

Perspective

# Mitochondrial signal transduction

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## SUMMARY

The analogy of mitochondria as powerhouses has expired. Mitochondria are living, dynamic, maternally inherited, energy-transforming, biosynthetic, and signaling organelles that actively transduce biological information. We argue that mitochondria are the processor of the cell, and together with the nucleus and other organelles they constitute the mitochondrial information processing system (MIPS). In a three-step process, mitochondria (1) sense and respond to both endogenous and environmental inputs through morphological and functional remodeling; (2) integrate information through dynamic, network-based physical interactions and diffusion mechanisms; and (3) produce output signals that tune the functions of other organelles and systemically regulate physiology. This input-to-output transformation allows mitochondria to transduce metabolic, biochemical, neuroendocrine, and other local or systemic signals that enhance organismal adaptation. An explicit focus on mitochondrial signal transduction emphasizes the role of communication in mitochondrial biology. This framework also opens new avenues to understand how mitochondria mediate inter-organ processes underlying human health.

## INTRODUCTION

Our collective view of mitochondria evolved from that of dynamic cytoplasmic granules or “bioblasts”<sup>1</sup> to bean-shaped ATP-synthesizing chemiosmotic machines,<sup>2,3</sup> motivating the popular “powerhouse of the cell” analogy.<sup>4</sup> Subsequently, mitochondria became known as maternally inherited organelles<sup>5</sup> with their own genome, in which mutations can cause disease,<sup>6,7</sup> setting off the field of mitochondrial medicine. The imaging of living mitochondria dynamically exchanging proteins and DNA<sup>8</sup> and triggering death via propagating apoptotic waves<sup>9,10</sup> later sparked an era of mitochondria as dynamic organelles undergoing constant fusion/fission events, enabling functional complementation<sup>11,12</sup> and mitochondrial quality control.<sup>13</sup> Most recently, the omics era and a new quantitative handle on intermediate metabolism have cast mitochondria as biosynthetic and signaling organelles<sup>14,15</sup> that produce signals influencing cell and organism behaviors via metabolite/mitokine signaling,<sup>16–18</sup> mito-nuclear crosstalk,<sup>19–22</sup> and remodeling of the epigenomic machinery across species.<sup>23–26</sup> Through these theoretical transitions, mitochondria have become the most studied organelle in the biomedical sciences (Figure 1).

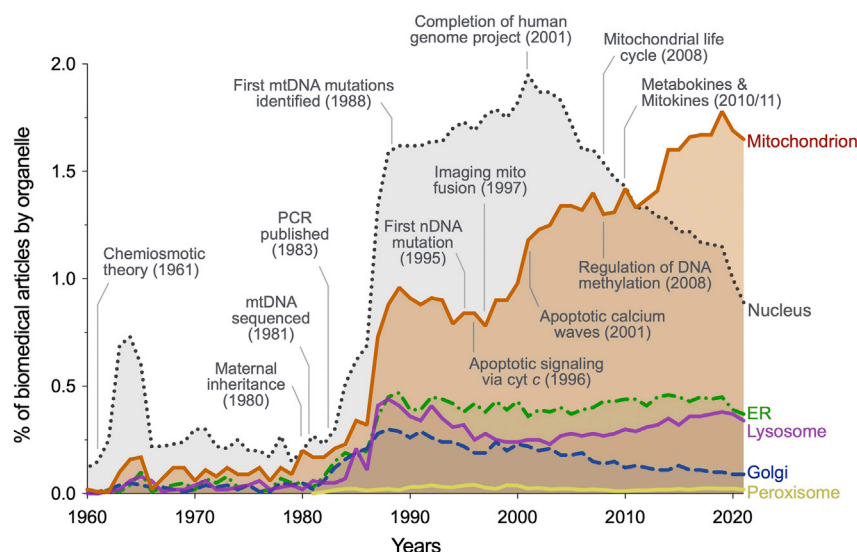
Outside of their intracellular roles as organelles, mitochondria also operate beyond the confines of the cell.<sup>27</sup> They undergo physical transfer from cell to cell,<sup>28–30</sup> influence neurotransmitter metabolism and inter-cellular communication at the neural synapse,<sup>31,32</sup> synthesize all circulating steroid hormones that ensure sexual reproduction and species survival in mammals,<sup>33</sup> and as

discussed below, even contain receptors for systemic hormones.<sup>34</sup> These discoveries are not only blurring cellular boundaries, but also revealing mitochondria in a light that emphasizes communication—i.e., the bidirectional transfer of information from organelle to organism—as a natural aspect of their biology.

It is a particularly exciting time for mitochondrial biology. Community resources like MitoCarta<sup>35</sup> and MitoCoP,<sup>36</sup> together with spectacular theoretical advances in mitochondrial research, have brought insights to diverse fields across the biological sciences and medicine.<sup>37</sup> This includes, but is not limited to, immunometabolism, behavioral neuroscience, and psychobiology. As examples, energy metabolism in general and mitochondria in particular play permissive and instructive roles in stem cell differentiation and in the acquisition of immunometabolic phenotypes,<sup>38</sup> influence whether animals are socially dominant or submissive,<sup>39</sup> and influence how multiple organ systems in mice respond to evoked stress.<sup>40</sup> In humans, mitochondrial energy production capacity also appears to dynamically respond to subjective psychosocial experiences,<sup>41,42</sup> providing a foundation to begin understanding the mind-mitochondria connection. These discoveries are contributing to mechanistically linking sub-cellular bioenergetic processes to physiological, health-related organismal phenotypes. Thus, scientific progress not only among mitochondrial biology but also more broadly in the biomedical sciences<sup>43</sup> has been and likely will continue to be catalyzed by increasingly accurate and integrative models of mitochondrial behavior.

In this perspective, we argue that as we move toward increasingly accurate mechanistic models of the role of





**Figure 1. Modern historical landmarks in mitochondrial research illustrate the need for an integrative view of this multifaceted organelle**

Proportion of biomedical publications by organelle, corrected for total published articles across biomedicine. Selected discoveries that challenged prior views about mitochondria are noted, as well as some historical landmarks for context. Figure adapted from Picard et al.<sup>390</sup> with data retrieved from <https://pubmed.ncbi.nlm.nih.gov/> on February 12, 2022.

mitochondria in human health, we need an understanding of mitochondrial behavior extending far beyond energetics. As echoed by others,<sup>44,45</sup> the “powerhouse” analogy promotes an overly simplistic picture of this beautifully complex organelle. The outdated mechanical analogy is too unidimensional to guide integrative scientific thinking. The challenge ahead is to integrate current prevailing perspectives of mitochondria as inherited, dynamic, energy-transforming, signaling organelles whose influence extends to all cellular compartments, and to the whole organism. Here we propose that our existing knowledge of mitochondrial biology can be integrated under the common framework of mitochondrial signal transduction. Consequently, a more integrative and accurate analogy portrays mitochondria as the processor of the cell—or more precisely as the mitochondrial information processing system (MIPS).

### WHY MITOCHONDRIAL SIGNAL TRANSDUCTION?

Signal transduction involves input-to-output transformation. It is a generalizable process in biology, taking place across all living, complex adaptive systems. Signal transduction allows single cells to sense, migrate toward, and respond to stimuli,<sup>46</sup> and enables organelle networks to interact and accomplish complex cellular operations that isolated organelles could not accomplish.<sup>47</sup> At the organ level, signal transduction also allows the brain to receive, integrate, and process multiple streams of sensory information to generate a coherent internal representation of the outside world.<sup>48</sup> This process is analogous to the way in which an antenna or sensor coupled to information processing systems—as in a cell phone, for example—receives and converts simple signals (e.g., radio waves) into intelligible outputs of different kinds (e.g., sounds, images, etc.). In the same way, cellular signal transduction systems such as the MIPS convert complex combinations of ions, proteins, nutrients, and energetic states into goal-driven genetic programs, which guide the reorganization of meta-

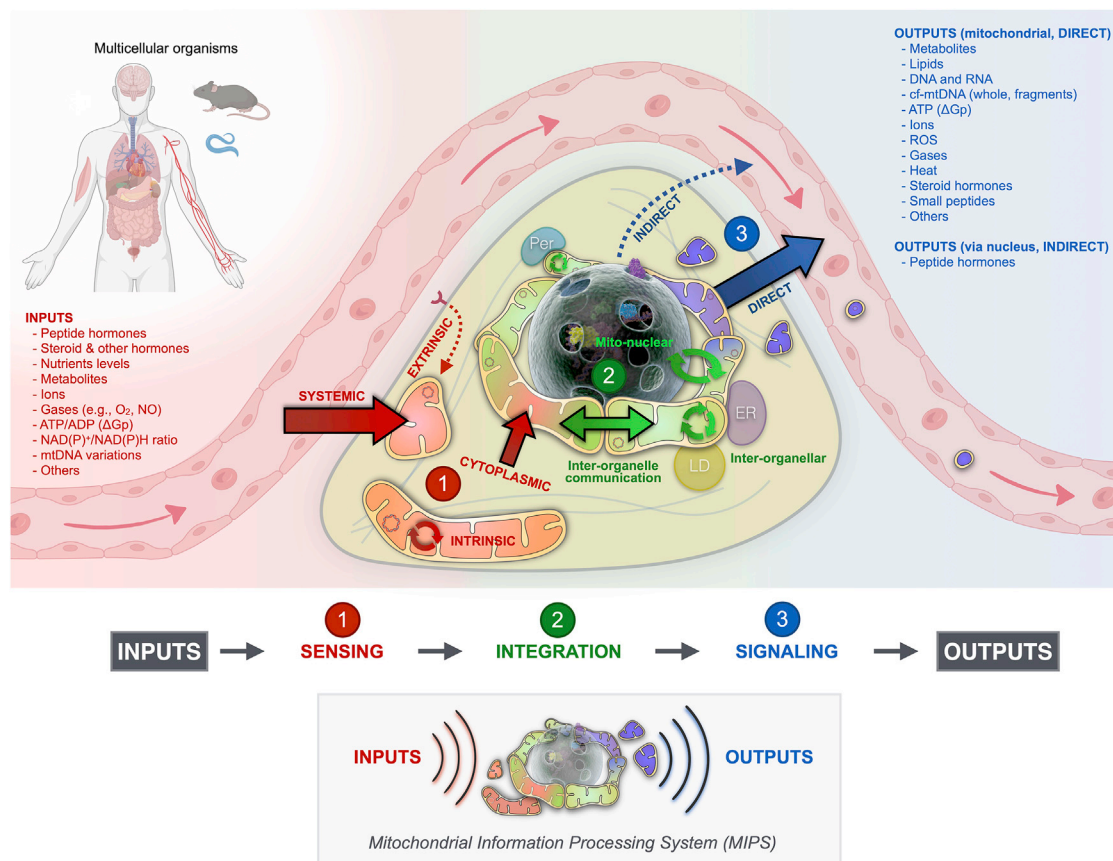
bolic pathways and drive adaptive behaviors (to grow and divide, contract, secrete, die, etc.). Signal transduction allows cells and organisms to respond and adapt to environmental demands.

Through evolution, the endosymbiotic incorporation of mitochondria marked the transition from a selfish unicellular world to a multicellular reality.<sup>49</sup> In multicellular organisms, a vital priority became the metabolic coordination and cell-to-cell cooperation toward a shared common goal—to sustain the organism. Cellular cooperation produced a sort of “social contract” among increasingly specialized cells.<sup>50</sup> Thus unified as an organism, cells make decisions not based solely on their individual states hardwired in the genome, but based on the collective state of the organism established through information exchange and communication between organs, cells, and organelles.<sup>51</sup> This collective principle implies that to ensure survival, cellular and organismal decisions must be matched to the local and systemic energetic constraints, which are reflected in the bioenergetic state of mitochondria.<sup>52,53</sup> For an organism, achieving faster responses to changing bioenergetic conditions means faster transitions to new optimal states. This, in turn, maximizes energetic efficiency and minimizes the risk of damage.<sup>54</sup> Therefore, the role of mitochondria in optimizing cellular and organismal behavior toward health—defined as optimal responsiveness to challenges<sup>55</sup>—requires mechanisms that transduce information, from organelle to organism.

### THE PILLARS OF MITOCHONDRIAL SIGNAL TRANSDUCTION

Before reviewing the specific molecular components and mechanisms that support signal transduction within the MIPS, we describe some general features of signal transduction. Signal transduction within the MIPS is an extension of the traditional process of receptor-mediated detection of (extra)cellular signals, signal amplification, and transduction into downstream secondary messengers (Figure 2). Mitochondrial signal transduction consists of three main processes:

- (1) Sensing: The ability of mitochondria to detect metabolic and hormonal inputs, and to transform these inputs into morphological, biochemical, and functional mitochondrial states.



**Figure 2. Three-step model of mitochondrial signal transduction**

As the mitochondrial information processing system (MIPS), mitochondria are input integrators and output generators. Within the cytoplasm, mitochondria are topologically positioned at the interface between incoming signals from the outside extracellular space and the inside compartment of the nucleus where the (epi) genome is stored. In a three-step process, mitochondria receive, integrate, and generate signals that contribute to cellular and organismal adaptation. All mitochondria have the potential to perform sensing, integration, and signaling steps. Here the contributions to signal transduction are color-coded and matched to specific topologies for illustrative purposes. ER, endoplasmic reticulum; LD, lipid droplet; Per, peroxisome; mtDNA, mitochondrial DNA; NO, nitric oxide;  $\Delta G_p$ , phosphorylation potential.

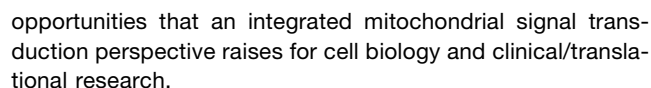
- (2) Integration: The pooling of multiple inputs into common effectors driven by the exchange of information among mitochondria and other organelles, and influenced by the current state of the mitochondrial network and of the cell.
- (3) Signaling: The production of mitochondrial outputs, or signals, that transmit information locally to direct metabolic pathway fluxes and influence other organelles, including nuclear gene expression, and systemically to regulate the physiology and organismal behavior.

The flow of information through the MIPS proceeds sequentially as follows: incoming signals are sensed by molecular receptors and biological structures on/within mitochondria (sensing), which exchange with each other molecular signals and labile states such as membrane potential via fusion/fission processes and other (non)physical interactions (integration), and which simultaneously release signaling factors such as metabolites, cofactors, proteins, nucleic acids, and heat that propagate information beyond the mitochondrial membranes (signaling). Figure 3 illustrates the repertoire of

known mitochondrial substrates and mechanisms available for signal transduction. This broad repertoire emphasizes communication at different levels of biological organization, including protein-protein interactions (molecular), inter-organelle communication (sub-cellular organelles), autocrine or inter-cellular paracrine transfer (cells), and endocrine information transfer (organs and systems).

In the following three sections, we first review the known molecular machinery responsible for mitochondrial input sensing (step 1), the processes enabling information integration within mitochondrial networks (step 2), and the resulting signals that communicate mitochondrial states intracellularly and systemically (step 3). To avoid the natural tendency to emphasize only specific well-known examples that would naturally narrow the spectrum of physiological processes to which mitochondrial signal transduction may apply, we discuss the mechanisms involved in the sensing, integration, and signaling stages sequentially. Recognizing that mitochondria are not all created equal, we then discuss tissue-specific mitochondrial features relevant to signal transduction. We close by considering outstanding questions and



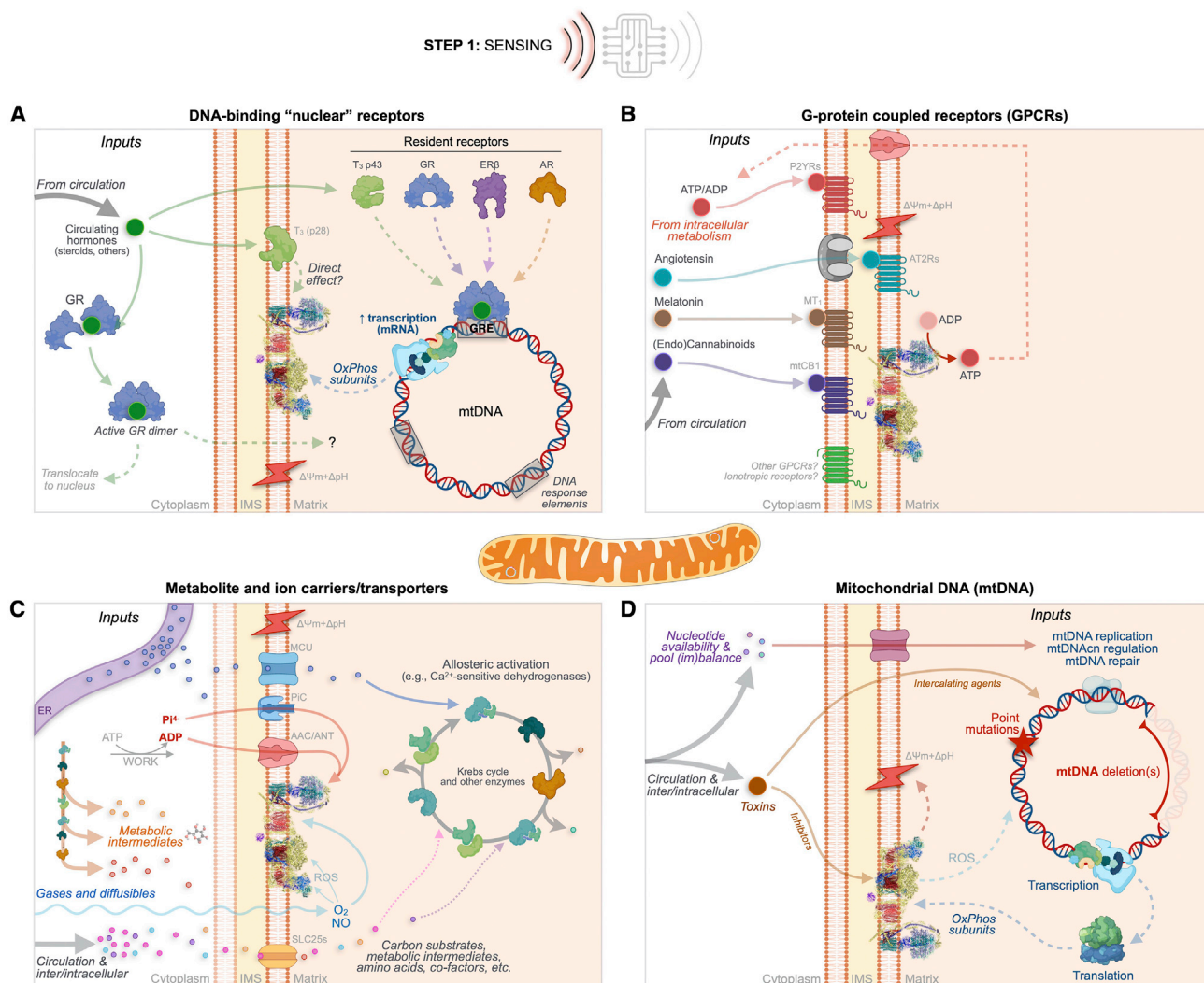


The main molecular features that enable mitochondrial sensing include traditional ligand-activated receptors, transporters, and biochemical reactions such as the oxidative phosphorylation (OxPhos) system within the outer and inner mitochondrial membranes (OMM and IMM) and the mitochondrial genome (Figure 4). These components enable mitochondria to rapidly and selectively sense changes in specific biochemical inputs. In the same way that a capsaicin receptor allows a sensory cell on the taste bud to depolarize and convert the spicy molecule into an action potential (recognizable as information by the brain), the mitochondrial sensing machinery converts simple inputs into biochemical, functional, and/or morphological changes (eventually converted to outputs that cells recognize). Mitochondrial inputs range in their nature from atoms, gases, and ions to small molecules and metabolites, proteins, lipids, DNA, and temperature, as well as physical interactions with surrounding organelles (Figure 3). In this section, we cover the molecular machinery that allows the MIPS to selectively sense and respond to extrinsic and intrinsic inputs (MIPS step 1 of 3).

Mitochondria contain ligand-activated transcription factors traditionally known as “nuclear” receptors. These receptors are expressed and generally reside in the cytoplasm or directly in mitochondria, homo- or hetero-dimerize upon ligand binding, and then translocate either to the nucleus or to the mitochondrial matrix where they interact with target DNA sequences. Limitations exist around the experimental evidence underlying the localization of these receptors within mitochondria, calling for further research using rigorous designs and sensitive molecular approaches.<sup>56</sup> The most well-documented examples include receptors for thyroid hormones, sex hormones (estrogen and androgen), and stress-related glucocorticoids (Figure 4A).

nuclear gene expression and directly on mitochondria. In isolated mitochondria, respiratory chain activity is modulated by triiodothyronine ( $T_3$ ) without changes in protein synthesis—taking place *in vitro* or “*in organello*”—substantiating the direct sensitivity of mitochondria to circulating thyroid hormones.<sup>57</sup> Two putative resident mitochondrial thyroid hormone receptors have been described. The p28 receptor likely resides in the IMM, lacks a DNA-binding domain, and binds  $T_3$  with high affinity.<sup>58</sup> It appears responsible for the rapidity (within 2 min *in vitro*, <30 min *in vivo*) with which mitochondrial respiration responds to thyroid hormone stimulation.<sup>59,60</sup> The other p43 receptor is a 43 kDa member of the c-Erb  $\alpha 1$  DNA-binding family residing in the mitochondrial matrix.<sup>61</sup> Mitochondria respond to  $T_3$  by increasing mitochondrial DNA (mtDNA) transcription and changing the ratio of mRNA and rRNA.<sup>62,63</sup> Most studies on mitochondrial  $T_3$  response have been performed on liver mitochondria, but mitochondrial thyroid sensing may exhibit high tissue specificity.<sup>64</sup> In mice, manipulating the expression of the mitochondrial  $T_3$  receptor p43 in different tissues triggers selective transcriptional and enzymatic effects on mtDNA-encoded Ox-Phos components (e.g., not on complex II proteins or activity),<sup>65</sup> as well as broad cellular and physiological effects,<sup>66</sup> highlighting the likely physiological significance of mitochondrial thyroid hormone sensing.

There are two isoforms of the estrogen receptor related to mitochondria: ER $\alpha$  and ER $\beta$ . They have relatively low homology for their ligand-binding domains and *trans*-activational domains, and



**Figure 4. MIPS step 1: Sensing**

As in excitable cells where a broad variety of chemical inputs (e.g., neurotransmitters) converge onto membrane potential variations, extrinsic and intrinsic MIPS inputs trigger molecular changes that converge into morpho-functional mitochondrial states. Mitochondria sense extrinsic and intrinsic information through four main classes of mechanisms.

(A) Canonical DNA-binding “nuclear” receptors for steroid hormones including glucocorticoids (GC), estrogen (ER), and androgen (AR) exist in mitochondria or can translocate upon ligand binding.

(B) G protein-coupled receptors (GPCRs) embedded within mitochondrial membranes including the angiotensin (AT<sub>1</sub>R and AT<sub>2</sub>R), the cannabinoid (mtCB1), melatonin (MT<sub>1</sub>), and purine (P2YRs) receptors, and possibly others (e.g., GPR35).

(C) Metabolite and ion carriers/transporters such as the ADP/ATP carrier protein (AAC, also adenine nucleotide translocator [ANT]) and the SLC25 family of transporters. Also shown are some gases and ions that either freely diffuse through the IMM or whose import/export is mediated by other carriers/transporters. (D) Acquired sequence variation in the mtDNA sequence, including mutations and deletions that cause functional changes within the OxPhos system. The top path shows nucleotide availability/imbalance, and the bottom path shows exogenous toxins that can interfere with electron transport chain function and secondarily cause mtDNA instability.

AAC/ANT, ADP/ATP carrier or adenine nucleotide translocator; AT<sub>2</sub>Rs, angiotensin receptors; AR, androgen receptor; ERβ, estrogen receptor beta; GR, glucocorticoid receptor; GRE, glucocorticoid response element (used here as an example for other gene regulatory elements); IMS, intermembrane space; MCU, mitochondrial calcium uniporter; mtCB1, mitochondrial cannabinoid receptor; MT<sub>1</sub>, melatonin 1 receptor; mtDNA, mitochondrial DNA; NO, nitric oxide; O<sub>2</sub>, molecular oxygen; OxPhos, oxidative phosphorylation; P2YRs, purine receptors; ROS, reactive oxygen species; SLC25s, solute carriers family 25; T<sub>3</sub>, triiodo-thyronine; ΔpH+ΔΨm, mitochondrial proton motive force.

therefore have strongly divergent functions.<sup>67</sup> ERα primarily influences nuclear gene expression, including activation of mitochondrial biogenesis and other aspects of mitochondrial biology,<sup>68</sup> whereas ERβ translocates to mitochondria and is found at high levels in murine and human mitochondria from neurons and cardiomyocytes.<sup>69</sup> Under baseline conditions in cancer cell lines, the

majority of ERβ is primarily located in mitochondria.<sup>70</sup> Activation of mitochondrial ERβ results in anti-apoptotic effects by disrupting Bad-Bcl-X(L) and Bad-Bcl-2 interactions.<sup>71</sup> Estrogen receptor signaling may also increase mitochondrial OxPhos capacity in genetically compromised cells from patients with Leber’s hereditary optic neuropathy (LHON), either directly by acting on the

mitochondrial OxPhos system or indirectly by reducing oxidative stress.<sup>72</sup>

Mitochondria sense androgenic hormones via the canonical androgen receptor (AR). In cultured prostate cells, a substantial fraction of total AR may localize to mitochondria.<sup>73</sup> The mitochondrial AR influences mtDNA levels, mtDNA transcription and translation, and RC protein abundance and complex activity.<sup>73</sup> This enables both genetic and functional mitochondrial responses to circulating androgen levels. In the human sperm midpiece, both the AR and ER $\beta$  localize to mitochondria.<sup>74</sup> Mitochondrial responses to estrogens and androgens via these DNA-binding receptors may in part account for sexually dimorphic mitochondrial features and functions.<sup>75,76</sup>

Mitochondria also contain the glucocorticoid receptor (GR) and thus can respond to glucocorticoid hormones, including the psychological stress mediator cortisol and corticosterone. Two major GR isoforms have been defined, GR $\alpha$  (predominant, ~90% of transcripts) and GR $\beta$  (minor, ~10%), which differ only in their distal domain from exon 9 alternative splicing.<sup>77</sup> Another isoform, GR $\gamma$ , is produced through alternative splicing (includes an intronic codon between exon 3 and 4) and differs from GR $\alpha$  and GR $\beta$  isoforms by a single amino acid.<sup>78</sup> Within mitochondria, the active GR receptors interact with mtDNA glucocorticoid response element (GRE) sequence motifs to influence mitochondrial rRNA synthesis<sup>79</sup> and gene expression. The mtDNA contains 8 putative GREs: 2 within the D loop,<sup>80</sup> 1 in the 12S rRNA, 1 in tRNA<sup>Leu</sup>(UUR), 3 in COX I, and 1 in COX III.<sup>81</sup> Compared to the nuclear genome, which contains approximately 680 GREs (1 GRE for every ~37 protein coding gene), the abundance of GREs in mtDNA is substantially higher, at 1 mtDNA GRE for every ~1.6 protein coding gene.<sup>82</sup> Mitochondrial glucocorticoid sensing is likely primarily mediated by GR $\gamma$ ,<sup>83</sup> acting on D loop GREs to promote expression of all polycistronic rRNA and mRNA genes.<sup>84</sup> It is worth noting that reduced mitochondrial GR localization may occur under certain pathological states, potentially hindering the mitochondrial sensing of glucocorticoid levels.<sup>85</sup>

Thus, conserved DNA-binding receptors allow mitochondria to sense the broad class of metabolism-regulating, sex- and stress-related hormones conveying systemic information about the state of the organism to core biochemical and genetic elements within the MIPS.

### G protein-coupled receptors

The mitochondrial sensory system also includes one of the evolutionarily more recent innovations, the G protein-coupled receptors (GPCRs). Mitochondrial GPCRs sit in the OMM and IMM and are specific to hormones such as angiotensin II,<sup>86</sup> melatonin,<sup>87</sup> endocannabinoids,<sup>88</sup> and purines.<sup>89</sup> Mitochondria-localized GPCRs influence core mitochondrial functions including ion uptake, OxPhos, nitric oxide synthesis, apoptotic signaling, and reactive oxygen species (ROS) production,<sup>90</sup> illustrating their potentially broad action spectrum (Figure 4B).

Angiotensin type 1 and 2 receptors (AT<sub>1</sub>R and AT<sub>2</sub>R) are present on both the nuclear membrane and on the IMM.<sup>91</sup> Despite lacking a canonical mitochondrial targeting sequence, transfection of full-length GFP-tagged AT<sub>2</sub>R naturally results in their mitochondrial localization.<sup>86</sup> Mitochondrial AT<sub>2</sub>R appears to colocalize with its endogenous ligand Angiotensin II (thus forming an

endogenous renin-angiotensin system) in several cell types including (from most to least abundant) mouse hepatocytes, cardiomyocytes, and renal tubule cells, and in human monocytes.<sup>91</sup> Functionally, activation of AT<sub>2</sub>R on isolated mitochondria was found to increase nitric oxide production by ~25% with a proportional decrease in complex I-driven O<sub>2</sub> consumption capacity,<sup>86</sup> supporting the physiological effects of the angiotensin GPCR on mitochondrial oxidative capacity.

Mitochondria also contain the functional melatonin GPCR MT<sub>1</sub>. In mouse brain neuron mitochondria, MT<sub>1</sub> is located on the OMM with its signal transduction apparatus coupled to cyclic AMP in the intermembrane space.<sup>87</sup> In isolated mitochondria, activation of MT<sub>1</sub> by melatonin could partially inhibit permeability transition and subsequent cytochrome c (Cyt c) release, and expectedly conferred downstream protection from ischemic injury, permeability transition pore (PTP) opening, and subsequent cell death.<sup>87</sup> Other potential functions of mitochondrial melatonin signaling involve redox modulation as melatonin is a potent antioxidant,<sup>92</sup> and may also include stimulation of mitochondrial biogenesis.<sup>93</sup> However, further research in neurons and other cell types is needed to disentangle the effects of MT<sub>1</sub> signaling on mitochondria versus on the plasma membrane.

Another functional GPCR localized to mitochondria is the type I cannabinoid receptor (mtCB1). mtCB1 is expressed in neurons,<sup>88</sup> astrocytes,<sup>94</sup> and skeletal muscle myofibers.<sup>95</sup> Similar to CB1 localized at the plasma membrane of neuronal synapses where their signaling inhibits neurotransmitter release, mtCB1 receptors on the OMM signal through intra-mitochondrial G $\alpha$ i protein activation and inhibition of soluble-adenylyl cyclase (sAC),<sup>34</sup> which inhibits protein kinase A (PKA)-dependent phosphorylation of target OxPhos subunits.<sup>34</sup> Functionally, mitochondrial endocannabinoid sensing through CB1 reduces complex I activity and mitochondrial respiration.<sup>88</sup> In mice, the effects of mtCB1 signaling affect neuronal function and memory formation,<sup>34</sup> suggesting that mitochondrial endocannabinoid sensing also has downstream effects on the behavior of the organism.

Mitochondria sense and respond to cytoplasmic purine (ATP, ADP, and AMP) levels via the purine GPCRs P2Y<sub>1</sub> and P2Y<sub>2</sub>. These receptors, whose precise sub-organellar location remains unclear, have been suggested to be coupled to phospholipase C (PLC) and downstream regulation of the mitochondrial calcium uniporter (MCU).<sup>89</sup> In hepatocytes, activation of mitochondrial P2Y<sub>1</sub> stimulates Ca<sup>2+</sup> uptake whereas activation of P2Y<sub>2</sub> inhibits uptake.<sup>96</sup> A receptor of the same family, the P2X<sub>7</sub> ionotropic purinoceptor (P2Y7R), also appears to be present in both the plasma membrane and in the OMM of cultured mouse and human cells.<sup>97</sup> Deletion of P2X<sub>7</sub> reduces transmembrane potential and respiratory capacity and leads to the accumulation of NADH (i.e., reductive stress) likely secondary to complex I inhibition.<sup>97</sup> The presence of surface ADP-sending GPCRs in mitochondria, along with the ADP/ATP carrier and F<sub>1</sub>/F<sub>0</sub> ATP synthase system (discussed below), illustrates the potential value of redundant mechanisms allowing the MIPS to sense particularly critical cytoplasmic signals such as the cytoplasmic ADP:ATP ratio.

Other receptors may also localize to the MIPS. For example, GPCRs internalized from the plasma membrane, such as the kynurenic acid-activated GPR35, may translocate to the OMM and modulate the OxPhos system under stress conditions.<sup>98</sup> The ligand-gated ion channel  $\alpha$ 7 nicotinic acetylcholine receptor



(nAChR) on the OMM may also enable mitochondria to sense acetylcholine (and nicotine) to modulate  $\text{Ca}^{2+}$  transients, permeability transition, and mtDNA release.<sup>99,100</sup> Additional work is required to discover and validate additional mitochondrial receptors, and to determine the ligand specificity and functional significance of mitochondrial GPCRs as components of the mitochondrial sensing machinery.

### Metabolite signaling

In a process highly integrated with cytoplasmic micronutrient sensors such as mTORC1 and AMPK, mitochondria sense and respond to metabolite levels and availability through specific carriers and transporters embedded within the IMM (Figure 4C). One of the classic MIPS inputs that triggers responses among the OxPhos system and multiple downstream mitochondrial processes is the phosphorylation potential ( $\Delta\text{Gp}$ ), reflected simplistically in ADP levels.<sup>101</sup> An increase in cytoplasmic ADP concentration (or more accurately a decrease in  $\Delta\text{Gp}$ , or ATP:ADP ratio) is rapidly transmitted to the mitochondrial matrix via monomers of the ADP/ATP carrier spanning the IMM.<sup>102</sup> This sets the rotary  $\text{F}_0\text{F}_1$  ATP synthase into motion, transiently dissipating the protonmotive force ( $\Delta\text{pH} + \Delta\psi_{\text{m}}$ ; pH gradient + mitochondrial membrane potential).<sup>103</sup> In this single step, the shift in ATP/ADP levels causes a consequential thermodynamic shift that sends biochemical ripples sequentially accelerating (1) proton pumping and electron flow by respiratory chain complexes I, III, and IV; (2) the reduction of  $\text{O}_2$  to  $\text{H}_2\text{O}$  by Cyt c oxidase (complex IV); and (3) the oxidation of reducing equivalents NADH and  $\text{FADH}_2$  at complexes I and II, respectively, thus (4) driving the oxidation of metabolic intermediates in the TCA/Krebs cycle, which in turn (5) increases  $\text{CO}_2$  production and (6) increases the diffusion gradient for the uptake of cytoplasmic substrates/metabolites into the matrix.<sup>101</sup> In isolated mitochondria, this ADP-induced change in state from low flux to actively respiring mitochondria is associated with profound conformation changes of the mitochondrial cristae membranes—from the orthodox to condensed state.<sup>104</sup> This simple example illustrates the breadth and complexity of ultrastructural and biochemical responses that a single input, such as ADP, can elicit within the MIPS.

Mitochondrial metabolite uptake and sensing are broadly enabled by >50 proteins from the SLC25 family of transporters, known as the mitochondrial carrier system (MCS).<sup>105</sup> These transmembrane IMM proteins are essential to both MIPS sensing and also to fulfill the biosynthetic functions of mitochondria, allowing the export of the ingredients for growth and repair, i.e., several amino acids and one-carbon carrying folate forms, carboxylic acids, fatty acids, cofactors, inorganic ions, and nucleotides to the whole cell.<sup>106</sup> In relation to mitochondrial sensing, the MCS provides sensory capacity to small molecule metabolite carriers including pyruvate (MPC1/2),<sup>107,108</sup> dicarboxylate (malate-phosphate, DIC) and tricarboxylate (citrate-malate, CTP) carriers, aspartate-glutamate (AGC1/2), the phosphate carrier<sup>105</sup> and NAD carrier,<sup>109</sup> and many others. Some mitochondrial transporters are often alternatively expressed variants of genes encoding plasma membrane carriers, as for the mitochondrial glutamine transporter that allow mitochondria to respond directly to cytoplasmic glutamine levels.<sup>110</sup> In cancer cells, the diverse MCS enables mitochondria to sense cytoplasmic levels of key amino acids via the TCA cycle, and to un-

dergo secondary morphological changes.<sup>111</sup> Thus, metabolite carriers and transporters allow the MIPS to sense and respond to inputs from numerous metabolic pathways.

We also note that the bioenergetic state of the cell exerts regulatory control on dozens of mitochondrial enzymatic reactions. As a result, high ATP:ADP and NADH:NAD<sup>+</sup> ratios favor anabolism and promote export of metabolites, shifting metabolism toward non-oxygen-dependent glycolysis and biosynthesis.<sup>112</sup> In cases of OxPhos defects, NADH accumulates and reduce the availability of NAD<sup>+</sup>, causing reductive stress,<sup>113,114</sup> driving the flux of metabolic pathways toward mitochondrial anabolic pathways contributing to disease.<sup>115,116</sup> The collective action of metabolic inputs on mitochondrial OxPhos and the NADH:NAD<sup>+</sup> ratio also translates into the availability of downstream substrates, including coenzyme A (CoA) and NADP(H). Changes in CoA availability affect the conversion of acetyl to acyl-CoA used to post-translationally acetylate and functionally modulate not only cytoplasmic enzymes and nuclear chromatin,<sup>117</sup> but also dozens of mitochondrial proteins.<sup>118</sup> Together, these biochemical dial systems provide a set of molecular cascades that dynamically integrate and convert biochemical inputs into functional mitochondrial recalibrations,<sup>119</sup> thereby allowing the MIPS to sense a broad array of biochemical inputs about the dynamic bioenergetic state of the cell.

### Electrophysiology of mitochondria: Ion signaling

Mitochondria sense and respond to ions, which is a logical consequence of the relatively high membrane potential across the IMM, generating a large diffusion potential for charged atoms and molecules (Figure 4C). One of the most studied examples of ionic mitochondrial sensing is calcium ( $\text{Ca}^{2+}$ ). The MCU enables rapid  $\text{Ca}^{2+}$  uptake within seconds.<sup>120,121</sup> Mitochondrial  $\text{Ca}^{2+}$  uptake from the cytoplasm and ER triggers rapid changes in mitochondrial physiology through post-translational modifications (PTMs) of dehydrogenases that increase TCA cycle activity<sup>122</sup> and results in membrane potential changes.<sup>123</sup> Although genetic ablation of the MCU is not lethal in most mouse strains, its loss prevents mitochondria from sensing surrounding  $\text{Ca}^{2+}$  levels<sup>123</sup> and may impair mitochondrial fusion during cell-cycle division,<sup>124</sup> highlighting the significance of mitochondrial  $\text{Ca}^{2+}$  sensing on the MIPS.

Mitochondrial  $\text{Ca}^{2+}$  is also linked with sodium ( $\text{Na}^+$ ) signaling. The organellar  $\text{Ca}^{2+}$  levels are affected by the activity of the mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCLX). NCLX sits in the IMM and extrudes matrix  $\text{Ca}^{2+}$  in exchange for cytoplasmic  $\text{Na}^+$ .<sup>125</sup> Entry of sodium into the cytosol during an action potential in neurons, or in response to glucose stimulation in the pancreatic beta cell, triggers the extrusion of calcium from the mitochondria, preventing mitochondrial calcium overload and subsequent cell death.<sup>126</sup> By lowering the intra-mitochondrial  $\text{Ca}^{2+}$  levels ( $[\text{Ca}^{2+}]_{\text{mito}}$ ) in cardiomyocytes and brown adipocytes, NCLX activity thereby decreases intra-mitochondrial calcium levels and directly regulates mitochondrial PTP (mPTP) dynamics and downstream signaling.<sup>127,128</sup> But in addition to decreasing  $[\text{Ca}^{2+}]_{\text{mito}}$  levels, NCLX activity rapidly elevates matrix  $[\text{Na}^+]$  levels,<sup>129</sup> likely preventing collapse of the MIPS through the flux of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ions.

Mitochondria sense and also respond to surrounding concentrations of various atoms and ions including magnesium (Mn),

inorganic phosphate (Pi), chloride (Cl), iron (Fe), and possibly others including lithium (Li),<sup>130</sup> although the underlying mechanisms for the most part are not resolved. Mitochondria are also sensitive to divalent gases such as nitric oxide (NO), which acts directly on complexes I and IV by chemically modifying sensitive residues, thereby modulating respiration.<sup>131</sup> One fairly well-studied input is molecular oxygen (O<sub>2</sub>), which is sensed directly by a combination of complex I-<sup>132,133</sup> and complex III-derived<sup>134</sup> bursts of ROS during hypoxia. In low-oxygen conditions (hypoxia), acidification of the mitochondrial matrix in a complex I-dependent manner solubilizes intra-mitochondrial calcium-phosphate deposits, which increases soluble [Ca<sup>2+</sup>]<sub>mito</sub> (even in the absence of MCU). In mouse embryonic fibroblasts, hypoxia activates NCLX and causes a 2- to 3-fold increase in mitochondrial matrix Na<sup>+</sup>, which interacts with IMM phospholipids to decrease membrane fluidity and promote superoxide formation by semiquinone,<sup>129</sup> transforming information about oxygen availability into molecular information within the MIPS. Mitochondrial O<sub>2</sub> sensing undoubtedly complements cytoplasmic sensors such as the hypoxia-inducible factor 1 alpha (HIF-1α) pathway that primarily act on the nucleus.<sup>135</sup> Overall, several evolutionarily ancient ion channels, transporters, and mechanisms based on chemical modifications thus ensure that mitochondria can sense and rapidly respond to their surrounding intracellular ionic environment.

### Intrinsic mtDNA defects

In addition to cytoplasmic signals sensed through canonical receptors and carriers, mitochondria also dynamically recalibrate their structure and internal processes in response to *intrinsic* signals, such as those arising from the mitochondrial genome (Figure 4D). The mtDNA codes for 37 canonical genes, including 13 protein-coding mRNA sequences,<sup>136</sup> plus small mitochondria-derived peptides (MDPs).<sup>137</sup> As the mtDNA can be affected by external factors (e.g., mutagens, nucleotide availability) and produce outputs (RNA, proteins) that influence and shape the OxPhos system and downstream mitochondrial behaviors, the mtDNA is a component of the mitochondrial sensing system.

Defects in the mtDNA sequence, which are either inherited<sup>138</sup> or acquired,<sup>139</sup> alter the synthesis of OxPhos subunits, which consequently impair respiratory capacity, OxPhos function, and ΔΨ<sub>m</sub>, culminating in disease.<sup>140,141</sup> Because most biochemical reactions taking place within mitochondria are directly or indirectly linked to OxPhos and ΔΨ<sub>m</sub>, including substrate and ion uptake, mtDNA perturbations have widespread consequences for several metabolic pathways.<sup>113,142</sup> Within skeletal muscle, for example, acquired somatic mtDNA deletions can disrupt OxPhos enzyme activities and trigger local mitochondrial proliferation when in proximity to a nucleus<sup>143</sup> (see section on [mitochondrial signaling](#)). Milder mtDNA variants are also sensed, are translated in metabolite levels,<sup>144</sup> and result in variation in cellular and organismal phenotypes that influence lifespan and disease risk.<sup>145,146</sup> Moreover, because some mutagens and toxins may preferentially affect the mtDNA relative to the nuclear DNA—in part owing to the negatively charged matrix compartment (which attracts positively charged molecules), rapid replication, poor repair, or other factors—the mitochondrial genome maintenance and expression systems may act as a cellular “sentinel” of genotoxic stress.<sup>147</sup>

Certain intrinsic mitochondrial inputs such as nucleotide availability and genotoxic molecules may not influence any of the mitochondrial biochemical processes directly, and may in fact only be sensed through the (replicating) mtDNA. For example, low nucleotide availability impairs OxPhos function specifically through the decline in mtDNA copy number,<sup>148</sup> which may or may not induce nucleotide salvage or cytoplasmic nucleotide synthesis pathways. We propose that the mtDNA replisome is an actively communicating structure, which tunes its function based on cellular nucleotide pools and the local mtDNA expression machinery. The actual signals contributing to mtDNA communication within the MIPS are still poorly understood.

### Summary of mitochondrial sensing

Mitochondria are equipped with a surprisingly wide variety of receptors and molecular features that give them the ability to sense hormonal, metabolic, ionic, genetic, and other inputs. With such sensitivity to a broad spectrum of inputs, the MIPS senses both the local biochemical conditions surrounding each organelle and systemic neuroendocrine signals produced in distant anatomical locations of the organism: by other cells, within other organs. Mitochondrial behavior is therefore not only driven by changes in nuclear gene expression—which produce the sensing components—but also more acutely and reversibly by biochemical and endocrine inputs that dynamically modulate their biochemical, genetic, ultrastructural, and physiological properties.

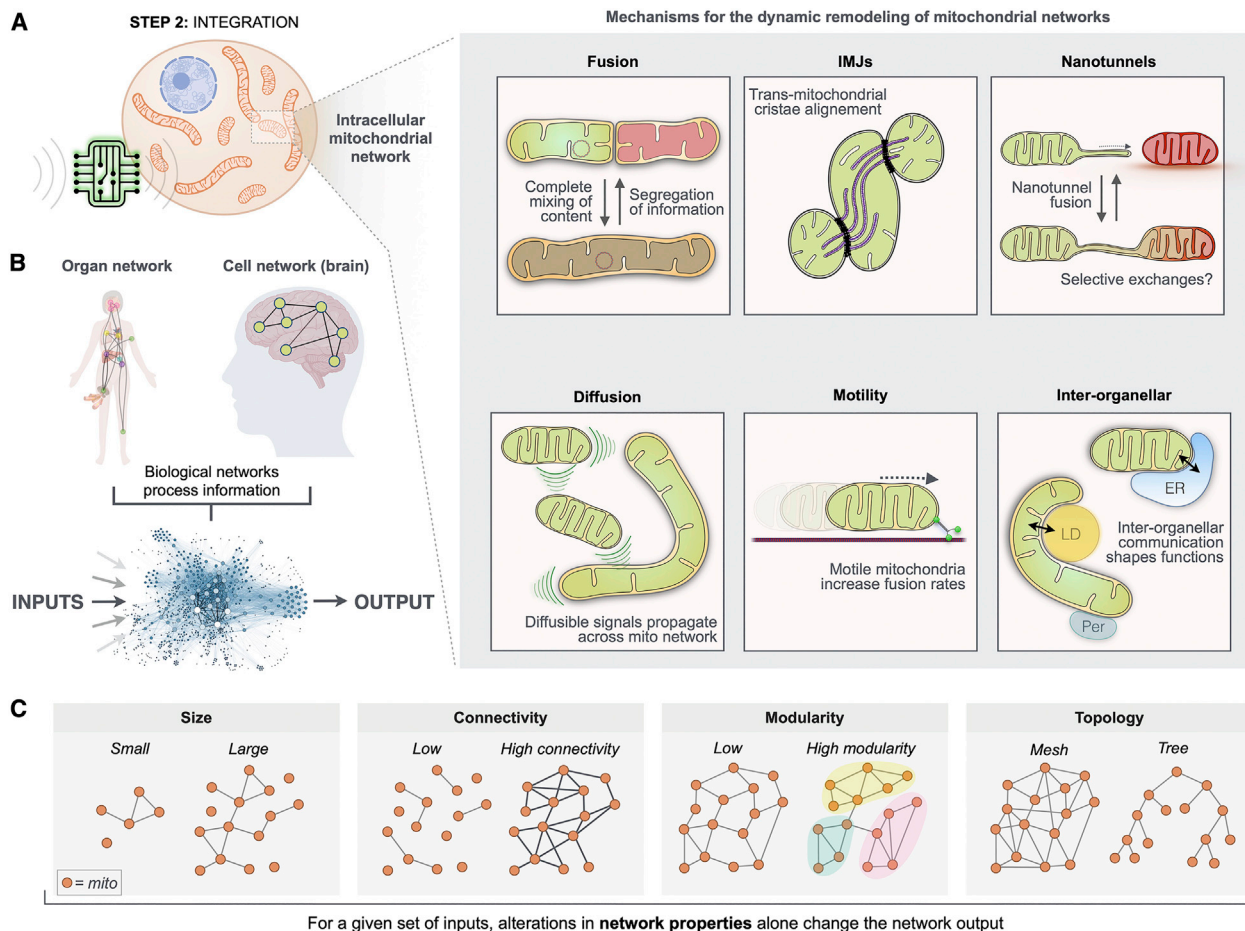
The evolutionary co-opting of a variety of DNA-binding receptors, GPCRs, and transporters suggests that increasing the range of inputs that mitochondria were capable of sensing must have positively contributed to the organism’s adaptive capacity. As a result, the diverse mitochondrial sensing machinery has been evolutionarily selected and likely also enriched in mitochondrial membranes relative to other organelles. Defining the full spectrum of inputs directly sensed by the MIPS across different cell types is an outstanding research challenge. Expanding our understanding of the inputs that directly shape mitochondrial biology could illuminate new disease pathways, independent or upstream of OxPhos or other well-defined disease-causing mechanisms.

Next, we turn our attention to dynamic factors that physically and functionally connect sensing mitochondria as interactive networks capable of signal processing and integration.

### MITOCHONDRIAL SIGNAL INTEGRATION

In its simplest form, signal integration is the process by which inputs are converted into common second messengers containing transformed information about the inputs. For example, within cells, multiple cell surface receptors converge on the production of common chemical secondary messenger molecules such as cyclic AMP or Ca<sup>2+</sup>, which in turn trigger broad-acting downstream response(s).<sup>149</sup> Because secondary messengers are shared products for multiple receptors, multiple stimuli converge on the same signaling hubs. Another good example of this concept takes place in neurons: dozens of neurotransmitters and modulators signal via ionotropic and metabotropic receptors to converge on a single cellular property—the plasma membrane potential. The temporal combination of inputs determines





**Figure 5. MIPS step 2: Signal integration**

The physical and functional binding of multiple energized units (mitochondria) into sparsely connected networks naturally gives rise to signal integration.

(A) Mechanisms of mitochondrial network remodeling and inter-organellar communication (mito-mito, mito-other organelles) among the MIPS.

(B) Conceptual representation of the organism's organ network and of the brain, where information from one group of units (e.g., neurons) is transmitted to other units, giving rise to computational agents. Information processing is not a private property of brains; it is a generalizable property of all life forms.

(C) Four examples of network properties that may be used to define the organization of mitochondrial collectives processing biochemical, metabolic, endocrine, and other inputs into coherent outputs.

whether or not an action potential is generated.<sup>150</sup> As a result, in neural networks (as in mitochondrial networks) membrane potential serves as an integrating hub for signal transduction. The convergence of inputs onto chemical second messengers and membrane potential thus allows cells to produce coherent, integrated, and robust responses simultaneously shaped by multiple inputs.

Another core concept for signal integration is that large-scale functional networks bind small competent units into larger scale computational agents.<sup>51</sup> Cells and organs integrate and compute information as cell collectives.<sup>151</sup> For instance, in the brain no single neuron (unit) can accomplish the sophisticated brain computations required to coordinate and sustain the rest of the body. Glial cells and neurons accomplish remarkable feats of integration through cell-to-cell communication, creating a functional collective (the brain) that naturally integrates or computes information.<sup>48</sup> Similarly, mitochondria are functionally linked and operate as “social” collectives within the cell cytoplasm.<sup>152</sup> For our purposes, integration refers to the functional

computations (i.e., the transformation of inputs into outputs) that take place within the MIPS between the sensing and signaling steps.

A third and final relevant concept to signal integration states that computational processes are influenced by the structural properties of the network itself—i.e., how individual units are arranged and connected relative to other ones.<sup>153,154</sup> The interactions between mitochondria, defined as the probability of direct information exchange between individual organelles, is termed “connectivity.” Across physical, biological, and social networks, the extent and nature of the connectivity between units largely define the network properties<sup>155,156</sup> (Figure 5). Thus, the information processing capacity of the MIPS, created by networks of co-existing mitochondria, must be determined jointly by both the intrinsic properties of mitochondria (their sensing machinery, mtDNA, OxPhos system, etc.) and their functional connectivity with one another.

Below we discuss how physical inter-organellar interactions, diffusible signals, dynamic morphological transitions, motility,

and sub-cellular positioning dynamically define the architecture and connectivity of mitochondrial networks, which are the basis for mitochondrial signal integration (MIPS step 2 of 3).

### Mechanisms of homologous mitochondrial communication

Several types of physical interactions enable transient information exchanges among mitochondria. Mitochondrial “kiss-and-run” involves the partial fusion of mitochondrial membranes among motile mitochondria in plants<sup>157</sup> and cultured mammalian cells.<sup>13,158</sup> These rapid interactions occur in the span of seconds to minutes and require the OMM mitofusins (MFN1/2) and IMM optic atrophy 1 (OPA1). Kiss-and-run events enable the exchange of proteins and membrane potential,<sup>158</sup> and possibly mtDNA nucleoids, although likely only in some cell types.<sup>159</sup>

Inter-mitochondrial junctions (IMJs) are close OMM-OMM contact sites anatomically similar to cell-cell gap junctions. The juxtaposition of highly electron-dense mitochondrial membranes, originally visualized in cardiomyocytes<sup>160</sup> between electrically connected mitochondria,<sup>161</sup> increases in frequency with cellular energy demand (e.g., exercise<sup>162</sup>) and with mitochondrial volume density.<sup>163</sup> At IMJs, which are evolutionarily conserved from mollusks to mammals, internal cristae membranes exhibit a remarkable degree of coordination (i.e., cristae alignment) across the two juxtaposed mitochondria, revealing the exchange of information between the two linked organelles.<sup>163</sup> Artificially linking energized mitochondria *in vitro* via synthetic linkers was sufficient to recapitulate IMJs and trigger cristae remodeling,<sup>163</sup> and the iron-sulfur cluster containing OMM protein MitoNEET may be one of the IMJ tethering proteins.<sup>164</sup> Functionally, IMJs may provide the physical basis for the propagation of membrane potential and other physico-chemical signals even in the absence of protein exchanges and complete mitochondrial fusion.<sup>165,166</sup>

Mitochondrial nanotunnels are thin ~100-nm-wide double-membrane protrusions that arise from donor mitochondria, extend over distances up to several microns, and can interact and fuse with a receiver mitochondrion.<sup>167</sup> Nanotunnels transport matrix proteins and therefore represent a mechanism of protein sharing and communication even between non-adjacent mitochondria.<sup>168</sup> In cultured cells, nanotunnels can be induced by the pulling action of the kinesin motor protein Kif5b.<sup>169</sup> *In vivo*, the existence of nanotunnels has been limited to tissues where mitochondrial motility is restricted such as in the densely packed cytoplasm of human skeletal muscles<sup>170</sup> and rat cardiomyocytes,<sup>171</sup> suggesting that physically constrained mitochondria that cannot encounter diverse fusion partners reach out to other functional mitochondria via nanotunnels. In patients with mitochondrial disease, mitochondria with compromised OxPhos function due to mtDNA mutations were found to have ~6-fold more nanotunnels than in healthy controls.<sup>170</sup> This suggests that mitochondrial nanotunnels may preferentially arise or stabilize between mitochondria with impaired OxPhos capacity as a mean of functional complementation,<sup>167</sup> or as a mean of increasing the effective functional connectivity among the mitochondrial network of the MIPS. Among other biological networks, enhancing the structural connectivity between individual units alters global network properties and can enhance robustness and computational/cognitive properties.<sup>172,173</sup>

Mitochondria also communicate via diffusible signals. One well-described example of diffusion-based mito-mito communication is ROS-induced ROS release (RIRR). Among the relatively uncluttered cytoplasm of cultured cells, mitochondria can generate and propagate waves of ROS production progressing through sequential PTP opening at rates of ~5  $\mu\text{m}/\text{min}$ .<sup>174</sup> This soluble form of signaling relies mostly on the physical proximity of mitochondria. In cardiomyocytes, proximity-based propagation depends on the production and diffusion of superoxide anions ( $\text{O}_2^{\cdot-}$ ) and  $\text{H}_2\text{O}_2$ .<sup>175</sup> Similarly, mitochondria can propagate waves of apoptotic signaling by sequentially undergoing permeability transition: waves are propagated by groups of mitochondria that sequentially uptake and release  $\text{Ca}^{2+}$ , which neighboring mitochondria then uptake and release, and so on.<sup>10</sup> The mito-mito transmission of information via diffusible signals within the MIPS may also be facilitated by some of the physical structures described above, particularly inter-organellar tethers.

### Mitochondrial dynamics: Fusion and fission

Mitochondrial fusion is a well-described process whereby two adjacent and generally motile mitochondria encounter each other and interact via the outwardly protruding domains of mitofusins (MFN1/2) and accessory proteins, leading to the sequential merging of the OMM and IMM of both organelles.<sup>176</sup> After fusion, the two original mitochondria form a unified organelle with a continuous matrix and membrane system. Organelle fusion allows the exchange of all matrix, IMM, IMS, and OMM components, including mtDNA, proteins and RC complexes, lipids, metabolites, ions, and membrane potential.

Experiments tracking the diffusion of photo-activable green fluorescent protein (mtPAGFP) in cultured mammalian cells<sup>177</sup> and *in vivo*<sup>178</sup> show that fusing mitochondria readily exchange molecular material.<sup>179</sup> In cardiomyocytes cultured *ex vivo*, mtPAGFP is exchanged through kiss-and-run fusion and nanotunnels and becomes distributed to the entire mitochondrial network within ~10 h.<sup>168</sup> In immortalized cell lines, the rate of mitochondrial fusion for each organelle is significantly faster, at one fusion event every ~5–20 min.<sup>13</sup> Mitochondrial membrane fusion therefore leads to the mixture and homogenization of mitochondrial protein distribution (i.e., mitochondria are more similar to each other). On the other hand, ablation of mitochondrial fusion by double Mfn1/2 silencing in mouse embryonic fibroblasts drastically increases mitochondrial heterogeneity (some mitochondria have a lot of protein x, others have little of it) within the MIPS.<sup>12</sup> *Ex vivo* studies of post-mitotic tissues and cells have made it clear that mitochondria in post-mitotic cells have lower fusion rates than cancer cells and immortalized cell lines (e.g., Eisner et al.<sup>180</sup>). Moreover, the cytoplasm of certain tissues can inhibit *ex vivo* mitochondrial fusion rates.<sup>181</sup> But in post-mitotic cells in which mitochondrial movement is restricted by cytoskeletal elements, fusion and fission can take place without displacement of mitochondria. This can be viewed as fire-doors in a long corridor—rapidly and reversibly modulating the network connectivity.

The functional relevance of dynamics to mitochondrial signal transduction is that larger mitochondria with larger matrix volume and lower surface-area-to-volume ratio respond differently to incoming signals. One example is the ability of mitochondria to handle histamine-induced rises in cytoplasmic  $[\text{Ca}^{2+}]$ . Relative to

small fragmented mitochondria, larger tubular mitochondria in the same cell uptake  $\text{Ca}^{2+}$  at a similar rate but recover more quickly (within 30 s).<sup>182</sup> In response to hyperglycemia, mitochondrial fragmentation precedes hyperglycemia-induced ROS production.<sup>183,184</sup> Hyperglycemia increases ROS production within ~30 min, and fragmented mitochondria produce ~50% more ROS than filamentous mitochondria in the same cell.<sup>182</sup> Again, the sequential events of sensing and responses illustrate how the functional responses of mitochondria to environmental inputs and stimuli are not rigidly set by genetically encoded states, but rather dynamically regulated by shape changes that remodel the network properties of the MIPS. Distinct fission signatures (i.e., where the fission event occurs along the mitochondrial tubule) are associated with the fate—degradation or biogenesis—of the resulting mitochondrial fragments,<sup>185</sup> possibly influencing long-term network properties.

The mitochondrial network also responds to metabolic signals. Mitochondrial fusion and fission are modulated by the cellular metabolic state,<sup>186</sup> and in turn regulate mtDNA stability *in vitro* and *in vivo*.<sup>187</sup> For example, the MIPS responds to substrate deprivation by undergoing MFN-dependent fusion,<sup>188,189</sup> whereas metabolic oversupply can inhibit mitochondrial fusion and lead to higher DRP1-dependent fragmentation in cultured cells<sup>184,190,191</sup> and in skeletal muscle *in vivo*.<sup>192</sup> Morphological changes underlie intra-mitochondrial functional changes that optimize coupling efficiency (i.e., the coupling of oxygen consumption to ATP synthesis) to best match the dynamic metabolic state.<sup>186,193</sup> In brown adipocytes, mitochondrial fission decreases coupling efficiency in a DRP-1 and free fatty acid-dependent mechanism,<sup>194</sup> reflecting an intra-mitochondrial morpho-functional response that increases fatty acid utilization and heat production. Like neural connections that come and go through activity-dependent sprouting and pruning,<sup>195</sup> mitochondrial interactions and connections also persist and vanish over variable time periods, modulating information flow within the MIPS network.

### Motility

Mitochondrial motility refers to the ability of mitochondria to travel to and from different parts of the cell. Motility influences MIPS structure as mitochondria stretch into their common tubular structure by adhering to cytoskeletal elements such as microtubules and actin filaments. When mitochondria fall off the cytoskeleton, they lose their tubular shape. Motility of an individual mitochondrion is also the strongest predictor of mitochondrial fusion.<sup>196</sup> Remarkably, the highest probability for a successful meeting between two mitochondria to develop into a fusion event is when one mitochondrion is moving while the other is stationary. The lowest probability is when both mitochondria have been stationary, even if they are juxtaposed.<sup>177</sup> On the other hand, fission is commonly followed with movement of the two daughter mitochondria so that they are not juxtaposed anymore (e.g., Kleele et al.<sup>185</sup>). The two fission products can therefore subsequently interact, possibly fuse, and thus share their content and more labile states with other units within the network. Both microtubules and actin filaments play a role in mitochondrial fission; for example, forcing the depolymerization of microtubules prevents the cytoplasmic redistribution of mitochondria in response to stress.<sup>197</sup> Directional motility is facilitated by cytoskeletal ele-

ments, but non-directional Brownian movement also appears to be a contributor to mitochondrial motility.<sup>198</sup>

Motility is influenced by the sensing of environmental signals.<sup>199</sup> The molecular sensors responsible for transducing metabolic and biochemical signals into motility implicate a complex of proteins that connect mitochondria to the motor machinery, the dynein and kinesin. Dynein and kinesin-1 walk the mitochondria on microtubules and thus any movement requires their attachment to the mitochondrial surface.<sup>200</sup> The molecular complex connecting mitochondria to these motor proteins includes Miro and Milton, whose regulations have been well defined in neurons.<sup>201</sup> When mitochondria enter an area with high calcium concentrations, Miro detaches from the motor proteins, resulting in the mitochondria falling off the cytoskeleton and becoming stationary; as a result, mitochondria stop their movement and accumulate in areas with increased calcium, where they can contribute to calcium buffering.<sup>202</sup> Similarly, Milton (Trak1) is inactivated by GlucNAC when glucose concentrations increase, leading to a similar arrest of mitochondrial movement in neurons in response to hyperglycemia.<sup>203</sup> In cultured cells, inter-mitochondrial tethering events similar to IMJs (without fusion) regulated by lysosomes<sup>204</sup> occur ~10× more frequently than fusion/fission events, limiting mitochondrial motility and therefore regulating mitochondrial distribution within the cytoplasm.<sup>205</sup> Overall, motility is a mechanism that dynamically redistributes mitochondria and together with fusion and fission determines the network structure of the intracellular mitochondrial collective.

### Communication with other organelles

The MIPS engages in functional interactions with the ER lysosomes, peroxisomes, lipid droplets, and likely other organelles. This topic has been elegantly reviewed elsewhere.<sup>206,207</sup> Mitochondrial metabolism is directly supported by surrounding organelles that provide various substrates, lipid intermediates, and ionic signals that not only supply substrates, but also communicate information about the overall state of the cell. In particular, input from the nucleus provides hundreds of proteins that sustain and confer mitochondria with both their molecular sensory machinery and the machinery for fusion/fission dynamics and motility that influence their propensity to adopt certain network configurations.

Mitochondrial cortisol synthesis is exemplary of this inter-organelle inter-dependence, requiring the transfer of cholesterol from lipid droplets to mitochondria and its import across mitochondrial membranes, followed by shuttling of steroidogenic intermediates from mitochondria to the ER, and from the ER back to the mitochondrial matrix, where cortisol is finally synthesized.<sup>208</sup> The synthesis of the mitochondrial IMM phospholipid cardiolipin similarly involves the shuttling of lipid intermediates between mitochondria and ER at mitochondria-associated membranes (MAMs) through the ERMES<sup>209</sup> and ER-mitochondria complex (EMC).<sup>210</sup> Punctual, localized, and pulsatile redox-based communication between mitochondria and the ER can also propagate signals from single mitochondria to the ER and other mitochondria.<sup>211</sup> These examples illustrate the functional inter-dependence of mitochondria and other organelles, and the existence of conserved mechanisms for information exchange, propagating the state of the MIPS to other organelles, and vice versa.

### Summary of mitochondrial signal integration

After describing the molecular machinery allowing mitochondria to sense and dynamically respond to intracellular and systemic inputs, here we have discussed the mechanisms allowing mitochondria to communicate and exchange information among each other and with other organelles. As the MIPS physically and functionally interacts as a mitochondrial collective with other organelles, it generates distributed representations of the biochemical and energetic conditions of the cells and organism. In turn, these capacities to sense and integrate information are adaptive, allowing mitochondria to tune and optimize their morpho-functional states to changing intracellular and environmental conditions.

Note that soluble communication mechanisms undoubtedly complement more complete forms of mitochondrial communication involving the merging and more-or-less complete union of mitochondria through membrane fusion. If diffusible signaling and transient protein exchange are analogous to “kiss-and-run,” more stable physical mitochondrial contacts such as IMJs and nanotunnels may reflect “engage-and-hold,” whereas complete mitochondrial fusion is analogous to “marry-and-mix.” Thus, mitochondrial interactions can be relatively transient (ion efflux lasts a few milliseconds), selective (nanotunnels connect with only one acceptor mito), and reversible (inter-organellar tethers can dissociate). The nature of these interactions is consistent with other plasticity mechanisms in biology, such as those modulating synaptic function within neural networks, which similarly integrate inputs and compute information.<sup>212</sup>

However, the ultimate unit of evolution and adaptation is not the mitochondrial network or the individual cell. It is the cell collective that constitutes the organism. Therefore, the goal of mitochondrial sensing and integrating information must be to optimize adaptation and health of the organism itself. Biologically, this becomes possible if the information sensed and integrated by the MIPS is then communicated to the cell and to the rest of the organism. This logic brings us to consider how mitochondrial inputs are converted and transmitted into meaningful cellular and organismal outputs or signals, through mitochondrial signaling (MIPS step 3 of 3).

### MITOCHONDRIAL SIGNALING

Several well-established and emerging signaling pathways link mitochondrial behavior to gene expression within the cell nucleus. Moreover, beyond the cell, the MIPS releases signals in the systemic circulation, influencing metabolic processes in neighboring cells and distant target organs. Several elements of mitochondrial signaling have been extensively covered elsewhere, such as apoptotic signaling,<sup>213</sup> ROS-mediated signaling,<sup>214,215</sup> and metabolic intermediates.<sup>216</sup> Here we only briefly cover these areas and expand the discussion of mitochondrial signaling to a broader spectrum of mitochondrial outputs that serve as intracellular and/or systemic outputs, including small metabolites, proteins, DNA, steroid hormones, and non-molecular signals including heat (Figure 6). We also discuss how the potency and specificity of signal transduction may be influenced by the sub-cellular localization of signaling mitochondria.

### Apoptotic signaling

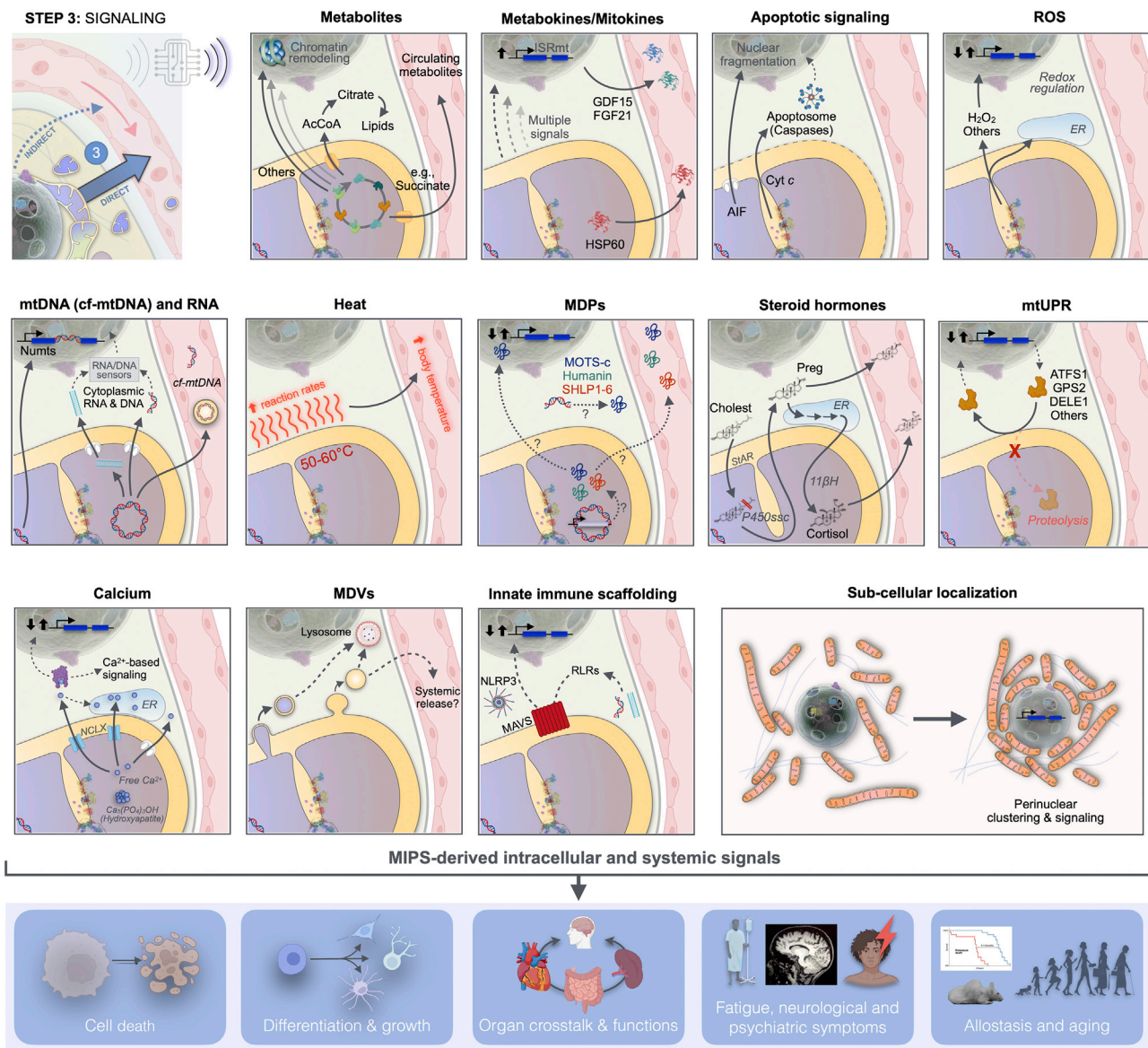
The first use of the term “mitochondrial signaling” appeared in 1999 in relation to the release of the pro-apoptotic mitochondrial output Cyt c.<sup>217</sup> Cyt c is a small heme protein normally residing in the IMS where it shuttles electrons between OxPhos complexes III and IV. However, in response to the convergence of specific inputs such as ROS, high  $[Ca^{2+}]$ , and low [ATP], especially among a fragmented and poorly connected mitochondrial network,<sup>218</sup> mitochondria undergo permeability transition through PTP opening.<sup>219</sup> PTP opening triggers the cytoplasmic release of Cyt c where it interacts with and activates pro-caspases,<sup>9</sup> as well as other mediators of the intrinsic apoptotic pathway, including the apoptosis inducing factor (AIF) and the endonuclease EndoG that translocates to the nucleus and fragments the nuclear genome, and Smac/Diablo (reviewed in Wang and Youle<sup>220</sup>). In cancer cells, Cyt c released during non-lethal permeability transition (i.e., “flickering mode”) can also play non-apoptotic signaling roles involving the activation of the nuclear ATF4-dependent integrated stress responses (ISRs; see below).<sup>221</sup> To prevent the assembly of pro-apoptotic molecular complexes at the OMM, mitochondria can also recruit anti-apoptotic proteins from the Bcl2 family. Functionally, PTP opening is closely linked to mitochondrial  $Ca^{2+}$  release and signaling, which is under the control of increasingly well-defined cristae-regulating mechanisms.<sup>222</sup> Thus, the MIPS contains a number of powerful cellular life-or-death signals coordinately released based on their integrated representation of biochemical conditions both within mitochondria and the cytoplasm.<sup>223</sup>

### Mitochondrial metabolite signaling

Mitochondria speak the language of the epigenome. It is likely that the endosymbiosis of mitochondria and the MIPS preceded the development of the histone code, such that current epigenetic nuclear mechanisms have developed to couple gene expression to the metabolic state of the cell in the context of mito-nuclear communication.<sup>224</sup> As a result, the nuclear genome is densely wrapped with abundant histone proteins (mainly H2A, H2B, H3, and H4) that contain hydrophilic tails, which are heavily post-translationally modified by the metabo-chemical perinuclear and nuclear environment.<sup>225</sup>

Most substrates or cofactors required by histone-modifying enzymes to alter histone structure and downstream gene expression are direct products of mitochondrial metabolism.<sup>226,227</sup> These include metabolites from the TCA cycle<sup>216</sup> and from one-carbon metabolism.<sup>228</sup> For example, the methylation of histones and DNA by histone methyltransferases (HMTs) and DNA methyltransferases (DMTs), respectively, requires S-adenosylmethionine (SAM) derived from serine metabolism as part of the folate cycle and one-carbon metabolism pathway.<sup>116,229</sup> On the other hand, the reverse demethylation reaction requires the cofactor  $\alpha$ -ketoglutarate ( $\alpha$ KG), a TCA cycle metabolite. Several mitochondrial-derived metabolites are involved in PTMs of histones (and other proteins). These include lactate (i.e., lactylation),<sup>230</sup> a metabolite derived from glycolysis that increases in concentration when mitochondrial OxPhos is impaired; dopamine (i.e., dopaminylation),<sup>231</sup> whose catabolism via the OMM-bound monoamine oxidase involves the respiratory chain;<sup>232</sup>  $\beta$ -hydroxybutyrate (i.e.,  $\beta$ -hydroxybutyration),<sup>233</sup> a





**Figure 6. MIPS step 3: Signaling**

Mitochondria synthesize and release signals evolved to influence cellular and organismal functions. Mitochondrial signals arise from various mitochondrial compartments and reach the cytoplasm, nucleus, and other organelles, where they induce cell-autonomous responses. These responses are transmitted to the systemic circulation either directly as mitochondria-derived metabolites and mitokines, or indirectly through transcriptional regulation of nuclear genes encoding metabokines or other hormone-like mediators. Mito-nuclear signaling is a form of signal amplification and integration. The MIPS converts metabolic signals into extracellular proteinaceous, secreted factors, allowing mitochondria to signal their state well beyond the confine of the cell in which they reside.

AcCoA, acetyl coenzyme A; AIF, apoptosis inducible factor; ATFS1, activating transcription factor associated with stress-1; cf-mtDNA, cell-free mitochondrial DNA; Cholest, cholesterol; Cyt c, cytochrome c; DELE1, DAP3-binding cell death enhancer 1; ER, endoplasmic reticulum; FGF21, fibroblast growth factor 21; GDF15, growth differentiation factor 15; GPS2, G-protein pathway suppressor 2; HSP60, heat shock protein 60; ISRmt, integrated stress response; MAVS, mitochondrial antiviral signaling; MDVs, mitochondria-derived vesicles; MDPs, mitochondria-derived peptides; NLRP3, NLR family pyrin domain containing 3; Numts, nuclear mitochondrial DNA segments; Preg, pregnenolone; P450ssc, side chain cleavage enzyme cytochrome P450; RLRs, RIG-I-like receptors; StAR, steroidogenic acute regulatory protein; UPRmito, mitochondrial unfolded protein response; 11βH, 11β-hydroxylase (mitochondrial cytochrome P450 11B1).

ketone body synthesized in the mitochondrial matrix under low carbohydrate conditions; and many others.

Mitochondrial metabolites are epigenome-modifying MIPS outputs. mtDNA-depleted Rho0 cells were initially used to demonstrate that mitochondrial outputs alter nuclear DNA methylation.<sup>26</sup> In a similar model comparing a series of human cell lines with varying mutation load (i.e., heteroplasmy) of the

pathogenic mtDNA 3243A>G mutation, heteroplasmy ranging from 0% to 100% influenced in a dose-response manner DMT gene expression and global transcriptional signatures.<sup>23</sup> In the same model, mtDNA heteroplasmy altered acetyl-CoA and αKG levels and yielded downstream changes in H4K16ac and H3K9me3 status.<sup>23a</sup> Acute mtDNA depletion in immortalized cells also triggered a physiologically meaningful decrease in

the mitochondrial acetyl-CoA pool and downstream histone acetylation,<sup>235</sup> illustrating the range of epigenomic effects of the MIPS. Finally, a longitudinal study in primary human fibroblasts tracking DNA methylation changes over several months showed that both genetic and pharmacological OxPhos defects caused conserved, age-related hyper- and hypomethylation at thousands of genomic locations encoding developmental programs and cell-cell signaling components.<sup>236</sup> A publicly available multi-omic, longitudinal dataset is available to explore the influence of bioenergetic perturbations on the epigenome and transcriptome of aging human fibroblasts.<sup>237</sup> Together, these findings illustrate some mechanisms whereby intrinsic mtDNA-related and OxPhos inputs are transduced into epigenome-re-modeling outputs.

However, one point that remains largely unclear is how MIPS-induced molecular and epigenetic modifications are temporally, spatially, and molecularly targeted, as well as their functional consequences on gene expression and cellular phenotypes.<sup>226</sup> Furthermore, this scientific challenge is compounded by the existence of multiple active TCA cycle enzymes directly in the nucleus.<sup>238</sup> The presence of mitochondrial enzymes in the nucleus, mostly documented to date in cancer cell lines, suggests that at least in some cell types, mitochondria may not be the only source of chromatin-modifying metabolites.

In recent years, other mitochondrial metabolites and molecular features have emerged as broad-acting intracellular signals. For example, the levels of TCA cycle metabolites succinate and fumarate are regulated by electron flux through the OxPhos system and more directly by the enzymes fumarate hydratase and succinate dehydrogenase (comprehensively reviewed in Martinez-Reyes and Chandel<sup>216</sup>). These metabolites are released in the cytoplasm, where they regulate signaling pathways involved in hypoxia sensing, immune activation, inflammation, and oncogenic transformation. TCA cycle metabolites also are enzymatically converted to metabolic derivatives such as itaconate and 2-hydroxyglutarate, among others. Itaconate is produced from the TCA cycle metabolite aconitate by aconitate decarboxylase and then acts either on intra-mitochondrial enzymes, for example by inhibiting succinate dehydrogenase,<sup>239</sup> or on transcription factors in the cytoplasm/nucleus, for example by inhibiting NF- $\kappa$ B signaling.<sup>240</sup> Two isomers of 2-hydroxyglutarate (2-HG) are produced from  $\alpha$ KG by the mitochondrial or cytoplasmic malate dehydrogenases (MDH2, MDH1, respectively) in an NADH-dependent manner, and also promoted by acidic pH.<sup>241</sup> In the nucleus, 2-HG then inhibits the demethylation of histone tails and DNA by the ten-eleven translocation hydroxylases (TET1-3) and plays important roles in cell fate transitions that affect oncogenesis<sup>242</sup> and immune activation.<sup>243</sup> Besides soluble metabolites, larger mitochondrial lipids also play important signaling roles.<sup>244</sup> For example, the IMM lipid cardiolipin participates in a variety of cell signaling events, translocating to the OMM during stress and serving as a signaling platform relevant to mitophagy, apoptotic signaling, and other functions.<sup>245</sup>

In addition to TCA cycle flux, NADH/NAD<sup>+</sup> ratio, and pH, the presence of carrier proteins on the IMM can influence MIPS metabolite signaling. For example, in mesenchymal stem cells age-related changes in the citrate carrier expression regulate the cytoplasmic export of acetyl-CoA to drive histone acetylation

levels, increase chromatin accessibility, and influence stem cell differentiation.<sup>246</sup> Thus, the nature and strength of mitochondrial outputs are likely regulated not only by rapidly changing fluxes through specific intra-mitochondrial metabolic pathways, but also by the relatively stable, albeit malleable, composition and abundance of IMM carriers and transporters.

Beyond the cell, metabolites also act in a cell-non-autonomous manner. A well-studied example is succinate, an obligatory mitochondrial TCA cycle intermediate that accumulates in equilibrium with the coenzyme Q redox state influenced by oxygen tension,  $\Delta$ pH+ $\Delta$  $\Psi_m$ , and ATP demand.<sup>247</sup> Succinate has been reported to signal extracellularly and perhaps systemically through at least one cell surface GPCR, the succinate receptor 1 (SUCNR1), on immune and other cell types to regulate inflammatory processes.<sup>248</sup> On target immune (and possibly other) cell types, succinate may also be imported via the monocarboxylate transporter 1 (MCT1), where it acts intracellularly to inhibit TCA cycle activity and signal transduction pathways inhibiting interferon secretion.<sup>249</sup> Thus, metabolite outputs from the MIPS collectively have broad-acting cell-autonomous and cell-non-autonomous effects on the epigenome, nuclear gene expression, and cell behavior.

One other mitochondrial metabolite is worth special mention for its well-known role in circadian biology: melatonin (N-acetyl-*l*-methoxytryptamine). Melatonin is an evolutionarily ancient bacterial molecule preceding endosymbiosis that has strong antioxidant properties (reviewed in Reiter et al.<sup>92</sup>). Mitochondria not only contain the MT1 melatonin GPCR, but also synthesize melatonin from the amino acid L-tryptophan (with serotonin as an intermediate) via two enzymatic reactions catalyzed by enzymes in the mitochondrial matrix (arylalkylamine N-acetyltransferase [AANAT] and acetyl serotonin methyltransferase [ASMT]).<sup>87</sup> Like other mitochondrial metabolites, systemic melatonin concentration exhibits strong diurnal variation (almost undetectable during the day, peaking at night; e.g., Paul et al.<sup>250</sup>). It modulates sleep/wake cycles in some animals,<sup>251</sup> and its oral consumption in humans may modulate sleep onset.<sup>252</sup> Thus, mitochondria-derived melatonin potentially acts locally in an “automitocrine” and cell-autonomous manner, in a paracrine manner between cells/neurons, as well as systemically via the bloodstream,<sup>253</sup> illustrating the broad reach of MIPS-derived metabolites/hormones in mammalian physiology.

Together, mitochondria-derived metabolic outputs represent complementary signals that integrate and transduce the bioenergetic state of the MIPS into signals intelligible to core cellular signal transduction machinery that orchestrate a broad array of cellular and organismal behaviors.

### Mitochondrial ROS signaling

ROS are diffusible molecules, particularly hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) produced from the dismutation of superoxide anion (O<sub>2</sub><sup>•−</sup>) by the matrix and IMS antioxidant systems. Mitochondrial ROS originate predominately from OxPhos complexes I and III<sup>254</sup> and travel to the cytoplasm and nucleus where they trigger redox-sensitive gene-regulatory processes.<sup>197,255</sup> Mitochondrial ROS signaling<sup>215</sup> and guidelines for their measurements<sup>256</sup> have previously been reviewed in detail, so here we mainly focus on recent developments in this area.

Mitochondrial ROS regulate various internal mitochondrial states and systemic signaling. For example, in brown adipose tissue mitochondrial ROS production post-translationally modifies UCP1 at Cys253 to increase uncoupling and enable thermoregulation, whereas pharmacological depletion of mitochondrial ROS with MitoQ prevented IMM uncoupling and heat production.<sup>257</sup> In the mitochondrial matrix of heme-synthesizing mitochondria in adipocytes, H<sub>2</sub>O<sub>2</sub> oxidizes bilirubin to form biliverdin, which is exported from mitochondria by the ATP binding cassette (ABC) transporter ABCB10.<sup>258</sup> And in secretory pancreatic beta cells, glucose-stimulated insulin secretion is similarly driven by H<sub>2</sub>O<sub>2</sub> accumulation,<sup>259</sup> illustrating how mitochondrial ROS signals within the mitochondrion and intracellularly to trigger the release of systemic endocrine signals such as insulin.

Likely owing to the central role of oxygen in the evolution of aerobic creatures, mitochondria-derived ROS have broad effects on nuclear transcriptional regulation. In cultured cells, elevated ROS production secondary to respiratory chain dysfunction, or mimicked with the addition of the mitochondria-targeted redox cycling agent paraquat (MitoPQ), was sufficient to activate proteins of the mitogen-activated protein kinase (MAPK), including JNK signaling, which induces a secondary signal, namely nuclear chromatin release into the cytoplasm.<sup>260</sup> Similarly, eliciting high levels of temporally controlled ROS specifically in mitochondria using a chemoptogenetic tool elevated nuclear hydrogen peroxide levels and induced telomere damage.<sup>261</sup> In mice, silencing the mitochondrial matrix antioxidant enzyme manganese superoxide dismutase (MnSOD) during development showed that mitochondria-derived ROS activated the cytoplasmic/nuclear Nrf2 and PPAR $\gamma$ /PGC-1 $\alpha$  pathway, leading to lasting adaptive hormetic responses that persist in adult animals.<sup>262</sup> Similar results were obtained in mice treated with low-dose rotenone (a complex I inhibitor that increases mitochondrial ROS emission) during embryonic and post-natal development, which altered nuclear DNA methylation and modified coat color.<sup>263</sup> In aging human fibroblasts, mitochondrial signaling via ROS is also necessary and sufficient to activate the Nf- $\kappa$ B pathway and senescence features, including the senescence-associated secretory profile (SASP).<sup>264</sup> In fact, experimentally depleting mitochondria from human fibroblasts by using a Parkin-overexpression/FCCP treatment prevented the acquisition of senescence characteristics,<sup>265</sup> providing compelling evidence that MIPS signaling—including but likely not limited to ROS—is required to trigger complex cellular states like senescence. Moreover, the SASP can propagate senescence phenotypes to neighboring bystander cells both *in vitro*<sup>264</sup> and *in vivo*,<sup>266</sup> illustrating one of many pathways whereby mitochondrial signaling propagates systemically in a cell-non-autonomous manner to influence organismal behavior and lifespan.<sup>17,267</sup>

Besides mito-nuclear signaling, ROS production by individual mitochondria also locally contributes to communication with the ER.<sup>211</sup> Even in distant neural arborizations, far from the nucleated cell body, mitochondrial ROS contribute to local synaptic activity.<sup>268</sup> In response to plasma membrane photodamage, mitochondria at the site of injury were also shown to respond in a DRP1-dependent manner by increasing repair-promoting ROS production.<sup>269</sup> Thus, the site-specific roles of mitochondrial ROS across sub-cellular locations illustrate the significance and potential specificity of localized ROS outputs from the MIPS as drivers of gene regulation and cellular functions.

### Mitochondria synthesize sex and stress hormones

One of the most powerful types of mammalian hormones are steroid molecules, broadly categorized into three major classes: (1) the sex-defining testosterone, estrogens, and progestins produced in the gonads; (2) the stress hormones that promote stress adaptation via metabolic and salt balance regulation including glucocorticoids and mineralocorticoids produced in the adrenal glands; and (3) neurosteroids produced in the nervous system.<sup>270,271</sup> Their release is regulated by trophic pituitary hormones from the brain (adrenocorticotrophic hormone, ACTH; follicular stimulatory hormone, FSH; and luteinizing, LH) mediated by GPCR-coupled cyclic AMP- protein kinase A (cAMP-PKA) or Ca<sup>2+</sup>-PKC signaling in steroid-producing cells.<sup>208</sup> In steroidogenic tissues, the rate-limiting step to synthesize all steroid hormones takes place within mitochondria.

Mitochondria produce steroid hormones from cholesterol, the initial substrate to all steroids. The import of cholesterol through the OMM and IMM requires microtubule and microfilament dynamics as well as protein synthesis<sup>272</sup> and is accomplished by the steroidogenic acute regulatory protein (StAR, from the *STAR1* gene) in the OMM.<sup>273</sup> Whereas mitochondrial import of StAR through the TIM/TOMM translocator complex leads to its proteolytic degradation, stabilization of StAR at the OMM,<sup>274</sup> in association with Tom22 and VDAC2,<sup>275,276</sup> delivers cholesterol to the matrix-facing IMM side chain cleavage enzyme cytochrome P450 (CYP450scc) protein. CYP450scc then catalyzes the rate-limiting reaction for steroidogenesis, which convert cholesterol into pregnanolone, the common precursor to all steroid hormones.<sup>33</sup> Pregnanolone synthesized in the matrix is then exported to the ER where other enzymes sequentially catalyze its transformation into progesterone and other steroid intermediates.<sup>208</sup> In steroidogenic mitochondria from the adrenal cortex zona fasciculata cells, the downstream steroid intermediate returns to the mitochondrial matrix, possibly through the MAM, where the terminal reaction is catalyzed by the mitochondrial matrix enzyme 11 $\beta$ -hydroxylase (11 $\beta$ H, also “mitochondrial cytochrome P450 11B1” encoded by *CYP11B1* in humans) that produces cortisol.<sup>277</sup> Mitochondrial synthesis of systemically acting steroids occurs rapidly within minutes, and its synthesis arrest is equally rapid.<sup>208</sup> The rapid, redox-sensitive, protein import-dependent regulation of this process illustrates how multiple intrinsic factors can influence mitochondrial steroidogenic outputs.

The evolutionary basis for positioning steroidogenesis in mitochondria remains uncertain but may have involved the uniquely reducing conditions of the mitochondrial matrix.<sup>278</sup> The conversion of cholesterol to pregnanolone by P450scc requires the reductive action of 3 high-energy NADPH molecules. As a result, the loss of the matrix-facing NADPH-generating enzyme nicotinamide nucleotide transhydrogenase (NNT) inhibits steroidogenesis, causing hypocortisolemia in mice and humans.<sup>279</sup> Developmentally, steroid hormones drive energetically expensive transcriptional and physiological programs that must incur substantial cellular energetic costs in target tissues. As a result, it is possible that to optimize fitness, these hormones should only be produced in proportion with the energetic capacity of target tissues. Assuming that the function of mitochondria is partially harmonized across both source steroidogenic and target catabolic energy-consuming tissues,



we postulate that the mitochondrial localization of steroidogenesis enzymes may reflect the product of system-level adaptation aiming to couple mitochondrial bioenergetic capacity and steroid hormone signaling across the organism.

### Mitochondrial genome signaling: Intracellularly

The circular mtDNA is typically contained in the mitochondrial matrix, insulated from the cytoplasm by two membranes. However, the enclosure of mtDNA within the mitochondrial IMM and OMM is naturally disrupted under certain physiological conditions. This includes mtDNA instability caused by the partial loss of the mtDNA-associated protein TFAM, which triggers mtDNA-dependent antiviral gene expression programs in the nucleus.<sup>22</sup> mtDNA release is a relevant signaling mechanism because both the cytoplasm and the extracellular surface of immune and non-immune cells harbor DNA sensors that recognize mtDNA fragments as a damage-associated molecular pattern (DAMP).<sup>280</sup> DNA (viral, bacterial, and mtDNA) is sensed by multiple innate immune receptors including cGAS (cyclic GMP-AMP synthase), TLR9 (toll-like receptor 9), and the NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) and AIM2 (absent in melanoma) inflammasomes. Sensing of mtDNA triggers signaling cascades that either converge on cytokine- and interferon-producing transcription factors including IRF3/7, MAPK, and NF- $\kappa$ B or engage Caspase-1 for processing and secretion of IL-1 $\beta$  and IL-18.<sup>281</sup> The cytosolic release of mitochondrial double-stranded RNA (mt-dsRNA) can also act as a DAMP and is detected by the RIG-I-like (RLR) receptors RIG-I and MDA5. Once engaged, these sensors translocate to mitochondria and activate the mitochondrial antiviral signaling protein (MAVS), which assembles as filaments on the mitochondrial surface<sup>282</sup> in a membrane potential-dependent manner to act as an antiviral signaling platform.<sup>283,284</sup>

Current thinking around innate immune signaling suggests that the mitochondrial network is both a source of stimulatory ligands and acts as the major signaling hub for the four major pattern recognition receptor families (TLRs, NOD, RLRs, and cytosolic DNA sensors [CDSs]).<sup>285</sup> Most of the effects of both exogenous (bacterial, viral) nucleic acids and endogenous mtDNA/mtRNA signaling are likely mediated via these pathways. As an example of mitochondrial signal transduction, when mitochondria in human fibroblasts and cancer cells detect the genotoxic agent doxorubicin, mtDNA damage (sensing) eventually leads to the cytoplasmic release of mtDNA fragments (signaling), which trigger nuclear DNA repair mechanisms in the nucleus and cGAS-STING-dependent activation of interferon-stimulated gene (ISG) expression.<sup>147</sup>

Regarding the mechanism(s) responsible for facilitating mtDNA extrusion into the cytoplasm, two molecular pathways have been described. One mechanism tested in mouse embryonic fibroblasts and mice with lupus-like disease involves VDAC oligomerization in the OMM, stabilized by short mtDNA fragments, forming a large-scale pore that enables the cytoplasmic extrusion of 100- to 400-bp-long mtDNA fragments.<sup>286</sup> Similarly, in bone marrow-derived macrophages 500- to 650-bp-long mtDNA fragments are cleaved from the circular genome by the mitochondrial protein flap-structure-specific endonuclease 1 (FEN1) and released in a VDAC-dependent manner into the cytoplasm where it activates the inflammasome

and cGAS-STING signaling.<sup>287</sup> The other described mechanism of cytoplasmic mtDNA extrusion consists of BAX/BAK-mediated pore formation in the OMM, followed by herniation of the IMM at the surface of mitochondria during apoptosis.<sup>288</sup> Under conditions of genotoxic stress, BAX/BAK-mediated herniation also appears to release ds-mtRNAs to activate ISGs in the nucleus.<sup>289,290</sup> A third non-specific mechanism may involve the rupture of mitochondrial membranes, possibly secondary to swelling, which, for example, may occur in skeletal muscle of patients with primary mtDNA mutations.<sup>291</sup>

Another, more permanent way in which the mitochondrial genome can carry information to the nucleus is via the translocation of mtDNA segments to the nucleus followed by their insertion within the coding sequence as nuclear mtDNA insertions (NUMTs, pronounced “*nu-mites*”).<sup>292</sup> This process, termed “numtogenesis,” has traditionally been understood as horizontal gene transfer, having occurred multiple times during the evolution of single-celled and multicellular organisms.<sup>293</sup> As a result, multiple germline NUMTs are shared across individuals. In the case of mitochondria, mtDNA gene transfer is also likely to explain how the majority of the genes from the original proteobacterium’s genome have migrated to the nucleus such that >98% of the mitochondrial proteome is now encoded in the nucleus.<sup>294</sup>

But the transfer of mtDNA sequences to the nucleus may also occur over a cell’s lifespan. In yeast, mitochondria lacking the mitochondrial protease Yme1 (yeast mtDNA escape 1), a member of the AAA family of ATPase that degrades IMS/IMM proteins to regulate mitochondrial cristae dynamics,<sup>295</sup> produce 77-fold more mito-nuclear transfer of NUMTs along with a 50% reduction in lifespan.<sup>296</sup> Similarly, in cancerous mammalian cells, mitochondria without the human homolog YME1L1 generate ~4-fold more NUMTs, recapitulating the abnormally elevated number of NUMTs in ovarian tumors<sup>297</sup> and other cancer types.<sup>298</sup> Numtogenesis may also occur at a steady rate over days to weeks in healthy replicating primary human fibroblasts *in vitro*, and over a person’s lifespan in brain tissue (unpublished data). Whether the effects of NUMTs on nuclear genome instability and cellular aging is a bona fide, regulated form of MIPS signaling remains to be established.

### Mitochondrial genome signaling as extracellular, cell-free mtDNA

mtDNA copies are often well in excess of the number of copies required to transcriptionally sustain OxPhos.<sup>299</sup> An emerging notion suggested by Shadel et al. is that the mtDNA molecules do not only supply RNA and OxPhos subunits but in fact exist as sentinels of genotoxic stress and other insults.<sup>147</sup> This suggests that the hundreds of mtDNA genomes in each cell may represent a pool of signaling molecules—in the same way the neurotransmitters are produced and stored in presynaptic boutons, awaiting release.

Beyond mtDNA detected in the cytoplasm and nucleus, a substantial amount of circulating cell-free mtDNA (cf-mtDNA; as well as nuclear DNA, cf-nDNA) is released extracellularly, detectable in various biofluids from healthy individuals (reviewed in Trumpff et al.<sup>300</sup>). In blood (serum or plasma, which contain different cf-mtDNA levels<sup>301</sup>) and cerebrospinal fluid, cf-mtDNA is elevated in some although not all individuals with primary OxPhos



defects,<sup>302–304</sup> during pregnancy,<sup>305</sup> after physiological stress such as exercise,<sup>306</sup> minutes after acute psychological stress,<sup>306,307</sup> and hours to days after intensely stressful life events,<sup>308</sup> highlighting the dynamic release of cf-mtDNA. In saliva, cf-mtDNA also increases several-fold during the morning sleep-wake transition.<sup>309</sup> Notably, in critically ill individuals cf-mtDNA levels are also dramatically elevated, and cf-mtDNA abundance (copies per mL) is a strong predictor of mortality,<sup>310</sup> highlighting the potential physiological significance of cf-mtDNA in humans.

Because the majority of the initial work on cf-mtDNA was conducted in the context of sepsis and inflammatory disorders, and given that the molecular features of the mitochondrial genome and associated proteins are bacterial in origin, the pro-inflammatory aspects of cf-mtDNA signaling have been emphasized.<sup>311</sup> The role of mitochondrial signaling in the control of inflammation has been expertly reviewed elsewhere<sup>285</sup> and leaves little doubt that intracellularly, cf-mtDNA is immunogenic.

However, in relation to extracellular cf-mtDNA in biofluids, emerging evidence suggests that (1) the majority of blood and saliva circulating cf-mtDNA in human plasma may not be naked (required to be accessible to DNA receptors) but rather contained within sedimentable cargo, possibly as circulating whole mitochondria,<sup>309,312,313</sup> and (2) cf-mtDNA is abundant in healthy individuals who do not show signs of systemic inflammation. Therefore, a critical re-appraisal of the evidence reveals that by itself, circulating cf-mtDNA in its physiological forms in human blood is unlikely pro-inflammatory.<sup>300</sup> Cf-mtDNA levels in blood, cerebrospinal fluid,<sup>314</sup> and saliva<sup>309</sup> also do not consistently correlate with inflammatory markers. The physiological role of cf-mtDNA in general remains unclear. Technically, how mtDNA fragments are physiologically released from the matrix to the cytoplasm, into the extracellular space, and into biofluids also remains to be established.

Together, these findings highlight the influence of mtDNA signaling beyond autocrine/cytoplasmic mito-nuclear signaling. Emerging work in multiple biofluids implicates paracrine (cell-to-cell) and potential endocrine (systemic) roles of cf-mtDNA signaling among the repertoire of signaling mechanisms available to transduce information from the MIPS to the organism.

### Non-apoptotic nuclear-encoded proteins sequestered in mitochondria

A different class of mitochondrial outputs includes a group of nuclear-encoded proteins that are normally imported and degraded by functional energized mitochondria but fail to be imported when mitochondria are de-energized (i.e., depolarized). In non-mammalian systems and cultured cells, stress conditions that induce mitochondrial depolarization inhibit protein uptake and cause their accumulation in the cytoplasm where they act as transcription factors.<sup>27</sup> Based on initial studies using unfolded protein stress in the mitochondrial matrix,<sup>315</sup> this response was coined the mitochondrial unfolded protein response (mtUPR). The mtUPR involves close physical contact and functional interactions between mitochondria and the ER.<sup>316</sup>

Known pathways involving nuclear-encoded proteins include ATFS-1 (activating transcription factor associated with stress-1, in *C. elegans*),<sup>19</sup> GPS2 (G-protein pathway suppressor 2, in mammalian cells),<sup>20</sup> and DELE1 (DAP3-binding cell death

enhancer 1, in mammalian cells).<sup>317,318</sup> In an OMA1 protease-dependent manner, the mitochondrial network acts as an active sink that normally shunts these proteins and prevents their interactions with nuclear transcription factors including EIF2 $\alpha$ .<sup>317,318</sup> Via these proteins, mitochondrial depolarization promotes ATF4 and ATF5 expression, the master regulators of the mtUPR.<sup>319,320</sup> Interestingly, inhibiting mitochondrial translation interferes with cytoplasmic translation and triggers ATF4/ATF5-dependent signaling, marking the interconnectedness of intra-mitochondrial and cytoplasmic protein homeostasis.<sup>321</sup> The ATF4/ATF5 transcription factors overlap with those of the mitochondrial ISR (ISRmt) well defined in mammals. However, in cultured human cells at least, different mitochondrial perturbations selectively induce the mtUPR and ISRmt in a relatively mutually exclusive manner.<sup>322</sup> This result suggests that the mtUPR and ISRmt stress response pathways have either evolved separately or diverged in their specificity, highlighting the existence of at least two well-defined nuclear transcriptional programs induced by MIPS signaling in mammals.

### Systemic mitochondrial signaling via the nucleus

MIPS signaling induces nuclear programs that remodel catabolic and anabolic biosynthetic pathways within the cell, and also shapes metabolism systemically. In mammalian models, intrinsic mtDNA transcriptional and translational defects,<sup>16,115,323</sup> inhibition of autophagy,<sup>324</sup> IMM uncoupling,<sup>325</sup> and both pharmacological and genetic OxPhos defects<sup>236,326</sup> are transduced to the nucleus via mechanisms that at least in part involve mitochondria-derived signals inducing the ATF4- and ATF5-regulated ISRmt. In cultured human cells, loss of hundreds of nuclear-encoded mitochondrial genes, although not all, selectively triggers the ISRmt.<sup>322</sup>

In animals, the ISRmt produces two main nuclear-encoded systemic signals: fibroblast growth factor 21 (FGF21) and growth differentiation factor 15 (GDF15)—two proteins with overlapping but distinct systemic metabolic effects.<sup>327</sup> In mouse models with molecular alterations in skeletal muscle mitochondria, the muscle-derived FGF21 protein travels to distant tissues where it is necessary for some tissue-specific effects such as white adipose tissue browning<sup>328</sup> and glucose uptake and mitochondrial biogenesis in the dorsal hippocampus.<sup>18</sup> However, FGF21 is dispensable for other systemic effects such as glucose tolerance, insulin resistance, anorexia, and weight regulation.<sup>328–330</sup> The other well-studied metabolite GDF15 is most highly expressed in secretory tissues (syncytiotrophoblasts, epithelial cells, and glandular cells; <https://www.proteinatlas.org/>) and has wide-ranging systemic effects linking mitochondrial OxPhos dysfunction, metabolism, and inflammation.<sup>331</sup> Mitochondrial translation defects (Crif1KO) in skeletal muscle<sup>329</sup> or in adipocytes,<sup>332</sup> or chronic skeletal muscle mitochondrial uncoupling (uncoupling protein 1 [UCP1] overexpression),<sup>333</sup> induce both FGF21 and GDF15 secretion from the affected tissues, where GDF15 is required to increase systemic energy expenditure and other behavioral and physiological recalibrations.

Consistent with the functional interplay of the MIPS and the organism, ISRmt signaling occurs at least in part via a periphery-to-brain signaling axis. Mice and humans express the receptor for GDF15 GFRAL most highly in brain tissue (e.g., Mullican et al.<sup>334</sup>), but GFRAL may be expressed in many tissues at low

levels (<https://www.proteinatlas.org/>) and is stress inducible in other cell types including macrophages.<sup>335</sup> Therefore, GDF15-GFRAL signaling provides a mechanism whereby mitochondrial outputs from peripheral tissues use the brain—an organismal integration center—to transduce the functional state of a tissue's mitochondria to regulate systemic metabolism.<sup>336,337</sup> Adding to this systemic signaling picture, in mice GDF15 signaling upregulates hypothalamic corticotropin-releasing hormone (CRH)<sup>338</sup> and activates the downstream HPA axis and secretion of corticosterone, a mitochondria-derived hormone released in response to psychosocial stress.<sup>339</sup> Consistent with the production of metabolites in response to intra-mitochondrial defects converging on OxPhos capacity, FGF21 and GDF15 are circulating biomarkers of subgroups of mitochondrial diseases in adults and children.<sup>113,304,340–342</sup>

Thus, the secreted nuclear-encoded metabolites FGF21 and GDF15 convey information about the state of the MIPS in one tissue/organ to the whole organism. However, the contributions of these stress-induced, nuclear-encoded mitochondrial signaling outputs to the maintenance of human health or to disease progression remain only partially explored.

### Mitochondria-derived peptides

The discovery of alternative open reading frames (ORFs) within the mtDNA sequence led to the identification of MDPs released within the cell systemic circulation (for a comprehensive review, see Reynolds et al.<sup>343</sup>). Eight MDPs have been reported: Humanin, a 24-amino-acid peptide encoded within the 16S rRNA gene initially discovered to have neuroprotective effects in neuronal cultures<sup>344</sup> and subsequently linked to longevity across invertebrates, small mammals, and humans;<sup>345</sup> 6 small humanin-like peptides (SHLP1–6) that functionally overlap with humanin;<sup>346</sup> and MOTS-c (mitochondrial ORF within the twelve S rRNA type-c), a 16-amino-acid peptide initially identified to promote insulin sensitivity and prevent age-related insulin resistance in mice.<sup>347</sup> When the mtDNA is selectively depleted with chronic ethidium bromide treatment, or mtDNA transcription is selectively inhibited with actinomycin, the expression of MDPs is lost, confirming their origin in the mitochondrial genome.<sup>347</sup> However, where MDPs are transcribed and translated (mitochondrial matrix or cytoplasm) remains uncertain.

Functionally, once in the cytoplasm, MOTS-c translocates to the nucleus in an AMP-activated protein kinase (AMPK)-dependent manner, where it regulates stress-induced gene expression and promotes cell survival.<sup>21</sup> In mice, MOTS-c and Humanin are also found in blood and act in a cell-non-autonomous manner to apparently regulate systemic metabolism.<sup>348</sup> In humans, like other mitochondrial outputs, MOTS-c<sup>349</sup> and Humanin<sup>350</sup> levels increase dynamically in skeletal muscle and in circulation upon exercise. Thus, in addition to nDNA-encoded metabolite proteins FGF21 and GDF15, mitochondria release mtDNA-encoded peptides that act as both intracellular and systemic signaling mediators. The range of physiological functions for MDPs is only beginning to be uncovered, but MOTS-c may increase running capacity (i.e., endurance) in mice and improve resilience to metabolic starvation in cultured myotubes.<sup>349</sup> Other mitochondrial resident proteins, including heat shock protein 60 (HSP60)<sup>351</sup> and TFAM,<sup>352,353</sup> have been identified in blood, suggesting that several canonical mitochondrial proteins are

released systemically by the MIPS where they have metabolic, inflammatory, or other systemic signaling roles.

### Mitochondrial heat signaling and thermodynamic gradients

Temperature is a powerful effector of biological change. Without heat, biological processes do not proceed. For instance, growth and degradation are greatly reduced at 4°C, whereas optimal temperatures accelerate enzyme kinetics, membrane fluidity, and organismal development.<sup>354</sup> Therefore, the diffusion of heat and the ensuing changes in biochemical activities represent a form of signaling, where information about the state of an organelle is transferred from one sub-cellular compartment to another. Because—according to the second law of thermodynamics—the flow of heat always proceeds from warmer to colder locations, the flow of information also must preferentially (although not exclusively) occur from warmer to colder structures.

Among the cell and the organism, mitochondria are the warmest compartment and the major heat source. Body temperature in endotherms is primarily derived from respiratory chain activity.<sup>355</sup> Exemplary of this phenomenon, mitochondria in brown adipocytes express high levels of UCP1 that increases proton leak across the IMM, accelerating upstream heat-producing biochemical reactions in a Ca<sup>2+</sup>-dependent manner.<sup>356</sup> Using temperature-sensitive fluorescent probes, initial studies found that the warmest cellular compartments in cultured cells were the nucleus (which is surrounded by perinuclear mitochondria) and mitochondria.<sup>357</sup> Uncoupling of OxPhos with the uncoupler FCCP increases mitochondrial matrix temperature by 6°C–9°C, as would be expected from relieving the electrochemical energy gradient across the IMM and subsequent cascading acceleration of biochemical reactions upstream from the OxPhos system.<sup>358</sup> Accordingly, the biochemical activity of mammalian mitochondrial respiratory chain enzymes was found to be maximal around 50°C.<sup>359</sup> Consistent with this finding, refined live-cell imaging with mitochondria-targeted temperature-sensitive probe showed that mitochondria function at internal temperatures around 50°C, well above the core body temperature of 37°C,<sup>359</sup> suggesting that the MIPS radiates heat-based signals into the cell. Thus, the temperature gradient between mitochondria (warmest compartment) and other sub-cellular structures likely provides mitochondria with a thermodynamically privileged position in signal transduction.

### Sub-cellular mitochondrial localization

The sub-cellular positioning of cellular structures influences their functions and ability to signal to other organelles. Within the cytoplasm, the MIPS is topologically positioned at the interface between the naturally inert nuclear genome and the dynamic extracellular environment. In many cell types, mitochondria directly contact or hover only hundreds of nanometers away from the nucleus. At the nuclear surface, diffusible mitochondrial signals can travel through nuclear pores to reach the nucleoprotein complex of the chromatin.<sup>360</sup> To travel 1 μm—from the mitochondrial IMS to the chromatin—the theoretical isotropic diffusion time for a small 40 kDa protein is 0.02 s,<sup>361</sup> whereas small metabolites like ATP<sup>362</sup> or amino acids<sup>363</sup> travel ~20–100 times

faster, closing the 1  $\mu$ m gap in less than 1 ms. Physical proximity, particularly at high temperature, favors rapid communication.

The position of the mitochondrial network within the cytoplasm can influence signaling behavior. In response to stressors such as hypoxia in cultured endothelial cells, mitochondria redistribute and cluster around the nucleus within <3 h where they promote a pro-oxidant intranuclear state.<sup>197</sup> Inhibiting mitochondrial motility with the microtubule depolymerizing agent nocodazole or dynein knockdown effectively prevents perinuclear clustering.<sup>197</sup> In this case, the reduced physical proximity appeared to decrease the potency of mito-nuclear signaling, hindering HIF-1 $\alpha$  binding to the nDNA hypoxia response element nucleotide sequence. The formation of physical contact sites between mitochondria and the nuclear envelope by the OMM-based translocator protein (TSPO) also enables cholesterol redistribution to the nucleus and initiates pro-survival nuclear transcriptional programs that are blunted without mito-nuclear proximity,<sup>255</sup> highlighting the influence of proximity and physical interactions in mito-nuclear signaling.

Mitochondrial positioning also shapes cell behavior away from the nucleus. In developing neurons, mitochondrial positioning at specific locations along axons determines the location of branch points.<sup>364</sup> In ganglion cell dendrites of the retina, mitochondrial positioning at terminal branch points and presynaptic sites also stabilizes mature dendritic structures.<sup>365</sup> In neurons, both within presynaptic boutons<sup>31</sup> and at specific locations near dendritic synapses, mitochondria contribute both to local ATP synthesis and Ca<sup>2+</sup> handling, locally influencing neurotransmitter release and, as a direct result, cell-cell communication.<sup>366</sup> Thus, the regulated topological positioning of mitochondria within the cell can minimize diffusion distances and likely optimize the potency and/or nature of MIPS outputs, illustrating how mitochondrial positioning guides signaling and various cellular functions.

### Summary of mitochondrial signaling

In addition to their elaborate sensing (step 1) and networking (step 2) capabilities, mitochondria also possess a wide array of signaling (step 3) mechanisms that transduce mitochondrial states to the cell and organism. The nature of these signals includes ions, metabolites, chemical species (e.g., ROS), DNA, and proteins. These outputs carry—possibly with a fairly high degree of specificity based on their circumstantial combinations—information about various aspects of mitochondrial biology to the cell nucleus and other organelles. At specific sub-cellular locations and in specific cell types, adaptive mitochondrial signals are transformed into transcriptional, cellular, and humoral physiological responses that inform and influence organismal functions. Less specific factors, including temperature and physical distances, may modulate the potency of these signals. Emerging evidence also suggest that systemic MIPS outputs may include mitochondria-derived vesicles (MDVs),<sup>367</sup> extracellular vesicles with mitochondrial cargo,<sup>368,369</sup> and even whole mitochondria<sup>313</sup> that travel from source cells/tissues to functionally impact distant target organ systems. Finally, the description of overlapping molecular connections between mitochondria and target nuclear genetic programs, such as the mtUPR and ISRmt, emphasizes the evolved sensitivity of the mammalian genome to MIPS outputs. Notably, several mitochondrial outputs reach the bloodstream, the biofluid that metabolically connects all cells

and organs, giving systemic organismal access to MIPS signaling.

### TISSUE SPECIFICITY IN MITOCHONDRIAL FUNCTIONS AND BEHAVIORS

Although we have so far considered mitochondria as a more-or-less uniform family of organelles, mitochondria are not all created equal. From their shared origin in the oocyte,<sup>370</sup> mitochondria undergo profound specialization as different cell types and tissues mature during embryonic development. This gives rise to somatic mitochondria that differ in their protein composition<sup>371,372</sup> and functions (respiratory properties, ROS production, PTP sensitivity,  $\beta$ -oxidation capacity).<sup>373</sup> These developmentally acquired characteristics represent tissue-specific mitochondrial phenotypes. Analogous to functionally and molecularly distinct cell types, there are functionally and molecularly distinct mitochondrial types, or "mitotypes."<sup>374</sup>

Different tissues and cell types contain markedly different mitotypes that likely influence MIPS signal transduction. For example, cardiomyocyte mitotypes in the heart are optimized for ATP synthesis, adrenal cortex mitotypes specialize for steroidogenesis, and liver mitotypes specialize for ketogenesis, serine metabolism, and anaplerosis. Even within a given organ, neighboring cell types can acquire distinct mitotypes. For example, mitochondria from adjacent oxidative versus glycolytic skeletal muscle fiber types acquire vastly different proteomes that match their functional specialization.<sup>375</sup> Likewise, circulating human immune cell subtypes (B cells, naive or activated T cells, monocytes, neutrophils, etc.) exhibit markedly different OxPhos profiles and mtDNA copies per cell.<sup>374</sup> And in the mouse brain, mitochondria exhibit regional<sup>376</sup> as well cell-type-specific functional and molecular diversity.<sup>372</sup> These divergent cell-type-specific mitochondrial features, along with cell-specific metabolic requirements, may explain why in mice spongiform neurodegeneration is caused by the loss of mtDNA specifically in astrocytes but not in neurons, for example.<sup>377</sup> In mitochondrial disease, large intracellular and cell-to-cell heterogeneity in mitochondrial phenotypes also develops as mutant mitochondria proliferate,<sup>378</sup> such that adjacent muscle fibers can show different stages or even opposite responses to OxPhos defects.<sup>379</sup>

Diverse mitotypes also populate different sub-cellular compartments within individual cells.<sup>201</sup> In neurons, the cell body (i.e., soma) and synaptic boutons have remarkably different mitochondrial proteomes.<sup>380</sup> Similarly, muscle fibers contain two mitotype sub-populations (subsarcolemmal [SS] and intermyofibrillar [IMF]) with quantitatively distinct proteomes.<sup>381</sup> Adipocytes also are populated by at least two main mitotypes: mitochondria proximal to lipid droplets (i.e., peridroplet, or PDMs) and mitochondria not in immediate contact with lipid droplets.<sup>382</sup> These two adipocyte-specific mitotypes exhibit distinct bioenergetic, proteomic, and fusion dynamics. Thus, the organism is composed of a wide spectrum of molecularly and functionally specialized mitotypes.

### Relevance of mitochondrial functional specialization to sensing, integration, and signaling

Mitochondrial sensing (MIPS step 1): in multicellular organisms, individual cell types express a narrow set of cell-type-specific

receptors and are therefore sensitive to a narrow set of inputs. For example, the sensory organs in animals exhibit specific sensitivities to a narrow set of inputs: the eyes sense light but do not perceive sound nor taste, and neither the inner ear nor the tongue respond to light. Each set of neurons within sense organs specifically responds to select inputs. Similarly, the expression levels of dozens of mitochondrial genes and proteins<sup>35,383</sup> including mitochondrial sensory components—transporters, receptors, enzymes—are relatively specialized across both mouse and human tissues (A.S. Monzel, personal communication). The functional specialization of mitotypes across tissues and cell types may thus produce unique mitochondrial sensory systems tailored for specific inputs in different cell types.

Mitochondrial signal integration (MIPS step 2): several aspects of mitochondrial morphology, dynamics, and motility vary between tissues and cell types.<sup>201</sup> In human and mouse skeletal muscle, SS/perinuclear mitotypes are spheroids whereas IMF mitotypes that intersperse sarcomeres have a branched, elongated morphology.<sup>170</sup> In neurons, despite sharing a continuous cytoplasm, somatic mitochondria form a partially connected network surround the cell nucleus, elongated branched dendritic mitochondria extend for tens of microns, whereas axonal and synaptic mitochondria are mostly punctate.<sup>384</sup> In relation to motility, skeletal muscle mitochondria are remarkably stationary<sup>180</sup> compared to neuron axonal and dendritic mitochondria that exhibit greater motility.<sup>385</sup> From first principles, these profound differences in the morphology, topology, and dynamic properties of the MIPS predict that mitochondrial information transfer, integration, and computation differ between tissues and cell types. Although currently technically challenging, improving imaging (e.g., Wolf et al.<sup>386</sup>) and experimental (e.g., Berry et al.<sup>387</sup>) technologies combining spatial and temporal resolution should eventually allow us to map and empirically manipulate information flow through mitochondrial networks.

Mitochondrial signaling (MIPS step 3): tissue-specific mitotypes influence both the nature and magnitude of MIPS outputs and cellular responses. For example, susceptibility to PTP opening,  $\text{Ca}^{2+}$  buffering capacity, and ROS emission characteristics differ remarkably between glycolytic and oxidative skeletal muscle mitotypes.<sup>373</sup> This has implications for inter-organelle crosstalk. The ISRmt response also is induced cell-specifically; proliferating myoblasts and myotubes exhibit different ISRmt responses to the same mitochondrial perturbations.<sup>326</sup> In the mouse brain, progressive mtDNA depletion in Twinkle-KO astrocytes but not neurons induces the ISRmt.<sup>377,388</sup> Mitochondrial activation of the ISRmt and its interaction with other pathways, such as the mTORC1, is also tissue-specific.<sup>115,379,388,389</sup> Thus, MIPS signaling as well as the cellular responses to MIPS signals both differ between cell types and tissues. Our current understanding of how mitotypes from different tissues differentially signal intracellularly and systemically is in its infancy.

### OUTSTANDING QUESTIONS

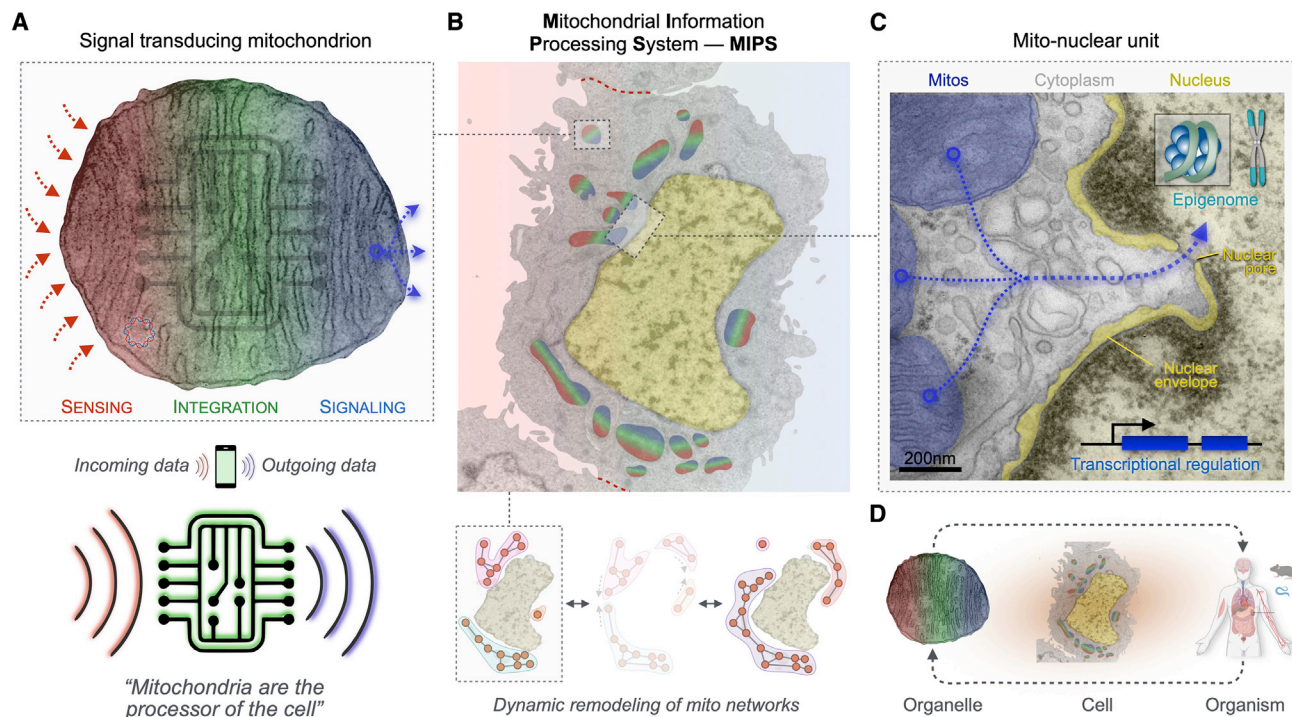
To understand how mitochondria contribute to organismal health and disease, several important challenges remain. As re-

viewed above, the MIPS performs several functions that ensure rapid cellular and systemic responses commensurate with the energetic and biochemical state of the organism. As a result, mitochondria contribute to healthy cellular and physiological adaptation. Clinically, it is clear that mitochondrial diseases involve primary genetic defects affecting molecular processes other than the OxPhos machinery (i.e., not all mitochondrial diseases are disorders of ATP deficiency).<sup>390</sup> Certain tissues and organs also specifically become affected, whereas others are relatively spared.<sup>391</sup> How do non-energetic mitochondrial functions influence health and disease?

The three-step mitochondrial signal transduction framework described here raises several questions, some of which are listed below. Providing answers to these and other emerging questions would advance our understanding of the instructive role that the mitochondria play in human health. Because tissue-specific mitochondrial phenotypes (i.e., mitotypes) are the integrators of cell, tissue, and organismal metabolic inputs, and because MIPS outputs modulate not only a large fraction of the human genome but also complex animal behaviors, the following questions broadly concern the biological and biomedical sciences.

- Are defects in the sensing, integration, and signaling functions of the MIPS sufficient to perturb physiological adaptation in the organism, leading to disease? Communication between cells is essential to maintaining organismal integrity, and perturbing communication alone is sufficient to cause health disorders.<sup>392,393</sup> For example, impaired mitochondrial fusion (and/or inter-organelle interactions) causes human disease.<sup>394,395</sup> Is impaired mitochondrial signal transduction—in the absence of OxPhos defects—a cause of metabolic and other types of health disorders?
- How are specific mitochondrial functional impairments communicated to the cell? Different molecular defects converging on downstream OxPhos deficiency can cause different gene-regulatory signals,<sup>322</sup> cellular responses,<sup>23</sup> and disease manifestations.<sup>140</sup> In general, critical biological processes tend to exhibit redundancy (several effectors exist to sense or communicate the same inputs), which, coupled to a diversity of downstream interacting genetic programs (e.g., innate immunity, ISRmt, mtUPR), affords a diversity of potential cellular and organismal responses. Are MIPS output signals, or combinations thereof, cell- or tissue-specific? What determines the exact transcriptional responses they elicit?
- How generalizable or species-specific are mitochondrial signal transduction mechanisms? Biological mechanisms and therapeutic processes in rodents and invertebrates often align only partially with humans,<sup>396,397</sup> and important differences also exist between mouse strains,<sup>398</sup> for example. Furthermore, different cell types harboring distinct mitotypes may exhibit different responses to metabolic stressors.<sup>379</sup> Systematic exploration of human health and disease states, as well as experimental models for specific molecular features that mimic as closely as possible human physiology or pathology, will increase the likelihood that our findings in model systems will be of biologic, diagnostic, or therapeutic value in humans.





**Figure 7. Core MIPS components for mitochondrial signal transduction**

(A) All mitochondria contain the machinery and capacity to sense, integrate, and signal information. Based on evidence reviewed above, mitochondria emerge as “the processor of the cell.”

(B) Electron micrograph of a cultured cell (human 143B) with mitochondria (blue, green, and red) and nucleus (yellow) highlighted; dotted red lines indicate cell-cell boundaries. The functions of the MIPS are determined by intrinsic properties of individual mitochondria and surrounding organelles, constantly shaped by the dynamic remodeling of organelle networks over minutes to hours. Topologically, the MIPS sits at the interface of the extracellular space harboring endocrine, metabolic, and biochemical signals, and of the (epi)genome in the nucleus.

(C) The mito-nuclear unit enhances and amplifies the effects of mitochondrial outputs, tapping into a rich variety of evolved nuclear DNA-encoded (epi)genetic stress response pathways that communicate local mitochondrial states to the organism. This includes the signaling of peripherally derived metabolites on the brain.

(D) The framework of mitochondrial signaling from organelle to organism. In multicellular animals, the MIPS is a core regulatory element acting both subcellularly and systemically to optimize adaptation and organismal health.

- Did the role of mitochondria as an information processing system contribute to the evolutionary turning point of endosymbiosis? Argument accounting for the role of mitochondria as a harbinger of multicellular, complex life includes the protection from oxygen<sup>399</sup> and a rise in energy supply,<sup>400</sup> although these possibilities have been challenged.<sup>401</sup> Communication and information exchange via optimized biological structures—epitomized at the scale of the organism by the nervous system<sup>360</sup>—afforded an unprecedented acceleration and complexification of social behaviors among animals. This raises the possibility that the acquired ability of cells to sense their environment, efficiently transduce information, and communicate with each other via the MIPS may have been a decisive factor in the evolution and diversification of multicellular life.

## SUMMARY

The past decades of mitochondrial research have produced remarkable advances in our knowledge of how mitochondria function and behave within the cell. More recently, accumulating

evidence revealed how mitochondria communicate extensively with other organelles, between cells, and even across organ systems. Integrating these notions under a common framework suggests that a central role of mitochondria is to transduce information, functioning as a distributed information processing system. The most advanced known form of signal transduction occurs in the brain, which efficiently integrates sensory inputs to develop precise internal representations of the outside world, and secondarily deploys optimal organismal responses and behaviors that promote adaptation and survival. We propose that mitochondria perform a similar, albeit more primitive, form of signal transduction. The MIPS integrates the constant flow of molecular and non-molecular inputs about the energetic and metabolic states of the system, and secondarily deploys in collaboration with the nucleus an array of outputs that guide cellular and organismal adaptation (Figure 7).

The mitochondrial signal transduction framework highlights how mitochondria simultaneously contribute central roles to energy transformation and biosynthesis, as well as to signal transduction. This framework also helps to situate our increasingly precise, mechanistic, and reductionistic investigations of mitochondrial features, activities, functions, and behaviors within the context of the organism and its environment. The view of

mitochondria as a distributed information processing system—or as a “portal” positioned at the interface of the outside environment and the inner world of the cell’s (epi)genome—integrates all historical domains of mitochondrial biology. As a result, the mitochondrial signal transduction framework emphasizes the need for knowledge integration across sub-fields of mitochondrial science. This also highlights the many ways in which multiple domains of mitochondrial biology beyond energetics may be linked to organismal health.

Improving human health is the shared goal of the biomedical community. This collective effort involves building increasingly accurate theories, models, and testable hypotheses about the processes that not only falter in advanced stages of diseases, but also those that enable optimal adaptation so that health is achieved. Health is the ability to deploy optimal responses to challenges.<sup>55</sup> Organismal health emerges from the functional interconnections and crosstalk between cellular and physiological systems, behaviors, and psychosocial states that regulate biology, and vice versa.<sup>402–405</sup> As we begin to map the basis of health beyond the absence of disease,<sup>393,406</sup> it appears crucial to mechanistically decipher two prominent forces related to mitochondria. The first is energy, which brings otherwise inert genes to life and powers the functions of cells and organs. The second is communication or signal transduction, which connects and thus turns parts into wholes. Signal transduction turns cells into cell collectives and binds organs together as an organism. The organism—not the cell—is the ultimate evolutionary unit upon which selection pressures act and where health manifests.

Therefore, articulating the role of multifaceted mitochondria in signal transduction across the organism can help us achieve our shared goal of improving human health in three main ways: (1) by broadening and prioritizing the health-relevant mitochondrial biology questions to test, (2) by selecting the ideal human study design or animal model systems in which to address them, and (3) by connecting more effectively new molecular, cellular, and physiological discoveries in mitochondrial biology to human health.

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### DECLARATION OF INTERESTS

O.S.S. is a co-founder of Capacity and Inspire Bio.

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