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LETTER

From evolutionary bystander to master manipulator: the emerging roles for the mitochondrial genome as a modulator of nuclear gene expression

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The importance of mitochondria in disease and organismal fitness is well established and recognized, but our current understanding of mitochondrial biology and genetics often struggles to pinpoint mechanisms through which mitochondria exert their influence. Recent findings may radically change our perception of mitochondria's capacity to have an impact on organismal fitness, revealing the presence of regulatory genetic elements that reside within the mitochondrial genome (mtDNA), but have effects on nuclear gene expression. These elements have remained unnoticed for decades, but their discovery and our growing appreciation for their breadth of impacts on disease and other phenotypes of interest may elevate the mitochondrial genome from bystander to master manipulator.

MITOCHONDRIA: CORNERSTONES OF EUKARYOTIC LIFE

The birth of the mitochondrial-eukaryote union marks one of most important stepping stones in the evolution of eukaryotic life.[1] Foremost, this union awarded the eukaryotic cell with a highly efficient means of energy generation in the form of oxidative phosphorylation (OXPHOS). However, the mitochondrion also has core roles in cytosolic calcium homeostasis, the generation of reactive oxygen species (ROS) and mediating cellular apoptosis, thus the mitochondrial network is a central anchor point to organismal fitness.

A hallmark of this symbiosis is the coexistence of two separate genomes within the cell with the primary genome maintained in the cell nucleus (nuDNA) and a diminutive secondary genome contained within the organelle's compartment (mtDNA). Whereas the overwhelming majority of an organism's genes are encoded in the nucleus, mtDNA harbors only a few, but essential, genes required for gene translation including 22 transfer-RNAs, 2 ribosomal RNAs and 13 genes encoding subunits of the OXPHOS pathway. These mitochondrial-encoded proteins form an integral part of the mitochondrial proteome that in its entirety consists of over 1000 nuclear-encoded proteins, requiring extensive anterograde (nucleus to organelle) and retrograde (organelle to nucleus) mito-nuclear signaling to coordinate accurate assembly of the OXPHOS machinery and to maintain mitochondrial functionality.[2] The concept of mito-nuclear crosstalk is not new, and the need for coordinated expression of genes encoded in the mitochondrial and nuclear genomes is currently best illustrated by the process of mitochondrial biogenesis (the process by which new mitochondria are formed). Mitochondria replicate by recruitment of new proteins that are added to the pre-existing protein complexes, and grow and divide through the process of mitochondrial fission and fusion.[3–7] The nuclear-encoded peroxisome-proliferator-activated receptor gamma coactivator-1 alpha (*PPARGC1A*) gene is perhaps most closely regarded as a master regulator for mitochondrial biogenesis. The protein product of *PPARGC1A* stimulates the regulatory activity of other key nuclear-encoded transcription factors that are essential for OXPHOS, mtDNA replication, mtDNA transcription, import and assembly of proteins, and fatty acid oxidation.[6,8] Mito-nuclear crosstalk is thus a well established and essential process in the maintenance of cellular homeostasis.

MITOCHONDRIA IN DISEASE AND FITNESS

Perturbation of the mito-nuclear interactions that coordinate the assembly of the mitochondrial proteome generally attracts grave consequences, and is reflected in a wealth of medical and biological studies, revealing the tight relationship between mitochondrial functionality and disease expression or fitness status.[9] Conventionally, it is thought that such perturbations are most frequently caused by heritable or somatic mutations in both the mitochondrial and/or nuclear genomes, leading to alterations in the nuclear transcriptional or translational machinery such that the biochemical properties of proteins or accurate protein synthesis is attenuated. For humans alone, ~500 potentially disease-causing mtDNA variants are known,[10] affecting both the coding and noncoding regions of the mitochondrial genome, and it is estimated that approximately one in 200 healthy individuals carries a pathogenic mtDNA mutation that could cause disease to the offspring of female carriers.[11] Various human diseases result from mutations to specific mtDNA sites, as is the case for the myoclonic epilepsy with ragged red fibers syndrome,[12] whereas other disease states are caused by single point mutations at one of a multitude of sites in the mtDNA, for example, Leber's hereditary optic neuropathy.[13] In the majority of instances, however, despite its small size and the ease with which mitochondrial genomes are sequenced (and mutations revealed), the search for disease-causing or fitness-reducing mutations in the mitochondrial genome is often marked by a struggle to link consequence to cause. Prominent amongst these is type 2 diabetes mellitus (T2DM), the most common disease found in an aging population.[14] Although the etiology of the disease has been outlined in detail and mitochondrial dysfunction has long been recognized as a key factor, it is still unclear how mitochondria exactly exert their central role. The struggle to unambiguously link single or multiple changes in the mtDNA to alterations in organismal fitness or disease expression suggests the additional layers on which mtDNA may exert control on its nuclear counterpart.

HOW MITOCHONDRIA MAY EXERCISE PARTIAL CONTROL OF NUCLEAR GENE EXPRESSION

Our current understanding of mito-nuclear crosstalk suggests that this coordinated bigenomic communication is predominantly initiated and regulated by the nucleus. However, recent findings have revealed additional mechanisms through which the mitochondrial genome may instead exert partial control over its nuclear counterpart, and thus directly have an impact on organismal fitness that may further be pivotal to disease expressivity. One such mechanism is the

epigenetic methylation of nuDNA, a process well documented for its role in the control of gene expression.[15] Intriguingly, patterns of nuDNA methylation are associated with specific mitochondrial haplogroups.[16] Thus, the methylation status of nuclear-encoded genes may change alongside alterations in the mutational pattern of the mitochondrial genome, a constraint that is further amplified by varying mtDNA copy number. For example, Xie *et al.*[17] found that reduced cellular mtDNA content invokes a mitochondrial–nuclear retrograde response that induces hypermethylation of key nuclear-encoded genes in both prostate and breast cancer cells. Of key interest, however, was the finding that the restoration of cellular mtDNA content to normal levels can reverse the hypermethylation process, suggesting a unique interaction between mtDNA copy number and genomic DNA methylation.[17,18] A further study by Bellizzi *et al.*[16] found that isogenic nuclear cybrid cells containing the mtDNA haplogroup J had significantly increased the levels of global DNA methylation in comparison to other mtDNA haplogroups. An insight into the role of mitochondrial activity involved with nuclear epigenetic methylation was gained by O'Hagan *et al.*[19] who found that ROS induced the recruitment of DNA methyltransferases (DNMT1 and DNMT3B) and a histone deacetylase (SIRT1) to form a unique protein complex that initiated methylation of CpG-rich DNA regions. A further report found that SIRT1 directly modulates the activity of DNMT1 and this interaction is critical for controlling the gene silencing activity of DNMT1.[20] These data suggest that the two epigenetic transcriptional controlling mechanisms of the cell (that is, DNA methylation and histone acetylation/deacetylation) are linked and that this process may be initiated by mitochondrial malfunction through an aberrant increase in ROS production. This is interesting because increases in ROS production and hypermethylation of CpG-rich DNA regions are typical biomarkers found in tumor cells.[15,21–23]

Emerging evidence further suggests that mtDNA sequence variations are capable of inducing mitochondrial–nuclear retrograde adaptive responses that function to maintain homeostasis. For example, a recent study found that inherited mtDNA variations associated with T2DM can induce nuclear compensatory gene expression patterns to maintain normal cellular function. Hwang *et al.*[24] found that isogenic nuclear cybrid cells containing T2DM-susceptible or T2DM-resistant mtDNA haplogroups were normal for mitochondrial and cellular metabolism. However, gene expression profiling between the two cell populations revealed significant differential expression for key genes involved with OXPHOS and glycolysis. The T2DM-susceptible mtDNA haplogroup cybrid cells demonstrated downregulation of OXPHOS and upregulation of glycolytic genes in comparison to the T2DM-resistant mtDNA cells. These data indicate that cellular adaptation in the form of expression of key nuclear genes is occurring directly in response to mtDNA variation. In addition, these data also intriguingly suggest that T2DM may develop as a consequence of cells becoming defective in their nuclear compensatory adaptive activity (that is, altered or loss of epigenetic DNA methylation of key genes).

Collectively, these findings suggest that changes in mtDNA, either through heritable or somatic mutations, may have the capacity to significantly have an impact on nuclear gene expression, and with that exert effects on disease status and organismal fitness.

A second mechanism by which mitochondria may exert further control of their nuclear counterpart stems from the discovery of a small signal peptide (humanin) encoded in the 16S RNA of the human mitochondrial genome.[25] Only 24 amino acids in length, humanin is translated in the cytoplasm, and is both an intracellular and extracellular secreted protein, entering the bloodstream to target distant receiving tissues. Seemingly acting as an antiapoptotic agent, humanin is hypothesized to be of fundamental importance for cell function and has been identified as a pivotal element in several age-related disease phenotypes, including Alzheimer's disease[26] and T2DM.[27] Interestingly, humanin levels are reduced in aged individuals and it is, therefore, tempting to speculate that such reduced levels may be indicative of age-related disease onset.[27,28] The humanin mtDNA sequence also maps to 13 nuclear-located regions with 10 of these being functionally relevant, indicating the existence of putative tissue-specific isoforms. These regions show high conservation with chimpanzee and establish a direct link between the mtDNA and nuclear function.[29] Humanin may, therefore, represent the first peptide identified in a new class of mitochondrial signal peptides (termed mitokines) that are hypothesized to be the key elements of mitochondrial communication.[30]

Recent evidence has indicated that mitochondrial mutations can induce the secretion of key factors that initiate cell adaptation to distal receiving cells in response to mitochondrial stress. Durieux *et al.*[30] generated *Caenorhabditis elegans* mitochondrial cytochrome *c* oxidase (COX) mutant models to determine the impact of single tissue mitochondrial impairment on the function of an entire organism. The mutant animals were targeted to generate a COX-specific mutation only in neuronal cells, with all other tissues remaining normal. Although neuronal COX malfunction was evident, mitochondrial malfunction was also found in the intestinal cells of the mutant animals, even though these cells did not contain the COX mutation. This mechanism may be a consequence of secreted proteins (mitokines) that are initiated in response to mitochondrial malfunction that then circulate to specific distal receiving cells to alter mitochondrial activity.[30] These data are in support of murine studies that suggest circulating serum factors from donor mice can significantly influence tissue function in recipient mice. For example, intravenous injection of blood plasma isolated from old mice can inhibit neuronal function in young healthy mice,[31] and circulating factors in young serum can reverse the age-related decline in muscle stem cells and liver cells in old mice.[32] These data may be indicative of putative mitokines in serum that induce compensatory adaptation of distal receiving cells.

Small RNAs (sRNAs) are the third regulatory system that appears to be underpinned by mitochondrial–nuclear crosstalk. Best known for their roles in translational regulation and gene silencing, sRNAs have emerged as critical genetic regulatory elements, affecting a broad range of fundamental physiological processes and disease. Coding of these sRNAs was thought to be limited to the nucleus, until recent transcriptomic analysis of the human mitochondrial DNA revealed the presence of 31 sRNAs encoded within the 22 mitochondrial tRNAs.[33] While the functions of these newly discovered elements are currently unknown, they are hypothesized to act in a manner akin to their nuclear counterparts to regulate gene expression and thus to regulate genetic pathways. These elements may, therefore, have the potential to significantly affect not only mitochondrial, but also nuclear gene expression.

Collectively, this surge of new information identifying hitherto unknown direct and indirect mechanisms, through which the mitochondrial genome can and may have an impact on the nuclear genome, opens an exciting new chapter in mitochondrial genetics. Considering these findings, it is reasonable to speculate that mitochondrial sRNA, humanin and ROS may act as key signaling factors to the nucleus to initiate gene expression through alterations in the nuclear epigenome. Mitochondrial DNA mutations may, therefore,

alter the production of these factors and lead to a breakdown in the mito-nuclear crosstalk signal, and to increased disease susceptibility. If further research confirms the presence and function of these genetic regulatory elements, such findings may radically change our perception of the organelle's role on gene expression and may provide in many instances the missing link to connect consequence to cause in disease and fitness phenotypes. Equally in biological and medical research, the mitochondrial noncoding region has been neglected methodically and dismissed as inconsequential to fitness and disease status, a view that needs urgent revision. The recently released ENCODE (Encyclopedia of DNA Elements) data[34] have initiated a paradigm shift in the way we view the noncoding regions of nuDNA, and we likely need a similar shift in thinking when it comes to our understanding of the diminutive, but mysterious, mitochondrial genome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Lang BF, Gray MW, Burger G: Mitochondrial genome evolution and the origin of eukaryotes. *Annu Rev Genet* 1999; **33**: 351–397.
2 Rand DM, Haney RA, Fry AJ: Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol Evol* 2004; **19**: 645–653.
3 Youle RJ, van der Bliek AM: Mitochondrial fission, fusion, and stress. *Science* 2012; **337**: 1062–1065.
4 Chan DC: Mitochondria: dynamic organelles in disease, aging, and development. *Cell* 2006; **125**: 1241–1252.
5 Frazier AE, Kiu C, Stojanovski D, Hoogenraad NJ, Ryan MT: Mitochondrial morphology and distribution in mammalian cells. *Biol Chem* 2006; **387**: 1551–1558.
6 Ryan MT, Hoogenraad NJ: Mitochondrial-nuclear communications. *Annu Rev Biochem* 2007; **76**: 701–722.
7 McBride HM, Neuspiel M, Wasiak S: Mitochondria: more than just a powerhouse. *Curr Biol* 2006; **16**: R551–R560.
8 Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM: A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998; **92**: 829–839.
9 Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; **39**: 359–407.
10 Ruiz-Pesini E, Lott MT, Procaccio V *et al*: An enhanced MITOMAP with a global mtDNA mutational phylogeny. *Nucleic Acids Res* 2007; **35**: D823–D828.
11 Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF: Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 2008; **83**: 254–260.
12 Mancuso M, Filosto M, Mootha VK *et al*: A novel mitochondrial tRNAPhe mutation causes MERRF syndrome. *Neurology* 2004; **62**: 2119–2121.
13 Abu-Amero KK, Bosley TM: Mitochondrial abnormalities in patients with LHON-like optic neuropathies. *Invest Ophthalmol Vis Sci* 2006; **47**: 4211–4220.
14 King H, Aubert RE, Herman WH: Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998; **21**: 1414–1431.
15 De Smet C, Lurquin C, Lethe B, Martelange V, Boon T: DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. *Mol Cell Biol* 1999; **19**: 7327–7335.
16 Bellizzi D, D'Aquila P, Giordano M, Montesanto A, Passarino G: Global DNA methylation levels are modulated by mitochondrial DNA variants. *Epigenomics* 2012; **4**: 17–27.
17 Xie CH, Naito A, Mizumachi T *et al*: Mitochondrial regulation of cancer associated nuclear DNA methylation. *Biochem Biophys Res Commun* 2007; **364**: 656–661.
18 Smiraglia DJ, Kulawiec M, Bistulfi GL, Gupta SG, Singh KK: A novel role for mitochondria in regulating epigenetic modification in the nucleus. *Cancer Biol Ther* 2008; **7**: 1182–1190.
19 O'Hagan HM, Wang W, Sen S *et al*: Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* 2011; **20**: 606–619.
20 Peng L, Yuan Z, Ling H *et al*: SIRT1 deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its activities. *Mol Cell Biol* 2011; **31**: 4720–4734.
21 Schagdarsurengin U, Richter AM, Hornung J, Lange C, Steinmann K, Dammann RH: Frequent epigenetic inactivation of RASSF2 in thyroid cancer and functional consequences. *Mol Cancer* 2010; **9**: 264.
22 Xia C, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, Jiang BH: Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Res* 2007; **67**: 10823–10830.
23 Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK: Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. *Cancer Res* 2008; **68**: 1777–1785.
24 Hwang S, Kwak SH, Bhak J *et al*: Gene expression pattern in transmitochondrial cytoplasmic hybrid cells harboring type 2 diabetes-associated mitochondrial DNA haplogroups. *PLoS One* 2011; **6**: e22116.
25 Tajima H, Niikura T, Hashimoto Y *et al*: Evidence for in vivo production of Humanin peptide, a neuroprotective factor against Alzheimer's disease-related insults. *Neurosci Lett* 2002; **324**: 227–231.
26 Zhang W, Li Z, Hao J *et al*: S14G-humanin improves cognitive deficits and reduces amyloid pathology in the middle-aged APPswe/PS1dE9 mice. *Pharmacol Biochem Behav* 2012; **100**: 361–369.
27 Muzumdar RH, Huffman DM, Atzmon G *et al*: Humanin: a novel central regulator of peripheral insulin action. *PLoS One* 2009; **4**: e6334.
28 Lee C, Yen K, Cohen P: Humanin: a harbinger of mitochondrial-derived peptides? *Trends Endocrinol Metab* 2013; e-pub ahead of print 7 February 2013; doi:10.1016/j.tem.2013.01.005.
29 Bodzioch M, Lapicka-Bodzioch K, Zapala B, Kamysz W, Kiec-Wilk B, Dembinska-Kiec A: Evidence for potential functionality of nuclearly-encoded humanin isoforms. *Genomics* 2009; **94**: 247–256.
30 Durieux J, Wolff S, Dillin A: The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 2011; **144**: 79–91.
31 Villeda SA, Luo J, Mosher KI *et al*: The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 2011; **477**: 90–94.
32 Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA: Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 2005; **433**: 760–764.
33 Mercer TR, Neph S, Dinger ME *et al*: The human mitochondrial transcriptome. *Cell* 2011; **146**: 645–658.
34 Dunham I, Kundaje A, Aldred SF *et al*: An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012; **489**: 57–74.