Published in final edited form as:
Mitochondrion. 2014 November ; 19PB: 231–237. doi:10.1016/j.mito.2014.03.008.

Minireview: DNA Replication in Plant Mitochondria

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Abstract

Higher plant mitochondrial genomes exhibit much greater structural complexity as compared to most other organisms. Unlike well-characterized metazoan mitochondrial DNA (mtDNA) replication, an understanding of the mechanism(s) and proteins involved in plant mtDNA replication remains unclear. Several plant mtDNA replication proteins, including DNA polymerases, DNA primase/helicase, and accessory proteins have been identified. Mitochondrial dynamics, genome structure, and the complexity of dual-targeted and dual-function proteins that provide at least partial redundancy suggest that plants have a unique model for maintaining and replicating mtDNA when compared to the replication mechanism utilized by most metazoan organisms.

Keywords

mtDNA replication; plant mtDNA recombination; plant mitochondrial genome; mtDNA polymerase; Twinkle primase/helicase

1. Introduction

Plant mitochondria are semi-autonomous organelles that contain genomes and essential genes for mitochondrial function. Over time many genes have migrated to the nucleus, and mitochondrial genomes in most organisms contain an essential but incomplete set of genes required for their function (Schuster and Brennicke, 1994; Knoop, 2004; Christensen, 2013). All known proteins required for plant mitochondrial DNA (mtDNA) maintenance are encoded within the nuclear genome of the cell. Many of these proteins are dual-targeted to both mitochondria and plastids (Table 1; Christensen et al., 2005; Carrie et al., 2009). However, replication of the mitochondrial DNA (mtDNA) is required to provide templates for genes that must be expressed to maintain optimal cellular metabolic functions.

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Plant mitochondrial genomes on average are larger and exhibit a higher level of complexity than those found in most metazoan mitochondrial genomes. Regardless of these differences, the relative type and number of mitochondrial encoded genes exhibits low variability between all organisms. However, despite an abundance of information that is available on plant mitochondrial genomes, gene expression, and mtDNA sequences (Schuster and Brennicke, 1994; Mackenzie and McIntosh, 1999; Liere et al., 2011) the mechanisms that control mtDNA replication and genome copy numbers are not understood. MtDNA levels can vary significantly in plant tissues of different ages, cell types, and locations (Preuten et al., 2010; Woloszynska et al., 2012; Cupp and Nielsen, 2013). This suggests that mechanisms are in place to regulate plant mtDNA replication and genome copy number.

2. Plant mitochondrial genomes and mtDNA replication

2.1. Complexity of plant mitochondrial genomes

Mitochondrial genomes vary widely in size among organisms, ranging from a very compact 16,500 bp in humans and other vertebrates to 30,000–90,000 bp in yeast and other fungi. Higher plants maintain an even broader range of mitochondrial genome size, from 208,000 bp to about 11.3 megabase pairs (Knoop, 2004; Gualberto et al., 2013). The increased sizes of plant mitochondrial genomes are mostly due to repeated sequences, AT-rich non-coding regions, and many large introns and non-coding DNA sequences rather than being due to a large increase in the number of genes. MtDNA has been shown to be associated with specific proteins that form nucleoid complexes within the mitochondrial matrix. These complexes provide both a scaffolding structure for mtDNA molecules and a targeted location where the nuclear-encoded DNA replication proteins can replicate the mitochondrial genome (Dai et al., 2005).

2.2. Comparison with animal mtDNA replication

Animal mtDNA is a circular molecule that is replicated by a nuclear-encoded DNA polymerase (Polγ). Replication occurs by a unidirectional displacement loop (D-loop) mechanism following initiation from the control region utilizing a processed transcript as primer. After replication of the first strand is nearly two-thirds completed, the other DNA strand is copied by staggered replication (Bogenhagen and Clayton, 2003). Animal mtDNA also replicates by a bidirectional replication mechanism termed strand-coupled replication or by a third mechanism termed RITOLS (Bowmaker et al., 2003; Fish et al., 2004; reviewed by McKinney and Oliveira, 2013). All three models of human mtDNA replication utilize the same origins of replication, but vary in the amount of RNA retained in the newly synthesized DNA strand. Strand-coupled replication infers that Okazaki fragments must occur as intermediates in new strand synthesis. However, a mitochondrial DNA primase has never been conclusively identified in human mitochondria. It has been proposed that partially processed transcripts synthesized by mitochondrial RNA polymerase may serve as primers and remain in the newly made DNA, eliminating the need for a DNA primase (McKinney and Oliveira, 2013; Reyes et al., 2013).

2.3. Potential plant mitochondrial DNA replication mechanisms

In contrast to animal models, plant mitochondrial genomes are unique in structural complexity. Traditionally, plant organelle genomes have been represented as circular molecules. However, most efforts to examine the structure of plant mitochondrial genomes have not confirmed the presence of the predicted master circle molecules. These genomes are often large, linear, and undergo DNA rearrangements and substoichiometric shifting (Arrieta-Montiel et al., 2009; Woloszynska et al., 2012; Christensen, 2013). It is now widely accepted that plant mtDNA exists primarily as subgenomic molecules that are predominantly linear (Bendich, 1993; Backert et al., 1997; Christensen 2013; Oldenburg and Bendich, 1996; 1998; 2001) with minor structures found in low abundance forming sigma-like lariats with linear tails, rosette-like structures, catenane-like molecules, and linear molecules with branching (Backert et al., 1996; Bendich, 1996; Backert and Borner, 2000). These characteristics make a D-loop replication mechanism less plausible for plant mtDNA, although there is one early reference reporting such a mechanism for replication origins in petunia (deHaas et al., 1991). However, there have been no further publications to confirm this mechanism in plants. The major differences in mitochondrial genome structure between plants and most metazoans suggest that plants may utilize different mechanisms for mtDNA replication that may be linked with DNA recombination.

In plants and fungi it has been shown that mtDNA may replicate by a recombination-dependent and/or rolling circle mechanism similar to bacteriophage T4 DNA replication (Backert et al., 1996; Backert and Borner, 2000; Oldenburg and Bendich, 2001; Manchekar et al., 2006; 2009; Mosig, 1998). In yeast (Sena et al., 1986; Maleszka et al., 1991; Lockshon et al., 1995) and the malarial parasite (Preiser et al., 1996) mtDNA replication is directly linked to recombination. It is still unclear whether specific replication origins are present in plant mtDNA, and if so, where they are located. In addition, nothing is known about the mechanism(s) that control mitochondrial genome copy number and that maintain the integrity of genetic information when the majority of mitochondria in plants appear to have less than a full genome equivalent (see section 4).

2.4. Location and timing of plant mtDNA replication

Several studies have documented a gradient of mtDNA levels in plant tissues that declines during plant development (Fujie et al., 1993; Preuten et al., 2010; Woloszynska et al., 2012; Oldenburg et al., 2013). MtDNA synthesis appears to be most active in rapidly dividing meristematic cells found at the root tip (Fujie et al., 1993) and in young cotyledon and leaf cells (Oldenberg and Bendich, 2013), while mtDNA levels in aging and senescent leaves rapidly declines (Preuten et al., 2010). In addition, a steady increase in number of mitochondria and size of cells has been observed in actively growing plant tissues, while mtDNA levels and mitochondrial size decline in aging tissues (Preuten et al., 2010). Mitochondria of rice egg cells contain ten-fold higher amounts of mtDNA as compared to rice root or leaf protoplasts (Takanashi et al., 2010), suggesting that mtDNA replication is very active in eggs. In roots, mtDNA synthesis appears to be limited to regions near the quiescent center at the lower part of the root meristem (Fujie et al., 1993). Outside of this region mtDNA levels fall to lower than stoichiometric levels per mitochondrion, suggesting that in the total plant most mitochondria have less than a full genome equivalent as a result

of mitochondrial division without continued mtDNA replication. This potential problem may be solved by the frequent fusion and fission of mitochondria mentioned above and in section 4.

While the presence of less than a full genome equivalent per mitochondrion would seem problematic, Woloszynska et al. (2012) have provided evidence that levels of the main mitochondrial genome change during plant development and with age and organ type. They propose that in rapidly dividing cells mtDNA replication maintains the full genome, but during the transition to mature and senescing cells DNA recombination may become active, as it is within these nondividing cells that the substoichiometric molecules appear to form in abundance as levels of the full mtDNA decrease (Woloszynska et al., 2012). However, it is unclear what is responsible for maintenance of the mtDNA in most cells while having rapid amplification of mtDNA in the cells of meristematic tissues.

3. Enzymes involved in plant mitochondrial DNA replication

The majority of proposed mechanisms for plant mtDNA replication and maintenance have been derived from comparison to yeast and mammalian mitochondrial mechanisms. In mammals there are three nuclear-encoded enzymes required for mtDNA replication: DNA Polγ (DNA polymerase gamma), Twinkle helicase, and single-stranded DNA binding proteins (McKinney and Oliveira 2013). Lethal mutations occur when either Polγ or Twinkle genes become nonfunctional (Wanrooij et al., 2004). However, similar results are not observed in plants. Following is a discussion of several proteins shown to localize to mitochondria that are likely involved in plant mtDNA replication. The proteins and basic information are listed in Table 1. Not all potential replication proteins are mentioned, and DNA repair mechanisms and repair proteins are not discussed, as they have been covered nicely in a recent review by Gualberto et al. (2013).

3.1. Plant organelle DNA polymerases

Arabidopsis and other plant species encode two nearly identical bacterial-like DNA polymerases that are dual-targeted to mitochondria and plastids termed DNA polymerase IA (PolIA) and IB (PolIB) (Elo et al., 2003; Christensen et al., 2005; Ono et al., 2007; Carrie et al., 2009; Moriyama et al., 2011). Structural analyses of these DNA polymerases indicate that they are distinct from the animal mtDNA polymerase gamma and other animal nuclear DNA polymerases, and that they show greatest phylogenetic relationship with bacterial PolI and other family A DNA polymerases (Ono et al., 2007). The tobacco organelle DNA polymerase has been expressed in bacteria, purified and characterized, and has been shown to have typical bacterial DNA polymerase I activity (Ono et al., 2007). More recently, another group has suggested that the plant organellar DNA polymerases have unique properties when compared to bacterial DNA polymerases and should have their own designation, which they have proposed as “plant organellar polymerases,” or POPs (Moriyama et al., 2011).

Recently Parent et al. (2011) characterized the two DNA polymerase genes as having divergent roles in *Arabidopsis*, and determined that DNA PolIB exhibits enzymatic functions involved in ptDNA repair. They found that allelic mutations in polIB increase

susceptibility to DNA damaging agents and organellar genotoxicity, while mutations in DNA PolIA showed no susceptibility. No altered plant growth phenotype relative to wild-type plants was observed for mutants in DNA PolIA (Parent et al., 2011). They provided little additional data for these mutants, although they suggest that the two DNA polymerases have different roles. In our studies we observed reductions in growth rate and seed production for allelic mutations in the DNA PolIA gene (Cupp and Nielsen, unpublished observations). This suggests that the two DNA polymerases have specific functions in the organelles, or at least that there are temporal and/or spatial expression differences that may be important in plant development.

We have conducted similar analysis with polIB mutants in *Arabidopsis*, and have found that although there are some minor growth phenotypes in seedlings in early stages of plant growth and development, by 7 days post-imbibition there are no differences in either plastid numbers or plastid DNA (ptDNA) copy numbers relative to wild-type plants of the same age. In contrast, there is a 30% reduction in mtDNA copy number and changes in both respiration and photosynthesis in these mutants (Cupp and Nielsen, 2013). While T-DNA allelic insertions significantly reduced expression of the polIB gene, we found that expression of the polIA gene is increased by 70% in the polIB knockout mutant. The ability of polIB mutant plants to maintain mitochondria and develop to maturity and produce viable seeds suggests that the two genes are at least partially redundant. These observations suggest that DNA polIB plays an important role in mtDNA replication and/or maintenance, and when knocked out by mutation, the increased expression of the DNA polIA homologue is unable to fully compensate to replicate mtDNA to wild type levels (Cupp and Nielsen, 2013). In addition to the 30% reduction in relative mtDNA abundance, PolIB homozygous allelic mutants exhibit a significant increase in the number of smaller sized mitochondria per cell. Under the same conditions, no phenotypic differences are observed in chloroplast size or number. These phenotypic differences may suggest that mutations in polIB lead to a reduction in mitochondrial fusion or to an increase in mitochondrial fission.

Some form of signal from the mitochondria to the nucleus may play a role in altering growth and development in the *Arabidopsis* PolIB mutant. This mutant has a reduction in mtDNA levels (but no effect on ptDNA levels) along with changes in mitochondrial numbers and size and alterations in respiration and photosynthesis and starch synthesis (Cupp and Nielsen, 2013). These mutants develop to maturity but have a significant reduction in number of seeds produced, possibly due to alteration in flower development (Cupp and Nielsen, 2013). This suggests that the reduction in mtDNA level, which is the direct effect of the elimination of DNA PolIB expression, may trigger a signal for changes in plant gene expression not only in the mitochondria, but also in chloroplasts and the nucleus. The coordination of mitochondrial structure and development with cellular needs has been discussed in a recent review (Welchen et al. 2014). These authors discuss the potential role of dual targeting of proteins, which includes many of the replication proteins discussed here, in cross-talk between the mitochondria, chloroplast, and nucleus in plants.

Quantitative RT-PCR analysis of RNA recovered from different wild type *Arabidopsis* seedling tissues showed different expression patterns for each DNA polymerase gene. While both are expressed in all tissues, PolIB expression was higher in tissues of higher cell

density and that involve rapid cell expansion including roots, the shoot apex, pistals and flower. In contrast, PolIA is expressed at higher relative levels in rosette leaves (Cupp and Nielsen, 2013).

In maize there are also two dual-localized organelle DNA polymerases, but it appears that the *w*2 DNA polymerase gene is responsible almost completely for ptDNA replication, as mutants in this gene result in a 100-fold reduction in ptDNA copy number and plastid gene expression (Udy et al., 2013). In contrast, these mutants show only a slight reduction in mtDNA levels, and they suggest that the other organellar DNA polymerase may function in mtDNA replication in maize.

This leads to several questions that need to be addressed: Is each DNA polymerase responsible for replication of organelle DNA at different times in plant development or in different tissues? Since DNA PolIB appears to have a greater role in mtDNA maintenance, does DNA PolIA play a greater role in ptDNA replication and maintenance, or does it have some other function? What is the mechanism by which changes in DNA levels in one organelle in the DNA PolI mutant leads to a signal for adjustment(s) in the plant to maintain homeostasis?

3.2. DNA primase or other priming enzyme

Due to the large size and complexity of plant mitochondrial genomes it is likely that priming of mtDNA replication is necessary. An ortholog of the bacteriophage T7 gp4 protein with DNA primase and DNA helicase activity has been identified in *Arabidopsis* (Diray-Arce et al., 2013). In the phage protein the N-terminal domain has the DNA primase activity, followed by a linker region that connects to the C-terminal helicase domain (Shutt and Gray, 2006). Orthologs of this protein are found in all eukaryotes except for fungi, including yeast, and phylogenetic analysis of the protein has been reported by Shutt and Gray (2010). In metazoans the ortholog has been named Twinkle (T7 gp4-like protein with intramitochondrial nucleoid localization), and while it has the entire coding region, numerous amino acid changes have occurred in the primase domain, so this protein has only DNA helicase activity (Shutt and Gray, 2006). In contrast, the *Arabidopsis* gene encodes a protein that retains the conserved amino acids and motifs required for both enzymatic activities (Diray-Arce et al. 2013).

The *Arabidopsis* Twinkle protein is reported to be dual targeted to mitochondria and chloroplasts (Carrie et al., 2013). Currently, no other DNA primase has been characterized that is localized to plant mitochondria. Interestingly, we have analyzed two separate T-DNA homozygous allelic mutation lines and have observed that, unlike in animal mitochondria models, no detrimental effects on plant phenotype occur even though both Twinkle protein levels (western blot analysis) and gene expression levels (RT-qPCR) have been shown to be knocked down (Cupp and Nielsen, unpublished observations).

Arabidopsis has a second gene that is a truncated version of the full-length Twinkle gene. This shorter gene encodes a protein that has retained only the DNA primase domain but lacks the linker and DNA helicase domains that are found in the full Twinkle protein (Diray-Arce et al., 2013). However, western blot analysis using an antibody that recognizes both

proteins indicates that this smaller protein is located in the chloroplast, and activity of this protein has not been shown (Cupp, Arce and Nielsen, unpublished observations). Much like the Twinkle allelic mutations described above we found no significant differences in plant phenotype when the truncated gene is knocked out (Cupp and Nielsen, unpublished observations).

As an alternative to the Twinkle protein, it is possible that one of the nuclear-encoded mitochondria-localized RNA polymerases (Hedtke et al., 2000; Liere et al., 2011; Carrie et al., 2013) may provide the DNA priming activity for mtDNA replication, similar to what has been inferred for animal mtDNA replication (Wanrooij et al., 2008). One of these RNA polymerases is localized only to mitochondria, and the other is localized to mitochondria and chloroplasts (Liere et al., 2011; Carrie et al., 2013). This potential role of mtRNA polymerase in priming mtDNA replication deserves analysis.

3.3. DNA helicase

Replication requires a DNA helicase to unwind the DNA for copying by DNA polymerase. The *Arabidopsis* ortholog of the bacteriophage T7 gp4 protein has both DNA primase and DNA helicase activities (Diray-Arce et al., 2013). Mutations in animal Twinkle have been shown to cause a reduction in mtDNA copy number and have been associated with several mitochondrial-associated diseases in humans and mice. These diseases were determined to be directly linked to the loss of DNA helicase activity (Spelbrink et al., 2001; Tyynismaa et al., 2004; 2005; Wanrooij et al., 2004). As mentioned in the previous section, Twinkle mutant plants have no clear phenotype, indicating that in contrast to the role of Twinkle in animal mitochondria, there is no absolute requirement for this gene in *Arabidopsis*. In humans there is a second DNA helicase, DNA2 nuclease/helicase, localized to the nucleus and mitochondria (Duxin et al., 2009; Zheng et al., 2008). The *Arabidopsis* genome encodes an ortholog of DNA2 (Table 1). However, this protein has not been characterized as to its localization in the plant cell, although plant protein localization prediction algorithms suggest it may localize to mitochondria, chloroplasts and the nucleus. It is possible that DNA2 or another protein may be localized to mitochondria to provide helicase function for mtDNA replication in addition to the Twinkle ortholog. This also requires detailed study.

3.4. Recombinase

As mentioned above, DNA recombination may play a central role in plant mtDNA replication. We have characterized mtDNA recombination in soybean (Manchekar et al., 2006) and turnip (Manchekar et al., 2009). Complex structures indicative of DNA recombination structures have been observed by electron microscopy and two-dimensional agarose gel electrophoresis of mtDNA from both species. MtDNA recombination would require one or more DNA recombinases. Two nuclear-encoded orthologs to bacterial RecA have been characterized in *Arabidopsis*. One is localized to mitochondria while the other is dual-localized to mitochondria and chloroplasts (Khazi et al., 2003; Gualberto et al., 2013). In addition to RecA-mediated recombination it is possible that, under conditions of stress to the plant, Rad51, which normally functions in the nucleus, may be recruited to mitochondria to facilitate mtDNA replication. If so, the plant Rad51 would function similarly to what has

been recently reported in human cells (Sage and Knight, 2013). This is an interesting area that deserves further study.

3.5. Single-stranded DNA binding protein (SSB)

There are at least two types of single-stranded DNA binding proteins (SSB) that appear to be involved in mtDNA replication, recombination and/or repair (Edmondson et al., 2005; Zaegel et al., 2006). One (SSB1) is very similar to bacterial SSB, which coats unwound DNA at the replication fork to prevent reannealing during the replication process. This protein has been shown to stimulate the activity of RecA (Edmondson et al., 2005), and has recently been shown to be dual-targeted to mitochondria and chloroplasts (Moore and Nielsen, unpublished data). The second class of proteins has been termed organellar single-stranded DNA binding proteins (OSB) and are distinct in function from typical bacterial SSB proteins. These proteins are unique to plant organelles (Zaegel et al., 2006). Mutants in mitochondrial-localized OSB1 and OSB4 exhibit an accumulation of aberrant mtDNA sequences that result from ectopic recombination (Zaegel et al., 2006; Gualberto et al., 2013). OSB1 is suggested to play a role in recombination surveillance to prevent transmission of aberrant mtDNAs to new mitochondria (Zaegel et al., 2006).

3.6. Topoisomerases

Topoisomerases are required for relieving tension induced in double-stranded DNA ahead of and behind the replication forks. Dual-localized topoisomerases (type I and II) have been characterized in *Arabidopsis* (Wall et al., 2004; Carrie et al., 2009). A type II topoisomerase with bacterial-like DNA gyrase activity was characterized, and T-DNA insertion mutants for this gene were shown to have an embryo lethal phenotype (Wall et al., 2004). More research in this area is warranted to determine the number and types of DNA topoisomerases that function in plant mtDNA replication.

4. Plant mitochondrial fission/fusion and segregation of mtDNA

It has been clearly shown that some mitochondria can be heteroplasmic and contain less than a full genome equivalent in plants (on an average per organelle basis) (Satoh et al., 1993; Kanazawa et al., 1994; Kmiec et al., 2006; Preuten et al., 2010). This is likely affected by frequent fission and fusion of plant mitochondria (Arimura et al., 2004). For example, long filamentous mitochondria may represent multiple mitochondria fused together or mitochondria that have failed to divide. Mixing of mtDNA from fragmented mitochondrial genomes most likely occurs within these filamentous organelles, and may provide a compartment where genes encoded within the mixed mtDNA samples can be combined to form a complete genome for mtDNA replication. These compartments may also serve as a location for selective amplification of genes required during different stages of tissue development or during a higher cellular demand for energy (Muise and Hauswirth, 1995; Woloszynska et al., 2006). These observations raise the question as to where plant mtDNA is replicated, when mtDNA is replicated, and what mechanisms influence or control mtDNA replication.

Mitochondrial fusion dynamics play an important role in inheritance and maintenance of mtDNA (Westermann 2010). For example, yeast cell mitochondria that have lost their ability to undergo fusion show a rapid loss of mtDNA and in addition to corresponding defects in cellular respiration. Fusion defective mitochondria in mammalian cells also lead to mitochondrial dysfunction. On the other hand, cells that are defective in mitochondrial fission accumulate large mitochondrial networks in one part of the cell, leaving other parts of the cells without functional mitochondria (Westermann, 2010).

MtDNA replication is not directly linked to the plant cell cycle, and mtDNA copy numbers can vary widely depending on the tissue and stage of development (Preuten et al., 2010). However, the structure and organization of the mitochondrial genome appears to vary during the cell cycle. A mitochondrial cage-like structure surrounding the cell nucleus has been observed within the meristem regions of plants and in animal stem cells (Segui-Simarro et al., 2008; Antico Arciuch et al., 2012). This centralized structure is maintained throughout the cell cycle and has been observed to divide with the cell upon anaphase and cytokinesis. In preparation for cell division this large cage-like mitochondrion divides into two structures, after which fission leads to the generation of small mitochondria (Segui-Simarro et al., 2008). It has been proposed that this centralized mitochondrion is a location where the majority of the mitochondrial genome is replicated and maintained within the shoot apical meristem (Logan, 2010). A centralized mitochondrion that encages the nucleus would provide an optimal location and distance for nuclear encoded gene products to localize into the mitochondria after translation in the cytoplasm. It has been proposed that the bulk of mtDNA replication occurs in the large, fused mitochondria, and that recombination is more active in the smaller organelles (Woloszynska et al., 2012).

Peripheral mitochondria have been observed to bud out from this centralized mitochondrion structure and re-fuse at specific stages within the cell cycle (Segui-Simarro et al., 2008). Presumably, mtDNA molecules of various copy number and structural complexity segregate into these smaller peripheral mitochondria upon fission, and it is possible that this may be the reason that some mitochondria have less than a full genome equivalent (Preuten et al. 2010). There is also evidence that mitochondrial gene copy numbers may vary in different tissues, while overall the genome integrity is maintained from individual to individual (Logan, 2006; Preuten et al. 2010).

5. Summary and Future Perspectives

Plant mtDNA replication appears to be most active in rapidly dividing cells in meristematic tissues, and thus is correlated with plant development but apparently not in direct relation with energy needs of the plant (Preuten et al., 2010). The complexity of plant mitochondrial genomes, including the presence of substoichiometric DNA molecules in some mitochondria, has made the characterization of mtDNA replication difficult. The main question that remains is the specific mechanism(s) by which plant mtDNA is replicated, including the presence and location of potential replication origins. It is possible that DNA recombination may play a major role in copying and maintaining the mtDNA, but it is also quite possible that mtDNA replicates by more than one mechanism in plants. The study of plant lines with mutations in one or more replication protein genes, and their effect on

mtDNA rearrangements and recombination structures observed by electron microscopy and other techniques may help in understanding the mechanism of plant mtDNA replication.

Complicating our understanding of the role of replication proteins in mtDNA replication is the apparent presence of two related proteins for each essential function in *Arabidopsis* and other plants. There are two DNA polymerases, two potential DNA helicases, two mechanisms for priming replication, at least two recombinase proteins and more than two SSB proteins. This may provide back-up activity for many or all of the replication protein activities, in contrast to the situation in animals. The generation of double mutants will likely be required to analyze the roles of each potential replication protein in plant mtDNA replication. Much remains to be learned about the unique aspects of replication of plant mitochondrial genomes.

Acknowledgments

Support for plant mtDNA replication research in the Nielsen laboratory has been provided by the U.S. National Institutes of Health and by Mentoring Environment Grants from Brigham Young University. We appreciate the contributions of several undergraduate students who have worked on various *Arabidopsis* DNA replication protein mutants in the Nielsen lab.

Abbreviations

mtDNA	mitochondrial DNA
Polγ	nuclear-encoded mitochondrial DNA polymerase gamma
D-loop	displacement loop

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Highlights

- Plant mitochondrial genomes replicate by a unique mechanism(s).
- All plant mtDNA replication proteins are nuclear-encoded.
- Many of these proteins are dual-localized to mitochondria and chloroplasts.
- There appears to be partial redundancy for some replication protein functions.
- Mutations in some replication proteins have complex effects on plant development.

Table 1

Nuclear-encoded (putative) mitochondrial DNA replication proteins in *Arabidopsis thaliana*

Function	Protein name	Arabidopsis gene designation	Closest homology[2]	Localization[3]	References
DNA polymerase	PolIA or Pol gamma 2[1]	At1g50840	Bacterial	M, P	Elo et al., 2003 Ono et al., 2007 Carrie et al., 2009
	PolIB or Pol gamma 1[1]	At3g20540	Bacterial	M, P	Elo et al., 2003 Ono et al., 2007 Carrie et al., 2009
Helicase	Twinkle	At1g30680	Phage	M, P	Diray-Arce et al., 2013 Shutt and Gray, 2006
	DNA 2?	At1g08840	Mammalian	?	Duxin et al., 2006 Zheng et al., 2008
Priming	Twinkle	At1g30680	Phage	M, P	Diray-Arce et al., 2013 Shutt and Gray, 2006
	RNA polymerase	At5g15700	Phage	M, P	Liere et al., 2011 Carrie et al., 2013
Single-strand DNA binding	SSB1	At4g11060	Bacterial	M, P	Edmondson et al., 2005 Moore and Nielsen, unpublished data
	OSB1	At3g18580	Bacterial-like, but unique to plants	M	Zaegel et al., 2006
	OSB3	At5g44785	Unique to plants	M, P	Zaegel et al., 2006 Carrie et al., 2013
	OSB4	At1g31010	Unique to plants	M	Zaegel et al., 2006
Recombination	RecA2	At2g19490	Bacterial	M, P	Shedge et al., 2007
	RecA3	At3g10140	Bacterial	M	Khazi et al., 2003 Shedge et al., 2007
Topoisomerase	Topoisomerase I	At4g31210	Bacterial	M, P	Carrie et al., 2013
	DNA gyrase	At3g10690	Bacterial	M, P	Wall et al., 2004
DNA ligase	LIG1	At1g08130	Bacterial	M, N	Gualberto et al., 2013

[1]These proteins have been given two separate names, first based on similarity to animal DNA polymerase gamma, but with further phylogenetic analysis the PolIA and PolIB designations are now generally accepted (Parent et al., 2011).

[2]Closest homology based on published phylogenetic analysis according to the references listed.

[3]Localization is indicated to mitochondria (M), plastid (P), nucleus (N), or unknown (?).