



Wellcome Film Project

Chromosome Recombination in Bacteriophage Lambda

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Colour

Duration: 00:08:38:21

00:00:00:00

<Opening titles>

<Brian W Bainbridge over animated diagram showing structure of bacteriophage lambda, then table listing processes to be covered in lecture>

Bacteriophage lambda is a virus which attacks the gut bacterium *Escherichia coli*, or *E. coli*, for short. The aim of this programme is to illustrate chromosome behaviour in this bacteriophage which is usually simply referred to as phage lambda.

It has two alternative life cycles: a virulent cycle when the host is killed and phage is released or the lysogenic cycle when the phage chromosome integrates into the host chromosome.

Here is a summary of the processes which will be covered. These are: circularisation, integration, excision, illegitimate excision, linearization and transducing phage and heterogenotes.

<Bainbridge over electron micrograph of phage lambda and animated diagram of phage lambda chromosome>

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Phage lambda has a simple symmetrical head and a flexible tail. Here is a negatively stained electron micrograph of the medically intact phage particle. Inside the head is a linear chromosome illustrated here as a double-stranded DNA molecule. There are 30 to 40 genes and 4 of these A, J, N and R are shown to illustrate the orientation of the chromosome. In the centre is the attachment site involved in the integration process. P prime and P are different phage sequences, or arms, which are either side of a core sequence of 15 base pairs represented here simply by a dot. At each end of the phage chromosome are single-stranded regions of 12 bases which are complementary to each other. These are the sticky ends involved in rejoining to produce a circle. Circularisation results in the linear chromosome forming a circle, the sticky ends join together and a ligase enzyme seals the gaps.

<Bainbridge over electron micrograph of thin section of E. coli cells, then animated diagram showing phage lambda chromosome>

This occurs in the bacterial cell and the attachment site pairs with the equivalent attachment site on the bacterial chromosome. B prime and B are different bacterial sequences on either side of the core sequence of 15 base pairs, which is also found in the phage attachment site. This is the actual sequence of 15 base pairs. Next we see the circular phage chromosome and the circular bacterial chromosome which are about to pair. The phage integrates into the bacterial chromosome near to the galactose locus.

<Intertitle>

Integration

<Bainbridge over animated diagram showing integration of the phage chromosome by recombination>

Integration of the circular phage chromosome now occurs by recombination. This is not an exact reciprocal event and there are 2 staggered breaks, 7 base pairs apart.

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The single-strand regions which result, rejoin in a cross-shaped fashion to form a figure of 8. The figure of 8 resolves into a circular molecule in which the phage chromosome is integrated into the length of the bacterial chromosome. The order of genes on the integrated chromosome is now different from the order on the original phage chromosome.

In this state the phage chromosome is known as a pro-phage and persists in this state from cell division to cell division. This is very similar to the occurrence of latent viruses in other systems, such as herpes virus in man which can remain latent and recur at later stages.

<Intertitle>

Excision

<Bainbridge over Petri dish showing phage excision>

The reverse of the integration process is known as excision. And this results in the lytic cycle with the release of phage particles. These can attack bacteria to produce clear areas known as plaques on bacterial lawns. This is a Petri dish containing both bacterial lawn and phages which have resulted in phage plaques. Ultraviolet light can be used to induce phage excision and the lytic cycle in bacteria carrying the pro-phage.

00:05:15:00

<Intertitle>

Illegitimate excision

<Bainbridge over animated diagram showing illegitimate excision of phage chromosome>

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On rare occasions, abnormal or illegitimate recombination can occur. This is similar to excision but recombination takes place at a site away from the common core site. This results in insertion of bacterial DNA into the phage chromosome and the transfer of phage genes into the bacterial chromosome. The phage which is produced may be defective if essential genes are lost or it may still produce plaques if non-essential genes have been removed.

<Intertitle>

Linearization

<Bainbridge over animated diagram showing linearization of phage chromosome>

The circular phage chromosome then undergoes linearization and is packaged into a phage particle. This phage can now transfer the galactose plus gene into another bacterium. It is called the transducing phage. This type of phage was used extensively to clone the lactose operon genes before the advent of genetic engineering.

Infection of a gal negative bacterium by such a transducing phage results in integration, not at the normal attachment site, but at the gal locus. Integration occurs and a pro-phage is produced which is flanked by gal plus and gal negative markers. This bacterium is heterozygous and is known as a heterogenote.

<Intertitle>

Heterogenote

<Bainbridge over animated diagram showing transducing phage, then earlier table listing processes covered in lecture>



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Excision of this pro-phage can again occur to produce a predominantly gal plus transducing phage. This is known as a high frequency transducing system.

To summarise, these processes of integration and excision depend on specific recombination between phage and bacterial chromosome. Errors can occur by illegitimate recombination during excision to insert bacterial DNA into the phage chromosome. The phage produced by this process can re-infect another bacterium to produce transduction and a heterozygous bacterium results which is known as a heterogenote. This heterogenote can be induced to produce high levels of transducing phage.

<End credits>