Genetic rearrangements in leukaemia and lymphoma / J.M. Goldman, D.G. Harnden.

LEUKAEMIA AND LYMPHOMA RESEARCH 2

Genetic Rearrangements in Leukaemia and Lymphoma

EDITED BY
J. M. Goldman
D. G. Hamden

Published for the
Leukaemia Research Fund
by Churchill Livingstone

Author:	GOLDMAN (J.M.) & HAMDEN (D.G.) eds.
Title:	Genetic rearrangements in leukaemia and lymphoma

Acc. No.	Class Mark	Date	Volume
005257	WH 250	1986	

Genetic Rearrangements in Leukaemia and Lymphoma

Leukaemia and Lymphoma Research
Published annually on behalf of the Leukaemia Research Fund (United Kingdon)

Already published
VOLUME 1
Goldman & Jarrett: Mechanisms of Viral Leukaemogenesis

Volume 2
LEUKAEMIA AND LYMPHOMA RESEARCH

Genetic Rearrangements in Leukaemia and Lymphoma

J.M. Goldman DM, FRCP, MRCPath
Consultant Physician, Royal Postgraduate Medical School,
Hammersmith Hospital, London, UK

D.G. Harnden PhD, FRCPath, FIBiol
Director, Paterson Laboratories, Christie Hospital and
Holt Radium Institute, Manchester, UK

Published on behalf of the
Leukaemia Research Fund
43 Great Ormond Street
London WC1N 3JJ

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1986

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by
Churchill Livingstone Inc., 1560 Broadway, New York,
N.Y. 10036, and by associated companies, branches
and representatives throughout the world.

First published 1986

ISBN 0-443-03102-9

British Library Cataloguing in Publication Data
Goldman, John M.
Genetic rearrangements in leukaemia and lymphoma.
—(Leukemia—Genetic aspects 2. Lymphomas
—Genetic aspects
I. Title II. Harnden, D. G. III. Series
616.99'419042 RC463

Library of Congress Cataloging in Publication Data
Genetic rearrangements in leukaemia and lymphoma.
Leukaemia and lymphoma research; v. 2)
"Published on behalf of the Leukaemia Research
Fund."
1. Leukemia—Genetic aspects. 2. Lymphomas—Genetic
aspects. 3. Genetic recombination. 4. Oncogenes.
I. Goldman, J. M. (John Michael) II. Harnden, D. G.
III. Leukaemia Research Fund. IV. Series. [DNLM:
1. Leukemia—familial & genetic. 2. Lymphoma—familial
& genetic. 3. Oncogenes. 4. Recombination, Genetic.
W1 LE927 v.2/WH 525 G328]
RC643.G45 1986 616.99'419071 85-16580

Printed in Great Britain by
Butler & Tanner Ltd, Frome and London

Preface

This volume is the second in the series *Leukaemia and Lymphoma Research* initiated in 1984. We have devoted this volume to a consideration of the genetic rearrangements that have been identified in the various haematological malignancies and to changes in position and expression of the associated oncogenes. The first chapter by David Harnden is based on his paper for the Annual Guest Lecture and surveys some of the constitutional chromosomal abnormalities which appear to predispose to cancer and leukaemia. Herman van den Berge and Avery Sandberg in the second chapter have catalogued the specific cytogenetic abnormalities associated with leukaemia (and lymphoma) cells and have related these to predominant morphological features of these diseases. Alan Sakaguchi in the third chapter has described the techniques that are valuable in mapping oncogenes to particular chromosomal sites in animals and man and has reviewed inter alia concepts of the development of the *src* family of oncogenes. Perhaps of all chapters the fourth, that by Janet Rowley, best exemplifies the speed of the progression from knowledge of chromosomal changes, in this case the Ph′ chromosome, towards an understanding of the alterations in associated oncogenes, notably c-*abl*. This view may be challenged since as little as two years ago the translocations involving c-*myc* in the majority of cases of Burkitt's lymphoma, fully documented in the eighth chapter by Gilbert Lenoir, looked the more likely candidate to shed new light on mechanisms of malignant transformation.

The remaining three chapters in the volume are equally important. George Klein has reviewed the evidence for chromosomal translocations and oncogene involvement in experimental murine plasmacytomas. Terry Rabbitts has written on gene rearrangements in the production of antibodies and the T-cell receptor – the latter a field that has progressed so rapidly that the concept of homology between immunoglobulin and T-receptor genes was scarcely considered when the chapter was commissioned. Chapter 7 by Howard Temin provides a historical perspective and attempts also to look to the future.

There is little doubt that this volume comes at a timely moment. It should appeal to research workers and clinical haematologists with a special interest in the cytogenetics and molecular biology of malignant disease but it should also have a much wider audience. Any reader who wants a modern insight into

aspects of the new biology and its possible contribution of our understanding of the malignant process in general should find this book instructive and we hope also easy to read.

1986

J.M.G.
D.G.H.

The Leukaemia Research Fund

The Leukaemia Research Fund is the only national charitable foundation in Britain devoting all its resources to research and patient care in leukaemia and the related blood diseases. Founded in 1960, it is the third largest cancer organisation in Britain and is a member of the United Kingdom Co-ordinating Committee on Cancer Research. The Fund is advised by a distinguished Medical and Scientific Advisory Panel.

The Fund finances an expanding programme of research and has recently set up the Leukaemia Research Fund Centre at the Institute of Cancer Research in London for the study of the molecular and cellular biology of human leukaemia. It has also introduced a large scale progressive aetiology study in Britain with particular emphasis on the biology of the diseases, and has funded pioneering work in Britain on bone marrow transplantation. In addition, the Fund is involved in the clinical support of patients and provides a full information service. Its academic work, including international symposia, workshops and lectures, is complemented by an active policy of world-wide collaboration. The Leukaemia Research Fund Annual Guest Lecture is delivered by a scientist or doctor who has made a major contribution to knowledge of leukaemia and lymphomas.

Contributors

Herman Van Den Berghe
Professor of Human Genetics, University of Leuven, Belgium

David G. Harnden
Director, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK

George Klein
Department of Tumour Biology, Karolinska Institute, Stockholm, Sweden

Gilbert Lenoir
Division des Cancerogenes, Cliniques et Biologiques, IARN, Lyon, France

Terry H. Rabbitts
MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, UK

Janet D. Rowley
Professor, Department of Medicine, University of Chicago, USA

Alan Y. Sakaguchi
Research Assistant Professor, Departments of Medicine, and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas, USA

Avery A. Sandberg
Departments of Genetics and Endocrinology, Division of Medicine, Roswell Park Memorial Institute, Buffalo, New York, USA

Rebecca Taub
Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA

Howard M. Temin
American Cancer Society Research Professor, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin, USA

Contents

Genetic rearrangements in leukaemia and lymphoma

It is unfortunately commonplace, when considering one particular aspect of the aetiology or natural history of a disease, to focus on one area without taking into account the broad framework within which the disease process may develop. To do this is particularly important for neoplastic diseases since, not only are we dealing with a multiplicity of diseases, but also we know that a great many different factors may be involved in the aetiology of even one particular type of the disease. Therefore, in the following discussions, while it will be necessary to select specific examples and consider some aspects more than others, we must be prepared to accommodate information from a variety of different sources. Any proposed role for genetic change in leukaemia and lymphoma must be capable of encompassing our knowledge of the biology of leukaemogenic viruses, and the mode of action of both ionizing radiation and of carcinogenic chemicals in leukaemogenesis. It must allow for the fact that just as there are many steps from a mutant germ cell to an abnormal individual in the population, there is also a long way from a somatic cell modified by an extrinsic agent to a clinically recognizable cancer. While extrinsic factors may be important in later stages of disease development, we know that factors such as age, immune status, and hormone balance also play a crucial role. Any model for the role of genetic change must be able to accommodate the undoubted influence of these aetiological factors.

An understanding of the interaction between these factors is crucial. The clastogenic and mutagenic action of environmental agents may be radically altered by the genetic make up of the individual. Thus if we are to understand why a particular chemical is leukaemogenic, we must study the variation between individuals in the exposed population, as well as the properties of the specific chemical in bringing about cellular transformation. Similarly, it is not sufficient to recognize a genetic change in a leukaemic cell and assume that this is responsible for the neoplastic phenotype; we must ask how this change relates to the changes known to be induced by leukaemogenic agents. Are such changes preferentially induced, or are they selected because they confer new properties on the cells? If they are selected, what are the properties of the cells which lead to selection and what are the roles of the various factors – such as hormone balance, immune status and cell–cell interactions – which influence this

selection process? In other words, we must have not only a knowledge of the factors involved but a conceptual framework which attempts to describe the basic steps in the biology of the disease. At its simplest, we may recognize an 'initiation' event which converts a normal cell into a potentially malignant cell; and a further series of events, collectively referred to as 'progression', by which the modified cell progresses towards frank neoplasia. An important corollary is that progression is not inevitable. The potentially malignant cell may die or be killed by some sort of surveillance mechanism, or else the latent period – which elapses between initiation and clinical cancer – may exceed the lifespan of the individual.

Genetic changes may be important at several different points in this process:

1. Inherited variation may predispose cells to undergo an initiation event. It is, in practice, hard to differentiate between those inherited factors which in some way modify or block initiating factors, from those which act on the initiation itself.

2. The initiation event is now widely accepted to be of a genetic nature. Whatever other changes may occur in the transformed or potentially malignant cell – be they membrane changes, mitochondrial changes or modifications of other cellular organelles – it is hard to envisage how such changes may be carried from one cell generation to the next unless accompanied by either structural or functional modification in the genetic material of the cell.

3. Subsequent progression may depend on genetic change in two different ways. First, the induction of genetic variation may be important; and the initiation event must confer properties upon the potentially malignant cell, or cells, which give a selective advantage. Though much evidence suggests that many tumours are of clonal origin, this does not exclude the possibility that the clonal population is achieved by selection of one cell from amongst a population of highly variable initiated cells. Second, the selected clone may itself be unstable and this may increase the variability of the population within which selection is occurring. At one extreme, such variation may lead to more successful variants and hence progression or at the other to instability incompatible with life of the cell.

Therefore, the following discussion is set within the framework of a multifactorial aetiology and a sequential development of the disease, with genetic change being viewed as one of the many aetiological factors which operates (potentially) at several different levels in the progression of the disease. For many years ideas evolved almost independently and information accumulated in several different areas. However, in the past few years they have come to fit together in a most remarkable and exciting manner. I will now consider:

1. Genetic predisposition to the development of neoplasia;
2. Visible chromosome changes within leukaemic cells; and
3. The recognition of specific cellular genes (oncogenes) of special importance in the development of neoplasia.

I hope to show how these areas now fit together to increase our understanding of neoplasia.

INHERITED PREDISPOSITION

While environmental factors are important in all neoplastic diseases these external agencies are acting on a very variable population. Part of this variation will be genetically determined and it is axiomatic that all neoplasms will have a genetic component. Even if this is not immediately obvious in the majority of cases, the existence of a proportion of situations in which an inherited component is clearly important simply emphasizes that not only does the genetic component exist but that it is so important that gross variations of genetic control mechanisms are not readily tolerated. In many cases, the mode of operation of the genetic susceptibility is quite unknown. The ensuing discussion therefore considers three possible mechanisms and then goes on to examine several specific examples.

Mechanisms of inherited susceptibility

There are three levels at which inherited susceptibility might operate. First, if it is assumed that leukaemogenesis involves genetic rearrangement in the progenitor cell, then the inherited genetic abnormality may itself be part of that rearrangement. Cells carrying such a rearrangement would then be more prone to become neoplastic simply because they require one step less in the multistep pathway to neoplasia. Second, a constitutional mutation may predispose the cells to cancer by making it more probable that an initiation event will occur. Third, genetic susceptibility may have nothing to do with the initiation stage but be associated with an increase in the probability that an initiated cell may progress to further stages, either because the cell is made more variable or the cellular environment is made more favourable to the continued growth of the aberrant clone.

Direct Inheritance

If a constitutional mutation is itself part of the neoplastic pathway, either it must be a recessive gene at the cellular level or the probability of subsequent events must be very low indeed. For a tissue with a reasonable number of cells, a dominant cancer gene could be incompatible with differentiation or at least maintenance of normal function. At first this seems a paradox, since some of the cancer susceptibility genes appear to be inherited in a dominant manner[1]. However, there is recent strong evidence to back the concept that even in those instances where the familial pattern is one of dominant inheritance, the inherited gene is recessive at the cellular level. In the case of retinoblastoma – where Knudson[2] has postulated that two successive mutations may be necessary for neoplasia to develop – it has been shown in patients heterozygous

for marker genes close to the putative retinoblastoma gene locus on chromosome 13, that the retinoblastoma tumours themselves are homozygous for these genes, suggesting deletion or gene inactivation[3]. Chromosome deletion, monosomy and somatic recombination account for the unexpected homozygosity found in some cases, and it is suggested that the other cases (without an obvious cause) are due to point mutation or gene conversion. Such findings are compatible with the idea that two genetic events involving homologous loci are required for neoplasia. Following Knudson's ideas, sporadic cases would require both events to be acquired in a single somatic cell, whereas familial cases would inherit one event and acquire the second. Murphee and Benedict[4] hypothesize that recessive inheritance of one abnormal locus followed by a further genetic event at the homologous locus, or two successive somatic events, may result in the loss of a suppressor or regulatory function, which would normally control the proliferation of specific cell populations and thus lead to retinoblastoma. These attractive ideas might also extend to Wilms' tumour, where unexpected homozygosity in the tumour has been found in heterozygous patients involving the short arm of chromosome 11 – a region which is known to carry deletions in the specific group of Wilms' tumour patients with aniridia and genitourinary defects[5].

However, there is no evidence as yet to suggest that such mechanisms are important in leukaemia and lymphoma. There are relatively few inherited conditions which predispose to leukaemia or lymphoma (Table 1.1) and none of these is inherited in a dominant manner. In the dominantly inherited cancers, there is usually a focal tissue abnormality which precedes the development of

Table 1.1 Inherited conditions predisposing to leukaemia and lymphoma

Chromosome breakage syndromes		
Ataxia-telangiectasia	AR	lymphoma, lymphoid leukaemia
Bloom's syndrome	AR	lymphoma, lymphoid leukaemia
Fanconi's anaemia	AR	AMML
Immunodeficiency		
Agammaglobulinaemia (Bruton)	XR	lymphoma, lymphoid leukaemia
Common variable	AR	lymphoma
Severe combined	AR	lymphoma, lymphoid leukaemia
	XR	lymphoma
Adenosine deaminase deficiency	AR	lymphoid leukaemia
Wiskott-Aldrich syndrome	AR	lymphoma
X-linked lymphoproliferative syndrome (Duncan's disease)	XR	B cell lymphoma
Chediak-Higashi syndrome	AR	? lymphoma
Chromosome disorder		
Down's syndrome trisomy 21		acute leukaemia
Miscellaneous		
Kostmann's infantile agranulocytosis	AR	acute monocyte leukaemia
Glutathione reductase deficiency	AR	leukaemia (unspecified)

AR: Autosomal recessive.
XR: X-linked recessive.
AMML: Acute myelomonocytic leukaemia.

frank neoplasia. A focal abnormality in the haematopoietic system would rapidly become systemic, but there seems no *a priori* reason why haematopoietic cells should behave any differently from other somatic cells in this respect. The absence of dominantly inherited forms may indicate that this kind of mechanism is unlikely for both leukaemias and lymphomas.

Increased probability of initiation

There is little evidence that any inherited cancer prone condition depends upon an unusual interaction between a carcinogenic agent and the DNA of the cell. Variation depends rather upon the way in which the carcinogen is handled prior to interaction with the DNA and upon the way in which induced DNA damage is handled. Both are clearly under genetic control. Many potentially carcinogenic chemicals require metabolic activation for the carcinogenic properties to be realized. There are many examples of genetic control of the activation process, e.g. the inducibility of enzymes controlling the metabolism of polycyclic hydrocarbon carcinogens[6]. Until recently there had been little evidence of leukaemia induction in man by chemical carcinogens. However, convincing reports of acute leukaemia following long-term cytotoxic therapy[7] now make it particularly important to elucidate the mechanisms by which drugs induce leukaemias in a proportion of patients.

Similarly, there is a clear association between the degree of skin pigmentation and the induction of all types of skin cancer. Therefore the effect of UV irradiation is modified before it reaches the target cell by a genetically controlled characteristic. Once again, however, there is no evidence for a role for UV in leukaemogenesis but the principle is still an important one.

In some inherited human cancer prone diseases, an unusual susceptibility to an environmental agent is thought to be due to events occurring after the primary interactions between the agent and the cellular DNA. In particular, defects in repair of DNA lesions have been implicated. In xeroderma pigmentosum, for example, defects of DNA repair, increased mutagenesis and carcinogens following exposure to UV light are clearly associated[8]. In other diseases, where defects of DNA repair have been suggested as explanations of increased susceptibility to leukaemia or lymphoma, the association is not clear cut. The complexities are best studied by considering one specific example – ataxia telangiectasia (see below).

Susceptibility to progression

Once again there is compelling circumstantial evidence that the progression of a focus of neoplastic cells towards malignancy will be influenced by factors under genetic control, for example, hormone balance, immune response and so on. There are, however, remarkably few well documented cases. For example, there is good evidence that patients with inherited immunodeficiency syndromes have an unusual tendency to develop leukaemias and lymphomas.

This may be mediated by an effect on progression but proof is lacking. Much of the evidence comes from the work of the Immune Deficiency Cancer Registry in the University of Minnesota[9,10]. The main categories of genetically determined immune deficiency disease (GDID) recorded in the Minnesota registry are: ataxia telangiectasia (A-T), Wiskott-Aldrich syndrome (WAS), common variable immune deficiency (CVI), selective IgA deficiency, X-linked (Bruton) agammaglobulinaemia, severe combined immunodeficiency, selective IgM deficiency, immunodeficiency with normal or elevated immunoglobulins, and a miscellaneous group. All clearly show evidence of an increased incidence of neoplasia. A-T, WAS and CVI account for a high proportion of the total cases – 34.5, 17.8 and 25.3 per cent respectively (77.6 per cent of a total of 292 cases). Taking the group as a whole, 58 per cent of all the reported malignancies are lymphomas, and 17 per cent are leukaemias. Epithelial tumours account for 23 per cent. Bearing in mind the relative proportion of these neoplasms in the population at large, this clearly represents a major excess of leukaemias and lymphomas. In children, the most likely tumour is non-Hodgkin's lymphoma, while in adults non-Hodgkin's lymphoma and carcinomas occur in about equal proportions.

Regardless of whether the defect is primarily in cell-mediated or in humoral immunity, the neoplasia affects particularly the lymphoid system. If the increase in malignancy in immune deficient patients is due to failure of a surveillance mechanism, one would have expected a wider spectrum of malignancy. One must therefore propose either that surveillance is only important for tumours of the lymphoid system, or that these genetic mechanisms are operating in an entirely different way. For example, it is possible that the primary defect which leads to immune deficiency also leads to an enhanced probability of a neoplastic focus arising specifically within the lymphoid system.

It is apparent that any attempt to specify the mechanism by which inherited susceptibility operates is, at present, fraught with difficulties.

Chromosome disorders associated with susceptibility to leukaemia and lymphoma

There are many reports of associations between constitutional chromosome abnormalities, leukaemia and lymphoma. For example, Langlands and Maclean[11] reported a patient with a t(14;21) translocation, who had two successive primary lymphoid neoplasms. Acute lymphoblastic leukaemia has been reported[12] in a patient with a t(4;14) translocation, acute myelogenous leukaemia has been reported[13] in a patient with a ring chromosome, and there are many other examples. While it would be unwise to deny a connection between the constitutional abnormality and the disease process, the absence of a consistent association makes this less likely. There are, however, some examples where a small number of cases may be significant. The report of first two[14] and later three[15] cases of chronic lymphocytic leukaemia (CLL)

associated with deletion of the G-group chromosome is particularly interesting. Such a chromosome is usually considered a 'normal variant' and is not widely associated with CLL, but in this particular family it may be significant.

The only situation in which there is a clear-cut association between a constitutional chromosome abnormality and leukaemia is Down's syndrome[16,17]. The types of leukaemia observed in Down's syndrome are the same as in other children and this has led to the suggestion[18] that specific genes located on chromosome 21 might be important in the development of both lymphocytic and non-lymphocytic leukaemia. However, recent developments in the oncogene field have not yet elucidated this problem – it would clearly be a profitable area for further study.

Single gene disorders associated with susceptibility to leukaemia and lymphoma

Familial cases and rare syndromes

There are many reports of familial associations of leukaemia or lymphoma. Some of these, especially reports of two cases only, will be chance association but others are clearly more significant. For example, multiple cases of chronic lymphocytic leukaemia in a single family are often reported. Blattner et al.[19] describe a particularly interesting family with three sibs all with CLL combined with immune defects. Similarly, Greene et al.[20] reported that in 526 consecutive patients with T cell lymphomas he found 21 first degree relatives with lymphoproliferative and haemopoietic malignancies. This suggests a strong genetic component, but the possible role of viruses (such as, human T-cell leukaemia viruses – HTLV) in these patients would also have to be considered.

Some rare syndromes are associated with leukaemia. Kostmann's infantile agranulocytosis is a rare recessive disease, in which acute lymphoblastic leukaemia has been reported in two sibs[21] and Gilman et al[22] have reported two cases of this disease with acute monocytic leukaemia. Malignant reticuloendotheliosis in a single family has been reported by Falletta et al.[23] – since 17 males were affected in two generations this appears to be a genuine X-linked inheritance of a haemopoietic malignancy. The Poland syndrome (unilateral aplasia of part of the pectoralis major muscle, together with syndactyly and brachydactyly) has been associated with leukaemia in six instances[24], but McKusick [25] regards most instances of this syndrome to be of sporadic occurrence.

Chromosome breakage syndromes

The most extensive information relates to the three recessively inherited syndromes, in which the occurrence of spontaneous chromosome breakage has been unequivocally demonstrated and where there is an increased incidence of malignant disease, including leukaemias and lymphomas[26]. These are Bloom's

syndrome, Fanconi's anaemia and ataxia-telangiectasia. Other diseases have been reported by some authors to be associated with both chromosome abnormalities and leukaemia, for example incontinentia pigmenti[27], but the reports are so sparse that they will not be considered further.

Bloom's syndrome. This rare disease has been very thoroughly documented by German and his colleagues in a remarkable series of papers[28–31]. Very briefly these patients are of short stature, have a characteristic facies, are sun sensitive and show susceptibility to infection linked to an impairment of immune function[32]. They have a very characteristic pattern of spontaneous chromosome aberrations in cultured lymphocytes, skin fibroblasts and marrow cells. Though many different types of aberration occur, the high incidence of symmetrical chromatid exchanges between homologous loci is particularly interesting. Similarly, the lymphocytes, fibroblasts and bone marrow cells all show dramatically elevated spontaneous levels (approximately 10-fold) of sister chromatid exchanges (SCE). One curious finding is that a proportion of lymphocytes in some patients have normal spontaneous levels of SCE's[29].

Bloom's patients have an unusually high susceptibility to develop malignant diseases in many different tissues, but there is a marked preponderence of leukaemias and lymphomas[26,32]. In 99 patients with Bloom's syndrome, there were 26 malignant neoplasms which included: five non-lymphoid leukaemias; four lymphoid leukaemias; one Hodgkin's disease and seven non-Hodgkin's lymphomas. There is no firm evidence to suggest a specific aetiology. There is some evidence of UV sensitivity at the cellular level, but this is not generally agreed[33]. There is better agreement on sensitivity to ethyl methanesulphonate (EMS). Krepinsky et al.[34] found Bloom's cells sensitive to the induction of chromosome aberrations and SCE's by EMS, while Arlett and Harcourt[35] found increased sensitivity to cell killing by EMS. The defective immunity may play a part in the increased susceptibility to malignant disease, but the particular increase in lymphoid neoplasms may suggest a specific disturbance of the lymphoid system.

Fanconi's anaemia is characterised by a progressive pancytopenia, short stature, and skeletal abnormalities especially hypoplasia of the thumb and radius. The spontaneous occurrence of chromosome aberrations is well documented, being characterized particularly by non-homologous chromatid interchanges[36]. Cells from Fanconi patients are particularly sensitive to the chromosome damaging and cell killing effects of DNA crosslinking agents, such as mitomycin C[37] and diepoxybutane[38]. These patients undoubtedly show an unusually high incidence of leukaemia, especially acute myelomonocytic leukaemia, and also of other tumours, especially liver tumours. Some reports show regression of liver tumours following cessation of treatment with drugs such as oxymetholone[39]. Such observations, and the chronological pattern of reports of malignancy in Fanconi patients, led German[26] to suggest that the possibility that the increased risk of malignancy in Fanconi patients is due to the use of anabolic steroids and other drugs cannot yet be ruled out.

Ataxia-telangiectasia (A–T). The features of this disease make it particularly

suitable for a discussion of the relationship between chromosome aberrations and leukaemia and lymphoma. A recent monograph gives all the background details[40]. An outline of the characteristics of this rare autosomal disease is given in Table 1.2. The major clinical problem is the progressive cerebellar ataxia with an onset at about 18 months of age. The telangiectasia – which occurs somewhat later, and is distributed on the bulbar conjuctivae, the ears and sometimes the butterfly area of the face, the hands and the feet – is a useful sign in differential diagnosis. A variety of other clinical problems have been reported in a proportion of these patients.

Table 1.2 An outline of the characteristics of ataxia–telangiectasia

Cerebellar degeneration	Atrophy of lymphoid tissue
Ataxia, choreoathetosis, absence of reflexes, grimacing	Defect of cellular immunity, IgA and IgE deficiencies, susceptibility to infection
Radiosensitivity	Telangiectasia
Drug sensitivity	Elevated serum α-fetoprotein
Defect of DNA repair Spontaneous chromosome breakage	Elevated serum carcino-embryonic antigen, Insulin resistance, Ovarian follicular agenesis, Premature ageing.
Susceptibility to malignant disease	

Attention was first drawn to the excess of leukaemias in the disease by Hecht et al[41] and has been documented in detail by Spector et al.[42]. An outline of their results is given in Table 1.3. It is clear that there is an excess of patients with lymphoreticular malignancies. Out of 108 cases of malignant disease 11 per cent had Hodgkin's disease, 44 per cent had non-Hodgkin's lymphoma (including three cases of Burkitt's lymphoma) and 24 per cent had leukaemias (mostly lymphocytic) while only 20 per cent had solid tumours of other kinds (cf. 35 per cent in Bloom's syndrome). The ages at which these malignancies occur indicates clearly an increase in all types but a particularly large excess of lymphoid neoplasms.

It is important to note that A–T patients are sensitive to ionizing radiation at the clinical level[43], at the cellular level[44] and at the chromosomal level[45]. A–T cells are also unusually sensitive to radiomimetic drugs, such as bleomycin[46] streptomycin and neocarzinostatin[47]. However, reports of sensitivity to other agents are conflicting and either await confirmation or have been discounted.

These observations all suggest an inability to repair DNA strand breakage. However, specific studies have failed to show any defect of rejoining of either single or double strand breaks[48] but recent evidence suggests that the fidelity of repair of strand breaks may be low[49]. Reports on the fundamental nature of the basic defect in A–T are still conflicting, although there clearly is a defect in capacity to repair DNA damage, as measured by potential lethal damage repair[50]. One important area of agreement is that A–T cells do not respond to

Table 1.3 Neoplasms in ataxia-telangiectasia (abbreviated from Spector *et al.*[42])

	Cases	Age (years)
Hodgkin's disease	12	
Burkitt's lymphoma	3	
Other non Hodgkin's lymphoma	45	
Acute lymphocytic leukaemia	20	
Acute leukaemia (unspecified)	5	
Chronic lymphocytic leukaemia	1	
Brain tumour	3	2, 6, 13
Carcinoma of stomach	8	14, 14, 19, 21, 22, 26, 26, 26
Dysgerminoma	3	16, 16, 16
Basal cell carcinoma	3	16, 21, 25
Carcinoma — Larynx	1	12
Carcinoma — Parotid	1	16
Carcinoma — Liver	2	24, 26
Carcinoma — Breast	1	30
Total	108	

N.B. Six patients also had a second neoplasm

low doses of ionizing radiation by reducing replication synthesis of DNA as do normal cells[51,52]. Therefore after exposure to ionizing radiation, it is possible for A–T cells to continue to synthesize DNA, while the DNA still contains primary radiation induced damage – this would then be converted into lethal damage without there being any chance for the repair processes to function. The use of recombinant DNA plasmids for studying repair capacity in mammalian cells may provide a tool for more detailed analyses of this problem[48].

Thus the study of the repair of DNA lesions has still not clarified the cancer susceptibility in A–T. There is, however, another feature of particular interest in these patients, namely the spontaneous occurrence of chromosome aberrations. Both chromatid and chromosome aberrations occur spontaneously, but the most dramatic feature is the occurrence of clones of T-lymphocytes with specific chromosome rearrangements[53]. They have been observed to increase with time[53,54,55], have a doubling time of approximately 6 months and may come to represent 80–90 per cent of all phytohaemagglutinin responsive cells. In the majority of cases, no haematological abnormality is observed during this period of clonal expansion. These clones therefore have a proliferative advantage but proliferate at the expense of similar cells so that the total white count and the differential are not disturbed.

While the precise chromosomal rearrangement varies from clone to clone, there is a consistent involvement of specific sites. In particular, band 14q12 is involved in the majority of the rearrangements. This site exchanges with a

limited number of other loci, notably 14q32 (either by inversion on the same chromosome or translocation with the homologous chromosome), 7p14,7q35 and Xq27. This pattern of rearrangement, involving one particular site exchanging with a limited number of other sites, bears a striking resemblance to the rearrangements involving chromosome 8 and other chromosome loci in B-cell lymphomas and chromosome 21 and other chromosome loci in chronic myeloid leukaemia.

Thus the question arises as to whether or not there is any connection between these clones and the development of lymphoid neoplasms. There is good evidence in some cases that a chromosome marker, observed before leukaemia has developed, is also present in the leukaemic cells (see ref. 56, cases 1, 2 and 3). These were all chronic or subacute T-cell leukaemias and it may be significant that each case involved the loss of the proximal portion of chromosome 14.

Taylor[55] reported a case of A–T with a complex clone involving among other rearrangements an inversion of chromosome 14, inv(14)(p12;q32), and isochromosome for the long arms of chromosome 8. At that time this patient was haematologically normal but later he developed a lymphocytosis and appeared to be gradually developing into a chronic T-cell lymphocytic leukaemia (Taylor, personal communication). This is of particular interest since Zech et al.[57] described inv(14)(p11;q32) and isochromosomes for the long arms of chromosome 8 in several cases of chronic T-cell lymphocytic leukaemia. However, in a case described by Wake et al[58], it is clear that the leukaemic cells did not have the marker observed in a pre-existing clone. In one case of Burkitt's lymphoma in an A–T patient, the blood lymphocytes – prior to diagnosis of the lesion – were normal, while the tumour cells carried the classic t(8;14) translocation.

It would therefore be reasonable to regard the clones not only as potentially neoplastic, but also as an indication that abnormal lymphoid cell proliferation is likely to occur in these patients. In such a patient, another clone of lymphoid cells – more aggressive than the ones observed during the non-neoplastic phase of the disease – may therefore emerge.

Conclusions

From a consideration of these diseases it may be concluded that:

1. At least some inherited genes, which lead to leukaemia and lymphoma, are associated with events which regularly lead to genetic rearrangements.
2. Some of these rearrangements are either highly specific or involve particular regions of particular chromosomes.
3. Such rearrangements are associated with clonal proliferation.
4. Clonal proliferation itself may not be sufficient for frank neoplasia – suggesting that further changes must be superimposed on the original event.

CHROMOSOME ABERRATIONS IN LEUKAEMIC CELLS

The occurrence of gross chromosome abnormalities in malignant cells has been recognized for a long time. Around the turn of the century, Theodor Boveri (working in Wurtzburg in Germany) made a series of observations on which he based his ideas on the origin and nature of cancer, which were published as a monograph in 1914[59] (also see Wolf[60]). This is a truly remarkable work in that many of his conclusions and speculations about genetic change and neoplastic disease are now being shown to be remarkably accurate, three-quarters of a century later. A summary of some of his more important conclusions is given in Table 1.4. It is clear that he regarded leukaemia and solid tumours as belonging to the same group of diseases. It seems especially interesting that he should have regarded genetic change as being causal and that among his ideas was the suggestion that homozygosity for an aberrant gene might be a means of generating an abnormal population of cells – a situation which (as we have already seen) has now been shown to be a probable mechanism in retinoblastoma.

It is all the more surprising therefore that some of the controversies aroused by Boveri's work have remained unresolved until today. The central argument has revolved around the significance of visible chromosome changes in cancer cells. Are these changes causal? Are they secondary changes but nevertheless important in the development of the neoplastic process? Or are they epiphenomena quite irrelevant to neoplasia?

Table 1.4 Predictions by Boveri

1. Genomic rearrangements are causal
2. Tumours have a monoclonal origin
3. Specific aberrations will be associated with particular neoplasms
4. Some cancers with apparently normal chromosomes will prove to be pseudo diploid
5. State of differentiation of tissue will be critical
6. Genomic changes are caused by exogenous agents
7. Some apparently normal cancers will prove to be pseudo diploid
8. Leukaemias will be similar to solid cancers

Technical problems

The principal reason is certainly technical. While it was possible for Boveri to recognize that chromosome abnormalities could explain the development of a neoplasm almost 100 years ago, more than 50 years elapsed before technical innovations led to greatly improved human chromosome preparations. In the case of the leukaemias the bone marrow culture technique of Ford, Jacobs and Lajtha[61] followed very quickly by the culture techniques for peripheral blood lymphocytes[62], proved to be a major turning point. The quality that can be achieved with current banding methods is quite remarkable. However, even

now it is recognized that in preparations which show normal cells to have very sharp distinct chromosomes, the chromosomes of leukaemic cells may be 'fuzzy' and/or short and stubby, so that preparations of adequate quality are often hard to achieve. A second difficulty is that, even with preparations of good quality, the complexity of the abnormalities observed is often so great that it is not possible to identify the precise nature of the rearrangements involved. When complex arrangements are identified they frequently appear to be random and therefore no particular significance can be assigned to them.

Over the past few years, however, the painstaking collection of data on well documented cases by individuals[63] or by groups e.g. *The International Workshops on Chromosomes in Leukaemia*[64–67] have led to the recognition of the fact that amongst this almost bewildering array of chromosome changes there are many examples of specificity which clearly are of great significance. In this respect the study of the leukaemias and lymphomas is far ahead of the study of solid tumours where the technical problems are even greater.

Highly specific rearrangements

The first specific rearrangement recognized in a leukaemia was the Philadelphia (Ph^1) chromosome – an unusually small acrocentric chromosome first described in chronic myeloid leukaemia by Nowell and Hungerford in 1960[68] and which we now know to be a translocation between chromosomes 9 and 22 – t(9;22)(q34;q11). In passing, it may be of interest to record that the name Ph^1 was not given by Nowell and Hungerford but by Baikie et al.[69] following a convention laid down at the first human chromosome nomenclature conference held in Denver in 1960.

Since then, many specific associations have been recognized and some of the important ones are listed in Table 1.5. This is not the place to discuss individual aberrations in detail, but it is worth discussing the reasons why it has taken rather a long time to make sense of these specificities. There was a great surge of interest, following the discovery of the Ph^1 chromosome, by those who believed that this might be a way of getting at causal mechanisms in leukaemia. Little progress was made and the arguments against an aetiological significance for chromosome aberrations in leukaemia looked quite powerful. In particular, a relatively high proportion of cases of leukaemia were found to have apparently normal chromosomes (Table 1.6). If this was the case, how can a particular significance be attached to those aberrations – even if apparently highly specific – which are observed in identical or similar cases? A second problem was that even within a single case, some of the cells might be abnormal but others quite normal. It has been shown that this admixture of normal and abnormal cells was of clinical significance in leukaemia – cases in which all the cells have abnormal chromosomes tend to have a worse prognosis for all kinds of leukaemia[66]. However, this posed conceptual problems when considering the role of these abnormalities in the biological evolution of the leukaemic cell.

Table 1.5 Examples of the specific association between subtypes of leukaemia, lymphoma and chromosomal abnormalities

		Proportion of Cases (%)
CGL	t (9;22) (q34;q11)	90
AML (M2)	t (8;21) (q22;q22)	9
Burkitt and B cell lymphoma and leukaemias	t (8;14) (q24;q32) t (8;22) (q24;q11) t (2;8) (p12;q23)	100
AML (not subtype specific)	t (6;9) (p23;q34)	8 cases
APL	t (15;17) (q25;q22)	41 (?100)
AMoL	t (9;11) (p21;q23)	3 cases
ALL (non B non T)	t (9;22) (q34;q11)	16
ALL (not defined by FAB or markers)	t (4;11) (q21;q23)	5

ALL: Acute lymphoblastic leukaemia.
AML: Acute myeloid leukaemia.
AMoL: Acute monocytic leukaemia
APL: Acute promyelocytic leukaemia
CGL: Chronic granulocytic leukaemia

The problem of 'normal' cases.

In the past few years, a clearer understanding of these problems has been emerging and there are several reasons for this. First, the technical quality of preparations from leukaemic cells has improved considerably. New techniques have been developed for examining prophase or very early metaphase chromosomes[70,71]. Even though the chromosomes of leukaemic cells do not band easily, these techniques have led to the recognition of abnormalities in cells previously thought to be normal. Yunis has suggested that all[72], or most,[73] patients with acute non-lymphocytic leukaemia (ANLL) have chromosome abnormalities in bone marrow preparations.

In the earlier paper, Yunis makes it quite clear that abnormalities can be detected using the methotrexate cell synchronization technique in cases which, using conventional procedures, appear normal (Table 1.7). In the later paper, abnormalities were found in 49 out of 51 patients studied, of which 46 had clonal abnormalities. Therefore, together with the earlier paper, where all 24

Table 1.6 Frequency of abnormal chromosomes in leukaemias

	Cases (untreated)	Normal	Some normal	Abnormal
ANLL[a]	241	102	80	59
ALL[b]	330	112	153	65

[a] First International Workshop, 1978[64]
[b] Third International Workshop, 1981[66]
ANLL: Acute non-lymphocytic leukaemia
ALL: Acute lymphoblastic leukaemia

Table 1.7 Chromosomes of acute non-lymphocytic leukaemia

	Cases (untreated)	Normal	Some normal	Abnormal
'Synchronized'[a]	24 (18)	0	15	9
Direct	24 (18)	11	10	3

[a] Yunis *et al.*[74]

cases were found to be abnormal, 73 out of 75 patients with ANLL have been found to have abnormalities. Previous studies had shown that approximately half of the cases of acute lymphoblastic leukaemia (ALL) and ANLL had normal chromosomes. It will be very important to get confirmation of Yunis's results from other laboratories, who can reproduce the exceptionally high quality of Yunis's preparations. If this proves to be the case, this will remove one of the major problems in ascribing aetiological significance to chromosome aberrations in leukaemias.

Probably the most important point that has been emerging over the past few years is that specific chromosome abnormalities are associated with specific sub-classes of disease. At the present time, it is not sufficient to look at, say, all cases of ANLL or of non-Hodgkin's lymphoma and expect to find a common aberration. For example, chromosome aberrations including the t(8;14) or other rearrangements involving chromosome 8 (Table 1.8) are found in only 4.9 per cent of the cases of ALL, but if subgroups are defined by the French-American-British (FAB) classification or by the presence of cell surface markers, 71 per cent (15/21) of L3 cases and 75 per cent (12/16) of B cell cases have such abnormalities (*Third International Workshop on Chromosomes in Leukaemia*[66]).

This is further backed up by the observation that when adequately studied, all B cell lymphomas and B cell ALL's appear to have one of the following translocations: t(2;8), t(8;14), or t(8;22)[75,76]. One has to be just a little cautious,

Table 1.8 Translocations involving chromosome 8 in ALL (Reproduced from the Third Workshop on Chromosomes in Leukaemia[66].)

		Cases	t(8; 14) or other t8	Percent
All	ALL	346	17	4.9
	FAB L1	125	1	0.8
	L2	157	0	0
	L3	21	15	71.4
	Markers:			
	'Null'	132	0	0
	T	34	0	0
	B	16[a]	12	75

[a] Other 4 had 14q+
ALL: Acute lymphoblastic leukaemia

since there are still some cases which do not seem to have any abnormality[77]. However, in this particular case[33], the assumption was made that divisions of unstimulated peripheral blood cells were representative of the malignant population.

Such subclassification is becoming apparent in many cases and led Yunis[73] to suggest that the type of aberration found in the majority of ANLL patients could be predicted on the basis of FAB classification and moreover, that cytogenetic studies in such cases are now an essential part of the diagnosis and assessment of prognosis. The recognition of the association of specific chromosome regions with specific leukaemia types will also facilitate the assignment of genes controlling differentiation and development of haemopoietic cells to specific chromosomal loci.

The problem of normal cells

Yunis[73] also touched on the second problem, namely the fact that only some of the cells studied show the specific abnormality. In some cases, direct examination of the bone marrow showed only normal cells, whereas short term culture revealed at least a proportion of abnormal cells. This both explains some of the so-called 'normal' cases and gives an indication of the explanation of cases with mixtures of normal and abnormal cells. Cells with normal chromosomes are likely to be normal cells from other lineages not involved in the leukaemic process and not yet displaced from the marrow by the leukaemic cells. This concept first emerged from the study of the chromosomes of cases of acute promyelocytic leukaemia (APL), where a specific translocation t(15;17) has been recognized for some years. At the *Second International Workshop on Chromosomes in Leukaemia*[65] a curious variation in geographic distribution, (see Table 1.9) of the anomaly was apparent, which suggested that perhaps ethnic or perhaps different environmental factors were involved in the aetiology of the disease. However, recent work by several groups[78] has drawn attention to the probability that this distribution is a technical artefact. Chromosomal preparations from APL patients have been made by different techniques in different laboratories, some direct and some by short term (24–48h) culture. Short term culture gives a higher proportion of positive cells and it has been shown very elegantly that two different populations of cells are being studied – the chromosomally normal erythroblasts and the chromosomally abnormal promyelocytes. In short term culture, metaphases from the promyelocytes predominate and laboratories using short term culture have a high proportion of positive cases. The direct method, usually thought be to preferable in the study of the chromosomes of leukaemic patients, can fail to reveal the abnormal population. When comparisons are made between laboratories using similar techniques, consistent results are obtained and moreover all cases of APL have the t(15;17) translocation.

While not all of the currently recognized specific aberrations can be defined in terms of clinical or haematological parameters, it seems possible that in

Table 1.9 Geographical distribution of cytogenetic categories of acute promyelocytic leukaemia (APL) cases (reproduced from the Second International Workshop on Chromosomes in Leukaemia[65].)

	Cytogenetic category		
Country (city)	Normal	t(15q+;17q−) or t(15q+;17q−) plus other changes	Other changes only
USA (Chicago)	0	6	0
USA (Buffalo)	13	1	4
USA (Rochester, MN)	2	0	0
Belgium	2	16	0
France	4	8	0
Finland	7	0	2
Australia	5	0	1
England	1	0	0
West Germany	3	0	0
Italy	3	0	0
Japan	0	2	0
Total	40	33	7

future further subdivisions will be recognized which coincide with the cytogenetic subgroups. However, it is important not to force this subgrouping too far, since in the case of chronic myeloid leukaemia, some cases which clearly fit the broad haematological definition do not have the Philadelphia chromosome and clearly the Ph[1] chromosome does occur in some cases of ALL.

Less specific aberrations

Not all the aberrations which have been described are quite as specific as those considered above. A rather more diffuse pattern emerges from an overall consideration of chromosome abnormalities in neoplasia. Mitelman and Levan[79] showed that deletion of certain chromosome segments, e.g. 5q22–34, 6q24–qter and 7q33–qter, occurs very frequently across a wide spectrum of neoplasms including leukaemias, lymphomas and other solid tumours. Similarly, triplication of a segment of a particular chromosome, e.g. the long arm of chromosome one, occurs in acute myeloid leukaemias[80] and also in many other neoplasms. This clearly prompts the question – which genes occur in those regions that when duplicated (in some cases) or deleted (in others) are so important for neoplasia? Molecular techniques described elsewhere in this volume are beginning to give answers to this question.

Complex abnormalities

Lastly, it is very important to remember that, while these specific or regional abnormalities may occur in isolation, they are often picked out from a complex background of other abnormalities. There is some correlation with the stage of

the disease. In chronic myeloid leukaemia the presence of aberrations other than the Ph[1] chromosome, while not always indicative of blastic crisis, often signals the onset of the acute phase – the emergence of particular abnormalities, such as the isochromosome for the long arm of 17 or a second Ph[1] chromosome, are particularly sinister. However, evidence of change from one sample to the next serial sample is more significant. In all kinds of leukaemias and lymphomas there is a tendency for greater complexity to accompany more advanced disease. It seems probable that some of these further abnormalities, e.g. iso(17)q, though not the primary lesion, are very important in determining the progression of the disease. In other cases the complex rearrangements may reflect a decrease in the stability of the genome rather than specific events of particular significance in the disease process.

Conclusions

1. Highly specific rearrangements are associated with specific subclasses of leukaemia and lymphoma.
2. Certain chromosome regions seem important in a wide range of neoplasia including leukaemia.
3. A point may be reached in the evolution of the disease, where a complete breakdown in the stability of the genome occurs.

ACTIVATION OR INDUCTION OF SPECIFIC GENES

The foregoing discussion therefore focuses attention on specific genes, chromosome loci or regions, which are associated in some way with either abnormal cellular proliferation or with frank malignancy. From what we know of the neoplastic process we may predict the broad categories of function in which such genes might be involved. For example:

1. Genes which control signals for entry into the cell cycle.
2. Genes which control the synthesis of enzymes or other macromolecules critical for progression through the cell cycle.
3. Genes which control the response to differentiation signals or which themselves code for such differentiation signals.
4. Genes which are concerned with other forms of intercellular communication.

On the other hand it seems improbable that genes concerned with specific products of differentiated cells such as pigments or structural proteins will be candidates for 'cancer genes'.

If 'cancer genes' are genes which control vital normal cellular function, it follows that there must be some modification – either mutational or regulatory – which leads to an abnormal product or an abnormal level of expression.

There are a host of questions that require answers. Are the chromosome abnormalities that we see in leukaemia in any way associated with the process of modification or activation? If they are, are they selected from amongst

randomly occurring genetic changes or are there regions of the chromosomes where such modifications are more likely to occur? If the observed aberrations are derived by selection, we have a range of clastogens which could be leukaemogens. If the changes are induced specifically, is there a particular agent which can modify the chromosome in a particular and repeatable way? Or is it possibly a reflection of some functional association between chromosome regions?

In the early 1960's there was a lot of interest in viruses as a cause of human leukaemia but there was remarkably little evidence, in spite of what was by that time the overwhelming evidence of virus involvement in murine and avian leukaemia. One particular observation made at this time linked in my mind genetic change in leukaemia and the potential of viruses as agents of specific genetic change – a patient with Klinefelter's syndrome who was a 46XX/47,XXY mosaic, also had chronic myeloid leukaemia[81] and there were both normal cells and Ph^1 positive cells in both cell lines. The most probable explanation was that the Ph^1 chromosome had arisen on two separate occasions, in the same individual. One can, therefore, postulate a directed exchange rather than the improbable occurrence by chance of two identical genetic lesions in the same individual. At that time it seemed to me that the involvement of a virus in this process would be one way to explain this phenomenon. This led me to become involved in the study of viruses as specific chromosome breaking agents[82] and so to the study of cellular transformation by oncogenic viruses.

Cellular transformation by viruses

The phenomenon of cellular transformation by oncogenic viruses depends, in most instances, on the delivery of a specific region of the virus genome to the cell which may be integrated into the host cell genome (adenovirus, polyoma virus), or is enabled to persist in a free replicating episomal form (EB virus, papilloma viruses). The cell acquires new properties, which resemble in many respects those of malignant cells, but the behaviour of these cells *in vivo* will depend upon the precise cell–virus interaction and upon the response of the host to the transformed cell – for example, not all virus transformed cells are malignant. Studies with both DNA virus and RNA virus have shown that the transforming activity usually resides in a small and clearly defined region of the virus genome. Two recent reviews of polyoma virus as a model for a DNA virus[83] and avian retrovirus as a model for RNA viruses[84] give up-to-date summaries. So far as leukaemia and lymphoma are concerned, the only viruses known to be directly involved are the RNA retroviruses and members of the DNA herpesvirus group. The mechanism by which the herpes viruses interact with cells is poorly understood partly, at least, because of the large complex genome and partly because of the absence of a suitable *in vitro* model system. The involvement of EB virus in the aetiology of Burkitt's tumour is well documented; but, as yet, the regions of the genome which are involved in cellular transformation are not clearly defined.

Therefore attention must focus, at present, on the RNA retroviruses. Specific types of leukaemia in several species are associated with particular RNA retroviruses, which have very precise target cell specificities. They may be divided broadly into two classes:

1. Those which induce rapid cellular transformation and which, on injection into an appropriate host, will cause leukaemia after a brief latent period; and
2. Those slowly transforming viruses, which do not normally transform cells in culture and which cause leukaemia in the intact animal, only after a prolonged latent period.

Rapidly transforming viruses contain transforming sequences (v-onc genes), while slow transforming viruses do not. Most, if not all, normal cells contain regions of the DNA with close sequence homology to these viral onc genes. These cellular sequences have been termed proto-oncogenes or c-onc genes. Since the cellular onc-genes contain introns and the v-onc genes do not, it is implied that the v-onc genes have been derived in an evolutionary sense from the cellular sequences by a process involving reverse transcription. The c-onc genes do not themselves have the capacity for cellular transformation. It is clear that activation must occur and also that this may arise in a number of different ways. There are clear examples of activation by gene mutation, gene deletion, introduction of promoting or enhancing sequences, and possibly gene amplification. The c-onc genes are therefore good candidates for cancer genes in the sense described earlier. They are apparently genes controlling vital, normal cellular functions whose mutation or misregulation are associated with neoplastic progression.

This is not the place to go into detail of the nature of onc genes, but rather to consider their possible role in human leukaemia. Attention was drawn to the proximity of the onc genes to points of chromosome exchange and Table 1.10 gives a list of human onc genes, which have been located at specific chromosomal sites – either by *in situ* hybridization, or by somatic cell genetic techniques involving hybridization of cells containing specific translocations. Some of the localizations are quite precise, while some are, at present, only tentative. It is clear that a number of these onc genes are located at or close to the break points of the specific translocations observed in human leukaemias. In particular, c-*myc* is located at the breakpoint on chromosome 8 in the t(8;14) and the other translocations involved in B-cell lymphomas[8]. It is of great interest that the secondary break points on chromosomes 14, 2 and 22 are close to the loci coding for the immunoglobulin heavy chain, the κ light chain and the λ light chains respectively [86,87]. An exactly analogous situation exists in the mouse, where rearrangements involve chromosome 15 and the chromosomes which carry the immunoglobulin genes (Table 1.11). The direct involvement of the c-*myc* gene and the immunoglobulin loci in these exchanges is quite clear from the description of the junction fragments of DNA which contain sequences of both genes[88,89]. The precise mechanisms involved are not yet clear.

The breakpoint on chromosome 9 in the t(9;22) translocation in chronic myeloid leukaemia (CML) is close to the location of the c-*abl* gene; indeed it is

Table 1.10 Chromosomal locations of human oncogenes

Oncogene	Chromosomal site	Specific chromosome site	Neoplasm
n-*ras*	1		
c-*myb*	6q 21	6q –	Lymphoma
c-Ki *ras*1	6		
c-*mos*	8q 22	t(8;21) (q22;q22)	AML
c-*myc*	8q 24	t(2;8) (p12;q24)	B cell
		t(8;14) (q24;q32)	Lymphoma
		t(8;22) (q24;q11)	
c-*abl*	9q 34	t(9;22) (q34;q11)	CML
c-Ha *ras*1	11p 14.1	11p 13	(Wilms' aniridia)
or	11p 15	t(3;11)	Renal cell carcinoma
c-Ki *ras*2	12p 12.1		
or	12p 24.2		
c-*fes*	15q 24-25	t(15;17) (q25;q22)	APL
c-*src*	20q		
c-*sis*	22q	t(9;22) (q34;q11)	CML
		t(8;22) (q24;q11)	B cell lymphoma
c-Ha *ras*2	X		

AML: Acute myeloid leukaemia
CML: Chronic myeloid leukaemia
APL: Acute promyelocytic leukaemia (FAB M3)

clear that the c-*abl* gene is translocated along with the terminal portion of chromosome 9 on to the small abnormal chromosome 22, the Ph[1] chromosome[90]. Similarly, the c-*sis* gene normally located on chromosome 22 is translocated to the abnormal chromosome 9. There is some evidence of abnormal expression of the translocated c-*abl* gene in CML[91]. It is not clear why this exchange should be important in CML. What is clear, however, is that this exchange on its own is not sufficient for cellular proliferation, let alone neoplasia, since the translocation is present also in cells of the erythroid series in CML patients, which remain largely under normal regulatory control. This could suggest that

Table 1.11 Immunoglobulin genes and chromosomal translocations in lymphoid neoplasms

Man	Chain	Heavy	14q32
		κ Light	2p13
		λ Light	22q
	Translocations		t(2;8), t(8;14), t(8,22)
	Commonest trisomy		8
Mouse	Chain	Heavy	12
		κ Light	
		λ Light	
	Translocations		t(12,15) λ or κ producers t(6;15) κ producers only
	Commonest trisomy		15

activation of a gene may only be important, if this occurs in a cell which is differentiated along a specific pathway.

However, we must be very careful not to assume that in gross chromosomal terms the proximity of an onc gene locus to a rearrangement breakpoint is a close association in both causal and molecular terms. To take an example outside the leukaemias – the deletion, at band 11p13, associated with the Wilms' tumour aniridia syndrome, does not include the locus of the c-Ha-*ras*2 gene which is known to be located on the short arm of chromosome 11[92,93].

In view of our work on ataxia–telangiectasia, it is tempting to speculate that there is an onc gene at 14q12 and that the exchange involving this locus also involves other chromosomal sites – the loci of genes which are activated in T cell differentiation. If this is correct, it is a further example of activation leading to only part of the neoplastic phenotype.

Conclusions

1. Specific cellular genes are critical for the development of neoplasia.
2. Their modification is associated with leukaemia.
3. Modified genes may: (a) acquire modification in situ, or (b) be inserted by a virus.
4. Modification of these genes may be only one part of the neoplastic process.

GENERAL CONSIDERATIONS

It seems likely that any external influence which causes genomic rearrangement will predispose the patient to the development of leukaemia (or cancer). In the first place the changes that are induced may be random, but later selection occurs for specific rearrangements because of the very special nature of the alterations in cell behaviour that they bring about. The possibility that rearrangements occur in particular regions of the genome – either because of the specificity of an exogenous agent (such as a virus), or because of a functional relationship between such regions – cannot at present be excluded. Oncogenic viruses are probably a special case where a rearranged primed onc gene is inserted direct into the genome. However, in other cases the chromosomal rearrangement is almost certainly involved in the inappropriate activation or alteration of the function of normal cellular genes controlling important cellular processes. While the understanding of the basic biology is important, there may also be long term implications for therapy. Any understanding of the function of the genes involved in these arrangements should enable specific therapies to be targeted at those particular processes. This gives the possibility of regulation of the leukaemic cell without necessarily destroying it.

A final stage may involve the total breakdown in cell control of chromosomal structure and replication. Beyond that point only cell killing is likely to effect any control on the leukaemic process.

REFERENCES

1. Harnden D, Morten J, Featherstone T 1984 Dominant susceptibility to cancer in man. Advances in Cancer Research 41: 185–255
2. Knudson A G 1971 Mutation and cancer. Statistical study of retinoblastoma. Proceedings of the National Academy of Science USA 68: 820–823
3. Cavenee W K et al 1983 Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305: 779–784
4. Murphee A L, Benedict W F 1984 Retinoblastoma: Clues to human oncogenesis. Science 223: 1028–1033
5. Koufos A, Hansen M F, Lampkin B C, Workman M L, Copeland N G, Jenkins N A, Cavenee W K 1984 Loss of alleles at loci on human chromosome 11 during genesis of Wilms' tumour. Nature 309: 170–172
6. Nebert D W, Felton J S 1976 Importance of genetic factors influencing the metabolism of foreign compounds. Federation Proceedings 35: 1133–1141
7. Kapadia S B, Krause J R, Ellis L D, Pan S P, Wald N 1980 Induced acute non-lymphocytic leukaemia following long-term chemotherapy. Cancer 45: 1315–1321
8. Andrews A D 1983 Xeroderma pigmentation. In: German J (ed) Chromosome mutation and neoplasia. Alan R Liss, New York, p. 63–83
9. Spector B D, Perry G S III, Kersey J H 1978 Genetically determined immunodeficiency diseases (GDID) and malignancy. Report from the Immunodeficiency Cancer Registry. Clinical Immunology and Immunopathology 11: 12–29
10. Filipovich A H, Spector B D, Kersey J H 1980 Immunodeficiency in humans as a risk factor in the development of malignancy. Preventative Medicine 9: 252–259
11. Langlands A O, Maclean N 1976 Lymphoma of the thyroid. An unusual clinical course in a patient possessing a 14/21 translocation. Cancer 38: 259–267
12. Garson O M, Milligan W J 1974 Acute leukaemia associated with an abnormal genotype. Scandinavian Journal of Haematology 12: 256–262
13. Bobrow M, Emerson P M, Spriggs A I, Ellis H L 1973 Ring-1 chromosome, chromosome, microcephalic dwarfism and acute myeloid leukaemia. American Journal of Diseases of Children 126: 257–260
14. Gunz F W, Fitzgerald P H, Adams A 1962 An abnormal chromosome in chronic lymphatic leukaemia. British Medical Journal 2: 1097–1099
15 Fitzgerald P H, Homer J W 1969 Third case of chronic lymphocytic leukaemia in a carrier of the inherited Ch′ chromosome. British Medical Journal 3: 752–754
16. Holland W W, Doll R, Carter C O 1962 The mortality from leukaemia and other cancers among patients with Down's syndrome (Mongols) and among their parents. British Journal of Cancer 16: 177–186
17. Miller R W 1970 Neoplasia and Down's syndrome. Annals of the New York Academy of Science 171: 637–644
18. Rowley J D 1981 Down's syndrome and acute leukaemia: increased risk may be due to trisomy 21. Lancet ii: 1020–1022
19. Blattner W A, Dean J H, Fraumeni J F 1979 Familial lymphoproliferative malignancy; clinical and laboratory follow up. Annals of Internal Medicine 90: 943–944
20. Greene MH et al. 1982 Lymphomas and leukaemia in the relations of patients with mycosis fungoides. Cancer 49: 737–741
21. Lui V, Ragab A H, Findley H, Frauen B 1978 Infantile genetic agranulocytosis and acute lymphocytic leukaemia in two sites. Journal of Paediatrics 92: 1028
22. Gilman P A 1972 Infantile genetic granulocytosis. Two cases of acute monocytic leukaemia. Birth Defects Original Article Series 8(3): 55–58
23. Falletta J M et al. 1973 A fatal X-linked recessive reticulo-endothelial syndrome with hypergammaglobulinaemia: X-linked recessive reticuloendotheliosis. Journal of Paediatrics 83: 549–556
24. Bolande R P 1977 Childhood tumours and their relationship to birth defects. In: Mulvihill J H, Muller R W, Fraumeni J F (eds) Genetics of human cancer. Raven Press, New York, p 43–75
25. McKusick V A 1983 Mendelian Inheritance in Man. 6th Edn. John Hopkins Press, Baltimore
26. German J 1983 Patterns of neoplasia associated with chromosome breakage syndromes. In: German J (ed) Chromosomes Mutation and Neoplasia. Alan R Liss, New York, p 97–134

27. Rivera R, Cangir A, Strong L 1975 Incontinentia pigmenti (Bloch-Sulzberger Syndrome) associated with acute granulocytic leukemia. Southern Medical Journal 68: 1391–1394
28. German J, Archibald R M, Bloom D 1968 Chromosomal breakage in a rare and probably genetically determined syndrome in man. Science 148: 506–507
29. German J, Schonberg S, Louie E, Chaganti R S K 1977 Bloom's syndrome. IV. Sister chromatid exchanges in lymphocytes. American Journal of Human Genetics 29: 248–255
30. German J, Bloom D, Passarge E 1979 Bloom's syndrome. VII. Progress report for 1978. Clinical Genetics 15: 361–367
31. German J 1983 Bloom's syndrome. X. The cancer proneness points to chromosome mutation as a crucial event in human neoplasia. In: German J (ed) Chromosomes Mutation and Neoplasia. Alan R Liss, New York, p 347–358
32. Passarge E 1983 Bloom's syndrome In: German J (ed) Chromosomes Mutation and Neoplasia. Alan R Liss, New York, p 11–22
33. German J, Schonberg S 1980 Bloom's syndrome. IX. Review of cytological and biochemical aspects. In: Gelboin H V (ed) Genetic and environmental factors in experimental and human cancer. Japan Scientific Society Press, Tokyo, p 175–186
34. Krepinsky A B, Heddle J A, German J 1979 Sensitivity of Bloom's syndrome lymphocytes to ethyl methanesulfonate. Human Genetics: 151–156
35. Arlett C F, Harcourt S A 1978 Cell killing and mutagenesis in repair deficient human cells. In: Hanawalt P C, Friedberg E C, Fox C F (eds) DNA repair mechanisms. Academic Press, New York, p 633–636
36. Schroeder T M, German J 1974 Bloom's syndrome and Fanconi's anaemia: Demonstration of two distinctive patterns of chromosome disruption and rearrangement. Humangenetik 25: 299–306
37. Sasaki M A 1975 Is Fanconi's anaemia defective in a process essential to the repair of DNA cross links? Nature 257: 501–503
38. Auerbach A D, Adler B, Chaganti R S K 1981 Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. Pediatrics 67: 128–135
39 Obeid D A, Hill F G H, Harnden D G, Mann J R, Wood B S B 1980 Fanconi anaemia: Oxymetholone, liver tumours and chromosome aberrations associated with leukemic transition. Cancer 46: 1401–1404
40. Bridges B A, Harnden D G (eds) 1982 Ataxia telangiectasia: A cellular and molecular link between cancer neuropathology and immune deficiency. John Wiley, Chichester
41. Hecht F et al. 1966 Leukaemia and lymphocytes in ataxia telangiectasia. Lancet ii: 1193
42. Spector B D, Filipovich A H, Perry G S, Kersey J H 1982 Epidemiology of cancer in ataxia-telangiectasia. In: Bridges B A, Harnden D G (eds) Ataxia telangiectasia – a cellular and molecular link between cancer, neuropathology and immune deficiency. John Wiley, Chichester p 103–138
43. Cunliffe P N, Mann J R, Cameron A H, Roberts K D, Ward H W C 1974 Radiosensitivity in ataxia telangiectasia. British Journal of Radiology 48: 374–376
44. Taylor A M R et al. 1975 Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. Nature, 258: 427–429
45. Taylor A M R, Metcalf J A, Oxford J M, Harnden D G 1976 Is chromatid type damage in ataxia telangiectasia after irradiation at G_0 a consequence of defective repair? Nature 260: 441–443
46. Taylor A M R, Rosney C M, Campbell J B 1979 Unusual sensitivity of ataxia telangiectasia cells to bleomycin. Cancer Research 39: 1046–1050
47. Shiloh Y, Tabor E, Becker Y 1982 The response of ataxia telangiectasia homozygous and heterozygous skin fibroblasts to neocarzinostatin. Carcinogenesis 3: 815–820
48. Lehman A R, Stevens S 1977 The production and repair of double strand breaks in cells from normal humans and from patients with ataxia telangiectasia. Biochimica et Biophysica Acta 474: 49–60
49. Cox R, Masson W K, Debenham P G, Webb M B T 1984 The use of recombinant DNA plasmids for the determination of DNA repair and recombination in cultured mammalian cells. British Journal of Cancer 49: Suppl. VI 67–72
50. Cox R, Masson W K, Weichselbaum R R, Nove J, Little J B 1981 Repair of potentially lethal damage in X-irradiated cultures of normal and ataxia-telangiectasia human fibroblasts. International Journal of Radiation Biology 39: 357–365
51. Lavin M F, Ford M D, Houldsworth J 1982 DNA replication in ataxia telangiectasia

cells after exposure to ionizing radiation. In: Bridges B A, Harnden D G (eds) Ataxia-telangiectasia: A cellular and molecular link between cancer, neuropathology and immune deficiency. John Wiley, Chichester, p 319–326
52. Edwards M J, Taylor A M R 1982 Unusual response of DNA synthesis in ataxia telangiectasia cell lines following γ irradiation. In: Bridges B A, Harnden D G (eds) Ataxia telangiectasia: a cellular and molecular link between cancer, neuropathology and immune deficiency. John Wiley, Chichester, p 327–338
53. Hecht F, Kaiser McCaw B 1982 Ataxia telangiectasia: chromosomes before cancer. In: Bridges B A, Harnden D G (eds) Ataxia-telangiectasia: a cellular and molecular link between cancer, neuropathology and immune deficiency. John Wiley, Chichester, p 235–243
54. Harnden D G 1977 The relationship between induced chromosome aberrations and chromosome abnormality in tumour cells. In: Human genetics. Proceedings of the Vth international congress of human genetics. Excerpta Medica, Amsterdam, p 355–366
55. Taylor A M R 1982 Cytogenetics of ataxia-telangiectasia. In: Bridges B A, Harnden D G (eds) Ataxia telangiectasia: a cellular and molecular link between cancer, neuropathology and immune deficiency, John Wiley, Chichester, p 53–82
56. Kaiser-McCaw B, Hecht F 1982 Ataxia telangiectasia: chromosomes and cancer. In: Bridges B A, Harnden D G (eds) Ataxia telangiectasia: a cellular and molecular link between cancer, neuropathology and immune deficiency. John Wiley, Chichester pp 243–257
57. Zech L, Gahrton G, Hammarström L, Juliusson G, Mellstedt H, Robert K H, Smith C E 1984 Inversion of chromosome 14 marks human T cell chronic lymphocytic leukaemia. Nature 308: 858–860
58. Wake N, Minowada J, Park B, Sandberg A A 1982 Chromosomes and causation of human cancer and leukemia XLVIII. T cell acute leukemia in ataxia telangiectasia. Cancer Genetics and Cytogenetics 6: 345–357
59. Boveri T 1914 Zur Frage der Entstehung maligner Tumoren. Gustav Fischer, Jena
60. Wolf U 1974 Theodor Boveri and his book 'On the problems of the origin of malignant tumours'. In: German J (ed) Chromosomes and Cancer. John Wiley, New York p 3–20
61. Ford C E, Jacobs P A, Lajtha L G 1958 Human somatic chromosomes. Nature 181: 1565–1568
62. Moorhead P S, Nowell P C, Mellman W J. Batipps D M, Hungerford D A 1960 Chromosome preparations of leucocytes cultured from human peripheral blood. Experimental Cell Research 20: 613–616
63. Mitelman F 1983 Catalogue of chromosome aberrations in cancer. Cytogenetics and Cell Genetics 36: 4–515
64. First International Workshop on Chromosomes in Leukaemia 1978 Chromosomes in acute non-lymphocytic leukaemia. British Journal of Haematology 39: 311–316
65. Second International Workshop on Chromosomes in Leukemia 1980 Cancer Genetics and Cytogenetics 2: 89–113
66. Third International Workshop on Chromosomes in Leukemia 1981 Cancer Genetics and Cytogenetics 4: 95–142
67. Fourth International Workshop on Chromosomes in Leukemia 1984 A prospective study of acute non lymphocytic leukemia. Cancer Genetics and Cytogenetics 11: 249–360
68. Nowell P C, Hungerford D A 1960 A minute chromosome in human CML. Science 132: 1497
69. Baikie A G, Court Brown W M, Buckton K E, Harnden D G, Jacobs P A 1960 A possible specific chromosome abnormality in human chronic myeloid leukaemia. Nature 188: 1165–1166
70. Dutrillaux B 1975 Obtention simultanée de plusiers marquages chromosomique sur les mêmes préparations, après traitment par le Brd U. Humangenetik 30: 297–306
71. Yunis J J 1979 High resolution of human chromosomes. Science 191: 1268–1270
72. Yunis J J 1982 Comparative analysis of high resolution chromosome techniques for leukemic bone marrows. Cancer Genetics and Cytogenetics 7: 43–50
73. Yunis J J 1984 Recurrent chromosomal defects are found in most patients with acute non-lymphocytic leukemia. Cancer Genetics and Cytogenetics 11: 125–137
74. Yunis J J, Bloomfield C D, Ensrud K 1981 All patients with acute non-lymphocytic leukemia may have a chromosomal defect. New England Journal of Medicine 305: 135–139

75. Berger R, Bernheim A 1982 Cytogenetics studies on Burkitt's lymphoma-leukemia. Cancer Genetics and Cytogenetics 7: 231–244
76. Bernheim A, Berger R, Lenoir G 1983 Cytogenetic studies on Burkitt's lymphoma cell lines. Cytogenetics and Cell Genetics 8: 223–229
77 Douglas E C, Magrath I T, Terebelo H 1982 Burkitt cell leukemia without abnormalities of chromosomes 8 and 14. Cancer Genetics and Cytogenetics 5: 181–185
78. Berger R, Bernheim A, Daniel M-T, Flandrin G 1983 t(15;17) in a promyelocytic form of chronic myeloid leukemia blastic crisis. Cancer Genetics and Cytogenetics 8: 149–152
79. Mitelman F, Levan G 1981 Clustering of aberrations to specific chromosomes in human neoplasms. IV A survey of 1871 cases. Hereditas 95: 79–139
80. Rowley J D 1977 Mapping of human chromosomal regions related to neoplasia: evidence from chromosomes 1 and 17. Proceedings of the National Academy of Sciences USA 74: 5729–5733
81. Court Brown W M, Harnden D G, Jacobs P A, MacLean N, Mantle D J 1964 Abnormalities of the Sex Chromosome Complement in Man. MRC Special Report Series. No. 305
82. Harnden D G Viruses chromosomes and tumors: The interaction between viruses and chromosomes. In: German J (ed) Chromosomes and Cancer. John Wiley, New York, p 151–190
83. Griffin B E, Dilworth S M 1983 Polyomavirus: An overview of its unique properties. Advances in Cancer Research 39: 183–268
84. Enrietto P J, Wyke J A 1983 The pathogenesis of oncogenic avian retroviruses. Advances in Cancer Research 39: 269–314
85. Dalla-Favera R, Breuni M, Erikson J, Patterson D, Gallo R C, Croce C M 1982 Human c-*myc* onc gene is located in the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proceedings of the National Academic of Sciences USA 79: 7824–7827
86. Taub R et al. 1983 Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proceedings of the National Academy of Sciences USA 79: 7837–7841
87. Adams J M, Gerondakis S, Webb E, Corcoran L M, Cory S 1983 Cellular *myc* oncogene is altered by chromosomal translocation to an immunoglobulin locus in murine plasmacytoma and is rearranged similarly in human Burkitt lymphoma. Proceedings of the National Academy of Sciences USA 80: 1982–1986
88. Dean M, Kent R B, Sonhenshein G E 1983 Transcriptional activation of immunoglobulin α heavy-chain genes by translocation of the c-*myc* oncogene. Nature 305: 443–446
89. Neuberger M S, Calabi F 1983 Reciprocal chromosome translocation between c-*myc* and immunoglobulin γ2b genes. Nature 305: 240–243
90. de Klein A et al. 1982 A cellular oncogene is translocated to the Philadelphia chromosome in chronic myeloid leukaemia. Nature 300: 765–767
91. Collins S J, Groudine M T 1983 Rearrangement and amplification of c-*abl* sequences in the human chronic myelogenous leukemia cell line K562. Proceedings of the National Academy of Sciences USA 80: 4813–4817
92. Huerre C, Despoisse S, Gilgenkrantz S, Lenoir G M, Junien C 1983 c-Ha-*ras*1 is not deleted in aniridia Wilms' tumour association. Nature 305: 638–641
93. de Martinville B, Francke U 1983 The c-Ha-*ras* 1, insulin and β globin loci map outside the deletion associated with aniridia-Wilms' tumour. Nature 305: 641–643

A survey of chromosome rearrangements in leukaemia and lymphoma

INTRODUCTION

This chapter will concern itself with the chromosomal (karyotypic, cytogenetic) changes in leukaemia and lymphoma, which can serve as differential diagnostic features, and with the association of these changes with the molecular events, which may be responsible – at least partially – for the oncogenetic events. The chapter will also review the primary (specific) chromosome changes, which may play an important role in the biological behaviour of these diseases. The major thrust of the chapter will be to acquaint the reader with: the impressive number of chromosome changes in human leukaemia and lymphoma, their value in the classification and characterization of these diseases, and the use of these karyotypic changes, both clinically and in some of the basic approaches, to decipher the nature of human leukaemia and lymphoma.

Some historical aspects

The first specific chromosomal change in human neoplasia, or for that matter in neoplasia of any species, was described about 25 years ago[1] – not long after the correct number of chromosomes in the human was established and the normal karyotype defined[2], i.e. the Ph[1] chromosome in chronic myeloid leukaemia (see Ch. 4). Subsequently, other specific karyotypic changes characterizing different diseases were described (e.g. –2 in meningioma), but their number remained relatively small because the origin and identity of abnormal and normal chromosomes could not be established with certainty. The situation changed radically when banding techniques were introduced in 1970[3,4]. Nevertheless, the cytogenetic data in human neoplasia obtained before that date served as an important base for banding studies and already suggested possible unique chromosomal features in various types of human neoplasia.

Some findings in the prebanding era

An example of the recognition of a unique karyotypic change in leukaemia without using banding techniques was described by Trujillo *et al.*[5], and

consisted of –C, +D, +E, –G and carried with it a relatively good prognosis. Subsequently, with banding techniques this apparently complicated set of karyotypic changes was shown to be due to one cytogenetic event, i.e. t(8;21)[6,7]. Other karyotypic changes recognized in the prebanding era included monosomy of chromosome 7, a cytogenetic event usually associated with a preleukaemic state and a number of accompanying phenotypic manifestations[4]. Though a number of other karyotypic changes were described in leukaemia and lymphoma before the introduction of banding, these could not be defined rigorously until the introduction of banding techniques which elucidated the precise details of the chromosomal involvement.

Banding and the establishment of specific karyotypic changes in leukaemia and lymphoma

The introduction of various banding techniques made possible the recognition and identification of individual chromosomes, subchromosomal structures and changes[4]. Banding thus afforded cytogeneticists the opportunity to define the chromosomes precisely – the bands and subbands involved in various translocations, and the location and extent of deletions, insertions, additions and breaks. Furthermore, refinement in cellular *in vitro* methodologies, particularly methotrexate synchronization[8,9] led to: (a) higher yields of metaphases and hence of cytogenetic results, (b) improved quality of chromosome preparations, and (c) the discovery of an increasing number of conditions with specific cytogenetic changes. Thus, the utilization of banding approaches resulted in an almost explosive increase in the number of specific (primary) chromosomal changes described in leukaemia and lymphoma, with new entities and subentities being added regularly.

METHODOLOGICAL CONSIDERATIONS

Source of material

The source of the material used for cytogenetic examination is of crucial importance in establishing the karyotypic changes in human leukaemia and lymphoma. As a rule, bone marrow is the best source of cells in most leukaemias and the involved lymph nodes or lymphomatous tumours are preferred in lymphomas[4]. A few exceptions to this rule will be discussed later. It must be stressed here that the chromosomal changes are confined to the affected cells and are not found in the other somatic cells of the body, for example fibroblasts or muscle cells. However, when the constitutional karyotype of an individual needs to be ascertained, then examination of phytohaemagglutinin stimulated T lymphocytes or cultured fibroblasts is the best approach.

Direct versus non-direct methods

In the acute leukaemias there is still controversy regarding the value and interpretation of direct examination of the marrow cells in comparison with

results following short term (48–72 h) culture. In some cases, it appears that the culture approach yields clonal chromosome changes in a significantly higher percentage of leukaemias than does direct examination[10,12], though this has not been the universal experience[13]. On the other hand the direct approach is necessary in some circumstances, such as when the presence or absence of karyotypically normal cells (see below) needs to be established. In our opinion, it may be best to analyse marrow cells using both approaches, since this is likely to yield maximal information in cases of acute leukaemia. Even with both approaches, a small percentage of cases with acute leukaemia (particularly those with acute lymphoblastic disease) do not have enough dividing cells in the marrow for cytogenetic analysis upon repeated attempts. It appears that such cases of leukaemia may have a rather poor prognosis.

In the lymphomas, direct examination of the cells or examination following short term incubation appear to be the best approaches to establishing the karyotypic changes[4,14]. Attempts to utilize various mitogenic agents for stimulating the abnormal B and/or T-cells (Table 2.1) in lymphoma have generally not proved useful except for chronic lymphocytic leukaemia (CLL). It is hoped that agents with specificity for one or other lymphomatous cell type will be discovered, which would increase the yield of metaphases and thus the number of lymphomas in which karyotypic changes can be established.

Table 2.1 Mitogenic stimulators of blood lymphocytes

Mitogen	Cell type stimulated	Concentrations used
Phytohaemagglutinin (PHA)	T-lymphocytes	25–100 μg/ml
Pokeweed mitogen (PWM)	B and T-lymphocytes	up to 150 μg/ml
Concanavalin (Con A)	T-lymphocytes (mouse)	1–5 μ/ml
Calcium ionophore–A23187	B and T-lymphocytes	5×10^{-7} μmol/l (0.5–2.0 μg/ml)
Sodium metaperiodate	T-lymphocytes	2–4 mmol/l
EB virus (EBV)	B-lymphocytes	10–20% supernatant; 1:9 v/v of culture
Lipopolysaccharide W (*E. coli* 055:B5)	B-lymphocytes	100 μg/ml
Staphylococcus genus of bacteria Cowan I protein	B-lymphocytes	100 μg/ml
Conditioned medium from PHA stim. T cells (PHA induced soluble factors)	B-lymphocytes	1:2 dilution
Protein A	B-lymphocytes	20–100 μg/ml
T-cell growth factor (Interleukin-2)	T-lymphocytes	10% medium

Most informative approaches

Although leukaemic and lymphoma cells circulating in the blood can serve occasionally as sources for establishing the karyotypic changes in a particular patient, in general examination of these cells does not yield the quantitative and qualitative information which examination of bone marrow material affords. Under some circumstances blood cells may be the only material available for cytogenetic analysis and every effort should then be made to establish the karyotypic changes of these cells. However, when bone marrow material and lymphomatous tumours or lymph nodes are available, these should definitely be examined in preference to blood. Under some circumstances, such as the extramedullary development of the blastic phase of chronic myeloid leukaemia (CML), examination of circulating cells may yield information comparable to that of the marrow and may even on occasion reveal karyotypic changes not apparent in the marrow. In such cases the abnormal cells of the blastic phase are apparently generated outside the bone marrow and find their way into the circulation prior to being established in the marrow. A similar situation may hold for the development of a leukaemic phase in lymphoma, in which the abnormal cells may sometimes be found in large numbers in the circulation at a time when the lymphomatous tumour may not be readily available for cytogenetic analysis or when the bone marrow is essentially uninvolved.

The most informative approaches, as far as banding techniques in particular are concerned, in establishing the karyotypic changes in any human leukaemia and lymphoma depend on the type of information being sought[4]. Generally, Q- and G-banding are sufficient for establishing most of the changes encountered in these diseases – though when complicated translocations exist, the application of R- (reverse) and C-banding is recommended for establishing the exact chromosomes involved in a translocation and the possible presence of more than one centromere. The role of various DNA and other molecular probes in ascertaining subcellular changes not revealed by existing banding techniques will undoubtedly expand in the future, though at present such techniques are available only in a limited number of laboratories.

New and future directions

The recent introduction into cytogenetics of molecular probes capable of establishing the presence, location and activation of genes – particularly those of cancer genes (oncogenes) – has further expanded the scope and breadth of information gained from chromosome analysis. Some of the newer findings in this area will be discussed below. It is now accepted that the use of molecular techniques will allow the recognition of karyotypic events (e.g. translocations, oncogene locations) not readily ascertained from presently available cytogenetic methodologies and will undoubtedly result in another quantum increase in the information on leukaemia and chromosomes in cancer, akin to

the major advances brought about by the introduction of other techniques in the past.

KARYOTYPIC FINDINGS IN CHRONIC MYELOID LEUKAEMIA (CML)

The Philadelphia (Ph^1) chromosome

A discussion of the karyotypic changes in human leukaemia and lymphoma must begin with CML (Table 2.2). First, this condition holds a historical place in this field since it was the first human disease in which a specific cytogenetic change, the Ph^1 chromosome, was established; secondly, there is more information available on the chromosome changes in this disease and in its various phases than in any other human condition[4] (see also Ch. 4).

Even though the relative specificity of the clinical significance of the Ph^1 in CML was recognized before the introduction of banding methods[4], the exact identity and origin of this abnormal chromosome were not established until 1973, when banding techniques then revealed that it was an abbreviated chromosome 22[15] which was involved in what appeared to be a reciprocal and balanced translocation with a chromosome 9 – i.e. t(9;22)(q34;q11) – in about 95 per cent of cases with CML[16]. As more data accumulate, it appears more and more likely that Ph^1-negative CML is quite separate from Ph^1-positive disease and that, in fact, CML may be defined by the presence of the Ph^1 chromosome. The Ph^1-negative disease may include a number of subentities, including chronic monocytoid or myelomonocytic leukaemia.

The Ph^1 chromosome is invariably an abbreviated chromosome 22 with the break occurring at band q11. This has been defined both cytogenetically and on a molecular basis[17,18]. In about five per cent of cases, it appears that chromosomes other than 9 are involved in the Ph^1 translocation, some involving only one other chromosome (variant simple Ph^1 translocations)[19]. Thus far, every chromosome in the human set (except the Y) has been shown to be involved in variant Ph^1 translocations of one type or another. A comparison of the survival and other haematological and clinical features of the cases with variant Ph^1 translocation with those with the standard Ph^1 translocation has generally not revealed any major differences[19,20] – which points to the crucial role played by the initial change on chromosome 22 in establishing the nature and course of the disease.

Table 2.2 Specific and non-random chromosome changes in CML

t(9;22)(q34;q11)	CML
i(17q)	Blastic phase of CML
+8	Blastic phase of CML
+19	Blastic phase of CML

The introduction of various DNA probes has led to the exciting finding of a possible translocation of an oncogene (c-*abl*) from chromosome 9 to the Ph[1] chromosome[21-24]. Recent research has shown[21] that in variant translocations, in which microscopically the chromosomes 9 appear to be normal and apparently not involved in Ph[1] translocation, molecular probing has revealed the presence of the c-*abl* oncogene on the Ph[1] – indicating that a molecular translocation, which is not discernible cytologically, has still occurred. This field is now being actively investigated and the significance and exact incidence of this and other molecular events will undoubtedly be established in the future.

Cytogenetics of the blastic phase

In the majority of cases with CML, the Ph[1] is found in all the cells of the marrow, thus apparently involving cells of the megakaryocytic, erythroblastic and granulocytic series[4]. This would suggest that the defect occurs in a common precursor (or precursors) of these cells. Although a small proportion of cases with CML show karyotypic changes in the chronic phase similar to those observed in the blastic phase (i.e. +8, +Ph[1], +19), generally the disease is characterized by the presence of Ph[1] as the sole karyotypic anomaly for a number of years before the blastic phase appears. The blastic phase, which has many of the biological and clinical characteristics of acute leukaemia, may be heralded by the appearance of additional chromosomal changes weeks, months or occasionally one year before the appearance of the distinctive haematological and clinical features. The most common changes observed in the blastic phase are those already mentioned, +Ph[1], +8 or +19. In addition, an isochromosome for the long arm of chromosome 17, i(17q), is rather characteristic for the blastic phase, but it seldom appears without other additional karyotypic changes.

The blastic phase of CML may assume either myeloblastic or lymphoid cytological characteristics. Though cytogenetically these two entities have not yet been defined[4], it is possible that the lymphoid phase is associated with fewer karyotypic aberrations, in addition to the Ph[1], than the myeloblastic one. Under some circumstances, the cells generated by extramedullary tissues may enter the circulation before being established in the marrow – in these cases examining the circulating leukaemic cells may in fact offer more information than the bone marrow. This is one of the few exceptions in which examination of extramedullary cells may offer the cytogeneticist and the clinician more information than examining the marrow cells. It should be pointed out that the Ph[1] chromosome involves cells which are of bone marrow origin and does not generally involve the remaining tissues of the body – including such cells as lymphocytes and fibroblasts.

CYTOGENETIC ASPECTS OF THE ACUTE LEUKAEMIAS

The acute leukaemias have been divided on the basis of their cellular morphology into two categories: acute nonlymphocytic leukaemia (ANLL) and

acute lymphoblastic leukaemia (ALL). Each category has been further subdivided according to the FAB classification[25] primarily according to morphological criteria. Recently, immunological and enzymatic characteristics of the leukaemic cells have added another dimension in defining the nature of the leukaemic cells, though they have not led to a definition of the specific subentities within the major FAB groups. However, some correlations between the immunological and enzymatic characteristics of the leukaemic cells and the clinical, particularly prognostic, aspects of some acute leukaemias have been reported.

A major contribution of cytogenetics has been the further definition of specific subtypes of leukaemias within ANLL and ALL[16,26–28]. These are shown in Tables 2.3 and 2.4. It is notable that each of these leukaemias is associated with a unique cytogenetic event. Moreover, prognostic and other clinical features, and in some cases some cellular and haematological features, characterize each of the leukaemic subentities which have been defined cytogenetically. For example, ALL with t(4;11) is possibly a pluripotent stem cell disorder with lymphoid as well as monocytoid differentiation[27,29–32] and pre-B-cell ALL with t(1;19)(q23;q13) may have a much poorer prognosis than cases of ALL without this translocation[33]; and ANLL cases with either an inversion of chromosome 16 or 16q- have an associated eosinophilia[34,35]. In addition to defining the diagnostic and clinical features of these leukaemias, the involved chromosomes may reflect the possible location of genes responsible for eosinophil or basophil production and physiology. There is also evidence that chromosome 3, particularly its short arm, may be related to megakaryocytic physiology and platelet production[37,38] – cases of leukaemia with involvement of

Table 2.3 Specific and non-random chromosome changes in ANLL

Type of anomaly	Type of ANLL	Frequency of occurrence*
t(3;5)(q21;q31)	ANLL (M2)	? < 5 %
t(6;9)(p23;q34)	AML (M2)	? < 5 %
t(8;21)(q22;q22)	AML (M2) with Auer rods	7 %
t(9;11)(p21;q23)	AMoL (M5)	? < 5 %
t(9;22)(q34;q11)	ANLL Ph¹+	< 3 %
t(11;21)(q22;q21)	ANLL (M4)	
t(11;19)(q23;q12 or p12)	ANLL (M5)	
t(15;17)(q22;q12)	APL (M3)	†
3p–, 3q–	Secondary leukaemia	
5q–, –5	Secondary leukaemia	17.9 %
7q–(q33q36), –7	Secondary leukaemia	17.9 %
+8	ANLL	
11q–(q23)	M4, M5 (M2)	
12p–	Secondary leukaemia	
12q–	ANLL	
inv(16)(p13q22) or 16q–(q22)	ANLL with eosinophilia (M4)	? < 5 %
21q–	ANLL	
+22	ANLL	

*Calculated on the basis of a prospective study of 6 cases examined at the FWCL[28]
†APL cases without t(15;17) are rare

Table 2.4 Specific and non-random chromosome changes in acute lymphoblastic leukaemia (ALL)

t(1;19)(q21;q13)	ALL (L1)
t(1;19)(q23;p13.3)	Pre-B-cell ALL
t(2;8)(p11–13;q24)	ALL (L3)
t(4;11)(q21;q23)	ALL — possibly myelomonocytic
t(8;14)(q24;q32)	ALL (L3)
t(8;22)(q24;q11)	ALL (L3)
t(9;22)(q34;q11)	ALL — Ph^1 + (L1 and L2)
t(11;14)(q13;q32)	ALL
6q–	ALL
9p–	T-cell ALL
12p–(p12)	ALL
14q+(q32)	Adult T-cell acute leukaemia
+21	ALL

chromosome 3 may show megakaryocyte immaturity and abnormalities of platelet morphology, number and function.

The cytogenetic delineation of acute leukaemia subentities has indicated that some of the cases thought to belong to a single FAB group are in fact heterogeneous and are possibly aetiologically and clinically distinct (Tables 2.3 and 2.4). This is particularly true, for example, of the M2 group of ANLL. A similar situation may apply to preleukemia and myelodysplastic disorders (Table 2.5).

Table 2.5 Chromosome changes in myelodysplastic and myeloproliferative syndromes and preleukaemic states

t(1;3)(p36;q21)	Myelodysplastic syndrome
t(1;7)(p11;p11)	Dysmyelopoietic disorder (induced?)
t(2;11)(p21;q23)	Dysmyelopoietic preleukaemia
t(3;17)(q26;q22)	Acute disease in myeloproliferative disorders?
t(6;9)(p23;q34)	Myeloproliferative diseases
t(11;21)(q22;q21)	Myeloproliferative diseases
5q– (interstitial)	Refractory anaemia
–7	Preleukaemia with infection
+8	Preleukaemia
20q–	Polycythaemia vera
21q–	Preleukaemia

Acute nonlymphocytic leukaemia (ANLL)

The FAB classification of the group of leukaemias known as ANLL and the cytogenetic findings in these are shown in Table 2.6. It is clear that cytogenetically each FAB group contains within it two or more entities, thus demonstrating that the cytogenetic approach is capable of differentiating certain leukaemias not readily distinguished by cytological, immunological, enzymatic or clinical criteria. Undoubtedly, more subgroups within these

Table 2.6 FAB classification of acute nonlymphocytic leukaemia and common chromosome changes

Classification	Cytological characteristics	Common chromosomal changes
M1	Myeloblastic leukaemia without maturation	includes t(9;22)-Ph[1]-positive AML
M2	Myeloblastic leukaemia with maturation	t(8;21) with Auer rods; t(6;9) with possible marrow basophilia; involvement of chromosomes 3,5,7 & 12 in secondary leukaemia t(3;5)(q26;q22)
M3	Hypergranular promyelocytic leukaemia	t(15;17)
M4	Myelomonocytic leukaemia	chromosome 11 often involved; inv(16)(p13q22) or 16q-(q22)
M5	Monocytic leukaemia	t(9;11) and 11q-; t(11;19)(q23;q12 or p12)
M6	Erythroleukaemia	Ph[1]-positive EL, often AA and MAKA[a]; high chromosome count

[a] AA – cytogenetically only abnormal cells
EL – erythroleukaemia
MAKA – major karyotypic abnormalities

leukaemias will be established on cytogenetic grounds; already the evidence indicates that ALL consists of a much larger number of entities than was realized in the past. It is interesting that each of these cytogenetically defined leukaemic subentities shows rather consistent cytological, laboratory and clinical aspects, indicating that we are dealing with a relatively homogeneously defined leukaemic group. Thus, acute myeloblastic leukaemia (AML) with t(8;21) has certain features which commonly characterize the patients with this disease[4,26,28,38,39]. The same can be said for other entities, such as AML associated with t(6;9)[40–42], Ph[1]-positive ANLL[4,28] and acute promyelocytic leukaemia associated with t(15;17)[4,28,43,44].

A quantitative evaluation of the chromosome changes in ANLL has revealed definite correlations with prognostic features[7,16,26,45,46]. Thus, it has been shown that those cases in which the bone marrow contains no cytogenetically normal cells (AA patients) tend to have a much shorter survival than patients who have some normal cells in the marrow (AN patients) (Table 2.7). It must be stressed that some of the AA cases undoubtedly have some normal cells in the marrow, but an inordinately large number of karyotypes would have to be examined to reveal their presence. Nevertheless, this qualitative observation of normal and abnormal cells in the marrow appears to correlate with prognostic aspects of the various leukaemias[26], including ALL[27,47]. It would appear that the AA cases would be prime candidates for bone marrow transplantation, when this procedure becomes safer and more generally applicable. The short survival of

Table 2.7 Median survival of ANLL patients according to karyotypic status

Number of patients	Karyotypic classification	Median survival (mos.)
136	NN	7.9
79	AN	5.9
51	AA	2.4

NN – All normal karotypes
AN – Some abnormal and some normal karyotypes
AA – All abnormal karyotypes

AA cases may be related to the eradication of the leukaemic cell population in the marrow, together with the inability of the marrow to regenerate normal elements – as appears to occur in cases of AN or NN[4,48].

Another important correlation with the qualitative aspects of the karyotypic changes is the presence of major karyotypic abnormalities (MAKA)[49] versus those cases with only minor karyotypic abnormalities (MIKA), consisting of only one or two cytogenetic events. Generally, MAKA cases tend to have a much shorter survival, due to failure of the patients to respond to therapy or achieve long complete remissions, whereas MIKA cases respond to therapy more readily and achieve long lasting complete remission. This situation appears to apply in particular to cases of erythroleukaemia (M6), which are often of the MAKA variety[4].

The presence of double minute chromosomes (DMS) appears to be associated with a poor prognosis when they are seen in the leukaemic cells of the marrow[50–52] – though such experience has not been universal[48].

Acute lymphoblastic leukaemia (ALL)

The cytogenetic findings in acute lymphoblastic leukaemia (ALL) (Tables 2.4 and 2.8) have also led to a definition of subgroups within the categories established according to the FAB classification[27]. Thus, as mentioned previously, ALL with t(4;11) may be a common progenitor stem cell disorder capable of differentiating also as a myelomonocytoid leukaemia, at least in a subgroup of patients. Other subentities within ALL have recently been recognized, such as a subgroup of L1 with t(1;19)(q21;q13)[53], a subentity of T-cell ALL with t(11;14)(p13;q13)[55], pre-B cell ALL with t(1;19)(q23;p13)[54], and 9p- in T-cell ALL[55]. Undoubtedly other subgroups will be described, which will have definite correlations with clinical and haematological features. Thus, cytogenetic findings have added to our diagnostic armamentarium in ALL, as well as in ANLL.

Other cytogenetic parameters in ALL, related to prognostic and diagnostic parameters, include the number of chromosomes in the leukaemic cells, i.e. the higher the chromosome number the better the prognosis, and the presence of normal cells in the marrow[56,57]. The latter situation is apparently associated

Table 2.8 FAB classification of acute lymphoblastic leukaemia and common chromosome changes

Classification	Cytological characteristics	Common chromosomal changes
L1	Small blasts Nucleoli not present Scanty cytoplasm Regularly shaped nucleus	6q-; t(1;19)(q23;q13) t(11;14)(q13;q32) high chromosome counts Ph[1]-positive ALL
L2	Large blasts; heterogeneous in size 1–2 large nucleoli Moderately abundant cytoplasm Irregularly shaped nucleoli	includes Ph[1]-positive ALL; near-haploid ALL; t(4;11); 12p-
L3	Large and homogeneous blasts 1–2 prominent nucleoli Cytoplasmic vacuolation Moderately abundant cytoplasm Regularly shaped (oval or round) nucleus	t(8;14) t(2;8) t(8;22)

with a better prognosis than when such cells are not encountered during the usual cytogenetic analysis. As in the case of ANLL, in about one third of the patients with ALL the chromosome picture appears to be normal – at least with current cytogenetic methodologies. It is possible that the 'cytogenetic' changes in these so-called diploid ALL cases occur at the molecular level, involving an oncogene or other genetic material whose nature can only be established with specialised probes. Thus, it will be of interest to ascertain in the future whether any true chromosomally normal leukaemias really exist.

Ph[1]-positive ALL, though it may affect other varieties of ALL, appears to be most commonly associated with the pre-B cell type of ALL. As more and more cases of Ph[1]-positive ALL are studied, this correlation will hopefully be established on a firmer basis than exists now.

CHROMOSOME CHANGES IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

B- and T-cell mitogenic stimulants

Chromosome changes in chronic lymphocytic leukaemia (CLL) were not established on a firm basis until mitogens (Table 2.1), capable of stimulating CLL cells into mitosis became available[58–60]. Thus, before such mitogens were introduced, the usual results in B-cell CLL consisted of diploid metaphases probably consisting of phytohaemagglutinin (PHA) stimulated normal T-cells present among the leukaemic cells[4].

PHA is capable of stimulating normal T-cells into mitosis but stimulation of malignant T-cells is more difficult. The use of a T-cell growth factor[61] may induce leukaemic cells of T-type CLL to enter mitosis.

In both chronic and acute T-cell leukaemias, with the exception of the inv(14q) mentioned below, specific karyotypic changes have not been established. This may be due to the failure to stimulate the cells with the primary change into mitosis, or to the existence of a chromosomal change beyond the resolution of presently used cytogenetically techniques and possibly requiring molecular DNA probing for its recognition.

B- and T-cell CLL

With the availability of various mitogenic agents capable of stimulating B-cells, including the leukaemic type, the karyotypic picture in B-cell CLL has become much clearer (Table 2.9). The most common change appears to be trisomy 12 (+12) and a 14q+ is the second commonest aberration[58-60]. The relation of these cytogenetic changes to prognostic aspects of CLL has been studied[62] – the appearance of additional chromosomal changes, besides the trisomy 12, carries a poorer prognosis and implies a more aggressive phase of the disease than in patients without the additional karyotypic changes[63]. Much remains to be accomplished cytogenetically in B-cell CLL and undoubtedly the refinement and use of various mitogenic agents will greatly enhance the success of establishing the karyotypic changes and their relationship to various clinical parameters.

The chromosomal picture in T-cell CLL (as well as the acute variety of the disease) has not been established with certainty. Reports indicate considerable variability in the karyotypes, though some changes appear to occur more frequently than others[64]. Nevertheless, a specific chromosome change at least in some cases of T-cell CLL has been established recently[65] – i.e. inversion of the long arm of chromosome 14, inv(14)(q11q32). Such an inversion has also been reported in a case of adult acute T-cell leukaemia[66] and in a cell line originating from a case of multiple myeloma[67]

Chromosome studies in adult acute T-cell leukaemia (ATL), a disease associated with the human T-cell leukaemia virus (HTLV-I), have not yielded a specific karyotypic change. One group in Japan has reported frequent involvement of chromosome 7 (+7) and to a lesser extent that of 14 (14q+)[68]. Another Japanese group[66] on the other hand stressed the lack of consistent chromosome abnormalities in ATL, though some common changes affecting chromosomes 1 (duplication of the segment q21q32), 3 (translocations,

Table 2.9 Specific and non-random chromosome changes in chronic lymphocytic leukaemia (CLL)

t(6;12)(q15;p13)	Prolymphocytic (B-cell) leukaemia
t(11;14)(q13;q32)	CLL
3p-(p13)	Prolymphocytic (B-cell) leukaemia
+12	CLL
14q+(q32)	CLL
inv(14)(q11q32)	T-cell CLL

trisomy), 6 (deletions of the long arm), 10, 14 (14q+) and 18, in the order of their frequency, were seen. The donor chromosomes for the 14q+ were Yq, 5p, 5q, 9q, 10q and 12q. To see whether the use of specific mitogenic stimulators for the involved T-cell will lead to the establishment of the specific changes in ATL, or to learn whether the specific karyotypic event is incorporation of a virus into a specific locus of the genome with the cytogenetic changes described to date being of a secondary nature, one will have to await more definitive studies in the future.

Prolymphocytic leukaemia (PLL)

Prolymphocytic leukaemia of the B-cell type appears to be characterized, at least in a subgroup of cases, by t(6;12)[69]. The T-cell variety of prolymphocytic leukaemia is accompanied by a variety of chromosome changes[64] and it will be interesting to see whether the specific change described in T-cell CLL is also observed in T-prolymphocytic leukaemia.

KARYOTYPIC CHANGES IN LYMPHOMA

Burkitt lymphoma (BL)

The karyotypic changes in lymphoma have been recently more clearly defined, partly due to the introduction of a new formulation for the classification of human lymphomas[70], which has made correlations with karyotypic findings easier and simpler. Generally, these chromosome changes have been studied against the background of the cytogenetic abnormalities described in BL. In the latter disease, a 14q+ anomaly was discovered shortly after the introduction of banding techniques[71] and subsequently shown to be due to a translocation between chromosomes 8 and 14[72] (see Ch. 8). This translocation has now been more clearly defined and the bands and subbands involved in t(8;14) are now known to be 8q24.13 and 14q32.33[73]. The translocation is thus reciprocal and balanced. Variant translocations, which always involved chromosome 8 at band q24, were also described. These involved either chromosome 2 or 22, i.e. t(2;8) or t(8;22)[74]. It should be pointed out that secondary chromosome changes, in addition to these primary events, have almost invariably been found in these tumours; and these secondary changes may dictate some of the anatomical and biological features of the disease – for example, the frequent involvement of the neck and head region in endemic areas of the disease and their less frequent involvement in the nonendemic areas. The karyotypic changes in BL have served as an important basis for establishing some of the molecular events in the disease; these include the translocation of an oncogene, c-*myc*[75,76], from chromosome 8 and the restricted expression of specific light chains of surface immunoglobulins[74,77]. Thus, in the disease characterized by a translocation between chromosome 2 and 8 only kappa light chains are expressed, whereas in the case of t(8;22) only lambda light chains are expressed[74,77]. Similar

information on the production of heavy chain immunoglobulins and translocation on to chromosome 14 have also been described. The findings are discussed in greater detail in Chapters 5 and 8.

However, recently published findings raise a number of questions regarding presently held views in BL. Thus, it has been reported[28] that non-endemic BL may be associated with a 6q- anomaly without evidence of involvement of chromosomes 8, 14, 2 or 22. The authors raise questions about the definition of BL, at least in cytogenetic terms, and of the involvement of various DNA sequences as a mechanism in the genesis of BL. In another report[79] cell lines derived from a homosexual patient with probable acquired immunodeficiency syndrome (AIDS) and BL with a consistent t(8;22) produced kappa light chains rather than the expected lambda chains. The findings indicated to the authors that the translocation in BL may occur as an event separate from immunoglobulin gene rearrangement or that the proposed hierarchical sequence of immunoglobulin gene rearrangement is not always adhered to. Furthermore, the authors[79] indicated that in cells containing a translocation between the long arm of chromosome 8 and a chromosome bearing an immunoglobulin gene, activation and expression of the c-*myc* proto-oncogene may occur regardless of the immunoglobulin gene that is expressed.

The frequent involvement of chromosome 1 in the karyotypic changes of BL – as it is in other lymphomas, leukaemias and cancers – was related to EBV status. All nine BL cell lines not associated with EBV were shown to contain an abnormality of the long arm (bands 1q23–q24) of chromosome 1. The authors hypothesized[80] that genetic information resembling that contained within the viral genome was present on the long arm of chromosome 1 and may bear upon the relationship between BL cell proliferation and EBV.

Malignant lymphomas (other than Burkitt's)

In lymphomas other than those of the Burkitt variety, certain karyotypic changes appear to be commonly associated with a particular type of disease (Table 2.10)[14,37,81,82]. Thus, t(8;14) of the same genesis as in BL appears often in the diffuse type of disease – including small noncleaved non-Burkitt lymphoma, as well as immunoblastic lymphoma. On the other hand, t(14;18)(q32.3;q21.3) appears to be commonly associated with follicular types

Table 2.10 Types of lymphoma and chromosome changes

t(8;14)(q24;q32)	Diffuse large cell
t(14;18)(q32;q21)	Follicular
t(11;14)(q13;q32)	Diffuse small cell
del(6)(q21)	Large cell, diffuse
+7	Diffuse, large cell or follicular
11q-	Diffuse small cell
+12	Diffuse small cell

of lymphoma – such as, small cleaved, mixed small and large cleaved cell lymphomas. This translocation has also been seen in rare cases of diffuse large cell lymphoma. In addition, 6q-(q21q25) has been commonly associated with diffuse large (noncleaved) cell lymphoma, some cases of follicular lymphoma of the mixed small and large cell variety, rare cases of T-cell lymphoma, transformed small cell lymphocytic lymphoma, and immunoblastic lymphoma. The anomalies t(11;14)(q13.1;q32.3) and +12 have been seen most commonly in small cell lymphocytic lymphoma or, as mentioned previously, in CLL. These chromosome changes are retained when a small cell lymphocytic lymphoma transforms to a diffuse large cell variety. As yet, no non-random or specific chromosome changes have been established in diffuse cleaved cell lymphoma, lymphoblastic lymphoma, or diffuse T- or B-cell mixed cell lymphoma. The involvement of 9p13, either in translocation or inversions, is of interest in some cases of T-cell mixed cell lymphomas[14].

In more recent reports on a large series of lymphomas[81,82], it was shown that the most frequent numerical chromosome changes were 12, 18, 7 and 21 (in order of frequency) – +12 was seen most frequently in small-cell lymphocytic lymphoma, a disease which has much in common with CLL and in which +12 is also the most frequent karyotypic change[59,60]. Structural abnormalities most frequently involved 14q, 18q, 6q, 1p and 8q (in order of frequency). The long arm of chromosome 14, which is often involved in translocations, was involved in more than 70 per cent of the lymphomas. The most frequent translocation was t(14;18)(q32;q21), followed by t(8;14)(q24;q32) and t(1;14)(q42;q32). Deletions most frequently involved chromosome 6 at band q21 or q23. There are also correlations between the karyotype and the histology and some of the immunological aspects of the lymphomas[75,83].

Correlations with some phenotypic aspects

As more and more data are collected on the karyotypic changes in lymphoma and correlated with various histological, haematological, immunological and clinical features, it is likely that a subclassification of this group of diseases, similar to that already established in the acute leukaemias, will emerge and will be of considerable aid in deciphering the nature of these diseases. It should be possible to relate the cytogenetic findings to the diagnostic and prognostic features, and to specify optional therapeutic approaches for each lymphoma, when a sufficient body of data has been accumulated and analyzed.

DIAGNOSTIC AND PROGNOSTIC ASPECTS OF CHROMOSOME CHANGES IN LEUKAEMIA AND LYMPHOMA

Specific (primary) changes

It was stated above that specific (primary) karyotypic changes in human neoplasia are apparently a *sine qua non* for the development of malignant transformation[84,85]. Such events may or may not involve oncogenes, but appear

to be of crucial importance in initiating the malignant process within a cell. However, the primary karyotypic change is frequently followed by the development of secondary chromosome changes, which may also play an important role in the aetiology of the leukaemia or lymphoma[86].

Primary versus secondary chromosome changes

As already discussed above, most leukaemias and lymphomas are characterized by primary (specific) chromosome changes, which have been tabulated. These primary changes are possibly related to, if not necessary for, the process of malignant transformation, which occurs in the affected cells and eventually results in the clinically evident manifestations of these diseases, when the bulk of the abnormal cells affect the normal physiology. Generally, a leukaemia or lymphoma is at its lowest level of malignancy when only the primary chromosome change exists. Later, when other secondary karyotypic changes appear[86,87], the disease becomes more aggressive and often resistant to therapy. In some instances, these secondary changes are of a non-random variety (e.g. +Ph^1, +8 and iso17q in CML). In other cases, such as in the lymphomas, the secondary changes are often of a complex nature and differ from one patient to another. Thus, in future it will be important to ascertain which of these secondary karyotypic changes is related to the phenotypic manifestations of the leukaemia or lymphoma, so that a better understanding of these diseases may be achieved. It is also possible that the secondary karyotypic changes may influence some of the clinical and phenotypic manifestations – for example, the anatomical distribution of BL in the neck and head in Africa as contrasted to that found in the Western World. Some of the phenotypic variations among lymphomas with the same primary karyotypic change, e.g. t(14;18), may also be due to the secondary changes which may be reflected in such features as whether the cells are cleaved or noncleaved, large or small, or in the organs affect by lymphoma.

CONCLUDING REMARKS

During the past 20 years, and especially during the last decade, a considerable number of characteristic chromosome changes have been discovered in leukaemia and lymphoma. It is likely that further research will disclose more, though probably less frequently occurring, changes in acute leukaemias. For lymphoma, new specific associations may be found when better culture and mitogenic methods are established and the widely diverse nature of the proliferations is better defined.

It is obvious that some leukaemias do not show consistent chromosome abnormalities. However, the vast majority – especially those which are clearly induced by environmental agents or chemotherapy – show a variety of rather specific changes.

Some of these are clearly so-called primary changes occurring as the sole

anomaly, usually very early in the disease. Others appear much later as additional secondary abnormalities, and may contribute to a diversity of changes in behaviour, including differential sensitivity to therapeutic agents. Both primary and secondary karyotypic changes may be relevant to oncogenes and may be instrumental in their activation, as shown elsewhere in this volume.

In the meantime, these specific chromosome changes are of great help to the clinician in assessing diagnosis, treatment and prognosis for their patients.

REFERENCES

1. Nowell P C, Hungerford D A 1960 A minute chromosome in human granulocytic leukemia. Science 132: 1497
2. Tijo JH, Levan A 1956 The chromosome number of man. Hereditas 42: 1–6
3. Standardization in human cytogenetics, Paris Conference 1971 1975 Birth defects: Orig Art Ser, Vol XI, No 9. The National Foundation, New York
4. Sandberg A A 1980 The chromosomes in human cancer and leukemia. Elsevier North-Holland, New York
5. Trujillo J M, Cork A, Ahearn M J, Youness E L, McCredie K B 1979 Hematological and cytological characterization of the 8;21 translocation. Blood 52: 695–706
6. Rowley J D 1973 Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet 16: 109–112
7. Sakurai M, Sandberg A A 1973 Prognosis in acute myeloblastic leukemia: Chromosomal correlation. Blood 41: 93–104
8. Yunis J J 1976 High resolution of human chromosomes. Science 191: 1268–1270
9. Hagemeijer A, Smit E M E, Bootsma D 1979 Improved identification of chromosomes of leukemic cells in methotrexate-treated cultures. Cytogenet Cell Genet 23: 208–212
10. Yunis J J, Bloomfield C D, Ensrud B S 1981 All patients with acute nonlymphocytic leukemia may have a chromosomal defect. New Engl J Med 305: 135–139
11. Knuutila S, Vuopio P, Elonen E, Siimes M, Kovanen R, Borgstrom G H, de la Chapelle A 1981 Culture of bone marrow reveals more cells with chromosomal abnormalities than the direct method in patients with hematologic disorders. Blood 58: 369–375
12. Fitzgerald P H, Morris C M, Giles L M 1982 Direct versus cultured preparation of bone marrow cells from 22 patients with acute myeloid leukemia. Hum Genet 60: 281–283
13. Berger R, Bernheim A, Le Coniat M, Vecchione D, Pacot A, Flandrin G 1983 Cytogénétique et leucémies aigues non lymphoblastiques intéret des cultures à court terme. Nouv Rev Fr Hematol 25: 81–86
14. Yunis J J, Oken M M, Kaplan M E, Ensrud K M, Howe R R, Theologides A 1982 Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. New Engl J Med 307: 1231–1236
15. Rowley J D 1973 A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243: 290–293
16. First International Workshop on Chromosomes in Leukemia. Helsinki, Finland, Aug 25–28, 1977. 1978 Cancer Res 38: 867–868
17. Geurts van Kessel A H M, ten Brinke H, Boere W A M, den Boer W C, de Groot P G, Hagemeijer A, Meera Khan P, Pearson P L 1981 Characterization of the Philadelphia chromosome by gene mapping. Cytogenet Cell Genet 30: 83–91
18. de Klein A et al. 1982 A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765–767
19. Sandberg A A 1980 Chromosomes and causation of human cancer and leukemia. XL. The Ph[1] and other translocations in CML. Cancer 46: 2221–2226
20. Potter A M, Watmore A E, Cooke P, Lilleman J S, Sokol R J 1981 Significance of non-standard Philadelphia chromosomes in chronic granulocytic leukaemia. Br J Cancer 44: 51–54

21. Bartram C R et al. 1983 Translocation of the human c-*abl* oncogene occurs in variant Ph-positive but not Ph-negative chronic myelocytic leukaemia. Nature 306: 277–280
22. Groffen J et al. 1983 C-*sis* is translocated from chromosome 22 to chromosome 9 in chronic myelocytic leukemia. J Exp Med 158: 9–15
23. Heisterkamp N, Stephenson J R, Groffen J, Hansen P F, de Klein A, Bartram C R, Grosveld G 1983 Localization of the c-*abl* oncogene adjacent to a translocation breakpoint in chronic myelocytic leukaemia. Nature 306: 239–242
24. Groffen J, Stephenson J R, Heisterkamp N, de Klein A, Bartram C R, Grosveld G 1984 Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36: 93–99
25. Bennett J M, Catovsky D, Daniel M T, Flandrin G, Galton D A G, Gralnick H R, Sultan C 1976 Proposals for the classification of the acute leukaemias. Br J Hematol 33: 451–458
26. Second International Workshop on Chromosomes in Leukemia. Leuven, Belgium, Oct 2–6, 1979. 1980 Cancer Genet Cytogenet 2: 89–113
27. Third International Workshop on Chromosomes in Leukemia. Lund, Sweden, July 21–25, 1980. 1981 Cancer Genet Cytogenet 45: 95–142
28. Fourth International Workshop on Chromosomes in Leukemia: A prospective study of acute nonlymphocytic leukemia. Chicago, Illinois, USA, Sept 2–7, 1982. 1984 Cancer Genet Cytogenet 11: 249–360
29. Arthur D C, Bloomfield C D, Lindquist L L, Nesbit, Jr. M E 1982 Translocation 4;11 in acute lymphoblastic leukemia: Clinical characteristics and prognostic significance. Blood 59: 96–99
30. Weh H J, Hossfeld D K 1982 Translocation t(4;11) in acute lymphocytic leukemia (ALL). Blut 44: 271–274
31. Kocova M, Kowalczyk J R, Sandberg A A 1985 Translocation t(4;11) acute leukemia: Three case reports and review of the literature. Cancer Genet Cytogenet 16: 21–32
32. Nagasaka M, et al. 1983 Four cases of t(4;11) acute leukemia and its myelomonocytic nature in infants. Blood 61: 1174–1181
33. Carroll A J, Crist W M, Parmley R T, Roper M, Cooper M D, Finley W H 1984 Pre-B cell leukemia associated with chromosome translocation 1;19. Blood 63: 721–724
34. Arthur D C, Bloomfield C D 1983 Partial deletion of the long arm of chromosome 16 and bone marrow eosinophilia in acute nonlymphocytic leukemia: A new association. Blood 61: 994–998
35. Le Beau M M, Larson R A, Bitter M A, Vardiman J W, Golomb H M, Rowley J D 1983 Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia. New Engl J Med 309: 630–636
36. Sweet D L, Golomb H M, Rowley J D, Vardiman J M 1979 Acute myelogenous leukemia and thrombocythemia associated with an abnormality of chromosome no. 3. Cancer Genet Cytogenet 1: 33–37
37. Bernstein R, Pinto M R, Behr A, Mendelow B 1982 Chromosome 3 abnormalities in acute nonlymphocytic leukemia (ANLL) with abnormal thrombopoiesis: report of three patients with a 'new' inversion anomaly and a further case of homologous translocation. Blood 60: 613–617
38. Swirsky D M, Li Y S, Matthews J G, Flemans R J, Rees J K H, Hayhoe F G J 1984 8;21 translocation in acute granulocytic leukaemia: cytological, cytochemical and clinical features. Br J Haematol 56: 199–213
39. Slater R M, Behrendt H, De Waal F C 1983 Chromosome studies on acute nonlymphocytic leukaemia in children. Pediatr Res 17: 398–405
40. Vermaelen K, Michaux J-L, Louwagie A, Van Den Berghe H 1983 Reciprocal translocation t(6;9)(p21;q33): new characteristic chromosome anomaly in myeloid leukemias. Cancer Genet Cytogenet 10: 125–131
41. Schwartz S, Jiji R, Kerman S, Meekins J, Cohen M M 1983 Translocation (6;9)(p23;q34) in acute nonlymphocytic leukemia. Cancer Genet Cytogenet 10: 133–138
42. Sandberg A A, Morgan R, McCallister J A, Kaiser-McCaw B, Hecht F 1983 Acute myeloblastic leukemia (AML) with t(6;9)(p23;q34): a specific subgroup of AML? Cancer Genet Cytogenet 10: 139–142
43. Fraser J, Hollings P E, Fitzgerald P H, Day W A, Clark V, Heaton D C, Hamer J W, Beard M E J 1981 Acute promyelocytic leukemia: cytogenetics and bone-marrow culture. Int J Cancer 27: 167–173

44. Fitzgerald P H, Morris C M, Fraser G J, Giles L M, Hamer J W, Heaton D C, Beard M E J 1983 Nonrandom cytogenetic changes in New Zealand patients with acute myeloid leukemia. Cancer Genet Cytogenet 8: 51–66
45. Berger R, Bernheim A, Flandrin G, Daniel M T, Valensi F, Ochoa M H, Marty M, Schaison G, Boiron M 1983 Valeur pronostique des anomalies chromosomiques dans les leucémies aigues non lymphoblastiques. Nouv Rev Fr Hematol 25: 87–90
46. Bernard P H, Reiffers J, Boisseau M R, Broustet A 1980 Leucémies aigues granuleuses de l'adulte: valeur pronostique du caryotype médullaire. Nouv Press Medicale 9: 499–503
47. Morse H G, Odom L F, Tubergen D, Hays T, Blake M, Robinson A 1983 Prognosis in acute lymphoblastic leukemia of childhood as determined by cytogenetic studies at diagnosis. Med Pediat Oncol 11: 310–318
48. Li Y S, Khalid G, Hayhoe F G J 1983 Correlation between chromosomal pattern, cytological subtypes, response to therapy, and survival in acute myeloid leukemia. Scand J Haematol 30: 265–277
49. Sakurai M, Sandberg A A 1976 Chromosomes and causation of human cancer and leukemia. XI. Correlation of karyotypes with clinical features of acute myeloblastic leukemia. Cancer 37: 285–299
50. Cooperman B S, Klinger H P 1981 Double minute chromosomes in a case of acute myelogenous leukemia resistant to chemotherapy. Cytogenet Cell Genet 30: 25–30
51. Marinello M J, Bloom M L, Doeblin T D, Sandberg A A 1980 Double minute chromosomes in human leukemia. New Engl J Med 303: 704
52. Hartley S E, Toolis F 1980 Double minute chromosomes in a case of acute myeloblastic leukemia. Cancer Genet Cytogenet 2: 275–280
53. Michael P M, Levin M D, Garson O M 1984 Translocation 1;19—A new cytogenetic abnormality in acute lymphocytic leukemia. Cancer Genet Cytogenet 12: 333–341
54. Williams D L, Look A T, Melvin S L, Roberson P K, Dahl G, Flake T, Stass S 1984 New chromosomal translocations correlate with specific immunophenotypes of childhood acute lymphoblastic leukemia. Cell 36: 101–109
55. Kowalczyk J, Sandberg A A 1983 A possible subgroup of ALL with 9p–. Cancer Genet Cytogenet 9: 383–385
56. Secker-Walker L M, Swansbury G J, Hardisty R M, Sallan S E, Garson O M, Sakurai M, Lawler S D 1982 Cytogenetics of acute lymphoblastic leukaemia in children as a factor in the prediction of long-term survival. Br J Haematol 52: 398–399
57. Williams D L, Tsiatis A, Broduer G M, Look A T, Melvin S L, Bowman W P, Kalwinsky D K, Rivera G, Dahl G V 1982 Prognostic importance of chromosome number in 136 untreated children with acute lymphoblastic leukemia. Blood 60: 864–871
58. Moller G 1972 Lymphocyte activation by mitogens. Transplant Rev 11: 1–267
59. Morita M, Minowada J, Sandberg A A 1981 Chromosomes and causation of human cancer and leukemia. XLV. Chromosome patterns in stimulated lymphocytes of chronic lymphocytic leukemia. Cancer Genet Cytogenet 3: 293–306
60. Gahrton G, Robert K H 1982 Chromosomal aberrations in chronic B-cell lymphocytic leukemia. Cancer Genet Cytogenet 6: 171–181
61. Poisez B J, Ruscetti F W, Mier J W, Woods A M, Gallo R C 1980 T-cell lines established from human T-lymphocytic neoplasias by direct response to T-cell growth factor. P.N.A.S. USA 77: 6815–6819
62. Robert K H, Gahrton G, Friberg K, Zech L, Nilsson B 1982 Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. Scand J Haematol 28: 163–168
63. Han T, Ozer H, Sadamori N, Emrich L, Gomaz G A, Henderson E S, Bloom M L, Sandberg A A 1984 Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. New Engl J Med 310: 288–292
64. Ueshima Y, Rowley J D, Variakojis D, Winter J, Gordon L 1984 Cytogenetic studies on patients with chronic T-cell leukemia/lymphoma. Blood 63: 1028–1038
65. Zech L, Gahrton G, Hammarstrom L, Juliusson G, Mellstedt H, Robert K H, Smith C I E 1984 Inversion of chromosome 14 marks human T-cell chronic lymphocytic leukaemia. Nature 308: 858–860
66. Miyamoto K, Tomita N, Ishii A, Nonaka H, Kondo T, Tanaka T, Kitajima K 1984 Chromosome abnormalities of leukemia cells in adult patients with T-cell leukemia. JNCI 73: 353–362

67. Togawa A, Inoue N, Miyamoto K, Hyodo H, Namba M 1982 Establishment and characterization of a human myeloma cell line (KMM-1). Int J Cancer 29: 495–500
68. Ueshima Y, Fukuhara S, Hattori T, Uchiyama T, Takatsuki K, Uchino H 1981 Chromosome studies in adult T-cell leukemia in Japan: significance of trisomy 7. Blood 58: 120–125
69. Sadamori N, Han T, Minowada J, Bloom ML, Henderson ES, Sandberg A A 1983 Possible specific chromosome change in prolymphocytic leukemia. Blood 62: 729–736
70. 'The non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. 1982 Cancer 49: 2122–2135
71. Manolov G, Manlova Y 1972 Marker band in one chromosome 14 from Burkitt lymphomas. Nature 237: 33–34
72. Zech L, Haglund U, Nilsson K, Klein G 1976 Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. Int J Cancer 17: 47–56
73. Yunis J J 1984 Clinical significance of high resolution chromosomes in the study of actute leukemias and non-Hodgkin's lymphomas. In: Fairbanks V F (ed) Current Hematology, vol. 3. John Wiley and Sons, Inc, p 353–391
74. Sandberg A A 1984 Cytogenetic abnormalities in lymphoid neoplasias. In: Magrath IT, O'Conor G T, Ramot B (eds) Pathogenesis of leukemias and lymphomas: environmental influences. Raven Press, New York, p 263–282
75. ar-Rushdi A, Nishikura K, Erikson J, Watt R, Rovera G, Croce C M 1983 Differential expression of the translocated and the untranslocated c-*myc* oncogene in Burkitt lymphoma. Science 222: 390–393
76. Leder P, Battey J, Lenoir G, Moulding C, Murphy W, Potter H, Stewart T, Taub R 1983 Translocations among antibody genes in human cancer. Science 222: 765–771
77. Bernheim A, Berger R, Lenoir G 1981 Cytogenetic studies on African Burkitt's lymphoma cell lines: t(8;14); t(2;8) and t(8;22) translocations. Cancer Genet Cytogenet 3: 307–315
78. Berger R, Bernheim A, Sigaux F, Valensi F, Daniel MT, Flandrin G 1985 Two Burkitt lymphomas with chromosome 6 long arm deletions. Cancer Genet Cytogenet 15: 159–168
79. Magrath I, Erikson J, Whang-Peng J, Sievers H, Armstrong C, Benjamin D, Triche T, Alabaster O, Croce C M 1983 Synthesis of kappa light chains by cell lines containing 8;22 chromosomal translocation derived from a male homosexual with Burkitt's lymphoma. Science 222: 1094–1098
80. Berger R, Bernheim A 1984 Existe-t-il une équivalence fonctionnelle entre anomalies du bras long du chromosome 1 et présence du virus d'Epstein-Barr dans les lignées continues de lymphome de Burkitt? CR Acad Sc Paris 298: 143–145
81. Bloomfield C D, Arthur D C, Frizzera G, Levine E G, Peterson B A, Gajl-Peczalska K J 1983 Nonrandom chromosome abnormalities in lymphoma. Cancer Res 2957–2984
82. Yunis J J, Oken M M, Theologides A, Howe R B, Kaplan M E 1984 Recurrent chromosomal defects are found in most patients with non-Hodgkin's lymphoma. Cancer Genet Cytogenet 13: 17–28
83. Kaneko Y, Rowley J D, Variakojis D, Haren J M, Ueshima Y, Daly K, Kluskens L F 1983 Prognostic implications of karyotype and morphology in patients with non-Hodgkin's lymphoma. Int J Cancer 32: 683–692
84. Sandberg A A 1983 A chromosomal hypothesis of oncogenesis. Cancer Genet Cytogenet 8: 277–285
85. Sandberg A A 1984 Chromosomal changes and cancer causation: Chromatin's reawakening. In: Fortner J G, Rhoads J E (eds) Accomplishments in cancer research. J. B. Lippincott Co., Philadelphia, p 157–169
86. Sandberg A A 1982 Chromosomal changes in human cancer: Specificity and heterogeneity. In: Owens Jr. A H, Coffey P S, Baylin S B (eds) Tumor cell heterogeneity: origins and implications, vol. 4. Academic Press, Inc, New York, p 367–397
87. Sandberg A A Secondary chromosome changes in cancer: their nature and significance. Submitted. Cancer Genet Cytogenet (In press)

Chromosome localization of cellular oncogenes

INTRODUCTION

It is now generally accepted that the transforming sequences (v-oncs) of most rapidly oncogenic retroviruses are derived from normal cellular genes[1]. Each v-onc is presumed to be a pirated copy of a cellular counterpart (generally termed c-onc for lack of a better name) acquired during infection of the host with a weakly oncogenic leukaemia virus devoid of cellular derived sequences[1–4]. Incorporation of the c-onc into the retroviral genome is believed to proceed by a series of poorly understood recombinational events that may occur at the chromosomal level and/or during reverse transcription of viral RNA[4,5]. This process is accompanied by structural changes in the c-onc (deletions, additions, and base pair substitutions) that may ultimately alter the function of the v-onc[6]. Consequently, the degree to which c-oncs and v-oncs are functionally equivalent is not clearly understood[6].

However, the fact that cellular genes could function as retroviral oncogenes, albeit with structural modifications, raised the possibility that aberrant expression of c-oncs, as might be observed in cancer cells, could be important for phenotypic expression of malignancy. The one clearly recognized type of abnormality in malignant cells involves structural and numerical aberrations in chromosomal content[7]. In several instances these chromosomal abnormalities take the form of highly specific translocations, perhaps the best known of which is the Philadelphia chromosome of chronic myelogenous leukaemia (see Ch. 4).

A major question is whether the chromosome aberrations observed in cancer cells play an aetiological role in tumourigenesis and whether c-oncs are involved. An approach that our group, as well as others, undertook to examine one facet of this complex question was to determine the native chromosomal locations of c-oncs in man and mouse. Such studies were made possible by an amalgamation of several techniques: the molecular cloning of retroviral oncogenes[1] the ability to detect single copy sequences in genomic DNA[8] and the availability of interspecies somatic cell hybrids for gene mapping[9,10]. The evolutionary conservation of c-oncs allowed the detection of homologous sequences in organisms as phylogenetically distant as man and *Drosophila*[11–15], and even in yeast[16–19]. The presence of evolutionarily conserved c-oncs in

phylogenetically diverse species connoted important roles in cellular physiology common to all eukaryotes[20], and demonstrated that the significance of c-oncs was not confined to the boundaries of malignant transformation.

In this chapter, a brief account is given of some of the methodologies that have been used to construct chromosomal maps of c-oncs, what this information has told us about the evolution of certain c-onc gene families in different species, and how the search for c-oncs has extended to species other than man and mouse. No attempt is made at a comprehensive description of the structure and function of c-oncs, somatic cell hybridization and in situ hybridization methodologies, the chromosome mapping of each c-onc in man and mouse, or their relationships to the chromosome abnormalities in cancer cells. For these topics the reader is referred to other comprehensive reviews[1,4,6,7,9,10,21] and to other chapters in this volume.

METHODS FOR C-ONC GENE LOCALIZATION

Somatic cell hybrids

Many of the chromosome assignments of human and mouse c-oncs have been accomplished by the use of interspecies somatic cell hybrids that are an efficient approach to gene localization[9,10,22,23]. The method is based upon the observation that hybrid cells derived from the fusion of cells from two different species will preferentially lose chromosomes of one of the parental cell types during subsequent cell proliferation. This 'directional' loss of chromosomes is influenced by the phenotypic properties of the parental cells. For example, when normal diploid human leukocytes or fibroblasts are fused to immortal mouse cells (fibroblasts and cells of other tissue origins have been used), human chromosomes are preferentially lost from the cell hybrids. In mouse–Chinese hamster cell hybrids, formed from immortal Chinese hamster cells and diploid mouse cells, the mouse chromosomes are preferentially lost[22,23]. To facilitate the isolation of interspecies cell hybrids, a selection medium such as 'HAT' (hypoxanthine, aminopterin, and thymidine) can be employed for fusions involving, for example, immortal mouse cells deficient in thymidine kinase or hypoxanthine phosphoribosyl transferase and diploid human cells[24,25]. Each cell hybrid clone should therefore retain a subset of chromosomes of one parent. If enough cell hybrids are isolated and characterized, a set in which the whole genome of one of the parental cells is collectively represented can be established.

The problem of determining which of the chromosomes of interest has been retained by each cell hybrid clone can be solved by one of several methods. First, by analyzing homogenates of cell hybrid clones, it is possible to detect (by histochemical or other assays) the presence of enzymes that are markers for specific chromosomes (for a comprehensive list of markers useful for identifying human and mouse chromosomes see refs. 9 and 10).

In the second method, chromosomes can be identified by staining techniques

which unveil characteristic banding patterns on metaphase chromosomes (see ref. 26 for examples). Direct karyotyping can also reveal chromosome breaks and rearrangements with a resolution of perhaps a single chromosome band, something that might be missed if only marker enzymes are used to characterize cell hybrids. Since most marker enzymes commonly used to characterize cell hybrids are widely distributed in the genomes of man and mouse, chromosome breaks and other rearrangements can sometimes go undetected if characterization is not supplemented by karyotyping.

A third method that can be used to document the chromosome content of cell hybrids employs cloned DNA sequences for specific genes or for undefined, 'anonymous' fragments. Over 900 genes and undefined DNA fragments have been chromosomally assigned in man and mouse principally by Southern filter hybridization analysis of cell hybrid DNA[27]. Currently, there are one or more cloned sequences that have been assigned to each human chromosome; and several human chromosomes can be characterized by cloned genes that reside on the short and long arms (e.g. human chromosome 11 that encodes insulin, β-globin, c-Ha-*ras*1, lactate dehydrogenase B, calcitonin, parathyroid hormone, and apolipoprotein AI and CIII genes[27]). Hence several human chromosomes can be analyzed at a relatively high resolution for breaks and rearrangements.

If a cloned probe is available, gene assignment by Southern filter hybridization analysis[8] of cell hybrid DNA can be efficiently accomplished. Cell hybrid clones are grown in mass culture, harvested, and used to prepare genomic DNA, cellular homogenates for enzyme and analysis, and metaphase chromosome spreads for karyotyping. All of the analyses are performed on cells of the same culture passage to ensure that results of these analyses are correlated to each other. The genomic DNA from a cell hybrid clone should be a faithful representation of the sequences contained in the chromosomes of the cell hybrid. And, by analyzing several independent cell hybrid clones, subsets of DNA sequences from different chromosomes can be probed by Southern analysis. Chromosome assignment of a human c-*src* gene to chromosome 20 serves to exemplify this approach. For our studies we utilized a subfragment of a cloned Rous sarcoma virus genome that contained sequences specific for the v-*src* oncogene[28]. This probe hybridized principally to a 28 kb EcoRI fragment of human DNA and to 15.5 and 14 kb EcoRI fragments of mouse DNA[28]. These distinct signature fragments were then utilized to locate human c-*src* to chromosome 20 by correlating the human chromosome profiles of the cell hybrids to the presence or absence of c-*src* sequences (discussed in more detail later).

In situ hybridization

In situ hybridization can be used as an alternative to or in combination with the cell hybrid technique[29-31]. In this method, a cloned fragment of DNA is hybridized directly to metaphase chromosomes. Basically, the method entails hybridizing radiolabelled DNA or RNA to metaphase chromosomes fixed on a

slide. Autoradiographic emulsion is used to detect the silver grains resulting from β-emission from the radiolabelled probe. The accumulation of the grains over distinct bands of the metaphase chromosome indicates the position of a given gene. Refinements in the *in situ* technique have allowed the detection of single copy genes. The improvements include:

1. The use of cloned genes allowing radiolabelling to fairly high specific activity with tritium.
2. The use of dextran sulphate in hybridization to promote network formation and thereby increase the signal.
3. The use of prophase and prometaphase chromosomes with up to 1000 bands to more precisely localize genes along a chromosome[31].

Usually somatic cells (i.e. peripheral blood) are used as a source of chromosomes, but meiotic chromosomes have been obtained from sperm[32,33]. The meiotic chromosome technique, although yielding a high degree of resolution, suffers from the difficulties of obtaining human material.

An example of *in situ* hybridization to prometaphase chromosomes is given in Figure 3.1, which shows representative human chromosomes 11 hybridized to four different probes: insulin, β-globin, c-Ha-*ras*1, and parathyroid hormone. To assign a gene to a specific band of a chromosome, a statistical analysis of the grains over the chromosome must be performed. Although the modal distribution of grains for a specific probe may vary, usually 10–20 per cent of grains are found at a specific locus[29–31].

GENERAL FEATURES AND CHROMOSOME ARRAY OF THE *SRC* FAMILY OF C-ONCS

Viral oncogenes structurally and sometimes functionally related to v-*src* comprise a group of genes greater in number than initially expected[1,34,35]. Several v-oncs were initially believed to be unrelated because they did not cross-hybridize under relatively stringent conditions that tolerate only limited mismatch in DNA duplexes[36,37]. However, as molecular clones of v-oncs were isolated and sequenced, the deduced amino acid sequences of the putative transforming proteins revealed a kinship contrary to earlier conclusions. The predicted amino acid sequences of the products encoded by the v-oncs *src*, *yes*, *erbB*, *fes*, *fps*, *abl*, *raf*, *mil*, *fms*, *ros*, *fgr*, and *mos* suggest that they represent divergent members of a multigene family whose ancestor may have been present at the inception of eukaryotic organisms[34] (see Ch. 7).

Each of the members of this v-onc family shares the greatest degree of homology in the protein domain encompassing the tyrosine kinase activity of v-*src*[34]. However, it should be emphasized that only certain members of this family are associated with a tyrosine kinase activity (*src*, *yes*, *abl*, *fes*, *fps*, and *ros*) whereas other members appear to lack this activity (*mos*, *raf*)[1,35,38]. The v-*mos* gene is currently the most distantly related member of this family sharing only 26 per cent amino acid identity with v-*src*[39], the prototype of this group. At the other extreme, v-*yes* shares 82 per cent amino acid identity with v-*src*[40]. The

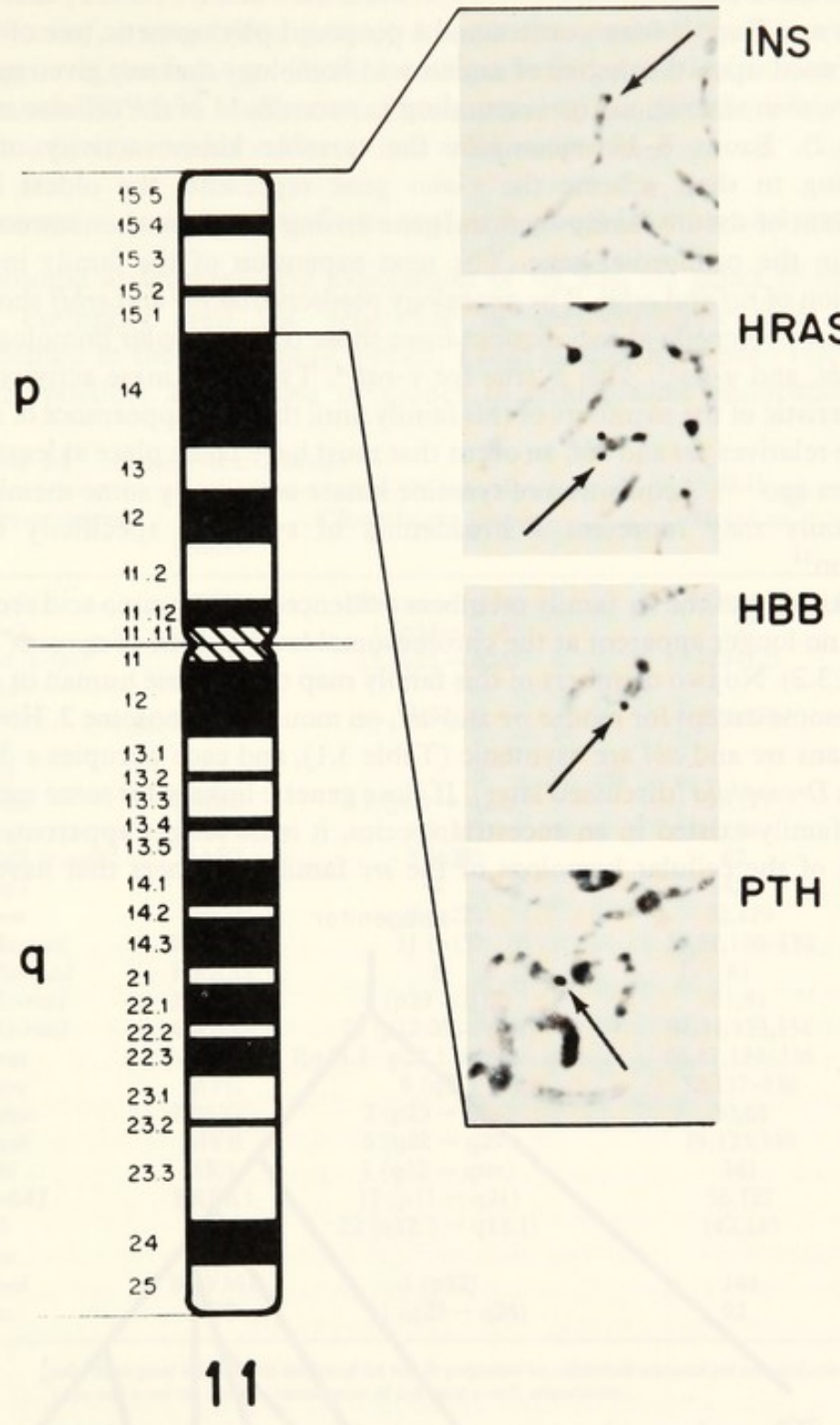

Fig. 3.1 Localization by *in situ* hybridization of insulin (*INS*), c-Ha- *ras* (*HRAS1*), β-globin (*HBB*), and parathyroid hormone (*PTH*) genes to the p15 region of human chromosome 11. Representative partial metaphase spreads showing chromosomes 11 are presented. Arrows point to silver grains marking hybridization site of probes. An ideogram of human chromosome 11 is shown on the left, with the region of hybridization of all four probes bracketed. See Zabel et al[31] for details of *in situ* hybridization.

other members of this family share amino acid identities with v-*src* that range between these two extremes[40].

Mark and Rapp[34] have constructed a proposed phylogenetic tree of the *src* family based upon the degree of amino acid homology that any given member has to v-*src* in the regions corresponding to exons 8–11 of the cellular *src* gene (Fig. 3.2). Exons 8–11 encompass the tyrosine kinase activity of *src*[41]. According to their scheme the v-*mos* gene represents the oldest known descendant of the *src* family ancestral gene arising at a time when introns were absent in the primordial gene. The next expansion of the family involved generation of *raf* and *erbB*. The genealogy predicts that *raf* and *erbB* should be present in *Drosophila* at loci distinct from those of the cellular homologs of v-*src*, v-*abl*, and v-*fes*[34]. This is true for v-*raf*[34]. Tyrosine kinase activity is not characteristic of the members of this family until the later appearance of *src* and its close relatives *yes* and *abl*, an event that must have taken place at least 800×10^6 years ago[11,12]. Acquisition of tyrosine kinase activity by some members of this family may represent a broadening of substrate specificity during evolution[34].

The kinship of the *src* family members evidenced at the amino acid sequence level is no longer apparent at the chromosomal level in man or mouse (Tables 3.1 and 3.2). No two members of this family map to the same human or mouse chromosome except for mouse *src* and *abl*, on mouse chromosome 2. However, in humans *src* and *abl* are asyntenic (Table 3.1), and each occupies a distinct locus in *Drosophila* (discussed later). If close genetic linkage for some members of this family existed in an ancestral species, it is no longer apparent.

Each of the cellular homologs of the *src* family members that have been

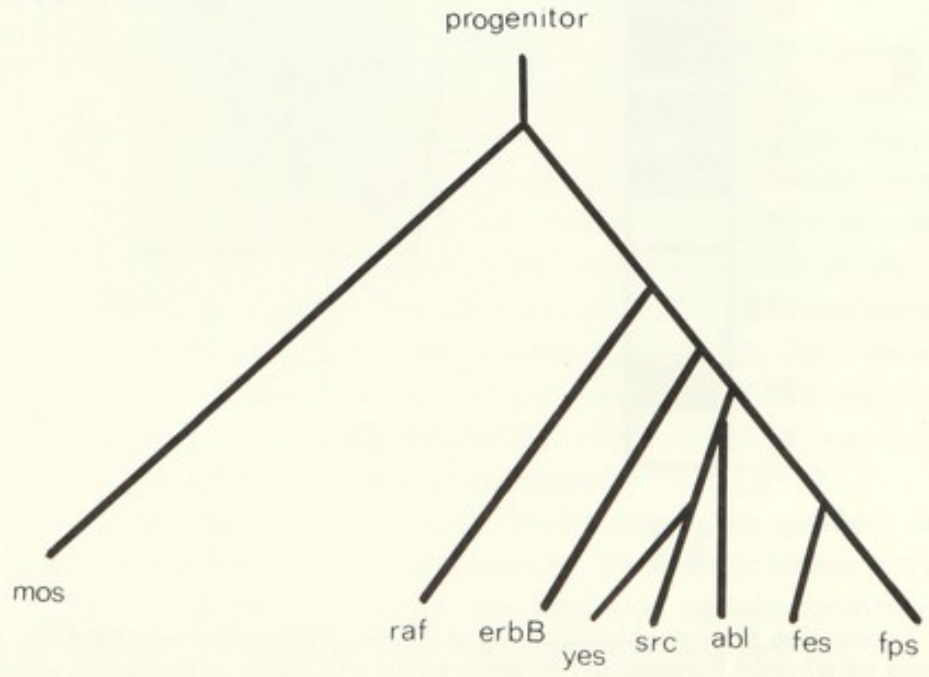

Fig. 3.2 Proposed phylogeny of *src* family members based upon deduced amino acid sequence of the putative transforming product of v-*raf* (amino acids 35–251). For point of reference, the divergence of *fps* (chicken) and *fes* (cat) is assumed to have occurred $200–225 \times 10^6$ years ago. (Adapted from Mark and Rapp[34].)

mapped, occupies a constant chromosomal position in normal human and mouse cells (Tables 3.1 and 3.2). Each represents a single locus, except for two forms of c-*raf*, a transcribed gene on human chromosome 3 and a processed pseudogene on chromosome 4[42]; for c-*src* represented as two loci, one on human chromosome 20[43] and another on chromosome 1p (C. Westbrook, personal communication); and for c-Ha-*ras* and c-Ki-*ras* genes.

Chromosome assignment of *src* and *erb* genes in man and mouse

Chromosome assignment and localization of *src* and *erb* genes in man and mouse exemplify well the repertoire of methodologies that can be implemented in the mapping of c-oncs. These studies have been relevant to studying possible associations of c-oncs to chromosome abnormalities in

Table 3.1 Human c-onc locations

Proto-oncogene	Gene symbol	Chromosome (region)	References[a]
c-*src1*	SRC1	20 (cen → q131)	28,43,122
c-*src2*	SRC2	1 (p34 → p36)	122
c-*fes* (c-*fps*)[b]	FES	15 (q25 → q26)	123–125
c-*yes*			
c-*abl*	ABL	9 (9q34)	124,126
c-*fgr*			
c-*ros*			
c-*erbB*	ERBB	7 (pter → q22)	56,127
c-*fms*	FMS	5 (q34)	128
c-*raf1* (c-*mil*)[b]	RAF1	3 (p25)	42
c-*raf2*	RAF2	4	42
c-*mos*	MOS	8 (q22)	32,129
c-Ha-*ras1*	HRAS1	11 (p15)	33,81,130–132
c-Ha-*ras2*	HRAS2	X	81
c-Ki-*ras1*	KRAS1	6 (p23 → q12)	81,91
c-Ki-*ras2*	KRAS2	12 (p12.05 → pter)	81,91,133,134
N-*ras*	NRAS 1	1(p13.1 p22.1 or p21 cen)	66,67,134–136
c-*myc*	MYC	8 (q24)	32,137–139
N-*myc*	NMYC	2 (p23 → p24)	30,64
c-*myb*	MYB	6 (q22 → q25)	29,123,140
c-*ski*	SK1	1 (q12 → qter)	141
c-*erbA1*	ERBA1	17 (p11 → q21)	56,127
c-*sis*	SIS	22 (q12.3 → q13.1)	142,143
c-*fos*			
Blyml	BLYM1	1 (p32)	144
c-*ets*	ETS	11 (q23 → q24)	92

[a] only those gene assignments are listed for which published or submitted manuscripts are available.
[b] c-*fps* and c-*mil* are chicken homologues of c-*fes* and c-*raf1*, respectively.

disease, and for examining evolutionary relationships of c-oncs to other syntenic markers.

Assignment of c-*src*

The gene termed v-*src* is the oncogene of Rous sarcoma virus (RSV), an avian retrovirus first isolated by Peyton Rous in 1911[44]. The v-*src* gene occupies a

Table 3.2 c-onc locations in rodents

Gene	Mouse chromosomes	References
c-*src*	2	58,94
c-*fes* (c-*fps*)	7	58,145
c-*abl*	2	97
c-*erbB*	11	56
c-*mos*	4	146
c-Ha-*ras1*	7	94,145
c-Ki-*ras2*	6	94
c-*myc*	15	138,139
c-*myb*	10	94
c-*erbA1*	11	56
c-*sis*	15	147
N-*ras*	3	148
	Rat chromosome	
c-*myc*	7	149

1578 bp region of the RSV genome and can specify a polypeptide product of 526 amino acids (see ref. 45 for the complete nucleotide sequence of the Prague C strain of RSV). The v-*src* product is a 60 000 dalton phosphoprotein termed $pp60^{v\text{-}src}$ that phosphorylates tyrosine residues in substrates[38]. Expression of a functional $pp60^{v\text{-}src}$ product is required for the initiation and maintenance of transformation of cells in vitro and sarcomagenesis in vivo. A protein similar in properties to $pp60^{v\text{-}src}$ is present in normal chicken cells, as well as in cells from species phylogenetically distant from vertebrates[1].

Consistent with the aforementioned observations was the earlier demonstration that normal chicken cells contained sequences homologous to v-*src*[46] – a finding later extended to mammalian species[20] and invertebrates[11]. The c-*src* genes detected appeared to be present in one or two copies per haploid genome and were thus low copy genes. In the chicken, c-*src* appeared to occupy a unique chromosomal location, unlinked to other retroviral related sequences[1]. These properties of c-*src* suggested that it should be possible to define its unique chromosomal location in humans and mouse – two species for which somatic cell hybrids could be used for gene localization.

A fragment of a molecular clone of RSV[47] containing v-*src* was annealed to DNA by the Southern filter hybridization method under conditions which allowed imperfectly matched DNA duplexes to form[28]. Using cellular DNAs cut with a variety of restriction enzymes, unique signature fragments of cellular *src* genes were detected in human, mouse, and Chinese hamster DNAs[28], as well as the DNAs of rat, sheep, cow, and monkey (unpublished observations). A 28 kb EcoRI fragment of human DNA annealing with v-*src* could be distinguished from 15.5 and 14 kb fragments of mouse DNA, and from 22 and 2.1 kb fragments of Chinese hamster DNA[28] (Fig. 3.3). This 28 kb human *src*-specific fragment cosegregated in human–mouse hybrids with human chromosome 20 exclusively when compared to all other human autosomes and

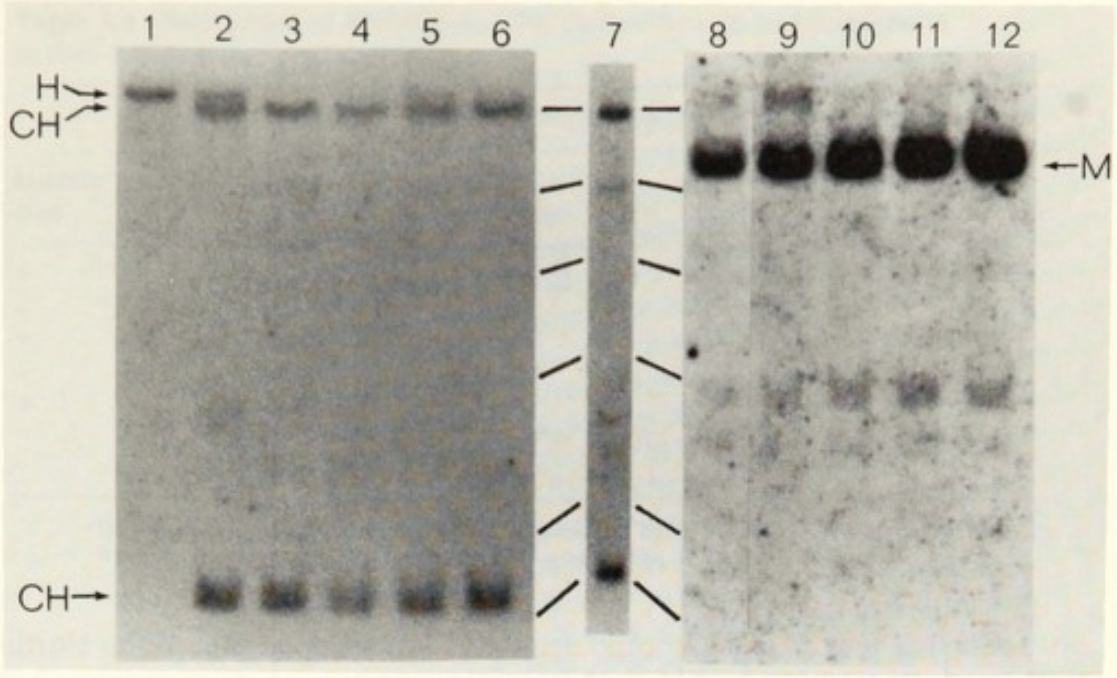

Fig. 3.3 Hybridization of v-*src* to EcoRI cleaved DNAs from human, mouse, Chinese hamster cells, and human–rodent somatic cell hybrids. Parental DNAs from human diploid fibroblasts, Chinese hamster CHW–1102 fibroblasts, and mouse A9 fibroblasts are in lanes 1, 7, and 8, respectively. Human–Chinese hamster somatic cell hybrid DNAs are in lanes 2–6. Human–mouse somatic cell hybrid DNAs are in lanes 9–12. Size of hybridizing fragments are: Human (H), lane 1, 28 kb; Chinese hamster (CH), lane 7, 22 and 2.1 kb; Mouse (M), lane 8, 15.5 and 14 kb (not resolved in this photograph). Cell hybrid DNAs in lanes 2 and 9 contain the 28 kb human c-*src* fragment. Cell hybrid DNA in lane 5 is very weakly positive for the 28 kb human c-*src* fragment. The ends of hatch marks point to the positions of bacteriophage lambda HindIII size markers.

the X chromosome (Table 3.3). Regional localization of human c-*src* on chromosome 20 was pursued because of structural abnormalities associated with 20q in a variety of myeloproliferative disorders. Regional localization of human c-*src* was accomplished using a set of human–rodent cell hybrids carrying translocations of human chromosome 20[48]. Following the 28 kb EcoRI fragment (or a 15.4 kb BglII fragment), human c-*src* segregated concordantly with 20q but not 20p, and was more precisely localized to the region 20cen→q13.1[43] (Fig. 3.3 and Table 3.4). A c-*src* gene on 20q has also been detected by *in situ* hybridization methodology along with a homologous sequence on 1p (C. Westbrook, personal communication), although the *src* related sequence on 1p might be a pseudogene (J.M. Bishop, personal communication). Our previous study of human c-*src* suggested the presence of *src* related sequences unlinked to chromosome 20[28]. However, the 870 bp PvuII fragment of v-*src* used to localize c-*src* to 20cen→q13.1 hybridizes almost exclusively to the 28 kb EcoRI fragment under the conditions of hybridization used[43] (Fig. 3.3).

These data place c-*src* on the same chromosome arm as deletions in 20q (in the region of q11) seen in myeloproliferative disorders[49]. However, c-*src* is distant from a deletion at 20p12.2 reported to be present in some persons with

Table 3.3 Segregation of c-*src* with human chromosomes in human–rodent hybrids

		Chromosome[1]																							
	c-*src*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Translocations
ICL-15	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	+	–	–	
DUA-5BSAgA	–	–	–	+	–	+	–	–	–	–	–	+	–	–	+	–	–	+	+	–	–	+	–	–	
XER-9	–	–	+	–	+	–	–	–	+	–	+	–	+	–	+	–	–	+	+	–	–	+	–	–	
NSL-9	+	–	–	–	–	+	–	–	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	–	17/9
ATR-13	–	+	+	+	+	–	+	+	+	–	+	–	+	–	+	+	–	+	+	–	–	–	–	–	5/X
REW8ICSAz4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	
WIL-14	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	
DUA-1CSAzF	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
EXR-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11/X;X/11
XER-7	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	–	–	–	–	–	+	11/X
XER-11	+	+	–	+	+	–	+	+	+	–	+	–	+	–	–	+	+	+	+	–	+	–	+	–	11/X;X/11
% discordancy[2]		45	22	33	64	50	43	38	14	56	28	28	30	30	30	61	40	43	40	36	0	34	48	50	

[1] A "+" indicates the presence of the chromosome in >10% of metaphases scored
[2] Per cent discordancy for c-*src* in an additional 30 enzyme characterized cell hybrids

Table 3.4 Segregation of human c-*src* with chromosome 20 translocations in human–rodent somatic cell hybrids

		Region of chromosome 20 present*		
Human c-*src*	Chromosome 20	20p	20q	20q13.1 → qter
+	–	–	+	
–	–	+	–	
+	+	+	–	
–	–	+	–	
+	–	+	+	
–	–	+	–	
–	–			+

*The region 20p is selectively retained in HAT medium as a Xq20p translocation. The region 20q13.1 → qter is selectively retained in HAT medium as a 17;20 translocation.

multiple endocrine neoplasia II[50]. Mouse chromosome 2, that encodes c-*src*, also frequently undergoes deletions in radiation-induced myeloid leukaemias[51]. It has also been shown that v-*src* can induce increased cell proliferation and renewal in haematopoietic cells[52]. However, whether aberrations of human chromosome 20 or mouse chromosome 2 in myeloid disorders reflect changes at c-*src* loci is altogether unknown.

Chromosomal assignment of c-*erb* genes

Rapidly transforming retroviruses carrying two host derived sequences pose unique problems for explaining their mode of capture by the virus – especially if the genes are unlinked in the host cell genome – and also for deciphering how the two encoded products might interact in the cell. One such virus is avian erythroblastosis virus (AEV), a rapidly transforming retrovirus that induces erythroid leukaemias and sarcomas in infected birds and transforms erythroblasts and fibroblasts *in vitro*[1,4]. Portions of the AEV genome have been substituted with two genetic loci (*erbA* and *erbB*) that have been derived from the chicken genome and are responsible for viral oncogenesis[53]. Transformation by AEV arrests the development of erythroid progenitor cells, and it is possible that the chicken cellular *erbA* and *erbB* genes might function normally in the control of haematopoietic cell differentiation. Chicken c-*erbB* is activated in avian leukosis virus–induced erythroblastosis by promoter insertion[54] akin to the activation of c-*myc* in lymphomas induced by leukosis virus[1]. Recent studies using in vitro mutagenesis techniques indicate that the ability of AEV to transform both fibroblasts and erythroblasts resides solely in the v-*erbB* locus[55]. However, the v-*erbA* gene may enhance the effect of v-*erbB* in leukaemogenesis although it is incapable of transforming cells independently.

In as much as AEV is composed of two independent cell-derived sequences of chicken origin, determination of the molecular and chromosomal organization of the chicken c-*erbA* and c-*erbB* genes has been pursued. The

chicken c-*erbA* and c-*erbB* genes are carried on distinct cellular DNA fragments[53] and the genes appear to be unlinked altogether (see later). The chromosomal topography of c-*erb* genes in other species, namely man and mouse, have now also been explored[56]. In man, c-*erbB* has been localized to 7pter→q22 and c-*erbA* to 17p11→q21 (Table 3.1).

The location of c-*erb* genes on chromosomes 7 and 17 was of interest, as certain regions of human chromosomes 7 and 17 encode genes whose mouse homologues appear to be syntenic. These include genes for β-glucuronidase, malate dehydrogenase, argininosuccinate lyase, and phosphoserine phosphatase on human chromosome 7, which have homologues on mouse chromosome 5[27]. Genes for thymidine kinase (*TK*, human; *Tk*, mouse) and galactokinase (*GALK*, human; *Glk*, mouse) are closely linked on human chromosome 17 and mouse chromosome 11[27]. Conserved linkage groups in man and mouse that include several proto-oncogenes have been described, so we explored the possibility that such a relationship existed for mouse c-*erbA* and c-*erbB* genes.

A panel of mouse–Chinese hamster cell hybrids was consistently negative for c-*erbA* and c-*erbB* – suggesting that both genes were located on mouse chromosome 11, the only mouse chromosome not represented in the panel. For direct proof for such an assignment, the microcell hybrid methodology developed by Fournier and Ruddle[57] was used. This technique allows the isolation of interspecies microcell hybrids containing single mouse chromosomes, which have been useful for gene mapping studies. One such microcell hybrid clone, F(11)F, containing only mouse chromosome 11 encoding Tk was used along with F(11)FR, counterselected with BrdUrd, which lacked mouse chromosome 11.

The c-*erbA* and c-*erbB* genes were both present in F(11)F and coordinately absent in F(11)FR, thus establishing synteny for both genes in the mouse, in contrast to their asynteny in man and chickens (Fig. 3.4A and 3.4B). The proximity of mouse c-*erbA* and c-*erbB* genes to two other mouse chromosome 11 markers, *Tk* and *Glk*, has been examined in PBH mouse–Chinese hamster hybrids. During passage in culture PBH hybrids had apparently suffered deletions in mouse chromosome 11. The possibility that the breakage events had separated these syntenic markers was explored and confirmed by filter hybridization of PBH DNA.

Whereas each of six PBH clones examined retained *Tk*, only some were Glk^+, indicating that chromosome breakage had separated these two syntenic markers[56]. Two of the six PBH hybrids were positive for mouse c-*erbA*, whereas all six were negative for mouse c-*erbB* (Fig. 3.5A and 3.5B). Thus, chromosome breakage had also separated c-*erbA* and c-*erbB*, suggesting that they are not tightly linked. Mouse c-*erbA* and *Glk* were present but independent of each other suggesting that they might reside on opposite sides of the *Tk* locus. However, the PBH hybrids had not been characterized karyotypically[56]. Consequently, multiple, complex breaks in mouse chromosome 11 could have occurred in PBH hybrids, so that the topographical relationship of the four

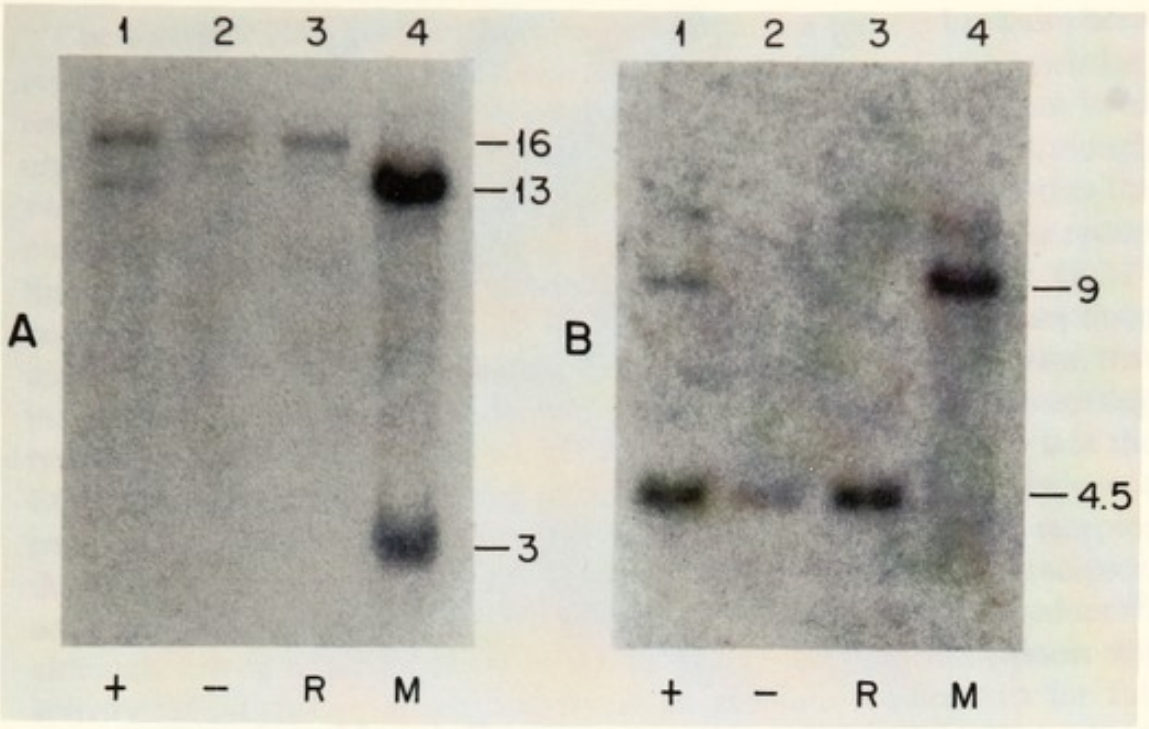

Fig. 3.4 Hybridization of rat–mouse microcell hybrid DNA to v- *erbA* (A) and v- *erbB* (B). Cellular DNAs in A (cleaved with HindIII) and B (cleaved with XbaI) are: lane 1, rat–mouse microcell line F(11)F containing only mouse chromosome 11; lane 2, hybrid F(11)FR, derived from F(11)F by counterselection using BrdUrd; lane 3, rat; and lane 4, mouse.
A. The v- *erbA* probe hybridizes strongly to a 13 kb mouse HindIII DNA fragment and less intensely with a 3 kb fragment (lane 4). A 16 kb fragment is detected in rat DNA (lane 3). The 13 kb mouse DNA fragment was coordinately present or absent with mouse chromosome 11 (lanes 1 and 2).
B. The v- *erbB* probe hybridizes with a 9 kb XbaI fragment of rat DNA (lane 4) and with a 4.5 kb XbaI fragment of rat DNA (lane 3). The 9 kb mouse c-*erbB* DNA fragment was present or absent together with mouse chromosome 11 (lanes 1 and 2). (From Zabel et al[56], with permission.)

genes on mouse chromosome 11 remains to be established. Two complementary approaches that could be utilized to answer this question are: in situ hybridization and linkage analysis in recombinant inbred strains of mice; or the segregation of polymorphic DNA markers detected by molecular clones of the genes of interest[29,31,58].

Jansson et al.[59] have isolated DNA sequences homologous to v-*erbA* and v-*erbB* from a human genomic library. The existence of two distantly related c-*erbA* genes (A1 and A2) in human and mouse was suggested by their study. The c-*erbA1* locus is most homologous with v-*erbA* and appears to be the gene assigned to human chromosome 17 and mouse chromosome 11[56]. It is not known if c-*erbA2* is linked to c-*erbA1*.

An interesting comparison of markers now including c-*erb* genes on human chromosomes 7 and 17 and mouse chromosome 11 can be made. Several other human chromosome 17 markers – *TK*, *GALK*, and sarcomeric myosin heavy chain genes *MYHSA1*, *MYHSA2* and *MYHSE1* – reside on mouse chromosome 11[27]. A possible relationship between genes on human chromosomes 7 and 17 may also exist, since another cluster of myosin heavy

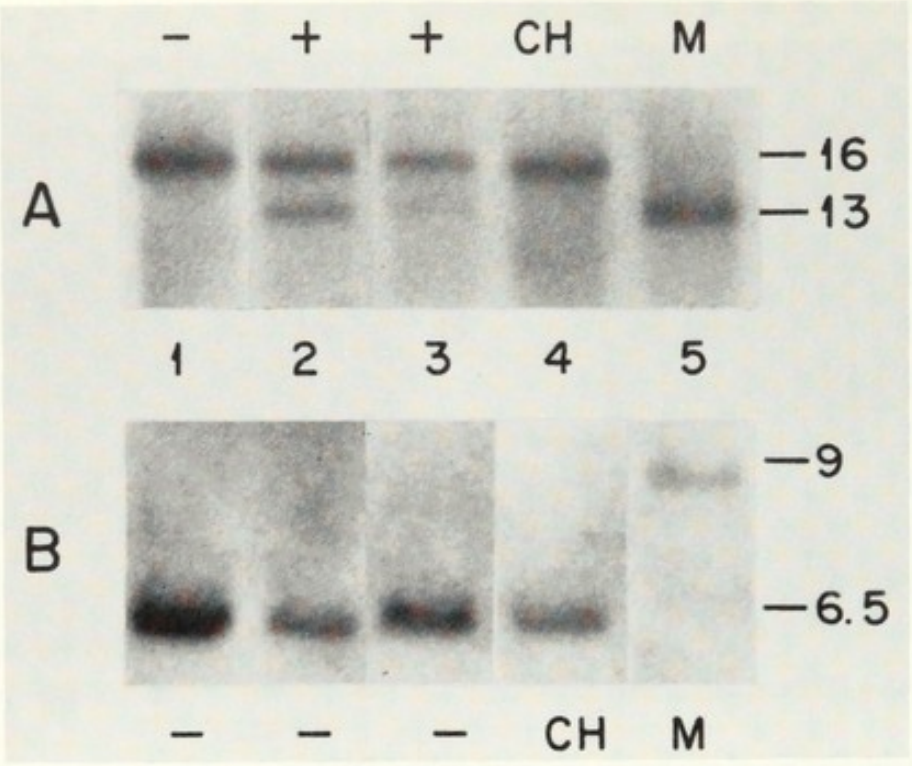

Fig. 3.5 Cellular DNAs from mouse–Chinese hamster hybrids (PBH) cleaved with HindIII (A) or XbaI (B) and hybridized to v- *erbA* (A) and v- *erbB* (B). The PBH hybrids contain fragments of mouse chromosome 11 and were also tested for mouse chromosome 11 markers thymidine kinase (*Tk*) and galactokinase (*Glk*). The results of the filter hybridization enzyme analyses for three of the six PBH hybrids shown are as follows: lane 1, PBH-1 (Tk^+, Glk^-, c- $erbA^+$, c- $erbB^+$); lane 2, PBH-5 (Tk^+, Glk^-, c- $erbA^+$, c- $erbB^-$); lane 3, PBH-15 (Tk^+, Glk^-, c- $erbA^+$, c- $erbB^-$). Lanes 4 and 5 contain Chinese hamster DNA and mouse DNA, respectively. (From Zabel et al.[56] with permission.)

chain genes (*MYH4*) has been localized on chromosome 7[27] and members of the collagen gene family have been mapped to human chromosomes 7 and 17[27]. Certain genes on mouse chromosome 11 have probably become dispersed to human chromosomes 7 and 17 during the 80×10^6 years of evolutionary time separating these two species. If the collagen gene family located on human chromosomes 7 and 17 is on mouse chromosome 11, it would support the notion of evolutionary dispersion of several mouse chromosome 11 genes to human chromosomes 7 and 17.

The chromosomal localization of c-*erbA* and c-*erbB* in humans is a useful starting point in analyzing possible rearrangements of these genes in malignancies with aberrations of chromosomes 7 and 17[7,60]. One such consistent aberration involving chromosome 17 is observed in acute promyelocytic leukaemia; t(15;17)(q22;q21)[61]. In one case of acute promyelocytic leukaemia that we examined, the c-*erbA* gene was not rearranged (unpublished observations: the acute promyelocytic leukaemia cells were kindly provided by J. Rowley). However, by analogy to the c-*myc* model in Burkitt's lymphoma in which c-*myc* is not always rearranged, further studies of c-*erbA* in acute promyelocytic leukaemia seem warranted.

Characteristic chromosome abnormalities in murine cancers have also been reported, some of which involve c-onc (see Ch. 6). However, to my knowledge, rearrangements or translocations of mouse chromosome 11 have not been reported as being prominent in mouse haematopoietic neoplasms[51]. Although v-*erbA* may enhance leukaemogenesis by v-*erbB*[55], it is unknown whether the normal c-*erbA* and c-*erbB* gene products might interact in the cell. The recent findings that human c-*erbB* encodes an epidermal growth factor (EGF) receptor is relevant in this regard[62]. The human EGF receptor possesses three major domains: a domain extending from the outer plasma membrane that binds EGF, a transmembrane domain, and a cytoplasmic domain possessing tyrosine kinase activity[62]. The v-*erbB* oncogene of AEV appears to lack the EGF binding domain, prompting suggestions that the oncogene represents a truncated chicken EGF receptor and behaves as a deregulated EGF receptor that is constitutively switched on[62]. However, in contrast to the EGF receptor, no tyrosine kinase activity has yet been detected for the v-*erbB* product[1,35], although several other members of the *src* family of oncogenes possess this activity[1,21,38]. Tyrosine kinase activity is an absolute requirement for full transformation of cells by the v-*src* oncogene[1,38]. Although no function has been ascribed to the c-*erbA* product, the carboxyl half of v-*erbA* is reported to bear 27 per cent amino acid homology with carbonic anhydrase II, a cytosolic enzyme[63]. However, human and chicken c-*erbA* genes exist as loci distinct from carbonic anhydrase[63].

C-ONCS UNAFFILIATED WITH RETROVIRUSES

Most of the c-oncs in Table 3.1 were identified as homologues of retroviral oncogenes when c-oncs were used to screen cellular genomic DNA[1,4,21]. However, at least three putative c-oncs are not represented among retroviral isolates carrying onc-genes. These three are N-*ras*, N-*myc* and B-*lym*1. N-*myc* is located at the distal end of the short arm of chromosome 2[30,64] whereas N-*ras* and B-*lym*1 are syntenic on chromosome 1p[65–67] (Table 3.1).

N-*myc* was identified as an amplified sequence in many neuroblastomas that was partially homologous to the c-onc c-*myc* in the 5′ domain[68]. N-*myc* has been located in heterogeneous staining regions (commonly known as 'HSR's') located in chromosomal regions distinct from its native location on chromosome 2p[30,64]. It has been suggested that translocation of N-*myc* may frequently accompany its amplification in neuroblastomas[30], and its location at the tip of chromosome 2 might exacerbate a tendency to undergo rearrangements. N-*ras* was identified as the transforming sequences in sarcoma tumour lines and in a leukaemia cell line[66,69], whereas B-*lym*1 was identified as a transforming gene in B-cell lymphoma[70] by the method of DNA mediated transfection of mouse NIH-3T3 cells[71]. N-*ras* and B-*lym*1 are also related to previously recognized genes. N-*ras* is probably derived from the same progenitor gene that gave rise also to c-Ha-*ras* and c-Ki-*ras* (discussed in more detail later). Human B-*lym*1 shares 6 of 39 amino acids (approx. 15 per cent) in

the N-terminal region of transferrin, an 80 000 dalton iron binding protein in serum encoded by a gene on the long arm of human chromosome 3[72]. Transferrin belongs to a family of iron binding proteins that have evolved by gene duplication in which the N- and C-terminal halves bear a high degree of homology[72]. A melanoma surface antigen p97 appears also to be a member of this family[73]. Transferrins are essential growth factors for cells in culture. While structural homology often suggests functional homology, there is presently no evidence that B-*lym*1 is an iron binding protein that is present in the serum or that it binds to specific receptors on the cell surface, properties associated with transferrin.

THE *RAS* FAMILY OF C-ONCS

Organization in mammals

The *ras* genes were first brought to view as the oncogenes of the Harvey and Kirsten murine sarcoma viruses (Ha-MSV and Ki-MSV respectively) isolated by Dr Jennifer Harvey in 1964[2] and Dr Werner Kirsten in 1967[3] respectively. In both instances these highly oncogenic retroviruses were identified during the course of experiments in which the weakly oncogenic Moloney murine leukaemia virus (MoMuLV, a mouse type C retrovirus) was passaged in rats. The rat-passaged virus was noted to cause a rapid onset of malignancy in mice with a pathology different from Moloney leukaemia virus.

Subsequently, it was demonstrated that Harvey and Kirsten murine sarcoma viruses represented recombinants between MuLV and genetic information present in normal rat cells. The genomes of Ha-MSV and Ki-MSV have been molecularly cloned and their transforming sequences defined[74,75]. Ha-MSV and Ki-MSV contain oncogenes termed v-Ha-*ras* and v-Ki-*ras* respectively; they are related to each other, encode serologically related 21 000 dalton guanine nucleotide binding proteins, and detect low copy homologous sequences in the genomes of humans, rats, mice, and phylogenetically distant species[74,75].

In humans, at least five *ras* related sequences have been characterized: two v-Ki-*ras* related genes, c-Ki-*ras*1 and c-Ki-*ras*2; two v-Ha-*ras* related genes, c-Ha-*ras*1 and c-Ha-*ras*2; and N-*ras* ('N' for neuroblastoma – the tumour cells in which a mutated form of the gene was detected by DNA transfection of mouse 3T3 cells).

Structural analysis of molecular clones of *ras* genes strongly supports the idea of common ancestry and has illuminated possible functions for these genes. N-*ras*, c-Ha-*ras*1, and c-Ki-*ras*2 have a similar exon–intron structure and location of splice junctions, indicating that each has evolved from a common ancestral gene that possessed at least four coding exons and was spliced in a similar manner[76–80]. In the case of human and rat c-Ki-*ras*2 genes, the presence of an additional diverged fourth exon provides the possibility of producing two alternative transcripts[78,79]. Nucleotide sequence analysis of human c-Ki-*ras*1 has revealed numerous base pair substitutions, insertions and deletions

compared to the v-Ki-*ras* coding sequence[78]. Thus, c-Ki-*ras*1 is a pseudogene incapable of encoding a functional p21 polypeptide[78]. It has been surmised that c-Ki-*ras*1 arose by reverse transcription of an abbreviated mRNA transcript in which the 3′ end of exon 3 had become fused to one of the two alternative fourth coding exons seen in c-Ki-*ras*2 (exon 4B)[79]. This processed cDNA copy may have undergone germline reinsertion into the genome. In man c-Ki-*ras*1 is located in the p23→q12 region of human chromosome 6 distinct from the location of c-Ki-*ras*2 at 12p12.05→pter (Table 3.1). Asynteny for c-Ha-*ras*1 on chromosome 11p and c-Ha-*ras*2 on the X chromosome has also been demonstrated, accompanied with the speculation that c-Ha-*ras*1, which is devoid of introns, may represent a processed gene[81]. In contrast to c-Ki-*ras*1, the c-Ha-*ras*2 gene is capable of transforming NIH-3T3 cells in transfection assays when ligated to a retroviral long terminal repeat sequence, suggesting that its coding sequence is intact[1]. The presence of homologous c-Ha-*ras*2 genes devoid of introns in both humans and rats suggests that they arose from a duplication event that occurred before these two species diverged[1]. An interesting duplication event of *ras* genes in mouse and hamster appears to have taken place relatively recently in evolution[82]. *Mus pahari* (but not other species of *Mus*) contain at least 10 copies of c-Ha-*ras*, whereas Chinese hamsters have approximately six copies of c-Ki-*ras* (Syrian hamsters only have a single copy). It is not known if the amplified sequences are all syntenic.

N-*ras*, c-Ha-*ras*1, and c-Ki-*ras*2 share common similarities and differences in their protein coding domains[76–80]. In spite of the fact that c-Ha-*ras*1 and c-Ki-*ras*2, and N-*ras* have probably been asyntenic for at least 80×10^6 years (c-Ki-*ras*2, c-Ha-*ras*1, and N-*ras* are also asyntenic in mouse and separated from man evolutionarily for 80×10^6 years), there has been a remarkable conservation of N-terminal amino acid residues. In the regions encoding amino acid residues 1–120 and 129–166 there is 90 per cent identity for N-*ras*, c-Ha-*ras*1, and c-Ki-*ras*2. There is 100 per cent amino acid identity in positions 1–80. Although the regions encoding amino acids 171–185 bear little homology in these three genes, the divergent regions of c-Ha-*ras*1 and c-Ki-*ras*2 share 95 per cent identity with their respective viral oncogene counterparts. It thus appears that these similar and divergent regions of the *ras* genes have been maintained by selective pressure – implying that the conserved N-terminal region serves an important function common to all three *ras* polypeptides and that this region cannot tolerate even minimal amino acid changes. Indeed, it is within the regions of the 12th and 61st amino acids that base pair changes have a profound effect on the function of cellular and viral *ras* polypeptides, endowing such altered products with transforming ability in vitro[83–87]. The N-terminus is also thought to be involved in GTP binding, a property common to *ras* polypeptides that may be important for their function[88].

The extent of the selective pressure operating on *ras* genes appears to be similar to that operating for certain other unlinked genes that have a common ancestry. For example, the genes for lactate dehydrogenase A and B, located on the short arms of human chromosomes 11 and 12 respectively, share

approximately 75 per cent amino acid identity[89]. The c-Ha-*ras*1 and c-Ki-*ras*2 genes also share approximately 80 per cent amino acid identity.

c-Ki-*ras*2 and c-Ha-*ras*1: result of chromosome duplication?

Certain of the human chromosomes are suggested to have had a common origin based upon similarities in banding patterns and content of paralogous genes[90]. For example, human chromosomes 16 and 17 encode mitochondrial and soluble forms of thymidine kinase respectively, and human chromosomes 11 and 12 encode lactate dehydrogenase isozymes A and B respectively[27]. Ohno has proposed that these presently recognized similarities in gene content of certain human chromosomes (and mouse chromosomes for that matter) could be the result of a tetraploidation that occurred 300×10^6 years ago in an ancestral species[90]. In particular it has been proposed that human chromosomes 11 and 12 shared a common ancestor. It is therefore of interest to compare paralogous genes that reside on human chromosomes 11 and 12. Lactate dehydrogenase A is located at 11p12.03 p12.08, whereas the B isozyme is located at 12p12.2[27]. The c-Ha-*ras*1 c-onc has been localized to the region 11p by analysis of somatic cell hybrids, with a probable site of 11p14.1→p15, as shown by in situ hybridization (see Table 3.1). Thus the short arms of chromosomes 11 and 12 share two pairs of paralogous genes that probably evolved from common ancestral genes[91]. Since the regions that subsume these genes represent only a portion of chromosomes 11 and 12, one can speculate that at least a partial duplication of a region common to the short arms of both chromosomes may have occurred in an ancestral species. No examples of paralogous genes on the long arms of human chromosomes 11 and 12 are currently available to extend this comparison. However, it is interesting to note that structural aberrations in the long arms of chromosomes 11 and 12 have been reported in haematopoietic malignancies[60], and a c-onc termed c-*ets*, homologous to one of the putative transforming sequences of the avian retrovirus E26, recently has been assigned to 11q23→q24[92]. Whether this suggests that a paralogous c-onc resides on the long arm of chromosome 12 is purely conjectural.

COMPARATIVE MAPPING OF C-ONCS

Comparisons of the sort made above, when interpreted with caution, are likely to reveal useful information concerning the extent of chromosomal rearrangements that have occurred during speciation. One reason for this is that c-oncs in general appear to have evolved very early in vertebrate evolution and their chromosomal positions may be landmarks for very ancient chromosome regions and linkage groups. For example, v-Ha-*ras* and v-Ki-*ras* detect distinct cellular sequences in man, mouse, rats, and chickens, thus indicating that their cellular homologues were generated by a gene duplication event that occurred prior to the divergence of birds and mammals more than

300×10^6 years ago[93]. It might be expected that at least some linkage groups involving c-oncs in man would be conserved in other mammalian species.

Chromosomal locations of c-oncs in species other than man that are amenable to genetic analysis have now been examined. Localization of c-oncs in the mouse has been accomplished by somatic cell hybrid analysis and recently by examining the segregation of DNA polymorphisms recognized by c-oncs in recombinant inbred strains of mice[58]. In the mouse c-Ki-*ras* and c-Ha-*ras*1 homologues reside on mouse chromosomes 6 and 7 respectively[96]. As is true in humans, lactate dehydrogenase B and A are linked to *ras* genes on mouse chromosomes 6 and 7 respectively[27]. The event that may have given rise to the duplication of the chromosomal regions containing *ras* genes and lactate dehydrogenase isozymes is thus suggested to have occurred before man and mouse diverged over 80×10^6 years ago. The situation with regards to N-*ras* is equally speculative. N-*ras* probably shared a common ancestor with c-Ki-*ras*2 and c-Ha-*ras*1, yet is located on chromosome 1p (Table 3.1). Are other isozymes of lactate dehydrogenase linked to N-*ras*? Did N-*ras* participate in the possible duplication of the chromosome region containing c-Ha-*ras*1 and c-Ki-*ras*2? Further comparative mapping of genes closely linked to the *ras* genes in man, mouse and other species may help to answer these questions.

The chromosomal location of c-oncs in mammalian species other than man has yielded additional clues about the involvement of c-oncs in certain types of neoplasm. For example, plasmacytomas in both mouse and rat involve transposition of c-*myc* to immunoglobulin loci. That these gene rearrangements involved chromosome translocations was indicated when c-*myc* was assigned to mouse 15 and rat 7, the sites of translocations in plasmacytomas (see Ch. 6).

CONSERVATION OF C-ONC SYNTENY GROUPS

Comparative mapping of c-oncs in different species has been of interest since c-oncs appear to be evolutionarily very old. By mapping c-oncs in species other than man, additional information has been gathered concerning the evolution of c-onc gene families and gene linkage groups. Through the work of many laboratories, a number of homologous genes have been mapped in humans and mice[27]. As predicted by Ohno[90], genes located on the X–chromosome have been retained as a conserved linkage group throughout mammalian evolution because of the dosage compensation needed in X–chromosome inactivation. Several genes are linked or syntenic on the X–chromosome in many species of mammals – including rodents, ungulates and primates[27].

In addition to the X–chromosome, a number of autosomal linkage groups have been described which have remained together during evolution. It is not surprising that the primates have many conserved linkage groups[95], since even the chromosomal banding patterns of many primate species are very similar[96]. The differences in chromosomal banding patterns among species reflect the chromosomal rearrangements that have taken place in the evolution of

primates. As the evolutionary distance between species increases, a concomitant increase in the number of chromosomal rearrangements is observed.

Some linkage groups have remained together for long periods of evolution. For example, several genes on the short arm of human chromosome 1 (for example, genes for phosphogluconate dehydrogenase, enolase-1 and phosphoglucomutase) are linked in such divergent species as man, monkey, mouse, cattle and sheep[27]. Extensive comparison of human to *Mus musculus* has revealed a number of conserved linkage or syntenic groups. These observations can now be extended to the location of c-oncs.

Table 3.2 lists the c-oncs which have been mapped in mouse – all of which have also been mapped in humans. Some of the genes comprise conserved syntenic groups (see Table 3.5), while others cannot be excluded from being in conserved regions because of a lack of suitable markers. Two c-oncs from the *src* family, c-*src* and c-*abl*, reside on the same chromosome in mouse[94-97]. However, the two genes appear to reside in different syntenic groups: c-*src*, which is syntenic with adenosine deaminase and inosine triphosphatase on human

Table 3.5 Conserved syntenic groups observed between man and mouse

Gene	Human chromosome	Mouse chromosome
c-*src*	20	2
adenosine deaminase	20	2
inosine triphosphatase	20	2
c-*abl*	9	2
adenylate kinase-1	9	2
c-*fes*	15	7
isocitrate dehydrogenase (mitochondrial)	15	7
c-Ki-*ras2*	12	6
glyceraldehyde phosphate dehydrogenase	12	6
triose phosphate isomerase	12	6
lactate dehydrogenase B	12	6
c-Ha-*ras1*	11	7
insulin	11	7
β-globin	11	7
lactate dehydrogenase	11	7
parathyroid hormone	11	7
calcitonin	11	7
c-*erbA1*	17	11
thymidine kinase	17	11
galactokinase	17	11
c-*sis*	22	15
arylsulfatase A	22	15
diaphorase-1	22	15
N-*ras*	1	3
amylase-1	1	3
amylase-2	1	3
nerve growth factor	1	3

chromosome 20, is also syntenic with these genes in mouse; and c-*abl* which is syntenic with adenylate kinase-1 on human chromosome 9 is syntenic with the mouse homologous gene (Table 3.5). Studies with inbred mice indicate that c-*abl* is more proximal on murine chromosome 2 than c-*src*[58]. If the c-*src*, adenosine deaminase and inosine triphosphatase trio truly represents a conserved synteny group in man and mouse, then a major rearrangement has occurred during the 80×10^6 years of evolution separating humans and mice, since adenosine deaminase and c-*src* are separated from inosine triphosphatase by the human chromosome 20 centromere[43]. Assignment of S-adenosyl homocysteine hydrolase (located at 20cen→q13.1, and proximal to adenosine deaminase) in the mouse would provide additional information to assess the topographical extent of this synteny group in man and mouse.

CHROMOSOMAL LOCATION OF C-ONCS IN CHICKENS

Chromosomal location of c-oncs has been pursued in chickens, as this species has yielded many of the known oncogenes of acutely transforming retroviruses[1,4]. Several avian retroviruses contain two host derived sequences believed to be important in malignant transformation of infected cells: these include avian erythroblastosis virus (v-*erbA* and v-*erb*)[4,53]; leukaemia virus MH2 (v-*myc* and v-*mht*)[98,99]; and erythroblastosis virus E26 (v-*myb* and v-*ets*)[100,101]. The existence of such viruses with tripartite structure raises the questions as to whether the particular transduced pairs of genes are closely situated in the chicken genome, and puts to test current models for retroviral transduction of cellular genes[4,5].

In the case of avian erythroblastosis virus, carrying v-*erbA* and v-*erbB* sequences, analysis of molecular clones of the corresponding chicken genomic sequences suggested that the genes were not contiguous and might be entirely unlinked[53]. One can speculate that capture of the cellular genes could have occurred by two independent recombinational events, or by incorporation of both genes that had previously become linked as a result of a chromosome translocation[4]. Avian leukaemia virus has been observed to integrate near the c-*erbB* gene in some erythroleukemias[54] and c-*erbA* and c-*erbB* have become linked in one instance (quoted in ref. 102). Recent studies indicating that c-*erbA* and c-*erbB* are asyntenic in chickens are therefore of interest[102].

Two principal methods have been used to determine the native chromosomal positions of chickens c-oncs in somatic cells. The first method is by chromosome flow sorting combined with Southern filter hybridization of DNA from purified chromosome fractions[102–104], and the second method is *in situ* hybridization[105,106] (see the quoted references for details of these methods).

The chromosome locations of chicken c-oncs determined by chromosome flow sorting are summarized in Table 3.6. The results indicate that just as in other species examined in any detail to date (humans, mouse, *Drosophila*), c-oncs in chickens can be dispersed amongst several chromosomes – although some may be syntenic (for example, c-*erbB* and c-*myc* on chicken chromosome

Table 3.6 Chromosomal localization of c-oncs in chickens

Chicken c-onc	Chromosome	References
c-*src*	microchromosomes	103,104
c-*myb*[1]	intermediate size, probably 3	104
c-*myc*[1]	large chromosome, probably 2	104
c-*fps*	microchromosomes	104
c-*erbA*	microchromosomes	104
c-*erbB*[1]	large chromosome, probably 2	104

[1]In contrast, these chicken c-oncs are reported to be on small chromosomes or microchromosomes by *in situ* hybridization[105,106].

2). The asynteny of c-*erbA* and c-*erbB* (microchromosomes v. chromosome 2, respectively) is interesting because it suggests that these genes may be captured by retroviruses independently of each other. The strain H isolate of AEV possesses only v-*erbB* sequences – apparently supporting the idea of independent transduction[107].

It should be mentioned that *in situ* hybridization studies suggested that chicken c-*myb*, c-*myc* and c-*erbB* all occur on small or microchromosomes[105,106] contrasting with the results obtained by chromosome flow sorting. Whether the discrepancy might arise by translocations in the continuous lymphoid cell lines used to prepare sorted chicken chromosomes has been considered but deemed unlikely[102].

The v-*erbB* gene is believed to be a member of the *src* family of v-oncs based upon extensive nucleotide sequence homology in the region encoding the tyrosine kinase activity, although such activity has not yet been detected in the v-*erbB* product[35,102]. Thus these two members of the *src* family (and probably other members) have already evolved into two separate and distinct loci in chickens. This notion is supported by the existence of distinct but related *Drosophila* c-oncs homologous to the *src* family on the basis of nucleotide sequence homologies (see below).

C-ONC HOMOLOGUES IN INVERTEBRATE SPECIES

The conservation of c-onc genes in phylogenetically diverse species implies similar, but not necessarily identical, roles in cellular physiology, and has provided the impetus to search for c-onc homologues in lower eukaryotic species[12,16]. There are a number of advantages in studying c-oncs in such organisms as yeast and *Drosophila* – two species that have yielded c-onc homologues[11–19]. Their genomes are much less complex than those of mouse and humans, and powerful genetic tools can be exploited to study c-onc expression during development. For example, studies of gene expression in both diploid and haploid cells is possible in yeast along with the ability to perform facile gene replacement via homologous recombination using genetically engineered vectors[17]. *Drosophila* are also amenable to developmental studies of c-onc expression and there are also methods available for gene

transfer in this species[108]. In addition, genes of these species usually possess fewer and smaller introns – compared to their mammalian counterparts – simplifying structural analysis.

The *src* family in *Drosophila*

Drosophila was shown to contain sequences homologous to vertebrate c-oncs by reduced stringency filter hybridization studies that allow stabilization of partially mismatched sequences[11]. Among the v-onc clones that detected distinct sequences were v-*src*, v-*abl*, v-Ha-*ras*, and v-*fes*, but only v-*abl* hybridized to a single restriction fragment of *Drosophila* DNA. Detection of v-onc homologous sequences in *Drosophila* argues that these genes must have evolved more than 800×10^6 years ago, antedating the divergence of the Annelid–Anthropod and the Echinoderm–Chordate superphyla, as pointed out by Shilo and Weinberg[11].

At least three genes homologous to v-*src* are present in *Drosophila*[12,14,15] (Table 3.7). Two of these, termed D*src* and D*ash*, have been sequenced[15]. D*src* is 60 per cent homologous in nucleotide sequence with v-*src* and 53 per cent homologous with v-*abl*. D*ash* is 70 per cent homologous in nucleotide sequence with v-*abl* and D*ash* is 58 per cent homologous with v-*src*[15] (Table 3.8). D*ash*

Table 3.7 Chromosomal localization of v-onc homologues in *Drosophila*

Homologue	Chromosome position	References
[1]D*ash*	73B	12,14,15
D*src*	64B	12,14,15
S13	29A	14
D*ras1*	85D	13
D*ras2*	64B	13
D*ras3*	62B	13

[1]D*ash* and *Dsrc* correspond to *S16* and *S24*, respectively, of Simon, et al[14].

and D*src* hybridized to their corresponding v-onc homologues, specifically to regions that encode the viral tyrosine kinase activity[15]. D*ash* hybridizes to the tyrosine kinase domains of both v-*src* and v-*abl*, indicating that these domains are probably derived from a common progenitor gene.

It has been conjectured that D*ash* may be similar in structure and function to the primordial gene that gave rise to c-*src* and c-*abl*[15]. However, the dissimilarity of D*ash* and D*src* suggests that if gene duplications are responsible for expanding the size of the *src* family of c-oncs, they represent ancient events[15]. A third v-*src*-related gene in *Drosophila*[14] also hybridizes with v-*fps*, the oncogene of the Fujinami sarcoma virus – an avian retrovirus. There is 41 and 43 per cent amino acid homology of v-*fps* with D*src* and D*ash*, respectively, and it has been suggested that this third *src* related gene (designated S13[14]) is the *Drosophila* homologue of the vertebrate c-*fps* gene[14,15].

Table 3.8 Homologies of the *src* family members in *Drosophilia*

Homologue	Compared to	Nucleotide	Amino acid
D*ash*	v-*abl*	70%	74%
	v-*src*	58%	
	v-*fes*		43%
D*src*	v-*abl*	53%	
	v-*src*	60%	54%
	v-*fps*	41%	

The hypothesis that the *Drosophila* genes related to vertebrate v-oncs represent distinct loci is also supported by chromosome mapping studies[12,14] (Table 3.7). *In situ* hybridization studies of the *Drosophila* c-oncs to polytene chromosomes indicate that D*ash*, D*src*, and *S13* are not contiguous[12,14]. D*ash* was localized to position 73B on the left arm of chromosome 3, D*src* to position 64B on chromosome 3L, and *S13* to position 29A. Since each of the *Drosophila* c-oncs possesses homology with the tyrosine kinase encoding domains of one or more of the vertebrate c-oncs, they appear to constitute divergent members of a multigene superfamily generated by gene duplications during speciation of metazoans. This scenario also appears to apply to another family of c-onc present in *Drosophila*, namely the *ras* genes.

The *ras* family in *Drosophila*

Three genes in *Drosophila* homologous to v-Ha-*ras* have been isolated and chromosomally assigned[13]. Each gene occupies a distinct locus on chromosome 3, and therefore they are not fragments of a single gene (D*ras1* maps to band 85D, D*ras2* to band 64B, and D*ras3* to band 62B – see Table 3.7). D*ras1* and D*ras2* have been sequenced and can encode polypeptides of 189 and 186 amino acids, respectively, which are very similar in size to vertebrate *ras* gene products[13]. D*ras3* also appears to be transcribed[13]. D*ras1* and D*ras2* share 75 per cent and 50 per cent amino acid homology to human c-Ha-*ras*, respectively. D*ras1* also shares regions of similarity and divergence to c-Ha-*ras*1 in the same manner that each of the vertebrate *ras* genes display. For example, in the first 80 amino acids of the *ras* gene products, in which human N-*ras*, c-Ha-*ras*1, and c-Ki-*ras*2 are completely homologous, D*ras1* shares 95 per cent homology. D*ras1* shares identical amino acids at positions 12 and 61 of the vertebrate *ras* gene products (glycine and glutamine respectively), indicating strong selective pressure for retention of these residues. One should remember that base pair substitutions of vertebrate *ras* genes lead to activation of transforming potential. Currently it is not known if alterations of D*ras1* at amino acids equivalent to positions 12 or 61 would confer transforming ability onto the gene product.

In contrast to D*ras1*, D*ras2* appears not to be highly conserved in amino acid sequence at the N-terminus compared to vertebrate *ras* genes. It has been

proposed that the conserved N-terminus of *ras* genes is involved in GTP binding, a property common to vertebrate *ras* genes[88]. D*ras2* might be incapable of binding GTP. D*ras2* differs at amino acid positions 12 and 61 from the vertebrate *ras* p21 products, but shares alanine at position 59. However, changes at all three residues in vertebrate *ras* genes are thought to activate transforming potential[83–87].

In situ hybridization of D*ras* probes to polytene chromosomes of *Drosophila* salivary glands has shown that D*ras2* at 64B is within 20 bands of the *Drosphila src* gene also at 64B[13]. D*ras1* at 85D is close to a testes specific β-tubulin gene at 85D4–7[13]. The latter localization is intriguing since a yeast *ras* gene with weak homology to vertebrate *ras* was shown to be 350 bp away from a yeast β-tubulin gene[18].

Since at least two mammalian *ras* genes in human and mouse are in proximity of lactate dehydrogenase isozyme genes[91,94], it would be of interest to compare the location of *Drosophila ras* genes to those of lactate dehydrogenase isozymes. The latter, to my knowledge, have not been localized in *Drosophila*.

Since all three genes appear to be transcribed, the possible presence of *ras* pseudogenes in *Drosophila* is currently unanswered[13]. In their screening of a *Drosophila* genomic library, Neuman-Silberberg *et al.*[13] reported finding only representatives of these three *ras* genes. Therefore, if *ras* pseudogenes exist in *Drosophila*, they have diverged to the point where they are no longer easily detectable by the conditions of hybridization used.

Yeast *ras* genes

Although initial experiments did not reveal the presence of c-onc homologues in yeast or slime moulds[11], recently *ras* homologues have been isolated from yeast and characterized[16–19]. The yeast *RAS1* and *RAS2* genes, in general, recapitulate the structural features which characterize vertebrate and *Drosophila ras* genes. Yeast *RAS1* and *RAS2* encode polypeptides of 309 and 322 amino acids, respectively[16]. Homology between the yeast and mammalian *ras* genes is colinear from amino acid residue 10 in yeast and from amino acid residue 3 in the human Ha-*ras* protein. There is nearly 90 per cent homology in the ensuing 80 amino acids amongst these polypeptide sequences[16]. The yeast *ras* products differ from the vertebrate *ras* counterparts in having an unusually long, divergent carboxy terminus, ending with a short conserved sequence as in mammalian *ras* proteins[16]. Both yeast *RAS1* and *RAS2* are homologous at residues corresponding to positions 12, 13, 59, 61, and 63 of the mammalian Ha-*ras* protein, again emphasizing the selective pressure on these amino acids and their importance in the normal functions of the proteins. Changes at these positions lead to activation of transforming potential of mammalian *ras* genes[83–87,109]. There is now evidence that yeast *RAS2* genetically engineered to substitute valine for glycine at position 19 (corresponding to position 12 of mammalian *ras* proteins) has a dominant phenotypic effect on some unknown process involved in successful sporulation[17].

Chromosomal localization and activity of yeast *RAS1* and *RAS2*

In an elegant series of experiments Kataoka *et al.*[17] have exploited powerful genetic methodologies to chromosomally assign yeast *RAS1* and *RAS2*. They relied on the phenomenon of site directed transformation[110] and the destabilization of chromosomes carrying the 2μ circle FLP recombination site in the presence of the FLP gene product[111]. By crossing yeast strains containing recessive markers for yeast chromosomes and the FLP gene product, the chromosomal locations of the *RAS* genes were determined[17] (Table 3.9). *RAS1* and *RAS2* were localized to yeast chromosomes 15 and 14, respectively. Tetrad analysis established linkage to previously assigned yeast markers[17]. *RAS1* was located proximal to the centromere 7 cM from *ade2* and 63 cM from *his3*. *RAS2* was localized 2 cM from *met4*, and 80 cM from the centromere.

Gallwitz *et al.*[18] have described a third *ras* homologous gene (*YP2*) expressed in yeast, which is more distantly related and diverged from the *RAS1* and *RAS2* genes isolated by Powers *et al.*[16]. *YP2*, sharing 38 per cent amino acid homology within the region encoding residues 4–165 of the viral *ras* proteins, was discovered situated between yeast tubulin and actin genes located on chromosome 6 (Table 3.9). The interesting situation of the proximity of *YP2* to within 350 bp of a yeast β-tubulin gene, and the localization of *Drosophila* D*ras1* near a testis-specific β-tubulin gene has been pointed out[13], although D*ras1* and *YP2* are considerably diverged from each other. In contast to D*ras1*,

Table 3.9 Chromosomal localization of *ras* homologues in yeast

Locus	Chromosome	Linked markers	Reference
RAS1	15	*his3, ade2*	17
RAS2	14	*met4, pet8*	17
YP2	6	actin and tubulin	18
CDC28	2L	*tyr1*	112

YP2 possesses serine at position 17 (comparable to position 12 of vertebrate *ras* genes), i.e. the same amino acid substitution occurring in the v-Ki-*ras* oncogene[75]. How this amino acid difference relates to the normal functions of *YP2* compared to D*ras1* is not known.

Still another yeast gene, *CDC28*, homologous to a vertebrate v-onc has been reported[112]. *CDC28*, located on chromosome 2L and linked to *tyr1*, complements a temperature sensitive defect in cell division in yeast, a property that allowed its isolation and characterization (see Table 3.9). The deduced amino acid sequence of *CDC28* bears a 25 per cent identity with the bovine cyclic AMP-dependent protein kinase and 22 per cent with the v-*mos* oncogene, the latter two genes believed to be members of the *src* superfamily[113]. Thus it is not surprising that *CDC28* bears a similar homology to other *src* family members: 25.5 per cent with v-*fes*; 25 per cent with v-*yes*; 24.5 per cent with v-

fps; and 24 per cent with v-*raf*[112]. At equivalent positions *CDC28* shares a lysine residue thought to be the ATP-binding site of bovine protein kinase, and a threonine residue at position 196 that is phosphorylated in the bovine protein kinase[113]. Three of the glycine residues in the conserved sequence Leu-Gly-X-Gly-X-Phe-Gly-X-Val, which occurs upstream of the conserved lysine residues of bovine protein kinase and several members of the *src* family[113,114], are also present in *CDC28*[112]. The highly significant homology of *CDC28* to vertebrate oncogenes implies a common ancestry and *CDC28* might be similar in structure to the primordial gene for all *src* family members. The involvement of *CDC28* in cell division in yeast is interesting compared to vertebrate cells transformed by retroviruses, in which regulatory events that control progression through the cell cycle are not honoured.

SUMMARY

Less than a decade ago a cellular homologue of the retrovirus oncogene v-*src* was demonstrated in the chicken genome[46] and shown to be highly conserved during vertebrate evolution[20]. Those studies provided a paradigm and the impetus for isolating and characterizing the cellular homologue of v-*src* and those of other retroviral oncogenes that now number over 20[1,21]. The search for c-oncs, which initially began in vertebrates, has now extended across enormous phylogenetic distances into such species as *Drosophila* and yeast in the hope that these simpler organisms will reveal clues of the functions of c-oncs and that these functions will be relevant to their roles in mammals.

Chromosomal mapping studies have demonstrated that c-onc members of multigene families that arose by gene duplication have maintained structural similarity despite being asyntenic for millions of years (e.g., *ras* genes). Structural similarities of c-oncs in phylogenetically diverse species separated evolutionarily by enormous periods of time attest to the selection pressure operating on c-oncs and also to their probable universal roles in metazoan organisms.

The application of parasexual genetic mapping techniques has allowed construction of chromosomal maps of varying detail of c-oncs in no less than six different species (man, mouse, rat, chicken, *Drosophila*, and yeast). Besides providing information on the evolution of c-oncs in different species, chromosomal mapping has practical utility in understanding chromosomal aberrations. The chromosomal maps of c-*oncs* in man and mouse have provided a background against which chromosomal translocations and the aberrations seen in tumour cells can be compared. In man and mouse – and even in *Drosophila* and yeast – chromosomal mapping of c-oncs has been a way to determine if a given c-onc coincided with the position of a known mutation or chromosomal aberration. Such information might either yield clues as to the function of the c-oncs or implicate the c-oncs in the disease or phenotype with which the chromosomal aberration is associated. On the other hand, exclusion of a c-onc from a chromosomal region associated with disease would tend to

absolve the c-onc from being the direct target of the aberration. Such considerations underlie current attempts to determine if the c-Ha-*ras1* c-onc located on 11p14.1 or p15 might be implicated in Wilms' tumour – a childhood cancer of the kidney that is frequently associated with aniridia and an interstitial deletion of the distal end of band 11p13[115]. Only some of the deletions examined in Wilms' tumour cells have resulted in loss of a c-Ha-*ras1* homologue in tumour cells[116–119]. However, some investigators suggest that it is premature to exclude the possible involvement of c-Ha-*ras1* in this cancer. There is a striking similarity between chromosome aberrations seen in cancer and the location of c-oncs determined by mapping studies[60,120]. As these abnormalities appear to be nonrandomly distributed amongst human chromosomes[60], the possibility remains that in the future additional c-oncs may be implicated in these abnormalities.

Less than four years ago not one of the presently recognized c-oncs had been chromosomally assigned in man or mouse. Today, in man alone, c-oncs have been located on 16 different chromosomes and some chromosomes carry more than a single c-onc. In two cases, c-oncs have been implicated in cancers associated with consistent chromosome translocations. However, for many c-oncs their possible roles in neoplastic disease remain undefined and we are reminded that cancer is far from being understood[121]. It also remains problematic whether in some instances c-oncs might represent loci that in mutant forms are associated with familial cancer syndromes which number more than 30[115]. Continued mapping of c-oncs will provide useful molecular markers for subchromosomal regions that might eventually be linked to familial cancers and/or to chromosomal aberrations observed in cancer cells.

ACKNOWLEDGEMENTS

I would like to thank numerous collaborators and others who generously supplied cloned oncogene probes. My own work was supported by Biomedical Research Support Grants, American Cancer Society Institutional Awards, National Cancer Institute Core Grant, and Public Health Service Research Grant CA39186 from the National Cancer Institute. I am indebted to M. Stankovich for secretarial assistance, S. Naylor for critical reading of the manuscript and B. Bowman for use of facilities.

REFERENCES

1. Bishop J M 1983 Cellular oncogenes and retroviruses. Annual Review of Biochemistry 52: 301–354
2. Harvey J J 1964 An unidentified virus which causes the rapid production of tumours in mice. Nature 204: 1104–1105
3. Kirsten W H, Mayer L A 1967 Morphologic responses to a murine erythroblastosis virus. Journal of the National Cancer Institute 39: 311–335
4. Graf T, Stehlein D 1982 Avian leukemia viruses: Oncogenes and genome structure. Biochimica et Biophysica Acta 651: 245–271

5. Temin H 1982 Viruses, protoviruses, development and evolution. Journal of Cellular Biochemistry 19: 105–118
6. Duesberg P 1983 Retroviral transforming genes in normal cells? Nature 304: 219–226
7. Sandberg A A 1980 The Chromosomes in Human Cancer and Leukemia, New York: Elsevier North Holland
8. Southern E M 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98: 503–517
9. Ruddle F H, Creagan R P 1975 Parasexual approaches to the genetics of man. Annual Review of Genetics 9: 407–486
10. Shows T B, Sakaguchi A Y, Naylor S L 1982 Mapping the human genome, cloned genes, DNA polymorphisms and inherited disease. Advances in Human Genetics 12: 341–452
11. Shilo B-Z, Weinberg R A 1981 DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences USA 78: 6789–6792
12. Hoffman-Falk H, Einat P, Shilo B Z, Hoffman F M 1983 *Drosophila melanogaster* DNA clones homologous to vertebrate oncogenes: evidence for a common ancestor to the *src* and *abl* cellular genes. Cell 32: 589–598
13. Neuman-Silberberg F S, Schejter E, Hoffman E M, Shilo B Z 1984 The *Drosophila ras* oncogenes: structure and nucleotide sequence. Cell 37: 1027–1033
14. Simon MA, Kornberg TB, Bishop JM 1983 Three loci related to the *src* oncogene and tyrosine-specific protein kinase activity in *Drosophila*. Nature 302: 837–839
15. Hoffman F M, Fresco L D, Hoffman-Falk H, Shilo B Z 1983 Nucleotide sequences of the *Drosophila src* and *abl* homologs conservation and variability in the *src* family oncogenes. Cell 35: 393–401
16. Powers S, Kataoka T, Fasano D, Goldfarb M, Strather J, Broach J, Wigler M 1984 Genes in *S. cerevisiae* encoding proteins with domains homologous to the mammalian *ras* proteins. Cell 36: 607–612
17. Kataoka T, Powers S, McGill C, Fasano O, Strathern J, Broach J, Wigler M 1984 Genetic analysis of yeast *RAS1* and *RAS2* genes. Cell 37: 437–445
18. Gallwitz D, Dorath C, Sander C 1983 A yeast gene encoding a protein homologous to the human C-*has$bas* proto-oncogene product. Nature 306: 704–707
19. DeFeo-Jones D, Scolnick E, Koller R, and Dhar R 1983 *ras*-related gene sequences identified and isolated from *Saccharomyces cerevisiae*. Nature 306: 707–709
20. Spector D H, Varmus H E, Bishop J M 1978 Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. Proceedings of the National Academy of Sciences USA 75: 4102–4106
21. Gilden R V, Rice N R, McAllister R M 1984 Oncogenes. Gene Analysis Techniques 1: 23–33
22. Lalley P A, Minna J D, Francke U 1978 Conservation of autosomal gene synteny groups in man and mouse. Nature 274: 160–163
23. Francke U, Lalley P A, Moss W, Ivy J, Minna J D 1977 Gene mapping in *Mus musculus* by interspecific cell hybridization: Assignment of the genes for tripeptidase-1 to chromosome 19, dipeptidase-2 to chromosome 18, acid phosphatase-1 to chromosome 12, and adenylate kinase-1 to chromosome 2. Cytogenetics and Cell Genetics 19: 47–84
24. Szbalski W S, Szbalska E H, Ragni G 1962 Genetic studies with human cell lines. National Cancer Institute Monograph 7: 75–88
25. Littlefield J W 1964 Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. Science 145: 709–710
26. Yunis J J, Sawyer J R, Ball D W 1978 Characterization of banding patterns of metaphase-prophase G-banded chromosomes and their use in gene mapping. Cytogenetics and Cell Genetics 22: 679–683
27. Human Gene Mapping Workshop VII 1984 Cytogenetics and Cell Genetics 32: 3–393
28. Sakaguchi A Y, Naylor S L, Shows T B 1983 A sequence homologous to Rous sarcoma virus v-*src* is on human chromosome 20. Progress in Nucleic Acid Research and Molecular Biology 29: 291–293
29. Harper M, Franchini G, Love J, Simon M I, Gallo R L, Wong-Staal F 1983

Chromosomal sublocalization of human c-*myb* and c-*fes* cellular onc genes. Nature 304: 169–171

30. Schwab M, Varmus H E, Bishop J M, Grzeschik K-H, Naylor S L, Sakaguchi A Y, Brodeur G, Trent J 1984 Chromosomal localization in normal human cells and neuroblastomas of the gene related to c-*myc*. Nature 308: 288–291
31. Zabel B U, Naylor S L, Sakaguchi A Y, Bell G I, Shows T B 1983 High resolution chromosomal localization of human genes for amylase, proopiomelanocortin somatostatin, and a DNA fragment (*D3S1*) by *in situ* hybridization. Proceedings of the National Academy of Science USA 80: 6932–6936
32. Neel B G, Jahnwar S C, Chaganti R S K, Hayward W S 1982 Two human c-onc genes are located on the long arm of chromosome 8. Proceedings of the National Academy of Science USA 79: 7842–7846
33. Jhanwar S C, Neel B G, Hayward W S, Chaganti R S K 1983 Localization of c-*ras* oncogene family on human germ-line chromosomes. Proceedings of the National Academy of Sciences USA 80: 4794–4797
34. Mark G E, Rapp U R 1984 Primary structure of v-*raf*: relatedness to the *src* family of oncogenes. Science 224: 285–295
35. Yamamoto T, Nishida T, Miyajima N, Kawai S, Doi T, Toyoshima K 1983 The *erbB* gene of avian erythroblastosis virus is a member of the *src* gene family. Cell 35: 71–78
36. Yoshida M, Kawai S, Toyoshima K 1980 Uninfected avian cells contain structurally unrelated progenitors of viral sarcoma genes. Nature 287: 653–654
37. Shibuya M, Hanafusa H, Balduzzi P C 1982 Cellular sequences related to three new *onc* genes of avian sarcoma virus (*fps, yes, ros*) and their expression in normal and transformed cells. Journal of Virology 42: 143–152
38. Brugge J, Chinkers M 1983 Tyrosine specific protein kinases. Annual Reports in Medicinal Chemistry 18: 213–224
39. Van Beveren C et al. 1981 Nucleotide sequence and formation of the transforming gene of a mouse sarcoma virus. Nature 289: 258–262
40. Kitamura N, Kitamura A, Toyoshima K, Hirayama Y, Yoshida M 1982 Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus. Nature 297: 205–208
41. Levinson A D, Courtneidge S A, Bishop J M 1981 Structural and functional domains of the Rous sarcoma virus transforming protein ($pp60^{src}$). Proceedings of the National Academy of Sciences USA 78: 1624–1628
42. Bonner T, O'Brien S J, Nash W G, Rapp U R, Morton C C, Leder P 1984 The human homologs of the *raf* (*mil*) oncogene are located on human chromosomes 3 and 4. Science 223: 71–74
43. Sakaguchi A Y, Mohandas T, Naylor S L 1985 (in press) A human c-*src* gene resides in the cen→q131 region of chromosome 20. Cancer Genetics and Cytogenetics
44. Rous P 1911 A sarcoma of the fowl transmissible by an agent separable from the tumor cells. Journal of Experimental Medicine 13: 397–411
45. Schwartz D E, Tizard R, Gilbert W 1983 Nucleotide sequence of Rous sarcoma virus. Cell 32: 853–869
46. Stehelin D, Varmus H E, Bishop J M, Vogt P K 1976 DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature 260: 170–173
47. DeLorbe W J, Luciw P A, Goodman H M, Varmus H E, Bishop J M 1980 Molecular cloning and characterization of avian sarcoma virus circular DNA molecules. Journal of Virology 36: 50–61
48. Mohandas T, Sparkes R S, Suh E J, Hershfield M S 1984 Regional localization of the human genes for S-adenosyl homocysteine hydrolase (cen→q131) and adenosine deaminase (q131→qter) on chromosome 20. Human Genetics 66: 292–295
49. Davis M P Dewald G W, Pierre R V, Hoagland H C 1984 Hematologic manifestations associated with deletions of the long arm of chromosome 20. Cancer Genetics and Cytogenetics 12: 63–71
50. Babu V R, Van Dyke D L, Jackson C E 1984 Chromosome 20 deletion in human multiple endocrine neoplasia types 2A and 2B: a double blind study. Proceedings of the National Academy of Sciences USA 81: 2525–2528

51. Hayata I et al. 1983 Chromosomal aberrations observed in 52 mouse myeloid leukemias. Cancer Research 43: 367–373
52. Boettiger D, Anderson S, Dexter T M 1984 Effect of *src* infection on long-term marrow cultures: increased self-renewal of hemopoietic progenitor cells without leukemia. Cell 36: 763–773
53. Vennstrom B, Bishop J M 1982 Isolation and characterization of chicken DNA homologous to the two putative oncogenes of avian erythroblastosis virus. Cell 28: 135–143
54. Fung Y K, Lewis W S, Crittenden L B, Kung H J 1983 Activation of the cellular oncogene c-*erbB* by LTR insertion: molecular basis for induction of erythroblastosis by avian leukosis virus. Cell 33: 345–355
55. Frykberg L, Palmieri S, Beug H, Graf T, Hayman M J, Vennstrom B 1983 Transforming capacities of avian erythroblastosis virus mutants deleted in the *erbA* and *erbB* oncogenes. Cell 32: 227–238
56. Zabel B U, Fournier R E K, Lalley P A, Naylor S L, Sakaguchi A Y 1984 Cellular homologs of the avian erythroblastosis virus *erbA* and *erbB* genes are syntenic in mouse but ayntenic in man. Proceedings of the National Academy of Sciences USA 81: 4874–4878
57. Fournier R E K, Ruddle F H 1977 Microcell-mediated transfer of murine chromosomes into mouse, Chinese hamster, and human somatic cells. Proceedings of the National Academy of Sciences USA 74: 319–323
58. Blatt C, Harper M E, Franchini G, Nesbitt M N, Simon M I 1984 Chromosomal mapping of murine c-*fes* and c-*src* genes. Molecular and Cellular Biology 4: 978–981
59. Jansson M, Philipson L, Vennstrom B 1983 Isolation and characterization of multiple human genes homologous to the oncogenes of avian erythroblastosis virus. European Molecular Biology Organization Journal 2: 561–565
60. Mittelman F 1984 Restricted number of chromosomal regions implicated in aetiology of human cancers and leukaemia. Nature 310: 325–327
61. Rowley J D, Golomb H M, Dougherty C 1977 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukaemia. Lancet i: 549–550
62. Downward et al. 1984 Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. Nature 307: 521–527
63. Debuire B et al. 1984 Sequencing the *erb*-A gene of avian erythroblastosis virus reveals a new type of oncogene. Science 224: 1456–1459
64. Kohl N E, Kanda N, Schreck R R, Latt S A, Gilbert F, Alt F W 1983 Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 35: 359–367
65. Morton C C, Taub R, Diamond A, Lane M A, Cooper G M, Leder P 1984 Mapping of the human B-*lym*1 transforming gene activated in Burkitt lymphomas to chromosome 1. Science 223: 173–175
66. Hall A, Marshall C J, Spurr N K, Weiss R A 1983 Identification of transforming gene in two human sarcoma cell lines as a new member of the *ras* family located on chromosome 1. Nature 303: 396–400
67. deMartinville B, Cunningham J M, Murray M J, Francke U 1984 The N-*ras* oncogene assigned to chromosome 1 (p31→cen) by somatic cell hybrid analysis. Cytogenetics and Cell Genetics 37: 531
68. Schwab M et al. 1983 Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature 305: 245–248
69. Gambke C, Signer E, Morini C 1984 Activation of N-*ras* gene in bone marrow cells from a patient with acute myeloblastic leukaemia. Nature 307: 476–478
70. Diamond A, Devine J M, Cooper G M 1984 Nucleotide sequence of a human B*lym* transforming gene activated in a Burkitt's lymphoma. Science 225: 516–519
71. Cooper G M 1982 Cellular transforming genes. Science 217: 801–806
72. Yang F et al. 1984 Human transferrin: cDNA characterization and chromosomal location. Proceedings of the National Academy of Sciences USA 81: 2752–2756
73. Brown J P, Hewick R M, Hellstrom I, Hellstrom K E, Doolittle R F, Dreyer W J 1982 Human melanoma-associated antigen p97 is structurally and functionally related to transferrin. Nature 296: 171–173
74. Dahr R, Ellis R W, Shih T Y, Oroszlan S, Shapiro B, Maisel J, Lowy D, Scolnick E

1982 Nucleotide sequence of the p21 transforming protein of Harvey murine sarcoma virus. Science 217: 934–937
75. Tsuchida N, Ryder T, Ohtsubo E 1982 Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. Science 217: 937–939
76. Capon D, Ellson Y, Levinson A, Seeburg P, Goeddel D 1983 Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature 302: 33–37
77. Fasano O, Taparowsky E, Fiddes J, Wigler M, Goldfarb M 1983 Sequence and structure of the coding region of the human H-*ras*-1 gene from T24 bladder carcinoma cells. Journal of Molecular and Applied Genetics 2: 173–180
78. McGraph J P et al. 1983 Structure and organization of the human Ki-*ras* proto-oncogene and a related processed pseudogene. Nature 304: 501–506
79. Shimizu et al. 1983 The structure of the K-*ras* gene of the human lung carcinoma cell line Calu-1. Nature 304: 497–500
80. Taparowsky E, Shimizu K, Goldfarb M, Wigler M 1983 Structure and activation of the human N-*ras* gene. Cell 34: 581–586
81. O'Brien S J, NaSh W G, Goodwin J L, Lowy D R, Chang E H 1983 Dispersion of the *ras* family of transforming genes to four different chromosomes in man. Nature 302: 839–842
82. Chattopadahyay S K, Chang E H, Lander M R, Ellis R W, Scolnick E M, Lowy D R 1982 Amplification and rearrangement of *onc* genes in mammalian species. Nature 296: 361–363
83. Tabin C et al. 1982 Mechanism of activation of a human oncogene. Nature 300: 143–148
84. Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M 1982 Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature 300: 762–765
85. Capon D et al. 1983 Activation of Ki-*ras*2 gene in human colon and lung carcinoma by different point mutations. Nature 304: 507–513
86. Reddy E, Reynolds R, Santos E, Barbacid M 1982 A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature 300: 149–152
87. Yuasa Y, Srivastava S, Dunn C, Rhim J, Reddy E, Aaronson S 1983 Acquisition of transforming properties by alternative point mutations within c-*bas/has* human proto-oncogene. Nature 30: 775–779
88. Shih T Y, Papageorge A G, Stokes P E, Weeks M O, Scolnick E M 1980 Guanine nucleotide-binding and autophosphorylating activities associated with the $p21^{src}$ protein of Harvey murine sarcoma virus. Nature 287: 686–691
89. Doolittle R F 1981 Similar amino acid sequences: Chance or common ancestry? Science 214: 149–159
90. Ohno S 1973 Ancient linkage groups and frozen accidents. Nature 244: 259–260
91. Sakaguchi A Y et al. 1984 Regional localization of two human cellular Kirsten *ras* genes on chromosomes 6 and 12. Molecular and Cellular Biology 4: 989–993
92. deTaisne C, Gegonne A, Stehelin D, Bernheim A, Berger R 1984 Chromosome localization of the human proto-oncogene c-*ets*. Nature 310: 581–582
93. Ellis R W et al. 1981 The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature 292: 506–511
94. Sakaguchi A Y, Lalley P A, Zabel B U, Ellis R W, Scolnick E M, Naylor S L 1984 Chromosome assignments of four mouse cellular homologs of sarcoma and leukemia virus oncogenes. Proceedings of the National Academy of Sciences USA 81: 525–529
95. deGrouchy J, Turleau C, Finaz C 1978 Chromosomal phylogeny of the primates. Annual Review of Genetics 12: 289–328
96. Dutrillaux B 1979 Chromosomal evolution in primates: Tentative phylogeny from *Microcebus murinus* (prosimian) to man. Human Genetics 48: 251–314
97. Goff S P, D'Eustachio P, Ruddle F H, Baltimore D 1982 Chromosomal assignment of the endogenous proto-oncogene c-*abl*. Science 218: 1317–1319
98. Jansen H W, Ruckert B, Lurz R, Bister K 1983 Two unrelated cell-derived sequences in the genome of avian leukemia and carcinoma inducing retrovirus MH2.

European Molecular Biology Organization Journal 2: 1969–1975
99. Kan N C, Flordellis C S, Garon C F, Duesberg P H, Papas T S 1983 Avian carcinoma virus MH2 contains a transformation specific sequence, *mht*, and shares the *myc* sequence with MC29, CMII, and OK10 viruses. Proceedings of the National Academy of Sciences USA 80: 6566–6570
100. Leprince D et al. 1983 A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. Nature 306: 395–397
101. Nunn M F, Seeburg P H, Moscovici C, Duesberg P H 1983 Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature 306: 391–395
102. Symonds G, Stubblefield E, Guyaux M, Bishop J M 1984 Cellular oncogenes (c-*erb*-A and c-*erb*-B) located on different chicken chromosomes can be transduced into the same retroviral genome. Molecular and Cellular Biology 4: 1627–1630
103. Hughes S H et al. 1979 Gene localization by chromosome fractionation: Globin genes are on at least two chromosomes and three estrogen inducible genes are on three chromosomes. Proceedings of the National Academy of Sciences USA 76: 1348–1352
104. Padgett T G, Stubblefield E, Varmus H E 1977 Chicken macrochromosomes contain an endogenous provirus and microchromosomes contain sequences related to the transforming gene of ASV. Cell 10: 649:657
105. Tereba A, Lai M M C 1982 Cell oncogenes are located on large microchromosomes in chicken cells. Virology 116: 652–657
106. Wong T C, Tereba A, Vogt P K, Lai M M C 1981 Characterization of the transformation-specific sequences of avian erythroblastosis virus in normal vertebrate cells. Virology 111: 418–426
107. Yamamoto T, Hihara H, Nishida T, Kawai S, Toyoshima K 1983 A new avian erythroblastosis virus, AEV-H, carries *erb*-B gene responsible for the inducation of both erythroblastosis and sarcomas. Cell 34: 225–232
108. Rubin G M, Spradling A C 1982 Genetic transformation of *Drosophila* with transposable element vectors. Science 218: 348–353
109. Fasano O, Aldrich T, Tamanoi F, Taparowsky E, Furth M, Wigler M 1985 Analysis of the transforming potential of the human H-*ras* gene by random mutagenesis. Proceedings of the National Academy of Sciences USA (In press)
110. Orr-Weaver T L, Szostak J W, Rothstein R J 1981 Yeast transformation: a model system for the study of recombination. Proceedings of the National Academy of Sciences USA 78: 6354–6358
111. Falco S C, Yuyang L, Broach J, Botstein D 1983 Genetic properties of chromosomally integrated 2 micron plasmid DNA in yeast. Cell 29: 573–584
112. Lorincz A T, Reed S I 1984 Primary structure homology between the product of yeast cell division control gene CDC28 and vertebrate oncogenes. Nature 307: 183–185
113. Barker W C, Dayhoff M O 1982 Viral *src* gene products are related to the catalytic chain of mammalian cAMP-dependent protein kinase. Proceedings of the National Academy of Sciences USA 79: 2836–2839
114. Kamps M P, Taylor S S, Sefton B M 1984 Direct evidence that oncogenic tyrosine kinases and cyclic AMP-dependent kinases have homologous ATP binding sites. Nature 310: 589–592
115. Knudson A G Jr. 1981 Human cancer genes. In: Arrighi F E, Rao P N, Stubblefield E (eds) Genes chromosomes and neoplasia, Raven Press, New York, p. 453–462
116. Huerre C, Despoisse S, Gilgenkrantz S, Lenoir G, Junien C 1983 c-Ha-*raS*1 is not deleted in aniridia-Wilms' tumour association. Nature 305: 638–641
117. de Martinville B, Francke U 1983 The c-Ha-*ras*1, insulin and β-globin loci map outside the deletion associated with aniridia-Wilms' tumour. Nature 305: 641–643
118. Reeve A E, Housiaux P J, Gardner R J M, Chewings W E, Grindley R M, Millow L J 1984 Loss of a Harvey *ras* allele in sporadic Wilms' tumour. Nature 309: 174–176
119. Fearon E R, Vogelstein B, Feinberg A P 1984 Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumours. Nature 309: 176–178
120. Rowley J D 1983 Human oncogene locations and chromosome aberrations. Nature 301: 290–291
121. Temin H M 1983 We still don't understand cancer. Nature 302: 656

122. Le Beau M M, Westbrook C A, Diaz M O, Rowley J D 1985 (submitted) *In situ* hybridization studies of c-*src*: evidence for two distinct loci on human chromosomes no. 1 and 20
123. Dalla-Favera R, Franchini G, Martinotti S, Wong-Stahl F, Gallo R C, Groce C M 1982 Chromosomal assignment of the human homologues of feline sarcoma virus and avian myeloblastosis onc genes. Proceedings of the National Academy of Sciences USA 79: 4714–4717
124. Heisterkamp N et al. 1982 Chromosomal localization of cellular homologues of two viral oncogenes. Nature 299: 747–749
125. Harper M E, Franchin G, Love J, Simon M I, Gallo R L, Wong-Staal F 1983 Chromosomal sublocalization of human c-*myb* and c-*fes* cellular onc genes. Nature 304: 169–171
126. deKlein A et al. 1982 A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765–767
127. Spurr N K 1984 Chromosomal localization of the human homologues to the oncogenes *erb*A and B. European Molecular Biology Organization Journal 3: 159–163
128. Groffen J, Heisterkamp N, Spurr N K, Dana S L, Wasmuth J J, Stephenson J R 1984 Chromosomal localization of the human c-*fms* oncogene. Nucleic Acids Research 11: 6331–6339
129. Prakash K, McBride O W, Swan D C, Tronick S R, Aaronson S A 1982 Molecular cloning and chromosomal mapping of a human locus related to the transforming gene of Moloney murine sarcoma virus. Proceedings of the National Academy of Sciences USA 79: 5210–5214
130. deMartinville B, Giacalone J, Shih C, Weinberg R A, Francke U 1983 Oncogene from human EJ bladder carcinoma is located on the short arm of chromosome 11. Science 219: 489–501
131. McBride O W, Swan D C, Santos E, Barbacid M, Tronick S R, Aaronson S A 1982 Localization of the normal allele of T24 human bladder carcinoma oncogene to chromosome 11. Nature 300: 773–774
132. Zabel B U, Naylor S L, Sakaguchi A Y, Shows T B 1984 Gene mapping by in situ hybridization. Cytogenetics and Cell Genetics 37: 615–616
133. Sakaguchi A Y, Naylor S L, Shows T B, Toole J J, McCoy M, Weinberg R A 1983 Human c-Ki-*ras*2 proto-oncogene on chromosome 12. Science 219: 1081–1083
134. Ryan J J, Barker P E, Shimizu K, Wigler M, Ruddle F H 1983 Chromosomal assignment of a family of human oncogenes. Proceedings of the National Academy of Sciences USA 80: 4460–4463
135. Davis M, Malcolm S, Hall A 1984 The N-*ras* oncogene is located on the short arm of chromosome 1. Cytogenetics and Cell Genetics 37: 448–449
136. McBride O W, Swan D, Tronick S, Moore D, Aaronson S A 1984 Chromosomal localization of N-*ras*, K-*ras*1, K-*ras*2, and *myb* oncogenes in human cells. Cytogenetics and Cell Genetics 37: 534–535
137. Dalla Favera R, Riegni M, Erikson J, Patterson D, Gallo R C, Croce C M 1982 Human c-*myc* onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proceedings of the National Academy of Sciences USA 79: 7824–7827
138. Sakaguchi A Y, Lalley P A, Naylor S L 1983 Human and mouse cellular *myc* protooncogenes reside on chromosomes involved in numerical and structural aberrations in cancer. Somatic Cell Genetics 9: 391–405
139. Taub R et al. 1982 Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma. Proceedings of the National Academy of Sciences USA 79: 7837–7841
140. Zabel B U, Naylor S L, Grzeschik K-H, Sakaguci A Y 1984 Regional assignment of human proto-oncogene c-*myb* to 6q21–qter. Somatic Cell and Molecular Genetics 10: 105–108
141. Balazs I, Grzeschik K-H, Stavnezer F 1984 Assignment of the human homolog of a chicken oncogene to chromosome 1. Cytogenetics and Cell Genetics 37: 410–411
142. Dalla-Favera R, Gallo R, Gialongo A, Croce C M 1982 Chromosomal localization of the human homolog (c-*sis*) of the simian sarcoma virus onc gene. Science 218: 686–688
143. Swan D C, McBride O W, Robbins K C, Keithley D A, Reddy E P, Aaronson S A

1982 Chromosomal mapping of the simian sarcoma virus oncogene analog in human cells. Proceedings of the National Academy of Sciences USA 79: 4691–4695

144. Morton C C, Taub R, Diamond A, Lane M A, Cooper G M, Leder P 1984 Mapping of the human B*lym*1 transforming gene activated in Burkitt lymphomas to chromosome 1. Science 223: 173–175

145. Kozak C A, Sears J F, Hoggan M D 1983 Genetic mapping of the mouse oncogenes c-Ha-*ras*1 and c-*fes* to chromosome 7. Journal of Virology 47: 217–220

146. Swan D, Oskarsson M, Keithley D, Ruddle F H, D'Eustachio P, Van de Woude G F 1982 Chromosomal localization of the Moloney sarcoma virus cellular (*c-mos*) sequence. Journal of Virology 44: 752–754

147. Kozak C A, Sears J F, Hoggan M D 1983 Genetic mapping of the mouse proto-oncogene c-*sis* to chromosome 15. Science 221: 867–869

148. Ryan J, Hart C P, Ruddle F H 1984 Molecular cloning and chromosome assignment of murine N-*ras*. Nucleic Acids Research 12: 6063–6072

149. Sumegi J, Spira J, Bazin H, Szpirer J, Levan G, Klein G 1983 Rat c-*myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t(6;7) chromosomal translocation. Nature 306: 497–498

The Philadelphia chromosome translocation

INTRODUCTION

The study of the chromosome pattern in the affected cells of a number of human tumours has been one of the most exciting areas in cancer research over the last 25 years. Major advances in our understanding of the specificity of some of the abnormalities have been achieved in the last decade with the application of chromosome banding techniques. For over 20 years, the cytogenetic analysis of patients with chronic myelogenous leukaemia (CML) has provided the paradigms for our interpretation of the role of chromosome abnormalities.

The study of the chromosome pattern in human leukaemias can be divided into three periods – the first two each cover ten years, from 1960–1970 and from 1970–1980. During the first period, the chromosome abnormalities seen in leukaemic cells were identified without banding techniques and therefore they included the changes in morphology that were detectable in unbanded preparations as well as abnormal modal chromosome numbers. The most significant observation was the identification of the Philadelphia (Ph^1) chromosome in leukaemic cells from patients with CML. In 1960, when this abnormality was discovered by Nowell and Hungerford[1], it appeared to represent a deletion of about half of the long arm of one G group chromosome – whether pair no. 21 or no. 22 was affected could not be determined. This observation led to a search for similar abnormalities closely associated with other types of malignant haematological diseases. The results were disappointing in that although the abnormalities seemed to be consistent in any particular patient, the patterns varied greatly from one patient to another. Thus the accepted notion was that the Ph^1 was a unique example of a consistent karyotypic abnormality, and the general rule was one of marked variability in karyotype. This, in turn, led most investigators to assume that chromosome changes were secondary phenomena not fundamentally involved with the process of malignant transformation.

The evidence obtained during the second period showed that these assumptions were not correct. With the use of banding techniques, the correct interpretation of the origin of the Ph^1 chromosome was discovered. Moreover, other specific abnormalities were found to be associated with certain

leukaemias and lymphomas. It should be emphasized that the data presented in this chapter have been gathered primarily during the period 1974–1983. The observations and conclusions are based on our improved ability to identify abnormal chromosomes using banding techniques. However, most of the studies during this period used chromosomes that were relatively contracted, and the banding pattern was often fuzzy and poorly defined.

We are now in the early stages of the third period of cancer cytogenetics that will be characterized by substantial improvements in the quality of the chromosome preparations available for analysis. These technical improvements have already been used to detect very subtle translocations, inversions and deletions that were previously overlooked. In addition, as will be emphasized in the latter sections of this chapter, the application of the techniques of *in situ* hybridization and of recombinant DNA analysis to leukaemic cells has already provided evidence that challenges some of our earlier assumptions. Those new methodologies hold the promise of translating cytogenetic aberrations detected at the level of light microscopy into a precise understanding of the genes involved in these rearrangements and of the alterations in gene function that occur as a consequence of the chromosome abnormalities.

THE Ph[1] TRANSLOCATION

Chronic myelogenous leukaemia (CML)

Chromosome banding techniques were first used in the cytogenetic study of leukaemia for identification of the Ph[1] chromosome. Caspersson *et al.* [2] and O'Riordan *et al.* [3] reported independently that the Ph[1] chromosome was a no. 22q-. Since quinacrine fluorescence revealed that the chromosome present in triplicate in Down's syndrome was no. 21, the abnormalities in Down's syndrome and CML were shown to affect different pairs of chromosomes.

The question of the origin of the Ph[1] (22q-) chromosome was answered in 1973, when Rowley[4] reported that the Ph[1] chromosome resulted from a translocation, rather than a deletion as many investigators had previously assumed. The first report presented data on nine Ph[1] positive patients, who all possessed additional dully fluorescing chromosomal material at the end of the long arm of no. 9 (9q+). This additional material was approximately equal in length to that missing from the Ph[1] chromosome and it had staining characteristics similar to those of the distal portion of the long arm of no. 22. Therefore, it was proposed that the abnormality in CML was an apparently balanced reciprocal translocation t(9;22)(q34;q11) (Fig. 4.1). Subsequent measurements of the DNA content of the affected pairs (9 and 22) showed that the amount of DNA added to no. 9 was equal to that missing from the Ph[1]. Thus there is no detectable loss of DNA in this chromosome rearrangement[5]. Other studies with fluorescent markers or chromosome polymorphisms have shown that the same no. 9 and the same no. 22 are involved in each CML cell[6] of a

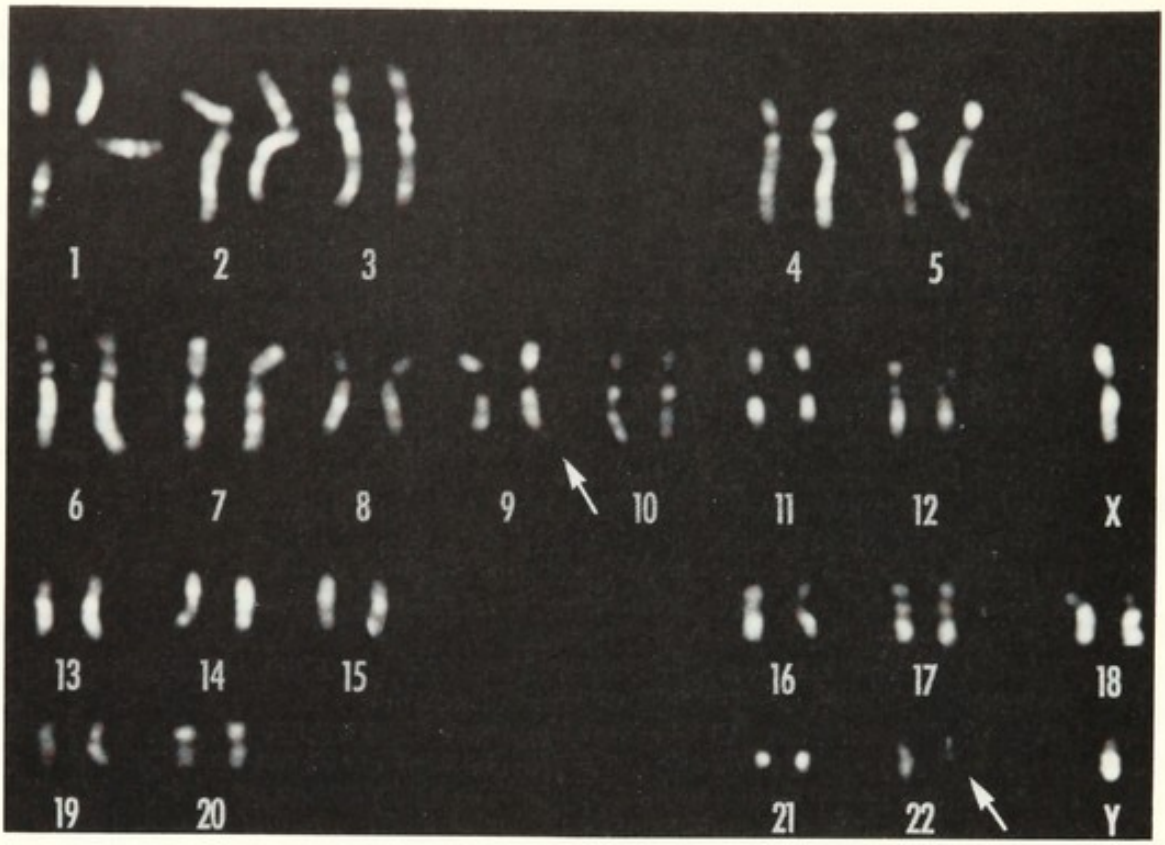

Fig. 4.1 Karyotype of a metaphase cell from a bone marrow aspirate obtained from an untreated male with chronic myeloid leukaemia. The chromosomes were stained with quinacrine mustard and photographed with ultraviolet fluorescence. The Philadelphia chromosome (Ph^1) is the chromosome on the right in pair 22 (arrow). The material missing from the long arm of this chromosome (22q-) is translocated to the long arm of chromosome 9 (9q+) on the right in pair 9 (arrow); and is the additional pale band that is not present on the normal chromosome 9.

particular patient. These observations confirm earlier work based on enzyme markers, indicating that CML cells originated from a single cell[7]. The original report on the translocation and the paper confirming it described only the t(9;22)[4,8] translocation. Within the first two years after the discovery of the t(9;22), it became apparent that variant translocations may also occur in addition to the typical t(9;22) type[9]. Until recently, these were thought to be of two types: one was a simple translocation involving no. 22 and some chromosome other than no. 9; and the other was a complex translocation involving three or more different chromosomes and, with the exception of a few cases, two of the chromosomes involved were nos. 9 and 22[10–12]. There are a few reports of patients who were said not to have had a translocation. There are also reports of complex translocations that involved the addition of fairly large chromosomal segments onto the Ph^1 chromosome, so that it became a larger chromosome than was typically seen, which was called a 'masked' Ph^1 [10–12].

Although our interpretations of some of these rearrangements has altered within the last year or so, it seems worthwhile to review the evolution of the analysis of variant translocations because of the lessons that can be learned.

In a review[13] of 1129 Ph^1 positive cases, 1036 (92 per cent) had the usual t(9;22) and 93 patients had an unusual translocation (Table 4.1) – in 42 patients it appeared to be simple, in 46 it was complex and in 5 patients only the Ph^1 was

Table 4.1 Incidence of variant translocations in Ph¹+ CML

References	Number of patients with				Total
	t(9;22)	Simple t*	Complex t	Other t	
Rowley and Testa[13]	1036 (92%)	42 (3.7%)	46 (4.1%)	5 (0.2%)	1129
Rowley (unpublished)	209 (95%)	2 (1%)	9(4%)	0	220
Carbonell *et al.*[14]	107 (98%)	0	2 (2%)	0	109
Ishihara *et al.*[15]	497 (96.6%)	2 (0.7%)	15 (2.9%)	0	514

*t = translocations

detected with no obvious piece of 22q translocated to another site. It should be noted that these five patients were all described in the 1970s when chromosome preparations were sometimes of a less than adequate quality. The data from three groups not included in the previous review are also presented in Table 4.1[14,15]. When individual laboratories or groups of laboratories are considered, the incidence of variant translocations ranges from 2 to 5 per cent. Variant translocations account for about 20 per cent of the cases of CML in Mitelman's Catalogue[16] but this probably represents preferential reporting of these patients. Although the earlier review[13] suggested that simple and complex locations occurred with equal frequency, in the light of more recent data this does not appear to be the case. About 1/4 to 1/3 of cases appear to have simple translocations and 2/3 to 3/4 of cases have complex translocations.

In a detailed analysis of the other chromosomes that participated in the translocation, Mitelman and Levan[12] noted that there was a nonrandom distribution of chromosomes involved in the simple and complex translocations that differed between the two types. Simple translocations tended to involve nos. 17, 12, 19, 10, 14, and 16, in order of their frequency, whereas complex translocations involved 3, 2, 1, 7, 4 and 14. A current map of the breakpoints in patients with simple and complex translocation is presented in Figure 4.2, which is derived from patients listed in Mitelman's Catalogue[16] as well as unpublished data from my laboratory. Of 67 patients whose cells appeared to have a translocation involving no. 22 and only one other chromosome, seven had a breakpoint in 12p13, six in 17q25 and four breakpoints each were located in 10q26 or 19q13. Bands 7p22, 11p15, and 14q32 were each involved in a translocation three times. Chromosomes 1, 8, 9 and the Y did not participate in simple translocations. The location of breakpoints seen in 121 patients with complex translocation was very different: 10 breaks occurred in 3p21, seven in 11q13, six in 10q22, and five breakpoints each were located in 3q21, 6p21 and 17q21. Translocations involving 1q22, 4q31, 5q13, 14q24, and 15q15 each occurred in four patients. It seems certain that this clustering of breaks to a few bands (particularly those with at least five or more breaks) is biologically important, although the interpretation of those nonrandom breaks is at present obscure.

In their analysis of the breakpoints in simple compared with complex translocations, Mitelman and Levan[12] noted that 19 of 23 breakpoints in the

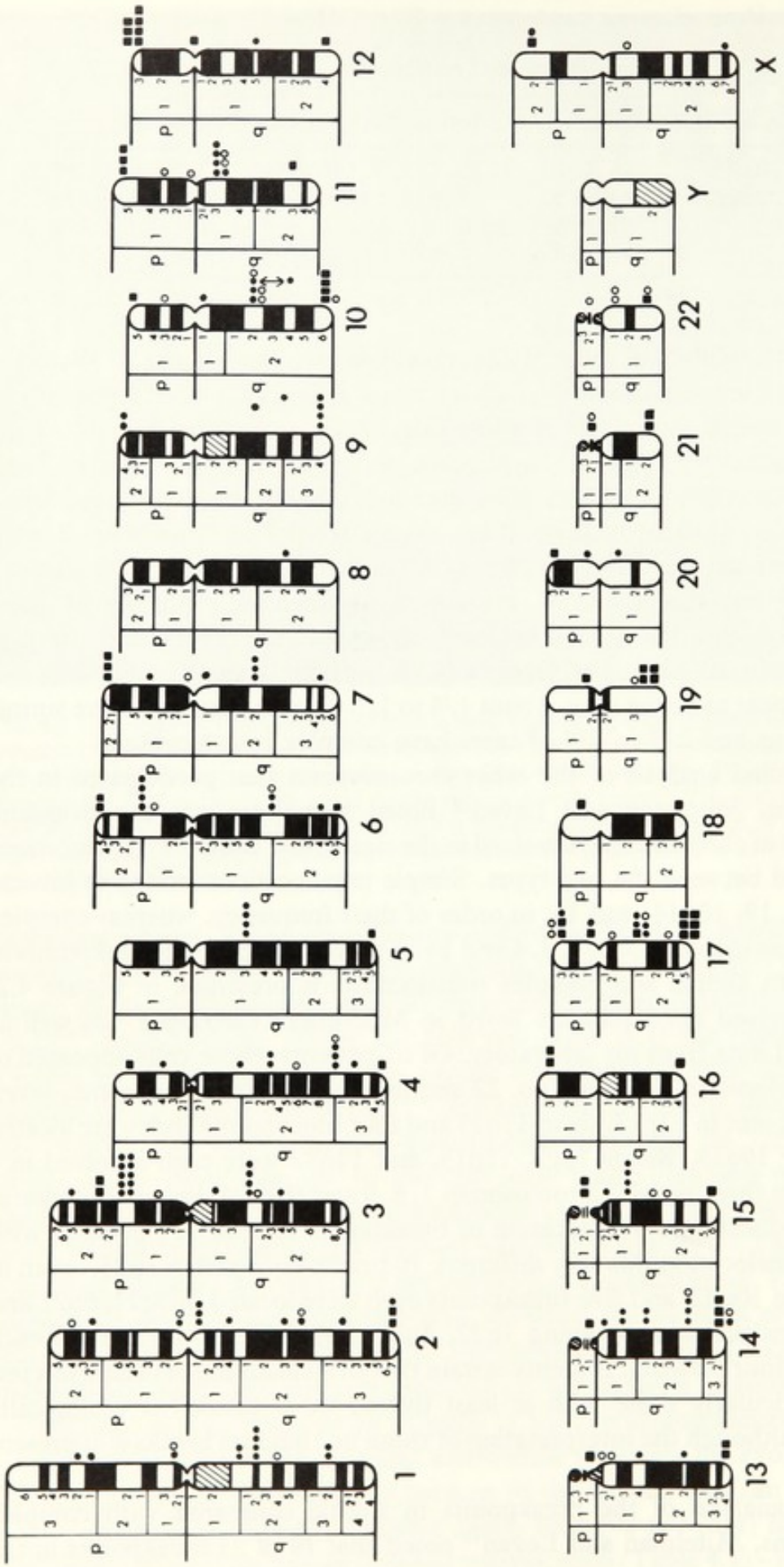

Fig. 4.2 Diagram of human karyotype with the breakpoints in variant translocations: the simple translocations in 67 patients, not previously thought to involve no. 9, are represented by closed circles (●); and the three-way translocations in 100 patients by squares (■). The open circles (○) identify breakpoints in translocations involving four or more chromosomes, which were seen in 21 patients. Four patients had 2 breaks involving no. 9 and one patient had an insertion of no. 10 (indicated by arrows) into part of no. 22 on the 9q+ chromosome.

simple translocations were located in terminal bands. The reverse distribution was observed in the complex translocations, in which only three of the 33 break sites were located in terminal bands. As can be seen from Figure 4.2, only six of the 67 breaks in the simple translocations failed to be located in a terminal band. In 121 patients with complex translocations, 16 breakpoints excluding those on no. 9, involved terminal bands – however, 11 of these occurred in patients who had four or more chromosomes involved in very complex rearrangements. Thus, these data confirm the original observation of Mitelman and Levan.

We now understand why this strange distribution of breakpoints was observed. A detailed explanation will be presented later in this chapter; for the moment, it is sufficient to say that we now know that there are no simple translocations that do not involve no. 9. Thus the break of no. 9 at 9q34 was overlooked because most of the preparations had short, contracted chromosomes and a translocation involving the movement of a terminal band (12p13 for example) to 9q34 with the translocation of no. 9 to the Ph^1 chromosome could not be detected. A partial karyotype from one patient can illustrate this point (Fig. 4.3). The upper part of Figure 4.3 shows a partial karyotype of the typical t(9;22) and the lower part contains pairs 9, 14, and 22 from a patient whom we initially considered to have a t(14;22)(q32;q11). Reassessment of the photographs of banded cells after the publication of the findings of Hagemeijer *et al.* [17], revealed that band 9q34 of the two no. 9s stained differently, with one somewhat darker – presumably representing a translocation from 14q32 that we had overlooked. This change is so subtle that one would not have had much confidence several years ago in classifying this as a three-way translocation.

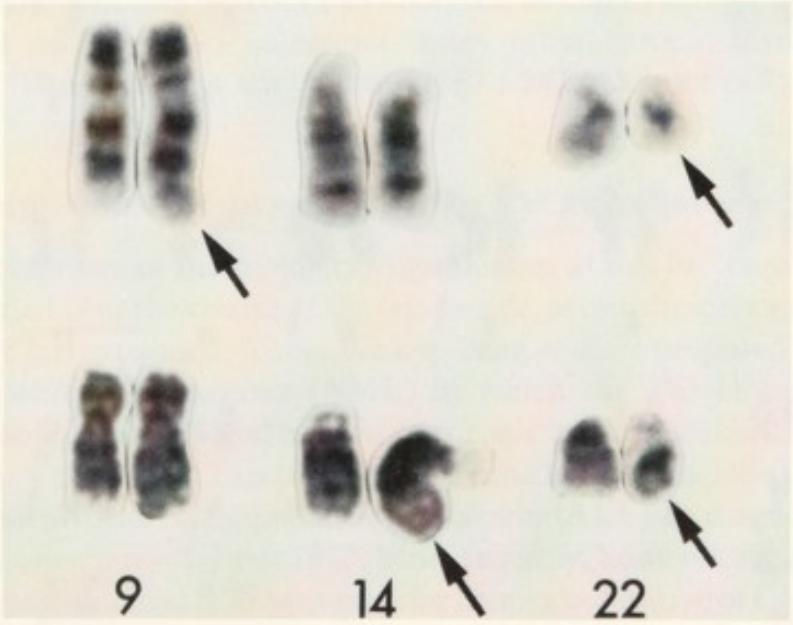

Fig. 4.3 Partial karyotype from a patient initially considered to have t(14;22)(q32;q11), as depicted in the upper part. Reassessment of banded cells showed that the two no. 9s stained differently, with the right hand one somewhat darker – presumably representing a translocation from 14q32 (see text).

In the complex translocations, the breakpoints in the chromosomes other than no. 9 were generally so obvious that there was no confusion about the nature of the translocation. An example of a complex translocation is illustrated in Figure 4.4. This patient was initially thought to have two independent translocations, one involving 10 and 22 and another involving 9 and 17. However, a more likely explanation is that there has been a four-way translocation of the end of 9q to 22q, with 22q moving to 10p, 10p moving to 17q and 17q21 to qter translocating to 9q34. The karyotype is 45,X–Y, t(9;22;10;17)(q34;q11;p13;q21). De Klein and Hagemeijer[18] have evidence that in at least one case of a masked Ph^1 that they studied with *in situ* hybridization, 9q34 moves to the 22q- chromosome in the usual location and that the other chromosome attaches distally to chromosome 22.

The consistency of the break points in chromosomes 9 and 22 is a matter of considerable biological importance. Verma and Dosik[19] suggested that the breakpoint in no. 22, determined with the R-banding technique, appeared variable. However, the data from the genetic analysis of the t(9;22), which will be discussed later, show that the breakpoint on no. 22 occurs within a very short region, while that on no. 9 occurs over a larger segment – one that would not be detectable at the level of the light microscope.

Although the review of the various types of translocations presented thus far has implied that once a translocation has occurred it is stable, evidence from

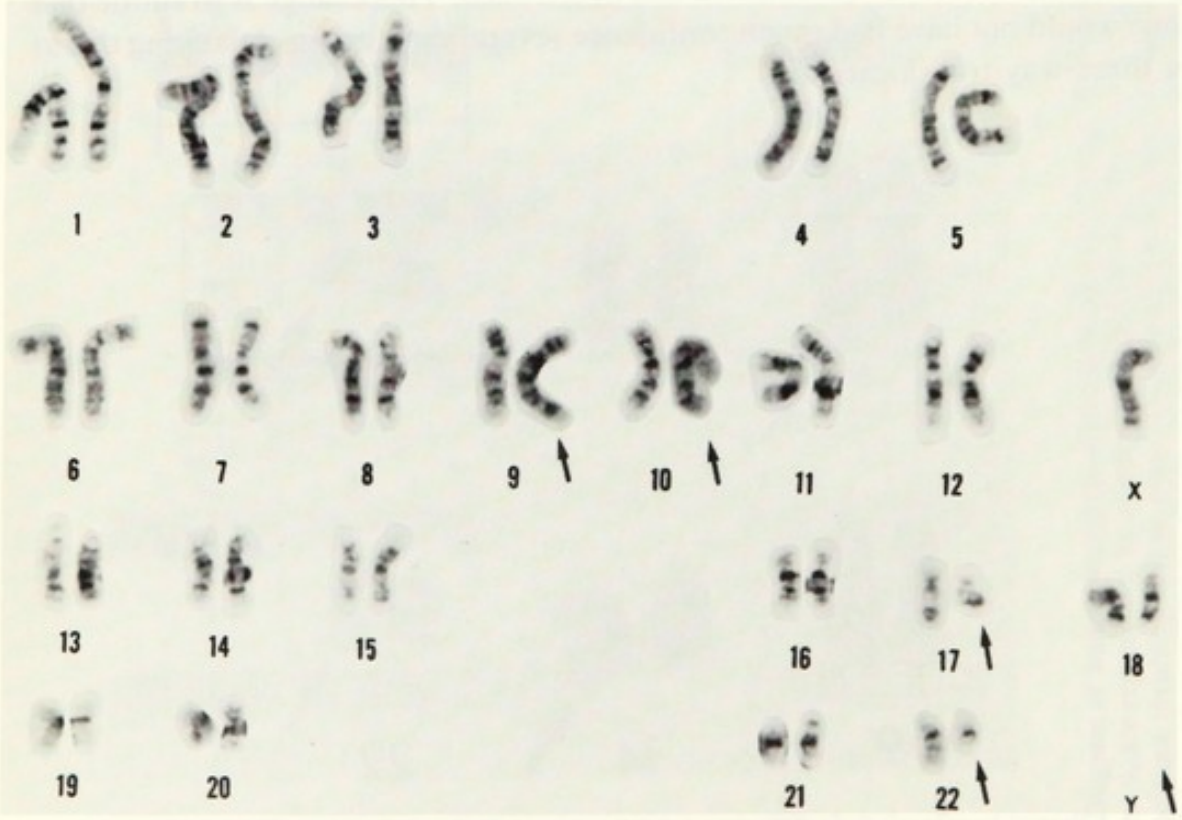

Fig. 4.4 Karyotype of cell from patient initially thought to have two separate translocations; more probably there was a single 4-way translocation, so the actual karyotype is 45,X-Y, t(9;22;10;17) (q34;q11;p13;q21).

several laboratories indicates that this is not always the case. Thus, three of the 17 cases reported by Ishihara *et al.* [15] as having variant translocations also had some cells with only a t(9;22). For example, one patient had only a t(9;22) in the first three cytogenetic studies; but later studies showed that an additional translocation had occurred between the 9q+ chromosome and no. 17. The 22q segment of the 9q+ was moved to 17p, which resulted in a 17p+ chromosome; thus it appeared as if the patient had a simple t(17;22)(p13; q11). Berger *et al.* [20] have reported a different modification of a t(9;22) – a patient, who was only studied in blast crisis, had some cells with t(9;22) and others with a further rearrangement involving the 9q+ and 1q21 resulting in a 3-way translocation. Because a number of patients with complex translocations have been studied only in the blast phase, it is possible that some of them were examples of secondary modifications of a typical t(9;22).

Clinical significance of the Ph[1] in CML

In the 1960s, a number of investigators who were studying patients that appeared to have CML, often found a small proportion of patients whose marrow cells lacked a Ph[1] chromosome. These patients, with Ph[1] negative (Ph[1]–) CML, accounted for 5 to 20 per cent of various series[4,11,21]. Paradoxically, it was noted that these patients who often had a normal karyotype, had a shorter survival than did those with the Ph[1] chromosome. We have recently reviewed the clinical, morphological and cytogenetic data on 25 patients who were said to have Ph[1]- CML[22]. The bone marrow slides have now been reclassified according to the recent French–American–British (FAB) classification of the myelodysplastic syndromes (MDS)[23]. After reclassification, only one of the patients was accepted as CML, and all the others fell within one of the MDS categories, most often chronic myelomonocytic leukaemia. Thus our data suggest that Ph[1]- CML does not exist as a distinct entity.

Acute lymphoblastic leukaemia with a Ph[1] chromosome

Our interpretation of the biological significance of the Ph[1] chromosome has been modified over the course of the last decade, as our clinical experience with this marker has expanded. Thus, Whang-Peng *et al.* [21] proposed that cases of acute myeloblastic leukaemia (AML) in which the Ph[1] chromosome was present should be reclassified as cases of chronic myeloid leukaemia presenting in blast transformation. This proposal was broadened to include the cases that appeared to be acute lymphoblastic leukaemia (ALL) at diagnosis and was generally accepted until about 1977. More recently, however, the tendency has been to refer to patients who have no prior history suggestive of CML as having Ph[1] positive acute leukaemia[10]. It is becoming increasingly evident that the observed interrelations of Ph[1]+ leukaemia are complex indeed, and that the distinctions between some categories, which are difficult to make, will be determined by the arbitrary judgement of the investigator.

When one reviews the descriptions of the patients who were first seen with what appeared to be acute leukaemia with a Ph[1] chromosome, it is apparent that a clear demarcation between morphological types is difficult. Thus, while some patients have a high percentage of lymphoblasts, others have a high percentage of myeloblasts, and still others have a mixture of myeloblasts and lymphoblasts. In some instances, cells from the latter patients have been analysed for cell surface markers. In case 7 of Chessells *et al.* [24] for example, 30 per cent of cells, including the smaller blasts, reacted positively with a common-ALL antiserum, and 60 per cent of cells, including the larger blasts, did not react with this antiserum but were positive with an antimyeloid antiserum. The factors that influence the expression of these various clinical forms, which can sometimes be seen in the same patient at different stages in the disease, are currently unknown.

Although there are several reports of series of patients with ALL whose karyotypes have been analysed at diagnosis, the largest number were reviewed at the Third International Workshop on Chromosomes in Leukemia[25] and included 330 patients – 173 adults and 157 children (15 years or younger). Thirty-nine patients had a Ph[1] chromosome, including 30 adults and 9 children. The incidence of Ph[1]+ patients with ALL was 5.7 per cent for children and 17.3 per cent for adults; the incidence previously reported was 2.0 per cent for children[24] and 25 per cent for adults[26]. Thus, the Ph[1] chromosome is the most frequent chromosome rearrangement in adult ALL. Thirty-six patients reviewed in the Workshop had the typical t(9q+;22q–), and the remaining 3 had variant translocations; therefore the incidence of the variant form was 8 per cent, which is similar to that observed in CML patients. One child had a t(6;22)(p23;q11) and two adults had either a t(21;22)(q23;q11) or a t(3;9;22)(p24;q34;q11). Among 56 cases of Ph[1]+ ALL included in Mitelman's Catalogue[16], 6 patients (11%) had a variant translocation. All of these had what were called simple translocations with breakpoints in 1p36, 1q24, 11p15, 12p13, 14q32, and 21q22; the last patient was also included in the Workshop review.

All of the Ph[1] patients reviewed in the Third Workshop, whose cells were studied, had non-T, non-B ALL. Cells from 8 of 9 children were L1; 7 adults had L1 cells and 21 had L2 cells, and no Ph[1]+ patients had L3 cells, according to FAB criteria.

Ph[1] positive acute non-lymphocytic leukaemia (ANLL)

The incidence of Ph[1]+ in patients with ANLL is difficult to determine, but it appears to be rare. Among the 660 patients with *de novo* ANLL included in the Fourth International Workshop on Chromosomes in Leukemia[27], only 2 patients had a t(9;22) (0.3 per cent). One other patient had a 22q- chromosome, with no obvious translocation, as part of a very complex karyotype.

Fifty cases of Ph[1]+ ANLL are listed in Mitelman's Catalogue[16] – 49 had a

t(9;22) and one had a t(12;22)(p13;q12), which gives a frequency of variant translocations of 2 per cent.

Both Workshop patients were classified as M1 by FAB criteria: among 29 Ph^1 + patients, whose FAB type was included in the Catalogue, 24 were M1 or M2 and 5 were M4 or M5. The subtype with the largest number of patients was M1.

IMPLICATIONS OF THE Ph^1 TRANSLOCATION

The nature of the Ph^1+ cell

The question as to which cells in CML contain a Ph^1 chromosome has not been completely resolved. It was shown relatively early that the Ph^1 chromosome was present in granulocytic, erythroid and megakaryocytic cells[11]. It was assumed that the Ph^1 chromosome was not present in lymphoid cells because most peripheral blood cells stimulated to divide by the mitogen phytohaemagglutinin lacked a Ph^1 chromosome. As techniques of cell separation improved and immunological markers were developed, it was established that some B cells were also Ph^1+. In blast crisis, some blasts had intracytoplasmic IgM, which is characteristic of pre-B cells. Martin *et al.* [28], showed that B cells from patients in the chronic phase were also Ph^1. He established Epstein-Barr virus (EBV) transformed B-lymphoblastoid cell lines from a patient with Ph^1+ CML, who was also heterozygous for glucose-6-phosphate dehydrogenase (G6PD) isoenzymes. Nine of the 74 cell lines were Ph^1+ and of G6PD type B, which was the isoenzyme type of the myeloid leukaemia clone. Moreover, each cell line expressed a single immunoglobulin class, thus providing further evidence for their monoclonal nature. Bernheim *et al.* [29] also showed, more directly, that some B cells have a Ph^1 chromosome. When cells with surface immunoglobulins (SIgM+) were stimulated with pokeweed mitogen or nocardia opaca antigen, seven of eight patients had a Ph^1 chromosome. In unstimulated cultures, all cells were Ph^1+ and lacked SIgM; they were considered to be granulocytes or monocytes.

How and when consistent translocations occur

I considered these questions in great detail in 1980[30] and will state only some pertinent conclusions. We do not know how consistent structural rearrangements occur, but there are at least two possibilities. The rearrangements may be random, but selection may act to eliminate the vast majority that do not provide the cell with a proliferative advantage. Alternatively, certain changes may occur preferentially and thus may be the ones we see. Although there is some evidence that proximity between chromosomes within the cell may be a contributing factor, I personally favour the first alternative. In my view, the fact that the immunoglobulin gene undergoes extensive DNA rearrangements as B cells mature is unrelated to the t(8;14) in Burkitt's lymphoma. Based on our present

knowledge (or lack thereof), the genes involved in the translocations in myeloid leukemias do not undergo similar DNA rearrangements.

An equally important question is, when in the process of malignant transformation of a particular cell do translocations or other chromosome aberrations occur? All available evidence from the study of carcinogenesis suggests that this is a multistage process. As is best illustrated by the blast crisis of CML, some chromosome changes occur as part of the further evolution of the malignant phenotype and they are, therefore, relatively late events. But what about the occurrence of the t(9;22) in CML, for example? In an individual patient, does the Ph^1 occur in a single normal cell which becomes the progenitor of the leukaemic clone, or is there expansion of a clone, possibly a leukaemic one, in which a translocation occurs in one of these already abnormal cells? This question is not easily resolved because of the lack of independent markers for the leukaemic cell. Fialkow and his colleagues[31] recently presented additional evidence supporting the latter concept. Of 74 EBV-induced B-cell lines established from a patient with CML, 65 were Ph^{1+}, and of these 65 lines, 18 were G6PD type A and 45 were type B. Since the expected ratio was 1:1, this difference in frequency was significant ($p < 0.001$). Moreover, karyotypic analysis of the Ph^{1-} cell lines revealed chromosome abnormalities (some of which were clonal) in 8 of 33 evaluable lines with B-type enzyme, compared to 0 of 14 lines with the A-type G6PD ($p < 0.05$). They concluded that this patient had an abnormal population of B-lymphoid cells that were Ph^{1-} and had the same G6PD phenotype as the Ph^{1+} leukaemic clone, and that these genetically unstable B cells arose from the same clone of cells that gave rise to the Ph^{1+} leukaemia. This notion is supported by observation of rare patients with CML, whose marrow cells appeared to be Ph^{1-} at diagnosis but later became Ph^{1+}. On the other hand, the observation of Kamada *et al.* [32] that atomic bomb survivors (who were followed regularly) had virtually 100 per cent Ph^{1+} cells prior to the development of clinically evident CML, indicates that the Ph^1 aberration can be a very early event in the disease process. Fialkow and Singer[33] described two other patients who were G6PD heterozygotes, one with myelodysplasia and one with ANLL who was thought to be in complete remission. In both patients, the malignant clone had an abnormal karyotype. In the patient with myelodysplasia, 21 of 24 EBV-transformed B-cell lines had the same G6PD phenotype as the malignant clone; however, none was chromosomally abnormal. In the other patient, the chromosomally normal myeloid cells during remission had the same G6PD phenotype as did the leukaemic cells prior to therapy.

The data from these three patients imply that the initial event is expansion of a chromosomally normal clone, and that the chromosome abnormality occurs in a cell belonging to this clone. What is required for resolution of this issue is a reliable marker for leukaemic cells, or for preleukaemic 'initiated' cells, that is independent of the karyotype. One could then correlate the karyotype with this marker to determine how many cells with a normal karyotype were positive for the marker.

Defining the critical recombinant chromosome

It is now apparent that the sites of consistent translocations pinpoint chromosome segments that contain genes critically involved in malignant transformation. Isolation and analysis of these segments of DNA have a high scientific priority. There are two recombinant chromosomes in each translocation, and it would appear useful to determine which is the critical recombinant, that is, which of the two chromosomes contains the essential gene rearrangement[34]. As was discussed earlier, the t(9;22) is also found in complex translocations, and one can use these variants to determine whether one recombinant chromosome is constant in both the typical and complex form. A comparison of these two types indicated that in both the complex and typical form the end of no. 9 consistently moved to the Ph^1 chromosome, whereas the end of 22q could and did move to a large number of sites. At the time of this analysis in 1982, the 4 per cent of patients whose cells had a simple translocation not obviously involving no. 9 were an enigma. As we now know, no. 9 is also involved in these translocations; and thus the common cytogenetic event in every Ph^1+ patient is the movement of 9q34 to 22q11.

THE MOLECULAR ANALYSIS OF THE Ph^1 TRANSLOCATION

One of the most exciting revelations in the past few years has involved the cellular oncogenes and their chromosome location. Much of the excitement derives from the observation that many proto-oncogenes are located in the bands that are involved in consistent translocations[35,36]. Although the first translocation to be deciphered at the molecular level was the t(8;14) in Burkitt's lymphoma, the analysis of the Ph^1 translocation has also provided some important insights both into the limitations of cytogenetic analysis at the level of light microscopy and into the alterations in proteins that can result from these translocations.

Identifying the gene on chromosome no. 9

The use of somatic cell hybrids was crucial to the clarification of the genes involved in the t(9;22). These hybrids were established over a period of years by the investigators at Erasmus University in the Netherlands[37]. The hybrids were obtained from 8 different CML patients. Because human chromosomes are lost preferentially, it was possible to isolate clones with only a few human chromosomes. Moreover, clones were obtained that contained different combinations of the relevant human chromosomes, namely 9, 9q+, 22, 22q-. These clones could be assayed for the expression of genes on no. 9 or no. 22, as well as for the karyotype of the hybrid cells. Using this strategy, the group in the Netherlands could show that adenylate kinase -1 (localized on 9q34) and the genes from no. 22 – mitochondrial aconitase, arylsulfatase A, and N-acetyl-2-D-galactosaminidase – all segregated with the 9q+ and none provided the evidence of a reciprocal translocation from 9[37].

Within 2 years, a new marker was available, which provided positive proof that the t(9;22) was reciprocal. The critical gene was the proto-oncogene, c-*abl*, known to be located on chromosome 9 and localized more precisely to band 9q34[38]. The human c-*abl* sequences homologous to the v-*abl* gene were cloned and were shown to be dispersed over a region of around 40 kilobases (kb) but the precise 5′ and 3′ limits of the human c-*abl* gene have not yet been established. Analysis of the somatic cell hybrids containing either 9, 9q+ 22, or 22q- chromosomes showed that c-*abl* was present in cells with the normal 9 or with the 22q-, but that it was absent in cells with the 9q+ or the normal 22[39]. This clearly established that the t(9;22) was a reciprocal translocation and it was the first proof of this fact. It was estimated that the amount of chromosome 9 that was translocated to no. 22 was less than 5000 kb. At the present time, all of the genetic markers that have been analysed show the same pattern of segregation, indicating that the breakpoints in the t(9;22) are consistent. However, this does not mean that they are identical. Although data on only two CML patients have been published, the breakpoints on no. 9 are at least 35 kb apart and are both some distance from the 5′ end of the cellular sequences that are homologous to v-*abl*. As noted earlier, neither the 5′ or 3′ ends of the c-*abl* gene have been isolated. Heisterkamp and her colleagues were able to identify a fragment from a preparation of total human DNA that hybridized to a c-*abl* probe that was 5′ to the sequences homologous to v-*abl* [40]. This fragment was used to provide small DNA probes that were then used to examine human DNA from various sources. There was no evidence for rearrangement of this fragment in normal DNA, whereas that from one Ph^1+ CML patient showed an altered-size restriction fragment. The other probes were used to localize the point of rearrangement, which should be at the junction of chromosomes 9 and 22. They were able to isolate a 6.0 kb BglII fragment that contained the junction, and further to develop a probe from this 6.0 kb fragment that contained sequences from chromosome 22. This probe for no. 22 – a 1.2 kb HindIII–BglII fragment (1.2 HBg) – was used to obtain a 5.0 kb BglII fragment from normal cellular DNA that was localized to chromosome 22.

Identifying the gene on chromosome no. 22

In another series of experiments, it was possible to use this 1.2 HBg fragment to localize the breakpoints in the DNA of a number of CML patients[41]. The data from 17 patients with CML indicated that the breakpoints in all patients occurred within a 5.8 kb segment of no. 22, which has been called a breakpoint cluster region or bcr (Fig. 4.5). Thus, although the breakpoint on no. 9 is variable, but probably within a limited segment, the break on no. 22 appears to be relatively restricted. The nature of the gene at the site of the break on no. 22 is unknown. The bcr region was normal in fibroblasts isolated from the CML patients, as well as from leukaemic cells from patients with Ph^1- CML. These results indicate that the involvement of the bcr is specific for the Ph^1+ cells. At about this same time, another oncogene (c-*sis*) was located on the distal part of

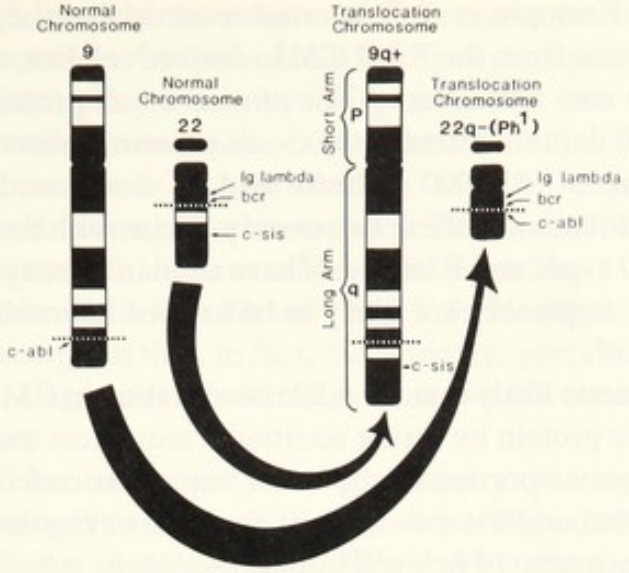

Fig. 4.5 Schematic representation of the reciprocal translocation involving nos. 9 and 22 with relative positions of the translocated oncogenes c-*abl* and c-*sis*. bcr = breakpoint cluster region.

no. 22 at band 22q13. Although the c-*sis* gene moves with the portion of no. 22 translocated from the Ph^1 chromosome, there is no evidence that it has any functional role in CML. Another gene on no. 22 is that for the lambda immunoglobulin light chain (Igλ). It has been localized to band 22q11, but it is closer to the centromere than the breakpoint in CML. In a cell line established from a patient with CML in blast crisis, K562, it has been shown that both c-*abl* and Igλ are amplified 4 to 8 times[42,43]. However, there is no evidence that the Igλ gene is involved in the breakpoint of the t(9;22)[40].

Functional consequences of the t(9;22)

Canaani and his colleagues have examined CML cells for the expression of c-*abl* [44]. In 5 patients with Ph^1+ CML, enhanced expression of c- *abl* was not observed; however, a new RNA transcript of 8 kb was detected in addition to the normal transcripts of 6 and 7 kb. A similar 7 kb c- *abl* RNA transcript was identified by Heisterkamp *et al.* in K562[40]; however, since they have detected the break in no. 9 at about 100 kb 5′ to known c-*abl* sequences and thus about 100 kb from the bcr on no. 22, it is remarkable that the translocation results in this altered RNA. Recent work however suggests that this new RNA may be derived from a large fused transcript of the *abl* and bcr RNAs that is then spliced to exclude varying amounts of the 3′ end of the bcr RNA and of the 5′ end of the *abl* RNA, thereby producing a new mRNA of a size remarkably consistent from patient to patient[44a,44b]. Work on the c-*abl* protein detected in K562 showed that a new chimeric protein was also formed from this large segment of DNA.

Several lines of evidence suggest that the transforming activity of the Abelson murine leukaemia virus (A-MuLV) is closely correlated with the tyrosine-specific kinase activity of the v- *abl* coded protein. Although the v- *abl* protein was derived from the c- *abl* protein and they are closely related, they differ in that the c- *abl* protein is not detectably phosphorylated on tyrosine *in*

vivo or *in vitro*[45]. Konopka *et al.*[46] reported recently that they have detected an altered c- *abl* protein from the K562 CML-derived cell line, which has tyrosine kinase activity *in vivo* and *in vitro*. The normal c- *abl* protein has a molecular weight of 145 000 daltons whereas the c- *abl* protein isolated from K562 has a molecular weight of 210 000 daltons and is designated P210; the data comparing the structures of these two c- *abl* proteins with those of v- *abl* (P160) suggest that P210 c- *abl* and P160 v- *abl* have similar carboxy-terminal regions. Thus the altered sequences are likely to be located internally or at the amino terminus of P210[46].

Therefore, it seems likely that the 9;22 translocation in CML cells could have created a chimeric protein by fusing additional sequences coded by the gene on no. 22 with the major portion of the c- *abl* sequences coded for on no. 9. We have only a minimal understanding of the function or regulation of the normal c- *abl* protein. A segment of a cloned human c- *abl* gene is highly homologous to v- *abl* and to other genes with tyrosine kinase activity. It is possible that the c- *abl* protein kinase activity requires interaction with a specific substrate to be detected.

The Ph[1] translocations revisited

The results of the molecular analysis of the t(9;22) and the availability of DNA probes specific for the c- *abl* sequences on no. 9 that move to the Ph[1] have led to new insights into the subtle rearrangements that can occur in this translocation.

The work of Hagemeijer and her colleagues[17] in the Netherlands, aided by the cooperation of European cytogeneticists who sent unique patient material that was critical in the clarification of the genetic rearrangements in CML, is presented in detail elsewhere[18] and only the essential points will be covered here. In two cases with the typical t(9;22), c- *abl* was localized to the Ph[1] chromosome by *in situ* hybridization. The genetic analysis of some unusual translocations was done using somatic cell hybrids, whereas others were defined with the use of *in situ* hybridization. In two patients with a complex 3-way translocation obviously involving nos. 9 and 22, the same movement of c- *abl* to the Ph[1] was demonstrated with *in situ* hybridization. However, their results are most significant in the analysis of the 'simple' two-way translocation, not obviously involving no. 9. In each of 3 patients, whose cells were thought to have simple variants – t(4;22)(p16;q11), t(12;22)(p13;q11) and t(7;22)(p22;q11) – *in situ* hydridization clearly demonstrated the presence of c- *abl* on the Ph[1]. Moreover, with careful high-resolution banding, Hagemeijer[18] could show that there was a distinguishable difference in the two no. 9's and that one of them could be identified as participating in a complex three-way translocation. These data strongly indicate that there is no such thing as a simple two-way translocation. Also, as illustrated in Figure 4.3, because the breakpoints in the apparently simple translocations involve the ends of the other chromosomes, whose size and staining characteristics are similar to band 9q34, these subtle 3-way rearrangements can not be reliably identified. Finally, one other enigma

has been resolved, namely the nature of the rearrangement leading to the masked Ph[1] chromosome. Analysis of cells from one such patient of Dr. Gödde-Salz was recently completed by Hagemeijer[18]; this patient had a t(6;22)(p21;q11) with translocation of 6p to the Ph[1], which resulted in a large marker chromosome. *In situ* hybridization revealed that c-*abl* was also translocated to the abnormal Ph[1] chromosome. The c-*abl* DNA is located in the middle of the abnormal Ph[1] in its usual site adjacent to 22q11 – the piece of 6p is distal to no. 9 and the Ph[1] is thus 22pter→22q11::9q34→'9qter'::6p21→6pter. It is a reasonable assumption that, in fact, the telemeric portion of no. 9 moves to 6p21. In this case, Hagemeijer could not discern a visible change in either of the no. 9 chromosomes.

Hagemeijer and her colleagues[18] have preliminary data regarding two other conundrums, namely Ph[1]+ ALL and some cases of Ph[1]- CML involving no. 9 but apparently not no. 22. In two cases of Ph[1]+ ALL, they have evidence that the breakpoint is located outside of the bcr region that is involved in CML. If this observation is confirmed, it will be possible to distinguish reliably between Ph[1]+ ALL and CML in lymphoid blast crisis. In two such patients, preliminary data suggest that rearrangements in the bcr had occurred. Further analysis of one patient who appeared to have a t(9;12) revealed that sequences from no. 22 were adjacent to the c-*abl* gene of chromosome 12, therefore a small amount of no. 22 had moved to no. 12 as well, although it could not be detected at the level of light microscopy. It should be noted that in Ph[1]- CML not involving no. 9, there is no evidence for rearrangement at the DNA level of either nos. 9 or 22[47].

The cloned probes for the DNA near to the junction sites on both nos. 9 and 22 provide powerful tools for the analysis of patients with CML and related diseases, whose karyotypes have been difficult to explain on the basis of our earlier concepts. Moreover, from a practical point of view, it will be possible to screen CML patients for the presence of a break in the bcr and thus to diagnose Ph[1]+ CML with Southern blots. This technique cannot, at least at present, identify patients with complex rearrangements.

CONCLUSIONS

The progress made in the 25 years from the discovery of the Ph[1] chromosome to its almost completed genetic analysis is truly remarkable. This has been possible because of the collaborative efforts of many scientists around the world. It has required the efforts of clinicians, pathologists, cytogeneticists, virologists, and now molecular geneticists. There is still a great deal that remains to be discovered; to achieve a full understanding continues to require the efforts of us all.

REFERENCES

1. Nowell P C, Hungerford D A 1960 A minute chromosome in human granulocytic leukemia. Science 132: 1497

2. Caspersson T, Gahrton G, Lindsten J, Zech L 1970 Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. Experimental Cell Research 63: 238–244
3. O'Riordan M L, Robinson J A, Buckton K E, and Evans H J 1971 Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia (Ph[1]) by fluorescence. Nature 230: 167–168
4. Rowley J D, 1973 A new consistent chromosomal abnormality in chronic myelogenous leukaemia. Nature 243: 290–293
5. Mayall B H, Carrano A V, Moore D H, and Rowley J D 1977 Quantification by DNA-based cytophotometry of the 9q+/22q chromosomal translocation associated with chronic myeloid leukemia. Cancer Research 37: 3590–3593
6. Gahrton G, Lindsten J, and Zech L 1974 Involvement of chromosomes 8, 9, 19 and 22 in Ph[1] positive and Ph[1] negative chronic myelocytic leukemia in the chronic or blastic stage. Acta Medica Scandinavica 196: 355–360
7. Fialkow P J 1974 The origin and development of human tumors studied with cell markers. New England Journal of Medicine 291: 26–35
8. Van den Berghe H 1973 The Ph[1] chromosome: translocation to chromosome 9. Lancet ii: 1030
9. Hayata I, Sakurai M, Kakati S, Sandberg A A 1975 Banding studies of chronic myelocytic leukemia, including five unusual Ph[1] translocations. Cancer 36: 1177–1191
10. Rowley J D 1980 Ph[1] positive leukemia, including chronic myelogenous leukaemia. Clinics in Haematology 9: 55–86
11. Sandberg A A 1980 The Chromosomes in Human Cancer and Leukemia. Elsevier-North Holland, New York
12. Mitelman F, and Levan G 1981 Clustering of aberrations to specific chromosomes in human neoplasms. IV. A survery of 1 871 cases. Hereditas 95: 79–139
13. Rowley J D, and Testa J R 1983 Chromosome abnormalities in malignant hematologic diseases. In: Advances in Cancer Research pp. 103–148. Academic Press Inc., New York
14. Carbonell F, Benitez J, Prieto F, Badia L, Sánchez-Fayos J. 1982 Chromosome banding patterns in patients with chronic myelocytic leukemia. Cancer Genetics and Cytogenetics 7: 287–297
15. Ishihara T et al 1983 A summary of cytogenetic studies in 534 cases of chronic myelocytic leukemia in Japan. Cancer Genetics and Cytogenetics 9: 81–91
16. Mitelman F 1983 Catalogue of chromosome aberrations in cancer. Cytogenetics and Cell Genetics 36: 1–515
17. Hagemeijer A, Bartram C R, Smit E M E, van Agthoven A J, Bootsma D 1984 Is the chromosomal region 9q34 always involved in variants of the Ph[1] translocation? Cancer Genetics and Cytogenetics 13: 1–16
18. de Klein A, Hagemeijer A 1985 Cytogenetic and molecular analysis of the Ph[1] translocation in chronic myeloid leukemia. In: Rowley J D (ed) Consistant chromosome aberrations and oncogenes in human tumours. Cancer Surveys 3: 515–529
19. Verma R S, Dosik H 1980 Heteromorphisms of the Philadelphia (Ph[1]) chromosomes in patients with chronic myelogenous leukaemia (CML) I. Classification and clinical significance. British Journal of Haematology 45: 215–222
20. Berger R, Bernheim A, Bussel A 1981 Chromosome Philadelphie par t(9;22) et t(1;9;22) en mosaïque. Nouvelle Revue Française d'Hématologie 23: 209–212
21. Whang Peng J, Henderson E S, Knutsen T, Freireich E J, Gart J J 1970 Cytogenetic studies in acute myelocytic leukemia with special emphasis on the occurrence of Ph[1] chromosome. Blood 36: 448–457
22. Pugh W C, Pearson M, Vardiman J W, Rowley J D 1985 Philadelphia chromosome-negative chronic myelogenous leukaemia: a morphological reassessment. British Journal of Haematology 60: 457–467
23. Bennett J M et al 1982 Proposals for the classification of the myelodysplastic syndromes. British Journal of Haematology 51: 189–199
24. Chessells J M, Janossy G, Lawler S D, Secker-Walker L M 1979 The Ph[1] chromosome in childhood leukaemia. British Journal of Haematology 41: 25–41
25. The Third International Workshop on Chromosomes in Leukemia. 1981 Cancer Genetics and Cytogenetics 4: 95–142
26. Bloomfield C D, Lindquist L L, Brunning R D, Yunis J J, Coccia P F 1978 The

Philadelphia chromosome in acute leukemia. Virchow's Archiv B of Cell Pathology 29: 81–92
27. Fourth International Workshop on Chromosomes in Leukemia. 1984 Cancer Genetics and Cytogenetics 11: 251–360
28. Martin P J, Najfeld V, Hansen J A, Penfold G K, Jacobson R J, Fialkow P J 1980 Involvement of the B-lymphoid system in chronic myelogenous leukaemia. Nature 287: 49–50
29. Bernheim A, Berger R, Preud'homme J L, Labaume S, Bussel A, Barot-Ciorbaru R 1981 Philadelphia chromosome positive blood B lymphocytes in chronic myelocytic leukemia. Leukemia Research 5: 331–339
30. Rowley J D 1980 Chromosome abnormalities in cancer. Cancer Genetics and Cytogenetics 2: 175–198
31. Fialkow P J, Martin P J, Najfeld V, Penfold G K, Jacobson R J, Hansen J A 1981 Evidence for a multistep pathogenesis of chronic myelogenous leukemia. Blood 58: 158–163
32. Kamada N, Uchino H 1982 Chronologic sequence in appearance of clinical and laboratory findings characteristics of chronic myelocytic leukemia. Blood 51: 843–850
33. Fialkow P J, Singer J W 1985 Tracing development and cell lineages in human hemopoietic neoplasia. In: I L Weissman (ed.), Leukemia. Dahlem Konferenzen. Springer-Verlag, Berlin 203–222
34. Rowley J D 1982 Identification of the chromosome region involved in human hematologic malignant disease. Science 216: 749–751
35. Rowley J D 1983 Human oncogene locations and chromosome aberrations. Nature 301: 290–291
36. Rowley J D 1984 The biological implications of consistent chromosomes rearrangements. Cancer Research 44: 3159–3168
37. Geurts van Kessell A H M, et al 1981 Characterization of the Philadelphia chromosome by gene mapping. Cytogenetics and Cell Genetics, 30: 83–91
38. Heisterkamp N et al 1982 Chromosomal localization of human cellular homologues of two viral oncogenes. Nature 299: 747–749
39. Hagemeijer A, Bootsma D, Spurr N K, Heisterkamp N, Groffen J, Stephenson J R 1982 A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765–767
40. Heisterkamp N et al 1983 Localization of the c- *abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. Nature 306: 239–242
41. Groffen J, Stephenson J R, Heisterkamp N, de Klein A, Bartram C R, Grosveld G 1984 Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36: 93–99
42. Collins S J, Groudine M T 1983 Rearrangement and amplification of c- *abl* sequences in the human chronic myelogenous leukemia cell line K-562. Proceedings of the National Academy of Sciences USA 80: 4813–4817
43. Selden J R et al 1983 Amplified Cλ and c- *abl* genes are on the same marker chromosome in K562 leukemia cells. Proceedings of the National Academy of Sciences USA 80: 7289–7292
44. Canaani E, Gale R P, Steiner-Saltz D, Berrebi A, Aghai E, Januszewicz E 1984 Altered transcription of an oncogene in chronic myeloid leukaemia. Lancet i: 593–595
44a. Shtivelman E, Lifshitz B, Gale R P, Canaani E 1985 Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukaemia. Nature 315: 550–554
44b. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G 1985 Structural organization of the *bcr* gene and its role in the Ph′ translocation. Nature 315: 758–761
45. Ponticelli A S, Whitlock C A, Rosenberg N, Witte O N 1982 In vivo tyrosine phosphorylations of the Abelson virus transforming protein are absent in its normal cellular homolog. Cell 39: 953–960
46. Konopka J B, Watanabe S M, Witte O N 1984 An alteration of the human c- *abl* protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell 37: 1035–1042
47. Bartram C R et al 1983 Translocation of the human c- *abl* oncogene occurs in variant Ph^1-positive but not Ph^1-negative chronic myelocytic leukaemia. Nature 306: 277–280

Human antibody and T-cell receptor genes: rearrangement in normal and abnormal cells

INTRODUCTION

In this chapter I will review the field concerning the two sets of genes which undergo rearrangement and sequence changes in human B-cells and T-cells. These are the antibody or immunoglobulin (Ig) genes in B-cells and the analogous antigen receptor genes in T-cells. I will also describe work that deals with the rearrangement of these genes in leukaemias and, in the case of B-cells, the associated aberrant rearrangements i.e. the chromosomal translocations. The article is not a comprehensive review of the literature (much of the work on the analogous systems in mouse and other species will only be scantily referred to), but it is intended to illustrate the state of the field at present and hopefully show the aspects of these intricate gene systems that are still to be explored. [Reviews dealing with mouse immunoglobulin can be found in refs 1 and 2.]

THE ANTIBODY PROTEIN AND THE ANTIBODY GENE LOCI

Antibodies are made up of four immunoglobulin polypeptide chains called light (L) or heavy (H) chains, according to their relative molecular weights. The four chains are held together by disulphide bridges in the manner schematically illustrated in Figure 5.1. A detailed description of these proteins can be found elsewhere[3]. The polypeptide sequences are divisible into N-terminal variable (V) regions, which constitute the part of the antibody molecules capable of combining with antigen (i.e. the antibody combining site) and C-terminal constant (C) regions. The C-region sequence contains the domains responsible for more conserved functions, such as complement-binding during the immune response. There are two types of L-chains with different C-region sequences; these are called kappa (κ) and lambda (λ) chains. The H-chains are divided into five classes (designated μ, δ, γ, ϵ, and α) again defined by the constant portion of the polypeptide; there are also four γ subclasses (γ_1, γ_2, γ_3, γ_4) and two α subclasses (α_1 and α_2), which are distinguished by amino acid substitutions. The various H-chain classes and subclasses share the same set of V-region sequences (see below), and the H-chain genes are present in a single gene locus located on the long arm of chromosome 14 at 14q32[4,5]. The light chains are encoded on two separate autosomes. The λ chain genes are on the

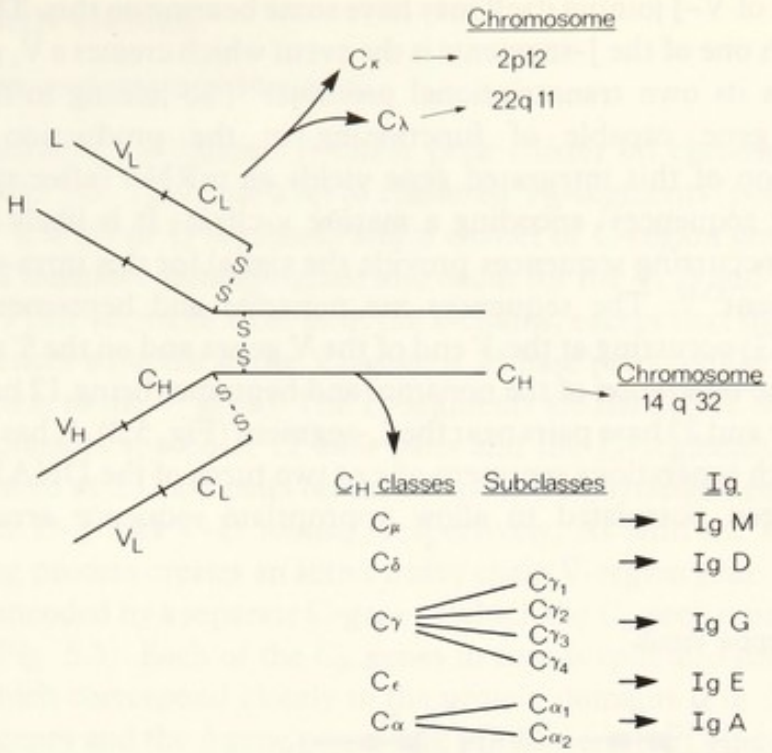

Fig. 5.1 Diagram of an antibody molecule showing the origin of the human classes and subclasses.

long arm of chromosome 22 at 22q11[6] and the κ-chain genes are on the short arm of chromosome 2 at 2p12[6,7]. Each of the three immunoglobulin gene loci are self-contained for the production of the complete polypeptide but there is considerable DNA rearrangement necessary before the functional immunoglobulin genes are formed. Only when there is productive rearrangement of an H-chain and a L-chain gene within a particular cell can a functional antibody molecule result.

STRUCTURE AND REARRANGEMENT OF THE HUMAN κ-CHAIN GENES

A mature chain is produced from a variable region gene (encoding amino acids 1 to 95), one of five joining, or J, segments (encoding amino acids 96 to 108), and a single C-gene (encoding the remaining part of C-region). The five J-segments are separated from each other by intervening sequences of about 300 bases and from the C-gene by a larger intervening sequence of 2.5 kb[8] (Fig. 5.2). A small intervening sequence also occurs in the V-gene itself[9], within a leader sequence at the N-terminus. The location of this 90 base pair interruption is usually at codon minus 4/5. There is thought to be a small number of V_κ-genes[10] and these are probably clustered in one part of chromosome 2. It was originally thought that a DNA deletion between the V and J segments accompanies the VJ joining. However, studies in mouse indicate that, although the intermediate DNA must certainly be removed from between the V and J regions, there may be a re-integration of this DNA into the genome at an unknown position[11]. The

mechanism of V–J joining itself may have some bearing on this. The joining of a V-gene with one of the J-segments is the event which creates a V_κ gene. Each V_κ gene carries its own transcriptional promoter[12], so joining to the J-segment creates a gene capable of functioning in the production of mRNA. Transcription of this integrated gene yields an mRNA (after splicing of the intervening sequences) encoding a mature κ-chain. It is likely that specific, repeatedly occurring sequences provide the signal for this intra-chromosomal rearrangement[13,14]. The sequences are nonamer and heptomers (consensus CACAGTG) occurring at the 3′ end of the V genes and on the 5′ side of each J-segment, the separation of the nonamer and heptamer being 12 base pairs near the V_κ-gene and 23 base pairs near the J_κ-segment (Fig. 5.2). It has been pointed out that such separations represent one or two turns of the DNA helix and that this has been postulated to allow appropriate sequence arrangement for joining[15].

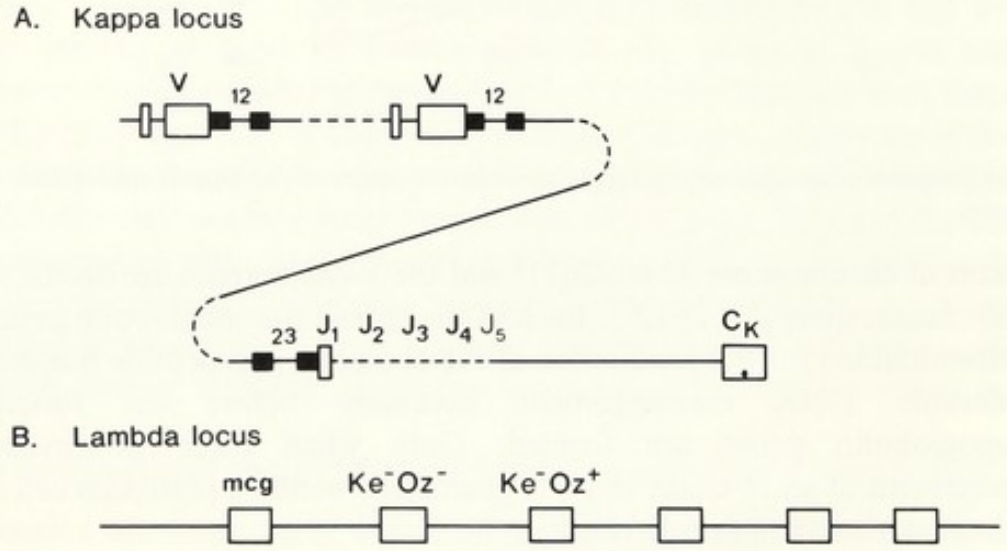

Fig. 5.2 Organization of human immunoglobulin light chain genes. A. The κ-locus on chromosome 2. The black boxes near the V and J segments represent the signal sequences thought to be involved in joining. The sequence between V and J is not transcribed in B-cells and that between J and C is intervening sequence. B. The Cλ locus consists of six linked C genes, of which three are known to correspond with allotypes of Cλ.

CURRENT KNOWLEDGE OF THE HUMAN λ-LIGHT CHAIN LOCUS

Knowledge of the Vλ-genes is very limited and, thus far, only one isolated Vλ gene has been reported[16]. The complexity of Vλ genes remains to be ascertained. The Cλ locus on the other hand has been shown to consist of six clustered C-genes[17] of which three are known to correspond to allotypic Cλ chains of known sequence variation (Fig. 5.2). The lack of data on the human λ system leaves open many questions regarding the origin of the diversity of λ chains. The analogous genes in the mouse are much better characterised and reviews on this subject can be found[1,2].

THE H-CHAIN GENES

Organization and expression of C_μ

The organization of the human H-chain gene cluster on chromosome 14 is shown in Figure 5.3. There are a set of clustered V_H-segments (possibly of the order of 100[18]), a set of D segments and a cluster of C-region genes[19,20]. The heptamer and nonamer joining signals also occur for the V, D and J segments; and the 12–23 rule seems to exist as in the κ-chains, except that the spacing of the two sequences adjacent to the V_H-gene is 23 base pairs (unlike the 12 base pair spacing next to the V_κ gene). The D-segments are flanked on both sides by the signal sequences spaced at 12 base pairs and the J_H-segments have these sequences spaced at 23 base pairs on their 5′ side. This arrangement allows for the process of D–J and V–D joining, respectively. As with the κ-chain, the V–D–J joining process creates an active heavy chain V-region gene. Each of the C-regions is encoded by a separate C-gene of which the C_μ gene is nearest to the J-segments (Fig. 5.3). Each of the C_H genes in turn is split into small exons in the DNA, which correspond closely to the protein domains (Fig. 5.4); the γ-genes, the α genes and the δ gene possessing in addition short hinge segments. The C_H exons are linked together in the mRNA by RNA splicing of the intervening sequences. V–D–J joining creates a transcription unit, which includes firstly the C_μ gene giving rise to the μ heavy chain (illustrated in Fig. 5.5). When the cell has joined such a μ gene and joined a L-chain gene, the antibody produced is IgM. Usually (as in B-cells) this IgM is attached to the surface as membrane-bound immunoglobulin. The IgM is anchored in the membrane by a carboxy terminal sequence (usually designated μm) which comes from a downstream pair of exons (not indicated in Fig. 5.4, which only shows the exons for the secreted protein). The μm exons represent an alternative pathway to RNA splicing enzymes. When the secreted form of IgM is made, the two C-terminal exons are spliced out of the mRNA and therefore simply not included in either mRNA or protein.

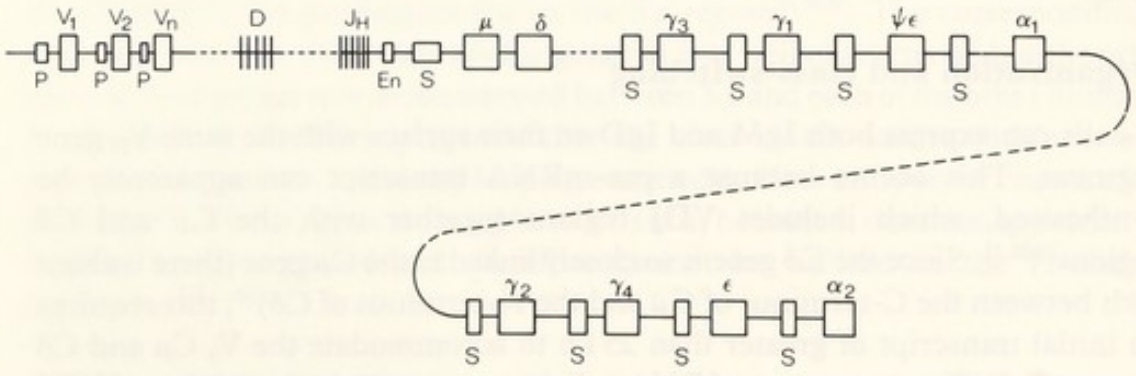

Fig. 5.3 Arrangement of the human heavy chain locus. The locus occurs on chromosome 14. P represents V gene promoter, En represents the major transcriptional enhancer and S represents the sequences involved in the class switch recombination. $\psi\epsilon$ is a partially deleted pseudo-epsilon gene.

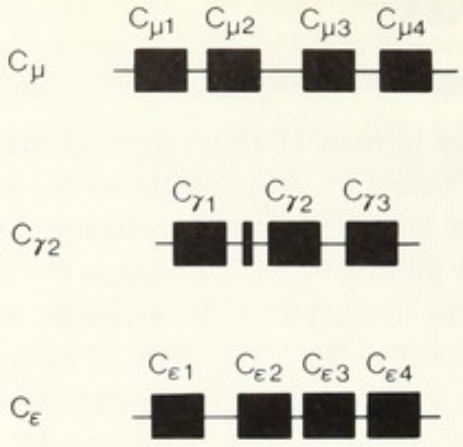

Fig. 5.4 Exon-intron structure of human heavy chain constant region genes.

The active μ-chain gene is controlled by a transcriptional enhancer located just downstream from the J_H segments, in the large intervening sequence from J_H to $C\mu$[21]. This enhancer is cis-acting and works on the local promoter of the V-gene joined to a J-segment. It is thought that this local activating effect is the explanation for the transcriptional inactivity of the non-rearranged germ-line V-genes.

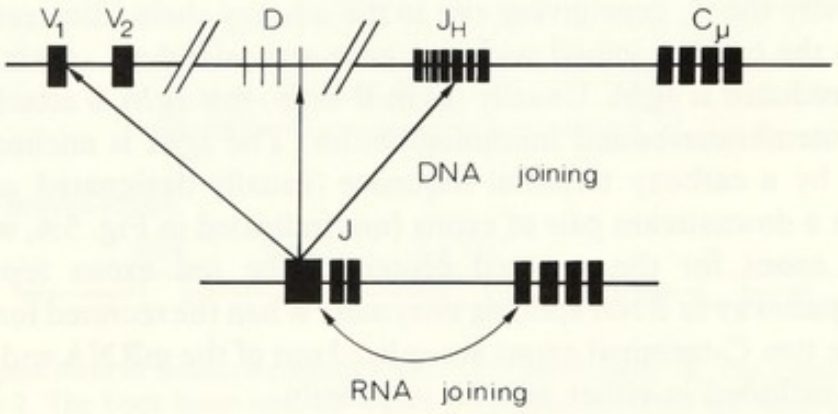

Fig. 5.5 Mechanism of creation and expression of an active μ heavy chain gene (secreted form).

Organization and class-switching

B-cells can express both IgM and IgD on their surface with the same V_H gene segment. This occurs because a pre-mRNA transcript can apparently be synthesised, which includes VDJ regions together with the $C\mu$ and $C\delta$ regions[20,22,23]. Since the $C\delta$ gene is so closely linked to the $C\mu$ gene (there is about 5 kb between the C-terminus of $C\mu$ and the N-terminus of $C\delta$)[20], this requires an initial transcript of greater than 25 kb to accommodate the V, $C\mu$ and $C\delta$ segments. Different patterns of RNA splicing must explain the joining of VDJ with $C\mu$ or $C\delta$ to generate the two types of mRNA for μ or δ H-chains.

The switch between μ and δ chains can occur without further DNA rearrangement. There is a different type of class switch, which does involve a

second type DNA rearrangement, called switch recombination. This process appears unique to heavy chain genes and involves the relocation of an integrated VDJ from the $C\mu$ to one of the other C_H genes. The human H-chain C-genes are organized as a group with the order shown in Figure 5.3. The two groups of genes ($C\gamma_3C\gamma_1\psi\epsilon\alpha_1$ and $C\gamma_2C\gamma_4\epsilon\alpha_2$) each occupy about 80 kb of DNA but it is not known what distance these two groups are apart. The characteristic order of the human C_H genes in the two similar groups suggests that massive DNA duplications occurred to produce the current organization[19]. The difference in organization of the human C_H cluster and the mouse C_H gene cluster makes several mechanisms for this duplication equally feasible. It is interesting that of all the C_H genes, the C_ϵ and C_δ genes are least well conserved between mouse and man[24–27]. This implies that the functional parts of these molecules are restricted to small portions, or that (as perhaps in the case of $C\delta$) the function has changed. Furthermore, there is one pseudo-gene (ψ) of the γ class whose location is as yet unknown[28,29]. Within the C_H locus there is another ψ-gene (ψ_ϵ), which is partly deleted in its N-terminal part[19,25,30] and is found between γ_1 and α_1 genes. A further ψ_ϵ-gene occurs on chromosome 9 and is a so-called processed gene from which the introns are missing, presumably removed by a process of reverse transcription in the evolutionary past.

The H-chain class switch is mediated by sequences aptly named switch sequences (shown as 'S' in Fig. 5.3). The final result of switching is that, for example, part of $S\mu$ becomes joined with the switch sequence adjacent to another C_H gene. The VDJ sequence, previously expressed with $C\mu$, is now expressed with the next C_H gene. The transcriptional enhancer is carried along with the VDJ segment, thus being utilised by the different C_H genes. Further switching could subsequently occur from the newly formed transcription unit (VDJC) to a C_H gene further downstream by similar recombination processes. The process of class switching occurs by deletion of the intermediate DNA[31–34] and is probably mediated through short tandemly-repeated sequences located near each C_H gene[19,35]. ($C\delta$ is the exception because it seems to lack a functional $C\delta$ portion, although the human $C\delta$ gene does appear to have a vestigial S-like sequence[27].) The sequence that probably mediates the class switch is a repeat of $G_3CT(GAGCT)n$ predominating in the $S\mu$ region[20,35,36]. The corresponding sequence near the other human C_H genes is less well characterized, although cross hybridization was demonstrated between $S\mu$ and each of the other human S regions[19]. It has been proposed that the class switch occurs within these repetitive sequences, either by looping out mechanisms or by sister chromatid exchange[37]. The ψ_γ gene is thought to be such, because it does not apparently contain an S segment and therefore cannot be utilized in the H-chain expression[29].

Organization and gene conversion

Immunoglobulin genes are mobile not only in the sense of somatic rearrangement but also in evolutionary terms. The difference in the C_H gene

organization in mouse[38] and man, reflects different duplication events that occurred in the evolution of these loci. Different evolutionary events have apparently resulted in the human α chain allotypes[39]. The α_2 gene of man is present in two allelic forms (allotypes) designated, in the protein, A2m(1) and A2m(2). These are derived from two allelic forms of α gene, one of which has apparently been involved in a gene conversion event with an α_1 gene[40]. In this putative process (Fig. 5.6) part of an α_1 gene was copied into the C-terminal part of the α_2 gene (indicated by the black region in the figure). The resulting 'converted' gene has its beginning and end like the ancestral α_2 gene and its middle like α_1 (the α_1 donor being unchanged by this process). This dynamic process provides a clear example of how the eukaryotic genes evolve.

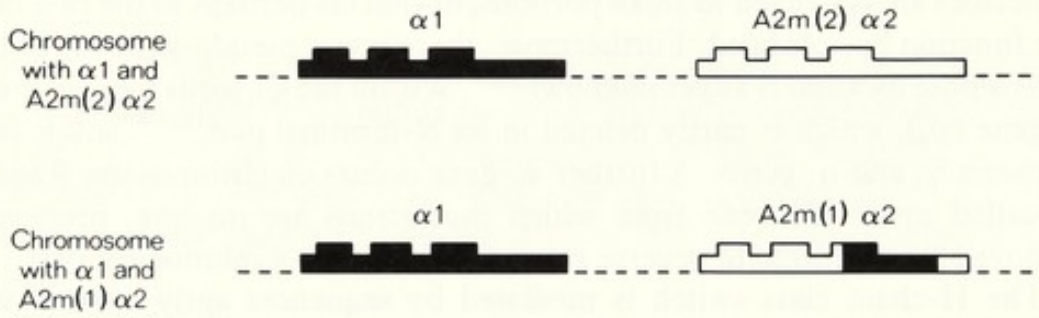

Fig. 5.6 Organization and sequence arrangement of the heavy chain α_2 allotypic genes. Two chromosomes are represented: one encodes α_1 and A2m(2)α2 genes; and the other encodes α_1 and A2m(1) α2 genes. The sequence relationship of the α_1 and α_2 genes is indicated by the open and closed areas. The part of the A2m(1)α2 gene which has undergone a putative gene conversion with an α_1 gene is indicated by the closed area.

THE ORIGIN OF V-REGION DIVERSITY

Diversity of the antibody-combining site (i.e. the V-regions) is generated in three ways:

1. Multiple inherited V-genes;
2. Somatic mutation of these V-genes; and
3. Junctional diversity at V–D, D–J and V–J joining sites.

Theoretically, if there are X inherited L-chain genes and Y H-chain genes there should be $X \times Y$ possible combinations and thus at least this number of antibody-combining sites. It remains to be proved whether all combinations of L and H chains can make productive antibodies. This inherited diversity is amplified by somatic mutation. It has been shown by studies on inbred mice that both L chain and H chain V-genes can be altered in individual B-cells, i.e. somatically. (This aspect of mouse immunoglobulin genes is reviewed in reference 2.) The relatively low number of human germ-line V-genes[10] is possibly indicative of a high rate of somatic mutation acting on these genes. Therefore, somatic mutation seems to contribute to the considerable diversification of V-genes in man and in mouse.

A major source of V-region sequence diversity is the so-called junctional

diversity. This occurs in the joining of V-gene segments with J-segments or D-segments and D with J-segments. It was first recognized in mouse κ-chain genes[13,14] and Table 5.1 illustrates an example. Here the joining of Vκ gene ($V_\kappa 41$) with J_1 can generate four different codons, which can give rise to three different amino-acids at residue 96. This residue occurs at the end of the third region of maximum variability in V_κ-sequences (the third hypervariable region). Inspection of Table 5.1 shows that junctional diversity comes from in-frame flexibility of the recombination site; out-of-frame joining can occur, which could result in an aberrant rearrangement making non-productive proteins. Given that combinational diversity can occur by V,D and J joining, there is clearly considerable potential for amino-acid changes in V-regions.

Table 5.1 Junctional diversity in V_κ genes.

	Codon 96	
$V_\kappa 41$	- - - C C C - - -	1 = T G G Trp
		2 = C G G Arg
	1 2 3 4	3 = C C G Pro
	↓ ↓ ↓ ↓	4 = C C C Pro
J_1	- - - T G G - - -	

The three bases indicated by the horizontal line are involved in the joining which can occur at one of the four positions to make the four codons shown on the right hand side.

HIERARCHY OF ANTIBODY REARRANGEMENT IN HUMAN B-CELLS

Ordered Ig gene rearrangements and allelic exclusion

B-lymphocytes that express immunoglobulin, derive from pluripotential marrow stem cells. The stem cells differentiate to pre-B-cells prior to final differentiation to B-cells. The latter cells synthesize Ig from only one of the allelic sets of Ig genes (allelic exclusion). The H-chain locus rearranges first, without necessarily L-chain gene arrangement, to produce cytoplasmic μH chains. Allelic exclusion seems to result from the fact of a high probability of aberrant (i.e. non-functioning) rearrangements occurring. This aberrant joining also applies to the L-chain loci. The process of making a B-cell with a functional rearrangement seems to involve a series of hit-and-miss events. A H-chain gene rearranges first and if a productive allele results then H-chain rearrangement seems to stop. However, if a non-productive allele results, the other allele can rearrange. The cell either has two non-productive rearrangements (whence it is destined to oblivion), or one productive and one non-productive rearrangement, in which case the cell moves on to L-chain rearrangement – as does the cell which productively rearranged a H-chain gene after the first attempt – where the same process of elimination occurs. In human B-cells κ-chain gene rearrangement precedes λ-chain[41] because κ-producing cells retain germ-line λ gene arrangement. However, λ-producing cells can

have deleted or rearranged κ genes[41–43]; this κ-chain rearrangement is not obligatory in λ-producing cells however[43]. B-cells can of course also switch H-chain class as described above.

Ig rearrangement in human B-cell leukaemia

A number of studies have shown that all types of B-cell leukaemias exhibit H and L-chain rearrangements[41,43,44] and there is no obvious significant pattern of rearrangements emerging at this stage. Chronic lymphocytic (CLL) and acute lymphoblastic (ALL) leukaemias of B-cell type show Ig rearrangement, as do at least some common-ALL cases and hairy cell leukaemia. Furthermore, chronic granulocytic leukaemia (CGL) can also show Ig rearrangements in blastic crisis[45]. These results confirm the B-cell origin and monoclonality of the various leukaemia disorders. So far, fresh T-cell leukaemia samples have failed to show Ig gene rearrangements but some human T-cell lines do have H-chain rearrangements, usually aberrant D–J rearrangements[46]. Studies on four cases of prolymphocytic leukaemia of B-cell type (B-PLL) show that both H chain genes are rearranged[43], and this may support the view that B-PLL is a more mature cell than that of CLL, where generally only a single H-chain rearrangement occurs. On the whole, it appears that individual B-cell leukaemias represent random cell malignancies in populations because of the heterogenous sizes observed in the rearranged genes of the many leukaemias now studied[43]. In principle, however, demonstrating the presence of unique Ig gene rearrangement (or T cell receptor gene rearrangement) patterns in a presumptive malignant clinical sample should prove extremely useful in the diagnosis and treatment of haemopoietic neoplasms.

GENE REARRANGEMENTS IN HUMAN T-CELLS

The human T-cell receptor molecules

T-cells recognize antigens in a specific way using the so-called T-cell receptor. This is a heterodimer of α and β chains whose characteristics indicate the presence of V and C regions analogous to the immunoglobulins. Recent DNA cloning experiments in man[47,48] and mouse[49,50] have shown that at least one chain (β) derives from a set of genes remarkably like the immunoglobulin genes. (The β-chain genes are located on chromosome 7 in man[51]). In man there is a set of V_β-genes, which join to D_β and to J_β segments[52] located upstream of two tandemly-linked C_β genes (about 10 kb apart – see Figure 5.7)[48]. There is considerable sequence homology in the two human C_β genes – except in the 3′ untranslated portion, which is very divergent[53]. The V, D and J segments are flanked by nonamer and heptamer sequences characteristic of the Ig locus[52]. It has been shown that a D segment occurs upstream of the J segments[54] and that this D_β can join to a J_β without V_β involvement. This D–J rearranged gene seems to be transcribed into RNA but, as yet, it is not known whether a protein is made from this transcript.

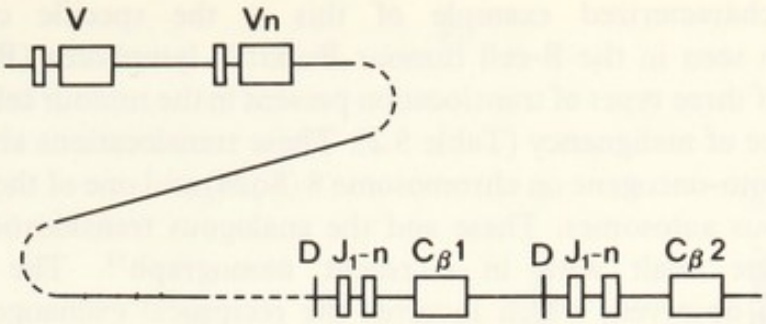

Fig. 5.7 Organization of the human T-cell reception β chain locus.

Although at a preliminary stage, the data so far strongly indicate that the V_β diversity at the germ-line level (i.e. inherited) is significant because multiple V-genes can be detected in non-T cell DNA[48,52] and the sequences of β-chain cDNA clones show considerable differences[53].

Presumably the origin of the two C_β genes is in a recent duplication event, but it is far from clear why two such related C_β genes should be needed; the conservation of this arrangement in mouse[55] and man[48] is, however, presumably significant. Early data on the possibility of somatic mutation in V-genes indicate that this may not occur as in the Ig genes[52]. The presence of two J_β loci and the concomitant possibility of creating junctional diversity might compensate for a low rate of somatic variation. Rearrangement of both C_β genes has been shown in human T-cell lines, leukaemias and clones[48]. The rearrangement of a $C_\beta 2$ gene can involve the deletion of $C_\beta 1$ gene[48].

T-cell receptor rearrangement in human leukaemias

Rearrangement of the β-chain allele is more or less restricted to the T-cell lineage as there are few examples of β rearrangement in B-cells. All types of T-cell leukaemias reported (T-acute lymphocytic, T-CLL, T-PLL, Sezary syndrome and adult T-cell leukaemia) have shown β-chain rearrangements, whether it be in the common childhood leukaemias or adult leukaemias[46]. Possibly because of the lack of case-law, there is no clear pattern of $C_\beta 1$ versus $C_\beta 2$ involvement nor of events on the allelic chromosomes, as all combinations of possible rearrangement genotype exist in the various leukaemia states. Further β-chain rearrangement seems to be an early event in T-cell differentiation, since thymocytes have rearranged C_β genes[56]. T-cell lines with helper or cytotoxic/suppressor phenotypes have also been examined and show C_β rearrangements[57].

CHROMOSOMAL TRANSLOCATIONS INVOLVING IMMUNOGLOBULIN LOCI

Burkitt's lymphoma

It seems that the rearrangements used by the Ig loci are usurped very occasionally in events that finally lead to the formation of lymphoid tumours.

The best characterized example of this is the specific chromosomal translocation seen in the B-cell tumour Burkitt's lymphoma (BL). There is always one of three types of translocation present in the tumour cells of patients with this type of malignancy (Table 5.2). These translocations always involve the c-*myc* proto-oncogene on chromosome 8 (8q24) and one of the three Ig loci on the various autosomes. These and the analogous translocation in mouse myelomas are dealt with in a recent monograph[58]. The commonest translocation observed, which involves the reciprocal exchange of material from the end of one of each pair of chromosomes, is t(8;14) and is found in about 90% of cases. It is particularly prevalent in the endemic Burkitt's lymphoma of the African malarial belt. The most common result of the BL translocation t(8;14) is that the c-*myc* gene is carried with the piece of chromosome 8 (very close to the break point) to become located near to a constant region gene of the IgH locus[59–61]. This is diagramatically shown in Figure 5.8B, which also shows the normal chromosome 8 with the unaltered c-*myc* gene. The situation with the so-called variant BL (i.e. those with translocations involving L-chain loci) is somewhat different. In these cases, the c- *myc* gene itself does not move from the abnormal chromosome 8 (Fig. 8A)[62,63]. Instead parts of the immunoglobulin loci are transported to the chromosome 8, downstream of the c-*myc* gene. The t(2;8) seems to involve a break within the κ-chain locus bringing C_κ (and possibly some V_κ genes) to the downstream region of c- *myc* gene[62,64]. Similarly the t(8;22) involves the relocation of the Cλ gene to the downstream region of c- *myc* [65,66].

Table 5.2 Chromosomal translocations involving Ig loci in Burkitt's lymphoma

Translocation	Approximate % of cases	Breakpoints	Gene involved
(8;14)	90	8q24 14q32	c-*myc* IgH
(2;8)	5	8q24 2p12	c-*myc* Igκ
(8;22)	5	8q24 22q11	c-*myc* Igλ

The common feature of these aberrant chromosomal rearrangements is a breakpoint near the c- *myc* gene and the association of Ig genes, particularly the C-gene, near to the various breakpoints. The commonest breakpoint on chromosome 14, in the t(8;14), is near to the first C_H gene of the locus[59] (i.e. Cμ), and frequently the breakpoint can be found in the tandemly repeated switch sequences (Fig. 5.9) discussed earlier, which are normally involved in class switching. This gives a clue to the mechanism of this aberrant rearrangement, since it strongly suggests that the normal process of Ig gene rearrangement can erroneously (though rarely) use the wrong sequences. There

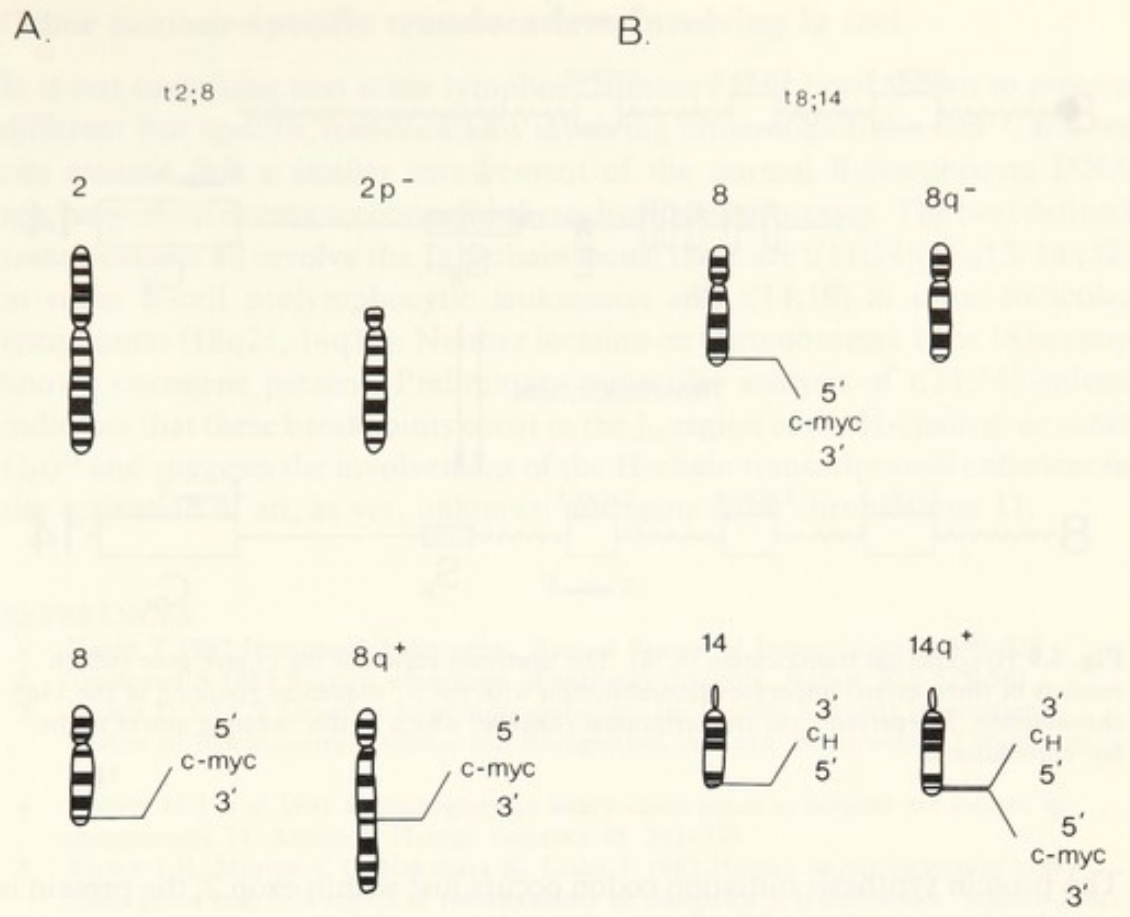

Fig. 5.8 Representation of specific chromosomal translocations in Burkitt's lymphoma cells with t(2;8) or t(8;14). **A.** t(2;8) translocation in which the κ-chain locus is disrupted by a break on 2p (resulting in an abnormal 2p chromosome) and in which most of chromosome 2 short-arm moves behind (3′) of the c- *myc* gene (to give an abnormal chromosome 8q+). **B.** t(8;14) translocation. The abnormal 8q- in this case does not carry the c-*myc* gene. Instead the abnormal 14q+ gains the piece of chromosome 8, including the c-*myc* gene.

is, however, a wide variety of different breakpoints near the c- *myc* gene in BL[67,68] and there does not seem to be any particular sequence predisposition for this translocation. Rather it appears that the translocation is a random event which occasionally results in activation of the c-*myc* gene. This erroneous joining, when it occasionally involves the c-*myc* locus, can lead to the development of malignant lymphoma. It is not so clear how the variant BL lymphoma (i.e. involving the L-chain) arises, but it is assumed that again the normal processes of Ig gene rearrangement are erroneously involved.

Mechanism of c- *myc* gene activation by chromosomal translocation

A previous chapter has dealt with possible activating mechanisms of the c- *myc* gene and I will only discuss this in specific relation to the enforced proximity of the immunoglobulin loci after translocation. The c- *myc* gene has three exons, of which the first is a transcribed but untranslated exon of unknown function[58].

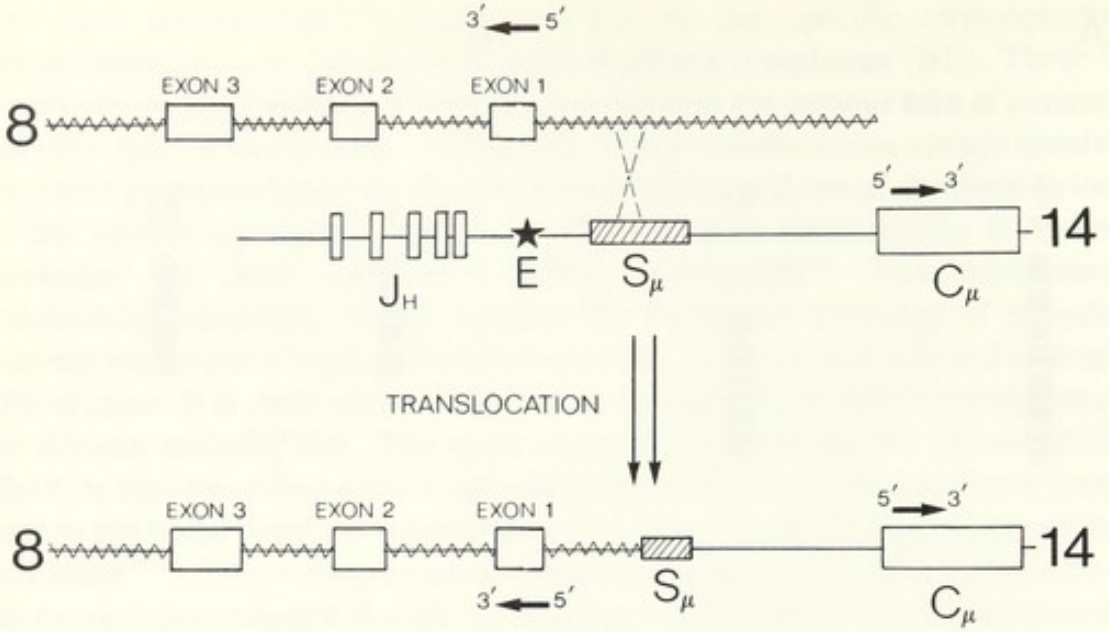

Fig. 5.9 Hypothetical translocation (8;14). The upstream region of the c- *myc* gene (which consists of three exons) undergoes recombination with the S_μ sequences resulting in the 14q+ chromosome. E represents the transcriptional enhancer which in this example moves to the 8q- chromosome.

The protein synthesis initiation codon occurs just within exon 2; the protein is encoded in exons 2 and 3 in the part homologous with the sequence present in the transforming retrovirus v- *myc* (present in the avian myelocytomatosis virus). The normally expressed gene, transcribed in actively growing cells, is a harmless proto-oncogene (or a potential oncogene); and the process of translocating this gene is thought to be the event that activates c- *myc* to become an oncogene. How does the re-positioning of the c- *myc* gene near to an Ig gene (or vice versa in the variant Burkitt's lymphomas) cause the activation? It would seem logical to assume that one of the features that is important for the Ig genes is brought to bear on the c- *myc* gene after or during translocation. It is known that somatic mutation can occur in c- *myc* genes[68–70], although coding region changes cannot be generally important since some translocated genes have an unmutated coding sequence[68,70]. Similarly, although some translocated c-*myc* genes are controlled by the IgC_H transcription enhancer[71], in many cases this enhancer is lost from the translocated c-*myc* gene[21] (as illustrated in Figure 5.9). It is apparent, however, that loss of normal transcriptional control is one consequence of translocation[72]. The observation of Ig enhancer control of translocated c-*myc* genes[71] may therefore be an extreme example of the transcriptional control exerted by the Ig loci. A more general explanation may lie in the existence of hitherto undiscovered elements near to Ig genes (which we might call 'maintainer' elements), which are normally responsible for unrestricted transcription of Ig genes but which exert their effect on the c-*myc* gene after translocation. Such putative elements would also explain why Ig genes are continuously transcribed in B-cells and plasma cells.

Other tumour-specific translocations involving Ig loci

It is not surprising that other lymphoid tumours have been shown to possess different but specific translocations involving immunoglobulin loci[73], and we can assume that a similar involvement of the normal B-lymphocyte DNA rearrangement events accounts for these disastrous processes. The best defined translocations all involve the IgH chain locus; these are t(11;14)(11q13; 14q32) in some B-cell prolymphocytic leukaemias and t(14;18) in some follicular lymphomas (18q21, 14q32). Neither location on chromosomes 11 or 18 has any known oncogene present. Preliminary molecular analysis of t(11;14) indeed indicates that these breakpoints occur in the J_H region of the H-chain gene (near $C\mu$)[74] and suggests the involvement of the H-chain transcriptional enhancer in the activation of an, as yet, unknown oncogene from chromosome 11.

REFERENCES

1. Honjo T 1983 Immunoglobulin genes. Annual Review of Immunology 1: 499–528
2. Tonegawa S 1983 Somatic generation of antibody diversity. Nature 302: 575–581
3. Secher D S 1979 Structure of immunoglobulins. In: Lennox E S (ed) International Review of Biochemistry: Defense and Recognition, 2nd edn. Butterworth, London, pp 1–48
4. Hobart M J et al 1981 Immunoglobulin heavy chain genes in humans are located in chromosome 14. Annals of Human Genetics 45: 331–335
5. Kirsch I R, Morton C C, Nakahara K, Leder P 1982 Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. Science 216: 301–303
6. McBridge O W, Hieter P A, Hollis G F, Swan D, Otey M C, Leder P 1982 Chromosomal location of human kappa and lambda immunoglobulin light chain constant region genes. Journal of Experimental Medicine 155: 1480–1490
7. Malcolm S, Barton P, Murphy C, Ferguson-Smith M A, Bentley D L, Rabbitts T H 1982 Localisation of human immunoglobulin kappa light chain variable region genes to the short arm of chromosome 2 by in situ hybridisation. Proceedings of the National Academy of Sciences USA 79: 4957–4961
8. Hieter P A, Maizel J V, Leder P 1982 Evolution of human immunoglobulin K J region genes. Journal of Biological Chemistry 257: 1516–1522
9. Bentley D L, Rabbitts T H 1980 Human immunoglobulin variable region genes – DNA sequences of two V genes and a pseudogene. Nature 288: 730–733
10. Bentley D L, Rabbitts T H 1981 Human V_κ immunoglobulin gene number: implications for the origin of antibody diversity. Cell 24: 613–633
11. Steinmetz M, Altenburger W, Zachau H G 1980 A rearranged DNA sequence possibly related to the translocation of immunoglobulin gene segments. Nucleic Acids Research 8: 1709–1720
12. Bentley D L, Farrell P J, Rabbitts T H 1982 Unrearranged immunoglobulin variable region genes have a functional promoter. Nucleic Acids Research 10: 1841–1856
13. Sakano H, Hüppi K, Heinrich G, Tonegawa S 1979 Sequences at the somatic recombination sites of immunoglobulin light chain genes. Nature 280: 288–294
14. Max E E, Seidman J G, Leder P 1979 Sequences of five potential recombination sites encoded close to an immunoglobulin K constant region gene. Proceedings of the National Academy of Sciences USA 76: 3450–3454
15. Early P, Huang H, Davis M, Calame K, Hood L 1980 An immunoglobulin heavy chain variable region gene is generated from three segments of DNA. Cell 19: 981–998
16. Anderson M L M, Szajnert M F, Kaplan J C, McColl L, Young B D 1985 Isolation of a human immunoglobulin lambda light chain variable gene from a recombinant library of chromosome 22. Nucleic Acids Research (In press)
17. Hieter P A, Hollis G F, Korsmeyer S J, Waldmann T A, Leder P 1981 Clustered arrangement of immunoglobulin λ constant region genes in man. Nature 294: 536–540

18. Matthyssens G, Rabbitts T H 1980 Structure and multiplicity of human immunoglobulin heavy chain variable region genes. Proceedings of the National Academy of Sciences USA 77: 6561–6565
19. Flanagan J G, Rabbitts T H 1982 Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ϵ and α genes. Nature 300: 709–713
20. Rabbitts T H, Forster A, Milstein C P 1981 Human immunoglobulin heavy chain genes: evolutionary comparisons of Cμ, Cδ and Cγ genes and associated switch sequences. Nucleic Acids Research 9: 4509–4524
21. Rabbitts T H, Forster A, Baer R, Hamlyn P 1983 Transcription enhancer identified near the human Cμ immunoglobulin heavy chain gene is unavailable to the translocated c-*myc* gene in a Burkitt's lymphoma. Nature 306: 806–809
22. Maki R et al 1981 The role of DNA rearrangement and alternative RNA processing in the expression of immunoglobulin delta genes. Cell 24: 353–365
23. Moore K W, Rogers J, Hunkapiller T, Early P, Nottenburg C, Hood L 1981 Expression of IgD may use both DNA rearrangement and RNA splicing mechanisms. Proceedings of the National Academy of Sciences USA 78: 1800–1809
24. Flanagan J G, Rabbitts T H 1982 The sequence of a human immunoglobulin epsilon heavy chain constant region gene, and evidence for three non-allelic genes. EMBO Journal 1: 655–660
25. Max E E, Battey J, Ney R, Kirsch I R, Leder P 1982 Duplication and deletion in the human immunoglobulin ϵ genes. Cell 129: 691–699
26. Ishida N, Ueda S, Hayashida H, Miyata T, Honjo T 1982 Sequence of mouse immunoglobulin epsilon gene: comparison with the human epsilon gene sequence. EMBO Journal 1: 1117–1123
27. Milstein C P, Deverson E V, Rabbitts T H 1984 The sequence of the human immunoglobulin μ-δ intron reveals possible vestigial switch segments. Nucleic Acids Research 12: 6523–6535
28. Krawinkel U, Rabbitts T H 1982 Comparison of the hinge-coding segments in human immunoglobulin gamma heavy chain genes and the linkage of the gamma 2 and gamma 4 subclass genes. EMBO Journal 1: 403–407
29. Takahashi N, Ueda S, Obata M, Nikaido T, Nakai S, Honjo T 1982 Structure of human immunoglobulin gamma genes: implications for evolution of a gene family. Cell 29: 671–679
30. Nishida Y, Miki T, Hisajima H, Honjo T 1982 Cloning of human immunoglobulin ϵ chain genes: evidence for multiple Cϵ genes. Proceedings of the National Academy of Sciences USA 79: 3833–3837
31. Honjo T, Kataoka T 1978 Organisation of immunoglobulin heavy chain genes and allelic deletion model. Proceedings of the National Academy of Sciences USA 75: 2140–2144
32. Rabbitts T H, Forster A, Dunnick W, Bentley D L 1980 The role of gene deletion in the immunoglobulin heavy chain switch. Nature 283: 351–356
33. Coleclough C, Cooper D, Perry R P 1980 Rearrangement of immunoglobulin heavy chain genes during B-lymphocyte development as revealed by studies of mouse plasmacytoma cells. Proceedings of the National Academy of Sciences USA 77: 1422–1426
34. Cory S, Adams J M 1980 Deletions are associated with somatic rearrangement of immunoglobulin heavy chain genes. Cell 19: 37–51
35. Dunnick W, Rabbitts T H, Milstein C 1980 An immunoglobulin deletion mutant with implications for the heavy chain switch and RNA splicing. Nature 286: 669–675
36. Kataoka T, Miyata T, Honjo T 1981 Repetitive sequences in class switch recombination regions of immunoglobulin heavy chain genes. Cell 23: 357–368
37. Rabbitts T H, Hamlyn P H, Matthyssens G, Roe B A 1980 The variability, arrangement and rearrangement of immunoglobulin genes. Canadian Journal of Biochemistry 58: 176–187
38. Shimizu A, Takahashi N, Yaoita Y, Honjo T 1982 Organisation of the constant region gene family of the mouse immunoglobulin heavy chain. Cell 28: 499–506
39. van Loghem E, de Lange G, Koistinen K 1976 The first isoallotype of human IgA proteins. An antigenic determinant occurring as allotype in the IgA2 subclass and as isotype in the IgA1 subclass. Scandinavian Journal of Immunology 5: 161–164

40. Flanagan J, Lefranc M P, Rabbitts T H 1984 Mechanisms of divergence of the human immunoglobulin $\alpha 1$ and $\alpha 2$ constant region gene sequences. Cell 36: 681–688
41. Hieter P A, Korsmeyer S J, Waldmann T A, Leder P 1981 Human immunoglobulin κ light chain genes are deleted or rearranged in λ-producing cells. Nature 290: 368–370
42. Bentley D L, Rabbitts T H 1983 Evolution of immunoglobulin V genes. Evidence indicating that recently duplicated human Vκ sequences have diverged by gene conversion. Cell 32: 181–189
43. Foroni L, Catovsky D, Rabbitts T H, Luzzatto L 1984 DNA rearrangement of immunoglobulin genes with phenotypic markers in B cell malignancies. Molecular Biology and Medicine, 2 63–79
44. Korsmeyer S J, Hieter P A, Ravetch J V, Poplack D G, Waldmann T A, Leder P 1981 Developmental hierarchy of immunoglobulin gene rearrangement in human leukemia pre-B cells. Proceedings of the National Academy of Sciences USA 78: 7096–7100
45. Bakhshi et al 1983 Lymphoid blast crises of chronic myelogenous leukemia represent stages in the development of B cell precursors. New England Journal of Medicine 309: 826–831
46. Rabbitts T H et al 1985 Heterogeneity of T-cell receptor β-chain rearrangements in human leukemia and lymphomas. In preparation
47. Yanagi Y, Yoshihai Y, Leggett K, Clark S P, Aleksander I, Mak T W 1984 A human T cell specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature 308: 145–149
48. Sims J E, Tunnacliffe A, Smith W S, Rabbitts T H 1984 The complexity of human T cell receptor β chain variable and constant region genes 312: 541–545
49. Hedrick S M, Nielsen E A, Kavaler J, Cohen D I, Davis M M 1984 Sequence relationships between putative T cell receptor polypeptides and immunoglobulins. Nature 308: 153–158
50. Saito H, Kranz D M, Takagaki Y, Hayday A C, Eisen H N, Tonegawa S 1984 Complete primary structure of a hetero dimeric T-cell receptor deduced from cDNA sequences. Nature 309: 757–762
51. Caccia N et al 1984 The T cell receptor β chain genes are located on chromosome 6 in mice and chromosome 7 in humans. Cell 37: 1091–1099
52. Siu G et al 1984 The human T-cell antigen receptor is encoded by variable, diversity and joining gene segments that rearrange to generate a complete V-gene. Cell 37: 381–391
53. Tunnacliffe A, Kefford R, Milstein C, Forster A, Rabbitts TH Proceedings of the National Academy of Sciences USA (in press)
54. Clark S P, Yoshikai Y, Siu G, Taylor S, Hood L, Mak T W 1984 Identification of a diversity segment of the human T cell receptor β chain and comparison to the analogous murine element. Nature 311: 387–384
55. Gascoigne N R J, Chien Y-H, Becker D M, Kavaler J, Davis M M 1984 Genomic organisation and sequence of T cell receptor β-chain constant and joining region genes. Nature, 310: 387–391
56. Yoshikai Y, Yanagi Y, Suciu-Foca N, Mak T W 1984 Presence of T cell receptor mRNA in functionally distinct T cells and elevation during intrathymic differentiation. Nature 310: 506–508
57. Toyonaga B, Yanagi Y, Suciu-Foca N, Minden M, Mak T W 1982 Rearrangements of T cell receptor gene YT35 in human DNA from thymic leukaemia T cell lines and functional T cell clones. Nature 311: 385–387
58. Potter M, Melchers F, Weigert M (eds) 1984 Current Topics in Microbiology and Immunology Vol 113, Springer-Verlag
59. Taub R et al 1982 Translocation of the c- *myc* gene into the immunoglobulin heavy chain locus in human Burkitt's lymphoma and murine plasmacytoma cells. Proceedings of the National Academy of Sciences USA 79: 7837–7841
60. Hamlyn P H, Rabbitts T H 1983 Translocation joins c- *myc* and immunoglobulin γ_1 genes in a Burkitt lymphoma revealing a third exon in the c- *myc* oncogene. Nature 304: 135–139
61. Dalla-Favera R, Martinotti S, Gallo R C, Erikson J, Croce C M 1983 Translocation and rearrangements of the c- *myc* oncogene locus in human undifferentiated B-cell lymphomas. Science 219: 963–967

62. Erikson et al 1983 Translocation of an immunoglobulin K locus to a region 3′ of an unrearranged c- *myc* oncogene enhances c- *myc* transcription. Proceedings of the National Academy of Sciences USA 80: 7581–7585
63. Davis M, Malcolm S, Rabbitts T H 1984 Chromosome translocation can occur on either side of the c- *myc* oncogene in Burkitt lymphoma cells. Nature 308: 286–288
64. Rabbitts T H, Baer R, Davis M, Forster A, Hamlyn P H, Malcolm S 1984 The c- *myc* gene paradox in Burkitt's lymphoma chromosomal translocation. Current Topics in Microbiology and Immunology 113: 166–171
65. Hollis G F et al 1984 A variant translocation places the λ immunoglobulin genes 3′ to the c- *myc* oncogene in Burkitt's lymphoma. Nature 307: 752–755
66. Croce C M et al 1983 Transcriptional activation of an unrearranged and untranslocated c- *myc* oncogene by translocation of a Cλ locus in Burkitt lymphoma cells. Proceedings of the National Academy of Sciences USA 80: 6922–6926
67. Bernard O, Cory S, Gerondakis S, Webb E, Adams J M 1983 Sequence of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B lymphoid tumours. EMBO Journal 2: 2375–2383
68. Rabbitts T H, Forster A, Hamlyn P, Baer R 1984 Effect of somatic mutation within translocated c- *myc* genes in Burkitt's lymphoma. Nature 309: 592–597
69. Rabbitts T H, Hamlyn P H, Baer R 1983 Altered nucleotide sequence of a translocated c- *myc* gene in Burkitt lymphoma. Nature 306: 760–765
70. Taub R et al 1984 Activation and somatic mutation of the translocated c- *myc* gene in Burkitt lymphoma cells. Cell 36: 339–348
71. Saito H, Hayday A C, Wiman K, Hayward W S, Tonegawa S 1983 Activation of the c- *myc* gene by translocation: A model for translational control. Proceedings of the National Academy of Sciences USA 80: 7476–7480
72. Rabbitts P H et al 1985 Cell cycle expression of c– *myc* mRNA and protein. EMBO. In press.
73. Yunis J J 1983 The chromosomal basis of human neoplasia. Science 221: 227–235
74. Erikson J, Finan J, Tsujimoto Y, Nowell P C, Croce C M 1984 The chromosome 14 breakpoint in neoplastic B cells with t(11;14) translocation involves the immunoglobulin heavy chain locus. Proceedings of the National Academy of Sciences USA 81: 4144–4148

Chromosome rearrangement and oncogenes in mouse plasmacytomas

The idea that chromosome translocations, regularly associated with a given type of tumour, may act by bringing an oncogene under the influence of a highly active chromosome region first arose from cytogenetic studies on mouse plasmacytomas (MPC)[1,2,3]. This hypothesis was fully confirmed by subsequent molecular studies, showing that the MPC-associated specific chromosomal translocations bring the c-*myc* oncogene under the influence of the IgH-locus or, more rarely, the kappa-gene (for reviews see references 4–6). Closely homologous translocations were found in human Burkitt lymphomas (BL).

The purpose of this chapter is to review the cytogenetic and molecular features of the MPC system. We shall also discuss the comparative aspects of the MPC- and BL-associated translocations and the likely position of the *myc*/Ig-juxtaposition in the tumour development scenario. We shall also briefly deal with the chromosomal and molecular features of plasmacytomas in some other species.

BIOLOGY

The induction, genetics, immunological and virological aspects of MPC have been reviewed recently[7]. We shall therefore only deal with points that may be directly relevant for the interpretation of the chromosomal translocations and their place within the tumour scenario.

The experimental study of murine plasmacytomas was pioneered by Michael Potter and his associates. These tumours are induced by intraperitoneal injection of mineral oil (pristane) into inbred BALB/c or NZB mice. Susceptibility is essentially restricted to these strains. Some other, chemically inert and non-metabolizable materials that remain in the peritoneal space like plexiglass discs or diffusion chambers are also active. They all induce the formation of chronic granuloma tissue.

BALB/c mice develop plasmacytomas after a latency period of 6–12 months after the intraperitoneal injection of mineral oil. However, plasmacytoma cells can be detected in the ascitic fluid as early as 20 days after the first injection, and most tumours begin to appear after 180 days. The latency period could not be decreased by changing the dose of the pristane[8], but the tumourigenic process

was greatly accelerated by infecting the pristane injected mice with Abelson virus[9].

The induction of the pathological peritoneal granuloma tissue was shown to play an important role both for the development[10] and maintenance[11] of the primary plasmacytomas. Development of the plasmacytomas in the early granuloma tissues could be detected directly on histological examination[10].

Studies on the transplantability of primary plasmacytomas (PCs) showed that they are conditioned, rather than autonomous, tumours in the terminology of Furth[12]. Primary MPCs usually do not grow after transplantation into the normal peritoneal cavity of syngeneic mice[13]. Conditioning of the recipients by intraperitoneal injection with pristane on the day of transplantation, or before, makes them susceptible to the transplant, as a rule. This effect is related to the influx of macrophages into the peritoneum[11]. There is suggestive evidence that the chronic inflammatory tissues generated by the oil granuloma may release a B-cell growth factor[14]. On repeated passage in conditioned mice, the PCs may lose their dependence on conditioning factors and become transplantable to normal syngeneic mice.

Bacterial factors, probably acting via antigenic stimulation, may play a contributory role in the genesis of the plasmacytomas. Germ-free BALB/c mice injected with mineral oil are resistant to plasma cell tumours, or develop them only after very long latency periods[15]. The predominance of IgA as the main immunoglobulin product of the MPCs suggests that the tumours originate from B-lymphocytes that normally participate in gut secretion. Germinal centre lymphocytes in the Peyer's patches are mostly IgA producers. They are believed to have been stimulated by antigens in the gut. In contrast, most NZB plasmacytomas are IgG producers. It is noteworthy that the autoimmunity of the NZB mice is associated with hyperactivity of the IgG producing cells.

CYTOGENETICS

Mineral oil induced plasmacytomas are usually aneuploid, with the majority in the near-tetraploid and a minority in the near diploid range[16]. Shepard *et al.* first showed that a transplanted plasmacytoma line contained a reciprocal translocation between chromosomes 6 and 15, whereas three others contained identical reciprocal translocations between chromosome 12 and 15[17–19]. The breakpoint in chromosome 15 was at the same site in both kinds of translocations.

Ohno *et al.*[1] found one of the two characteristic translocations, either 6;15 or 12;15, in seven pristane induced primary plasmacytomas. The breakpoints on chromosome 15 were the same as observed earlier, located between the D3 and E bands. In subsequent karyotypic studies on early transfer generation mineral oil induced plasmacytomas in BALB/c mice or their congenic substrains, 85 per cent were found to have the 12;15 translocation, which was therefore

designated as 'typical'; whereas 11 per cent had a 6;15 translocation, referred to as 'variant'[1,2,7].

The translocations always affected only one chromosome 15 in diploid cells and two of the four in tetraploid cells. The typical 12;15 and the variant 6;15 translocations were not found in the same cell. The plasmacytomas induced by the combination of pristane oil and Abelson virus, which arose after drastically reduced latency periods, contained the same translocations as the tumours induced by the pristane oil alone, but they had a slightly higher frequency of the 6;15 variant translocation – 10 of 18 tumours had 12;15, whereas 5 had 6;15[20]. Three had no translocations, but two of the three had an interstitial deletion of chromosome 15 instead[21]. The deletion was localized to the interface of bands D2/3 and within band D2. One of the two chromosomes 15 had a deletion in near-diploid, and two of four in near tetraploid cells.

The translocation breakpoint of chromosomes 15, 12 and 6 could be determined more accurately by high resolution banding[22]. This technique has also confirmed the reciprocal nature of the 12;15 translocation, not readily identifiable by conventional banding. The breakpoint on chromosome 15 was located between bands D2 and D3 (D2/3) and on chromosome 12 it was within band F1. In the typical translocation, the F2 band of chromosome 12 was translocated to D2/3 on chromosome 15. The high resolution banding (HRB) technique also unequivocally confirmed that the breakpoint of chromosome 15 in the variant 6;15 translocation was identical with the breakpoint in the typical 12;15 translocations. The breakpoint on chromosome 6 was located at band C2.

Chromosome 6 carries the kappa chain, whereas the distal region of chromosome 12 contains the IgH complex. Its localization corresponds to the breakpoint in the typical translocations. This, together with the previously known fact that the distal part of chromosome 15 carries a gene that plays an important role in mouse leukemia development[23], prompted our hypothesis that the translocations act by bringing an oncogene located in the distal part of chromosome 15 to the region of an active immunoglobulin locus[1,3].

The high susceptibility of the BALB/c strain to MPC induction raised the question whether this might be due to some peculiarity of the oncogene carrying segment of the BALB/c derived chromosome 15. This was examined in plasmacytomas induced in (BALB/c × AKR) × BALB/c backcross mice, carrying cytogenetically distinguishable chromosomes no 15[22]. There was no significant preference for the BALB/c derived chromosome as the translocation donor. This indicates that the susceptible gene(s) are not within the translocated region of chromosome 15.

The HRB-study[22] has also included three MPCs of special interest. They were induced in (BALB/c × AKR 6;15) × BALB/c backcross mice that segregated a Robertsonian translocation chromosome, derived from the fusion of chromosomes 6 and 15 at the centromere. While seven other tumours induced in this cross had the usual 12;15 translocations, the three exceptional tumours had variant translocations of the reciprocal (6;15) type. Due to the fact that 6 and 15 were fused at the centromere, this translocation was generated by

two breaks in the single Robertsonian 6;15 chromosome, followed by the pericentric inversion of the liberated fragment. Karyotypic analysis confirmed the independent origin of the three tumours. Thus, the extraordinary event of pericentric inversion occurred during the genesis of 3 different tumours. High resolution banding showed that the same bands were involved as in the usual reciprocal 6;15 translocation. The repeated occurrence of the precisely analogous pericentric inversion adds further weight to the argument that the translocations represent an essential step in the generation of the plasmacytomas.

MOLECULAR BIOLOGY

Typical translocation (12;15)

The study of the molecular biology of MPC started from different points in different laboratories, but converged rapidly towards closely similar pictures. Kirsch *et al.*[24] found that five of six MPCs contained a rearranged DNA fragment originating from somewhere outside the IgH cluster, which was joined directly to the α-switch region. Susanne Cory, Jerry Adams and their associates in Melbourne, Australia[25] and Kenneth Marcu's group at Stony Brook, USA[26] demonstrated a similar association in a variety of MPCs. Both groups suspected that the foreign sequences may have been moved to the IgH region by the chromosomal translocations. The Australian group called them LyR (lymphoma rearranging) sequences, whereas the American group coined the term NIARD (non-immunoglobulin associated rearranging DNA). They also showed that in some plasmacytomas the sequences were directly linked to the α-region. The Australians reported that rearranged and germ line LyR sequences were simultaneously present in 25 of 28 plasmacytomas. They related this to the known presence of an equal number of translocated and normal chromosomes 12 and 15 in the tumours[1]. Resting or activated B cells and pre-B cell leukaemias induced by the Abelson virus contained only germline sequences. Rearranged LyR sequences were also present in three of five B-cell leukaemias and in three of 18 T-cell leukaemias. The LyR rearrangement was often but not always accompanied by elevated transcript levels, compared with cells that contained only germline sequences.

Using two independently generated series of somatic hybrids, both groups have subsequently shown that the exogenous sequences originate from chromosome 15[27,28]. A similar conclusion was reached by Hood's group[29]. They showed that the 5′ side of the allelically excluded Cα gene was joined to chromosome-15-derived sequences (designated NIRD) in two IgA-producing MPCs. NIRD was rearranged in the majority of all plasmacytomas tested (including IgG, IgM and IgA producers) but rearrangement to the Sα switch region was only found in IgA producers. However, in the larger material of Adams *et al.*[25], some of the IgG and IgM producers also showed a rearrangement to the Cα region, but not as frequently as the IgA producers. Calame *et al.*[29] suggested that the DNA rearrangement associated with the 12;15 translocation occurs at the time when B cells undergo C_H switching.

Only a few months after this initial development, all the involved groups reached consensus in identifying 'NIARD', 'NIRD' and 'LYR' with the murine c-*myc* gene. This has verified the postulated involvement of an oncogene and its juxtaposition to the IgH locus. The molecular studies have also confirmed the postulated homology between the translocations associated with MPC and human Burkitt's lymphoma (for reviews see refs. 30 and 31).

Shen-Ong *et al.*[32] were pioneers in identifying the rearranging sequences as c-*myc*. They found that *myc* was rearranged in six of seven MPCs. The recombination event interrupted the 5′ end of the gene. In five of them, *myc* was juxtaposed to the α heavy-chain locus in a 5′ to 5′ configuration, meaning that the two genes were transcribed in the opposite direction from two different strands. The distance between the two genes varied between 2 and 4 kb. In the sixth tumour, *myc* has recombined with an unknown DNA sequence. The DNA recombination event was accompanied by the production of a novel 2.1 kb species of *myc* mRNA that was 0.4 kb shorter than the normal cellular transcript. Adams *et al.*[33] reported closely similar results, but they also showed that the plasmacytoma translocation breakpoints were in the neighbourhood of, or within, the 5′ terminal c-*myc* exon – generating c-*myc* mRNA with different 5′ termini, originating from the DNA strand opposite to the strand used for the α heavy-chain mRNA. Clearly, the translocation separated the c-*myc* gene from its normal promoter and 5′ regulatory machinery, and placed it under some regulatory element associated with the immunoglobulin gene.

The identity of NIARD with c-*myc* was also shown by Marcu *et al*[34]. In contrast to Shen-Ong *et al*[32] and Adams *et al*[33], they emphasized that the *myc* transcript levels in the plasmacytomas were 10 to 20 fold higher than in normal BALB/c spleen. In their view, the location of the DNA rearrangement sites, as reflected by the transcripts, 'collectively argues' that the translocation has led to the activation (or more efficient utilization) of a new promoter site.

Subsequent work in the same and other laboratories has confirmed using a larger volume of material that the relative orientation of c-*myc* gene and the IgH locus was always head-to-head and that the translocation has affected the non-functionally rearranged, allelically excluded, immunoglobulin gene. Switch recombination (S) regions and particularly Sα remained the most frequent recipients of the transposed *myc* gene. The adjacent Cα locus was sometimes in a germline configuration but more frequently it had been rearranged by a switch recombination[35]. However, in NZB-derived plasmacytomas the switch region was not normally Sα[36]. Within a particular switch region, the exact recombination breakpoint could vary by more than 2 kb. Moreover, some *myc*-translocations were found to hit the IgH area outside the switch regions, at least in the human BL system[37].

Analysis of the breakpoint of the c-*myc* gene in different plasmacytomas showed that nearly all mapped within an 1.1 kb region spanning the non-coding first exon of c-*myc*[35].

The preferential involvement of the switch regions suggests that switch recombination enzymes may be involved in generating the translocations.

However, the existence of variant translocations in both the MPC and the BL-system that lead to the juxtaposition of c-*myc* and one of the light chain loci, implies that functionally analogous translocations may arise independently of the switch recombination machinery. No switch region homology could be detected within the breakpoint region of murine c-*myc*, thus ruling out homologous recombination as the translocation mechanism[35]. Similar findings were reported for the human BL-system[31].

One of the first hypotheses to explain the role of c-*myc* in BL-cell neoplasia suggested an altered c-*myc* product. However, the coding exons of c-*myc* were regularly intact in all translocations. In several cases, where the sequence of the polypeptide has been determined, it was identical to the germline sequence[6,36]. It is more likely that the essential change is concerned with the regulation of transcription, as discussed later.

The reciprocal nature of the chromosome 15 translocations has been confirmed by cloning chromosome junctions from four plasmacytomas[38]. The reciprocal structures can be designated as C_HS_H-*myc* and 5′*myc*-S_H structures. The H locus targets included Sα fused to Sμ, Sμ fused to Sγ_2b, and the germline Sγ region. The fusion regions contained deletions, extraneous nucleotides and one duplication. A translocation model was formulated, in which staggered single-stranded breaks on each chromosome were followed by single-strand excision or polymerization prior to ligation to the other chromosomes.

In another analogous study[39], the reciprocal rearrangement between the *myc*-gene on chromosome 15 and the immunoglobulin γ2a switch region on chromosome 12 was found to remove 11 base pairs of the c-*myc* sequence and 300 base pairs of the Sγ2a sequence recombination. No structural features were found that could explain the *myc*/Ig rearrangements.

Nucleotide sequencing confirmed the complete identity of the coding sequence between the normal murine and the plasmacytoma associated, translocated *myc*-gene[40]. The conclusion that there was no need for mutations in the translocated *myc*-gene was confirmed by S1 nuclease analysis performed on the c-*myc* RNAs[36].

The variant (6;15) translocation

Only a single paper deals with the molecular basis of the variant translocation[41]. The analyses have been hampered by the absence of any detectable *myc*-rearrangement on Southern blots and by the fact that the kappa locus has not been localized within chromosome 6. To circumvent this difficulty, Cory *et al*[41] clone both C-kappa genes from the clones which contained chromosome 15 sequences and therefore had to represent the 15:6 junction. Recombination had occurred with the $J_\kappa C_\kappa$ intron. The location of the breakpoints did not support the possibility that the $V_\kappa J_\kappa$ joining enzymes participated in the translocation event.

A 67 kb segment of chromosome 15 spanning the ABPC4 breakpoint did not overlap with the *myc*- locus. Therefore, the ABPC4 breakpoint must be located

at a distance of at least 55 kb from the *myc*-promoters. The survey of other 6:15 plasmacytomas indicated that several of them, although not all, had the same breakpoint as ABPC4. The affected site was termed as the *pvt* (plasmacytoma variant translocation) locus. The possibility was considered that it may represent another oncogene than *myc*. However, the *pvt* sequences did not hybridize to any known oncogene, including c-sis that maps to chromosome 15. Since *myc* is highly expressed in variant translocations, Cory *et al* suggested that the translocation to the *pvt* locus represents a novel way of activating *myc* from a distance, probably by some long range enhancer associated with the Ig light chain locus.

DELETION PLASMACYTOMAS

One of the exceptional MPCs, ABPC45, which carries no translocation but has an interstitial deletion near the usual translocation breakpoint 15D2/3[21], was recently analysed at the molecular level[42]. Cloning and sequencing of the rearranged c-*myc* carrying fragment revealed, surprisingly, that the 5′-end of *myc* was facing a sequence head-to-head, as in the usual translocations. There was no detectable morphological change on chromosome 12 by high resolution banding. Further sequencing beyond the 3′ end of Sα did not lead into a Cα site, as in the usual 12;15 translocations, but into a region that was also derived from chromosome 12 and contained J_H, the Ig-enhancer, and Cμ, oriented opposite to the Sα sequence, tail-to-tail.

The most likely explanation of this extraordinary finding is a double translocation, in conjunction with a specific sequence inversion. Conceivably, the usual 12;15 translocation may have occurred as the first step. In a second step, a similar exchange may have taken place between the two partners that have entered the first translocation, leading to a somewhat more imprecise rearrangement, including the inversion of a chromosome 12-derived segment. An alternative explanation could be based on transposable genetic elements. Whatever the final explanation turns out to be, the existing evidence strongly emphasizes the special ability of the Ig-genes to translocate to and/or to activate the c-*myc* locus constitutively. The findings on the deletion PCs also reaffirm that the activation of c-*myc* may be a common feature of all mouse plasmacytomas – including the apparent exceptions, where the translocation does not appear to be detectable at first sight. This makes the analogy between MPC and the human BL system (where translocations occur in 100 per cent of the cases that have been examined so far) even closer.

RETROVIRAL INSERTION IN PLASMACYTOID LYMPHOSARCOMA

A different type of oncogene activation has been detected in plasmacytoid lymphosarcomas, induced by the Abelson virus (ABPL)[43]. The typical plasmacytomas that arise after combined administration of Abelson virus and

pristane oil carry the usual translocations[20]. Abelson virus alone induces pre-B-lymphomas that are purely diploid[44]. The ABPL group, a morphologically distinct subset of lymphosarcomas that arise after the combined administration of pristane oil and Abelson-virus, is characterized by plasmacytoid cytoplasm with very little immunoglobulin production. They do not contain the typical plasmacytoma-associated translocations[43]. In contrast to the translocation carrying ABPCs and the Abelson-virus induced pre-B lymphomas that had high levels of v-*abl* transcripts, the ABPLs had no detectable v-*abl* transcription. This group was also distinguished by the abundance of an unusually large c-*myb* RNA[43]. In contrast to the other 2 groups, c-*myb* was also rearranged at the DNA level in the ABPL group. In contrast to the ABPCs, c-*myc* was only found in the germline configuration. Subsequent molecular analysis has shown that the rearrangement of the c-*myb* gene was due to the insertion of a deleted, LTR carrying Moloney murine leukaemia virus upstream from the 5′-most c-*myb* exon[45,46]. There was no evidence that the v-*abl* oncogene participated in the induction of these tumours, although it could not be excluded that the Abelson virus may trigger off the chain of events leading to the neoplastic development by some kind of hit and run mechanism.

Both *myc* and *myb* code for DNA-binding nuclear proteins. Transfection experiments suggest that both can immortalize normal diploid cells[47]. It is intriguing that the activation of c-*myb* by retroviral insertion is involved in generating plasmacytoid lymphosarcomas, whereas the v-*abl* oncogene transforms pre-B lymphocytes, probably by direct action, without requiring any chromosomal change. In the mature plasma cell of the BALB/c mouse, the combined action of Abelson virus and mineral oil generates plasmacytomas at a precipitously increased rate and with shortened latency periods. Apparently, the constitutive activation of c-*myc* by its juxtaposition to one of the three Ig-loci and the highly transcribed v-*abl* oncogene achieve this in cooperation. These findings (schematically summarised in Table 6.1) illustrate two important points. First, multiple oncogenes may interact in generating a given tumour, and some of them may substitute for each other. Second, different oncogenic events – such as, infection with a transducing virus; activation of a resident oncogene by retroviral insertion in its neighbourhood; and constitutive activation of a cellular oncogene by chromosomal translocation – occur with different probabilities in different cell types. At least some of these differences may be determined at the level of target cell differentiation. It is conceivable, for instance, that the likelihood of the *myc*/Ig translocation is greatest at the time of the normal DNA rearrangement during B-lymphocyte differentiation. Retroviral insertion near c-*myb* and/or the oncogenic consequences of this event may be most prevalent around the time of lymphocyte–plasmacyte transition. The exquisite and nearly unique sensitivity of the pre-B-cell to the direct oncogenic effect of *abl* must reflect a distinctive interaction between this oncogene and some specific differentiation-related feature of the target cell. It is well known that the transforming potency of certain oncogenes may vary greatly depending on the lineage and stage of target cell differentiation[48]. Also,

Table 6.1 Schematic summary of Abelson virus associated oncogenic events in the murine B-cell series (after Mushinski et al.[43,45,46])

Tumour	Cell type	Mineral oil	Oncogene activity[a]			Mechanism of oncogene activation
			v-*abl*	c-*myb*	c-*myc*	
ABLS	Pre-B	-	++	low	low	Transducing virus
ABPL	Malignant B-lymphocyte	+	-	+++ (re-arr.)[b]	low	Retroviral (M-MuLV) insertion
ABPC	Plasma cell	+	++	low	+++ (re-arr)	Chromosomal translocation (*myc*), in combination with transducing virus

[a] Transcript levels
[b] *Rearr:* rearrangement at the DNA level

differentiation inducing signals may overcome the transforming signals of activated oncogenes in certain situations.[49]

COMPARATIVE ASPECTS OF THE *myc*/Ig TRANSLOCATIONS IN THE MURINE (MPC) AND THE HUMAN (BL) SYSTEM

The regular occurrence of *myc*/Ig juxtaposition in the development of both MPC and BL can only be interpreted to mean that the translocations represent an essential, rate-limiting step in the development of these tumours rather than some secondary epiphenomenon.

The close homology between the human and the murine system is most remarkable. There are only some minor differences in detail. In MPC, Sα is the most frequently involved translocation-acceptor site within the IgH locus, whereas in BL it is Sμ. Since most MPCs make IgA, while most BLs make IgM, there appears to be a functional correlation at first sight; however, this impression is deceptive because the translocation affects the abortively rearranged, allelically excluded, IgH locus and not the chromosome responsible for the production of the functional heavy chain. Also, there are numerous exceptions to the correspondence between the switch region effected by the translocation and the heavy chain product of the cell. The actual relationship can be best explained by assuming that events related to the heavy chain switch recombination mechanism tend to affect both homologous chromosomes. MPCs originate from the B-cells of the peritoneal cavity. A large majority of the B lymphocytes in this region are IgA producers. They are believed to proceed to this ultimate station of the heavy chain deletion process, after receiving the appropriate T-cell derived switch signals. Moreover, the

likelihood of the '*myc* translocation accident' is probably greatest at the time of the normal DNA rearrangement events. Therefore, the fact that most IgA producing MPCs have a *myc* to Sα transposition suggests that the Sμ/Sα recombination switch cannot distinguish between the functionally and the nonfunctionally rearranged chromosome, but tends to affect both.

BLs originate from less mature B-cells that have not yet started to delete their heavy chains, as a rule. This may be the reason why Cμ is the most frequent translocation acceptor site. Switch recombination enzymes may be involved in favouring the *myc*/Ig juxtaposition, prior to the deletion of Cμ. One would then expect that a more distant C_H switch site can be involved as well, as least occasionally. This is actually the case and the most thoroughly explored example is the BL line Raji, where the *myc*- rearrangement affects the Cγ2a region [50].

In both BL and MPC, the typical translocations that involve the heavy chain locus occur much more frequently than the variants that affect one of the light chain loci. The ratio is approximately 8:2. It is possible that the switch recombination enzymes or some other structural or functional features of the IgH locus may account for the increased probability of *myc*-juxtaposition, compared to the light chain loci. Alternatively, the timing of the heavy versus the light chain rearrangement during B lymphocyte differentiation may be crucial, in that the probability of neoplastic conversion would be greater if the *myc*/Ig juxtaposition occurred at the time of the IgH rearrangement rather than at the somewhat later time point of light chain rearrangement.

The comparative aspects of the translocation breakpoint in the region of the c-*myc* gene are also interesting. It has been the universal rule in all cases of MPC and BL so far studied that the two coding exons, nos. 2 and 3, are not affected by the break. This suggests that a functionally intact *myc*- protein is essential for the tumourigenic event. All other features of the *myc*- chromosome breakage are highly variable. It can occur outside or inside the gene. When it occurs inside, it may affect the first non-coding exon or the intron immediately downstream of it. This is the most frequent type of break in the MPCs, where most breakpoints are distributed within a 2 kb region[35]. In the BLs, only about 40 per cent of the breakpoints are within the *myc* gene itself. They are in and around the same exon 1–intron 1 region as in MPC, but with a wider spread. The majority of the typical 8;14 translocation carrying BLs have a breakpoint upstream of *myc* either between the 5′ end of the gene and the nearest EcoRI restriction site or outside the restriction site, more than 17 kb away. In the latter case, the translocation is not accompanied by a *myc*- rearrangement on the Southern blot. This can also occur in the MPCs but it is more of a rarity.

These slight but interesting differences suggest that the likelihood of the break is somewhat different for the BL, compared to the MPC precursor cell. Alternatively, it is conceivable that the IgH region of the relatively less mature BL cell can activate the *myc* gene over greater distances than the IgH complex of the more mature plasma cell. It has to be noted that the IgH enhancer, which is normally located between C_H and J_H[51], is only retained between the

juxtaposed IgH and *myc* sequences in a few exceptional tumours[52]. More usually, the enhancer region moves with the reciprocal translocation fragment. If that fragment contains the decapitated non-coding exon 1 of *myc*, transcription is turned on at a high level – but only in B-cells[53]. While this illustrates the power of the B-cell specific IgH enhancer, it is clear that the coding exons of the oncogene are activated by some other mechanism.

The less common variant translocations provide an interesting contrast. There are two types of variant translocations in the BL system: 8;2 that involves the kappa locus and 8;22 that relates to the lambda locus. The mouse system has only one variant, 6;15, that affects the kappa locus on chromosome 6. So far, no plasmacytoma associated translocations have been found that would involve the lambda carrying mouse chromosome 16. This may or may not be related to the lower diversity of the murine, compared to the human, lambda locus and its much more infrequent involvement in functional immunoglobulin production. If this difference reflects a lower probability of murine lambda rearrangement, and if the rearrangement provides a major risk for the illegitimate *myc* translocation event, it may account for the species difference with regard to the occurrence of the *myc*/lambda translocation.

Table 6.2 Comparative features of a BL and MPC-associated translocation

BL	*MPC*
Typical (IgH) much more frequent than variant (light chain) translocation	The same
Sμ preferred acceptor site	Sα preferred
myc-breakpoint highly variable, 60% or more are outside the gene	*myc*-breakpoints in typical translocation within a narrow area (exon *1* or first intron) with occasional exceptions.
Two types of variant translocations, affecting the kappa or the lambda region	Only one type of variant translocation, affecting the kappa region
Pseudofunctional correlation between the light chain chromosome affected by the translocation and the light chain product	The same
No *myc*-rearrangement in variant translocations	The same
Variant translocations break downstream of the *myc* gene	Unknown
Additional factors: EBV, immunosuppression, second oncogene?	Granuloma dependence. Secondary changes: IAP activation of c-*mos*, 10;6 (kappa) translocation, cooperative action of v-*abl* and translocation in ABPC.

IAP: – Intrausternal A particle
ABPC: – Abelson virus-related plasmacytomas

The variant translocations show a peculiar 'pseudofunctional correlation'. All variant 6;15 producing mouse plasmacytomas so far studied make a kappa light chain[2]. All 8;2 variant BLs that make any light chains produce kappa, whereas all light chain producer 8;22s except one, make lambda[54,55].

In the relatively few cases that have been studied at the molecular level, the variant translocations were found to involve the non-functionally rearranged light chain locus. The correlation between the affected chromosome and the light chain product must therefore have some indirect explanation, perhaps related to the close temporal sequence of the functional and non-functional rearrangement within each pair of light chain carrying homologous chromosomes, as contrasted to the more disparate timing of the kappa versus lambda rearrangement.

The variant translocations were not found to be associated with any c-*myc* rearrangement on the Southern blot, in either the human or the mouse In the human system it has been conclusively shown that the breakpoint of the variant translocations is downstream of the c-*myc* gene in all cases so far studied[56,57]. In contrast to the typical translocation, c-*myc* stays in its original location on chromosome 8, and the C_κ or C_λ sequences attach to it from below, in a head–to–tail orientation. The mechanism of downstream activation and the maximum permissible distance are not known.

In the variant MPC translocations, there is no information as yet on the location of the breakpoint in relation to the *myc* gene. Some comparative aspects of the BL versus MPC associated translocations are summarized in Table 6.2.

THE SCENARIO OF BL AND MPC DEVELOPMENT

Most, if not all, tumours develop by a multi-step process[58,59]. Some steps can now be characterized at the molecular level. Due to the information that exists on the natural history of MPC and BL, including the molecular characterization of the activating (immunoglobulin) and the oncogene (*myc*) partner, both B-cell tumours provide interesting models. Their dissection may pave the way towards the exploration of other tumour systems.

The fact that the Ig/*myc* juxtapositions are limited to these two tumours is puzzling. Neither human multiple myelomas nor murine B-cell lymphomas carry corresponding translocations[60,61]. The only obvious common denominator between BL and MPC can be found in their long preneoplastic history, with chronic proliferation of the preneoplastic target cell at risk as a characteristic feature. In MPC, the proliferation is due to the chronic oil granuloma, which is a prerequisite for tumour development in itself. In the EBV-carrying high endemic form of BL, the combination of the highly transforming virus and chronic hyper- or holoendemic malaria may provide the chronic stimulus for the serial division of the preneoplastic target cell[62].

It may be appropriate to consider the BL scenario first, since somewhat more is known about it. At least 3 different genetic systems may be involved in the

genesis of BL. Epidemiological evidence has shown that the early EBV-infection of African children at a relatively high multiplicity, contributes to the causation of the tumour[63]. This is corroborated by the fact that 97 per cent of the African Burkitt lymphomas carry multiple EBV genome copies in all their cells[64].

With the exception of a single atypical BL-derived line[59], 100 per cent of the Burkitt lymphomas so far studied were found to carry one of three specific *myc*/Ig juxtaposing translocations. This applies not only to the EBV-carrying African form but also to the sporadic non-endemic cases that occur all over the world. It is possible that a third oncogene system may be involved as well – as suggested by the transformability of NIH-3T3 fibroblasts with the DNA of BL-derived lines. The transforming sequences are not related to EBV or to *myc*. Their identity is currently controversial, however.

How do these genes interact in generating Burkitt's lymphoma?

EBV is a uniquely potent immortalizing agent for human B-cells. Three regions of the viral genome (LT-1, 2 and 3) are regularly transcribed in immortalized cells[65]. The LT-1 region that codes for the nuclear antigen EBNA-2[66] may be involved in the activation of the normal B-lymphocyte. The LT-2 region that codes for EBNA-1[67] may be involved in the maintenance of transformation and/or the repression of the lytic viral cycle, which is in itself a prerequisite for the continuous proliferation of the virally transformed B-lymphocyte.

Recent experiments by Gordon *et al*[68] suggest that at least part of the immortalizing effect of EBV is due to the activation of an autocrine loop in B-lymphocytes. Both EBV transformed B-cell lines of normal origin (LCL) and tumourigenic BL-lines release a BCGF-like growth factor, but while LCLs are dependent on it, BL cells are not. Some of the BL lines are responsive to it, but others have lost their responsiveness. This course of events is strongly reminiscent of the earlier literature on the evolution of tumours in many hormone dependent tissues. 'Conditioned tumours' that can only grow in animals that have the same hormonal imbalance as their original host tend to change by mutation-like events to hormone independence with maintained responsiveness and, in a later step, to hormone unresponsiveness[12].

What is the role of the *myc*/Ig translocation in this scenario? A large variety of interpretations have been proposed such as abnormally elevated transcription rates, abnormal transcript size, changed promoter utilization; and changed translational control (for review see ref. 30). Each claim appeared to be valid for some BL or MPC lines, and at the molecular level, the two systems were discussed interchangeably. However, none of them was applicable to all or even the majority of the tumours. Perhaps there are several alternatives and there is no unifying mechanism.

Alternatively, the crucial event may be more subtle than a relatively crude quantitative or qualitative change. The concept of dysregulation is receiving increasing attention. It postulates that the transposed *myc*-gene becomes

subordinated to the *cis*- control of the immunoglobulin gene and must therefore behave as if it was part of the Ig locus itself. The transcription of the normal *myc*-gene is regulated in relation to cell proliferation[69,70]. Terminally differentiating cells turn off c-*myc* transcription. The translocated c-*myc* is not able to do so. As a result, the cells may remain continuously in the cycle.

Although there is no direct evidence for this hypothesis, it is supported by comparative studies on the behaviour of the translocated *myc* gene and its normal allele in MPCs and BLs. It has been found repeatedly in both systems that the translocated gene is highly transcribed, whereas its normal counterpart is switched off (for review see ref. 30). This is compelling evidence for the differential regulation of the two genes. It may be noted that the turn off of the normal allele in the two tumours is the only exception to the general rule that the c-*myc*-gene is highly transcribed in actively proliferating B-cells.

The relative growth factor independence of the BL-cells may be due to the locking of the Ig-juxtaposed *myc*-gene in a continuously active position. As a result, it may act as a 'pacemaker' from within – prompting the continuous re-entry of the cells into the cycle, thereby obviating the need for exogenously supplied BCGF or other positive growth signals.

In the natural history of African BL, EBV-transformation of the target cell is probably the first event[71]. We have postulated that an environmental co-factor, perhaps chronic hyper-endemic malaria[72], may act by increasing the size of the target cell population at risk. Heavy malarial infection may facilitate the development of the lymphoma by two routes, polyclonal B-cell activation, and through T-cell immunosuppression, impairing the ability of the host to reject the EBV carrying cells. Since the EBV-infected B-cell is more long-lived than its EBV-negative counterpart, it can be expected to pass through a larger number of divisions before it is eliminated by terminal differentiation or by immunological mechanisms. The probability of all genetic aberrations increases in direct relationship with the number of cell divisions. This includes the risk of the *myc*/Ig juxtaposition. Once the *myc*/Ig juxtaposition occurs by an act of illegitimate recombination – probably favoured by the DNA rearrangement during normal B-cell differentiation – the resulting subclone can grow into an autonomous tumour.

It is more difficult to develop detailed scenarios for MPC since no transforming virus is known to be involved. It is nevertheless noteworthy that transduction of the v-*abl* oncogene by Abelson virus considerably increased the frequency and reduced the latency period of pristane oil induced MPCs[20].

Mineral oil induced MPCs often fail to grow in syngeneic recipients, unless the hosts are pretreated with pristane oil. It is likely that this is due to the dependence of the cells on a growth factor produced by some component of the granuloma system. After serial transplantation in conditioned mice, they often acquire the ability to grow in untreated syngeneic recipients as well. It will be important to explore whether this additional step towards autonomy can be related to the activation of other oncogenes. Potentially relevant secondary events have been observed, but no attempts were made to relate them to any

functional characteristic of the tumour. Nevertheless, it seems reasonable to attach considerable significance to oncogene activation events that have been observed in more than one tumour. The following examples may be mentioned.

1. Perlmutter *et al*[73] found a 6;10 recombinant in the NS-1 murine plasmacytoma line that also contained the typical 12;15 translocation. This recombination, detected exclusively at the molecular level, has led to the juxtaposition of C_κ and a single copy element derived from chromosome 10. A corresponding 6;10 translocation was also found in a second MPC. The chromosome 10-derived sequence was identical in both tumours. It was not homologous to any known oncogene, it was transcribed at a high level, and homologous sequences could be detected in three different species: mouse, rabbit and human. It is possible that the 6;10 translocation reflects a secondary oncogene activation event that occurs in the course of tumour progression.

2. Rearrangement of the c-*mos* oncogene found in some mouse plasmacytomas may be another case in point. It may be particularly significant in view of the fact that c-*mos* is not transcribed in normal tissues at all[74] and only very rarely in tumours. In the XRPC24 plasmacytoma that carries a typical 12;15 translocation and has an already activated c-*myc*, the c-*mos* gene was highly transcribed as well. The rearranged cloned sequence, designated as rc-*mos* could transform NIH-3T3 cells in transformation assays[75,76]. The 5′ end of the gene contained an intracisternal A particle (IAP) gene in a head-to-head orientation. IAP sequences are known to behave as movable genetic elements. It was suggested that the insertion of the IAP sequences may be responsible for the activation of c-*mos* by analogy with the Hayward model of oncogene activation by retroviral promoter insertion[77]. IAP gene integration into the c-*mos* occurred also in the NS-1 plasmacytoma, another 12;15 translocation carrier[76]. However, the molecular details were different in the two tumours, both with regard to orientation and distance between the IAP and c-*mos*. In NS-1 c-*mos* was not detectably transcribed.

3. Chromosome 11 trisomy is another frequent secondary change in murine plasmacytomas[20].

Oncogene activation in plasma cell tumours of other species

The inbred Louvain strain of rats develops a high incidence of spontaneous immunocytomas[78]. Our group found that these tumours regularly contain a translocation that arose by a reciprocal exchange between the distal segments of chromosomes 6 and 7[79]. Using a panel of segregating rat/mouse somatic hybrids, we have localized the rat c-*myc* gene to chromosome 7 and showed that it was rearranged in the majority of the tumours[80]. This suggests that the translocation may be similar to the murine plasmacytomas. However, as yet no immunoglobulin sequences have been localized to rat chromosome 6.

We have found no *myc* rearrangement in human multiple myelomas[61]. However, *myc* was highly amplified in one plasma cell leukaemia. This is of considerable interest, in view of the demonstrated occurrence of *myc*-

amplification in for example, colorectal carcinomas, hepatomas and lung carcinomas[81]. In small cell lung carcinoma system, *myc* amplification could be related to the progression of the tumour towards a more highly malignant phenotype[82]. Plasma cell leukaemia is known as the most highly malignant form of human plasma cell tumour.

REFERENCES

1. Ohno S, Babonits M, Wiener F, Spira J, Klein G, Potter M 1979 Nonrandom chromosome changes involving the Ig gene-carrying chromosomes 12 and 6 in pristane-induced mouse plasmacytomas. Cell 18: 1001–1007
2. Wiener F, Babonits M, Spira J, Klein G, Potter M 1980 Cytogenetic studies on IgA/lambda-producing murine plasmacytomas: Regular occurrence of a t(12;15) translocation. Somatic Cell Genetics 6: 731–738
3. Klein G 1981 The role of gene dosage and genetic transposition in carcinogenesis. Nature 294: 313–318
4. Klein G 1983 Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. Minireview. Cell 32: 311–315
5. Perry R P 1983 Consequences of *myc* invasion of immunoglobulin loci: Facts and speculation. Cell 33: 647–649
6. Cory S 1983 Oncogenes and B-lymphocyte neoplasia. Immunology Today 4: 205–207
7. Potter M, Wiener F, Mushinski J F 1984 Recent developments in plasmacytomagenesis in mice. Advances in Viral Oncology 4: 139–162
8. Potter M 1977 Antigen binding myeloma proteins of mice. Physiological Reviews 52: 631–719
9. Potter M, Sklar M D, Rowe W P 1973 Rapid viral induction of plasmacytomas in pristane primed BALB/c mice. Science 182: 592–594
10. Potter M, MacCardle R C 1964 Histology of developing plasma cell neoplasia induced by mineral oil in BALB/c mice. Journal of National Cancer Institute 33: 497–515
11. Cancro M, Potter M 1976 The requirement of an adherent cell substratum for the growth of developing plasmacytoma cells in vivo. Journal of Experimental Medicine 144: 1554–1566
12. Furth J 1953 Conditioned and autonomous neoplasms. Cancer Res. 13: 477–492
13. Potter M, Pumphrey J G, Walters J L 1972 Growth of primary plasmacytomas in the mineral oil conditioned peritoneal environment. Journal of the National Cancer Institute 49: 305–308
14. Metcalf D 1974 The serum factor stimulating colony formation in vitro by murine plasmacytoma cells: Response to antigens and mineral oil. Journal of Immunology 113: 235–243
15. McIntire K R, Princler G L 1969 Prolonged adjuvant stimulation in germ-free BALB/c mice. Development of plasma cell neoplasia. Immunology 17: 481–487
16. Yosida T H, Imai H T, Moriwaki U 1970 Chromosomal alteration and development of tumors. XXI. Cytogenetic studies of primary plasma cell neoplasms induced in BALB/c mice. Journal of the National Cancer Institute 45: 411–418
17. Shepard J S, Wurster-Hill D H, Pettengill O S, Sorenson G D 1974 Giemsa banded chromosomes of mouse myeloma in relationship to oncogenicity. Cytogenetics and Cell Genetics 13: 279–304
18. Shepard J S, Pettengill O S, Wurster-Hill D H, Sorenson G D 1974 Karyotype marker formation and oncogenicity in mouse plasmacytomas. Journal of the National Cancer Institute 56: 1003–1011
19. Shepard J S, Pettengill O S, Wurster-Hill D H, Sorenson D 1978 A specific chromosome breakpoint associated with mouse plasmacytomas. Journal of the National Cancer Institute 61: 225–256
20. Ohno S et al 1984 Chromosomal translocations activating *myc* sequences and transduction of v-abl are critical events in the rapid induction of plasmacytomas by pristane and Abelson virus. Journal of Experimental Medicine 159: 1762–1777

21. Wiener F et al 1984 Hemizygous interstitial deletion of chromosome 15 (band D) in three translocation-negative murine plasmacytomas. Proceedings of the National Academy of Sciences USA 83: 1159–1163
22. Wiener F et al 1984 High resolution banding analysis of the involvement of strain BALB/c- and AKR-derived chromosomes no 15 in plasmacytoma-specific translocations. Journal of Experimental Medicine 159: 276–291
23. Wiener F, Ohno S, Spira J, Haran-Ghera N, Klein G 1978 Cytogenetic mapping of the trisomic segment of chromosome 15 in murine T-cell leukaemia. Nature 275: 658–660
24. Kirsh I R, Ravetch J V, Kwan S-P, Max E E, Ney R L, Leder P 1981 Multiple immunoglobulin switch region homologies outside the heavy chain constant region locus. Nature 293: 585–587
25. Adams J M, Gerondakis S, Webb E, Mitchell J, Bernard O, Cory S 1982 Transcriptionally active DNA region that rearranges frequently in murine lymphoid tumors. Proceedings of the National Academy of Sciences USA 79: 6966–6970
26. Harris L J, Lang R B, Marcu K B 1982 Non-immunoglobulin associated DNA rearrangements in mouse plasmacytomas. Proceedings of the National Academy of Sciences USA 79: 4175–4179
27. Cory S, Gerondakis S, Adams J M 1983 Interchromosomal recombination of the cellular oncogene c-*myc* with the immunoglobulin heavy chain locus in murine plasmacytomas is a reciprocal exchange. EMBO Journal 2: 697–703
28. Harris L J, D'Eustachio P, Ruddle F H, Marcu K B 1982 DNA sequence associated with chromosome translocations in mouse plasmacytomas. Proceedings of the National Academy of Sciences USA 79: 6622–6626
29. Calame K, Kim S, Lalley P, Hill R, Davis M, Hood L 1982 Molecular cloning of translocations involving chromosome 15 and the immunoglobulin Ca gene form chromosome 12 in two murine plasmacytomas. Proceedings of the National Academy of Sciences USA 79: 6994–6998
30. Croce C M, Klein G 1985 Chromosome translocation and human cancer. Scientific American 252: 57–60
31. Leder P et al 1983 Translocations among antibody genes in human cancer. Science 222: 765–771
32. Shen-Ong G L C, Keath E J, Piccoli S P, Cole M D 1982 Novel *myc* oncogene RNA from abortive immunoglobulin gene recombination in mouse plasmacytomas. Cell 31: 443–452
33. Adams J M, Gerondakis S, Webb E, Corcoran L M, Cory S 1983 Cellular *myc*- gene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. Proceedings of the National Academy of Sciences USA 80: 1982–1986
34. Marcu K B, Harris L J, Stanton L W, Erikson J, Watt R, Croce C 1983 Transcriptionally active c-*myc* oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B cell neoplasia. Proceedings of the National Academy of Sciences USA 80: 519–523
35. Cory S, Gerondakis S, Corcoran L M, Bernard O, Webb E, Adams J M 1984 Activation of the c-*myc* oncogene in B lymphoid and T lymphoid tumors. Current Topics in Microbiology and Immunology 113: 161–165
36. Yang J O, Mushinski J F, Stanton L W, Fahrlander P D, Tesser P S, Marcu K B 1984 Modes of c-*myc* oncogene activation in murine plasmacytomas. Current Topics in Microbiology and Immunology 113: 146–153
37. Erikson J, Finan J, Nowell P C, Croce C M 1982 Translocation of immunoglobulin. V_H genes in Burkitt lymphoma. Proceedings of the National Academy of Sciences USA 79: 5611–5615
38. Gerondakis S, Cory S, Adams J M 1984 Translocation of the *myc* cellular oncogene to the immunoglobulin heavy chain locus in murine plasmacytomas is an imprecise reciprocal exchange. Cell 36: 973–982
39. Stanton L W, Yant J-O, Eckhardt L A, Harris H J, Birshtein B K, Marcu K B 1984 Products of a reciprocal chromosome translocation involving the c-*myc* gene in a murine plasmacytoma. Proceedings of the National Academy of Sciences USA 81: 829–833
40. Stanton L W, Fahrlander P D, Tesser P M, Marcu K P 1984 Nucleotide sequence comparison of normal and translocated murine c-*myc* genes. Nature 310: 423–425
41. Cory S et al 1985 Modes of activation of the c-*myc* oncogene in B and T lymphoid

tumors. In: Gene Expression during normal and malignant differentiation, Academic Press (In press)
42. Fahrlander P, Sümegi J, Yong J Q, Wiener F, Marcu K, Klein G 1985 Proceedings of the National Academy of Ssciences, USA (in press)
43. Mushinski J F, Potter M, Bauer S R, Reddy E P 1983 DNA rearrangement and altered RNA expression of the c-*myb* oncogene in mouse plasmacytoid lymphosarcomas. Science 220: 795–798
44. Klein G, Ohno S, Rosenberg N, Wiener F, Spira J, Baltimore D 1980 Cytogenetic studies on Abelson-virus-induced mouse leukemias. International Journal of Cancer 25: 805–811
45. Lavu S, Mushinski J F, Shen-Ong G L C, Potter M, Reddy E P 1984 Structural organization of mouse c-*myb* locus and mechanism of its rearrangement ABPL-2 tumor line induced by pristane and Abelson murine leukemia virus. Current Topics in Microbiology and Immunology 113: 37–40
46. Shen-Ong G L C, Reddy E P, Potter M, Mushinski J F 1984 Destruction and activation of the c-*myb* locus by M-MULV insertion in plasmacytoid lymphosarcomas use by pristane and Abelson viruses. Current Topics in Microbiology and Immunology 113: 41–46
47. Land H, Parada L F, Weinberg R A 1983 Cellular oncogenes and multiple carcinogenesis. Science 222: 771–778
48. Bishop J M 1983 Cellular oncogenes and retroviruses. Annual Review of Biochemistry 52: 301–354
49. Collins S, Groudine M 1982 Amplification of endogenous *myc* related DNA sequences in a human myeloid leukaemia cell line. Nature 298: 659–681
50. Rabbitts T H, Forster A, Hamlyn P, Baer R 1984 Effect of somatic imitation within translocated c-*myc* genes in Burkitt's lymphoma. Nature 309: 592–594
51. Gillies S D, Morrison S L, Oi V T, Tonegawa S 1983 A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell 33: 717–729
52. Hayday A C et al 1984 Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus. Nature 307: 334–340
53. Nishikura K, Abbas Ar-Rushdi, Erikson J, Watt R, Rovera G, Croce C M 1983 Differential expression of the normal and of the translocated human c-*myc* oncogenes in B-cells. Proceedings of the National Academy of Sciences USA 80: 4822–4826
54. Klein G, Lenoir G 1982 Translocation involving Ig-locus-carrying chromosomes: a model for genetic transposition in carcinogenesis. In: (Klein G, Weinhouse S, ed.) Advances in Cancer Research pp 381–387
55. Lenoir G M, Preud'homme J L, Bernheim A, Berger R 1982 Correlation between immunoglobulin light chain expression and variant translocation in Burkitt's lymphoma. Nature 298: 474–476
56. Emanuel B S, Selden J R, Chaganti R S K, Suresh J, Nowell P C, Croce C M 1984 The 2p breakpoint of a 2;8 translocation in Burkitt lymphoma interrupts the V_κ locus. Proceedings of the National Academy of Sciences USA 81: 2444–2445
57. Erikson J et al 1983 Translocation of an immunoglobulin kappa locus to a region 3′ of an unrearranged c-*myc* oncogene enhances c-*myc* transcription. Proceedings of the National Academy of Sciences USA 80: 7581–7585
58. Foulds L 1958 The natural history of cancer. Journal of Chronic Diseases 69: 2–37
59. Klein G, Klein E 1984 Oncogene activation and tumor progression. Commentary. Carcinogenesis 5: 429–435
60. Wiener F, Spira J, Babonits M, Klein G 1982 Non-random duplication of chromosome 15 in T-cell leukemias induced in mice heterozygous for reciprocal and Robertsonian translocations. International Journal of Cancer 30: 479–487
61. Sümegi J, Hedberg T, Perlmann C and Klein G 1985 Amplification of c-*myc* oncogene in a human plasma cell leukemia (Submitted)
62. Klein G, Klein E 1984 The changing faces of EBV research. In: Melnick J L, Hummeler K, eds. Progress in Medical Virology. S. Karger, Basel 30: 87–106
63. Geser A, de Thé G, Lenoir G, Day N E, Williams E H 1982 Final case reporting from the Ugandan prospective study of the relationship between EBV and Burkitt's lymphoma. International Journal of Cancer 29: 397–400
64. Klein G 1975 Studies on the Epstein-Barr virus genome and the EBV-determined

nuclear antigen in human malignant disease. Cold Spring Harbor Symposium on Quantitative Biology 39: 783–790
65. Kieff E, Danbaugh T, Hummel M, Heller M 1983 Epstein Barr virus transformation and replication. In: (Klein G, ed.) Advances in Viral Oncology 3: 133–182. Raven Press, New York
66. Rymo L and Klein G 1985 Expression of a second Epstein-Barr virus determined nuclear antigen in mouse cells after gene transfer with a cloned fragment of the viral genome. Proc Natl Acad Sci USA, in press
67. Summers W P, Grogan E A, Shedd D, Robert M, Shun-Ren Liu, Miller G 1982 Stable expression in mouse cells of nuclear neoantigen after transfer of a 3.4-megadalton cloned fragment of Epstein-Barr virus DNA. Proceedings of the National Academy of Sciences USA 79: 5688–5692
68. Gordon J, Åhman P, Rosén A, Ernberg I, Ehlin-Henriksson B, Klein G 1985 Capacity of B-lymphocytic lines of diverse tumor origin to produce and respond to B-cell growth factors: A progression model for B-cell lymphoma genesis. International Journal of Cancer 35: 251–256
69. Campisi J, Gray H E, Pardee A B, Dean M, Sonenshein G E 1984 Cell cycle control of c-*myc* but not c- *ras* expression in host following chemical transformation. Cell 36: 241–247
70. Kelly K, Cochran B H, Stiles C D and Leder P 1983 Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell 36: 603–610
71. Klein G 1979 Lymphoma development in mice and humans: diversity of initiation is followed by convergent cytogenetic evolution. Proceedings of the National Academy of Sciences USA 76: 2442–2446
72. Burkitt D P 1969 Etiology of Burkitt's lymphoma–an alternative hypothesis to a vectored virus. Journal of the National Cancer Institute 42: 19–28
73. Perlmutter R M, Klitz J L, Pravtcheva D, Ruddle F, Hood L 1985 A novel 6;10 chromosomal translocation in the murine plasmacytoma NS-1. Nature (In press)
74. Müller R, Slamon D J, Tremblay J M, Cline M J, Verma I M 1982 Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature 299: 640–644
75. Rechavi G, Givol D, Canaani E 1982 Activation of a cellular oncogene by DNA rearrangement: possible involvement of an IS-like element. Nature 300: 607–611
76. Canaani E et al 1983 Activation of the c- *mos* oncogene in a mouse plasmacytoma by insertion of an endogenous intracisternal A-particle genome. Proceedings of the National Academy of Sciences USA 80: 7118–7122.
77. Hayward W S, Neel B G, Astrin S M 1981 Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature 290: 475–480
78. Bazin H, Deckers C, Becker A, Heremaus J F 1972 Transplantable immunoglobulin secreting tumors in rats. I. General features of LOU/Ws1 strain rat immunocytomas and their monoclonal proteins. International Journal of Cancer 10: 568–580
79. Wiener F, Babonits M, Spira J, Klein G, Bazin H 1982 Non-random chromosomal changes involving chromosome 6 and 7 in spontaneous rat immunocytomas. International Journal of Cancer 29: 431–437
80. Sümegi J, Spira J, Bazin H, Szpirer J, Levan G, Klein G 1983 Rat c-*myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t(6;7) chromosomal translocation. Nature 306: 497–498
81. Alitalo K, Schwab M, Lin C C, Varmus H E, Bishop J M 1983 Homogenously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuro-endocrine cells from a human colon carcinoma. Proceedings of the National Academy of Sciences USA 80: 1707–1711
82. Little C D, Nau M M, Carney D N, Gazdar A F, Minna J D 1983 Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. Nature 306: 194–196

Oncogenes and cancer

The hypothesis that specific genetic changes are important in carcinogenesis has long been important in cancer research. However, it is only with the recent characterization of oncogenes that this hypothesis has had a real experimental foundation.

DEFINITION OF ONCOGENES

The concept that specific genes cause cancer was implicit in the early experiments of Peyton Rous. Rous discovered several viruses that caused cancer in chickens, and he noted that each different virus caused a different type of tumour[1,2]. These viruses were the first isolates of what came to be called Rous sarcoma virus. They and others like them are now referred to as highly oncogenic retroviruses. (Retroviruses are so-called because their replication involves transcription of viral RNA back into DNA – the reverse of the usual flow of information in living systems.)

After an assay for Rous sarcoma virus was developed, which involved transformation of the morphology of chicken cells in culture, it was found that different mutants of Rous sarcoma virus caused different morphologies of the infected cells in a focus of transformed cells[3]. A gene called *morph* was defined as the viral gene that controlled the morphology of the infected cells that became transformed. This transformation of morphology of cells in culture is completely analogous to neoplastic transformation in a chicken. Thus, this gene was the first viral oncogene to be recognized and is now called *src* for sarcomagenic.

The *src* gene was further defined by studies of transformation-defective variants of Rous sarcoma viruses (*td*-RSV) and from studies of mutants of Rous sarcoma virus that were temperature-sensitive for the transforming function that is the infected cells were transformed at 35°C but not at 41°C. Finally, nucleic acid hybridization analysis provided molecular evidence for the existence of the *src* gene. At the same time, similar nucleic acid hybridization experiments established the existence of the *mos* and Ki-*ras* oncogenes in Moloney and Kirsten murine sarcoma viruses, respectively[4].

The existence of viral oncogenes is now most clearly established by the study

of molecular clones of highly oncogenic retroviruses. For example, we have been working with a highly oncogenic retrovirus that causes rapid lethal lymphomas in young chickens, reticuloendotheliosis virus strain T (Rev-T). This virus is replication-defective and is normally found associated with the replication-competent retrovirus – reticuloendotheliosis virus strain A (Rev-A) – which acts as a helper by supplying information for viral proteins.

Most highly oncogenic retroviruses are replication-defective as a result of loss or alteration of viral genes and are found with a helper virus. Rous sarcoma virus is an exception; it is replication-competent. Usually, replication-competent retroviruses are non-oncogenic or weakly oncogenic. Comparison of their genomes shows that the highly oncogenic retrovirus Rev-T differs from the weakly oncogenic Rev-A by a large substitution and a large deletion (Fig. 7.1). Experiments involving specific alterations in the Rev-T genome, followed by recovery and assay of the virus bearing these alterations, established that the substitution contained the viral oncogene[5,6]. Thus, a viral oncogene is defined as a gene in a highly oncogenic retrovirus that codes a product that causes neoplastic transformation. The complete oncogene actually includes both the coding sequences and the sequences controlling transcription and translation. However, the oncogene is often referred to as if it were only the coding sequences, since the sequences controlling transcription and translation are present within the viral sequences surrounding the oncogene coding sequences.

Nucleic acid hybridization and nucleotide sequencing further established that the gene substitution in Rev-T is not homologous to the viral oncogenes carried in other highly oncogenic retroviruses[7,8]. Therefore, the viral oncogene in Rev-T has been called *rel*. While the rel protein has not been isolated, its amino acid sequence has been predicted from the nucleotide sequence (see later).

Similar experiments, although not always as complete, have established the existence of about 20 different viral oncogenes, as listed in Table 7.1[9]. They are

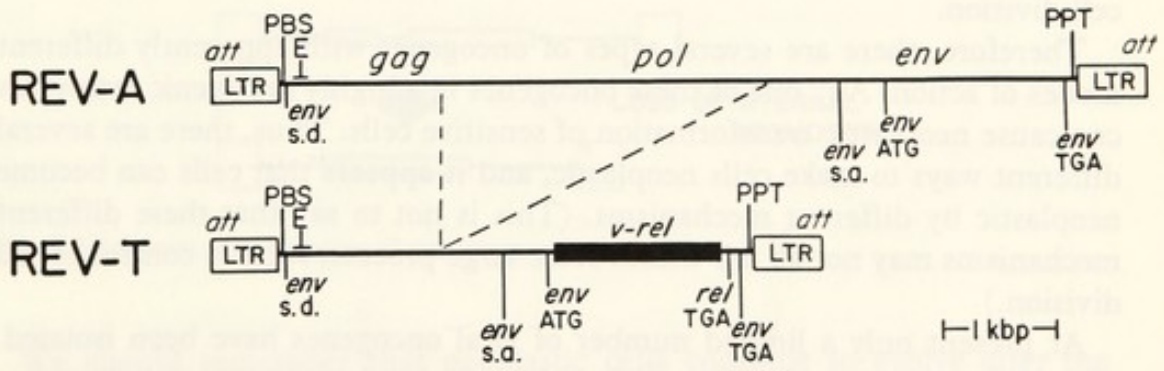

Fig. 7.1 Genomes of replication-competent helper retrovirus Rev-A and replication-defective highly oncogenic retrovirus Rev-T. Symbols are: open box, LTRs with controls for RNA synthesis; *att*, attachment sequence; line, other virus sequences; thick bar, substitution of viral oncogene *rel;* PPT and PBS, sequences needed for viral DNA synthesis; E, encapsidation sequence; *gag, pol,* and *env,* three genes coding for virion proteins; s.d. and s.a., splice donor and splice acceptor; and kbp, kilobase pairs.

Table 7.1 Viral oncogenes

Viral oncogenes	Species of origin	Classification
src	chicken	tyrosine protein
yes	chicken	kinase activity
fps/fes	chicken/cat	
abl	mouse	
ros	chicken	
fgr	cat	
erb-B	chicken	
mos	mouse	sequence related
fms	cat	to *src*
raf/mil	mouse/chicken	
rel	turkey	?
Ha-*ras/bas*	rat/mouse	GTP-binding
Ki-*ras*	rat	activity
myc	chicken/cat	having a nuclear
myb	chicken	location
fos	mouse	
myb-ets	chicken	
sis	monkey/cat	other
ski	chicken	

grouped, according to similarities of sequence and/or function, into families: *src*- related; *ras*- like; those having a nuclear location, and others. (*rel* has been tentatively placed in the *src*- related family, on the basis of weak similarities of some regions of the projected *rel* protein to the *src* protein[10].) This grouping of viral oncogenes into families may indicate in some cases a similar mode of action of the oncogenes. However, it probably only reflects the conservative nature of evolution – this is, the divergent evolution of genes from a small number of precursor genes, or possibly genes involved in similar processes, for example, cell division.

Therefore, there are several types of oncogenes with apparently different modes of action. Any one of these oncogenes in a highly oncogenic retrovirus can cause neoplastic transformation of sensitive cells. Thus, there are several different ways to make cells neoplastic, and it appears that cells can become neoplastic by different mechanisms. (This is not to say that these different mechanisms may not all fall within some large process, such as control of cell division.)

At present only a limited number of viral oncogenes have been isolated. Moreover, several viral oncogenes have been isolated more than once from one species of animal: for example, there are three isolates of *src* and four of *fps* from chickens. Additionally some oncogenes have been isolated from more than one species of animal: for example, *myc* from chickens and cats, and *abl* from mice and cats[11–15]. Thus, it appears that there may be only a limited number of potential viral oncogenes. One would guess that perhaps twice the present

number of 20 is an upper limit, since the already known viral oncogenes are now found more frequently than new ones.

Some highly oncogenic retroviruses contain two gene substitutions. For example, avian erythroblastosis virus contains *erb*-A and *erb*-B and MH2 virus contains *myc* and *mil*[16–18]. In these cases, one substituted gene may be the oncogene and the other a modifier, as in avian erythroblastosis virus or both may be oncogenes, as in MH2 virus[19–23].

So far, I have been describing viral oncogenes. Viral oncogenes, as I shall discuss later, do not include all oncogenes. They only include oncogenes that are dominant and can be expressed in a highly oncogenic retrovirus. That is, they are oncogenes that transform normal cells when a single copy is introduced into the genome of sensitive target cells in a retrovirus.

The study of experimentally modified retroviruses has indicated that there is strong selection during virus replication for the absence of internal promoters, intervening sequences, and 3′ poly(A) addition signals (Fig. 7.2)[24–27]. Thus, any oncogene with one or more of the following characteristics required for transforming activity will not be found in a highly oncogenic retrovirus: not dominant, very large, requires intervening sequences, requires own promoter, requires other upstream sequences, requires own 3′ sequences.

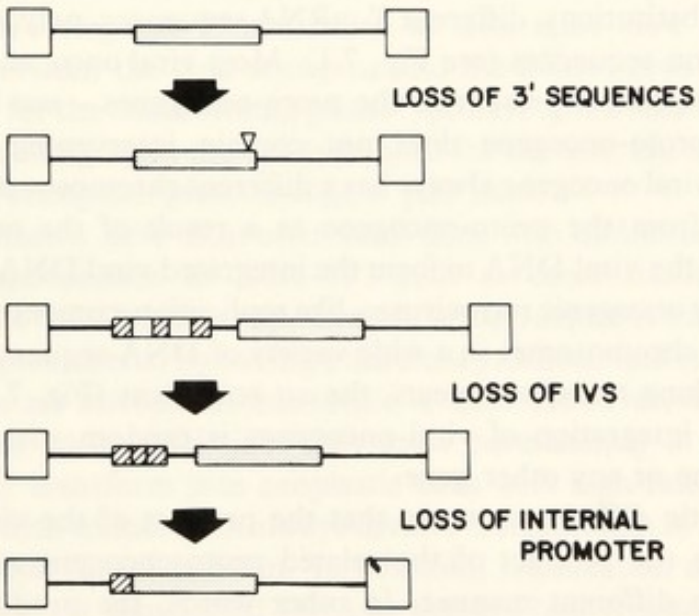

Fig. 7.2 Selection in replication of retrovirus vectors.

We should remember that neoplastic cells continue to evolve after the original neoplastic transformation. Thus, they can and do accumulate further genetic changes including some related to transformation – that is, initially transformed cells progress to greater malignancy (see below). Stated another way, highly malignant cells will contain more genetic changes than necessary to cause neoplastic transformation of a normal cell.

PROTO-ONCOGENES

All viral oncogenes are derived from homologous cellular sequences called proto-oncogenes, as was first discovered for *mos,* Ki-*ras,* and *src* [28]. Proto-oncogenes are normal cellular genes, usually expressed in some normal cells as mRNA and protein, and are usually highly conserved evolutionarily. In fact some, for example Ha-*ras,* are found even in yeast and *Drosophila* [29]. A proto-oncogene has been defined for each viral oncogene. That is, a highly oncogenic retrovirus is a recombinant between a replication–competent retrovirus and a proto-oncogene.

Because of the evolutionary conservation of proto-oncogenes, it has been concluded that they are important normal cellular genes. This conclusion is supported by the findings that the proto-oncogene for *sis* is the gene for the β chain of platelet derived growth factor, and that *erb*-B is homologous to the 3′ two-thirds of the gene for the epidermal growth factor receptor[30,31]. In addition, *fgr* has been shown to be partially related to the gene for cytoplasmic actin, and the *src*- family is related to genes involved in the control of the cell cycle in yeast and to bovine cyclic-AMP dependent protein kinase[32,33].

In all cases, there are genetic differences between the viral oncogene and the homologous proto-oncogene. The differences include at a minimum: a different transcriptional enhancer and promoter; 5′ mRNA sequences, usually amino acid substitutions, different 3′ mRNA sequences, poly(A) addition site, and termination sequences (see Fig. 7.1). Most viral oncogenes have lost the intervening sequences present in the proto-oncogenes – *mos* is an exception because its proto-oncogene does not contain intervening sequences. In addition, the viral oncogene always has a different chromosomal location in the infected cell from the proto-oncogene as a result of the non-homologous integration of the viral DNA to form the integrated viral DNA – the provirus. That is, highly oncogenic retroviruses, like replication-competent retroviruses, integrate into chromosomes in a wide variety of DNA sequences by using the ends of their long terminal repeats, the *att* sequences (Fig. 7.1)[34]. Thus, the chromosomal integration of viral oncogenes is random with respect to the proto-oncogene or any other gene.

These genetic differences mean that the product of the viral oncogene is different from the product of the related proto-oncogene and/or that it is regulated in a different manner. In other words, the product of the viral oncogene is usually both qualitatively and quantitatively different from the product of the related proto-oncogene.

DIFFERENCES BETWEEN REPLICATION-COMPETENT WEAKLY ONCOGENIC RETROVIRUSES AND REPLICATION-DEFECTIVE HIGHLY ONCOGENIC RETROVIRUSES

In addition to the viral oncogene substitution or addition, there are other differences between the replication-competent putative parental retrovirus and

the highly oncogenic retrovirus derived from it. These differences are the presence of additional deletions in the remaining viral sequences, as in Rev-T and Moloney murine sarcoma virus, and less frequently, the presence of additional sequences, as in Rous sarcoma virus[35–38]. (Rous sarcoma virus contains several sets of additional sequences around the *src* oncogene, some of unknown origin). Experiments have indicated that these additional changes may also be important for the transforming ability of the highly oncogenic retroviruses. For example, restoring the deleted sequences in Rev-T results in loss of the transforming ability of the resultant virus. Further mutations must occur to restore the transforming ability of the virus[39,40]. Thus, the deletion in viral sequences is necessary for the transforming activity of Rev-T. Since *rel* is expressed by a subgenomic message[41], the deletion probably has an effect on the efficiency of splicing. The alterations found in other highly oncogenic retroviruses may relate to other processes in addition to splicing.

NUMBER OF CHANGES NEEDED FOR NEOPLASTIC TRANSFORMATION

Highly oncogenic retroviruses cause cancer by introducing a multiply altered proto-oncogene (viral oncogene) into new locations in sensitive target cells. Thus, there are numerous differences between the oncogene and the *in situ* original proto-oncogene. Experiments to determine how many of these differences between the viral oncogene and the homologous proto-oncogene are necessary for the transforming phenotype have given somewhat discordant results – depending upon the cell type used for the test, the means of testing, and the viral oncogene/proto-oncogene pair used.

The experiments have most often been done with molecularly cloned genes assayed by transfection of NIH-3T3 cells or other similar preneoplastic permanent line cells. In these experiments, several copies of the cloned DNA of a proto-oncogene altered by having a different promoter and enhancer or a base pair mutation are introduced into sensitive cells. However, the sensitive cells are themselves not normal since they grow permanently in cell culture and spontaneously transform into neoplastic cells with high rates. Furthermore, experiments with transfection always involve translocation of the gene studied from its original *in situ* location. In addition, transfection usually results in amplification of the transfected DNA as well as a high frequency of mutation, since transfection itself is a mutagenic process[42–45].

With these restrictions, it appears that transfection of NIH-3T3 or some other preneoplastic cell lines by proto-oncogenes with a 'single alteration' – that is, either a single base pair mutation or a different 5′ or 3′ control region – can cause transformation with the proto-oncogenes of *mos*, *ras*, and *fos*, but not with proto-*src*[46–49]. However, studies with more normal cells using transfection by cloned DNA and infection by retrovirus vectors and constructed viruses carrying proto-oncogenes or their derivatives, indicate that 'single' differences like these are not sufficient for transformation of normal cells, even with proto-

ras. These studies indicate that multiple differences between a single oncogene and its homologous proto-oncogene or else several oncogenes are needed in one cell for transformation of normal cells[50–54].

The differences between these two types of results are easy to reconcile. The cells used in the successful transfection experiments were already preneoplastic and contained several genetic changes along the path to full transformation. On the other hand, infection or transfection of normal cells requires more than one change to make a fully transformed cell[55].

Thus, multiple genetic changes in both the proto-oncogene and the replication-competent retrovirus are necessary for the formation of the viral oncogene-highly oncogenic retrovirus complex.

OTHER ONCOGENES

Most cancers are not caused by highly oncogenic retroviruses. However, the concept of the oncogene has been generalized from the viral oncogenes discussed above. Oncogenes, in general, have been defined as genes in cancer cells that are altered structurally or functionally compared to genes in normal cells and that either: (a) transform NIH-3T3 or other cells after transfection, or (b) have homology to viral oncogenes, or (c) are transcriptionally activated by retrovirus proviruses in certain tumour cells. They are called oncogenes by analogy to viral oncogenes, although in no case is it possible to establish that they are responsible for the tumours in which they are found.

The first non-viral oncogene was found by transfection of NIH-3T3 cells. Then it was shown to be related to the viral *ras* oncogene originally described in Harvey murine sarcoma virus[56]. Transfection assays were also used in the isolation of the B*lym* oncogene from chicken bursal lymphomas induced by replication–competent avian leukosis viruses, although B*lym* has not been isolated as a viral oncogene. Oncogenes discovered as a result of their homology to viral oncogenes include N- *myc,* which is related to the viral *myc* oncogene[57]. The oncogenes identified as being transcriptionally activated by retrovirus proviruses in certain tumours induced by weakly oncogenic replication-competent retroviruses include *int*-1 and -2 in mouse mammary carcinomas induced by murine mammary tumour virus[58–60] and different genes found in murine leukaemia virus-induced tumours[61–63]. None of these oncogenes that have been newly identified from being activated in tumours by retrovirus proviruses are related to any viral oncogenes. In addition, some known proto-oncogenes have been found near the provirus in tumours induced by replication-competent retroviruses[64].

All non-viral oncogenes, like the viral oncogenes discussed above, differ from the related proto-oncogenes. The non-viral oncogenes are defined by their difference from the related normal cell gene. For example, the oncogene may have a base pair mutation (Ha-*ras*), be amplified (N-*myc*), be translocated (c-*myc*), or have a new transcriptional control region (*int* -1). Translocation and amplification are the most important ways that chromosomal changes are

involved in the activation of proto-oncogenes in cancer. (Chromosomal changes are also involved in other ways in the causation and progression of cancer).

ANTI-ONCOGENES

All of the oncogenes discussed so far can be dominant, can act with dominant oncogenes, or are related to dominant oncogenes. For example, a viral oncogene will transform a sensitive cell when only one copy of the virus is present. Also, the transfected oncogenes appear to transform NIH-3T3 cells even when only a single copy is present and the oncogenes activated by nearby integration of a weakly oncogenic retrovirus also appear to be in single copies in tumour cells.

By contrast, study of certain human childhood tumours – namely retinoblastomas and Wilms' tumours – has suggested the existence of recessive genes affecting cancer incidence. These genes have been identified and partially localized by the occurrence of homozygosity or hemizygosity of mutant loci at particular chromosomal sites in these tumours[65,66]. The active gene(s) in these regions can be called anti-oncogenes under the assumption that their normal action suppresses or otherwise blocks the action of the dominant oncogenes discussed above. As such, their action would be mimicked by the suppressing action of certain *cis*-acting sequences in retroviruses (see above) and *trans*-acting factors in certain differentiated cells, which block the action of viral oncogenes.

There is evidence for the existence of such suppressor-type genes. For example, infection of chicken macrophage cells by Rous sarcoma virus does not lead to cell transformation even though the viral oncogene product $pp60^{src}$ is made and active[67]. Furthermore, another highly oncogenic retrovirus, avian myeloblastosis virus, can transform the same macrophages which are resistant to transformation by Rous sarcoma virus. This resistance could be explained by the presence of an inhibitor (the product of an anti-oncogene) of the neoplastic action of *src* or perhaps, more likely, by the absence of the proper target for the action of the product of *src*. In the former case, mutations that prevented synthesis of the inhibitor would be necessary for the oncogene to act and transform cells; and in the latter case, mutation of the genes producing the molecules that are targets for the oncogene product would make cells resistant to the oncogene.

Tumour promotion may be related to inactivation of anti-oncogenes. That is, to transform a normal cell into a neoplastic cell, there must be mutations in both copies of each anti-oncogene, in addition to the dominant mutations needed to convert proto-oncogenes into oncogenes. Once there is one mutant allele of an anti-oncogene it is easiest to use a form of mutation other than base pair mutation to inactivate the other one – for example, chromosome loss, gene conversion, mitotic crossing over, etc.[68]. Most of the latter changes are visible as chromosomal alterations and can be seen either with the microscope or with the new DNA mapping techniques using chromosome specific DNA clones.

Alternatively, tumour promotion could represent expansion of a cell population with a single activated oncogene (an initiated population) thereby increasing the probability of further mutations required for neoplastic transformation. Conceivably both mechanisms are important in different systems.

MULTI-STEP NATURE OF CANCER

All cancers, whether induced by highly oncogenic retroviruses or by other agents – such as replication-competent retroviruses, DNA tumour viruses, or chemical and physical agents – involve multiple genetic differences between the cancer cells and the normal precursor cells. For highly oncogenic retroviruses these differences appeared during the course of their evolution from replication-competent retroviruses and proto-oncogenes followed by the subsequent introduction of the multiply changed proto-oncogene (now part of a viral genome) into the cell genome by infection. For replication-competent retroviruses, these differences involve mutation of the virus during multiple cycles of infection, followed by infection of sensitive target cells and activation of one or more proto-oncogenes in the cell genome by insertional activation[69,70]. For those DNA tumour viruses whose mechanism of carcinogenesis is understood, there is introduction of several viral oncogenes into a cell by infection followed by further chromosomal and other mutational changes to give fully transformed cells[71,72]. For non-viral cancers, the multiple genetic differences appear during the latent period of the tumour and probably include both activation of proto-oncogenes and inactivation of anti-oncogenes, although there is as yet only circumstantial evidence for this.

EVOLUTIONARY INEVITABILITY OF CANCER

Thus, cancer is the result of the accumulation in cells of mutations in genes controlling normal cell growth and division. (Of course not all mutations in these genes will be carcinogenic). Therefore, cancer is an unavoidable result of the existence of mutational processes (spontaneous and environmentally-induced) in somatic and germ-line cells affecting genes that directly control normal cell growth and other genes controlling these genes. Since the existence of both some genetic variation and of genes controlling normal cell growth is necessary to life, some cancer seems inevitable. Of course, cancer caused by environmental factors such as cigarette smoking is clearly preventable.

Similarly, no special forms of selection or variation are required to explain the evolution of highly oncogenic retroviruses from replication-competent retroviruses. Given the existence of retroviruses which integrate into cell DNA, and of rearrangements in chromosomes and of RNA transcription and processing, the transduction of random cell DNA by retroviruses must occur – that is, incorporation of random cell DNA into the retrovirus genome will occur at some small frequency. When the cell DNA that is picked up by a retrovirus

contains sequences of a proto-oncogene and is expressed, a highly oncogenic retrovirus may result. (Additional genetic changes will be needed to form the highly oncogenic retrovirus, as mentioned above. But since normal retrovirus replication rapidly generates variants, these changes will occur spontaneously.) However, the successful completion of the entire process of formation of a highly oncogenic retrovirus is rare, as shown by the infrequent occurrence of such viruses.

A variety of different kinds of genetic changes involving proto-oncogenes and anti-oncogenes have been reported. These include: base pair mutations, including transitions, transversions, small deletions and small insertions, as well as gene amplifications, translocations, homologous and non-homologous recombination, and abnormal RNA processing or transcription. Transposition of an intra-cisternal A-particle gene has also been reported to activate a proto-oncogene[73]. Thus, no single type of mutation is uniquely involved in forming cancer.

There are some small suggestions of specificity in mutation in some proto-oncogenes. For example, transfection of DNA from a random collection of human tumours and tumour cell lines has shown that approximately 20 per cent of tumours may have an altered *ras* gene[74]. In addition, although experimental work indicates that base pair mutations at several different sites can lead to activation of the Ha-*ras* gene, mutation at only one site was found in one set of nine rat mammary tumours induced by a chemical carcinogen[75,76]. However, Ha-*ras* activation has been found in preneoplastic lesions and also to occur only during the evolution of a cancer[77,78]. Thus, there does not appear to be any simple correlation between mutation of the *ras* oncogene and any one type or stage of cancer. This result may indicate that the activated proto-oncogene can play different roles in different tumours, is a result of progression, is a highly mutable gene, or is an epiphenomenon in cancer cells.

EVOLUTIONARY ATTEMPTS TO CONTROL THE EFFECTS OF MUTATIONS IN PROTO-ONCOGENES

The problem of controlling cancer for an organism is similar to the problem involved in controlling a parasite. The cancer cell, however, is an endogenous parasite. Since the cancer cell is not foreign, the organism cannot use most of the mechanisms developed to protect against foreign parasites, unless the cancer cell develops new 'foreign' antigens. The problem for an organism in controlling cancer is analogous to the problem for an individual cell of controlling movable genetic elements. In both cases, one component of the biological system is aberrant; that is, a cancerous cell is parasitic in an organism and a movable genetic element is parasitic in a cell genome.

A movable genetic element is a DNA sequence that has a selective advantage compared to other cellular DNA sequences, in the sense that it leaves more copies in subsequent cell generations than other cellular DNA sequences, resulting in an increase with time in the copy number per cell of a movable

genetic element[79]. The selective advantage of movable genetic elements comes from their ability to transpose and the ability of most to replicate during transposition. The presence of movable genetic elements is not essential to the cells they inhabit, although it may be advantageous – for example, by carrying drug resistance genes or increasing variation. However, cellular movable genetic elements are very successful in evolution. Many different types of movable genetic elements are known, and the percentage of the cell genome made up of such elements or their by-products may be very large, over 20 per cent in some cells[80,81].

The structure of cellular movable genetic elements usually has a small specific nucleotide sequence at both ends, which is used as a recognition signal for transposing the element[82]. In addition, most such elements have one or more open reading frames coding for a 'transposase'. (These features are similar to those seen in the retrovirus genomes in Fig. 7.1.) In addition, several features of these elements and some viruses are homologous, indicating that cellular movable genetic elements and some viruses are evolutionarily related[83,84]. For example, the terminal sequences of many eukaryotic cellular movable genetic elements and all retroviruses end in TG..cA.

It appears that cellular movable genetic elements are an inevitable result of the requirement in DNA for recognition signals for enzymes and DNA-binding proteins and also of mutations in these signals and in genes for the proteins. That is, a part of the normal cell genome mutates leading to sequences that can transpose and, thus, over-replicate[85,86]. This evolution is reminiscent of the inevitable occurrence of cancer because of the existence of certain types of genes and their variation.

A key genetic question about cellular movable genetic elements and about cancer is what, if any, kind of controls have cells and organisms evolved to counteract these mutant parts of themselves, or are any controls a result of selection of mutations in the element or cancer itself? That is, since the appearance of cellular movable genetic elements and of cancer cannot be avoided by organisms, has there been any selection for genes that control them?

For prokaryotic cellular movable genetic elements there is little evidence of cellular controls[87]. However, there is in *Drosophila* a suppressor of the action of a cellular movable genetic element named gypsy[88].

For cancer, we have described the existence of anti-oncogenes. In this context the question posed in this section can be rephrased as: Are there specific cellular or organismal control mechanisms that suppress the action of altered proto-oncogenes (oncogenes), or are there only normal control mechanisms for proto-oncogenes?

There does not appear to be great specificity in the relationship of specific oncogenes to specific tumours or in the alterations in proto-oncogenes. Also cancer is usually a disease that occurs after the age of reproduction. Therefore, it is unlikely that there was selection for mutations specific for suppression of proto-oncogenes which can be altered to become oncogenes. Thus, probably only the normal control mechanisms for proto-oncogenes exist to control

oncogenes. When the product of the oncogene is similar to that of the proto-oncogene or when the proto-oncogene product is altered but the controlling sequences are the same, these controls may still operate on the oncogene or its product. These controls may be very strong. The apparent suppressing action of normal blastoderms on teratocarcinoma cells might involve such a mechanism[89]. One might suppose that the existence of cancer has led to some weak selection of tighter controls on the action of proto-oncogenes than there would have been without cancer. Thus, one might predict that proto-oncogenes will be found to be under tighter control than many other genes that cannot mutate into oncogenes.

SUMMARY

Oncogenes are altered genes found in cancer cells and in certain highly oncogenic retroviruses. These genes are related to certain normal cell genes called proto-oncogenes, but differ from them both qualitatively and quantitatively. A variety of genetic mechanisms are involved in this variation. In addition, there are other genes which are altered in cancer cells and apparently suppress the action of the oncogenes. Mutation in these genes occurs during the evolution of highly oncogenic retroviruses or the latent period of a tumour. Chromosomal changes are important both in these changes and in the further evolution of tumours in tumour progression as well as possibly in potential cellular responses to neoplasia.

Acknowledgements

I thank members of my laboratory and Dr. R. G. Temin for helpful comments on this manuscript. The research in my laboratory is supported by U.S. Public Health Service Grants CA-22443 and CA-07175. I am an American Cancer Society Research Professor.

Note added in proof

We and others have now shown that the rel protein is a 59 000 dalton cytoplasmic phosphoprotein. We have also shown that the deletion in Rev-T increases the efficiency of splicing and thus, the expression of the rel protein.

REFERENCES

1. Rous P 1911 A sarcoma of the fowl transmissible by an agent separable from the tumor cell. Journal of Experimental Medicine 13: 397–411
2. Rous P, Murphy A B 1914 On the causation by filterable agents of three distinct chicken tumors. Journal of Experimental Medicine 19: 52–69
3. Temin H M 1960 The control of cellular morphology in embryonic cells infected with Rous sarcoma virus *in vitro*. Virology 10: 182–197
4. Bishop J M, Varmua H 1982 Functions and origins of retroviral transforming genes. In: Weiss R, Teich N, Varmus H E, Coffin J (eds.), RNA tumor viruses. The molecular

biology of tumor viruses, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, ch 9, p 999–1108
5. Chen I S Y, Temin H M 1982 Substitution of 5′ helper virus sequences into non- *rel* portion of reticuloendotheliosis virus strain T suppresses transformation of chicken spleen cells. Cell 31: 111–120
6. Temin H M, Miller C K 1984 Insertion of oncogenes into retrovirus vectors. Cancer Surveys. 3: 229–246
7. Stephens R M, Rice N R, Hoebsch R R, Bose H R Jr., Gilden R V 1983 Nucleotide sequence of v- *rel:* the oncogene of reticuloendotheliosis virus. Proceedings of the National Academy of Sciences USA 80: 6229–6233
8. Wilhelmsen K C, Eggleton K, Temin H M 1984 Nucleic acid sequences of the oncogene v- *rel* in reticuloendotheliosis virus strain T and its cellular homolog, the proto-oncogene, c- *rel.* Journal of Virology 52: 172–182
9. Bishop J M 1983 Cellular oncogenes and retroviruses. Annual Review of Biochemistry 52: 301–354
10. Feng D F, Johnson M S, Doolittle R F 1985 Aligning amino acid sequences: comparison of commonly used methods. In press
11. Bishop, op. cit. Reference 9
12. Levy L S, Gardner M B, Casey J W 1984 Isolation of a feline leukaemia provirus containing the oncogene *myc* from a feline sarcoma. Nature 306: 853–856
13. Mullins J I, Brody D S, Binari R C Jr., Cotter S M 1984 Viral transduction of c-*myc* gene in naturally occurring feline leukaemia. Nature 308: 856–858
14. Neil J C et al 1984 Transduction and rearrangement of the *myc* gene by feline leukaemia virus in naturally occurring T-cell leukaemia. Nature 308: 814–820
15. Besmer P, Hardy Jr W D, Zukerman E E, Bergold P, Lederman L, Snyder H W 1983 The Hardy-Zukerman 2-FeSv, a new feline retrovirus with oncogene homology to Abelson-MuLV. Nature 303: 825–828
16. Vennstrom B, Bishop J M 1982. Isolation and characterization of chicken DNA homologous to the two putative oncogenes of avian erythroblastosis virus. Cell 28: 135–143
17. Kan N C, Flordellis C S, Garon C F, Duesberg P H, Papas T S 1983 Avian carcinoma virus MH2 contains a transformation-specific sequence, *mht,* and shares the *myc* sequence with MC29, CMII, and OK10 viruses. Proceedings of the National Academy of Sciences USA 80: 6566–6570
18. Leprince D et al 1983 A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. Nature 306: 395–397
19. Sealy L, Moscovici G, Moscovici C, Bishop J M 1983 Site-specific mutagenesis of avian erythroblastosis virus: v-*erb*-A is not required for transformation of fibroblasts. Virology 130: 179–194
20. Frykberg L, Palmieri S, Beug H, Graf T, Hayman M J, Vennstrom B 1983 Transforming capacities of avian erythroblastosis virus mutants deleted in the *erb*- A or *erb*- B oncogenes. Cell 32: 227–238
21. Debuire B 1984 Sequencing the *erb*A gene of avian erythroblastosis virus reveals a new type of oncogene. Science 224: 1456–1459
22. Jansen H W, Lurz R, Bister K, Bonner T I, Mark G E, Rapp U R 1984 Homologous cell-derived oncogenes in avian carcinoma virus MH2 and murine sarcoma virus 3611. Nature 307: 281–284
23. Sutrave P, Bonner T I, Rapp U R, Jansen H W, Patschinsky T, Bister K 1984 Nucleotide sequence of avian retroviral oncogene v-*mil:* homologue of murine retroviral oncogene v-*raf.* Nature 309: 85–88
24. Emerman M, Temin H M 1984 High frequency deletion in recovered retrovirus vectors containing exogenous DNA with promotors. Journal of Virology 50: 42–49
25. Shimotohno K, Temin H M 1982 Loss of intervening sequences in genomic mouse alpha-globin DNA inserted in an infectious retrovirus vector. Nature 299: 265–268
26. Sorge J, Hughes S H 1982 Splicing of intervening sequences introduced into an infectious retroviral vector. Journal of Molecular and Applied Genetics 1: 547–559
27. Shimotohno K, Temin H M 1981 Formation of infectious progeny virus after insertion of herpes simplex thymidine kinase gene into DNA of an avian retrovirus. Cell 26: 67–77, 1981

28. Bishop, op. cit. Reference 9
29. Tatchell K, Chaleff D T, DeFeo-Jones D, Scolnick E M 1984 Requirement of either of a pair of *ras*- related genes of *Saccharomyces cerevisiae* for spore viability. Nature 309: 523–527
30. Owen A J, Pantazis P, Antoniades H N 1984 Simian sarcoma virus-transformed cells secrete a mitogen identical to platelet-derived growth factor. Science 225: 54–56
31. Downward J et al 1984 Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. Nature 307: 521–527
32. Naharro G, Robbins K C, Reddy E P 1984 Gene product of v-*fgr* oncogene: hybrid protein containing a portion of actin and a tyrosine-specific protein kinase. Science 223: 63–66, 1984
33. Feng et al, op. cit. Reference 10
34. Panganiban A T, Temin H M 1983 The terminal nucleotides of retrovirus DNA are required for integration but not for virus production. Nature 306: 155–160
35. Schwartz D E, Tizard R, Gilbert W 1983 Nucleotide sequence of Rous sarcoma virus. Cell 32: 853–869
36. Takeya T, Hanafusa H 1983 Structure and sequence of the cellular gene homologous to the RSV *src* gene and the mechanism for generating and transforming virus. Cell 32: 881–890
37. van Beveran C, van Straaten F, Galleshaw J A, Verma I M 1981 Nucleotide sequence of the genome of a murine sarcoma virus. Cell 27: 97–108
38. Chen I S Y, Mak T W, O'Rear J J, Temin H M 1981 Characterization of reticuloendotheliosis virus strain T DNA and isolation of a novel variant of reticuloendotheliosis virus strain T by molecular cloning. Journal of Virology 40: 800–811
39. Chen and Temin, op. cit. Reference 5
40. Temin and Miller, op. cit. Reference 6
41. Chen I Y K, Wilhelmsen K, Temin, H M 1983 Structure and expression of c-*rel*, the cellular homolog to the oncogene of reticuloendotheliosis virus strain T. Journal of Virology 45: 104–113
42. Perucho M, Hanahan D, Wigler M 1980 Genetic and physical linkage of exogenous sequences in transformed cells. Cell 22: 309–317
43. Calos M P, Lebkowski J S, Botchan M R 1983 High mutation frequency in DNA transfected into mammalian cells. Proceedings of the National Academy of Sciences USA 80: 3015–3019
44. Razzaque A, Mizusawa H, Seidman M M 1983 Rearrangement and mutagenesis of a shuttle vector plasmid after passage in mammalian cells. Proceedings of the National Academy of Sciences USA 80: 3010–3014
45. Razzaque A, Chakrabarti S, Joffee S, Seidman M 1984 Mutagenesis of a shuttle vector plasmid in mammalian cells. Molecular and Cellular Biology 4: 435–441
46. Oskarsson M, McClements W L, Blair D G, Maizel J V, Vande Woude G F 1980 Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcoma virus. Science 207: 1222–1224
47. DeFeo D et al 1981 Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. Proceedings of the National Academy of Sciences USA 78: 3328–3332
48. Verma I 1984 From c-*fos* to v-*fos*. Nature 308: 317
49. Parker R C, Varmus H E, Bishop J M 1984 Expression of v-*src* and chicken c-*src* in rat cells demonstrates qualitative differences between $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$. Cell 37: 131–139
50. Ruley H E 1983 Adenovirus early region IA enables viral and cellular transforming genes to transform primary cells in culture. Nature 304: 602–607
51. Newbold R F, Overell R W 1983 Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-*ras* oncogene. Nature 304: 648–651
52. Land H, Parada L F, Weinberg R A 1983 Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304: 596–602
53. Iba H, Takeya T, Cross F R, Hanadua T, Hanafusa H 1984 Rous sarcoma virus variants that carry the cellular *src* gene instead of the viral *src* gene cannot transform chicken embryo fibroblasts. Proceedings of the National Academy of Sciences USA 81: 4424–4428

54. Wilhelmsen K C, Tarpley W G, Temin H M 1984 Identification of some of the parameters governing transformation by oncogenes in retroviruses. In: The Cancer Cell. 2. pp 303–308 Cold Spring Harbor Laboratory – Cold Spring Harbor, New York
55. Newbold R F, Overell R W, Connell J R 1982 Induction of immortality is an early event in malignant transformation of mammalian cells by carcinogens. Nature 299: 633–635
56. Cooper G M 1982 Cellular transforming genes. Science 217: 801–806
57. Lee W-H, Murphree A L, Benedict W F 1984 Expression and amplification of the N-*myc* gene in primary retinoblastoma. Nature 309: 458–460
58. Nusse R, Varmus H E 1982 Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31: 99–109
59. Nusse R, van Ooyen A, Cox D, Fung Y K T, Varmus H 1984 Mode of proviral activation of a putative mammary oncogene (*int*- 1) on mouse chromosome 15. Nature 307: 131–136
60. Dickson C, Smith R, Brookes S, Peters G 1984 Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region *int* 12. Cell 37: 529–536
61. Lemay G, Jolicoeur P 1984 Rearrangement of a DNA sequence homologous to a virus-cell junction in several Moloney murine leukemia virus-induced rat thymomas. Proceedings of the National Academy of Sciences USA 81: 38–42
62. Tsichlis P N, Strauss P G, Hu L F 1983 A common region for proviral DNA integration in MoMuLV induced rat thymic lymphomas. Nature 302: 445–449
63. Cuypers H T et al 1984 Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. Cell 37: 141–150
64. Neel B G, Hayward W S, Robinson H L, Fang J, Astrin A M 1981 Avian leukosis virus-induced tumors have common proviral integration sites and synthesize discrete new RNAs: Oncogenesis by promoter insertion. Cell 23: 323–334
65. Godbout R, Dryja T P, Squire J, Gallie B L, Phillips R A 1983 Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. Nature 304: 451–453
66. Solomon E 1984 Recessive mutation in aetiology of Wilms' tumour. Nature 309: 111–112
67. Lipsich L, Brugge J S, Boettiger D 1984 Expression of the Rous sarcoma *src* gene in avian macrophages fails to elicit transformed cell phenotype. Molecular and Cellular Biology 4: 1420–1424
68. Cavenee W K et al 1983 Chromosomal mechanisms revealing recessive alleles in retinoblastoma. Nature 305: 779–784
69. Cooper G M, Neiman P E 1981 Two distinct candidate transforming genes of lymphoid leukosis virus-induced neoplasms. Nature 292: 857–858
70. Thomas C Y, Khiroya R, Schwartz R S, Coffin J M 1984 Role of recombinant ecotropic and polytropic viruses in the development of spontaneous thymic lymphomas in HRS/J mice. Journal of Virology 50: 397–407
71. Cuzin F 1984 The polyoma virus oncogenes. Coordinated functions of three distinct proteins in the transformation of rodent cells in culture. Biochemica Biophysica Acta 781: 193–204
72. Asselin C, Gelinas C, Branton P E, Bastin M 1984 Polyoma middle T antigen requires cooperation from another gene to express the malignant phenotype *in vivo*. Molecular and Cellular Biology 4: 755–760
73. Cohen J B, Ungar T, Rechavi G, Canaani E, Givol D 1983 Rearrangement of the oncogene c-*mos* in mouse melanoma NS1 and hybridomas. Nature 306: 797–799
74. Albino A P, Le Strange R, Oliff A I, Furth M E, Old L J 1984 Transforming *ras* genes from human melanoma: a manifestation of tumour heterogeneity? Nature 307: 69–72
75. Fasano O, Aldrich T, Tamanoi F, Taparowsky E, Furth M, Wigler M 1984 Analysis of the transforming potential of the human H- *ras* gene by random mutagenesis. Proceedings of the National Academy of Sciences USA 81: 4008–4012
76. Sukumar S, Notario V, Martin-Zanca D, Barbacid M 1983 Induction of mammary carcinomas in rats by nitroso-methyl urea involves malignant activation of H- *ras*- 1 locus by single point mutations. Nature 306: 658–661
77. Balmain A, Ramsden M, Bowden G T, Smith J 1984 Activation of the mouse cellular Harvey- *ras* gene in chemically induced benign skin papillomas. Nature 307: 658–659

78. Tainsky M A, Cooper C S, Giovanella B C, Vande Woude G F 1984 An activated *ras*^{N} gene: detected in late but not early passage human PA1 teratocarcinoma cells. Science 225: 643–645
79. Temin H M, Engels W 1984 Movable genetic elements and evolution. In: Pollard J W (ed) Evolutionary prospects in the 1980s. John Wiley, Chichester, ch 4, p 173–201
80. Kleckner N 1977 Translocatable elements in prokaryotes. Cell 11: 11–23
81. Temin and Engels, op. cit. Reference 79.
82. Way J C, Kleckner N 1984 Essential sites at transposon Tn*10* termini. Proceedings of the National Academy of Sciences USA 81: 3452–3456
83. Temin H M 1980 Origin of retroviruses from cellular movable genetic elements. Cell 21: 599–600
84. Campbell A 1981 Evolutionary significance of accessory DNA in bacteria. Annual Review of Microbiology 35: 55–83
85. Orgel L E, Crick F H C, Sapienza C 1980 Selfish DNA. Nature 288: 645–646
86. Berg D E, Berg C M 1983 The prokaryotic transposable element Tn5. Bio-Technology 15: 417–435
87. Shapiro J (ed) 1983 Mobile genetic elements. Academic Press, New York
88. Modolell J, Bender W, Meselson M 1983 *Drosophila melanogaster* mutations suppressible by the suppressor of Hairy-wing are insertions of a 7.3 kilobase mobile element. Proceedings of the National Academy of Sciences USA 80: 1678–1682
89. Bradley A, Evans M, Kaufman M H, Robertson E 1984 Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309: 255–256

Chromosomal translocations and oncogenes in Burkitt's lymphoma

The genes responsible for malignant cell transformation were first identified after dissection of oncogenic viruses such as the retro- and papovaviruses. The isolation of cloned genes and characterization of their products and functions provided a molecular basis for understanding the process of neoplastic transformation. The finding, in the late seventies, that most of these viral genes designated as oncogenes (v-onc) are picked up by the virus through recombination with the cellular genome suggested that the biological activity of oncogenic viruses could be due to activation or deregulation of specific genes under the control of viral elements[1]. In humans, however, the majority of cancers do not appear to be virally induced. The biological function of the cellular homologues of the v-onc, called proto-oncogenes (c-onc), thus remains to be elucidated. The question is how and whether such sets of genes, which have normal, well-controlled physiological activity during cellular proliferation and development, can be activated in cancer cells. A first answer was provided by the isolation of 'activated oncogenes' from a human tumour, by DNA-mediated transfer onto NIH-3T3 cells, showing that the malignant phenotype was produced by expression of a c-onc gene altered by a single point mutation[2].

Another fruitful approach to the study of the genetic mechanisms involved in the activation of cellular oncogenes is analysis of chromosomal rearrangements characteristic of some neoplasia. During the past decade, a number of investigators have demonstrated that certain types of cancer are characterized by specific somatic chromosomal anomalies, such as translocations, deletions, inversions, monosomy and trisomy. These are found in particular in association with neoplasia of the haematopoietic system, for which a number of consistent, specific karyotype markers have been described[3]. The almost inevitable association between certain types of translocations in myeloid and lymphoid cancers, such as the t(9;22) in chronic myelogenous leukaemia[4,5] and the t(8;14) in Burkitt's lymphoma (BL)[6,7], suggests that chromosomal rearrangement is causally related to the development of a malignant cell clone and is not merely a secondary event.

During the past three years, it has become possible to study the molecular consequences of these translocations, following molecular cloning of a number of cellular oncogenes and their mapping on the human genome. This technique

has opened new avenues for studying some of the mechanisms of oncogene deregulation in cancer and for defining molecular markers of certain types of cancer.

One of the first models used was BL. It was shown that, following a chromosomal translocation, the expression of an oncogene designated as c-*myc* was altered by juxtaposition with a DNA segment carrying an immunoglobulin (Ig) gene[8]. In this review we will summarize most of the recent findings on the molecular and biological consequences of the chromosomal translocations observed in BL. Taking into consideration the history of the discoveries, we will also discuss how these 'unique gene interactions' could contribute to the process of malignant transformation together with other causally associated factors, such as the Epstein-Barr virus (EBV).

BURKITT'S LYMPHOMA

This cancer, in which the significance of chromosomal translocations has been studied so extensively over the last three years, is a useful model for several reasons. During a recent symposium (the proceedings of which have been published by the IARC*), an attempt was made to review comprehensively information on the clinical, epidemiological and laboratory characteristics of this cancer, and these can be summarized briefly as follows.

The tumour now called BL was initially described 25 years ago as a distinct clinicopathological entity occurring with high frequency in children from Central Africa[9,10]. From cytological and pathological points of view, the initial description is still valid[11,12]; BL is a malignant lymphoma comprising a monomorphic outgrowth of undifferentiated lymphoid cells, with little variation in size and shape, an amphophilic cytoplasm with clear vacuoles, and a non-cleaved nucleus containing two to five basophilic nucleoli. At low magnification, a 'starry sky' pattern is frequently observed, caused by macrophage infiltration of this rapidly growing tumour[13]. In the new working formulation of non-Hodgkin's lymphoma, BL belongs to the high-grade, malignant lymphoma, small non-cleaved cell group[14]. BL consists of a clonal proliferation of lymphocytes from the B-cell subset, all of which synthesize heavy-chain Ig, predominantly of the μ subtype[15]. Ig light chain expression is also observed in most cases, but not in all BL tumours[16].

Initial epidemiological observations suggested a viral aetiology, and holoendemic malaria was considered a possible causal co-factor. When in 1964 the Epstein-Barr virus was first isolated from a Burkitt culture[17] and later shown to be almost universally associated with the African tumour, BL was considered to be one of the first human cancers with a probable viral aetiology.

*Burkitt's lymphoma: a human cancer model. Lenoir G M, O'Conor G, Olweny C L M, (eds) 1985 IARC Scientific Publications No. 60, Lyon (see ref 98)

BL epidemiology

The incidence of BL shows very important geographical variations. The tumour occurs throughout the world and is one of the most frequent cancers (if not the most frequent) in African children[18]. Outside Africa (in Europe and the USA), it represents less than 3% of childhood cancers (for review, see ref 19); however, in all regions it represents a significant proportion of malignant non-Hodgkin's lymphomas (30 to 40%)[20,21]. Although epidemiological studies have identified areas of high incidence (Central Africa, New Guinea) and low incidence (Europe, USA), there is still a lot of uncertainty about the incidence of BL in areas such as South America, Asia and North Africa.

Role of EBV

The association between EBV and BL and other neoplastic and non-neoplastic diseases has been the subject of extensive epidemiological and laboratory investigations during the last two decades.

Even though EBV was initially isolated from a BL tumour[17] and was later shown to be a probable causal factor in BL on epidemiological grounds[22], the virus cannot be considered the sole cause of BL. The DNA of this ubiquitous human herpetovirus (the virus infects latently 95–98% of the adult population throughout the world) can be detected in malignant cells of the great majority (96%) of BL cases occurring in high-incidence areas[23]. However, in the remaining 4% and in up to 85% of cases occurring in some low-incidence areas (Europe, USA), viral markers are not present in malignant cells[19].

It was shown in laboratory studies that EB viral infection of human B lymphocytes is followed by continuous in-vitro multiplication of infected cells, leading to the establishment of continuous lymphoblastoid cell lines (LCL)[24]. This phenomenon is called cellular 'immortalization'. When LCL and BL cell lines are compared, it is clear that some of the malignant properties of the BL lines (e.g. induction of tumours in immunosuppressed animals, growth in semi-solid medium) are absent in LCL[25,26]. This suggests that even though the virus is capable of triggering cell proliferation, other complementary mechanisms are responsible for the malignant phenotype of BL cells. As early as 1972, it was shown that one of the key differences between BL and LCL is the almost invariable presence of non-random chromosomal anomalies in BL malignant cells and their consistent absence from non-malignant EBV-immortalized cells (see Ch. 6).

BL cell lines

One of the characteristic features of BL, which was of great assistance in laboratory investigations on this cancer, is the relative ease with which malignant cells can be cultivated in vitro. Using simple tissue culture procedures, most BL tumour cells can be established as permanent cell lines –

an almost unique phenomenon in the field of human cancer[27]. Most of the major advances in laboratory studies of BL aetiology were made using BL cell lines: for the initial discovery of EBV (1964)[17], for the first description of the 14q+ chromosomal marker (1972)[6] and more recently (1982) for molecular studies implicating c-*myc* oncogene transposition[8].

It was thought for some years that the relative ease with which BL cell lines could be established was limited to EBV-associated cases. Our recent experience in low-incidence areas such as France, where most BL tumours do not contain the EB viral genome, shows that malignant cells can be grown in culture in most cases[28]. This property can thus be considered a characteristic feature of Burkitt-type lymphomas, independent of their association with EBV.

The phenotype of Burkitt's lymphomatous cells has been well characterized, corresponding to expression by B lymphocytes of surface Ig in most cases. The stage of maturation varies from one tumour to another – from an almost pre-B phenotype (cytoplasmic IgM) to a more mature phenotype with expression of IgD and IgG, and even IgA[29]. However, the cells never fully differentiate to a plasmacytic phenotype.

CHROMOSOMAL TRANSLOCATIONS

Cytogenetic studies on BL that were carried out before the introduction of banding techniques gave conflicting results, without identification of specific chromosomal markers (for review, see ref 30).

In 1972, however, at a time when most investigations were focusing on the role of EBV in BL aetiology, G. and Y. Manolov used the newly developed banding techniques to demonstrate that BL cells are characterized by an abnormal chromosome 14, which has an extra band on the extremity of its long arm, 14q+[. This work was rapidly confirmed by others[31] and was expanded by Zech et al[7] and Kaiser-McCaw et al[32] who demonstrated that the extra material on chromosome 14 originated from the long arm of chromosome 8. The t(8;14) was thus discovered. Another important fact was noted by these two groups: the cytogenetic anomaly was also observed in non-EBV-associated BL, suggesting that the translocation was a characteristic marker of the tumour, independent of its association with the virus. This translocation was never observed in non-malignant EBV-immortalised LCL.

In 1979 a study of cases originating from so-called 'non-endemic' areas (now better defined as low incidence areas) led to a new, important cytogenetic discovery: BL cases without the 14q+ marker but with two new translocations, designated variant translocations. The t(2;8) was initially described in cases from Japan, Belgium and the Federal Republic of Germany[33–35]. In France, in the meantime, Berger et al reported a case of BL with a t(8;22) translocation[36], which was followed immediately by three other cases. The important feature was that for all three translocations – t(8;14) (q24;q32), t(2;8) (p11;q24) and t(8;22) (q24;q11) – the breakpoint on chromosome 8 was cytogenetically the same, 8q24. Soon after, BL cases with variant translocations were also reported

from high-incidence areas, i.e. in Africa[37], indicating that independent of the geographic origin of the patient or the EBV-association of the tumour, the characteristic cytogenetic anomaly of BL is a translocation of chromosome 8 (band q24) with chromosome 2, 14 or 22 (see Fig. 8.1)[38–40]. Cytogenetically, the translocations are considered to be reciprocal and balanced[41].

In order to estimate the relative frequency of the three types of BL translocation, we recently summarized the data obtained on 51 of our BL lines (15 African, 13 North-African and 23 Caucasian). t(8;14) is by far the most frequent translocation observed in BL (76% of the cases), only one fourth of the tumours presenting with a variant translocation. t(8;22) and t(2;8) are not equally distributed, the former being at least twice as frequent as the latter (see Table 8.1).

Table 8.1 Estimation of the relative proportion of the three types of BL translocations based on the analysis of 51 BL cell lines established at IARC

Ethnic origin of the patient	t(8;14)	t(8;22)	t(2;8)
Caucasian	18	5	0
African (Central)	11	2	2
North African	10	1	2
Total	39	8	4
%	76%	16%	8%

How consistently are chromosomal translocations involving chromosome 8q24 found in BL? On the basis of cytogenetic studies performed on BL cell lines, there is apparently no exception. More than 100 lines have been analysed, and all carried one of the three characteristic translocations. One unique, extensively studied cell line derived from an EBV-genome-negative African B cell lymphoma and designated BJAB was found to have no anomaly involving chromosome number 8[7] and was considered an example of BL lacking the translocation. However, on the basis of several criteria, such as clinical history, morphology and surface phenotype, the diagnosis of BL in this case remains very uncertain. Normal karyotypes have also been reported after analysis of fresh BL (lymphomas or leukaemias); however, as pointed out by Berger et al (1983)[42], this might be due to infiltration of chromosomes of non-malignant cells. Very recently, the same authors reported two cases of BL with no specific translocation but with several other chromosomal anomalies, including a 6q- marker[43].

Are t(8;14), t(8;22) and t(2;8) specific to BL? In his review, Mitelman indicated that the t(8;14) has been found in non-Burkitt malignant lymphoproliferations[44]. However, it is not very frequent. The study of Sigaux et al[45] indicates that, on the basis of morphometric measurements of malignant

cells, rearrangement of 8q24 with chromosome 14 is found mainly in lymphomas that show a morphological continuum extending from BL to immunoblastic lymphomas. They might, in fact, all correspond to cell proliferation of the B-cell lineage.

EBV does not appear to be capable of inducing the BL translocations directly. No such chromosomal rearrangement is observed in lymphoid cells during infectious mononucleosis in vivo. Furthermore, in-vitro LCL obtained by immortalization of non-malignant lymphocytes have normal karyotypes at the time of their establishment; when they are kept in culture even for several years, the chromosomal changes that are observed are never BL-type translocations[46]. In BL patients, the translocations are observed only in malignant cells and never in other haematopoietic cell types such as in stem cells. The association is thus very different from that of t(9;22) or Ph[1] with chronic myelogenous leukaemia (CML), where it has been shown that Ph[1] can be (i) an early event in the disease process, observed, for example, before clinical evidence of CML in atomic bomb survivors[47], and (ii) detected in non-myeloid cell lineages, such as B lymphoid cells[48], indicating that the t(9;22) is most probably already present in stem cells.

MOLECULAR CONSEQUENCES OF THE TRANSLOCATIONS

At the end of 1980, following the cytogenetic demonstration that the same segment of chromosome 8, 8q24, is consistently involved in the three types of translocations in BL, it was clear that a molecular approach should be envisaged, aimed at characterizing the DNA fragments involved. The possibility of a molecular approach was also stimulated by the finding that in BL cells – Ig-producing cells – the chromosomes that exchanged material with chromosome 8 are those that carry the Ig heavy- and light-chain genes.

Involvement of Ig genes

In 1981, following the Gene Mapping Conference, it became apparent that the three Ig-gene-carrying chromosomes in humans are those involved in BL in translocations with chromosome 8. A few months later, it was demonstrated that the loco-regional location of these genes on each chromosome is compatible with the site of the breakpoint: the Ig heavy chain gene cluster was situated on chromosome band 14q32[49–50], the kappa light-chain genes on chromosome band 2p11[51] and the lambda light-chain on band 22q11[52] (see Fig. 8.1).

Furthermore, a correlation was found between the type of translocation and the type of light-chain expressed by the malignant cells: for example, BL cells with t(8;22) make lambda chains rather than kappa chains[53]. A similar correlation had been observed by Ohno in murine plasmacytoma[54], suggesting a direct implication of an active Ig locus in the translocations[55].

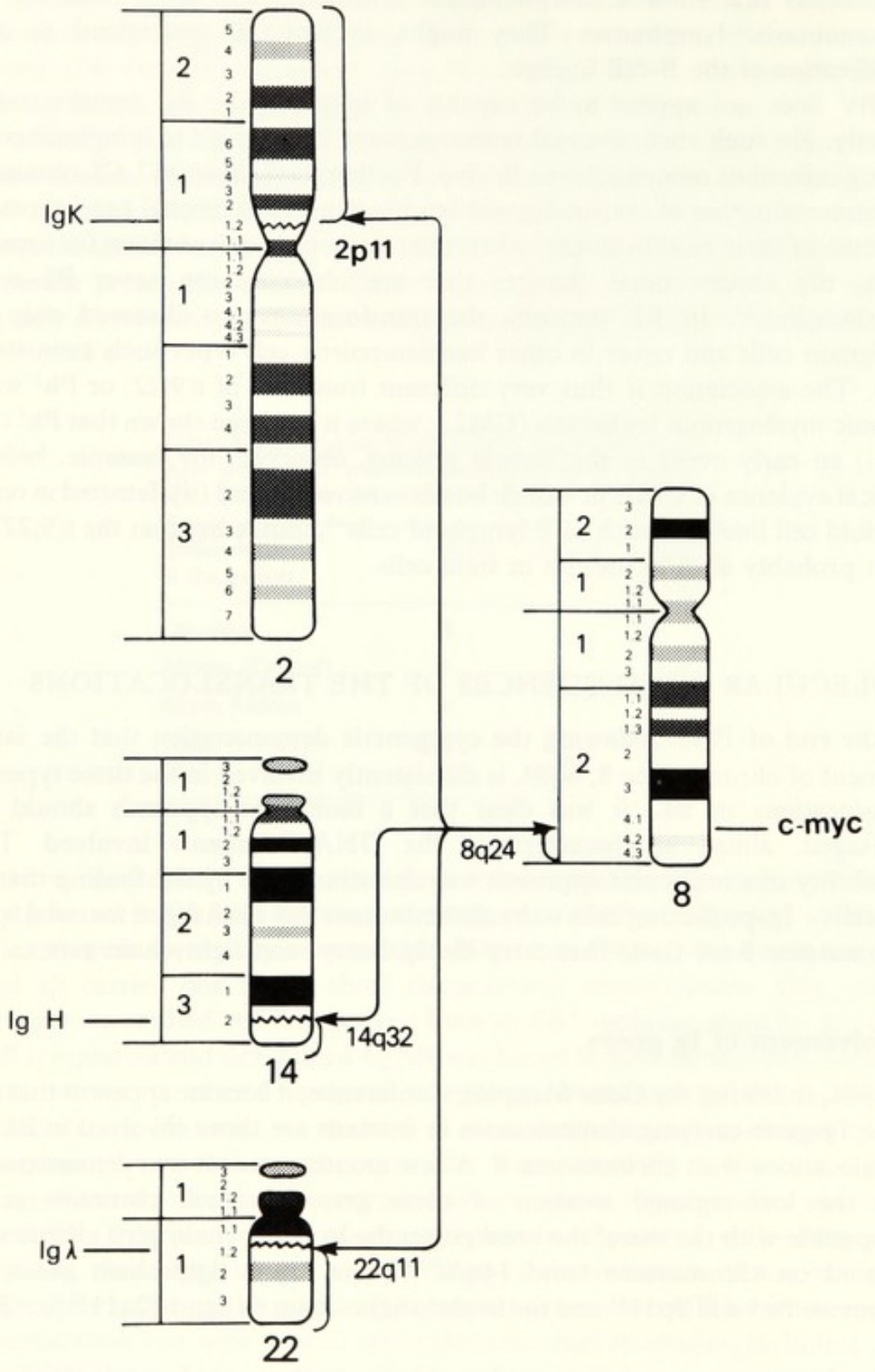

Fig. 8.1 Diagrammatic representation of the human chromosomes involved in the specific translocations of BL. The arrows point to breakpoints at which chromosomes 2, 14 and 22 reciprocally exchange chromosomal segments with chromosome 8.

Using somatic cell hybrids made between BL cells with the typical t(8;14) and mouse plasmacytoma, Croce and his co-workers were able to prove that the heavy-chain locus was directly involved in this translocation: hybrid cells that retained the 14q+ chromosome were found to carry the human Ig heavy chain constant region (C_H) genes, while hybrid cells that retained the 8q- chromosome contained the Ig heavy-chain variable (V_H) genes[56]. It was demonstrated that, at least in the cases studied, the break on chromosome 14 directly involves the heavy-chain locus, separating the variable and constant regions. Later, using the same approach, Croce's group obtained similar results for variant translocations. The chromosome breakpoint on chromosomes 2 and 22 splits the light-chain kappa and lambda loci respectively, and, as a consequence, the constant light-chain genes move to the 8q+ chromosome markers[57–59].

The breakpoints on chromosomes 14, 2 and 22 were thus localized at the gene level. Was it possible to localize them more precisely at the molecular or nucleotide level, and to identify the segment of chromosome 8 involved in the exchange?

c-myc oncogene is involved in chromosomal translocations in BL

The identification of the cross-over point of the translocation on chromosome 8 was facilitated by the finding in mouse plasmacytoma of aberrantly rearranged fragments of DNA that were joined to the Ig switch region[60–62]. The finding by several groups that in these fragments the IgA constant gene and a cellular oncogene, c-*myc*, were physically linked in a single fragment of DNA suggested a similar phenomenon in BL[63]. The c-*myc* oncogene was localized to human chromosome 8 band q24 by several investigators, and was thus considered to be potentially the oncogene involved in chromosomal translocations in BL[8,64,65]. However, localization of the c-*myc* gene to chromosome band 8q24 approximates its actual location only within 10^7 base pairs. The role of c-*myc* in BL could be further elucidated only by identification of the chromosomal breakpoint on chromosome 8 at the molecular level.

Using a molecular probe for the c-*myc* gene in a Southern blot hybridization of DNA from normal and BL cells, rearrangements were detected within a few thousand base pairs of the c-*myc* gene. Our studies in P. Leder's laboratory demonstrated that most BL cells with the t(8;14) translocation showed rearrangement near c-*myc* [8] (Fig. 8.2). However, only two out of approximately 10 t(2;8) or t(8;22) BL cell lines showed rearrangement near c-*myc*. We considered that perhaps the translocation breakpoint in these other cell lines was near c-*myc* but further than could be detected using genomic blot studies.

The heavy-chain class switching region is frequently the site of the chromosomal translocation in BL cells

Presumably the rearrangement of the c-*myc* gene detected in genomic blot studies of t(8;14) BL cells was due to its translocation into the Ig heavy-chain

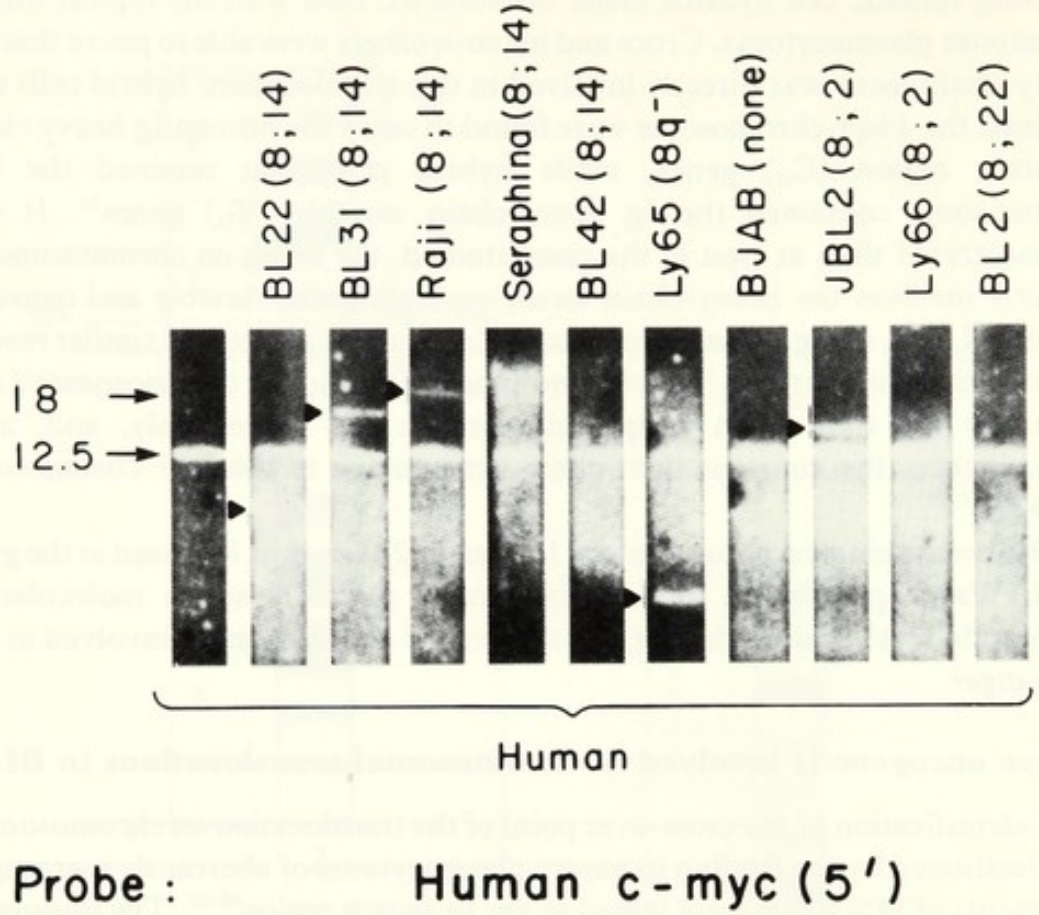

Fig. 8.2 Demonstration of rearranged c-*myc* containing fragments in BL cells: Southern blot analysis of DNA derived from control (c) and BL cell lines that have been digested with restriction enzyme EcoR1 and probed with a human c-*myc* DNA fragment. The normal 12.5 kb EcoR1 fragment is indicated by an arrow and the rearranged c-*myc* fragments are indicated by triangles. The designation of each Burkitt's cell line is indicated above each line with the type of translocation.

gene locus. As has been described (see Ch. 5), the Ig gene loci undergo somatic rearrangements in antibody-producing cells. Since BL cells are B cells at a stage of intermediate development, producing varying amounts of antibodies, both heavy- and light-chain genes could be rearranged. However, genomic Southern blots studies showed uncharacteristic as well as characteristic rearrangements in heavy-chain gene regions in some t(8;14) cells. Further studies demonstrated that these unusual rearrangements were due to translocation of the c-*myc* oncogene into the Ig heavy-chain locus, often within the heavy-chain class switching region.

Segments of DNA from several t(8;14) cell lines containing both the c-*myc* gene and the Ig gene region were isolated using molecular cloning techniques (Fig. 8.3)[8,66–68]. Analysis of these segments of DNA in several laboratories showed that the translocation breakpoint is variable both with respect to the c-*myc* gene and to the Ig gene locus. While the t(8;14) translocation breakpoint frequently involves the IgM switching region, in some cases it has been shown to occur further upstream within joining (J) region segments[69] or further

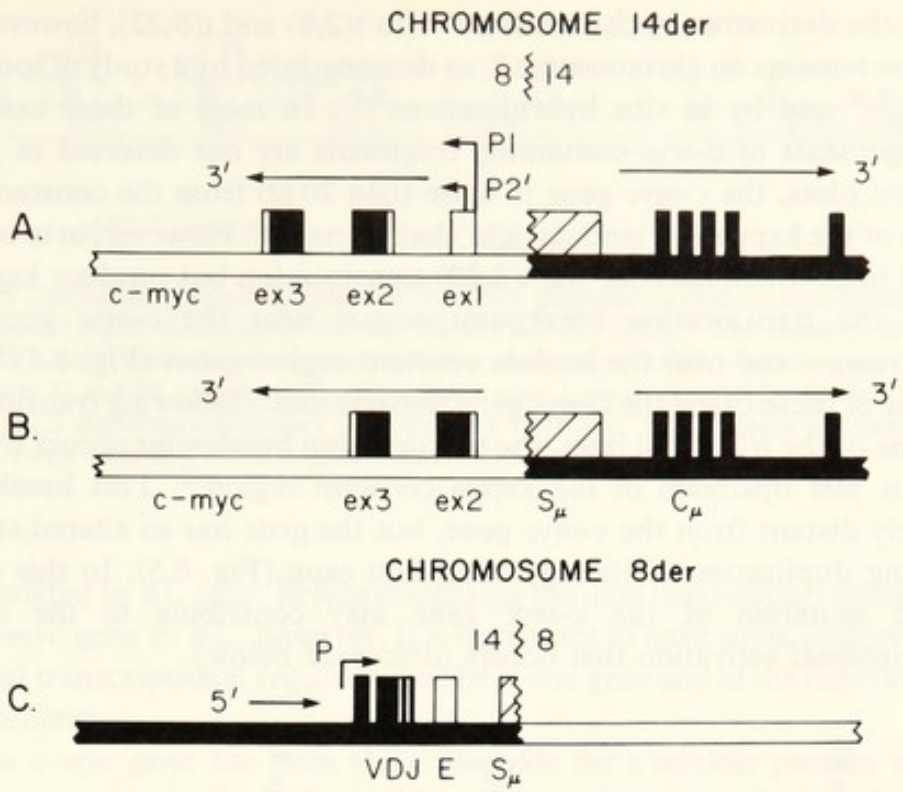

Fig. 8.3 Diagrammatic representation of DNA fragments formed by translocation between chromosomes 8 and 14. (A) Map of the genes as they rearranged the Burkitt line BL 22[70] The coding sequences are indicated by boxes. The filled boxes have amino-acid coding function. The hatched box is the switch signal. The transcriptional direction of each segment is given by the arrow above it. (B) Map of the genes as they rearranged in the Burkitt line LY65[69]. (C) Hypothetical representation of the reciprocal product that one would expect in the translocation shown in (A). E segment refers to the position of the suspected human IgH enhancer sequence.

downstream within the IgG locus[67]. Diagrams of two of these translocation breakpoints, both involving the IgM switching region, are shown in Fig. 8.3. The normal c-*myc* gene has three regions or exons, which are present in the processed mature *myc* messenger RNA. The first of these coding regions is untranslatable into protein and presumably has some role in the regulation of c-*myc* transcription. Following the translocation, the c-*myc* gene is oriented in a head-to-head fashion with the Ig region segments (Fig. 8.3A). In this case, the c-*myc* gene remains intact following the translocation, but frequently, the three-exon structure of the gene is interrupted by the translocation, the first, noncoding exon being removed to the reciprocal translocated chromosome (Fig. 8.3B). The translocation is almost exactly reciprocal, occurring within nonhomologous regions of DNA and resulting in the loss of only a small number of nucleotide base pairs at the translocation breakpoint[70,71].

In variant translocations, the breakpoint is often distant from c-*myc*

Because of the orientation of the c-*myc* gene on chromosome 8 and the Ig genes on chromosome 14, the t(8;14) translocation results in movement of the c-*myc*

gene to the derivative 14 chromosome[65]. In t(2;8) and t(8;22), however, the c-*myc* gene remains on chromosome 8, as demonstrated by a study of somatic cell hybrids[57,58] and by in situ hybridizations[72,73]. In most of these cases, since rearrangements of c-*myc*-containing fragments are not detected in genomic Southern blots, the c-*myc* gene is more than 20 kb from the constant-region portion of the kappa and lambda light chain gene loci. However, in two unusual BL cell lines which harbour the t(8;22) translocation but produce kappa light chains, the translocation breakpoint occurs near the c-*myc* gene – just downstream – and near the lambda constant-region genes (Fig. 8.4)[74,75]. In at least one of these cases, the c-*myc* gene remains intact following translocation[74].

In one of the t(2;8) cell lines, the translocation breakpoint occurs within the Ig locus just upstream of the kappa constant region[72]. This breakpoint is relatively distant from the c-*myc* gene, but the gene has an altered structure, including duplication of its regulating first exon (Fig. 8.5). In this cell line, somatic mutation of the c-*myc* gene may contribute to the observed transcriptional activation that occurs (discussed below).

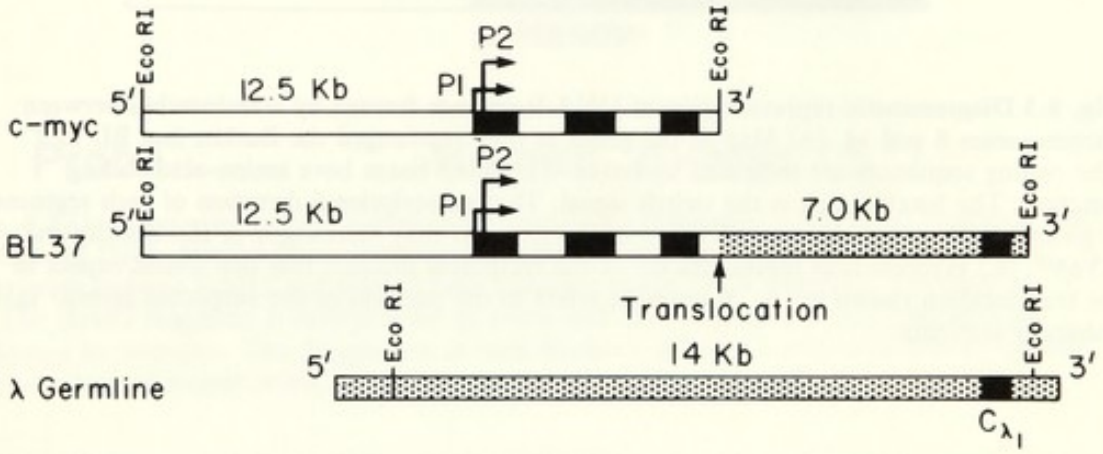

Fig. 8.4 Diagrammatic representation of the DNA fragment formed by translocation between chromosome 8 and chromosome 22 in the Burkitt cell line BL 37[74]. Upper lane shows the 12.5 kb EcoR1 germ-line c-*myc* fragment. The second lane represents the 19.5 kb EcoR1 clone corresponding to the breakpoint between chromosomes 8 and 22, with a 12.5 kb *myc* part and a 7 kb fragment containing Cλ gene. Third lane Cλ gene is in germ line configuration.

The wide variation in the location of translocation breakpoints (Fig. 8.6) within the Ig loci and with respect to c-*myc* makes it difficult to suggest a mechanism by which gross rearrangement of chromosomal material has occurred. What is clear is that the oncogene is being rearranged into recombinationally and transcriptionally active loci in these B cells.

The function of c-*myc* in normal cells

If the translocation of the c-*myc* gene is important in the tumourigenesis of BL cells, as the consistency of the translocation would imply, then it should be possible to demonstrate that the c-*myc* gene is transcriptionally activated or

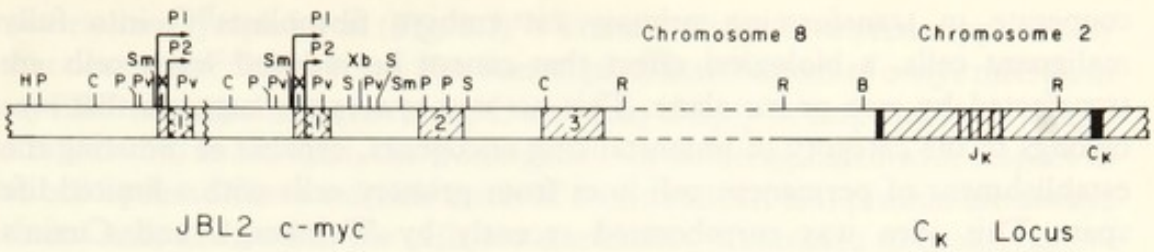

Fig. 8.5 Diagrammatic representation of the DNA fragment formed by translocation between chromosome 8 and chromosome 2 in the Burkitt cell line JBL2[72]. The locations of the c-*myc* exons 1, 2 and 3 are indicated by striped boxes and the two mRNA initiation sites, P1 (promoter 1) and P2 (promoter 2) within c-*myc* exon 1 are designated by arrows. The position of the JBL2 duplication boundary is shown by a wavy line. The Cκ locus is situated 3′ to the altered c-*myc* gene at a distance greater than 20 kb.

deregulated in BL cells. Before discussing the data that support deregulation of the c-*myc* gene in BL, however, it is important to have some understanding of normal transcriptional regulation of the c-*myc* gene and of the function of the c-*myc* protein.

The c-*myc* gene has been shown to code for a nuclear protein with DNA binding properties[76]. It is highly conserved over evolution, homologous sequences having been found in organisms as simple as yeast and *Drosophila,* but specific details of their function are not available. The c-*myc* protein may have a housekeeping function, in that c-*myc* mRNA is normally present in most of the cells that have been examined[77]. Transcription of the c-*myc* gene appears to be cell cycle-specific, since c-*myc* mRNA is not present in the G_0 phase of the cell cycle but appears shortly after cells enter the G_1 phase. For instance, when quiescent fibroblasts are stimulated with platelet derived growth factor (PDGF) or when lymphocytes are stimulated with lymphocyte mitogens, the level of c-*myc* mRNA and protein increases dramatically within two hours[77]. In order to study the biological activity of c-*myc* more directly, transfections of various DNA preparations containing activated *myc* oncogene were performed. The results indicate that the *myc* oncogene is not capable of transforming mouse NIH-3T3 cells like mutated *ras* genes do; however *ras* and *myc* genes can

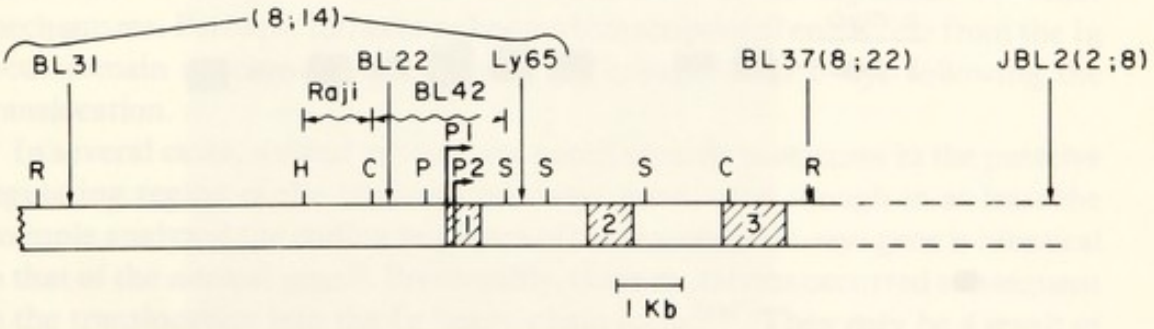

Fig. 8.6 Location of translocation breakpoint with respect to the c-*myc* gene in 7 BL cell lines. The 3 c-*myc* exons are numbered and located with respect to various restriction sites.

cooperate in transforming primary rat embryo fibroblasts[78,79] into fully malignant cells, a biological effect that cannot be induced when cells are transfected by *myc* or *ras* alone. This *ras-myc* synergism suggests that *myc* belongs to the category of immortalizing oncogenes, capable of inducing the establishment of permanent cell lines from primary cells with a limited life span. This idea was corroborated recently by Weinberg's and Cuzin's groups[79,80].

All of these results suggest that c-*myc* plays a very important role in promoting normal cellular growth. Alterations of c-*myc* structure and expression have been observed in many tumours other than BL, including a promyelocytic leukaemia HL60 line and a colon carcinoma colo 320 line[81,82] (in which the c-*myc* gene can be detected in multiple copies) and in chicken bursal lymphomas[83] (in which c-*myc* transcription is stimulated by an integrated viral promoter). The role of altered expression of c-*myc* in these diverse tumours may be similar in that in all cases c-*myc* may be acting to promote abnormal cellular proliferation.

Activation of c-myc in BL

The nature of the activation or deregulation of the c-*myc* gene in these tumours may vary. Likewise, c-*myc* may be activated by a variety of mechanisms even within a single tumour type, such as BL, where enhanced transcription of c-*myc* may occur because of its proximity to Ig transcriptional enhancers[84] or, alternatively, because of structural alterations within the c-*myc* gene itself.

When the levels of c-*myc* mRNA in BL cells are measured and compared with those in other cells, they are higher in BL cells than in normal fibroblasts, but about the same or at most increased by two to five times relative to EBV-

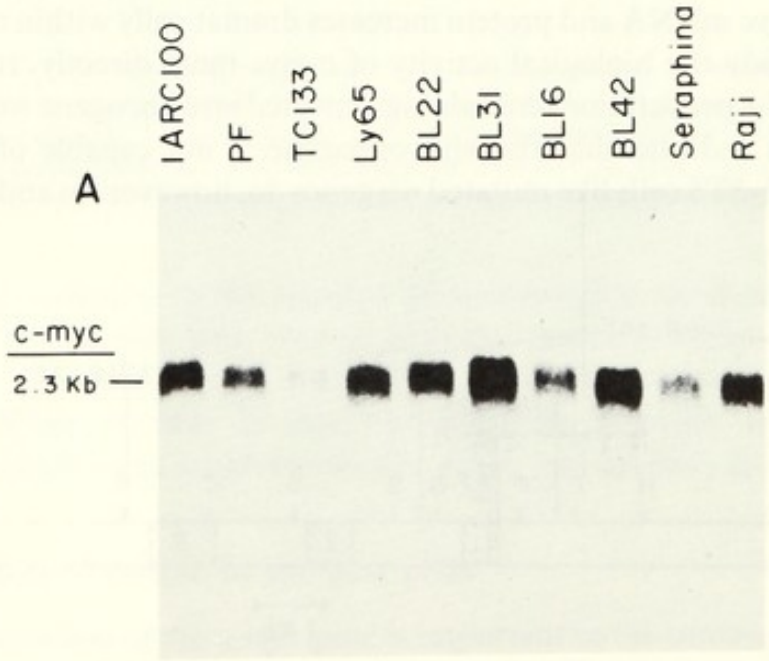

Fig. 8.7 Northern blot analysis of c-*myc* mRNA in lymphoblastoid cells (IARC 100 and PF), fibroblasts (TC 133) and various BL cell lines (as indicated).

immortalized lymphocytes (Fig. 8.7)[69]. These findings have been supported by other observers[85,86], but it has also been argued that the level of c-*myc* mRNA in *both* EBV immortalized cells and BL cells may simply be inappropriately high. Alternatively, in BL cells, the c-*myc* gene may be deregulated with respect to the cell cycle so that moderate levels of c-*myc* mRNA and protein are always present and, as a result, the cells are continually stimulated to proliferate.

One indication of deregulation, other than the slightly elevated levels of c-*myc* mRNA, is an alteration in the utilization of the two c-*myc* promoters that is observed in BL cells. Normally, as shown in Fig. 8.6, the c-*myc* gene can be transcribed from either of two promoters. In fibroblasts and in EBV-immortalized cells, the second of these promoters, P2, is utilized preferentially, whereas in BL cells P1 is used at least equally frequently. Since the first exon region in both P1- and P2-derived transcripts is not translated into protein, both code for the same protein product; however, the presence of increased P1 transcripts in BL cells suggests that abnormal transcriptional activities are acting on the translocated c-*myc* [69].

A more striking observation that indicates that the c-*myc* gene on the translocated chromosome has been deregulated is the finding that, in most BL cells, virtually all of the c-*myc* mRNA has been derived from the c-*myc* gene present on the translocated chromosome and not from the structurally normal c-*myc* gene on the unaffected chromosome 8[69,86]. This suggests a model in which the c-*myc* gene on the translocated chromosome is insensitive to the normal repression of c-*myc* transcription[71,87].

This proposed insensitivity of the translocated c-*myc* gene in BL to normal regulation may be due both to its location within the Ig locus and to alterations in its structure. In most t(8;14) translocations, the c-*myc* gene sits in the heavy-chain Ig region in cells in which active transcription of this region occurs following functional rearrangements of the variable-region segment and its accompanying transcriptional promoter. In BL cells, the c-*myc* gene promoter have rearranged into this locus, albeit in a head-to-head orientation with Ig sequences. It can easily be imagined that this rearrangement might activate the c-*myc* gene; however, in all but a few cases the heavy-chain transcriptional activator or 'enhancer' is no longer present on chromosome 14 following the translocation, but has been removed to the reciprocal chromosome product[70,88]. Activation of c-*myc* in these cases would have to be explained by other mechanisms. Perhaps, hitherto unknown transcriptional enhancers from the Ig locus remain on chromosome 14 and are brought near c-*myc* following the translocation.

In several cases, we and others have noted somatic mutations in the putative regulating region of the translocated c-*myc* gene, even though in at least the example analyzed the coding sequence of the rearranged c-*myc* gene is identical to that of the normal gene[70]. Presumably, these mutations occurred subsequent to the translocation into the Ig heavy-chain locus[69,89]. They may be a result of the proximity of c-*myc* to Ig sequences, which normally appear to have a very high rate of mutation[90]. More striking examples of alterations to the structure of

the c-*myc* gene following translocations are those in which the regulating portion of the c-*myc* gene has been truncated or eliminated from the two protein coding regions. These are the most commonly observed types of translocation in mouse plasmacytomas, and are also commonly seen in BL cells with t(8;14). Another interesting example is the t(2;8) translocation, in which the c-*myc* gene is somewhat removed from the known transcriptional enhancing regions of the Ig kappa locus but has undergone duplication of its putative regulating exon[72]. These observations and those of others indicate that the c-*myc* gene may be deregulated in BL cells as a result both of transcriptional enhancement by proximity to the Ig locus and of alterations in the structure of the c-*myc* gene. It should be noted that in the t(8;14) translocation the chromosome break occurs in the 5′ region of c-*myc*, whereas in both t(2;8) and t(8;22) it occurs in the 3′ region[57,58,72,74,91]. However, in all three types of translocation, the *myc* gene moves to become adjacent to the 5′ region of the Ig constant gene (Fig. 8.8). This position on the human chromosome, in the place of a variable Ig gene, might be critical for the appearance of somatic mutations.

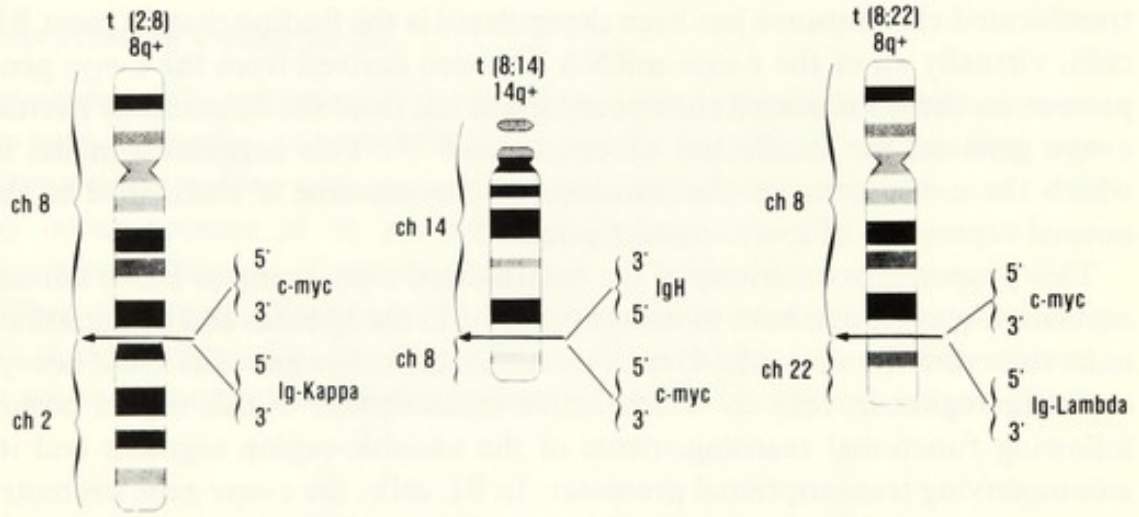

Fig. 8.8 Summary of the critical junctions in BL translocations. In BL with the t(8;14) translocation the c-*myc* gene is translocated to chromosome 14 and becomes adjacent 5′ to the Ig heavy-chain constant region (14q+). In BL with t(8;22) or t(2;8) translocations, c-*myc* remains on the involved chromosome 8 but the genes for the Ig light-chain constant region (Igλ and κ) translocate to a region 3′ to the c-*myc* (8q+). Again as a consequence of the translocation, c-*myc* becomes adjacent 5′ to the Ig constant region.

The fact that, in most variant translocations, the *myc* gene involved is situated at a distance (10 to 50 kb) from the Ig constant gene involved suggested to C. Croce that activation of the *myc* gene could be achieved under the influence of what he defined as stage-specific 'long range enhancers'[92].

CONCLUSIONS AND PERSPECTIVES

Initial studies on BL stressed the importance of the interaction of various disciplines, such as epidemiology, virology and cell biology, for a

comprehensive analysis of the pathogenesis of this cancer. More recently, the addition of cytogenetic techniques, with the powerful tools of modern molecular biology, has proven to be instrumental in dissecting out certain fundamental aspects of human neoplasia, by identifying the genes involved in the tumourigenic process. What is remarkable in the case of BL is that the genes in all four chromosomal regions involved in BL translocations are clearly identified: the c-*myc* oncogene on 8q24 and the Ig loci on 14q32, 2p11 and 22q11. This situation is at present unique in the field of human cancer.

Why do these specific loci, which recombine between each other, have such dramatic consequences? Physiologically, the Ig loci have to undergo site- or region-specific recombination that is critical for the synthesis of antibodies. It can be postulated that factors, or an association of factors (EBV and malaria, for example), that induce profound polyclonal B cell activation in vivo increase the risk of abnormal genetic recombination involving the Ig loci, especially if the stimulated cells are in the process of rearranging their Ig genes. The fact that, in vitro, EBV-immortalized lymphoid cells never develop these translocations might be due to the fact that they have already rearranged their Ig genes functionally. The c-*myc* locus on chromosome 8 does not seem to correspond to a fragile site on the human chromosome. Furthermore, the breakpoint observed in BL varies: 5′ or 3′ to the gene, within the gene (between the first and second exon) or to at least 30 kb downstream. However, the various translocations in BL have at least one feature in common: the c-*myc* gene moves to become adjacent to an Ig gene-constant region (5′ to it), which is normally occupied by the Ig variable regions (Fig. 8.8).

How is *myc* gene expression activated or deregulated? Various hypotheses have been proposed and presented in this review: however, in the absence of knowledge about the physiological role of *myc* and regulation of its expression, a precise assessment of the biological consequence of BL translocations will remain difficult to make. The elegant studies of Croce et al[92] using somatic cell hybrids suggested that regulation of the expression of a c-*myc* gene (translocated or not) also depends largely on the differentiated state of the cell harbouring the oncogene.

Little more than 25 years after its initial identification, can the scenario of BL be described? In Africa, some of the actors were identified following epidemiological surveys. Hyper-holoendemic malaria is considered as one of the BL causal factors, and high anti-EBV titres have been proven to be markers of BL risk. Excessive B cell proliferation induced by EBV in conjunction with the immunosuppressive effect of the parasitic disease increases the risk of appearance of malignant cell clones following *myc* deregulation by specific chromosomal translocations. Outside Africa, in the so-called low-incidence areas, the risk factors have yet to be identified, even though the chromosomal changes are also observed consistently. Do activated oncogenes other than *myc* play a role in the malignant process, perhaps by complementing *myc* action? The latter was postulated by Diamond et al following the discovery of the B *lym* gene, a gene capable of transforming NIH-3T3 cells[93]. However, these

experiments have yet to be reproduced by others. Activated *ras* genes have also been detected in BL tumours but only rarely (two cases)[94,95].

Until recently, only two main methods were available for identifying cellular oncogenes: either through isolation of their viral counterparts, or by isolation from malignant transformed NIH-3T3 after DNA mediated transfection. Information gained from molecular studies of BL chromosomal translocations may make it possible to envisage new strategies for oncogene identification. For instance, it has been suggested that by taking advantage of specific chromosomal translocations to the Ig locus in other B-cell neoplasia and to T-cell receptor genes in T-cell lymphomas and leukaemias the genes involved in the neoplastic process could be isolated if they moved to become adjacent to the Ig or T cell receptor locus. Some results already obtained in some B-cell neoplasia with either t(11;14) or t(14;18) are very encouraging in this regard[96,97].

Acknowledgements

Several experiments reported in this review were performed in the laboratory of Dr P Leder, Department of Genetics, Harvard Medical School, Boston MA 02115 with grant support from E J D Pont de Nemours and Company Inc and from the American Business Cancer Research Foundation.

REFERENCES

1. Bishop J M 1983 Cellular oncogenes and retroviruses. Annual Review of Biochemistry 52: 301–354
2. Tabin C, Bradley S, Bargmann C, Weinberg R, Papageorge A, Scolnick E, Dhar R, Lowy D, Chang E 1982 Mechanisms of activation of a human oncogene. Nature 300: 762–765
3. Yunis J 1983 The chromosomal basis of human neoplasia. Science 221: 227–236
4. Nowell P C, Hungerford D A 1960 A minute chromosome in human granulocytic leukemia. Science 132: 1497
5. Rowley J D 1973 A new consistent chromosomal abnormality in chronic myelogenous leukaemia. Nature 243: 290–293
6. Manolov G, Manolova Y 1972 Marker band in one chromosome 14 from Burkitt lymphomas. Nature 237: 33–34
7. Zech L, Haglund U, Nilsson K, Klein G 1976 Characteristic chromosomal abnormalities in biopsies and lymphoid cell lines from patients with Burkitt and non-Burkitt lymphomas. International Journal of Cancer 17: 47–56
8. Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P 1982 Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt's lymphoma and murine plasmacytoma cells. Proceedings of the National Academy of Sciences USA, 79: 7837–7841
9. Burkitt D P 1958 A sarcoma involving the jaws in African children. British Journal of Surgery 46: 218–223
10. Burkitt D P 1962 A children's cancer dependent on climatic factors. Nature 194: 232–234
11. O'Conor G T 1961 Malignant lymphoma in African children. II. A pathological entity. Cancer 14: 270–283
12. Wright D H 1963 Cytology and histochemistry of the Burkitt's lymphoma. British Journal of Cancer 17: 50–55
13. Berard C W, O'Conor G T, Thomas L B, Torloni H (eds) 1969 Histopathological definition of Burkitt's tumour. Bulletin of the World Health Organization 40: 601–607

14. Rosenberg S A (Chairman) 1982 National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Summary and description of a working formulation for clinical usage. Cancer 49: 2112–2135
15. Klein E, Klein G, Nadkarni J S, Nadkarni J J, Wigzell H, Clifford P 1967 Surface IgM specificity on cells derived from a Burkitt's lymphoma. Lancet 2: 1068–1070
16. Preud'homme J L, Flandrin G, Daniel M T, Brouet J C 1975 Burkitt's tumor cells in acute leukemia. Blood 46: 990–992
17. Epstein M A, Achong B G, Barr Y M 1964 Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1: 702–703
18. Burkitt D P 1983 The discovery of Burkitt's lymphomas. Cancer 51: 1777–1786
19. Lenoir G M, Philip T, Sohier R 1984 Burkitt-type lymphomas—EBV association and cytogenetic markers in cases from various geographic locations. In: Magrath I T, O'Conor G T, Ramot B (eds) Pathogenesis of leukemias and lymphomas: environmental influences. Raven Press, New York, p 283–295
20. O'Conor G T, Rappaport H, Smith E B 1985 Childhood lymphoma resembling 'Burkitt tumor' in the United States. Cancer 18: 411–417
21. Philip T, Lenoir G M, Bryon P A, Gerard-Marchant R, Souillet G, Philippe N, Freycon F, Brunat-Mentigny M 1982 Burkitt-type lymphoma in France among non-Hodgkin malignant lymphomas in Caucasian children. British Journal of Cancer 45: 670–678
22. de-Thé G, Geser A, Day N E, Tukei P M, Williams E H, Beri D P, Smith P G, Dean A G, Bornkamm G W, Feorino P, Henle W 1978 Epidemiological evidence for a causal relationship between Epstein-Barr virus and Burkitt's lymphoma: Results of a Ugandan prospective study. Nature 274: 756–761
23. Geser A, Lenoir G, Anvret M, Bornkamm G W, Klein G, Williams E H, Wright D H, de-Thé G 1983 Epstein-Barr virus markers in a series of Burkitt's lymphoma from the West Nile District of Uganda. European Journal of Cancer and Clinical Oncology 19: 1394–1404
24. Pope J H, Horne M K, Scott W 1968 Transformation of foetal human leukocytes in vitro by filtrates of a human leukaemic cell line containing herpes-like virus. International Journal of Cancer 3: 857–866
25. Nilsson K, Ponten J 1975 Classification and biological nature of established human hematopoietic cell lines. International Journal of Cancer 15: 321–341
26. Nilsson K, Giovenella B C, Stehlin J S, Klein G 1977 Tumorigenicity of human hematopoietic cell lines in athymic nude mice. International Journal of Cancer 19: 337–344
27. Epstein M A, Barr Y M 1964 Cultivation in vitro of human lymphoblasts from Burkitt's malignant lymphoma. Lancet 1: 252–253
28. Lenoir G M, Vuillaume M, Bonnardel C 1985 The use of lymphomatous and lymphoblastoid cell lines in the study of Burkitt's lymphoma. In: Lenoir G M, O'Conor G, Olweny C L M (eds) Burkitt's lymphoma: a human cancer model. IARC Scientific Publications No. 60, Lyon, p 309–318
29. Preud'homme J L, Dellagi K, Gugliemi P, Vogler L B, Danon F, Lenoir G M, Valensi F, Brouet J C 1985 Immunologic markers of Burkitt's lymphoma cells. In: Lenoir G M, O'Conor G, Olweny C L M (eds) Burkitt's lymphoma: a human cancer model. IARC Scientific Publications No. 60, Lyon, p 47–64
30. Berger R, Bernheim A 1985 Cytogenetics of Burkitt's lymphoma-leukaemia: a review. In: Lenoir G M, O'Conor G, Olweny C L M (eds) Burkitt's lymphoma: a human cancer model. IARC Scientific Publications No. 60, Lyon, p 65–80
31. Jarvis J E, Ball G, Rickinson A B, Epstein M A 1974 Cytogenetic studies on human lymphoblastoid cell lines from Burkitt's lymphomas and other sources. International Journal of Cancer 14: 716–721
32. Kaiser-McCaw B, Epstein A L, Kaplan H S, Hecht F 1977 Chromosome 14 translocation in African and North American Burkitt's lymphoma. International Journal of Cancer 19: 482–486
33. Miyoshi I, Hiraki S, Kimura I, Miyamoto K, Sato J 1979 2/8 translocation in a Japanese Burkitt's lymphoma. Experientia 35: 742–743
34. Van den Berghe H, Parloir A, Gosseye S, Englebienne V, Cornu G, Sokal G 1979 Variant translocation in Burkitt lymphomas. Cancer Genetics and Cytogenetics 1: 9–14

35. Bornkamm G W, Kaduk B, Kachel G, Schneider U, Fresen K O, Schwanitz G, Hermanek P 1980 Epstein-Barr virus positive Burkitt's lymphoma in a German woman during pregnancy. Blut 40: 167–177
36. Berger R, Bernheim A, Weh H J, Flandrin G, Daniel M T, Brouet J C, Colbert N 1979 A new translocation in Burkitt's tumor cells. Human Genetics 53: 111–112
37. Bernheim A, Berger R, Lenoir G 1981 Cytogenetic studies on African Burkitt's lymphoma cell lines t(8;14), t(2;8) and t(8;22). Cancer Genetics and Cytogenetics 3: 302–315
38. Miyoshi I, Hamasaki K, Miyamoto K, Nagase K, Narahawa K, Kitajima K, Kimura I, Sato J 1981 Chromosome translocations in Burkitt's lymphoma. New England Journal of Medicine 304: 734
39. Bernheim A, Berger R, Lenoir G 1983 Cytogenetic studies on Burkitt's lymphoma cell lines. Cancer Genetics and Cytogenetics 8: 223–229
40. Mark-Vendel E, Philip T, Ladjadj Y, Aboulola M, Lenoir G M 1983 Chromosomal translocations in Algerian Burkitt's lymphoma. Lancet 8353: 788
41. Manolova Y, Manolov G, Kieler J, Levan A, Klein G 1979 Genesis of the 14q+ marker in Burkitt's lymphoma. Hereditas 90: 5–10
42. Berger R, Bernheim A, Daniel M T, Valensi F, Flandrin G 1983 Cytological types of mitoses and chromosome abnormalities in acute leukemia. Leukemia Research 7: 221–236
43. Berger R, Bernheim A, Sigaux F, Valensi F, Daniel M T, Flandrin G 1985 Two Burkitt's lymphomas with chromosome 6 long arm deletions. Cancer Genetics and Cytogenetics 15: 159–167
44. Mitelman F 1981 Marker chromosome 14q+ in human cancer and leukemia. Advances in Cancer Research 34: 141–170
45. Sigaux F, Berger R, Bernheim A, Valensi F, Daniel M T, Flandrin G 1984 Malignant lymphomas with band 8q24 chromosome abnormality: a morphologic continuum extending from Burkitt's to immunoblastic lymphoma. British Journal of Haematology 57: 393–405
46. Shade M, Woodward M A, Steel C M 1980 Chromosome aberrations acquired in vitro by human B-cell lines. II Distribution of break points. Journal of the National Cancer Institute, 65: 101–109
47. Kamada N, Uchino H 1982 Chronologic sequence in appearance of clinical and laboratory findings characteristic of chronic myelocytic leukemia. Blood 51: 843–850
48. Bernheim A, Berger R, Preud'homme J L, Labaume S, Bussel A, Barot-Ciorbaru R 1981 Philadelphia chromosome positive blood B lymphocytes in chronic myelocytic leukemia. Leukemia Research 5: 331–339
49. Croce C M, Shander M, Martinis J, Cicurel L, D'Ancona G G, Dolby T W, Koprowski H 1979 Chromosomal location of the genes for human immunoglobulin heavy chains. Proceedings of the National Academy of Sciences USA 76: 3416–3419
50. Kirsch I R, Morton C C, Nakahara K, Leder P 1982 Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. Science 216: 301–303
51. Malcolm S, Barton P, Murphy C, Ferguson-Smith M A, Bentley D L, Rabbits T H 1982 Localization of human immunoglobulin K light chain variable region genes to the short arm of chromosome 2 by in situ hybridization. Proceedings of the National Academy of Sciences USA 79: 4957–4961
52. McBride O Wm Heiter P A, Hollis G F, Swan D, Otey M C, Leder P, Potter M 1982 Chromosomal location of human kappa and lambda immunoglobulin light chain constant region genes. Journal of Experimental Medicine 155: 1480–1490
53. Lenoir G, Preud'homme J L, Bernheim A, Berger R 1982 Correlation between immunoglobulin light chain expression and variant translocation in Burkitt's lymphoma. Nature 298: 474–476
54. Ohno S, Babonits M, Wiener F, Spira J, Klein G, Potter M 1979 Nonrandom chromosome changes involving the Ig gene-carrying chromosomes 12 and 6 in pristane-induced mouse plasmacytomas. Cell 18: 1001–1007
55. Klein G 1981 The role of gene dosage and genetic transplantation in carcinogenesis. Nature 294: 313–318
56. Erikson J, Finan J, Nowell P C, Croce C M 1982 Translocation of immunoglobulin V_H

genes in Burkitt lymphoma. Proceedings of the National Academy of Sciences USA 79: 5611–5615

57. Croce C M, Thierfelder W, Erikson J, Nishikura K, Finan J, Lenoir G M, Nowell P C 1983 Transcriptional activation of an unrearranged and untranslocated c-myc oncogene by translocation of a Cλ locus in Burkitt lymphoma cells. Proceedings of the National Academy of Sciences USA 80: 6922–6926
58. Erikson J, Nishikura K, Ar-Rushdi A, Finan J, Emanuel B, Lenoir G, Nowell P C, Croce C M 1983 Translocation of an immunoglobulin K locus to a region 3′ of an unrearranged c-*myc* oncogene enhances c-*myc* transcription. Proceedings of the National Academy of Sciences USA 80: 7581–7585
59. de la Chapelle A, Lenoir G, Boué J, Boué A, Gallano P, Huerre C, Szajner M F, Jean-Pierre M, Lalouel J M, Kaplan J C 1983 Lambda Ig constant region genes are translocated to chromosome 8 in Burkitt's lymphoma with t(8;22). Nucleic Acids Research 11: 1133–1142
60. Kirsh I R, Ravetch J V, Kwan S P, Max E E, Ney R L, Leder P 1981 Multiple immunoglobulin switch region homologies outside the heavy constant region locus. Nature 293: 585–587
61. Adams J M, Gerondakis S, Webb E, Mitchell J, Bernard O, Cory S 1982 Transcriptionally active DNA region that rearranges frequently in murine lymphoid tumors. Proceedings of the National Academy of Sciences USA 79: 6966–6970
62. Harris L J, Lang R B, Marcu K B 1982 Non-immunoglobulin-associated DNA rearrangements in mouse plasmacytomas. Proceedings of the National Academy Sciences USA 79: 4175–4179
63. Shen-Ong G L, Keath E J, Piccoli S P, Cole M D 1982 Novel *myc* oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell 31: 443–452
64. Neel B G, Jhanwar S C, Chaganti R S K, Hayward W S 1982 Two human c-onc genes are located on the long arm of chromosome 8. Proceedings of the National Academy of Sciences USA 79: 7842–7846
65. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo R C, Croce C M 1982 Human c-*myc* oncogene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proceedings of the National Academy of Sciences USA 79: 7824–7827
66. Dalla-Favera R, Martinotti S, Gallo R C, Erikson J, Croce C M 1983 Translocation and rearrangements of the c-*myc* oncogene locus in human undifferentiated B-cell lymphomas. Science 219: 963–967
67. Hamlyn P H, Rabbitts T H 1983 Translocation joins c-*myc* and immunoglobulin γ1 genes in a Burkitt lymphoma revealing a third exon in the c-*myc* oncogene. Nature 304: 135–139
68. Adams J M, Gerondakis S, Webbe E, Corcoran M M, Cory S 1983 Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. Proceedings of the National Academy of Sciences USA 80: 1982–1986
69. Taub R, Moulding C, Battey J, Murphy W, Vasicek T, Lenoir G M, Leder P 1984 Activation and somatic mutation of the translocated c-*myc* gene in Burkitt lymphoma cells. Cell 36: 339–348
70. Battey J, Moulding C, Taub R, Murphy W, Stewart T, Potter H, Lenoir G, Leder P 1983 The human c-*myc* oncogene: Structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell 34: 779–787
71. Leder P, Battey J, Lenoir G, Moulding C, Murphy W, Potter H, Stewart T, Taub R 1983 Translocations among antibody genes in human cancer. Science 222: 765–771
72. Taub R, Kelly K, Battey J, Latt S, Lenoir G M, Tantravahi U, Tu Z, Leder P 1984 A novel alteration in the structure of an activated c-*myc* gene in a variant t(2;8) Burkitt lymphoma. Cell 37: 511–520
73. Rappold G A, Hameister H, Cremer T, Adolph S, Henglein B, Freese U K, Lenoir G M, Bornkamm G W 1984 c-*myc* and immunoglobulin kappa light chain constant genes are on the 8q+ chromosome of three Burkitt lymphoma lines with t(2;8) translocations. The EMBO Journal 3: 2951–2955
74. Hollis G F, Mitchell K F, Battey J, Potter H, Taub R, Lenoir G M, Leder P 1984 A variant translocation places the lambda immunoglobulin genes 3′ to the c-*myc* oncogene in Burkitt's lymphoma. Nature 307: 752–755

75. Magrath I, Erikson J, Whang-Peng J, Sieverts H, Armstrong G, Benjamin D, Triche T, Alabaster O, Croce C M 1983 Synthesis of kappa light chains by cell lines containing an 8;22 chromosomal translocation derived from a male homosexual with Burkitt's lymphoma. Science 222: 1094–1098
76. Hann S R, Eisenman R N 1984 Proteins encoded by the human c-*myc* oncogene: Differential expression in neoplastic cells. Molecular and Cellular Biology 4: 2486–2497
77. Kelly K, Cochran B H, Stiles C D, Leder P 1983 Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell 85: 603–610
78. Land H, Parada L F, Weinberg R A 1983 Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304: 596–602
79. Land H, Parada L F, Weinberg R A 1983 Cellular oncogenes and multistep carcinogenesis. Science 222: 771–778
80. Mougneau E, Lemieux L, Rassoulzadegan M, Cuzin F 1984 Biological activities of v-*myc* and rearranged c-*myc* oncogenes in rat fibroblast cells in culture. Proceedings of the National Academy of Sciences USA 81: 5758–5762
81. Dalla Favera R, Wong-Staal F, Gallo R C 1982 Onc gene amplification in promyelocytic leukaemia cell line HL-60 and primary leukaemic cells of the same patient. Nature 299: 61–63
82. Alitalo K, Schwab M, Lin C C, Varmus H E, Bishop J M 1983 Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. Proceedings of the National Academy of Sciences USA 80: 1707–1711
83. Hayward W S, Neel B G, Astrin S M 1981 Activation of a cellular oncogene by promoter insertion in ALV induced lymphoid leukosis. Nature 290: 475–480
84. Hayday A C, Gillies S D, Saito H, Wood C, Wiman K, Hayward W S, Tonegawa S 1984 Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus. Nature 307: 334–340
85. Erikson J, Ar-Rushdi A, Drwinga H L, Nowell P C, Croce C M 1983 Transcriptional activation of the translocated c-*myc* oncogene in Burkitt lymphoma. Proceedings of the National Academy of Sciences USA 80: 820–824
86. Nishikura K, Ar-Rushdi A, Erikson J, Watt R, Rovera G, Croce C M 1983 Differential expression of the normal and the translocated human c-*myc* oncogenes in B cells. Proceedings of the National Academy of Sciences USA 80: 4822–4826
87. Rabbitts T H, Forster A, Hamlyn P, Baer R 1984 Effect of somatic mutation within translocated c-*myc* genes in Burkitt's lymphoma. Nature 309: 592–597
88. Rabbitts T H, Forster A, Baer R, Hamlyn P H 1983 Transcription enhancer identified near the human Cμ immunoglobulin heavy chain gene is unavailable to the translocated c-*myc* gene in a Burkitt lymphoma. Nature 306: 806–809
89. Rabbitts T H, Hamlyn P H, Baer R 1983 Altered nucleotide sequences of a translocated c-*myc* gene in Burkitt lymphoma. Nature 306: 760–765
90. Tonegawa S 1983 Somatic generation of antibody diversity. Nature 302: 575–581
91. Davis M, Malcolm S, Rabbitts T H 1984 Chromosome translocation can occur on either side of the c-*myc* oncogene in Burkitt lymphoma cells. Nature 308: 286–288
92. Croce C M, Nowell P C 1985 Molecular basis of human B cell neoplasia. Blood 65: 1–7
93. Diamond A, Cooper G M, Ritz J, Lane A 1983 Identification and molecular cloning of the human B-*lym* transforming gene activated in Burkitt's lymphomas. Nature 305: 112–116
94. Murray M, Cunningham J, Parada L, Dautry F, Lebowitz P, Weinberg R A 1983 The HL-60 transforming sequence: a *ras* oncogene coexisting with altered *myc* genes in hematopoietic tumors. Cell 33: 749–757
95. Lenoir G M, Land H, Parada L F, Cunningham J M, Weinberg R A 1984 Activated oncogenes in Burkitt's lymphoma. Current Topics in Microbiology and Immunology 113: 6–14
96. Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell P C, Croce C M 1984 Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. Science 224: 1403–1406
97. Tsujimoto Y, Finger L R, Yunis J, Nowell P C, Croce C M 1984 Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226: 1097–1099

Index

1987-1
88-1