Buffering mitochondrial DNA variation

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Reactive oxygen species (ROS) are traditionally viewed as the toxic by-product of cellular respiration. A new study suggests a homeostatic role for ROS in maintaining stable respiratory phenotypes across genetic variants of the mitochondrial genome.

At only 16.5 kb, the size of the mitochondrial genome says nothing of its complexity or its ability to surprise us even 25 years after its initial sequencing[1]. Dozens of inborn errors of mitochondrial metabolism have been attributed to rare, single mitochondrial DNA (mtDNA) mutations that affect cellular respiration[2], but a longstanding question in mitochondrial biology is how common variations in mtDNA influence phenotype. Indeed, common genetic variation in human mtDNA has recently been associated with cold weather adaptation, aging and complex diseases[3]. Cells harboring different mtDNA haplotypes, however, typically show comparable rates of cellular respiration and growth, calling into question the validity of these associations. On page 1261 of this issue, Raquel Moreno-Loshuertos and colleagues[4] report a new role for ROS that may have previously masked the phenotypic consequences of mtDNA variation.

To directly compare mtDNA haplotypes in the presence of an identical nuclear background, Moreno-Loshuertos *et al.* use a well-established cytoplasmic hybrid (cybrid) technology[5]. Cybrids are created by fusing enucleated donor cells containing mitochondria of the desired genotype to recipient cells devoid of endogenous mtDNA. The authors create cybrids each containing one of four fully sequenced mouse mtDNA haplotypes. As mtDNA encodes only genes required for oxidative phosphorylation (OXPHOS), penetrant genetic variations should be observed as alterations in cellular respiration. However, as observed by other groups[6], these four cell lines were indistinguishable in their rate of oxygen consumption, a coarse indicator of OXPHOS function.

Unveiling a phenotype

To stop with only measurements of respiration would not do justice to the intricacies of the OXPHOS system. First, although OXPHOS is the primary source of ATP *in vivo*, numerous studies have suggested that in standard, high-glucose cell culture conditions, the majority of ATP derives from glycolysis. Second, although most mitochondrial oxygen consumption is efficiently coupled to the production of ATP, a small percentage of oxygen is reduced by wayward electrons to produce potentially dangerous ROS. In fact, mitochondria are the primary source of ROS in the cell (**Fig. 1**).

With these insights in mind, Moreno-Loshuertos and colleagues revisited the cybrids and phenotyped them carefully under perturbed conditions. They first grew their cybrids in galactose medium, a carbon source that forces cells to rely on OXPHOS, and they noted that two cybrid lines showed a slight growth defect. These same two cybrids possessed higher steady-state ROS levels and mtDNA copy numbers (high-ROS cybrids) than the others (low-ROS cybrids). Surprisingly, when the cybrids were grown in the presence of antioxidants that scavenge intracellular ROS, the high-ROS cybrids, but not the low-ROS cybrids, showed reduced mtDNA copy number and diminished respiratory performance. Therefore, ROS are not only a differential marker of these cybrid genotypes but also seem to have a functional role in maintaining a stable rate of cellular respiration.

Sending out an ROS

ROS are traditionally viewed as a cellular hazard that can damage proteins, lipids and DNA. However, more recent studies have suggested regulatory roles for ROS in the cell (**Fig. 1**). ROS signaling can affect cellular

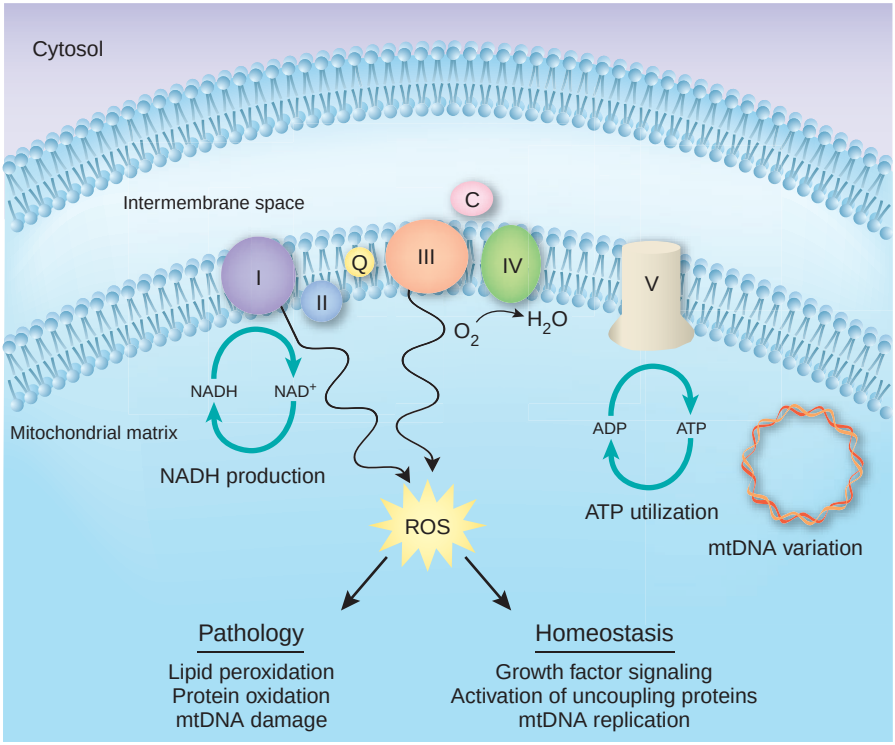

Figure 1 The generation and consequences of ROS in mitochondria. Mismatches between NADH production and the use of ATP can stress the electron transport chain (complexes I–IV) and modulate the production of ROS. According to Moreno-Loshuertos *et al.*, the mtDNA haplotype can also influence steady-state ROS generation in the cell. Although ROS are traditionally viewed as toxic agents contributing to cellular pathology, emerging evidence suggests that ROS are also critical in cellular homeostasis.

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energetics by acutely regulating ATP production via activation of uncoupling proteins[7]. Moreover, ROS are required for transducing growth signals via certain receptor tyrosine kinases[8]. Moreno-Loshuertos *et al.* offer the beginnings of a model suggesting that steady-state cellular ROS production is at least partially established by inherited variation in mtDNA. The authors note that the high-ROS cybrids share only one mtDNA polymorphism, a polypyrimidine insertion in the mitochondrial $tRNA^{Arg}$ gene, which is not present in the low-ROS cybrids. They speculate that this insertion may influence the translational fidelity of OXPHOS proteins, thus leading to reduced OXPHOS capacity. However, the decline in OXPHOS due to this genetic variant seems to be compensated with higher ROS production that in turn drives mtDNA replication.

Although the model is appealing, several questions remain unanswered. The molecular basis for differential ROS production between the mtDNA haplotypes requires further exploration, as does the exact mechanism by which ROS serve to normalize respiration. Although ROS enhance mtDNA replication, the authors were unable to detect differential expression of mtDNA-encoded proteins. Also, key enzymes of the TCA cycle are downregulated in high-ROS cybrids, seemingly contradicting the hypothesized compensatory role for ROS. Finally, it is important to remember that the current study focuses on a handful of mouse cybrids, and it remains an open question whether their model can be extended to other cybrids or to common human mtDNA variants.

A long and winding road

The study by Moreno-Loshuertos *et al.* reminds us of the long road between genotype and the expressed phenotype. Some mtDNA variants can reduce OXPHOS function, but this reduction can be compensated by enhanced ROS production. Therefore, ROS serve to mask the phenotypic consequences of mtDNA variation, thus adding ROS to the small list of molecular mechanisms that ensure stable cellular phenotypes in the face of genotypic variation[9]. If confirmed *in vivo*, such 'buffering' could provide insight into the clinical heterogeneity of mitochondrial diseases. For instance, this mechanism might explain the tissue-specific pathology that is often observed with mtDNA disease, presumably owing to tissue-dependent ROS scavenging mechanisms. Additionally, individuals whose mtDNA haplotype influences ROS homeostasis might modify the inherited risk of developing complex diseases[10]. We now eagerly await further studies that test whether there are mechanistic links between common mtDNA variation, ROS homeostasis and *in vivo* phenotypes.

1. Anderson, S. *et al. Nature* **290**, 457–465 (1981).
2. Dimauro, S. & Schon, E.A. *N. Engl. J. Med.* **348**, 2656–2668 (2003).
3. Wallace, D.C., Ruiz-Pesini, E. & Mishmar, D. *Cold Spring Harb. Symp. Quant. Biol.* **68**, 479–486 (2003).
4. Moreno-Loshuertos, R. *et al. Nat. Genet.* **38**, 1261–1268 (2006).
5. King, M.P. & Attardi, G. *Science* **246**, 500–503 (1989).
6. Battersby, B.J. & Shoubridge, E.A. *Hum. Mol. Genet.* **10**, 2469–2479 (2001).
7. Echtay, K.S. *et al. Nature* **415**, 96–99 (2002).
8. Sundaresan, M., Yu, Z.X., Ferrans, V.J., Irani, K. & Finkel, T. *Science* **270**, 296–299 (1995).
9. Rutherford, S.L. *Nat. Rev. Genet.* **4**, 263–274 (2003).
10. Houstis, N., Rosen, E.D. & Lander, E.S. *Nature* **440**, 944–948 (2006).

R-spondin1 tips the balance in sex determination

Blanche Capel

Female-to-male sex reversal is an extremely rare and puzzling phenomenon. A new study identifies mutations in the gene encoding R-spondin1 in XX sex-reversed individuals and suggests that antagonistic pathways in the bipotential gonad regulate sex determination.

On page 1304 of this issue, Pietro Parma and colleagues[1] describe a recessive mutation in the gene encoding R-spondin1 (*RSPO1*) that results in complete female-to-male sex reversal associated with palmoplantar hyperkeratosis (PPK) and predisposition to squamous cell carcinoma of the skin. The authors mapped the gene in a consanguineous family informative for linkage analysis of the PPK trait, and they identified a single nucleotide insertion leading to a frameshift and stop codon in *RSPO1*. They confirmed the identity of the gene in a second independent case of the syndrome in which the affected XX male (shown to have a different haplotype) was found to carry a homozygous deletion within *RSPO1*. These data provide strong evidence that *RSPO1* is the gene responsible for this complex syndrome.

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Sry and Sox9

The existence of XX individuals who develop a testis and complete female-to-male sex reversal, yet carry no *SRY* gene, has been puzzling. The Y-linked gene *SRY* encodes a DNA-binding protein that triggers testis development in the bipotential gonad[2]. Initially, it was expected that SRY would be found to bind to many downstream targets affecting various aspects of the testis developmental program. It was difficult to imagine how a single mutation in an XX individual could lead to the activation of all SRY-mediated pathways.

However, subsequent research has shown that a closely related autosomal gene, *SOX9*, is upregulated immediately downstream of *SRY* and can also activate the testis pathway. Gain or loss of function of *Sox9* in mice mimics the effects of *Sry*: activation of *Sox9* in the gonads of XX embryos leads to testis development and female-to-male sex reversal[3], whereas deletion of the gene from XY gonads leads to ovary development[4]. These findings suggest that *Sox9* may be the only requisite target of *Sry*. The puzzle of how to activate the testis pathway in XX individuals then boils down to a question of how to stabilize *Sox9* expression in gonadal cells.

Fgf versus Wnt

Fgf9 is required to stabilize *Sox9* in the gonad. In $Fgf9^{-/-}$ XY mice, *Sox9* is initially activated; however, its expression is not maintained. In the absence of *Fgf9* and *Sox9*, the XY gonad switches to the ovarian pathway, characterized by *Wnt4* expression. In reciprocal experiments in $Wnt4^{-/-}$ XX mice, both *Sox9* and *Fgf9* are activated. These findings strongly suggest that an antagonistic relationship between Fgf and Wnt signaling is translated into two opposite outcomes: the activation or repression of *Sox9*