Acta Biochimica Polonica

Vol. 48 No. 1/2001

145–155

QUARTERLY

Review

Uncoupling proteins in mitochondria of plants and some microorganisms★✪

Wiesława Jarmuszkiewicz✉

Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland

Received: 20 November, 2000; revised: 24 January, 2001; accepted: 20 February, 2001

Key words: mitochondria, uncoupling protein, fatty acid, alternative oxidase

Uncoupling proteins, members of the mitochondrial carrier family, are present in mitochondrial inner membrane and mediate free fatty acid-activated, purine-nucleotide-inhibited H^+ re-uptake. Since 1995, it has been shown that the uncoupling protein is present in many higher plants and some microorganisms like non-photosynthetic amoeboid protozoon, *Acanthamoeba castellanii* and non-fermentative yeast *Candida parapsilosis*. In mitochondria of these organisms, uncoupling protein activity is revealed not only by stimulation of state 4 respiration by free fatty acids accompanied by decrease in membrane potential (these effects being partially released by ATP and GTP) but mainly by lowering ADP/O ratio during state 3 respiration. Plant and microorganism uncoupling proteins are able to divert very efficiently energy from oxidative phosphorylation, competing for $\Delta\mu_H^+$ with ATP synthase. Functional connection and physiological role of uncoupling protein and alternative oxidase, two main energy-dissipating systems in plant-type mitochondria, are discussed.

According to the chemiosmotic energy-transduction concept, in respiring mitochondria electron transport through the respiratory chain is coupled to pumping of protons from the mitochondrial matrix, generating a proton electrochemical gradient ($\Delta\mu_H^+$) across the inner membrane [1] (Fig. 1). This protonmotive force drives the protons back into the matrix through the F_1F_0-ATP synthase resulting in phosphorylation of ADP and supports energy requiring processes,

★Presented at the 36th Meeting of the Polish Biochemical Society, Poznań, 2000.

✪This research was supported by the State Committee for Scientific Research (KBN, Poland) under grant 6 P04A 005 18.

✉Wiesława Jarmuszkiewicz, Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, A. Fredry 10, 61-701 Poznań, Poland; phone: (48 61) 829 4531; fax: (48 61) 829 4503; e-mail: wiesiaj@main.amu.edu.pl

Abbreviations: AOX, alternative oxidase; BAT, brown adipose tissue; BHAM, benzohydroxamate; BSA, bovine serum albumin; CAT, carboxyatractyloside; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; FFA, free fatty acids; LA, linoleic acid; PUMP, plant uncoupling mitochondrial protein; ROS, reactive oxygen species; state 3, respiration in the presence of ADP, phosphorylating respiration; state 4, resting respiration, respiration in the absence of ADP; UCP, uncoupling protein; $\Delta\Psi$, transmembrane electrical potential; $\Delta\mu_H^+$, proton electrochemical gradient.

like ion and substrate transport. In this way $\Delta\mu_H^+$ performs useful work. However, the proton electrochemical gradient built up by respiration can be dissipated by leak reactions catalyzed by endogenous proton-conductance pathways including mitochondrial uncoupling protein (UCP) [2]. UCP, located in the inner mitochondrial membrane, mediates free fatty acid (FFA)-activated, purine nucleotide-inhibited H^+ re-uptake.

Plant-type mitochondria (plant and some microorganism mitochondria) contain two energy-dissipating systems: (i) respiratory chain electron carriers that dissipate redox energy instead of building $\Delta\mu_H^+$, i.e., additional, insensitive to rotenone, external and internal NAD(P)H dehydrogenases and an alternative ubiquinol cyanide-resistant oxidase (AOX) [3]; and (ii) mentioned above uncoupling protein that dissipates energy contained in the proton electrochemical gradient [4, 5] (Fig. 1). Although these two energy-dissipating systems act at two different levels of the overall energy transduction pathway, they lead to the same final effect, i.e., decrease in the ATP synthesis yield.

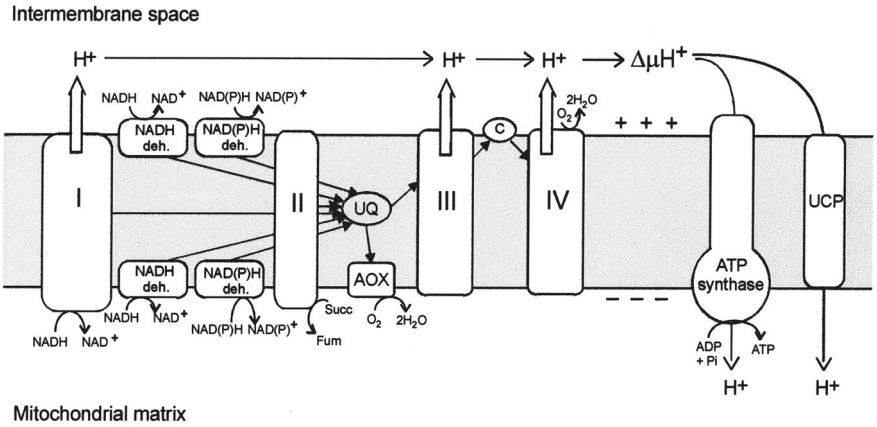

Figure 1. Schematic representation of the plant-type inner mitochondrial membrane.

Respiratory chain: I, complex I (NADH dehydrogenase); II, complex II (succinate dehydrogenase); III, complex III (cytochrome *bc1*); IV, complex IV (cytochrome *c* oxidase); c, cytochrome *c*; UQ, ubiqinone; deh., dehydrogenase; Succ, succinate; Fum, fumarate; AOX, alternative oxidase. Proton electrochemical gradient ($\Delta\mu_H^+$) consumers: ATP synthase; UCP, uncoupling protein. Other $\Delta\mu_H^+$ consumers, like ion and metabolite transporters, are not shown.

DISCOVERY OF NON-MAMMALIAN UNCOUPLING PROTEINS

Since its discovery in the late seventies, the first mammalian uncoupling protein called "thermogenin" or BAT UCP (renamed UCP1) has been believed to exist only in the brown adipose tissue (BAT) of mammals, and so, to be a late evolutionary acquisition required especially for transient thermogenesis in newborn, cold-acclimated, and hibernating mammals [6, 7]. Recent discovery of several novel uncoupling proteins in other mammalian tissues (UCP2 in several tissues, UCP3 in BAT and skeletal muscle, UCP4 and UCP5 in brain) showed that uncoupling protein is more widespread in tissues of higher animals than previously believed and could have various physiological roles [8–12].

In 1995, the first evidence for the existence of a UCP-like protein in plants (also called PUMP, plant uncoupling mitochondrial protein) was provided by respiration and membrane potential ($\Delta\Psi$) analysis of isolated potato mitochondria [4]. Furthermore, a 32 kDa protein, named PUMP has been isolated from potato tuber and reconstituted in proteoliposomes revealing FFA-mediated H^+ transport similar to that described for UCP1 [13]. Uncoupling protein has been subsequently detected immunologically in several climacteric fruits, i.e., those that exhibit a respiratory burst during ripening [14]. The existence of a UCP-like protein in plants was confirmed by the cloning of the first plant UCP cDNA, present in *Solanum tuberosum* (StUCP), encoding a peptide with high similarity to mammalian uncoupling proteins

[15]. Homologous cDNAs from *Arabidopsis thaliana* (AtUCP1 and AtUCP2) were subsequently cloned, indicating that two different uncoupling proteins exist in this plant [16, 17]. However, no tissue-specific expression of plant UCP is known yet.

In 1999, a UCP-like protein from *Acanthamoeba castellanii* (AcUCP), a non-photosynthetic soil amoeboid protozoon, was identified using antibodies raised against plant UCP and functionally characterized [18]. The presence of UCP in *A. castellanii,* which in molecular phylogenesis appears on a branch basal to the divergence points of plants, animals, and fungi [19], allowed hypothesizing that UCPs emerged, as specialized proteins for H^+ cycling, before the major radiation of phenotypic diversity in eukaryotes, maybe even earlier after acquisition of mitochondria in Eukarya and could occur in the whole eukaryotic world [18]. The first evidence and characterization of a UCP-like protein in fungi kingdom, namely in non-fermentative yeast *Candida parapsilosis* (CpUCP) [20], and its suspected presence in malaria parasite *Plasmodium berghei* [21] seem to confirm this hypothesis.

UCPs AS MEMBERS OF MITOCHONDRIAL CARRIER FAMILY

Structure

UCPs belong to the superfamily of anion carriers of the mitochondrial inner membrane [6]. The alignment of the amino-acid sequences of StUCP and AtUCP in comparison with mammalian UCPs sequences shows that these proteins are significantly similar (41% identity for both with UCP1, and 43% and 46% identity for StUCP and AtUCP with UCP2) [5, 22, 23]. UCPs are around 300-amino acid proteins with a predicted molecular mass of approx. 32 kDa. They exhibit a triplicated structure consisting of three domains of about 100 residues, each containing a signature motif that is also present in other mitochondrial carriers. Moreover, specific sequence motifs of UCPs, including plant UCPs, have been recently found [23]. These UCP signatures are thought to be involved in fatty acid anion binding and translocation. As in all other mitochondrial carriers, the predicted transmembrane topology for plant and mammalian UCPs reveals the existence of six transmembrane α-helices linked by polar loops.

Uncoupling mechanism of UCP

Two mechanisms describing H^+ re-uptake by UCP and FFA action are still under discussion (Fig. 2). In one model UCP is a true H^+ carrier and fatty acids are not translocated through the membrane. FFA bind to the carrier in such a way that their carboxyl groups serve as H^+ donors/acceptors to resident carboxyl groups (Asp or Glu). Histidine residues are proposed to be the final H^+ relay that liberates the proton into the mitochondrial matrix [6, 24]. In the other model, UCP acts as a fatty acid anion carrier, which transports the deprotonated fatty acid from the matrix to the cytosol where it becomes protonated. After protonation, fatty acid crosses the membrane by diffusion resulting in a H^+ re-uptake by a fatty acid cycling process [25, 26]. Recently, it has been proposed that the mechanism of the uncoupling activity of UCP1 could change according to the absence or presence of fatty acids and also according to FFA concentration. In the absence or presence of small amount of FFA, UCP works mainly as a H^+ carrier, while with increase in FFA content, it works dominantly as FFA carrier allowing the completion of a protonophoric cycle of fatty acids in the membrane [22]. Whatever is the true mechanism, UCP activity short-circuits the proton gradient generated by the respiratory chain.

Mitochondrial uncoupling by other carriers

Understanding of modulation of size of the proton gradient and the level of coupling of respiration by uncoupling protein is complicated as other members of the mitochondrial carrier family seem to mediate, at least in animal mitochondria, FFA-induced mitochondrial uncoupling [27], mainly in a high energy state (high $\Delta\mu_{H^+}$) of mitochondria. The FFA-dependent H^+ re-cycling is a side function of these carriers and can be inhib-

ited by their translocated substrates or specific inhibitors. Thus, the FFA-induced uncoupling could be mediated, at least in part, by the ATP/ADP antiporter [28], the aspartate/glutamate antiporter [29], the dicarboxylate carrier [30], and phosphate carrier [31], likely at high membrane potential but unlikely during phosphorylating respiration, when they are mainly employed in the import of ADP, glutamate, dicarboxylates, and phosphate, respectively. Furthermore, in plant mitochondria, some pieces of evidence in favour of the main responsibility of UCP in the FFA-induced H^+ re-cycling have been obtained during study of post-harvest tomato fruit ripening [32]. Indeed, a good correlation was established between UCP protein concentration in the mitochondrial membrane and the effect induced by linoleic acid (LA, an abundant fatty acid) in tomato fruit mitochondria.

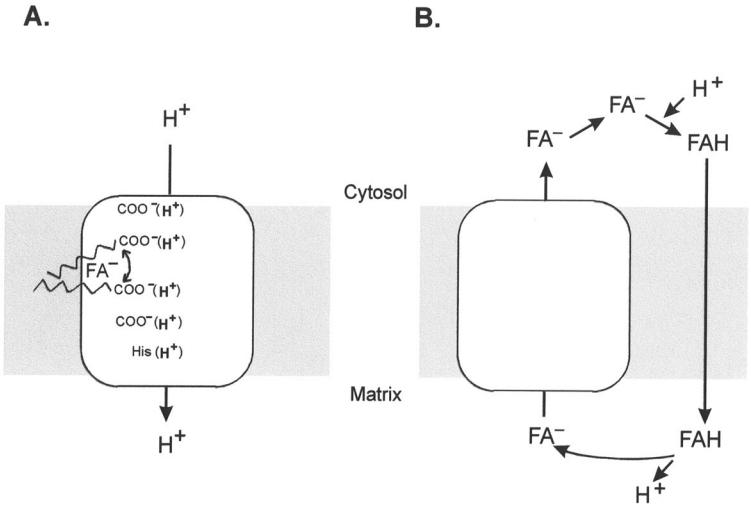

Figure 2. Two possible mechanisms of the action of uncoupling proteins showing H^+ transport and the participation of fatty acids.

A. True H^+ carrier model. Carboxylic groups of free fatty acids participate in the formation of the proton translocation pathway of UCP. B. Free fatty acid recycling model. UCP acts as fatty acid anion carrier participating in a protonophoric cycle of fatty acids. FA^-, fatty acid anion; FAH, protonated fatty acid.

FUNCTIONAL CHARACTERIZATION OF PLANT AND MICROORGANISM UCPs

The action of mammalian UCP is to mediate FFA-activated, purine nucleotide-inhibited H^+ re-uptake driven by $\Delta\Psi$ and pH (both constituting $\Delta\mu_H{}^+$). Using known activators of UCP1, mainly LA, mitochondrial uncoupling mediated by UCP was functionally characterized in mitochondria of some plants, i.e., potato tuber [4, 33], tomato and avocado fruits [34, 35], durum wheat seedlings [36], and two monocellular eukaryotes (non-photosynthetic amoeba *A. castellanii* and non-fermentative yeast *C. parapsilosis*) [18, 20]. Addition of FFA to isolated respiring mitochondria results in mitochondrial uncoupling revealed by an increase in resting (state 4) respiration, a decrease in membrane potential and a decrease in the ADP/O ratio (Fig. 3). Further additions of GTP (or ATP), that inhibits UCP, or BSA (fatty acid-free), that adsorbs FFA, cancel FFA-induced respiration and restores $\Delta\Psi$ (Fig. 3A). In the absence of FFA, coupling of respiring mitochondria, if they are not sufficiently depleted of endogenous FFA during preparation procedure, can be also improved by BSA and purine nucleotides. These effects are attributed to the presence in mitochondrial membrane of UCP similar to the mammalian mitochondrial UCP. In amoeba mitochondria, AcUCP (UCP of *A. castellanii*) was weakly inhibited by purine nucleotide di- or triphosphates contrarily to CpUCP (UCP of *C. parapsilosis*) [18, 20]. Sensitivity to these inhibitors seems to vary also within plant species. For instance, significant inhibition was observed in mitochondria of potato

tuber, postclimacteric avocado fruit, durum wheat, while weak inhibitory effect was observed in mitochondria of green tomato fruit and tential (16 μM, for durum wheat mitochondria) is much lower than the apparent K_m determined in reconstituted systems (83 μM) [13, 18, 36, 38].

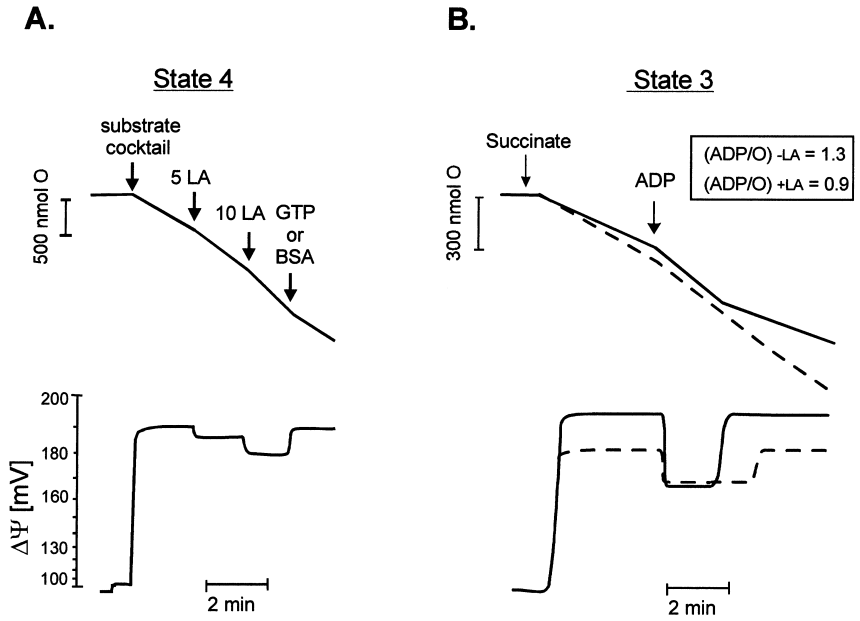

Figure 3. Influence of linoleic acid (LA) on respiratory rates and the transmembrane potential.

A. Mitochondria of *C. parapsilosis* (depleted of FFA) were incubated in the presence of 1.5 mM BHAM and 1 μM carboxyatractyloside. Additions: substrate cocktail (5 mM malate, 5 mM succinate, 5 mM pyruvate, and 1 mM NADH), 5 and 10 μM LA, 2 mM GTP, and 0.5% BSA. B. Mitochondria of green tomato fruit (depleted of FFA) were incubated in the presence of 5 μM rotenone and 1.5 mM BHAM, in the absence (solid lines) or presence (dashed lines) of 4 μM LA. Additions: 10 mM succinate, and pulse ADP. For details see [20, 39].

preclimacteric avocado fruit [4, 34–36]. As characterised by photoaffinity labelling, pH-sensitive purine nucleotide binding site of potato tuber mitochondria closely corresponds to the UCP of brown adipose tissue mitochondria [37]. In isolated potato mitochondria, interaction of UCP with FFA was also observed using swelling technique. FFA induced H^+-dependent purine nucleotide-sensitive mitochondrial swelling in potassium acetate medium in the presence of valinomycin [33]. Furthermore, potato and tomato UCP reconstituted into liposomes also reveal FFA-mediated H^+ transport similar to that described for UCP1 [13]. The activity of reconstituted protein was also downregulated by purine nucleotides (ATP and GTP). Comparison of respiratory experiments in isolated mitochondria with H^+ flux measurements in reconstituted systems indicates that the concentration of LA causing a half-maximum effect on respiration (8–10 μM for green tomato fruit and amoeba mitochondria) or on membrane po-

In tomato, amoeba (*A. castellanii*) and yeast (*C. parapsilosis*) mitochondria, a single force-flux relationship was observed for state 4 respiration with increasing concentration of LA or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and for state 3 respiration with increasing concentration of oligomycin or carboxyatractyloside (CAT) in the presence of benzohydroxamate (BHAM), an inhibitor of the alternative oxidase [18, 20, 34]. Modulation of the force ($\Delta\Psi$) either by phosphorylation potential (with inhibitor of ATP synthase), by H^+ permeability (with FCCP) or by LA addition leads to the same modification of the flow (respiratory rate) that reaches the same maximal value in the three conditions (Fig. 4). These observations indicate that LA has a pure protonophoretic effect like the artificial protonophore (FCCP), not acting directly on the activity of the respiratory chain, and that it is able to induce full uncoupling.

Although, in tomato, amoeba *A. castellanii* and yeast *C. parapsilosis* mitochondria, LA does not stimulate state 3 respiration (which is probably limited by the rate of electron supply by dehydrogenases to the respiratory chain), its uncoupling effect is evidenced by a decrease in ADP/O ratio and respiratory control [18, 20, 34, 38, 39] (Fig. 3B). LA concentration-dependent decrease in the ADP/O ratio indicates a progressive decrease in oxidative phosphorylation efficiency with increasing LA concentration. Thus, UCP is active during phosphorylating respiration (state 3) and is potentially able to decrease the yield of ATP synthesis, converting the osmotic energy into heat.

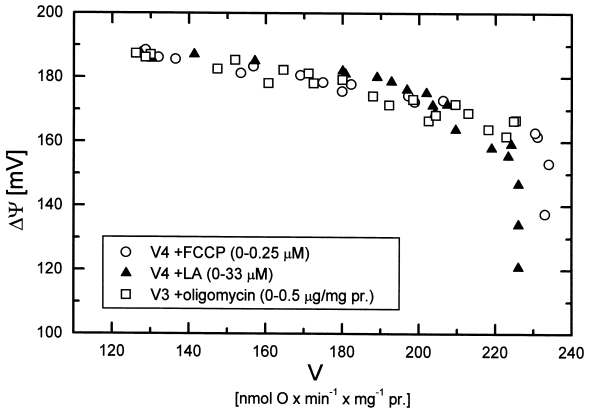

Figure 4. Relation between membrane potential (ΔΨ) and mitochondrial respiration in the presence of BHAM.

Mitochondria of *A. castellanii* were incubated in the presence of 10 mM succinate, 5 μM rotenone, 1.5 mM BHAM, and 170 μM ATP. State 4 (V4) was measured in the presence of 2 μg/mg of protein oligomycin with increasing concentration of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) or linoleic acid (LA). State 3 (V3) was measured in the presence of 2 mM ADP with increasing concentration of oligomycin. For details see [18].

UCP AND ATP SYNTHASE AS COMPETITORS FOR MITOCHONDRIAL $\Delta\mu_H^+$

Both UCP and ATP synthase are able to consume $\Delta\mu_H^+$ built up by the mitochondrial respiratory chain. They may be considered as two branching pathways: UCP as the $\Delta\mu_H^+$ energy-dissipating path, and ATP synthase as the $\Delta\mu_H^+$ energy-conserving path. The ADP/O method [40] has been applied to calculate the contributions of UCP activity and ATP synthesis in state 3 respiration of green tomato fruit mitochondria (fully depleted of FFA), using pair measurements of ADP/O ratios in the absence or presence of various LA concentrations [38, 41, 42]. Measurements made in the presence of BHAM (to exclude the oxygen consumption by AOX) and with increasing concentration of n-butyl malonate (an inhibitor of succinate uptake in order to decrease the rate of the quinone-reducing pathway i.e. succinate oxidation) allowed description of evolution of the UCP and ATP synthase contributions when the phosphorylating respiration decreases. As result, at fixed LA concentration, UCP activity remained constant and ATP synthesis decreased linearly [42] (Fig. 5). Using higher concentration of LA, UCP activity increased at the expense of ADP phosphorylation without an increase in the respiration. These results show how efficiently UCP activity can divert energy from oxidative phosphorylation, especially when state 3 respiration is progressively inhibited.

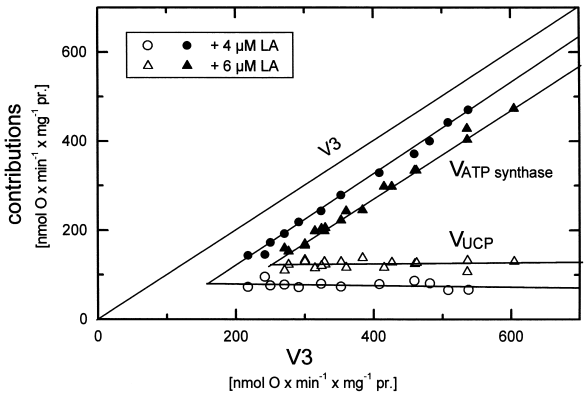

Figure 5. Partitioning of state 3 respiratory rate (V3) between uncoupling protein activity (V_{UCP}) and ATP synthesis (V_{ATP} synthase).

Data obtained from green tomato fruit mitochondria in the presence of two concentrations of LA. Oxidation rate of succinate (state 3 in the presence of 1.5 mM BHAM) was gradually decreased by increasing concentrations of n-butyl malonate (5–35 mM). As described in [42], contribution of ATP synthase (V_{ATP} synthase) is equal to V3 × $(ADP/O)_{+LA}/(ADP/O)_{-LA}$ and contribution of UCP (V_{UCP}) is equal to V3–V_{ATP} synthase. ADP/O ratios were determined in the absence or presence of 4 or 6 μM LA. For details see [42].

UCP AND AOX AS FREE-ENERGY DISSIPATING SYSTEMS

Functional connection

Mitochondria of plants and some microorganisms like amoeba *A. castellanii* possess two free-energy dissipating systems, UCP that dissipates $\Delta\mu_H^+$ and AOX that dissipates redox energy instead of building $\Delta\mu_H^+$, and can use them to modulate yield of ATP synthesis. AOX is a cyanide- and antymicin-resistant ubiquinol oxidase that catalyses the reduction of oxygen to water with electrons derived directly from ubiquinol, bypassing the energy-conserving sites (i.e. proton-translocating complexes III and IV) of the cytochrome pathway [3, 43].

In plant mitochondria, LA, that activates UCP, inhibits the cyanide-resistant respiration mediated by AOX [44–46]. It was shown for green tomato fruit mitochondria that increasing concentration of LA progressively decreases the AOX activity (Fig. 6) [46]. These results show how an increase in FFA level *in vivo* could affect both energy-dissipating systems but in opposite directions: AOX could be progressively switched off by an increase in the FFA content in cells when, simultaneously, UCP is switched on. They also indicate that in plant mitochondria AOX and UCP would never work together at their maximal activity [32, 46].

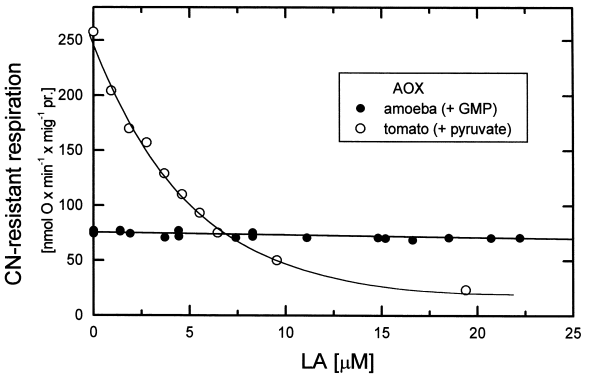

Figure 6. Cyanide-resistant respiration *versus* LA concentration in tomato and amoeba mitochondria.

Mitochondria were incubated in the presence of 10 mM succinate, 5–15 μM rotenone, and 2–2.5 μg/mg of protein oligomycin. Cyanide-resistant respiration was measured in the presence of 1–1.5 mM cyanide and in the presence of activators of the alternative oxidase: 0.15 mM pyruvate and 1 mM dithiothreitol for tomato mitochondria and 0.6 mM GMP for amoeba *A. castellanii* mitochondria. Increasing concentrations of LA (0–22 μM) were applied. For details see [18, 46].

As AOX and UCP activities seem to be mutually excluded *in vitro* in plant mitochondria, they could work sequentially during the life of plant cells according to their particular physiological state. As thermogenesis occurs during ripening [47, 48] and as FFA concentration increases in post-growing stage [49, 50], ripening of fruits provides an interesting model to study a relationship between AOX and UCP. Experiments following evolution of AOX and UCP activities and the amounts of immunodetectable proteins during post-harvest tomato fruit ripening indicate that AOX is active mainly during the growing period while UCP start working in post-growing state. Progressive increase in the cytosolic FFA content during fruit ripening could activate UCP but at the same time it inhibits AOX [32, 46, 51]. Therefore, the main function of UCP could be the modulation of $\Delta\mu_H^+$, according FFA concentration, while AOX bypassing proton pumping complexes, favours the cycling of cofactors necessary for biosynthesis and plant growth [51].

Contrary to plant mitochondria, in amoeba *A. castellanii* mitochondria possessing the plant-type branched respiratory chain, the two energy-dissipating systems are not co-regulated through FFA as shown by the insensitivity of GMP-stimulated amoeba AOX activity to LA (Fig. 6) and their cumulative uncoupling effect on ADP/O [18]. This observation does not mean that amoeba UCP and AOX are not co-regulated at all. Indeed, as both energy-dissipating systems lead to the same energetic result that is a decrease in the ATP synthesis, their metabolic usefulness can only be found in different physiological roles, and this point imposes a co-regulation perhaps through GMP/GDP/ GTP ratios [18].

Physiological role

An obvious physiological function of UCP and AOX can be recognised in specialised plant and animal thermogenic tissues as heat generation related to increase in temperature: in spadices of

Araceae during reproductive processes (AOX activity) [52] and in the mammalian brown adipose tissue (UCP activity) [7]. In unicellular microorganisms and in non-thermogenic plant tissues, the role of UCP and AOX is not fully understood. Since the activity of UCP and AOX and their protein expression in plants are induced by cold stress [15, 53–55], free energy dissipation by both systems could produce heat as a side event [56, 57]. Therefore, plant UCP and AOX may be concerned with response to the cold and the resistance to chilling. On the other hand, in non-thermogenic plant tissues, UCP and AOX could have a subtle role in the energy metabolism control working as safety valves when overloads in redox potential or/and in phosphate potential occur [51, 56, 57]. These overloads are consequences of imbalance between the reducing substrate supply and energy and carbon demands for biosynthesis, both being coupled by the respiratory chain activity. As operation of UCP can directly induce a drop in the phosphate potential, and as operation of AOX can directly diminish the rise of the reducing power, providing NAD^+ to the Krebs cycle and glycolysis, they could theoretically correct the imbalance [51, 56, 57].

One important physiological role of the energy dissipating systems can be related to the cell protection against mitochondrial production of reactive oxygen species (ROS) [27]. First, it has been demonstrated that the plant AOX may prevent formation of ROS by mitochondria at the level of ubiquinol especially when the cytochrome pathway is slowed down [58–62]. In the case of plant UCP, it was first shown in potato tuber mitochondria, that the inhibition of UCP activity significantly increases mitochondrial hydrogen peroxide generation, while UCP substrates, such as LA, reduce this generation [63]. Furthermore, in durum wheat mitochondria, it was shown that exogenous ROS could increase the effect of LA on the membrane potential [36]. Recently, it has been suggested that in mitochondria of pea stem, isolated from actively growing tissues, two energy dissipating systems, UCP and AOX could accomplish their protective effects against oxidative damage as a function of the type of tissue and the developmental state of the plant [64].

In conclusion, UCP and AOX, two main energy-dissipating systems in mitochondria of plant and some microorganism, appear to have a central role in the balance of cell energy metabolism related to the regulation of ATP production, control of the $NADH/NAD^+$ ratio, and limitation of the level of mitochondrial ROS production. Moreover, the presence of both UCP and AOX in the non-photosynthetic ameboid protozoon *A. castellanii*, which in the molecular phylogenic tree appears on a branch basal to the divergence points of plants, fungi, and animals leads to the following hypothesis. Appearance of intracellular (mitochondrial) oxidative phosphorylation was very soon accompanied by the emergence not only of UCP, a protein carrier specialized in FFA-mediated H^+ recycling, but also of AOX, an energy-dissipating oxidase that both can modulate the tightness of coupling between respiration and the ATP synthesis, thereby maintaining a balance between energy supply and demand in the cell [18].

REFERENCES

1. Mitchell, P. (1961) Coupling of phosphorylation to electron and proton transfer by a chemiosmotic type of mechanism. *Nature* **191**, 144–148.
2. Stuart, J.A., Brindle, K.M., Harper, J.A. & Brand, M.D. (2000) Mitochondrial proton leak and uncoupling proteins. *J. Bioenerg. Biomembr.* **31**, 517–525.
3. Mackenzie, S. & McIntosh, L. (1999) Higher plant mitochondria. *Plant Cell* **11**, 571–585.
4. Vercesi, A.E., Martins, I.S., Silva, M.A.P., Leite, H.M.F., Cuccovia, I.M. & Chaimovich, H. (1995) PUMPing plants. *Nature* **375**, 24.
5. Laloi, M. (1999) Plant mitochondrial carriers: An overview. *Cell. Mol. Life Sci.* **56**, 918–944.
6. Klingenberg, M. (1990) Mechanism and evolution of the uncoupling protein of brown adipose tissue. *Trends Biochem. Sci.* **15**, 108–112.
7. Nicholls, D.G. & Rial, E. (1999) A history of the first uncoupling protein, UCP1. *J. Bioenerg. Biomembr.* **31**, 399–418.

8. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Boillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D. & Warden, C.H. (1997) Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15**, 269–272.

9. Boss, O., Samec, S., Paolonigiacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. & Giacobino, J.-P. (1997) Uncoupling protein-3: A new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* **408**, 39–42.

10. Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J.S. & Lowell, B.B. (1997) UCP3: An uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem. Biophys. Res. Commun.* **235**, 79–82.

11. Mao, W.G., Yu, X.X., Zhong, A., Li, W.L., Brush, J., Sherwood, S.W., Adams, S.H. & Pan, G. (1999) UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. *FEBS Lett.* **443**, 326–330.

12. Sanchis, D., Fleury, Ch., Chomiki, N., Goubern, M., Huang, Q. L., Neverova, M., Grégoire, F., Easlick, J., Raimbault, S., Lévi-Meyrueis, C., Miroux, B., Collins, S., Seldin, M., Richard, D., Warden, C., Bouillaud, F. & Ricquier, D. (1998) BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast. *J. Biol. Chem.* **273**, 34611–34615.

13. JeSek, P., Costa, A.D.T. & Vercesi, A.E. (1997) Reconstituted plant uncoupling mitochondrial protein allows for proton translocation *via* fatty acid cycling mechanism. *J. Biol. Chem.* **272**, 24272–24278.

14. JeSek, P., Engstová, H., Sačková, M., Vercesi, A.E., Costa, D.T., Arruda, P. & Garlid, P. (1998) Fatty acid cycling mechanism and mitochondrial uncoupling proteins. *Biochim. Biophys. Acta* **1365**, 319–327.

15. Laloi, M., Klein, M., Riesmeier, J.W., Muller-Rober, B., Fleury, C., Bouillaud, F. & Ricquier, D. (1997) A plant cold-induced uncoupling protein. *Nature* **389**, 135–136.

16. Maia, I.G., Benedetti, C.E., Leite, A., Turcinelli, S.R., Vercesi, A.E. & Arruda, P. (1998) AtPUMP: An *Arabidopsis* gene encoding a plant uncoupling mitochondrial protein. *FEBS Lett.* **429**, 403–406.

17. Watanabe, A., Nakazano, M., Tsutsumi, N. & Hirai, A. (1999) AtUCP2: A novel isoform of the mitochondrial uncoupling protein of *Arabidopsis thaliana*. *Plant Cell Physiol.* **40**, 1160–1166.

18. Jarmuszkiewicz, W., Sluse-Goffart, C.M., Hryniewiecka, L. & Sluse, F.E. (1999) Identification and characterization of a protozoan uncoupling protein in *Acanthamoeba castellanii*. *J. Biol. Chem.* **274**, 23198–23202.

19. Gray, M.W., Burger, G. & Lang, B.F. (1999) Mitochondrial evolution. *Science* **283**, 1476–1481.

20. Jarmuszkiewicz, W., Milani, G., Fortes, F., Schreiber, A.Z., Sluse, F.E. & Vercesi, A.E. (2000) First evidence and characterization of an uncoupling protein in fungi kingdom: CpUCP of *Candida parapsilosis*. *FEBS Lett.* **467**, 145–149.

21. Uyemura, S.A., Luo, S., Moreno, S.N.J. & Docampo, R. (2000) Oxidative phosphorylation, Ca^{2+} transport, and fatty acid-induced uncoupling in malaria parasites mitochondria. *J. Biol. Chem.* **275**, 9709–9715.

22. Ricquier, D. & Bouillaud, F. (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem. J.* **345**, 161–179.

23. JeSek, P. & Urbanková, E. (2000) Specific sequence motifs of mitochondrial uncoupling proteins. *IUBMB Life*, **49**, 63–70.

24. Winkler, E. & Klingenberg, M. (1994) Effect of fatty acids on H^+ transport activity of the reconstituted uncoupling protein. *J. Biol. Chem.* **269**, 2508–2515.

25. Skulachev, V.P. (1991) Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. *FEBS Lett.* **264**, 246–248.

26. Garlid, K.D., Orosz, D.E., Modriansky, M., Vassanelli, S. & JeSek, P. (1996) On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. *J. Biol. Chem.* **271**, 2615–2620.

27. Skulachev, V.P. (1998) Uncoupling: New approaches to an old problem of bioenergetics. *Biochim. Biophys. Acta*, **1363**, 100–124.

28. Andreyev, A.Y., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Tsofina, L.M., Volkov, N.L. & Vygodina, T.V. (1989) The ATP/ADP-antiporter is involved in the uncoupling

effect of fatty acids on mitochondria. *Eur. J. Biochem.* **182**, 585–592.

29. Smartsev, V.N., Mokhova, E.N. & Skulachev, V.P. (1997) The pH-dependent reciprocal changes in contribution of ADP/ATP antiporter and aspartate/glutamate antiporter to the fatty acid-induced uncoupling. *FEBS Lett.* **412**, 179–182.

30. Więckowski, M. & Wojtczak, L. (1997) Involvement of the dicarboxylate carrier in the protonophoric action of long-chain fatty acids in mitochondria. *Biochem. Biophys. Res. Commun.* **232**, 414–417.

31. Sačková, M., Krämer, R. & JeSek, P. (2000) Interaction of mitochondrial phosphate carrier with fatty acids and hydrophobic phosphate analogs. *Int. J. Biochem. Cell Biol.* **32**, 499–508.

32. Almeida, A.M., Jarmuszkiewicz, W., Khomsi, H., Arruda, P., Vercesi, A.E. & Sluse, F.E. (1999) Cyanide-resistant, ATP synthesis-sustained, and uncoupling protein-sustained respiration during postharvest ripening of tomato fruit. *Plant Phy*siol. **119**, 1323–1329.

33. JeSek, P., Costa, A.D.T. & Vercesi, A.E. (1996) Evidence for anion-translocating plant uncoupling mitochondrial protein in potato mitochondria. *J. Biol. Chem.* **271**, 32743–32748.

34. Jarmuszkiewicz, W., Almeida, A.M., Sluse-Goffart, C.M., Sluse, F.E. & Vercesi, A.E. (1998) Linoleic acid-induced activity of plant uncoupling mitochondrial protein in purified tomato fruit mitochondria during resting, phosphorylating, and progressively uncoupled respiration. *J. Biol. Chem.* **273**, 34882–34886.

35. Saviani, E.E., da Silva, A. & Martins, I.S. (1998) Role of F_1F_0-ATP synthase and uncoupling protein in mitochondrial respiratory uncoupling of ripened avocado fruits. *Plant Physiol. Biochem.* **36**, 575–582.

36. Pastore, D., Fratianni, A., Pede, S.D. & Passarella, S. (2000) Effects of fatty acids, nucleotides and reactive species on durum wheat mitochondria. *FEBS Lett.* **470**, 88–92.

37. Saviani, E.E., da Silva, A., Jr. & Martins, I.S. (1997) Photoaffinity labelling of the uncoupling protein from potato tuber mitochondria. *Plant Physiol. Biochem.* **35**, 701–706.

38. Jarmuszkiewicz, W., Almeida, A.M., Vercesi, A.E., Sluse, F.E. & Sluse-Goffart, C.M. (2000) Proton re-uptake partitioning between uncoupling protein and ATP synthase during benzohydroxamic acid-resistant state 3 respiration in tomato fruit mitochondria. *J. Biol. Chem.* **275**, 13315–13320.

39. Jarmuszkiewicz, W., Hryniewiecka, L., Sluse-Goffart, C.M. & Sluse, F.E. (2000) Efficiency of *Acanthamoeba castellanii* uncoupling protein in energy-dissipating processes; in *Animating the Cellular Map* (Hofmeyr, J.-H., Rohwer, J.M., Snoep, J.L., eds.) pp. 119–123, Stellenbosch University Press.

40. Jarmuszkiewicz, W., Sluse-Goffart, C.M., Hryniewiecka, L., Michejda, J. & Sluse, F.E. (1998) Electron partitioning between the two branching quinol-oxidizing pathways in *Acanthamoeba castellanii* mitochondria during steady-state state 3 respiration. *J. Biol. Chem.* **273**, 10174–10180.

41. Sluse, F.E., Jarmuszkiewicz, W., Almeida, A.M., Vercesi, A.E., Hryniewiecka, L. & Sluse-Goffart, C.M. (1998) Determination of actual contributions of energy-dissipating pathways in mitochondrial respiration: the ADP/O method; in *Plant Mitochondria: from Gene to Function* (Möller, I.M., Gardestrom, P., Glimelius, K., Glaser, E., eds.) pp. 519–523, Backhuys Publishers, Leiden.

42. Sluse, F.E., Jarmuszkiewicz, W., Almeida, A.M., Vercesia, A.E. & Sluse-Goffart, C.M. (2000) Proton partitioning between ATP synthase and uncoupling protein during cytochrome pathway state 3 respiration in tomato fruit mitochondria; in *Animating the Cellular Map* (Hofmeyr, J.-H., Rohwer, J.M., Snoep, J.L., eds.) pp. 135–140, Stellenbosch University Press.

43. Vanlerberghe, G.C. & McIntosh, L. (1997) Alternative oxidase: From gene to function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 703–734.

44. Kay, C.J. & Palmer, J.M. (1985) Solubilization of the alternative oxidase of cuckoo-pint (*Arum maculatum*) mitochondria. *Biochem. J.* **228**, 309–318.

45. Minagawa, N., Sakajo, S. & Yoshimoto, A. (1992) Effects of unsaturated fatty acids on CN-resistant respiration of mitochondria isolated from *Hansenula anomala*. *Biosci. Biotechnol. Biochem.* **56**, 1342–1343.

46. Sluse, F.E., Almeida, A.M., Jarmuszkiewicz, W. & Vercesi, A.E. (1998) Free fatty acids regulate the uncoupling protein and alternative oxidase activi-

ties in plant mitochondria. *FEBS Lett.* **433**, 237–240.

47. Kumar, S., Patil, B.C. & Sinha, S.K. (1990) Cyanide-resistant respiration is involved in temperature rise in ripening mangoes. *Biochem. Biophys. Res. Commun.* **168**, 818–822.

48. Kumar, S. & Sinha, S.K. (1992) Alternative respiration and heat production in ripening banana fruits (*Musa paradisiaca* var. *Mysore Kadali*). *J. Exp. Bot.* **43**, 1639–1642.

49. Güçlü, J., Paulin, A. & Soudain, P. (1989) Changes in polar lipids during ripening and senescence of cherry tomato (*Lycopersicn esculentum*): Relation to climacteric and ethylene increases. *Plant Physiol.* **77**, 413–419.

50. Rouet-Mayer, M.A., Valentova, O., Simond-Cote, E., Daussant, J. & ThPvenot, C. (1995) Critical analysis of phospholipid hydrolyzing activities in ripening tomato fruits: Study by spectrofluorimetry and high-performance liquid chromatography. *Lipids* **30**, 739–746.

51. Sluse, F.E. & Jarmuszkiewicz, W. (2000) Activity and functional interaction of alternative oxidase and uncoupling protein in mitochondria from tomato fruit. *Braz. J. Med. Biol. Res.* **33**, 259–268.

52. Meeuse, B.D.J. (1975) Thermogenic respiration in Aroids. *Annu. Rev. Plant Physiol.* **26**, 117–126.

53. Elthon, T.E., Stewart, C.E., McCoy, C.A. & Bonner, W.D., Jr. (1986) Alternative respiratory path capacity in plant mitochondria: Effect of growth temperature, the electrochemical gradient, and assay pH. *Plant Physiol.* **80**, 378–383.

54. Duque, P. & Arrabaca, J.D. (1999) Respiratory metabolism during cold storage of apple friuit. II. Alternative oxidase is induced at the climacteric. *Physiol. Plant.* **107**, 24–31.

55. Nantes, I.L., Fagian, M.M., Catisti, R., Arruda, P., Maia, I.G. & Vercesi, A.E. (1999) Low temperature and aging-promoted expression of PUMP in potato tuber mitochondria. *FEBS Lett.* **457**, 103–106.

56. Jarmuszkiewicz, W., Sluse-Goffart, C.M., Vercesi, A.E. & Sluse, F.E. (2000) Alternative oxidase and uncoupling protein: Thermogenesis *versus* cell energy balance. *Biosci. Rep.* (in press).

57. Sluse, F.E. & Jarmuszkiewicz, W. (2000) Dynamics of H^+ fluxes in mitochondrial membrane; in *Handbook of Plant Growth: pH as a Master Variable in Plant Growth* (Rengel, Z., ed.) Marcel Dekker, Inc. New York (in press).

58. Purvis, A.C. & Shewfelt, R.L. (1993) Does the alternative pathway ameliorate chilling injury sensitive plant tissues? *Physiol. Plant.* **88**, 712–718.

59. Purvis, A.C. (1997) Role of the alternative oxidase in limiting superoxide production by plant mitochondria. *Physiol. Plant.* **100**, 165–170.

60. Wagner, A.M. & Moore, A.L. (1997) Structure and function of the plant alternative oxidase: Its role in the oxygen defence mechanism. *Biosci. Rep.* **17**, 319–333.

61. Popov, V.N., Simonian, R.A., Skulachev, V.P. & Starcov, A.A. (1997) Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. *FEBS Lett.* **415**, 87–90.

62. Maxwell, D.P., Wang, Y. & McIntosh, L. (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8271–8276.

63. Kowaltowski, A.J., Costa, A.D.T. & Vercesi, A.E. (1998) Activation of the potato plant uncoupling mitochondrial protein inhibits reactive oxygen species generation by the respiratory chain. *FEBS Lett.* **425**, 213–216.

64. Casolo, V., Braidot, E., Chiandussi, E., Macri, F. & Vianello, A. (2000) The role of mild uncoupling and non-coupled respiration in the regulation of hydrogen peroxide generation by plant mitochondria. *FEBS Lett.* **474**, 53–57.