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Essay

Evolution of Mitochondria as Signaling Organelles

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Mitochondria have primarily been viewed as bioenergetic and biosynthetic organelles that autonomously co-exist within the cell. However, the past two decades have provided evidence that mitochondria function as signaling organelles, constantly communicating with the cytosol to initiate biological events under homeostatic and stress conditions. Thus, the signaling function of the mitochondria may have been selected by nature from the inception of the early eukaryote, as discussed in this essay.

Mitochondria are popularly known as the "powerhouse" of the cell, as they typically generate the bulk of ATP used to maintain homeostasis and survival of mammalian cells (Pagliarini and Rutter, 2013). Additionally, mitochondria play an essential, albeit underappreciated, role in the biosynthesis of macromolecules, such as lipids, heme, and iron-sulfur clusters that have been studied for decades. These two major roles of the mitochondria, the production of energy and support of biosynthesis, make them central to diverse biological outcomes including proliferation, differentiation, and adaptation to stress. Classically, it is thought that the nucleus integrates commands to initiate cellular actions, and changes in mitochondrial metabolism occur simply as a consequence of changes in the nucleus. Mitochondria themselves are rarely considered to dictate commands or provide signals to change biological outcomes. However, should the cell commit to a process like proliferation or differentiation without adequate functioning mitochondria, then it would likely undergo a metabolic crisis resulting in cell death or senescence. Therefore, for optimal cell function, a health status feedback from the mitochondria should exist to act as a checkpoint prior to cellular action. This feedback is analogous to the fuel gauge on a car, which instructs the driver the distance he may drive.

Indeed, recent evidence indicates that mitochondria provide signals that may function as an early checkpoint prior to the propagation of diverse biological outcomes. This idea raises a few fundamental questions: (1) What are the primary mechanisms of communication from the mitochondria to the rest of the cell? (2) How does this mechanism of communication relate to the evolution of mitochondria from bacteria? (3) How does the cell respond to signals indicating the presence of unhealthy mitochondria?

To begin to answer these fundamental questions, it may be informative to first consider how the mitochondria became an essential part of eukaryotes. Unlike prokaryotes, all eukaryotic cells have evidence of possessing mitochondria during some point in their lifespan. Mitochondria likely originate from α-proteobacteria, which developed an endosymbiotic relationship with the host archaeon (Gray, 2012). The nature and benefit of this symbiosis is hotly debated. A long-held belief is that the α-proteobacteria originally provided ATP or detoxified reactive oxygen for their archaeon host. However, mitochondria possess abundant electron donors, which can provide electrons to oxygen to form reactive oxygen species (ROS) like superoxide, hydroxyl radicals, and hydrogen peroxide. Therefore, the mitochondria may actually toxify more than detoxify oxygen.

A second, speculative explanation of the original symbiosis may be that the α-proteobacteria and its host provided important metabolites for each other. As described in Müller and Martin's "hydrogen hypothesis" (Martin and Müller, 1998), eukaryotes may have evolved from a metabolic relationship between α-proteobacteria and a methanogenic archaeon (Martin and Müller, 1998). The α-proteobacteria produced hydrogen (H_2), carbon dioxide (CO_2), and possibly acetate as waste products, which the archaeon could utilize to conduct methanogenesis. As H_2 and CO_2 became limiting in the environment, the archaeon became dependent on the α-proteobacteria. Thus, there was strong selective force for the archaeon to localize near the α-proteobacteria or it would starve. Ultimately, the archaeon and α-proteobacteria fused, whereby the archaeon would provide organic compounds to the α-proteobacteria for continued generation of CO_2, H_2, and acetate. As this metabolic symbiosis evolved into the first eukaryotic cell, the mitochondria (i.e., α-proteobacteria) and their new host (i.e., archaeon) became dependent on each other and thus needed to develop mechanisms to communicate. To this day, these mechanisms are likely essential for eukaryotic cellular function.

In thinking about what modes of communication would have first evolved, it is important to consider that the host would want to assess mitochondrial function when it has diminished and not necessarily when it has completely ceased. This would allow the host to make the necessary decisions based upon the availability of mitochondrial function. One possible early messenger may be acetate, one of the original waste products of α-proteobacteria. In the cytosol, acetate is readily converted to acetyl-coA, which can be used for protein acetylation. In both prokaryotes and eukaryotes, protein lysine acetylation is prevalent and utilized to control diverse cellular functions including metabolism. Recent evidence in yeast indicated that acetate-derived acetyl-coA is a key regulator of the cell cycle through histone acetylation (Shi and Tu, 2015). To regulate histone acetylation, metazoans generate acetyl-coA from both acetate and citrate, a mitochondrial TCA cycle intermediate.

An alternative or additional messenger may be mitochondrial ROS. The rising oxygen levels in the eukaryotic environment likely selected for the emergence of the modern mitochondrial respiratory chain, which consumes oxygen to produce water and the ROS superoxide. There are two observations that support this idea. First, α-proteobacteria, such as *Paracoccus denitrificans*, have a similar respiratory chain to modern mitochondria and release superoxide as byproducts of respiratory chain flux under aerobic conditions (Henry and Vignais, 1980). Second, ROS, notably hydrogen peroxide, are known to oxidize specific cysteine residues within transcription factors activating prokaryotic genes (D'Autréaux and Toledano, 2007). Despite the historical notion that ROS are toxic to the cell, low levels of mitochondrial ROS in eukaryotes appear to fluctuate in response to stress and subsequently promote adaptation through protein oxidation. Thus, acetylation by acetyl-coA and oxidation of proteins by ROS are two plausible signals emanating from early eukaryotic mitochondria that may convey the initial and subtle changes in mitochondrial fitness to the rest of the cell (Figure 1).

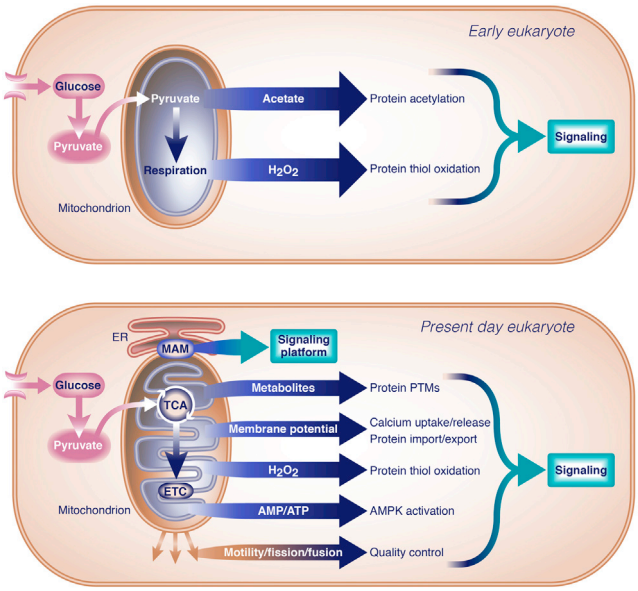

Figure 1. Multiple Modes of Mitochondrial-Dependent Signaling
Early eukaryote mitochondria generated acetate and hydrogen peroxide (H_2O_2) to induce protein acetylation and protein thiol oxidation as signaling mechanisms to communicate their fitness to the rest of the cell. Present-day mitochondria have multiple mechanisms to communicate their fitness, including the release of metabolites and ROS, activation of AMPK, peptides, as well as changes in inner mitochondrial membrane potential and calcium. Mitochondrial-associated membranes (MAMs) with other organelles such as the endoplasmic reticulum serve as a signaling platform. Mitochondrial dynamics (motility/fission/fusion) ensure the mitochondria deliver their appropriate signals in the proper location within cells as well as provide mechanisms to assess mitochondrial quality control.

As highlighted here, in metazoans, there are multiple other mechanisms of communication between the mitochondrion and the rest of the cell (Figure 1). Cytochrome *c* release from the mitochondria to the cytoplasm to induce cell death is a salient example of this communication. However, the release of cytochrome *c* is largely controlled by cytosolic factors. The intrinsic mitochondrial mechanisms that communicate their fitness to the rest of the cell include the release of TCA cycle metabolites and ROS, activation of AMPK, the discharge or uptake of calcium into the mitochondria, and changes in mitochondrial membrane potential. These mechanisms may function jointly—as the mitochondrial respiratory chain flux declines, the release of ATP, ROS, and TCA cycle metabolites may also decline. Decreased ATP and concurrent increased AMP activate AMPK, causing a switch from an anabolic to a catabolic state. Decreased ROS diminishes the activation of signaling pathways necessary for cell proliferation, differentiation, and metabolic adaptation (Sena and Chandel, 2012). A decrease in the TCA cycle intermediate citrate may result in decreased lipid biosynthesis necessary for cell growth as well as a decrease in protein acetylation (Wellen and Thompson, 2012). Furthermore, a decrease in mitochondrial respiratory chain flux reduces mitochondrial membrane potential. As a consequence, calcium uptake is decreased and the efficient import or export of proteins known to impact cell signaling from the mitochondria is prevented (Haynes et al., 2013; Patron et al., 2013). The inability of the mitochondria to buffer cytosolic increases in calcium levels can put the cell at risk for cell death. An interesting emerging idea is that mtDNA is released to regulate normal immune responses (West et al., 2015); peptides encoded by mtDNA such as humanin and MOTS-c (mitochondrial open reading frame of the 12S rRNA-c) may be released to prevent neurodegeneration and metabolic syndrome. A remarkable finding is that invoking mild mitochondrial stress initiates mitochondrial dependent signaling that promotes adaptive mechanisms to subsequent detrimental stress, termed mitohormesis (Yun and Finkel, 2014).

Beyond release of a specific entity from the mitochondria, the outer mitochondrial membrane serves as a scaffold for signaling complexes, notably immune responses and control of cell death. Mitochondrial membranes also associate with other organelle membranes, such as endoplasmic reticulum, referred to as mitochondria-associated membranes (or MAMs), to control signaling. It remains to be determined whether mitochondria communicate among themselves within a cell to coordinate signaling events, analogous to quorum sensing in bacteria. Recent studies have also demonstrated that mitochondrial dynamics (motility and fission/fusion) are important regulators of cellular signal transduction (Labbé et al., 2014). Mitochondrial motility ensures that signals are disseminated at the proper location. Mitochondrial fission and fusion are mechanisms by which mitochondrial quality control is assessed. Mitochondria undergoing fission are often eliminated from cells through mitophagy, while mitochondria undergoing fusion are protected from mitophagy.

Given the importance of functional mitochondria to the overall health of the cell, mechanisms must exist to prune dysfunctional mitochondria. What defines a dysfunctional mitochondrion? Conventionally, it is defined as a mitochondrion that has ceased to generate ATP. However, recent evidence suggests

that mitochondria can maintain biosynthetic function in the absence of ATP generation. In other words, some cells have the ability to derive all of their ATP from glycolysis and rely on mitochondrial biosynthesis for normal proliferation. Cessation of mitochondrial biosynthesis induces a catabolic state, whereby mechanisms are initiated to acquire the building blocks for macromolecules through other means such as autophagy or from the extracellular environment. However, collapse of mitochondrial membrane potential causes termination of mitochondrial protein import and export, and the generation of iron-sulfur cluster and heme proteins are compromised, which ultimately impairs cell functions (Veatch et al., 2009). Naturally, there are mechanisms to either preserve mitochondria, by inducing the mitochondrial heat shock proteins through the mitochondrial unfolded protein response (mtUPR), or eliminate mitochondria, those lacking a mitochondrial inner membrane potential, through mitophagy. This is in part mediated by a Parkinson-related protein called Parkin, which ubiquitinates mitochondria with a low mitochondrial membrane potential targeting them for degradation (Youle and Narendra, 2011). If this mechanism to clear dysfunctional mitochondria fails, low-membrane-potential mitochondria may accumulate and fail to efficiently import or export proteins from the mitochondria such as iron-sulfur clusters and hemes.

A pertinent question is whether the mitochondrion and the rest of the cell are still in a symbiotic relationship in mammalian cells. It is clear that the mitochondrion fulfills the biosynthetic and bioenergetic needs of the cell, but what needs does the cell fulfill of the mitochondrion? As noted above, mitochondria require a membrane potential across their inner membrane or risk being destroyed by mitophagy. Thus, substrates such as pyruvate are imported into the mitochondria to generate reducing equivalents NADH and $FADH_2$ that feed the electron transport chain (ETC) for the generation of an inner mitochondrial membrane potential. If the ETC is inhibited, such as in cells under ischemic conditions, the inner mitochondrial membrane potential is sustained by reversal of the mitochondrial ATP synthase. This process requires ATP import in the mitochondria. Cells even under nutrient restricting conditions ensure mitochondria receive adequate substrates primarily by inducing autophagy, which generates amino acids that feed the TCA cycle. A failure to provide substrates to maintain mitochondrial inner membrane potential can put the cells at risk of cell death by releasing cytochrome *c*. Furthermore, contents of the mitochondrial matrix, such as mtDNA and peptides generated from mitochondrial proteins, can activate improper immune responses to elicit a detrimental organismal inflammatory response in mammals. Thus, mitochondria are constantly fed substrates to sustain their inner mitochondrial membrane potential.

Going forward, new experimental approaches will be needed to distinguish the three distinct functions of mitochondria—bioenergetic, biosynthesis, and signaling—on biology, physiology, and pathophysiology. Current pharmacologic and genetic techniques utilized to inhibit mitochondrial proteins or ETC complexes can perturb all three functions. For example, ETC inhibition at a specific complex can result in simultaneous decrease in ATP production, biosynthetic activity of the TCA cycle, and ROS generation for signaling, making the interpretation challenging as to why ETC inhibition results in a specific phenotype. Moreover, the precise targets within the cytosol that propagates mitochondrial-dependent signal transduction as well as how the cytosolic factors control mitochondrial-dependent signaling remains to be uncovered. The deciphering of molecular determinants that govern mitochondrial-dependent signaling potentially can help alleviate stress induced pathologies.

In summary, mitochondria evolved to form a metabolic symbiosis with the cell, which necessitates communication between each symbiotic participant. There are likely multiple mechanisms yet to be uncovered that allow mitochondria to communicate their functional status to the rest of the cell. In the absence of this communication the cell would initiate activity without knowing whether it possesses sufficient energy and/or biosynthetic capacity. Therefore, cellular decision-making is driven not only by extracellular signals, but importantly, also by intracellular signals emitted from the mitochondria.

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