

# The efficiency and plasticity of mitochondrial energy transduction

M.D. Brand<sup>1</sup>

MRC Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 2XY, U.K.



## Keilin Memorial Lecture

Delivered at the SECC, Glasgow,  
on 20 July 2005

Martin Brand

## Abstract

Since it was first realized that biological energy transduction involves oxygen and ATP, opinions about the amount of ATP made per oxygen consumed have continually evolved. The coupling efficiency is crucial because it constrains mechanistic models of the electron-transport chain and ATP synthase, and underpins the physiology and ecology of how organisms prosper in a thermodynamically hostile environment. Mechanistically, we have a good model of proton pumping by complex III of the electron-transport chain and a reasonable understanding of complex IV and the ATP synthase, but remain ignorant about complex I. Energy transduction is plastic: coupling efficiency can vary. Whether this occurs physiologically by molecular slipping in the proton pumps remains controversial. However, the membrane clearly leaks protons, decreasing the energy funnelled into ATP synthesis. Up to 20% of the basal metabolic rate may be used to drive this basal leak. In addition, UCP1 (uncoupling protein 1) is used in specialized tissues to uncouple oxidative phosphorylation, causing adaptive thermogenesis. Other UCPs can also uncouple, but are tightly regulated; they may function to decrease coupling efficiency and so attenuate mitochondrial radical production. UCPs may also integrate inputs from different fuels in pancreatic  $\beta$ -cells and modulate

insulin secretion. They are exciting potential targets for treatment of obesity, cachexia, aging and diabetes.

## Why should we care how much ATP mitochondria make?

How many ATP molecules can mammalian mitochondria make from ADP for each oxygen atom they consume during substrate oxidation and oxidative phosphorylation? This number is known as the P/O ratio. In the context of the chemiosmotic theory, this question can be broken down into two smaller ones: how many protons do mitochondria pump out for each oxygen atom consumed (the H<sup>+</sup>/O ratio) or electron pair transferred from a electron donor to an acceptor (the H<sup>+</sup>/2e ratio), and how many protons must flow back to the matrix for each ADP molecule phosphorylated to ATP (the H<sup>+</sup>/ATP ratio)? These may be simple questions, but attempts to answer them go back to the roots of bioenergetics and biochemistry. Although much progress has been made, we still do not have the final answers.

Much of an animal's physiology and ecology is driven by its energetics, and animal life is dominated by the processes of acquiring nutrients from plants or other animals, ensuring a suitable supply of oxygen to oxidize them, and making ATP in sufficient quantities and rates to survive, reproduce and keep ahead of competitors. The P/O ratio is very important in these relationships: most biochemical processes consume a fixed amount of ATP per cycle, so the value of the P/O ratio affects how much oxygen is needed to run such processes. If we obtained a little more or a little less ATP per oxygen, then we might alter the constraints under which our lungs, heart and circulation, digestive system and muscles labour. In terms of one of the major pathologies of westernized humans, if we could make less ATP for a given nutrient intake, the danger of obesity would be less. Similarly, the ecology of animals is intimately tied in to their energy budgets: it is no accident that energy-efficient reptiles outnumber energy-expensive mammals in hot desert environments.

What determines the P/O ratios (and the H<sup>+</sup>/O and H<sup>+</sup>/ATP ratios) that biology uses? P/O ratios could, in principle, have any value, just as the gears on a bicycle can be set to any gearing ratio. The trade-off is the same: if you make large amounts of ATP at high P/O ratio, there is only a fixed amount of power available from substrate oxidation, so you obtain it at very low phosphorylation potential. You can

**Key words:** mitochondrion, oxidative phosphorylation, P/O ratio, proton leak, uncoupling protein (UCP).

**Abbreviations used:** ANT, adenine nucleotide translocase; H<sup>+</sup>/O ratio, H<sup>+</sup>/2e ratio, H<sup>+</sup>/ATP ratio, number of protons translocated across the mitochondrial inner membrane for each oxygen atom reduced to water, pair of electrons transferred from donor to acceptor, or ATP synthesized; P/O ratio, number of ATP molecules made by mitochondria from ADP for each oxygen atom consumed during substrate oxidation and oxidative phosphorylation; ROS, reactive oxygen species; UCP, uncoupling protein.

<sup>1</sup>email martin.brand@mrc-dunn.cam.ac.uk

cycle further and faster in high gear. Conversely, if biology operated at very small P/O ratios, not much ATP would be made, but it could be delivered at very high potential. You can cycle up steep hills in low gear. Evolution will have favoured P/O ratios that deliver ATP at a phosphorylation potential appropriate to the reactions that it drives. The second aspect of the problem is the mechanistic constraints. Very large P/O ratios are difficult to achieve because the energy needs to be divided up into tiny packets before ATP is made (this is, of course, what metabolism achieves when glucose is burnt, and the energy is released in relatively small packets rather than all at once as it is in simple combustion). To get energy from glucose oxidation in still smaller packets would require more complex chemistry and biochemistry, which will limit the attainment of very large P/O ratios. Very small P/O ratios are difficult to achieve because the energy needs to be accumulated in a large reservoir before being used to make a small amount of ATP. To accumulate the energy from the oxidation of several glucose molecules to make a single ATP would also require complex machinery and energy pools at high potential that might be hard to maintain.

Knowing the values of P/O, H<sup>+</sup>/O and H<sup>+</sup>/ATP ratios is crucially important to quantitative biological energetics. There are still many studies in which the yield of ATP is calculated from the oxygen consumed, often with a simple (but incorrect) assumption of a P/O ratio of 3, or 2.5. The values are also crucial for mechanistic bioenergetics, to set the constraints for, and to help unravel, the mechanisms of the redox proton pumps and the ATP synthase. A central requirement of any proposed mechanism of proton pumping by complex I of the electron-transport chain, for example, is that it translocates the correct number of protons across the membrane.

### What is the maximum amount of ATP made by mitochondria per oxygen atom consumed?

The history of this question has been dogged by the implicit assumption that the answer should be a small integer. We now know that this is not so, but we may still be subject to this alluring expectation. Integral coupling stoichiometries would be expected if the coupling mechanism involved simple one-to-one chemical reactions, so P/O ratios might be 1, 2 or 3. This is the historical sequence: an initial assumption that one ATP would be made per oxidation reaction as there is in substrate-linked glycolytic oxidation reactions, followed by empirical discovery that more than one ATP (and later more than two ATPs) is made per oxygen, suggesting a sequential chain of ATP synthesis reactions. The outmoded textbook result of three coupling sites for ATP synthesis and a P/O ratio of 3 is based on this implicit assumption of integral stoichiometries (reviewed in [1–3]). A big advantage of these old-fashioned integral coupling ratios is that they are conceptually simple and very easy to remember, which may partly explain their tenacity in the face of overwhelming empirical evidence that they are wrong. A more modern

view [2,3] takes into account the chemiosmotic theory, and expects the P/O ratio to be a function of other small integers; the H<sup>+</sup>/O ratio that describes the formation of the protonmotive force, divided by the H<sup>+</sup>/ATP ratio that describes its consumption. Other linked reactions that also translocate protons during oxidative phosphorylation from physiological substrates, such as the protons used in shuttling NADH equivalents from the cytoplasm to the electron-transport chain, have to be accounted for as well, adding more small integers to the calculations. However, we should not lose sight of the possibility that the coupling mechanisms of the proton pumps may turn out to be at least partially field-driven, resulting in continuously variable values of the coupling stoichiometries, with no integral values even at the level of individual reactions.

It is generally agreed that cytochrome *c* oxidase, complex IV, pumps two protons per oxygen atom reduced, and also translocates two electrons from cytochrome *c* on the cytoplasmic face to the matrix face of the membrane, where two protons are consumed in the generation of water. These reactions give an H<sup>+</sup>/2e ratio of 2 and a charge/2e ratio of 4, with four protons removed from the matrix side of the membrane and two protons appearing on the cytoplasmic side. Although the broad outlines are clarifying, the exact mechanism of proton pumping by the oxidase is still unclear [4]. The cytochrome *bc*<sub>1</sub> complex, complex III, uses the Q-cycle [4], and translocates protons with an H<sup>+</sup>/2e ratio of 4, but a charge/2e ratio of only 2 since two protons per electron pair are released on the cytoplasmic side by scalar reactions. It is the charge/2e ratio, rather than the H<sup>+</sup>/2e ratio, that mainly determines the amount of ATP that can be made at a coupling site, since the protonmotive force is expressed mostly as a membrane potential, rather than as a pH gradient. The cytochrome *bc*<sub>1</sub> complex and cytochrome *c* oxidase together translocate six protons per oxygen and six charges per oxygen atom consumed. The coupling stoichiometry of NADH-Q oxidoreductase, complex I, is less certain. Despite the paucity of direct evidence, most workers assume that it translocates four protons per electron pair and four charges per electron pair. The mechanism of proton pumping is not established. Thus the full chain from NADH to oxygen is believed to translocate ten protons per oxygen atom consumed, and the chain from succinate to oxygen translocates six protons per oxygen consumed. This value of 6 for the H<sup>+</sup>/O ratio of mitochondrial succinate oxidation was originally found empirically nearly 30 years ago [5], and is part of a consensus about the H<sup>+</sup>/O ratios of the mitochondrial electron-transport chain that has now remained stable for more than a decade [2].

The value of the H<sup>+</sup>/ATP ratio is less certain. A value of approx. 4, made up of three protons per ATP on the ATP synthase and one proton per ATP on the phosphate and adenine nucleotide carriers, is supported by equilibrium thermodynamic measurements in broken mitochondria, but exceeds the values from thermodynamic or kinetic experiments in intact mitochondria. Until recently, the main support for a total of four protons transported for each ATP synthesized by

mitochondria was that it fitted the accepted  $H^+/O$  and  $P/O$  ratios. More recently, theoretical arguments based on a model of the ATP synthase in which rotation is driven by protons passed on by sequential  $c$ -subunits in the membrane sector,  $F_o$ , allied to the observation of three ATP synthase active sites and ten  $c$ -subunits in the yeast enzyme [6], suggest that ten protons may need to pass through the ATP synthase for every three ATPs made. Together with the requirement for one proton used to import ADP and phosphate and export each ATP, this predicts an overall  $H^+/ATP$  coupling stoichiometry of 13/3 in mammalian mitochondria. For a graphic representation of the mechanisms that give rise to this coupling ratio, see <http://www.tcd.ie/Biochemistry/IUBMB-Nicholson/swf/ATPSynthase.swf>. This  $H^+/ATP$  ratio of 4.33 is slightly greater than the consensus integral value of 4 that was previously accepted [2], necessitating a downwards recalculation of the maximum  $P/O$  ratios that mitochondria can achieve. Because of the inherent difficulties in making sufficiently accurate measurements of  $P/O$  ratios, there is a general view that values based on experiment but fine-tuned by theory are superior to those based on measurement alone. Whether this is a valid approach should eventually be resolved by more detailed experimentally supported models of the proton-translocation reactions in complex I and the ATP synthase.

We now have a new consensus view among mitochondrial bioenergeticists, based as much on theory as it is on observation, and therefore subject to further revision in the future (see also [3,4]). For NAD-linked substrates, the  $H^+/O$  ratio is 10, the  $H^+/ATP$  ratio is 13/3, and the maximum  $P/O$  ratio is near to 2.31 (Table 1). It is important to stress that the  $P/O$  ratio in this model is not an approximation to any integer, but is a fraction of relatively small integers, in this case it is simply  $10 \times (3/13)$ , which has the value  $2.30769(230769)_\infty$ . For succinate and other FAD-linked substrates, the  $H^+/O$  ratio is 6, the  $H^+/ATP$  ratio is 13/3, and the maximum  $P/O$  ratio is near to 1.38 (Table 1). However, textbook authors and researchers not directly involved in the arguments about stoichiometries have remained cautious in adopting the new values, sometimes still preferring to use the older, apparently less controversial,  $P/O$  ratios. This is unfortunate, since the ramifications of these lower  $P/O$  ratios are considerable in cellular bioenergetics. In particular, the yield of ATP during metabolism is approx. 20–30% less than the classical  $P/O$  ratios suggest, and calculations based on the classical values can lead to large errors in the calculated energy budgets of cells and organisms.

Using the new consensus values, the maximum possible yield of ATP from the complete mitochondrial oxidation of palmitate is just under 96.5 ATP molecules per palmitate, not 129 as given by standard textbooks, with a maximum overall  $P/O$  ratio of only approx. 2.1, not 2.8 as conventionally supposed (Table 1). The maximum possible yield of ATP from the complete oxidation of glucose in a cell that uses the malate–aspartate shuttle for transfer of reducing equivalents into the mitochondrial matrix will be just over 28.9 ATP molecules per glucose (27.5 if the glycerol phosphate shuttle

is used instead), not 38 as given by standard textbooks, with a maximum  $P/O$  ratio of only 2.41 (2.29 if the glycerol phosphate shuttle is used), not 3 as sometimes supposed (Table 1) [2].

## Slip reactions

The values for  $P/O$  ratios given above are the maximum attainable according to our current understanding of the coupling mechanisms. However, if the proton pumps themselves are not perfectly coupled, they may slip, particularly at high protonmotive force or high rate, resulting in less protons pumped by the electron-transport-chain complexes for each electron pair transferred or less ATP made by the ATP synthase for each proton driven through it. Such slip reactions lead to lower and more plastic overall  $P/O$  ratios than those discussed above.

It is not difficult to envisage slip reactions in the mechanisms of the proton-pumping machinery [7]. In complex III, if occasional electrons bypass the  $b$  haems, less protons will be pumped. It seems that complex III is well-designed to minimize this possibility, perhaps by requiring physical movement of part of the iron–sulphur protein to distribute electrons correctly [4], but such mechanisms are unlikely to be perfect. In complex IV, blocking the channels that allow protons access to the central mechanism may not fully prevent electron flow, but generates a slipping enzyme. In the ATP synthase, the rotational mechanism may not always be strictly coupled to proton flow, particularly when nucleotide concentrations are low [8], leading to less than maximal ATP production.

Analysis of slip reactions is very useful for probing the mechanisms of proton pumping by bioenergetic complexes. Whether it occurs under physiological conditions in cells remains uncertain: we have provided evidence that it does not [9,10], but others have argued that it does [7].

## Leak reactions lower effective $P/O$ ratios

A second way that  $P/O$  ratios may fall below their maximum values is by leaks of protons across the coupling membrane, so that pumped protons leak back and are not available to drive ATP synthesis. Such proton leaks are major pathways that profoundly alter the bioenergetics of mitochondria within isolated cells [11–13].

It is well known that isolated mitochondria leak protons: oxygen consumption is readily measurable in the absence of ATP synthesis, and it drives a futile steady-state cycle of proton pumping and proton leak. Surprisingly, the proton leak is high in mitochondria within intact cells such as isolated primary hepatocytes, where, despite large species-dependent differences in the proton conductance of isolated mitochondria [14,15], it makes up a very similar proportion of total cellular respiration rate (approx. 20%) across a range of vertebrate and invertebrate species (Table 2). This makes it the single most important energy sink in these cells *in vitro*. In perfused rat muscle, the contribution made by proton leak is even greater, measured as 35% in contracting preparations

**Table 1 | Maximum extra-mitochondrial ATP yields and P/O ratios for the oxidation of succinate or pyruvate plus malate by isolated mammalian mitochondria, and oxidation of glucose, glycogen or palmitate by mammalian cells**

(a) Succinate enters mitochondria on the dicarboxylate carrier in exchange for malate. It is oxidized to fumarate, reducing the FAD in succinate dehydrogenase to FADH<sub>2</sub>, which is oxidized by the electron-transport chain causing the translocation of six protons from the matrix to the intermembrane space. The fumarate is converted into malate by fumarase and exits in exchange for incoming succinate. The six protons re-enter the matrix through the ATP synthase with an H<sup>+</sup>/ATP ratio of 13/3 (see text), causing phosphorylation of a little more than 1.384 ATP molecules per oxygen atom reduced to water. (b) Pyruvate enters mitochondria on the pyruvate carrier (electroneutrally with a proton that is eventually exported with citrate) and is oxidized to acetyl-CoA by pyruvate dehydrogenase, and malate is oxidized to oxaloacetate by malate dehydrogenase, forming two NADH and citrate, which exits the mitochondria on the tricarboxylate carrier in exchange for incoming malate. The two NADH are oxidized, causing pumping of 20 protons, generating just over 4.615 ATP with a P/O ratio of just above 2.307. (c) Glucose is oxidized in cells by glycolysis to two pyruvate, using two ATP, but generating four ATP and two NADH, which enter the mitochondria by the glutamate aspartate (Glu/Asp) shuttle for reducing equivalents, driven by re-entry of two of the 20 subsequently pumped protons, or by the glycerol phosphate ( $\alpha$ GP) shuttle, allowing the reducing equivalents to enter the electron-transport chain without passing through complex I, so pumping 12 protons, eight less than normal for NADH. The two pyruvate from glycolysis are fully oxidized by the TCA (tricarboxylic acid) cycle, generating eight NADH and two FADH<sub>2</sub>, which pump 92 protons, and two GTP, which can formally be converted into ATP and exported to the cytoplasm, using two pumped protons. The sum of 108 (or 102) pumped protons yield just over 24.9 (23.5) ATP, giving total ATP yields and P/O ratios as shown. (d) Glycogen oxidation requires one fewer ATP per glucose unit than glucose oxidation does, as glucose 6-phosphate is generated without ATP consumption, yielding higher overall P/O ratios. (e) Palmitate activation to palmitoyl-CoA generates AMP from ATP, effectively using two ATP. Palmitoyl-CoA enters the matrix electroneutrally as palmitoyl carnitine on the carnitine transporter.  $\beta$ -Oxidation to acetyl-CoA yields seven NADH and seven FADH<sub>2</sub>, then oxidation of eight acetyl-CoA in the TCA cycle yields 24 NADH, eight FADH<sub>2</sub> and eight GTP. The overall P/O ratio is just under 2.1. Oxidation of other fatty acids gives slightly different yields and P/O ratios.

Reaction	Activation	Aerobic glycolysis		TCA cycle and $\beta$ -oxidation		Overall yield		Total ATP	P/O <sub>max</sub>	
	ATP	H <sup>+</sup>	ATP	H <sup>+</sup>	ATP	H <sup>+</sup>	ATP			
(a) Succinate + [O] → malate + H <sub>2</sub> O				+6		+6		6/(13/3) = 1.38462	1.385	
(b) Pyruvate + malate + 2[O] → citrate + 2H <sub>2</sub> O				+20		+20		20/(13/3) = 4.61538	2.308	
(c) Glucose + 12[O] → 6CO <sub>2</sub> + 6H <sub>2</sub> O	-2	+20	+4	+92	-2	+2	+110	+4		
Glu/Asp shuttle		-2					-2		108/(13/3) + 4 = 28.92308	2.410
$\alpha$ GP shuttle		-8					-8		102/(13/3) + 4 = 27.53846	2.295
(d) Glycogen <sub>(n)</sub> + 12[O] → glycogen <sub>(n-1)</sub> + 6CO <sub>2</sub> + 6H <sub>2</sub> O	-1	+20	+4	+92	-2	+2	+110	+5		
Glu/Asp shuttle		-2					-2		108/(13/3) + 5 = 29.92308	2.494
$\alpha$ GP shuttle		-8					-8		102/(13/3) + 5 = 28.53846	2.378
(e) Palmitate + 46[O] → 16CO <sub>2</sub> + 16H <sub>2</sub> O	-2			+400	-8	+8	+392	+6	392/(13/3) + 6 = 96.46154	2.097

**Table 2** | Respiration driving mitochondrial proton leak in cells and tissues from different organisms

System	Percentage of respiration	Reference(s)
Rat hepatocytes	20–26	[17,46]
Rat muscle	35–50	[17,47]
Rat basal metabolic rate	20–25	[17,47]
Mammal hepatocytes (mouse, ferret, sheep, pig, horse)	Approx. 20	[48]
Avian hepatocytes (finch, sparrow, starling, currawong, pigeon, duck, goose, emu)	Up to 21	[49]
Crocodylian hepatocytes	Up to 13–30	[50]
Lizard hepatocytes	Up to 30	[51]
Frog hepatocytes	Up to 20–25	[52]
Lamprey hepatocytes	Up to 25–50	[53]
Snail hepatopancreas cells	Up to 15–25	[54]

and 50% in resting muscle. The sum of the proton-cycling rates in different organs of the rat suggests that up to 20–25% of its basal metabolic rate may be devoted entirely to driving this futile cycle (Table 2).

In hepatocytes, non-mitochondrial oxygen consumption accounts for another 20% of the total oxygen-consumption rate, so only approx. 60% of the oxygen used by these cells is used for ATP synthesis. Thus the effective P/O ratio for oxidation of palmitate in isolated liver cells is only approx. 1.3 ( $2.1 \times 0.6 = 1.26$ ) and the effective P/O ratio for oxidation of glucose is only approx. 1.4 ( $2.4 \times 0.6 = 1.44$ ). These values for the effective P/O ratio will depend on ATP demand, since the proportion of cellular respiration that is used to drive the proton leak often decreases as more ATP is made. They must be measured or calculated for any particular cellular system before attempts are made to estimate ATP production from oxygen-consumption rate. Whether effective P/O ratios are as low *in vivo* as they are *in vitro* remains to be established: recent measurements of P/O ratios *in vivo* [16] suggest that, at least in muscle, coupling may be better than it is in perfused, highly oxygenated preparations [17], although both types of experiment are demanding and can be criticized on technical grounds.

The pathway of the basal proton conductance of mitochondria has been elusive. Liposomes made from mitochondrial phospholipids are considerably less proton-permeable than native mitochondria [18], and proton conductance is the same in liposomes prepared from mitochondria with very different endogenous proton conductances [19], therefore simple diffusion through bulk regions of the membrane bilayer does not explain the proton conductance, and other components of the mitochondrial membrane must be involved. As well as the basal proton conductance discussed above, mitochondria have an inducible proton conductance catalysed (in brown

adipose tissue) by UCP1 (uncoupling protein 1). However, neither UCP1 nor the related UCP2 and UCP3 catalyse the basal proton conductance, since it remains the same in mitochondria from UCP-knockout mice [20]. Recent studies have thrown light on this problem: basal proton conductance is halved in muscle mitochondria from mice in which ANT1 (adenine nucleotide translocase) has been ablated, and varies linearly as the carboxyatractylate-inhibited ANT content of *Drosophila* mitochondria is manipulated genetically, suggesting strongly that a substantial part of the basal proton conductance of mitochondria is caused by the presence (not the activity) of ANT in the membrane [21].

## UCPs

In addition to the basal proton conductance, some mitochondria contain specialized UCPs whose function is to catalyse a regulated inducible proton conductance. The classic example is UCP1, the most abundant protein in the mitochondria of brown adipose tissue of small rodents and newborn humans, which executes adaptive thermogenesis [22,23]. In response to cold, the sympathetic nervous system releases noradrenaline in brown adipose tissue, activating protein kinase A. Chronically, protein kinase A activation causes hypertrophy of brown adipose tissue and up-regulates the expression of UCP1. Acutely, it stimulates hormone-sensitive lipase, which hydrolyses triacylglycerol stores to release fatty acids. The fatty acids act as a substrate for electron transport, and simultaneously activate the proton conductance of UCP1, resulting in uncoupling of electron transport from ATP synthesis. The consequent uncoupled respiration allows rapid fatty acid oxidation and produces heat, which is distributed to the rest of the body by the circulation.

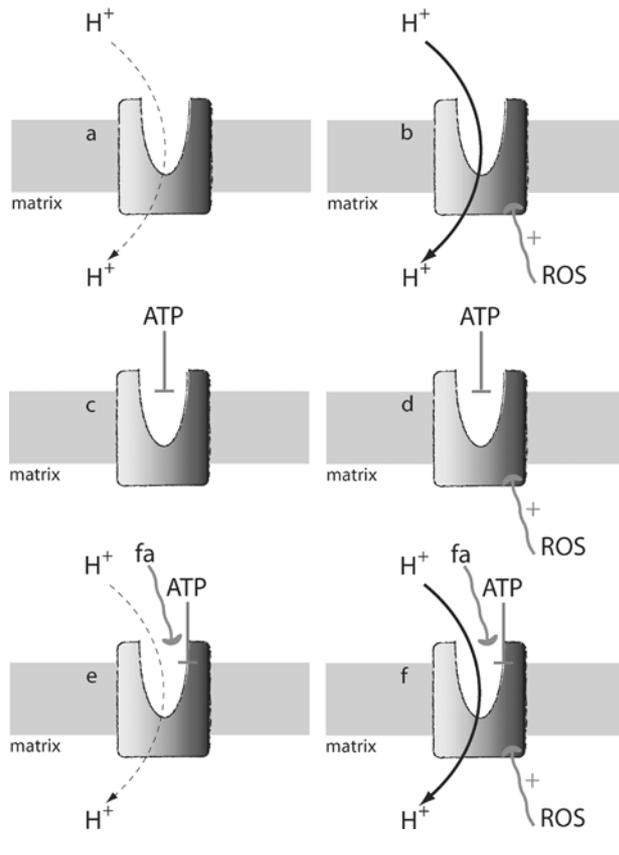
The regulation of UCP1 is apparent from the properties of isolated brown adipose tissue mitochondria. As isolated, they are uncoupled by the activity of UCP1. If both BSA (a chelator of contaminating fatty acids) and purine nucleotides such as GDP or ATP are added, UCP1 activity is prevented and coupling appears [24,25]. The inhibition by nucleotides is competitively overcome by fatty acids [24,26,27], leading to the model for UCP1 activation shown in Figures 1(a), 1(c) and 1(e). Isolated brown adipose tissue mitochondria have a modest UCP1-mediated proton conductance owing to the absence of inhibitory nucleotides (Figure 1a). In the resting brown adipose cell, however, endogenous concentrations of nucleotides, chiefly ATP, keep UCP1 fully inhibited (Figure 1c). Under noradrenaline stimulation, the concentration of fatty acids rises sharply, and fatty acids compete with nucleotides, overcoming their inhibitory effect and allowing uncoupling through UCP1 (Figure 1e).

## Activation of the proton conductance of UCP2 and UCP3

UCP1 is found in brown adipose tissue, and does not contribute to the proton conductance of mitochondria from

**Figure 1 | Model for the regulation of UCPs**

In the absence of physiological concentrations of ATP, proton conductance is modest (UCP1) or very low (other UCPs) (**a**), but can be activated by ROS (**b**) [55–57]. In the presence of ATP, proton conductance is inhibited whether ROS are absent (**c**) or present (**d**) [29,32,58]. Fatty acids prevent inhibition by ATP [24,26,27], so, in the presence of ATP and fatty acids (fa), proton conductance is modest or absent (**e**) unless ROS are present to allow activity (**f**). Thus, at physiological concentrations of ATP, UCPs only uncouple if both ROS and fatty acids are present (**f**).



tissues such as skeletal muscle. The discovery of the UCP1 homologues, UCP2 and UCP3, in tissues such as lung, spleen, brain and skeletal muscle raised the possibility that they too contribute to inducible proton leak [28]. However, UCP2 and UCP3 do not uncouple unless they are first activated [13]. The nature of the activators remained unknown until the discovery that generation of exogenous superoxide was able to cause a UCP-dependent nucleotide-sensitive proton conductance in mitochondria isolated from skeletal muscle (UCP3) [29], brown adipose tissue (UCP1) [29] or kidney (UCP2) [29,30]. Other activators include a carbon-centred radical generator, reactive alkenals such as hydroxynonenal, and fatty acid or alkenal analogues, and inhibitors of activation include carbon-centred radical quenchers [31–35].

These considerations led to a working model of physiological UCP activation in which superoxide produced by the electron-transport chain attacks the polyunsaturated acyl chains of membrane phospholipids to generate carbon-centred fatty acyl radicals, propagating chain reactions that

produce lipid hydroperoxides and ultimately form reactive alkenals such as hydroxynonenal, the proximal UCP activator [32–35]. In this model, activation of UCP2 and UCP3, and perhaps also UCP1, by this ROS (reactive oxygen species) pathway is essential before their proton conductance is competent. As shown in Figure 1, the UCPs would be inactive (or only weakly active) in the absence of ROS, but once the ROS pathway was activated, regulation by nucleotides and fatty acids would be the same for UCP2 and UCP3 as discussed above for UCP1, and the fully active state (Figure 1f) would be achieved *in vivo* only when the UCP was activated by the ROS pathway and inhibition by endogenous ATP was overcome by raised fatty acid concentrations.

**Physiological roles of UCP2, UCP3 and related UCPs**

What is the function of the plasticity of the coupling of mitochondrial energy transduction revealed by the inducible, regulated uncoupling activity of the UCPs? There are three situations where tight coupling appears to be sacrificed in favour of UCP-regulated looser coupling [36].

The first, and probably ancestral, function of regulated uncoupling by UCPs may be to attenuate the production of damaging ROS by the mitochondrial electron-transport chain [34–36]. Mitochondrial ROS production is thought to be central to oxidative stress, cellular senescence and aging. Under conditions of high oxygen tension and low ATP demand in the presence of high concentrations of substrate (particularly fatty acids [37]), respiratory control of the electron-transport chain leads to high protonmotive force and a reduced state of the electron carriers. These conditions strongly favour the production of superoxide by complex I following reverse electron transport, and by complex III and glycerol phosphate dehydrogenase. ROS production by complex I is exquisitely sensitive to protonmotive force [38], so the mild uncoupling induced by ROS activation of UCPs limits protonmotive force and attenuates ROS production. In this model, UCPs would have little effect on oxidative phosphorylation when ATP demand is high, but would cause mild uncoupling and would slightly lower the effective P/O ratio when a tissue such as muscle is resting but still perfused with substrate and oxygen and poised to do work.

The second, probably more recently evolved, function of regulated uncoupling by UCPs is thermogenesis in response to cold or excess caloric intake. This is the main function of UCP1 in brown adipose tissue in rats and mice, as discussed above. However, mice in which UCP2 or UCP3 have been knocked out have normal adaptive thermogenesis and are not obese, implying that, unlike UCP1, UCP2 and UCP3 have no role to play in adaptive thermogenesis. Nevertheless, UCP3-knockout mice are resistant to the thermogenic effects of 3,4-methylenedioxymethamphetamine (the recreational drug ecstasy) [39], suggesting that it is possible for UCP3 to be recruited pharmacologically in mammalian muscle to give significant heat production. It is not clear whether there are physiological situations where muscle UCP3 is significantly

thermogenic, but it remains a potential target for drugs to increase peripheral energy expenditure and so help to treat obesity.

Birds represent a separate lineage of warm-blooded vertebrates, and have developed adaptive thermogenesis without having brown adipose tissue or UCP1. Instead, they probably use skeletal muscle for non-shivering adaptive thermogenesis. Avian UCP is expressed in muscle, and its expression and activity are strongly induced in mitochondria from king penguins when they first acquire adaptive thermogenesis following exposure to cold water [40]. This suggests that uncoupling by avian UCP may contribute to adaptive thermogenesis in bird muscle, although a primary role in protection against oxidative stress has not yet been ruled out.

The third function of regulated uncoupling by UCPs appears to be in signalling. The best example comes from the interaction between UCP2 and glucose-stimulated insulin secretion in the  $\beta$ -cells of pancreatic islets. The main function of  $\beta$ -cells is to monitor blood glucose and release insulin when glucose is high. They do this, at least in part, by exploiting an unusual bioenergetic design [41]: when glucose rises, it is oxidized faster, raising the mitochondrial protonmotive force and the cytoplasmic phosphorylation potential. High ATP and low ADP inhibit plasma membrane  $K_{ATP}$  channels, depolarizing the plasma membrane and allowing voltage-gated calcium channels to open. The rise in cytoplasmic calcium triggers exocytosis of insulin-containing vesicles. Activation of UCP2 partially short-circuits this pathway by causing mild uncoupling. A plausible regulator of UCP2 function is fatty acid oxidation, which raises  $\beta$ -cell ROS production, activates the proton conductance of UCP2 and attenuates insulin secretion. This may be a pathological side effect of a protective mechanism that limits ROS production and islet damage during lipotoxicity or hyperglycaemia, but helps cause Type II diabetes in humans following a Western lifestyle [30,42–45]. It may also be a physiological mechanism to regulate the use of fatty acids and glucose as fuels [35,36].

## Conclusions

The coupling efficiency of oxidative phosphorylation is crucial to constraining mechanistic models of the molecular catalysts involved, and central to the physiology, pathology and ecology of energy metabolism. Despite 50 years of work, we still lack a full and detailed understanding of this fascinating problem. The plasticity of mitochondrial coupling efficiency appears to be very important, with regulated uncoupling by mitochondrial UCPs underpinning physiological processes such as adaptive thermogenesis, prevention of oxidative damage and insulin secretion. It may also play a major part in slowing aging, in protecting against neurological and circulatory diseases caused by oxidative stress, and in dysfunctions of energy metabolism in obesity and diabetes.

collaborators, who have made huge contributions to the work described here.

## References

- Ernster, L. (1993) *FASEB J.* **7**, 1520–1524
- Brand, M.D. (1994) *Biochemist* **16**, 20–24
- Hinkle, P.C. (2005) *Biochim. Biophys. Acta* **1706**, 1–11
- Rich, P.R. (2003) *Biochem. Soc. Trans.* **31**, 1095–1105
- Brand, M.D., Reynafarje, B. and Lehninger, A.L. (1976) *J. Biol. Chem.* **251**, 5670–5679
- Stock, D., Leslie, A.G. and Walker, J.E. (1999) *Science* **286**, 1700–1705
- Kadenbach, B. (2003) *Biochim. Biophys. Acta* **1604**, 77–94
- Feniouk, B.A., Mulikjanian, A.Y. and Junge, W. (2005) *Biochim. Biophys. Acta* **1706**, 184–194
- Porter, R.K. and Brand, M.D. (1995) *Biochem. J.* **310**, 379–382
- Ainscow, E.K. and Brand, M.D. (1995) in *BioThermoKinetics in the Post Genomic Era* (Larsson, C., Pahlman, I.L. and Gustafsson, L., eds.), pp. 192–195, Chalmers Reproservice, Goteborg
- Brand, M.D. (1990) *Biochim. Biophys. Acta* **1018**, 128–133
- Brand, M.D., Chien, L.F., Ainscow, E.K., Rolfe, D.F.S. and Porter, R.K. (1994) *Biochim. Biophys. Acta* **1187**, 132–139
- Brand, M.D., Brindle, K.M., Buckingham, J.A., Harper, J.A., Rolfe, D.F.S. and Stuart, J.A. (1999) *Int. J. Obes.* **23** (suppl. 6), S4–S11
- Porter, R.K. and Brand, M.D. (1995) *Am. J. Physiol.* **269**, R226–R228
- Brand, M.D., Turner, N., Ocloo, A., Else, P.L. and Hulbert, A.J. (2003) *Biochem. J.* **376**, 741–748
- Marcinek, D.J., Schenkman, K.A., Ciesielski, W.A. and Conley, K.E. (2004) *Am. J. Physiol.* **286**, C457–C463
- Rolfe, D.F.S., Newman, J.M., Buckingham, J.A., Clark, M.G. and Brand, M.D. (1999) *Am. J. Physiol.* **276**, C692–C699
- Brookes, P.S., Rolfe, D.F.S. and Brand, M.D. (1997) *J. Membr. Biol.* **155**, 167–174
- Brookes, P.S., Hulbert, A.J. and Brand, M.D. (1997) *Biochim. Biophys. Acta* **1330**, 157–164
- Esteves, T.C. and Brand, M.D. (2005) *Biochim. Biophys. Acta* **1709**, 35–44
- Brand, M.D., Pakay, J.L., Ocloo, A., Kokoszka, J., Wallace, D.C., Brookes, P.S. and Cornwall, E.J. (2005) *Biochem. J.*, doi:10.1042/BJ20050890
- Nicholls, D.G. and Locke, R.M. (1984) *Physiol. Rev.* **64**, 1–64
- Cannon, B. and Nedergaard, J. (2004) *Physiol. Rev.* **84**, 277–359
- Nicholls, D.G. and Rial, E. (1999) *J. Bioenerg. Biomembr.* **31**, 399–406
- Rial, E., Aguirregoitia, E., Jimenez-Jimenez, J. and Ledesma, A. (2004) *Biochim. Biophys. Acta* **1608**, 122–130
- Huang, S.G. (2003) *Arch. Biochem. Biophys.* **412**, 142–146
- Shabalina, I.G., Jacobsson, A., Cannon, B. and Nedergaard, J. (2004) *J. Biol. Chem.* **279**, 38236–38248
- Ricquier, D. and Bouillaud, F. (2000) *Biochem. J.* **345**, 161–179
- Echtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B., Cadenas, S., Stuart, J.A., Harper, J.A., Roebuck, S.J., Morrison, A., Pickering, S. et al. (2002) *Nature (London)* **415**, 96–99
- Krauss, S., Zhang, C.Y., Scorrano, L., Dalgaard, L.T., St-Pierre, J., Grey, S.T. and Lowell, B.B. (2003) *J. Clin. Invest.* **112**, 1831–1842
- Rial, E., Gonzalez-Barroso, M., Fleury, C., Iturrizaga, S., Sanchis, D., Jimenez-Jimenez, J., Ricquier, D., Goubern, M. and Bouillaud, F. (1999) *EMBO J.* **18**, 5827–5833
- Echtay, K.S., Esteves, T.C., Pakay, J.L., Jekabsons, M.B., Lambert, A.J., Portero-Otin, M., Pamplona, R., Vidal-Puig, A., Wang, S., Roebuck, S.J. and Brand, M.D. (2003) *EMBO J.* **22**, 4103–4110
- Murphy, M.P., Echtay, K.S., Blaikie, F.H., Asin-Cayuela, J., Cocheme, H.M., Green, K., Buckingham, J.A., Taylor, E.R., Hurrell, F., Hughes, G. et al. (2003) *J. Biol. Chem.* **278**, 48534–48545
- Brand, M.D., Buckingham, J.A., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Murphy, M.P., Pakay, J.L., Talbot, D.A. and Echtay, K.S. (2004) *Biochem. Soc. Symp.* **71**, 203–213
- Brand, M.D., Affourtit, C., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Pakay, J.L. and Parker, N. (2004) *Free Radical Biol. Med.* **37**, 755–767
- Brand, M.D. and Esteves, T.C. (2005) *Cell Metab.* **2**, 85–93
- St-Pierre, J., Buckingham, J.A., Roebuck, S.J. and Brand, M.D. (2002) *J. Biol. Chem.* **277**, 44784–44790
- Lambert, A.J. and Brand, M.D. (2004) *Biochem. J.* **382**, 511–517
- Mills, E.M., Banks, M.L., Sprague, J.E. and Finkel, T. (2003) *Nature (London)* **426**, 403–404
- Talbot, D.A., Duchamp, C., Rey, B., Hanuise, N., Rouanet, J.L., Sibille, B. and Brand, M.D. (2004) *J. Physiol.* **558**, 123–135

Current work in my laboratory is supported by the Medical Research Council and the Wellcome Trust. I thank all my colleagues and

- 41 Rutter, G.A. (2001) *Mol. Aspects Med.* **22**, 247–284
- 42 Zhang, C.Y., Baffy, G., Perret, P., Krauss, S., Peroni, O., Grujic, D., Hagen, T., Vidal-Puig, A.J., Boss, O., Kim, Y.B. et al. (2001) *Cell* **105**, 745–755
- 43 Joseph, J.W., Koshkin, V., Saleh, M.C., Sivitz, W.I., Zhang, C.Y., Lowell, B.B., Chan, C.B. and Wheeler, M.B. (2004) *J. Biol. Chem.* **279**, 51049–51056
- 44 Chan, C.B., Saleh, M.C., Koshkin, V. and Wheeler, M.B. (2004) *Diabetes* **53** (suppl. 1), S136–S142
- 45 Krauss, S., Zhang, C.Y. and Lowell, B.B. (2005) *Nat. Rev. Mol. Cell Biol.* **6**, 248–261
- 46 Nobes, C.D., Brown, G.C., Olive, P.N. and Brand, M.D. (1990) *J. Biol. Chem.* **265**, 12903–12909
- 47 Rolfe, D.F.S. and Brand, M.D. (1996) *Am. J. Physiol.* **271**, C1380–C1389
- 48 Porter, R.K. and Brand, M.D. (1995) *Am. J. Physiol.* **269**, R1213–R1224
- 49 Else, P.L., Brand, M.D., Turner, N. and Hulbert, A.J. (2004) *J. Exp. Biol.* **207**, 2305–2311
- 50 Hulbert, A.J., Else, P.L., Manolis, S.C. and Brand, M.D. (2002) *J. Comp. Physiol. B* **172**, 387–397
- 51 Brand, M.D., Couture, P., Else, P.L., Withers, K.W. and Hulbert, A.J. (1991) *Biochem. J.* **275**, 81–86
- 52 Brand, M.D., Bishop, T., Boutilier, R.G. and St-Pierre, J. (2000) in *Life in the cold* (Heldmaier, G. and Klingenspor, M., eds.), pp. 413–430, Springer, Berlin
- 53 Savina, M.V. and Gamper, N.L. (1998) *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **120**, 375–383
- 54 Bishop, T. and Brand, M.D. (2000) *J. Exp. Biol.* **203**, 3603–3612
- 55 Echtay, K.S., Winkler, E. and Klingenberg, M. (2000) *Nature (London)* **408**, 609–613
- 56 Echtay, K.S., Winkler, E., Frischmuth, K. and Klingenberg, M. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1416–1421
- 57 Esteves, T.C., Echtay, K.S., Jonassen, T., Clarke, C.F. and Brand, M.D. (2004) *Biochem. J.* **379**, 309–315
- 58 Echtay, K.S. and Brand, M.D. (2001) *Biochem. Soc. Trans.* **29**, 763–768

---

Received 17 June 2005