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Plant Uncoupling Mitochondrial Protein and Alternative Oxidase: Energy Metabolism and Stress

Jiří Borecký[1] and Aníbal E. Vercesi[1,2]

Energy-dissipation in plant mitochondria can be mediated by inner membrane proteins via two processes: redox potential-dissipation or proton electrochemical potential-dissipation. Alternative oxidases (AOx) and the plant uncoupling mitochondrial proteins (PUMP) perform a type of intrinsic and extrinsic regulation of the coupling between respiration and phosphorylation, respectively. Expression analyses and functional studies on AOx and PUMP under normal and stress conditions suggest that the physiological role of both systems lies most likely in tuning up the mitochondrial energy metabolism in response of cells to stress situations. Indeed, the expression and function of these proteins in non-thermogenic tissues suggest that their primary functions are not related to heat production.

KEY WORDS: PUMP; AOx; mitochondria; energy metabolism; stress.

MITOCHONDRIAL ENERGY METABOLISM

In eukaryotic cells, mitochondria generate most of the cellular ATP. Redox free-energy is stepwisely utilized by complex I (NADH dehydrogenase), complex III (cytochrome bc_1), and complex IV (cytochrome *c* oxidase) of the electron transport chain, to pump protons from the matrix out to the intermembrane space, producing an electrochemical proton potential ($\Delta\mu_H+$) across the inner mitochondrial membrane. This proton potential is the driving force used by the ATP synthase (Complex V) to phosphorylate ADP (Mitchell, 1961). It can also be directly used to drive other processes, such as ion transport and ATP^{4-}/ADP^{3-} exchange across the inner mitochondrial membrane. The brown adipose tissue possesses a specialized mechanism to convert $\Delta\mu_H+$ into heat by a process called nom-shivering thermogenesis (Nicholls and Locke, 1984). Finally, changes in $\Delta\mu_H+$ may also be involved in defense mechanisms under situations of oxidative stress (Kadenbach, 2003).

[1]Laboratório de Bioenetgética (NMCE), FCM, Universidade Estadual de Campinas (UNICAMP), CP 6111, 13083-970, Campinas, SP, Brazil.

[2]To whom correspondence should be addressed. E-mail: anibal@unicamp.br

It is known that the rates of both respiration and ATP synthesis can be regulated by substrate availability (reducing equivalents, ADP, and O_2) and by the degree of coupling between respiration and phosphorylation. The regulation by substrates is complex, since their concentrations are rarely optimal because the energy metabolism of eukaryotes has to cope with variable energy demands. These include, coupling between the rate of respiration and the rate of ATP utilization, increasing thermogenesis at low temperatures, efficient utilization of nutrients under starvation, degradation of excess food intake, and stimulation of ATP synthesis under stress conditions.

Two distinct processes can regulate the coupling between respiration and phosphorylation: redox potential-dissipating systems (intrinsic regulation) represented by alternative oxidases (AOx) or proton electrochemical potential-dissipating systems (extrinsic regulation) represented by uncoupling proteins (UCP). The mechanism of intrinsic regulation prevents $\Delta\mu_H+$ formation during respiration, whereas extrinsic regulation is mediated by electrochemical proton dissipation without ATP synthesis (Vercesi *et al.*, 1995; Ježek *et al.*, 2001; Vercesi, 2001; Kadenbach, 2003), This mini-review focuses on the possible roles of plant alternative oxidase, and plant uncoupling mitochondrial protein (PUMP) on plant mitochondrial energy metabolism.

ALTERNATIVE OXIDASE

AOx are mitochondrial enzymes widespread within the eukaryotic world with exception of animals; AOx proteins were identified in various plants (Elthon *et al.*, 1989; Siedow and Umbach, 1995; Day and Wiskich, 1995), fungi (Lambowitz *et al.*, 1989, Sakajo *et al.*, 1991), and other organisms (Clarkson *et al.*, 1989; Jarmuszkiewicz *et al.*, 1997). Gene searches lead to the discovery of *AOx* gene superfamily that consists of at least two families of genes encoding AOxs (Considine *et al.*, 2002). Members encoding AOxs of type 1 (AOx1 family) were identified in both monocotyledonous and dicotyledonous plants, while *AOxs* of type 2 (AOx2 family members) were identified only in dicotyledonous plants (Considine *et al.*, 2002). Very recently, the existence of the third AOx family (AOx3 family) was inferred in *Arabidopsis* (Borecký J., Nogueira, F.T.S, Maia, I.G., Vercesi, A.E., and Arruda, P., unpublished results). Expression of *AOx1* genes seems to be closely related to stresses, while AOx2 family members are expressed in a constitutive manner. Expression of the *Arabidopsis* member of AOx3 family was downregulated after exposure of *Arabidopsis* plantlets to low temperature (4°C), indicating that AOx3 plays possibly a distinct physiological role in energy metabolism.

AOx functions as a dimer and catalyzes ubiquinol-oxygen oxidation/reduction that is not linked to proton pumping and thus does not contribute to $\Delta\mu_H+$ formation (Vanlerberghe and McIntosh, 1997; Sluse and Jarmuszkiewicz, 1998; Affourtit *et al.*, 2001a; Milani *et al.*, 2001). The activity of AOx has a very intricate nature. Firstly, it depends on substrate availability, i.e. total ubiquinol (UQ) concentration in the membrane as well as on actual O_2 concentration in the cell. AOx becomes active only when the UQ pool is 40–50% reduced, and then its activity increases sharply and non-linearly with higher reduction level of UQ (Moore *et al.*, 1988; Dry *et al.*, 1989). Furthermore, the apparent affinity of AOx for O_2 depends on

species, tissues, and age of the plants investigated (K_m ~ 1–20 μM; Ikuma *et al.*, 1964; Bendall and Bonner, 1971; Millar *et al.*, 1994; Ribas-Carbo *et al.*, 1994) and is much lower than the cytochrome *c* oxidase affinity (K_m ~ 0.1–0.15 μM; Barzu and Satre, 1970; Bendall and Bonner, 1971; Rawsthorne and LaRue, 1986). Moreover, the apparent K_m of AOx for O_2 seems to vary with ubiquinol reduction level: the more reduced the ubiquinol pool the lower is the AOx affinity for O_2 (Ribas-Carbo *et al.*, 1994). AOx dimer can be linked with a disulfide bridge and thus is also sensitive to its proper redox state, with the reduced AOx dimer being four- to five-fold more active than the oxidized form (Umbach and Siedow, 1993; Umbach *et al.*, 1994; Moore *et al.*, 1995). Reduction of the AOx dimer can occur during oxidation of Krebs cycle substrates (isocitrate and malate) that reduce $NADP^+$ in plant mitochondria (Vanlerberghe *et al.*, 1995). Thus, it has been proposed that the AOx reduction state depends on NADPH generation and involves NADPH-reduced glutathione or the thioredoxin coupling system (Umbach and Siedow, 1993; Day and Wiskich, 1995; Siedow and Umbach, 1995; Vanlerberghe *et a1.*, 1995). AOx is not sensitive to inhibitors of cytochrome pathway such as cyanide, antimycin A, or miyxothiazol, but is inhibited by primary hydroxamic acids (Schonbaum *et al.*, 1971) such as benzohydroxamate (BHAM) and by fatty acids (Minagawa *et al.* 1992b, Sluse and Jarmuszkiewicz, 2000). In some tissues, AOx can be stimulated by α-keto acids, such as pyruvate (Millar *et al.*, 1993; Day *et al.*, 1995).

The only confirmed function for AOx is the thermogenic respiration in *Arum* sp. and other species, where heat produced during anthesis volatilizes aromatic compounds to attract pollinators (Meeuse and Buggeln, 1969; Meeuse, 1975; Raskin *et al.*, 1987; Seymour *et al.*, 2003). Thermogenic activity of AOx was described mainly in spadices of *Arum maculatum* (Moore and Siedow, 1991), *Symplocarpus foetidus* (Berthold and Siedow, 1993), and *Sauromatum guttatum* (Rhoads and McIntosh, 1991). Energy for thermogenesis is provided by an increase in mitochondrial respiration through the AOx and is controlled by salicylic acid. However, *AOx1* expression is induced by salicylic acid both in thermogenic and in non-thermogenic plants (Raskin *et al.*, 1987, 1989; Rhoads and McIntosh, 1991; Van Der Straeten *et al.*, 1995; Maxwell *et al.*, 2002).

In non-thermogenic plants, the supposed role for AOx is to minimize the production of ROS as described below (Vanlerberghe and McIntosh, 1997; Maxwell *et al.*, 1999; Parsons *et al.*, 1999; Yip and Vanlerberghe, 2001). The experiments with transgenic tobacco (*Nicotiana tabacum*) lacking or overexpressing AOx also suggested that AOx is involved in the hypersensitive response to virus infection and may prevent programmed cell death induced by downregulation of the cytochrome pathway (Ordog *et al.*, 2002; Vanlerberghe *et al.*, 2002). AOx is effectively induced by artificial chemical inhibition of the cytochrome pathway by poisons such as cyanide and antimycin A (Vanlerberghe *et al.*, 1994; Wagner and Wagner, 1997). Gilliland *et al.* (2003) suggested that induction of *AOx* by cyanide/antimycin A as well as by salicylic acid is indirect, via the increase of ROS production that is known to upregulate *AOx1* expression (Minagawa *et al.*, 1992a; Vanlerberghe and McIntosh, 1996).

It is well known that cold stress activates AOx in non-thermogenic plants (Vanlerberghe and Mclntosh, 1997; McIntosh *et al.*, 1998; Zhou and Solomos, 1998; Calegario *et al.*, 2003). Recent results demonstrate that the rates of CO_2 output in potato tubers stored at 5°C increased steadily during the first 4 day period, reaching

a plateau level that was maintained up to 10 days, when compared to potato tubers stored at 25°C (Fig. 1). Under these conditions, AOx activity in mitochondria isolated from potato tubers stored at 5°C for 10 days was about 10-fold higher than that from tubers stored at 25°C (Fig. 2). Despite this large increase in the respiration rate, the temperature of the tubers was maintained at 5°C. Similar results were previously obtained by Zhou and Solomos (1998), when potato tubers were transferred from 10°C to 1°C. They found that the rate of CO_2 output increased, reaching a peak in respiration rate threefold higher within 12 days at 1°C. Kannerworff and van der Plas (1994) also observed a higher O_2 consumption in tulip bulbs stored at 5°C than in bulbs stored at 20°C.

Purvis and Shewfelt (1993) postulated that cold-induction of AOx occurs in plants to prevent superoxide overproduction by mitochondria. Wagner and Wagner (1997) reported that both the total coenzyme Q content and the relative amount of its oxidized form increased in mitochondria isolated from cold-treated *Petunia hybrida*. They observed the same effect after antimycin A treatment, suggesting that it is not the cold treatment *per se*, but the stress conditions that cause induction of the AOx.

PLANT UNCOUPLING MITOCHONDRIAL PROTEIN

The first uncoupling protein (originally termed UCP and currently UCP1) was identified in brown adipose tissue in 1976 (Ricquier and Kader, 1976) and its cDNA was cloned 9 years later (Bouillaud *et al*., 1985). Vercesi *et al*. (1995) discovered a UCP plant counterpart (the plant uncoupling mitochondrial protein, PUMP) in potato tubers, the gene of which was identified by Laloi *et al*. (1997) in potato flowers and later by Maia *et al*. (1998) in *Arabidopsis thaliana*. Using antibodies raised against potato PUMP or against recombinant PUMP isolated from *E. coli*

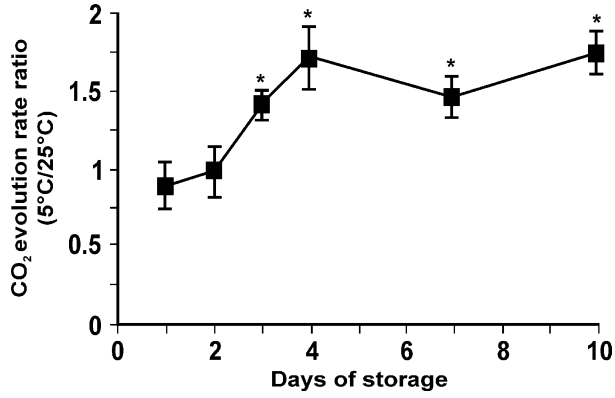

Fig. 1. Ratio between CO_2 evolution rates of intact potato tubers stored at 5 ± 1°C and tubers stored at 25 ± 1°C. Each point represents the mean value $\pm$ SD of four determinations, each one using a group of four intact potato tubers. Significant differences ($p < 0.01$) between CO_2 evolution rates of cold- and warm-treated potato tubers are indicated by asterisks.

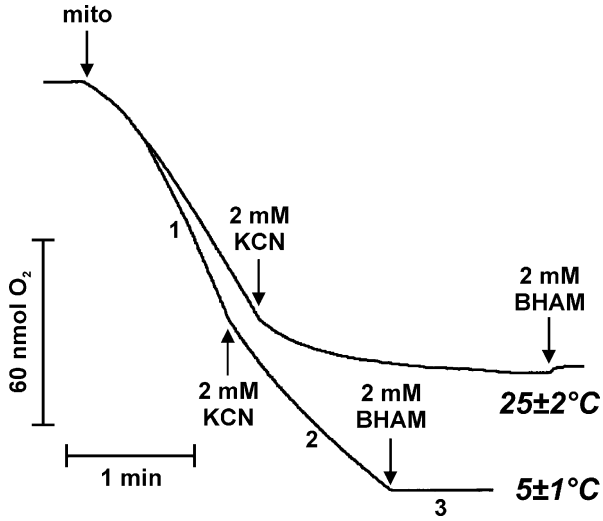

Fig. 2. Determination of AOx capacity in mitochondria isolated from potato tubers stored for 4 days at 5 ± 1°C or 25 ± 2°C. Mitochondria (0.5 mg/ml) were incubated in a standard reaction medium (28°C) in the presence of 2.5 μg/ml oligomycin, 300 μM propranolol, 2 μM atractyloside, 0.1 mM ATP, 0.5% BSA, 1 mM DTT, and 0.15 mM pyruvate. Additions of 2 mM KCN and 2 mM BHAM were done where indicated. Slope 1 refers to the total respiration rate, a difference between slope 1 and slope 2 refers to CN-sensitive respiration and between slope 2 and slope 3 to AOx capacity.

expression system (Borecký *et al.*, 2001a) this protein was also detected in many plants such as tomato, spinach, carrot, cauliflower, broccoli and turnip (Ježek *et al.*, 2000), or fruits such as banana, mango, apple, strawberry, papaya, melon, orange, pineapple, pear and peach (Ježek *et al.*, 2001). Within 3 years after PUMP discovery, genes encoding four additional animal UCPs (UCP2, Fleury *et al.*, 1997; UCP3, Boss *et al.*, 1997; UCP4, Mao *et al.*, 1999; BMCP1/UCP5, Sanchis *et al.*, 1998) and also two *Arabidopsis* genes encoding PUMP (AtPUMP1, Maia *et al.*, 1998; AtUCP2, Watanabe *et al.*, 1999) were identified. In the last few years, one to two different PUMP sequences were identified within the plant kingdom in skunk cabbage (Ito, 1999), mango (Considine *et al.*, 2001), tomato (Accession Number AF472619), wheat (Murayama and Handa, 2000), rice (Watanabe and Hirai, 2002), and maize (Brandalise *et al.*, 2003). Several reviews of these UCP members were published in recent years (Borecký *et al.*, 2001; Hourton-Cabassa *et al.*, 2004; Ledesma *et al.*, 2002; Ricquier and Bouillaud, 2000a; Sluse and Jarmuszkiewicz, 2002). Recently, our group (Borecký J., Nogueira F.T.S., Maia I.G., Vercesi A.E., and Arruda, P., unpublished results) identified a probably complete PUMP family consisting of five to six members present either in dicots (*Arabidopsis thaliana*; At-PUMP1–6, Accession numbers AJ223983, AB021706, AK117673, NM 118590, NM_127816, NM_120984) or monocots (*Saccharum* spp.; SsPUMP1–5, Accession numbers AY644460 to AY644464). Interestingly, distinct expression patterns among gene family members were observed between monocots and dicots and during

chilling stress (Borecký J., Nogueira F.T.S., Maia I.G., Vercesi A.E., and Arruda, P., unpublished results). These findings suggest that the members of each energy-dissipating system are subject to different cell or tissue/organ transcriptional regulation (Nogueira *et al*., this issue).

Uncoupling proteins are supposed to be found in all eukaryotic organisms (Borecký *et al*., 2001b). The only exception known is *Saccharomyces cerevisiae* that does not possess any form of UCP (ElMoualij *et al*., 1997). During the last years, UCP gene sequences were also found in birds (Vianna *et al*., 2001; Hirabayashi *et al*., 2005; Talbot *et al*., 2004), ectothermic vertebrates, such as frog (*Xenopus octopus;* Klein *et al*., 2002), carp (*Ciprinus carpio*) and zebrafish (*Danio rerio*; Stuart *et al*., 1999), insects (*Drosophila melanogaster;* Fridell *et al*., 2004) and in the primitive eukaryotic organism *Caenorhabditis elegans* (CeUCP, Accession number AAB54239). UCP has also been identified in mitochondria from *Acanthamoeba castellanii* (Jarmuszkiewicz *et al*., 1999), *Dictyostelium discoideum* (Jarmuszkiewicz *et al*., 2002), *Candida parapsilosis* (CpUCP, Jarmuszkiewicz *et al*., 2000), *Candida albicans* (Cavalheiro *et al*., 2004), and in trophozoites of the malaria parasite *Plasmodium Berghei* (Uemura *et al*., 2000). The existence of UCPs in protozoa, fungi, plants, insects, and fishes suggests that uncoupling proteins emerged early during evolution as a distinct member of the mitochondrial anion carrier family (MACF), probably before the divergence of plant, animal, and fungi kingdoms.

Biochemical properties of PUMP and fungal UCPs were found to be comparable to those of UCP1 (Ježek *et al*., 1996, 1997, 1998; Vercesi *et al*., 1997, 1998; Jarmuszkiewicz *et al*., 1998, 2000; Kowaltowski *et al*., 1998; Sluse *et al*., 1998; Almeida *et al*., 1999; Borecký *et al*.,; Costa *et al*., 1999; Nantes *et al*., 1999). Antibodies raised either against potato PUMP, isolated from solubilized mitochondrial proteins by chromatography on hydroxyapatite column (Nantes *et al*., 1999), or against recombinant AtPUMP1, isolated from E. *coli* expression system (Borecký *et al*., 2001a), were both able to detect potato and tomato PUMP as well as AtPUMP1. These results indicate that potato and tomato PUMP and AtPUMP1 share a high homology. A similarly high degree of homology among proteins encoded by *LePUMP, StUCP*, and *AtPUMP1* can be seen in phylogenetic analyses (Borecký *et al*., 2001b; Hourton-Cabassa *et al*., 2004).

Uncoupling proteins represent the foremost form of extrinsic regulation of oxidative phosphorylation efficiency. UCPs as well as PUMPs permit the return of extruded protons back to the mitochondrial matrix without ATP synthesis, dissipating the energy of $\Delta\mu_H+$ as heat. The result is an uncoupling of respiration from ATP synthesis, a process that gave the name to this class of proteins. Two possible models of proton transport mechanisms were hypothesized: a proton-buffering model, where H^+ is transported directly by the UCP (Klingenberg, 1990) and a protonophore model, where H^+ transport is carried by a free fatty acid (FA) cycling. In this model, UCP mediates passage of FA anions to the outer monolayer of the inner mitochondrial membrane, where FA anions become protonated and neutral FA can readily move back across the membrane by a flip-flop mechanism, while carrying H^+ (Garlid *et al*., 1996).

Activity of animal as well as plant and fungal uncoupling proteins seems to be regulated by activators, such as free fatty acids (Klingenberg, 1990; Garlid *et al*.,

1996; Ježek *et al*., 1997), UQ and its redox state (Echtay *et al*., 2000, 2001; Jarmuszkievicz *et al*., 2004), and ROS (Echtay *et al*., 2002a; Considine *et al*., 2003), in addition to inhibitors such as purine nucleotides that inhibit UCPs/PUMPs in a pH-dependent manner (Borecký *et al*., 2001a; Rafael *et al*., 1994). As mentioned above, the presence of free FA is necessary to activate UCP/PUMP. Studies on UCP1 (Ježek and Garlid, 1990) and AtPUMP1 (Borecký *et al*., 2001a) activation by a variety of FA demonstrated a tendency that longer, more unsaturated FAs activate UCP1 and AtPUMP1 more effectively. The activating function of UQ remains unclear. Echtay *et al*. (2000, 2001) reported that UQ activates UCP1–3. In contrast, Jabùrek and Garlid (2003) found that the activation was not due to UQ, but due to the presence of dichlormethane, a solvent used for UQ additions. However, Echtay and Brand (2001) found an activation of GDP-sensitive proton conductance of kidney mitochondria by UQ. To their surprise, the addition of superoxide dismutase strongly decreased UQ activation of mitochondrial proton conductance. For this reason, they conclude that UQ can activate UCP indirectly, via increasing superoxide production. Indeed, superoxide was found to activate UCP1–3 (Echtay *et al*., 2002a) and also potato PUMP (Considine *et al*., 2003). The proposed mechanism involves the activation of uncoupling proteins by superoxide from the matrix side of the inner mitochondrial membrane (Echtay *et al*., 2002b), where superoxide releases iron from proteins containing iron–sulfur centers such as aconitase. Iron (Fe^{2+}) reacts with superoxide forming hydroxyl radical that promotes generation of carbon-centered radicals that initiate lipid peroxidation, yielding breakdown products, such as 4-hydroxy-2-*trans*-nonenal, that activate UCPs (Murphy *et al*., 2003; Echtay *et al*., 2003) and PUMP (Smith *et al*., 2004). These results suggest conservation of basic mechanisms of UCP and PUMP activation. Recently, our group reported a different way in which UQ may regulate UCP activity (Jarmuszkiewicz *et al*., 2004). Instead of UCP activation by UQ, we found that the inhibition of FFA-induced UCP activity by GTP could be under control by the redox state of endogenous membranous UQ. The activity of muscle UCPs became GTP-sensitive when the UQ reduction level was below 64% (in state 3 respiration) and fully inhibited when it was below 57%. Thus, we propose that the UQ redox state could be a metabolic sensor that modulates the purine nucleotide inhibition of FFA-activated UCPs in muscle and probably other mitochondria.

The finding of uncoupling proteins in non-thermogenic plants raised the question of their physiological role, originally considered to be solely heat production in non-shivering thermogenesis in hibernating mammals. Recent hypotheses favor a more general role—the regulation of energy metabolism in mitochondria (Ježek and Garlid, 1998; Skulachev, 1998; Ricquier and Bouillaud, 2000; Jarmuszkiewicz *et al*., 2001) in order to avoid an extremely high $\Delta\mu_H+$, which can lead to excessive production of reactive oxygen species (Skulachev, 1998; Brandalise *et al*., 2003a; Considine *et al*., 2003).

In chilling-sensitive plants, such as sugarcane (*Saccharum* sp.; Tai and Lentini, 1998), oxidative stress is a major component of chilling stress (Pinhero *et al*., 1997). ROS, i.e., hydrogen peroxide, superoxide, and hydroxyl radicals, can react with DNA, lipids and proteins, to cause severe cellular damage (Sato *et al*., 2001). ROS-detoxification systems are composed of multiple enzymes, whose activity varies according to the level of stress in different cell compartments (Iba, 2002). Since

mitochondria represent one of the major sources of ROS during cold stress, PUMP and AOx may act as ROS production regulators in this organelle (Kowaltowski *et al.*, 1998; Maxwell *et al.*, 1999). In this regard, the results found by our group (Brandalise *et al.*, 2003b) demonstrate that overexpression of *AtPUMP1* in transgenic tobacco plants led to a significant increase in tolerance to oxidative stress promoted by exogenous hydrogen peroxide as compared to nontransgenic control plants. Induction of expression of maize *PUMP* (*ZmPUMP*) by menadione, which generates superoxide, suggested that *PUMP* expression *in vivo* may be controlled by ROS levels (Brandalise *et al.*, 2003a). Hence, we might consider that the purpose of the cold-stress-induced PUMP expression and activity is to balance the potentially increasing ROS production. Interestingly, analysis of the 1.0 kb promoter region upstream to transcription initiation site of *Arabidopsis PUMP4*, *PUMP5*, and *AOxla* using PLACE (Higo *et al.*, 1999) and PlantCARE (Lescot *et al.*, 2002) indicated the presence of several copies of a TCTCC core sequence. This sequence is recognized by the *ADR1* transcriptional factor, involved in oxidative processes and also activates peroxisomal proteins (Simon *et al.*, 1991).

The thermogenic function is ascribed up to now only for UCP1 from mammalian brown adipose tissue (Nicholls, 1979). Recently, evidences for muscle type UCP were identified in hummingbirds (HmUCP, Vianna *et al.*, 2001) involved in thermogenesis associated with rewarming period after torpor. The existence of thermogenic activity was suggested in muscles from other birds (Hirabayashi *et al.*, 2005, Talbot *et al.*, 2004). Interestingly, endoplasmic reticulum of brown adipose tissue (de Meis, 2003) as well as white muscle cells (Barata and de Meis, 2002) possess Ca^{2+}-ATPase (SERCA) able to hydrolyse ATP without transport of Ca^{2+} that also results in heat production. The authors suggest that dissipation of energy conserved in the ATP molecule by the uncoupled $Ca2^{+}$-ATPase can increase respiration rates to restore cytosolic ATP concentration, thus increasing heat production through a pathway alternative to UCP1-mediated thermogenesis.

The participation of all other isoforms in the process of thermogenesis is uncertain. Mammalian UCP2 and UCP3 seem to be involved in regulation of body weight (Arsenijevic *et al.*, 2000) or ROS production (Li *et al.*, 2001). Thermogenic roles of UCP2 (hypothetically in fever) or UCP3 have not yet been established (Ricquier and Bouillaud, 2000; Ježek, 2002). In plants, expression of *Arabidopsis* PUMP genes encoding the PUMP1, PUMP4, and PUMP5 isoforms are reported to be induced by cold (Maia *et al.*, 1998; Borecký J., Nogueira F.T.S., Maia I.G., Vercesi A.E., and Arruda, P., unpublished results), while PUMP2 and PUMP3 expression seems to be low and insensitive to cold. Recent studies showed that the content of potato PUMP (StPUMP) also increased in potato tubers exposed to cold (5°C, Calegario *et al.* 2003). Simultaneously, cold storage for 4 days (Fig. 3) yielded mitochondria with well-pronounced response to recoupling by 5 mM ATP plus 1% BSA after preincubation with 10 μM linoleic acid. On the contrary, ATP had no recoupling effect on control mitochondria from warm-stored potato tubers. The BHAM-insensitive respiration rate of mitochondria from 4 day cold-stressed potato tubers was also higher (114.5 nmol O_2 min^1) than in controls (97.1 nmol O_2 min^{-1}). After ATP and BSA additions, these respiration rates decreased to the same levels (66.2 and 63.3 nmol O_2/min/mg protein) for cold stress and controls, respectively.

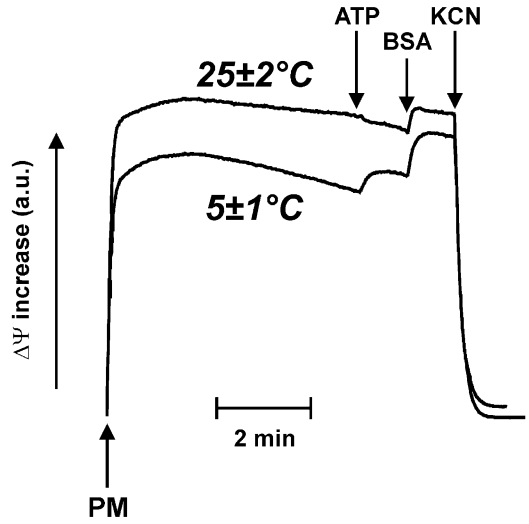

Fig. 3. Electrical transmembrane potential ($\Delta\Psi$) of mitochondria isolated from potato tubers stored for 4 days at 25 ± 2 or 5 ± 1°C. Potato mitochondria ("PM"; 0.5 mg/ml) were incubated in a standard medium (28°C) containing 2 μg/ml oligomycin, 7 μM atractyloside, 300 μM propranolol, 0.1 mM ATP, and 10 μM linoleic acid (LA). Additions of 5 mM ATP, 0.1% BSA, and 1 mM KCN were done where indicated. $\Delta\Psi$ was estimated as a function of changes in safranin O fluorescence (arbitrary units, here referred to as a.u.).

Thus PUMP-sustained O_2 consumption demonstrated a 44% increase in PUMP capacity induced by cold stress. However, the temperature of tubers was equal to ambient temperature, suggesting that higher functional capacity of StPUMP did not trigger thermogenesis.

CONCLUSIONS

Both intrinsic and extrinsic regulations of oxidative phosphorylation in plants are performed through energy dissipation that leads to the same final effect, i.e. increase in heat production. Nevertheless, energy-dissipating systems can manifest thermogenesis only at extremely high functional capacity. Only expression levels of UCP1 in brown adipose tissue of mammals and AOx1 in spadices of *Arum maculatum* (Moore and Siedow, 1991), *Symplcarpus foetidus* (Berthold and Siedow, 1993), and *Sauromatum guttatum* (Rhoads and McIntosh, 1991) seem to be high enough to promote thermogenesis.

A possible thermogenic role of PUMP came from observations of cold-stress-stimulated transcription of PUMP genes in roots and flowers of potato (*StUcP;* Laloi *et al*., 1997) and *Arabidopsis thaliana* (*AtPUMP1*; Maia *et al*., 1998). However, cold stress elevates the production of ROS that also induce transcription of PUMP genes (Brandalise *et al*., 2003a).

Most plants, with exception of thermogenic plants, do not produce enough metabolic heat to raise the temperature of bulk tissue (Moynihan *et al*., 1995; Breidenbach *et al*., 1997; Calegario *et al*., 2003). Nevertheless, a slow heat release must be concomitant to other relevant PUMP and AOx functions such as metabolic regulation (Vanlerberghe and Mclntosh, 1997; Sluse *et al*., 1998a; Affourtit *et al*., 2001a; Ježek *et al*., 2001) and/or prevention of excessive ROS formation (Kowaltowski *et al*., 1998; Moller, 2001; Popov *et al*., 1997; Wagner and Moore, 1997).

REFERENCES

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

Affourtit, C., Krab, K., and Moore, A. L. (2001) Control of plant mitochondrial respiration. *Biochim. Biophys. Acta* **1504:**58–69.

Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Couplan, E., Alves-Guerra, M. C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000) Disruption of the uncoupling protein 2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet*. **26:**435–439.

Barata, H. and de Meis, L. (2002) Uncoupled ATP hydrolysis and thermogenic activity of the sarcoplasmic reticulum Ca^{2+}-ATPase: coupling effects of dimethyl sulfoxide and low temperature. *J. Biol. Chem*. **277:**16868–16872.

Barzu, O. and Satre, M. (1970) Determination of oxygen affinity of respiratory systems using oxyhemoglobin as oxygen donor. *Analytic. Biochem*. **36:**428–433.

Bendall, D. S. and Bonner, W. D. (1971) Cyanide-insensitive respiration in plant mitochondria. *Plant Physiol*. **47:**236–245.

Berthold, D. A. and Siedow, J. N. (1993) Partial purification of the cyanide-resistant alternative oxidase of skunk cabbage (*Symplocarpus foetidus*) mitochondria. *Plant Physiol*. **101:**113–119.

Borecký, J., Maia, I. G., Costa, A. D., Jezek, P., Chaimovich, H., de Andrade, P. B., Vercesi, A. E., and Arruda, P. (2001a) Functional reconstitution of *Arabidopsis thaliana* plant uncoupling mitochondrial protein (AtPUMP1) expressed in *Escherichia coli*. *FEBS Lett*. **505:**240–244.

Borecký, J., Maia, I. G., and Arruda, P. (2001b) Mitochondrial uncoupling proteins in mammals and plants. *Biosci. Rep*. **21:**201–211.

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

Bouillaud, F., Ricquier, D., Thibault, J., and Weissenbach, J. (1985) Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein. *Proc. Natl. Acad. Sci. USA* **82:**445–448.

Brandalise, M., Maia, I. G., Borecký, J., Vercesi, A. E., and Arruda, P. (2003a) ZmPUMP encodes a maize mitochondrial uncoupling protein that is induced by oxidative stress. *Plant Sci*. **165:**329–335.

Brandalise, M., Maia, I. G., Borecký, J., Vercesi, A. E., and Arruda, P. (2003b) Overexpression of plant uncoupling mitochondrial protein in transgenic tobacco increases tolerance to oxidative stress. *J. Bioenerg. Biomembr*. **35:**203–209.

Calegario, F. F., Cosso, R. G., Fagian, M. M., Almeida, F. V., Jardim, W. F., Ježek, P., Arruda, P., and Vercesi, A. E. (2003) Stimulation of potato tuber respiration by cold stress is associated with an increased capacity of both plant uncoupling mitochondrial protein (PUMP) and alternative oxidase. *J. Bioenerg. Biomembr*. **35:**211–220.

Cavalheiro, R. A., Fortes, F., Borecký, J., Faustinoni, V. C., and Schreiber, A. Z. (2004) Respiration, oxidative phosphorylation, and uncoupling protein in *Candida albicans*. *Braz. J. Med. Biol. Res*. **37:**1455–461.

Clarkson, A. B., Bienen, E. J., Pollakis, G., and Grady, R. J. (1989) Respiration of bloodstream forms of the parasite *Trypanosoma brucei brucei* is dependent on a plant-like alternative oxidase. *J. Biol. Chem.* **264:**17770–17776.

Considine, M. J., Holtzapffel, R. C., Day, D. A., Whelan, J., and Millar, A. H. (2002) Molecular Distinction between Alternative Oxidase from Monocots and Dicots. *Plant Physiology* **129:**949–953.

Considine, M. J., Daley, D. O., and Whelan, J. (2001) The expression of alternative oxidase and uncoupling protein during fruit ripening in mango. *Plant Physiol.* **126:**1619–1629.

Considine, M. J., Goodman, M., Echtay, K. S., Laloi, M., Whelan, J., Brand, M. D, and Sweetlove, L. J. (2003) Superoxide stimulates a proton leak in potato mitochondria that is related to the activity of uncoupling protein. *J. Biol. Chem.* **278:**22298–22302.

Costa, A. D. T., Nantes, I. L., Ježek, P., Leite, A., Arruda, P., and Vercesi, A. E. (1999) Plant Uncoupling Mitochondrial Protein Activity in Mitochondria Isolated from Tomatoes at Different Stages of Ripening. *J. Bioenerg. Biomembr.* **31:**527–533.

Day, D. A. and Wiskich, J. (1995) Regulation of alternative oxidase activity in higher plants. *J. Bioenerg. Biomembr.* **27:**379–385.

De Meis, L. (2003) Brown adipose tissue Ca2 + -ATPase: uncoupled ATP hydrolysis and thermogenic activity *J. Biol. Chem.* **278:**41856–41861.

Dry, J. B., Moore, A. L., Day, D. A., and Wiskich, J. T. (1989) Regulation of alternative pathway activity in plant mitochondria: nonlinear relationship between electron flux and the redox poise of the quinone pool. *Arch. Biochem. Biophys.* **273:**148–157.

Echtay, K. S. and Brand, M. D. (2001) Coenzyme Q induces GDP sensitive proton conductance in kidney mitochondria. *Biochem. Soc. Trans.* **29:**763–768.

Echtay, K. S., Winkler, E., Frischmuth, K., and Klingenberg, M. (2001) Uncoupling proteins 2 and 3 are highly active H (+) transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). *Proc. Natl. Acad. Sci. USA* **98:**1416–1421.

Echtay, K. S., Esteves, T. C., Pakay, J. L., Jekabsons, M. B., Lambert, A. J., Portero-Otin, M., Pamplona, R., Vidal-Puig, A. J., Wang, S., Roebuck, S. J., and Brand, M. D. (2003) A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J.* **22:**4103–4110.

Echtay, K. S., Roussel, D., St-Pierre, J., Jekabsons, M. B., Cadenas, S., Stuart, J. A., Harper, J. A., Roebuck, S. J., Morrison, A., Pickering, S., Clapham, J. C., and Brand, M. D. (2002a) Superoxide activates mitochondrial uncoupling proteins. *Nature* **415:**96–99.

Echtay, K. S., Murphy, M. P., Smith, R. A., Talbot, D. A., and Brand, M. D. (2002b) Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. *J. Biol. Chem.* **277:**47129–47135.

Echtay, K. S., Winkler, E., and Klingenberg, M. (2000) Coenzyme Q is an obligatory cofactor for uncoupling protein function. *Nature* **408:**609–613.

ElMoualij, B., Duyckaerts, C., Lamotte-Brasseur, J., and Sluse, F. E. (1997) Phylogenetic classification of the mitochondrial carrier family of Saccharomyces cerevisiae. *Yeast* **13:**573–581.

Elthon, T. E., Nickels, R. L., and McIntosh, L. (1989) Monoclonal antibodies to the alternative oxidase of higher plant mitochondria. *Plant Physiol.* **89:**1311–1317.

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) Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15:**269–272.

Fridell, Y. W., Sanchez-Blanco, A., Silvia, B. A., and Helfand, S. L. (2004) Functional characterization of a *Drosophila* mitochondrial uncoupling protein. *J. Bioenerg. Biomembr.* **36:**219–228.

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

Gilliland, A., Singh, D. P., Hayward, J. M., Moore, C. A., Murphy, A. M., York, C. J., Slator, J., and Carr, J. P. (2003) Genetic modification of alternative respiration has differential effects on antimycin A-induced versus salicylic acid-induced resistance to tobacco mosaic virus. *Plant Physiol* **132:**1518–1528.

Higo, K., Ugawa, Y., Iwamoto, M., and Korenaga, T. (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acid Res.* **27:**297–300.

Hirabayashi, M., Ijiri, D., Kamei, Y., Tajima, A., and Kanai, Y. (2005) Transformation of skeletal muscle from fast to slow-twitch during acquisition of cold tolerance in the chick. *Endocrinology* **146:**399–405.

Hourton-Cabassa, C., Rita Matos, A., Zachowski, A., and Moreau, F. (2004) The plant uncoupling protein homologues: a new family of energy-dissipating proteins in plant mitochondria. *Plant Physiol. Biochem.* **42:**283–290.

Iba, K. (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance *Annu. Rev. Plant Biol.* **53:**225–245.

Ikuma, H., Schindler, F. J., and Bonner, W. D. (1964) Kinetic analysis of oxidases in tightly coupled plant mitochondria. *Plant Physiol.* **39:**S–1x.

Ito, K. (1999) Isolation of two distinct cold-inducible cDNAs encoding plant uncoupling proteins from the spadix of skunk cabbage (*Symplocarpus foetidus*) *Plant Sci.* **149:**167–173.

Jabůrek, M. and Garlid, K. D. (2003) Reconstitution of recombinant uncoupling proteins: UCP1, -2, and -3 have similar affinities for ATP and are unaffected by coenzyme Q10. *J. Biol. Chem.* **278:**25825–25831.

Jarmuszkiewicz, W., Sluse-Goffart, C. M., Vercesi, A. E., and Sluse, F. E. (2001) Alternative oxidase and uncoupling protein: thermogenesis versus cell energy balance. *Biosci Rep.* **21:**213–222.

Jarmuszkiewicz, W., Navet, R., Alberici, L. C., Douette, P., Sluse-Goffart, C. M., Sluse, F. E., and Vercesi, A. E. (2004) Redox State of Endogenous Coenzyme Q Modulates the Inhibition of Linoleic Acid-Induced Uncoupling by Guanosine Triphosphate in Isolated Skeletal Muscle Mitochondria. *J. Bioenerg. Biomembr.* **36:**493–502.

Jarmuszkiewicz, W., Behrendt, M., Navet, R, and Sluse, F. E. (2002) Uncoupling protein and alternative oxidase of *Dictyostelium discoideum*: occurrence, properties and protein expression during vegetative life and starvation-induced early development. *FEBS Lett.* **532:**459–464.

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

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

Jarmuszkiewicz, W., Wagner, A. M., Wagner, M. J., and Hryniewiecka, L. (1997) Immunological identification of the alternative oxidase of *Acanthamoeba castellanii* mitochondria. *FEBS Lett.* **411:**110–114.

Ježek, P. (2002) Possible physiological roles of mitochondrial uncoupling proteins—UCPn *Int. J. Biochem. Cell Biol.* **34:**1190–1206.

Ježek, P, Borecký, J, Žáčková, M, Costa, A. D., and Arruda, P. (2001) Possible basic and specific functions of plant uncoupling proteins (pUCP). *Biosci Rep.* **21:**237–245.

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

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

Ježek, P. and Garlid, K. D. (1990) New substrates and competitive inhibitors of the Cl^- translocating pathway of the uncoupling protein of brown adipose tissue mitochondria. *J. Biol. Chem.* **265:**19303–19311.

Ježek, P. and Garlid, K. D. (1998) Mammalian mitochondrial uncoupling proteins. *Int. J. Biochem. Cell Biol.* **30:**1163–1168.

Ježek, P., Žáčková, M., Košařová, J., Rodrigues, E. T., Madeira, V. M., and Vicente, J. A. (2000) Occurrence of Plant-Uncoupling Mitochondrial Protein (PUMP) in Diverse Organs and Tissues of Several Plants. *J. Bioenerg. Biomembr.* **32:**549–561.

Kadenbach, B. (2003) Intrinsic and extrinsic uncoupling of oxidative phosphorylation *Biochim. Biophys. Acta.* **1604:**77–94.

Kannerworff, W. A. and van der Plas, L. H. W. (1994) Respiration of bulb scale fragments of tulip after storage at 5°C . *Plant Sci.* **104:**31–38.

Klein, S. L., Strausberg, R. L., Wagner, L., Pontius, J., Clifton, S. W., and Richardson, P. (2002) Genetic and genomic tools for *Xenopus* research: The NIH Xenopus initiative. *Dev. Dyn.* **225:**384–391.

Klingenberg, M. (1990) Mechanism and evolution of the uncoupling protein of brown adipose tissue *Trends Biochem. Sci.* **15:**108–112.

Kowaltowski, A. J., Costa, A. D. T., and 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.

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

Lambowitz, A. M., Sabourin, J. R., Bertrand, H., Nickels, R., and McIntosh, L. (1989) Immunological identification of the alternative oxidase of Neurospora crassa mitochondria. *Mol. Cell. Biol.* **9:**1362–1364.

Ledesma, A., de Lacoba, M. G., and Rial, E. (2002) The mitochondrial uncoupling proteins. *Genome Biol.* **3:**3015–1–30159.

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouze, P., and Rombauts, S. (2002) PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acid Res.* **30:**325–327.

Li, L.-X., Skorpen, F., Egenberg, K., Jorgensen, I. H., and Grill, V. (2001) Uncoupling protein 2 participates in cellular defence against oxidative stress in clonal ß-cells. *Biochem. Biophys. Res. Commun.* **282:**273–277.

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

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

Maxwell, D. P., Nickels, R., and McIntosh, L. (2002) Evidence of mitochondrial involvement in the transduction of signals required for the induction of genes associated with pathogen attack and senescence. *Plant J*. **29:**269–279.

Maxwell, D. P., Wang, Y., and McIntosh, L. (1999) The alternative oxidase lowers mitochondrial reactive oxygen species production in plant cells. *Proc Natl Acad Sci USA* **96:**8271–8276.

McIntosh, L., Eichler, T., Gray, G., Maxwell, D., Nickels, R., and Wang, Y. (1998) Biochemical and genetic controls exerted by plant mitochondria. *Biochim. Biophys. Acta* **1365:**278–284.

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

Meeuse, B. J. D. and Buggeln, R. G. (1969) Time, space, light and darkness in the metabolic flare-up of the *Sauromatum* appendix. *Acta Bot. Neerl.* **18:**159–172.

Milani, G., Jarmuszkiewicz, W., Sluse-Goffart, C. M., Schreiber, A. Z., Vercesi, A. E., and Sluse, F. E. (2001) Respiratory chain network in mitochondria of *Candida parapsilosis*: ADP/O appraisal of the multiple electron pathways. *FEBS Lett*. **508:**231–235.

Millar, A. H., Bergersen, F. J., and Day, D. A. (1994) Oxygen affinity of terminal oxidases in soybean mitochondria. *Plant Physiol. Biochem.* **32:**847–852.

Millar, A. H., Wiskich, J. T., Whelan, J., and Day, D. A. (1993) Organic acid activation of the alternative oxidase of plant mitochondria. *FEBS Lett*. **329:**259–262.

Minagawa, N., Koga, S., Nakano, M., Sakajo, S., and Yoshimoto, A. (1992a) Possible involvement of superoxide anion in the induction of cyanide-resistant respiration in Hansenula anomala. *FEBS Lett*. **302:**217–219.

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

Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism *Naturwissenschaften* **191:**144–148.

Møller, I. M. (2001) Plant mitochondria and oxidative stress: Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Species *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52:**561–591.

Moore, A. L., Umbach, A. L., and Siedow, J. N. (1995) Structure-function relationships of the alternative oxidase of plant mitochondria: a model of the active site. *J. Bioenerg. Biomembr.* **27:**367–377.

Moore, A. L. and Siedow, J. N. (1991) The regulation and nature of the cyanide resistant alternative oxidase of plant mitochondria. *Biochem. Biophys. Acta* **1059:**121–140.

Moore, A. L., Dry, I. B., and Wiskich, J. T. (1988) Measurement of the redox state of the ubiquinone pool in plant mitochondria. *FEBS Lett*. **235:**76–80.

Moynihan, M. R., Ordentlich, A., and Raskin, I. (1995) Chilling-Induced Heat Evolution in Plants. *Plant Physiol.* **108:**995–999.

Murayama, S. and Handa, H. (2000) Isolation and characterization of cDNAs encoding mitochondrial uncoupling proteins in wheat: wheat UCP genes are not regulated by low temperature. *Mol. Gen. Genet*. **264:**112–118.

Murphy, M. P. and Brand, M. D. (1988) The stoichiometry of charge translocation by cytochrome c oxidase and the cytochrome bc_1 complex of mitochondria at high membrane potential. *Eur. J. Biochem*. **173:**645–651.

Murphy, M. P., Echtay, K. S., Blaikie, F. H., Asin-Cayuela, J., Cocheme, H. M., Green, K., Buckingham, J. A., Taylor, E. R., Hurrell, F., Hughes, G., Miwa, S., Cooper, C. E., Svistunenko, D. A., Smith, R. A., and Brand, M. D. (2003) Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. *J. Biol. Chem*. **278:**48534–48545.

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

Nicholls, D. G. (1976) Hamster brown-adipose-tissue mitochondria Purine nucleotide control of the ion conductance of the inner membrane, the nature of the nucleotide binding site. *Eur. J. Biochem*. **62:**223–228.

Nicholls, D. G. and Locke, R. M. (1984) Thermogenic mechanisms in brown fat. *Physiol. Rev*. **64:**1–64.

Ordog, S. H., Higgins, V. J., and Vanlerberghe, G. C. (2002) Mitochondrial alternative oxidase is not a critical component of plant viral resistance but may play a role in the hypersensitive response. *Plant Physiol*. **129:**1858–1865.

Parsons, H. L., Yip, J. Y. H., and Vanlerberghe, G. C. (1999) Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. *Plant Physiol*. **121:**1309–1320.

Pinhero, R. G., Rao, M. V., Paliyath, G., Murr, D. P., and Fletcher, R. A. (1997) Changes in Activities of Antioxidant Enzymes and Their Relationship to Genetic and Paclobutrazol-Induced Chilling Tolerance of Maize Seedlings. *Plant Physiol*. **114:**695–704.

Popov, V. N., Simonian, R. A., Skulachev, V. P., and Starkov, A. A. (1997) Inhibition of the alternative oxidase stimulates H2O2 production in plant mitochondria. *FEBS Lett*. **415:**87–90.

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

Rafael, J., Pampel, I., and Wang, X. (1994) Effect of pH and $MgCl_2$ on the binding of purine nucleotides to the uncoupling protein in membrane particles from brown fat mitochondria. *Eur. J. Biochem*. **223:**971–980.

Raskin, I., Ehmann, A., Melander, W. R., and Meeuse, B. J. D. (1987) Salicylic acid: a natural inducer of heat production in arum lilies. *Science* **237:**1601–1602.

Raskin, I., Turner, I. M., and Melander, W. R. (1989) Regulation of heat production in the inflorescences of an *Arum* lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. USA* **86:**2214–2218.

Rawsthorne, S. and LaRue, T. A. (1986) Metabolism under microaerobic conditions of mitochondria from cowpea nodules. *Plant Physiol*. **81:**1097–1102.

Rhoads, D. M. and McIntosh, L. (1991) Isolation and characterization of a cDNA clone encoding an alternative oxidase protein of *Sauromatum guttatum* (Schott). *Proc. Natl. Acad. Sci. USA* **88:**2122–2126.

Ribas-Carbo, M., Berry, J. A., Azcon-Bieto, J., and Siedow, J. N. (1994) The reaction of the plant mitochondrial cyanide-resistant alternative oxidase with oxygen. *Biochim. Biophys. Acta* **1188:**205–212.

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

Ricquier, D. and Bouillaud, F. (2000b) Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J Physiol*. **529:**3–10.

Ricquier, D. and Kader, J. -C. (1976) Mitochondrial protein alteration in active brown fat: a soidum dodecyl sulfate-polyacrylamide gel electrophoretic study. *Biochem. Biophys. Res. Commun*. **73:**577–583.

Sakajo, S., Minagawa, N., Komiyama, T., and Yoshimoto, A. (1991) Molecular cloning of cDNA for antimycin A-inducible mRNA and its role in cyanide-resistant respiration in Hansenula anomala. *Biochim. Biophys. Acta* **1090:**102–108.

Sanchis, D., Fleury, C., Chomiki, N., Goubern, M., Huang, Q., Neverova, M., Gregoire, F., Easlick, J., Raimbault, S., Levi-Meyrueis, C., Miroux, B., Collins, S., Seldin, M., Richard, D., Warden, C., Bouillaud, F., and 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.

Sato, Y., Murakami, T., Funatsuki, H., Matsuba, S., Saruyama, H., and Tanida, M. (2001) Heat shock-mediated APX gene expression and protection against chilling injury in rice seedlings. *J. Exp. Bot.* **52:**145–151.

Schonbaum, G. R., Bonner, W. D., Storey, B. T., and Bahr, J. T. (1971) Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. *Plant Physiol.* **47:**124–128.

Seymour, R. S., White, C. R., and Gibernau, M. (2003) Environmental biology: heat reward for insect pollinators. *Nature* **426:**243–244.

Siedow, J. N. and Umbach, A. L. (1995) Plant mitochondrial electron transfer and molecular biology. *Plant Cell* **7:**821–831.

Simon, M., Adam, G., Rapatz, W., Spevak, W., and Ruis, H. (1991) The Saccharomyces cerevisiae ADR1 gene is a positive regulator of transcription of genes encoding peroxisomal proteins. *Mol. Cell Biol.* **11:**699–704.

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

Sluse, F. E. and Jarmuszkiewicz, W. (1998) Alternative oxidase in the branched mitochondrial respiratory network: an overview on structure, function, regulation, and role. *Braz. J. Med. Biol. Res.* **31:**733–747.

Sluse, F. E. and 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.

Sluse, F. E. and Jarmuszkiewicz, W. (2002) Uncoupling proteins outside the animal and plant kingdoms: functional and evolutionary aspects. *FEBS Lett.* **510:**117–120.

Sluse, F. E., Almeida, A. M., Jarmuszkiewicz, W., and Vercesi, A. E. (1998) Free fatty acids regulate the uncoupling protein and alternative oxidase activities in plant mitochondria. *FEBS Lett* **433:**237–234.

Smith, A. M., Ratcliffe, R. G., and Sweetlove, L. J. (2004) Activation and function of mitochondrial uncoupling protein in plants. *J. Biol. Chem.* **279:**51944–51952.

Stuart, J. A., Harper, J. A., Brindle, K. M., and Brand, M. D. (1999) Uncoupling protein 2 from carp and zebrafish, ectothermic vertebrates. *Biochim. Biophys. Acta* **1413:**50–54.

Talbot, D. A., Duchamp, C., Rey, B., Hanuise, N., Rouanet, J. L., Sibille, B., and Brand, M. D. (2004) Uncoupling protein and ATP/ADP carrier increase mitochondrial proton conductance after cold adaptation of king penguins. *J. Physiol.* **558:**123–135.

Tai P. Y. P., Lentini R. S., (1998) Freeze Damage of Florida Sugarcane. In: Sugarcane handbook (Anderson D. L. Ed.), Ed. 1. pp. 1–3, Florida Cooperative Extension Service, University of Florida, Florida.

Uemura, S. A., Luo, S., Moreno, S. N. J., and Docampo, R. (2000) Oxidative Phosphorylation, Ca^{2+} Transport, and Fatty Acid-induced Uncoupling in Malaria Parasites Mitochondria. *J. Biol. Chem.* **275:**9709–9715.

Umbach, A. L. and Siedow, J. N. (1993) Covalent and non-covalent dimers of the cyanide-resistant alternative oxidase protein in higher plant mitochondria and their relationship to enzyme activity. *Plant Physiol.* **103:**845–854.

Umbach, A. L., Wiskich, J. T., and Siedow, J. N. (1994) Regulation of alternative oxidase kinetics by pyruvate and intermolecular disulfide bond redox status in soybean seedling mitochondria. *FEBS Lett.* **348:**181–184.

Van Der Straeten, D., Chaerle, L., Sharkov, G., Lambers, H., and Van Montagu, M. (1995) Salicylic acid enhances the activity of the alternative pathway of respiration in tobacco leaves and induces thermogenicity. *Planta* **196:**412–419.

Vanlerberghe, G. C. and McIntosh, L. (1996) Signals Regulating the Expression of the Nuclear Gene Encoding Alternative Oxidase of Plant Mitochondria. *Plant Physiol.* **111:**589–595.

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

Vanlerberghe, G. C., Day, D. A., Wiskich, J. T., Vanlerberghe, A. E., and McIntosh, L. (1995) Alternative oxidase activity in tobacco leaf mitochondria: dependence on tricarboxylic acid cycle-mediated redox regulation and pyruvate activation. *Plant Physiol.* **109:**353–361.

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

Vanlerberghe, G. C., Vanlerberghe, A. E., and McIntosh, L. (1994) Molecular genetic alteration of plant respiration (silencing and overexpression of alternative oxidase in transgenic tobacco). *Plant Physiol.* **106:**1503–1510.

Vanlerberghe, G. C., Robson, C. A., and Yip, J. Y. H. (2002) Induction of Mitochondrial Alternative Oxidase in Response to a Cell Signal Pathway Down-Regulating the Cytochrome Pathway Prevents Programmed Cell Death. *Plant Physiol.* **129:**1829–1842.

Vercesi, A. E. (2001) The discovery of an uncoupling mitochondrial protein in plants *Biosci. Rep.* **21:**195–200.

Vercesi, A. E., Martins, I. S., Silva, M. A. P., Leite, H. M. F., Cuccovia, I. M., and Chaimovich, H. (1995) PUMPing plants. *Nature* **375:**24.

Vercesi, A. E., Chaimovich, H., and Cuccovia, I. M. (1997) A plant uncoupling mitochondrial protein, PUMP. *Rec. Res. Devel. Plant Physiol.* **1:** 85–91.

Vercesi A. E., Ježek P., Costa A. D. T., Kowaltowski A. J., Maia I. G., Arruda P., (1998) A plant uncoupling mitochondrial protein. In: Plant Mitochondria: From Gene to Function., (Moller I. M., Gardeström P., Glimelius K., and Glaser E., eds.), Backhuys Publishers, Leiden, The Netherlands, pp. 435–440.

Vianna, C. R., Hagen, T., Zhang, C.-Y., Bachman, E., Boss, O., Gereben, B., Moriscot, A. S., Lowell, B. B., Bicudo, J. E. P. W., and Bianco, A. C. (2001) Cloning and functional characterization of an uncoupling protein homolog in hummingbirds. *Physiol. Genomics* **5:**137–145.

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

Wagner, A. M. and Wagner, M. J. (1997) Changes in mitochondrial respiratory chain components of petunia cells during culture in the presence of antimycin A. *Plant Physiol.* **115:**617–622.

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

Watanabe, A. and Hirai, A. (2002) Two uncoupling protein genes of rice (*Oryza sativa* L.): molecular study reveals the defects in the pre-mRNA processing for the heat-generating proteins of the sub-tropical cereal. *Planta* **215:**90–100.

Yip, J. Y. H. and Vanlerberghe, G. C. (2001) Mitochondrial alternative oxidase acts to dampen the generation of active oxygen species during a period of rapid respiration induced to support a high rate of nutrient uptake. *Physiol. Plant.* **112:**327–333.

Zhou, D. B. and Solomos, T. (1998) Effect of hypoxia on sugar accumulation, respiration, activities of amylase and starch phosphorylase, and induction of alternative oxidase and acid invertase during storage of potato tubers (*Solanum tuberosum* cultivar Russet Burbank) at 1°C . *Physiol. Plant.* **104:**255–265.