

Mitochondrial Proton Leak and the Uncoupling Proteins

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An energetically significant leak of protons occurs across the mitochondrial inner membranes of eukaryotic cells. This seemingly wasteful proton leak accounts for at least 20% of the standard metabolic rate of a rat. There is evidence that it makes a similar contribution to standard metabolic rate in a lizard. Proton conductance of the mitochondrial inner membrane can be considered as having two components: a basal component present in all mitochondria, and an augmentative component, which may occur in tissues of mammals and perhaps of some other animals. The uncoupling protein of brown adipose tissue, UCP1, is a clear example of such an augmentative component. The newly discovered UCP1 homologs, UCP2, UCP3, and brain mitochondrial carrier protein 1 (BMCP1) may participate in the augmentative component of proton leak. However, they do not appear to catalyze the basal leak, as this is observed in mitochondria from cells which apparently lack these proteins. Whereas UCP1 plays an important role in thermogenesis, the evidence that UCP2 and UCP3 do likewise remains equivocal.

KEY WORDS: Proton leak, uncoupling proteins, UCP1, UCP2, UCP3, BMCP1, thermogenesis, sequence homology, mitochondria, respiration.

THE PHENOMENON OF PROTON LEAK

Oxygen is consumed by mitochondria as electrons are passed along the electron transport chain with the concomitant translocation of protons from the mitochondrial matrix to the cytosol. ATP is generated from ADP and P_i as protons pass down their electrochemical gradient through the F_oF₁-ATPase. It has long been recognized that the F_oF₁-ATPase is not the only route by which the mitochondrial electrochemical proton gradient is dissipated (Nicholls, 1974). When the F_oF₁-ATPase is not working, either in the absence of ADP, or in the presence of oligomycin (which blocks the passage of protons through the F_o portion of the ATPase), all of the protons pumped out of the matrix pass back into the mitochondria through other proton conductance routes. Flux through this nonproductive

proton conductance pathway has been termed "proton leak." The respiration required to maintain protonmotive force against this proton leak is represented by the state-4 respiration rates of mitochondria.

In early work, the proton conductance was believed to be an artifact of damage to mitochondria, sustained in the act of isolating them from cells and tissues. It was thought that the shearing forces used to disrupt cellular membranes and thus free the mitochondria for experimentation were responsible for altering the physical properties of the mitochondrial inner membranes, allowing the passage of protons that could not occur in normal, intact mitochondria. Certainly, observations that more vigorous methods of mitochondrial isolation did result in mitochondria with greater proton conductance seemed to confirm this view.

An important first step in determining whether proton leak was indeed an experimental artifact, or a natural phenomenon, was, therefore, the measurement of proton conductance in mitochondria that had not been subjected to any procedures involving mechanical or chemical disruption of the cell. The approach to

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this problem was to transfer existing techniques for measuring mitochondrial function *in vitro* to *in situ*, by making the same measurements in intact cells. This was achieved using a rat hepatocyte model and measuring mitochondrial membrane potential at different rates of cellular oxygen consumption in undisrupted cells (Nobes *et al.*, 1990). These experiments showed that proton leak across the inner membrane was at least as great when mitochondria were functioning in intact cells as it was in mitochondria stirred in an electrode chamber. A basal proton conductance of the mitochondrial inner membrane was thus shown to be a feature of undamaged mitochondria operating in their natural environment, the cytosol.

THE IMPORTANCE OF PROTON LEAK

The above approach also allowed the quantification of the rates of oxygen consumption required to sustain the proton leak and comparison of this cost with overall rates of cellular oxygen consumption. The results of these calculations are perhaps surprising. The energy consumed by proton leak represents about 25% of the energy budget of isolated rat liver cells (Nobes *et al.*, 1990), making it the single most expensive component of cellular energy metabolism. How does this information from an isolated cell model translate to the whole organism? In rats, liver represents only a small proportion of total tissue mass and among tissues is not the greatest oxygen consumer (Rolfe and Brand, 1996). Of all rat tissues, the greatest oxygen consumer is skeletal muscle. Thus, a meaningful estimate of the significance of mitochondrial proton leak to whole animal energetics required that similar measurements be made in muscle. This was achieved using perfusions of the rat hindquarter (Rolfe and Brand, 1996). The results of these experiments suggested that the contribution of proton leak to metabolic rate in muscle is, in fact, greater than the approximately 25% observed for isolated hepatocytes. In resting muscle, about 50% of metabolic rate appears to be dedicated to countering the leak of protons into the mitochondrial matrix.

Both the hepatocyte model and the perfused muscle used in these initial experiments represent "at rest" states, as both systems are capable of reaching much greater rates of oxygen consumption under natural conditions of physiological stress. Thus, while proton leak may have accounted for a large proportion of metabolic rate in these resting systems, perhaps it is a trivial

component under conditions where greater workloads were imposed. This idea was examined in each model system (Rolfe *et al.*, 1999). The hepatocyte model was made to work harder by imposing upon it the additional burden of driving the ornithine–urea cycle and gluconeogenesis at high rates and thereby increasing the rate of ATP turnover twofold. Similarly, the skeletal muscle was subjected to vigorous contraction. The results of these experiments indicate that, even as metabolic rate increases to accommodate greater workloads, proton leak continues to consume a large proportion, approximately 35% of the overall available energy (Rolfe *et al.*, 1999).

Taken together, these experiments demonstrate that proton leak is enormously important to the overall energy budget of the rat and, by inference, of mammals. Calculations indicate that the energy required to counter proton leak in a rat accounts for 20–25% of the standard metabolic rate of the whole animal. This makes the phenomenon of proton leak the single most energetically important component of basal energy metabolism in the rat. What is the purpose of this seemingly wasteful energy consumption?

PROTON LEAK AND THERMOGENESIS

It has often been assumed that the high contribution of proton leak to the metabolic rate of a mammal represents the cost of endothermy (warm-bloodedness), i.e., that the function of this futile cycle of proton pumping and leaking is to increase the amount of heat produced within cells, allowing mammals to raise their internal temperature up to values well above those of their surroundings. However, experiments with an ectotherm (cold-blooded) model, the bearded dragon, suggest that the relationship of mitochondrial uncoupling to metabolic rate may not be this simple. The oligomycin-insensitive respiration rate, or state-4 rate (representing proton leak), in lizard hepatocytes is only about 20% of the state-4 rate of rat hepatocytes (Brand *et al.*, 1991). However, bearded dragon hepatocytes consume oxygen at only one-fourth the rate of rat hepatocytes (Brand *et al.*, 1991). Thus, while it is true that the rate of proton leak is much reduced in the lizard relative to the rat, the cost of the proton leak, as a proportion of metabolic rate, is similar in the lizard and the rat. In both animals, proton leak accounts for 20–25% of metabolic rate. Thus the contribution of proton leak to metabolic rate appears to be conserved. This observation is important, as it suggests

that endothermy in the rat is not achieved simply by making the futile cycle of proton pumping and leaking a greater proportion of metabolic rate. Rather, it suggests that rates of all metabolic processes are increased in concert in the rat and that, in mammals, more systemic heat is produced at many steps in energy metabolism by augmenting the rates of each.

Evidence that the proportional contribution of proton leak to standard metabolic rate is conserved also comes from other experimental systems. Porter and Brand (1995a) compared proton leak rate as a proportion of total oxygen consumed in hepatocytes from a range of mammals of different body size. As body size increases, the rate of oxygen consumption in hepatocytes decreases (Porter and Brand, 1995a). The rate of proton leak is found to also decrease, so that it is maintained at approximately the same proportional value (about 25%) relative to total hepatocyte oxygen consumption (Porter and Brand, 1995a).

This observation can be extended at the level of whole animals. When mitochondria isolated from animals (ectothermic and endothermic vertebrates) with a range of whole body standard metabolic rates are compared, one finds that state-4 proton leak correlates reasonably well ($r = 0.75$) with standard metabolic rate (Brookes *et al.*, 1998). Thus, there is evidence that, across a wide range of ectothermic and endothermic vertebrates, the proportional contribution that proton leak rate makes to standard metabolic rate may be similar.

If the function of proton leak is not primarily thermogenesis, what is it for? One of the more appealing hypotheses is that it serves to limit the production of reactive oxygen species by the electron transport chain and so protect against aging (Skulachev, 1998).

THE MECHANISM OF PROTON CONDUCTANCE

The experiments outlined above confirm the importance of the process of proton leak to energy metabolism. However, despite many years of study, the mechanism(s) by which proton leak occurs remains unknown. Much debate has centered around whether the proton conductance is protein-catalyzed, or merely a physical property of the inner mitochondrial membrane. The latter hypothesis appeared to receive support from studies which demonstrated correlations between the phospholipid fatty acyl chain composition of the mitochondrial membranes and proton conduc-

tance (Brand *et al.*, 1991; Porter and Brand, 1995b; Porter *et al.*, 1996; Brookes *et al.*, 1998). In mammals, the proton leak rates of mitochondria correlate both with the surface area of the inner membrane and with specific indexes of phospholipid fatty acyl composition (Porter *et al.*, 1996; Brookes *et al.*, 1998).

The hypothesis that phospholipid fatty acyl composition determines proton conductance has been rigorously tested. In experiments on liposomes constructed from phospholipids of mitochondria of a range of animals, whose mitochondria had a range of proton permeabilities (Brookes *et al.*, 1997a), virtually no differences were found between the proton leak rates of any of the liposomes. Further, it has been observed that liposomes made from rat liver mitochondrial membrane phospholipids support a proton leak rate that is only about 5% of that of the intact mitochondria (Brookes *et al.*, 1997b). Thus, removal of membrane-bound proteins appears to result in the removal of the vast majority of the proton conductance property of mitochondrial inner membrane. These results are, however, consistent with at least two distinct possibilities: (1) the physical properties of the membrane (e.g., packing effects, surface charge, radius of curvature) are altered by the removal of all of the proteins; or (2) the protein (or proteins) which catalyze proton conductance have been removed.

There is, in fact, some evidence that the correlations between phospholipid fatty acyl composition and proton leak could exert themselves through specific proteins. Some of the specific fatty acyl compositional changes, which correlate with apparent increases in UCP1 activity in mouse brown adipose tissue (Claninin *et al.*, 1996), are the same as those identified as correlates of increased proton leak rate in mammalian mitochondria (Porter *et al.*, 1996).

MITOCHONDRIAL UNCOUPLING PROTEINS (UCPs)

In 1997, a discovery in the field of molecular biology opened the door for a fundamental reexamination of the mechanism of the proton conductance. Up until this time, the only known uncoupling protein was thermogenin (since designated UCP1), which is a demonstrated uncoupler of mitochondrial respiration (Nicholls, 1979). However, this protein could not be involved in the proton conductance observed in liver mitochondria because it is not expressed in liver. UCP1 occurs only in brown adipose tissue, where it catalyzes

a substantial proton conductance whose physiological purpose is known to be thermogenesis (Nicholls and Locke, 1984; see Lowell, 1998 for recent review). Fleury *et al.* (1997) and Gimeno *et al.* (1997) independently discovered and described an "uncoupling protein 2" (UCP2), a protein with a notably high (59%) amino acid identity to UCP1. An important feature of UCP2 was that it appeared to be expressed in nearly all cell types and could thus be a candidate for the mechanism of the ubiquitous basal proton conductance. This initial interpretation has since been revised somewhat. For example, UCP2 mRNA originally localized to the liver has been shown to be present only in the Kupffer cells, and not hepatocytes. Under normal conditions, very low or undetectable levels of UCP2 mRNA are measurable in hepatocytes (Carrettero *et al.*, 1998). UCP2 was transfected into yeast cells on an inducible expression vector (Fleury *et al.*, 1997; Gimeno *et al.*, 1997). When the protein is expressed, a drop in mitochondrial membrane potential is observed. In addition, measurements on isolated mitochondria were made (Fleury *et al.*, 1997) which showed lowered coupling (respiratory control ratios) in UCP2-containing mitochondria. These observations, combined with the striking similarity of the protein to UCP1, were taken as evidence for the protein's uncoupling function. Subsequently and in similar fashion, a plant UCP (stUCP) (Laloi *et al.*, 1997) and a mammalian UCP3 were found (Boss *et al.*, 1997). More recently, a possible fourth mammalian uncoupling protein—brain mitochondrial carrier protein (BMCP1; Sanchis *et al.*, 1998) has been identified. Because of the significance of the putative UCPs to the field, it is important to critically examine all of these studies.

The mitochondrial inner membrane potential results from the balance of the opposing functions of proton expulsion from the matrix and reentry through leak pathway(s) and/or the ATP synthase. Observation of a drop in membrane potential is not diagnostic of increased membrane proton conductance, as any change that disrupts electron transport and, therefore, proton pumping, can also give this result. It is not possible to evaluate from the data provided in Fleury *et al.* (1997) and Gimeno *et al.* (1997) whether the drop in membrane potential may have resulted from an inhibition of the electron transport side of the steady state or stimulation on the leak side. Unpublished results (Ricquier, 1998) indicate, however, that state-3 rates are not compromised in the UCP2 expressing mitochondria, which suggests that lowered membrane

potential may indeed result from an augmentation of the leak pathway (or ATP turnover).

An important concern with the yeast model used to measure uncoupling of respiration is the presence, in the line of yeast (laboratory strain W303) used in many of the above studies, of an ATP-inducible proton conductance pathway (Prieto *et al.*, 1992). The laboratory W303 strain of yeast, and a number of other laboratory strains, are distinct from wild-type yeast (Roucou *et al.*, 1997), in that physiologically relevant concentrations of ATP induce an augmentation of proton conductance in the mitochondria (Prieto *et al.*, 1992). While this inducible leak is apparently suppressed by millimolar concentrations of inorganic phosphate under some *in vitro* conditions (Prieto *et al.*, 1995), it is not clear to what extent it operates *in vivo*. If ATP levels in the cell vary in response to protein expression, induction of this pathway could increase or suppress proton leak rates secondarily. This may be relevant to the interpretation of the results of Paulik *et al.* (1998), who showed that UCP2 expression in a W303 strain of yeast caused an increase in oxygen consumption rate (measured as heat production), but the apparent increase in UCP2 expression in adipocytes, which, of course, lack the ATP-inducible pathway, did not. A possible influence of yeast strain (genetic background) is also evident in the results of Gimeno *et al.* (1997), where the magnitude of the depression of mitochondrial membrane potential is greater when UCP2 is expressed in W303-derived yeast cells than when expressed in yeast of the S288C genetic background. The existence of secondary effects related to the ATP-inducible proton leak pathway could be tested by comparing UCP homologs with other proteins that might be expected to alter cellular and mitochondrial ATP concentrations. Recently, such an experimental control has been employed by Sanchis *et al.* (1998). These authors found that expression of 2-oxoglutarate carrier caused none of the effects observed when the hUCP homolog BMCP1 was expressed. Nonetheless, it may be that the W303 strain of laboratory yeast are an inappropriate model for studies of proton leak, given the presence of this secondary, and inducible, proton leak pathway.

SEQUENCE HOMOLOGY AND OCCURRENCE OF UCPs IN INVERTEBRATES

Protein sequence similarity searches played an important role in the discovery of the new UCP1 homo-

logs, which were initially identified on the basis of their high amino acid sequence identity to the previously known brown fat uncoupling protein, thermogenin (UCP1) (Fleury *et al.*, 1997; Gimeno *et al.*, 1997; Boss *et al.*, 1997). In humans, UCP2 and UCP3 have 59 and 58% amino acid identity, respectively, to human UCP1 (hUCP1). Allowing for conservative amino acid substitutions, similarities are 75 and 73%, respectively.

What should be considered “high” sequence identity and is there a minimal level of sequence identity typical of two proteins with identical function? This question is probably not strictly answerable simply on the basis of similarity of amino acid sequences. However, it may be useful to gain some perspective on these relationships by examining the sequence identities of other pairs of proteins. UCP1 amino acid sequences are known for a number of mammals, including rabbits, guinea pigs, rats, mice, and cows. The UCP1s of these animals are 83, 79, 79, 79, and 81% identical, respectively, to hUCP1. This could tell us either that these animals are all closely enough related that little mutational change has occurred over the evolutionary distance separating the species, or alternatively, it could suggest that about 80% sequence identity represents some functionally vital conservation.

On the other hand, another possible new human uncoupling protein BMCP1 (brain mitochondrial carrier protein 1) has recently been identified and characterized (Sanchis *et al.*, 1998), which has only 34, 38, and 39% amino acid identity to hUCP1, hUCP2, and hUCP3, respectively. Paradoxically, this protein also shares 39% identity with a mitochondrial 2-oxoglutarate carrier, the closest identified relative to the hUCPs based on amino acid sequence comparisons. Even this latter number only marginally rises above the approximately 20–35% identities, which are typically observed between members of the mitochondrial transporter family of proteins. We can draw some tentative conclusions based on these facts. The mammalian UCP1s, which are known to function virtually identically, share 80% amino acid sequence identity. The human UCP homologs, whose functions remain ambiguous, share between 34 and 59% identity with UCP1.

These numbers are useful in determining criteria, admittedly somewhat arbitrary, for further database sequence searches. We have used such searches to look for UCP homologs amongst proteins of invertebrates. It has been observed repeatedly that mitochondria iso-

lated from a wide range of eukaryotic cells have an appreciable proton conductance, based on widely reported state-4 rates of respiration. This state-4 rate, indicating proton leak, is present in single and multicellular invertebrates as well as ectothermic (cold-blooded) vertebrate and warm-blooded nonmammals (e.g., birds). In short, the phenomenon of proton conductance is apparently ubiquitous in the animal kingdom. Thus, it seems reasonable that, if the UCPs catalyze this basal proton leak in mammals, they should have homologs in other animals. Here we address this question using sequence similarity searches.

The recent publication of complete genome sequences of two eukaryotes, the yeast, *Saccharomyces cerevisiae*, and the parasitic nematode worm, *Caenorhabditis elegans*, allowed an exhaustive and complete search for UCPs in these organisms, as in both cases, databases of the predicted amino acid sequences of the entire complement of cellular proteins are available. We also used two other eukaryote protein databases which are available in incomplete form. They are based on the approximately 55% sequenced genome of the fission yeast, *Schizosaccharomyces pombe*, and the 25% sequenced fruitfly, *Drosophila melanogaster*, genome.

We used the BLAST (Basic Local Alignment Search Tool) method of sequence comparison (Altschul *et al.*, 1990). This algorithm compares a given amino acid sequence to all such sequences in a database and computes a similarity score based not only on amino acid sequence identity, but also the probabilities of particular amino acid substitutions, lengths of sequences compared, opening of gaps in the sequence to allow for deletions and insertions, and the imposition of penalties associated with the extension of gaps.

BLAST searches of the protein databases of the yeast *S. cerevisiae* and *C. elegans* produced no compelling evidence for the existence of “mammal-like” UCPs. To illustrate BLAST scores associated with homologous proteins, which are known to perform the same function in different organisms, we performed individual searches with the human adenine nucleotide translocator (hANT), human phosphate transporter (hPT), human 2-oxoglutarate/malate carrier (h2OXO), human tricarboxylate transporter (hTCT), and yeast phosphate transporter (yphos) sequences. A BLAST score associated with 100% sequence identity is also shown by running a protein (yeast phosphate transporter) against itself in Table I.

Table I. BLAST Sequence Similarity Scores from Searches with the Amino Acid Sequences of Mitochondrial Transporter Family Proteins in the *Saccharomyces cerevisiae* Database^{a,b}

	YLR348C DIC1 dicarboxylate carrier	YKL120W PMT similarity to uncoupling protein	YBR291C CTP1 citrate transport protein	YJR095W ACR1 succinate-fumarate carrier	YBR085W AAC3 ADP/ATP carrier	YBL030C PET9 ADP/ATP carrier	YER053C strong similarity to phosphate carrier	YJR077C MIR1 phosphate transporter
hUCP1	366	354	238	262	182	178	135	163
hUCP2	352	323	249	278	221	230	110	122
hUCP3	363	326	238	291	234	243	125	126
stUCP	349	161	249	274	200	202	136	164
hANT	155	245	191	197	740	738	102	108
h2OXO	451	354	208	300	182	183	131	153
hTCT	152	211	534	427	164	163	130	194
hphos	116	144	157	170	114	105	719	573
yphos	148	108	167	195	119	108	555	1575

^a <http://speedy.mips.biochem.mpg.de/mips/yeast/index.html>

^b Key to abbreviations: human uncoupling proteins 1, 2 and 3 (hUCP 1, hUCP2, hUCP3); human adenine nucleotide translocator (hANT); human phosphate transporter (hphos); human 2-oxoglutarate/malate carrier (h2OXO); human tricarboxylate transporter (hTCT); plant uncoupling protein (stUCP); and yeast phosphate transporter (yphos). Yeast proteins shown represent the two highest-scoring yeast proteins for each search. Names of *S. cerevisiae* proteins are as they appear in the database. Numbers in bold represent highest scoring proteins.

In the *S. cerevisiae* database, the UCPs are most similar to a yeast protein identified as a dicarboxylate carrier (Table I). However, the human protein h2OXO is more similar to this protein. The similarity of the UCPs to the yeast protein identified as a putative uncoupling protein is no greater than the similarity of h2OXO to this protein. Human proteins with apparent homologs in the yeast database (i.e., hANT, h2OXO, hTCT, and hphos) score between 451 and 740. The

highest scoring UCP homologs, on the other hand, give BLAST scores of 349 to 366. Thus, in the yeast protein database, no protein is found which is notably similar to the UCPs.

A similar pattern emerges when the searches are repeated on the *C. elegans* database (Table II). The highest scoring protein, against the UCPs (scoring 410–428), in the database is the protein identified as a 2-oxoglutarate/malate carrier (B0432.4). However,

Table II. BLAST Sequence Similarity Scores from Searches with the Amino Acid Sequences of Mitochondrial Transporter Family Proteins in the *Caenorhabditis elegans* Database^{a,b}

	B0432.4 CE07742 2oxoglutarate/ malate carrier	K07B1.3 CE11880 proton channel	K11G12.5 CE02827 2-oxoglut. carrier	K11H3.3 CE00474 putative tricarbox transporter	K01H12.2 CE03454 ADP/ATP carrier	T01B11.4 CE12898 ADP/ATP translocase	F01G4.6 CE09162 phosphate carrier precursor	C33F10.12 CE04144 phosphate carrier
hUCP1	441	399	371	252	198	199	172	160
hUCP2	428	364	360	225	144	144	149	148
hUCP3	422	416	370	222	176	172	162	134
stUCP	410	358	368	229	227	225	165	129
hANT	149	191	161	128	978	976	107	92
h2OXO	1055	327	503	212	179	180	95	79
hTCT	157	112	136	811	156	156	150	111
hphos	153	130	129	140	132	129	1076	996
yphos	161	122	92	196	121	79	561	510

^a At www.sanger.ac.uk.

^b Abbreviations are as for Table I. Yeast proteins shown represent the four highest-scoring yeast proteins for UCP searches and the two highest scoring yeast proteins for other searches. Names of *C. elegans* proteins are as they appear in the database.

Table III. BLAST Sequence Similarity Scores from Searches with the Amino Acid Sequences of Mitochondrial Transporter Family Proteins in the *Schizosaccharomyces pombe* Database^{a,b}

	SPAC4G9 .20C putative mito carrier protein	SPAC19G12.05 hypothetical 31.6KD protein	SPBC12D12.05 C putative mito carrier protein	SPBC530 .10C (ANC1) ADP,ATP carrier	SPCC1442 .03 putative mito carrier protein	SPBC27B12 .09C FAD carrier
hUCP1	207	201	198	180	164	154
hUCP2	251	205	203	234	144	151
hUCP3	198	185	221	225	167	139
stUCP	197	236	205	206	151	148
hANT	119	117	265	692	117	94
h2OXO	188	199	163	147	161	140
hTCT	264	539	183	236	222	204
hphos	118	124	121	102	163	169
yphos	114	123	121	103	157	157

^a At www.sanger.ac.uk.

^b Abbreviations are as in Table I. Names of *S. pombe* proteins are given as they appear in the database.

when compared to human 2OXO, this protein scores 1055. The UCPs actually score lower (358–416) when compared to a protein entered in the database as a putative proton channel. All other comparisons with putative *C. elegans* homologs of human or yeast proteins score notably higher, from 510 for the comparison of yphos with C33F10.12, to 978 for the comparison of hANT with K01H12.2. Thus, on the basis of amino acid sequence similarities, there is no evidence for the presence in the *C. elegans* database of a protein with high homology to the UCPs.

Table IV. BLAST Sequence Similarity Scores from Searches with the Amino Acid Sequences of Mitochondrial Transporter Family Proteins in the Fruitfly *Drosophila melanogaster* Database^{a,b}

	Y12495 DMCOLT 2	Y10618 DMADPAT-PT 2
hUCP1	211	176
hUCP2	239	230
hUCP3	222	208
stUCP	228	238
hANT	176	1243
h2OXO	210	147
hTCT	257	187
hphos	221	155
yphos	143	143

^a At www.fruitfly.org.

^b Abbreviations are as in Table I. Names of fruitfly proteins are as they appear in the database. Note: no other proteins scored > 70.

In the partially complete databases, there are also no convincing UCP homologs. In the fission yeast protein database (Table III) the highest scoring match with the UCPs actually scores higher when compared with hTCT. The second highest UCP match in the yeast database is even more similar to hTCT, at a BLAST score of 539. These results are, of course, more ambiguous, as about 45% of the genome remains as yet unsequenced. It is possible that UCP homologs are present in the unsequenced portion of the genome. Note that fission yeast phosphate transporter genes appear to have not yet been sequenced. Nor is there a strong homologue to h2OXO.

No strong homologies to the UCPs are found in the fruitfly database (Table IV). However, a large portion of fruitfly proteins are not yet in the database, as evidenced by the presence of only two apparent members of the mitochondrial transporter protein family in this database. No other proteins in the database scored greater than 70 when compared to any of the human or yeast protein sequences.

Therefore, it must be concluded that no proteins with high sequence similarity to the UCPs exists in the yeast species *S. cerevisiae*, or in the invertebrate worm *C. elegans*. These results could indicate that the UCPs of nonmammals are simply indistinguishable, on the basis of amino acid sequence, from other mitochondrial transporter family proteins with different functions. However, it seems equally likely that the lack of identifiable UCP homologs is indicative of a lack of UCPs in these nonmammalian eukaryotes. This

leads us to a consideration of the possible role of the mammalian UCPs in catalyzing the proton leak observed in mammalian mitochondria.

As outlined above, mitochondria from all organisms probably have appreciable proton leak. Yet, apparently only mammalian mitochondria have UCPs. This suggests that there may be two components of proton leak in animal cells—a basal leak rate, which is not mediated by UCPs, and, at least in mammals, possibly an augmentative leak catalyzed by UCPs (Brand *et al.*, 1999). A leading hypothesis for the function of UCPs has been that they play an important role in mammalian thermogenesis. Some insight into the validity of this interpretation is possible, based on a number of recent studies linking changes in thermogenic status to UCP2 and/or UCP3 mRNA levels (Gong *et al.*, 1997; Boss *et al.*, 1998a,b; Boyer *et al.*, 1998; Lin *et al.*, 1998; Samec *et al.*, 1998; Sanchis *et al.*, 1998). No consistent or convincing correlation is found between the UCP2 or UCP3 mRNA levels and physiological states where thermogenesis is altered. This may indicate that the function of UCP1 homologs is not thermogenesis. However, it could also be that their activities are simply controlled in other ways. Perhaps UCP2 and UCP3 exist in active and inactive forms, turned on and off in response to the organism's thermogenic requirements. Alternatively, the activity of either protein may be modulated by effectors which signal that increased heat production is required. Unpublished results (Ricquier, 1998) suggest that UCP2 activity can, indeed, be modulated by metabolite effectors. Finally, it will be interesting to determine whether or not UCP1 homologs are present in ectothermic vertebrates. If their importance lies in thermogenesis than one would expect them to be absent in these animals.

CONCLUSIONS

In the absence of ATP synthesis, mitochondria from all eukaryotic cells continue to conduct protons from the cytosol into the mitochondrial matrix at appreciable rates. In cells and tissues where both proton leak rates and cellular respiration have been measured, this proton leak has been found to account for a substantial proportion, some 25–50%, of standard metabolic rate. The mechanism(s) by which proton leak occurs remains obscure, however. The absence, in non-mammals, of proteins with strong amino acid sequence identities to the human UCPs suggests that the proton leak observed in yeast and in invertebrates is not due

to proteins with strong sequence homology to human UCPs. It is likely that, in the mitochondria of all eukaryotic cells, including mammals, some basal level of proton leak exists, which occurs through, as yet, unknown mechanism(s). Recent studies suggest that, in mammals, some plants, and perhaps other organisms, this basal rate of proton leak may be augmented by some means. Clearly, the newly discovered UCP1 homologs UCP2, stUCP, UCP3, and BMCP1 are candidates for this augmentative proton leak. However, further experimental evidence, especially the demonstration of uncoupling in model systems other than W303 yeast, is required to verify this hypothesis. If the UCP1 homologs do indeed catalyze the augmentative leak rate in mammals, their purpose may not be thermogenesis.

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