Concept Clearance

Branch: Exposure, Response, and Technology Branch

Council Period: February 19-20, 2014

Concept Title: Mitochondria, Energetics, Epigenetics, Environment and DNA Damage Response (MEEED)

Cells respond to environmental stressors through several key pathways, including response to reactive oxygen species (ROS), nutrient and ATP sensing, DNA damage response, and epigenetic regulation of gene expression. Mitochondria (mt) play a central role in these pathways, not only through energetics and ATP production but also through metabolites generated in the tricarboxylic acid (TCA) cycle, and mt-nuclear signaling related to mt morphology, biogenesis, fission/fusion, mitophagy, apoptosis, and epigenetic regulation. Recent studies have highlighted the extent of the integration of mt functions with other cellular pathways, notably epigenetic regulation/transcriptional control and DNA damage response (DDR).

Mt can affect epigenetic regulation of nuclear-encoded genes through generation of TCA metabolites, including acetyl CoA, ATP, NAD+, and α-ketoglutarate, which are required for proteins involved in histone modifications (e.g., acetylation and methylation) and DNA methylation (e.g., TET enzymes). Recent studies suggest that both endogenous and exogenous stressors (e.g., toxic metals, pesticides and air pollutants) can induce altered epigenetic patterns including histone modifications and altered DNA methylation of nuclear-encoded genes, possibly through mt-mediated responses to changes in energetics and/or ROS signaling. Mt are also tightly integrated with cellular responses to DNA damage to both mtDNA and nuclear DNA. For example, base excision repair (BER) is a critical pathway for removing oxidative and alkylation damage in both mtDNA and nuclear DNA. Another key DDR protein, ATM, which is involved in sensing and response to DNA double strand breaks, is now known to function in redox sensing, insulin signaling, cellular energy balance, and mt homeostasis. For example, ATM-null mice showed altered mt morphology, elevated ROS levels, and decreased ETC activity and ATP production. In another example, the mt fission protein Drp1 plays an important role in normal cell cycle progression; inhibition of Drp1 causes cell cycle disruption, with G2 arrest, abnormal DNA content, aneuploidy, and other chromosome abnormalities.

NIEHS sponsored a workshop on March 25, 2013 exploring the overlap of mt processes with these key stress response pathways. Speakers covered an overview of mt functions including the susceptibility of mt to environmental toxicants, the integration of nuclear and mt functions in epigenetic regulation, the crosstalk and signaling between mt and DDR processes, and a systems biology-type approach for investigating the role of mt functions in cellular responses to stress. Workshop recommendations included 1) developing better reagents to track the production and fate of individual reactive oxygen species 2) expanding fluxomics capabilities to track individual metabolites (e.g., from the TCA cycle) at steady state conditions using isotopically labeled substrates 3) utilizing appropriate experimental models, including yeast, Drosophila, and zebrafish, and mouse genetics resources as well as in vitro systems (e.g., iPS cells) and human population studies (e.g., childhood cancer survivors , patients treated with AZT and other RT inhibitors) to address integrated mt-cellular responses to stress and 4) applying systems-type approaches for understanding mt-cell signaling networks (e.g., global protein acetylation changes, mapping the mt proteome, altered DNA methylation of both mtDNA and nDNA, and the use of differential genetic networks to map altered function and biological networks in response to environmental stress).

A focused research program on the cross-talk between mt and other cellular response pathways, including a deeper understanding of mt-nuclear signaling, will lead to a more comprehensive understanding of how cells sense and respond to environmental stress and will form a more solid basis for developing early biomarkers for environmentally-related diseases. Based on recommendations from the MEEED workshop, we propose a

two-phase approach. The first phase will focus on development of technology and tools for tracking specific ROS, and expanded fluxomics capability, development and enhancement of in vivo and in vitro models for mt-nuclear interactions in response to environmental perturbations, and pilot studies of mt proteomics, global protein acetylation changes, and epigenetic alterations. The second phase will encompass larger research projects for systems-level investigation of the integrated responses to environmental stress between mt functions and other cellular pathways, including epigenetic regulation, DDR, nutrient sensing, and ROS sensing and signaling. A key element of this research will involve understanding organ- or cell-specific differences in mt-cellular interactions in response to environmental stress.

Phase I for MEEED will solicit R21/R33 grants for technology development (e.g., improved reagents for tracking specific ROS, and expanded fluxomics capability), pilot testing, initial mt proteomics, global protein acetylation, and epigenetics studies, etc.– $2M for 6-8 awards for 3-4 years. Transition from R21 to R33 awards will be decided based on successful completion of milestones for the development and testing of technologies or enhanced model systems for investigating mt-cellular crosstalk. R33 projects should focus on application and validation of these technologies using environmental stressors. Phase 2 will solicit R01 grants for systems-level investigation of the integrated responses to environmental stress between mt functions and other cellular pathways (including epigenetic regulation, DDR, nutrient sensing, and ROS sensing and signaling) - $3M, 5-7 awards for 5 years. Although there has been substantial progress in recent years in omics capabilities, including global proteomics, metabolomics, and epigenetic regulation (both chromatin remodeling and DNA methylation), enhanced reagents and approaches are needed to precisely track ROS, mt metabolites, and mt-mediated changes in response to cellular stress. Developing new tools and approaches, including refined in vivo and in vitro models, will be a critical step to enable larger studies of the cross-talk between mt and other cellular response pathways. Phase 2 can build on technologies developed in the R21/R33 projects. These larger studies could include investigating mt-cellular pathway interactions in human studies of susceptible populations (e.g., childhood cancer survivors, progeria patients, and HIV patients treated with nucleoside analogs), comprehensive studies of mt-mediated cellular response pathways using animal or in vitro models with global metabolomics, proteomic, protein acetylation, or epigenetics approaches. Studies may focus on multiple mt endpoints, including effects on energetics and ATP production, metabolite generation from the TCA cycle, mt morphology and dynamics (e.g., fusion, fission, mitophagy, or biogenesis), ROS sensing and signaling, apoptosis, or nutrient or ATP sensing. Research will be expected to probe mt-cellular interactions using appropriate environment stressors, including but not limited to toxic metals, pesticides, air pollution components, mt toxicants, and inhibitors of electron transport chain complexes.

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