

The role of mitochondrial uncoupling proteins in lifespan

Marcelo O. Dietrich · Tamas L. Horvath

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Abstract The increased longevity in modern societies raised the attention to biological interventions that could promote a healthy aging. Mitochondria are main organelles involved in the production of adenosine triphosphate (ATP), the energetic substrate for cellular biochemical reactions. The production of ATP occurs through the oxidative phosphorylation of intermediate substrates derived from the breakdown of lipids, sugars, and proteins. This process is coupled to production of oxygen reactive species (ROS) that in excess will have a deleterious role in cellular function. The damage promoted by ROS has been emphasized as one of the main processes involved in senescence. In the last decades, the discovery of specialized proteins in the mitochondrial inner membrane that promote the uncoupling of proton flux (named uncoupling proteins–UCPs) from the ATP synthase shed light on possible mechanisms implicated in the buffering of ROS and consequently in the process of aging. UCPs are responsible

for a physiological uncoupling that leads to decrease in ROS production inside the mitochondria. Thus, induction of uncoupling through UCPs could decrease the cellular damage that occurs during aging due to excess of ROS. This review will focus on the evidence supporting these mechanisms.

Keywords Aging · Mitochondria · Free radical · Oxidative phosphorylation · ATP

The last two centuries have seen an increase in longevity in Western civilizations, mainly due to advances in medicine. This has been accompanied by an increase in the incidence of chronic diseases related to growing older. Thus, understanding the mechanisms implicated in the aging process has become a significant component in the treatment of these age-related diseases. Diverse hypotheses have been proposed to elucidate the biological properties of aging. One important theory that attempts to explain the harmful events that occur during aging concerns the role of reactive oxygen species (ROS) in promoting cellular damage, including damage to DNA, proteins, and cellular membranes. These deleterious effects could accumulate over the years leading to cellular dysfunction, and eventually, death. The predominant organelle involved in the production of ROS, and in its elimination, is the mitochondria. Mitochondria are located in the cytosol of eukaryotic cells, and are also involved in the production of energy. Indeed, the presence of mitochondria in eukaryotic cells is essential for survival, and only a few subtypes of cells exist with minimal cellular functions that can survive without mitochondria-derived ATP production. Curiously enough, this organelle is responsible for the production of the most important substrate for cellular function, ATP, but also for the most harmful molecules, ROS. Thus, an appropriate balance between ATP production and ROS buffering is essential to maintain cellular homeostasis. This review will focus on the role of mitochondria uncoupling proteins (UCP), specifically

M. O. Dietrich · T. L. Horvath (✉)
Section of Comparative Medicine,
Yale University School of Medicine,
New Haven, CT 06520, USA
e-mail: tamas.horvath@yale.edu

M. O. Dietrich
Programa de Pós-graduação em Bioquímica,
Department of Biochemistry,
Universidade Federal do Rio Grande do Sul,
Porto Alegre, RS 90035, Brazil

T. L. Horvath
Departments of Obstetrics,
Gynecology and Reproductive Sciences,
Yale University School of Medicine,
New Haven, CT 06520, USA

T. L. Horvath
Neurobiology, Yale University School of Medicine,
New Haven, CT 06520, USA

UCP2, in the maintenance of this balance, emphasizing its role in the aging process.

Mitochondrial oxidative phosphorylation: the core of energy production

Metabolic intermediates derived from the breakdown of proteins, fatty acids, and sugars enter the mitochondria and are the substrates for the oxidative machinery to produce energy in the form of ATP. The oxidation of these substrates produces free energy, which is stored in special reduced carriers, such as flavin adenine dinucleotide (FADH₂) and nicotinamide adenine dinucleotide (NADH). These carriers will donate electrons to the mitochondrial respiratory complex located in the inner mitochondrial membrane. Complexes of enzymatic proteins, called complexes I, II, III, IV, and V compose the respiratory machinery. The donation of electrons from reduced carriers (NADH and FADH₂) to complexes I and II starts the process of pumping protons from the mitochondrial matrix into the mitochondrial intermembrane space, generating an electrochemical gradient, where the mitochondrial membrane potential ($\Delta\Psi_m$) is one of its components. Utilizing additional electron carriers, such as coenzyme Q and cytochrome C, complexes III and IV of the respiratory chain are activated and further increase the $\Delta\Psi_m$. The presence of protons in the intermembrane space together with the difference in membrane potential between this space and the mitochondrial matrix is the driving force for the conversion of adenosine diphosphate (ADP) to ATP by the ATP synthase (F_O-F₁ subunits, complex V). The phosphorylation of ADP to ATP is coupled to a flux of protons through the ATP synthase, which will pump the protons back to the mitochondrial matrix, reducing the difference in the mitochondrial membrane potential. The activity of the ATPase complex V is a rate-limiting step in the generation of ATP through mitochondrial respiration. Different metabolic conditions can variously modulate the coupling of mitochondrial respiration to the production of ATP. However, even in the best coupled condition, the machinery involved in the mitochondrial production of ATP is not perfectly efficient. Indeed, there are forms of dissipation of energy within the inner membrane of the mitochondria, for example, the “proton leak” which is not coupled to ATP production, and will decrease the efficiency of the respiratory complex.

Uncoupling the ATP synthase pump

The proton leak shuttles the protons from the intermembrane space back to the mitochondrial matrix reducing the

number of protons flowing through the ATP synthase. Since the protons located in the intermembrane space are derived from oxidative metabolism, the proton leak dissipates the energy from the oxidative substrates. There are several forms of proton leak, as for example the passage of protons through pores in the lipids of the mitochondrial membrane. Another form of “proton leak” in the inner mitochondrial membrane is carried by specialized uncoupling proteins [30]. UCPs are members of the superfamily of mitochondrial anion transporters that contains about 40 proteins, including different carriers and transporters. The key strategy of UCPs is to regulate the flux of protons through the ATP synthase. Indeed, the phosphorylation of ADP by ATP synthase is regulated by the levels of ATP, which can in turn inhibit mitochondrial respiration. Thus, uncoupling the proton flux is an adaptative pathway to avoid inhibition of the mitochondrial respiration.

Mitochondrial uncoupling proteins

The first UCP described was UCP1, which has been shown to control the brown adipose tissue (BAT)-regulated thermogenesis [39]. In the BAT, the proton leak through UCP1 is a tightly regulated mechanism that utilizes energy from the oxidation of intermediate substrates to generate heat. The BAT-regulated thermogenesis by UCP1 is important in several physiological conditions, for example, during cold exposure, arousal from hibernation, in newborns and in overfed mammals [15]. More recently, it has been shown to be functional in adult humans, as well [17, 52].

Our knowledge to date concerning the role of UCPs is based mostly on studies utilizing UCP1 as the classic model. Structurally, UCPs have a tripartite configuration that contains three repeats of approximately 100 amino acids. Each repeat has two hydrophobic regions that extend across the inner mitochondrial membrane corresponding to alpha helices. These two alpha helices are connected by a long hydrophilic loop oriented towards the matrix side of the inner mitochondrial membrane, whereas the amino and carboxyl termini extend into the intermembrane space [36]. The functional UCP is a homodimer [28] that, therefore, contains 12 alpha helices. This complex is believed to form a hydrophilic channel, the access of which is regulated by gates formed by the hydrophilic loops [3]. The presence of 12 alpha helices in the functional structure of UCPs is a common feature to most of the anion carriers and channel proteins, and unifies the UCP family, even though the amino acid structure of some UCPs has very low identity.

UCPs are expressed in a myriad of species, including mammals and plants. In mammals, five different UCP homologues have been described, UCP1-5, that contain

different levels of identity. Compared to UCP1, the archetypal UCP, UCP2 [22] and UCP3 [12] have high levels of amino acid identity (59% and 57%, respectively). UCP4 [34] and UCP5 [45] (also known as BMCP1) have low levels of identity (30% and 33%, respectively).

Distribution of UCPs

UCP1, the classic UCP, is expressed exclusively in the BAT. UCP2 is more broadly expressed [40, 41, 44], occurring in many tissues, including the pancreatic β -cells [54], cells of the immune system [4], and several neuronal populations in different brain nuclei [43]. In the brain, UCP2 is highly expressed in the hypothalamus in several nuclei important for the coordination of basic autonomic functions. Additionally, UCP2 is also expressed in high levels in the nucleus of the solitary tract (important for autonomic regulation), in neurons of the limbic system (important for higher cognitive tasks), and in dopaminergic neurons in the midbrain (which are impaired in Parkinson's disease) [18, 26, 41, 42, 53].

UCP3 is highly expressed in the skeletal muscle, and to a lesser extent in the heart and BAT [12, 24, 51]. Interestingly, in skeletal muscle, UCP3 is the only UCP that is expressed at the protein level [12]. Indeed, the expression of UCP homologues in different tissues is highly modulated at the posttranscriptional level, since in many cases the messenger RNA (mRNA) was identified, but the protein levels were not [41, 44, 46].

The role and distribution of the other two UCPs, UCP4, and UCP5, are more obscure. Their expression is more specific to the central nervous system, where both UCP4 [34] and UCP5 [27, 45] have a more widespread pattern of expression than UCP2. Further research to understand the basic aspects of these UCPs is warranted.

ROS production in the mitochondrial respiratory chain

The oxidative stress hypothesis to explain senescence proposes that reducing the production of reactive oxygen species within the mitochondria will concomitantly decrease their deleterious effects [8, 48]. Classically, complexes I and III have been described as the main producers of ROS (superoxide) in the mitochondria. Complex I seems to produce most of the superoxide, even though the relative contribution of other complexes to total superoxide production may vary greatly depending on the tissue analyzed [6, 7]. The high levels of superoxide produced by complex I utilizes reverse electron transport from the oxidation of succinate in complex II. Worthy of note is the observation

that superoxide is produced mainly on the matrix side of the inner membrane, and is unable to cross it. Thus, due to its highly deleterious effects, it is essential that mechanisms exist to detoxify superoxide in the mitochondrial matrix. The most important of these mechanisms is the dismutation of superoxide ($O_2^- \rightarrow O_2 + H_2O_2$), a process that occurs spontaneously. However, in biological tissues this process is not sufficiently fast enough to avoid its damaging effects to other molecules. In this scenario, the presence of superoxide dismutase (SOD) enzymes that accelerate this process is essential to maintain cellular homeostasis. In fact, the lack of either of the two major forms of SOD (SOD1 and SOD2) has a major impact on longevity in several organisms due to increased superoxide-induced cellular damage [31]. In addition to the production of superoxide in the respiratory chain, growing evidence indicates that other enzymes located within the mitochondria are capable of producing ROS and can play a role in the process of aging, as well [2, 50].

Thus, the development of strategies to decrease ROS production or damage is a key target for the study of senescence given the free radical theory. Copious amounts of research have been done in an attempt to elucidate the effect of various antioxidant agents against the damage promoted by ROS. This review will not analyze these sets of data, but will focus on the evidence that uncoupling the proton flux can minimize the production of superoxide, acting at the step before the damage occurs.

Interplay between superoxide and UCPs

The role of uncoupling in modulating the production of free radicals in the mitochondria was proposed a decade ago [13, 38, 47]. In the first report, Negre-Salvayre [38] and colleagues showed that the inhibitory effect of guanosine diphosphate (GDP) in mitochondrial uncoupling was accompanied by an increase in membrane potential and H_2O_2 production. The authors correlated their findings in tissues that showed low and high expressions of UCP1 or UCP2, demonstrating a lack of effect of GDP in tissues with low (or virtually absent) expression of both UCPs [38]. The authors concluded that inhibition of uncoupling by GDP was responsible for increasing the membrane potential due to the loss of proton leak, and it led to increased ROS production. Subsequently, in plant mitochondria, Kowaltowski et al. [29] corroborated these observations showing that activity of mitochondrial uncoupling proteins decreases membrane potential and inhibit the formation of ROS at the level of the semiquinone forms of coenzyme Q.

Years later, the effect of ROS to activate UCPs in mammalian cells was shown by research headed by Martin

Brand and colleagues. They revealed strong evidence that superoxide (or one of its related metabolites) was capable of activating the proton conductance of UCPs on the matrix side of the inner mitochondrial membrane [20]. The superoxide-induced uncoupling required fatty acids and was inhibited by purine nucleotides [19]. The mechanism by which superoxide activates UCPs is still not completely understood, but accumulating evidence suggests that lipid peroxidation by ROS generates reactive alkenals which will, in turn, activate UCPs [20, 37]. These observations suggest that the interaction between ROS and UCPs functions as a mechanism to decrease levels of free radical concentrations inside the mitochondria and to reduce the consequent deleterious effects.

The role of UCPs in senescence

The studies highlighted above have immediate implications in several medical fields that postulate a role for mitochondrial free radical production as an underlying cause of many pathological processes and aging. Thus, a description of the putative cascade of events by which UCPs could regulate ROS production and damage inside the mitochondria can be drawn. This cascade of events could unfold as follows. An increase in cell metabolism due to high energy demand generates a concomitant increase in metabolic intermediates. These intermediates are used inside the mitochondria for oxidative phosphorylation of ADP to produce ATP. This process occurs through the mitochondrial respiratory chain in the inner membrane of the mitochondria and also generates superoxide, mainly by complexes I and III. The flux of electrons through the complexes in the electron transport chain is coupled to a flux of protons from the mitochondrial matrix to the intermembrane space, thereby increasing the difference in membrane potential. This difference in membrane potential is the driving force for ATPase to generate ATP from ADP + Pi. The superoxide radicals produced in complexes I and III cause local lipid peroxidation in the inner mitochondrial membrane. This peroxidation generates aldehydic lipid peroxidation intermediates that act directly upon UCPs (2 and 3) to stimulate proton leak. Additionally, other molecules such as fatty acids (and other endogenous activators) promote a mild uncoupling activity. This uncoupling of proton flux through the ATPase decreases the difference in membrane potential, thereby reducing the rate of ATP formation and decreasing the production of superoxide in complexes I and III. However, the presence of uncoupling activity promotes an ideal milieu for constant ATP production, since in the absence of uncoupling, high ATP production due to increased differences in membrane potential can cause inhibition of mitochondrial respiration. Additionally, high

levels of oxidative phosphorylation can cause increases in superoxide formation, leading to undesired damage to the mitochondrial membrane, DNA and protein complexes. Also relevant to note, the mild uncoupling activity that is taken into account in this model differs from a high (almost complete) uncoupling activity that would halt the proton flux through the ATP synthase, and consequently the production of ATP.

Several lines of evidence suggest that the cascade of events outlined above are involved in aging, and that uncoupling the proton flux through UCPs (mainly UCP2) is a critical pathway in the regulation of senescence. This idea was first raised by Martin Brand [13] and was called the “uncoupling-to-survive” hypothesis of aging. In support of this idea, studies indicated that a stronger resistance to oxidative damage by ROS was positively correlated with enhanced longevity in several species [21, 32], including mammals [25]. In 2004, another correlative study published by Speakman et al. [49] found that mice in the upper quartile of metabolic intensity (measured by food assimilation divided by body weight) lived longer, had higher resting oxygen consumption, and higher mitochondrial uncoupling when compared to mice in the lowest quartile. Even though this study was based purely on correlations, it provided the first experimental evidence in support of the role of mitochondrial uncoupling in extending lifespan. Subsequent work has shown that calorie restriction, the only known intervention that reliably increases lifespan [11], induces an increase in the expression of both mRNA and protein levels of UCP2 and UCP3 in mice [9, 10, 35]. When Swiss albino mice were treated with low doses of the uncoupling agent 3,4-dinitrophenol, they exhibited a phenotype that resembled that of calorie restriction: decreased ROS production, reduced oxidative damage, improved metabolic parameters, and increased longevity [14].

The first attempt to study the direct effects of UCPs in senescence came in 2005 by Fridell and collaborators [23]. In this work, the authors overexpressed the human UCP2 (hUCP2) in adult fly neurons, and found an extension of lifespan in both male and female flies. In these transgenic flies, they found an increase in state 4 respiration (the respiration that occurs in the absence of ADP due to proton leak), and a decrease in ROS production and oxidative damage. Additionally, the long-lived hUCP2 flies showed no changes in physical activity or reproduction [23], thus, emphasizing the specificity of the transgenic fly in enhancing lifespan. In 2006, another elegant study by Conti et al. [16] showed the effect of selective overexpression of UCP2 in neurons and its role in longevity. The authors generated a transgenic mouse that overexpressed UCP2 selectively in the hypocretin neurons (Hcr-UCP2) of the lateral hypothalamus (LH). They found that these mice have reduced body temperature and increased lifespan

compared to controls, an effect that is similar to that seen during calorie restriction. The authors provided evidence that this effect was due to elevated temperature in the central thermostat, which is located in a region adjacent to the LH [16].

Altogether, these data strongly implicate UCPs (mainly UCP2) in playing a role in the regulation of lifespan and confirms the “uncoupling-to-survive” hypothesis. However, in 2008 and 2009, three additional studies came out and provided more detailed data on the effects of UCP2 and UCP3 with regard to mammalian longevity. In an elegant study, Andrews and Horvath [1] showed that in rodents (rats and mice) mitochondrial uncoupling-induced (measured as the ratio between free fatty acids) respiration, and state 4 respiration increased during aging, an effect that was accompanied by increased levels of ROS and lipid peroxidation. When comparing both species (mice vs rats), the authors found that rats had significantly lower levels of mitochondrial uncoupling, ROS production, and lipid peroxidation. They correlated these results with the differences in longevity between rats and mice. In the same report, the authors studied the effect of UCP2 manipulations on the lifespan of mice utilizing different genetic approaches. First, wild-type mice were compared to UCP2 knockout mice (UCP2^{-/-}). In this model, the UCP2^{-/-} mice had a significantly shorter survival age. In another experiment, mice that overexpressed hUCP2 (hUCP2-Tg) were compared to their wild-type controls. In this set of data, the authors reported that hUCP2-Tg mice showed a delayed time of the first death, even though they had the same survival age as their controls. Subsequently, the authors backcrossed the UCP2 mutants with mice knocked out for superoxide dismutase 2 (SOD2). SOD2^{-/-} mice have a severe phenotype, living no longer than 3 weeks [31]. The double knockout mice (SOD2^{-/-}; UCP2^{-/-}) had a significantly reduced lifespan when compared to SOD2^{-/-}; UCP2 wild-type mice. The crossing of SOD2^{-/-} with hUCP2-Tg mice increased the survival age compared to wild-type controls.

Two other studies lead by McDonald and collaborators [5, 35] elicited some contradictions on the topic of UCP2–3 and longevity. In these reports, the authors analyzed the phenotype of transgenic mice overexpressing UCP2/3, and also UCP2^{-/-} and UCP3^{-/-} mice. Overall, the authors reported that UCP3^{-/-} mice presented minimal or no effects on their survival rate. When both UCP2/3 were overexpressed, the transgenic mice showed a slight increase in mean survival. However, the most conflicting result of these reports was the finding that UCP2^{-/-} mice showed no difference in lifespan when compared to their wild-type littermates. This is in total opposition to what was found by Andrews and Horvath [1], and may be due to slight differences in their protocols [33].

Concluding remarks

In the last few years, growing evidence has been established to corroborate the theory of “uncoupling-to-survive,” in which mitochondrial uncoupling is a key mechanism involved in the production and buffering of ROS and their associated damage. The major UCP involved in these processes seems to be UCP2, which is widely distributed throughout many tissues including the brain. In addition to the mechanisms highlighted in this review, there are other putative roles for the UCPs, such as the regulation of fatty acid metabolism, which will likely be implicated in the mechanisms of aging as well. Other functions of UCPs, including calcium transport, have been targets of intense debate, and much more investigation will be necessary to draw credible conclusions about the importance of UCPs in these processes.

Even though research in the field of mitochondrial uncoupling is progressing quickly and strong evidence exists to suggest a role of UCP manipulation in treating several pathological states, no good strategies have been put forward to date to be able to pharmacologically treat patients with specific and potent compounds. Thus, this remains a hot topic with more research needed that in all likelihood will provide significant advances in our understanding of several physiological and pathological states.

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