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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.

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

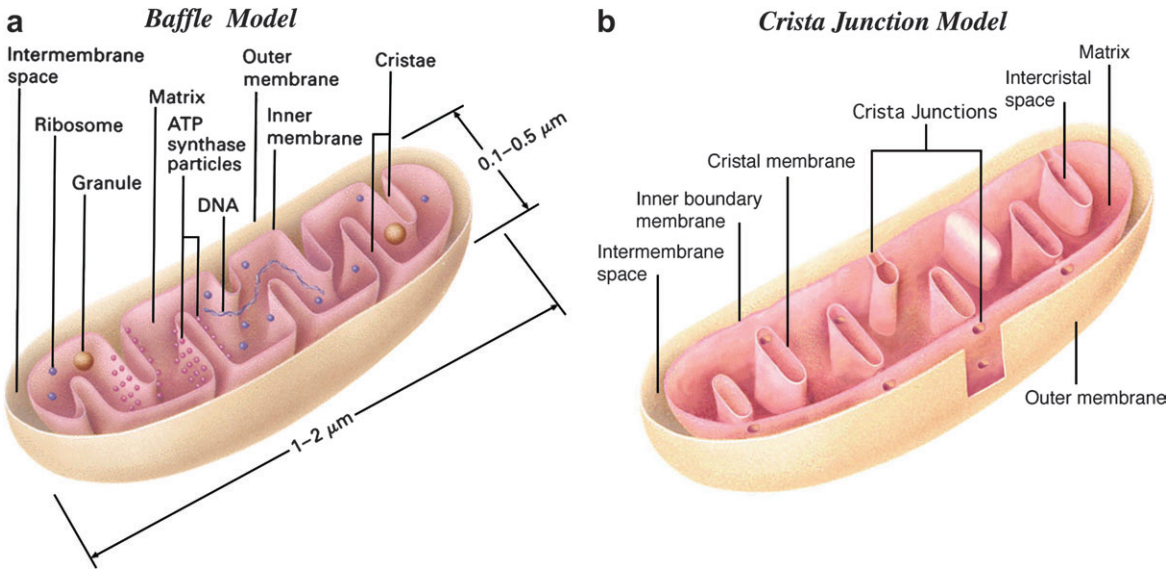


**Fig. 1.** Models of mitochondrial membrane structures. (a) Infolding or 'baffle' model, which is the representation most commonly depicted in textbooks (reproduced from Lodish *et al*., 1995, Fig. 5–43, with permission from WH Freeman). This model originated with Palade in the 1950s and has been prominent 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

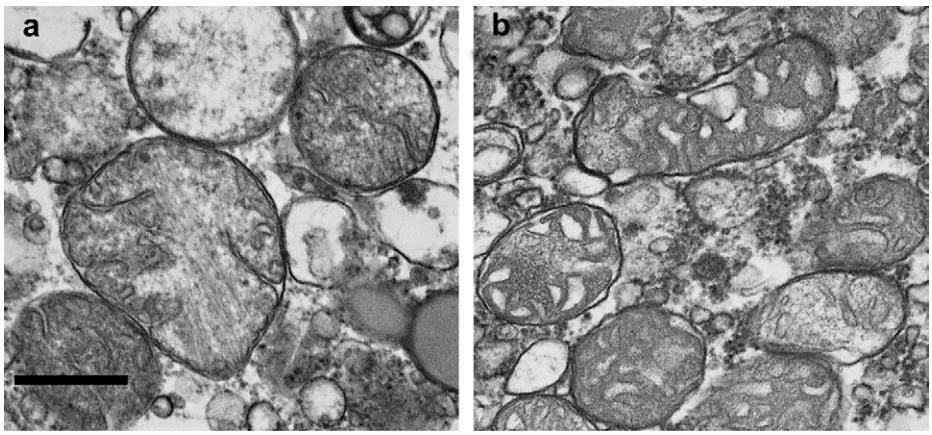


**Fig. 2.** Conformation of internal structure in mitochondrial purified from germinating maize embryos. Transmission electron micrographs of mitochondria after subcellular fractionation of embryos excised from seed imbibed for either (a) 24 h, orthodox conformation or (b) 48 h, condensed conformation. 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.

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