J Bioenerg Biomembr (2008) 40:435–443
DOI 10.1007/s10863-008-9178-2

The mitochondrial ADP/ATP carrier: functional and structural studies in the route of elucidating pathophysiological aspects

Véronique Trézéguet · Ludovic Pélosi · Guy J. M. Lauquin · Gérard Brandolin

Received: 4 July 2008 / Accepted: 23 July 2008 / Published online: 1 November 2008

Abstract The mitochondrial ADP/ATP carrier plays a central role in aerobic cell energetics by providing to the cytosol the ATP generated by oxidative phosphorylation. Though discovered around 40 years ago owing to the existence of unique inhibitors and in spite of numerous experimental approaches, this carrier, which stands as a model of the mitochondrial solute carriers keeps some long-standing mystery. There are still open challenging questions among them the precise ADP/ATP transport mechanism, the functional oligomeric state of the carrier and relationships between human ADP/ATP carrier dysfunctioning and pathologies. Deciphering the 3D structure of this carrier afforded a considerable progress of the knowledge but requires now additional data focused on molecular dynamics from this static picture. State of the art in this topic is reviewed and debated in this paper in view of better comprehending origin of the discrepancies in these questions and, finally, the multiple physiological roles of this carrier in eukaryotic cell economy.

This work was supported by the University of Bordeaux2, the Région Aquitaine, the University Joseph Fourier of Grenoble, the Région Rhône-Alpes, the Commissariat à l'Energie Atomique, and the Centre National de la Recherche Scientifique.

V. Trézéguet (✉) · G. J. M. Lauquin
Laboratoire de Physiologie Moléculaire et Cellulaire,
Institut de Biochimie et Génétique Cellulaires-UMR 5095,
CNRS-Université Bordeaux2,
1, rue Camille Saint-Saëns,
33077 Bordeaux Cedex, France
e-mail: vero.trezeguet@ibgc.u-bordeaux2.fr

L. Pélosi · G. Brandolin
Laboratoire de Biochimie et Biophysique des Systèmes Intégrés (BBSI), Institut de Recherches en Technologies et Sciences du Vivant (iRTSV), UMR 5092,
CNRS-CEA-Université Joseph Fourier,
38054 Grenoble cedex 9, France

Keywords ADP/ATP carrier · Mitochondria · Carboxyatractylate · Bongkrekic acid · Pathophysiology

Introduction

Major metabolic pathways occur within mitochondria, which is the primary site of ATP synthesis. This organelle is surrounded by two membranes (outer and inner) that are therefore important sites for the regulation of metabolic functions, though at different degrees. The mitochondrial Carrier Family (MCF) members are integral membrane proteins that transport various metabolites through the mitochondrial inner membrane, the real isolating barrier between cytosol and matrix mitochondria. Among them the mitochondrial ADP/ATP carrier (Ancp) was the first to be identified around 40 years ago (Bruni et al. 1964). It plays a key role in the energetic cell metabolism because it exchanges ATP and ADP, product and substrate of the mitochondrial ATP synthase, respectively. Ancp is the most abundant among MCF members (up to 10% of mitochondrial proteins in beef heart mitochondria) and has to cope with high nucleotide amounts in order to fulfill cell energetic requirements.

The field of Ancp studies has emerged and dominated because this carrier can be efficiently and specifically inhibited by two classes of natural poisons, of which the chemical natures are very different. Atractyloside (ATR) and carboxyatractyloside (CATR) are heteroglucosides produced by the thistle *Atractylis gummifera*, a plant growing in the Mediterranean countries. ATR and CATR are nonpermeant and bind exclusively on the cytosolic side of Ancp, with a K_d in the nanomolar range. Bongkrekic (BA) and isobongkrekic (isoBA) acids are polyunsaturated long chain fatty acid derivatives secreted by the bacteria

Pseudomonas cocovenenans, now reassigned to *Burkholderia cocovenenans*. They are permeant inhibitors given the appropriate pH conditions. They bind exclusively on the matrix side of Ancp also with K_d values in the nanomolar range.

Mutually exclusive binding of CATR and BA afforded evidence that Ancp adopts at least two conformations, referred to as CATR and BA conformations, in equilibrium in the membrane in the absence of the inhibitors. They were differentiated on the basis of numerous experimental approaches (for a review see Brandolin et al. 1993). Only transportable nucleotides can trigger the interconversion between those two conformations in the absence of inhibitors, suggesting that the BA and CATR conformations are involved in ADP/ATP transport.

The three-dimensional structure of bAnc1p, model member of the MCF, has been solved by X-ray crystallography at a 2.2 Å resolution (Pebay-Peyroula et al. 2003). So far, it is the only one obtained for MCF members. The structure is that of a monomer in complex with CATR. Ancp has the general shape of a basket delineated by six tilted transmembrane segments (TMS 1 to 6). The odd-numbered ones are sharply kinked at the level of proline residues. On the matrix side, three hydrophilic loops contain small amphipatic helices that are parallel to the membrane surface and that tightly surround the carrier. As a result, Ancp has a basket shape that opens toward the intermembrane space and CATR lies deeply inside its cavity. Interactions between the carrier and CATR involve several basic residues located mainly in TMS 2 to 4, hydrophobic residues and also hydrogen bonds some of which involve water molecules.

Ancps (around 300 amino acids) are encoded by the nuclear genome and are imported into mitochondria through the TOM/TIM machinery (for a review, see Bolender et al. 2008). The number of isoforms is highly variable with the organism. In human and beef, four isoforms have been identified (for a review, see for example Dahout-Gonzalez et al. 2006). In *Saccharomyces cerevisiae*, only one out of three isoforms, *ScANC2*, is essential for the cells to grow on nonfermentable carbon sources. All of them are dispensable on fermentable carbon sources.

Evolutionary conservation analysis of Ancps

Identification of regions of Ancps conserved over a wide phylogenetic range was performed using the Consurf web-server (http://consurf.tau.ac.il; Landau et al. 2005). A multiple sequence alignment of 116 Ancp sequences was generated with ClustalX (Chenna et al. 2003) and conservation scores computated with a Bayesian method were mapped on the crystal structure of bAnc1 (1okc; Glaser et al. 2003).

The most variable residues are mainly located in the carrier periphery facing the lipid membrane (Fig. 1A and B) while the protein core including the cavity open to the intermembrane space exhibits most of the conserved residues. The extra-membrane loops connecting the transmembrane segments including the three small helices facing the matrix are also enriched in variable amino acids. This result was not unexpected as the propension of conserved residues to locate mainly within the protein core of transmembrane proteins has been set out as a predictive

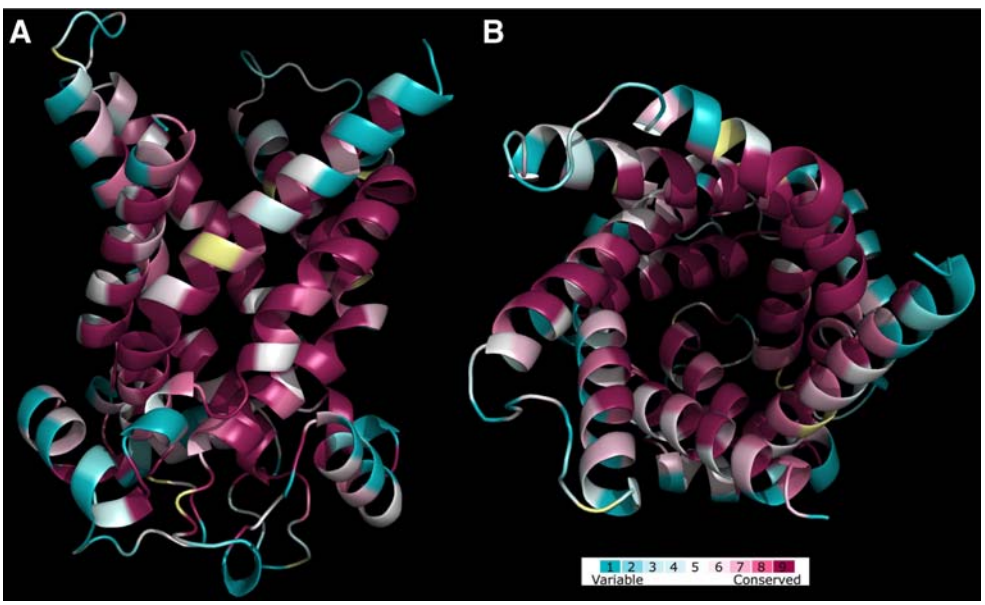

Fig. 1 Evolution conservation mapping of Ancps. The evolutionary conservation scores were projected on the bAnc1p crystal structure represented as a ribbon diagram with the program PYMOL (DeLano, WL, 2002, http://www.pymol.org). **A** Lateral view **B** View from the intermembrane space. The amino acids are *colored* according to their conservation grades using a color scale from turquoise-through-maroon indicating variable-through-conserved. Colored in *yellow* are the amino acids for which insufficient data is available from the multiple sequence alignment

rule for modeling transmembrane segments (Fleishman et al. 2006, Fleishman and Ben-Tal 2006).

The oligomeric state of the Ancps

The idea that the bovine Ancp was organized as a dimer emerged from the finding that the bAnc1p•CATR complex isolated in detergent contained 1 mol of CATR bound per 2 mol of Ancp (Block et al. 1980; Hackenberg and Klingenberg 1980). Investigation of the oligomeric state was further carried out by biochemical and biophysical approaches as well as molecular biology techniques (for a review, see for example Nury et al. 2006). Because Ancp is a paradigm of the MCF, studies were extended to other members, including the uncoupling protein (Klingenberg and Appel 1989; Lin and Klingenberg 1982), the oxoglutarate (Bisaccia et al. 1993; Palmisano et al. 1998), and the citrate (Kotaria et al. 1999; Capobianco et al. 2002) carriers. The most convincing demonstration was brought about by Schroers et al. (1998) who evidenced unambiguously the association of differently tagged phosphate carrier monomers in heterodimers. Taken together, these results led to the widely accepted concept of the dimeric organization of the mitochondrial carriers.

This model was challenged upon elucidation of the 3D structure of the bAnc1p·CATR complex which revealed only carrier monomers in the crystal lattice (Pebay-Peyroula et al. 2003). However protein–protein interactions between adjacent monomers, seemingly mediated by cardiolipins, were visualized in crystals obtained from "low salt" preparations of bAnc1p (Nury et al. 2005). This emphasizes the critical role of experimental conditions used for the handling of membrane proteins, particularly regarding the possible dissociating effect of ionic strength and elevated detergent concentrations during the crystallisation process. Therefore reconciling the biochemical and structural data requires to establish the existence of an asymmetric dimeric bAnc1p·CATR complex consisting of interacting but structurally distinct monomers, only one of them carrying CATR. It is postulated that the latter was crystallized following dissociation of the dimer whereas the unloaded monomer escaped crystallization, possibly because of its unlocked conformation. Although formation of the crystalline bAnc1p dimers did not comply to the CATR-binding stoichiometry, it illustrated the potential role of cardiolipins in mediating monomer-monomer interactions. This could explain the requirement for cardiolipins of reconstituted functional ScAnc2p (Hoffmann et al. 1994).

The dogma of a dimeric organization of mitochondrial carriers was recently refuted from a set of experiments carried out with ScAncp. Using analytical ultracentrifugation it was shown to exist only as monomers in detergent solution (Bamber et al. 2006), unlike bAnc1p (Hackenberg and Klingenberg 1980). Providing the oligomeric state of ScAncp was not modified during sample preparation, which required several days of dialysis to remove excess of detergent, this result may indicate that the isolated beef and yeast carriers display different oligomeric states. However, it cannot be ruled out that weaker protein-lipid interactions of ScAncp have lead to the dissociation of dimers, depending on the nature of the detergent.

Oligomerization of ScAncp was also investigated by negative dominance studies at the molecular level. ScAncp, which is inactivated by thiol specific reagents, and the corresponding cysteine-less mutant, which is a fully active carrier, were co-expressed in various molar ratios (Bamber et al. 2007a). The transport rates of the combined Ancps were then assessed in a semi-reconstituted system in the presence of a cysteine alkylating reagent. It was found that the ADP/ATP transport of the cysteine-less carrier was unaffected by inhibition of the co-expressed wild type ScAncp, indicating nucleotide transport was not sustained by a functional dimer. This interpretation should be more convincing if deduced from higher transport rates and therefore improved accuracy in measurements.

No stable dimer was detected following the differential purification of tagged or untagged ScAncp co-expressed in defined molar ratios in yeast, whatever the nature of the detergent used for solubilization (Bamber et al. 2007b). However, the conclusion that the two forms worked independently as monomers in the membrane has ignored the dynamic status of ScAncp in the membrane, which was demonstrated in following the ability of ScAncp monomers to readily associate and dissociate (Dyall et al. 2003).

Mechanistic and structural aspects of the Ancp function

Spectrofluorometric experiments carried out with naphthoyl-ADP (N-ADP), a fluorescent derivative of ADP, demonstrated that the CATR and the BA conformers of the beef heart Ancp co-exist in equilibrium and therefore do not undergo an "induced-fit" transition (Fiore et al. 1998). Further, both the CATR and BA conformers were found to bind externally added ADP or ATP, a result which obviously contradicts the "single reorienting site" mechanism of ADP/ATP transport. According to it, a single binding site for nucleotides and inhibitors may be alternatively open to the inside ("m" state of Ancp) and to the cytosol ("c" state) during the transport process (Klingenberg 2007). As a consequence, the CATR and BA conformations are clearly not equivalent to the postulated "c" and "m" states. Moreover, the single site mechanism cannot be reconciled with the existence of several adenine nucleotide binding sites, demonstrated for either the bovine or the yeast membrane-embedded ADP/ATP carriers (Block and Vignais 1984; Clémençon et al.

2008). These findings strongly support the transitory occurrence of a ternary complex made up of one transport unit and two bound nucleotides during the transport process (Duyckaerts et al. 1980).

The 3D structure of the monomeric bAnc1p·CATR complex is consistent with a model of transport in which the ADP/ATP exchange would be achieved by two adjacent monomers, one binding a nucleotide coming from the matrix compartment and the other one from the intermembrane space. According to this model, a cross talk between the two subunits, possibly mediated by tightly bound cardiolipins (Nury et al. 2005), in a dimeric assembly of the carrier is obviously required and probably responsible for half-of-the-sites reactivity for CATR binding leading to the stoichiometry of one CATR bound per two monomers of Ancp (Riccio et al. 1975).

It is difficult to deduce the location of the nucleotide binding sites from the structure of the constrained bAnc1p·CATR complex which has lost the dynamic properties of the CATR conformer (see above). However the patches of positive charges present within the cavity probably attract and bind nucleotides coming from the outside. This is consistent with the photolabeling of a central region of bAnc1p within the cavity with a nucleotide derivative (Dalbon et al. 1988). The binding of nucleotides to Ancp has to induce conformational changes that trigger the one-to-one exchange. We have hypothesized that these changes are linked to molecular events such as salt bridges disruptions and/or transmembranes helices organization re-arrangements (Pebay-Peyroula and Brandolin 2004).

Genetic approaches to unraveling the ADP/ATP transport mechanism

Structure-function studies of the ADP/ATP carrier obviously required the compulsory contribution of genetic approaches to investigate the effect of point mutations on transport activity. Most of them have been achieved using yeasts which contain endogenous mitochondrial ADP/ATP carriers and which are suited for the screening of the functional state of carrier mutants because of their ability to grow under either fermentative or respiratory conditions. Site-directed mutagenesis has been mostly applied with the aim at locating strategic amino acid residues expected to play a role in the transport mechanism but although promising, this technique has not often afforded a clear-cut interpretation of data. Different from the approach which consists in inactivating transport in mutants, we have addressed to a fluorometric approach based on the use of tryptophan-directed mutants that were selected for their ability to keep full transport activity (Le Saux et al. 1996; Roux et al. 1996).

Mutated forms of ScAnc2p able to sustain the growth of yeast on non-fermentable carbon sources were characterized with respect to ADP/ATP transport in isolated mitochondria or in proteoliposomes. It should be noticed that whereas the reconstituted systems allow the investigation of the intrinsic functional characteristics of a given mutated carrier, they do not afford the relevant comparison of absolute rates of transport because of possible differential insertion of mutated proteins into proteoliposomes.

Obviously the correlation of the effects of mutations on the actual inactivation of the carrier at the level of mitochondria must take into account the possible inhibition of the biogenesis and the import of the carrier into mitochondria. This implies the compulsory determination of the carrier content which is classically achieved based on either immunochemical techniques or measurements of binding of specific inhibitors, providing they are not affected by the mutation.

In a different way, attempts to segregate mutational effects on the production and on the function of the carrier have been performed after its heterologous expression in bacteria, then isolation and reconstitution. Results confirmed that the removal of positively charged residues strongly affected the nucleotide exchange activity but they also evidenced the role of some mutations in the folding of the carrier (Heimpel et al. 2001).

The mutagenesis approach has been extended to the search of intragenic second-site revertants spontaneously generated in yeast from inactivated mutants of the carrier (Klingenberg 1993) Despite the fact that in some cases the number of revertants was unexpectedly high, as illustrated in the case of the R254I primary mutation, this approach anticipated the existence of charge pairs able to build a network, as we found after determination of the 3D structure of the bovine carrier.

The first mutation in ScAnc2p that has been investigated, R96H, was obtained by random mutagenesis of yeast cells; it prevents their growth on glycerol (Kolarov et al. 1990; Klingenberg 1993) but in this case the transport capacity of the ADP/ATP carrier, assessed with a reconstituted system, is only partially impeded. Experiments with isolated mitochondria led to similar conclusion (Postis et al. 2005). A number of other substitutions, listed in Nury et al. (2006) have been carried out, some of them have been characterized with respect to the ADP/ATP transport function. This led to the conclusion of the general sensitivity of ScAnc2p to the elimination of charged residues, some of which being acidic but most of them positively charged. Whether these residues are directly involved in functioning of the carrier (for example arginine and lysine being implicated in the binding of nucleotides) or swings between different conformational states, or contribute to the proper folding of the carrier could not be determined.

Current standing: the ScAnc2p vs bAnc1p structure

Following the elucidation of the 3D structure of the bAnc1p·CATR complex it was discovered that residues of ScAnc2p whose mutation was deleterious for transport activity corresponded to the residues tightly interacting with CATR, thus leading to the hypothesis that ADP and CATR binding sites overlap. This assumption is based on the implicit statement that ScAnc2p and bAnc1p display similar folding, consistent with the fact they share 47% sequence identity. In contrast to this postulate it has been observed marked topographical differences between the yeast and the bovine carriers. The major evidences came from limited proteolysis experiments of the membrane-bound carriers (Marty et al. 1992; Dahout-Gonzalez 2005). For example we have shown that bAnc1p in mitoplasts in the presence of CATR is remarkably resistant to trypsin proteolysis, remaining uncleaved even after long time exposition to this protease, whereas in contrast the ScAnc2p carrier is readily digested under similar conditions. These results clearly show that the peptide segments of bAnc1p and ScAnc2p disclosed on the cytoplasmic face of the membrane are not equivalent—because directly related to their primary sequences—a conclusion that was strongly supported by additional labeling experiments combined to site-directed mutagenesis (Dahout-Gonzalez et al. 2005). The fragments generated from ScAnc2p proteolysis belong to a central loop which would be exposed to the matrix, assuming that ScAnc2p and bAnc1p share similar 3D structure. It was therefore proposed that the cleavable loop of ScAnc2p, whereas connecting the ends of TM helices in the matrix compartment, protrudes within the membrane. The existence of re-entrant loops has already been hypothesized on the basis of labeling experiments in the case of the ADP/ATP carrier (Bogner et al. 1986; Mayinger et al. 1989; Majima et al. 1993; Dianoux et al. 2000), and of the mitochondrial carnitine/acylcarnitine carrier (Indiveri et al. 2002). This has also been clearly established from the elucidation of the high resolution structures of a glutamate transporter (Yernool et al. 2004) and of a potassium channel (Doyle et al. 1998).

The involvement of the intramembrane loop in the ADP/ATP transport function was first inferred from its CATR-sensitive photolabeling with an ADP derivative (Dianoux et al. 2000). It was demonstrated in a further study that this loop undergoes conformational changes during the transport cycle of the carrier, as probed by limited proteolysis and by chemical modification with alkylating reagents such as EMA (Dahout-Gonzalez et al. 2005).

It should be emphasized that no intruding loop was detected in the crystal structure of the bAnc1p•CATR complex, suggesting it does not perfectly describe the structure of ScAnc2p. However assuming the dimeric organization of the carrier in the mitochondrial membrane, we cannot rule out some still unexpected features of the monomers which do not bind CATR.

Appraisal of the pore function of Ancp

Because it is implicated in cell apoptosis and necrosis and in ischemia-reperfusion linked damages in brain and heart, the mitochondrial permeability transition (MPT) has important physiological relevance and was ascribed to a permeability transition pore (PTP) opening (Leung and Halestrap 2008). The possible role of Ancp in MPT was suggested by the effects of atractyloside and bongkrekic acid observed during patch clamp experiments of isolated mitochondria in the presence of Ca^{2+}. Following a wealth of efforts devoted to characterize PTP properties and protein composition, cyclophilin D, Ancp and VDAC (mitochondrial porin) were proposed as central components. Additional proteins, including hexokinase II, creatine kinase as well as Bcl-2 family members and the benzodiazepine receptor, were suspected to belong to the pore, based on co-purification and reconstitution experiments, although some of which may be flawed (Beutner et al. 1998). Besides, the demonstration of the modulation of mitochondrial cyclosporin A-sensitive Ca^{2+} leakage by quinone compounds was a strong indication that also complex I participates in the MPT (Fontaine et al. 1998). New insights have been provided by genetic knockout of individual putative PTP components (Juhaszova et al. 2008). Cyclophilin D acts as a central regulator of the pore function, being the exclusive target of cyclosporin A (CsA) and, unexpectedly, VDAC is not an essential component, ruling out the classical model of the PTP architecture.

Involvement of Ancp itself remains largely controversial opposing contradictory findings. For example, convincing results have shown that recombinant Ancp expressed in bacteria behaved exactly as the PTP (Brustovetsky et al. 2002). On the other hand, several genetic approaches have seemingly refuted the participation of Ancp. First, a high conductance channel similar to the PTP was characterized in yeast mitochondria in which the *ANC* genes have been deleted (Lohret et al. 1996). Second, it was recently demonstrated that CsA sensitive MPT could still be induced by Ca^{2+} ions in mouse liver mitochondria in which the Ancp was genetically inactivated (Kokoszka et al. 2004). Yet, it was observed that the Ca^{2+} concentration required for PTP activation was higher than that used with mitochondria from non-transgenic animals, arguing for a regulatory role of Ancp. Alternatively, in the absence of Ancp other MCF members, such as the mitochondrial phosphate carrier, could take over the pore function (Leung and Halestrap

2008), implicating the carriers behave as unselective channels under conditions that trigger pore opening.

Pathologies associated with ADP/ATP carrier deficiencies

In human, four *ANC* isoforms encode Ancp and their expression has tissue-specific pattern. Their confusing nomenclature is made explicit in Dahout-Gonzalez et al. (2005). *HANC1* is specific to heart, skeletal muscles and brain, organs with restricted mitotic regeneration; *HANC2* is ubiquitous but transcribed at low levels; *HANC3* is growth-regulated and highly transcribed in proliferating cells with high energetic demand such as kidney, liver and spleen (Stepien et al. 1992) but also in cancer cells (Chevrollier et al. 2005). *HANC4* was identified from an expressed sequence tag library and its transcripts detected mainly in testis, liver and brain (Dolce et al. 2005).

HANC1 is expressed at low levels in proliferating cells and its over-expression induces apoptosis (Bauer et al. 1999) by depleting the nuclear factor kappa B (Zamora et al. 2004). Advantage has been taken from that by Jang et al. (2008a) who observed significant tumor growth inhibition after transfection of a nude mouse with *HANC1*. Interestingly, these authors used also siRNA interference to inactivate *HANC3* expression in cultured human breast cancer cells and in a nude mouse. They observed induction of apoptotic cell death and significant in vivo tumor regression (Jang et al. 2008b).

Ancp, along with the phosphate carrier (Picp), is directly linked to mitochondrial energy production through the ATP synthase to which those carriers provide its substrates. Therefore any dysfunction of Ancp is expected to result in reduced energy production in tissues were it is expressed (for a review, see Sharer 2005). Besides, oxidative phosphorylation disorders are known to induce heterogeneous clinical aspects. This was also true for *ANC1* invalidation in mouse resulting in an animal presenting the characteristic features of myopathy and cardiomyopathy (cardiac hypertrophy, mitochondrial over-proliferation in skeletal and heart muscles, ragged-red fibers, elevated levels of serum lactate...; Graham et al. 1997) Yin et al. (2005) further showed that *Ant1*$^{-/-}$ mice presented chronic external ophthalmoplegia associated with normal ocular motility, probably because *HANC1* defect was compensated for by increase in *HANC3* transcripts.

HANC1 expression is modified in several cases of mitochondrial myopathies such as myoclonic epilepsy associated with ragged-red fibers, myopathy, encephalopathy, lactic acidosis and stroke-like episodes and Kearn–Sayre syndrome (Heddi et al. 1993). This reflects the intricate coordination of nuclear and mitochondrial OXPHOS gene expression to compensate for respiratory deficiencies. Getting insights into the mechanisms involved may take advantage of global analyses of nuclear and mitochondrial transcriptomes and proteomes.

Sengers syndrome is characterized by congenital cataract, hypertrophic cardiomyopathy, mitochondrial myopathy and lactic acidosis. HAnc1p content has been determined in unrelated or sibling patients. It is strongly reduced or undetectable in muscles and this is associated with a low or null ADP/ATP transport activity (Jordens et al. 2002; Morava et al. 2004). However genetic analyzes suggested that *HANC1* was not the primary cause of the defect and the authors hypothesized that rather it was one or several genes involved in *HANC1* transcription, translation or post-translational modification and processing.

Autoantibodies against HAnc1p, the heart and muscle-specific isoform, were detected in patients suffering from dilated cardiomyopathy (DCM; Schultheiss and Bolte 1985), associated with nucleotide transport inhibition and increase in HAncp content (Schultheiss 1992; Sylven et al. 1993). Detailed analyses of DCM patients evidenced no *HANC1* mutation but rather an isoform shift in the pattern of *HANC* gene expression (Dörner et al. 2006). hAnc1p was over-produced and *HANC3* was down-regulated, accounting for a 50% reduced mitochondrial ADP/ATP transport activity in DCM patients as compared to the controls. Indeed, HAnc3p has a higher V_{Max} value than HAnc1p (De Marcos Lousa et al. 2002) with K_M for ADP values in the micromolar range. Though increased, the amount of HAnc1p would not be sufficient to compensate for HAnc3p defect. This can be understood considering first that *HANC3* expression is substantially higher in tissues that predominantly express *HANC1* and are also high-energy consumers (Dörner et al. 2006) and, second, that *HANC3* knockout in mouse is lethal, pointing out the importance of this isoform (Kokoszka et al. 2004). An *HANC1* point mutation was detected in a patient presenting mitochondrial myopathy and cardiomyopathy that converted the conserved A123 into D (Palmieri et al. 2005). The mutation is recessive and induces large mtDNA deletions. The mutant HAnc1p reconstituted in liposomes from biopsy of the patient is inactive.

In the above-reported pathologies, *HANC* point mutation was evidenced in only one case, and the HAncp defect was due instead to so far unknown factors, contrary to what has been described for adPEO (autosomal dominant progressive external ophthalmoplegia). Those patients suffer from chronic external ophthalmoplegia, generalized myopathy and exercise intolerance. Four different nuclear loci are involved in adPEO, which is one of the most frequent nuclear gene defect associated with multiple mtDNA deletions. Direct involvement of HAnc1p mutations is described in one sporadic (V289M) and in four familial

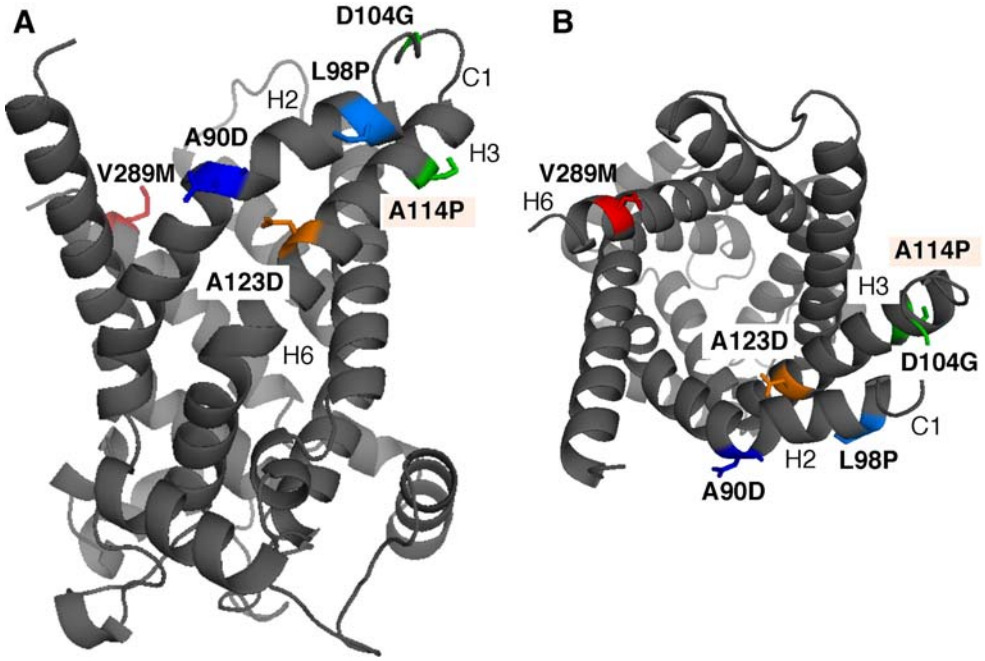

Fig. 2 Mutations associated with HAnc1p deficiencies. **A** Lateral view of the structural model of beef Anc1p (cartoon representation with PYMOL) showing the positions equivalent to the six amino acid substitutions found to date in patients presenting adPEO and cardiomyopathy (A123D). **B** View from the cytoplasmic side. Mutations D90A and L98P are located in transmembrane α-helix H2, A114P, A123D in H3, V289M in H6 and D104G in the cytosolic loop CI. Only two of those amino acids are pretty well conserved among all known Ancp sequences (A90=99%; A123=97%); the other ones present various conservation degrees: D104=72%; A114=64%; V289=53%; L98 or I98=49% (Nury et al. 2006)

(A90D, A114P, L98P, D104G) cases of adPEO (Fig. 2; Kaukonen et al. 2000; Napoli et al. 2001; Komaki et al. 2002; Siciliano et al. 2003; Deschauer et al. 2005). Three of the mutated residues (A90D, L98P and A114P) are located at the membrane-protein interface in the bovine Anc1p structure; all of them are located near the cytosolic side of the protein (Fig. 2).

Deciphering the consequences of HAnc1p defect is compromised in cultured cells from adPEO patients since (1) they present no phenotype, (2) *HANC1* is not expressed in any cultured cells even in myoblasts (Kaukonen et al. 2000) and (3) over-expression of *HANC1* induces apoptosis (Bauer et al. 1999). Therefore, De Marcos Lousa et al. (2002) expressed the mutated genes (A114P and V289M) in *S. cerevisiae*. Such an approach was made possible because the wild type *HANC* genes could restore yeast growth on nonfermentable carbon sources allowing determination of the ADP/ATP transport kinetic properties of the corresponding proteins. However, the mutant carriers were not produced in *S. cerevisiae*, preventing further characterization. Yet growth was rescued by introducing a second mutation (V181M) that corresponded to a spontaneous mutant of *HANC1* expressed in yeast responsible for a notably increased yeast growth (De Marcos Lousa et al. 2005). In the structure of bAnc1p, V181 is oriented toward two acyl chains of a cardiolipin molecule tightly bound to the carrier (Pebay-Peyroula et al. 2003). We can therefore hypothesize that three of the adPEO mutations are involved in Ancp-membrane lipid interactions. These mutations might either loosen or, on the contrary, tighten the interactions of Ancp with the surrounding lipids, thus impeding the conformational changes inherent to nucleotide transport. These assumptions need to be largely sustained by structural data of the uninhibited Ancp.

Considering the overall amino acid conservation between HAnc1p and ScAnc2p and their similar roles in cells, amino acids corresponding to adPEO were mutated at equivalent positions in ScAnc2p (Kaukonen et al. 2000; Chen 2002; De Marcos Lousa et al. 2002, 2005; Fontanesi et al. 2004; Palmieri et al. 2005; Lodi et al. 2006). For example, A114 of HAnc1p corresponds to A128 of ScAnc2p. Yet A128P has variable effects depending on the authors, from complete inactivation of the protein to subtle modification of transport kinetic parameters. Similar variability of results was obtained when studying the V289M mutation. Therefore though promising such an approach needs improvements to be routinely used and to provide relevant data interpretation. We are investigating other organisms closer to humans than yeast that may be more appropriate for such studies.

How HAnc1p defect induces unexpectedly mtDNA depletion is still not clearly understood. Ancp involvement in maintenance of mitochondrial dNTP pool has been evoked (Kaukonen et al. 2000) as well as a putative role in mitochondrial permeability (Chen 2002) and increased

oxidative stress as a consequence of impaired OXPHOS resulting in mitochondrial membrane and DNA damages (Fontanesi et al. 2004). This needs further investigations associated with deciphiring genes and processes involved in mtDNA maintenance and segregation.

Conclusions

Despite many years of efforts the current state of knowledge does not afford an unequivocal view of the detailed mechanism of mitochondrial ADP/ATP transport. Fundamental points such as the functional oligomeric organization of Ancp are still debated and although the 3D structure of this model transport protein has illuminated some of its structure-function relationships it does not yet allow a relevant molecular analysis of Ancp dysfunctionning. Indeed, understanding at the molecular level consequence of HAnc1p mutations is challenging to further investigate possible Ancp activators. This may be an interesting alternative to gene therapy that is more applicable to tumor regression induction than to regain of OXPHOS.

References

Bamber L, Harding M, Butler PJ, Kunji ER (2006) Proc Natl Acad Sci U S A 103(44):16224–16229

Bamber L, Harding M, Monne M, Slotboom DJ, Kunji ER (2007a) Proc Natl Acad Sci U S A 104(26):10830–10834

Bamber L, Slotboom DJ, Kunji ER (2007b) J Mol Biol 371(2):388–395

Bauer MK, Schubert A, Rocks O, Grimm S (1999) J Cell Biol 147 (7):1493–1502

Beutner G, Ruck A, Riede B, Brdiczka D (1998) Biochim Biophys Acta 1368(1):7–18

Bisaccia F, De Palma A, Dierks T, Kramer R, Palmieri F (1993) Biochim Biophys Acta 1142(1–2):139–145

Block MR, Vignais PV (1984) Biochim Biophys Acta 767(2):369–376

Block MR, Pougeois R, Vignais PV (1980) FEBS Lett 117(1):335–340

Bogner W, Aquila H, Klingenberg M (1986) Eur J Biochem 161 (3):611–620

Bolender N, Sickmann A, Wagner R, Meisinger C, Pfanner N (2008) EMBO Rep 9(1):42–49

Brandolin G, Le Saux A, Trézéguet V, Lauquin GJM, Vignais PV (1993) J Bioenerg Biomembr 25(5):459–472

Bruni A, Luciani S, Contessa AR (1964) Nature 201:1219–1220

Brustovetsky N, Tropschug M, Heimpel S, Heidkamper D, Klingenberg M (2002) Biochemistry 41(39):11804–11811

Capobianco L, Ferramosca A, Zara V (2002) J Protein Chem 21 (8):515–521

Chen XJ (2002) Hum Mol Genet 11(16):1835–1843

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Nucleic Acids Res 31(13):3497–3500

Chevrollier A, Loiseau D, Chabi B, Renier G, Douay O, Malthiery Y, Stepien G (2005) J Bioenerg Biomembr 37(5):307–317

Clémençon B, Rey M, Dianoux AC, Trézéguet V, Lauquin GJM, Brandolin G, Pelosi L (2008) J Biol Chem 283(17):11218–11225

Dahout-Gonzalez C, Ramus C, Dassa EP, Dianoux AC, Brandolin G (2005) Biochemistry 44(49):16310–16320

Dahout-Gonzalez C, Nury H, Trézéguet V, Lauquin GJM, Pebay-Peyroula E, Brandolin G (2006) Physiology (Bethesda) 21:242–249

Dalbon P, Brandolin G, Boulay F, Hoppe J, Vignais PV (1988) Biochemistry 27(14):5141–5149

De Marcos Lousa C, Trézéguet V, Dianoux AC, Brandolin G, Lauquin GJM (2002) Biochemistry 41(48):14412–14420

De Marcos Lousa C, Trézéguet V, David C, Postis V, Arnou B, Pebay-Peyroula E, Brandolin G, Lauquin GJM (2005) Biochemistry 44 (11):4342–4348

Deschauer M, Hudson G, Muller T, Taylor RW, Chinnery PF, Zierz S (2005) Neuromuscul Disord 15(4):311–315

Dianoux AC, Noël F, Fiore C, Trézéguet V, Kieffer S, Jaquinod M, Lauquin GJM, Brandolin G (2000) Biochemistry 39:11477–11487

Dolce V, Scarcia P, Iacopetta D, Palmieri F (2005) FEBS Lett 579 (3):633–637

Dörner A, Giessen S, Gaub R, Grosse Siestrup H, Schwimmbeck PL, Hetzer R, Poller W, Schultheiss HP (2006) Eur J Heart Fail 8 (1):81–89

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) Science 280(5360):69–77

Duyckaerts C, Sluse-Goffart CM, Fux JP, Sluse FE, Liebecq C (1980) Eur J Biochem 106(1):1–6

Dyall SD, Agius SC, De Marcos Lousa C, Trézéguet V, Tokatlidis K (2003) J Biol Chem 278(29):26757–26764

Fiore C, Trézéguet V, Le Saux A, Roux P, Schwimmer C, Dianoux AC, Noël F, Lauquin GJM, Brandolin G, Vignais PV (1998) Biochimie 80(2):137–150

Fleishman SJ, Ben-Tal N (2006) Curr Opin Struct Biol 16(4):496–504

Fleishman SJ, Harrington SE, Enosh A, Halperin D, Tate CG, Ben-Tal N (2006) J Mol Biol 364(1):54–67

Fontaine E, Ichas F, Bernardi P (1998) J Biol Chem 273(40):25734–25740

Fontanesi F, Palmieri L, Scarcia P, Lodi T, Donnini C, Limongelli A, Tiranti V, Zeviani M, Ferrero I, Viola AM (2004) Hum Mol Genet 13(9):923–934

Glaser F, Pupko T, Paz I, Bell RE, Bechor-Shental D, Martz E, Ben-Tal N (2003) Bioinformatics 19(1):163–164

Graham BH, Waymire KG, Cottrell B, Trounce IA, MacGregor GR, Wallace DC (1997) Nat Genet 16(3):226–234

Hackenberg H, Klingenberg M (1980) Biochemistry 19(3):548–555

Heddi A, Lestienne P, Wallace DC, Stepien G (1993) J Biol Chem 268 (16):12156–12163

Heimpel S, Basset G, Odoy S, Klingenberg M (2001) J Biol Chem 276(15):11499–11506

Hoffmann B, Stockl A, Schlame M, Beyer K, Klingenberg M (1994) J Biol Chem 269(3):1940–1944

Indiveri C, Giangregorio N, Iacobazzi V, Palmieri F (2002) Biochemistry 41(27):8649–8656

Jang JY, Choi Y, Jeon YK, Aung KC, Kim CW (2008a) BMC Cancer 8:160

Jang JY, Choi Y, Jeon YK, Kim CW (2008b) Breast Cancer Res 10(1): R11

Jordens EZ, Palmieri L, Huizing M, van den Heuvel LP, Sengers RC, Dorner A, Ruitenbeek W, Trijbels FJ, Valsson J, Sigfusson G, Palmieri F, Smeitink JA (2002) Ann Neurol 52(1):95–99

Juhaszova M, Wang S, Zorov DB, Nuss HB, Gleichmann M, Mattson MP, Sollott SJ (2008) Ann N Y Acad Sci 1123:197–212

Kaukonen J, Juselius JK, Tiranti V, Kyttala A, Zeviani M, Comi GP, Keranen S, Peltonen L, Suomalainen A (2000) Science 289 (5480):782–785

Klingenberg M (1993) J Bioenerg Biomembr 25(5):447–457
Klingenberg M (2007) Biochimie 89(9):1042–1048
Klingenberg M, Appel M (1989) Eur J Biochem 180(1):123–131
Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC (2004) Nature 427(6973):461–465
Kolarov J, Kolarova N, Nelson N (1990) J Biol Chem 265(21):12711–12716
Komaki H, Fukazawa T, Houzen H, Yoshida K, Nonaka I, Goto Y (2002) Ann Neurol 51(5):645–648
Kotaria R, Mayor JA, Walters DE, Kaplan RS (1999) J Bioenerg Biomembr 31(6):543–549
Landau M, Mayrose I, Rosenberg Y, Glaser F, Martz E, Pupko T, Ben-Tal N (2005) Nucleic Acids Res 33(Web Server issue): W299–W302
Le Saux A, Roux P, Trézéguet V, Fiore C, Schwimmer C, Dianoux AC, Vignais PV, Brandolin G, Lauquin GJM (1996) Biochemistry 35(50):16116–16124
Leung AW, Halestrap AP (2008) Biochim Biophys Acta 1777(7, 8):946–952
Lin CS, Klingenberg M (1982) Biochemistry 21(12):2950–2956
Lodi T, Bove C, Fontanesi F, Viola AM, Ferrero I (2006) Biochem Biophys Res Commun 341(3):810–815
Lohret TA, Murphy RC, Drgon T, Kinnally KW (1996) J Biol Chem 271(9):4846–4849
Majima E, Koike H, Hong YM, Shinohara Y, Terada H (1993) J Biol Chem 268(29):22181–22187
Marty I, Brandolin G, Vignais PV (1992) Biochemistry 31:4058–4065
Mayinger P, Winkler E, Klingenberg M (1989) FEBS Lett 244 (2):421–426
Morava E, Sengers R, Ter Laak H, Van Den Heuvel L, Janssen A, Trijbels F, Cruysberg H, Boelen C, Smeitink J (2004) Eur J Pediatr 163(8):467–471
Napoli L, Bordoni A, Zeviani M, Hadjigeorgiou GM, Sciacco M, Tiranti V, Terentiou A, Moggio M, Papadimitriou A, Scarlato G, Comi GP (2001) Neurology 57(12):2295–2298
Nury H, Dahout-Gonzalez C, Trézéguet V, Lauquin G, Brandolin G, Pebay-Peyroula E (2005) FEBS Lett 579(27):6031–6036
Nury H, Dahout-Gonzalez C, Trézéguet V, Lauquin GJM, Brandolin G, Pebay-Peyroula E (2006) Annu Rev Biochem 75:713–741
Palmieri L, Alberio S, Pisano I, Lodi T, Meznaric-Petrusa M, Zidar J, Santoro A, Scarcia P, Fontanesi F, Lamantea E, Ferrero I, Zeviani M (2005) Hum Mol Genet 14(20):3079–3088
Palmisano A, Zara V, Honlinger A, Vozza A, Dekker PJ, Pfanner N, Palmieri F (1998) Biochem J 333(Pt 1):151–158
Pebay-Peyroula E, Brandolin G (2004) Curr Opin Struct Biol 14 (4):420–425
Pebay-Peyroula E, Dahout-Gonzalez C, Kahn R, Trézéguet V, Lauquin GJM, Brandolin G (2003) Nature 426(6962):39–44
Postis V, De Marcos Lousa C, Arnou B, Lauquin GJM, Trézéguet V (2005) Biochemistry 44(45):14732–14740
Riccio P, Aquila H, Klingenberg M (1975) FEBS Lett 56(1):133–138
Roux P, Le Saux A, Fiore C, Schwimmer C, Dianoux AC, Trézéguet V, Vignais PV, Lauquin GJM, Brandolin G (1996) Anal Biochem 234:31–37
Schroers A, Burkovski A, Wohlrab H, Krämer R (1998) J Biol Chem 273(23):14269–14276
Schultheiss HP (1992) Basic Res Cardiol 87(Suppl 1):311–320
Schultheiss HP, Bolte HD (1985) J Mol Cell Cardiol 17(6):603–617
Sharer JD (2005) IUBMB Life 57(9):607–614
Siciliano G, Tessa A, Petrini S, Mancuso M, Bruno C, Grieco GS, Malandrini A, DeFlorio L, Martini B, Federico A, Nappi G, Santorelli FM, Murri L (2003) Neuromuscul Disord 13(2):162–165
Stepien G, Torroni A, Chung AB, Hodge JA, Wallace DC (1992) J Biol Chem 267(21):14592–14597
Sylven C, Lin L, Jansson E, Sotonyi P, Fu LX, Waagstein F, Hjalmarsson A, Marcus C, Bronnegard M (1993) Cardiovasc Res 27(7):1295–1299
Yernool D, Boudker O, Jin Y, Gouaux E (2004) Nature 431:811–818
Yin H, Stahl JS, Andrade FH, McMullen CA, Webb-Wood S, Newman NJ, Biousse V, Wallace DC, Pardue MT (2005) Invest Ophthalmol Vis Sci 46(12):4555–4562
Zamora M, Merono C, Vinas O, Mampel T (2004) J Biol Chem 279 (37):38415–38423