Expanding Our Understanding of mtDNA Deletions

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Clonal expansion of mtDNA deletions compromises mitochondrial function in human disease and aging, but how deleterious mtDNA genomes propagate has remained unclear. In this issue (Gitschlag et al., 2016) and in a recent *Nature* publication, *C. elegans* studies implicate the mitochondrial unfolded protein response (UPR^{mt}) and offer mechanistic insights into this process.

The first mitochondrial genetic defect identified was the large-scale mtDNA deletion, which remains a commonly identified cause of disease, accounting for ~12% of adult mitochondrial disorders (Gorman et al., 2015). Large-scale mtDNA deletions due to nuclear defects affecting mtDNA maintenance may account for another 20% of mitochondrial disease cases. The formation and propagation of large-scale mtDNA deletions are therefore important biological and clinical questions. Two recent papers in *Nature* (Lin et al., 2016) and in this issue of *Cell Metabolism* (Gitschlag et al., 2016) shed some light on this question by using a *Caenorhabditis elegans* (*C. elegans*) strain harboring an mtDNA deletion.

A challenge of mtDNA genetics is the multi-copy nature of the mitochondrial genome in individual cells, such that both normal and mutant mtDNA molecules, including selfish genomes with no advantage for cellular fitness, coexist in a state known as "heteroplasmy." mtDNA deletions are functionally recessive; high levels of heteroplasmy (>60%) are required before a biochemical phenotype appears. In human tissues, we also see a mosaic of cells with respiratory chain deficiency related to different levels of mtDNA deletion (Sciacco et al., 1994). Interestingly, cells with high levels of mtDNA deletions in muscle biopsies show evidence of mitochondrial proliferation (the so-called ragged red fibers; Figure 1), a compensatory mechanism likely triggered by mitochondrial dysfunction. In such circumstances, deleted mtDNA molecules in a given cell will have originated clonally from a single mutant genome (Brierley et al., 1998). This process is therefore termed "clonal expansion."

The accumulation of high levels of mtDNA deletions is challenging to explain, especially given that mitophagy should provide quality control to eliminate dysfunctional mitochondria. Studies in human tissues do not allow experimental manipulation, but large-scale mtDNA deletion models in *C. elegans* have proved to be helpful, showing some conserved characteristics that match the situation in humans, as well as some divergences. Gitschlag et al. (2016) and Lin et al. (2016) use a *C. elegans* strain with a heteroplasmic 3.1 Kb mtDNA deletion to demonstrate the importance of the mitochondrial unfolded protein response (UPR^{mt}) in allowing clonal expansion of mutant mtDNAs to high heteroplasmy levels. Both papers demonstrate that wild-type mtDNA copy number is tightly regulated, and that the mutant mtDNA molecules hijack endogenous pathways to drive their own replication.

According to data by both groups, the expansion of mtDNA deletions involves nuclear signaling to upregulate the UPR^{mt} and increase total mtDNA copy number. In the paper by Gitschlag et al. (2016), normal and mutant mtDNA copies were quantified by digital droplet PCR (ddPCR), a method with reported improved sensitivity over real-time PCR for low heteroplasmy levels (Belmonte et al., 2016). The increase in mutant and total copy number is in agreement with data in human tissues, where mtDNA defects cause compensatory upregulation of mtDNA (Giordano et al., 2014). The nature of the mito-nuclear signal in this *C. elegans* model may have been partly elucidated by Lin et al., who demonstrate that the transcription factor ATFS-1 (activating transcription factor associated with stress-1), which fails to be imported by depolarized mitochondria, mediates UPR^{mt} activation by mtDNA deletions (Lin et al., 2016).

A long-standing hypothesis proposes that deleted mtDNA molecules clonally expand because they replicate more rapidly due to their smaller size (Wallace, 1992). To address this question, Gitschlag et al. examined the behavior of a second, much smaller mtDNA deletion molecule. They found no evidence for a replicative advantage of the smaller genome, and clonal expansion to similar levels as the larger deletion (Gitschlag et al., 2016). In human skeletal muscle, mtDNA deletions of different sizes also undergo clonal expansion to the same degree (Campbell et al., 2014). Furthermore, point mutations that do not change the size of the total mtDNA molecule also successfully expand to deleterious levels, indicating that clonal expansion is not driven by genome size. Thus, similar mechanisms may be operating across organisms. In the worm, this involves mito-nuclear signaling and activation of the UPR^{mt}.

Where these papers differ is the conclusions drawn about the effector pathway involved. Gitschlag et al. present genetic data indicating that UPR^{mt} allows the mutant mtDNA molecules to accumulate by reducing mitophagy. Lin et al. demonstrate that the UPR^{mt} induces mitochondrial biogenesis and promotes organelle dynamics (fission and fusion). Both papers show that by downregulating the UPR^{mt} response, mtDNA deletion levels

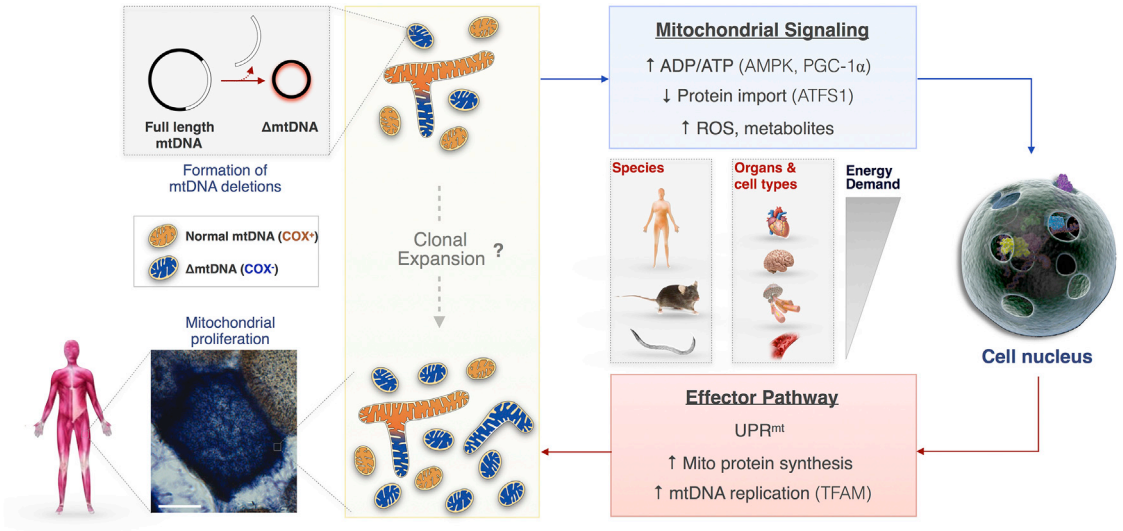

Figure 1. Potential Mechanisms for Clonal Expansion of mtDNA Molecules with Large-Scale Deletions

mtDNA deletions (ΔmtDNA) are generated from the loss of a genomic segment generally affecting multiple genes and are a common cause of human disease. They are present in a state of heteroplasmy, where both full-length (normal) and deleted mtDNA molecules coexist in the cell cytoplasm. The resulting functional impairment triggers the release of mitochondrial signals that reach the nucleus. These signals activate the transcription of genes involved in effector responses, such as the mitochondrial unfolded protein response (UPR^{mt}) in *C. elegans*; upregulation of proteins of the respiratory chain; and proteins, including the transcription factor of mitochondria (TFAM), involved in mtDNA transcription and replication. In humans, mice, and worms, increased replication of deleted mtDNA molecules is associated with an expansion of mitochondrial mass and total mtDNA copy number. This manifests histologically in human muscle fibers as ragged red fibers, illustrating the hyperproliferation of mitochondria deficient in mtDNA-encoded cytochrome *c* oxidase (COX, brown), but positive for nuclear-encoded succinate dehydrogenase (SDH, blue) activity. Scale bar, 25 μm.

fall, which may allow a therapeutic approach in humans.

Could there be a similar mechanism in humans, especially since some features detected in *C. elegans* are also present in human tissues, including the increase in mitochondrial biogenesis and the lack of relationship between mitochondrial genome size and expansion? It is likely that there will be a similar mechanism to preserve deletions since, as in the worm, deletions persist and accumulate in human tissues, despite an active autophagic quality-control process. Although the UPR^{mt} has not been characterized in humans as it has in the worm, and no equivalent protein to ATFS-1 has been identified in mammals, proteins such as CHOP, HSP-60, ClpP, and mtHSP70 appear to serve similar functions in mammals as those in *C. elegans* and suggest that a similar mechanism may be present.

The *C. elegans* system is relevant to human pathology since mtDNA deletions are largely seen in post-mitotic tissue, being rapidly lost from mitotic tissue. Human tissues have been studied at the single-cell level, and this has revealed that mtDNA deletions are distributed as a mosaic of affected and non-affected cells. In the worm, cellular and sub-cellular studies cannot be performed as easily. It is therefore unclear if in heteroplasmic worms (whole body), every cell harbors the same level of mtDNA heteroplasmy, or if some cells have disproportionately elevated heteroplasmy levels and some others are spared. Differences in the segregation of mtDNA defects such as the m.3243A>G mutation have been demonstrated in human tissues and correlate with energy demand (Picard and Hirano, 2016), suggesting that clonal expansion may be driven by tissue- and cell-type-specific mechanisms.

Mapping the mechanisms whereby mtDNA deletions propagate to deleterious levels will be important to understand human mitochondrial disease. While searching for universal pathways, it is important to consider inherent species and tissue differences (Figure 1). Is the selfish mitochondrial genome usurping endogenous pathways to take over the cell, as suggested by Gitschlag et al.? Does this mechanism apply to human pathology? Or are we witnessing another facet of a highly adaptable symbiotic relationship between the cell and its resident mitochondria, guided by species- and tissue-specific mechanisms? These two papers provide fascinating insight into mitochondrial biology, and time will tell how relevant these observations are to human disease.

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