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Transport Function and Regulation of Mitochondrial Uncoupling Proteins 2 and 3*

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Uncoupling protein 1 (UCP1) dissipates energy and generates heat by catalyzing back-flux of protons into the mitochondrial matrix, probably by a fatty acid cycling mechanism. If the newly discovered UCP2 and UCP3 function similarly, they will enhance peripheral energy expenditure and are potential molecular targets for the treatment of obesity. We expressed UCP2 and UCP3 in *Escherichia coli* and reconstituted the detergent-extracted proteins into liposomes. Ion flux studies show that purified UCP2 and UCP3 behave identically to UCP1. They catalyze electrophoretic flux of protons and alkylsulfonates, and proton flux exhibits an obligatory requirement for fatty acids. Proton flux is inhibited by purine nucleotides but with much lower affinity than observed with UCP1. These findings are consistent with the hypothesis that UCP2 and UCP3 behave as uncoupling proteins in the cell.

Uncoupling protein 1 (UCP1)[1] of brown adipose tissue mitochondria occupies a special place in bioenergetics, because it is the exception that proves the rule of Mitchell's elegant chemiosmotic theory (1), a protein designed to short circuit the redox proton pumps in order to generate heat and dissipate energy. UCP1 was identified from functional studies on brown adipose tissue mitochondria (2) and was one of the first membrane proteins to be sequenced (3).

For many years it was thought that UCP was expressed solely in mammalian brown adipose tissue; however, it now turns out that Nature has engineered at least five uncoupling proteins. In 1995, a plant uncoupling protein was discovered and later sequenced (4, 5), and 2 years later, UCP2 and UCP3 were identified (6–9). UCP4 was recently described as a brain-specific UCP (10). UCP2 maps to regions of human chromosome 11 and mouse chromosome 7 that have been linked to hyperinsulinemia and obesity, and it is hypothesized that UCP2 is the peripheral target for energy dissipation in the regulation of body weight. UCP2 is ubiquitously expressed in mammalian tissues, whereas UCP3 is expressed primarily in glycolytic skeletal muscle in humans and may account for the thermogenic effect of thyroid hormone (11). These aspects of this rapidly emerging area of research have been nicely reviewed by Boss *et al.* (12).

Virtually nothing is known about the transport functions of UCP2 and UCP3, and their putative physiological functions have been deduced primarily from their striking sequence identities with UCP1 (12). To address this problem, we expressed human UCP2 and UCP3 in *Escherichia coli*, where they accumulated in inclusion bodies. Following detergent extraction, we reconstituted the proteins into liposomes and measured H^+ and K^+ fluxes. Purified UCP2 and UCP3 both catalyzed electrophoretic flux of protons and alkylsulfonates, and proton flux exhibited an obligatory requirement for fatty acids. We also found that FA-dependent proton transport by UCP2 and UCP3 was inhibited by purine nucleotides, albeit with lower apparent affinities for nucleotides than those observed with UCP1. From these results, we conclude that UCP2 and UCP3 are functional uncoupling proteins and that their biophysical properties are consistent with a physiological role in energy dissipation.

EXPERIMENTAL PROCEDURES

Expression of UCPs in Saccharomyces cerevisiae—UCPs were expressed in yeast as described previously (13). Briefly, the *Sac*I/*Sph*I fragments from M13mp19 plasmid containing wild-type rat UCP1 cDNA were subcloned into *Sac*I/*Sph*I-cut pCGS110 *E. coli*/*S. cerevisiae* shuttle vector. The *S. cerevisiae* strain JB516 (MATa, ura3, ade1, leu2, his4, gal$^+$) was transformed with the shuttle vector construct and plated on uracil-lacking selective plates. The resulting yeast transformants were grown at 30 °C in selective medium, and overexpression of UCP1 was induced by the addition of 0.2% galactose (13). Similar protocols were followed for UCP2 and UCP3.

Expression of UCP2 and UCP3 in E. coli—Human UCP2 and human UCP3 open reading frames were amplified by PCR and inserted into the *Nde*I and *Not*I sites of the pET21a vector (Novagen). From DNA sequencing, the constructs are predicted to encode proteins with an amino acid sequence identical to the wild-type UCP2 or UCP3 proteins (6–9). Plasmids were transformed into the bacterial strain BL21 (Novagen). Transformed cells were grown at 30 °C to A_{600} = 0.6 and then induced with 1 mM isopropyl-β-D-thiogalactopyranoside at 30 °C for 6 h. Cells from a 700-ml culture were lysed in a French press in 20 ml of lysis buffer (10 mM Tris, pH 7, 1 mM EDTA, 1 mM dithiothreitol); the lysate was centrifuged at 27,000 × *g* for 15 min; and the pellet was resuspended in 20 ml of lysis buffer and centrifuged at 1000 × *g* for 3 min. 1-ml aliquots of the supernatant were centrifuged at 14,000 × *g* for 15 min in a microcentrifuge, and the resulting pelleted inclusion bodies were stored frozen at −70 °C.

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A preliminary account of these findings was reported in abstract form (33).

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[1] The abbreviations used are: UCP, uncoupling protein; FA, fatty acid(s); SLS, sodium lauroylsarcosinate; TEA^+, tetraethylammonium cation; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

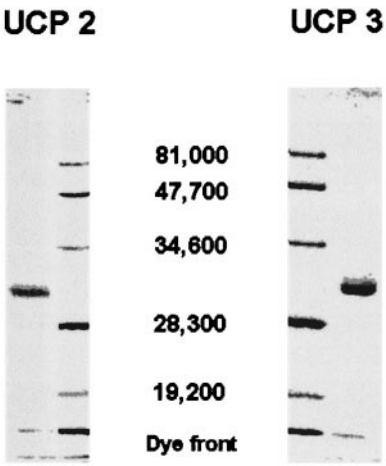

FIG. 1. **Purified, reconstituted UCP2 and UCP3.** Coomassie Blue-stained SDS-polyacrylamide gels from proteoliposomes containing UCP2 and UCP3. The proteins were expressed in *E. coli*, extracted from inclusion bodies, and reconstituted into liposomes. 10 μg of delipidated proteins were loaded onto each lane of the gel parallel to M_r standards.

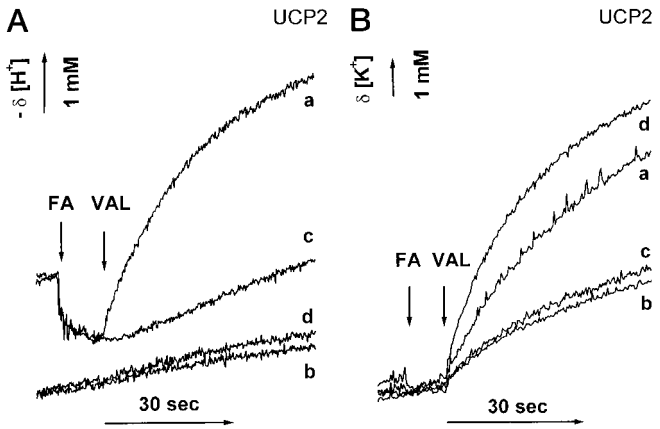

FIG. 2. **FA-dependent proton and undecanesulfonate transport via UCP2.** *A*, traces follow changes in intraliposomal acid ($\delta[H^+]$), which were determined from quenching of 6-methoxy-*N*-(3-sulfopropyl)quinolinium fluorescence by the anion of TES buffer (17). *Trace a*, 40 μM palmitate and 0.1 μM valinomycin were added sequentially. *Trace b*, valinomycin was added without FA. *Trace c*, liposomes *without* UCP2; FA and valinomycin were added sequentially. *Trace d*, 40 μM undecanesulfonate and valinomycin were added sequentially. (Note that *traces a* and *c* are offset for clarity). *B*, traces follow changes in total intraliposomal K^+ ($\delta[K^+]$), which were measured using potassium-binding benzofuran isophthalate fluorescence. Assay conditions and additions for each trace were identical to those described for *A*. Except for *trace c*, liposomes contained UCP2. H^+ efflux was driven by an inward K^+ gradient. These data are representative of more than 20 experiments on 10 different UCP2 reconstitutions.

Extraction of UCP2 and UCP3 from Inclusion Bodies—We modified published protocols (14, 15) for solubilization of *E. coli* inclusion bodies. The pelleted inclusion bodies (about 2 mg of protein) were suspended and washed three times in wash buffer (tetraethylammonium (TEA^+) salts of 0.15 M phosphate, 25 mM EDTA, 1 mM ATP, and 1 mM dithiothreitol, pH 7.8). The final pellet was solubilized in 0.4 ml of 50 mM TEA-TES, pH 7.2, containing 1.5% sodium lauroylsarcosinate (SLS). The extract was supplemented with 10 mg/ml asolectin and 3% octylpentaoxyethylene (C_8E_5 detergent) and then dialyzed for 15 h against 3 × 400 ml of extraction buffer (TEA^+ salts of 50 mM TES and 1 mM EDTA, pH 7.2) to remove SLS. In the first two dialysis periods (1 and 13 h), the extraction buffer was supplemented with 1 mM dithiothreitol and 0.03% sodium azide. These were removed from the final dialysis (1 h). Aliquots of the dialyzed extract, containing about 0.2 mg of protein, were stored at −20 °C.

Reconstitution of Uncoupling Proteins into Liposomes—Reconstitutions were carried out as described previously for UCP1 (16). Egg yolk phosphatidylcholine (UCP1) or soybean phospholipids (UCP2 and UCP3) were supplemented with cardiolipin (2 mg/ml), dried, and stored under nitrogen. Internal medium (TEA^+ salts of TES (30 mM), SO_4 (80 mM), and EDTA (1 mM), pH 7.2) was added to give a final concentration of 40 mg of phospholipid/ml of proteoliposome stock. The mixture was vortexed and sonicated to clarity in a bath sonicator, and detergent (10% C_8E_5), protein extract, and fluorescent probe were added. The final mixture (1.1 ml) was applied onto 2 ml of Bio-Bead SM-2 (Bio-Rad) column to remove the detergent. After 2 h of incubation, the column was centrifuged, and the resulting proteoliposomes were applied onto a new 2-ml Bio-Bead column, incubated for 30 min, and centrifuged. The formed vesicles (1 ml) were passed through a Sephadex G-25–300 column to remove external probe.

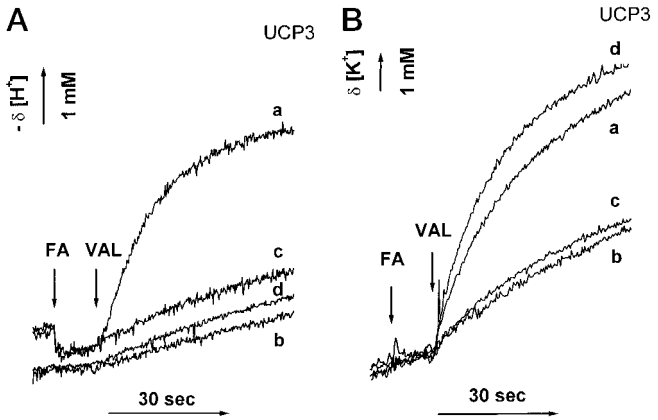

FIG. 3. **FA-dependent proton and undecanesulfonate transport via UCP3.** The traces shown were obtained under assay conditions identical with those described in Fig. 2 for UCP2, except that 40 μM laurate was used instead of palmitate. *A*, traces follow changes in intraliposomal acid ($\delta[H^+]$). *B*, traces follow changes in total intraliposomal K^+ ($\delta[K^+]$). Except for *trace c*, liposomes contained UCP3. These data are representative of more than 20 experiments on 10 different UCP3 reconstitutions.

TABLE I

K_i values for nucleotide inhibition of the uncoupling proteins

Experiments were carried out under identical assay conditions at pH 7.2 as described in the legend to Fig. 4.

Nucleotide	UCP1	UCP2	UCP3
	μM		
ATP	125 ± 5	760 ± 50 μM	650 ± 36 μM
GTP	20 ± 3	~1 mM	~1.7 mM
GDP	17 ± 2	~1.2 mM	~1 mM

Fluorescence Measurements of Ion Fluxes—Ion flux in proteoliposomes was measured using ion-specific fluorescent probes and an SLM Aminco 8000C spectrofluorometer. Measurements of H^+ fluxes were obtained from changes in 6-methoxy-*N*-(3-sulfopropyl)quinolinium fluorescence due to quenching by the anion of TES buffer (17). Measurements of K^+ fluxes, reflecting the movement of ionic charge across the membrane, were obtained from changes in potassium-binding benzofuran isophthalate fluorescence (16, 18). Internal and external media contained K^+ or TEA^+ salts of TES buffer (30 mM), SO_4 (80 mM), and EDTA (1 mM), pH 7.2. TEA^+ internal medium and K^+ external medium were used for the experiments of Figs. 3 and 4 to measure electrophoretic H^+ efflux, while these cations were reversed for the experiments of Figs. 5 and 6 to measure nucleotide inhibition. Each proteoliposome preparation was individually calibrated for fluorescent probe response, and its internal volume was estimated from the volume of distribution of the fluorescent probe (16).

Chemicals and Reagents—Potassium-binding benzofuran isophthalate and 6-methoxy-*N*-(3-sulfopropyl)quinolinium were purchased from Molecular Probes, Inc. (Eugene, OR). Undecanesulfonate was purchased from Research Plus, Inc. Asolectin (45% L-α- phosphatidylcholine) was purchased from Avanti Polar Lipids, Inc. Sulfuric acid was purchased from Fisher. Materials for UCP1 expression in yeast were from sources listed previously (17). All other chemicals were from Sigma. Purine nucleotides were adjusted to pH 7.2 with Tris base.

RESULTS

Isolation and Reconstitution of UCPs Expressed in E. coli—The development of a functional yeast expression system al-

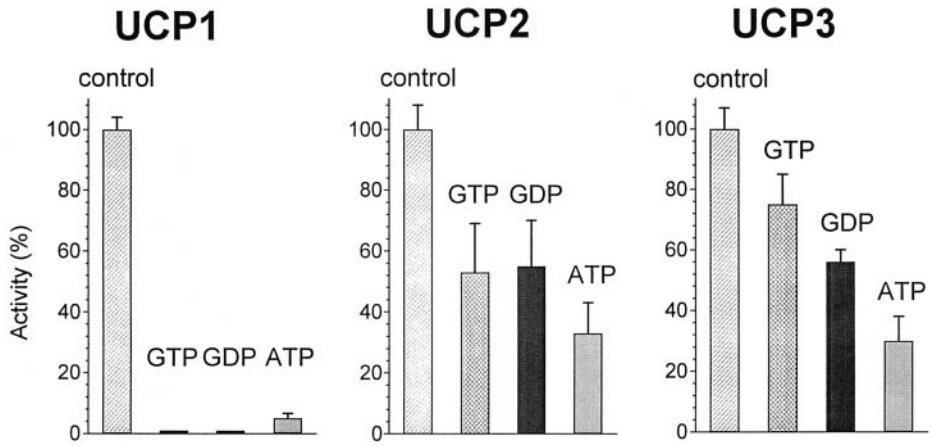

FIG. 4. **Sensitivities to nucleotide inhibition of UCP1, UCP2, and UCP3.** The *bars* represent residual, FA-induced proton flux in the presence of 1 mM nucleotide. 100% inhibition was estimated by extrapolation of inhibitor concentration plots obtained with ATP, as in Fig. 5. 50 μM laurate was used for UCP1 and UCP3, and 50 μM palmitate was used for UCP2. H^+ influx was driven by an outward K^+ gradient in the presence of 30 nM valinomycin. Assay pH was 7.2.

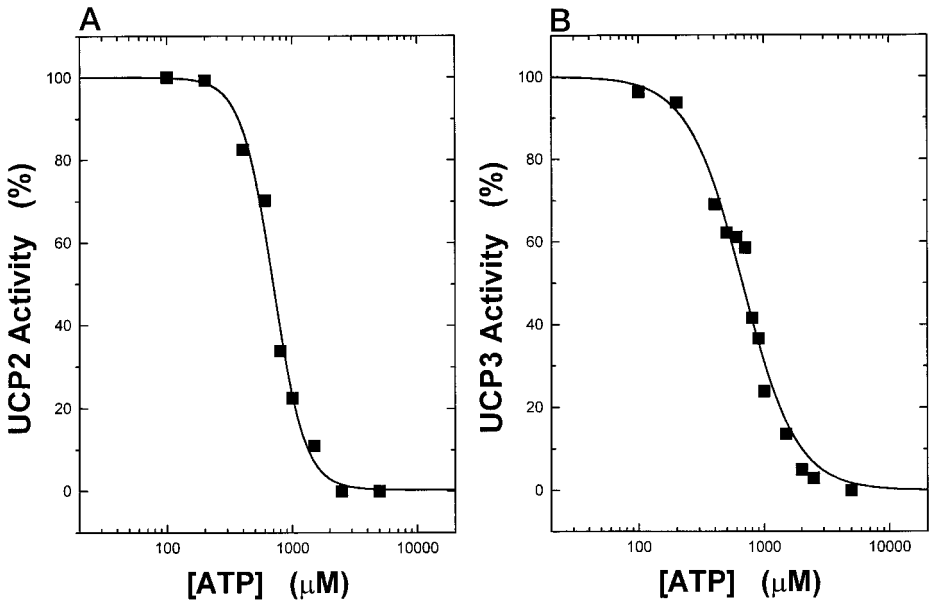

FIG. 5. **Concentration dependence of nucleotide inhibition of UCP2 and UCP3.** *A*, ATP inhibition of UCP2-mediated H^+ influx, in the presence of 50 μM palmitate, pH 7.2. The K_i for ATP inhibition is 710 μM. *B*, ATP inhibition of UCP3-mediated H^+ influx, in the presence of 50 μM laurate, pH 7.2. The K_i for ATP inhibition is 670 μM. The curves are representative of three independent preparations of UCP2 and -3.

lowed us to investigate structure-function relationships of UCP1 using site-directed mutagenesis (19, 20). Similarly, we attempted to use yeast expression for human UCP2 and UCP3 in order to study their function. As opposed to UCP1, however, both UCP2 and UCP3 expressed in lower quantities in yeast and were difficult to purify. Expression in *E. coli* yielded high amounts of UCP2 and UCP3, which accumulated in inclusion bodies. However, when the proteins were extracted using SLS detergent and reconstituted into liposomes, the proteins were found to be inactive (not shown). We obtained functionally active protein by supplementing the extract with asolectin and octylpentaoxyethylene and subjecting the extract to prolonged dialysis against SLS-free buffer. The dialysis may have reduced the SLS concentration, but this was not assayed. The proteoliposomes typically had an internal volume of 1.2 μl/mg of lipid and contained 3–5 μg protein/mg of lipid. To estimate the purity of reconstituted UCP2 and UCP3, the proteoliposomes were delipidated and subjected to SDS-polyacrylamide gel electrophoresis, with the results shown in Fig. 1.

Undecanesulfonate and Fatty Acids Induce Electrophoretic Fluxes in Liposomes Reconstituted with UCP2 and UCP3—The representative ion flux traces in Fig. 2 show that UCP2 catalyzes FA-dependent, electrophoretic proton flux. The traces in Fig. 2*A* follow H^+ movement across the membrane. It can be seen that FA induce a strong H^+ flux (Fig. 2*A*, *trace a*) that is absent in the absence of FA (*trace b)* and does not occur in liposomes without protein (*trace c).* Undecanesulfonate, an analogue of laurate, does not support H^+ transport (*trace d*). The traces in Fig. 2*B* follow K^+ movement. It can be seen that charge movement across the membrane exactly matches FA-induced H^+ flux (*trace a*), confirming that the H^+ flux is electrophoretic. Undecanesulfonate induced a strong K^+ flux (*trace d)*, demonstrating that the sulfonate anion is transported by UCP2.

A rapid intraliposomal acidification ensues upon the addition of FA (Fig. 2*A*, *traces a* and *c*). This is due to flip-flop of the protonated FA and acid-base equilibration (21). It is an electroneutral process, as evidenced by the lack of a corresponding K^+ jump upon the addition of FA (Fig. 2*B*).

The representative ion flux traces in Fig. 3 were obtained with UCP3, and they are qualitatively identical in every detail with those of UCP2. These results are highly reproducible and are representative of more than 20 assays from 10 or more preparations of each UCP.

Preliminary results from a kinetic study of UCP2 and UCP3 (not shown) indicate that there may be quantitative differences in FA preference among the UCPs. The K_m values for FA are similar among all three UCPs (10–20 nmol of FA/mg of lipid), and the V_{max} values for palmitate are also similar (10–30 μmol/mg·min). However, the V_{max} for laurate is much lower in UCP2 than in UCP1 or UCP3, indicating a preference for long-chain FA by UCP2.

Inhibition of UCPs by Purine Nucleotides—A second essential property of UCP1 is inhibition of fatty acid-induced proton

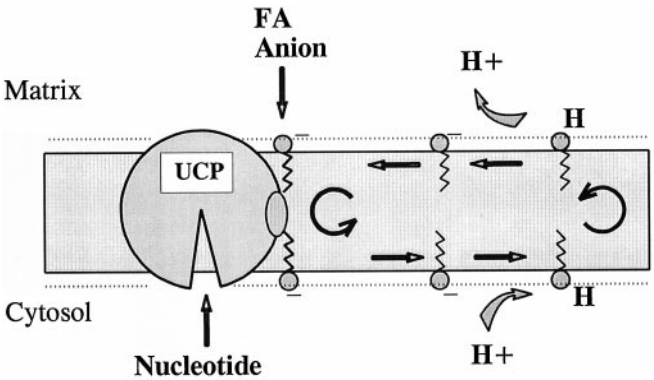

FIG. 6. **The UCP-catalyzed protonophoretic cycle.** The diagram shows an inner membrane segment containing UCP1. The complete uncoupling cycle consists of the following five steps. (i) FA anion partitions in the lipid bilayer with its head group at the level of the acyl glycerol linkages and *below* the surface of the phospholipid head groups. This location is shielded from the aqueous, which causes the pK_a values of FA in membranes to be 3–4 units higher than their values in solution (34). There is no significant flux of FA anion, because the bilayer energy barrier is too high (35). (ii) The FA anion diffuses laterally in the bilayer to reach a subsurface binding site on UCP that is shielded from the bulk aqueous phase (36). (iii) The energy barrier to FA anion transport is lowered by a weak binding site located about halfway through the UCP transport pathway (37). The electric field created by redox-linked proton ejection drives the anionic head group to the energy well. (iv) The FA carboxylate group is transported to the other side of the membrane and then diffuses laterally away from the conductance pathway. The preference of UCP for hydrophobic anions (36) indicates that the hydrophobic FA tail remains in the bilayer during transport. (v) The FA is protonated, and the protonated FA rapidly flip-flops again, delivering protons electroneutrally to the mitochondrial matrix and completing the cycle.

fluxes by purine nucleotides. To our surprise, we found striking differences in nucleotide sensitivity among the UCPs, as evidenced by the data in Fig. 4 comparing inhibition by 1 mM GDP, ATP, and GTP. To date, we have identified ATP as the most potent inhibitor of UCP2 (Fig. 5*A*) and UCP3 (Fig. 5*B*), although the apparent K_i values for ATP inhibition are considerably lower than that for UCP1 (Table I). UCP2 and UCP3 are notably less sensitive to GDP or GTP, which are potent inhibitors of UCP1.

DISCUSSION

Electrophoretic proton flux is the *sine qua non* of an uncoupling function. The data in Figs. 3 and 4 show that UCP2 and UCP3 meet this primary criterion, thereby establishing them as uncoupling proteins in function as well as in name. Indeed, the transport properties of UCP2 and UCP3 are qualitatively *identical* with those of UCP1 with respect to transport of protons and alkylsulfonates (21, 22).

The finding that FA are obligatory for proton flux mediated by UCP2 and UCP3, just as they are for UCP1 (22, 23), has important implications for the biophysical transport mechanism of UCPs, an issue that is not entirely resolved. We favor the FA protonophore model, shown in Fig. 6, in which UCPs contain a transport pathway for the anionic head groups of FA and alkylsulfonates. The head group is driven from one membrane leaflet to the other by the electric field generated by electron transport. When the FA carboxylate reaches one side, it picks up a proton and rapidly flip-flops back to release the proton to the other side. The UCPs thus catalyze a protonophoretic cycle, leading to uncoupling of oxidative phosphorylation (21).

An alternative model by Klingenberg and co-workers (24) proposes that UCP1 transports protons, that the transport pathway contains histidines, and that FA function as nonstoichiometric cofactors to buffer intrachannel protons. In a major advance, Bienengraeber *et al.* (25) demonstrated that substitution of two histidines (H145Q,H147N) in UCP1 caused selective loss of H^+ transport and concluded that these histidines constitute part of the proton conducting pathway. The authors go on to predict that UCP2, which contains neither histidine, will not conduct protons and that UCP3, which contains only one histidine, will conduct protons only weakly. In our view, the mutagenesis results are equally consistent with the FA protonophore model and suggest that the histidines in UCP1 form part of the surface binding site in the FA anion transport pathway. UCP2 and UCP3 possess ample basic residues in this region to fulfill such a role (26). Our interpretation therefore predicts that UCP2 and UCP3 will catalyze FA-dependent proton transport, and our results thus provide independent support for the FA protonophore model (Fig. 6).

Transport of the head group of undecanesulfonate also supports the FA protonophore model. Undecanesulfonate is a close analogue of laurate and is a competitive inhibitor of laurate-induced H^+ transport in UCP1 (22). The sulfonate group is transported across the membrane by all three UCPs; however, alkylsulfonates do not support H^+ transport. The reason for this failure is that sulfonates are very strong acids and, consequently, cannot deliver protons by electroneutral flip-flop across the bilayer (21). Thus, alkylsulfonates share the anion transport pathway in UCP1 with FA, but they cannot complete the protonophoretic cycle. The fact that the anionic head group of alkylsulfonates is *transported across the membrane* is a serious problem for the buffering model, because there is no known physicochemical mechanism that would permit alkylsulfonate anion transport and prohibit FA anion transport.

Inhibition by purine nucleotides is also an essential property of UCP1. Since FA have no effect on the K_i for nucleotide inhibition (22), it is generally agreed that transport and inhibition take place on different domains. The nucleotide binding domain in UCP1 is extensive and reasonably well characterized. The sugar-base moiety reacts with three residues located on the matrix segment that connects helices 5 and 6, an interaction that may confer selectivity among nucleotides (27–29). A glutamate, Glu^{190}, in the fourth transmembrane helix is the pH sensor for nucleotide binding (30). Three arginines, located in the transmembrane helices 2, 4, and 6, are required for nucleotide inhibition and have been shown to bind the nucleoside phosphates (20). Site-directed mutagenesis studies have led to a three-stage binding-conformational change model for nucleotide binding and inhibition in UCP1 (20).

It is noteworthy that the seven residues involved in nucleotide inhibition are largely conserved in UCP2, UCP3, and plant uncoupling protein, suggesting not only that these proteins would be regulated by nucleotides but also that regulation would be similar among the UCPs. Surprisingly, there are striking differences in nucleotide sensitivity among the UCPs, with UCP2 and UCP3 being only weakly sensitive to GDP, for example (Fig. 4, Table I). Similarly, plant uncoupling protein was also only weakly sensitive to purine nucleotides (31).

The physiological significance of variations in nucleotide inhibition is unclear, because it is not known how any of the UCPs are opened *in vivo*. In the case of UCP1, a common view is that uncoupling is initiated by dissociation of ATP (32). In our view, nucleotide debinding is an unlikely opening mechanism; to regulate important physiological processes, Nature normally relies on specific signaling pathways and not on the law of mass action. Regulation may involve post-translational modification of the proteins; however, no such signaling pathway has yet been demonstrated in the opening of any of the UCPs.

A major value of studies such as these on isolated, reconsti-

tuted UCPs is that they permit direct comparison with similar studies obtained using UCP1. In this regard, our most noteworthy finding is that the three mammalian UCPs are qualitatively identical in mediating FA-dependent proton transport. Studies on whole cells and isolated mitochondria containing native UCP2 and UCP3 are urgently needed to advance the field. It is hoped that the biophysical approach described here will prove useful as a guide to studies on the native system.

REFERENCES

1. Mitchell, P. (1961) *Nature* **191,** 144–148
2. Nicholls, D. G., and Locke, R. M. (1984) *Physiol. Rev.* **64,** 1–64
3. Aquila, H., Link, T. A., and Klingenberg, M. (1985) *EMBO J.* **4,** 2369–2376
4. Vercesi, A. E., Martins, I. S., Silva, M. A. P., and Leite, H. M. F. (1995) *Nature* **375,** 24
5. Laloi, M., Klein, M., Reismeier, J. W., Muller-Rober, B., Fleury, C., Bouillaud, F., and Ricquier, D. (1997) *Nature* **389,** 135–136
6. Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A., and Tartaglia, L. A. (1997) *Diabetes* **46,** 900–906
7. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) *Nat. Genet.* **15,** 269–272
8. Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dullo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997) *FEBS Lett.* **408,** 39–42
9. Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B (1997) *Biochem. Biophys. Res. Commun.* **235,** 79–82
10. Mao, W., Yu, X. X., Zhong, A., Li, W., Brush, J., Sherwood, S. W., Adams, S. H., and Pan, G. (1999) *FEBS Lett.* **443,** 326–330
11. Gong, D.-W., He, Y., Karas, M., and Reitman, M. (1997) *J. Biol. Chem.* **272,** 24129–24132
12. Boss, O., Muzzin, P., and Giacobino, J.-P. (1998) *Eur. J. Endocrinol.* **139,** 1–9
13. Murdza-Inglis, D. L., Patel, H. V., Freeman, K. B., Ježek, P., Orosz, D. E., and Garlid, K. D. (1991) *J. Biol. Chem.* **266,** 11871–11875
14. Fiermonte, G., Walker, J. E., and Palmieri, F. (1993) *Biochem. J.* **294,** 293–299
15. Schroers, A., Burkovski, A., Wohlrab, H., and Kramer, R. (1998) *J. Biol. Chem.* **273,** 14269–14276
16. Garlid, K. D., Paucek. P., Sun, X., and Woldegiorgis, G. (1995) *Methods Enzymol.* **266,** 331–348
17. Orosz, D. E., and Garlid, K. D. (1993) *Anal. Biochem.* **210,** 1–15
18. Ježek, P., Mahdi, F., and Garlid, K. D. (1990) *J. Biol. Chem.* **265,** 10522–10526
19. Murdza-Inglis, D. L., Modriansk´, M., Patel, H. V., Woldegiorgis, G., Freeman, K. B., and Garlid, K. D. (1994) *J. Biol. Chem.* **269,** 7435–7438
20. Modriansk´, M., Murdza-Inglis, D. L., Patel, V., Freeman, K. B., and Garlid, K. D. (1997) *J. Biol. Chem.* **272,** 24759–24762
21. Garlid, K. D., Orosz, D. E., Modriansk´, M., Vassanelli. S., and Ježek, P. (1996) *J. Biol. Chem.* **271,** 2615–2620
22. Ježek, P., Orosz, D. E., Modriansk´, M., and Garlid, K. D. (1994) *J. Biol. Chem.* **269,** 26184–26190
23. Strieleman, P. J., Schalinske, K. L., and Shrago, E. (1985) *J. Biol. Chem.* **260** 13402–13405
24. Klingenberg, M., and Huang, S-G. (1999) *Biochim. Biophys. Acta* **1415,** 271–296
25. Bienengraeber, M., Echtay, K. S., and Klingenberg, M. (1998) *Biochemistry* **37,** 3–8
26. Garlid, K. D., Jabůrek, M., and Ježek, P. (1998) *FEBS Lett.* **438,** 10–14
27. Winkler, E., and Klingenberg, M. (1992) *Eur. J. Biochem.* **203,** 295–304
28. Mayinger, P., and Klingenberg, M. (1992) *Biochemistry* **31,** 10536–10543
29. Huang, S.-G., and Klingenberg, M. (1995) *Biochemistry* **34,** 349–360
30. Winkler, E., Wachler, E., and Klingenberg, M. (1997) *Biochemistry* **36,** 148–155
31. Ježek, P., Costa, A. D. T., and Vercesi, A. E. (1996) *J. Biol. Chem.* **271,** 32743–32749
32. LaNoue, K. F., Strzelecki, T., Strzelecka, D., and Koch, C. (1986) *J. Biol. Chem.* **261,** 298–305
33. Garlid, K. D., Jabůrek, M., Vařecha, M., Gimeno, R. E., and Tartaglia, L. A. (1999) *Biophys. J.* **76,** A1
34. Hamilton, J. A., and Cistola, D. P. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 82–86
35. Garlid, K. D., Beavis, A. D., and Ratkje, S. K. (1989) *Biochim. Biophys. Acta* **976,** 6199–6205
36. Ježek, P., and Garlid, K. D. (1990) *J. Biol. Chem.* **265,** 19303–19311
37. Garlid, K. D. (1990) *Biochim. Biophys. Acta* **1018,** 151–154