REVIEW ARTICLE

Zebrafish as a model system for mitochondrial biology and diseases: a review

Q29 Q1 **S. L. STEELE, S. V. PRYKHOZHIJ, and JASON N. BERMAN**

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Animal models for studying human disease are essential to the continuing evolution of medicine. Rodent models are attractive for the obvious similarities in development and genetic makeup compared with humans, but have cost and technical limitations. The zebrafish (*Danio rerio*) represents an excellent alternative vertebrate model of human disease because of its high conservation of genetic information and physiological processes, inexpensive maintenance, and optical clarity facilitating direct observation. This review highlights recent advances in understanding genetic disease states associated with the dynamic organelle, the mitochondrion, using zebrafish. Mitochondrial diseases that have been replicated in the zebrafish include those affecting the nervous and cardiovascular systems, as well as red blood cell function. There are a large number of studies involving genes associated with Parkinson's disease, as well as many of the genes associated with heme synthesis and anemia. Gene silencing techniques, including morpholino knockdown and TAL-effector endonucleases have been exploited to demonstrate how loss of function can induce human diseaselike states in zebrafish. Moreover, modeling mitochondrial diseases has been facilitated greatly by the creation of transgenic fish with fluorescently labeled mitochondria for *in vivo* visualization of these structures. In addition, behavioral assays have been developed to examine changes in motor activity and sensory responses, particularly in larval stages. Zebrafish are poised to advance our understanding of the pathogenesis of human mitochondrial diseases beyond the current state of knowledge and provide a key tool in the development of novel therapeutic approaches to treat these conditions. (Translational Research 2013;■:1–20)

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Abbreviations: 2,5-DHBA = 2,5-dihydroxybenzoic acid; ALS = amyotrophic lateral sclerosis; ATP = adenosine triphosphate; Bcl-2 = B-cell lymphoma 2; CMT2 = Charcot-Marie-Tooth 2; CNS = central nervous system; COX = cytochrome *c* oxidase; DA = dopaminergic; ETFDH = electron transfer flavoprotein dehydrogenase; HIF1α = hypoxia-induced factor 1α; hpf = hours postfertilization; HSC = hematopoietic stem cell; IMM = inner mitochondrial membrane;

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IMS = intermembrane space; KBP = KIF1-binding protein; LRRK2 = leucine-rich repeat kinase 2; MADD = multiple acyl-CoA dehydrogenase deficiency; MD = mitochondrial disease; MDS = myelodysplastic syndromes; MFN = Mitofusin; MFRN = mitoferrin; mRNA = messenger RNA; mtDNA = mitochondrial DNA; OMM = outer mitochondrial membrane; OPA1 = optic atrophy 1; oxphos = oxidative phosphorylation; PD = Parkinson's disease; PP2Cm = protein phosphatase 2C family member; ROS = reactive oxygen species; SA = sideroblastic anemia; SOD1 = superoxide dismutase 1; VCF = velocardiofacial

Mitochondria are found in all nucleated cells and originally arose from the endosymbiotic uptake of an alpha-proteobacterium by a unicellular eukaryotic host some 1.5 billion years ago. The subsequent expansion of modern eukaryotes can possibly be explained by the increased bioenergetic capacity provided by mitochondria to expand the proteome and therefore cell complexity, and ultimately the evolution of such things as the cell cycle and multicellularity.[1] Indeed, mitochondria are famously known as the "powerhouses" of the cell, generating a (ATP) via oxidative phosphorylation (oxphos). Mitochondria have 2 membranes, the outer mitochondrial membrane (OMM) and the highly convoluted inner mitochondrial membrane (IMM) sandwiching the intermembrane space (IMS). The process of oxphos depends on 5 transmembrane enzyme complexes of the electron transport chain located within the IMM that generate the electrochemical gradient between the IMS and the mitochondrial matrix required for ATP synthesis (reviewed in Nunnari and Suomalainen[2]). Mitochondria have their own unique circular genome (mitochondrial DNA [mtDNA]) encoding 22 transfer RNAs and 2 ribosomal RNAs, as well as 13 proteins, all of which are subunits of enzymes of the electron transport chain.[3] However, multiple proteomics studies have identified or predicted more than 1000 proteins associated with mammalian mitochondria (eg, Pagliarini et al[4]), the majority of which are encoded by nuclear genes.

With their substantial protein network, it is not surprising that these organelles are not only limited to energy production but also are involved in a variety of other cellular processes including (but not limited to) apoptosis, calcium storage, signaling events, and metabolism. Apoptosis can be initiated and accelerated when cytochrome *c* and other proapoptotic factors are released from mitochondria that have become permeable as a result of the formation of pores in the OMM, a process regulated by the B-cell lymphoma 2 (Bcl-2) family member proteins. Cytochrome *c* in the cytoplasm initiates a proapoptotic cascade and caspase activity in the cell. For a thorough review of mitochondrial-mediated apoptotic cascades, see Elmore.[5] Apoptosis can also be initiated independently of these pathways by mitochondrial reactive oxygen species (ROS), which are generated as a by-product of oxphos. The free radical superoxide anion ($O_2 \cdot^-$) is a highly toxic and reactive ROS produced mainly in the mitochondria by electron "leakage" at complexes I–III of the electron transport chain. It is the precursor for the slightly less reactive hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH\cdot$) species. Levels of these potentially toxic oxphos byproducts are controlled by the presence of antioxidant enzymes, such as superoxide dismutase, as well as the respiration rate of individual cell types and modification of components of the electron transport chain. ROS act as signaling molecules and are involved in normal cell proliferation and differentiation pathways. However, abnormally high ROS levels cause protein and DNA damage within the cell and are associated with a number of human pathologies, including diabetes mellitus, neurodegeneration, inflammation, and cancer (for reviews see Turrens,[6] Murphy,[7] and Pedersen[8]).

Mitochondria are highly dynamic and mobile structures. Their function is affected not only by the proteins found within, but also by their number, cellular localization, and movement within the cell. Mitochondrial locomotion occurs along both microtubules and actin microfilaments in an ATP-dependent manner via their association with molecular motor proteins such as kinesins and dyneins. The anterograde and retrograde movement of mitochondria is particularly well studied in polarized cells such as neurons and yeast. Increased mitochondrial movement into the synapse (in neural cells) and into budding daughter cells (in yeast) is thought to be a result of increased localized metabolic demand in these areas (for reviews, see Frederick and Shaw,[9] and Saxton and Hollenbeck[10]). Mitochondria also undergo regular fusion and fission events during which both IMS and matrix contents are exchanged between mitochondria, and new mitochondria are produced. These events are critical for the exchange of both mtDNA and oxphos substrates between healthy mitochondria, ultimately helping to maintain mtDNA homogeneity and the availability of substrates for cellular respiration. When mitochondrial fusion and fission events become unbalanced or eliminated (eg, by loss of function of the proteins required for 1 or both of these processes), the morphology, DNA stability, and respiratory capacity of mitochondria can be impacted negatively (for reviews, see Chan[11] and Galloway and Yoon[12]).

As we describe in this review, the effects of mitochondrial dysfunction extend well beyond the level of the cell to the whole organism and are involved in the