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Molecular, Functional, and Pathological Aspects of the Mitochondrial ADP/ATP Carrier

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In providing the cell with ATP generated by oxidative phosphorylation, the mitochondrial ADP/ATP carrier plays a central role in aerobic eukaryotic cells. Combining biochemical, genetic, and structural approaches contributes to understanding the molecular mechanism of this essential transport system, the dysfunction of which is implicated in neuromuscular diseases.

Most of the energy usable by aerobic eukaryotic cells is provided by the hydrolysis of ATP into ADP and inorganic phosphate. Therefore, the continuous synthesis of ATP is required to sustain the cellular energetic economy: each human being recycles the equivalent of his/her own mass of ATP every day. ATP is regenerated in the mitochondrial matrix through the oxidative phosphorylation process (FIGURE 1). The continuous uptake of electron-rich substrates, phosphate, and ADP, as well as the release of ATP into the cytosol require their crossing over mitochondrial membranes. Nonspecific porins mediate this transport through the outer membrane (7). In contrast, the inner membrane displays highly selective permeability, and hydrophilic metabolites can only cross this lipid bilayer thanks to specific carrier proteins. As a consequence, mitochondrial carrier proteins not only provide mitochondria with essential physiological metabolites but also play an important role in regulating the balance between intra- and extra-mitochondrial compartments (e.g., redox potential, phosphate potential). Solutes transported by theses carriers differ considerably in size and structure. They range from the smallest cation, the proton, to one of the largest anions, ATP. At physiological pH, most metabolites are anions, but some of them, including carnitine, ornithine, and glutamine, are zwitterions. To date, ~20 carriers have been identified on the basis of their activity, assessed either in intact mitochondria or in reconstituted proteoliposomes (51). Some of them are involved in specialized metabolic pathways such as gluconeogenesis and urea synthesis. For a number of them, isoforms displaying tissue-specific distribution have been identified (51). In addition, the distribution of carriers is tissue dependent, e.g., heart mitochondria, which lack the citrate carrier. On the basis of common biochemical characteristics, such as the presence in their sequence of the amino acid signature P-X-[D/E]-X-X-[K/R] found three times, ~100 residues apart, mitochondrial carriers have been classified as members of the mitochondrial carrier family (68).

The ADP/ATP carrier was the first mitochondrial transport system to be discovered. Ideas in the field of solute exchange between closed intracellular compartments were first introduced in the mid-1950s from the pioneering work by Werkeiser and Bartley (69). They provided experimental evidence for the existence of two distinct compartments in mitochondria, differing in their accessibility to compounds of various molecular weights. These two compartments were later assigned to the mitochondrial matrix and the intermembrane space. During the course of the study of the ATP production by the oxidative phosphorylation process, it was demonstrated that adenine nucleotides were distributed in two pools located in the intermembrane space and in the mitochondrial matrix (63). Later, Pressman postulated that these two pools could exchange their nucleotides (55). In the early 1960s, it was found that atractyloside (ATR), a natural poison, competitively inhibited the phosphorylation of ADP added to mitochondria but not that of matrix ADP. This finding led to the obvious conclusion that, other than the coupling mechanism, an ATR-sensitive extra step was implicated in the phosphorylation of external ADP. The existence of a specific ADP/ATP transport system was fully established from the inhibition by ATR of the binding of external ADP to mitochondria (10) and from the Michaelis-Menten kinetics of transmembrane nucleotide exchange.

ADP/ATP transport proceeds according to an exchange-diffusion mechanism with a one-to-one stoichiometry, thus maintaining the adenine nucleotide pool at a constant level in the matrix compartment. Only the free forms of these nucleotides are transported. Under the conditions of oxidative phosphorylation, external ADP^{3-} enters mitochondria in exchange for ATP^{4-} released into the intermembrane space. This exchange is not charge compensated, and, as a consequence, it is driven by the membrane potential. It has been evaluated that the energy consumption required for ADP/ATP exchange amounts to ~30% of the energy delivered by mitochondrial respiration (22).

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Genes and Tissue-Specific Expression

The mitochondrial carriers are encoded by nuclear genes resulting from ancestral gene duplications, thus producing polypeptide chains exhibiting three related motifs of ~100 residues each. Corresponding proteins are imported into the mitochondrial inner membrane by the TIM/TOM machinery (for a review, see Ref. 56). The number of isoforms identified so far encoding the ADP/ATP carriers depends on organisms and organs. In the yeast *Saccharomyces cerevisiae*, three highly similar genes have been isolated (1, 39, 42), with *ScANC2* being the only one required for cell growth on nonfermentable carbon sources such as glycerol or lactate. Four isoforms have been identified in human genome. The nomenclature of these genes is rather confusing and is given in Table 1 for clarification. Human isoforms of the carrier have different tissue distribution: *hANC1* is specific to heart and skeletal muscles, *hANC3* is recovered in proliferating cells, whereas *hANC2* is ubiquitous (64). The fourth isoform has been recently discovered in human, its transcript products being predominantly detected in liver, testis, and brain (18). Only three isoforms encoding ADP/ATP carriers have been identified so far in rodents. In mouse, gene inactivation of two of them allowed the establishment of model mouse lines suffering from mitochondrial myopathy (30, 38).

Unique Inhibitors

ATR did not only allow the identification of the ADP/ATP transport system but also has been of considerable interest for the detailed study of its kinetic properties and its specificity. ATR and carboxyatractyloside (CATR) are two natural heteroglucosides (FIGURE 2A) that are produced in some plants, such as *Atractylis gummifera*, a thistle growing in countries bordering the Mediterranean Sea (for a review, see Refs. 15, 65). Toxicity of ATRs has long been recognized, since at least the first century A.D., but they remain a cause of fatal poisoning in humans. This follows accidental ingestion of the sweetish rhizome of the thistle, which is easily confused with a wild artichoke. ATR and CATR have also been isolated from other plants from different genera, including *Callilepis laureola*, *Xanthium strumarium*, *Iphiona aucheri*, and *Wedelia glauca*. ATR poisoning has also been reported to result from inappropriate dosage of traditional herbal medicine of North and South Africa. Due to the inhibition of the ADP/ATP exchange across the mitochondrial inner membrane, the toxic effects of ATR result in hepatic necrosis and renal failure.

ATR and CATR are nonpermeant inhibitors that bind with high affinity (K_d in the nanomolar range) to carrier sites accessible from the intermembrane space. We have synthesized a number of fluorescent, spin-labeled, and photoactivatable derivatives of ATR that were of great benefit in structure-function studies of the carrier protein (for review, see Ref. 67). For example, radiolabeled derivatives of ATR have been used to characterize the carrier in yeast and plant mitochondria and to map their binding sites. Later, fluorescent analogs of ATR were synthesized to set up a fluorometric assay for the characterization of the carrier in pathological carrier-deficient muscle (26, 59).

The ADP/ATP carrier can also be very efficiently and specifically inhibited by bongkrekic acid (BA), a natural poison secreted by the bacteria *Pseudomonas cocovenenans* (35). BA is a polyunsaturated long-chain fatty acid derivative (FIGURE 2B) that interacts with

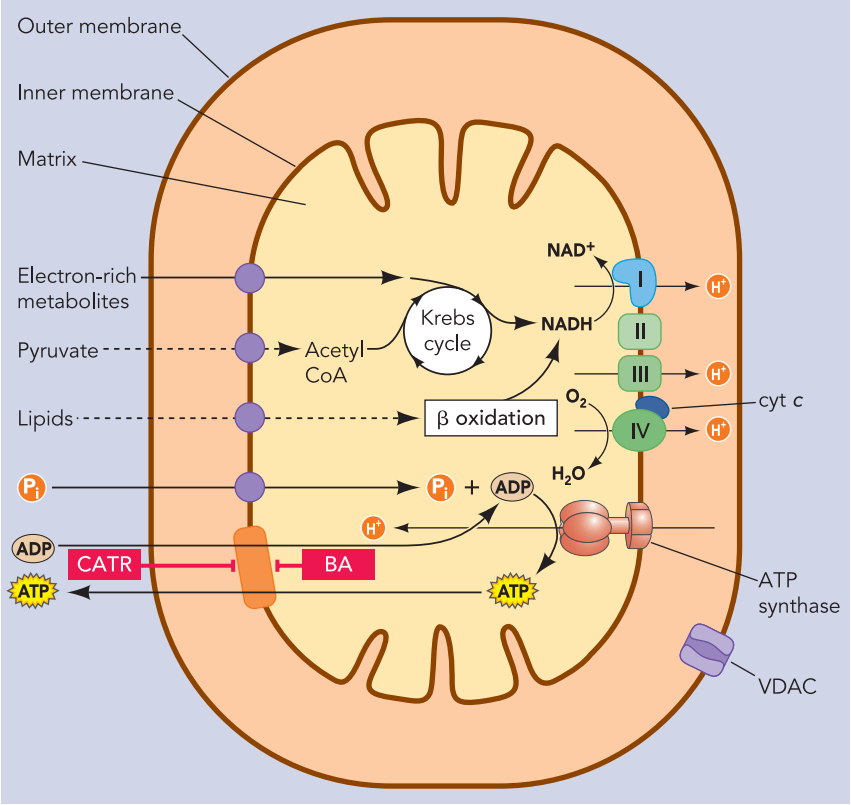

FIGURE 1. **Schematic representation of mitochondrial ATP synthesis and export**
Substrates originating from the degradation of lipids and electron-rich metabolites are oxidized by the complexes I, II, III, and IV of the respiratory chain. This process results in the building of a transmembrane proton gradient that is used, in turn, by the ATP synthase to produce ATP from ADP and phosphate. Phosphate ions are imported into mitochondria thanks to a P_i/OH^- exchanger. Channelling of ADP, entering mitochondria, and ATP exported toward the cytosol is operated by the ADP/ATP carrier (orange box). BA, bongkrekic acid; CATR, carboxyatractyloside; IM, inner mitochondrial membrane; OM, outer mitochondrial membrane; VDAC, voltage-dependent anion channel.

Table 1. Nomenclature of expressed human adenine-nucleotide carrier genes

Nomenclature				Chromosome Location
ANC1	*T1*	*ANT1*	*AAC1*	4q35.1
ANC2	*T2*	*ANT3*	*AAC3*	Xp22.33
ANC3	*T3*	*ANT2*	*AAC2*	Xq24
ANC4			*AAC4*	4q28.1

high affinity (K_d in the nanomolar range) with carrier sites accessible from the matrix compartment. Therefore, in contrast to ATRs, BA has to cross the mitochondrial inner membrane to produce its inhibitory effect on ADP/ATP transport.

It was shown that binding of ATRs and BA to the ADP/ATP carrier are mutually exclusive. This behavior demonstrated the existence of two conformational states of the carrier, referred to as the CATR and BA conformations since they are able to bind CATR and BA, respectively. These two conformations exist in equilibrium in the mitochondrial membrane, but in the presence of CATR or BA this equilibrium is shifted and results in the formation of very stable distinct CATR- or BA-carrier complexes that can be differentiated on the basis of their chemical and immunochemical reactivity and on their sensitivity to proteases (for a review, see Ref. 9). In the absence of inhibitors, only ADP and ATP are able to trigger the rapid interconversion between the CATR and the BA conformations, suggesting that this transition is involved in the transport process. This peculiar feature of the carrier has been especially advantageous for the study of the transport mechanism at a molecular level.

Recently, structural information was obtained for the ADP/ATP carrier in complex with ATR or CATR, using two-dimensional electron microscopy (41) and three-dimensional crystallography (53), respectively.

Molecular Aspects of the ADP/ATP Carrier-ATRs Interactions

The aglycone moiety of ATR and CATR (atractyligenin) is a nonvolatile diterpene of the kaurene family, with a perhydrophenanthrenic structure (FIGURE 2A). The glucidic moiety consists of a glucose linked to an isovaleric acid on C2' and to two residues of sulphuric acid in C3' and C4'. The hydroxyl group on C6' is free. The carbohydrate C1' is attached to the COOH-hydroxyl of atractyligenin via a glycosidic linkage. CATR differs from ATR in having an additional carboxyl group in *position 4* of atractyligenin. Structure-activity relationships of ATRs have been investigated using chemically modified derivatives. It was determined that all parts of the ATR molecule, except the C6' alcohol group, play a role in the inhibitory action of ATR. For example, removal of isovaleric acid or the sulfate groups leads to less effective compounds, and atractyligenin is 150 times less active than ATR. More striking, reduction to alcohol of the C4 carboxylic group produces atractylitriol, which is a nontoxic compound.

The molecular bases of the specific inhibitory properties of the CATR molecule, in line with its strong toxic actions, have been established only recently thanks to the elucidation by X-ray crystallography at high resolution of the structure of the bovine ADP/ATP carrier in complex with this inhibitor (53). The structure shows six tilted transmembrane α-helices, labeled H1 to H6, three of them being kinked at the level of proline residues (FIGURE 3). One of the most striking features of the carrier structure is the consequence of the tripartite structure on the three-dimensional polypeptide chain arrangement. The carrier protein is indeed organized by the assembly of three motifs of ~100 residues each, whose main chain backbone structures can be superimposed with root mean square deviations of <2 Å, thus resulting in the overall pseudo threefold symmetry of the carrier. Deviations to this pseudo-symmetry are probably necessary to the transport mechanism. Each motif consists of two hydrophobic transmembrane helices connected by a loop exposed to the matrix compartment and containing a short amphipathic α-helix lying parallel to the membrane surface. On the external side of the membrane, the three motifs are connected by two short loops. Both NH_2- and COOH-terminal extremities of the protein are oriented toward the intermembrane space. The bundle of transmembrane helices forms a wide cone-shaped cavity open toward the intermembrane space. This cavity is ~30 Å in depth and 20 Å in maximal diameter. Toward the matrix compartment, it is closed by a barrier of ~10 Å in thickness. The CATR molecule is trapped at the bottom of the cavity, but it is noticeably not centered onto the pseudo threefold symmetry axis, as it rather interacts with residues belonging to *transmembrane helices 2—5* (FIGURE 3B). The aglycone moiety is lying at the bottom of the cavity, whereas the disulfate glucose is pointing toward the intermembrane space. With

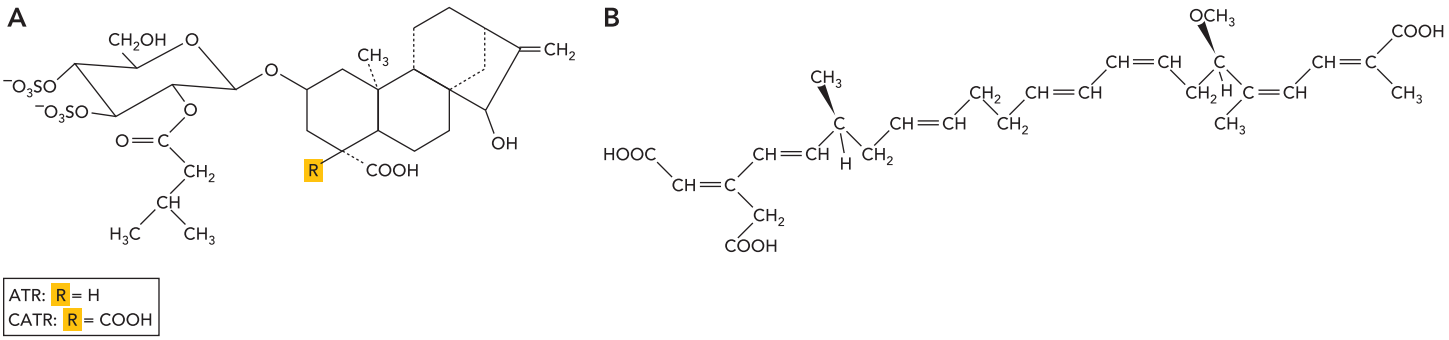

FIGURE 2. **Chemical structures of atractylosides and bongkrekic acid**
Chemical structures of atractylosides (*A*) and bongkrekic acid (*B*).

the notable exception of the primary alcohol group carried by the glucose moiety on C6', all of the CATR chemical groups are interacting with the protein. For example, the isovaleric chain on C2' is in van der Waals contact with side chains of T83, L127, V130, and I183, and the sulfate groups on C3' and C4' are interacting through hydrogen bonds with polar residues (N87, K91, and R187). In addition, the diterpene moiety stacks on the phenolic ring of Y186 and the hydroxyl group linked to C15 are connected through hydrogen bonds with D231 and R234. Moreover, the two carboxyl groups carried by the atractyligenin moiety of CATR are located in a polar environment composed of K22, R79, D134, R234, R235, and R279. One of them forms a salt bridge with R79, whereas the second interacts with R279 via a water molecule. In the case of ATR, which carries only one carboxyl group, the interactions with the peptide chain of the carrier are fewer, explaining why ATR exhibits a dissociation constant that is 10 times higher than that of CATR.

The high-resolution structure of the CATR-carrier complex also sheds light on a possible selectivity filter for substrates and on some putative molecular events implicated in the transport mechanism, such as salt bridge disruption and/or structural modification of transmembrane helices leading to the opening of the cavity (52, 58).

Oligomeric State of the Carrier and Molecular Mechanism of Transport

The oligomeric state of the functional membrane-embedded ADP/ATP carrier is still a matter of debate. However, as for many other members of the mitochondrial carrier family, such as the uncoupling protein (43), the phosphate carrier (60), or the citrate carrier (45), models implying dimeric assemblies are favored. For the ADP/ATP carrier, one of the most convincing arguments comes from the determination of the binding stoichiometry of either CATR or BA. Using radioactive ligands, a binding stoichiometry of one inhibitor per two carrier monomers was established, either for CATR (57) or for BA (2). From these results, a model in which inhibitor and/or nucleotide binding sites are localized at the interface of the two monomers was proposed (47). In such a case, the ligand pathway would be surrounded by 12 transmembrane helices. This hypothesis is now rejected in light of our high-resolution structural data, which show that CATR is trapped within the cavity made up of a single monomer. On the contrary, the structural data are consistent with a model of transport in which ADP/ATP exchange would rather be achieved by two adjacent carrier monomers, one binding a nucleotide coming from the matrix compartment and the other one from the intermembrane space. Such a mechanism implicates that both carriers are in tight vicinity and display transmembrane positive cooperativity for nucleotide binding. Numerous experimental data are consistent with a dimeric assembly of the carrier: ultracentrifugation analysis (31), small angle neutron scattering (8), cross-linking experiments (33), native gel electrophoresis (24), and covalent tandem dimer engineering (34, 66). It has been demonstrated more recently from the functional characterization of chimeras of yeast carriers in which the functional ADP/ATP carrier is fused to a nonfunctional subunit, that a cross talk between the two subunits is necessary for the exchange process to occur (54). From additional

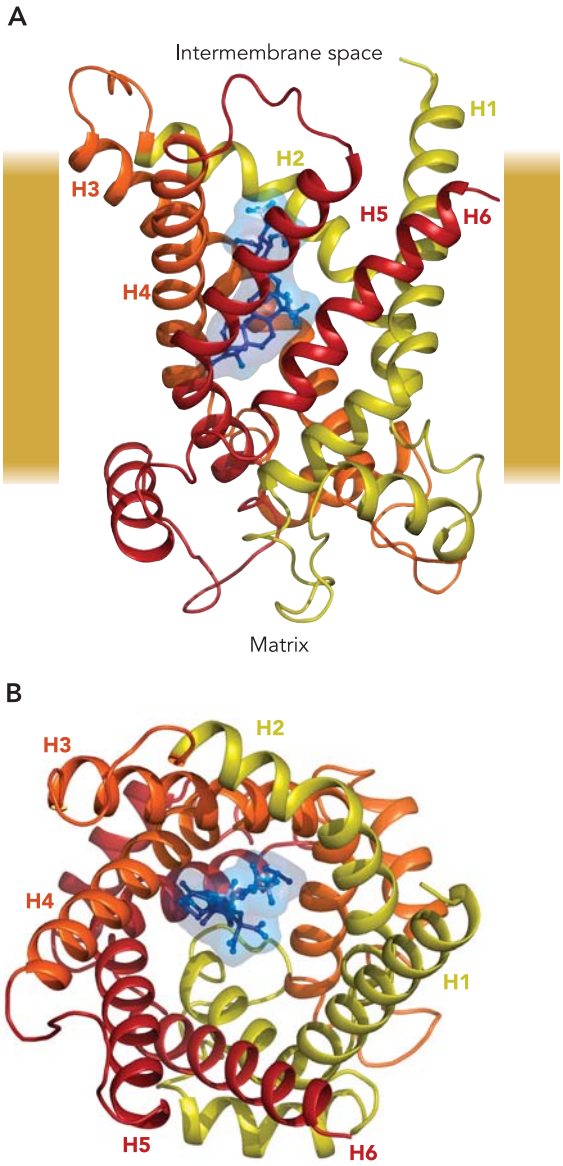

FIGURE 3. **Structure of the bovine ADP/ATP carrier in complex with carboxyatractyloside**
The six transmembrane helices (H1—H6) are depicted in ribbon representation. The first, second, and third motives of the protein are colored in yellow, orange, and red, respectively. CATR is shown in blue, in ball-and-stick representation, surrounded by its van der Waals surface. *A*: view parallel to the plane of the membrane. *B*: view from the intermembrane space.

structural data, we also postulate that this cross talk is possibly mediated by endogenous cardiolipins recovered in the protein crystals (50). From a functional point of view, a strong positive cooperativity in the binding of substrates coming from each side of the membrane has been suggested from kinetics studies (23). These results demonstrated that the ADP/ATP exchange can only occur when a ternary complex is formed, i.e., two nucleotides bound at the same time on a transport system. Then, nucleotide transport and release necessarily imply structural rearrangements, in which matrix loops are generally involved (46). In recent work performed on the yeast carrier, the central part of the second matrix loop was shown to actively participate to the exchange process through a swinging movement (14).

Pathophysiological Aspects of the ADP/ATP Carrier

Because of its key physiological function in the cell energy metabolism, defects in the ADP/ATP transport system result in severe disorders. Evidence that dysfunctioning of this system is implicated in pathologies was first provided by the elaboration of a knockout mouse deficient in the heart/muscle isoform of the carrier. Adult animals exhibited mitochondria proliferation in muscle together with cardiac hypertrophy and clinical presentation of myopathy (30). Deficiency of the ADP/ATP carrier has been reported in striated muscle of patients affected with myopathy (4) and in patients suffering from Sengers syndrome (36). In the latter case, the *hANC1* gene encoding the muscle isoform of the ADP/ATP carrier was not altered, indicating that the pathology could result either from impairment of the carrier transcription or from impeded import into the mitochondrial membrane.

Five-point mutations of hAnc1p, A89D, L98P, D104G, A114P, and V289M have been detected so far in patients affected with autosomal-dominant progressive external ophthalmoplegia (adPEO), an adult-onset pathology characterized by the weakness of external eye muscles (17, 37, 40, 49, 62). The functional consequences of these mutations were evaluated in yeast cells because cultured human cell lines proved to be unsuitable for *hANC1* overexpression (6). In a first approach, it was shown that the heterologous expression of *hANC1* was strongly impaired by either the A114P or the V289M mutation, thus rendering the mutated human genes unable to rescue the growth on nonfermentable carbon source of a yeast strain devoid of endogenous *ANC* genes (16). Following another strategy, Fontanasi et al. (27) introduced sequentially the L98P, A114P, and V289M mutations at corresponding positions in the yeast *ScANC2* gene. Results showed unambiguously a defective oxidative growth phenotype in yeast, thus demonstrating the pathological character of these mutations in humans. The consequences of these five-point mutations remain difficult to be explained on a structural basis even though bovine and human protein sequences are highly conserved.

It is well established that cancer cells display a glycolytic phenotype resulting from the downregulation of all mitochondria-encoded genes and impairment of the mitochondrial function. Neoplastic transformation is also associated with the puzzling overexpression of *hANC3*, a gene related to the yeast *ScANC3*, which is also induced under anaerobic conditions (12). Both genes possess in their promoter a sequence associated with negative regulation of transcription by oxygen. The authors hypothesized that hAnc3p imports glycolytic ATP into mitochondria in exchange for ADP exported from the matrix compartment. Such a process would sustain a basal mitochondrial activity, associated with the maintenance of a membrane potential, under the predominant glycolytic status of tumor cell proliferation (12).

Involvement of the ADP/ATP carrier in the pathophysiology of dilated cardiomyopathy was first assessed from the detection of anti-carrier antibodies in the sera of patients suffering from this disease (61). Further studies revealed a concomitant impairment of ADP/ATP carrier function in heart despite a higher total carrier protein content, which resulted from a shift in the isoform expression (19). It was demonstrated that an increased amount of hAnc1p isoform correlated to a decrease of hAnc3p isoform, with hAnc2p remaining at a constant level. Alterations in ADP/ATP carrier function and expression were also observed in inflammatory heart disease with enteroviral infection but not from other types of heart disease (20, 21).

The Permeability Transition Pore

The permeability transition of mitochondria is described as a generalized increase of permeability of the inner membrane to solutes with molecular masses up to 1,500 Da. It occurs as a result of Ca^{2+} accumulation or simply as a consequence of in vitro aging. The proposal that the permeability transition is linked to reversible opening of a pore rather than to unspecific membrane damage is based on the inhibitory effect of cyclosporin A (CsA) used at nanomolar concentrations (13, 29). Electrophysiological approaches carried out with isolated mitochondria led to the following findings: *1*) the pore is a high-conductance voltage-dependent membrane channel and *2*) there are a wide variety of factors and drugs able to trigger its opening, with the presence of calcium ions being essential for this event to occur (70).

Whereas the permeability transition pore (PTP) has been recently proposed to play a key role in the mitochondrial relay of apoptotic signals, its molecular nature remains unsolved (25). The PTP is believed to consist of mitochondrial proteins assembled in complexes with three central components: the inner mem-

brane ADP/ATP carrier, the matrix cyclophilin D, and the outer membrane voltage-dependent anion channel (VDAC or porin) (28). The initial idea that the ADP/ATP carrier could participate in forming the PTP emerged from the finding that its specific ligands, CATR and BA, are effectors of the mitochondrial permeability transition. However, this also provided evidence that there is no connection between the ADP carrier activity and the PTP status since CATR and BA, which are both potent inhibitors of the transport, trigger the opening and closing of the pore, respectively. Therefore, the effects exerted by the ADP/ATP carrier on PTP function likely lie on conformational grounds. The ADP/ATP carrier has been proposed to maintain close contact and possibly functional relationships with VDAC and proteins involved in apoptotic events such as Bax and Bcl-2 (28). Cyclophilin D has also been proposed to interact directly with the ADP/ATP carrier, and the fact that it was identified as the mitochondrial target of CsA indicated a possible mechanism for the CsA-mediated inhibition of PTP function (32).

A number of studies have been carried out with the aim of demonstrating unequivocally the involvement of the ADP/ATP carrier in PTP formation, but they have led to controversial conclusions. Results obtained by Brustovetsky et al. (11), based on patch-clamp experiments, clearly established the ability of the *Neurospora crassa* carrier to induce a Ca^{2+}-dependent channel in the membrane of proteoliposomes, excluding the contribution of PTP formation of other proteins originating from mitochondria. Although convincing, these results could not be reconciled with a previous report of a high conductance mitochondrial channel evidenced in yeast mitochondria in which *yANC* genes have been deleted (44). More recently, genetic strategies have been employed in mice to define the role of putative components of the mitochondrial PTP. Studies have essentially focused either on cyclophilin D or on the ADP/ATP carrier that were genetically eliminated in transgenic animals. Conclusions from three independent studies indicated that permeability transition can still be observed in mitochondria lacking cyclophilin D (3, 5, 48). In addition, Kokoszka et al. (38) have shown that mitochondria isolated from ADP/ATP carrier-deficient mice livers could undergo a CsA-sensitive Ca^{2+}-induced permeability transition, thus demonstrating that the carrier is not required for this process to occur. However, the capacity of mitochondria devoid of ADP/ATP carrier to accumulate Ca^{2+} ions before PTP opening was significantly increased, suggesting at the most its regulatory role in PTP function rather than a structural implication in the pore assembly.

Conclusion

The ADP/ATP carrier plays a key role in cell economy. Because of its unique properties, it has provided, in biochemical and genetic studies, advanced insights into the molecular basis of metabolite transport across biomembranes. A major progress in the knowledge of this carrier results from the recent determination at high resolution of the structure of the CATR-carrier complex. Solving the structure of the carrier in other conformational states would provide essential information to elucidate the molecular mechanism of adenine nucleotide exchange across the inner mitochondrial membrane and highlight the consequences of mutations involved in related genetic diseases. ■

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References

1. Adrian GS, McCammon MT, Montgommery DL, and Douglas MG. Sequence required for delivery and localization of the ADP/ATP translocator to the mitochondrial inner membrane. *Mol Cell Biol* 6: 626—634, 1986.
2. Aquila H, Eiermann W, Babel W, and Klingenberg M. Isolation of the ADP/ATP translocator from beef heart mitochondria as the bongkrekate-protein complex. *Eur J Biochem* 85: 549—560, 1978.
3. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, and Molkentin JD. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434: 658—662, 2005.
4. Bakker HD, Scholte HR, Van den Bogert C, Ruitenbeek W, Jeneson JA, Wanders RJ, Abeling NG, Dorland B, Sengers RC, and Van Gennip AH. Deficiency of the adenine nucleotide translocator in muscle of a patient with myopathy and lactic acidosis: a new mitochondrial defect. *Pediatr Res* 33: 412—417, 1993.
5. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, and Bernardi P. Properties of the permeability transition pore in mitochondria devoid of cyclophilin D. *J Biol Chem* 280: 18558—18561, 2005.
6. Bauer MK, Schubert A, Rocks O, and Grimm S. Adenine nucleotide translocase-1, a component of the permeability transition pore, can dominantly induce apoptosis. *J Cell Biol* 147: 1493—1502, 1999.
7. Benz R. *Structure and Function of Mitochondrial (Eukaryotic) Porins*. New York: Wiley, 2004.
8. Block MR, Zaccai G, Lauquin GJM, and Vignais PV. Small angle neutron sattering of the mitochondrial ADP/ATP carrier protein in detergent. *Biochem Biophys Res Commun* 109: 471—477, 1982.
9. Brandolin G, Le Saux A, Trézéguet V, Lauquin GJM, and Vignais PV. Chemical, immunological, enzymatic, and genetic approaches to studying the arrangement of the peptide chain of the ADP/ATP carrier in the mitochondrial membrane. *J Bioenerg Biomembr* 25: 459—472, 1993.
10. Bruni A, Luciani S, and Contessa AR. Inhibition by atractyloside of the binding of adenine-nucleotides to rat-liver mitochondria. *Nature* 201: 1219—1220, 1964.
11. Brustovetsky N, Tropschug M, Heimpel S, Heidkamper D, and Klingenberg M. A large Ca^{2+}-dependent channel formed by recombinant ADP/ATP carrier from *Neurospora crassa* resembles the mitochondrial permeability transition pore. *Biochemistry* 41: 11804—11811, 2002.
12. Chevrollier A, Loiseau D, Chabi B, Renier G, Douay O, Malthiery Y, and Stepien G. ANT2 isoform required for cancer cell glycolysis. *J Bioenerg Biomembr* 37: 307—317, 2005.
13. Crompton M, Ellinger H, and Costi A. Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. *Biochem J* 255: 357—360, 1988.

14. Dahout-Gonzalez C, Ramus C, Dassa EP, Dianoux AC, and Brandolin G. Conformation-dependent swinging of the matrix loop m2 of the mitochondrial *Saccharomyces cerevisiae* ADP/ATP carrier. *Biochemistry* 44: 16310—16320, 2005.

15. Daniele C, Dahamna S, Firuzi O, Sekfali N, Saso L, and Mazzanti LG. Atractylis gummifera poisoning: an ethnopharmacological review. *J Ethnopharmacol* 97: 175—181, 2005.

16. De Marcos Lousa C, Trézéguet V, Dianoux AC, Brandolin G, and Lauquin GJM. The human mitochondrial ADP/ATP carriers: kinetic properties and biogenesis of wild-type and mutant proteins in the yeast *Saccharomyces cerevisiae*. *Biochemistry* 41: 14412—14420, 2002.

17. Deschauer M, Hudson G, Muller T, Taylor RW, Chinnery PF, and Zierz S. A novel ANT1 gene mutation with probable germline mosaicism in autosomal dominant progressive external ophthalmoplegia. *Neuromuscul Disord* 15: 311—315, 2005.

18. Dolce V, Scarcia P, Iacopetta D, and Palmieri F. A fourth ADP/ATP carrier isoform in man: identification, bacterial expression, functional characterization and tissue distribution. *FEBS Lett* 579: 633—637, 2005.

19. Dorner A, Giessen S, Gaub R, Grosse Siestrup H, Schwimmbeck PL, Hetzer R, Poller W, and Schultheiss HP. An isoform shift in the cardiac adenine nucleotide translocase expression alters the kinetic properties of the carrier in dilated cardiomyopathy. *Eur J Heart Fail* 8: 81—89, 2006.

20. Dorner A, Pauschinger M, Schwimmbeck PL, Kuhl U, and Schultheiss HP. The shift in the myocardial adenine nucleotide translocator isoform expression pattern is associated with an enteroviral infection in the absence of an active T-cell dependent immune response in human inflammatory heart disease. *J Am Coll Cardiol* 35: 1778—1784, 2000.

21. Dorner A and Schultheiss HP. The myocardial expression of the adenine nucleotide translocator isoforms is specifically altered in dilated cardiomyopathy. *Herz* 25: 176—180, 2000.

22. Duszynski J, Bogucka K, Letko G, Kuster U, Kunz W, and Wojtczak L. Relationship between the energy cost of ATP transport and ATP synthesis in mitochondria. *Biochim Biophys Acta* 637: 217—223, 1981.

23. Duyckaerts C, Sluse-Goffart CM, Fux JP, Sluse FE, and Liebecq C. Kinetic mechanism of the exchanges catalysed by the adenine-nucleotide carrier. *Eur J Biochem* 106: 1—6, 1980.

24. Dyall SD, Agius SC, De Marcos Lousa C, Trézéguet V, and Tokatlidis K. The dynamic dimerization of the yeast ADP/ATP carrier in the inner mitochondrial membrane is affected by conserved cysteine residues. *J Biol Chem* 278: 26757—26764, 2003.

25. Ekert PG and Vaux DL. The mitochondrial death squad: hardened killers or innocent bystanders? *Curr Opin Cell Biol* 17: 626—630, 2005.

26. Fiore C, Trézéguet V, Le Saux A, Roux P, Schwimmer C, Dianoux AC, Noël F, Lauquin GJM, Brandolin G, and Vignais PV. The mitochondrial ADP/ATP carrier: structural, physiological and pathological aspects. *Biochimie* 80: 137—150, 1998.

27. Fontanesi F, Palmieri L, Scarcia P, Lodi T, Donnini C, Limongelli A, Tiranti V, Zeviani M, Ferrero I, and Viola AM. Mutations in AAC2, equivalent to human adPEO-associated ANT1 mutations, lead to defective oxidative phosphorylation in *Saccharomyces cerevisiae* and affect mitochondrial DNA stability. *Hum Mol Genet* 13: 923—934, 2004.

28. Forte M and Bernardi P. Genetic dissection of the permeability transition pore. *J Bioenerg Biomembr* 37: 121—128, 2005.

29. Fournier N, Ducet G, and Crevat A. Action of cyclosporine on mitochondrial calcium fluxes. *J Bioenerg Biomembr* 19: 297—303, 1987.

30. Graham BH, Waymire KG, Cottrell B, Trounce IA, MacGregor GR, and Wallace DC. A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat Genet* 16: 226—234, 1997.

31. Hackenberg H and Klingenberg M. Molecular weight and hydrodynamic parameters of the adenosine 5'-diphosphate/adenosine 5'-triphosphate carrier in Triton X-100. *Biochemistry* 19: 548—555, 1980.

32. Halestrap AP, McStay GP, and Clarke SJ. The permeability transition pore complex: another view. *Biochimie* 84: 153—166, 2002.

33. Hashimoto M, Majima E, Goto S, Shinohara Y, and Terada H. Fluctuation of the first loop facing the matrix of the mitochondrial ADP/ATP carrier deduced from intermolecular cross-linking of Cys56 residues by bifunctional dimaleimides. *Biochemistry* 38: 1050—1056, 1999.

34. Hatanaka T, Hashimoto M, Majima E, Shinohara Y, and Terada H. Functional expression of the tandem-repeated homodimer of the mitochondrial ADP/ATP carrier in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 262: 726—730, 1999.

35. Henderson PJ and Lardy HA. Bongkrekic acid. An inhibitor of the adenine nucleotide translocase of mitochondria. *J Biol Chem* 245: 1319—1326, 1970.

36. Jordens EZ, Palmieri L, Huizing M, van den Heuvel LP, Sengers RC, Dorner A, Ruitenbeek W, Trijbels FJ, Valsson J, Sigfusson G, Palmieri F, and Smeitink JA. Adenine nucleotide translocator 1 deficiency associated with Sengers syndrome. *Ann Neurol* 52: 95—99, 2002.

37. Kaukonen J, Juselius JK, Tiranti V, Kyttala A, Zeviani M, Comi GP, Keranen S, Peltonen L, and Suomalainen A. Role of adenine nucleotide translocator 1 in mtDNA maintenance. *Science* 289: 782—785, 2000.

38. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, and Wallace DC. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427: 461—465, 2004.

39. Kolarov J, Kolarova N, and Nelson N. A third ADP/ATP translocator gene in yeast. *J Biol Chem* 265: 12711—12716, 1990.

40. Komaki H, Fukazawa T, Houzen H, Yoshida K, Nonaka I, and Goto Y. A novel D104G mutation in the adenine nucleotide translocator 1 gene in autosomal dominant progressive external ophthalmoplegia patients with mitochondrial DNA with multiple deletions. *Ann Neurol* 51: 645—648, 2002.

41. Kunji ER and Harding M. Projection structure of the atractyloside-inhibited mitochondrial ADP/ATP carrier of *Saccharomyces cerevisiae*. *J Biol Chem* 278: 36985—36988, 2003.

42. Lawson JE and Douglas MG. Separate genes encode functionally equivalent ADP/ATP carrier proteins in *Saccharomyces cerevisiae*. Isolation and analysis of AAC2. *J Biol Chem* 263: 14812—14818, 1988.

43. Lin CS and Klingenberg M. Isolation of the uncoupling protein from brown adipose tissue mitochondria. *FEBS Lett* 113: 299—303, 1980.

44. Lohret TA, Murphy RC, Drgon T, and Kinnally KW. Activity of the mitochondrial multiple conductance channel is independent of the adenine nucleotide translocator. *J Biol Chem* 271: 4846—4849, 1996.

45. Ma C, Kotaria R, Mayor JA, Eriks LR, Dean AM, Walters DE, and Kaplan RS. The mitochondrial citrate transport protein: probing the secondary structure of transmembrane domain III, identification of residues that likely comprise a portion of the citrate transport pathway, and development of a model for the putative TMDIII—TMDIII′ interface. *J Biol Chem* 279: 1533—1540, 2004.

46. Majima E, Ikawa K, Takeda M, Hashimoto M, Shinohara Y, and Terada H. Translocation of loops regulates transport activity of mitochondrial ADP/ATP carrier deduced from formation of a specific intermolecular disulfide bridge catalyzed by copper-*O*-phenanthroline. *J Biol Chem* 270: 29548—29554, 1995.

47. Majima E, Koike H, Hong YM, Shinohara Y, and Terada H. Characterization of cysteine residues of mitochondrial ADP/ATP carrier with the SH-reagents eosin 5-maleimide and N-ethylmaleimide. *J Biol Chem* 268: 22181—22187, 1993.

48. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, and Tsujimoto Y. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 434: 652—658, 2005.

49. Napoli L, Bordoni A, Zeviani M, Hadjigeorgiou GM, Sciacco M, Tiranti V, Terentiou A, Moggio M, Papadimitriou A, Scarlato G, and Comi GP. A novel missense adenine nucleotide translocator-1 gene mutation in a Greek adPEO family. *Neurology* 57: 2295—2298, 2001.

50. Nury H, Dahout-Gonzalez C, Trézéguet V, Lauquin GJM, Brandolin G, and Pebay-Peyroula E. Structural basis for lipid mediated interactions between mitochondrial ADP/ATP carrier monomers. *FEBS Lett* 579: 6031—6036, 2005.

51. Palmieri F. The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflügers Arch* 447: 689—709, 2004.

52. Pebay-Peyroula E and Brandolin G. Nucleotide exchange in mitochondria: insight at a molecular level. *Curr Opin Struct Biol* 14: 420—425, 2004.

53. Pebay-Peyroula E, Dahout-Gonzalez C, Kahn R, Trézéguet V, Lauquin GJM, and Brandolin G. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature* 426: 39—44, 2003.

54. Postis V, De Marcos Lousa C, Arnou B, Lauquin GJM, and Trézéguet V. Subunits of the yeast mitochondrial ADP/ATP carrier: cooperation within the dimer. *Biochemistry* 44: 14732—14740, 2005.

55. Pressman BC. Intramitochondrial nucleotides. I. Some factors affecting net interconversions of adenine nucleotides. *J Biol Chem* 232: 967—978, 1958.

56. Rehling P, Brandner K, and Pfanner N. Mitochondrial import and the twin-pore translocase. *Nat Rev Mol Cell Biol* 5: 519—530, 2004.

57. Riccio P, Aquila H, and Klingenberg M. Purification of the carboxy-atractylate binding protein from mitochondria. *FEBS Lett* 56: 133—138, 1975.

58. Robinson AJ, and Edmund ERS, and Kunji RS. Mitochondrial carriers in the cytoplasmic state have a common substrate binding site. *Proc Natl Acad Sci USA* 103: 2617—2622, 2006.

59. Roux P, Le Saux A, Fiore C, Schwimmer C, Dianoux AC, Trézéguet V, Vignais PV, Lauquin GJM, and Brandolin G. Fluorometric titration of the mitochondrial ADP/ATP carrier protein in muscle homogenate with atractyloside derivatives. *Anal Biochem* 234: 31—37, 1996.

60. Schroers A, Burkovski A, Wohlrab H, and Krämer R. The phosphate carrier from yeast mitochondria. Dimerization is a prerequisite for function. *J Biol Chem* 273: 14269—14276, 1998.

61. Schultheiss HP and Bolte HD. Immunological analysis of auto-antibodies against the adenine nucleotide translocator in dilated cardiomyopathy. *J Mol Cell Cardiol* 17: 603—617, 1985.

62. Siciliano G, Tessa A, Petrini S, Mancuso M, Bruno C, Grieco GS, Malandrini A, DeFlorio L, Martini B, Federico A, Nappi G, Santorelli FM, and Murri L. Autosomal dominant external ophthalmoplegia and bipolar affective disorder associated with a mutation in the ANT1 gene. *Neuromuscul Disord* 13: 162—165, 2003.

63. Siekevitz P and Potter VR. Biochemical structure of mitochondria. II. Radioactive labeling of intra-mitochondrial nucleotides during oxidative phosphorylation. *J Biol Chem* 215: 237—255, 1955.

64. Stepien G, Torroni A, Chung AB, Hodge JA, and Wallace DC. Differential expression of adenine nucleotide translocator isoforms in mammalian tissues and during muscle cell differentiation. *J Biol Chem* 267: 14592—14597, 1992.

65. Stewart MJ and Steenkamp V. The biochemistry and toxicity of atractyloside: a review. *Ther Drug Monit* 22: 641—649, 2000.

66. Trézéguet V, Le Saux A, David C, Gourdet C, Fiore C, Dianoux A, Brandolin G, and Lauquin GJM. A covalent tandem dimer of the mitochondrial ADP/ATP carrier is functional in vivo. *Biochim Biophys Acta* 1457: 81—93, 2000.

67. Vignais PV, Block MR, Boulay F, Brandolin G, and Lauquin GJM. *Molecular Aspects of Structure: Function Relationships in Mitochondrial Adenine Nucleotide Carrier*. Cleveland, OH: CRC, 1985.

68. Walker JE. The mitochondrial transporter family. *Curr Opin Struct Biol* 2: 519—526, 1992.

69. Werkeiser WC and Bartley W. The study of steady-state concentrations of internal solutes of mitochondria by rapid centrifugal transfer to a fixation medium. *Biochem J* 66: 79—91, 1957.

70. Zoratti M and Szabo I. The mitochondrial permeability transition. *Biochim Biophys Acta* 1241: 139—176, 1995.