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The Problem with Mixing Mitochondria

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Mixing of mitochondrial DNAs (heteroplasmy) is unfavorable for reasons unknown. Sharpley et al. show that heteroplasmy has surprising genetic and behavioral effects in mice, even when each haplotype alone produces a normal phenotype. This interference is bioenergetic and may have contributed to the evolution of sexes.

Sex requires the fusion of two gametes, but there is no obvious reason why there should be two distinct sexes. Yet even unicellular eukaryotes that produce isogametes typically have two mating types. Why so choosy? One distinction between isogametes relates to the inheritance of organelles, notably mitochondria: one "sex" usually passes on mitochondria, the other does not. This distinction is even more marked in multicellular eukaryotes. Biparental inheritance of mitochondria produces zygotes with a mixture of mitochondrial DNA (mtDNA) types (heteroplasmy), which can lead to selfish conflict or mitonuclear incompatibilities (Hadjivasiliou et al., 2012). Developmental bottlenecks can also amplify one variant over another. If that type contains mutations, deletions or mitonuclear incompatibilities, the outcome is reduced fitness, for example mitochondrial disease. Whether mtDNA mutations occur frequently enough to explain the evolution of two sexes is uncertain. In this issue, Sharpley et al. (2012) show that mutations are not needed. Heteroplasmy alone leads to unexpected genetic and behavioral instabilities, even when the two mtDNA types appear to function equally well against the same nuclear background.

Mitochondrial DNA encodes a small proportion of respiratory proteins along with the tRNAs and rRNAs needed to synthesize these proteins within the mitochondrial matrix. As many as 100,000 copies are passed on in mammalian oocytes. Mutations in mtDNA have unpredictable consequences as they depend not only on the mutation itself but also on its proportion relative to total mtDNA, the nuclear background, and segregation in different tissues and organs. For decades, segregation was seen as random, but evidence for rapid loss of severe mtDNA mutations over generations (Fan et al., 2008; Stewart et al., 2008) challenged this view. Still, there is a big difference between eliminating harmful mtDNA mutations via a germline bottleneck and distinguishing between two apparently equivalent mtDNA haplotypes.

In a large study, involving 500 mice over 14 years of careful breeding experiments, Sharpley et al. (2012) show exactly this: different cells and tissues somehow distinguish between two equivalent mtDNA haplotypes, NZB and 129S6 mtDNA. By constructing embryos with varying proportions of NZB/129 heteroplasmy and generating heteroplasmic mice, they show that smallish proportions (~0%–15%) of either NZB or 129 mtDNA do behave in a stochastic manner, but mixtures of roughly 50:50 NZB:129 behave very differently. Various tissues show a systematic bias in segregation toward either NZB or 129 mtDNA (Figure 1). For example, in liver and kidney, 129 mtDNA is preferentially lost, whereas in ovarian tissue and oocytes, NZB mtDNA is preferentially lost, restoring 129 mtDNA homoplasmy over several generations. In contrast, heart, skeletal muscle, and brain maintain a stable heteroplasmic state over a lifetime. The reasons for such patterns of segregation remain unknown.

Although these patterns are unexpected, the most striking finding is that heteroplasmy itself causes behavioral and cognitive abnormalities in mice. Homoplasmic mice (with either NZB or 129 mtDNA against a congenic C57BL/6J nuclear background) are indistinguishable in terms of fertility, physical activity, cognitive function, and behavior: neither NZB nor 129 mtDNA is obviously better adapted to the shared nuclear background. Yet a 50:50 mixture of NZB

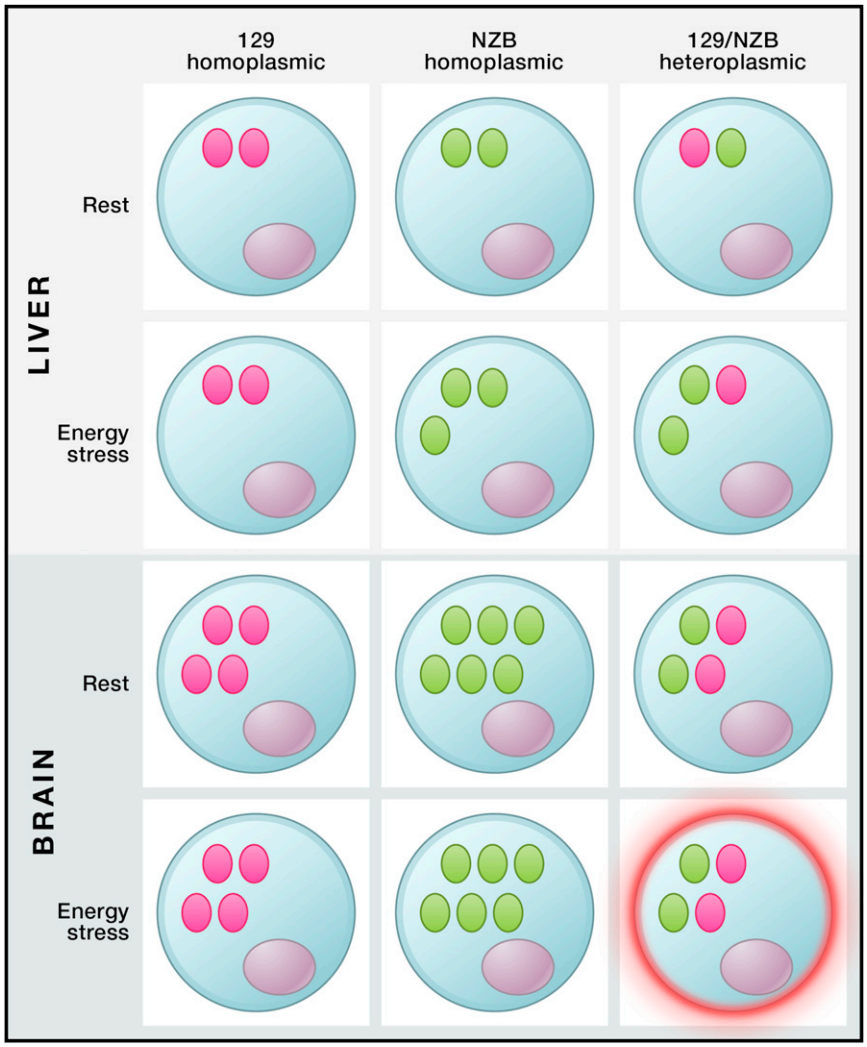

Figure 1. A Possible Explanation for Tissue-Specific mtDNA Segregation Patterns

Differences in mitochondrial density are predicted from Sharpley et al. (2012).

Liver: in homoplasmic cells, equivalent densities of 129 (pink) and NZB (green) mitochondria are sufficient for ATP synthesis under basal conditions. As the liver has a low dynamic range of ATP synthesis, increased energy demands causes stress. 129 mtDNA functions well against the nuclear background (purple), so ATP synthesis is rapid. NZB mtDNA is less well matched: ATP synthesis is slower, and ROS leak signals compensatory biogenesis, raising capacity to meet demand. In heteroplasmic cells, NZB mtDNA (with higher ROS leak) is preferentially amplified.

Brain: with its high energy demands, mitochondrial density is already increased during development. Increases in tissue demand lead to phosphorylation of respiratory complexes rather than biogenesis. Mitochondrial density is greater for NZB than 129 mtDNA, reflecting differences in rates of ATP synthesis. Thus homoplasmic cells can meet energy demands during stress owing to their differences in mitochondrial density. Heteroplasmic cells, however, fail to respond to energy needs, contributing to cognitive and behavioral deficits (red cell).

and 129 mtDNA has striking effects. Heteroplasmic mice are less active during the nocturnal foraging period, and eat less, giving them a lower respiratory exchange ratio. They show impaired spatial learning and retention, taking four times longer than their homoplasmic siblings to escape from a maze. And when subjected to stress, notably the forced-swim test, heteroplasmic mice are, curiously, more resistant to "behavioral despair."

What is going on at the molecular level? A possible clue comes from the work of José Antonio Enríquez and colleagues (Moreno-Loshuertos et al., 2006). They have shown that different mtDNA haplotypes can support equal rates of ATP synthesis but differ in reactive oxygen-species (ROS) leak and mtDNA copy number. ROS leak seems to optimize ATP synthesis by stimulating mitochondrial biogenesis (mtDNA copy number), an interpretation supported by the fact that antioxidants lower not only ROS leak but also mtDNA copy number and ATP synthesis. ROS leak, in effect, signals low capacity relative to demand, stimulating compensatory mitochondrial biogenesis. Different mtDNA haplotypes could then drive differences in tissue segregation relevant to aging (Lane, 2011). In addition to mtDNA haplotype, such segregation would depend on the nuclear background of the tissue (as mitochondria differ by up to half their proteome by tissue; Mootha et al., 2003), cell turnover, energy demands and dynamic range of ATP synthesis, and tissue architecture. Thus, heart and brain have high ATP requirements and a high dynamic range, with major constraints on tissue architecture (Phillips et al., 2012). Accordingly, variations in ATP synthesis are modulated by phosphorylation of respiratory complexes, rather than biogenesis (Acin-Perez et al., 2009). Little segregation of mtDNA is seen. In contrast, the liver has a low dynamic range and fewer architectural constraints. Here, variations in energy demand are met by biogenesis, hence the observed segregation of mtDNA haplotypes. This interpretation could explain the patterns of segregation of mtDNA in different tissues and is testable (Figure 1). Could it also explain why heteroplasmy produces cognitive and behavioral abnormalities in mice? Perhaps. If NZB and 129 mtDNA are intrinsically different in the rates of ATP synthesis they can support, 129, for example, being "fast" and NZB being "slow," these differences might need to be compensated for, during development, by stable differences in mitochondrial density (Figure 1).

Why would heteroplasmy be detrimental? If a mixture of "fast" and "slow" mtDNA haplotypes met normal energy requirements, there would be no compensatory increase in mitochondrial density during development. In the brain, where further mtDNA biogenesis is limited, neurons would then become compromised whenever energy

demands were high, possibly causing acute cognitive and behavioral abnormalities. If so, the greater tolerance to stress (latency to behavioral despair and agoraphobia) in heteroplasmic mice could be interpreted as a consequence of energy deficit. In fact, mice with a lysine codon deletion in the deubiquitinating enzyme USP46 exhibit a striking "loss of behavioral despair" in forced swim tests, as well as abnormalities in circadian rhythms (Zhang et al., 2011). As the ubiquitination system is sensitive to energy availability and associated with respiratory dysfunction in Parkinson's disease, it is not implausible that a respiratory deficit could affect deubiquitination, leading to the observed alterations in behavior. Whether subtle bioenergetic deficits play an important role in human psychiatric disorders and learning difficulties deserves serious consideration.

Although such behavioral changes are restricted to animals, their underlying cause may have played a wider role in organisms, including plants and fungi. By showing that heteroplasmy causes an energy deficit, Sharpley et al. (2012) just added another dimension to the long-standing questions of how and why uniparental inheritance of mitochondria arose and its relation to the evolution of two sexes.

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Turning on Brown Fat and Muscle Metabolism: Hedging Your Bets

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Developmental genes are essential in the formation and function of adipose tissue and muscle. In this issue of *Cell*, Teperino et al. demonstrate that noncanonical hedgehog signaling increases glucose uptake into brown fat and muscle. Modulation of developmental pathways may serve as a potential target for new treatments of diabetes and other metabolic disorders.

Obesity results from an imbalance between energy intake and energy expenditure. In obesity, the excess energy is stored as triglyceride in white adipose tissue (WAT). Mammals, including humans, also have active brown adipose tissue (BAT), which is specialized for energy expenditure. BAT has also been shown to share some common developmental origins with skeletal muscle, the other major tissue involved in thermogenesis (Kajimura et al., 2010).

Over the past two decades, the mechanisms underlying the differentiation of WAT and BAT have been elucidated and have been shown to involve a transcriptional cascade beginning with C/EBPβ, C/EBPδ, and Krox20, which induce C/EBPα and PPARγ, the major transcriptional regulators of adipose differentiation. In the case of brown fat, additional coactivators are involved, including PGC1α and PRDM16 (Kajimura et al., 2010). These pathways are regulated positively by a number of growth factors and hormones, especially insulin, IGF-1, and the BMPs, and negatively by the Wnt pathway (Fournier et al., 2012; Christodoulides et al., 2009). Recently, fundamental developmental genes, including several Hox and T-box genes, have also been shown to be involved in programming adipose development and function and contribute to differences in WAT and BAT, as well as differences between WAT in different anatomical