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REVIEW

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Sustaining Life: Maintaining Chloroplasts and Mitochondria and their Genomes in Plants

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Chloroplasts (members of the plastid family) and mitochondria are central to the energy cycles of ecosystems and the biosphere. They both contain DNA, organized into nucleoids, coding for critical genes for photosynthetic and respiratory energy production. This review updates the cellular and molecular biology of how chloroplasts, mitochondria, and their genomes in Angiosperms are maintained; particularly in leaf development and maternal inheritance. Maternal inheritance is the common form of transmission to the next generation. Both organelles cannot be derived *de novo*. Proplastids during very early leaf development develop into chloroplasts with their characteristic thylakoid structure, with the nucleoids associated with the thylakoids. In cell divisions in the leaf primordia and very early leaf development, mitochondria and plastids are duplicated, their nucleoids replicated and segregated, and the population of mitochondria and plastids segregated to daughter cells using the cytoskeleton. To maintain their nucleoids, mitochondria must undergo fusion as well as fission. Chloroplasts are transmitted to the next generation as proplastids where they are maintained in the egg cell but eliminated from the sperm cells. Mitochondria in the apical meristem undergo massive mitochondrial fusion (MMF†) prior to floral induction and subsequent maternal inheritance. MMF also occurs again in early germination. MMF encourages DNA repair and recombination, possibly as part of a quality control in each generation. As a further quality control in both chloroplasts and mitochondria, damaged organelles are removed by autophagy. Following consideration of the above, areas requiring further understanding are highlighted.

INTRODUCTION

Eukaryotic cells not only have a membrane bound nucleus, but also house mitochondrial genomes in the case of animals, and mitochondrial and chloroplast (a member of the plastid family) genomes in the case of plants. There is widespread agreement that these organelles were derived by endosymbiosis [1-3]. Current thinking favors a progenitor of eukaryotes from within the Archaea, with this last common ancestor of eukaryotes living about 1.8 billion years ago [2]. There is recent molecular evidence that these latter ancestors contained a profilin-regulated actin cytoskeleton [3]. This primitive cytoskeleton could have facilitated the phagocytosis of the aerobic bacteria that evolved into mitochondria and the cyanobacteria that evolved into chloroplasts. Molecular evidence supports the endosymbiosis of an α-proteobacterium progenitor of the mitochondrion about 1.5 billion years ago and of the

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†Abbreviations: AF, actin filament; cpDNA, chloroplast DNA; DAPI, 4′,6-diamidino-2-phenylindole; MMF, massive mitochondrial fusion; mtDNA, mitochondrial DNA; RDR, recombination – dependent replication; SAM, shoot apical meristem.

Keywords: Chloroplast DNA, chloroplast dynamics, chloroplast maintenance, plant mitochondrial dynamics, plant mitochondrial DNA, plant organelle inheritance, plant organelle nucleoids, proplastids

cyanobacterium progenitor of the chloroplast about 1.2 billion years ago [1].

To sustain our planet and human life as it exists today, plants with their three genomes and energy producing chloroplasts and mitochondria are essential, with photosynthesis powering ecosystems, human nutrition, and facilitating a stable biosphere. It has been known since the 19th century that nuclear chromosomes are transmitted via mitosis in the cell division process and undergo meiosis in the formation of the haploid gametes. These latter events are the basis of the understanding of modern genetics and their cell and molecular biology have been studied in great detail. The inheritance and transmission during development of mitochondria and chloroplasts, which house important genes, have received much less attention. Chloroplast DNA (cpDNA) encodes key thylakoid proteins for light-induced ATP production as well as the large subunit of the carbon dioxide fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which contains the active site. Mitochondrial DNA (mtDNA) encodes critical proteins of the cytochrome electron transport chain required for ATP production.

In cell division mitochondria and chloroplasts have to be duplicated, their genomes replicated and then the population of mitochondria and chloroplasts segregated to daughter cells. During reproduction these two organelles and their genomes in most cases have to be transmitted to only female gametes, for subsequent zygote formation [4].

This review provides an update of current understanding of how mitochondria and chloroplasts and their genomes are faithfully inherited by daughter cells in cell divisions, during plant development and in reproduction. The focus is on Angiosperms – the flowering plants.

CHLOROPLAST AND MITOCHONDRIAL DIVISION

As chloroplasts and mitochondria do not derive *de novo* they need to divide by fission to be maintained and increase populations in leaf development. Chloroplast division [5] (Figure 1a) is reasonably well understood [6,7]. Chloroplasts are the most important members of the plastid family and are derived from proplastids present in the leaf primordia [8,9]. In the mesophyll cells of the developing leaf, chloroplasts divide in the cell cycle and can continue into cell enlargement once division has ceased. The contractile "Z ring" positions in the center of the chloroplast at the division site and is associated with the inner chloroplast envelope. The "Z ring" consists of the two tubulin-like cytoskeletal GTPases, FtsZ1 and FtsZ2, which form heteropolymers on the stromal surface of the inner envelope membrane [6,7]. The FtsZ proteins are descended from cyanobacterial ancestors. The FtsZ proteins are the first structures to be assembled at the division site. This central positioning is regulated by the Min system consisting of ARC3 and Min proteins. The division machinery associated with the outer chloroplast envelope consists of two contractile rings, the DYNAMIN RELATED PROTEIN 5B (DRP5B) and the outer plastid dividing (PD) ring. In Arabidopsis two sets of paralogous proteins associated with the inner (ARC6 and PARC6) and outer membranes (PDV1 and PDV2) coordinate the FtsZ and DRP5B contractile rings to facilitate the constriction and fission process [10].

Proplastids are the chloroplast progenitors present in the zygote for the beginning of subsequent development and in the meristems [8,9]. Their division has been much less studied [6]. An indirect way of studying proplastid division is to see if all the genes expressed in the mesophyll cell with dividing chloroplasts are expressed in the shoot apical meristem (SAM). The expression of key chloroplast division genes has been detected in the SAM - the *FtsZ*, *DRP5B, ARC6,* and *Min* genes [6].

The DYNAMIN RELATED PROTEINS, DRP3A and DRP3B are involved in mitochondrial division. Fluorescently-labelled DRP3A and DRP3B localize to the outer membrane of the mitochondrial division sites before division occurs [11,12]. Other important mitochondrial division proteins are FISSION 1 and 2 (FIS1 and 2 also known as BIGYIN 1 and 2), ELONGATED MITOCHONDRIA1 (ELM1) and PEROXISOMAL AND MITOCHONDRIAL DIVISION FACTOR1 and 2 (PMD1 and 2). FIS1, FIS 2, and ELM1 localize to the outer mitochondrial membrane [11,12]. ELM1 is required to recruit the DRP proteins to the outer mitochondrial membrane while the FIS proteins interact with the DRP proteins to anchor them to the outer membrane. As in chloroplasts the dynamin related proteins drive constriction and fission of the organelle. PMD1 and PMD2 are also associated with the outer membrane and are required for mitochondria proliferation, but do not interact with DRP and FIS proteins.

GENOME ORGANIZATION INTO NUCLEOIDS

The chloroplast genome is about 150 kb (*Nicotiana tabacum* 156 kb, Arabidopsis 154 kb), coding for about 100 protein genes plus rRNA and tRNA genes [13,14]. The cpDNA can be visualized as a circular molecule when isolated, consistent with restriction endonuclease mapping [13]. However, based on pulsed-field gel electrophoresis it is argued that circular molecules only represent a small proportion of cpDNA [15]. Most of the cpDNA was found to be in linear or complex branched forms containing several chloroplast genomes, with the branched forms probably representing recombination

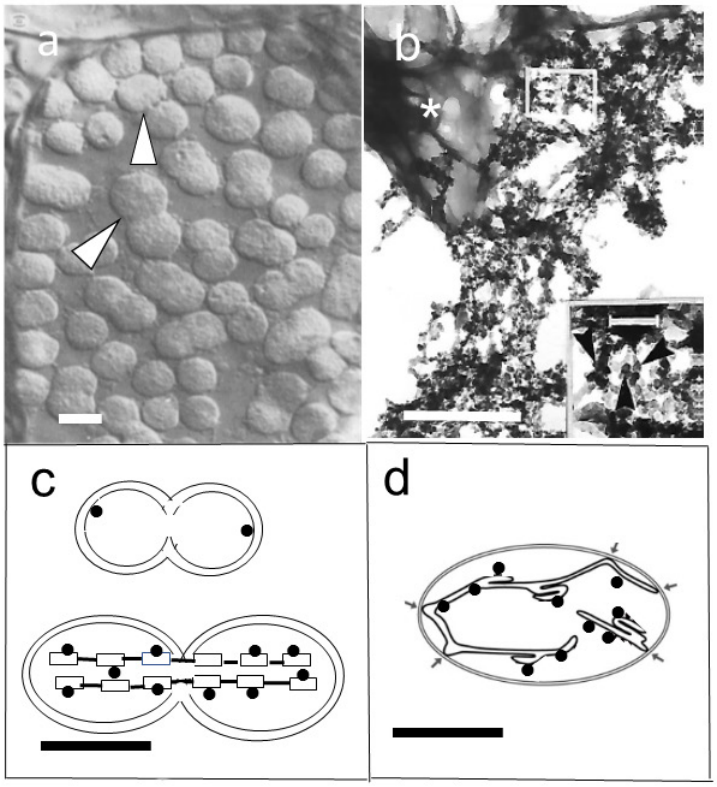

Figure 1. Chloroplast division and chloroplast nucleoids. (**a**) Partially synchronized chloroplast divisions (arrowheads) in mesophyll cell of a cultured spinach leaf disk grown in low intensity green light then transferred to high intensity white light. Nomarski Interference Contrast optics ([5], for details). Bar = 5 μm. (**b**) Thylakoid-associated (*) transcriptional networks with ribosome sized granules (inset). Network can be disrupted by DNAase II ([51], for details). Asterisk indicates thylakoid. Bar = 0.5 μm and inset = 100 nm. (**c**) Top diagram represents dividing proplastid, bottom diagram represents dividing chloroplast. Solid circles represent nucleoids associated with inner membrane of proplastid and associated with grana of chloroplast. Bar = 5 μm. (**d**) Diagram of differentiating chloroplast from proplastid at pro-granal stage as defined and represented by Liang *et al*. [49]. Solid circles represent nucleoids associated with thylakoids. Bar = 2.5 μm. Permissions: (a) reproduced from reference 5, Creative Commons License (b) reproduced from reference [51], ScienceDirect permissions.

involved in DNA replication and repair [15]. DNA replication is considered further in the next section. The cpDNA is organized into nucleoids which are structures containing up to several cpDNA molecules associated with proteins [16]. Staining with the DAPI (4′,6-diamidino-2-phenylindole) fluorochrome reveals the nucleoid as a brightly fluorescing roughly spherical or ovoid structure. The nucleoid-enriched proteome includes proteins associated with DNA replication, organization, and repair; transcription, as well as mRNA processing, splicing, and editing [17]. There is substantive evidence, based on a range of morphological studies with the light and electron microscope using ^{3}H-thymidine labelled cpDNA [18] and molecular studies [19,20], that in most chloroplasts the nucleoid is associated with the thylakoid membranes in the region of the inverted repeat [21]. The nucleoid is anchored to the thylakoid membrane by the MATRIX ATTACHMENT REGION FILAMENT-LIKE PROTEIN 1 (MFP1) as well as other candidates [19,20]. The expression pattern of MFP1 is closely associated with thylakoid development [19,20]. In chloroplasts there are many nucleoids spread throughout the chloroplast (20-30), associated with the thylakoids [16]. Proplastids have few or no internal membranes with much fewer cpDNA copies compared to chloroplasts resulting in one or a few nucleoids that are associated with the inner envelope [16,18]. In proplastids, a key anchoring protein is the PLASTID ENVELOPE NUCLEOID DNA BINDING PROTEIN (PEND) and MFP1 does not become dominant until thylakoids develop such that there is a reciprocal relationship between the two [19].

The structure of flowering plant mitochondrial genomes has some special features compared to human and animal mitochondrial genomes, which are much smaller.

Plant mitochondrial DNA in angiosperms commonly varies in size from 200 to 750 kb, but is 1,556 kb in *Cucumis sativus* [22,23]. In Arabidopsis the mitochondrial genome is 367 kb [14] compared to the 16.6 kb in humans. Plant mtDNA codes for 32 protein coding genes plus rRNA and tRNA genes [22]. The structural organization of the plant mtDNA is quite complex. Early models were based on a master circle from mapping studies and sub-genomic circles which formed a multipartite genome from direct repeats in the master circle [12,22-24]. More recent analysis indicates that physical forms of mtDNA are mainly linear with circular forms representing a small proportion [12,22-25]. A master circle in its physical form is likely to be rare [12], but sub-genomic circles have been visualized [26]. Mitochondrial DNA like cpDNA is also organized into membrane-bound nucleoids [22,25]. Individual nucleoids do not necessarily contain a complete genome [26] and a mitochondrion may have zero, one, or a few nucleoids [26-28]. This is discussed further in the section below on fusion and fission.

DNA REPLICATION AND NUCLEOID SEGREGATION IN CHLOROPLAST AND MITOCHONDRIAL DIVISION

There is no detailed understanding of cpDNA and mtDNA replication. However, based on the visualization of branched linear molecules using improved isolation and visualization procedures [25], available evidence is supportive of an RDR (recombination dependent replication) mechanism rather than the older theta and rolling circle models using a circle-based template [12,25]. The RDR mechanism is likely to have similarities to that used in bacteriophage T4 replication [12,25,29]. In this mechanism two linear DNA molecules are involved. There is single strand invasion from DNA molecule 1 into DNA molecule 2 which is followed by the 3' ends of each linear molecule serving as primers, with the homologous strands of the recombining DNA molecules acting as templates [12,25,29]. Very long molecules that have been observed could be produced by head to tail concatemers [23].

In chloroplasts, as the nucleoids are distributed throughout the chloroplast associated with the thylakoids this likely ensures equal segregation to the equally sized daughters. In Angiosperms this fairly equal segregation was shown by pulsing and chasing ^{3}H-thymidine labelled cpDNA into daughter chloroplasts [30].

Two genes have been implicated in the segregation of the chloroplast nucleoids. There is evidence for the involvement of DNA gyrase in nucleoid segregation. When DNA gyrase is silenced only a few nucleoids are present as opposed to the many scattered throughout the chloroplast in *Nicotiana benthamiana* controls [31]. In *Arabidopsis thaliana*, mutants of the Holliday junction resolvase MONOKARYOTIC CHLOROPLAST1 (MOC1) prevented nucleoid segregation [32]. These two genes provide some insight into how nucleoids are spread throughout the chloroplast. We have argued that this is linked to the membrane association in chloroplasts [18]. Support for this comes from studies with maize chloroplasts in the bundle sheath and mesophyll cells and *Phaseolus vulgaris* etioplasts [18]. In maize bundle sheath cells chloroplasts with unstacked parallel membranes have more and smaller nucleoids than the more complex grana-containing (stacked thylakoids) chloroplasts of the mesophyll cells. In etioplasts as the thylakoid membranes grow out from the prolamellar bodies the nucleoids locate along the growing thylakoid. The detail of how the molecular connection of the nucleoid to the growing membrane and physical segregation of the nucleoid involving DNA gyrase and MOC1 leads to the nucleoid segregation remains to be ascertained. The membrane fraction is also known to be enriched for replication forks [33]. When proplastids are considered, there are fewer studies, but again there is membrane association, and a membrane relationship to segregation could occur [18].

In dividing mitochondrion, daughter mitochondria can be of different sizes and some daughters lack nucleoids [27]. While nucleoid segregation could be similar to plastids, the folding of the inner membrane into the molecular rich cristae might make it difficult to distribute the nucleoids throughout the mitochondrion.

OVERCOMING NUCLEOID HETEROGENEITY IN MITOCHONDRIA – FUSION AND FISSION

Mitochondrial fusion followed by fission overcomes the heterogeneity of the nucleoid population, and also facilitates mixing of the mitochondrial components.

Mitochondrial fusion was inferred many years ago from novel mtDNA restriction patterns from fused protoplasts, used in the production of somatic hybrids [34]. Arimura *et al*. [27] made the first direct demonstration of mitochondrial fusion in plants using the photoconvertible protein Kaede where it was possible to demonstrate transient fusion of red and green mitochondria between mitochondrial pairs.

This question of mitochondrial nucleoid heterogeneity was also considered in a regenerating protoplast experimental system [35,36] shown in Figures 2 and 3, which demonstrated massive mitochondrial fusion (MMF). Mitochondria were labelled and tracked using a CoxIVGFP probe. The mitochondria from isolated protoplasts can be visualized going through a number of stages. Initially the mitochondria are clumped together then undergo fusion, forming very long rods (Figure 2a and 2b). Fusion is followed by fission into numerous small

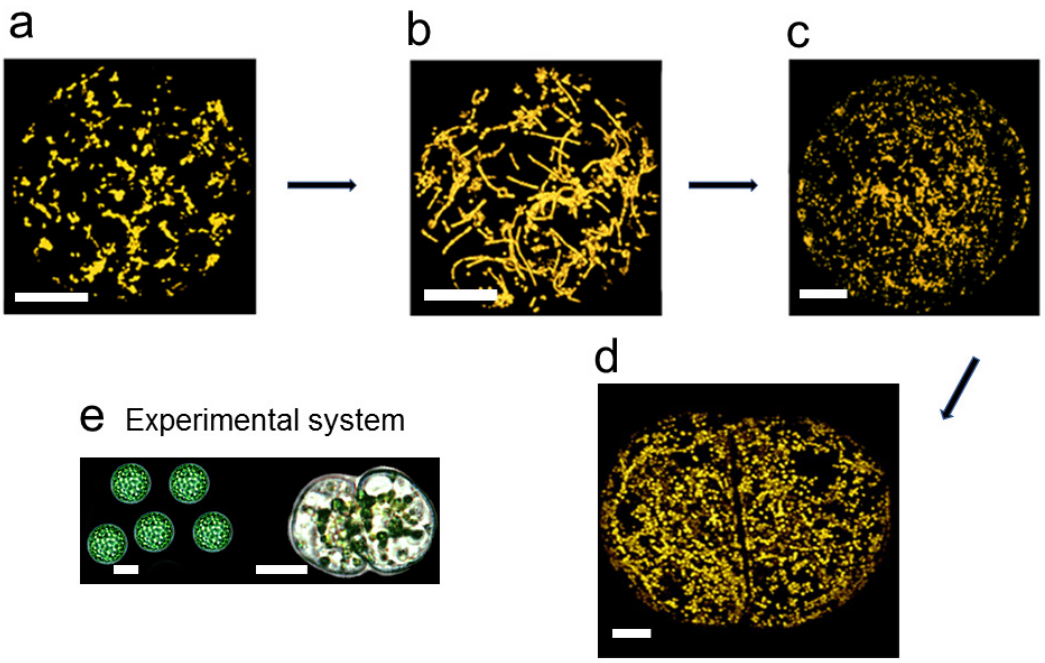

Figure 2. **Mitochondrial fusion and fission prior to first cell division in regenerating *Nicotiana tabacum* protoplasts. Visualized by GFP-expressing mitochondria.** (**a**) Mitochondria in freshly isolated protoplasts are small ovoid organelles. (**b**) Massive mitochondrial fusion forming highly elongated mitochondria. (**c**) After fusion there is fission, generating large numbers of small mitochondria. (**d**) Uniformly dispersed mitochondria enable unbiased inheritance at cell division. (**e**) The experimental system. Dividing protoplast shows clustering of chloroplasts around the nucleus. Bars (a), (b), (c), (d) 10 µm, Bars (e) = 20 µm. Figures (a), (b), (c), (d) based on Sheahan *et al*. [35] with permission from John Wiley and Sons.

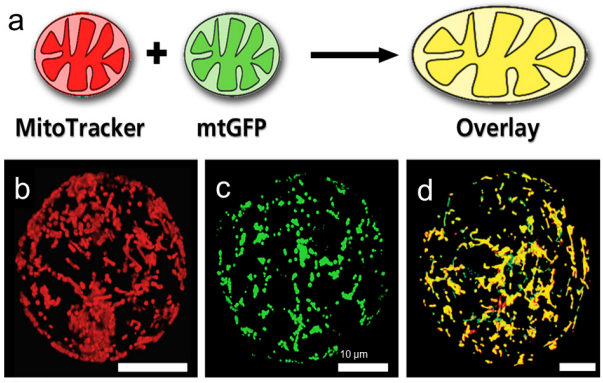

Figure 3. Mitochondrial fusion in cultured *Nicotiana tabacum* protoplasts. (**a**) Schematic of mitochondrial fusion demonstrated by co-localization of green (mtGFP) and red (MitoTracker) mitochondrial labels. (**b**) MitoTracker stained protoplasts with red mitochondria. (**c**) mtGFP-expressing protoplasts with green mitochondria. (**d**) Fused protoplasts with red and green mitochondria incubated 24 h showing co-localization of fused red and green mitochondria (yellow). Bars = 10 µm. Figures based on Sheahan *et al*. [36] with permission from John Wiley and Sons.

mitochondria (Figure 2c). Additional evidence for MMF was obtained by fusing protoplasts with different colored mitochondria (Figure 3). Mitochondria from one group of protoplasts fluoresced green using coxIVGFP and the mitochondria from the other group fluoresced red using MitoTracker and then the cells were fused. After cell fusion there was MMF and the population fluoresced uniformly yellow due to MMF and nucleoids were restored to most mitochondria. As pointed out by Logan [11] we can think of mitochondria of a plant cell existing as a discontinuous whole.

Unlike the situation in yeast, mammals, and humans, key proteins involved in mitochondrial fusion have not yet been identified in plants. The *fmt* (*friendly mitochondria*) mutant has allowed the identification of the FMT protein which has been proposed to mediate intermitochondrial association prior to fusion [12].

CELL DIVISION AND SEGREGATION OF THE ORGANELLES INTO DAUGHTER CELLS

Bearing in mind the differences in the nucleoid segregation between chloroplasts and mitochondria discussed above, it would be expected that nucleoids would replicate and segregate and then the organelles divide prior to cell division. A variety of studies carried out with culture systems and in leaf development indicate that there is not only a simple doubling of organelle DNA in the cell cycle in meristematic cells, as there is also a requirement to prepare for subsequent cell enlargement and the physiology of the organ [37,38]. Plastid DNA synthesis is intensive in the young leaf in the proplastid and early chloroplast development phase and ceases once cell division ceases, such that during cell expansion cpDNA synthesis is not coordinated with chloroplast division. For example, in spinach with small chloroplasts (20 per cell) in small leaves there are 200 cpDNA copies per chloroplast. In larger cells, after extensive cell expansion and increases in chloroplast numbers (130 to 170 per cell) with their well-developed thylakoids, there are 30 cpDNA copies per chloroplast [39]. In Arabidopsis proplastids, large nucleoids develop before thylakoid development starts [39]. This pattern of plastid synthesis, occurring in very early leaf development has been shown to be characteristic of a number of species [25].

Mitochondrial DNA synthesis in Arabidopsis starts earlier than cpDNA synthesis, being actively synthesized in the SAM and young leaf primordium [40] and then declines. In the SAM and young leaf primordium the DNA content per mitochondrion is more than 1Mbp and decreases to 170 kb in the mature leaf.

Plastid and mitochondrial DNA synthesis is then frequently out of step with the division of these organelles. There is also no evidence for a discrete phase of cpDNA synthesis in the plastid division cycle such as the nuclear DNA S phase in the cell cycle [37].

Although organelle division can be uncoupled from cell division in cell expansion, it must be coordinated with cell division to maintain plastid and mitochondrial numbers. There is evidence for specific genes that are involved in coordinating organelle and cell division. AtCDT1a and b, components of the nuclear DNA pre-replication complex, interact with the chloroplast division protein ARC6. Silencing of the AtCDT1 genes inhibits plastid division and alters nuclear DNA replication [41]. In eukaryotic algae there is evidence that the state of chloroplast division is relayed as a retrograde signal to cell cycle regulators, such that if chloroplast division is blocked then the cell cycle is arrested [42].

When cells divide, the complement of organelles present have to be segregated into the new daughter cells for organelle continuity, bearing in mind that all plant cells contain mitochondria and plastids. Figure 2 provides an example of how this is achieved for mitochondria in regenerating protoplasts. Following MMF and fission in cultured protoplasts the mitochondrial population disperses evenly throughout the cell, with equal segregation into daughter cells (Figure 2c and 2d). The key is the uniform dispersion throughout the cell by the cytoskeleton which enables partitioning for unbiased inheritance. Mitochondrial dispersion is primarily actin filament (AF) dependent with little or no role for microtubules [35]. The initial evidence for this was based on inhibitors, with the AF inhibitor Latrunculin B preventing dispersion but with the microtubule inhibitor Oryzalin having little effect. Subsequent work used the actin probe mGFP:ABD2 to visualize the actin filaments, with the mitochondria tracking along the actin filaments (Figure 4c and 4d) [43]. It seems likely that the movement is powered by myosin using ATP hydrolysis [38].

There is also unbiased inheritance of chloroplasts utilizing AFs rather than microtubules. However, the mechanism is somewhat different. Instead of the chloroplasts dispersing throughout the cell they cluster around the nucleus (Figure 4a and 4b) [35] and this facilitates the equal partitioning of the chloroplasts to the daughter cells. The AFs form baskets around the chloroplasts and translocate with a network of actin arrays to the nuclear periphery. The role of myosins, if any, in chloroplast movement is not clear [38,44]. Segregation of proplastids in the dividing cell has not been investigated in any detail but they do organize around the nucleus as for chloroplasts, suggesting a similar segregation mechanism [45].

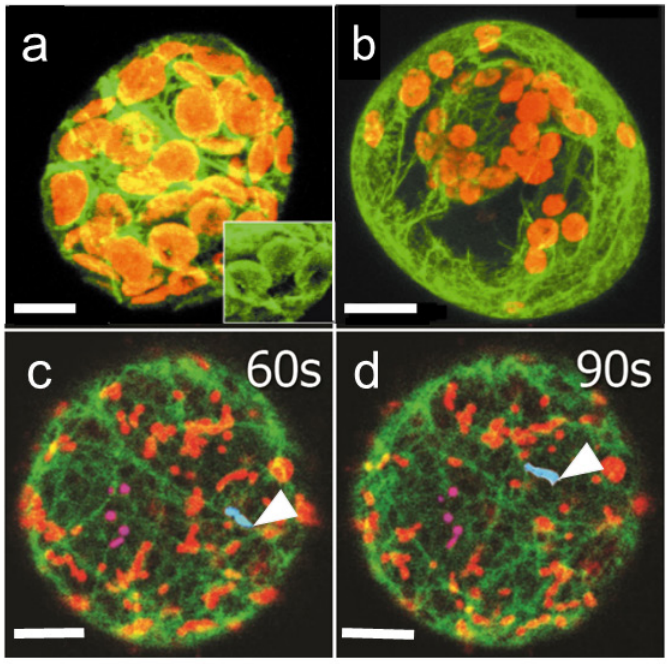

Figure 4. Chloroplast and mitochondrial movement driven by AF interactions in cultured protoplasts. (**a**) Prominent chloroplast-AF baskets (inset) become enmeshed in AF network (**b**) which drives chloroplasts to surround nucleus. (**c**) and (**d**) Time-lapse imaging of erratic (magenta) and vectorial (cyan, arrowheads) mitochondrial movement. Erratic movements appear to follow movement of underlying AFs while vectorial movement appears to be along static AFs. These movements distribute mitochondria throughout the cell. Bars = 10 μm. (a) and (b) Sheahan *et al*. [35] with permission from John Wiley and Sons. (c) and (d) Sheahan *et al*. [43] with permission from John Wiley and Sons.

THE MATERNAL INHERITANCE OF PLASTIDS AND MITOCHONDRIA

Plastids and mitochondria display uniparental-maternal, biparental and uniparental-paternal inheritance in angiosperms, but maternal inheritance is the predominant pattern [4,46]. This non-Mendelian inheritance was established using classic genetics many decades ago. It was confirmed by the DAPI staining of nucleoids showing their absence in the male germ line, as well as by inheritance studies using restriction endonucleases and Southern hybridization [4,47]. The absence of paternal organellar DNA can be accomplished by DNA degradation mechanisms or autophagy [4] so that cytoplasmic DNA is absent in sperm cells. Exclusion of organelles by asymmetric partitioning is another mechanism. Tanaka [48] studied *Lilium longiflorum* where maternal inheritance of cpDNA was due to the absence of plastids in the generative cell. The nucleus is asymmetrically positioned. The plastids are excluded from the periphery of the haploid nucleus by microtubules radiating out from the nucleus and are located at the other cell pole. There was no direct cytological evidence for AF involvement in this asymmetry. GFP-based actin labelling was not available at that time.

SHOOT APICAL MERISTEMS – INFLUENCING BOTH PLASTIDS AND MITOCHONDRIA IN THE PLANT DEVELOPMENT AND REPRODUCTION PHASES

The shoot apical meristem is of key importance in both a development and reproductive context for plastids and mitochondria. In plant developmental biology there has been an understandable major focus on the molecular regulation of stem cell maintenance, cellular differentiation, and the flowering transition in the apical meristem. Chloroplasts and mitochondria as semi-autonomous cytoplasmic DNA-containing organelles have more frequently been studied in leaf development and in *in vitro* cell division systems [38]. For the apical meristem, plastids and mitochondria have been considered separately below as there are some major differences, over and above their division and nucleoid segregation and their segregation mechanism at cell division.

(a) The plastids

The SAM is formed in the embryo and becomes encased in the seed. After germination, in the SAM of

the new plant, proplastids with their nucleoids are passed through cellular generations to maintain about 10 to 20 plastids per cell [6]. Leaf primordia develop from the SAM and form leaves, where in the presence of light the proplastids differentiate into chloroplasts. The mature chloroplast contains the thylakoids which house the photosystems which facilitate the energy-generating photosynthetic process, the nucleoids, the protein-synthesizing system as well as Rubisco. While there is a reasonable understanding of the molecular architecture of the thylakoids, the signals and mechanisms that allow the formation of the granal-thylakoid system from a proplastid has received less attention. However, there is recent improved understanding using an Arabidopsis cotyledon system, with mutants and electron tomography of cryo-fixed cells [49]. Five stages were proposed, from stage 1 tubulovesicular membranes to the stage 5 elaborate grana stacks and narrow grana-stroma membrane connections [49]. Stage 2 is the pre-granal thylakoids, stage 3 is the pro-granal stacks and stage 4 the grana-stroma thylakoid network. At every stage there are envelope-thylakoid contacts and from stage 2 polysomes are associated with the thylakoids. How the few tubulovesicular membranes of the proplastids form is not clear. The inner-membrane thylakoid contacts maybe indicative of an initial inner membrane origin of thylakoids [50]. The polysome associations are indicative of the need to assemble the thylakoid components. The dynamin-like protein FZL which facilitates the fusing of membrane compartments and the CURT1 membrane-bending proteins are both involved in the formation of the thylakoid network [49]. These essential proteins are in addition to the thylakoid proteins involved in photosynthesis; with characteristic locations in the thylakoid system. These are the ATP synthase, cytochrome b_6f, photosystems I and II (PSI and II), and light harvesting complex II (LHCII). PSII and its interacting partner LHCII are associated with the granal thylakoids and LHCII promotes granal thylakoid adhesion [49].

The Liang *et al*. study [49] also has implications on how the many nucleoids of the chloroplast become associated with the thylakoids. It is interesting to examine the way the ribosome association tracks thylakoid development from the proplastid so that ribosomes occur on the granal thylakoids adjacent to the stroma. Something similar might occur with the nucleoids (Figure 1c and 1d). There is evidence that DNA replication is membrane associated and the nucleoid partitioning can be linked to membrane growth [18,33]. Early electron micrographs showed a coupling of transcription and translation (Figure 1b) [51]. More recent molecular evidence supports this [52], though the majority of translation occurs post-transcriptionally [52]. With transcripts and ribosomes connected to nucleoids in turn linked to membranes at least some of the ribosomes depicted in the Liang *et al*. studies could be transcriptionally linked (Figure 1b) [18,51].

Two transcription factor families, the GATA NITRATE-INDUCIBLE CARBON-METABOLISM-INVOLVED (GNC) and GOLDEN TWO-LIKE (GLK) families have been implicated as potential master regulators of the development of proplastids into chloroplasts as well as chloroplast growth and division [53]. The GNC family acting as repressors and the GLK family acting as activators have independent and overlapping roles, based on protein-binding microarrays.

In a reproductive context the apical meristem is converted to a flower which includes the female egg which transmits the proplastids to the next generation after fertilization. If there are any green tissues containing chloroplasts in the SAM these are capable of dedifferentiating to proplastids similar to what occurs in plants mesophyll cells undergoing plant regeneration [54].

(b) The mitochondria

The mitochondria are present in the cytoplasm of the SAM with the proplastids and in the leaf primordia where the mitochondria and their genomes are maintained in the cells as discussed in the sections above. There are no major structural differentiation changes in mitochondria during leaf development, as occurs with the proplastids.

However, in a reproductive context there are major changes associated with the mitochondria. Segui-Simarro *et al*. [55] observed MMF where the mitochondria fuse to form a massive cage-like structure surrounding the nucleus which subsequently undergoes fission to form many small mitochondria. In a subsequent publication Segui-Simarro and Staehelin (Figure 5a and 5b) [9] pointed out that the SAM gives rise to the floral meristem and the female gametes which house the mitochondria. This allows mixing of the protein content, prevents undesired mutation, allows most mitochondria to contain DNA, and enhances the opportunity for intramolecular recombination.

After the zygote develops into an embryo and the seed is formed and a period of dormancy follows, the mitochondria are rather rudimentary in terms of cristae development. After germination autotrophic growth is initiated. Paszkiewicz *et al*. (Figure 5c and 5d) [56] showed that after imbibition at the testa rupture stage there is MMF in the form of a perinuclear tubuloreticular structure followed by fission to form numerous mitochondria, again giving the advantages of MMF. Once the seedling starts to grow there is still not 100 percent of mitochondria containing DNA and the frequent fusion and fission of mitochondrial pairs in somatic cells can occur as described by Arimura *et al*. [27]. While transient fusion and fission is available to maintain nucleoids and exchange proteins it is feasible that MMF either in clonal (from

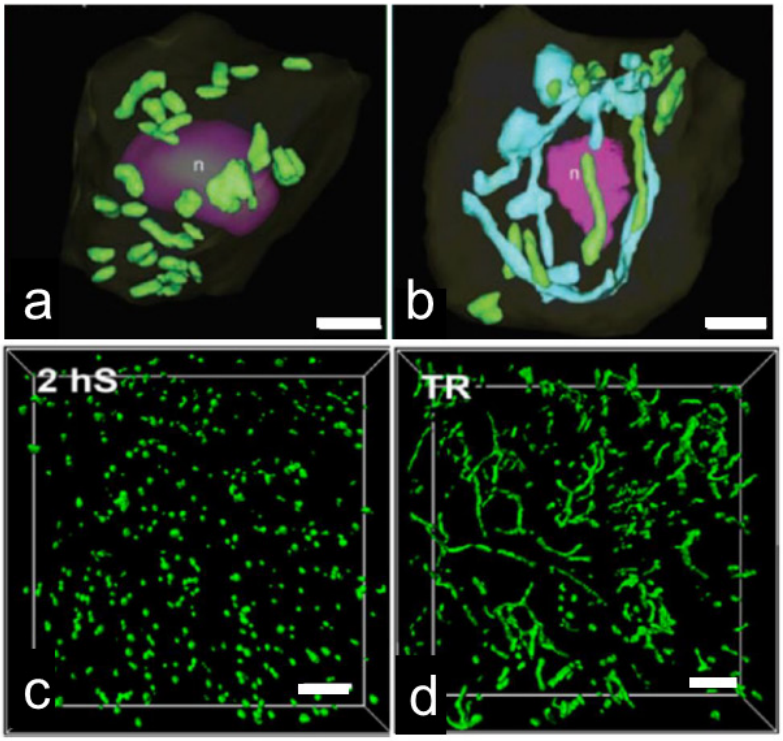

Figure 5. Massive mitochondrial fusion in two *in planta* examples. (**a**) Interphase cell from Arabidopsis stem showing round/oval-shaped mitochondria (green). (**b**) Prometaphase cell from SAM with some individual mitochondria (green) and mitochondria fused into a massive cage-like structure (blue). (**c**) Numerous discrete mitochondria in embryonic Arabidopsis cotyledon cell 2 h after imbibition. (**d**) Embryonic cotyledon cell at the testa rupture (TR) stage showing fused mitochondria with tuboreticular morphology. Compare with Fig 3a and 3b. (a) (b) Bars = 2 µm, (c) (d) Bars = 5 µm. (a) and (b) are from Segui-Simaro and Staehelin [9], Creative Commons Attribution. (c) and (d) are from Paskiewicz *et al*. [56] reproduced according to American Society of Plant Biologists permissions. For context of Figure 5 see [28].

isolated protoplasts) or sexual reproduction encourages DNA repair and recombination as part of a quality control in each generation [28]. Mitochondrial DNA repair and recombination are important processes in genome quality control [57,58]. Arimura [12] has suggested that MMF is associated with periods of active DNA synthesis. This is likely given when MMF occurs, but this requires direct demonstration.

Why is fusion characteristic of mitochondria but not plastids? Ultimately it is likely related to their different evolutionary pathways, however plastids are able to maintain quality genomes in each plastid without undergoing fusion/fission cycles. In both plastids and mitochondria DNA recombination and replication mechanisms are important but nucleoids can be maintained in individual plastids without fusion.

The 16.6 kb human mitochondrial genome is not as complex as the plant mitochondrial genome but fusion and fission are still essential. If this does not occur in neurons, then serious diseases such as Charcot-Marie-Tooth type 2A (CMT syndrome) can arise, whereby this peripheral motor neuropathy can cause loss of limb function [59]. This suggests that mtDNA quality control is facilitated even in a less complex genome, to maintain optimum mitochondrial function.

REMOVAL OF DAMAGED MITOCHONDRIA AND PLASTIDS

As mitochondria and plastids are not derived *de novo* maintaining the quality of these organelles is critical. There is increasing evidence that another mechanism is to selectively remove damaged mitochondria and chloroplasts, which would allow only quality organelles to undergo DNA replication and division.

Removal of damaged mitochondria [60] and chloroplasts [61] can be carried out by autophagy. Autophagasomes sequester the organelle and fuse with the vacuole for degradation. For mitochondria the process is known as mitophagy and for chloroplasts chlorophagy.

Mitochondrial damage indicated by a loss of membrane potential ($\Delta\psi_m$) or excessive ROS production (O_2^-),

sets mitophagy in train [62]. Mitophagy is a structured process regulated by AUTOPHAGY-RELATED (ATG) proteins [60]. Central to the process is the formation, by ATG proteins, of a cup-shaped vesicle called the phagophore which ultimately closes to form the autophagosome, the double membrane-enclosed compartment which fuses to the vacuole. Important components in this system are the ubiquitin-like proteins, ATG8 and ATG12, which in an ATP dependent process conjugate to the lipid phosphatidylethanolamine and the ATG5 protein [60].

In chloroplasts, damage can lead to chlorophagy. The initial signals that induce autophagy of whole chloroplasts are not fully understood but in UVB-induced damage, accumulation of O_2^- appears to be the signal [61]. The mechanism though not understood as well as mitochondria [63] involve similar mechanisms using ATG proteins. Chlorophagy can be prevented by atg mutants [61]. The ATG8 protein is also an important autophagy marker protein associated with the phagophore [61,63].

CONCLUSIONS AND FUTURE PROSPECTS

The integration of contemporary molecular biology and cell biology has enabled an improved understanding of how chloroplasts and mitochondria and their genomes are maintained in plant development and reproduction. These organelles have unique ways to maintain cpDNA and mtDNA quality. The framework of understanding now available provides a clearer view of what the outstanding questions are. There are still many genes to discover. The key genes responsible for plant mitochondrial fusion have not yet been identified and details of the molecular basis of the AF mitochondrial and chloroplast movement to enable segregation also requires further understanding. The specific nuclear genes that signal the onset of DNA replication and chloroplast and mitochondrial division need to be identified. In a number of cases intense DNA replication occurs in preparation for subsequent cycles of mitochondrial and chloroplast division. Mechanisms of integrating these latter events to the cell cycle and cell growth require more understanding. In this context a focus on the apical meristems is an important area influencing cell lineages and reproduction. The molecular structure of the chloroplast and mitochondrial nucleoid is still in its infancy and how this is linked to DNA replication and transcription, repair and recombination mechanisms requires more understanding.

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