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Plasticity of oxidative metabolism in variable climates: molecular mechanisms

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Abstract

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Keywords

variable, plasticity, mechanisms, molecular, climates, oxidative, metabolism

Disciplines

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

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Plasticity of Oxidative Metabolism in Variable Climates: Molecular Mechanisms*

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ABSTRACT

Converting food to chemical energy (ATP) that is usable by cells is a principal requirement to sustain life. The rate of ATP production has to be sufficient for housekeeping functions, such as protein synthesis and maintaining membrane potentials, as well as for growth and locomotion. Energy metabolism is temperature sensitive, and animals respond to environmental variability at different temporal levels, from within-individual to evolutionary timescales. Here we review principal molecular mechanisms that underlie control of oxidative ATP production in response to climate variability. Nuclear transcription factors and coactivators control expression of mitochondrial proteins and abundance of mitochondria. Fatty acid and phospholipid concentrations of membranes influence the activity of membrane-bound proteins as well as the passive leak of protons across the mitochondrial membrane. Passive proton leak as well as protein-mediated proton leak across the inner mitochondrial membrane determine the efficacy of ATP production but are also instrumental in endothermic heat production and as a defense against reactive oxygen species. Both transcriptional mechanisms and membrane composition interact with environmental temperature and diet, and this interaction between diet and temperature in determining mitochondrial function links the two major environmental variables that are affected by changing climates. The limits to metabolic plasticity could be set by the production of reactive oxygen species leading to cellular damage, limits to substrate availability in mitochondria, and a disproportionally large increase in proton leak over ATP production.

Introduction

Since the appearance of modern vertebrate lineages in the Mesozoic, physical conditions on earth have fluctuated dramatically. Periods of increased warmth in the Cretaceous were followed by cooling to the current low temperatures in the Pleistocene. Superimposed on climatic variations lasting many millions of years are shorter-term fluctuations, such as Milankovitch cycles of tens of thousands of years and shorter cycles (Huybers and Curry 2007). Evolution has occurred against this backdrop of instability. In fact, the inherent variability in climate probably represents one of the greatest selection pressures, and animals have evolved a range of mechanisms to compensate for environmental variability.

Short-term exposure to extreme heat or cold changes the pattern of expression of heat shock genes, thereby protecting cellular proteins from temperature-induced damage (Feder and Hofmann 1999). This stress response is fundamentally different, however, from longer-term (seasonal, evolutionary) fluctuations of temperature within a range that is not immediately damaging to cells but which nonetheless has a pronounced effect on cellular reaction rates (Guderley and St-Pierre 2002). Animals may respond to thermal change by thermoregulating to maintain a constant internal environment despite environmental variability and by modifying cellular functions to compensate for thermal variability. Compensation by individuals for changing environmental conditions is ecologically relevant because it buffers organisms from the impact of recurrent variability (e.g., seasonal changes) or catastrophic events (e.g., human-induced climate changes). Plastic responses within individuals (acclimation, acclimatization) typically occur in the direction opposing a thermodynamic effect and are therefore not a simple temperature-induced phenomenon (Fig. 1A). The magnitude of plastic responses of enzyme activities or locomotor performance, for example, can be as great or greater
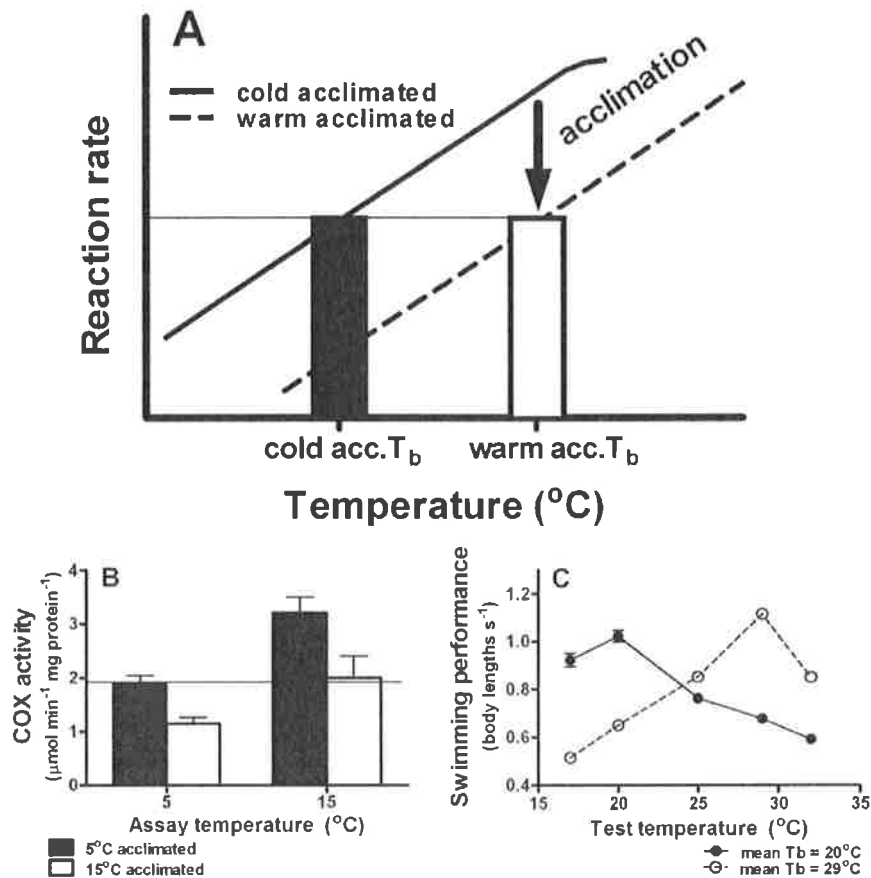
Figure 1. Within-individual plasticity (acclimation) results in relatively constant reaction rates (cf. *solid and open bars*) despite pronounced body temperature variations (*A*). Acclimation to warm temperatures (*dashed line*) causes a decrease in reaction rate compared with cold-acclimated rates (*solid line*) at the high acclimation temperature, thereby avoiding excessively high reaction rates. For example, cytochrome c oxidase (COX) activities in rainbow trout are identical at the different acclimation body temperatures of 5° and 15°C (*B*; data from Kraffe et al. 2007). Similarly, aerobically fueled critical-sustained swimming speed in crocodiles is maximal at the different mean acclimation body temperatures of 20° and 29°C (*C*; redrawn from Glanville and Seebacher 2006).

than differences that exist between populations of species experiencing different climatic conditions (Seebacher 2005). The capacity of individuals to modulate rate functions can therefore cushion the effect of environmental selection pressures. There are no absolute relationships between body temperature and reaction rates because reaction rates are modulated by several components, such as substrate concentrations, enzyme concentrations, different enzyme isoforms, and biological membrane composition (Guderley 2004b). Differences in body temperatures between species or individuals, or even between seasons within individuals, therefore do not necessarily reflect metabolic differences (Seebacher et al. 2003a; Glanville and Seebacher 2006).

One of the most fundamental animal functions is conversion of food into energy that is usable by cells. Energy metabolism therefore occupies a pivotal position between environmental resources and animal fitness through ATP production. Metabolic pathways are tightly regulated to maintain activity as con-

stant as possible when body temperatures change (Brand 1997; St-Pierre et al. 1998; Guderley and St-Pierre 1999; Seebacher et al. 2003a, 2003b). To maintain cellular function, ATP production must at least match ATP use by the energy-consuming processes that are perpetually occurring simply to keep the organism alive (Hulbert and Else 2000). In addition, to maintain fitness, it is essential to supply energy to activities such as locomotion, growth, and reproduction. Animals that do not enter a state of dormancy are particularly sensitive to any environmentally imposed decreases in ATP production, and the capacity of animals to up- or downregulate their metabolic pathways will determine their success in variable environments.

The mitochondrial electron transport chain and oxidative phosphorylation are the principal ATP-producing pathways. Oxidative metabolism in mitochondria is linked to a network of catabolic and regulatory processes that provide substrates to feed into the tricarboxylic acid cycle and electron transfer chain and that modulate flux. Oxidative metabolic pathways are

highly conserved among animals (Koch and Britton 2008), and any response to environmental variability is likely to be a quantitative adjustment rather than a de novo pathway. At a temporal scale, animals may respond immediately to changes in cellular energy balance by increasing expression of transcription factors and target genes as well as changing fatty acid composition of membranes within hours. Longer-term changes, such as responses to seasonal variation, may take several weeks to establish (Bouchard and Guderley 2003; Rogers et al. 2004) and may be associated with changes in thermoregulatory set points (Glanville and Seebacher 2006). At an evolutionary scale, populations and species living in different climates can nonreversibly adapt to prevailing conditions (Johnston et al. 1994, 1998). In addition to its effect on environmental temperatures, climate variation will also affect productivity through changes in rainfall, for example (Sillmann and Roeckner 2008). Changes in the quality and quantity of food resources can have a pronounced effect on metabolism. In most cases, temperature and resources will change concurrently and will have an interactive effect on metabolism.

Here we will review temporal responses of vertebrates to thermal and nutritional variation in the context of the regulatory mechanisms that determine mitochondrial function. We focus on the three broad areas of (1) transcriptional control, (2) membrane composition, and (3) mitochondrial uncoupling and proton leak. These mechanisms are highly conserved among living organisms (Smith and Morowitz 2004; Koch and Britton 2008) so that we do not attempt to delineate between particular animal groups, except for contrasts between endotherms and ectotherms. Our purpose is not to provide a comprehensive review of the literature but to summarize the main features of plastic responses in mitochondrial function, and we concentrate on our own work to highlight these.

Mechanisms of Metabolic Regulation

Transcriptional and Genetic Mechanisms

Mitochondrial capacities and densities can change in response to latitudinally, seasonally, or experimentally altered thermal environments (Wodke 1974; Johnston et al. 1998; Lucassen et al. 2003; Fangue et al. 2009) in taxonomically diverse animal groups (Hardewig et al. 1999; Seebacher et al. 2003a; Sinclair et al. 2006; Rogers et al. 2007). A good example are trout (*Oncorhynchus mykiss*), which respond to increasing water temperatures in summer by a decrease in the capacity of the red muscle to oxidize pyruvate and palmitoyl carnitine, which is associated with lower enzyme activities (citrate synthase, cytochrome c oxidase, and carnitine palmitoyl transferase; Fig. 1B). In addition, mitochondrial cristae surface density, but not mitochondrial volume density, decreases in summer-acclimatized trout (St-Pierre et al. 1998). The effect of these adjustments is that reaction rates and mitochondrial capacities remain relatively constant across seasons despite a difference of 15°C in body temperature between winter and summer (Guderley and St-Pierre 1999). Similar responses occur in whole-animal locomotor performance (Fig. 1C) and across latitudes where

populations of the same species living in warmer climates have lower mitochondrial densities and capacities compared with populations at higher latitudes (Johnston et al. 1998; Fangue et al. 2009). Hence, increasing environmental temperatures do not increase metabolic flux to levels that are excessively high. Temperature is not the only mechanism involved in setting seasonal changes in metabolic capacities. Photoperiod can serve as a major coordinator of seasonal cycles, and acclimatization of trout to short day lengths at warm (summer) temperatures increases the oxidative capacities of muscle mitochondria (Martin et al. 2009).

Mitochondrial densities and oxidative capacities are transcriptionally controlled. In particular, the co-activators PGC-1α and PGC-1β (Puigserver et al. 1998; Moyes 2003; St-Pierre et al. 2003) play important roles in metabolic regulation of vertebrates (Walter and Seebacher 2007; LeMoine et al. 2008; Scarpulla 2008; Seebacher et al. 2009) by modulating oxidative capacity of skeletal muscle, liver, and brown adipose tissue and by inducing mitochondrial biogenesis (St-Pierre et al. 2003; Scarpulla 2008). PGC-1α is also an important regulator of gluconeogenesis in liver (Herzig et al. 2001). Rather than binding directly to regulatory sequences on DNA to stimulate expression of structural genes, PGC-1α and PGC-β interact with transcription factors that regulate gene expression (Lin et al. 2005). Their principal targets include nuclear receptors of the PPAR family, which play a principal role in regulating fatty acid and glucose metabolism (Long et al. 2007; Benton et al. 2008a; LeMoine et al. 2008), and nuclear respiratory factors (NRF1 and NRF2). NRF1 and NRF2 control transcription of nuclear and mitochondrial genes involved in mitochondrial respiratory function (Moyes 2003; Ongwijitwat and Wong-Riley 2005), including the mitochondrial transcription factor Tfam, which regulates mitochondrial DNA copy number and gene expression (Scarpulla 2008). Conversely, transcriptional repressors such as RIP140 can have a negative effect on mitochondrial biogenesis. It is possible that RIP140 competes with PGC-1α for binding to transcription factor ligands, thereby regulating nuclear receptor function in skeletal muscle and adipocytes (White et al. 2008). DNA methylation may be an additional level of metabolic control, and at least in mouse adipose tissue, DNA methylation can repress expression of PPARγ (Fujiki et al. 2009).

Transcriptional regulation of mitochondrial capacities can be linked to thermal variability through thermosensory mechanisms. In mammals and reptiles, thermal variability in the environment is detected by thermal sensing proteins (transient receptor potential ion channels [TRPs]) that are associated with free nerve endings (Patapoutian et al. 2003). There are several TRPs that are gated by heat but which have different thermal sensitivities (Patapoutian et al. 2003). TRPs are associated with dorsal root ganglion and trigeminal neurons (Patapoutian et al. 2003) that provide the afferent thermosensory input to the hypothalamus (Morrison et al. 2008). For example, blocking of the high-temperature gated TRPV1 in rats causes hyperthermia (Gavva et al. 2007), and blocking of TRPV1 and the cool sensor TRPM8 abolishes thermoregulatory behavior in

crocodiles (Seebacher and Murray 2007). In mammals, the efferent thermoregulatory response is mediated by sympathoexcitatory neurons in the dorsomedial hypothalamus through sympathetic premotor neurons in the medulla (Nakamura et al. 2004). Sympathetic outflow from the hypothalamus can modulate cellular metabolism by stimulating $\beta2$ and $\beta3$ adrenergic receptors on the cell surface, which leads to increased transcription of PGC-1α (Puigserver et al. 1998; Morrison et al. 2008). Cold-induced expression of PGC-1α also leads to increased transcription of cytochrome c oxidase and F_0F_1-ATPase, which partially control the rate of oxidative phosphorylation (Puigserver et al. 1998).

Diet composition can affect mitochondrial capacities by influencing expression of transcription factors. For example, levels of malonyl-CoA, a fatty acid synthesis intermediate, in mice are an indicator of whole-body energy status. Increases in malonyl-CoA concentration in the hypothalamus are correlated with an increase in energy expenditure in skeletal muscle (Cha et al. 2007). The malonyl-CoA signal is transmitted by the sympathetic nervous system to induce PGC-1α expression in skeletal muscle by stimulation of β adrenergic receptors. Induction of PGC-1α leads to increased mitochondrial biogenesis and expression of key mitochondrial enzymes (Cha et al. 2007).

In goldfish, there is an interactive effect of diet and thermal acclimation on the regulation of oxidative capacities of skeletal muscle (LeMoine et al. 2008). Similar to trout, citrate synthase and cytochrome c oxidase activities decrease with warm acclimation. These enzyme activities are likely to be controlled by PGC-1β and NRF1, and their mRNA concentrations are also decreased in the warm-acclimated fish. PGC-1α and PPARα in goldfish are predictors of fatty acid synthesis, and both are increased in warm-acclimated fish. Interestingly, PGC-1α mRNA concentration is increased in the liver of fasted fish, which could indicate a role in gluconeogenesis. The mRNA concentrations of other transcription factors vary between fasted fish and fish on a high-fat diet, but responses are tissue specific (LeMoine et al. 2008). Nonetheless, both temperature and diet modify mRNA concentrations of the same transcriptional regulators, which could lead to synergistic or conflicting regulatory demands.

Oxidative capacities of tissues and whole organisms depend on the cellular mitochondrial content, which may be subject to evolutionary adaptation in vertebrates (Houle-Leroy et al. 2000; Moyes and Hood 2003; Dalziel et al. 2005). Similar to plasticity of mitochondrial content within individuals, it is likely that evolutionary changes in mitochondrial content are controlled transcriptionally by, for example, PGC-1α and NRF1 (Moyes 2003). Differences between species could arise from mutations in cis-regulatory regions of genes (Dalziel et al. 2005), which could lead to heritable differences in gene expression patterns (Oleksiak et al. 2002). In addition, evolutionary differences in nuclear content can alter constitutive expression patterns and therefore provide a heritable determinant of oxidative capacity (Dalziel et al. 2005).

Also at an evolutionary timescale, mitochondrial function may be determined by selection pressures on mitochondrial

DNA (mtDNA). The pattern of sequence evolution of mtDNA is fundamentally different from that of nuclear DNA because of its tendency for single-parent inheritance, low rate of recombination, and high rate of mutations (Neiman and Taylor 2009). Mitochondrial genomes contain genes coding for tRNA, rRNA, and subunits of the various complexes of the electron transport chain. These proteins fulfill essential functions in energy metabolism and are under positive selection (Das et al. 2004; Dalziel et al. 2006). Given the essential functions that mitochondria play in energy metabolism, Ca^{2+} cycling (Brookes et al. 2008), and apoptosis (Circu and Yee Aw 2008), it would be expected that changes in protein coding mtDNA sequences and their interaction with imported nuclear proteins will have pronounced phenotypic and ecological effects (Ellison and Burton 2006).

A good example of the consequences of selection on mtDNA for mitochondrial function stem from *Drosophila*. In *Drosophila simulans*, there are three distinct mtDNA haplotypes. Haplotypes possess different phenotypic characteristics, which influence the relative fitness of the groups in different environments (Ballard and James 2004; Ballard et al. 2008). There is a life-history trade-off where flies from the *si*II haplotype have greater egg size and fecundity and are more cold tolerant, which confers an advantage in environments with continuous and abundant resources. However, flies with a *si*III mtDNA haplotype are more starvation resistant and have higher cytochrome c oxidase activity (Ballard et al. 2008; Fig. 2A, 2B). However, whether differences between *Drosophila* mitochondrial haplotypes arise because of differences in mtDNA alone or because of interactions with nuclear proteins is not clear.

In humans, mtDNA haplotype has also been associated with differential fitness in different environments. Human mitochondrial H and T haplotypes have different disease susceptibilities and longevity, and it has been suggested that these phenotypic differences arise because of bioenergetic differences of mitochondria between the different haplotypes (Taylor and Turnbull 2005). Empirical data, however, do not confirm this prediction. Analysis of bioenergetic capacities and uncoupling of mitochondria inserted into cytoplasmic hybrids, which eliminates interference from the nuclear background, do not show differences between the haplotypes (Amo et al. 2008). A second hypothesis is that during human evolution greater uncoupling of mitochondria and the resultant increased heat production may have been selected for in populations that radiated into high latitudes (Ruiz-Pesini et al. 2004). However, experimental tests of the bioenergetics of mitochondria from different haplotypes showed that there are no differences between arctic and tropical mtDNA haplotypes in the overall kinetics of the oxidative phosphorylation system and, hence, heat production potential (Amo and Brand 2007; Fig. 2C).

Membrane Composition: Fatty Acids and Phospholipids

Membrane fatty acid composition affects the activity of membrane-bound proteins (Hulbert and Else 1999). This effect may in part be due to the increased kinetic energy transferred
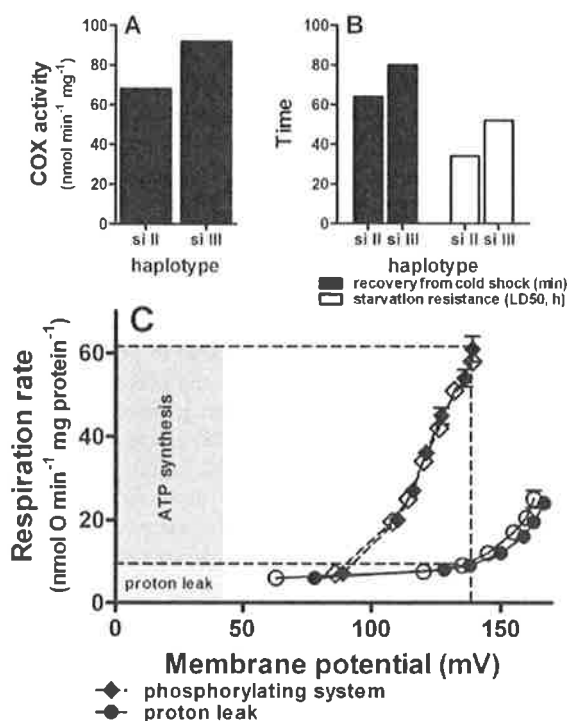
Figure 2. Phenotypic differences between mitochondrial DNA (mtDNA) haplotypes. In *Drosophila*, *si*II haplotypes have significantly greater COX activity than *si*III haplotypes (*A*). There is a trade-off between cold resistance (recovery from cold shock) and starvation resistance (50% survival [LD$_{50}$]; *B*); *si*II haplotypes recover faster from cold shock but are less starvation resistant than *si*III haplotypes (*A* and *B* redrawn from Ballard et al. 2008). In contrast, there are no phenotypic differences in mitochondrial bioenergetics between human mtDNA haplotypes from arctic (*solid symbols*) and tropical (*open symbols*) regions (*C*). Proton leak, and therefore heat production, of arctic populations is not greater than that of tropical populations (redrawn from Amo and Brand 2007).

to membrane proteins by more frequent collisions with freely moving polyunsaturated membrane phospholipids compared with more unsaturated phospholipids (Hulbert et al. 2005). For example, the activity of complex IV in the mitochondrial electron transport chain, cytochrome c oxidase, is affected by interactions between specific fatty acids of particular phospholipid classes, and its activity increases with the abundance of relatively minor fatty acid species (Kraffe et al. 2007; Guderley et al. 2008). The effect of membrane fatty acid saturation on enzyme activity is also pronounced for Na$^+$-K$^-$-ATPase. The activity of Na$^+$-K$^+$-ATPase, which constitutes about 20%–25% of resting metabolic rate, is severalfold lower in the relatively saturated membranes of ectotherms compared with the polyunsaturated membrane of endotherms (Turner et al. 2005). A similar pattern exists within endotherms where smaller animals with more polyunsaturated membranes also have greater Na$^+$-K$^+$-ATPase activity compared with larger animals (Turner et al. 2006). The role of membrane phospholipid composition in

determining enzyme activity has been demonstrated elegantly by reconstituting isolated cow Na$^+$-K$^+$-ATPase protein with a crocodile membrane, which resulted in a decrease in the activity of the cow protein to crocodile levels (Fig. 3*A*). Vice versa, transplanting crocodile Na$^+$-K$^+$-ATPase protein into a cow membrane significantly increases molecular activity (Wu et al. 2004; Fig. 3*B*).

Increasing membrane polyunsaturation is also correlated with increased proton leakage across the inner mitochondrial membrane (Brookes et al. 1998; Brand et al. 2003) and Na$^+$ and K$^+$ permeability in plasma membranes (Hulbert and Else 1999). Hence, oxidative capacity can be stimulated by the high Na$^+$-K$^+$-ATPase activity and the resulting ATP use that is required to maintain membrane potentials in membranes that are relatively leaky to ions (Else et al. 1996; Hulbert and Else 1999; Wu et al. 2004; Turner et al. 2005). At the same time, because mitochondrial membranes are relatively permeable to protons, the increased proton conductance as a consequence of the increased flux resulting from ATP demand by Na$^+$-K$^+$-ATPase activity will also result in increased heat production in endotherms (Walter and Seebacher 2009). By modulating both ATP-consuming and ATP-producing processes, membrane
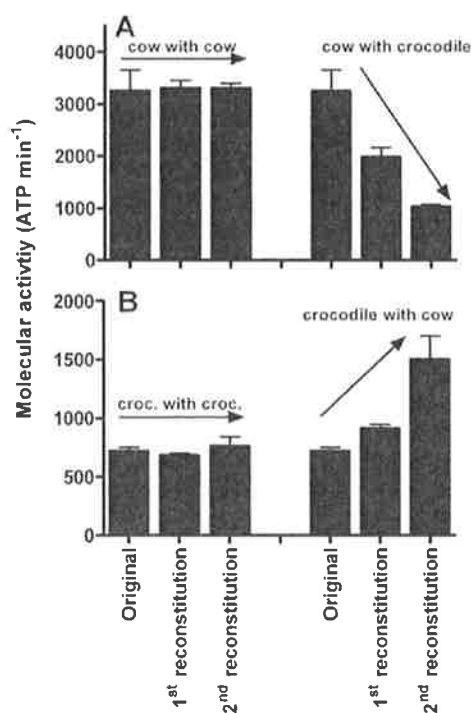
Figure 3. Molecular activities of cattle and crocodile Na$^-$-K$^+$-ATPase after species membrane crossover experiments. Molecular activity does not change after two reconstitutions of protein and membranes from the same species (*left*). Cow Na$^-$-K$^+$-ATPase activity decreases significantly when reconstituted with crocodile membrane (*A*, *right*). Conversely, crocodile protein activity increases when reconstituted with cow membrane (*B*, *right*). Redrawn from Wu et al. (2004).

composition can have a profound effect on the metabolic capacity of tissues and organisms.

Temperature affects membrane function because the liquid crystalline structure of the membrane acyl center changes from a fluid state to a gel-like structure as temperatures drop below a transition temperature. In its gel-like state, biological membrane function is compromised, and the activity of membrane-bound proteins is negatively affected (Hazel 1995). Increasing content of polyunsaturated fatty acids in the membrane will maintain membrane fluidity over a broader temperature range, and adjustments of membrane fluidity are thought to be an important response to temperature variation ("homeoviscous adaptation"; Sinesky 1974; Hazel 1995; Pernet et al. 2008). Warm acclimation may be accompanied by changes in the composition of phospholipid classes and subclasses in membrane (Kraffe et al. 2007; Overgaard et al. 2008). In addition, fatty acid composition of minor phospholipid classes changes with warm acclimation in trout, but the changes are not consistent between classes and do not mirror fatty acid changes of total phospholipids (Kraffe et al. 2007). These alterations in specific phospholipid classes emphasize the importance of considering the effect of minor phospholipid classes as well as total phospholipid fatty acid composition (Mitchell et al. 2007) on mitochondrial function. Phospholipid head groups of minor classes, such as cardiolipin, can have an important effect on the activity of proteins in mitochondrial membrane where proteins are separated by only very few phospholipids (Kraffe et al. 2007; Houtkooper and Vaz 2008).

Fatty acid composition in the diet can change the acyl chain composition of mitochondrial membranes and thereby affect oxidative capacities (Simandle et al. 2001; Hulbert et al. 2005; Guderley et al. 2008). In addition, dietary fatty acids and fat content of diet can modify metabolism by interacting with the PGC-1α regulatory system (Benton et al. 2008b). The interaction between diet and temperature in determining mitochondrial function through membrane modifications links the two major environmental variables that are affected by changing climates. In particular, changes in dietary n-3 fatty acid content can influence the n-3 content of plasma membranes in liver, heart, and brain of mammals (Hulbert et al. 2005). In trout, mitochondrial membrane fatty acid composition changes significantly between animals fed on diets of different fatty acid composition for 10 wk (Guderley et al. 2008). In parallel with changes in fatty acid composition, mitochondrial oxidative capacities change (Morash et al. 2008). The best predictors for changes in oxidative capacities are relatively minor fatty acids rather than general membrane characteristics such as percent unsaturation (Guderley et al. 2008). In addition to diet composition, energy content and food availability can have a pronounced effect on mitochondrial capacities of skeletal muscle and thereby locomotor performance (Guderley 2004a).

Uncoupling of Electron Transport from Oxidative Phosphorylation

Electron transport within complexes in the mitochondrial electron transport chain establishes a proton gradient across the inner mitochondrial membrane. The protonmotive force contained within this proton gradient provides the energy to phosphorylate ADP to ATP in complex V (F_0F_1-ATPase). However, the coupling of electron transport to oxidative phosphorylation is incomplete, owing to proton leakage across the mitochondrial inner membrane as well as "slip reactions" where proton pumps fail to transfer H^+ across the membrane (Brand 2005). A fraction of proton leak in isolated mitochondria is constitutive (basal), but a certain proton leak activity is inducible and mostly mediated by membrane-bound proteins. Movement of protons, either by passive leakage or by protein-mediated uncoupling, back into the mitochondrial lumen decreases the membrane potential and therefore the energy available to produce ATP (Skulachev 1998). However, proton leak and uncoupling have important functions in endothermic heat production and in reduction of reactive oxygen species (ROS) generation (Goglia and Skulachev 2003; Criscuolo et al. 2005; Echtay et al. 2005).

The rate of proton leak across the inner mitochondrial membrane is a principal determinant of tissue metabolic rate. Proton leak in mammals, for example, is severalfold greater than in ectothermic reptiles. This difference in basal proton leak is proportional to the greater resting metabolic rate of endotherms compared with ectotherms and can at least partly explain metabolic differences between these groups (Brand et al. 1991; Hulbert and Else 1999). The increased proton leak in mammals is positively correlated with a greater proportion of polyunsaturated long-chain fatty acids in the mammalian mitochondrial membrane (Brand et al. 1994). However, fatty acid–dependent proton leak accounts for only about 5%–25% of basal mitochondrial proton leak in intact mitochondria (Brookes et al. 1997). Most of the remaining basal proton leak is through the membrane-bound protein adenine nucleotide translocase (ANT). ANT contributes to basal proton conductance by a mechanism that is independent from its function as an exchanger of ADP and ATP across the mitochondrial membrane (Brand et al. 2005). In addition, ANT can be stimulated by free fatty acids and by alkenals that originate from peroxidation of mitochondrial membranes (Echtay et al. 2003; Brand et al. 2004). Unlike ANT, the different homologues of uncoupling protein (UCP) cause proton leak only when stimulated, for example, by alkenals or fatty acids (Brand and Esteves 2005). Of the different UCPs, UCP1 is specific for mammalian brown adipose tissue (Klingenspor et al. 2008). It functions in adaptive thermogenesis by stimulated uncoupling of the electron transport chain from oxidative phosphorylation so that a large proportion of the protonmotive force across the mitochondrial membrane is released as heat (Cannon and Nedergaard 2004; Morrison et al. 2008).

Birds, however, lack brown adipose tissue and UCP1. Nonetheless, repeated cold exposure (cold acclimation) in penguins increases uncoupling of skeletal muscle mitochondria (Talbot et al. 2004). Uncoupling in cold-acclimated penguins is mediated by increased mRNA abundance of avian UCP (avUCP) and ANT compared with controls (Talbot et al. 2004). Interestingly, fasting in penguins has the opposite effect to cold acclimation; that is, fasting reduces whole-animal oxygen con-

CLIMATE VARIABILITY
temperature + nutrition

Transcription
transcription factors
co-activators

Membranes
saturation
phospholipids
fatty acid classes

Mitochondrial Bioenergetics
protonmotive force
proton leak/phosphorylation
mitochondrial density

Reactive O Species
Proton leak Q₁₀
Oxygen limitation

RESPONSE TIME
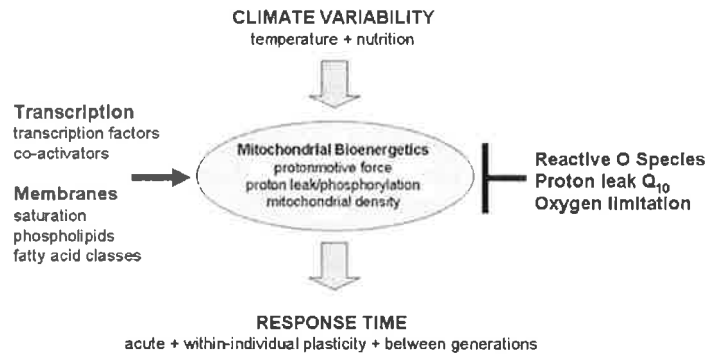acute + within-individual plasticity + between generations

Figure 4. Schematic summary of mitochondrial responses to climate variability. Changes in both temperature and nutrition affect mitochondrial bioenergetics. Two of the principal mechanisms by which mitochondrial responses are effected are (1) transcriptional control of mitochondrial density and mitochondrial proteins and (2) changes in membrane composition. Mitochondrial responses are limited by the production of reactive oxygen species that can damage or destroy the organelle, limitation in oxygen supply to mitochondria, and a disproportional increase in proton leak with increasing temperatures. Within this framework, responses at different temporal scales—acute, within-individual plasticity or acclimation, and changes between generations—are superimposed on one another.

sumption. The decrease in oxygen consumption while fasting is accompanied by a decrease in the abundance and activity of avUCP and in the PGC-1α mRNA concentration. Nonetheless, mitochondrial density and cytochrome c oxidase activity do not change with fasting (Rey et al. 2008a), which indicates reduced uncoupling and an increase in the efficacy of ATP production. Again, conflicting demands between the need for body temperature regulation and responses to dietary changes could compromise the fitness of the birds if lack of food and exposure to cold coincided.

UCP2 and UCP3 homologues occur in most animal groups (Sokolova and Sokolov 2005; Schwartz et al. 2008) and play no role in thermogenesis (Brand and Esteves 2005; Walter and Seebacher 2009). UCP2 and UCP3 cause mild stimulated uncoupling that may be important in alleviating the production of reactive oxygen species (Brand and Esteves 2005). The mRNA concentrations of UCP2 and UCP3 increase following cold acclimation in the liver of crocodiles (Schwartz et al. 2008), and reptilian UCP may function in preventing ROS damage following cold exposure in a lizard (Rey et al. 2008b). In addition, uncoupling and proton leak can change with nutrition and temperature acclimation in a toad and may act in adjusting mitochondrial efficiency to environmental demands (Trzcionka et al. 2008).

What Are the Limits to Plasticity?

Mitochondrial function is extremely plastic: oxidative capacities and densities can vary severalfold between muscle types of the same individual (Battersby and Moyes 1998) and over a relatively short time within the same tissue (Seebacher et al. 2003a; Lucassen et al. 2006). At an evolutionary timescale, mitochondria have adapted to function at temperatures ranging from $-1.8°C$ in polar waters to $+40°C$ in hot springs (Johnston et al. 1994; Hardewig et al. 1998; Seebacher et al. 2005). There are limits, however, and understanding how mitochondrial bioenergetics can constrain animal function is important, particularly in the short term, when nonevolutionary mechanisms are likely to be most important in responding to rapid (relative to life span) changes in climate. For example, if human activity induces a significant change in climate over the next 50 yr, the rate of change may be too rapid for evolutionary adaptation to occur in animals with generation times of several months or more (Smith and Eyre-Walker 2002). Hence, within-individual plasticity will represent the most crucial time frame for compensatory responses.

Constraints to mitochondrial function can be caused by damage to parts of the organelle or by limited substrate availability (Fig. 4). One of the most important mechanisms that can damage mitochondria is the production of reactive oxygen species (ROS; Brand et al. 2004). Reactive superoxide (O_2^-) is produced in the high-energy environment of the inner mitochondrial membrane and, in particular, by complexes I and III (Brand et al. 2004). Mitochondria possess a natural, enzymatic defense system. Superoxide dismutase turns superoxide into the less reactive hydrogen peroxide (H_2O_2). Catalase and glutathione peroxidase break down hydrogen peroxide into water and molecular oxygen ($H_2O + O_2$), thereby eliminating the threat from ROS. However, the system is not perfect, and secondary radicals, particularly hydroxyl radicals (OH), form from superoxide. These radicals react with the mitochondrial membrane, disrupting its proper function and producing reactive alkenals. Reactive alkenals stimulate ANT and UCPs, causing uncoupling of the electron transport chain and thereby decreasing membrane potential and the risk of further reactive oxygen production (Brand et al. 2004). Nonetheless, the efficacy of the enzymatic defense system and uncoupling is limited, and increasing membrane potentials beyond these limits will damage and eventually destroy the mitochondrion (Abele et al. 2002). Hence, there is a possible trade-off between ROS and ATP production, and ROS are linked to degenerative diseases

and longevity in mammals (Hulbert 2008). ROS production is certainly a candidate mechanism by which mitochondrial plasticity and thermal acclimation can be limited (Guderley 2004b). Whether ROS production actually limits acclimation or acclimatization under natural conditions is not clear, however.

An alternative hypothesis proposes that animals, and particularly marine fish, are negatively affected by warming because increasing temperatures limit oxygen delivery to the mitochondria as a result of greater cellular demand and decreased oxygen content of water (Pörtner and Knust 2007). However, oxygen transfer between the environment and blood depends to a large extent on differentials in oxygen partial pressures, which in water decrease only slightly with increasing temperature (from 20.3 kPa at 0°C to 18.4 kPa at 37°C; derived from Benson and Krause 1984; Graham 1987) and are therefore unlikely to limit the countercurrent oxygen transfer at the fish gill (Piiper and Scheid 1975). Oxygen delivery through the cardiovascular system of fish can be modulated to compensate for increasing temperatures so that in many species cardiac output is unlikely to be compromised over the temperature range that is forecast to occur as a result of human-induced climate change (Franklin et al. 2007; Steinhausen et al. 2008). In addition, with the onset of hypoxia, tissue oxygen affinity increases as a result of induced transcription of myoglobin isoforms in muscle, liver, gill, and brain (Sidell 1998; Fraser et al. 2006; Cossins et al. 2009). It is therefore unlikely that temperature-induced changes in oxygen supply are a principal constraint for mitochondrial function, at least over the range of predicted climate change.

A third possibility is that mitochondria become too inefficient to sustain cellular functions. If the thermal sensitivities of proton leak and oxidative phosphorylation differ, changing temperatures could compromise ATP production. The substrate oxidation system (TCA cycle, electron transport chain) establishes the protonmotive force and proton leak, and the phosphorylation system (F_0F_1-ATPase) dissipates it. If the thermal sensitivity (Q_{10}) of the proton leak is greater (i.e., it increases faster with increasing temperatures) than that of substrate oxidation and phosphorylation, the efficiency of ATP production will decrease as temperatures increase (Pörtner et al. 2005). These relationships constrain animal function as temperatures increase acutely (Abele et al. 2002). However, the effect of longer-term temperature increases on mitochondrial coupling (i.e., the ratio between oxygen consumed as a result of phosphorylating ADP to proton leak) is less clear, and the mitochondrial coupling ratio may change with acclimation temperatures (St-Pierre et al. 1998; Schnell and Seebacher 2008), and absolute temperature limits are likely to differ between species.

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