

Genes and Junk in Plant Mitochondria—Repair Mechanisms and Selection

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Abstract

Plant mitochondrial genomes have very low mutation rates. In contrast, they also rearrange and expand frequently. This is easily understood if DNA repair in genes is accomplished by accurate mechanisms, whereas less accurate mechanisms including nonhomologous end joining or break-induced replication are used in nongenes. An important question is how different mechanisms of repair predominate in coding and noncoding DNA, although one possible mechanism is transcription-coupled repair (TCR). This work tests the predictions of TCR and finds no support for it. Examination of the mutation spectra and rates in genes and junk reveals what DNA repair mechanisms are available to plant mitochondria, and what selective forces act on the repair products. A model is proposed that mismatches and other DNA damages are repaired by converting them into double-strand breaks (DSBs). These can then be repaired by any of the DSB repair mechanisms, both accurate and inaccurate. Natural selection will eliminate coding regions repaired by inaccurate mechanisms, accounting for the low mutation rates in genes, whereas mutations, rearrangements, and expansions generated by inaccurate repair in noncoding regions will persist. Support for this model includes the structure of the mitochondrial *mutS* homolog in plants, which is fused to a double-strand endonuclease. The model proposes that plant mitochondria do not distinguish a damaged or mismatched DNA strand from the undamaged strand, they simply cut both strands and perform homology-based DSB repair. This plant-specific strategy for protecting future generations from mitochondrial DNA damage has the side effect of genome expansions and rearrangements.

Key words: mitochondrial genome, mutation rate, DNA repair, junk DNA.

Introduction

Plant mitochondrial genomes have followed different evolutionary trajectories from their counterparts in animals and fungi. The genomes are very large (up to 11 Mb) but still have only 30–60 genes, thus most of the DNA is noncoding. The mutation rate measured in protein-coding regions and rRNA regions is very low, but the genomes are subject to major rearrangements and expansions (Palmer and Herbon 1988). The mutational burden hypothesis was proposed as an explanation for the paradox of low mutation rates and high expansion rates (Lynch et al. 2006; Lynch 2007), but exceptional species with both high mutation rates and high expansion rates have been found that defy this explanation (Cho et al. 2004; Parkinson et al. 2005; Sloan, Muller, et al. 2012; Sloan et al. 2012). After comparing the mitochondrial noncoding sequences of two *Arabidopsis thaliana* ecotypes that had been diverged for approximately 200,000 years, I proposed that coding and noncoding DNAs are repaired by

different mechanisms and thus have different mutation rates and spectra (Christensen 2013). Although coding regions are highly conserved, noncoding DNA has diverged so rapidly that over 200 kb of the *A. thaliana* mitochondrial genome is not alignable with any sequences outside the Brassicales family of plants, suggesting that it is nonfunctional junk (Brenner 1998; Christensen 2013). This also explains why noncoding DNA has not previously been used in mutational or phylogenetic studies—it evolves too quickly to be useful over evolutionary time scales. The model proposes that coding regions are repaired very accurately, likely by homologous recombination or gene conversion. Noncoding regions are repaired by inaccurate mechanisms of double-strand break (DSB) repair that produce rearrangements, chimeric genes, and genome expansion (Davila et al. 2011). Because there is no mechanism available for precisely removing junk DNA, it accumulates by Muller's ratchet (Muller 1964). The common feature in both coding

and noncoding DNA is DSB repair, leading either to homology-based accurate repair or to inaccurate repair with duplications expanding the genome.

Although this model explains the observed features of mitochondrial genomes, how the coding and noncoding DNA have such distinctly different mutation rates and spectra is still a mystery. One possible explanation is that the primary mechanisms of DNA repair are different in genes and in junk, and the only plausible mechanism for this is transcription-coupled repair (TCR) (Ganesan et al. 2012; Vermeulen and Fouteri 2013; Howan et al. 2014). The existence of cotranscribed genes in plant mitochondria provides an opportunity to test this hypothesis. In this work, I find the hypothesis of TCR to be unlikely and suggest a model for how mitochondrial genomes are repaired differently in genes and in junk.

Results

The hypothesis of TCR can be tested by examining both coding and noncoding transcribed regions, for example, the protein-coding regions and intergenic regions of cotranscribed genes. The model predicts that the mutation rate in the coding regions should be equal to the mutation rate in the intergenic regions. In *A. thaliana*, there are four gene clusters shown to be cotranscribed: *nad4L-atp4*, *rpl5-cob*, *nad3-rps12*, and *rps3-rpl16* (Hoffmann et al. 1999; Forner et al. 2007) and these same clusters are observed in a wide variety of angiosperms (Richardson et al. 2013). The lengths of the intergenic regions in these transcripts in *A. thaliana* are 266 bp, 1.9 kbp, 45 bp, and 0 bp (*rps3* and *rpl16* overlap by 134 bp), respectively (Davila et al. 2011). Because selection might be acting near the translation start and stop sites, the two larger intergenic regions are most suitable as a test of the hypothesis. In several species including *A. thaliana*, there is an *rps14* pseudogene between *rpl5* and *cob* (Aubert et al. 1992; Quinones et al. 1996; Figueroa et al. 1999; Ong and Palmer 2006). Because in some species *rps14* is a functional gene and in others it is a pseudogene in the intergenic region, several species were chosen for analysis all of which have a functional *rps14* gene. The *rpl5* and *rps14* genes are just a few nucleotides apart, so only the *rps14-cob* intergenic region was used. The species chosen were all legumes with completely sequenced mitochondrial genomes containing single copies of the *nad4L-atp4* and *rpl5-rps14-cob* clusters. Four legumes were chosen: The mung bean (*Vigna radiata*), the azuki bean (*Vigna angularis*), the pongam tree (*Millettia pinnata*), and the fava bean (*Vicia faba*). *Carica papaya* was chosen as outgroup (fig. 1).

The five coding regions, *nad4L*, *atp4*, *rpl5*, *rps14*, and *cob*, were aligned (supplementary fig. S1, Supplementary Material online), and the synonymous substitutions per synonymous site were measured using the concatenation of all five. The genes of plant mitochondria also show extensive RNA editing (Barkan and Small 2014). The edited sites were confirmed and

annotated in the *M. pinnata* genome (Kazakoff et al. 2012). All of these are C to U edits in the mRNA and most change the amino acid encoded. This alters the definitions of synonymous and nonsynonymous sites for two reasons. If an edit of a C to a U in the mRNA changes the amino acid codon, then a mutation in the genome at that site from a C to a T will be a synonymous change, but standard methods will count that position as a nonsynonymous site. Several examples are in this data set. Of the 48 edited cytosines in these 5 genes, all are conserved within the legumes, but 12 of those edited sites have mutated to T in *C. papaya* (see supplementary fig. S1, Supplementary Material online). Of those sites, 11 would be classified as nonsynonymous substitutions, but the editing in the legumes means that the differences in *C. papaya* are actually synonymous substitutions. Furthermore, the pentatricopeptide repeat (PPR) proteins that mediate editing recognize the RNA sequence upstream of the edit (Barkan and Small 2014), so changes in these positions will all be nonsynonymous if they affect editing efficiency, even if the amino acid sequence at the site of the mutation does not change. For this reason, the analysis was done twice: Once using the entire coding regions and again with any edited codons and the six preceding codons removed from the alignment. The intergenic regions between *nad4L* and *atp4* and between *rps14* and *cob* were also aligned (supplementary fig. S2, Supplementary Material online), and the mutation rate was determined using the concatenation of both alignments, including both transitions and transversions, but not indels. The rates are shown in table 1 and graphed in figure 2. The substitution rate in the intergenic region is higher than the synonymous substitution rate in the complete coding sequences. When edited sites are removed, the substitution rate in the intergenic sequence is still higher than in coding sequences but not statistically significant in most cases.

However, the substitution rate is only a small part of the story. The alignments also reveal frequent nucleotide losses and gains in the intergenic regions (particularly just upstream of *cob*). The intergenic regions have mutated much more extensively than the coding regions when indels are taken into account. As shown previously, most of the intergenic regions in plant mitochondria cannot even be aligned except between very closely related species (Christensen 2013). Without the flanking coding regions of *rps14* and *cob*, the intergenic region between them cannot be accurately aligned using these five species.

If TCR is the mechanism of repair in plant mitochondria, then the mutation rate in a transcribed intergenic region should be the same as the neutral mutation rate measured by synonymous substitutions in the coding regions of the same transcripts, and the frequency of indels in the intergenic regions should be low, as in the coding regions. Indels in the coding regions are rare and are always in-frame, whereas in the intergenic regions, there are more indels per nucleotide; therefore, the hypothesis of TCR is most likely incorrect.

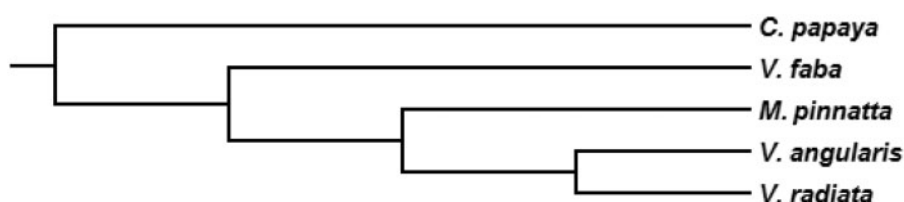


Fig. 1.—Phylogenetic relationships of the species studied. Tree showing the relationships between the four legumes used in this study and the outgroup *Carica papaya*. Based on Soltis et al. (2011).

Table 1
Mutation Rates in Coding and Intergenic Regions

Species 1	Species 2	CDS	CDS – edits	Intergenic
<i>Carica papaya</i>	<i>Vicia faba</i>	0.0735 ± 0.0107	0.0826 ± 0.0135	0.1009 ± 0.0092
<i>C. papaya</i>	<i>Millettia pinnata</i>	0.0570 ± 0.0093	0.0636 ± 0.0114	0.0873 ± 0.0095
<i>C. papaya</i>	<i>Vigna angularis</i>	0.0543 ± 0.0084	0.0677 ± 0.0117	0.0860 ± 0.0091
<i>C. papaya</i>	<i>V. radiata</i>	0.0543 ± 0.0085	0.0677 ± 0.0117	0.0896 ± 0.0094
<i>Vic. faba</i>	<i>M. pinnata</i>	0.0285 ± 0.0064	0.0280 ± 0.0080	0.0411 ± 0.0064
<i>Vic. faba</i>	<i>V. angularis</i>	0.0313 ± 0.0074	0.0411 ± 0.0097	0.0477 ± 0.0075
<i>Vic. faba</i>	<i>V. radiata</i>	0.0323 ± 0.0074	0.0424 ± 0.0097	0.0491 ± 0.0078
<i>M. pinnata</i>	<i>V. angularis</i>	0.0128 ± 0.0041	0.0189 ± 0.0063	0.0209 ± 0.0040
<i>M. pinnata</i>	<i>V. radiata</i>	0.0128 ± 0.0041	0.0189 ± 0.0063	0.0264 ± 0.0054
<i>V. angularis</i>	<i>V. radiata</i>	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0102 ± 0.0032

NOTE.—Synonymous substitution rates in the coding sequences (CDS), coding sequences with edited regions removed (CDS – edits), and intergenic regions are shown (\pm standard errors). Analyses were conducted using the Kumar model (Nei and Kumar 2000). The analysis involved 5 nt sequences. All positions containing gaps and missing data were eliminated. There were a total of 967 positions in the CDS data set, 712 positions in the CDS – edits data set, and 1,620 positions in the intergenic data set. Of these positions in the CDS data set, there were 51 variants within the 4 legumes, including 20 synonymous substitutions, 26 nonsynonymous substitutions, and 5 in-frame indels.

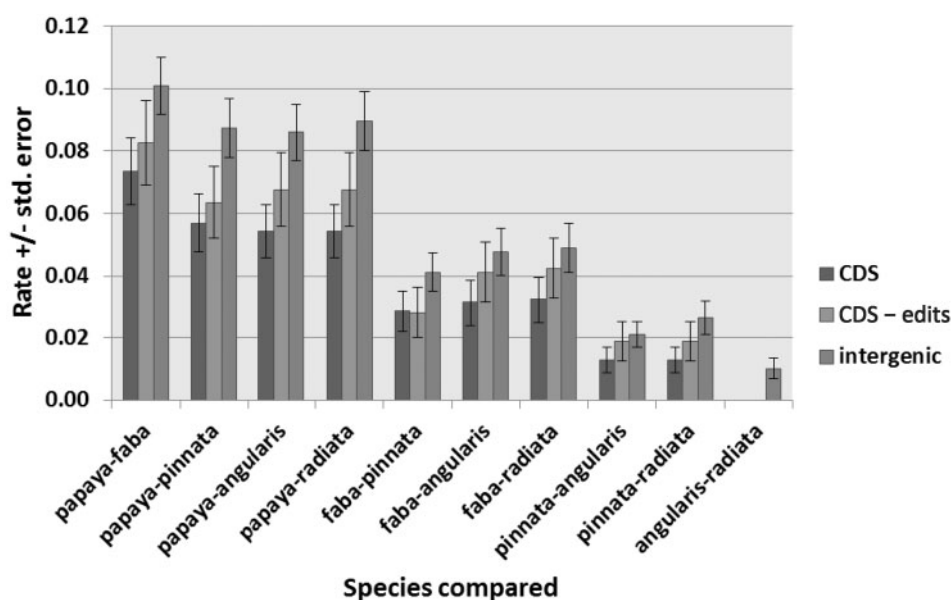


Fig. 2.—Mutation rates in coding regions (CDS) and noncoding regions. Synonymous substitution rates in the CDS of *nad4L*, *atp4*, *rpl5*, *rps14*, and *cob* and the coding regions without the edited regions (CDS – edits) were calculated as described in the text. Substitution rates in the intergenic regions between *nad4L* and *atp4* and between *rps14* and *cob* were also calculated as described in the text. Standard errors are shown.

Discussion

How do plant mitochondrial genomes and their repair systems produce genes with very low synonymous substitution rates, but intergenic regions with high substitution, indel, genome expansion, and rearrangement rates? One possibility was a different DNA repair pathway in genes and in junk, but the only plausible mechanism is TCR, which can be ruled out. The explanation must therefore be a combination of the available DNA repair pathways and selection on the DNA postrepair. Plant mitochondria have a short-patch base-excision repair system, at least for removal of uracil (Boesch et al. 2009), but there is no evidence for long-patch base-excision repair or nucleotide-excision repair (Gualberto et al. 2013). Genome evolution and the rearrangements seen in mutants suggest that DSB repair is an important process in plant mitochondria (Shedge et al. 2007; Arrieta-Montiel et al. 2009; Davila et al. 2011; Janicka et al. 2012; Miller-Messmer et al. 2012; Christensen 2013). DSB repair has multiple modalities that can produce either very accurate or inaccurate repair. One pathway, break-induced replication (BIR), can also result in large duplications, particularly if the break invades another DNA molecule at a homeologous site (Llorente et al. 2008; Cappadocia et al. 2010).

Other than short-patch base-excision repair, little is known about DNA repair proteins in mitochondria, except for the MSH1 protein, a mitochondrially targeted homolog of mismatch repair proteins. It has been suggested that the MSH1 protein plays a role in homology surveillance during DSB repair (Abdelnoor et al. 2003; Shedge et al. 2007; Arrieta-Montiel et al. 2009; Davila et al. 2011). Nuclear and bacterial mismatch repair systems include a strand-discrimination mechanism that directs endonuclease cleavage and repair to the newly synthesized DNA strand (Kunkel and Erie 2005; Ghodgaonkar et al. 2013). Homologs of the strand-discrimination components have not been identified in plant organelles; however, the MSH1 protein of higher plants is fused directly to an endonuclease domain (Abdelnoor et al. 2006). Sequence comparisons and modeling showed that the endonuclease domain is similar to the GIY-YIG homing endonuclease I-Tev1, which makes DSBs as a monomer (Mueller et al. 1995; Kleinstiver et al. 2013). This suggests a model for DNA repair in plant mitochondria of lesion recognition followed by double-strand breakage, catalyzed by MSH1 and other unknown nucleases. A DSB eliminates the need for a strand-discrimination system but requires a template.

If DNA damage (other than what can be repaired by short-patch base-excision repair, such as deaminated cytosine) is converted into DSBs, and these breaks are then processed by DSB repair mechanisms, there are a number of possible outcomes. Alternative pathways for processing the DSB will depend on whether a template molecule is available and whether the second broken end is captured by the repair event. If the two DNA ends are coordinated, nonhomologous

end joining can be very accurate, but otherwise it can lead to chimeric gene formation and duplications. BIR at a homologous region may lead to large duplications and can also shift the stoichiometry of different parts of the genome. BIR at a short region of homology (such as the 50–500 bp repeats) will lead to rearrangements and genome expansion; BIR at microhomologies of a few nucleotides can also produce chimeric genes. Homologous recombination or gene conversion will accurately repair the DSBs. The question still remains of how coding sequences are repaired so accurately while the noncoding regions experience rapid change.

The most likely explanation is that both types of DSB repair occur in all parts of the genome, but selection determines which outcomes we can observe (fig. 3). DSB repair can occur in either coding or noncoding DNA and can either be accurate or inaccurate. In noncoding DNA, accurate repair presumably occurs but is impossible to observe in alignments. Inaccurate repair leads to expansions, mutations, and rearrangements, which are observed. In coding DNA, mitochondria with inaccurately repaired essential genes may be eliminated from the cell, or not inherited, thus what we observe in coding DNA is repair that maintains gene function, explaining the low synonymous substitution and indel rate. Accurate, homology-based repair such as gene conversion can explain the observations in coding sequences. If a template is not available within a mitochondrion, mitochondrial fusion could occur to make a template DNA molecule available. This model, that most DNA repair is mediated via generating DSBs followed by the DSB repair pathways and selection for functional mitochondria within a cell, can explain the evolution of plant mitochondrial genomes.

An interesting additional question is why natural selection has favored this mechanism of DNA repair in plant mitochondria but not in animal mitochondria or the nucleus. Recent work showed that in animals the female germline sequesters a subset of mitochondria that are relatively inactive in producing reactive oxygen species and other DNA damaging agents, to minimize transmission of mitochondrial mutations (de Paula et al. 2013). Both plants and animals need to avoid the inheritance of accumulated mitochondrial mutations and appear to use different mechanisms to accomplish that. Plants do not have the luxury of specifying a germline, so converting damage into DSBs followed by accurate template-directed repair ensures that the genes will be faithfully inherited. The side effect of using DSB repair for nearly every type of damage is genome expansion and accumulation of chimeric genes, but the benefit of accurate transmission of mitochondrial genes to the next generation must outweigh the relatively minor cost of replicating a large mitochondrial genome. Finally, the mutational burden hypothesis does not appear to apply to plant mitochondria. In addition to mutations in the junk DNA apparently being mostly neutral, the specific repair mechanisms available do not lead to an inverse correlation between mutation rate and genome size. This model further

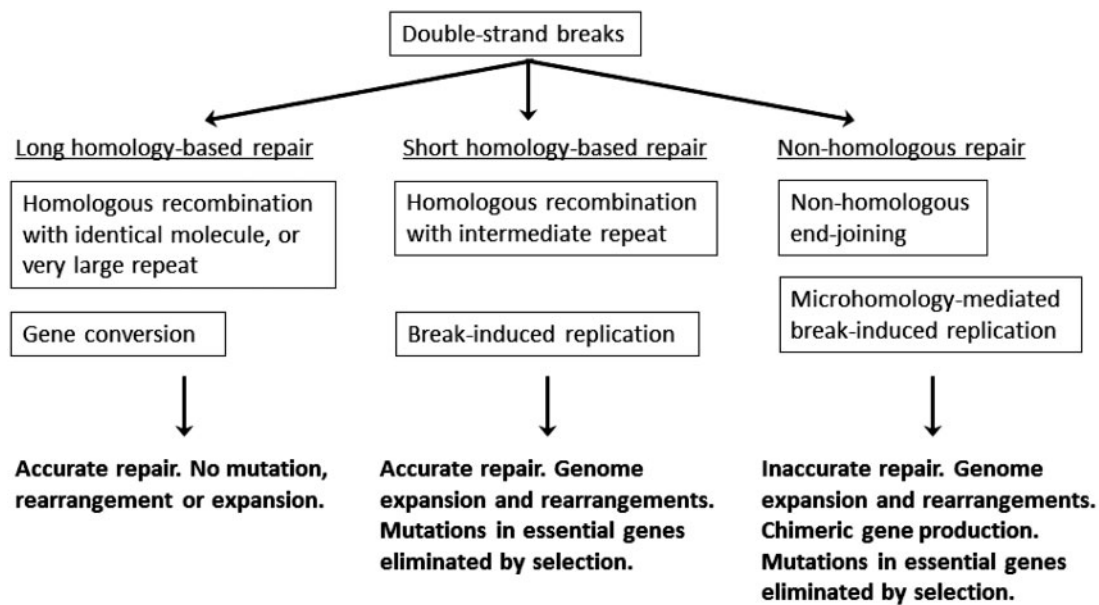


FIG. 3.—Model for mitochondrial DNA repair explaining differences between genes and junk. The diagram shows the fate of DSBs. These can be repaired by nonhomologous or template-based repair, and a template can either be a sister DNA molecule or be a short stretch of identity in a different context in the same or a different DNA molecule.

predicts that if mechanisms such as base-excision repair or mismatch repair are less effective or transiently lost in a lineage, DSB repair will produce genome expansions at the same time as base substitution rates increase. This also predicts a loss of editing sites and can explain the counterintuitive positive correlation between mutation rates and genome expansions in plant mitochondria.

Materials and Methods

Complete mitochondrial genome sequences used were accessions KC189947 for *V. faba* (Negruk 2013), JN872550 for *M. pinnata* (Kazakoff et al. 2012), AP012599 for *V. angularis* (Naito et al. 2013), HM367685 for *V. radiata* (Alverson et al. 2011), and EU431224 for *C. papaya* (Ming et al. 2008). Sequence manipulation to extract the specific genes and intergenic regions studied was done using the VectorNTI 11.5.0 package from Invitrogen.

Alignments were done using MUSCLE (Edgar 2004) as implemented in MEGA6 (Tamura et al. 2013). Alignments were prepared for figures using Jalview (Waterhouse et al. 2009). Synonymous substitution rates and standard error estimates were calculated by MEGA6, using the Kumar model (Nei and Kumar 2000) with all ambiguous positions removed for each sequence pair. Substitution rates in noncoding regions were calculated by MEGA6, using Kimura's two-parameter model (Kimura 1980), including both transitions and transversions, with all ambiguous positions removed for each sequence pair.

Supplementary Material

Supplementary figures S1 and S2 are available at *Genome Biology and Evolution* online (<http://www.gbe.oxfordjournals.org>).

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