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Plant mitochondrial DNA

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1. ABSTRACT

Plants possess mitochondrial genomes that are large and complex compared to animals. Nearly all animal mitochondrial genomes are about 16.5. kbp in length, whereas plant mitochondrial genomes range between 200-2,000 kbp. This is curious if we assume modern mitochondria originated from a common alpha-proteobacterial ancestor. Despite their size, plant mitochondrial genomes do not contain significantly more genes than their animal counterparts. Most of the additional DNA found in plant mitochondrial genomes consists of large introns, repeats and non-coding regions. Furthermore, plant mtDNA does not exist as large circular DNA molecules but mostly as a collection of linear DNA with combinations of smaller circular and branched molecules. Studies into these highly fragmented genomes heavily imply that recombination is the main mechanism driving replication of plant mtDNA.

2. INTRODUCTION

Plant and animal mitochondria look very similar to each other. In 1917, the botanist N. H. Cowdry wrote about the differences of plant versus animal mitochondria: "Their morphology is identical in plants and in animals. They assume no forms in the one, which are not present in the other. They undergo similar variations in size and shape in different tissues and in different cells in both. If it were possible to view mitochondria dissociated from their environment, it would be impossible to decide whether they came from plant or animal tissues, provided that they did not contain starch, pigment or some other easily recognizable substance, to serve as a clue. We have every reason to suppose that their chemical composition is much the same in both plants and animals, but here our knowledge is for the most part supposition and inference, since direct chemical analyses are obviously out of the question (1)." Although plant and animal mitochondria were obtained through similar endosymbiotic events, the genetic content of these organelles is very different. We now have access to tools which reveal that although plant and animal mitochondria look similar on the outside, they are very different on the inside.

Eukaryotic cells rely on mitochondria as they are the location for biological energy (ATP) production. Mitochondria, as well as chloroplasts, were once believed to be independent free living organisms that were engulfed by larger cells. After being engulfed, ancient mitochondria formed a symbiotic relationship with their host. A similar process can be observed currently in root-nodule systems of many land plants (2). Over time, ancient mitochondria lost more and more of their DNA as its genes were moved and incorporated into the nuclear genome of the hosts they inhabited. At some point, these changes caused mitochondria to lose the ability to survive on their own. Today, mitochondria and chloroplasts still possess and maintain their own DNA, remnants of their independent pasts. This residual DNA encodes only some of the genes required for respiration, photosynthesis, and other organelle functions (3, 4). Other proteins required for organelle function are encoded in the nucleus and must be imported. These include the machinery required for DNA replication, gene transcription and some proteins and tRNAs needed for translation.

3. PHYLOGENY

As mentioned above, mitochondria were once independent, free-living organisms. Comparisons between present-day mitochondria and *Rickettsiales*, an

order of small proteobacteria, are often made because analyses of *Rickettsiales* indicate high similarity to mitochondrial DNA patterns (5). This makes *Rickettsiales* the closest known living extant relative of present-day mitochondria (6). The most likely ancestor of modern mitochondria is an alpha-proteobacteria with an oxidative respiratory chain system. This predecessor likely evolved monophyletically into the mitochondria we recognize today (7).

In contrast to *Rickettsiales* many researchers believe that *Chlamydomonas*, unicellular green algae, possesses mitochondria that evolved separately from the mitochondria in plant systems. Analysis of plant mtDNA with *Chlamydomonas* mtDNA shows large sequence variations. The two mtDNAs are most likely unrelated and evolved independently of each other (8). However, comparing the mtDNA of the green alga *Chara vulgaris* with the moss *Marchantia polymorpha* shows remarkable similarity and provides evidence that *Charales* forms a sister group to mitochondria in land plants. The study further infers that the common ancestor to plant mitochondria harbored a gene-rich, intron-poor genome (3).

Analysis of plant mitochondrial rRNA sequences shows a closer relationship with eubacteria and chloroplasts than with mitochondria from other organisms (9). Unique rRNA genes have been discovered in plant mtDNA that are not found in non-plant mtDNA (10, 11).

If an organism similar to *Rickettsiales* was the progenitor of modern day mitochondria, it is interesting to see how the pattern of genome evolution differed in plants versus animals, which has been reviewed in detail by Knoop (3). Animals reduced the genome of the ancient proto-mitochondria to what it is today: gene-dense and intron poor. The opposite occurred in plants; the genome became gene-poor and intron rich. If the ancestor of plant mitochondria possessed a gene-rich genome, this implies an increase rather than reduction of introns in plant mtDNA. This may be in part due to a higher occurrence of recombination-driven DNA replication rather than the typical bi-directional replication from an origin sequence.

4. GENES AND GENOME SIZE

The most puzzling difference between plant and animal mitochondria is the relatively large size of plant mitochondrial genomes. Mitochondrial genomes are about 16.5. kb in almost all animals. These genomes have been heavily reduced, are very gene dense with no or few introns and little non-coding DNA. Plants, on the other hand, have large mitochondrial genomes that can vary anywhere from 200-2,000 kbp in size (Figure 1) (12, 13). Much of the increased sizes are due in part to repeated sequences, AT-rich non-coding regions, and large introns and non-coding sequences (2, 14). Plant mitochondrial genomes also contain fairly significant amounts of relatively short nuclear and chloroplast genomic sequences that appear to have been integrated at some time during evolution (2,3,8,9). Although these large plant mitochondrial genomes are mapped as circular DNA molecules, scientists have yet to observe a single circular molecule large enough to constitute the entire genome (15). Instead, mtDNA has primarily been observed as a collection of smaller linear or branched molecules (15-17).

Using mostly nuclear-encoded proteins, mitochondria contain all of the machinery for transcribing and translating genes encoded in the mtDNA. John Allen has proposed that the reason that mitochondria (and chloroplasts) retain their own genomes is for local control of gene expression in response to the unique local redox state within the organelle(s) (18). Despite the large size differences, plants do not encode a significantly greater number of genes in their mitochondrial genomes than animals do. Furthermore, while one plant may have a mitochondrial genome much larger or smaller than another, the number of genes encoded by each genome is fairly similar (Table 1). The genes that are encoded consist of rRNAs, tRNAs, ribosome synthesis proteins, and oxidative phosphorylation proteins (14, 19). Many plant mtDNA-encoded genes undergo RNA editing where specific C's in the RNA transcripts are deaminated to U's. In each of these cases the new sequence follows the universal genetic code to facilitate translation of the proper amino acid sequence. Mitochondrial RNA editing does not occur in mammals, and requires specific and complex mechanisms to create mature mRNAs for translation (2).

The fragmented nature of plant mitochondrial genomes also leads to frequent DNA recombination events. In addition, some mitochondrial encoded genes are split in the genome, requiring trans-splicing to join two separate transcripts together to create mature mRNA capable of being translated (3). In some instances, mtDNA rearrangements result in cytoplasmic male sterility, causing plants to lose the ability to make pollen (20).

Unlike nuclei, a single mitochondrion possesses many copies of its own genome. The number of copies per organelle can vary between 50-500 and depends on the tissue type and age of the plant (21, 22). For example, studies in maize have shown that as plants age, mtDNA copy numbers decline (23). Interestingly, human mtDNA copy numbers were shown to remain constant with age (24). An example of tissue dependency is root tissue which often contains many more genome copies per mitochondria versus leaf tissue, which often has a reduced copy number (25). Mitochondria also fuse together forming large tubular networks, allowing for easy transfer of genetic material from one mitochondrion to

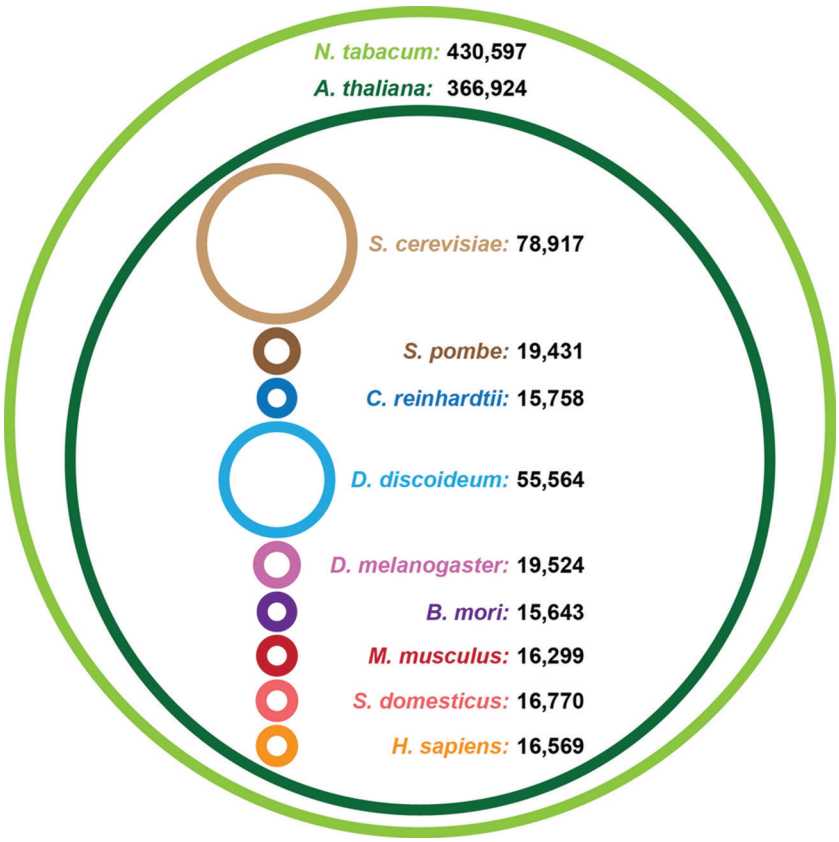

Figure 1. Scale comparison of mitochondrial genome size between different organisms. The two outermost circles represent the size of two plant mitochondrial genomes, *Nicotiana tabacum* and *Arabidopsis thaliana*. Note the great size disparity between plants and other organisms from different phyla such as yeast, insects, mammals, and humans. Most animal mitochondrial genomes are 16.5. kbp in length, and while larger size variation can occur in some organisms, plants still possess the largest known mitochondrial genomes of all living organisms.

another. This results in individual mitochondria within the same cell having different genome copy numbers (26).

5. PLANT MTDNA STRUCTURE

A great deal of variation exists in the actual structure of mtDNA. Due to the large size of these molecules a variety of techniques have been utilized in an attempt to determine the structure, including pulsed field gel electrophoresis (PFGE) and electron microscopy (15, 17, 27). In the vast majority of publications mtDNA is observed as a collection of linear molecules in varying sizes (15). In fact, there have only been two instances where circular DNA molecules large enough to be mitochondrial genomes have been observed at very low levels in the lower plant liverwort (28) and in *Vicia faba* (29).

A study performed by Lo *et al.* went into extensive detail concerning the structure of DNA in mung bean mitochondrial nucleoids. They observed plant mtDNA as supercoiled molecules, sub-genomic open circles of variable size, linear molecules, and other highly complex structures (30). It is worth noting that although all of these structures were observed, nearly 50% of all observed DNA took on the form of highly complex structures, followed by nearly 30% in linear form, 15% as variable sized open circles, and 5% as supercoiled DNA molecules. Similar observations were made in cultured tobacco cells (31).

6. NUCLEOIDS

Mitochondria and plastids lack nuclei but possess proto-nuclear structures called nucleoids. Nucleoids are a loose association of organellar DNA molecules, RNA transcripts, and DNA compacting proteins. Because they are simpler than nuclear chromatin, nucleoids are mostly homogenous, but individual nucleoids can consist of different subgenomic DNA molecules and proteins that differ from other nucleoids (30).

Mitochondria are unequally distributed, and many fail to stain with the DAPI DNA stain (32). In fact, it has been determined that some mitochondria contain less than a full genome equivalent (on an average basis) (33). If there was no mechanism in place to ensure that each mitochondrion received the appropriate DNA

Table 1. Size and number of genes in various organism's mitochondrial genomes

Species	mtDNA genome size	Genes encoded			Reference
		Protein	rRNA	tRNA	
Land plants					
Arabidopsis thaliana	366,924	33/117*	3	21	(14)
Beta vulgaris	368,801	27/140*	5	26	(51)
Brassica rapa	219,747	34/78*	3	18	(52)
Glycine Max	402,558	36/88*	3	19	(53)
Gossypium raimondii	676,078	39	6	30	(54)
Nicotiana tabacum	430,597	37/156*	4	23	(55)
Oryza sativa	491,515	33/54*	6	33	(56)
Triticum aestivum	452,528	35/39*	9	25	(57)
Zea Mays	569,630	39/163*	4	29	(58)
Fungi					
Ashbya gossypii	23,564	8	2	23	(59)
Neurospora crassa	64,840	22/28*	-	28	(60)
Saccharomyces cerevisiae	78,917	8	2	24	(61)
Schizosaccharomyces pombe	19,431	6/10*	2	25	(62)
Algae					
Chlamydomonas reinhardtii	15,758	8	14	3	(63)
Dictyostelium discoideum	55,564	33/42*	2	18	(64)
Animal					
Homo sapiens	16,569	13	2	22	(65)
Mus Musculus	16,299	13	2	22	(66)

and proteins, they would be in danger of losing genetic information which could lead to death or malfunction of the mitochondria. To remedy this problem, mitochondria frequently divide and fuse, sharing nucleoid molecules and ensuring that the proteins needed for normal function localize to the correct organelle (26). This process allows mitochondria to maintain their shape and size even if they do not possess an entire genome. This also permits healthy mitochondria to complement the activity of defective mitochondria.

7. REPLICATION MECHANISMS OF PLANT MTDNA

Because of the size and complexity of plant mitochondrial genomes, the exact mechanism for plant mtDNA replication remains unclear. Organellar DNA levels vary significantly in different ages and tissues depending on energy needs, tissue type, and stage of development (34-36). This suggests tightly controlled regulation of mtDNA and ctDNA replication and genome copy number. This regulation is not yet understood, but is likely distinct from mechanisms that tightly control replication of bacterial and eukaryotic nuclear genomes.

Plants most likely utilize multiple strategies in tandem to replicate their mitochondrial genomes (37). Some of these mechanisms include recombination-dependent replication (RDR) and/or a rolling circle mechanism similar to bacteriophage T4 DNA replication (23, 37, 38) as well as traditional bi-directional replication from specific origins of replication (Figure 2). This is much more complex than replication of animal mtDNA, which has been characterized in detail as a displacement-loop mechanism (39). Oftentimes, plant mtDNA can be observed as 'rosette' or 'starburst' structures. The leading theory is that these structures occur due to a high frequency of recombination and replication initiated from recombination events. Another theory poses that the center of the rosettes contain origins of replication, but rather than serving as sites for traditional bi-directional replication they are instead sites for initiation of DNA recombination (27).

With growing evidence that RDR may be a major mechanism for DNA replication in plant mitochondria, this infers that one or more recombinases facilitates DNA synthesis. Three bacterial RecA orthologs have been identified in the Arabidopsis nuclear genome; RecA1,

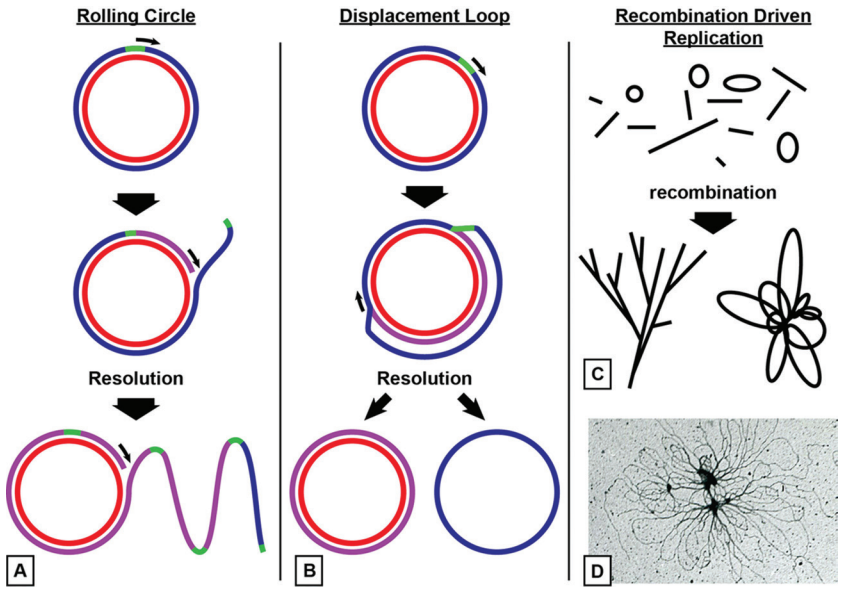

Figure 2. Methods commonly employed during replication of mitochondrial DNA. Rolling circle replication begins by nicking one of the double strands of DNA. Replication continues along the circle displacing one strand while synthesizing a single-stranded copy of the other DNA strand. When replication reaches the original origin it continues to displace DNA, creating a long concatameric DNA molecule that has many copies of the original genome end-to-end (A). In a displacement loop (B), replication occurs uni-directionally, displacing one strand of DNA until reaching the original origin of replication. At this point, double stranded and single stranded circular DNA molecules exist. The single stranded DNA circle is replicated soon after the first strand finishes or will begin replicating part way through the replication of the first displacement loop. This method is commonly observed in animal mitochondria. Recombination occurs when single stranded homologous regions of DNA invade double stranded DNA molecules causing the DNA to form a replication bubble. Recombination of plant mitochondrial DNA often leads to complex branched or looped replicating structures (C). The structures are often seen as DNA 'rosettes' in EM images (D) (unpublished image from the Nielsen laboratory).

RecA2, and RecA3 (40, 41). RecA1 is localized to the chloroplasts, RecA2 is dual-localized to mitochondria and chloroplasts, and RecA3 is found only in mitochondria. Strand invasion catalyzed by one of the RecA homologs followed by extension of new DNA synthesis by a mtDNA polymerase may be directly involved in plant mitochondrial genome replication.

Replication of mtDNA utilizes a bacteria-like, single subunit DNA polymerase. Structural and phylogenetic analysis of plant mtDNA polymerases reveal a similarity to bacterial DNA polymerase I (42). Most plants possess two nuclear-encoded copies of the DNA polymerase gene. In all plants where localization has been examined these enzymes are dual targeted to both mitochondria and chloroplasts (37).

8. SEGREGATION OF MTDNA MOLECULES DURING MITOCHONDRIAL DIVISION

As mentioned above, plant mitochondria frequently divide and fuse. Because of the subgenomic nature of plant mitochondrial genomes, totally random or stochastic distribution of mtDNA to new mitochondria would lead to many defective mitochondria that lack a complete genome. Mitochondria move along the actin cytoskeleton, and fuse to form networks that allow for mixing of the genetic information. Studies conducted by Sheahan *et al*. (32, 43) and reviewed by David Logan (44) suggest that massive mitochondrial fusion allows mixing and recombination of the mitochondrial genome prior to cell division, thus ensuring continuity of genetic information.

Regulation of genome copy number and division/transmission is under nuclear regulation. This has been demonstrated in yeast (45, 46) as well as *Arabidopsis* (47) and the common bean (48). In *Arabidopsis*, mutation of the gene MSH1 generally leads to an increased number of gene copies compared to wild-type plants (49). Interestingly, mutations in MSH1 lead to epigenetic changes that cause plants to grow larger compared to wild-type. This phenotype is passed down to progeny and the epigenetic changes persist even when cross-pollination removes the MSH1 mutation (50).

9. SUMMARY

Mitochondria are the remnants of an alpha-proteobacterial ancestor that was engulfed and incorporated into larger cells. Over time, this alpha-proteobacterium lost more and more of its genes to the nuclear genome of the larger host cell. Eventually, this occurred to the point that the bacterial cell could no longer survive on its own.

In animals, mtDNA has been heavily edited to become gene dense with little or no introns or non-coding DNA. Plants on the other hand have had an increase in the number of introns and non-coding DNA within their mitochondrial genomes. As a result, plant mitochondrial genomes are relatively huge compared to their animal counterparts. Despite their size, plant mitochondrial genomes code for relatively few genes. The number of genes does not go up with the increase in mitochondrial genome size.

Plant mitochondrial genomes have not been observed as circular DNA molecules. Instead they exist as a collection of linear pieces and smaller circular molecules. These subgenomic fragments most likely require recombination to be the main driver of plant mtDNA replication. A rolling circle or other mechanism may also be involved in replicating plant mtDNA.

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