PNAS COMMENTARY

Plant organellar genomes utilize gene conversion to drive heteroplasmic sorting

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While the nuclear genome only has two copies per cell, the genomes of organelles like mitochondria and plastids have an extra layer of complication in that multiple organellar copies exist within the same cell. Multiple genetically distinct genomes can exist within these organelles, resulting in a state of heteroplasmy in which the effect of organellar mutations depends on the variant reaching a critical level of copies within the cell to impact the cell's function. Heteroplasmy results in a microcosm of molecular evolution occurring in each cell of an organism. Allele frequencies can shift within a single cell, resulting in a change of which allele is dominant (1). In animals, genetic bottlenecks in the randomly sampled set of mitochondrial DNA copies play an important role in the fixation or elimination of mitochondrial variants (2). Genetic bottlenecks in animals occur as mitochondria are randomly distributed to developing oocytes from a germ cell (Fig. 1*A*). Since there is a large reduction in the number of mitochondria in these oocytes, the mitochondrial variants inherited by the oocytes can easily become skewed. The process of an organellar variant becoming fixed or eliminated is referred to as heteroplasmic sorting. However, less is known about the role of heteroplasmic sorting in the organellar genomes of plants, since they tend to have very low mutation rates (3), which makes studying the changing rates of de novo mutations more difficult. Additionally, genetic bottlenecking can occur within nonreproductive tissues, and the later germline separation of plants (4) might make this form of noninherited genetic bottlenecking more significant. In PNAS, the work of Broz et al. (5) explores how sorting of organellar variants can occur within *Arabidopsis thaliana* mitochondrial and plastid genomes. Broz et al. (5) posit that plant organelles utilize the MutS Homolog 1 (MSH1) to facilitate gene conversion which results in heteroplasmic sorting within their organelles (Fig. 1*B*).

To better understand how heteroplasmic sorting occurs within plants, Broz et al. (5) measured the rate of recessive single nucleotide variants sorting in female wild-type *Arabidopsis*. The *Arabidopsis* mitochondrial genomes sorted in a few generations with an effective bottleneck size of ~1.3. This estimated rate is quite rapid compared to the estimated rates of mitochondrial DNA sorting in animals, which range from ~5 to 10 in *Daphnia* (6) to ~170 in zebrafish (7). Unfortunately, the plastid genomes sorted too rapidly for the authors to analyze, but this shows that the rate of plastid sorting in plants is occurring much more rapidly than researchers expected.

How is *Arabidopsis* able to undergo this rapid heteroplasmic sorting? The authors (5) propose that the *Arabidopsis* organellar genomes utilize MSH1 to maintain this rapid sorting. MSH1 is believed to be at least partially responsible for maintaining the low rate of mutations in plant organellar genomes (3), as mutating *msh1* results in increased rates of mutation in plant mitochondrial and plastid genomes (8). MSH1 is predicted to be involved in mismatch repair, a process in which it creates double-strand breaks to facilitate the repair of mismatched or damaged bases through homologous recombination (9). The process of homologous recombination can lead to gene conversion in which a donor sequence is used as a template to repair the double-strand breaks and the cellular genomes become more homogenous. Additionally, genetic surveillance genes such as *msh1* tend to be more common in plants and organisms that lack a fixed body plan, such as corals and sponges (4). Since genetic bottlenecking is not documented in the organelles of plants and organisms that lack a fixed body plan, the team of Edwards et al. (4) previously

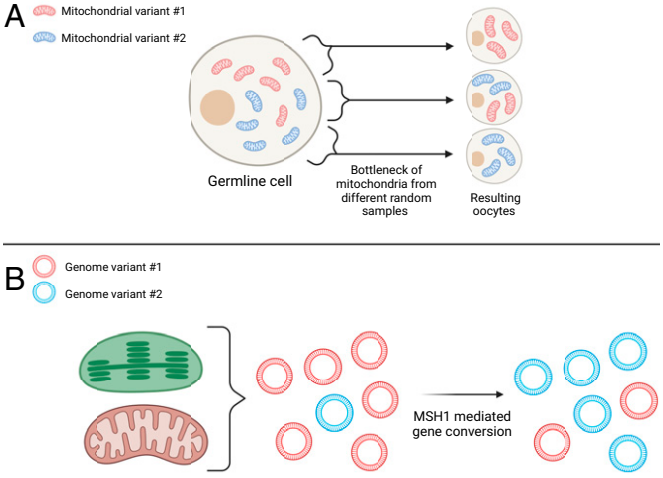

Fig. 1. (*A*) Schematic depicting the process of genetic bottlenecking in mitochondrial cells which can lead to different mitochondrial variants becoming dominant within the resulting oocytes. (*B*) Schematic depicting the proposed process from Broz et al. (5) in which the plastid and mitochondrial genomes of the plant *Arabidopsis* utilize MSH1-mediated gene conversion to change the genomic variants within these organelles; this process can lead to a different genomic variant becoming dominant within the organelle. Created with BioRender.com.

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proposed that gene conversion could be used as an alternative to genetic bottlenecking in organisms lacking early germline segregation.

To test that MSH1 is responsible for their finding of rapid heteroplasmic sorting rates in plant mitochondria, Broz et al. (5) utilize de novo mutations identified from organellar DNA pools of *msh1* mutant lines of *Arabidopsis.* Ten low-impact single nucleotide variants were selected for different family lines. The frequency of each single nucleotide variant was then measured over the next few generations. The authors discover that heteroplasmic sorting in these *msh1* mutant backgrounds occurs more slowly than in the wild-type background and with higher effective bottleneck sizes. Plastid genomes with the *msh1* mutant background sorted with an effective bottleneck size of ∼1, while *msh1* mutant mitochondrial genomes sorted with an effective bottleneck size of ∼4, showing that *msh1* is at least partially responsible for the rapid heteroplasmic sorting rates in plant organellar genomes.

Broz et al., posit that plant organelles' utilize the MutS Homolog 1 (MSH1) to facilitate gene conversion which results in heteroplasmic sorting within their organelles.

Lastly, Broz et al. (5) discover that there is a GC bias in the single nucleotide variant inheritance in mitochondrion and plastid genomes in both the wild-type and *msh1* mutant background. This is quite strange considering that organellar genomes are typically AT rich (10, 11), and most organisms experience an AT bias in their mutations (12). This GC bias might be some yet unknown effect of gene conversion or other heteroplasmic sorting mechanism.

Even with the findings of Broz et al. (5), there remain many unanswered questions about how this MSH1-mediated heteroplasmic sorting is occurring. *Arabidopsis* mitochondrial and plastid genomes sort more rapidly than most animal mitochondrial genomes, even in an *msh1* mutant background. Are there other heteroplasmic sorting mechanisms that plants use to achieve this high rate? Additionally, future research will need to validate that it is MSH1's proposed role in mismatch repair-driven gene conversion that drives the increased rate of heteroplasmic sorting in *Arabidopsis* organellar genomes.

Since Broz et al. (5) report that gene conversion occurs in organellar genomes, could there be other organisms in which this process is occurring? There is little known about whether and how mismatch repair occurs in organellar genomes beyond plants. There is an MSH1 protein in yeast (13); however, this yeast MSH1 is not orthologous to the plant MSH1 (14). While yeast MSH1 has been shown to localize to the mitochondrion and is involved in DNA repair (13), whether it plays a role in mismatch repair has yet to be elucidated. Recent molecular evolution research has identified yeast MSH1 homologs across hexacorals (14). Additionally, mismatch repair has been found in both rat liver mitochondrial lysate (15) and human mitochondrial extracts (16), but, in both cases, the mismatch repair activity seemed to be independent of any MSH protein. More research is needed to see whether these yeast MSH1 and MSH-independent means of mismatch repair can cause organellar gene conversion and whether this gene conversion can drive heteroplasmic sorting. The findings of Broz et al. (5) show that the mechanisms by which organellar genomes undergo heteroplasmic sorting are probably more complex than currently researched. It may be that genetic bottlenecks and gene conversion are just the start of the yet undiscovered mechanisms that drive organellar evolution.

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