

Uncoupling to survive? The role of mitochondrial inefficiency in ageing

M.D. Brand*

MRC Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 2XY, UK

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Abstract

Mitochondria are incompletely coupled, and during oxidative phosphorylation some of the redox energy in substrates is lost as heat. Incomplete coupling is mostly due to a natural leak of protons across the mitochondrial inner membrane. In rat hepatocytes the futile cycle of proton pumping and proton leak is responsible for 20–25% of respiration; in perfused rat muscle the value is 35–50%. Mitochondrial proton cycling is estimated to cause 20–25% of basal metabolic rate in rats. Proton cycling is equally prominent in hepatocytes from several different mammalian and ectotherm species, so it may be a general pathway of ecologically significant energy loss in all aerobes. Because it occurs in ectotherms, thermogenesis cannot be its primary function. Instead, an attractive candidate for the function of the universal and expensive energy-dissipating proton cycle is to decrease the production of superoxide and other reactive oxygen species (ROS). This could be important in helping to minimise oxidative damage to DNA and in slowing ageing. Mitochondria are the major source of cellular ROS, and increased mitochondrial proton conductance leads to oxidation of ubiquinone and decreased ROS production in isolated mitochondria. However, to date there is no direct evidence in cells or organisms that mitochondrial proton cycling lowers ROS production or oxidative damage or that it increases lifespan. © 2000 Elsevier Science Inc. All rights reserved.

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1. Oxidative phosphorylation

A fundamental property of living organisms is that they harness energy from their environment to drive thermodynamically unfavourable processes involving production of order and complexity. Such processes include maintenance, growth and reproduction.

* Tel.: +44-1223-252800; fax: +44-1223-252805.

E-mail address: martin.brand@mrc-dunn.cam.ac.uk (M.D. Brand).

Perhaps the best way to release energy at constant temperature and pressure is to allow electrons to flow from reductants to oxidants, and one of the earliest inventions of life was the use of such redox reactions in photosynthesis and metabolism. Electron transport chains that catalyse these redox reactions appeared very early in evolutionary history, and had advanced to a sophisticated level before the last common ancestor of present-day life forms. This is inferred from the observation that a homologous set of molecules, protein structures, reaction mechanisms and reaction pathways (including FeS centres, cytochromes, quinones and Q cycles) underlies energy metabolism in virtually all modern organisms. These basic mechanisms were, therefore, in place before oxygenic photosynthesis evolved, so they pre-date the oxidising atmosphere that dominates the biosphere today (Castresana and Saraste, 1995).

Atmospheric oxygen became available at high concentrations about two billion years ago when photosynthetic organisms evolved the ability to photolyse water and inorganic sinks for the liberated oxygen, such as oceanic iron, were exhausted. Non-photosynthetic organisms were then able to pass electrons to oxygen rather than to weaker acceptors like H_2S , organic acids and nitrate. They were able to extract much more energy from sugars and other reduced substrates than their anaerobic competitors. This benefit was accompanied by the problems of coping with the corrosive and reactive oxygen molecule, to prevent unwanted oxidation reactions. Life's transition to an aerobic atmosphere must have involved extensive redesign of structures and pathways. In this article I will examine the idea that a record of these events two billion years ago is preserved in a modern compromise that has been reached between the need for high efficiency of oxidative phosphorylation and the need to decrease the damaging and ageing effects of reactive oxygen species (ROS).

In eukaryotic mitochondria, electrons are fed into the electron transport chain from reduced substrates (such as glycerol phosphate, fatty acids, NADH or succinate). Large membrane-bound enzymes, such as glycerol phosphate dehydrogenase, the dehydrogenases of β -oxidation, NADH-Q oxidoreductase (Complex I) or succinate-Q oxidoreductase (Complex II) pass electrons down the gradient of redox potential to the mobile lipid-soluble carrier, ubiquinone (Q). From Q, the electrons pass down through Q-cytochrome *c* oxidoreductase (Complex III), cytochrome *c* (a second, water-soluble, mobile carrier) and cytochrome *c* oxidase (Complex IV) to the final acceptor, oxygen. Coupling of this series of energy-releasing oxidation reactions to the energy-demanding reactions of ATP synthesis (i.e. oxidative phosphorylation), is accomplished by a chemiosmotic mechanism. As electrons flow down their chemical gradient, Complex I, Complex III and Complex IV pump protons from the mitochondrial matrix to the intermembrane space, against their electrochemical gradient. This active proton pumping sets up a protonmotive force, which consists mostly of an electrical gradient (membrane potential), accompanied by a small chemical gradient (pH difference). The protonmotive force then drives protons back into the matrix through the mitochondrial ATP synthase, resulting in ATP synthesis. The resulting chemiosmotic proton circuit (the lower circuit in Fig. 1) is the mechanism of oxidative phosphorylation.

One of the most notable early successes of the chemiosmotic theory was its explanation of the action of uncoupling agents, which abolish the link between oxidation and phosphorylation, allowing electron transport to proceed without coupled ATP synthesis.

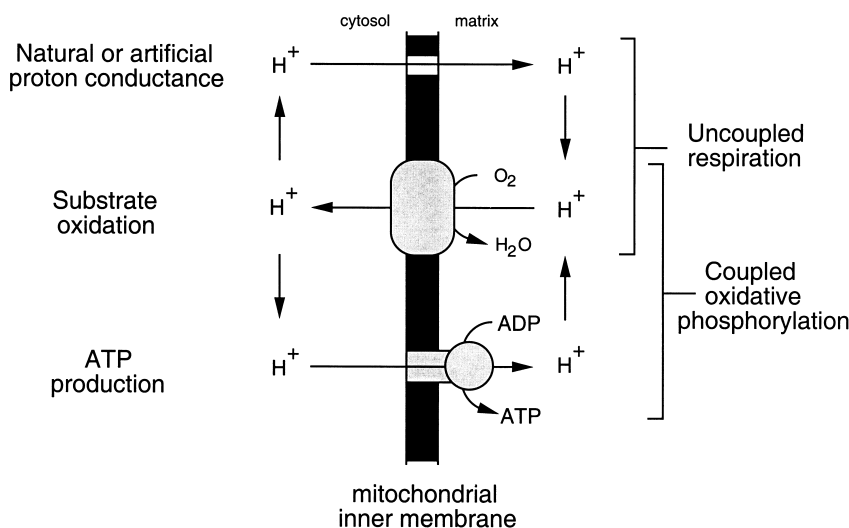


Fig. 1. The chemiosmotic proton circuit in the mitochondrial inner membrane. The lower circuit, consisting of substrate oxidation and the enzymes of ATP production, results in coupled oxidative phosphorylation. The upper circuit, consisting of substrate oxidation and natural or artificial proton conductance pathways, results in uncoupled respiration.

Uncoupling agents like dinitrophenol (DNP) are lipid-soluble weak acids that can cross the mitochondrial membrane in either the protonated or the unprotonated state. This sets up a catalytic cycle that dissipates the protonmotive force and so allows substrate oxidation to proceed without providing the driving force for coupled ATP synthesis (the upper circuit in Fig. 1).

2. The mitochondrial proton leak

Even in the absence of artificial uncoupling agents, there is a finite proton conductance of the inner membrane of isolated mitochondria (Nicholls, 1974; Brand, 1990; Brand et al., 1994; Brand et al., 1999; Rolfe and Brand, 1997; Stuart et al., 1999). This conductance allows a leak of protons back into the matrix, and so results in oxidative phosphorylation being less than fully coupled (i.e. simultaneous operation of both circuits in Fig. 1). For many years this endogenous uncoupling was assumed to be an artefact of mitochondrial isolation, but it is now clear that it is a natural pathway that operates in intact cells and tissues, such as hepatocytes, thymocytes and intact skeletal muscle (Nobes et al., 1990; Rolfe and Brand, 1996; Rolfe et al., 1999).

The proportion of respiration that is used to drive the energy-dissipating futile proton cycle across the mitochondrial inner membrane in rat hepatocytes is surprisingly high; measured as 20% in active hepatocytes and 25% in resting hepatocytes. In perfused rat muscle it is even greater, measured as 35% in contracting preparations and 50% in resting muscle. The sum of the proton cycling rates in different organs of the rat

suggests that 20–25% of its basal metabolic rate may be devoted entirely to driving this futile cycle (Table 1). Although the proton conductance of liver mitochondria isolated from mammals varies with body mass (Porter and Brand, 1993), the proportion of respiration of mammalian hepatocytes that is devoted to proton leak is remarkably constant at about 20% in species of widely different body mass ranging from mouse to horse (Porter and Brand, 1995). Although there is information on only a small number of species, proton cycling appears to be equally prominent in hepatocytes from several different ectotherms, including reptiles, amphibians and molluscs (Table 1). Thus, a high mitochondrial proton cycling rate that accounts for a significant proportion of hepatocyte respiration rate is characteristic of all species that have been investigated, so it may be a general phenomenon. There is no reason to expect that other tissues do not follow the same pattern as liver, so it is reasonable to speculate that the mitochondrial proton cycle makes up an important part of basal metabolic rate in all animals, as it does in rat. If so, it may be a general pathway of ecologically significant energy loss. These considerations suggest that the high energetic cost of running the mitochondrial futile proton cycle must be offset by some outcome of high benefit to a great variety of organisms, both endotherms and ectotherms.

The mechanism of the basal mitochondrial proton conductance is unclear. Membranes made from pure mitochondrial phospholipids have a proton conductance with the same properties as the proton conductance of intact mitochondria. However, the phospholipid liposomes are several-fold less proton permeable than native mitochondria, and proton conductance is the same in liposomes prepared from mitochondria with very different endogenous proton conductances, so it appears that simple diffusion through bulk regions of the membrane bilayer is not a sufficient explanation of proton conductance (Brookes et al., 1998). Other components of the mitochondrial membrane must be involved. There could be non-specific effects of membrane proteins, catalysis by several proteins such as the fatty acid-stimulated proton conductance of the adenine nucleotide carrier (Andreyev et al., 1989), or specific catalysis by proteins yet to be identified. In addition to the basal proton conductance discussed above, mitochondria have inducible proton conductance catalysed (in brown adipose tissue only) by uncoupling protein 1 (UCP1). Whether other UCP1 homologues, such as UCP2 and UCP3, are responsible for basal or inducible proton

Table 1
Percentage of respiration that drives mitochondrial proton leak in cells and tissues from different organisms

System	% of respiration	Reference
Rat hepatocytes	20–26	Nobes et al. (1990); Rolfe et al. (1999)
Rat muscle	35–50	Rolfe and Brand (1996); Rolfe et al. (1999)
Rat BMR	20–25	Rolfe and Brand (1996); Rolfe et al. (1999)
Mammal hepatocytes (mouse, ferret, sheep, pig, horse)	About 20	Porter and Brand (1995)
Lizard hepatocytes	Up to 30	Brand et al. (1991)
Frog hepatocytes	Up to 20–25	Brand et al. (2000)
Snail hepatopancreas cells	Up to 15–25	Brand et al. (2000)

conductance in other tissues is controversial and has yet to be established (see Brand et al., 1999; Stuart et al., 1999; Ricquier and Bouillaud, 2000).

3. A possible function of the mitochondrial proton leak in ageing

Several possible functions of mitochondrial proton leak have been suggested (Rolfe and Brand, 1997). Because the mitochondrial proton cycle appears to be such an important energy drain in such a wide range of organisms, the function (or functions) must be so important that organisms are prepared to pay a very high energetic price to maintain it. Suggested functions include thermogenesis, an improved ability to regulate energy metabolism, a safety valve for avoidance of dielectric breakdown of the membrane at excessive membrane potentials, an ability to continue carbon metabolism when ATP demand is low, regulation of body mass and the attenuation of free radical production. Clearly, the mitochondrial proton cycle is a thermogenic mechanism, because it stimulates respiration without energy conservation, and indeed the inducible proton leak catalysed by UCP1 in brown adipose tissue mitochondria is used for this purpose. However, the basal proton cycle occurs in cells from ectotherms as well as endotherms (Table 1), so thermogenesis cannot be its primary function in all species. Although the proton cycle could achieve the other suggested functions, most of these functions do not seem to be so important to organisms that they merit the huge ecological costs implied by the high proportion of basal metabolic rate that is devoted to the proton cycle. Of the suggested functions, only the attenuation of free radical production and the associated protection against cellular degeneration and ageing appears important enough to warrant the energetic costs that the proton leak imposes.

According to the free radical theory of ageing (see Beckman and Ames, 1998), the processes of oxidative metabolism in aerobic cells are accompanied by the reduction of oxygen to superoxide and other ROS such as hydrogen peroxide and hydroxyl radicals. ROS cause damage to cellular components, particularly nuclear and mitochondrial DNA, and this leads to impaired function and increased somatic mutation and hence to degeneration and ageing. Mitochondria are the main source of cellular ROS (Boveris and Chance, 1973; Nohl, 1994) because of an electron leak from ubiquinone ($\text{QH}\cdot$) or other carriers in the electron transport chain (Boveris et al., 1976). $\text{QH}\cdot$ produced during turnover of Complex I (Hansford et al., 1997) and Complex III (Nohl et al., 1996; Herrero and Barja, 1998) reacts with molecular oxygen to produce superoxide, which in turn produces other ROS. Because it is produced at the mitochondrial inner membrane, mitochondrial DNA is a major target of ROS-induced damage, and mitochondrial function may be an early casualty of the degenerative process. Evidence that this ROS production is related to ageing includes observations that ROS production is higher in mitochondria from animals with shorter maximum lifespan (see Herrero and Barja, 1998).

Cells have powerful antioxidant defences to protect themselves against damage by ROS, and most of the attention in this field has been focused on these antioxidants. Much less attention has been paid to the regulation of ROS production, despite the fact that prevention, rather than cure, would appear to be a more logical way to decrease oxidative damage. What determines the rate of mitochondrial ROS production? Agents

that increase respiration rate, such as uncouplers (which stimulate the upper circuit in Fig. 1) or ADP (which stimulates the lower circuit in Fig. 1) have long been known to decrease ROS production by isolated mitochondria (Boveris and Chance, 1973). However, inhibitors of electron transport can either increase or decrease ROS production depending on their site of action, so ROS production is not linearly related to the rate of electron transport. It does, however, show a steep dependence on the magnitude of the protonmotive force in isolated mitochondria (Liu, 1997; Korshunov et al., 1997). Uncouplers and ATP synthesis lower protonmotive force by increasing its consumption. The protonmotive force would affect ROS production by altering the redox state of Q; at high protonmotive force respiration slows, so electrons would accumulate on Q instead of passing down the electron transport chain to oxygen. This would increase the steady-state concentration of $\text{QH}\cdot$ and so increase the rate of mitochondrial ROS production. Inhibitors of electron transport lower protonmotive force, but they also affect the redox state of the Q pool: those that are expected to increase the oxidation of the Q pool decrease ROS production and those that are expected to increase the reduction of the Q pool increase ROS production. Thus the concentration of $\text{QH}\cdot$ in the electron transport chain is an important determinant of ROS production.

These observations suggest a role for the endogenous mitochondrial proton conductance in protecting against ROS production and ageing (Skulachev, 1996). Partial uncoupling by the basal proton leak will tend to lower the protonmotive force, so it will lead to a more oxidised Q pool and a lower concentration of $\text{QH}\cdot$. It will also tend to increase the oxygen consumption rate, so it will lower the oxygen tension around the mitochondria. Because superoxide production is a second order reaction, dependent on both $[\text{QH}\cdot]$ and $[\text{O}_2]$, the lower concentrations of $\text{QH}\cdot$ and oxygen will lead to a lower rate of ROS production and protection against oxidative damage. The same benefits could be achieved by alternative oxidase pathways that do not have coupled proton pumping: this mechanism is found in plant mitochondria and it is not clear why one method might be preferred over the other.

This hypothesis that mitochondrial proton leak functions primarily to protect against ROS production is attractive, and would explain the benefits of high endogenous proton leak rates in cells and organisms, despite the associated high energetic costs. The role of the antioxidant defences would then be to mop up residual ROS that escaped the primary control. However, all of the evidence in favour of the hypothesis has been obtained with isolated mitochondria, often under quite contrived conditions of excess electron supply, inhibition of Q oxidation, and depletion of endogenous antioxidants. There is no direct evidence in either cells or organisms that mitochondrial proton cycling lowers ROS production, that it decreases oxidative damage or ageing or that it increases maximum lifespan. Several laboratories, including my own, are currently engaged in a search for evidence that supports or refutes the hypothesis.

4. Why has evolution allowed mitochondrial ROS production to persist?

The answer to this question is not known, but we can speculate. Assume that the free radical theory of ageing is at least partially correct, and that lowering of ROS production to protect against oxidative damage is an important function of mitochondrial proton leak

and the cause of a substantial proportion of basal metabolic rate. With these assumptions, we would expect there to be strong evolutionary pressures to decrease basal metabolic rate and increase efficiency by minimising mitochondrial ROS production. Of course, it may be that there is value in a low rate of endogenous ROS production, leading to an enhanced mutation rate and increased evolutionary potential. Equally, ageing and death may not suit individuals, but they may be valuable to the species. But in either case, it would still seem better to achieve these objectives at lower energy cost by modifying the electron transport machinery to lower mitochondrial ROS production so that less uncoupling is required to achieve the same effect.

Is mitochondrial ROS production an inevitable consequence of aerobic energy transformations? As argued above, electron transport chains utilising $\text{QH}\cdot$ as an essential intermediate in the Q cycle may have evolved in a reducing atmosphere. Perhaps the basic principle of using ubiquinone as a component of the electron transport chain is flawed in an aerobic environment because it cannot be prevented from producing ROS, and ROS production is therefore an inevitable consequence of oxidative phosphorylation. The mechanism of Q reduction by Complex I is not known, but the mechanism and structure of Complex III are well understood (Trumpower, 1990; Xia et al., 1997; Saraste, 1999) and can be used as a basis for discussion. Are there features of its design that cannot be changed to prevent reaction of oxygen with $\text{QH}\cdot$ and so prevent superoxide production? The central function of Complex III is the pumping of protons, which it achieves by a Q-cycle mechanism (Fig. 2). QH_2 passes an electron to the Rieske FeS protein at centre 'o' to form $\text{QH}\cdot$. The second electron on $\text{QH}\cdot$ is passed to a different acceptor, haem b_L , and Q is released. This electron distribution is an integral part of the proton-pumping mechanism, and requires QH_2 and Q from the membrane pool to have access to centre "o". The crystal structure shows cavities that allow the access of ubiquinone, and also hydrophilic channels that allow proton ejection from centre "o" to the aqueous phase. Since oxygen is very lipid-soluble, it is hard to see how access for ubiquinone and protons could be retained if oxygen was to be excluded, so it appears that oxygen cannot be excluded from the active site. To ensure the correct distribution of electrons to the two different acceptors, the headgroup of the FeS protein appears to be hinged so that it moves 2 nm away from the Q-binding site in centre "o" after it accepts the first electron, forcing the second electron, held on the $\text{QH}\cdot$ now exposed within the site, to pass to haem b_L . It may be that evolution has not found a way to retain this essential electron distribution and proton pumping function of Complex III without at the same time allowing the oxygen access to the $\text{QH}\cdot$, with resultant superoxide and ROS production.

An alternative way of decreasing ROS production by mitochondria would be to restrict the supply of electrons to the electron transport chain, keeping the ubiquinone pool relatively oxidised and the steady-state concentration of $\text{QH}\cdot$ low. For example, the NADH/NAD ratio could be kept relatively oxidised. Mechanisms that could achieve this are well known: the flow of carbon to the mitochondria from catabolism is regulated by hormones and by ATP demand, and the dehydrogenases of the citric acid cycle are controlled by the matrix calcium concentration. However, cells and tissues appear to be set up to maintain a high mitochondrial protonmotive force (and a relatively high Q reduction state), with a high proton leak rate even when ATP synthesis rate is low

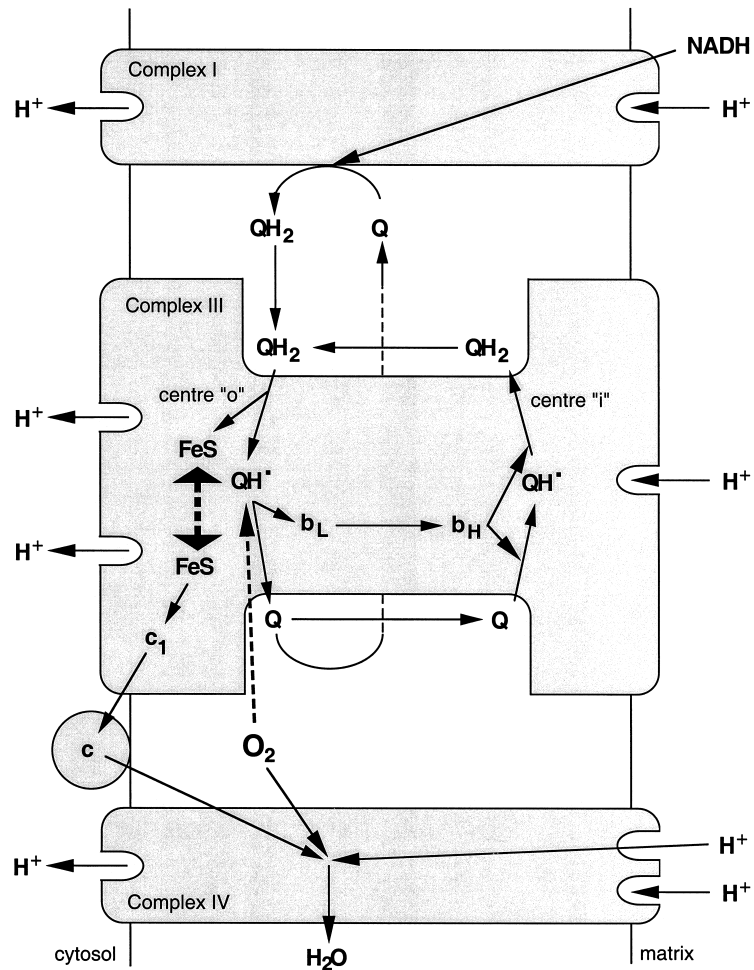


Fig. 2. The Q cycle. See the text for an explanation.

(Nobes et al., 1990; Rolfe et al., 1999). Nevertheless, it is interesting that the proton-motive force may actually rise slightly when resting rat muscle is stimulated to contract (Rolfe et al., 1999). It may be that electron supply needs to be kept high so that proton-motive force and the phosphorylation potential of ATP are also kept high under resting conditions. Essential ATP-consuming reactions continue in the resting state, in order to maintain protein turnover and plasma membrane ion gradients. A high phosphorylation potential would allow them to proceed uncompromised by any decrease in the chemical potential of their energy supply. However, we can speculate that the protective effect of caloric restriction against ageing does have a component due to a relatively oxidised ubiquinone pool as electron supply is restricted but proton leak continues, leading to a lower ROS production rate.

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