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## Harnessing the Power of Integrated Mitochondrial Biology and Physiology

### A Special Report on the NHLBI Mitochondria in Heart Diseases Initiative

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**Mitochondrial biology is the sum of diverse phenomena from molecular profiles to physiological functions. A mechanistic understanding of mitochondria in disease development, and hence the future prospect of clinical translations, relies on a systems-level integration of expertise from multiple fields of investigation. Upon the successful conclusion of a recent National Institutes of Health, National Heart, Lung, and Blood Institute initiative on integrative mitochondrial biology in cardiovascular diseases, we reflect on the accomplishments made possible by this unique interdisciplinary collaboration effort and exciting new fronts on the study of these remarkable organelles.**

Heart disease persists as a leading cause of mortality worldwide as the search for new diagnostic and therapeutic approaches continues. Recent research has converged on the importance of mitochondria in the life, death, and stress response of cardiac cells, spurring hope of new insights and therapies as we decipher the organizing principles of these organelles. Despite that the mitochondrial genome is  $\approx 16\,600$ -nucleotides long and its proteome is only  $\approx 1500$ -protein large, mitochondria represent a complex physiological system—where the genome, proteome, and metabolome interact to orchestrate key biological processes, including energy transduction,  $\text{Ca}^{2+}$  flux, redox balance, and cell death. A comprehensive, mechanistic understanding of mitochondria in heart diseases will require systems-level approaches on how alterations of biomolecules (ie, nucleotides, proteins, metabolites) trigger sophisticated interplay between bioenergetics, electrophysiology,  $\text{Ca}^{2+}$  homeostasis, and other subsystems.

Advances in systems biology over the past 15 years offers a tremendous opportunity to simultaneously monitor multiple

biomolecules in disease. At the same time, it creates an enormous challenge to integrate and substantiate massive amounts of molecular data into a meaningful synthesis of mitochondrial pathophysiology. This challenge demands integrative thinking and collaborative relationships among researchers whose areas of expertise were traditionally under the purview of separate disciplines. In light of this challenge, a National Heart, Lung, and Blood Institute (NHLBI) Working Group on Modeling Mitochondrial Dysfunction in Cardiovascular Disease was convened in 2007 to outline the future goals of mitochondrial studies in cardiovascular diseases. The resulting Executive Summary identified 3 key areas of scientific opportunities: (1) advance understanding of mitochondrial functions in health and disease using integrated genetic, proteomic, and functional data, (2) develop and manage new open-source computational models and experimental tools to enable cross-disciplinary investigations, and (3) accelerate the translation of basic mitochondrial research to in vivo systems to identify novel therapeutic targets. Consequently, an NHLBI Request for Application entitled *The Role of Cardiomyocyte Mitochondria in Heart Disease: An Integrated Approach* (HL-10-002) was announced in 2008. The NHLBI initiative brought together 6 teams (led by centers at UCSD, Aurora Healthcare, Sanford-Burnham, Johns Hopkins, U. Washington, and UCLA), which were tasked to form interdisciplinary collaborations and use innovative systems approaches to understand the role of cardiac mitochondria in health and disease.

As the initiation concludes, we are pleased to report that the ensuing productivity has been impressive, resulting in 132 publications, 1 patent, and numerous conference presentations

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### Nonstandard Abbreviations and Acronyms

<b>AF</b>	atrial fibrillation
<b>HF</b>	heart failure
<b>MPT</b>	mitochondrial permeability transition

during the 4-year award period. These works generated new projects that led to additional National Institutes of Health (NIH)-funded applications, the training of 35 trainees, and the inception of 7 lasting collaborations. We highlight several key accomplishments and scientific innovations here (Figure 1).

### Advancing Cross-Disciplinary, Systems Biology Studies of Cardiac Mitochondria

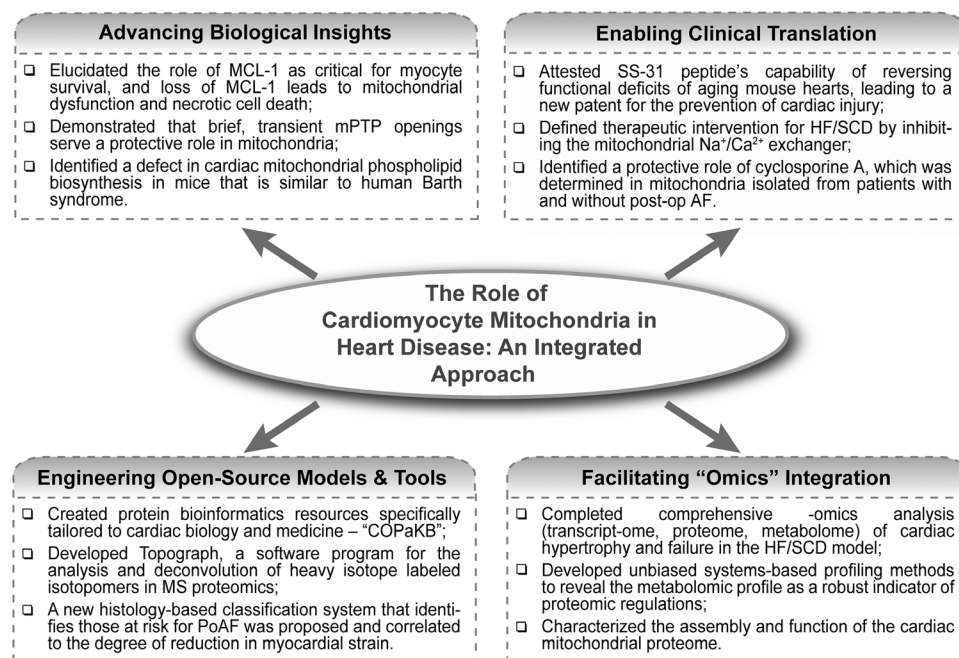
Several noteworthy discoveries were made through comprehensive omics analysis of various model systems, surveying the transcriptome, proteome, post-translational modifications, and metabolome of mitochondria. Highlights include (1) understanding cardiac energy production adaptations in response to physiological changes (eg, development of heart failure [HF]) by emphasizing the relationship between gene regulatory pathways, metabolite profiles, and energetics phenotypes<sup>1</sup>; (2) integrating mass spectrometry and functional data to simultaneously measure metabolites, bioenergetics, and turnover to identify predictive/diagnostic metabolic disease states<sup>2</sup>; (3) characterizing the mitochondrial proteome in normal versus HF to reveal protective alterations under mitochondrial reactive oxygen species production; (4) discovering new pathways that control cardiac mitochondrial phospholipid biosynthesis<sup>3</sup>; (5) interrogating the therapeutic potential of mitochondrial-targeted peptides to mitigate proteomic

changes in transverse aortic constriction models and enhance cardioprotection; and (6) developing new methods to quantify mitochondrial phosphorylation signaling.

In parallel, integrated physiology combining molecular profiles and functional measurements has made great strides in revealing the regulatory principles of mitochondrial physiology, including (1) the defensive role of transient mitochondrial permeability transition (MPT) in protecting mitochondria against cardiac stress (eg, elevated cytoplasmic  $\text{Ca}^{2+}$ )<sup>4</sup>; (2) the transcriptional regulatory circuitry controlling mitochondrial dynamics in cardiac development; (3) the functional consequences of mitochondrial reactive oxygen species in mtDNA damage/decline on mitochondrial biogenesis signaling; (4) the local dynamics of mitochondrial  $\text{Ca}^{2+}$  flux in disease<sup>5</sup>; (5) the prosurvival role of mitophagy following infarcts<sup>6</sup>; and (6) the prevention of sudden cardiac death by modulating mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchange.<sup>7</sup>

### Building Novel Open-Source Tools, Models, and Platforms

A second scientific priority of the initiative was to promote the integration of computational and informatics methods into the central thought process and analysis pipelines of the investigations. This development has spurred the construction of several readily accessible open-source computational platforms and models for the scientific community to explore mitochondrial biology. Several mathematical and computational models emerged to synthesize molecular data into higher organizational structures of mitochondria, including (1) computational models of mitochondrial pH regulation, electron transport, redox,  $\text{Ca}^{2+}$  flux, and  $\text{P}_i$ -dependent buffering; and (2) models of mitochondria temporal events (eg,  $\text{Ca}^{2+}$ , MPT, mitochondrial



**Figure 1 . Schematic overview of accomplished milestones: characterizing the role of mitochondria in heart diseases using integrated approaches.** AF indicates atrial fibrillation; COPaKB, Cardiac Organellar Protein Atlas Knowledgebase; HF, heart failure; MCL-1, myeloid cell leukemia 1; and SCD, sudden cardiac death.

reactive oxygen species) and spatial architecture of PKC $\epsilon$ -Src (protein kinase C epsilon-Src kinase) signaling pathways in nitric oxide-mediated cardioprotection.

Moreover, 2 computational platforms materialized in response to the need for specific tools to facilitate integrative sciences: (1) Topograph, a freely available software program to improve mass spectrometry discovery of protein turnover rates<sup>8</sup> (<https://goo.gl/dysfqm>); (2) COPaKB, a cardiac protein knowledgebase that serves as a protein bioinformatics resource tailored to cardiac biology and medicine<sup>9</sup> (<http://heartproteome.org/copa>). Looking ahead, we anticipate the continual development of bioinformatics resources to be instrumental for the study of cardiac mitochondria as integrated systems. A corollary of the omics revolution is the massive amount of large-scale data that are being generated every day. The aggregation, reanalysis, and meta-analysis of these data will be an exciting new front for the integrated approach. It is foreseen that mechanisms to cross-reference existing data sets generated by these other NHLBI-funded projects will represent an upcoming area of intense research.

### Bridging the Divide Between Basic Research and Clinical Intervention

In parallel, studies are making headway to bring integrative mitochondrial biology to the clinic. Treatments are under trial or development that target redox, cell death, and mitophagy pathways, whereas our understanding of their mechanisms of action continues to refine. Highlights include

1. Clinical studies of patient atrial samples after coronary bypass operations implicate mitochondrial alterations in the development of postoperative atrial fibrillation (AF). Atrial mitochondria are sensitized toward MPT in AF, suggesting new targets of intervention by cyclosporine A or metformin. Reduced respiratory complex I/II activities and mitochondrial metabolomic markers are being explored as features to complement blood biomarkers in predicting/stratifying patients at risk for postoperative paroxysmal/persistent AF. Newly defined roles of mitochondria in fibroblast proliferation/differentiation prompted further avenues of intervention, with the mitochondrial depolarizer 2,4-dinitrophenol shown to inhibit fibrotic growth in preclinical studies. These studies contributed to the ongoing development of a scoring system to monitor postop AF.
2. Bendavia/SS-31, a mitochondrial-protective tetrapeptide, is shown to protect cardiac function and reverse proteomic profiles in transverse aortic constriction and angiotensin II mouse models of HF. Mechanistic studies are exploring its similarities and differences with the mitochondrial catalase model and reveal a role of the peptide in improving state III respiration and reversing age-induced respiratory dysfunctions. As a result of this work, a new patent has resulted for protection from HF (US Appl 20110082084); the peptide is currently under Phase II trials for the prevention of injury after acute infarction, repair of renal artery stenosis, and improving skeletal muscle function in elderly subjects<sup>10</sup> (NCT 01572909/01755858/02245620).

3. A newly characterized BCL-2 family protein MCL-1 presents a promising diagnostic marker for cell death, mitochondrial dysfunction, and impaired autophagy.<sup>11</sup> Animal models and reagents are being generated to capitalize MCL-1 as a future therapeutic target to restore salutary mitophagy; whereas in noncardiac systems, BCL-2 family inhibitors that specifically counter the antiapoptotic properties of MCL-1 are under trials for cancer treatment.
4. On the imaging front, novel-imaging modalities, such as 3-dimensional speckle tracking echocardiography, were used to predict recurrence in patients with AF.<sup>12</sup> A toolbox of fluorescent bioprobes was developed to track hexokinase isoforms and their translocation from mitochondria to cytoplasm in real time, which can be used for spatiotemporal dynamics studies in living cells.

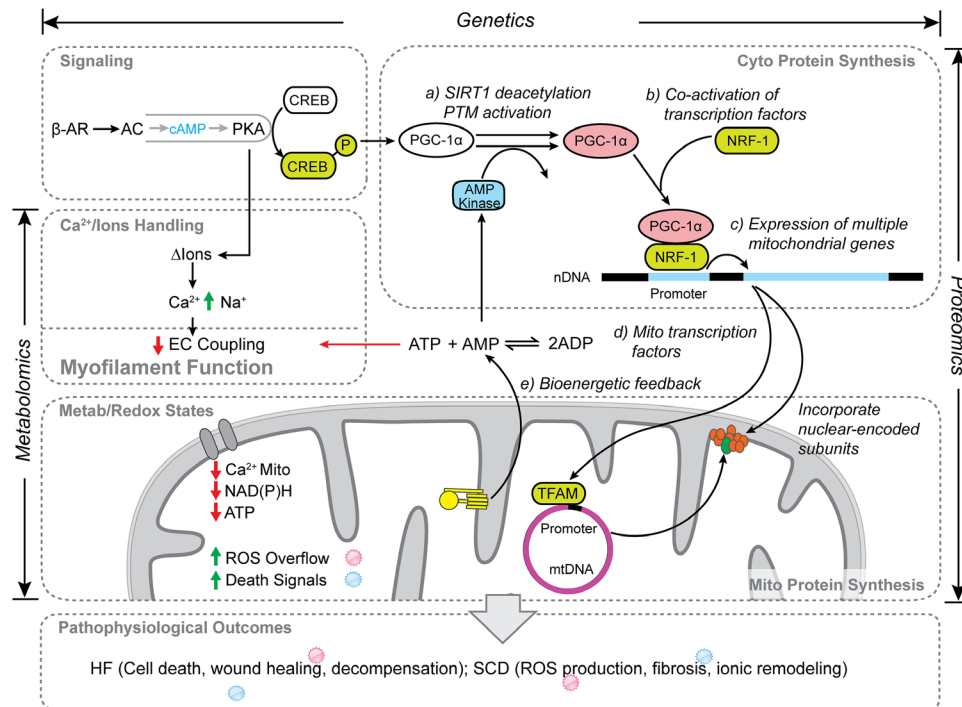
### Conclusion and Future Directions

The mitochondria in heart diseases initiative brought together 15 investigators whose expertise lies in diverse areas of redox biology, metabolism, transcriptional control, mass spectrometry, computational biology, clinical physiology, electrophysiology, and beyond. Many conceptual advances emerged from this work, which include the studies from multiple centers have consistently shown that changes in myocardial fuel utilization per se are not sufficient to drive HF. This suggests that the adult mammalian heart is capable of remarkable fuel utilization plasticity in a manner reminiscent of adaptive metabolic shifts in cancer. The projects also established the paradigm that alterations in ion homeostasis in the cytoplasm and mitochondrial matrix are the mechanisms behind impaired mitochondrial reactive oxygen species handling in addition to impaired energy supply/demand matching in HF.

Most importantly, these investigations unequivocally confirmed cardiac mitochondria as a rich source of future therapeutic targets in diverse cardiac diseases. This theme is now receiving great attention by increasing number of investigators in the field in search for transformative diagnostic/therapeutic avenues. For instance, mechanistic knowledge on mitochondrial redox balance, Ca<sup>2+</sup> flux, and MPT permits new strategies to correct specific defects and arrest disease progression. Newly identified molecular targets mediating ion transport across mito-membranes are leading to genetically engineered mouse models from several laboratories, which enable the testing of new concepts and hypotheses. The acknowledgment that defects in fuel/energy metabolism is key to dysfunction and failure of multiple cardiac subsystems is particularly transformative and has contributed to major new emphases with relevance to the development of novel therapeutics aimed at early HF, diabetes mellitus, and ischemic/reperfusion. Indeed, the once-esoteric field of bioenergetics has been transformed into a hot topic in cardiovascular research, drawing in diverse researchers interested in protein modification and turnover, organelle processing and autophagy, oxidative stress, necrotic and apoptotic cell death, energy balance, and ion homeostasis.

These works also reaffirm the power of systems-level investigations to identify unexpected hypotheses, pathogenic





**Figure 2 . Defining new network connections through multi-omics analysis, highlighting the role of mitochondria in heart failure.** EC indicates excitation-contraction; HF, heart failure; MCL-1, myeloid cell leukemia-1; mPTP, mitochondrial permeability transition pore; NRF-1, nuclear respiratory factor-1; PGC, peroxisome proliferator-activated receptor-gamma coactivator; PKA, protein kinase A; PTM, post-translational modification; ROS, reactive oxygen species; SCD, sudden cardiac death; SIRT1, sirtuin-1; and TFAM, mitochondrial transcription factor A.

mechanisms, and therapeutic targets. Complex network relationships are emerging that encompass multiple layers of details and molecules in cardiac mitochondria (Figure 2). Comparative analyses of genomic, transcriptomic, proteomic, and metabolomic profiles consistently conclude that the mitochondrial derangements so frequently observed in disease models cannot be predicted from single pathways alone, and that orthogonal multi-omics data are necessary if a more complete picture of the post-transcriptional and post-translational regulations of heart disease is to be pursued. This realization now compels investigators to delve deeper into the plasticity and dynamics of mitochondrial biomolecules with respect to their modifications, interactions, and spatiotemporal distributions in search of causal disease drivers. As simultaneous characterizations of multiple molecular parameters become the norm, the integration of previously insulated disciplines will enable more sophisticated questions to be asked, for example, whether global mitochondrial metabolite alterations could regulate post-translational modifications that contribute to vicious cycles of energy starvation or whether a systems biology strategy can be devised to screen for induced pluripotent stem cell-derived cardiomyocyte lines with preserved mitochondrial functions and resistance to injury.

The priority now is to work to translate the mechanistic insights obtained from the studies to new clinical interventions, such as mitochondrial targeted therapeutics, including both antioxidants and agents that target cardiolipin to enhance stability and function of the electron transport system. This work will require continuous collaboration and support similar to those

that materialized here. A consensus unanimously expressed by the investigators was that the organizational structure of the initiative has been especially propitious to foster cross-center/cross-disciplinary interactions. The frequent meetings and communications have stimulated conversations and organic exchanges of ideas that are driven by the common goal to understand a biological entity rather than the conventional boundaries of methodologies. By extension, these interactions created a fertile training environment, which conferred a collaborative mentality and scientific vision on the trainees who might be sustained throughout their research career. Future endeavors will likely also benefit from formal coordination of computational experts (eg, through specialized working groups) from the outset to promote the sharing of data science resources.

In summary, this initiative has resulted in opportunities extended to additional investigators of mitochondrial biology in both cardiovascular and noncardiovascular fields. Many regulatory principles in mitochondrial biology are important not only to heart diseases, but also to cancer and neurodegenerative disorders, which often present common molecular targets for intervention. Prime examples include bidirectional modulations of BCL-2 proteins to confer tumor suppression (promoting apoptosis) or cardioprotection (inhibiting); inhibition of MPT to mitigate ischemic/reperfusion and axonal degeneration; and the profound cardiac implications of mitophagy pathways originally expounded in familial Parkinsonism. Exciting opportunities exist that, through mutually beneficial exchange and collaborations with other mitochondrial experts, a new discipline of mitochondrial physiology can arise that has the same level of rigor and vigor as other current fields of biomedical

sciences and that will make substantial contributions to the medicine of multiple common disorders.

A list of 132 publications originating from this initiative can be found at <http://goo.gl/oJZYrb>.

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### Disclosures

None.

### References

1. Aubert G, Vega RB, Kelly DP. Perturbations in the gene regulatory pathways controlling mitochondrial energy production in the failing heart. *Biochim Biophys Acta*. 2013;1833:840–847. doi: 10.1016/j.bbamcr.2012.08.015.
2. Nemetlu E, Zhang S, Gupta A, Juranic NO, Macura SI, Terzic A, Jahangir A, Dzeja P. Dynamic phosphometabolomic profiling of human tissues and transgenic models by  $^{18}\text{O}$ -assisted  $^{31}\text{P}$  NMR and mass spectrometry. *Physiol Genomics*. 2012;44:386–402. doi: 10.1152/physiolgenomics.00152.2011.
3. Martin OJ, Lai L, Soundarapandian MM, Leone TC, Zorzano A, Keller MP, Attie AD, Muoio DM, Kelly DP. A role for peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth. *Circ Res*. 2014;114:626–636. doi: 10.1161/CIRCRESAHA.114.302562.
4. Korge P, Yang L, Yang JH, Wang Y, Qu Z, Weiss JN. Protective role of transient pore openings in calcium handling by cardiac mitochondria. *J Biol Chem*. 2011;286:34851–34857. doi: 10.1074/jbc.M111.239921.
5. Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL, Bers DM. Measuring local gradients of intramitochondrial  $[\text{Ca}^{2+}]$  in cardiac myocytes during sarcoplasmic reticulum  $\text{Ca}^{2+}$  release. *Circ Res*. 2013;112:424–431. doi: 10.1161/CIRCRESAHA.111.300501.
6. Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, Jimenez R, Petrosyan S, Murphy AN, Gustafsson AB. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem*. 2013;288:915–926. doi: 10.1074/jbc.M112.411363.
7. Liu T, Takimoto E, Dimaano VL, DeMazumder D, Kettlewell S, Smith G, Sidor A, Abraham TP, O'Rourke B. Inhibiting mitochondrial  $\text{Na}^{+}/\text{Ca}^{2+}$  exchange prevents sudden death in a Guinea pig model of heart failure. *Circ Res*. 2014;115:44–54. doi: 10.1161/CIRCRESAHA.115.303062.
8. Hsieh EJ, Shulman NJ, Dai DF, Vincow ES, Karunadharm PP, Pallanck L, Rabinovitch PS, MacCoss MJ. Topograph, a software platform for precursor enrichment corrected global protein turnover measurements. *Mol Cell Proteomics*. 2012;11:1468–1474. doi: 10.1074/mcp.O112.017699.
9. Zong NC, Li H, Li H, et al. Integration of cardiac proteome biology and medicine by a specialized knowledgebase. *Circ Res*. 2013;113:1043–1053. doi: 10.1161/CIRCRESAHA.113.301151.
10. Dai DF, Chen T, Szeto H, Nieves-Cintrón M, Kutuyavin V, Santana LF, Rabinovitch PS. Mitochondrial targeted antioxidant peptide ameliorates hypertensive cardiomyopathy. *J Am Coll Cardiol*. 2011;58:73–82. doi: 10.1016/j.jacc.2010.12.044.
11. Thomas RL, Roberts DJ, Kubli DA, Lee Y, Quinsay MN, Owens JB, Fischer KM, Sussman MA, Miyamoto S, Gustafsson AB. Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. *Genes Dev*. 2013;27:1365–1377. doi: 10.1101/gad.215871.113.
12. Mirza M, Caracciolo G, Khan U, Mori N, Saha SK, Srivathsan K, Altemose G, Scott L, Sengupta P, Jahangir A. Left atrial reservoir function predicts atrial fibrillation recurrence after catheter ablation: a two-dimensional speckle strain study. *J Interv Card Electrophysiol*. 2011;31:197–206. doi: 10.1007/s10840-011-9560-6.

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