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REVIEW ARTICLE

The mitochondrial compartment

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Abstract

Mitochondria are vital organelles that perform a variety of fundamental functions ranging from the synthesis of ATP through to being intimately involved in programmed cell death. Comprised of at least six compartments: outer membrane, inner boundary membrane, intermembrane space, cristal membranes, intracristal space, and matrix, mitochondria have a complex, dynamic internal structure. This internal dynamism is reflected in the pleomorphy and motility of mitochondria. Mitochondria contain their own DNA (mtDNA), encoding a small number of vital genes, but this role as a genetic vault is not compatible with the role of mitochondria in bioenergetics since electron transport results in the generation of reactive oxygen species (ROS) that induce lesions in the mtDNA. It is hypothesized that ROS shape the morphological organization of the higher plant cell mitochondrial population into a *discontinuous whole*, and that ROS are a selective pressure affecting the organization of the mitochondrial genome. This review describes how inter- and intra-mitochondrial compartmentalization underpins the biology of this complex organelle.

Key words: Cytoskeleton, discontinuous whole, division, dynamics, fusion, mitochondria, mitochondrial genome, morphology, mutants, ultrastructure.

Introduction

Mitochondria are highly dynamic, pleomorphic organelles composed of a smooth outer membrane surrounding an inner membrane of significantly larger surface area that, in turn, surrounds a protein-rich core, the matrix. Although mitochondria contain their own genome and protein-synthesizing machinery (Leaver *et al*., 1983; Unseld *et al*., 1997; Gray *et al*., 1999) they are only semi-autonomous: the majority of mitochondrial polypeptides are encoded in the nuclear genome, synthesized in the cytosol and imported into the mitochondria post-transcriptionally (Unseld *et al*., 1997; Whelan and Glaser, 1997; Duby and Boutry, 2002). The role of the mitochondrion in the synthesis of ATP formed by oxidative phosphorylation is well established (Saraste, 1999) and, in addition, mitochondria are involved in numerous other metabolic processes including the biosynthesis of amino acids, vitamin cofactors, fatty acids, and iron-sulphur clusters (Mackenzie and McIntosh, 1999; Bowsher and Tobin, 2001). Apart from the role of the mitochondrion in ATP synthesis and various biosynthetic pathways the mitochondrion is one of three cell compartments involved in photorespiration (Douce and Neuburger, 1999), is implicated in cell signalling (Vandecasteele *et al*., 2001; Logan and Knight, 2003), and has been shown recently to be involved in programmed cell death (Jones, 2000; Youle and Karbowski, 2005).

This review deals with the complex biology of the mitochondrion and describes how various levels of compartmentalization within the mitochondrion and cellular mitochondrial population as a whole (the chondriome) underpin the multiple functions of this vital organelle. Although focused on the higher plant mitochondrial compartment, frequent reference will be made to studies using non-plant model organisms. In some cases, this is simply due to a paucity of information about specific aspects of plant mitochondrial biology; in all cases it is because I believe the information is of fundamental relevance. A short article such as this can only provide a brief overview of the importance of compartmentalization to the life of the mitochondrion. A great deal has been left out (e.g. co-ordination of the mitochondrial and nuclear genomes, control of protein import, the mitochondrial proteome, biochemical defence against ROS, amongst

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Abbreviations: Cyt *c*, cytochrome *c*; GFP, green fluorescent protein; MMF, massive mitochondrial fusion; MPT, mitochondrial permeability transition; mtDNA, mitochondrial DNA; PCD, programmed cell death; PTP, permeability transition pore; ROS, reactive oxygen species; TCA, tricarboxylic acid; TPR, tetratrico peptide repeat.

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other topics) and it is possible, even likely, that my choices of topics to include might not be of interest to all with an interest in mitochondria but, in the end, this is a personal view of the mitochondrial compartment.

Compartmentalization and the chemiosmotic theory

The vast majority of biological energy (ATP) production is associated with energy-transducing membranes: the prokaryotic plasma membrane of bacteria and blue-green algae, the thylakoid membranes of chloroplasts, and the inner mitochondrial membrane. The energy-transducing membrane is central to the chemiosmotic theory that explains the basic mechanism of biological energy production, whereby ATP production is coupled to the controlled dissipation of a proton electrochemical gradient (proton motive force). The membrane allows compartmentalization of protons, via their vectorial transport across the membrane, by the action of a primary proton pump(s). In mitochondria the primary proton pumps comprise complexes I, III, and IV. These primary pumps generate a high gradient of protons that forces a secondary pump (the ATP synthase complex) to reverse, energized by the flow of protons 'downhill', thereby synthesizing ATP from ADP and Pi. Any proton leak across the membrane would cause a short-circuit, destroy the compartmentalization of protons and uncouple the proton motive force from the ATP synthase. The energy-transducing membrane must, therefore, be essentially closed and have a high resistance to proton flux.

The energy-transducing membrane of mitochondria, the inner mitochondrial membrane, is a highly pleomorphic structure. Although there are an almost endless variety of inner mitochondrial membrane morphologies in mitochondria from different species, from different cell types within the same species or from the same cell types but in different metabolic states (Munn, 1974), some generalizations can be made. Transmission electron microscopy led to the development of models of the internal structure of mitochondria. Palade's model (Palade, 1952), also called the baffle model, depicted the invaginations of the inner mitochondrial membrane, the cristae, as random, wide in-folds of the membrane (the typical text book image, Fig. 1) while Sjostrand suggested the cristae were composed of a stack of independent membranous lamellae (Sjostrand, 1953). It is clear from two ground-breaking research papers published in 1994 (Lea *et al*., 1994; Mannella *et al*., 1994), describing results obtained using high-resolution scanning electron microscopy or electron tomography, respectively, together with subsequent investigations, that neither model was entirely correct (Mannella, 2006).

The results obtained using advanced tomographic imaging techniques demonstrate that, at least in animal tissue, tubular rather than lamellar cristae predominate and that the morphology of cristae infers that they are

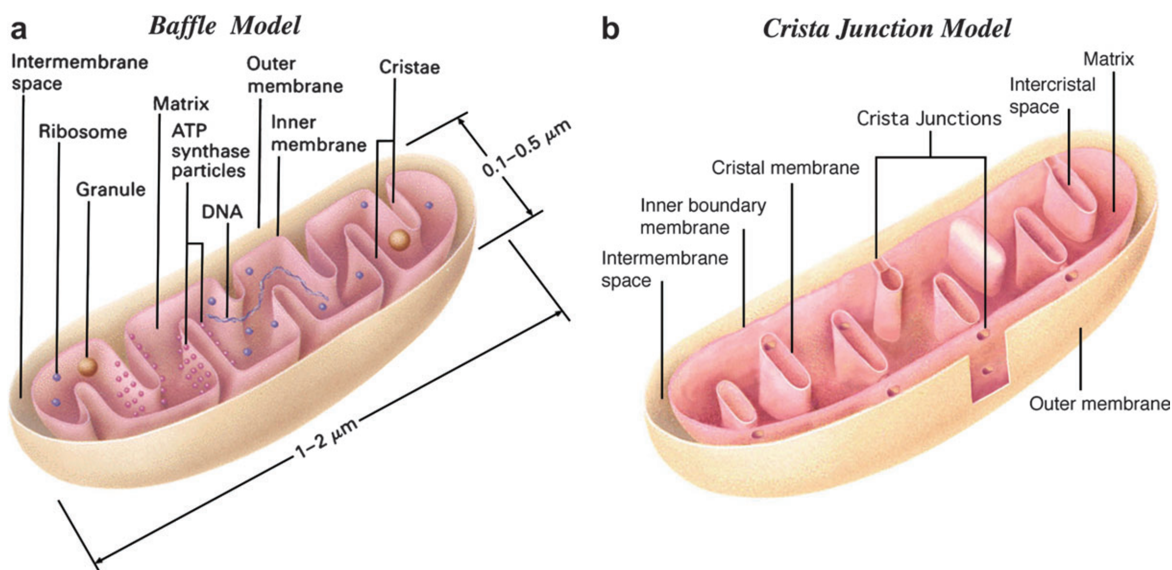

Fig. 1. Models of mitochondrial membrane structures. (a) Infolding or 'baffle' model, which is the representation most commonly depicted in textbooks (reproduced from Lodish *et al*., 1995, Fig. 5–43, with permission from WH Freeman). This model originated with Palade in the 1950s and has been prominent until recently. (b) Crista junction model, which supplants the baffle model for all mitochondria examined to date from higher animals. Electron tomography has been instrumental in providing the improved 3D visualizations of mitochondria *in situ* that have generated a new model for membrane architecture. Instead of the large openings connecting the intercristal space to the intermembrane space present in the baffle model, narrow tubular openings (crista junctions) connect these spaces in this model. Most cristae have more than one crista junction and these can be arranged on the same side of the mitochondrial periphery, or on opposite sides if the crista extends completely across the matrix. The model in (b) is courtesy of M Bobik and M Martone, University of California, San Diego. Reprinted from Perkins and Frey (2000). Copyright (2000), with permission from Elsevier. Additional annotations in (b) by the author.

structurally distinct from the rest of the inner mitochondrial membrane. An additional finding was confirmation that the cristae were connected to the inner boundary membrane (cortical inner mitochondrial membrane, parallel to the outer membrane) by membranous tubules, instead of the cristae being simple in-folds of the membrane as suggested by Palade (1952). Daems and Wisse (1966) first reported that cristae attach to the inner boundary membrane via narrow tubules termed pediculi, but this finding was not consistent with the baffle paradigm. Subsequently, it has been shown that the connections between the cristae and the inner boundary membrane, the term crista junction has superseded pediculi, have a preferred size and morphology and are independent of the source of the mitochondrion and the means of fixation (Mannella *et al*., 1994, 1997; Perkins *et al*., 1997*a*, *b*, 1998). Indeed, it has been proposed that crista junctions are a uniform structural component of all mitochondria (Perkins and Frey, 2000). For example, in rat liver mitochondria, crista junctions are 30–50 nm long although tubules three times that length have been measured, and in *Neurospora crassa* the slot-like crista junctions have been measured at up to 200 nm, although the average length is 30–40 nm (Frey *et al*., 2002; Perkins *et al*., 1997*a*).

The number of crista junctions and the morphology of the intercristal space have been shown to change with the metabolic state of the mitochondria (Hackenbrock, 1968; Mannella *et al*., 1994, 1997). In the orthodox state, corresponding to partial matrix expansion, the intercristal space is compressed and tubular with few cristae interconnections and one or two crista junctions with the inner boundary membrane. In the condensed state, corresponding to partial matrix contraction, the intercristal spaces are dilated and there are more numerous intercristal membrane connections and crista junctions. Hackenbrock (1968) demonstrated, by rapid fixation of purified mouse liver mitochondria in different respiratory steady-states, that mitochondria in state 3 (maximum respiratory rate in the presence of excess ADP and respiratory substrate) were in the condensed conformation, but reverted to an orthodox morphology after entering state 4 respiration (characterized by a reduction in respiration due to the depletion of ADP). Addition of ADP to these mitochondria caused a reversion to the condensed form within 35 s, followed by a gradual return to the orthodox conformation as all the ADP is phosphorylated.

Dry, quiescent maize embryos contain mitochondria with little internal membrane structure and an electron-light matrix (Logan *et al*., 2001). Upon imbibition, mitochondrial biogenesis is stimulated and within 24 h (protrusion of the radicle typically took place after 36–48 h imbibition) mitochondria in the embryo have a normal, orthodox, conformation (Fig. 2; Logan *et al*., 2001). By contrast, mitochondria isolated from germinated embryos (after 48 h imbibition) had a condensed conformation (Fig. 2; Logan *et al*., 2001). It is tempting to speculate that the switch from an orthodox to a condensed conformation during mitochondrial biogenesis is indicative of the changing biochemistry of the organelle as it switches from being reliant on the provision of electrons from external NADH dehydrogenases to the newly assembled TCA cycle (Logan *et al*., 2001).

A condensed morphology, large intercristal spaces with narrow crista junctions to the intermembrane space, has been shown by computer simulation to lead to a reduction in diffusion of ADP into the cristae, reduction in the transport of ADP across the inner mitochondrial membrane and, therefore, ATP production (Mannella, 2006). Adoption of an orthodox confirmation when the bulk ADP concentration is low might therefore act to minimize the negative effect on ATP production of limited diffusion of ADP through the crista junctions by concentrating the ADP within a smaller intercristal volume. The results of Hackenbrock (1968) and those from the computer

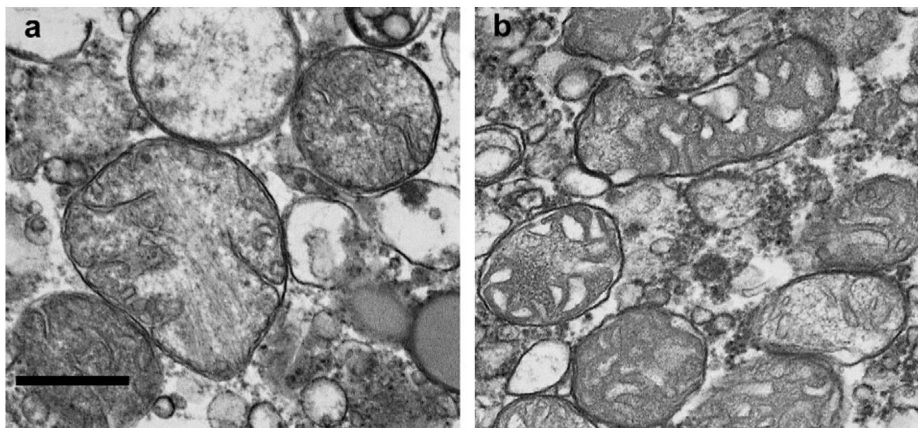

Fig. 2. Conformation of internal structure in mitochondrial purified from germinating maize embryos. Transmission electron micrographs of mitochondria after subcellular fractionation of embryos excised from seed imbibed for either (a) 24 h, orthodox conformation or (b) 48 h, condensed conformation. Scale bar = 500 nm. [Logan *et al*. (2001)].

simulation suggest that inner mitochondrial membrane remodelling, which affects the degree of compartmentalization, is a mechanism enabling the control of ATP production by mediating ADP availability (Mannella, 2006). Whether this control mechanism operates *in vivo* remains to be determined. What is clear from the above discussion is that at least six discrete mitochondrial compartments can be recognized on a structural basis: outer membrane, intermembrane space, inner boundary membrane, cristal membrane, intercristal space, and matrix.

Biogenesis of the cristal membranes is dependent on ETC biogenesis

The extent to which the structural organization and compartmentalization of the energy-transducing inner mitochondrial membrane to form three components (inner boundary membrane, cristal membranes and intercristal space) are reflected in, or indeed due to, a different protein complement of each compartment is not fully understood. It has been demonstrated recently, with bovine heart mitochondria, that approximately 94% of both Complex III and ATP synthase protein, as detected by immuno-gold labelling, resides in the cristal membrane, the remaining 6% is located in the inner boundary membrane (Gilkerson *et al*., 2003). The authors concluded that there is restricted diffusion of respiratory complexes through the crista junctions and that the cristae comprise a regulated functionally distinct subcompartment of the inner mitochondrial membrane (Gilkerson *et al*., 2003). A similar compartmentalization of cytochrome *c* oxidase in the cristae has been recorded in Jerusalem artichoke (Kay *et al*., 1985; Moller *et al*., 1987) and rat cardiac muscle and pancreas (Perotti *et al*., 1983) mitochondria, and in the cristae and inner boundary membrane of mouse liver mitochondria (Hiraoka and Hirai, 1992). In addition, indirect evidence to support the hypothesis that the cristal membrane is the site of oxidative phosphorylation comes from examination of Rho^0 cells that lack mitochondrial DNA (Gilkerson *et al*., 2000). Human mitochondrial DNA encodes 13 polypeptide components of the respiratory chain and, therefore, in Rho^0 cells, the oxidative phosphorylation machinery is incompletely assembled. This selective loss of only a small proportion of respiratory complex subunits has a dramatic effect on the internal structure of the mitochondria: the cristal membranes are greatly reduced and disorganized, yet the inner boundary membrane remains visibly unaltered (Gilkerson *et al*., 2000). This specific effect on the cristal membranes can be explained if the cristal membranes are functionally distinct from the inner boundary membrane and are dependent on the correct biogenesis of the respiratory chain for their own biogenesis.

Two supernumerary F_0-ATPase-associated subunits, g and Tim11p (also called e), that are not essential for growth in yeast and are restricted to mitochondria (Walker *et al*., 1991; Higuti *et al*., 1993; Collinson *et al*., 1994; Boyle *et al*., 1999), are involved in the dimerization of the F_1F_0-ATPase and cristae biogenesis and morphology (Paumard *et al*., 2002). However, although these subunits are conserved between yeast and mammals there are no significant homologues in *Arabidopsis*. In *S. cerevisiae*, absence of either subunit, g or Tim11p, results in the absence of cristae, although the inner boundary membrane is present (Paumard *et al*., 2002). A similar aberrant mitochondrial phenotype has been described in mutants of a large GTPase called Mgm1p (Wong *et al*., 2000), and it was proposed that Mgm1p is involved in inner membrane remodelling events in yeast (Wong *et al*., 2000). Subsequently, Mgm1p was identified independently by two groups (Herlan *et al*., 2003; McQuibban *et al*., 2003) as a substrate of a yeast rhomboid-type protease named Rbd1p (rhomboid) or Pcp1p (processing of cytochrome *c* peroxidase (Esser *et al*., 2002) and that cleavage of Rbd1p/Pcp1p regulates inner membrane remodelling (Herlan *et al*., 2003; McQuibban *et al*., 2003). Rbd1p/Pcp1p contains six transmembrane domains and is embedded in the inner mitochondrial membrane (McQuibban *et al*., 2003). Upon import of an Mgm1p precursor, the N-terminal hydrophobic region becomes tethered in the inner membrane at the site of the first transmembrane domain, by what is assumed to be a translocation-arrest mechanism, leaving the N-terminal mitochondrial targeting presequence exposed to the matrix (Herlan *et al*., 2003). Cleavage by the matrix-processing peptidase generates what is called the large isoform of Mgm1p (l-Mgm1p) (Herlan *et al*., 2003). Next, l-Mgm1p is further translocated into the matrix and the second transmembrane domain becomes inserted into the inner membrane, whereupon it undergoes further proteolytic cleavage by Rbd1p/Pcp1p producing a smaller isoform, s-Mgm1p, which is released into the intermembrane space and becomes associated with either the outer or inner mitochondrial membrane (Herlan *et al*., 2003). Both isoforms function in the maintenance of mitochondrial morphology and respiratory competence, but the mechanism controlling the ratio of l-Mgm1p to s-Mgm1p is unknown (Herlan *et al*., 2003). Recently, Amutha *et al*. (2004) integrated the Tim1p, Mgm1p, and Rbd1p/Pcp1p data by demonstrating that Mgm1p is an upstream regulator of Tim1p subunit stability, of the assembly of the F_1F_0-ATPase, and of cristae biogenesis. Homologues of Mgm1p and Rbd1p/Pcp1p genes are present in *Arabidopsis*: Mgm1p=members of the *Arabidopsis* dynamin-like gene family (Hong *et al*., 2003), the closest being DRP3B, At2g14120; Rbd1p/Pcp1p=At1g18600. At the time of writing, only DRP3B has been shown to be required for normal mitochondrial morphology (Arimura and Tsutsumi, 2002), but no information is available on the internal morphology of mitochondria in DRP3B mutants.

Contact sites

Contact sites were first described by Hackenbrock (1968) as specific regions where the outer membrane and inner boundary membrane are closely apposed, with no discernible space between them. It is now known that at least two types of contact site exist. One is as described by Hackenbrock, while in the second, the outer and inner boundary membranes are connected by bridge-like structures that maintain a constant separation between the membranes (Senda and Yoshinaga-Hirabayashi, 1998; Perkins *et al*., 2001). Senda and Yoshinaga-Hirabayashi (1998) suggested that the bridges might keep the outer and inner membranes apart thus maintaining the intermembrane space as a physically distinct compartment. The close apposition of the outer and inner boundary membranes as reported by Hackenbrock led him to suggest that these contact sites could facilitate the passage of solutes and small molecules between the cytosol and the matrix (Hackenbrock, 1968). Subsequently, it was demonstrated that translationally-arrested polysomes were selectively bound to the outer membrane surface at contact sites (Kellems *et al*., 1975) and that precursor proteins, trapped during translocation, were stuck within both outer and inner boundary membranes (Schleyer and Neupert, 1985; Schwaiger *et al*., 1987). Using chimeras composed of the N-terminal portion of a mitochondria-targeted precursor protein fused to a cytosolic protein which become trapped during translocation, Pon and colleagues were able to show that the partly translocated precursors are enriched at contact sites and that contact sites contain import activity (Pon *et al*., 1989). A similar approach, using arrested translocation intermediates, enabled the co-isolation of the translocase of the outer membrane (TOM) and the preprotein translocase of the inner membrane (TIM23 complex) (Dekker *et al*., 1997; Schulke *et al*., 1999).

A component of contact sites in *Arabidopsis* was identified recently. The translocase of the inner membrane 17 (*At*TIM17-2) was shown to link the inner and outer membranes by means of its C-terminal region that is also essential for protein import (Murcha *et al*., 2005). Interestingly, the *Arabidopsis* protein can complement a yeast TIM17 mutant, but only when the C-terminal region of 85 amino acids, not present in the yeast protein, is removed (Murcha *et al*., 2003). A new component of the *S. cerevisiae* TIM23 complex, Tim21, has been identified (Mokranjac *et al*., 2005). Tim21 is anchored in the inner boundary membrane and, via its C-terminal domain, specifically interacts with the TOM complex, possibly stabilizing the contact site (Mokranjac *et al*., 2005). It is possible that the C-terminal regions of *At*TIM17-2 and *S. cerevisiae* Tim21 perform a similar role in the respective organisms. The exact relationship between morphological contact sites and translocation contact sites is not known, i.e. whether all contact sites are also import sites or whether there is a subset of the contact sites, for example, the closely-apposed type, that function as sites of protein import while the bridge-type contact sites are structural only.

Compartmentalization within the matrix

The matrix contains the enzymes of the pyruvate dehydrogenase complex (PDC), TCA cycle, and glycine oxidative decarboxylation during photorespiration, and contains pools of metabolites including NAD, NADH, ATP, and ADP. However, little is known about how the different proteins and metabolites are distributed in the matrix. GFP targeted to the matrix of mitochondria in various types of animal cell is fully dispersed throughout the available space and FRAP (fluorescence recovery after photobleaching) studies have shown diffusion rates of GFP to be close to that of a protein in a dilute aqueous solution (Partikian *et al*., 1998). The fact that the measured diffusion rate of GFP in the matrix is only 3–4-fold less than in water led Partikian and colleagues to question the widely-held view that metabolite channelling, where the product of one enzyme is transferred, as substrate, directly to the next enzyme in the pathway, circumventing free aqueous-phase diffusion, is necessary. Instead, Partikian *et al*. (1998) suggested that the arrangement of metabolic pathways into metabolons, particles containing the enzymes of a part or the whole of a metabolic pathway (Robinson and Srere, 1985; Velot *et al*., 1997), enabled the establishment of an uncrowded, enzyme-free, aqueous space through which solutes could easily diffuse. PDC is a multienzyme complex considered to be a prototypical metabolon. Analysis of the distribution of protein fusions between GFP and PDC subunits in human fibroblasts revealed a discrete, heterogeneous distribution of PDC in the matrix (Margineantu *et al*., 2002*a*). Since human fibroblast mitochondria typically form a reticulum of tubules, the heterogeneous distribution of GFP fluorescence indicates hotspots of PDC along the mitochondrial tubules (Margineantu *et al*., 2002*a*). It will be very interesting to discover whether this heterogeneity is maintained under conditions that cause a fragmentation of the reticulum, i.e. will there be discrete mitochondria lacking PDC? Unfortunately, to my knowledge, nothing is known about the inter-mitochondrial distribution of PDC or the TCA-cycle complexes in the physically discrete mitochondria of higher plants.

Glycolysis

Recently, the application of proteomics has demonstrated that seven of the ten glycolytic enzymes are present in a mitochondrial fraction from *Arabidopsis* suspension culture cells, four of the seven (glyceraldehyde-3-P dehydrogenase, aldolase, phosphoglycerate mutase, and enolase) were also identified in the intermembrane space/outer

membrane fraction (Giege *et al*., 2003). The purified mitochondrial fraction was capable of metabolizing ^{13}C-glucose to ^{13}C-labelled TCA cycle intermediates, demonstrating that the full glycolytic pathway was present and active, and fusions of enolase or aldolase to yellow fluorescent protein demonstrated co-localization with Mitotracker Red stained mitochondria (Giege *et al*., 2003). Giege *et al*. (2003) concluded that the complete glycolytic pathway is associated with mitochondria (possibly as a structurally linked glycolytic metabolon) enabling pyruvate to be provided directly to the mitochondrion where it is a substrate for the matrix-localized PDC. The discoveries of a heterogeneous distribution of PDC along human mitochondrial tubules and the association of glycolysis with mitochondria in *Arabidopsis* raises the intriguing possibility of the two types of compartmentalization existing in the same organism. The glycolytic pathway, partly associated with the outer mitochondrial membrane, would then be adjacent to the matrix-located PDC thereby enabling the direct channelling of pyruvate from glycolyis to the TCA cycle. It is conceivable that this putative juxtaposition of glycolysis and PDC would occur at contact sites (or induce the formation of contact sites) thereby increasing the efficiency of pyruvate channelling.

Intrinsic control of mitochondrial morphology and motility

The conformation of the inner membrane, believed to be continuously variable between the two extremes detailed above (orthodox and condensed) and dependent on the energy state of the mitochondrion, has been shown to affect the external morphology and motility of mitochondria (Bereiter-Hahn and Voth, 1983). Change in the external morphology of mitochondria, the bending, branching, formation, and retraction of localized protrusions (Logan *et al*., 2004) that are typical of mitochondria in living cells have all been ascribed to the rearrangement of cristae (Bereiter-Hahn and Voth, 1994). However, the extent to which these shape changes are truly intrinsic, or involve the activity of molecular motors on the cytoskeleton, is not known. Bereiter-Hahn and Voth (1983) analysed shape changes and motility of mitochondria in endothelial cells from *Xenopus laevis* tadpole hearts. In the condensed state, mitochondria are immobile, while in the orthodox state they are motile (Bereiter-Hahn and Voth, 1983). Inhibition of electron transport or oxidative phosphorylation causes a decrease in mitochondrial motility and a concomitant transition to the condensed conformation (Bereiter-Hahn and Voth, 1983). Injection of ADP, which induces extreme condensation, also immobilizes mitochondria. In addition to their affect on mitochondrial motility, inhibitors of electron transport induce the formation of large disc-shaped mitochondria, an identical morphology is seen in tissues under anoxic conditions (Bereiter-Hahn and Voth, 1983). Low oxygen pressure, achieved by mounting cells at high density under a coverslip on a microscope slide, also induces the formation of disc-like mitochondria in tobacco suspension cultured cells (Van Gestel and Verbelen, 2002). Over a time period of 4 h (shorter at higher cell densities) the normal discrete mitochondria (0.5–5 μm in length) have fused to form a reticulum composed of linear and ring-shaped tubular sections interspersed with large plate-like structures (Van Gestel and Verbelen, 2002). Mitochondria in *Arabidopsis* leaf epidermal cells have been observed undergoing similar morphological transitions during prolonged (40 min) incubation of sections of leaf between slide and coverslip (DC Logan, unpublished observations). Interestingly, unlike the *Xenopus* mitochondria, tobacco suspension cell mitochondria did not change morphology in response to respiration inhibitors or uncouplers (KCN, dinitrophenol or carbonyl cyanide *m*-chlorophenylhydrazone) nor did oxidative stress induced by paraquat, menadion, hydrogen peroxide, or $CuSO_4$ induce changes in the normal mitochondrial morphology (Van Gestel and Verbelen, 2002). Van Gestel and Verblen suggest that this may be due to up-regulation of the alternative respiratory pathway which has been suggested to mitigate against ROS damage in plant cells (Van Gestel and Verbelen, 2002). However, paraquat and hydrogen peroxide do induce a change in the mitochondrial morphology in *Arabidopsis* leaf epidermal cells and mesophyll protoplasts (I Scott, AK Tobin, DC Logan, unpublished data, see below).

The effect of the metabolic status of the mitochondrion on mitochondrial morphology and motility has been suggested to help ensure the mitochondria are located where they are needed. Association of mitochondria with energy-requiring structures or organelles has been well described in a variety of systems (Munn, 1974; Tyler, 1992; Bereiter-Hahn and Voth, 1994). One classic example is the formation of the Nebenkern, a collar around the sperm axoneme formed during spermatogenesis and comprising two giant mitochondria formed by repeated fusion events (Hales and Fuller, 1996, 1997). In plant tissues containing chloroplasts, visualization of mitochondria stained with $DiOC_6$ or expressing GFP has shown the frequent close proximity of these two organelles (Stickens and Verbelen, 1996; Logan and Leaver, 2000). It is assumed that this facilitates exchange of respiratory gases and possibly metabolites, although direct evidence for this is lacking. In characean internode cells, it has been suggested that the spatiotemporal distribution of mitochondria within the cell promotes their association with chloroplasts (Foissner, 2004). A further example of mitochondrial association with energy-consuming structures is the association of mitochondria with the endoplasmic reticulum. One explanation for this association has recently been gaining acceptance. It has been demonstrated in HeLa cells that there are micro-domains of the mitochondrial reticulum

where it is in very close contact (<60 nm) with the ER (Rizzuto *et al*., 1998). The functional significance of these micro-domains has been explained on the basis of Ca^{2+} dynamics (Rutter and Rizzuto, 2000). For example, localized agonist-induced release of Ca^{2+} from the ER may stimulate uptake into the closely associated mitochondria where the transient increase in Ca^{2+} may modulate mitochondrial function.

Mitochondrial inheritance

Mitochondria cannot be created *de novo* and so mitochondrial propagation can only occur through the process of division of the parental organelle. It follows that individuals must inherit their mitochondria. For a recent review of mtDNA inheritance in plants, readers are directed to Barr *et al*. (2005). Mitochondrial inheritance is maternal in the majority of angiosperms (Mogensen, 1988) and therefore represents a form of extreme compartmentalization whereby paternal mtDNA is excluded or removed from the zygote. In contrast to the mechanism in mammals, wherein sperm mitochondria are ubiquitinated during spermatogenesis leading to their specific degradation in the zygote (Sutovsky *et al*., 1996, 1999*a*, 2003; Thompson *et al*., 2003), maternal inheritance in angiosperms appears to occur primarily as a result of the exclusion of paternal mitochondrial genomes from the male reproductive cells before fertilization (Nagata *et al*., 1999*a*, *b*; Sodmergen *et al*., 2002), although a mechanism similar to that active in mammals may also operate in angiosperms (Liu *et al*., 2004). In species that show biparental inheritance of mitochondria, the abundance of mtDNA increases during pollen development (Nagata *et al*., 1999*a*). The question of why the majority of eukaryotes display uniparental inheritance of mitochondria is addressed in the section below on the mitochondrial theory of ageing.

Mitochondrial inheritance in yeast is a highly ordered process and many proteins have been implicated in its control (for a review see Okamoto and Shaw, 2005). By contrast, little is known about the mechanisms controlling the inheritance and proliferation of mitochondria in multicellular organisms. During the human cell cycle, mitochondria switch between two predominant morphological states (Barni *et al*., 1996; Karbowski *et al*., 2001; Margineantu *et al*., 2002*b*). During the G_1 phase of the cell cycle, mitochondria fuse to form reticula, bringing the number of individual organelles to half the number prior to M phase (Karbowski *et al*., 2001). As cells proceed from G_1 to S phase mitochondrial numbers increase due to fragmentation (division) of the mitochondrial reticula (Barni *et al*., 1996; Karbowski *et al*., 2001; Margineantu *et al*., 2002*b*). A similar series of events occurs in plants (Sheahan *et al*., 2004, 2005). Using protoplast culture, Sheahan and co-workers showed that, prior to protoplast division, mitochondria undergo a period of extensive elongation as a result of what is termed massive mitochondrial fusion (MFF; see below) followed by a phase of mitochondrial fission which, in turn, is followed by the active and uniform dispersal of the mitochondrial population within the cell (Sheahan *et al*., 2005). The active and uniform dispersal of mitochondria throughout the cytoplasm ensures the unbiased distribution of mitochondria into each daughter cell following cytokinesis, thus the cellular inheritance of mitochondria is not a strictly random process.

Little is known concerning the mechanisms regulating the cellular distribution of mitochondria in higher plants, but disruption of the actin cytoskeleton affected the dispersal of mitochondria and resulted in a biased distribution of mitochondria in the daughter cells (Sheahan *et al*., 2004). Mutations of the *Arabidopsis FRIENDLY MITOCHONDRIA* (*FMT*) gene lead to a grossly altered cellular distribution of mitochondria (Logan *et al*., 2003). Disruption of *FMT* causes the mitochondria to form large clusters of ten or hundreds of organelles, although some mitochondria remain apparently normally distributed as singletons throughout the cytoplasm (Logan *et al*., 2003). *FMT* is a conserved eukaryotic gene but, apart from a short tetratricopeptide repeat (TPR) domain that is thought to function in protein–protein interactions, the FMT protein has no homology to proteins of known function. Disruption of *FMT* homologues in *Dictyostelium discoideum* (*cluA*) or *S. cerevisiae* (*CLU1*) causes aberrant mitochondrial phenotypes (Zhu *et al*., 1997; Fields *et al*., 1998). In the *cluA*$^-$ mutant of *D. discoideum* the mitochondria cluster near the cell centre (Zhu *et al*., 1997) while in the *S. cerevisiae clu1* mutant the mitochondrial tubules collapse to one side of the cell (Fields *et al*., 1998).

Although at the time of writing the role of the FMT protein is unknown, it is proposed that FMT is involved in the interaction of mitochondria with the microtubule cytoskeleton (Logan *et al*., 2003). Plant mitochondrial movement has been shown to rely on actin microfilaments (Olyslaegers and Verbelen, 1998; Van Gestel *et al*., 2002). However, microtubules have been shown to have a role in the positioning and/or tethering of mitochondria (Van Gestel *et al*., 2002). Mitochondria have been shown to associate with the microtubule-specific motor protein, kinesin (Khodjakov *et al*., 1998) that binds cargo at the tetratricopeptide repeat (TPR) domains in the kinesin light chains (Stenoien and Brady, 1997; Verhey *et al*., 2001). When mitochondria are moving on actin filaments they are presumably prevented from binding to microtubules until this is required to immobilize the mitochondria or effect small-scale adjustments to their position. Based on the phenotype of the *fmt* mutant and the presence of a conserved TPR domain in FMT it is hypothesized that when mitochondria are moving on actin filaments FMT binds to the kinesin-like receptor on the mitochondrion via the TPR domains thereby preventing any unwanted association with microtubules. This putative role of FMT/CluA as

a cap for the kinesin-like receptor on the mitochondrion leaves the microtubule-associated kinesin motor free to associate with other mitochondria or different types of cargo. Applying this hypothesis to the *fmt* mutant, in which receptor-capping would not occur, leaves the mitochondria free to bind to microtubules which prevents their movement on actin. A cluster of mitochondria then develops as mitochondria divide but are unable to move apart. This hypothesis is consistent with the lack of a clustered mitochondrial phenotype in Clu-mutants of *Caenorhabditis elegans* (M Clarke, personal communication) since, in this organism, large-scale mitochondrial movement is microtubule-based. Application of this receptor-capping hypothesis for FMT function to *D. discoideum* suggests an explanation for the interconnections between clustered mitochondria in the *D. discoideum cluA*$^-$ mutant reported by Fields *et al*. (2002). In *D. discoideum*, final separation of mitochondria following their division may require movement on the cytoskeleton and, therefore, when this is prevented in the case of the *cluA*$^-$ mutant, because of inexorable binding to microtubules, the mitochondria remain connected. The absence of a similar phenotype in *Arabidopsis* may simply reflect differences in the architecture of the actin cytoskeleton or the contribution of movement to the mitochondrial division process. This hypothesis for FMT function is supported by the observation that the pattern of mitochondrial distribution in *fmt* mutants mimics the effect of latrunculin-B, which promotes the rapid depolymerization of the actin cytoskeleton (DC Logan, unpublished observations). The *Arabidopsis* genome contains 61 identified kinesin genes, the highest number in any sequenced eukaryotic genome (Reddy and Day, 2001). However, no heavy chains have been identified in any plant, although some light chains have been predicted in the *Arabidopsis* genome (Reddy and Day, 2001). By contrast, there are many microtubule-associated processes unique to plants that are likely to require additional plant-specific microtubule-associated proteins including motors (Reddy and Day, 2001; Hussey, 2004). Yeast-two-hybrid screens are currently being used to identify proteins interacting with FMT *in vivo.*

Control of mitochondrial number, size, and gross morphology

Mitochondria are pleomorphic organelles and, at any point in time, the mitochondrial population of a given cell consists of a heterogeneous mix of mitochondrial morphologies. In higher plants, mitochondria are typically discrete, spherical to sausage-shaped organelles although more extreme morphologies are frequently seen (see www.st-andrews.ac.uk/~dclogan for movies of *Arabidopsis* leaf mitochondria and www.plantcellbiologyoncd.com for a range of movies of mitochondria in living plant tissue). The number of mitochondria per cell is variable, depending on cell type and physiological state, however, a typical *Arabidopsis* mesophyll cell contains approximately 200–300 discrete mitochondria while tobacco mesophyll protoplasts typically contain 500–600 (Sheahan *et al*., 2004). This basic organization of the higher plant mitochondrial population into discrete organelles sets plants apart from many other eukaryotes. For example, in *S. cerevisiae* there are typically 5–10 tubular mitochondria forming an extended reticular network beneath the cell cortex (Stevens, 1977). In various mammalian cell types the mitochondrial morphology is complex, consisting of a mixture of small discrete spherical mitochondria and elongate tubular regions that often form a reticulum. Mitochondrial pleomorphism in mammalian cells is known to be affected by culture conditions since different research groups working on the same cell lines have described different morphologies (Karbowski and Youle, 2003).

Mitochondrial fission

Mitochondrial shape, size, and number are controlled by the dynamically opposing processes of fission and fusion (for review see Logan, 2003; Okamoto and Shaw, 2005). Hermann *et al*. (1997) screened a population of UV-mutagenized *S. cerevisiae* for individuals that displayed aberrant mitochondrial morphologies as revealed by staining with $DiOC_6$. In one of the identified mutants, *mdm29*, the mitochondrial reticulum collapses into a bundle of tubules along one side of the cell. The *mdm29* mutation was mapped to *DNM1* (Otsuga *et al*., 1998), which encodes a protein structurally related to dynamin, a large GTPase required for membrane scission during exocytosis (Herskovits *et al*., 1993; van der Bliek *et al*., 1993; Damke *et al*., 1994, 1995; Hinshaw and Schmid, 1995). Independently, Smirnova *et al*. (1998) demonstrated that Drp1, another member of the dynamin superfamily of large GTP-binding proteins, is involved in the maintenance of normal mitochondrial morphology and distribution in humans. At around the same time as it was suggested that dynamin-like proteins function in mitochondrial fission, a second large GTP-binding protein, called *fuzzy onions* (*fzo*), was shown to be required for normal development of the Nebenkern (see above), during spermatogenesis in *Drosophila* (Hales and Fuller, 1997). Analyses of the *fzo1* mutation in yeast confirmed the role of this protein in mitochondrial fusion (Hermann *et al*., 1998; Rapaport *et al*., 1998); see below) and led to confirmation of the role of the dynamin-like protein, Dnm1p, in yeast mitochondrial fission (Bleazard *et al*., 1999; Sesaki and Jensen, 1999). The Fzo1p GTPase regulates mitochondrial fusion, and loss of Fzo1p function causes the mitochondrial network to fragment rapidly (Hermann *et al*., 1998). By contrast, mutation of *DNM1* causes mitochondria to lose their normal structure and, instead, form a large network of interconnected tubules

(Sesaki and Jensen, 1999). In *fzo1p dnm1p* double mutants the wild-type yeast mitochondrial phenotype is restored. This analysis elegantly demonstrated that a delicate balance of fission and fusion is required to maintain yeast mitochondrial shape, size, and number.

Dnm1p is a soluble protein but assembles on the outer mitochondrial membrane at sites where subsequent division occurs (Okamoto and Shaw, 2005). Three further proteins are required for yeast mitochondrial fission and have been shown to regulate the assembly of Dnm1p. Fis1p (Mozdy *et al*., 2000; Tieu and Nunnari, 2000; not to be confused with the *Arabidopsis* Fertilization Independent Seed 1 protein) is an evolutionarily conserved integral outer membrane protein tethered by a C-terminal hydrophobic domain and, unlike Dnm1p, is evenly distributed on the mitochondrial outer surface. The mitochondrial phenotype of yeast *FIS1* deletion mutants, in which the mitochondria have a net-like morphology, is identical to the *DNM1* deletion mutants and double *FIS1/DNM1* deletion mutants suggesting that Fis1p and Dnm1p act in the same pathway (Mozdy *et al*., 2000). Fis1p is required for targeting of cytosolic Dnm1p to the mitochondria (Mozdy *et al*., 2000; Tieu and Nunnari, 2000). The second protein that interacts with Dnm1p, independently identified by four research groups, is Mdv1p, a mitochondria-associated cytosolic protein that, like Dmn1p, is localized to the site of mitochondrial division (Cerveny *et al*., 2001; Fekkes *et al*., 2000; Mozdy *et al*., 2000; Tieu and Nunnari, 2000). Cells disrupted in *MDV1* showed aberrant mitochondrial morphology characterized by a net-like sheet of interconnected tubules, similar to the phenotype of *DNM1* deletion mutants. The third Dnm1p-interacting protein, Caf4p, was identified in an affinity screen aimed at identifying proteins interacting with Fis1p (Griffin *et al*., 2005). Caf4p interacts with each of the other members of the mitochondrial division apparatus: the N-terminal half of Caf4p interacts with Fis1p and Mdv1p; the C-terminal WD40 domain interacts with Dnm1p (Griffin *et al*., 2005).

Dynamin-like proteins are also required for mitochondrial division in *Arabidopsis*. Disruption of either of two *Arabidopsis* dynamin-like genes, *DRP3A* or *DRP3B*, results in an aberrant mitochondrial morphology characterized by an increase in the size of individual mitochondria and a concomitant decrease in the number of mitochondria per cell (Arimura and Tsutsumi, 2002; Arimura *et al*., 2004; Logan *et al*., 2004; I Scott, AK Tobin, DC Logan, unpublished results). The mutant mitochondria also have frequent constrictions along their length suggesting that they are arrested at the stage of membrane scission (Logan *et al*., 2004; I Scott, AK Tobin, DC Logan, unpublished results). In addition to DRP3A and DRP3B, a further two members of the *Arabidopsis* dynamin-like superfamily have been implicated in the control of mitochondrial morphology (Jin *et al*., 2003). DRP1C and DRP1E, members of the DRP1 subfamily with closest homology to soybean phragmoplastin (Gu and Verma, 1996), were reported to co-localize partially with mitochondria and disruption of either gene was reported to increase the proportion of mitochondria with an elongated morphology (Jin *et al*., 2003). However, DRP1C and DRP1E have also been localized to the developing cell plate and so these dynamin-like proteins may have a complex role in plant development.

Apart from dynamin-like proteins, only one other member of the plant mitochondrial division apparatus has been identified. An *Arabidopsis* functional homologue of hFis1/Fis1p was recently identified, and named *BIGYIN* (At3g57090) to reflect the mitochondrial phenotype in homozygous T-DNA insertion mutants (Scott *et al*., 2006). Disruption of *BIGYIN* leads to a reduced number of mitochondria per cell, coupled with a large increase in the size of individual mitochondria relative to wild type, a phenotype very similar to that of DRP3A and DRP3B mutants. The similarity between the DRP3A, DRP3B, and BIGYIN mutant phenotypes reflects the situation in yeast and mammalian cells where Dnm1p/Drp1 and Fis1/hFis mutants have indistinguishable mitochondrial morphologies. This suggests that, as in yeast and mammalian cells (Mozdy *et al*., 2000; Tieu *et al*., 2002; Stojanovski *et al*., 2004), in plant cells, dynamin-like (DRP3A, DRP3B) and Fis-type (BIGYIN) proteins act in the same pathway. The *Arabidopsis* genome contains two homologues of the yeast *FIS1* and human *hFIS1* genes and analysis of their protein sequence shows that *BIGYIN* shares highest homology with hFIS1 (26.7% identity, 48.3% similarity) and other *FIS1*-type genes from multicellular organisms, such as the *C. elegans FIS-2* gene (locus NM_001029389). Conversely, the second *Arabidopsis* Fis1-type gene, At5g12390, shares highest homology with yeast Fis1p (27.0% identity, 43.8% similarity). An analysis of the role of the second *Arabidopsis* Fis1-type gene, At5g12390, in mitochondrial fission is currently hampered by the lack of T-DNA mutants of this gene. However, future research using alternative reverse genetics approaches should help delineate the role of At5g12390 in mitochondrial dynamics and should reveal any redundancy between the two *Arabidopsis* Fis1-type genes. The *Arabidopsis* genome contains structural homologues of *CAF4*, to my knowledge, nothing is known concerning their function in mitochondrial fission. At the time of writing no structural homologues of *MDV1* have been identified in any multicellular eukaryote.

Mitochondrial fusion

As with mitochondrial division, much of our knowledge about mitochondrial fusion comes from studies using *S. cerevisiae*. However, as mentioned above, the first component of the mitochondrial fusion apparatus was discovered in *Drosophila*. Homologues of *fuzzy onions* are involved in

mitochondrial fusion in *S. cerevisiae* and multicellular animals (e.g. *C. elegans* and humans), but, to date, no structural homologues have been identified in any plant species (Logan, 2003; Okamoto and Shaw, 2005). Indeed, no plant genes mediating mitochondrial fusion have been identified. There are *Arabidopsis* homologues of Mgm1p within the large family of dynamin-like proteins, but the protein with the highest homology to Mgm1p is DRP3A, which is believed to be involved in mitochondrial fission. However, if the parsimonious explanation of the role of DRP3A as provided by Arimura *et al*. (2004) and Logan *et al*. (2004) is incorrect, it is possible that DRP3A functions as a negative regulator of mitochondrial fusion, although this role would contrast with the role of Mgm1p and its mammalian homologue, OPA1 (Misaka *et al*., 2002; Okamoto and Shaw, 2005).

Although no genetic components of the plant mitochondrial fusion apparatus have been identified there is no doubt that plant mitochondria fuse. Movies showing mitochondria fusing *in vivo* in a variety of plants have been available for a number of years (see movies at www.plantcellbiologyoncd.com), but it is only in the past couple of years that attempts have been made to investigate the process in detail. In an elegant study, Arimura *et al*. (2004) used a photoconvertible fluorescent protein called Kaede that can be induced to change irreversibly from green to red upon exposure to light of 350–400 nm. Using transient expression of mitochondria-targeted Kaede in onion cells, Arimura *et al*. (2004) were able to convert half of the mitochondria to fluoresce red and then visualize fusion between red and green mitochondria through the appearance of yellow mitochondria due to mixing of the matrix-localized fluorescent proteins. Although in onion cells the fusion was often transient, lasting only a few seconds, there was sufficient fusion between the mitochondria to convert them all to yellow within 1–2 h (Arimura *et al*., 2004).

As described in the section on mitochondrial inheritance above, extensive fusion of mitochondria (the MMF, Sheahan *et al*., 2005) occurs immediately prior to a phase of mitochondrial fission, followed by redistribution of the newly mixed mitochondrial population and subsequent cytokinesis. The MMF, which occurs within 4–8 h of the initiation of protoplast culture, requires an inner membrane electrochemical gradient, cytoplasmic protein synthesis and an intact microtubule cytoskeleton; MMF does not require ATP or an intact actin cytoskeleton (Sheahan *et al*., 2005).

Compartmentalization of mtDNA: the mitochondrion is a discontinuous whole

In using the term *mitochondrial population* I have so far ignored a recurring question in mitochondrial biology: does the cell contain one single mitochondrion or a population (i.e. greater than one physically discrete mitochondrion)? Of course this is too general a question to have one single succinct answer, but if one had to give an equally general answer it would be that, in most cells, most of the time, a population of mitochondria exists, with the number of discrete organelles within the population being dependent on species, cell type, physiological status, and, equally crucially, being dependent on the time when the observation of the number of discrete organelles is made. Accepting that the default status of the chondriome (all the mitochondria of a cell collectively) in higher plants is to exist as a population of discrete organelles, the question to be asked is are physically discrete mitochondria independent of each other? To answer this question it is necessary to understand a little about the organization of the mitochondrial genome.

Mitochondrial DNA is organized into nucleoids, mtDNA–protein complexes, located in the matrix, tethered to the inner mitochondrial membrane. The plant mitochondrial genome is a highly complex structure composed of small circular and large circularly permuted DNA molecules (Lonsdale *et al*., 1988; Bendich, 1993, 1996; Backert *et al*., 1995; Oldenburg and Bendich, 2001) that arise by active homologous inter- and intra-molecular recombination (Lonsdale *et al*., 1988). The high frequency of recombination results in the mitochondrial genome existing as a series of subgenomic, sometimes substoichiometric, DNA molecules that, once generated, may replicate autonomously (Lonsdale *et al*., 1988; Small *et al*., 1989; Janska and Mackenzie, 1993; Janska *et al*., 1998; Abdelnoor *et al*., 2003). However, it is clear that many physically discrete mitochondria within a plant cell contain less than a full genome (Lonsdale *et al*., 1988). To account for the observed complexity of the mitochondrial genomes of higher plants, Lonsdale *et al*. (1988) proposed that the mitochondria form a ‘dynamic syncytium’ and that the mitochondrial population within a cell is panmictic (via organelle fusion and fission) and, as a result, in a state of recombinational equilibrium. Inter-mitochondrial recombination has been demonstrated in tobacco cybrids (Belliard *et al*., 1979) and complementation between fused mitochondria is now believed to be a common mechanism to counter the accumulation of mtDNA mutations in mammalian mitochondria (Nakada *et al*., 2001; Ono *et al*., 2001). In addition, there is direct evidence for the heterogeneous distribution of nucleoids between physically discrete mitochondria in *Arabidopsis* (Arimura *et al*., 2004), tobacco (Sheahan *et al*., 2005), and in the mitochondrial reticulum of human cell lines (Margineantu *et al*., 2002*b*). The role of mitochondrial fusion in the redistribution of mitochondrial nucleoids has also been demonstrated (Arimura *et al*., 2004; Sheahan *et al*., 2005). However, in my opinion ‘dynamic syncytium’ is better suited as a description of a predominantly reticular chondriome (such as in *S. cerevisiae* and many mammalian cell types) than as a description of the higher plant

chondriome that normally comprises discrete organelles and rarely forms a syncytium. Instead, I suggest that the higher plant chondriome is better termed a 'discontinuous whole'. So, to return to the question: are morphologically discrete mitochondria independent of each other? The answer must be no, at least not genetically. But, are physically discrete regions of the chondriome functionally heterogeneous?

Form and function: compartmentalization and division of labour

In the previous sections I have reviewed what is known about compartmentalization of the mitochondrial population mainly from a structural perspective. In the remainder of this review I will try to bring much of the preceding information together into a discussion of how structural compartmentalization may underpin functional diversity and why compartmentalization is vital to the success of what is the oldest symbiosis in the history of the eukaryotic cell.

Changes in the protein composition of mitochondria have been reported to underpin changes in the development of various plant tissues. Mitochondrial biogenesis has been studied in many species during seed germination and, in parallel with the increase in respiratory activity in the germinating seed, changes in the mtDNA content, phospholipids, protein complement, and enzyme activities have been reported (Akazawa, 1956; Breidenbach *et al*., 1967; Solomos *et al*., 1972; Morohashi and Bewley, 1980*a*, *b*; Morohashi *et al*., 1981*a*, *b*; Rickwood *et al*., 1987; Ehrenshaft and Brambl, 1990; Logan *et al*., 2001). In germinating maize embryos two populations of mitochondria have been identified that differ in structural development, protein complement, and respiratory activity (Logan *et al*., 2001). However, to what extent these populations represent stages in the biogenesis of nascent pre-mitochondria in the dry seed into fully functioning, respiratory-efficient organelles during imbibition rather than populations of mitochondria with distinct functions is unknown. Dai (1998) reported different populations of mitochondria in etiolated mung bean seedlings. Using density gradient fractionation Dai *et al*. (1998) identified four mitochondrial populations: regular-sized organelles, poorly respiring morphologically distinct mitochondria, and two populations of small, non-respiring low-density organelles. Dai *et al*. (1998) suggested that the different populations of mitochondria may indicate different functional subclasses, but evidence for this is lacking. Thompson *et al*. (1998) studied the biogenesis of mitochondria during leaf development of barley. Their data indicate that the protein composition of mitochondria changes significantly during leaf development and they were able to identify three groups of mitochondrial proteins according to their developmental profile (Thompson *et al*., 1998). Within group A were proteins involved in electron transport, or synthesis and assembly of the electron transport chain. Group A proteins (e.g. ATPase subunits, cytochrome oxidase) accumulated during the early stages of leaf development, reaching maximum abundance at the end of the zone of elongation (20 mm from the basal meristem). Group B proteins (e.g. PDC E1α, isocitrate dehydrogenase, CPN-60) also accumulated with leaf age, but at a slower rate than group A proteins and reached peak abundances coincident with the stage of leaf development marked by a rapid increase in the abundance of photosynthetic proteins. Group C proteins (e.g. fumarase, GDC subunits) showed a steady increase in abundance throughout development. These distinct patterns of protein accumulation reflect the changing needs of the leaf blade as it develops and matures (Thompson *et al*., 1998). At early stages of development cells lacking photosynthetic capability rely on mitochondria for ATP production and thus group A proteins are vital. As the photosynthetic apparatus is assembled and activated, so is the need for the cells to be able to support demand for glycine oxidation, hence the increase in abundance of GDC subunits. Specific changes in the protein complement and activity of mitochondria have been correlated with other developmental processes. One of the best characterized is the increase in alternative oxidase (AOX) activity in the Arum lily spadex which results in a great increase in non-phosphorylating proton transport thereby generating the heat required to release the volatiles and attract pollinators (Meeuse and Raskin, 1988; Vanlerberghe and McIntosh, 1997). On the day of flower opening, AOX activity peaks and the respiratory rate of the spadex is equivalent to humming bird flight muscle and oxygen uptake is fully cyanide resistant (Elthon *et al*., 1989; Rhoads and Mcintosh, 1992).

In non-thermogenic tissues, AOX has been proposed to act as an energy-overflow that enables the TCA cycle to continue to function and provide intermediates for biosynthesis even under conditions when the cytochrome pathway has become limiting (Mackenzie and McIntosh, 1999). A related possible function of AOX is to protect the mitochondrion from reactive oxygen species (ROS; Moller, 2001). The production of ROS is an unavoidable consequence of aerobic metabolism (Chance *et al*., 1979) and the mitochondrial electron transport chain is a major site of ROS production in mammalian cells (Halliwell and Gutteridge, 1999) and in non-photosynthesizing plant cells (Puntarulo *et al*., 1991); the relative contributions of mitochondria and chloroplasts to the cellular ROS pool have been estimated by Foyer and Noctor (2003). ROS induce a variety of damage to DNA including base pair mutations, deletions, insertions, and various other lesions (Wiseman and Halliwell, 1996). Due to the proximity of the ROS producing ETC to mitochondrial nucleoids, mtDNA is prone to oxidative damage and mtDNA has been demonstrated to be more susceptible to ROS damage than

nuclear DNA (Mandavilli *et al*., 2002). Since ROS production is due to the interaction of molecular oxygen with reduced electron transport components, one way to minimize ROS production is to prevent overreduction of the ETC. The function of AOX as an alternative conduit for electrons when the cytochrome pathway is saturated thereby acts to prevent the generation of ROS. Indeed, Maxwell *et al*. (1999) demonstrated that cells overexpressing AOX had half as much ROS as controls, while cells in which AOX expression was suppressed contained five times as much ROS. A similar role in preventing the generation of ROS has been attributed to the uncoupling proteins (Kowaltowski *et al*., 1998). Dissipation of the proton concentration gradient across the inner membrane would reduce the membrane potential and thereby reduce the formation of ROS by the mitochondrion (Skulachev, 1996*b*; Korshunov *et al*., 1997).

Mitoptosis and PCD eliminate ROS-damaged mtDNA

ROS are known to have a direct effect on mitochondrial morphology. In many mammalian cell types, in which the chondriome is organized as a large reticulum, it has been shown that ROS cause the break-up of the reticulum into smaller, physically discrete, organelles. This process of coordinated mitochondrial fission, which is termed the thread-grain transition has been proposed as a means to isolate a damaged part of the reticulum which, if not repaired, is removed from the mitochondrial population (Skulachev, 1996*b*, 2002; Skulachev *et al*., 2004). This selective removal and destruction of damaged mitochondrial membranes and mtDNA, termed mitoptosis, by analogy with apoptosis, (Skulachev, 1998), is a form of organelle suicide, since the mitochondrion precipitates its own demise (Skulachev, 2002).

In addition to the induction of the thread-grain transition, *in vitro* ROS treatment of mitochondria causes what is termed a mitochondrial permeability transition (MPT) possibly by opening a megachannel in the inner mitochondrial membrane called the permeability transition pore (PTP), a pore permeable to compounds of molecular mass <1.5 kDa (Forte and Bernardi, 2005; Green and Kroemer, 2004). The MPT dissipates the proton motive force, causing the cessation of oxidative phosphorylation, protein import, and the correct organization of mitochondria-synthesized proteins in the inner membrane (Skulachev, 1996*a*, *b*, 1998, 2002). In addition, the increased permeability of the mitochondrion will cause the matrix to swell, the outer membrane to rupture and the release of death-inducing intermembrane space proteins into the cytosol (Wang, 2001).

A MPT, measured as a decrease in the mitochondrial membrane potential, has been reported to be an early indicator of PCD in *Arabidopsis* protoplasts in response to various cell death triggers, including an HR elicitor, ceramide, or protoporphyrin IX (Yao *et al*., 2004). Protoporphyrin IX is a molecule similar to the proposed substrate of ACD2, which is a red chlorophyll catabolite reductase (Mach *et al*., 2001); ACD2 (*accelerated cell death 2*) mutants show ectopic cell death and, therefore, the substrate is believed to an endogenous cell-death trigger (Yao *et al*., 2004). Swelling of potato mitochondria was induced by Ca^{2+} but not Mg^{2+}; Ca^{2+}-induced swelling was inhibited in the presence of cyclosporin A, an inhibitor of the PTP (Arpagaus *et al*., 2002). Ca^{2+}-induced swelling was also detected with oat mitochondria pretreated with the Ca^{2+} ionophore A23187 (Curtis and Wolpert, 2002). Furthermore, swelling led to disruption of the outer, but not inner, mitochondrial membrane and release of cytochrome *c* (cyt *c*) (Arpagaus *et al*., 2002) which is a trigger of classical apoptosis in animal cells (Wang, 2001). *In vivo*, cyt *c* release into the cytosol has been documented as an early event during PCD in cucumber (Balk *et al*., 1999), maize suspension culture cells (Stein and Hansen, 1999), tobacco protoplasts (Sun *et al*., 1999), and *Arabidopsis* suspension cells (Krause and Durner, 2004). However, the mechanisms underlying the MPT and cyt *c* release in plants are unknown; no links have been established *in vivo* between these two events. If the number (volume) of mitochondria being destroyed by mitoptosis is small relative to the volume of the cell, then the concentrations of intermembrane space proteins released are too low to induce PCD. However, once the proportion of mitochondria undergoing mitoptosis passes a given threshold then PCD will be induced (Skulachev, 2000, 2001; Skulachev *et al*., 2004). Indeed, it has been demonstrated that induction of apoptosis in mammalian cells by treatment with staursosporine leads to disintegration of the mitochondrial reticulum (Frank *et al*., 2001). Mitochondrial fragmentation at the onset of PCD appears to be mediated by the mitochondrial division apparatus since neither fragmentation, loss of inner membrane potential, release of cyt *c* nor cell death occurred in cells expressing dominant mutations of Drp1 (Frank *et al*., 2001; Lee *et al*., 2004). In addition, the pro-apoptotic protein Bax, co-localizes with Drp1 at the tips of mitochondrial tubules and at mitochondrial constriction sites (Karbowski *et al*., 2002). While there is no obvious Bax sequence homologue in plant genomes, heterologous Bax induces PCD in a predominantly ROS-dependent manner and localizes to *Arabidopsis* mitochondria *in vivo* (Baek *et al*., 2004).

It is tempting to hypothesize that the effect of ROS on chondriome structure may explain the organization of the higher plant chondriome as a discontinuous whole (I Scott, personal communication). Chloroplasts are major sites of ROS production in photosynthetic tissues and it is possible that the higher steady-state concentration of ROS in photosynthetic organisms is a selective pressure shaping chondriome structure. A shift in the equilibrium of the

dynamic syncytium during plant evolution to produce a more discontinuous chondriome would be advantageous since it would limit propagation of the ROS chain-reaction within mitochondria. A downside to the discontinuous whole arrangement over a reticular structure is that complementation of damaged mtDNA molecules via recombination would be less efficient, more often requiring mitochondrial fusion to provide mutation-free mtDNA from an undamaged discrete mitochondrion. The relatively large size and complex organization of the higher plant mitochondrial genome into numerous sub-genomic DNA molecules within each discrete organelle can be viewed as a mechanism to increase the likelihood that a discrete mitochondrion is able to repair itself without risking passing the mutation to other mitochondria. If repair of the damaged mitochondrion is not possible then that organelle is presumable targeted for destruction. It is hypothesized that, as with mammalian cells, if too many mitochondria become damaged then programmed cell death would be initiated. However, little is known about the role of mitochondrial dynamics in plant cell death.

The effect of exogenously applied ROS or ROS-inducing chemicals on *Arabidopsis* mitochondrial morphology was recently investigated (I Scott, AK Tobin, DC Logan, unpublished results). Within 1 h of exposing *Arabidopsis* mesophyll protoplasts to ROS (paraquat, H_2O_2) mitochondria in 60–70% of protoplasts undergo a morphology transition characterized by swelling to at least double their volume [a similar effect of paraquat and H_2O_2 was reported by Yoshinaga *et al*. (2005)]. No significant cell death occurs over the following 23 h, but after 48 h ROS treatment 70–90% of protoplasts have died, all containing mitochondria that underwent a morphology transition; the 10–30% of protoplasts that remain alive after treatment contain mitochondria of normal morphology. While it is concluded that the mitochondrial morphology transition is an early and specific indicator of subsequent cell death, it is not yet known whether the mitochondrial morphology transition is synonymous with the mitochondrial permeability transition and whether or not cells die via a programmed pathway (I Scott, AK Tobin, DC Logan, unpublished research).

The mitochondrial theory of ageing

The initiation of ROS production can lead to a chain-reaction whereby ROS damage leads to greater ROS production leading, in turn, to greater ROS-induced damage. This positive feedback-loop in mitochondria, whereby free-radical damage of mtDNA leads to a damaged electron transport chain, which leads to incorrect electron transfer, and thus damage to mtDNA, has negative effects on both the bioenergetic and genetic integrity of the mitochondrion. Such is the central role of the mitochondrion in the life of the cell that the accumulation of bioenergetically and genetically compromised mitochondria has been suggested to underpin the ageing process (Ernster, 1994; Shigenaga *et al*., 1994; Ozawa, 1995; Allen, 1996). There is a vast literature dealing with the mitochondrial theory of ageing and anything but a superficial treatment of this topic is out of the scope of this review, instead readers are directed to two recent reviews if they wish to learn more: Balaban *et al*. (2005) and Dufour and Larsson (2004).

The mitochondrial theory of ageing not only provides an explanation for uniparental inheritance of mitochondria (see section on mitochondrial inheritance) but also hints at a more general division of labour amongst mitochondria. Because of the ROS feedback-loop, there is a conflict between the mitochondrion's role as the site of oxidative phosphorylation and its role as the unit of its own dissemination. It follows, therefore, that one of the simplest means to decrease the mutation rate of mtDNA would be division of labour among mitochondria; some mitochondria fulfilling a bioenergetic function while others would be carriers of the mtDNA. Allen (1996) proposed that the conflict between respiration and genetic integrity could be solved by anisogamy and his hypothesis not only provided a means to ensure fidelity of transmission of mtDNA from parent to offspring, but it also could help explain the evolution of the sexes and some of their defining characteristics. Since offspring do not inherit their mitochondria from somatic cells, the division of labour must exist between germ-lines of different sex. As proposed by Allen (1996) the 'male' sex is defined as the sex that produces a large number of small mobile gametes and does not contribute to the offspring's mitochondrial genome. In the 'male', a combination of the numerous mtDNA replicative cycles needed to produce the large number of gametes, together with the high respiratory activity required of motile gametes would result in a relatively high number of mutations per gamete by the time fertilization could occur. The female sex, by contrast, produces a small number of large immobile gametes with a repressed bioenergetic function; the number of accrued mtDNA mutations is relatively low and therefore 'female' mitochondria are the source of the mitochondrial genome in the offspring. Bioenergetically active mitochondria of the male germ line and somatic cells in the offspring of both sexes arise from division of the female germ-line mitochondria and the switch from a genetic to a bioenergetic role is suggested to be irreversible (Allen 1996). By contrast, mitochondria in the female germ-line would be protected from oxidative damage by the absence of the ETC and by keeping organelle and mtDNA replication to a minimum.

Conclusions

Mitochondria are complex semi-autonomous organelles that provide the energy for life and the trigger for death.

Without compartmentalization, mitochondria would not be able to convert the potential energy stored in respiratory substrates into ATP. However, being a semi-autonomous organelle, containing its own DNA, is not compatible with the mitochondrion's role as provider of energy. This conflict of interests, between energy provider (hence ROS generator) and genetic vault, has been overcome in plants by a series of features including: (i) compartmentalization of the chondriome into a series of physically discrete mitochondria (a 'discontinuous whole'), (ii) the organization of the mitochondrial genome in a multipartite highly replicated structure and its distribution amongst the discrete mitochondria in (frequently) sub-genomic proportions, (iii) inter-mitochondrial complementation to mitigate against the accumulation of mtDNA lesions in any given physically discrete organelle, either by panmictic fusion of discrete mitochondria in non-dividing cells or during the MMF prior to cell division, and (iv) anisogamy in most angiosperms to ensure the inheritance of undamaged mitochondria and mtDNA. Future research using the latest advances in bioimaging and functional genomics will help to answer questions about the division of labour amongst the physically discrete members of the higher plant chondriome of somatic cells. For example, whether or not individual mitochondria within any given cell are specialized for bioenergetics, biosynthesis, ROS monitoring/signalling, or PCD induction.

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