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Review

Plant mitochondrial dynamics

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Abstract

Higher plant mitochondria are dynamic, pleomorphic organelles. The higher plant chondriome (all mitochondria in a cell collectively) is typically composed of numerous, physically discrete, mitochondria. However, frequent inter-mitochondrial fusion, enabling the mixing and recombination of mtDNA, ensures that the higher plant chondriome functions, at least genetically, as a discontinuous whole. Nothing is known about the genes controlling mitochondrial fusion in plants; there are no plant homologues of most of the genes known to be involved in fusion in other organisms. In contrast, the mitochondrial fission apparatus is generally conserved. Higher plant mitochondria use dynamin-like and Fis-type proteins for division; like yeast and animals, higher plants have lost the mitochondrial-specific form of the prokaryote-derived protein, FtsZ. In addition to being providers of energy for life, mitochondria provide a trigger for death. The role of mitochondrial dynamics in the initiation and promulgation of cell death is conserved in higher plants although there are specific differences in the genes and mechanisms involved relative to other higher eukaryotes.

Keywords: Cytoskeleton; Dynamin; FtsZ; Matrixules; Mitochondrial dynamics; Morphology; Mitochondrial fission; Mitochondrial fusion; Mitochondrial permeability transition; Programmed cell death

1. Introduction

Mitochondria are vital eukaryotic organelles. They were recognised over 50 years ago as the site of oxidative energy metabolism [1] and synthesise the majority of respiratory ATP in plants, animals and fungi. Mitochondria of higher plants are known to differ substantially from those of other eukaryotes in a number of respects [2]. These include the existence of an alternative oxidase [3], at least four extra NADH dehydrogenases, two internal-facing, two external facing, in addition to complex I [4] and, in leaf mitochondria, a high capacity for glycine oxidation [5]. The presence of plastids, as additional bioenergetic organelles, highlights a fundamental difference between plant mitochondria and those from other organisms, that is, their involvement in photorespiration. In addition to their crucial roles in respiration and photorespiration, mitochondria are involved in the production of many compounds, such as phospholipids, nucleotides and several amino acids.

Research into mitochondrial dynamics has been stimulated by the application of GFP-based cell imaging [6], enabling the unambiguous analysis of mitochondria, in real-time, non-invasively, and in living tissue, through the expression of nuclear transgene fusions of GFP to N-terminal mitochondrial signal sequences. The first report of the use of GFP as a mitochondrial marker (mito-GFP) was published in 1995 by Rizzuto et al. [7]. Targeting of GFP to plant mitochondria was subsequently demonstrated independently by several research groups, using the signal sequence of: (i) the yeast CoxIV subunit [8], (ii) the *Arabidopsis* F_1-ATPase gamma-subunit [9] or (iii) the *Arabidopsis* chaperonin-60 or *Nicotiana plumbaginifolia* F_1-ATPase beta-subunit [10]. The availability of mito-GFP constructs and lines heralded a new era of research into plant mitochondrial dynamics and underpinned many of the advances made in our understanding of the fundamentals of plant mitochondrial dynamics: from the discovery of DRP3B as a component of the plant mitochondrial division apparatus [11], through the identification of the first higher plant mitochondrial dynamics mutants [12], to the demonstration of mitochondrial fusion in non-dividing [13] and dividing [14] cells. These studies have shown that while there are similarities between the

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mechanisms and molecules involved in plant mitochondrial dynamics and those involved in mitochondrial dynamics in other eukaryotes there are more differences than similarities. My aim in this article is to present a comparative review of our knowledge of the genes, proteins and mechanisms controlling plant mitochondrial dynamics.

2. Evolution of the mitochondrial division apparatus

2.1. FtsZ is involved in mitochondrial division only in lower eukaryotes

It is now generally agreed that mitochondria evolved from free-living alpha-proteobacteria following a single endosymbiotic event around two billion years ago [15–18]. Since that time, eukaryotic cells have diversified into the many forms seen today. The evolution of mitochondria has resulted in the loss from yeast and all higher eukaryotes of the prokaryotic genes originally involved in division of the symbiont. Bacterial cytokinesis involves a suite of proteins and the earliest-acting and most phylogenetically widespread of these is FtsZ [19]. FtsZ is found in nearly all prokaryotes and assembles into a large oligomeric structure forming a contractile ring around the interior surface of the cell membrane [20]. However, while FtsZ is likely to be ubiquitous amongst prokaryotes it is absent from the mitochondria of yeast and higher eukaryotic cells [21–23]. Indeed, up until 2000, no eukaryote had been identified that contained mitochondria that used FtsZ as part of the division apparatus. The evolutionary missing-link, a eukaryote with FtsZ-using mitochondria, was discovered by Beech and colleagues [24]. By screening a cDNA library of the unicellular chromophyte (stramenopile/heterokont) alga *Mallomonas splendens* with a fragment of the *ftsZ1* gene of the alpha-proteobacterium *Sinorhizobium meliloti* [25], Beech et al. identified a single-copy nuclear gene named *MsFtsZ-mt* [24]. Phylogenetic analysis confirmed that MsFtsZ-mt is closely related to FtsZs of alpha-proteobacteria [24]. Later in 2000, a mitochondrial-type FtsZ homologue was reported in the genome of the red alga *Cyanidioschyzon merolae* [26]. Phylogenetic analysis suggested that this gene, named *CmftsZ1*, is a mitochondrial-type FtsZ gene and immunoblotting with antisera raised against bacterial-expressed *CmftsZ1* demonstrated that the protein was located within mitochondria [26]; subsequent research has identified a second *C. merolae* mitochondrial-type FtsZ gene (the genes are now named *CmftsZ1-1* and *CmftsZ1-2*) [27]. Two mitochondrial-type *ftsZ* genes (named *fszA* and *fszB*) have also been discovered recently in the amoeboid protist *Dictyostelium discoideum* [28].

During the course of evolution all mitochondrial-type FtsZ genes identified to date transferred from the mitochondrial genome to the nucleus and are now targeted back to mitochondria. The FtsZs in *C. merolae* and *M. splendens* and at least one of the two proteins in *D. discoideum* localise early-on with dividing mitochondria as patches or ring-like structures coincident with the division site [24,26,28]. For example, immunofluorescence localisation of MsFtsZ-mt in *M. splendens* demonstrated that MsFtsZ-mt was always associated with mitochondria in one of two locations: around the middle of the organelle or at the tips [24]. Genetic evidence for the involvement of mitochondrial-type FtsZ proteins in the control of mitochondria division was provided by Gilson et al. who demonstrated that disruption of either *fszA* or *fszB* in *D. discoideum* caused the normally spherical or rod-shaped mitochondria to be replaced by elongated tubular mitochondria, suggesting that fewer mitochondrial division events occurred in the mutants [28]. FszA and FszB are differentially localised within *D. discoideum* mitochondria: FszA localises to presumptive constriction sites whereas FszB localises to the end of the organelle [28], together mimicking the localisation of MsFtsZ-mt in *M. splendens.*

The discovery of mitochondrial FtsZ genes in *M. splendens*, *C. merolae* and *D. discoideum* has plugged a gap in the evolution of the mitochondrion from its free-living bacterial past to its present-day central role in mitochondriate eukaryotes [21] but still leaves unanswered the question: Why have higher eukaryotes lost mitochondrial FtsZ?

2.2. Plastids, the contractile ring, actomyosin and the loss of mitochondrial FtsZ

Mitochondrial FtsZ genes have been lost, independently, from both the higher plant lineage, after divergence of the green-plastid lineage from the red algae and Glaucophyta [11,29], and from the yeast and animal lineages, before diversification of the opisthokonts [11,19,29,30].

Like mitochondria, plastids have evolved from a prokaryotic endosymbiont, in this case a free-living cyanobacterium [15,31–34]. The evolutionary link to free-living cyanobacteria led to the suggestion that chloroplasts might share features of their division apparatus with modern free-living bacteria [35]. This hypothesis was proven correct when the first component of the chloroplast division apparatus to be identified was a homologue of FtsZ [36]. Subsequently, plastid-type FtsZ genes were identified in *M. splendens* [24] and *C. merolae* [26]. Plant protein homologues of FtsZ have been shown to be targeted to the chloroplast [36–38], to form a ring around the chloroplast midpoint [39] and, in plants expressing antisense constructs of FtsZ genes, chloroplast are larger than wild-type and there are fewer per cell [37]. In primitive plants, therefore, plastids and mitochondria divide using very similar mechanisms that include an FtsZ ring [30,40–42].

It is postulated that the dual role of FtsZ in mitochondria and chloroplast division was a limiting factor in plant evolution and was unsuited to the more complex mitochondrial and plastidic dynamics in chlorophyte algae and higher plants. The single cells of *C. merolae* contain only one chloroplast and one mitochondrion and organelle division occurs sequentially, prior to cell division [43], similarly, there is only one chloroplast in *M. splendens* (and 20–40 mitochondria, Beech, P.L., personal communication). In contrast, a typical *Arabidopsis* mesophyll cell contains approximately 120 chloroplasts [44] and many hundreds of mitochondria. Furthermore, higher plant mitochondria are highly dynamic, undergoing frequent division and

fusion events, even in non-dividing cells [11,12,45–47]. I suggest the loss of FtsZ as a component of the plant mitochondrial division apparatus in the green-plastid lineage, removed an insurmountable barrier to the development of a complex organellar architecture, multicellularity and, therefore, the evolution of the plant cell. Interestingly, mitochondrial FtsZ-type genes have also been lost independently from the Apicomplexans, parasitic protozoans that contain a vestigial plastid, the apicoplast, that originated via secondary endosymbiosis of a photosynthetic alga [19,48,49]. The use of FtsZ for division of both mitochondria and plastids would therefore appear to be an evolutionary dead-end that has been avoided independently at least twice.

As explained above, mitochondrial FtsZ was also lost independently from a common ancestor of modern day animals and fungi. As yeast and animals do not contain plastids the above hypothesis for the loss of FtsZ from higher plant genomes is not relevant. Instead I propose an alternative hypothesis centered on the evolution of the actomyosin cytoskeleton; the evolutionary development of the actomyosin cytoskeleton underpins cell and organism motility and locomotion [50]. Mitochondrial movement in plants, fission yeast (*Saccharomyces cerevisiae*), and in at least some protists (e.g., *D. discoideum*) is based predominantly on actin microfilaments [46,51–53], whereas in animals, mitochondrial movement is predominantly microtubule based [53]. I posit that the evolving role of actin and myosin as components of the contractile ring, formed during cytokinesis in yeast and animals, was incompatible with their roles as the predominant cytoskeletal proteins involved in mitochondrial movement. I suggest that these conflicting roles underpin the evolution of the microtubule cytoskeleton as the predominant network for mitochondrial movement in filamentous yeast and animals. That mitochondria still move predominantly on actin in *S. cerevisiae*, although an actomyosin contractile ring functions during budding, is likely due to the importance of the actin cytoskeleton in the inheritance of mitochondria in this organism. Since FtsZ is structurally related to eukaryotic tubulins and is believed to be their evolutionary progenitor [54] I suggest that the switch from actin to microtubules as the predominant mediator of mitochondrial motility was incompatible with the role of FtsZ in the division of mitochondria in close association with microtubules. In this hypothetical scenario the switch to microtubules from actin thus underpins the evolution of the actomyosin cytoskeleton (muscle) and locomotion.

3. Mitochondrial chondriome structure and organisation—the discontinuous whole

The higher plant chondriome (all the mitochondria in a cell collectively) is a highly dynamic structure composed predominantly of physically discrete organelles. This structure contrasts with that of most animal cell types and yeast cells where the chondriome is frequently organised into long tubules or reticula. Analysis of mitochondrial morphology in vivo, either by differential interference contrast microscopy, staining with low concentrations of DiOC6, a fluorescent lipophilic dye, and fluorescence microscopy or fluorescence microscopy of transgenic tissue expressing mitochondrial-targeted GFP, has shown higher plant mitochondria to be highly pleomorphic, although most frequently they are spherical to sausage-shaped organelles. The typical organisation of the higher plant chondriome into a population of hundred to thousands of physically discrete organelles has a knock-on effect on the organisation of the mitochondrial genome.

The plant mitochondrial genome is a large, complex structure relative to the smaller, simpler mitochondrial genomes in other eukaryotes. For example, the mitochondrial genome of animals varies from around 15 to 18 kbp (human = 16.6 kbp), and that of yeast from 18 to > 100 kbp (*S. cerevisiae* = 75 to 85 kbp), whereas the mitochondrial genome of higher plants varies from 208 kbp in white mustard (*Brassica hirta*) to 2500 kbp in muskmelon (*Cucumis melo*). However, the gene contents of the greatly expanded plant mitochondrial genomes approximate to those of ancestral protists [16]. A comparison of human and *Arabidopsis* mtDNA reveals that the relatively small human mitochondrial genome (16.6 kbp) encodes 13 polypeptides while the relatively large *Arabidopsis* mitochondrial genome (366.9 kbp) encodes 33 polypeptides, meaning *Arabidopsis* mtDNA only encodes 2.5 times as many proteins as in humans even though the genome is 22 times as large. It is clear, therefore, that the evolving plant genomes have experienced a large increase in non-coding sequence, unidentified open reading frames (ORFs), introns and intron ORFs through horizontal gene transfer from plastids and the nucleus [16,55,56].

The plant mitochondrial genome is composed of small circular and large, circularly-permutated DNA molecules [57–61] that are generated by active inter- and intra-molecular homologous recombination [59]. The organisation of the plant chondriome into a population of physically discrete organelles is likely to have a large influence on the organisation of the mitochondrial genome since recombination between mtDNA molecules in physically distinct mitochondria initially requires mitochondrial fusion, in addition to any other intra-mitochondrial processes. A high frequency of recombination results in the plant mitochondrial genome existing as a series of subgenomic, occasionally substoichiometric, DNA molecules that, once generated, may replicate autonomously [59,62–65]. Indeed, it has been demonstrated by means of a quantitative analysis of mtDNA content, mitochondrial genome size and mitochondrial number per cell, that many physically discrete mitochondria within a plant cell contain less than a full genome [59]. In order to explain the observed complexity of the mitochondrial genomes of higher plants, Lonsdale et al. proposed that the chondriome forms a *dynamic syncytium* and that the cellular mitochondrial population is panmictic, resulting in a state of recombinational equilibrium [59]. However, I suggest that *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*.

4. Mitochondrial fission and matrixules

Dynamin-like proteins are required for mitochondrial division in *Arabidopsis* and rice and have been shown to localise to the tips of discrete mitochondria and at constrictions sites [45,47,66]. Disruption of either of two *Arabidopsis* dynamin-like genes, *DRP3A* or *DRP3B*, results in an aberrant mitochondrial morphology characterised by an increase in the size of individual mitochondria and a concomitant decrease in the number of mitochondrial per cell [11,45,47] (Fig. 1). The mutant mitochondria also have frequent constrictions along their length suggesting that they are arrested at the stage of membrane scission [47]. In addition to DRP3A and DRP3B, two further two *Arabidopsis* dynamin-like proteins have been implicated in the control of mitochondrial morphology [67]. DRP1C and DRP1E, members of the DRP1 subfamily with closest homology to soybean phragmoplastin [68], were reported to partially locate to mitochondria and gene disruption of either gene was reported to increase the proportion of mitochondria with an elongated morphology [67]. DRP1C and DRP1E have also been localised to the developing cell plate and so these dynamin-like proteins may have a complex role in plant development.

As described above, mutants of *DRP3A* have a grossly altered mitochondrial morphology: mitochondria are either large spherical organelles or, more frequently, long tubular

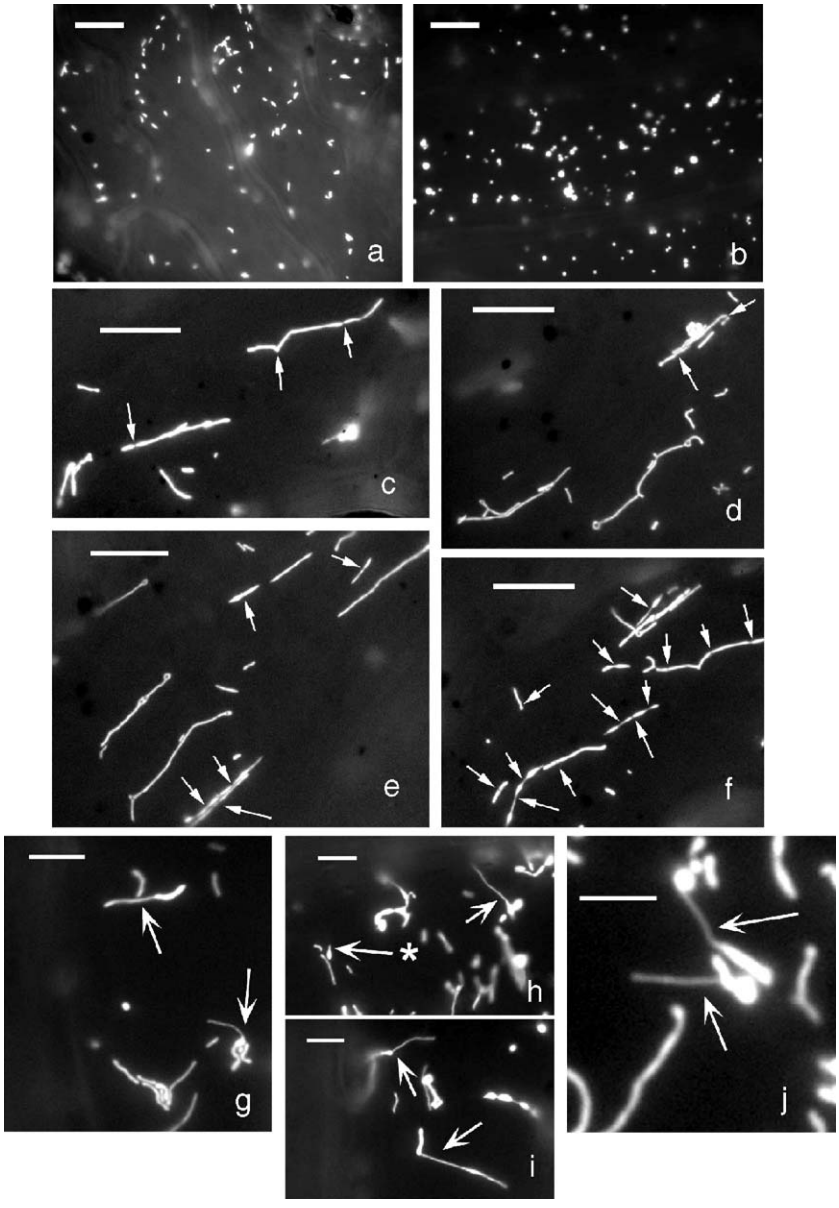

Fig. 1. Mitochondrial morphology in homozygous T-DNA insertion mutants of the *Arabidopsis* dynamin-like gene *DRP3A* (At4g33650). Epifluorescent micrographs of epidermal cell mitochondria in leaves from mitochondrial-GFP transformed plants. a, wild-type (line 43A9, [10]); b–j, plants homozygous for the T-DNA insertion. Images b, c, d, g and h are of line I; e, f, i and j are of line II. Arrows in c–f indicate constriction sites. Arrows in g–j indicate matrixules, *A spherical mitochondria starting to elongate with the initiation of matrixule formation. Scale bar = 10 μm in a–f and 5 μm in g–j. Reproduced from [47] with permission from Oxford University Press.

structures often with many constriction sites [47] (Fig. 1). A third mitochondrial phenotype, rarely seen in wild-type mitochondria but frequently observed in the *DRP3A* knockouts, is characterised by a thin protuberance extending from an individual mitochondrion up to many micrometers in length (Fig. 1g–j) that we termed matrixules [47], in keeping with the naming, as stromules, of similar structures seen to extend from chloroplasts [69]. The presence of mitochondria with numerous matrixules was proposed to be linked to the function of DRP3A as a component of the mitochondrial division machine [47]. It was hypothesised that, in advance of division, the mitochondria elongate by a process that pushes or pulls them through a constrictive collar, possibly similar to the mitochondrial division ring [70] although this has not been found in higher eukaryotes, encircling the mitochondria at one pole, which forces the mitochondria into an elongated morphology. It was suggested that the first portion of the mitochondria to be extruded in this way forms the matrixule that grows and expands as the mitochondrion passes through the collar. It has been demonstrated that following mitochondrial division in yeast, dynamin remains associated with one cut end of the mitochondrion [71]. Assuming a similar situation occurs in *Arabidopsis* and based on the mitochondrial morphology of the *DRP3A* mutant and the localisation of DRP3A to the tips of mitochondria [45], it is postulated that one of the roles of DRP3A is to remain associated with the mitochondrion after division, at what is now one pole of the daughter organelle, to prevent further division events that would otherwise lead to severe fragmentation of the mitochondria. The fact that fragmentation does not occur in the *DRP3A* knockout mutant suggests there may be reduced efficiency of the division process in the absence of DRP3A. Logan et al. hypothesised that in the *DRP3A* knockout mutant, the absence of DRP3A enables daughter mitochondria from recent division events to re-enter the division process, starting with elongation via collar-based extrusion and the initial formation of a matrixule. This hypothesis explains why, although matrixules are occasionally observed in wild-type cells, they are much more frequent in the cells of the *DRP3A* knockout [47].

Apart from dynamin-like proteins, only one other member of the plant mitochondrial division apparatus has been identified. We recently identified an *Arabidopsis* orthologue of hFis1/Fis1p, an evolutionarily conserved protein playing a vital role in mitochondrial division [72–74], that we named *BIGYIN* (At3g57090) to reflect the mitochondrial phenotype in homozygous T-DNA insertion mutants [75]; the *Arabidopsis* gene name Fis1 is already in use. Disruption of *BIGYIN* leads to a reduced number of mitochondria per cell, coupled to a large increase in the size of individual mitochondria relative to wild-type, a phenotype very similar to that of DRP3A and DRP3B mutants [75]. A GFP-BIGYIN fusion, driven by the CaMV 35S promoter, localises to mitochondria, peroxisomes and chloroplasts; it is not yet known if this multiple localisation pattern is maintained when expression is driven by the native promoter [Arimura, S., Tsutsumi, N., personal communication]. 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 [72,76,77], 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 reveal any redundancy between the two *Arabidopsis* Fis1-type genes. The *Arabidopsis* genome contains structural homologues of Caf4p, a member of the mitochondrial division apparatus in *S. cerevisiae* that interacts with Fis1p and Dnm1p [78], but nothing is known concerning their function in mitochondrial fission. No structural homologues of Mdv1p, an additional member of the *S. cerevisiae* mitochondrial division apparatus [79–81], have been identified in any multicellular eukaryote.

5. Mitochondrial fusion

No plant genes mediating mitochondrial fusion have been identified; there are no *Arabidopsis* homologues of Fzo1p or Ugo1p. There are homologues of Mgm1p within the large family of *Arabidopsis* dynamin-like proteins but the protein with the highest homology to Mgm1p is DRP3A, which is understood to be involved in mitochondrial fission (see Section 3). However, if the parsimonious explanation of the role of DRP3A as provided by Arimura et al. [45] and Logan et al. [47] 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 [82,83]. 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. Mitochondrial fusion has been clearly demonstrated recently in *Arabidopsis* cells by exploiting a photoconvertible fluorescent protein called Kaede which can be induced to change irreversibly from green to red upon exposure to light of 350–400 nm [13]. Transient expression of mitochondrial-targeted Kaede in onion cells enabled Arimura et al. to induce half of the mitochondria to fluoresce red and then visualise fusion between red and green mitochondria through the appearance of yellow mitochondria as a result of mixing of

the matrix-localised fluorescent proteins; there was sufficient fusion between the mitochondria to convert them all to yellow within 1 to 2 h [13].

Extensive fusion of mitochondria, called Massive Mitochondrial Fusion (MMF), also occurs prior to cell division [14]. MMF is followed by numerous fission events which refragment the chondriome prior to redistribution of the newly mixed mitochondrial population throughout the cytoplasm and subsequent cytokinesis [14]. A similar situation is known to occur in yeast during mating, when, upon cell fusion, the mitochondria fuse, mixing their DNA and matrix proteins [84,85]. 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 did not require ATP or an intact actin cytoskeleton [14].

Mitochondrial fusion, enabling the exchange and complementation of mtDNA molecules both during the MMF [14] and during the more frequent, but less extensive, fusion events that occur in non-dividing cells [13], is inherent in Lonsdale's dynamic syncytium hypothesis [59]. Mitochondrial fusion overcomes the physical barrier to the exchange and complementation of mtDNA molecules that accompanied the organisation of the higher plant chondriome into physically discrete organelles. The linked processes of fission and fusion are therefore responsible for maintenance of the plant chondriome as a discontinuous whole.

6. Mitochondria, cytoskeleton and cellular inheritance

In contrast to mitochondria in mammals and most yeast, but similar to mitochondria in *S. cerevisiae*, plant mitochondria move predominantly on the actin cytoskeleton [51,52] although movement on and tethering to microtubules probably also occurs depending on developmental state and physiology [52]. Since mitochondria cannot be created de novo, instead they propagate by means of division of the parental organelle, it follows that individuals inherit their mitochondria. For a recent review of mtDNA inheritance in plants, readers are directed to Barr et al. [86]. Mitochondrial inheritance in yeast is a highly ordered process and many proteins have been implicated in its control [82]. In contrast, little is known about the mechanisms controlling the cellular inheritance of mitochondria in multicellular organisms. During the human cell cycle, mitochondria switch between two predominant morphological states [87–89]. During the G1 phase of the cell cycle, mitochondria fuse to form reticula, bringing the number of individual organelles to half the number prior to M phase [88]. As cells proceed form G1 to S phase mitochondrial numbers increase due to fragmentation (fission) of the mitochondrial reticula [87–89]. A similar series of events has been shown to occur in plants [14,90]. Extensive mitochondrial fusion (MMF, see above) has been shown to enable the mixing of mtDNA previously held within physically discrete mitochondria. The active and uniform dispersal of mitochondria throughout the cytoplasm, after the fission events that follow MFF, ensures the unbiased distribution of mitochondria into each daughter cell following cytokinesis [14], thus the cellular inheritance of mitochondria is a stochastic rather than a strictly random process.

Little is known about the mechanisms regulating the cellular distribution of mitochondria in higher plants. However, the results of Sheahan et al. implicate the actin cytoskeleton since disruption of actin polymerisation affected the dispersal of mitochondria and resulted in a biased distribution of mitochondria in the daughter cells [90]. Mutations of the *Arabidopsis FRIENDLY MITOCHONDRIA* (*FMT*) gene lead to a grossly altered cellular distribution of mitochondria (Fig. 2k and l) [12]. 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 (Fig. 2k) [12]. *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 *D. discoideum* (*cluA*) or *S. cerevisiae* (*CLU1*) also causes aberrant mitochondrial phenotypes [91,92]. In the *cluA*$^-$ mutant of *D. discoideum*, the mitochondria cluster near the cell centre [91] while in the *S. cerevisiae clu1* mutant the mitochondrial tubules collapse to one side of the cell [92]. The only clues to the function of FMT come from the mitochondrial phenotype and the presence of the TPR domain. Mitochondrial association with microtubules has been shown to involve the microtubule-specific motor protein, kinesin [93] that binds cargo at the tetratricopeptide repeat (TPR) domains in the kinesin light chains [94,95]. Based on the above information, it was suggested that FMT is involved in the interaction of mitochondria with the microtubule cytoskeleton [12]. It is assumed that when mitochondria are moving on actin filaments they are prevented from binding to microtubules, until this is required to immobilise the mitochondria or effect small-scale adjustments to their position. With reference to the phenotype of the *fmt* mutant and the presence of a conserved TPR domain in FMT it is hypothesised 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 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 *C. elegans* (Clarke, M., 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. [96]. In *D. discoideum*, final separation of mitochondria following their division may

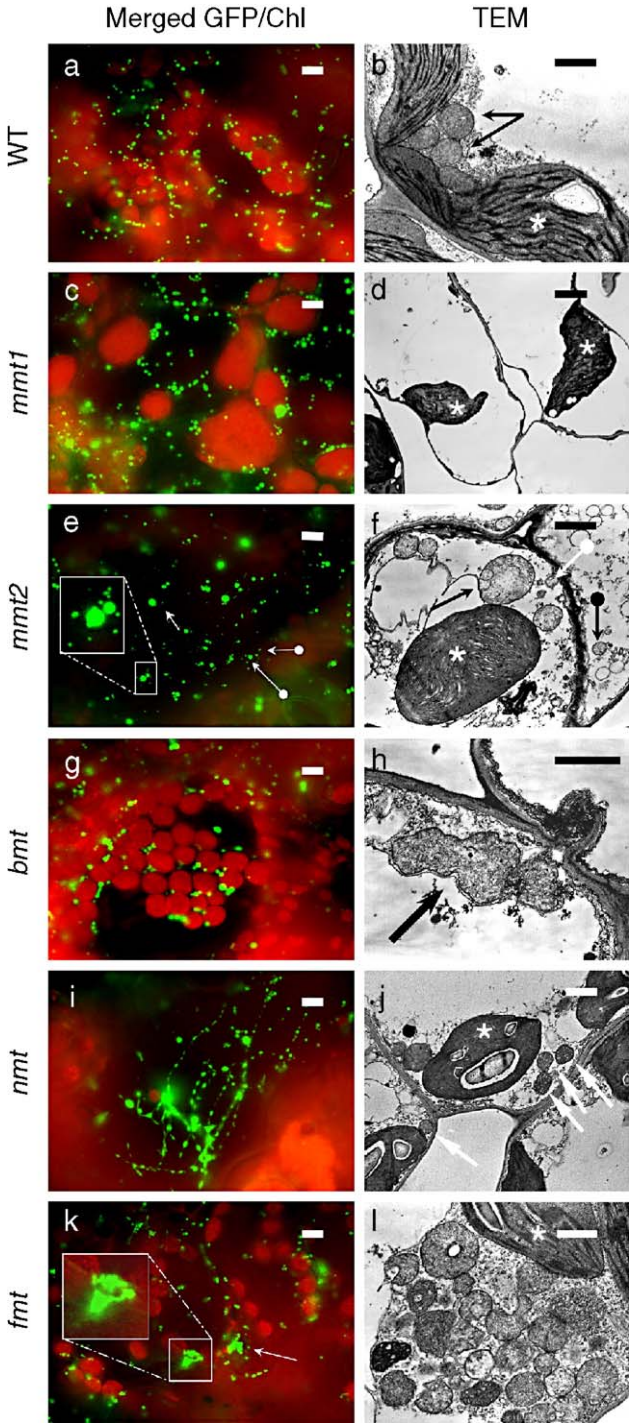

Fig. 2. Epifluorescent (left hand panels) and TEM micrographs (right hand panels) of wild-type (line 43A9, [10]) and mutant *Arabidopsis* leaf mitochondria. Epifluorescent micrographs are false-coloured for GFP (green) and chlorophyll (red). a and b, Wild-type, arrows = mitochondria, * = chloroplast. c and d, *mmt1* mutant, * = chloroplast. e and f mmt2 mutant, plain arrows = large mitochondria, arrows with circle = small mitochondria, the boxes indicate an area magnified to highlight the heterogeneity of mitochondria size within a single cell, * = chloroplast with dense mass of internal membranes. g and h *bmt* mutant, arrow = mitochondria. i and j nmt mutant, arrows indicate small mitochondria, * = chloroplast. k and l *fmt* mutant, arrow = large mitochondrial cluster, boxes indicate an area magnified to highlight a large cluster of mitochondria, * = chloroplast. Scale bars in epifluorescent images = 5 μm; in TEMs = 1 μm, except in d where the bar = 5 μm. Reproduced from [12] with permission from Blackwell Publishing.

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 fission 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 depolymerisation of the actin cytoskeleton (Logan, D.C., unpublished data). The *Arabidopsis* genome contains 61 identified kinesin-like genes, the highest number in any sequenced eukaryotic genome [97]. However, no heavy chains have been identified in any plant, although some light chains have been predicted in the *Arabidopsis* genome [97]. In contrast, there are many MT-associated processes unique to plants that are likely to require additional plant-specific microtubule associated proteins including motors [97,98]. Yeast-two-hybrid screens are currently being performed to identify proteins interacting with FMT in vivo.

7. Plant mitochondrial dynamics mutants

There are few *Arabidopsis* homologues of animal or yeast proteins playing a direct role in mitochondrial dynamics [46]. In some cases, this can be explained by the fundamental differences between yeast and plant mitochondrial morphology and reproductive biology. For example, the yeast protein Mdm1p is involved in the maintenance of the tubular mitochondrial morphology [99] so it is not surprising that there is no homologue in *Arabidopsis* where the numerous discrete mitochondria alternate between spherical and sausage-shaped structures. Similarly, in contrast to animal and plant cells, the budding yeast, *S. cerevisiae*, proliferates by budding whereby the mother cell produces a daughter bud that grows and eventually becomes an independent cell. An essential part of this process is the transport of mitochondria and other organelles into the daughter bud. Mmm1p, Mdm10p, Mdm12p and Mdm20p are all involved in the transmission of mitochondria to the daughter buds [53] and since cell proliferation in plants occurs by cell division, these genes are not required. Equally, the differences between yeast and plant cells mean that there are likely to be many proteins involved in mitochondrial dynamics that are either specific to multicellular organisms or plant-specific. To identify the genes, proteins and mechanisms controlling mitochondrial dynamics in higher plants an EMS-mutagenised *Arabidopsis* population (expressing mitochondria-targeted GFP, [10]) was screened for altered mitochondrial shape, size, number and distribution mutants [12]. Seven viable mutants with distinct mitochondrial phenotypes were identified (including *fmt*, see above) from a population of approximately 9,500 individuals (Fig. 2). In the first motley mitochondrial mutant (*mmt1*) the mitochondrial population is highly heterogeneous varying in size from one quarter to four times the average plan area of wild-type mitochondria (Fig. 2c, d). The size distribution of chloroplasts is also affected in the mutant, chloroplast plan areas in the mutant

range from 4 to 240 times the plan area in the wild-type [12]. The second motley mitochondrial mutant (*mmt2*) contains a highly heterogeneous mitochondrial population similar to *mmt1* (cf. Fig. 2c and e). Although gross chloroplast morphology remains normal in *mmt2* (Fig. 2f), transmission electron microscopy (TEM) demonstrated that the internal structure of the chloroplasts is severely altered (Fig. 2f). Chloroplasts in the *mmt2* mutant contain a large number of electron dense particles and a mass of densely packed membranes instead of the normal morphology of granal stacks connected by stromal lamellae. Mitochondria in the big mitochondrial mutant (*bmt*) have plan areas approximately two to four times wild-type and there are approximately half as many per microscope field-of-view (Fig. 2g and h). The presence of long, interconnected mitochondrial tubules extending to many tens of micrometers in length characterises the network mitochondrial mutant (*nmt*) (Fig. 2i). Examination of leaf tissue of *nmt* plants under the TEM showed that the reticular morphology was not maintained in the fixed tissue (Fig. 2j) instead, the mitochondrial tubules fragmented to form organelles as small as 1/16th the plan area of those in wild-type cells. Gene mapping of the *mmt1*, *mmt2*, *bmt* and *nmt* mutants indicates that these four loci are novel genes involved in mitochondrial dynamics since no mitochondrial development genes/mutants have been mapped to the regions containing the mutant loci, nor are any obvious candidate genes in these regions [12].

In an attempt to identify additional *Arabidopsis* mitochondrial morphology mutants, Feng et al. generated and screened an independent EMS-mutagenised population [100]. From 19,000 M2 individuals, 17 mutant lines were identified; in all cases, the mitochondria were either longer or larger than wild-type [100]. Gene-mapping of the mutant loci in seven of the 17 lines demonstrated that at least four different loci were involved. Three lines mapped close to DRP3A, two mapped close to *bmt* and one mapped close to *nmt*. The one remaining mutant locus mapped to the long arm of chromosome 4, a locus not previously implicated in the control of mitochondrial morphology, nor containing homologues of any genes known to affect mitochondrial morphology [100]. None of the mutant loci identified in this screen have been cloned.

8. Mitochondria and cell death

Programmed cell death (PCD) is a highly ordered and regulated cellular process requiring active gene expression and is essential for the maintenance and development of healthy tissues in multicellular organisms [101]. For example, PCD is vital for normal plant development, architecture and defence [102]. Examples of developmentally regulated PCD include leaf and petal senescence, the death of root cap cells during root growth, xylogenesis, and death of aleurone cells during cereal seed germination. In addition, a variety chemicals activate PCD in plants including hydrogen peroxide (H_2O_2), hypersensitive response (HR) elicitors (e.g., fumonisin [101], victorin [103]), signalling intermediates (e.g., Ca^{2+} [104]), and environmental stresses (e.g., UV irradiation [105], and temperature stress [104]). In spite of the fundamental importance of PCD in plants, little is known about the genes, proteins, and mechanisms underpinning the phenomenon. There are no plant homologues of the pro- and anti-apoptotic Bcl-2 family of proteins [106–108] but heterologous expression of mammalian Bax induced HR-like cell death in tobacco [109] and *Arabidopsis* [110]. Complementing these results, the expression of anti-apoptotic members of the Bcl-2 family (Bcl-x_L CED-9) in plants protects against cell death induced by UV irradiation, tobacco mosaic virus infection (HR), or treatment with paraquat—all treatments known to stimulate ROS production [111–113].

It has been demonstrated recently that heterologous Bax localises, at least partially, to plant mitochondria [114], mimicking the translocation of cytosol-localised Bax to mitochondria upon induction of apoptosis in mammalian cells [115]. Expression of heterologous Bax in *Arabidopsis* induces a change in the typical morphology of leaf mitochondria from elongate, bacilliform or sausage-shaped organelles to more condensed spherical organelles, accompanied by a cessation of movement [114]. One day later, mitochondrial swelling was observed, although not quantified, and a significant increase in cell death over the control was measured after a further 24 h [114]. A similar series of events has been found to occur in response to applied ROS or ROS-inducing chemicals [Scott, I., Tobin, A.K. and Logan, D.C., unpublished data]. Within 1 h of exposing *Arabidopsis* mesophyll protoplasts to ROS (paraquat or H_2O_2) mitochondria in 60–70% of protoplasts undergo a morphology transition characterised by swelling to at least double their volume. No significant cell death occurs over the following 20 h but by 48 h after 24 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. Paraquat or H_2O_2 treatment of whole leaves has a similar affect on mitochondrial morphology [Scott, I., Tobin, A. K. and Logan, D.C., unpublished data, [116]. While we conclude that the mitochondrial morphology transition is an early and specific indicator of subsequent cell death, we do not yet know whether the morphology transition is a manifestation of the mitochondrial permeability transition (MPT).

The MPT is an early event in many types of apoptosis in animals and there is accumulating evidence supporting the role of the MPT in the early stages of PCD in plants. Evidence for activation of the MPT in plants comes from in vitro experiments on oat [117] and potato mitochondria [118]. 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 permeability transition pore [118], a pharmacologically characterised megachannel connecting the outer and inner mitochondrial membranes in animal mitochondria and implicated in the MPT. Ca^{2+}-induced swelling was also detected with oat mitochondria pretreated with the Ca^{2+}-ionophore A23187 [117]. A MPT, measured as a decrease in the mitochondrial membrane potential, was 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. Protoporphyrin IX is a molecule similar to the proposed substrate of ACD2, which is a red chlorophyll

catabolite reductase [119]; ACD2 (accelerated cell death 2) mutants show ectopic cell death and therefore the substrate is believed to an endogenous cell-death trigger [120].

Taken together, the above evidence suggests that the mitochondrion is central to plant PCD and that the MPT, measured as a mitochondrial morphology transition (i.e., swelling), or by reduction in the mitochondrial membrane potential, is a common and early event that precedes visible morphological changes to the cell accompanying the progression of PCD. The role of the release of cytochrome *c* (cyt *c*) from the inter membrane space in plant PCD is less clear. Cyt *c* release from plant mitochondria has been demonstrated during PCD but whether cyt *c* release is as essential and ubiquitous a stage in plants as in animals is still a matter of debate. In vivo, cyt *c* release into the cytosol has been documented as an early event during PCD in cucumber [121], maize suspension culture cells [122], tobacco protoplasts [123] and *Arabidopsis* suspension cells [124]. In addition, cyt *c* release followed observation of the MPT in potato mitochondria, both of which were inhibited by cyclosporin A [118]. However: (i) cyclosporin A treatment during tracheary element PCD blocks death without blocking cyt *c* release [125]; (ii) the ceramide-induced MPT was partially prevented by cyclosporin A but cyt *c* release was not inhibited [120]; (iii) protoporphyrin IX-induced death was not correlated with cyt *c* release [120]. It has been suggested that, in plants, the release of cyt *c* may, in some cases, be an unavoidable consequence of the release of proteins that are active in plant PCD, e.g., nucleases [126].

As explained above, there are no plant sequence homologues of the Bcl-2 family of proteins. However, the *Arabidopsis* and rice genomes contain homologues of a negative regulator of PCD, Bax inhibitor 1 (BI-1) [110,127]. The rice and *Arabidopsis* homologues are able to suppress death induced by mammalian Bax in yeast [127] and the *Arabidopsis* gene also suppresses mammalian Bax-induced cell death in *Arabidopsis* [110]. Conservation of the role of BI-1 in plants, in the absence of Bax and other Bcl-2 family members, suggests that BI-1 has a general role in preventing PCD and that there is evolutionary conservation of parts of the PCD machinery. Indeed, it has been demonstrated that: (i) overexpression of AtBI-1 can mitigate against the death-inducing effects of the HR in rice suspension cells [128] and against H_2O_2- or salicylic acid-induced death in tobacco [129]; (ii) that heterologously expressed tomato, *Arabidopsis*, rice, and *Drosophila* BI-1 genes could protect against Bax- and partially against H_2O_2- and heat-shock-induced death of yeast [130]; (iii) in barley, overexpression of barley BI-1 protected against pathogen induced cell death [131]. Recently, in an elegant piece of research AtBI-1 was used as the core component in a successful genetic screen to identify *Arabidopsis* cell death-inducing genes [132]. The screen involved identification of *Arabidopsis* cDNAs that, upon expression in yeast, induced death in an AtBI-1-dependent manner (i.e., overexpression of AtBI-1 could suppress the death-inducing effect of the expression of the cDNA). Screening 20,000 cDNA clones enabled the identification of a gene named *Cdf1* (cell growth defect factor-1, At5g23040) that encodes a polypeptide of 258 amino acids with an estimated molecular mass of 28.8 kDa [132]. Cdf1 has no significant homology to proteins of known function nor does it contain any recognisable protein motifs [132]. Cdf1 has two homologues in the *Arabidopsis* genome, At3g51140 and At2g20929, which share 32.9% and 22.4% identity with Cdf1 [132]. There are similarities in the mode of action of Cdf1 and Bax in inducing yeast cell death. Both proteins induce similar changes to the internal structure of yeast cells, both proteins are ineffective against the Bax-resistant yeast mutant, BRM1, and perhaps most intriguing of all, both localise to yeast mitochondria [132]. It has yet to be determined whether Cdf1 has death-inducing activity in *Arabidopsis* or whether Cdf1 localises to *Arabidopsis* mitochondria.

9. Conclusions

Mitochondria evolved from free-living alpha-proteobacteria following a single endosymbiotic event over one billion years ago. Subsequent evolution has resulted in the development of significant differences in chondriome structure, and in the genes required to maintain chondriome structure, between plant, yeast, and animal lineages. The physical organisation of the plant chondriome as a discontinuous whole, as opposed to a reticular structure seen typically in many yeast and animal cells, has knock-on effects on the organisation of the plant mitochondrial genome, which is much more complex than that of opisthokonts. The physically discrete mitochondria in plant cells undergo frequent fusion events enabling inter-mitochondrial exchange and recombination of mtDNA, however, no components of the fusion apparatus have been identified in plants. Despite all the differences between plant mitochondria and those of other eukaryotes some aspects of mitochondrial dynamics have been conserved, including the maintenance of the chondriome by the dual processes of fission and fusion and the use of dynamin-like and Fis-type proteins for organelle division. Most significant of all, perhaps, is the conservation of the role of mitochondria as triggers of death.

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