Ch03.qxd 02/09/06 6:24 PM Page 39

3 Origin, Function, and Transmission of Mitochondria

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3.1 Introduction

Mitochondria have existed for more than a billion years, but it was not until the middle of the nineteenth century that they were actually recognised in cells, at first as a grainy appearance in the cell cytoplasm when observed by light microscopy. The anatomist Kölliker (1856) observed mitochondria in muscle cells in the 1850s, while Altman (1890) suggested that his "bioblasts" (granules microscopically observable throughout the cell) were symbionts, something Schimper (1883) had suggested for chloroplasts 7 years earlier, and this idea was taken further by Mereschkowsky (1905). The name mitochondrion was first coined by Benda (1898), and it comes from two Greek words, *mitos* (thread) and *chondros* (granule), which describes the appearance of mitochondria during spermatogenesis. In the following years, many people speculated on the role of mitochondria in the cell, with Warburg (1913) recognising the particulate nature of cell respiration and Keilin (1925) associating the cytochrome system with cellular structures. The first direct evidence for this functional association depended on isolation of the mitochondria from the rest of the cell, which became possible in the 1930s. The first isolations of mitochondria by cell fractionation were made by Bensley and Hoerr (1934), and, following this breakthrough, the path opened for study of the biochemical reactions occurring in mitochondria.

As the sites of energy conversion and cellular respiration, mitochondria became regarded as the "powerhouses" of the cell. However the possible origin of mitochondria was not looked at for some time, not really until the 1950s. It was in the early 1950s that Ephrussi (1950) and Mitchell and Mitchell (1952) observed that mitochondrial replication in yeast cells was controlled by non-Mendelian genetic factors, and slightly later that McLean et al. (1958) observed that mitochondria synthesise proteins. The discovery of mitochondrial DNA followed in the early 1960s, when a number of different groups (Luck and Reich 1964; Nass and Nass 1963a,b; Schatz et al. 1964) published their findings of both mitochondrial and chloroplast DNA.

While the endosymbiotic origin of mitochondria had been considered since the time of Mereschkowsky (Martin and Kowallik 1999), the advances in biochemical techniques in the 1960s led to a revival of the idea, and a new and enthusiastic following for it. The driving force behind this renewed

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Ch03.qxd 02/09/06 6:24 PM Page 40

interest was, of course, the discovery of organelle DNA, and the non-Mendelian inheritance of organelles. Margulis (1970) published a reformulation of the endosymbiotic theory in 1970, and endosymbiosis has subsequently become the accepted view of the origin of mitochondria. There is still disagreement about how this endosymbiosis arose, and which organisms it involved, but there is a general agreement now that the mitochondrion has descended ultimately from a free-living bacterium.

In the course of a little over 100 years, scientists have gone from the first observations of mitochondria to an understanding of their structure, function, inheritance, and origin. The mitochondrial genomes of over 250 different species are now known (Tsang and Lemire 2003), as are the effects of mutations in many mitochondrial genes. There are, however, basic questions still left to be answered. Which organisms contributed to the first eukaryotic cell? Why do mitochondria retain a genome? Can mitochondria still function without their own genomes? Why are only certain mitochondria passed on to the next generation?

3.2 Origins of Mitochondria

All developments seem to be from simple to more complex forms. Whether this is true or just an imaginary chain of events that fits more comfortably with our way of thinking remains to be seen. The anthropocentric view comes naturally to us. Cars, for example, "evolved" from simple horseless carriages to high-performance automotive vehicles. This "evolution", according to some proponents, is similar to the evolution of living organisms, including parameters such as natural selection. Nonetheless, the evolution of life is often thought to have occurred in a smooth and gradual manner. The first group of organisms on our planet, the prokaryotes, are generally the simplest. In principle, prokaryotes are "nothing more" than membrane-enclosed bags of enzymes capable of some biochemical trickery. In contrast, take eukaryotes, with ourselves as the glorifying example of how complex life can be. Clearly, "higher life" evolved from lowly creatures such as bacteria by gradually improving their simple architecture into more elaborate cells which include organelles such as nuclei and mitochondria.

The gradual transformation of a prokaryote into a primitive anucleate eukaryote is still considered the logical chain of events in many textbooks. Accordingly, this primitive eukaryote at one stage took up a free-living bacterium which converted into our modern-day mitochondrion. Such endosymbiosis theories for the evolution of eukaryotes at one stage involved amitochondriate (i.e. without mitochondria) eukaryotes. This hypothetical group received recognition in the now defunct kingdom of the Archezoa (Cavalier-Smith 1987). All studied members of this group have been shown to contain mitochondria of some sort (van der Giezen et al. 2005). This raises

Ch03.qxd 02/09/06 6:24 PM Page 41

the question as to why true amitochondriate eukaryotes, which would have been direct descendants of this anucleate eukaryote, do not seem to exist nowadays. Normally, one would expect intermediary stages of evolutionary development to be capable of producing a lineage of descendants even if the ancestors themselves become extinct. So, why do we not see truly amitochondriate eukaryotes nowadays? This could be for two reasons: either they never existed or they lost the battle of the "survival of the fittest". The latter scenario suggests that, although these organisms did evolve in a particular environmental niche, they no longer occupy this niche, either because it does not exist anymore or, again, because its former occupants lost out to more competitive eukaryotes. An easier way to explain the absence of intermediate forms is to suggest they never existed in the first place. Although this might run in the face of our convenient way of ordering things in a gradual progression from simple to more complex, it actually explains our observations without invoking subsequent events (selective culling of the amitochondriates). So, perhaps the origin of the eukaryotes evolved in a "big bang"-like fashion; with a momentous event.

Let us consider a counterintuitive but satisfying proposal for this event, and one that explains several key aspects in the evolution of eukaryotes. Firstly, the origin of eukaryotes and mitochondria was the same event. In addition, in contrast to general belief, the eubacterial organism that gave rise to the mitochondrion was not an obligate aerobe, far from it. Finally, again in contrast to general belief, the reason for the establishment of the mitochondrion was not energy production. The name of this heretical hypothesis? The hydrogen hypothesis (Martin and Muller 1998), which suggests that hydrogen, and not oxygen or energy, was the currency for the establishment of the mitochondrial endosymbiont. This suggests that the host was able to metabolise hydrogen.

Eukaryotic genome analyses have indicated that almost all informational genes (i.e. involved in genetics) are archaebacterial in origin (Rivera et al. 1998). In contrast, all operational genes, i.e. those involved in metabolism, are eubacterial in origin (Rivera and Lake 2004). Various analyses, for example cytochrome phylogenies, had already indicated that the origin of the mitochondrion might be sought amongst the α-proteobacteria (Schwartz and Dayhoff 1978). So, the players involved are an archaebacterial methanogenic host and an α-proteobacterial endosymbiont (Fig. 3.1). *Rickettsia* has been put forward as the α-proteobacterium which would be most closely related to the original endosymbiont. One reason is the similarity of its aerobic respiration to mitochondrial respiration. But here is a problem: methanogens are one of the most oxygen-intolerant prokaryotes, and cannot produce any energy in the presence of oxygen. So, in a last-ditch attempt, the mitochondrial endosymbiont is put forward as a saviour of the oxygen-sensitive host (Kurland and Andersson 2000). But why put an oxygen scavenger inside the host it is supposedly protecting from harm? One would not put the knights on the courtyard but put them up on the walls to fend off any enemy.

Ch03.qxd 02/09/06 6:24 PM Page 42

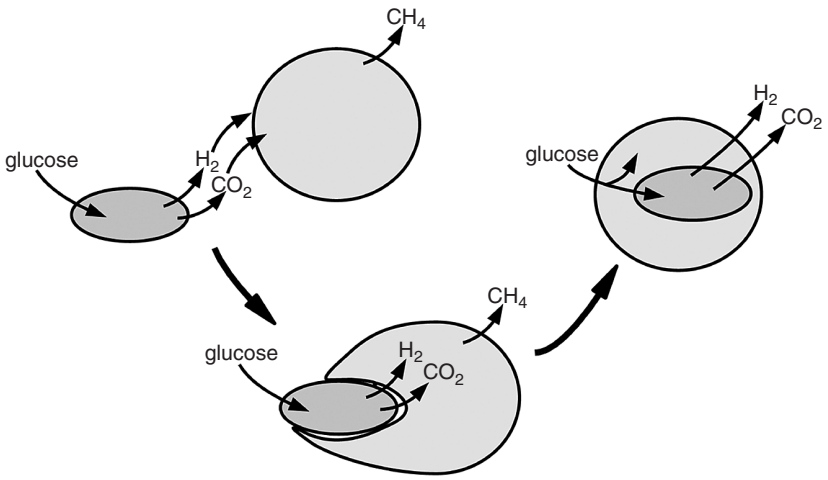

Fig. 3.1. The hydrogen hypothesis of Martin and Müller. The events leading to the establishment of the mitochondrial endosymbiont. *Top left*: A facultative anaerobic α-proteobacterium (*dark grey*) produces hydrogen which is taken up by an autotrophic methanogen (*light grey*). *Middle*: A more intimate relationship results into a larger surface area that can be used for interspecies hydrogen transfer. *Top right*: After eventually becoming fully incorporated, the proteobacterium initially kept producing hydrogen and in return received reduced organic compounds. (Martin and Muller 1998)

So, an α-proteobacterial host with a methogenic endosymbiont would make more sense if oxygen protection was the reason that forged the symbiosis. The hydrogen hypothesis does present the symbiosis as an interspecies hydrogen transfer gone too far. The endosymbiont remains an α-proteobacterium, but this time something more similar to *Rhodobacter*, capable of aerobic and anaerobic metabolism. It offered the methanogen molecular hydrogen and carbon dioxide, and the autotrophic host returned reduced organic compounds, which geared the endosymbiont's metabolism to new heights. Subsequent gene transfers forged the symbiosis for eternity.

It has been argued that anaerobic metabolism could not have been the driving force in times when atmospheric oxygen concentrations were rising (Kurland and Andersson 2000). The concentration of atmospheric oxygen around the time of the endosymbiosis (about 2,000 million years ago; Martin et al. 2003) was about 3%, or about 7 times less then the present-day concentration (Nisbet and Sleep 2001). Perhaps more importantly, large parts of ocean waters around these times were anoxic (Canfield 1998), and it is thought that these important evolutionary events would have taken place in the sea and not on the land as perhaps commonly thought. So, oxygen seems to have been an extremely unlikely factor to have influenced the establishment of the mitochondrial endosymbiont and hydrogen seems more important then ever imagined.

Ch03.qxd 02/09/06 6:24 PM Page 43

One problem discussing mitochondrial function is that there does not seem to be a typical mitochondrion. Mitochondria evolved over a period of 2,000 million years in an huge variety of organisms living under an enormous range of environmental conditions (van der Giezen and Tovar 2005). Mitochondria range from archetypal aerobic mitochondria, via various anaerobic versions and hydrogenosomes, to the most derived forms, mitosomes (Tielens et al. 2002). Nonetheless, currently we know of at least one function found in all mitochondrial varieties; iron–sulphur cluster assembly (Lill and Muhlenhoff 2005). This essential pathway produces iron–sulphur co-factors for both mitochondrial and cytosolic enzymes involved in electron transport, enzyme catalysis, and regulation of gene expression. The most aerobic of mitochondria are involved in oxidative phosphorylation using oxygen as terminal electron acceptor, while more anaerobic versions use alternative electron acceptors such as nitrate (Tielens et al. 2002). Hydrogenosomes, similarly to aerobic mitochondria, convert pyruvate to acetylcoenzyme A, however not using pyruvate dehydrogenase but by means of the oxygen-sensitive pyruvate:ferredoxin oxidoreductase (Embley et al. 2003). All these mitochondrial variants produce energy, be it by means of harvesting the electrochemical gradient generated via the respiratory chain or by substrate-level phosphorylation. Mitosomes on the other hand are not known to be directly involved in energy generation; currently, their function seems exclusively tied to iron–sulfur cluster assembly (van der Giezen et al. 2005)

3.3 Mitochondrial Genomes

As discussed by van der Giezen and Tovar (2005), mitochondria are an enormously diverse set of various organelles. Even if one is not willing to include the anaerobic varieties as being mitochondrial, the vast biochemical repertoire present in aerobic mitochondria alone is staggering. In addition to this biochemical heterogeneity, there exists a genetic heterogeneity as well. There does not exist such a thing as a mitochondrial genome. This genome can be as small as 5,967 bases in the case of *Plasmodium falciparum* (Feagin et al. 1991) and only code for three proteins (cytochrome B, cytochrome oxidase I and III) or as large as 490,000 bases for rice (Notsu et al. 2002). Strangely enough, although the rice mitochondrial genome is 80 times larger than the *Plasmodium* one, it does not code for 80 times as many genes. Although plant mitochondrial genomes tend to be the largest, the mitochondrial genome which actually contains the most genes is the one from the freshwater protozoon *Reclinomonas americana*, which contains 97 genes (Lang et al. 1997). The median is therefore something around 45 genes. If one takes a present-day α-proteobacterium (*Rhodobacter sphaeroides*, for example) which contains almost 4,000 genes, it becomes obvious that many genes of the original endosymbiont have been lost as a consequence of the symbiotic

Ch03.qxd 02/09/06 6:24 PM Page 44

interaction. These genes have either been lost owing to redundancy (the host already contained homologous genes) or been transferred to the host genome (Timmis et al. 2004). It has been estimated that up to 75% of a eukaryotic genome could actually originate from the endosymbiont (Esser et al. 2004). The remaining mitochondrial genes are involved in a limited set of functions; always respiration and translation (as evident in the case of *P. falciparum*), and occasionally also in transcription, RNA maturation, and protein import (Burger et al. 2003). The partially sequenced hydrogenosomal genome from the ciliate *Nyctotherus ovalis* does indeed code for parts of a mitochondrial electron transport chain (Boxma et al. 2005). Other hydrogenosomes and

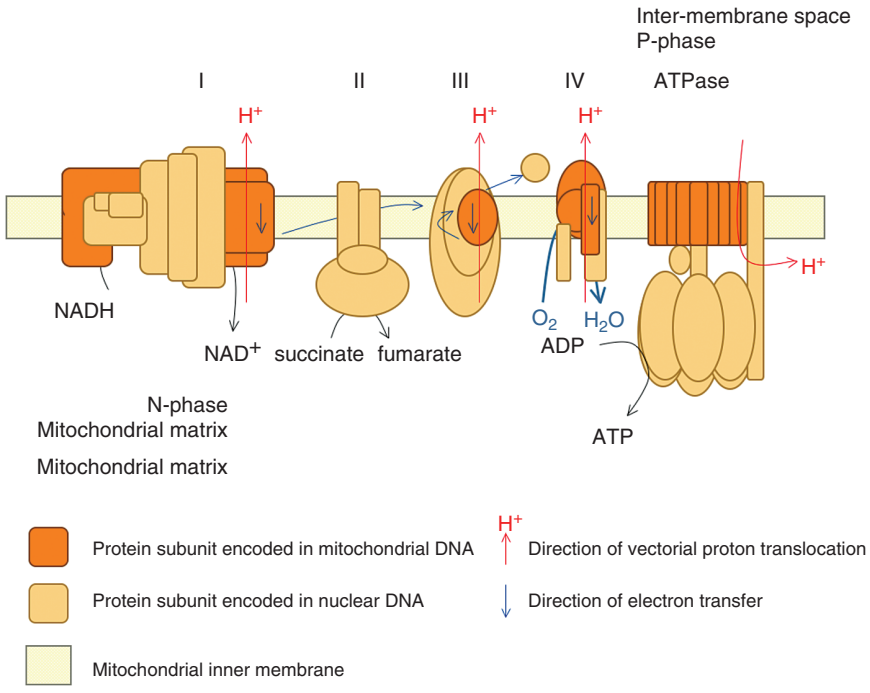

Fig. 3.2. Elements of energy transduction in respiration and oxidative phosphorylation in mitochondria. The mitochondrial inner membrane is shown in *yellow*. The principal complexes involved in energy transduction are complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (the cytochrome *b*–cytochrome c_1 complex), complex IV (cytochrome *c* oxidase), and the coupling ATPase. Vectorial electron transfer is depicted as *thin, dark-blue arrows*. Proton (hydrogen ion; H^+) translocation is depicted as *thin, red arrows*. Other chemical conversions are given *black arrows*. The major, variable environmental input is oxygen (O_2), shown in *blue*. Subunits of protein complexes are coloured according to the location of the genes encoding them. Mitochondria are usually pink or reddish-brown, the colour of cytochromes and iron–sulphur proteins, so *reddish-brown subunits* have genes in the mitochondrion and are synthesised in the mitochondrial matrix; *light-brown subunits* have genes in the nucleus, and are imported from the cytosol as precursors. The depiction of sites of synthesis is schematic only and corresponds roughly to the arrangement in vertebrates. (Adapted from Allen 1993a)

Ch03.qxd 02/09/06 6:24 PM Page 45

mitosomes as well do not seem to have kept their mitochondrial genome (van der Giezen et al. 2005), indicating that the mitochondrial genome's core function is respiration and oxidative phosphorylation (Fig. 3.2).

3.4 The Mitochondrial Theory of Ageing

Reactive oxygen species, generated largely by the mitochondrial electron transport chain, damage the mitochondrial proteins and DNA, and the mitochondrial theory of ageing, simply put, states that this damage leads to ageing and its associated degenerative diseases (Fig. 3.3). This theory was first put forward by Harman (1956), although earlier observations had linked life span to metabolic rate: the higher the metabolic rate, the shorter the life span (Pearl 1928). Although Harman's theory has been around for 50 years, and there is a lot of circumstantial evidence to support it, there remain many

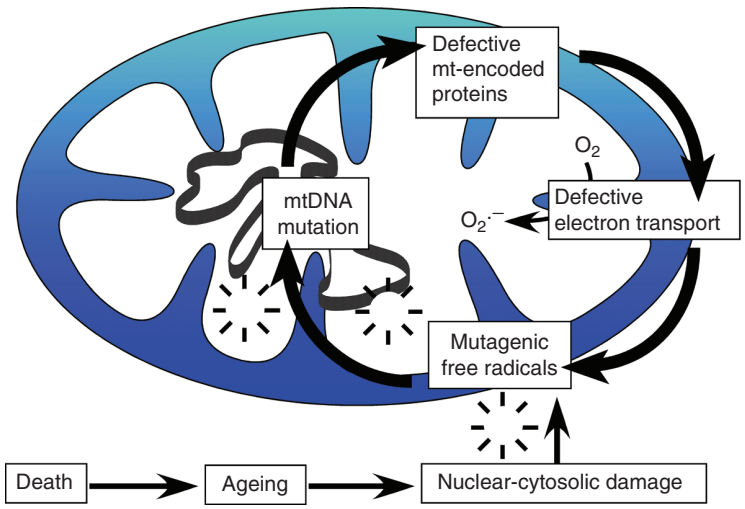

Fig. 3.3. Why we grow old and die: the mitochondrial theory of ageing. Free radicals (whose reactions are symbolised by a *star*), including the superoxide anion radical, $O_2^{\cdot -}$, are produced at a low frequency as by-products of respiratory electron flow in oxidative phosphorylation. Free-radical mutagenesis of mitochondrial DNA (*mtDNA*) then impairs the structure and function of respiratory chain proteins, in turn increasing the frequency of free-radical production. Univalent reduction of oxygen by semiquinone anion radicals may be an important initial step, since ubisemiquinone is an intermediate in protonmotive Q-cycles in oxidative phosphorylation, and readily reduces oxygen to the superoxide anion radical, $O_2^{\cdot -}$. Other oxygen free radicals and sites in the respiratory chain may also be involved. Direct damage to proteins and membranes may accelerate the cycle and initiate somatic degeneration. Mitochondria may minimise, but never eliminate, mutagenic electron transfers. For animal cells, this positive feedback loop, or "vicious circle", has been proposed as the primary cause of ageing. (Adapted from Allen 1996)

Ch03.qxd 02/09/06 6:24 PM Page 46

uncertainties. When Harman first put forward his hypothesis, it had not actually been shown that cells generated free radicals, and it was only in 1969, with the discovery of superoxide dismutase (McCord and Fridovich 1969), that this question was satisfactorily answered. It is now known that cells generate reactive oxygen species at many sites, the majority of these being within the mitochondria. The two major sites are believed to be sites I and III of the respiratory chain. Experiments increasing the redox potential of either site I or site III increase the rate of generation of free radicals (Chen et al. 2003; Kushnareva et al. 2002). Both of these complexes reduce ubiquinone (ubiquinol is also oxidised by complex III), and univalent reduction of oxygen probably occurs by electron transfer from the ubisemiquinone free radical, an intermediate in ubiquinone–ubiquinol oxidation and reduction_

$$UQ^{\cdot -} + O_2 \rightarrow UQ + O_2^{\cdot -}.$$

It is not known how much of the oxygen consumption of the cell is turned over to generating reactive oxygen species, but the figure is thought to be between 2 (Chance et al. 1979) and 0.2% (St-Pierre et al. 2002; Staniek and Nohl 2000). The cell has very efficient scavenging mechanisms, and so these figures may be underestimates.

How much damage mitochondrial DNA suffers as a result of reactive oxygen species generation is still an open question. Studies have shown (Shigenaga et al. 1994) that mitochondria from older animals are morphologically different, and produce more oxidants and less ATP than those from younger ones, but we do not actually know if damage to mitochondria causes ageing, or merely correlates with it. The field of ageing research – what causes ageing and how do we stop, slow, or even reverse it – is an active one. Almost everyone would like to be able to extend their life span. Long-lived mutants of the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and even mice have been established in the laboratory, as reviewed by Balaban et al. (2005), but all of these have defective mitochondria, slowing down energy production as well as ageing. These animals also seem to have a reduced reproductive capacity. It seems that reducing generation of reactive oxygen species does indeed slow ageing, but at what cost? These animals can survive under laboratory conditions, but it is unlikely that they could survive in nature. Perhaps our mortality is the price we have to pay for survival in the short term, and our immortality has been secured by reproduction. Mitochondrial DNA is kept in the most hostile environment in the cell. While the vast majority of genes from the original endosymbiont have been transferred to the nucleus or lost, a small core of genes persist in the mitochondrial matrix. There is strong evidence that damage to mitochondrial DNA by reactive oxygen species generated during oxidative phosphorylation contributes to ageing and death of an organism, and so it is reasonable to assume that there must be a very compelling reason for the organism to continue to keep DNA there.

Ch03.qxd 02/09/06 6:24 PM Page 47

3.5 Why Are There Genes in Mitochondria?

Mitochondria have descended, in evolution, from free-living bacteria (Gray and Doolittle 1982; Gray 1992; Martin et al. 2001). Before the bacterial origin of mitochondria was generally appreciated, there were attempts to account for mitochondrial biogenesis in terms of sequestration of nuclear DNA in the cytoplasm. These need not detain us. However, there is a more recent dogma: that mitochondria retain genes and genetic systems *because* they are descended from bacteria. This statement, while correct, is not a complete explanation. For one thing, there are clearly subcellular organelles, hydrogenosomes and mitosomes, which are also derived from bacteria, and which no longer possess their own, internal genetic systems (van der Giezen et al. 2005). Another objection to this otherwise reasonable first guess – mitochondria happen to be stuck with bacterial genes – is as follows: many mitochondrial proteins with homology to bacterial proteins are now encoded in the cell nucleus, and are successfully imported, post-translationally, as precursors, prior to processing and assembly into functional complexes (Schatz 1998). Indeed, the major respiratory chain complexes are hybrids as regards the location of the genes for their subunits (Fig. 3.2), and there is no indication that their nuclearly encoded subunits are any less bacterial in origin than the mitochondrially encoded ones.

Thus, even granted the endosymbiotic origin of mitochondria, the persistence of mitochondrial genes and genomes requires explanation: if most ancestral, bacterial genes have been successfully relocated to the cell nucleus, then why not all? What is it about mitochondrial genes, or their gene products, that has prevented their successful removal to the nucleus?

The textbook *The Molecular Biology of the Cell* (Alberts et al. 1994) states the problem very clearly, and the following quotation has been retained, unchanged, from the first edition (1983).

> Why do mitochondria and chloroplasts require their own separate genetic systems when other organelles that share the same cytoplasm, such as peroxisomes and lysosomes, do not? ... The reason for such a costly arrangement is not clear, and the hope that the nucleotide sequences of mitochondrial and chloroplast genomes would provide the answer has proved unfounded. We cannot think of compelling reasons why the proteins made in mitochondria and chloroplasts should be made there rather than in the cytosol.

There seems to be no explicit proposal for the most widely held hypothesis for the persistence of mitochondria genomes, but the hypothesis is implicit in many discussions of mitochondrial structure and function. For example, and in contrast to the open question posed by Alberts et al., *Cell and Molecular Biology Concepts and Experiments* (Karp 2002) provides what is probably still the current consensus view.

> Mitochondrial DNA is a relic of ancient history. It is a legacy from a single aerobic bacterium that took up residence in the cytoplasm of a primitive cell

Ch03.qxd 02/09/06 6:24 PM Page 48

that ultimately became an ancestor of all eukaryotic cells. Most of the genes of this ancient symbiont were either lost or transferred over the course of evolution to the nucleus of the host cell, leaving only a handful of genes to encode some of the most hydrophobic proteins of the inner mitochondrial membrane.

Thus, according to this "hydrophobicity hypothesis", proteins that are encoded and synthesised within organelles are characterised by shared, and extreme, hydrophobicity – all are intrinsic membrane proteins (Claros et al. 1995; Popot and de Vitry 1990; Von Heijne 1986).

This view amounts to mitochondrial genes being stuck where they are because of an insuperable difficulty if translocating hydrophobic proteins between subcelluar compartments. Yet there seems to be no evidence that hydrophobicity presents a particular barrier to protein import. For example, mitochondrial ADP–ATP carriers (AACs) are intrinsic to the mitochondrial inner membrane, have six transmembrane helices, and yet are encoded in the nucleus (Saraste and Walker 1982; van der Giezen et al. 2002).

3.6 Co-location of Gene and Gene Product Permits Redox Regulation of Gene Expression

This hypothesis states that mitochondria and chloroplasts contain genes whose expression must be under the direct, regulatory control of the redox state of their gene products, or of electron carriers with which their gene products interact (Fig. 3.4). These genes comprise a primary subset of organellar genes. The requirement for redox control of these genes then confers a selective advantage upon location of that gene within the organelle instead of in the cell nucleus. Mitochondrial and chloroplast genomes also contain genes for components of the their own, distinct, genetic systems. These genes comprise a secondary subset of organellar genes: genetic system genes. Retention of genetic system genes is necessary for the operation of redox control of expression of genes in the primary subset: bioenergetic genes. Without genes in the primary subset, the function of genetic system genes is eventually lost, and organelles lose their genomes.

This hypothesis of co-location for redox regulation of gene expression, CORR, was first outlined, in general terms, in a review on protein phosphorylation in regulation of photosynthesis (Allen 1992). The hypothesis was put forward in two articles (Allen 1993a, b), where the function of the location of organellar genes was proposed as redox regulation of gene expression. The term CORR was introduced more recently (Allen 2003a, b).

CORR applies equally to mitochondria and chloroplasts, and accounts for the fact that both of these organelles possess membrane-intrinsic electron transport systems along with discrete, extranuclear genetic systems. CORR rests on ten assumptions, or principles, as follows:

Ch03.qxd 02/09/06 6:24 PM Page 49

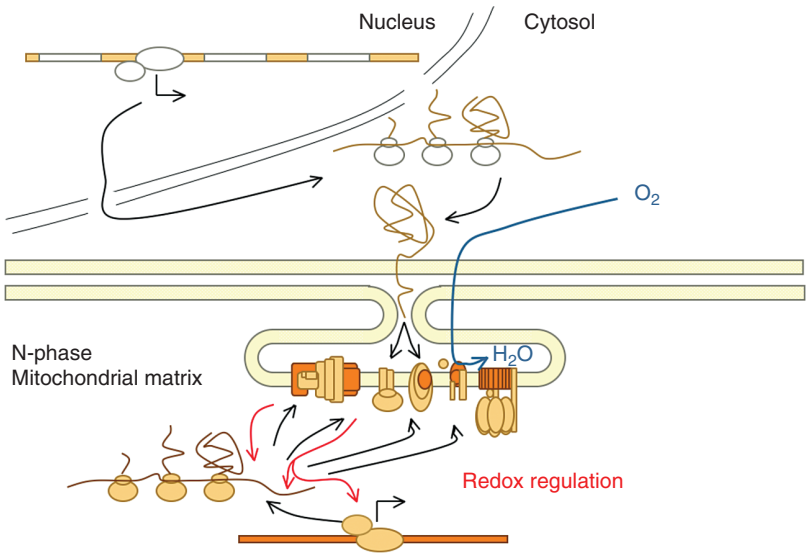

Fig. 3.4. Gene expression and principal pathways of biosynthesis of subunits of protein complexes involved in respiration and oxidative phosphorylation in animal mitochondria. *Reddish-brown DNA, RNA, and protein subunits* are located and synthesised in the mitochondrial matrix; *light-brown protein subunits* have genes (also *light brown*) in the nucleus, and are imported from the cytosol as precursors. *White genes* and ribosomal and protein subunits are nuclear-cytoplasmic and of archaebacterial origin. *Reddish-brown and light-brown genes* and ribosomal and protein subunits are of bacterial origin. The major, variable environmental input is oxygen (*blue*). It is proposed that it is beyond the ability of the nuclear-cytoplasmic system to respond rapidly and directly to changes in oxygen concentration or partial pressure, and so redox regulation of gene expression (*red arrows*) has been retained from the ancestral, bacterial endosymbiont. This redox regulation requires co-location of certain genes, with their gene products, within the mitochondrion. (Adapted from Allen 2003)

1. *Endosymbiotic origin*. As now generally agreed, mitochondria and chloroplasts evolved from free-living bacteria.
2. *Unselective gene transfer*. Gene transfer between the symbiont or organelle may occur in either direction and is not selective for particular genes.
3. *Unselective protein import*. There is no barrier to the successful import of any precursor protein, nor to its processing and assembly into a functional, mature form.
4. *Evolutionary continuity of redox control*. Direct redox control of expression of certain genes was present in the bacterial progenitors of mitochondria and chloroplasts, and was vital for selectively advantageous cell function before, during, and after the transition from bacterium to organelle. The mechanisms of this control have been conserved.
5. *Selective value of redox control*. For each gene under redox control (principle 4), it is selectively advantageous for that gene to be retained and expressed only within the organelle.

Ch03.qxd 02/09/06 6:24 PM Page 50

6. *Selective value of nuclear location for genes not under redox control*. For each bacterial gene that survives and is not under redox control, it is selectively advantageous for that gene to be located in the nucleus and expressed only in the nucleus and cytosol. If the mature gene product functions in chloroplasts or mitochondria, the gene is first expressed in the form of a precursor for import.
7. *Continued and contemporary operation of natural selection for gene location*. For any species, the distribution of genes between organelle (by principle 5) and nucleus (by principle 6) is the result of selective forces which continue to operate.
8. *Primary involvement in energy transduction is necessary for organelle gene location*. Those genes for which redox control is always vital to cell function have gene products involved in, or closely connected with, primary electron transfer. These genes are always contained within the organelle. Where primary energy transduction is lost completely, then organelles lose their genomes.
9. *Secondary involvement in energy transduction may be sufficient for organelle gene location*. Genes whose products contribute to the organelle genetic system itself, or whose products are associated with secondary events in energy transduction, may be contained in the organelle in one group of organisms, but not in another, depending on the physiology and biochemistry of photosynthesis and respiration in the species concerned.
10. *Nuclear encoding of redox-signalling components*. Components of the redox-signalling pathways upon which co-location for redox regulation depends are themselves not involved in primary electron transfer, and so their genes have been relocated to the nucleus, in accordance with principle 6.

At present, direct evidence for the redox control of organellar gene expression that is predicted CORR is stronger for chloroplasts than for mitochondria (Pfannschmidt et al. 1999). Redox effects on mitochondrial gene expression in vitro are largely confined, at present, to protein synthesis (Allen et al. 1995; Galvis et al. 1998). The search for a direct signalling pathway from the respiratory chain to mitochondrial DNA is likely to be an active area of future research (Allen et al. 2005; Lane 2005).

3.7 Maternal Inheritance of Mitochondria

As discussed previously, the mitochondrial theory of ageing rests on the observation that mitochondrial DNA is exposed to high levels of reactive oxygen species when the mitochondrion is performing its redox chemistry. These reactive oxygen species cause mutation. These mutations accumulate, gradually damaging the mitochondrion's ability to function. This happens in

Ch03.qxd 02/09/06 6:24 PM Page 51

all the cells of an organism, leading to the symptoms we know as ageing, and eventually to death. One of the problems facing any eukaryotic organism is how to ensure the next generation does not inherit the damage that it had suffered to its mitochondria. We can see that each generation starts off with new, healthy mitochondria, but how does this happen?

The theory that two sexes are derived from a division of labour between male and female germ line mitochondria was first proposed in 1996 (Allen 1996). Here, it was proposed that the mitochondria of the female germ line have a repressed bioenergetic function, and so they escape the damage to their DNA caused by mutagens generated by respiratory electron transport. The mitochondria are therefore able to replicate and pass to the next generation with minimal change. The male gametes need to have functional mitochondria, in order to generate the energy needed to reach the egg cell. The hypothesis proposes that these bioenergetically active, and therefore damaged, mitochondria will be prevented from entering the germ line.

As far as the mitochondria go, the most significant difference between the male and female is that the male mitochondria have to produce a lot of ATP to propel the sperm as quickly as possible towards the egg, and the female mitochondria do not really have to do much at all. If free radicals generated by an active electron transport chain are responsible for damage to mitochondrial DNA, then it is reasonable to assume that sperm mitochondria are likely to be more damaged than those in the egg. To take this idea one step further, perhaps the mitochondria in the egg cells are prevented from carrying out oxidative phosphorylation at all, in order to preserve as accurate a copy as possible of the DNA to pass to the offspring.

In the systems that have been studied, it can be seen that cells destined to become the female germ line are identified and set aside very early in development. In the female, these cells will go on to form the eggs, and will contain the mitochondria that will pass to the next generation. When one of these eggs is fertilised, the cells that will form the gametes for the next generation are set aside, and so on.

In the majority of organisms, and all mammals, the mitochondria are inherited from only one parent, and that is the mother (Law and Hutson 1992).It is thought that there are different mechanisms for excluding the male mitochondria, some organisms prevent entry to the egg cell, but in mammals it is an active destruction process. Sutovsky et al. (2000) have shown that the sperm cell mitochondria in cattle do get into the egg cell, and are subsequently targeted and destroyed, mostly between the four-cell and the eight-cell stage of the embryo. The male sperm are ubiquitinated within the oocyte cytoplasm, which provides a target for proteolysis. The germ line is determined at a very early stage of embryology. In the nematode worm *C. elegans* (Seydoux et al. 2001), the fate of every cell from the zygote onwards has been studied, and even from the four-cell stage, the germ line can be distinguished. In mammals, the germ line is segregated very early, before implantation in

Ch03.qxd 02/09/06 6:24 PM Page 52

fact. By the blastocyst stage, the germ line has already been segregated, and this is before the embryo becomes aerobic.

From these observations, it is possible to see how mitochondria could be passed from one generation to the next without ever having to fulfil their role as generators of cellular energy, but only having to act as templates. The energy for replication could come from the egg's helper cells, and once any mitochondria have an active respiratory chain they become unsuitable to act as a template for future generations (Fig. 3.5). If such mitochondria did get

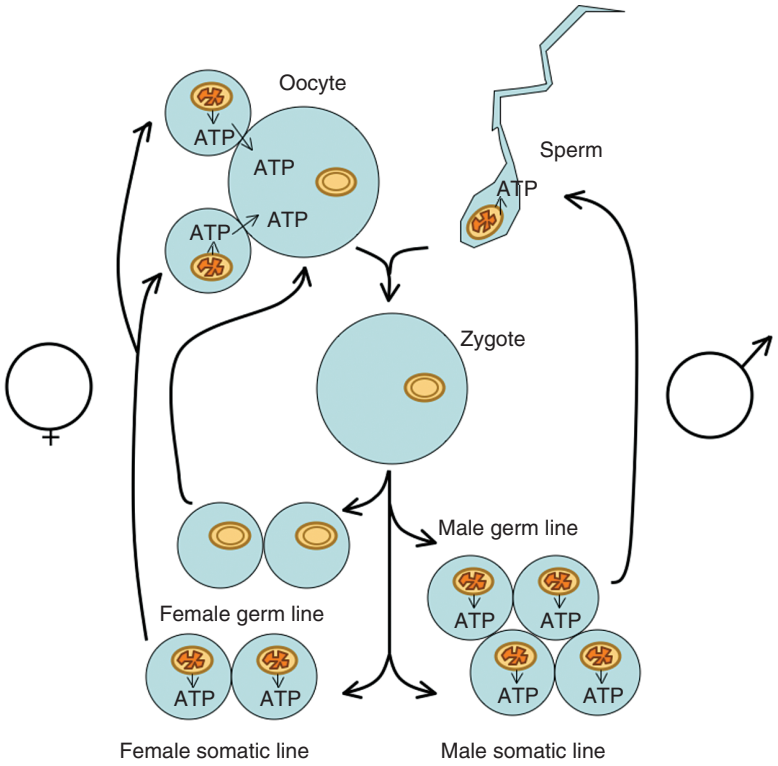

Fig. 3.5. Why our genes do not die with us: differentiation of male and female gametes for motility and for fidelity of mitochondrial genome replication. The probability of encounters of two gametes is ideally the same whether one or both are motile; therefore, one sex (male) may produce gametes that sacrifice the mitochondrial genome in favour of oxidative phosphorylation. The other sex (female) is then free to produce immobile gametes in which oxidative phosphorylation is repressed in promitochondria, and through which the mitochondrial genome is thus transmitted with increased fidelity. Promitochondria are sequestered early in development in the female germ line. Female oocytes obtain ATP from oxidative phosphorylation in the differentiated mitochondria of ancillary somatic cells (follicle cells in animals). Promitochondria persist in plants in meristematic cells, prior to differentiation of somatic and germ cells. In contrast, any ancillary germ cells (nurse cells in invertebrates) will also require imported ATP, since they share the oocyte's cytoplasm. Gamete differentiation may likewise rescue the chloroplast genomes of plants. Mitochondria and chloroplasts are thus maternally inherited. (Adapted from Allen 1996)

Ch03.qxd 02/09/06 6:24 PM Page 53

into the egg cell, and so form the basis of the inherited mitochondria, one would expect that the offspring would be born at a more advanced cellular age than normal. Although the data are sparse, it is thought that early reproductive cloning of mammals was achieved by fusing a somatic cell with an egg cell whose DNA had been destroyed. "Dolly" the sheep was the first cloned mammal, and her nuclear DNA was derived from a 6-year-old ewe. She showed signs of ageing very early on, and died at the age of 5 (usual life span would be 11 years), of a disease more usually associated with old age (Allen and Allen 1999).

3.8 Conclusions

From the time that mitochondria were first seen in cells, they have been an interesting enigma. The more we learn about them, the more questions are raised. There would seem to be enough unanswered questions in the field of mitochondrial function, genetics, and origins to engage researchers for a long time to come.

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