EXTENDED ABSTRACT

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Mitochondrial DNA Recombination, Repair and Segregation: Recent Scientific Data and Perspectives

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Abstract

Large deletions in the mitochondrial DNA (mtDNA) are the cause of dramatic neurodegenerative diseases in humans. Error-prone recombination-mediated repair has been proposed to be the main cause of deletion. Recent investigations indeed brought evidence for mtDNA recombination intermediates and active recombination in heart and brain mitochondria, as well as for microhomology-mediated end joining repair of mtDNA. These pathways are still poorly characterized and identification of recombination factors in mammalian mitochondria remains scarce. Recombination has been more extensively documented in plant mitochondria, where repeat-mediated homologous recombination generates subgenomes and alternative mtDNA configurations. These mechanisms are supported and tightly controlled by a series of nuclear-encoded and mitochondrially imported factors of prokaryotic origin that seem to be absent from mammalian organelles. Notably, knockdown of these factors in plant mutants promotes error-prone repair of the mtDNA. Mutated mtDNA copies in mammalian mitochondria can become preferentially amplified and segregate, leading to clonal expansion in tissues, organs or following generations. Similarly, minor alternative mtDNA configurations in plants can also segregate from the heteroplasmic state and become predominant. Knowledge gained in plants and introduction of plant factors into mammalian model systems might thus shed new light into the field.

List of abbreviations: *bp: base-pair; kb: kilo base pair; MMEJ: Microhomology-mediated end joining; mtDNA: Mitochondrial DNA; SSA: Single-strand annealing*

Introduction

Dynamics, maintenance and transmission of the mitochondrial DNA (mtDNA) are at the forefront of organellar genetics. These issues are of primary importance, as mutations and rearrangements in the mtDNA result in multiple, severe and incurable neurodegenerative diseases (1). Recombination and break repair pathways play a major role in these processes, but have mostly been documented at the genetic and molecular level in yeast and plant mitochondria (2,3). Conversely, the occurrence of recombination in mammalian mitochondria has been a matter of debate, although experimental evidence has been brought at least for some tissues (4,5). On the other hand, pathogenic mutations are usually heteroplasmic, but mutated mtDNA copies can undergo preferential amplification and/or segregation, leading to clonal expansion. We emphasize here the importance of understanding mitochondrial recombination and repair mechanisms to progress towards treating mtDNA diseases.

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The human mitochondrial genome is complex and dynamic

The human mitochondrial genome is usually presented as a compact circular DNA of 16.5 kb, with a single, short non-coding region carrying the promoters for transcription. It has no significant intergenic regions and the genes do not carry introns. Two origins of replication have been described, with a leading strand/lagging strand replication mechanism. Deeper analysis showed that, at least in tissues like heart or brain, the human mtDNA actually displays complex configurations, including circles, dimeric and oligomeric molecules, catenated networks, branched structures, tangled aggregates (5).

Human mitochondrial genome deletions and recombination

Over 300 pathogenic point mutations have been described, causing incurable neurodegenerative diseases, but further diseases result from large (1-10 kb) mtDNA deletions (6). Over 100 different pathogenic deletions have been indexed and most of them (~85%) are flanked by short direct repeats (from a few to over 10 bp), which suggests that they might result from mtDNA repair involving recombination. Prominent four-way junctions, recombination intermediates and illegitimate recombination products, called sublimons, have indeed been detected while analyzing human mtDNA (4,5). Recombination appears to proceed in mitochondria of oxyradical-rich tissues like heart, brain, and, to a lower extent, skeletal muscle. Oxidative damage can generate double-strand breaks, which are substrates for recombination repair. Oxidatively modified DNA is also prone to mispairing of repetitive elements during recombination. There seems to be a correlation of mtDNA deletions with recombination and repair, hence the importance of characterizing mitochondrial recombination and repair pathways and the factors involved. Plants are a good model for such studies, as recombination is specially active in plant mitochondria.

The dynamic plant mitochondrial genome

Plant mitochondrial genomes are much larger, mostly in the range of 200-700 kb, with a very low gene density and large intergenic regions, but they show a mixture of circular, linear, branched, and rosette-like forms resembling those of mammalian mtDNA (3,5,7). Plant mtDNA usually carries several large repeats (>500 bp) involved in frequent homologous recombination, which results in a dynamic structure distributed into sub-genomic forms. Mitochondrial genomes in plants also include numerous intermediate size repeats (100-500 bp) that recombine at low frequency. This is due to a tight surveillance by a series of nuclear-encoded and mitochondrially imported factors that inhibit ectopic recombination involving intermediate size repeats in normal growth conditions (3). On the contrary, in genotoxic conditions generating double-strand breaks, ectopic recombination involving intermediate size repeats and a further set of nuclear-encoded factors serves as a major process in double-strand break repair. Mutation of surveillance genes promotes mtDNA rearrangements through ectopic recombination, whereas mutation of repair genes impairs faithful homologous recombination-mediated repair and allows reorientation of the damaged mtDNA towards error-prone pathways mediated by microhomologies.

Recombination factors

The factors involved in the different steps of homologous recombination have been extensively characterized in bacteria. Most of these have no identifiable orthologs in human mitochondria, especially recombination mediators binding to single-stranded DNA, factors supporting Holliday junction migration and factors needed to rescue stalled replication forks. By contrast, we and others have shown in *Arabidopsis thaliana* that plant mitochondria have retained most of the recombination factors of prokaryotic origin (3). Two of these, ODB1 and RECG1, are described in more detail below.

Plant ODB1 in mtDNA recombination and repair

Plant ODB1 is a 177 amino acid polypeptide essentially constituted of a single strand annealing (SSA) domain similar to that of RAD52. The latter is a nuclear factor involved in DNA double-strand break repair mediated by homologous recombination. RAD52 binds single-stranded DNA ends, contributes to annealing of complementary DNA strands and recruitment of the DNA recombinase RAD51.

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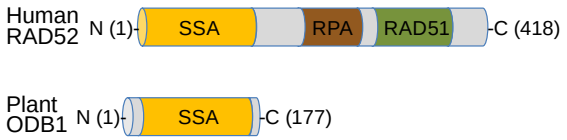
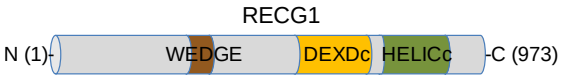


SSA=single strand annealing domain RPA=domain interacting with Replication Protein A
RAD51=domain interacting with RAD51

We showed that ODB1 localizes to mitochondria and chloroplasts (8). Whereas *odb1* mutants have no developmental phenotype in normal conditions, the *odb1* mutation results in mtDNA rearrangements in genotoxic conditions induced by ciprofloxacin treatment. ODB1 is a DNA-binding protein with high affinity for single-stranded DNA but with little sequence specificity (8). It promotes annealing of DNA complementary sequences. As a whole, plant ODB1 is a mitochondrial recombination mediator promoting faithful homologous recombination-dependent double-strand break repair. The absence of ODB1 favors error-prone pathways based on microhomology-mediated repair or single-strand annealing (8). No human ortholog of ODB1 has been documented and there is no evidence that human RAD52 can be targeted to mitochondria.

Plant-RECG1 in mtDNA recombination and repair

RECG1 is an ortholog of the bacterial RecG helicase and is present in all plants, including algae (9). We could modelize *Arabidopsis thaliana* RECG1 on the known structure of bacterial RecG bound to DNA. RECG1 is also dual-targeted to mitochondria and chloroplasts.



WEDGE=DNA-binding and substrate specificity domain
DEXDc=ATP and $MgCl_2$ binding domain
HELICc=Helicase domain

In normal growth conditions, *A. thaliana recG1* mutants have no visible developmental phenotype. Nevertheless, they accumulate products of ectopic recombination involving intermediate size repeats. RECG1 is thus involved in repressing mtDNA ectopic recombination (9). On the other hand, a *recG1* mutation promotes an increase in the stoichiometry of a single region in the mitochondrial genome. This increase results from a recombination event involving an intermediate size repeat. The recombination generates a circular episome that contains the *atp1* gene and replicates autonomously. Finally, a secondary recombination event based on another repeat generates a third mtDNA configuration in which the *atp1*-carrying episome has been reintegrated into the main genome in another location. Generation of this complex heteroplasmic mtDNA shows that plant RECG1 is a major contributor to the control of homologous recombination (9).

In genotoxic conditions generating double-strand breaks, *recG1* mutation abolishes the above-mentioned regular mtDNA repair based on ectopic homologous recombination involving intermediate size repeats, which results in a detrimental phenotype (9). Thus, plant RECG1 also contributes to repair of mtDNA double-strand breaks by faithful homologous recombination.

mtDNA segregation and transmission

As mentioned above, plant mtDNA recombination involving intermediate size repeats occurs only at low frequency. Substoichiometric levels of alternative mtDNA configurations can nevertheless be generated, leading to a heteroplasmic state. Minor alternative configurations can subsequently segregate in the progeny and become predominant through substoichiometric shifting (3). Ectopic recombination and recombination-mediated repair are thus major contributors to the rapid evolution of the plant mitochondrial genome. In these processes too, a parallel can be drawn with mammalian organelles. Initially, mutations in the human mtDNA are mostly heteroplasmic and the onset of clinical symptoms is determined by the ratio of wild-type to mutant mtDNA, with a typical threshold effect. But preferential amplification of the mutated mtDNA copies subsequently leads to clonal expansion and progressive tissue or organ-specific shift. The proportion of mutated mtDNA copies can also shift in daughter cells upon mitotic segregation. Finally, mtDNA genotypes may segregate between generations.

Plant RECG1 in mtDNA segregation

As mentioned above, recombination events resulting from *recG1* mutation generate a heteroplasmic state

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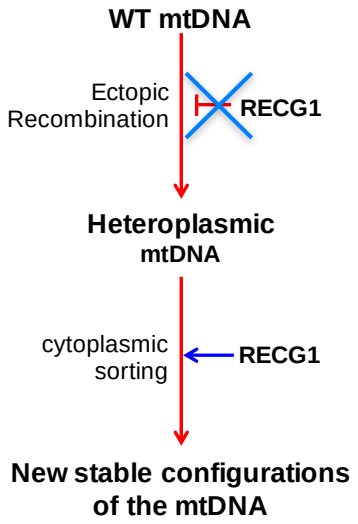
combining the wild-type mtDNA with two alternative mitochondrial genome configurations and a circular episome carrying the *atp1* gene. Backcrossing the *recG1* mutant with wild-type *A. thaliana* and screening the individuals of the F1 generation highlighted further tasks of RECG1 (9). First, re-introduction of the protein results in the disappearence of the *atp1*-carrying circular episome in the progeny, implying that RECG1 contributes to the suppression of replicative sub-genomes. Second, re-introduction of the protein elicits a segregation of the wild-type and the alternative mitochondrial genome configurations generated by recombination. As a consequence, the F1 progeny of the backcross comprises plants with a wild-type mtDNA, plants with an alternative mtDNA essentially deprived of *atp1* and plants with a new, stable and functional, mitochondrial genome configuration (9).



Conclusion

In plant mitochondria, ODB1 and RECG1 both promote faithful homologous recombination-dependent double-strand break repair, preventing error-prone repair based on microhomologies. No factors like ODB1 and RECG1, or factors with similar functions, seem to be present in human mitochondria and recent evidence suggests that the error-prone microhomology-mediated end joining (MMEJ) pathway might be the principal mediator of mtDNA double-strand break repair in mammalian mitochondria (10). One can speculate that therein lies the reason for the deletions in human mtDNA. Expression and mitochondrial targeting of plant ODB1 or RECG1 in human model cells would be an interesting approach to test this hypothesis. In addition, RECG1 contributes to the suppression of replicative sub-genomes in plant mitochondria and would potentially repress replication of deleted sub-genomes in human mitochondria. Finally, heterologous expression of RECG1 might also help to understand the mechanisms underlying segregation and clonal expansion of mutated genome copies in human cells.

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