Wellcome Film Project

Mitochondrial Biogenesis

The Scientific Basis of Medicine

Presented by Dr Thomas S Work.

University of London Audio-Visual Centre, 1973.

Introduced by Dr Ian Gilliland.
Produced by David R Clark.

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Black and White
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<**Opening credits**>

<**Dr Ian Gilliland to camera**>

Dr Work has been head of the Division of Biochemistry at the National Institute for Medical Research in London since 1955. His career began in Edinburgh with Professor George Barger and from thence he came to the Lister Institute in London. In addition to his own original contributions he has been editor of the Biochemical Journal, author of a book on the biochemical basis of chemotherapy and has edited a series of manuals on laboratory techniques. Dr Work has been particularly concerned with protein synthesis in mitochondria and particularly with the characterisation of mitochondrial ribosome and its function as a separate centre for protein synthesis independent of the cytoplasmic ribosome system. His work has been fundamental, along with that of others, in establishing that the mitochondrion is a semi-independent organelle with its own store of genetic information and possible

potential mutation. It is appropriate that the head of this distinguished division should give this discourse on mitochondrial biogenesis. Dr Work.

<**Dr Thomas S Work to camera, standing by large slide showing a hand holding glass of cyanide**>

If a man drinks a glass of cyanide, he dies very quickly and very suddenly**.** Why? Well, the cyanide combines with the cytochrome oxidase of his mitochondria and shuts down his energy production. The association between mitochondria and energy production is very obvious if we look at the cross section of some of those cells that have high energy requirements.

<**Work over slides showing various muscle fibres of the body which utilise mitochondria for energy**>

For example, the heart cell where the individual muscle fibres are interlayered with mitochondria. The insect flight muscle has perhaps the highest energy requirement of any known muscle and in this case the individual fibres are completely surrounded by mitochondria. In the kidney cell also there is considerable need for energy. The kidney cell has to pass large numbers of ions across membranes and to do this it requires the energy. You see here, in this slide, the kidney cell with the mitochondria interdigitated between the folded membranes. The spermatozoan also requires a lot of energy, it after all has to swim vigorously up the vagina and this energy is provided by mitochondria packed into the spermatozoal tail on each side of the fibre.

<**Work to camera then over diagram showing energy yields from sugar before and after oxidation with molecular oxygen; over a slide showing a cross-section of a cell undergoing division, then at higher magnifications to show mitochondria**>

The importance of mitochondria lies in their ability to use molecular oxygen directly. The enzyme cytochrome oxidase is the only enzyme in the body that can interact with molecular oxygen and it is confined entirely to the mitochondria. The advantage

of aerobic oxidation over anaerobic fermentation is very large indeed. For example, to ferment one pound of sugar we get an energy yield of about 125 Kcal. When we oxidise that same sugar with molecular oxygen to carbon dioxide and water, we achieve an energy yield at least 12 times as great.

The mitochondria, of course, are only one type among many types of organelle. Here is a cross-section of a cell just after the process of cell division has got underway and you see the two nuclei separating from one another and the various subcellular organelles moving off into the two new polar regions.

It's difficult to see the mitochondria at that scale, but if we go to this much higher scale magnification, we see up here in the left-hand corner the nuclear membrane, immediately adjacent to it are the mitochondria, these striated organelles. And as we move down the picture we can see the lysosomes, these dark objects here. And if we move right down, to the bottom left-hand corner, we can see a lot of membranous material, the Golgi complex. Even at that magnification we do not see everything.

Let's go to a still higher magnification. Here we see the rough endoplasmic reticulum and if we look at that at the maximum possible magnification, we see that it is studded with little black dots: these are the ribosomes, these little black dots. Now to give you some idea of the scale we're talking about, a ribosome contains at least 70 protein molecules and 3 nucleic acid molecules.

00:06:56:00

<**Work over 3-dimensional model of a ribosome, then to camera**>

We have here a model of the ribosome. This gives you some idea of the complexity of the structure. Here is our ribosome where each of the individual white balls represents a protein molecule and each of the wires here represents one of the nucleic acid molecules and you can perhaps see there its spiralised form.

Why do I regard the mitochondria as the most interesting among all these organelles? Well, it is because they can synthesise proteins independently of the rest of the cell. You will remember that some twelve years ago Crick, Watson, Monroe and others established that the DNA of the nucleus stored the information for cellular reproduction, that this DNA was copied in the form of messenger RNA by an enzyme transcriptase. This process of copying has been photographed by Oscar Miller in some most impressive photographs which you see here.

<**Work over photographs showing process of DNA copying; over earlier 3-dimensional model of ribosome; to camera; over diagram showing mitochondria passing down a sucrose gradient; over table showing levels of mitochondria in DNA; electron micrographs of non-nuclear DNA mitochondria**>

The spine of this feather-like object here is the DNA molecule, the gene. And the enzyme runs up, or rather a series of enzymes run up the spine, each one with an RNA molecule, the fronds sticking out, attached to the enzyme and becoming progressively longer as the enzyme moves up the DNA. Finally, the RNA is released and then passes into the cytoplasm and is attached to the ribosome.

We are not quite sure where the attachment occurs but the message probably passes through this hole in the middle of the ribosome. In doing so, it controls the sequence in which amino acids are put together in order to make the protein molecule.

Twelve years ago it was assumed that all the necessary information for the reproduction of the cell was in the nucleus and that all the DNA of the cell was in the nucleus. But in 1961, we had found that mitochondria separated from a cell homogenate by passing down a sucrose gradient could be shown, by the use of radioactive amino acids, to synthesise proteins quite independently of the other cell organelles.

Two years later, Nass working in Sweden and Luck working in New York both showed that mitochondria contained DNA. This DNA is rather different from the

nuclear DNA; it is a relatively small molecule, molecular weight about 10 million in the case of the animal cell as compared to 500 million or more for the nuclear DNA molecules and these are probably also linear. Whereas the mitochondrial DNA, as you'll see in this photograph here, taken by electron microscopy, is a circular molecule. You see the circle here, passing right round, a complete circle as we've seen earlier, a map of Australia, this is a double-stranded DNA molecule. Not only does the mitochondrion possess this DNA, it also possesses mitoribosomes, that is small ribosomes, and an inner and an outer membrane – you can see the construction of the mitochondrion diagrammatically in this cross section I have drawn here. The outer membrane is a smooth shell, but immediately inside that there is a heavily folded inner membrane and here you see the mitochondrial DNA and the mitoribosomes. Now, let us look in more detail at this membrane.

00:12:18:00

<**Work, standing, adds elements to a diagram to show the structure of the membrane containing all the cytochrome oxidase, then to camera**>

This is the membrane which contains all of the cytochrome oxidase. The cytochrome oxidase is not made merely by the mitochondrial genome, it is a cooperative effort. The messengers from the mitochondria and the mitochondrial ribosomes together with the messengers from the nuclear DNA and the cytoplasmic ribosomes combine to make polypeptides. Cytochrome oxidase contains six polypeptides: three made by the mitochondrial genomes and three by the nuclear so that we finish up with a multi-peptide structure which is partly mitochondrial in origin and partly nuclear in origin.

Now, how has this extraordinary degree of cooperation arisen? It seems likely to me that the answer lies in the evolutionary history of the mitochondrion. It is certainly not essential to have two genetic systems to make mitochondria because bacteria, sorry, to make cytochrome oxidase, because bacteria, with only one genetic centre make cytochrome oxidase very effectively.

The earth is thought to be, on the basis of geological evidence, about 5000 million years old.

<**Work over graphs diagrams comparing oldest organic deposits and earliest organisms of the earth**>

The oldest organic deposits are perhaps 3000 million years old, that is, the early shales and the early asphalt deposits. If these are analysed, we find they are of two different types. One type gives hydrocarbons of odd numbered sequence, that is the top graph in our picture here – you see this is the typical product of a biological synthesis. But if we look at some of the other asphalts, we find that there is no predominant molecular weight, that they are a complete mixture of all possible molecular weights. Now, this is precisely what one gets when hydrocarbons are synthesised by catalytic process in the laboratory. How did those pre-biotic compounds arise?

The geological evidence suggests also: the oldest living organisms of the bacterial type are perhaps 3000 million years old and the blue-green algae, rather less, perhaps 2500 million years old. These primitive organisms arose apparently by chance in a situation where organic material already existed on earth. How was this organic material formed? What was its nature? And how did the organisms come to develop as they did?

<**Work, seated, refers to tables listing early compounds necessary for the creation of life; to camera between tables**>

Well, about 20 years ago, a Californian student, Miller, carried out the sort of experiment that we should all carry out occasionally, a somewhat mad experiment. He knew that the pre-biotic earth had an anaerobic atmosphere consisting probably of such gases as hydrogen, carbon monoxide, carbon dioxide, methane, ammonia and possibly hydrocyanic acid. What Miller did was, he took these components, put them in a glass vessel and passed an electric discharge through the vessel. He then

analysed to see what he had got. And he, in fact, found his products were many of the compounds which were of importance in organisms today.

Since Miller's time this work has been carried quite a lot further and it has now been shown you can not only synthesise those originally observed by Miller, you can make all the amino acids, all the purines, all the pyrimidines and a number of sugars – in fact, you can make all the necessary precursors of the proteins in the nucleic acids. We have a situation, therefore, on the earth around about 3000 million years ago in which there were already rich deposits of those compounds which are necessary for the creation of life. And it seems likely, it seems in fact almost certain, that the first organisms were anaerobic simple organisms which made use of these compounds.

But, as in all biological situations, there would be competition for available resources and sooner or later one of these organisms acquired the ability to synthesise porphyrins. Calvin estimates, on the best available evidence, that this probably occurred around about 3000 million years ago and once an organism had acquired the ability to create a porphyrin it was no great step forward, with successive mutations, to acquire the ability to carry out photosynthesis. When an organism has achieved photosynthesis it has achieved a great biological advantage.

<**Work over tables showing the advantages of an early organism achieving photosynthesis**>

Firstly, it is able to synthesise compounds efficiently using the energy from the sun. Secondly, it is largely independent of pre-formed organic compounds. And thirdly, and perhaps most importantly, it is able to produce oxygen. Now oxygen is highly toxic to any anaerobe and when photosynthesis got underway there must have been a situation on the earth where the anaerobes were in danger of extinction and were fighting for their existence. At this stage it seems likely that some anaerobes set up house in symbiosis with a photosynthetic organism and so formed the very first of the nucleated eukaryotic cells. In doing this it achieved, in fact, two advantages. Firstly, it was able to carry out aerobic oxidation, but secondly, it had also acquired two genetic centres. This meant that not every mutation was likely to be lethal and so the

process of mutation and use of mutation and evolution could be speeded up. This is shown very clearly by the geological evidence.

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The first primitive eukaryotes, about 1000 million years ago, but the first multi-cellular organisms, 500 million years ago. And man, represented by this little white strip up here, has only been developing for 50 million years. In other words, evolution has proceeded very rapidly indeed.

<**Work, seated, to camera and then refers to chart detailing effects of antibiotic drugs on different protein syntheses, then to camera**>

What biological evidence is there to support this hypothesis? There is, in fact, quite a lot. Krohn in Holland and Lenane in Australia, both showed a few years ago that the antibiotic chloramphenicol, which inhibits bacterial protein synthesis, also inhibits mitochondrial protein synthesis, but has no effect on protein synthesis in eukaryotic ribosomes. So we get inhibition of bacterial protein synthesis, inhibition of mitochondrial protein synthesis but no inhibition of nuclear ribosomal synthesis.

The drug cycloheximide, on the other hand, specifically inhibits the nuclear cytoplasmic system, has no effect on mitochondria and no effect on bacteria. A second line of evidence, Marker, working in Cambridge, was recently able to show that all protein synthesis in bacteria begins with the methionine derivative formylmethionine and so does all protein synthesis in mitochondria. But protein synthesis in the nuclear cytoplasmic system begins with methionine.

Another line of evidence – the great bulk of the membrane of all animal cells is rich in cholesterol. Bacteria have membranes without any cholesterol and the inner membrane of the mitochondrion lacks cholesterol. The cytoplasmic ribosomes of the eukaryote all sediment at around 80 Svedberg units. Bacterial ribosomes are smaller, they sediment at about 70; mitochondrial ribosomes are also smaller.

<**Work over table listing organism sizes of mitochondrial DNA; to camera; over electron micrographs of mitochondrial mutation**>

Perhaps the most compelling evidence of all is the size of the mitochondrial DNA itself. In the most primitive eukaryotes, the yeasts and the moulds, the mitochondria have DNA molecular weight perhaps 50 or 60 millions. We go to the higher plants we found that the molecular weight has fallen to 40 millions. The primitive protozoa such as tetrahymena, it is down to 30 millions. In the frog it is down to 11 millions and in man it is 10 millions. To me this suggests that a process of tidying up has gone on over a period of development and that gradually, as we go to higher organisms, progressively genes have been transferred from the mitochondrion to the nucleus so as to make the most efficient use of the available DNA.

Certainly then, this is an attractive hypothesis. But can we prove it? I don't think we should expect to be able to prove it. The philosopher Karl Popper has frequently pointed out that a scientific hypothesis is not provable, that in fact it is the most satisfactory explanation of current knowledge and that if it is to do its job properly, it should suggest new approaches and new ideas. Now does this hypothesis have any medical implications? I think it does.

Mitochondria possess genes, genes are subject to mutation, so mitochondria are subject to mutation. The classic example of mitochondrial mutation is the conversion of the yeast cell from the normal aerobic form where it is exposed through the drug ethidium bromide, this converts the yeast cell to the very small petite form which has to get all its energy by fermentation.

If this process occurs in the yeast it seems likely it also occurs in ourselves. How many of our genetic diseases are due to mutation in mitochondria? We don't know. It seems likely that some of them may be. There is also the point that any drug which interferes with mitochondrial metabolism is likely to be dangerous. As an example of this I might quote to you about the drug chloramphenicol – as you know chloramphenicol is a dangerous antibiotic and quite a number of people have died from aplastic anaemia as a result of treatment with chloramphenicol. It seems likely

to me that this was because the chloramphenicol shut down the protein synthesis in their mitochondria.

<**Work to camera, seated in front of a cartoon illustration of a flock of birds with one standing alone to the side**>

The famous 19th century biologist, Bateson, in his book *On an Introduction to Genetics* says: “Treasure your exceptions for they will show the way to future progress.” Mitochondria contain 25 or 30 genes. The nucleus contains anything up to 50,000 genes so that mitochondria are the exceptional genes and I think that they will still hold some surprises for us.

<**End credits**>