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The role of mitochondrial respiration in physiological and evolutionary adaptation

Jayatri Das

Summary

Aerobic mitochondria serve as the power sources of eukaryotes by producing ATP through oxidative phosphorylation (OXPHOS). The enzymes involved in OXPHOS are multisubunit complexes encoded by both nuclear and mitochondrial DNA. Thus, regulation of respiration is necessarily a highly coordinated process that must organize production, assembly and function of mitochondria to meet an organism's energetic needs. Here I review the role of OXPHOS in metabolic adaptation and diversification of higher animals. On a physiological timescale, endocrine-initiated signaling pathways allow organisms to modulate respiratory enzyme concentration and function under changing environmental conditions. On an evolutionary timescale, mitochondrial enzymes are targets of natural selection, balancing cytonuclear coevolutionary constraints against physiological innovation. By synthesizing our knowledge of biochemistry, physiology and evolution of respiratory regulation, I propose that we can now explore questions at the interface of these fields, from molecular translation of environmental cues to selection on mitochondrial haplotype variation. ***BioEssays*** **28:890–901, 2006. © 2006 Wiley Periodicals, Inc.**

Introduction

In diverse eukaryotic taxa, aerobic mitochondria play a vital role as the energy production centers of cells. The mechanisms by which energy is harnessed and used to synthesize ATP in mitochondria have long been the focus of intense biochemical and biophysical study. More recently, our understanding of mitochondrial function has expanded to include physiological regulation of oxidative phosphorylation (OXPHOS), which in turn has yielded new insight on how this ancient pathway has evolved to meet the demands of changing environmental and physiological conditions.

With available data on biochemical function, protein structure, physiological regulation, and whole genome sequences from an ever-increasing number of species, we are now poised to answer new evolutionary questions about mitochondrial respiration. To illustrate the role of mitochondria in adaptation and diversification of higher animals, with examples from other taxa where appropriate, I first review the molecular mechanisms by which OXPHOS enzymes are regulated. I examine our current knowledge of the physiological regulation of mitochondrial respiration, i.e. *physiological adaptations* that allow an individual to adjust to environmental change. I then discuss how functional constraints have shaped the evolution of both mitochondrial and nuclear respiratory genes, i.e. *evolutionary adaptations* resulting from natural selection. I close by highlighting promising new directions of interdisciplinary research.

It should be noted that, despite the ubiquity of aerobic mitochondria in textbooks, OXPHOS is not the only means of energy production that has evolved in eukaryotes. In numerous anaerobic species, mitochondrial respiration utilizes terminal electron acceptors other than oxygen, producing waste products such as acetate, succinate and nitric oxide.[1] Other derivatives of mitochondria include hydrogenosomes, which produce hydrogen as a byproduct of ATP synthesis, and mitosomes, which share some functional similarities to mitochondria but produce no ATP; both generally lack organellar genomes.[2] Although the physiology and evolution of these alternatives to OXPHOS are fascinating, they are beyond the scope of this review and I limit my discussion here to aerobic mitochondria.

The cellular toolkit: mechanisms of regulating mitochondrial respiration

Initial work in the 1950s suggested a feedback-based regulatory mechanism of OXPHOS based on extramitochondrial

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Abbreviations: ACTH, corticotropin; ATPase, ATP synthase; COX, cytochrome c oxidase; CR, calorie restriction; CREB, cAMP-responsive element binding protein; eNOS, endothelial nitric oxide synthase; EST, expressed sequence tag; HRE, hormone response element; ND, NADH dehydrogenase; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PGC-1, PPARγ coactivator-1; PRC, PGC-1 related coactivator; RIα, protein kinase A regulatory subunit; ROS, reactive oxygen species; SD, succinate dehydrogenase; T_2, 3,5-diiodothyronine; T_3, 3,5,3′-triiodothyronine; TFAM, mitochondrial transcription factor A.

concentrations of ADP and P_i.[3,4] Application of the metabolic control theory developed by Kacser and Burns[5] and Heinrich and Rapoport[6] in the 1970s has since guided a quantitative approach to understanding a far more complex system of regulation. This method of analysis assumes that control of flux, i.e. oxygen consumption and/or ATP synthesis, is quantitatively distributed among all enzymes of the pathway rather than being concentrated in a single rate-limiting step. The role of each step in regulation can be determined by estimating the relative change in flux for each enzyme when it is inhibited in its isolated state versus in its pathway context, a value termed the flux control coefficient. In vivo, the flux control coefficients and relative regulatory importance of OXPHOS enzymes can vary—for example, between tissues or under different physiological conditions—due to changes in substrate supply, enzyme concentration or transmembrane electric potential.[7–9]

Here I present a broad overview of the different types of regulatory mechanisms operating on OXPHOS enzymes in order to introduce the biochemical toolkit available for physiological and evolutionary adaptation. These mechanisms include quantitative regulation of mitochondrial enzyme concentration and/or organelle density, qualitative alteration of enzyme activity and regulation of mitochondrial uncoupling.

The key enzymes: a brief overview of mitochondrial respiration

In aerobic respiration, mitochondrial enzymes accept electrons from electron carriers reduced in glycolysis and the tricarboxylic acid cycle. These electrons are ultimately transferred to O_2 to produce water. The series of redox reactions involves four enzyme complexes embedded in the inner mitochondrial membrane (Fig. 1). The electron carriers NADH and succinate donate electrons to NADH dehydrogenase (ND, Complex I, EC 1.6.5.3) and succinate dehydrogenase (SD, Complex II, EC 1.3.5.1), respectively. The electrons are then transferred to coenzyme Q to form ubiquinol, which donates them to the cytochrome bc_1 complex (Complex III, EC 1.10.2.2). Cytochrome c is the next electron acceptor and donates electrons to cytochrome c oxidase (COX, Complex IV, EC 1.9.3.1). In the final step, the electrons are accepted by molecular oxygen to produce water.

These four enzyme complexes use the energy released from electron transfer to pump protons out of the mitochondrial matrix into the intermembrane space, creating a pH gradient and transmembrane electric potential. A fifth enzyme complex, ATP synthase (ATPase, Complex V, EC 3.6.3.14), uses the energy stored in this electrochemical gradient to condense ADP and P_i into ATP. OXPHOS is most efficient when excess ADP is available, since all available oxygen is consumed in

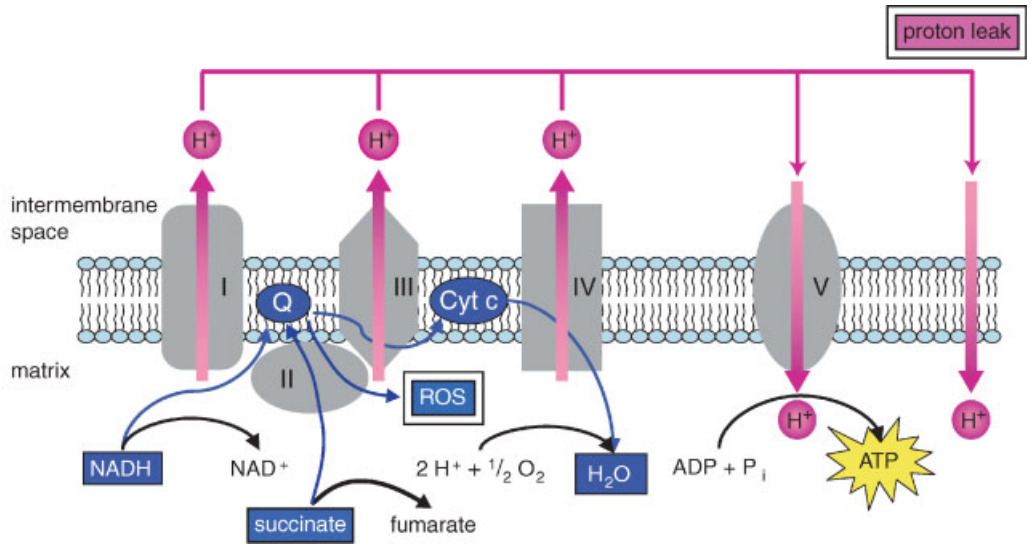

Figure 1. An overview of oxidative phosphorylation in the inner membrane of aerobic mitochondria. Complexes I (ND) and II (SD) transfer electrons from NADH and succinate, respectively, to coenzyme Q to form ubiquinol. Complex III (cytochrome bc_1 complex) catalyzes electron transfer to Complex IV (COX), which reduces molecular oxygen to form water. The path of electrons is indicated by blue arrows. Complexes I, III and IV use the energy from electron transfer to pump protons into the intermembrane space, creating an electrochemical gradient. The energy stored in this gradient allows complex V (ATPase) to produce ATP as the protons reenter the mitochondrial matrix. The path of protons is indicated by pink arrows. Electrons and protons can break this circuit at two points, indicated by black boxed text. Electrons can escape redox transfer and leak back into the matrix, reacting with oxygen to produce harmful ROS. Protons can bypass ATPase via uncoupling mechanisms, which can be used to maintain membrane potential or generate heat.

order to maintain membrane potential to drive ATP synthesis. When ADP is in limiting quantity, some of the molecular oxygen is reduced to form toxic reactive oxygen species (ROS).

In animals and many other eukaryotic taxa with aerobic mitochondria, all five complexes generally (though not universally) consist of multiple subunits, ranging from 4 subunits in SD to 46 subunits in mammalian ND.[10] While the majority are encoded by the nuclear genome with mitochondrial localization sequences in each gene, some OXPHOS genes have been maintained in mitochondrial genomes across taxa ranging from the protozoon *Reclinomonas americana*[11] to vertebrates: seven subunits of ND, three subunits of COX, one subunit of the cytochrome bc_1 complex and two subunits of ATPase. This division of genetic information necessitates intergenomic coordination of transcription and translation for assembly and function.

Quantitative regulation of mitochondrial enzyme levels

In metazoans, transcriptional regulation is the primary quantitative mechanism by which mitochondrial enzymes are maintained at steady-state levels or adjusted according to the need for respiratory function. Coordinated transcription of nuclear- and mitochondrially encoded proteins for mitochondrial biogenesis (synthesis of complete organelles) is a complex process, involving about 1000 genes. The molecular factors regulating this process, with hormonal cues activating downstream targets including general transcription factors, mitochondria-specific transcription factors and coactivators, have recently begun to be identified.[12]

Endocrine signals often initiate transcriptional regulation of respiration (Fig. 2). The presence of hormone response element (HRE) sequences in the mitochondrial genome as well as increased mitochondrial transcription in the presence of hormones suggest that mitochondrial gene expression may be directly stimulated by endocrine signals.[13] For instance, expression of mitochondrially encoded ND subunit 3 is upregulated by thyroid hormone 3,5,3′-triiodothyronine (T_3) in rat brain and heart, possibly via a thyroid hormone receptor binding site in the ND3 gene.[14] T_3 also increases transcription of both nuclear- and mitochondrially encoded subunits of cytochrome oxidase in rat liver.[15] Experiments to provide direct proof of this interaction are still in progress. Corticotropin (ACTH) has also been shown to increase mRNA levels of mitochondrially encoded genes involved in OXPHOS in bovine tissues, leading to increased enzyme activity.[16]

Regulation of nuclear-encoded mitochondrial gene expression in response to endocrine signals is controlled by transcription factors, nuclear respiratory factors (NRFs) and coactivators (Fig. 2).[12] Promoter analysis of nuclear-encoded mitochondrial genes reveals the presence of binding sites for the general zinc finger transcription factors Sp1 and YY1 as well as for the cAMP-responsive element binding protein (CREB). NRFs involved in transcriptional regulation are induced by coactivators such as PGC-1 (PPARγ coactivator-1) and PRC (PGC-1 related coactivator) that respond to hormonal cues.

NRFs also coordinate the regulation of mitochondrially encoded genes by activating mitochondrial transcription factor A (TFAM). NRFs induce expression of TFAM in the nucleus; the protein is then targeted to the mitochondria and stimulates mitochondrial transcription (Fig. 2).[13] In rat β-cells lacking PDX1, a transcription factor involved in insulin secretion, reduced expression of TFAM is associated with reduced mRNA levels of complex I and IV subunits and lower total ATP levels, despite apparent compensatory increases in

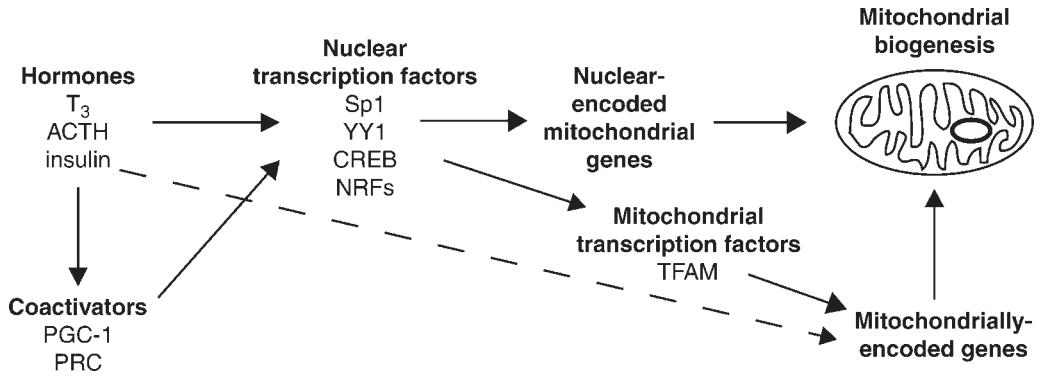

Figure 2. The regulatory pathway for mitochondrial biogenesis in animals. Endocrine signals, such as thyroid hormones, ACTH, or insulin can initiate mitochondrial biogenesis by activating transcription factors in the nucleus either directly or via coactivators. Nuclear factors upregulate expression of nuclear genes that encode mitochondrial proteins that are targeted to the organelle. NRFs also activate the mitochondrial transcription factor TFAM, which initiates transcription of the mitochondrial genome. Mitochondrially encoded genes may also be directly stimulated by hormones, but evidence of a direct interaction between mitochondrial DNA and hormone receptors has not yet been demonstrated (indicated by dashed line).

expression of nuclear-encoded subunits of complex II and V.[17]

While transcriptional regulation of mitochondrial genes is common among animals, post-transcriptional mechanisms may dominate in other taxa. For example, in yeast, where cells rely on fermentation in the presence of glucose, OXPHOS is repressed by glucose-induced degradation of mRNA for subunits of complex II.[18] Mitochondrially encoded genes in dicot plants contain a constitutive promoter, including an AT box and a conserved nonanucleotide motif,[19] but regulation of protein abundance is primarily post-transcriptional.[20] Mechanisms involved in developmental regulation include tissue-specific splicing, polyadenylation for targeted degradation and tissue-specific proteolysis. In nuclear-encoded genes, an upstream ''pollen-box'' motif in the promoter region has been shown to be required for tissue-specific transcriptional activation, but binding proteins have not been identified.[19]

Qualitative regulation by allosteric interactions

Mitochondrial respiration can be qualitatively regulated by endocrine signaling pathways through direct allosteric interactions. As the only component of the electron transport chain that does not operate near equilibrium, COX is the primary target of allosteric regulation. Based on experimental studies of COX in vertebrate systems, Ludwig and colleagues[21] have proposed a model for COX regulation in which the enzyme has two states of operation: a relaxed state under allosteric regulation by ATP and ADP, and an excited state that is regulated chemiosmotically by membrane potential. COX operates at a lower, more efficient rate in its relaxed state, while the excited state yields ATP at a higher rate but is less efficient and produces more ROS that are harmful when they accumulate over time. The switch from the relaxed state to the excited state may be induced by stress-hormone-initiated signaling cascades, substrates such as 3,5-diiodothyronine (T_2) or palmitate, or a high ratio of reduced to oxidized cytochrome c. Supporting this model of allosteric regulation, conformational changes in COX in the presence of T_2 have been characterized in vertebrates.[22] Deiodination of T_3 and subsequent regulation of COX by T_2 may be the nongenomic mechanism by which thyroid hormones increase basal metabolic rate when a temporary increase in energy is needed (at the cost of higher ROS production in the short term).[23]

OXPHOS is also qualitatively regulated by the antagonistic effects of insulin and glucagon. In mammalian cells, glucagon signaling induces cAMP-dependent phosphorylation of COX subunit I, decreasing V_{max} and increasing K_m for cytochrome c so as to inhibit enzyme activity even in the presence of the activator ADP.[24] In addition, the regulatory subunit of protein kinase A (RIα) downregulates cytochrome oxidase through a cAMP-dependent interaction between RIα and COX Vb.[25] As a reduced level of cAMP is a downstream effect of insulin signaling, insulin may increase cytochrome oxidase activity by reducing levels of cAMP and inhibiting the association of COX Vb and RIα.

Regulation of mitochondrial uncoupling

Mitochondrial uncoupling is the leak of protons from the intermembrane space back into the matrix, bypassing ATPase and converting the energy stored in membrane potential directly into heat (Fig. 1). Proton leak is an inherent biophysical property of lipid bilayers maintaining a proton motive force. Flux rate can vary depending on the phospholipid fatty acid composition of the membrane,[26] and can also be specifically regulated by uncoupling proteins (UCPs). In mammals, UCP1 is restricted to brown adipose tissue and was originally identified as a factor associated with adaptive thermogenesis.[27] Two additional homologs have since been identified in mammals; UCP2 is ubiquitously expressed[28,29] while UCP3 is primarily restricted to the skeletal muscles.[30] The regulatory roles of these proteins will be discussed in greater detail in the following sections in the context of their physiological functions. While homologs have also been found in plants,[31] birds[32] and fish,[33] the biochemical mechanisms and physiological regulation of UCP function in these taxa have not yet been fully characterized.

Physiological adaptation: modulating bioenergetics during environmental change

An organism's ability to progress through development, adopt alternative morphologies, or adapt to novel environments often depends on the amount of available energy. Data suggest that OXPHOS has the potential to be upregulated under stress (e.g. by ACTH, as discussed earlier) or other energy-intensive conditions as an adaptive physiological response; studies in *Drosophila* show that mitochondrial enzymes operate well below maximal rates of activity when producing normal levels of ATP,[34] and COX activity can vary up to tenfold between tissues in rats.[35] In addition, the limited number of factors that coordinate expression of nuclear-encoded mitochondrial genes suggests that regulation of mitochondrial biogenesis may be relatively straightforward.[36]

Regulation of respiratory output has been demonstrated to mediate a wide range of physiological changes. For example, in the wing-polymorphic cricket *Gryllus firmus*, flight muscle mitochondria in winged individuals have higher respiratory rates than those in wingless individuals.[37] Respiration is also thought to influence caste differentiation in the honeybee *Apis mellifera*, as queen larvae have higher metabolic rates and higher mitochondrial gene expression than workers.[38] In yeast, alternative isoforms of a nuclear-encoded subunit of COX are expressed under hypoxic and normal conditions,[39] where the ''hypoxic'' isoform confers a 3- to 4-fold increase in the catalytic constant.[40] Plants may take a different, quantitative approach to adapting to oxidative stress; in *Oryza*

sativa, respiratory rate is slowed through major downregulation of expression of respiratory genes.[41]

To illustrate in more detail how mitochondrial respiration is regulated under changing physiological conditions, I focus on two processes: cold acclimation and calorie restriction. These examples demonstrate the range of pathways that can be manipulated in a physiological context.

Cold acclimation

In both endotherms and ectotherms, the mitochondrial response during acclimation to cold temperatures commonly involves some combination of three strategies: quantitative changes in protein levels, qualitative changes in enzyme capacity or substrate affinity and regulation of uncoupling (Table 1). As an example of the first strategy, small mammals upregulate mitochondrial respiratory genes during hibernation, possibly compensating for protein damage.[42] There is evidence that frogs utilize the second and third strategies, maximizing respiratory efficiency in cold hypoxic conditions by increasing mitochondrial affinity for oxygen coupled with reducing proton leak by lowering membrane potential.[43,44] In rainbow trout, cold acclimation involves a quantitative increase in mitochondrial activity by increasing cristae density, complemented by a qualitative increase in enzyme activity per milligram of protein.[45]

What are the mechanisms underlying these strategies? Quantitative regulation of mitochondrial proteins can occur through changes in DNA copy number, transcription, translation, mRNA stability or protein stability. In mice, the thermogenic response is mediated by β-adrenergic receptors that induce the transcriptional coactivator PGC-1, which in turn upregulates NRFs that stimulate mitochondrial biogenesis.[46] In fish, respiratory enzyme levels increase under cold acclimation without proportional increases in mitochondrial DNA copy number or mRNA levels,[47] suggesting regulation of translation or protein degradation; however, mechanisms for these regulatory processes are not known. In contrast, insects survive seasonal cold temperatures by reducing respiration: overwintering larvae of the goldenrod gall fly and the arctic woolly bear caterpillar consume less oxygen and have decreased levels of mitochondrial DNA, though they maintain a pool of mitochondrial mRNAs possibly stabilized by polyadenylation.[48]

The second strategy, qualitatively increasing oxidative capacity in cold temperatures, is commonly accomplished in fish by remodeling mitochondrial membrane composition.[49–51] Increases in unsaturated fatty acids and phosphatidyl ethanolamine in the lipid bilayer maintain its flexibility, enhancing the function of membrane-bound protein complexes. In fact, phospholipid membrane properties are so effective at maintaining the activity of membrane-bound proteins that they largely mitigate the need for the type of evolutionary changes in protein structure that are often found in non-membrane enzymes in cold-adapted species.[52]

Mitochondrial uncoupling, the third strategy, is driven by the biophysical properties of lipid bilayers maintaining a proton motive force, so membrane composition plays an important role in modulating proton leak. In fish, cold exposure leads to an increase in unsaturated fatty acids as well as an increase in the proportion of membrane-destabilizing phosphatidyl ethanolamine to phosphatidyl choline.[53] These changes in membrane composition increase proton leak and enhance the activity of membrane-bound proteins in cold temperatures by accelerating the leak-pump cycle. In mammalian brown adipose tissue, proton leaks are specifically regulated by the uncoupling protein UCP1. In a well-characterized thermogenic

Table 1. Physiological adaptations to cold acclimation and calorie restriction

	Regulatory mechanisms	Mitochondrial response
Cold acclimation		
Mammals	Transcriptional activation by PGC-1 and NRFs[45]	Increased mitochondrial biogenesis
	Uncoupling to increase proton leak by UCP1[53]	Generation of heat
Fish	Translational activation or enhanced protein stability[46]	Increased mitochondrial enzyme levels
	Remodeling membrane composition and increased proton leak[48–50,52]	Enhanced enzyme activity
Insects	Reduction of mtDNA[47]	Reduced mitochondrial respiration
Calorie restriction		
Mammals	Transcriptional activation by eNOS, PGC-1[58,59]	Increased mitochondrial biogenesis, increased fat breakdown
	Uncoupling to increase proton leak by UCP2 and UCP3 and coactivators[65,66]	Maintenance of membrane potential and reduced oxidative damage
Yeast	Shift from anaerobic to aerobic respiration[62]	Increased mitochonchondrial respiration
	Uncoupling to increase proton leak[64]	Increased mitochondrial respiration, reduced oxidative damage

Quantitative regulatory mechanisms are important for enabling organisms to adjust quickly to changes in the environment. The list of examples here is not exhaustive but is generally representative of the mechanisms underlying a range of observed mitochondrial responses.

response, PGC-1, in conjunction with other transcriptional coactivators and the retinoic acid X receptor, induces transcription of UCP1 in brown fat adipocytes.[54] The increased proton leak short-circuits OXPHOS to generate heat.

Calorie restriction

Calorie restriction (CR) has drawn attention in recent years since the discovery of its role in extending lifespan in multiple model systems. Several models have been proposed regarding whether and/or how CR-induced respiratory changes affect longevity but have yet to be fully resolved.[55–57] Regardless of long-term consequences on lifespan, the short-term response to limited resource availability is an important adaptation in natural populations. While a classical view of respiratory regulation intuitively predicted that organisms would lower their metabolic rate under CR (thereby extending longevity due to reduced oxidative stress),[58] recent work has shown that the respiratory response to CR is a highly regulated process and far more complex (Table 1).

One response to low nutrition is an increase in mitochondrial biogenesis. In CR mice, this response is induced by upregulation of endothelial nitric oxide synthase (eNOS, EC 1.14.13.39).[59] The product of eNOS, nitric oxide, and its second messenger, cGMP, activate the PGC-1 pathway to induce mitochondrial proliferation.[59,60] As a short-term physiological adaptation, quantitative changes in the number of mitochondria may increase lipolysis and β-oxidation (which occurs in the mitochondrial matrix), allowing animals to draw on stored fats in times of low resource availability. Supporting this hypothesis, upregulation of eNOS also increases levels of SIRT1,[59] which mobilizes fats from white adipocyte tissue during CR.[61]

The effect of increased mitochondrial biogenesis on metabolic rate is unclear; in vitro, oxygen consumption decreases in cells treated with serum from CR mice (although ATP levels are maintained),[60] yet in vivo, metabolic rate increases when animals are kept on a CR diet for 3 to 12 months.[59] This contrast may represent a sequential shift in the metabolic response at the organ level as body composition changes over the duration of CR. In vivo experiments in other model systems also demonstrate an increase in metabolic rate correlated with sustained nutrient limitation. *C. elegans* cultured without bacterial food content consume more oxygen than those raised on a normal diet[62] while, in yeast, the increase in metabolic rate under CR is caused by a shift from anaerobic to more efficient aerobic respiration.[63]

Another mechanism by which CR affects respiratory regulation is through mitochondrial uncoupling.[57] In vitro, short-term CR increases proton leak and decreases mitochondrial membrane potential, linking respiratory rates in CR animals with lower rates of ROS production.[60,64] In yeast as well, increased respiration and reduced ROS generation under CR conditions can be replicated by the action of chemical uncoupling agents.[65]

In mammals, uncoupling proteins UCP2 and UCP3 have been implicated in metabolic regulation during CR.[66,67] Both require the presence of activating cofactors to catalyze proton transport; free fatty acids and ROS byproducts resulting from increased β-oxidation during CR are sufficient to initiate proton conductance.[66] UCP2 expression increases during fasting and UCP2-knockout mice have elevated insulin levels, supporting a model in which UCP2 regulates insulin secretion through control of a membrane-bound K^+ channel in β-cells that is sensitive to the [ATP]/[ADP] ratio.[68,69] However, Bordone and colleagues[70] demonstrated that SIRT1 can directly repress UCP2 to restore insulin secretion; this result has not yet been reconciled with data that show upregulation of both UCP2 and SIRT1 under CR. The putative function of UCP3 is even less clear; UCP3 has been postulated to transport fatty acid anions out of the matrix when lipid breakdown exceeds mitochondrial capacity,[67] but evidence for this process remains circumstantial.

These mechanisms of respiratory regulation by uncoupling proteins during CR appear unlikely to be universal. Although related proteins have been found in plants and ectothermic fish, no significant homologs of UCP2 or UCP3 exist in *S. cerevisiae* or *C. elegans*.[71] The in vivo pathway underlying CR-induced uncoupling in yeast has not yet been determined and, in contrast to the results in mice, *daf-2* mutant *C. elegans* with reduced levels of insulin receptor signaling exhibit decreased proton leak.[62]

Thus, mitochondrial respiration appears to be regulated during CR to maximize the use of stored lipids for metabolic fuel and to minimize the oxidative damage that accompanies this shift, but many gaps remain in our understanding of the regulatory network. For instance, it is unclear whether the previously discussed models of COX allosteric regulation involving endocrine signaling[21,24,25] are relevant to CR or if they are limited to more immediate "fight or flight" physiological responses. Also, as the regulatory mechanisms described above involve factors in lifespan extension (e.g. homologs of SIRT1 and the insulin receptor) that are conserved in non-mammalian systems, the potential conservation of their proximate roles in physiological adaptation to CR warrants further study as well.

Evolutionary adaptation: natural selection on mitochondrial respiratory genes

With our present understanding of the functional interactions of mitochondrially encoded genes and increasing amounts of sequence data available for comparative analysis, we are gaining a clearer picture of the forces of selection acting on the mitochondrial genome. In this section, I discuss how respiratory enzymes have evolved to accommodate the diversification of major eukaryotic taxa. I then describe

the coevolutionary constraints on mitochondrial genes imposed by the need for functional coordination with the nuclear genome. Finally, I discuss some intriguing examples of how respiratory genes can evolve to fulfill the physiological requirements of unique bioenergetic adaptations.

Evolution of taxon-specific function in higher eukaryotes

The subunit composition of each enzyme complex involved in OXPHOS has been determined in a variety of species. These data show that, while subunits encoded by the mitochondrial genome are conserved among the higher eukaryotes, the nuclear-encoded subunits are not universally present in all taxa. Based on studies in bacteria, yeasts and mammals, the number of nuclear-encoded subunits is often thought to correlate with the regulatory complexity of the enzyme.[21] Variation exists not only in the number but also in the identity of nuclear-encoded subunits. For example, multiple enzyme complexes have plant-specific subunits.[72–74] These unique subunits in plants are thought to be involved either in regulation and signaling or in peripheral activities such as processing peptidases[75] or synthesizing ascorbate[76] that have been integrated into mitochondrial enzymes.

High-resolution crystal structures of three of the five bovine enzyme complexes[77–79] provide a structural context in which to observe patterns of evolutionary constraints. Using this information to map conserved amino acids can detect protein domains that are essential for enzyme function and regulation across diverse taxa. Comparative analysis of COX, using the bovine structure and sequence data from plants, animals and yeasts showed that conserved sites fall into three categories: known functional sites that are conserved across taxa, known functional sites that are not conserved and conserved sites of unknown function.[72] In the first category, the unexpected discovery of some conserved functions, such as a vertebrate interaction site for thyroid hormone T_2 that is conserved in insects and yeasts, suggests new hypotheses for experimental studies on respiratory regulation. The second category of sites indicates which regions may be important for the evolution of physiological specialization. Biochemical classification of all conserved residues, including those of unknown function in the third category and analysis of their spatial distribution throughout the crystal structure further suggest that, in COX, clusters of interacting amino acids are conserved to maintain the structural foundation for evolving catalytic sites. Extending this type of analysis to other enzyme complexes may yield similar insights into evolutionary constraints and functional diversification across a wide range of species.

Coevolution of mitochondrial and nuclear genes

Given the evidence for Muller's ratchet (the process by which non-recombining lineages irreversibly accumulate deleterious mutations) in mitochondrial genomes,[80] why do respiratory genes persist in the mitochondria? The conservation of a nonrandom, consistent set of genes in aerobic mitochondria across fungi, animals and plants, in conjunction with data demonstrating no obvious obstacles to gene transfer from the mitochondrion to the nucleus, makes it unlikely that these genes are merely the last to be transferred to the nuclear genome in an ongoing evolutionary process.[81] Possible explanations include the difficulty of transporting highly hydrophobic proteins across the mitochondrial membrane, the non-standard genetic code used in the mitochondrial genome and—the strongest hypothesis—the need for redox control of mitochondrial gene expression.[81] Importantly, this division of genetic information between genomes imposes a coevolutionary constraint on respiratory genes.

When mitochondrial DNA is crossed into a foreign nuclear background, the resulting disruption in mitochondrial activity suggests that functional interactions between different subunits have coevolved. For example, in backcrosses between genetically isolated intraspecific populations of the copepod *Tigriopus californicus*, cytonuclear interactions contributed to a decrease in COX activity in the hybrid offspring,[82] and cytochrome c variants isolated from different populations yielded higher activity when paired with the enzyme from their respective source populations.[83] Positive selection may be restricted to this interaction, as other proteins involved in electron transport showed no evidence of selection despite sequence divergence between populations.[84]

Disruption of coevolved cytonuclear gene complexes generally increases with greater evolutionary distance, as demonstrated by introgression experiments in *Drosophila* showing lower COX activity in interspecific compared to intraspecific backcrosses.[85] Similarly, in ''xenomitochondrial cybrids'', where foreign mitochondrial genomes from six murid species were introduced into *Mus musculus* cells lacking mitochondrial DNA, the decrease in ATP production correlated with divergence between the species of the host cell and mitochondrial donor.[86] With interspecific hybrids, however, disruption of nuclear–mitochondrial interactions is no longer limited to alteration of enzyme efficiency but extends to inhibition of assembly of the enzyme complex itself. In experiments with rodent and primate xenomitochondrial cybrids, protein translation was not affected but steady-state levels of subunits decreased, indicating that reduced respiratory activity was due to problems in enzyme complex assembly.[87,88]

Complementing these experimental studies, sequence analysis shows that amino acids in contact with other subunits and non-contact residues differ in patterns of substitution, consistent with the hypothesis of cytonuclear coevolution. In mammalian COX subunits, residues on nuclear-encoded subunits evolve more slowly when in close proximity to mitochondrially encoded subunits; in contrast, mitochondrially

encoded residues in contact with other subunits evolve more rapidly than non-contacting amino acids, which may allow optimizing interactions with residues on nuclear-encoded subunits.[89] However, while such results begin to establish the molecular basis underlying the functional incompatibility of respiratory enzyme subunits discussed above, there is still only limited evidence as to whether these patterns of substitution are actually indicative of adaptive positive selection; rather, maintenance selection may be driving fixation of compensatory alleles.[90]

Adaptive evolution of respiratory genes

There is a growing number of examples of positive selection acting on individual subunits of mitochondrial enzymes, including the relatively frequent occurrence of duplication and subfunctionalization of subunits across taxa. For example, just as COX V (vertebrate subunit IV) has been duplicated in yeasts, with the different isoforms mediating the physiological response to hypoxia,[39] an analogous duplication has occurred with COX VII (vertebrate subunit VIc) in *Dictyostelium discoideum*.[91]

For other COX subunits, often a predominant isoform is expressed in multiple tissues while a duplicate copy is tissue-specific, such as root-specific COX VIb in plants[92] and muscle-specific isoforms of COX VIa, VIIa and VIII in mammals.[93] Rapid subfunctionalization for tissue-specific expression may be necessary to supply sufficient copies of components of multi-subunit protein complexes where stoichiometry is important. This hypothesis is supported by an analysis of the COX VIIa family in primates that reveals accelerated rates of nonsynonymous substitutions in the mitochondrial targeting presequences after gene duplication.[94] In fact, sometimes coding sequences of duplicated genes remain identical while divergence in the presequence is responsible for differentiation of spatial and temporal expression, as is the case with bovine ATP synthase.[95]

Testis-specific isoforms have also evolved multiple times, possibly to accommodate the high energy production necessary for sperm motility. In mammals, both cytochrome c and COX VIb (the nuclear-encoded subunit that interacts with cytochrome c) have testis-specific isoforms, consistent with a model of specific phosphorylation-dependent induction of testes COX.[96] Duplication and testis-specific expression of OXPHOS genes is more extensive in *D. melanogaster*, where 97 out of 100 expressed sequence tags (ESTs) originating from sixteen duplicated genes were found in testis-derived libraries, compared to less than 2% of ESTs from the parental copies.[97] Differences in biochemical activity between isoforms have yet to be characterized.

Molecular evolutionary analyses have also identified patterns of positive selection on OXPHOS genes that are important for physiological adaptation. In the glacially obligate ice worm *Mesenchytraeus solifugus*, the β and γ subunits of F1 ATPase have undergone rapid divergence from homologs in related mesophilic lineages.[98] The observed nonsynonymous substitutions result in smaller, less polar proteins with fewer charged amino acids, allowing molecular flexibility that likely enables ice worms to maintain unusually high levels of ATP production at very low temperatures. Presumably, the environment of the ice worm is so extreme that modification of the lipid bilayer composition is insufficient to maintain enzyme activity at this level.

Another case of molecular changes in respiratory genes underlying physiological innovation is the evolution of COX I in the aquatic carnivorous bladderworts *Utricularia*. In these plants, two consecutive amino acids, Leu_{113} and Ala_{114} located at the docking site of cytochrome c, have both been replaced by cysteine residues.[99] The formation of a disulfide bridge between these residues is theoretically possible, which could change the structure of COX I and alter the kinetics of the interaction to allow the high respiratory rate required for the ATP-dependent trapping mechanism.

A third example of positive selection is the rapid divergence of OXPHOS genes in anthropoid primates. Subunits of complexes I, III and IV as well as cytochrome c show elevated rates of nonsynonymous substitution in at least two of the anthropoid, catarrhine and hominid lineages.[100] The amino acid replacements tend to occur at sites involved in catalysis or substrate interaction, suggesting a coordinated change in enzyme kinetics and providing one of the few examples of potentially adaptive coevolution. For instance, the electrostatic charge of the cytochrome c binding site on COX, involving 57 residues from eight subunits, is dramatically reduced, complemented by two charge-altering changes on cytochrome c.[101] Although the biochemical effects of these substitutions have not yet been characterized, they are hypothesized to facilitate extended lifespan and enlarged brain size.

Conclusions and future directions of interdisciplinary research

Although OXPHOS involves the coordination of five multisubunit enzymes encoded by both the mitochondrial and nuclear genomes, a relatively limited number of molecular mechanisms are responsible for its regulation. Physiological adaptations to environmental conditions such as cold temperatures or nutrient limitation primarily depend on quantitative mechanisms that quickly adjust mitochondrial enzyme levels to maximize respiratory efficiency or mobilize alternative metabolic fuels. Evolutionary adaptations, subject to cytonuclear coevolutionary constraints, tend to involve qualitative changes in protein function or interaction resulting in physiological diversification. Synthesizing our current knowledge of molecular regulation, physiology and evolution

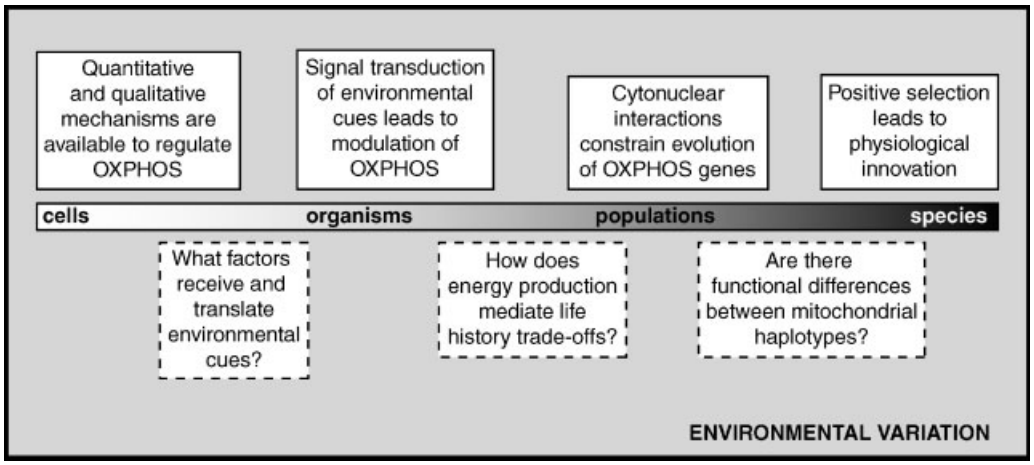

Figure 3. The role of mitochondrial respiration from cellular regulation to diversification of species. Function and regulation of OXPHOS is relevant to all levels of biological organization, from individual cells to organismal physiology to expansion of species range to the evolution of new taxa. Environmental variation at all scales (represented by the gray box) challenges organisms to adjust respiration and produce sufficient energy for survival and reproduction. The topics covered in this review, indicated in the solid boxes above the gradient, have generally focused on molecular characterization of cellular pathways, physiological responses to environmental change, or theoretical sequence analysis of evolutionary patterns. New questions, such as those in the dashed boxes below the gradient, arise from synthesis of these approaches at intermediate levels of biological organization and will help establish a comprehensive understanding of the evolutionary importance of mitochondrial respiration.

of mitochondrial respiration, we can now ask new questions at the intersection of these fields (Fig. 3).

What are the mechanisms by which environmental cues are received and translated to molecular signal transduction pathways?

A promising direction of research is the characterization of receptors for environmental cues—such as cold acclimation and calorie restriction, as well as cues such as altitude that are less well-studied at the molecular level—that activate regulatory pathways. While β-adrenergic receptors have been implicated in the cold acclimation response in vertebrates,[46] more work is needed to identify analogous receptors in other taxa. Intriguing candidate effectors are the thyroid hormones and the putative insect analog juvenile hormone. Although effects of thyroid hormone on transcription of mitochondrial genes have been well-studied,[13,102,103] their nongenomic effects are less clear and may be important for physiological regulation under starvation conditions;[104] for example, T_3 has been shown to upregulate the uncoupling proteins UCP2 and UCP3.[105] Conservation of the vertebrate T_2 interaction site on COX Va in insects and yeasts suggests that allosteric regulation may also occur with juvenile hormone and an unknown yeast analog.[72]

What role does molecular regulation of respiration play in mediating life history trade-offs?

In some instances, trade-offs occur in a background of constant respiratory rate. In the case of the parthenogenetic collembolan *Folsomia candida*, clones with low survival rates, fast growth rates and high fecundity have no difference in metabolic rate compared to clones with the opposite suite of traits.[106] In contrast, other trade-offs require additional energetic investment, such as the trade-off between flight capability and early reproduction in wing-polymorphic crickets, with winged individuals having a higher respiratory metabolism.[107] By what mechanisms are these decisions of energetic allocation made? Identifying the molecular basis of these investment strategies may help us understand how mechanisms of respiratory regulation have been exploited for physiological diversification.

How does variation in the mitochondrial genome influence physiological function?

The use of mitochondrial DNA, including coding regions of respiratory genes, for phylogeography has generated large datasets characterizing different haplotype groups in many species.[108] To what extent does this sequence variation underlie physiological differences? This question has begun to be investigated in humans, where associations of different haplotypes with predilection to various diseases has shed light on the compensatory adaptive value of mutations.[109,110] Comparative analyses of closely related taxa that differ in a given physiological trait, as well as adding life history data to existing phylogeographic information, will provide insight into the cause–effect relationships underlying the influence of natural selection on population structure and speciation.

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