Chloroplast DNAs Diversify Nuclear and Mitochondrial Genomes in Plants

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Abstract

Mitochondria and chloroplasts in eukaryotic cells originated more than a billion years ago when an ancestor of the nucleated cell engulfed two different prokaryotes in separate sequential events. Extant cytoplasmic organellar genomes contain very few genes compared with their candidate free-living ancestors, as most have functionally relocated to the nucleus. The first step in functional relocation involves the integration of cytoplasmic organllar DNA fragments into nuclear chromosomes and this process continues at high frequency with attendant genetic, genomic and evolutionary consequences. The frequency of DNA transposition from plastid (chloroplast) to nucleus has been measured experimentally in tobacco plants (*Nicotiana tabacum*) growing in ideal growth conditions.

To monitor the effects of environmental stress on the rate of DNA transfer from plastid to nucleus, two different transplastomic tobacco lines were used and it was shown that DNA migration from chloroplasts to the nucleus was markedly increased by mild heat stress. In addition, manually induced DNA double-strand breaks (DSBs) were made using the rare-cutting endonuclease I-SceI in tobacco and Arabidopsis and this system was used to investigate the role of DSBs repair during organellar DNA insertion into the nuclear genome. Integrants of none organelle DNA origin were found at the break points when plants grown at normal temperature. In contrast, insertions of mitochondrial DNA fragments occurred during the repair of induced DSBs were only observed in tobacco when plants were heat treated. This finding suggested that the frequency of mitochondrial DNA migration was also increased by mild heat stress.

To further investigate whether the DSB repair is involved in plastid DNA integration into the nuclear genome, 14 nuclear insertions of chloroplast DNA (*nupts*) that are unique to *Oryza sativa* subsp. *indica* were indentified. Comparisons with the nuclear pre-insertion loci (identified in the related subspecies, *O. sativa* subsp. *Japonica* which lacked these *nupts*) indicated that chloroplast DNA had integrated by non-homologous end joining. Combined with analyzing available DNase-seq data, this analysis also revealed that *nupts* were significantly more

frequent in open chromatin regions of the nucleus. The generality of this insertion site preference was tested in the chimpanzee genome by comparing nuclear loci containing integrants of mitochondrial DNA (*numts*) with *numt*-lacking preinsertion sites in the human genome. Mitochondrial DNAs also tended to insert more frequently into regions of open chromatin revealed by human DNase-seq and FAIRE-seq databases.

Chloroplast DNA movement is not limited to the nucleus and it is also found within the mitochondrial genome in most plants. However, the functions of these plastid-derived DNA tracts in mitochondrial genomes (also called *mtpt* for *mit*ochondrial *p*las*t*id DNA) have been considered to be limited to rare instances where plastid tRNA genes have replaced their mitochondrial counterparts, where short patches of mitochondrial genes evolved using their homologous plastidic copies by gene conversion, or where a new promoter region is created. In this thesis it is demonstrated that some *mtpts* contribute codons to unrelated mitochondrial protein-coding sequences and others may have a role in post-transcriptional RNA processing.

Declaration

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List of Publications

Wang, D., Lloyd, A.H. and Timmis, J.N. (2012) Environmental stress increases the entry of cytoplasmic organellar DNA into the nucleus in plants. *Proc Natl Acad Sci USA* **109**, 2444-244.

Wang, D., Lloyd, A.H. and Timmis, J.N. (2012) Nuclear genome diversity in somatic cells is accelerated by environmental stress. *Plant Signal Behav* **7**, 595-597.

Lloyd, A.H., Wang, D. and Timmis, J.N. (2012) Single molecule PCR reveals similar patterns of non-homologous DSB repair in tobacco and Arabidopsis. *PLoS One*, **7**, e32255.

Wang, D., Rousseau-Gueutin, M. and Timmis, J.N. (2012) Plastid sequences contribute to some plant mitochondrial genes. *Mol Biol Evol* **29**, 1707-1711.

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