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REVIEW

REGULATION OF MUSCLE MITOCHONDRIAL DESIGN

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Summary

Mitochondria are responsible for the generation of ATP to fuel muscle contraction. Hypermetabolic stresses imposed upon muscles can lead to mitochondrial proliferation, but the resulting mitochondria greatly resemble their progenitors. During the mitochondrial biogenesis that accompanies phenotypic adaptation, the stoichiometric relationships between functional elements are preserved through shared sensitivities of respiratory genes to specific transcription factors. Although the properties of muscle mitochondria are generally thought to be highly conserved across species, there are many examples of mitochondrial differences between muscle types, species and developmental states and even within single cells. In this review, we discuss (1) the nature and regulation of gene families that allow coordinated expression of genes for mitochondrial products and (2) the regulatory mechanisms by which mitochondrial differences can arise over physiological and evolutionary time.

Key words: mitochondria, muscle, design, biogenesis, mitochondrial DNA, symmorphosis, oxidative phosphorylation, NRF, OX BOX, respiration.

Introduction

In general, mitochondria (mt) provide most of the energy for muscles under aerobic conditions. The differences in oxidative capacity between tissues appear to be due primarily to variation in mitochondrial content, measured biochemically (enzyme activity), ultrastructurally (volume density) or genetically (mtDNA copy number). Comparisons of mitochondrial properties across vertebrates reveal a remarkable conservation of structure and function. Taylor and Weibel (1981) recognized the general similarity of muscle mitochondrial ultrastructural properties when they studied the locomotory muscles of African mammals with a wide range of metabolic rates. The apparent conservation of stoichiometric relationships between structural elements in the respiratory system, including mitochondrial volume, led to their hypothesis of 'symmorphosis', which states that biological structures are optimally designed to meet, but not to exceed, the functional requirements of biological systems (Taylor and Weibel, 1981; Weibel et al., 1981). Although symmorphosis has since been extended to other biological pathways (Diamond and Hammond, 1992; Vock *et al.* 1996; Weibel *et al.* 1996), convincing arguments have been presented against its fundamental tenets (Garland and Huey, 1987; Dudley and Gans, 1991). In this review, we examine the molecular basis for the apparent conservation of muscle mitochondrial structure and function across species, tissues and physiological states and propose mechanisms by which mitochondria change over physiological and evolutionary time. Although we focus on the nature of differences from a protein perspective, it is important to realize that the function of proteins is profoundly influenced by the nature of the membrane lipids.

Are all muscle mitochondria really created equal?

Oxygen is consumed in the terminal step of mitochondrial oxidative phosphorylation (OXPHOS) at cytochrome oxidase (COX). The maximal rate of oxygen consumption ($\dot{V}_{O_2max}$) of most mammals is correlated with the mitochondrial volume density of their skeletal muscle (see Suarez, 1996). This has been shown in comparisons between (i) similarly sized sedentary and athletic species (e.g. dog *versus* goat) and (ii) mammals of different sizes (i.e. allometric variation) (Hoppeler, 1990; Vock *et al.* 1996; Weibel *et al.* 1991; Suarez, 1996). When calculated on the basis of mitochondrial volume density, most species studied to date demonstrate a $\dot{V}_{O_2max}$ of 3–5 ml O_2 min^{-1} cm^{-3} mitochondria. More recent studies have shown that this relationship depends upon a relatively conservative mitochondrial ultrastructure, specifically the amount of inner mitochondrial membrane per mitochondrial volume. Several vertebrates (hummingbirds, Suarez *et al.* 1991; pronghorn antelope, Lindstedt *et al.* 1991; skipjack tuna, Moyes *et al.* 1992*b*) have 2–3 times more mitochondrial cristae surface area per cm^{-3} mitochondrial volume than do the mammalian quadrapeds previously studied. When all respiration data are expressed per unit cristae surface area,

rather than per mitochondrial volume, the observed mitochondrial respiration rates are very similar across a wide range of species (see Suarez, 1996). The combined studies of Taylor, Weibel and Hoppeler have led to a general belief that mitochondrial structure and function relationships are highly conserved across vertebrate muscles (see Schwerzmann et al., 1986). However, there are many examples of qualitative and quantitative differences in muscle mitochondria between fibre types and species, and even within a tissue.

Skeletal muscle demonstrates several fibre types that differ in a number of biochemical properties including mitochondrial content. Fast-twitch glycolytic fibres have fewer mitochondria than slow oxidative fibres, but there is some evidence that their mitochondria are qualitatively different as well. Mitochondria from fast glycolytic fibres have lower rates of oxidation of lipid substrates, which correlates with lower activities of lipid-oxidizing enzymes (Baldwin *et al.* 1972; Moyes *et al.* 1992*b*). When one considers vertebrate species other than mammals, there is even greater diversity in mitochondrial fuel preference, specifically in the ability to oxidize lipid-based substrates (Moyes *et al.* 1990). Muscles of some species (e.g. chondrichthians) lack the ability to oxidize fatty acids directly, instead relying upon liver-generated ketone bodies as their ‘lipid’ fuel (Moyes *et al.* 1992*a*).

Ultrastructural studies reveal the presence of two intracellular populations of mitochondria within striated muscle (both skeletal and cardiac). Subsarcolemmal mitochondria (SSmt) are located just under the cell membrane, whereas a second population occurs between the myofibrils (intermyofibrillar or IMFmt). SSmt account for 5–40 % of the total mitochondrial volume depending upon the taxa and the fibre type (see Suarez, 1996). The advantage of two distinct, specialized mitochondrial populations may relate to their location with respect to metabolite and O_2 gradients, benefits expected to accrue even if the two mitochondrial populations were identical *in vivo* (Suarez *et al.* 1991). There do appear, however, to be biochemical differences between these two populations when the mitochondria are isolated and studied *in vitro*. In general, IMFmt have a higher $\dot{V}_{O_2}$ and demonstrate a greater capacity to oxidize fatty acids (e.g. Palmer *et al.* 1985; Cogswell *et al.* 1993). It should be noted that the biochemical differences in muscle mitochondrial populations are not always observed (e.g. McKean, 1990; Sillau *et al.* 1990; Manneschi and Federico, 1995), nor have they been shown to differ in properties *in situ*. The origins of two distinct intracellular populations of mitochondria, if they exist, are not immediately clear given that biogenesis of both populations would be under the control of the same nuclei. Intracellular distributions of nuclei, cytosolic free ribosomes and mitochondria could contribute to the creation of heterogeneities in mitochondrial populations through differential diffusion of regulatory factors and mitochondrial pre-proteins. Distinct populations within a single fibre could also originate by differential processing of existing organelles rather than by intracellular differences arising during biogenesis.

The properties of muscle mitochondria change throughout the lifetime of the cell and organism. Under the appropriate hormonal influences, undifferentiated myoblasts enter a myogenic programme that is accompanied by mitochondrial proliferation. During myogenesis, the quantitative relationships between enzymes and ultrastructure change dramatically and with different time courses. The activities of selected matrix enzymes increase fivefold when mitochondrial volume density increases by only 50 %; cristae surface area and cristae enzyme activities increase in parallel, but to a lesser extent than do matrix enzyme activities (Moyes *et al.* 1997). Once the myogenic programme is complete, the adult muscle shows an impressive capacity for adaptive changes, which for the most part preserve the relationships between functional and structural elements (see Puntschart *et al.* 1995). Exercise training and chronic nerve stimulation produce parallel increases in mitochondrial enzyme activities, the quantities of mRNA species and mtDNA copy number (see Hood *et al.* 1994). The oxidative properties of isolated mitochondria from trained and sedentary individuals do not differ (Davies *et al.* 1981). As the adult, postmitotic muscle ages, a number of changes in mitochondrial structure and function occur. Changes in the activity of a number of mitochondrial enzymes and a decline in respiratory properties are considered to be part of the pathology of ageing (Ames *et al.* 1995). It is thought that the decline in mitochondrial prowess with ageing arises ultimately from the accumulation of free-radical-induced mtDNA mutations (Linnane *et al.* 1989).

As previously discussed, mitochondrial structure and function relationships are highly conserved across adult muscle types, species and physiological states. However, differences in mitochondrial properties and content do occur across regions of a cell, between tissues and species and over the lifetime of the tissue. Regulatory mechanisms by which mitochondrial properties are so highly conserved are often overlooked, yet these mechanisms allow for specialization and differences to emerge over evolutionary and physiological time.

Control of mitochondrial biogenesis

Mitochondrial biogenesis in response to physiological stimuli requires the appropriate expression of all nuclear genes encoding mitochondrial products and the coordination of nuclear gene expression with that of mtDNA. This represents a regulatory challenge as these genomes are in different subcellular compartments and are found at dramatically different gene copy numbers. Recent studies have shed light upon the mechanisms by which mitochondrial gene expression is coordinated over physiological time (see Hood *et al.* 1994; Scarpulla, 1996). Symmorphosis assumes that the conserved properties across species and physiological states arises through natural selection, resulting in near-optimal relationships between elements of the integrated system (Weibel *et al.* 1991). However, the molecular mechanism for the coordination of gene products observed during physiological adaptation may underlie the relationships across species. Rather than a product of optimal selection of

individual elements, as suggested by the symmorphosis hypothesis, the conservation of mitochondrial structure and function may be another example of *modularity*. There are several examples of profound changes in complex systems that arise by expression of ‘master control’ genes (see Wagner and Altenberg, 1996). Halder *et al.* (1995) provided a vivid example of modularity when the mis-expression of the *Drosophila eyeless* gene led to the appearance of ectopic eyes on body segments that would normally express wings, antennae and legs. Modularity is also evident in the myogenic programme, when complex changes are coordinated by very few gene products such as myogenin (Ludolph and Konieczny, 1995). Although no single ‘master control’ gene has been identified that controls mitochondrial biogenesis, a number of proteins have been identified which coordinate the expression of nuclear and mitochondrial respiratory genes.

Control of mtDNA expression

mtDNA is circular in structure and very densely packed with genes. Vertebrate mtDNA encodes 13 proteins, 22 tRNAs and two rRNAs (see Attardi and Schatz, 1988, for a review of mtDNA structure). mtDNA-encoded proteins contribute to four of the five OXPHOS complexes, including ND-1 to ND-6 (Complex I), cytochrome *b* (Complex III), COX 1, 2 and 3 (Complex IV or cytochrome *c* oxidase), and ATP 6 and ATP 8 (Complex V) (Fig. 1). Only Complex II is entirely nuclear-encoded, a feature than has been used to study the regulatory relationships during mitochondrial biogenesis (Moyes *et al.* 1997). Most of the protein-encoding genes and both rRNAs are located on the H strand. The regions coding for the two rRNAs are immediately downstream of the H strand transcription initiation site. Individual peptide-coding regions are punctuated with tRNA-coding regions. Transcription may be terminated following the synthesis of the two ribosomal subunits. Alternatively, it can continue until the entire H strand is transcribed. In general, mtDNA transcription is asymmetric, with the rate of transcription of mitochondrial rRNA being an order of magnitude faster than that of mitochondrial mRNA (Gelfand and Attardi, 1981). Coordinated transcription of 12 of the 13 peptide-encoding genes is achieved by the polycistronic nature of the transcript (the thirteenth peptide, ND6, is located on the L strand and its mRNA is present at much lower concentrations; Gillham, 1994). Individual full-length mRNAs are liberated when endonucleases excise tRNAs from the polycistron. Replication and transcription are controlled primarily at the displacement loop (D-loop), a

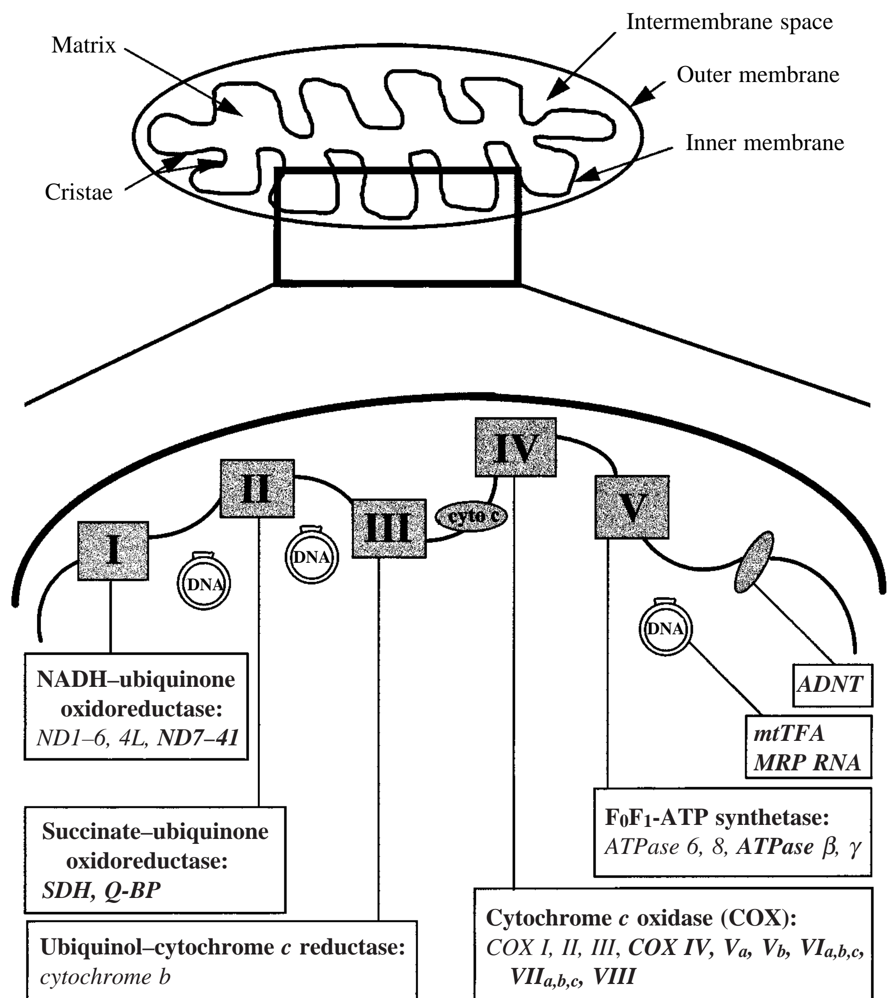

Fig. 1. Summary of basic mitochondrial ultrastructure, with special emphasis on the proteins forming the five complexes (I–V) involved in oxidative phosphorylation. Double circles represent copies of mitochondrial DNA, with the D-loop of one copy initiating replication (italized, mitochondrially encoded; bold/italicized, nuclear-encoded). cyto c, cytochrome *c*; ADNT, adenine nucleotide translocase; MRP, mitochondrial RNA processing; SDH, succinate dehydrogenase.

triplex region of mtDNA that contains all of the genetic regulatory elements. More information on mitochondrial genetics is presented in Clayton (1992).

It is generally thought that an increase in levels of mtDNA gene products under physiological stimuli is primarily met by mtDNA replication (i.e. increased copy number) rather than by changes in transcription *per se*. Chronic electrical stimulation, an extreme model of exercise training, results in a four- to fivefold increase in the number of functional copies of mtDNA (Williams, 1986). However, copy number may not be the only factor controlling mtDNA transcription within a tissue; increases in the relative amount of triplex mtDNA correlate with an increase in the amount of mitochondrial mRNA (cytochrome *b*) relative to mitochondrial rRNA. Tissues with higher aerobic capacity have a greater proportion of mtDNA in the triplex form (Annex and Williams, 1990). From an evolutionary perspective, it is not known whether species with higher metabolic rates show a similar trend.

Control of nuclear-encoded mitochondrial genes

The expression of nuclear genes is controlled by interactions between the upstream promoter and regulatory factors that bind to specific sequences of DNA (elements). Basal levels of gene expression are controlled by core promoter elements such as Sp1, CAAT or TATA boxes. These are short DNA sequences that define the position within the gene at which transcription is initiated (see Williams, 1990). Changes in transcription rates in response to physiological signals (e.g. exercise training) are mediated by transcription factors which bind reversibly to specific nucleotide sequences within the promoter (regulatory elements). These regulatory elements can be located hundreds of nucleotides up- or downstream of the transcription initiation site (Williams, 1990) and may be either stimulatory (enhancers) or inhibitory (repressors). The responsiveness of the gene is determined by the numbers, types and organization of regulatory elements in the promoter regions. While the nature of the promoter is determined genetically, the responsiveness to physiological conditions is controlled by the profile of transcription factors (relative concentrations and activities of activators and repressor proteins) (Attardi and Schatz, 1988).

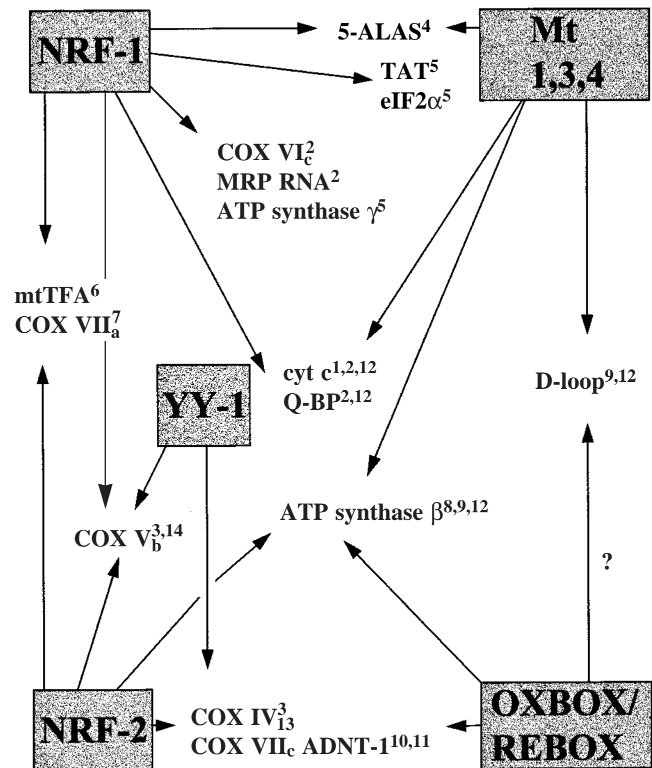

Fig. 2. Summary of the interactions between regulatory factors and known target genes. 1, Evans and Scarpulla (1989); 2, Evans and Scarpulla (1990); 3, Virbasius *et al.* (1993); 4, Braidotti *et al.* (1993); 5, Chau *et al.* (1992); 6, Virbasius and Scarpulla (1994); 7, Seelan *et al.* (1996); 8, Virbasius and Scarpulla (1991); 9, Haraguchi *et al.* (1994); 10, Li *et al.* (1990); 11, Chung *et al.* (1992); 12, Suzuki *et al.* (1991); 13, Seelan and Grossman (1997); 14, Basu et al. (1993).

In recent years, the regulatory regions of several genes encoding mitochondrial products have been characterized. The appearance of common enhancers led to the identification of transcription factors which help coordinate the expression of nuclear genes encoding mitochondrial products (Table 1; Fig. 2). It is thought that the shared sensitivity of many mitochondrial genes to only a few transcription factors creates a network of 'gene families' that can respond in a modular

Table 1. *Summary of transcription factors known to modulate the transcription rates of nuclear-encoded mitochondrial proteins and their sequence recognition sites*

Factor	Sequence recognition site (5′→3′)	Source
Nuclear Respiratory Factor 1 (NRF-1)	(T/C)GCGCA(T/C)GCGC(A/G)	Evans and Scarpulla (1989)
NRF-2	GCTCTTCCGGT	Virbasius and Scarpulla (1991)
OXBOX	GGCTCTAAAGAGG	Li *et al.* (1990)
REBOX	AAGAGGGC	Chung *et al.* (1992)
Mt 1	TTATTCAGGTGTGCT	Suzuki *et al.* (1989)
Mt 3	ATCTGGCT	Suzuki *et al.* (1991)
Mt 4	TGGTGATA	
Ying Yang 1 (YY-1)	CCAT	Basu *et al.* (1993)

fashion to physiological challenges. In its most simplistic sense, changes in mitochondrial structure, function and content can be imagined as arising through modest changes in these regulators, conserving the relationship between the components. For instance, NRF-1 elements occur in the regulatory region of genes encoding ubiquinone-binding protein (Q-BP), cytochrome *c*, COX V_b, VI_c and VII_a, and ATP synthase γ (Table 1; Fig. 2). Additional studies have revealed the presence of NRF-1 binding sites in the promoter regions of other nuclear genes encoding proteins essential for haem synthesis (5-ALAS; Braidotti *et al.* 1993), tyrosine catabolism (TAT; Chau *et al.* 1992) and translation initiation (eIF2α: Chau *et al.* 1992) (Fig. 2). Thus, it has been suggested that NRF-1 may modulate the expression of 'gene families' which code for polypeptides that are rate-limiting to their respective metabolic pathways (Scarpulla, 1996). However, regulation of coordination is more complex than described above in several respects: no single regulatory element appears on all mitochondrial genes, gene families display considerable overlap, and individual genes are often regulated by other factors.

Several nuclear genes encoding mitochondrial products have elements that suggest a sensitivity to two or more of the transcription factors thought to coordinate mitochondrial gene expression (Fig. 2). For example, the genes for COX V_b and VII_a having binding sites for both NRF-1 and NRF-2. The presence of enhancers common to multiple genes encoding OXPHOS proteins of different complexes probably facilitates their coordinated expression. Some mitochondrial genes also possess regulatory elements sensitive to more common signal transduction pathways, which may allow for independent regulation of transcription with respect to other OXPHOS genes. For example, the cytochrome c_1 gene contains cyclic-AMP-responsive elements (CREs) (Gopalakrishnan and Scarpulla, 1994) in addition to NRF-1 (Evans and Scarpulla, 1989, 1990) and Mt 1, 3 and 4 binding sites (Suzuki *et al.* 1989, 1991). Furthermore, stimulation of respiratory genes by known signal transduction pathways may be mediated indirectly by factors such as NRF-1. The cytochrome *c* gene lacks a thyroid-responsive element (TRE), but cytochrome *c* levels rise in response to increased levels of thyroid hormone. These observations prompted the suggestion that the undescribed NRF-1 promoter may itself possess TREs, imparting thyroid hormone sensitivity to the entire NRF-1-sensitive family of genes (see Scarpulla, 1996).

While additional transcription factors and regulatory elements remain to be identified, the discovery of NRF-1, NRF-2, OXBOX/REBOX, Mt 1, 3 and 4, and YY-1 proteins and/or elements has provided the first insight into the basic framework by which expression of genes critical to mitochondrial structure and function is coordinated.

Coordination of nuclear and mtDNA

The discovery of factors capable of coordinating the transcription rates of nuclear-encoded mitochondrial proteins has been crucial to our understanding of the process of mitochondrial biogenesis. While nuclear regulatory factors have the potential collectively to control the expression of all the relevant nuclear genes, it is unlikely that most of these factors could either gain access to the mitochondrial matrix or bind to mtDNA. An important breakthrough came when NRF-1 and NRF-2 elements were found in the regulatory region of nuclear genes encoding (i) mtTFA (mitochondrial transcription factor A), which controls the rate of mtDNA transcription (Virbasius and Scarpulla, 1994), and (ii) the RNA moiety of MRP (mitochondrial RNA processing) RNAase, a riboprotein involved in mtDNA replication (Evans and Scarpulla, 1990) (Figs 1, 2). The coordination of expression of mitochondrial and nuclear genes also appears to involve several transcription factors in addition to mtTFA. Mt 3 and 4 elements have been found within the D-loop of four mammalian species (Suzuki *et al.* 1991) and are also found upstream of the cytochrome c_1, Q-BP and ATP synthase β genes (see Fig. 2), all of which are located in the nucleus (Suzuki *et al.* 1989, 1995). Moreover, a sequence located adjacent to the bidirectional promoter of mtDNA exhibits partial homology to the REBOX element found in promoters of both the muscle-specific form of the adenine nucleotide translocase (ADNT-1) and the ATP synthase β genes (Haraguchi *et al.* 1994).

A number of studies using diverse approaches and models have concluded that the expression of mitochondrial genes during mitochondrial biogenesis is highly coordinated (see Hood *et al.* 1989). The discovery of the regulatory factors described above (Fig. 2) has reinforced the perception that respiratory gene expression is tightly coordinated temporally. However, when sampling intervals are sufficiently frequent, asynchronous changes in levels of mRNA for specific respiratory genes are observed during nerve stimulation (Annex *et al.* 1991), hyperthyroidism (Luciakova and Nelson, 1992) and differentiation-induced mitochondrial biogenesis (Moyes *et al.* 1997). From a functional perspective, it is more important that gene products, rather than transcription *per se*, change stoichiometrically during phenotypic adaptation. If coordination were achieved exclusively through transcriptional control, any stoichiometry would be lost through differential post-transcriptional events such as mRNA stability, translation, uptake and assembly of mitochondrial proteins. By extension, coordination at the level of transcription should not be expected to be stoichiometric if any gene-specific differences in post-transcriptional events exist. Although some nuclear-encoded genes are transcriptionally regulated (Williams et al., 1987; Hood *et al.* 1989; Williams, 1990), there are many examples of genes that depend upon post-transcriptional regulation (Izquierdo *et al.* 1995; Taylor and Pikó, 1995; Chrzanowski-Lightowlers *et al.* 1994). The concept of perfect coordination of nuclear and mitochondrial gene expression, like the basic tenet of symmorphosis, seems to rely upon unrealistic assumptions of optimality, a condition that need not be true to meet physiological demands (see Garland and Huey, 1987; Dudley and Gans, 1991). Nonetheless, there appear to be mechanisms which co-regulate expression of a number of respiratory genes in nuclear and mtDNA.

How are mitochondrial structure/function relationships altered?

The mechanisms by which mitochondrial gene expression can be changed over evolutionary time vary in important respects from the mechanisms by which cells respond to physiological challenges. While cells rely primarily on the activity of regulators to change the expression of nuclear genes, evolutionary changes in the gene's promoter can potentially alter both basal expression levels and sensitivity to the physiological regulators. Evolutionary processes may also result in changes in the coding region of the genes, allowing for proteins with altered functional properties. Although the field is relatively new, there is some evidence that each of these strategies is used to alter the quantitative and qualitative relationships of mitochondrial design. At the protein level, these regulatory changes may alter the stoichiometries between complexes or the levels of enzymes governing qualitative properties (e.g. fuel preference, redox shuttles).

Changing regulatory elements

The evolutionary strategies by which species achieve elevated mitochondrial levels may involve changing the numbers, types and arrangements of regulatory elements within the relevant promoters. Interspecific comparisons of the promoters of specific nuclear-encoded mitochondrial genes are rarely studied when trying to explain differences in the levels of mitochondrial enzymes between organisms. However, the effectiveness of potential evolutionary strategies can be surmised by comparing the promoters of different genes within a single species. The presence of multiple elements for the same transcription factor may confer a greater degree of sensitivity to that regulator. For instance, the multiple NRF-2 sites which appear on both COX IV (two sites) and COX V_b (four sites) increase the sensitivity to that transcription factor (Virbasius and Scarpulla, 1991). The evolutionary implication is that promoters of active species might be expected to possess more NRF-2 elements, for example, than the homologues of more sedentary species. Increases in basal mitochondrial gene expression need not be due solely to the addition of enhancer elements. Schulte *et al.* (1997) showed that differential rates of transcription of *LDH-B* genes between northern and southern populations of the teleost *Fundulus heteroclitus* may be explained by the loss of a functional repressor binding element in one allele. Functional differences between populations occur as a result of differential distribution of the allozymes.

Changing transcription factor levels or profiles

The efficacy of transcription factors in any physiological state is influenced by the concentration and activity as well as the presence of repressors that can compete for the same binding site or bind elsewhere. The strategy by which evolutionary changes might alter the function of transcription factors affecting respiratory genes depends upon their mechanism of action. For example, some transcription factors (e.g. NF-κB) exist as inactive protein complexes. Their effectors (e.g. reactive oxygen species) cause their release from an inhibitory binding protein (IκB), with subsequent binding to a regulatory element and transcriptional activation (see Sen and Packer, 1996). Other transcription factors are themselves transcriptionally regulated. Although Gopalakrishnan and Scarpulla (1995) studied the structure of the NRF-1 gene and its expression in a variety of rat tissues, its promoter region has yet to be described. It is thought that thyroid hormones may exert their effect on mitochondrial genes through the stimulation of NRF-1 expression (Scarpulla *et al.* 1986; Nelson *et al.* 1995) and, since the thyroid hormone receptor is a nuclear binding protein, this implies that NRF-1 may be transcriptionally regulated (Scarpulla, 1996). However, relatively little is known about the regulation of the activity and concentration of the mitochondrial transcription factors identified in Table 1. Until more is known about the relative importance of transcriptional, translational and post-translational regulation for these transcription factors, speculation about the nature of potential evolutionary differences in their regulation is somewhat premature.

Changing the nature of mitochondrial proteins

The previous two sections dealt with mechanism by which muscles change their levels of mitochondrial proteins. The function of existing proteins can be altered over the short term by allosteric or covalent regulation of existing proteins. Apart from changing the levels or activities of mitochondrial proteins, physiological and evolutionary processes may lead to changes in the proteins themselves. Longer-term changes in physiological demands, for example, differentiation and development, may lead to a genetic response in the form of a shift in the expression of isoforms. Over evolutionary time, mutations and gene duplications can lead to genes that encode proteins with more favourable properties.

Isoforms originate predominantly through gene duplication events, followed by mutations (Ohta, 1991). Rodents have a testicular cytochrome *c* isoform which has arisen in a very short evolutionary period and possesses structural features and regulatory properties distinct from the somatic isoform (Virbasius and Scarpulla, 1988). Alternatively, isoforms may arise from single-copy genes through alternative splicing of mRNA (e.g. Matsuda *et al.* 1993). Isoform switching is mediated by changes in the profile of transcription factors. Shifts from one isoform to another are, in general, common in muscle biochemistry, particularly with contractile proteins (see Booth and Thomason, 1991). However, there are relatively few examples of isoform shifts of mitochondrial proteins in vertebrates in response to physiological signals, other than those occurring during differentiation and development (Stepien *et al.* 1992; Seelan *et al.* 1996). Myogenesis is accompanied by a switch in the pattern of expression of COX and ADNT isoforms. Liver isoforms of nuclear-encoded COX subunits VI_a, VII_a and VIII give way to heart isoforms (Lomax *et al.* 1990; Seelan and Grossman, 1997). Although the kinetic differences between ADNT isoforms are not well understood, myogenesis includes a shift from ADNT-2, which predominates in glycolytic tissues, to ADNT-1, the muscle-

specific isoform (Stepien *et al.* 1992). Chronic hypoxia leads to reciprocal changes in expression of glycolytic (increase) and mitochondrial (decrease) enzymes (Webster *et al.* 1990), but there is little evidence for isoform shifts in vertebrate muscle. Although ADNT-1 is one of the mRNA species whose expression has been shown to decrease during hypoxia, it is not known whether hypoxia leads to a reciprocal increase in expression of ADNT-2. Yeast switch from COX V to COX V_b in response to hypoxia, which alters the V_{max} of the holoenzyme (see Poyton and McEwen, 1996). Although several isoforms of mitochondrial enzyme subunits exist, their regulation during physiological stress and the impact of any shifts is not well understood.

Isoform shifting provides a cell with plasticity by allowing it to respond to physiological or developmental cues by producing a protein demonstrating a more appropriate function. Evolutionary processes also give rise to changes in protein structure that could provide a species with a selective advantage. The rates at which nuclear genes accumulate mutations differ dramatically but, in general, mitochondrial genes evolve several times faster than nuclear genes (Neckelmann *et al.* 1987). While the existence of diversity in coding regions of several respiratory genes is known, there are relatively few studies which address the functional consequences of these differences. Complexes I and IV are so structurally complex that the function of several subunits is still unknown. The kinetics of Complex I *in vitro* appears to be more dependent upon the substrate used than the nature of the protein itself (Genova *et al.* 1995). While it is possible that interspecific differences in the structure of OXPHOS proteins endow kinetic advantages, this remains to be investigated.

Conclusions

Studies of the promoter regions of nuclear genes encoding mitochondrial products have provided valuable insight into the molecular mechanisms responsible for altering mitochondrial content in response to physiological signals and hypermetabolic stresses. While several of the transcription factors that are involved in the coordinated expression of the nuclear and mitochondrial genomes have been identified, much remains to be learned about the overall regulation of this process. Future studies will probably reveal additional regulatory elements critical to the coordination of gene expression that will strengthen our current understanding of how transcription factors modulate the expression of functionally linked ‘gene families’.

Although our understanding of the molecular mechanisms involved in altering mitochondrial properties in response to hypermetabolic stresses is growing, very little is known about the mechanism of changes in mitochondrial content and properties that occur over evolutionary time. This review has summarized the possible mechanisms for generating differences in mitochondrial properties over evolutionary time. Future studies might focus on interspecies comparisons of the regulatory elements of OXPHOS genes or levels of critical transcription factors, such as NRF-1.

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