Mitochondrial Uncoupling Proteins: New Perspectives for Evolutionary Ecologists

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ABSTRACT: Reactive oxygen species (ROS)–induced damage on host cells and molecules has been considered the most likely proximal mechanism responsible for the age-related decline in organismal performance. Organisms have two possible ways to reduce the negative effect of ROS: disposing of effective antioxidant defenses and minimizing ROS production. The unbalance between the amount of ROS produced and the availability of antioxidant defenses determines the intensity of so-called oxidative stress. Interestingly, most studies that deal with the effect of oxidative stress on organismal performance have focused on the antioxidant defense compartment and, surprisingly, have neglected the mechanisms that control ROS production within mitochondria. Uncoupling proteins (UCPs), mitochondrial transporters of the inner membrane, are involved in the control of redox state of cells and in the production of mitochondrial ROS. Given their function, UCPs might therefore represent a major mechanistic link between metabolic activity and fitness. We suggest that by exploring the role of expression and function of UCPs both in experimental as well as in comparative studies, evolutionary biologists may gain better insight into this link.

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Although the aim of evolutionary biology is to understand the evolutionary forces that over time have shaped the observed variation in individual traits and the ultimate consequences of such variation in terms of fitness (Endler 1986; Stearns 1992), increasing interest is currently devoted to the study of the proximal mechanisms that generate the interindividual variation (Zera and Harshman 2001). Understanding the proximal basis of interindividual variation for traits of interest can provide insights into both the adaptive nature of these traits and the constraints that set the limits to their evolution (Rose and Bradley 1998; Watt and Dean 2000; Barnes and Partridge 2003). For instance, in the past decade, much emphasis has been placed on understanding how physiological processes such as metabolic rate, endocrine homeostasis, and immune functioning can constrain the evolution and the expression of life-history traits (Folstad and Karter 1992; Zera and Harshman 2001).

Living organisms must gain energy from their environments to sustain their biological functions, particularly when these are thermodynamically unfavorable processes. In the mitochondria, evolution has succeeded in combining the production of energy as adenosine triphosphate (ATP; phosphorylation) with an efficient chain of redox reactions, with dioxygen as the final electron acceptor (oxidation).When oxidation is impaired or slows down, electrons cannot reach cytochrome oxidase, where dioxygen undergoes tetravalent reduction to produce water. In this case, single electrons react directly with molecular oxygen instead of passing to the next electron carrier of the chain, and they form highly damaging molecules (estimated as 0.1% to 4% of total oxygen consumption)—the reactive oxygen species (ROS; Cadenas and Davies 2000; Raha and Robinson 2001). Because of their reactive nature, ROSs can have profound effect on the main biomolecules such as DNA, proteins, and lipids and on gene regulation (Harman 1956; Finkel and Holbrook 2000). Given that ROSs

are unavoidable by-products of metabolic activities, organisms dispose of a number of antioxidant defenses to protect themselves, ranging from endogenously produced enzymes to food-acquired vitamins (Finkel and Holbrook 2000; Raha and Robinson 2000). Both ROS production and the availability of antioxidants can vary during the life cycle of an organism, giving rise to a dynamic process called oxidative stress. Oxidative stress has been defined as an unbalance between ROS production, the efficiency of the repair system, and the availability of antioxidants (Beckman and Ames 1998; Finkel and Holbrook 2000). The cumulative damage induced by oxidative stress has been suggested to be involved in the age-associated progressive decline in organismal performance, in particular, in apoptosis and DNA damage (Papa and Skulachev 1997; Raha and Robinson 2001) and, finally, in longevity (Sohal and Weindruch 1996). There is a wide range of taxonomic and species-specific variation in the rate of aging (Stearns 1992). Given that the more oxygen is consumed, the more ROSs are produced, early hypotheses suggested a direct link between metabolic activity and longevity, the so-called rate of living theory (reviewed in Beckman and Ames 1998). However, in spite of some evidence in support of this theory, several exceptions to this pattern exist, showing that the link between metabolism, ROS production, and life history is more complex than previously thought (Speakman et al. 2002). For instance, the conventional wisdom that metabolic rate is positively associated with ROS production has been challenged recently, as Speakman et al. (2004) found that mice with a higher metabolic rate have a reduced ROS production caused by uncoupled mitochondrial oxidative phosphorylation.

There are two ways organisms can protect themselves against oxidative stress. The first obvious way is to reduce ROS production; the second is to mobilize an effective network of antioxidant defense and repair systems. Interestingly, most efforts to understand the ultimate consequences of oxidative stress have focused on measurements of antioxidant defenses and of oxidative damage (Johnson et al. 1999). Antioxidants such as the superoxide dismutase (SOD) and other enzymes are usually able to maintain low body concentrations of ROS, and experimental work has shown that enhanced availability of antioxidants is generally associated with extended longevity, at least in nematodes *Caenorhabditis elegans* (Melov et al. 2000) and fruit flies *Drosophila melanogaster* (Bonilla et al. 2002). Nevertheless, these results should be viewed cautiously because the effect of antioxidants can be context dependent. For instance, whereas the overexpression of antioxidative enzymes (CuZn-SOD, Mn-SOD, and catalase) has been reported to extend life span in transgenic fruit flies (Orr and Sohal 1994), increases in the activities of CuZn-SOD and catalase were associated with a slight decline in longevity in wild-type flies (Orr et al. 2003). Surprisingly, evolutionary biologists interested in the evolution of senescence and age-associate syndromes have largely neglected the mechanisms that control ROS production. Given the dynamic nature of oxidative stress, we believe that a comprehensive understanding of the role of oxidative stress on the evolution of life-history traits requires taking into account the equilibrium between ROS production and antioxidant defenses.

To partly close this gap, in this article we will focus on a subfamily of mitochondrial carriers that are widely distributed in the animal kingdom, the uncoupling proteins (UCPs). These proteins are thought to share the ability to control mitochondrial ROS production (Skulachev 1997; Arsenijevic et al. 2000; Ricquier and Bouillaud 2000; Miwa and Brand 2003) and may, therefore, be involved in all oxidation-related processes, with potential implications for life-history evolution.

What Is an Uncoupling Protein?

The mitochondrion is delimited by two membranes with different properties. The inner membrane has a strictly controlled permeability. An electrochemical gradient is established by pumping a proton (H^+) from the mitochondrial matrix into the intermembrane space. The electron protonmotive force drives the proton back to the matrix across the protein complex of the inner membrane, the ATP synthase, leading to the phosphorylation of adenosine diphosphate (ADP) into ATP. The whole process defines the mitochondrial oxidative phosphorylation (fig. 1*A*; Nicholls and Rial 1984).

The mitochondrial respiration can also occur in the absence of ATP production (state 4 of respiration), suggesting that protons can cross the inner membrane by another pathway than the ATP synthase (Rolfe and Brand 1997). This phenomenon accounts for one-third to one-half of the resting energy expenditure (Brand 2000). In the mid-1980s, the coupling of oxidative phosphorylation was found to be impaired by a specific transporter in the brown adipose tissue (BAT) of rodents, the uncoupling protein 1 (UCP1; Nicholls and Locke 1984; Aquila et al. 1985; Bouillaud et al. 1986). A major consequence of the uncoupling activity of UCP1 is the dissipation of the oxidation energy produced by the respiratory chain as heat, no ATP being produced (fig. 1*B*; reviewed in Ricquier and Bouillaud 2000). Since then, the uncoupling protein family has grown, and several homologues (UCP2, UCP3) have been found in mammals and other eukaryotes (Boss et al. 1997; Fleury et al. 1997; Laloi et al. 1997; Ricquier and Bouillaud 2000; Raimbault et al. 2001).

All the uncoupling proteins belong to the superfamily of anion carriers of the mitochondrial inner membrane.

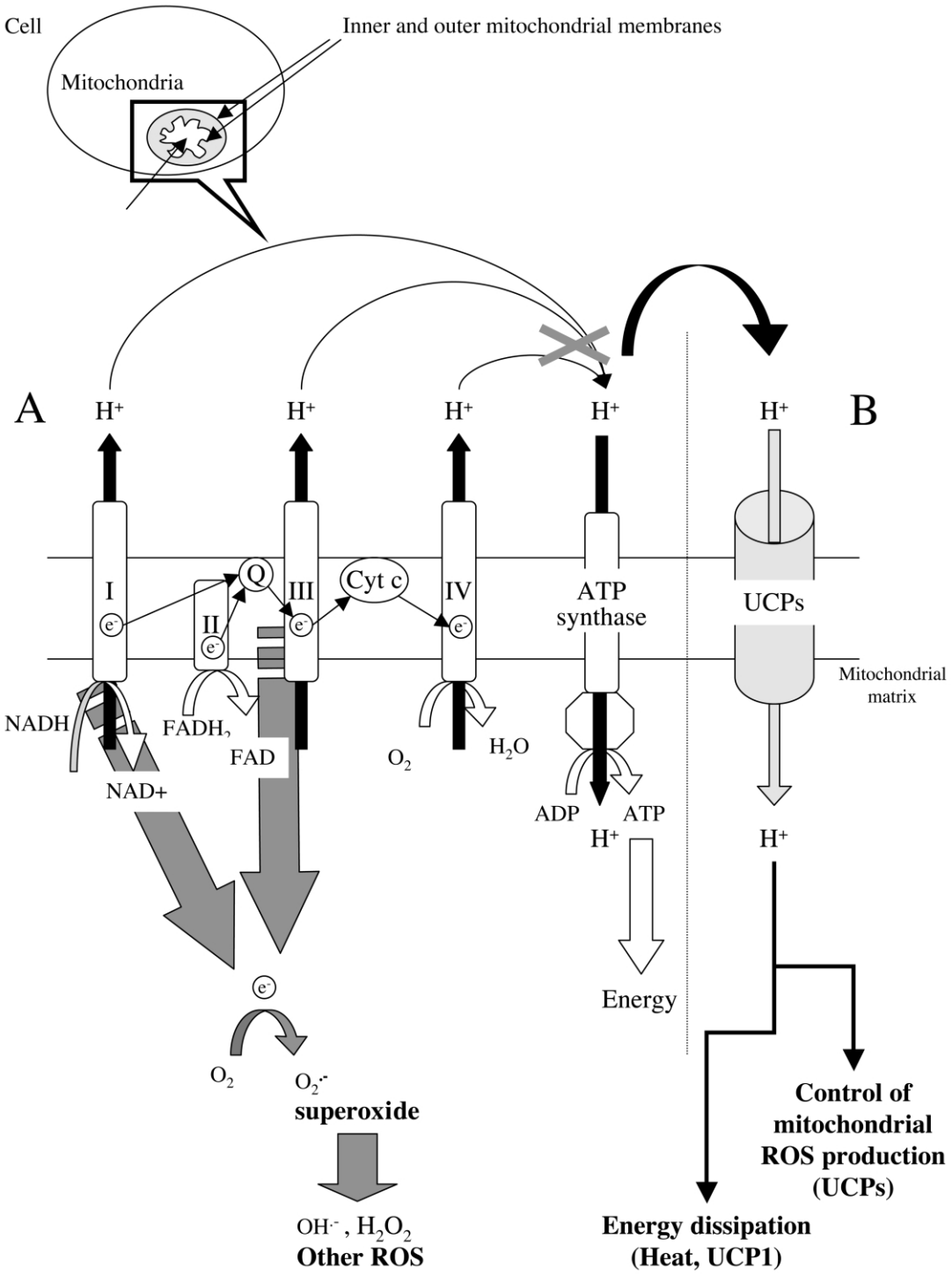

Figure 1: *A*, Mitochondrial respiratory chain depicted as a series of complexes (I, II, etc.) embedded in the inner mitochondrial membrane. Each complex accepts one electron from the previous one until the final acceptor, dioxygen (chemiosmotic hypothesis; Mitchell 1979). This electron transfer produces enough energy to export H^+, thus creating an electrochemical gradient used by the ATP (adenosine triphosphate) synthase for the phosphorylation of ADP (adenosine diphosphate) into ATP. *B*, Mitochondrial respiration produces reactive oxygen species (ROS) continuously,

They have a tripartite structure, a molecular mass between 31 and 36 kDa, and functional similarities (Ledesma et al. 2002). All anion carriers have about 30% of their amino acids in common, but this sequence homology does not necessarily imply a functional homology because each carrier ensures one specific metabolic transport (ADP, ATP, phosphate). Some mitochondrial carriers have an additional amino acid sequence signature, such as the conserved methionines in the ADP-ATP carrier (Pebay-Peyroula et al. 2003). The UCPs do not present such a specific signature, and therefore UCP1 homologues have been recognized based not only on their sequence homology but, more importantly, on the characterization of their uncoupling activity either in vivo or in vitro (table 1).

What Is the Physiological Role of Uncoupling Activity?

The thermogenic activity of UCP1 in BAT is now well established (Klingenberg 1990). Dissipation of energy as heat could obviously create a better intracellular thermal environment for enzymatic activities, thereby allowing the organism to be more independent of external thermal changes. The UCP1 knockout mice maintain a constant body temperature except in conditions of pronounced cold exposure (Enerback et al. 1997). The UCP1 is restricted to BAT, and several mammals do not have this particular thermogenic tissue (i.e., pigs), or they lose it at adulthood (i.e., humans; Alves-Guerra et al. 2002). These observations have led to the conclusion that the importance of UCP1 is restricted to a few mammalian species and BAT cold-induced thermogenesis (Alves-Guerra et al. 2002).

Energy-dissipating systems such as uncoupling processes can confer flexibility to the redox state and to the energy requirements of the cell (Ricquier and Bouillaud 2000; Sluse and Jarmuszkiewicz 2002). The mitochondrial respiratory machinery can be simplified as a box that utilizes reduced coenzymes or substrates (such as nicotinamide adenine dinucleotide [NADH] and succinate) to set up the H^+ gradient and to produce ATP. Without uncoupling activity, this system is rigid, and the control of the redox state of the cell is ADP/ATP dependent. By allowing the respiratory chain to use reduced coenzymes in an ADP/ATP-independent manner, uncoupling proteins allow the mitochondria to function as a safety valve when there is an unbalance between the supply of reducing substrates and energy production.

UCP1 Homologues: Evidence against a Thermogenic Role

In addition to the biochemical evidence that numerous UCP1 homologues do not have UCP1-like uncoupling activity, UCPs or UCP-like proteins have been found in organisms not known to exhibit endothermy or even thermogenesis. The plant uncoupling protein (PUMP or StUCP) was first discovered in the potato tuber *Solanum tuberosum* (Vercesi et al. 1995; Laloi et al. 1997), where the uncoupling activity was characterized (Hourton-Cabassa et al. 2002). PUMP was thereafter found in several other species (tomato *Lycopersicon esculentum*, *Arabidopsis thaliana*, maize *Zea mays*, and soya bean *Glycine maxima*; Ježek et al. 2001). Additionally, mitochondrial uncoupling activity has been reported in fungi *Candida parapsilosis* (Jarmuszkiewicz et al. 2000) and immunodetected (but not cloned) in a nonphotosynthetic soil amoeboid protozoan *Acanthamoeba castellanii* (Jarmuszkiewicz et al. 1999). Even though these data are far from being conclusive, they suggest an emergence of the UCP superfamily early in the diversification of eukaryotic lineages.

The physiological functions of UCP homologues in protozoa and fungi, but particularly in plants, have been discussed in several studies (reviewed in Ježek et al. 2001) and are still subject to much speculation (table 1). The large distribution of UCP homologues in different species and tissues (such as roots, leaves, seeds, and flowers) supports the view of several physiological, tissue-specific, and possibly even transient roles, depending on the life cycle of the organism. Among these functions, uncoupling activity in plants can be triggered by ROS and can downregulate ROS production by the respiratory chain (Kowaltowski et al. 1998; Pastore et al. 2000). Interaction with another system leading to uncoupling respiration (an alternative oxidase) may represent an efficient mechanism of ROS control in plants (see Sluse and Jarmuszkiewicz 2002; Hourton-Cabassa et al. 2004 for review).

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particularly at complexes I and III of the respiratory chain. This production is enhanced when the electrochemical gradient is high, and consequently, the transport of protons out of the matrix is more difficult (no ATP production, state 4 of respiration). At this moment, electron flow is interrupted, and reduced coenzymes (such as ubiquinone) may directly react with oxygen to generate superoxide, the first element of a chain reaction that produces several radical oxygen species. Therefore, a small decrease in the inner membrane potential (mild uncoupling) via uncoupling proteins (UCPs), induced by a H^+ return to the matrix independent of the ATP synthase, may favor a decrease in the electrochemical gradient and a decrease in the half-life of reduced coenzymes. In the brown adipose tissue of mammals, UCP1 induces a drastic uncoupling, allowing dissipation of the electrochemical gradient energy as heat. Cyt c, cytochrome c; FAD, flavine adenine dinucleotide; NADH, nicotinamide adenine dinucleotide; Q, ubiquinone; I, NADH-Q reductase; II, succinate dehydrogenase; III, cytochrome c reductase; IV, cytochrome c oxidase.

Table 1: Overview of published data on function, amino acid homology, and uncoupling activity of UCPs or UCP-like proteins across different taxa

Taxa and species	Experimental evidence for a putative function for UCPs	NCBI reference number	Homology with UCP1 (%)	Homology with UCP2 (%)	Measured uncoupling activity	References
Protozoan:						
Acanthamoeba castellanii	…	Not cloned	…	…	FFA dependent	Jarmuszkiewicz et al. 1999
Fungi:						
Candida parapsilosis	Redox balance (biosynthesis and energy supply)	Not cloned	…	…	FFA dependent	Jarmuszkiewicz et al. 2000
Plant:						
Solanum tuberosum	Involved in thermoregulation; fruit ripening; control of mitochondrial ROS production; plant senescence, seed dormancy, thermogenesis, etc.	CAB60277	44	47	?	Laloi et al. 1997
Mangifera indica		AAK70939	43	47	FFA and $O_2^{\cdot -}$ dependent	Considine et al. 2001
Triticum durum		Not cloned	…	…	…	Pastore et al. 2000
Arabidopsis thaliana, *Zhea mays*		AAL07121	42	50	FFA and ROS dependent	Ježek et al. 2001
Insect:						
Drosophila melanogaster	Related to lipid metabolism?	NP729738	27	25	…	Hanák and Ježek 2001
	Role in central nervous system?	AAF48769	35	33	FFA dependent	Fridell et al. 2004
Fish:						
Danio renio[a]	…	Q9W720	58	80	…	Stuart et al. 1999
Cyprinus carpio	Not related to thermogenesis	Q9W725	58	80	…	
Pagrus major	Relationship with ROS metabolism and apoptosis	AAL92117	60	74	…	Liang et al. 2003
Amphibian:						
Xenopus laevis	Undefined	AAO26203	28	34		Jastroch et al. 2004
Xenopus laevis	Undefined	AAH44682	58	83		Jastroch et al. 2004
Silurana tropicalis	Undefined	AAH63352	57	82		Jastroch et al. 2004
Bird:						
Gallus gallus	Thermogenesis	BAC15532	56	70	…	Raimbault et al. 2001; Toyomizu et al. 2002; Collin et al. 2003*a*
Euptomena macroura	Thermogenesis	AAK16829	56	70	Uncoupling in vitro	Vianna et al. 2001
G. gallus	Lipid utilization as fuel substrate					Evock-Clover et al. 2002; Collin et al. 2003*b*
Aptenodytes patagonicus	Relationship with ROS metabolism	AAT05613	60	74	ROS dependent	Talbot et al. 2003, 2004
Metatheria:						
Antechinus flavipes	Lipid metabolism, mammalian	AAS45212	57	73	…	Jastroch et al. 2004
Sminthopsis macroura	UCP2-like activity?	AAP45779	62			
Eutheria:						
Mus musculus	UCP1: thermogenesis	NP036814		62	Established	Nicholls and Locke 1984
Rattus norvegicus	UCP2: ROS metabolism	NP062227	58	89	FFA or ROS dependent, but controversial	Arsenijevic et al. 2000; Echtay et al. 2002
Homo sapiens	UCP3: ROS and lipid metabolism	NP037299	56	73		Vidal-Puig et al. 2000; Dulloo et al. 2001

Note: Sequence searches were performed in public databases using BLAST alignments (NCBI [National Center for Biotechnology Information] database). Comparisons of protein sequences were made with UCP1 (NP036814) or marsupial UCP2 (AAP45779). FFA, free fatty acids; ROS, reactive oxygen species.

[a] See Jastroch et al. (2005) for information on the recent discovery of an apparent nonthermogenic UCP1 gene in fish.

The thermogenic role of UCP1 homologues can also be questioned in animals. Conserved amino acid sequences of uncoupling proteins have been found in the genome of *Drosophila melanogaster*, thereby potentially extending the presence of the uncoupling protein family to insects (Hanák and Ježek 2001). However, there is no consensus that these primary sequences are specific to UCPs, given that it is difficult to distinguish UCPs structurally from other mitochondrial carriers. More recently, Fridell et al. (2004) characterized DmUCP5, a UCP protein close to the brain mitochondrial carrier 1 (BMCP1), with a preponderant expression in the central nervous system. However, DmUCP5 and BMCP1 share less than 30% of amino acid sequence identity with UCP1 and cannot be safely considered as uncoupling proteins. Moreover, DmUCP5 has only a weak uncoupling effect on mitochondrial potential, and its physiological role is still unclear (Schreiter et al. 2004). Closer homologues of UCP1 were discovered in several fish species (Stuart et al. 1999; Liang et al. 2003; Jastroch et al. 2005) and in amphibians (Jastroch et al. 2004), but little is known about the function of UCP homologues in these taxa (Stuart et al. 2001).

Uncoupling protein homologues have been found in both classes of endotherms, birds (Raimbault et al. 2001; Vianna et al. 2001) and mammals (metatheria: Jastroch et al. 2004; eutheria: Bouillaud et al. 1986; Fleury et al. 1997; Gimeno et al. 1997; Nedergaard and Cannon 2001). The function of the two mammalian UCP1 homologues—UCP2, which is expressed in several tissues (Fleury et al. 1997), and UCP3, which is present mainly in skeletal muscles (Boss et al. 1997)—is still debated, even if they are able to induce an increased proton leak in recombinant systems (see Bouillaud 1999). Mice whose UCP2 and UCP3 genes have been knocked out are not cold sensitive, which argues against a thermogenic role for these proteins (Arsenijevic et al. 2000; Vidal-Puig et al. 2000). Interestingly, these experiments also provided the first evidence of a role for uncoupling proteins in the control of ROS production. Macrophages of $UCP2^{-/-}$ mice produced more ROS (Arsenijevic et al. 2000), and the expression of UCP2 in macrophages is induced in response to oxidative stress (Pecqueur et al. 2001). The control of mitochondrial ROS production by UCP2 was confirmed recently by Fridell et al. (2005); human UCP2 overexpression in transgenic flies decreases cellular oxidative damages and is sufficient to extend life span.

Finally, the analysis of protein sequences of different mitochondrial carriers from several vertebrates also supports the view that UCPs might have different physiological functions. Compared with mitochondrial carriers known to have a high specificity of transport (such as the ADP-ATP carriers or phosphate carriers), proteins of the UCP family show a substantial degree of diversity (Jastroch et al. 2004). The mechanisms that generate such a diversity are multiple and are still under debate (Lynch and Katju 2004). Gene duplication is often regarded as one such mechanism, although other models have suggested that diversification of pleiotropically constrained functions could precede gene duplication (Lynch and Katju 2004). Interestingly, the UCP1 gene was found recently in ectothermic fish species, but with an apparent nonthermogenic function; it is therefore different from mammalian UCP1 (Jastroch et al. 2005). Definitely, work that goes beyond the scope of this review is needed to investigate the molecular mechanisms at the origin of UCP diversity.

At the organismal level, the uncoupling activity of UCPs is likely to be involved in the late appearance of age-related diseases (Blanc et al. 2003; Diano et al. 2003). For example, it has been suggested that free radical production is involved in the dysfunction of pancreatic β-cells and the pathogenesis of diabetes (Brownlee 2003; Krauss et al. 2003). The uncoupling activity of UCP2, which is expressed in the pancreas and was originally linked to hyperinsulinemia (Fleury et al. 1997), can blunt glucose-induced insulin secretion based on the resultant decrease in ATP levels inside β-cells (Zhang et al. 2001). Similarly, uncoupling proteins expressed in brain tissue may also play a neuroprotective role (Diano et al. 2003). Likewise, UCP2 seems to be protective against atherosclerosis (Blanc et al. 2003), another disease that usually appears later in life. More recently, overexpressed UCP1 in mouse heart was shown to protect mice against the ischemia-reperfusion process, which is also known to involve ROS-mediated tissue damage (Hoerter et al. 2004).

Uncoupling Protein in Birds: Current Status and Ecological Prospects

Birds live substantially longer than mammals of similar size despite greater overall exposure to oxidative stress caused by their higher metabolic rate, body temperature, and blood glucose levels (Holmes and Austad 1995). Two processes may be involved in an increased resistance to oxidative stress in birds: a more effective antioxidant defense and/or reduced ROS production (Papa and Skulatchev 1997). In support of this latter hypothesis, comparative studies reported reduced free radical production in birds (Herrero and Barja 1998; Perez-Campo et al. 1998), possibly mediated by an uncoupling of respiratory activity.

In 2001, a UCP homologue (avianUCP) was discovered in the chicken *Gallus gallus* (Raimbault et al. 2001) and in the hummingbird *Euptomena macroura* (Vianna et al. 2001). This protein shares 70% sequence homology with the mammalian UCP2 and UCP3 and is expressed in skeletal muscles (Raimbault et al. 2001), heart (Vianna et al.

2001), spleen, and brain and in a more limited way in kidney, lung, liver, and adipose tissue (Evock-Clover et al. 2002).

Since 2001, avianUCP has been discovered in other bird species: turkey *Meleagris gallopavo* and king penguin *Aptenodytes patagonicus* (Talbot et al. 2003), eider duck *Somateria mollissima* and zebra finch *Taeniopygia guttata* (D. Ricquier et al., unpublished data), and it may be involved in nonshivering thermogenesis (Raimbault et al. 2001; Vianna et al. 2001; Collin et al. 2003*a*) or favor lipid utilization during fasting (Evock-Clover et al. 2002; Collin et al. 2003*b*). However, the cold-induced uncoupled respiration observed in avian skeletal muscles is more related to the activity of another mitochondrial transporter (the ADP/ATP transporter) than to avianUCP (Toyomizu et al. 2002). Both cold exposure and fasting induce free fatty acid oxidation in birds (Barré et al. 1986; Cherel et al. 1988), thereby increasing ROS production by the respiratory chain (St-Pierre et al. 2002). Therefore, the biological activity of avianUCP could be related to the control of free radical production within the mitochondrial matrix. This hypothesis was supported by Talbot et al. (2003, 2004), who demonstrated that exposure of isolated mitochondria from avian muscle to exogenous superoxide triggered uncoupled respiration. In addition, superoxide-induced mitochondrial uncoupling could be regulated via lipid peroxidation by superoxide, which then would activate UCPs indirectly (Echtay et al. 2003; Murphy et al. 2003).

As in mammals, the uncoupling activity of avianUCP may be important in protecting tissue from ischemia injuries (Hoerter et al. 2004). This stems from a rapid switch between conditions of poor tissue oxygenation to high oxygen boosts that may enhance ROS production. For example, the ability of penguins to recover from long dives (Handrich et al. 1997) could involve a mild uncoupling process. Interestingly, another mitochondrial carrier, avian BMCP, presenting high homology with mammalian BMCP1 (D. Ricquier et al., unpublished data), indicates that mitochondrial carriers other than UCPs may protect the avian brain from oxidative damage.

The involvement of UCP1 homologues and avianUCP in the control of ROS production means that they are of particular value in the study of the link between oxidative stress and life-history traits (see fig. 2 for a schematic view of the potential link between UCPs, life-history traits, and fitness). In the next section of this review, we will briefly discuss some future directions on how the expression of avianUCP might affect aging, immune functioning, and sexual signals.

Perspectives and Future Directions

UCP Control of ROS Production and the Evolution of Aging

Senescence is defined as the progressive decline in performance with age, decline that includes somatic maintenance as well as reproductive activities. Evolutionary theories of aging share the same assumption: the intensity of natural selection declines with age because extrinsic mortality reduces the proportion of living individuals at old ages (see Kirkwood and Austad 2000 for a review). As a consequence, any mechanism that favors performance early in life at the expense of performance late in life should be selected for. At the proximal level, it has been suggested that oxidative stress and the associated ROS-induced damages might be the mechanism responsible for most of the degenerative processes associated with aging (Sohal and Weindruch 1996; Johnson et al. 1999). The finding that oxidative stress increases with age has been reported in several studies (Das et al. 2001; Sohal et al. 2002), and the reason for this enhanced sensitivity to ROS in old individuals resides partly in the decreased availability of antioxidant defenses (Klichko et al. 2004). Experimental manipulation of antioxidants has indeed been shown to improve life expectancy in *Caenorhabditis elegans* (Melov et al. 2000) and transgenic fruit flies (Orr and Sohal 1994) but not in wild-type flies (see Orr et al. 2003). A nonexclusive alternative, however, to explain the observed pattern of age-dependent oxidative stress would be that in parallel with a decrease in antioxidants, age is associated with an increase in mitochondrial ROS production. Age-dependent changes in both ROS production in mitochondria and UCP expression have been reported for animal models (Chaves et al. 2002; Radak et al. 2004). For instance, Kerner et al. (2001) reported a 68% reduction in UCP3 abundance in mitochondria from elderly compared with adult rats. Similarly, UCP1, UCP2, and UCP3 mRNA expressions were markedly reduced in the adipose tissue and skeletal muscle of old rats compared with young individuals (Iritani et al. 2002). Obviously, we need to extend these investigations to nonmodel species. Birds are good candidates for such studies because, as mentioned above, they seem to have special adaptations for cellular protection or regeneration (reviewed in Holmes and Austad 1995). Evidence is accumulating on the possible role of ROS production as a mechanism that explains species-specific variation in longevity (Barja 2004). We believe that an interesting avenue would be to study UCP expression as well as ROS production in mitochondria of individuals of different ages and in species with variable life expectancy.

Life-history traits do not evolve independently of each other; instead, they are linked in a matrix of genetic cor-

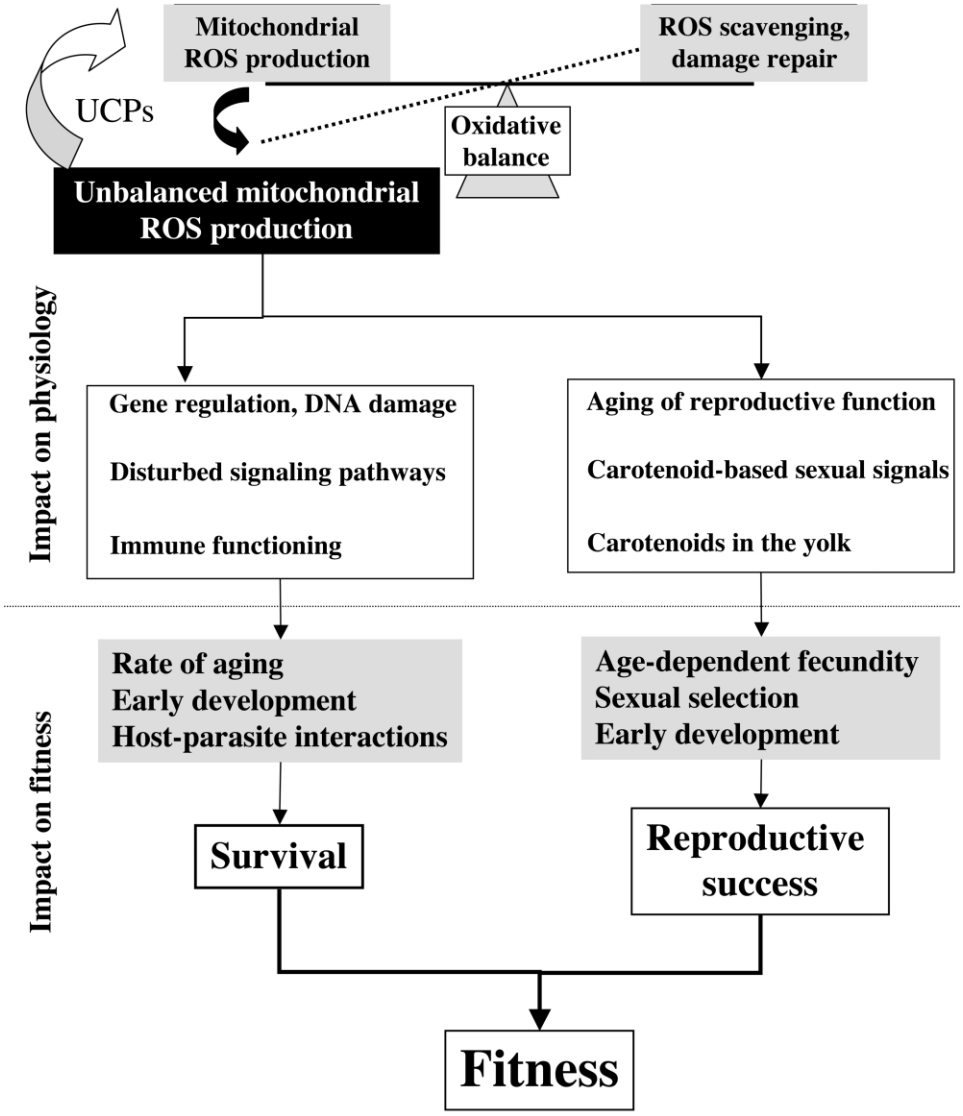

Figure 2: Schematic representation of the potential fitness consequences of an unbalanced ROS (reactive oxygen species) metabolism. Oxidative stress results when antioxidant defenses are overcome by pro-oxidant forces and ROSs are not removed efficiently; ROSs are also generated in normal physiological signaling pathways of growth factors and cytokines. Pro-oxidant perturbations have physiological and ecological consequences that affect individual fitness. Uncoupling proteins (UCPs) are involved in the control of ROS production, thereby representing an additional means for the regulation of oxidative balance.

relations that limit the evolution and the phenotypic expression of each trait (Stearns 1992). Therefore, a comprehensive study of aging cannot ignore the correlations that exist between longevity and the age-dependent pattern of investment in reproduction (age at first reproduction, number of reproductive attempts, reproductive effort per reproductive attempt). As for somatic maintenance, reproductive senescence is also slower in birds compared with mammals of similar size (Holmes et al. 2001). In females, this slow fertility decline could be due to an increased resistance of the ovarian follicular cells to aging-related damage (Holmes et al. 2003). In mice, the homologue UCP2 is expressed in ovarian and endometrial cells, and its gene expression increased before ovulation and during gestation (Rousset et al. 2003). Interestingly, $UCP2^{-/-}$ females produced litters with fewer offspring compared with their wild-type littermates (Rousset et al. 2003), and these authors suggested that the lack of UCP2 might lead to increased ROS production, which would inhibit ovarian functioning. So far, the question of a po-

tential role of avianUCP in reproductive senescence has not been investigated, and it is not even known if avianUCP is expressed in ovaries. However, an independent control of ROS production in the reproductive tract by avianUCP would provide a plausible explanation for the uncoupled process that may control longevity and reproductive senescence (Ricklefs et al. 2003).

Beyond the set of genetic correlations that limit their expression, life-history traits are particularly sensitive to the environmental conditions encountered by individuals. Whereas the effect of current environmental condition on reproductive output might seem obvious, correlational and experimental evidence consistently shows that past conditions also can have profound effects on life-history traits (Lindström 1999; Lummaa and Clutton-Brock 2002). A bad early start in life has been associated with reduced life span or increased risk of development of coronary disease and diabetes later in life (Metcalfe and Monaghan 2001). Similarly, it has been reported that antioxidant availability (carotenoids) can be modified by nutritional conditions in early life, establishing a link between environmental condition, oxidative stress, and the expression of carotenoid-based sexual signals (Blount et al. 2003*b*). As far as we know, the potential effect of bad early condition on ROS production and the underlying control mechanism has not been explored. The characterization of avianUCP expression in birds experiencing a range of environmental conditions during growth with a parallel assessment of mitochondrial ROS production and antioxidant availability might prove useful to close this gap. A longitudinal survey of individuals raised in such contrasted environments would provide evidence for the potential long-lasting effect of early conditions on UCP expression and ROS production.

UCPs and the Control of Infectious Diseases

Although ROSs are produced in mitochondria during normal respiratory activity, macrophages and other cells of the immune system release reactive oxygen species and free radicals as a response to pathogen insult (Janeway et al. 2005). The production of ROS and nitric oxide (NO) by immune cells is an essential part of the innate, nonspecific immune response. The innate immune response is a crucial component of the ability hosts have to control an infectious disease (Janeway et al. 2005). Because of their reactive and toxic nature, ROSs have the potential to harm and kill an invading pathogen. However, as mentioned above, they can also damage host cells. This sets the scene for a conflict between the costs and benefits of ROS production in macrophages and calls for a mechanism controlling the allocation rule to ROS production during and in the absence of a parasitic attack. The best evidence for an involvement of UCPs in the control of the innate immune response comes from an elegant study by Arsenijevic et al. (2000). Using UCP2-deficient mice, these authors were able to show that resistance to the protozoan *Toxoplasma gondii* was tightly associated with the expression of the UCP2 gene. Experimental infections showed that *T. gondii* had a lethal effect on wild-type mice, with 100% of individuals dead by day 51 postinfection, whereas $UCP2^{-/-}$ individuals were still alive at day 80 postinfection. More important, the number of *T. gondii* cysts was three times higher in wild-type mice than in $UCP2^{-/-}$ individuals. But does this enhanced resistance to *T. gondii* stem from a more effective macrophage activity? Macrophages isolated from $UCP2^{-/-}$ mice were indeed more effective in killing *T. gondii* tachyzoites in vitro compared with macrophages of the wild type, and they also produced more ROS. Therefore, the ability of $UCP2^{-/-}$ mice to better resist infection by *T. gondii* seems to be related to the preactivated status of macrophages due to their enhanced production of mitochondrial ROS (Bai et al. 2005; S. Rousset et al., unpublished data). From an evolutionary perspective, these results might appear puzzling. Why should wild-type mice express the UCP2 gene if it confers a susceptibility to a lethal pathogen? The answer to this question might be that UCPs do protect host cells and molecules from the deleterious overproduction of ROS. We should, therefore, expect that UCPs might control ROS production so as to maximize the ratio between the benefits of ROS during an infectious process and the costs in terms of damage to the host structures. We might, for instance, expect a conditional expression of the UCP2 gene, depending on the infectious status, with an underexpression of the gene when ROSs are needed to fight off a parasitic insult. To our knowledge, these predictions have not been tested.

Multifunction proteins, such as UCPs, involved in the control of mitochondrial efficiency of energy production, ROS production, and macrophage immunity could also contribute to modulating the energetic cost of immune activation (Lochmiller and Deerenberg 2000) and may help in understanding the mechanisms linking immune activation and fitness (Norris and Evans 2000; Bonneaud et al. 2003). For example, the characterization of UCP ligands that can regulate the expression and the activity of these proteins might help us to understand whether vertebrates may control the level of their immune response, particularly during periods of intense energetic demands such as reproduction or cold stress. Future studies should extend the characterization of UCP expression in immune cells of nonmodel species. This work should focus not only on macrophages, since UCPs have also been found in T- and B-lymphocytes, but on dendritic cells as well (S. Rousset et al., personal communication).

UCPs and the Expression of Carotenoid-Based Sexual Traits

Carotenoids are naturally occurring pigments that are usually red, orange, or yellow, depending on their molecular arrangement (the number of double bonds in the long chain of the molecule; Krinsky and Yeum 2003). They can be deposited in skin and proteinaceous structures as feathers or bills. In addition to their impact on the expression of sexual ornaments (Alonso-Alvarez et al. 2004), carotenoids seem to be valuable for the organism because they also have an immunomodulatory function (Chew and Park 2004). For instance, Blount et al. (2003*a*) found that female zebra finches had a marked preference for males who had access to food supplemented with carotenoids and who expressed a redder bill color and a better immune response. Similarly, Faivre et al. (2003) showed that an immune insult significantly reduced the expression of a carotenoid-based sexual trait in the European blackbird *Turdus merula* compared with a control group. These results are, therefore, strongly suggestive of a role of carotenoid-based sexual traits as a signal of male health (Lozano 1994). Carotenoids and some of their metabolic derivatives also have antioxidant properties (Mortensen et al. 1997; Krinsky and Yeum 2003), and therefore it has been suggested that allocation to sexual traits also might inform a potential partner about the male's ability to resist an oxidative burst (von Schantz et al. 1999). Variable levels of ROS production can, therefore, profoundly affect the allocation rule of carotenoids to sexual traits and, as such, modulate mating success and reproductive value of males. Controlling ROS production at the source within the mitochondrial respiratory chain would therefore be a complementary way to ensure a sufficient allocation of carotenoids to sexual signals.

Carotenoids and their metabolic derivatives can also directly interfere with the uncoupling activity of UCPs because retinoic acid has been shown to be a potent activator of uncoupling activity of avianUCP in vitro (Criscuolo et al. 2005). This result is particularly interesting because, in addition to providing information on antioxidant status, carotenoid-based sexual traits might also indicate the ability to control mitochondrial ROS production.

Another connection between UCPs and sexual selection stems from a possible sex-specific expression of uncoupling proteins. Because of the maternal inheritance of mitochondrial DNA (mtDNA), male mitochondrial defects cannot be selected on directly (Rand et al. 2001, 2004). Therefore, sex-specific expression of UCPs may play a role in modulating male-specific mtDNA diseases and male-specific allocation of carotenoids to sexual traits.

Conclusion

Uncoupling proteins are key regulators of cellular functions such as mitochondrial respiration and ATP production efficiency, thermogenesis, and free radical production. As shown by mammalian knockout models, UCPs can alter glucose tolerance, ROS production, immune efficiency, and reproductive output. The pleiotropic function of these proteins might, therefore, confer to UCPs a keystone role in the mechanism underlying the expression of life-history traits. In addition, integrating UCPs into an ecological and evolutionary perspective will offer a good opportunity to establish a link between molecular bioenergetics and evolutionary biology (Watt 1985), a promising exchange of concepts for both disciplines.

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