SUPPLEMENTARY MATERIAL

Adenine nucleotides transporters in organelles: novel genes and functions

Javier Traba[*,1], Jorgina Satrústegui[*] and Araceli del Arco[†,*]

*** Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa UAM-CSIC, CIBER de Enfermedades Raras (CIBERER), Universidad Autónoma de Madrid, Madrid, Spain.**

† Área de Bioquímica, Centro Regional de Investigaciones Biomédicas (CRIB), Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Toledo, Spain.

1 Correspondence to:

Javier Traba; Tel: 034-911964651; Fax: 034-911964420; E-mail: jtraba@cbm.uam.es

1) AAC in *Saccharomyces cerevisiae* (Table S1)

The ADP/ATP carrier in yeast exchanges ADP^{3-} with ATP^{4-} between the cytosol and mitochondrial matrix. It is thus essential for oxidative phosphorylation, but it is also essential for anaerobic growth (see below).

Expression

AAC2 is the main isoform and the only one which is detectable in growing yeast. The expression of *AAC1* and *AAC3* is very low and can not be detected by western blot [40]. *AAC3* is expressed at low levels and only in anaerobiosis (Sabova et al., 1993).

AAC2 expression is modulated by carbon source and oxygen availability. It is preferentially expressed with respiratory (non-fermentable) carbon sources and in aerobiosis. The expression of *AAC2* requires the HAP2/3/4 complex and heme (Betina et al, 1995).

AAC3 expression is also modulated by carbon source and oxygen availability. It is preferentially expressed with glucose and in anaerobiosis. Repression by oxygen is mediated by heme and the heme-dependent repressor factor ROX1 (Sabová et al, 1993). Repression by respiratory carbon sources is mediated by RAP1, a ubiquitous transcriptional regulator, and by ERA (ethanol-repression sequence) *cis*-acting elements (Sokolíková et al, 2000).

AAC1 expression is positively regulated by oxygen in a heme-independent manner. Unlike *AAC2* and *AAC3*, it is independent of carbon source (Gavurníková et al, 1996).

Disruption mutants

The *aac1* mutant shows no phenotype. It is able to grow on respiratory (non-fermentable) carbon sources at a normal rate. The *aac2* mutant is unable to grow on respiratory carbon sources, but it is able to grow on glucose in aerobiosis and anaerobiosis. It is unable to loose mitochondrial DNA (i.e. it becomes petite-negative). The *aac3* mutant shows no phenotype, as the *aac1* mutant (Kolarov et al, 1990).

The *aac1,3* double mutant is identical to the single *aac1* or *aac3* mutants and shows no phenotype [40]. The *aac1,2* double mutant is identical to the single *aac2* mutant. The *aac2,3* double mutant is unable to grow on respiratory carbon sources as the *aac2* mutant, but in addition, it is unable to grow in anaerobiosis (Kolarov et al, 1990). The *aac1,2,3* triple mutant is identical to the *aac2,3* mutant [40].

The *aac1* mutant in combination with the *ade2* mutation shows a red colony phenotype, which suggests that Aac1p could be involved somehow in vacuolar metabolism (Drgon et al, 1992).

ADP/ATP exchange between mitochondria and cytosol

Mitochondria normally function in ATP production by oxidative phosphorylation. During respiratory growth the ATP produced in the mitochondria is exported to the cytosol in exchange for ADP (fig. S2A). The AAC is thus essential for growth in respiratory carbon sources.

However, during anaerobic growth or in ρ^0 cells (i.e.: cells lacking mitochondrial DNA, which encodes subunits of the respiratory complexes and subunits of the F_0F_1-ATP synthase) respiration is not possible and mitochondria switch to ATP consumers. In these conditions ATP is utilized to build up a mitochondrial membrane potential and for the import and assembly of mitochondrial proteins. The AAC is essential for anaerobic growth or in ρ^0 cells, because the generation of a mitochondrial membrane potential depends on electrogenic exchange of cytosolic ATP^{4-} for mitochondrial ADP^{3-} coupled either to H^+-pumping through the F_0F_1-ATP synthase (in anaerobiosis, fig. S2B) or only to ATP hydrolysis by F_1-ATPase (in ρ^0 cells, fig. S2C).

In yeast cells growing aerobically on glucose the transcription of genes coding for mitochondrial proteins is repressed, and the contribution of respiration to glucose catabolism and energetics is very small. It has been proposed that the mitochondria would be ATP consumers also in this condition (Francis et al., 2007; [11,33]), importing ATP from the cytosol and hydrolyzing it through the F_0F_1-ATP synthase as in anaerobiosis (fig. S2B).

Function of the AAC isoforms

All three isoforms of the ADP/ATP carrier are able to perform ADP_{in}/ATP_{out} exchange, and are able to support respiratory growth when expressed at high levels from expression vectors. Their translocation properties are similar to those of Aac2p (Drgon et al, 1992). However, in growing yeast, the expression of *AAC1* and *AAC3* is negligible and can not be detected by western blot [40]. Thus, Aac2p is the main isoform of the ADP/ATP carrier, and is the only one which participates in respiratory growth and oxidative phosphorylation. It is also able to catalyse the inverse exchange (cytosolic ATP for mitochondrial ADP) in anaerobiosis, because the *aac1,3* mutant, in which Aac2p is the only ADP/ATP carrier, is still able to perform anaerobic growth [40].

Endogenous Aac3p and Aacp1 do not participate in respiratory growth or oxidative phosphorylation, because the *aac2* mutant is unable to grow in respiratory carbon sources. The *aac1,2* mutant, in which Aac3p is the only ADP/ATP carrier, is able to grow in anerobiosis (Kolarov et al, 1990), and thus Aac3p is able to catalyse the inverse exchange (cytosolic ATP for mitochondrial ADP), even though its endogenous expression is very low. This suggests that the requirement of ATP import to support anaerobic growth is not as demanding as ATP export to support respiration.

Role of AAC on yeast apoptosis

Apoptosis-like yeast cell death has many features in common with mammalian apoptosis. In the mitochondrial pathway the outer mitochondrial membrane permeabilization and release of pro-apoptotic proteins from the intermembrane space are crucial for death. Many of the pro-apoptotic proteins released are conserved from yeast to human, including cytochrome c, and AIF. The nature of the pore that releases these proteins is still unknown, both in yeast and in mammalian cells, however, and the identity of the proteins involved in its formation is highly controversial.

In yeast cells, unlike mammalian cells, the ADP/ATP carrier, but not its catalytic activity, was found to be required for mitochondrial outer membrane permeabilization and cytochrome c release in some forms of apoptosis (Pereira et al, 2007).

AAC in other yeast

Candida paralopsis: in this aerobic yeast there is only one AAC gene, *CpAAC1* (Nebohácová et al, 1999). *CpAAC1* is able to complement *aac2,3 Saccharomyces cerevisiae*, both in respiratory growth and in anerobic growth on glucose, suggesting that the protein is able to transport ATP in both directions. However, *Candida paralopsis* is a strict aerobic species with a low fermentative capacity. The expression of *CpAAC1* is strongly reduced in cells grown in glucose (in aerobic and semianaerobic conditions) and enhanced in non-fermentable sources.

Schizosaccharomyces pombe: in this aerobic, fermentative, petite-negative yeast there is only one AAC gene, *SpANC1* (Trézéguet et al, 1999). The expression of *SpANC1* is reduced in cells grown in low or high concentrations of glucose, even further reduced by semianaerobic conditions and enhanced in non-fermentable sources. Deletion of *SpANC1* yields viable cells, although *Schizosaccharomyces pombe* is a petite-negative yeast species (unable to loose mitochondrial DNA). However, growth is retarded in raffinose and prevented in respiratory carbon sources and in glucose in semianaerobiosis.

Kluyveromyces lactis: in this aerobic, respiratory, petite-negative yeast there is only one AAC gene, *KlAAC* (Trézéguet et al, 1999). The expression of *KlAAC* is reduced in cells grown in glucose, even further reduced by semianaerobic conditions and enhanced in non-fermentable sources. Deletion of *KlAAC* is lethal in some strains, but in others yields viable cells only in glucose, but not in galactose, raffinose, maltose or respiratory carbon sources. Semianerobic growth in glucose is also severily impaired. *AAC1*, *AAC2* and *AAC3* from *Saccharomyces cerevisiae* are able to complement the mutation, but none of them confer upon *Kluyveromyces lactis* the ability to grow under strict anaerobic conditions (Fontanesi et al, 2006). *KlAAC* from *Kluyveromyces lactis* can complement the petite-negative phenotype of *aac2 Saccharomyces cerevisiae* mutant as well as its inability to grow on nonfermentable carbon sources.

Yarrowia lipolytica: in this aerobic, dimorphic yeast there are three genes, *YlAAC1*, *YlAAC2* and *YlAAC3* (Mentel et al, 2005). *YlAAC1* is repressed in low oxigen, whereas *YlAAC3* is slightly stimulated and *YlAAC2* is unaffected. The expression of the AAC is identical in fermentable and respiratory carbon sources, probably because in this yeast there is no glucose repression. *Yarrowia lipolytica* is able to use proteins and peptides as carbon sources, and these sources produced a moderate repression of all three genes.

2) AAC in mammals (Table S2)

Expression

ANT1 is expressed mainly in heart and skeletal muscle. ANT2 is only expressed very weakly in the main tissues and abundantly in highly proliferative cells. The expression of ANT2 is regulated by Sp1 and the GRBOX (glycolysis regulated box) promoter sequence, a sequence which is homologous to a yeast *AAC3* promoter sequence (Giraud et al, 1998). ANT3 is expressed ubiquitously at a level depending on the respiratory activity of the tissues. ANT4 is expressed mainly in testis, and at lower levels in liver and brain (Dolce et al, 2005).

Mouse Ant1 is expressed at high levels in skeletal muscle and heart, similarly to human ANT1 (Levy et al, 2000). Mouse Ant2 is strongly expressed in all tissues but muscle, in marked contrast to human ANT2 (Levy et al, 2000). Mouse Ant4 is expressed mainly in testis.

Function of the AAC in mammalian cells

During respiratory growth the ATP produced in the mitochondria is exported to the cytosol in exchange for ADP. ANT1 and ANT3 appear to be the main isoforms involved in respiration and oxidative phosphorylation and export ATP produced in the mitochondrial matrix to the cytosol (fig. S2A).

However, in ρ^0 cells or in cells with mutations in key respiratory genes respiration is not possible and mitochondria switch to ATP consumers, as in yeast. The AAC is essential for growth in this conditions, because the generation of a mitochondrial membrane potential depends on electrogenic exchange of cytosolic ATP^{4-} for mitochondrial ADP^{3-} coupled either to H^+-pumping through the F_0F_1-ATP synthase (fig. S2B) or only to ATP hydrolysis by F_1-ATPase in ρ^0 cells (fig. S2C). Interestingly, mammalian ρ^0 cells become auxotrophs for uridine, as *de novo* synthesis of pyrimidines requires the regeneration of ubiquinone by the respiratory chain (Perales-Clemente et al., 2008). ANT2 is overexpressed in ρ^0 cells and in cells with mutations in mitochondrial DNA (Loiseau et al, 2002; Bonod-Binaud et al, 2001) and is the isoform proposed for the inverse exchange (cytosolic ATP for mitochondrial ADP). The capacity of

ANT2 to catalyse the inverse exchange was confirmed because it is able to support growth in anaerobiosis in the *aac1,2,3* yeast mutant (Giraud et al, 1998).

Many cancers and cell lines have a reduced mitochondrial function and rely mainly on glycolysis to generate ATP. It has been proposed that in these cells the mitochondria are ATP consumers, importing ATP from the cytosol and hydrolyzing it through the F_0F_1-ATP synthase (fig. S2B). Indeed, ANT2 is overexpressed in glycolytic cell lines and cancers (Chevrollier et al, 2005).

Role of AAC on the mitochondrial permeability transition

The mitochondrial permeability transition pore (PTP) is a non-specific pore that is formed in the inner mitochondrial membrane under conditions of calcium overload and/or oxidative stress. Its molecular composition is still debated (Leung and Halestrap, 2008), but it is implicated in necrotic cell death, for example, in situations of ischemia-reperfusion (Baines et al., 2005; Nakagawa et al., 2005; Schinzel et al., 2005). The ADP/ATP was thought to be a component of the PTP, along with the voltage-dependent anion channel (VDAC), members of the pro- and anti-apoptotic BAX-BCL2 protein family and cyclophilin D. Accordingly, the pore is regulated by AAC inhibitors and CyP-D inhibitors. Interestingly, the two AAC inhibitors have opposite effects on permeability transition, as CAT, which fixes the carrier in the *c* conformation, stimulates it, and BKA, which fixes the carrier in the *m* conformation, inhibits it.

However, liver mitochondria lacking ANT1 and ANT2 could still be induced to undergo permeability transition (Kokoszka et al, 2004), but more Ca^{2+} was required to activate it, and the pore became insensitive to AAC substrates (ADP) or inhibitors (CAT and BKA). Therefore, the ADP/ATP carriers should be now considered as PTP regulators, but not as key components. It has been proposed that, since the AAC is the most abundant protein in the inner mitochondrial membrane, its conformation is likely to affect the inner membrane properties. The *m* conformation (triggered by ADP or BKA) would be protective, while the *c* conformation (triggered by CAT) would stimulate PTP opening.

Knock out mice

Ant1-deficient mice exhibit many of the hallmarks of human oxidative phosphorylation (OXPHOS) disease (Graham et al, 1997). Histological and ultrastructural examination of skeletal muscle from these mutants revealed ragged-red muscle fibers and a dramatic proliferation of mitochondria, while examination of the heart revealed cardiac hypertrophy with mitochondrial proliferation. Mitochondria isolated from mutant skeletal muscle exhibited a severe defect in coupled respiration. Ant1 mutant adults also had a resting serum lactate level fourfold higher than that of controls, indicative of metabolic acidosis. Significantly, mutant adults manifested severe exercise intolerance. Therefore, Ant1 mutant mice have the

biochemical, histological, metabolic and physiological characteristics of mitochondrial myopathy and cardiomyopathy. On the other hand, Ant1-deficient mice are more resistant to excitotoxic insults (Lee et al., 2009), which agrees with the fact that the absence of AAC desensitizes the PTP to Ca^{2+} (se above).

Ant4-deficient male mice were infertile (Brower et al, 2007), probably because meiosis in male germ cells is arrested (Brower et al, 2009). A significant reduction in testicular size was also observed without any other distinguishable abnormalities.

AAC in disease

Mitochondrial disorders of oxidative phosphorylation (OXPHOS) comprise several diseases that can be caused by mutations in nuclear and mitochondrial genes. Many of them are also characterized by deletions in mitochondrial DNA, and involve muscle, eye, heart and brain pathology. Gain of function mutations in ANT1 have been reported to cause autosomal dominant progressive external ophthalmoplegia (adPEO). These mutations produce a leak of protons through the AAC protein (it becomes an unregulated channel) and thus uncouple the inner mitochondrial membrane and decrease the mitochondrial membrane potential. This produces severe defects in mitochondrial biogenesis, which secondarily cause mtDNA instability (Wang et al., 2008).

A single patient with a loss of function mutation in ANT1 has been found (Palmieri et al,. 2005). It presented with hypertrophic cardiomyopathy, mild myopathy with exercise intolerance and lactic acidosis. A muscle biopsy showed the presence of numerous ragged-red fibers. Thus, the phenotype of the patient resembles that of Ant1-deficient mice.

ANT2 is highly expressed in proliferative cells and its induction has been found in many human cancers, particularly breast cancer. Knock-down of ANT2 by siRNA has been shown to induce apoptosis and inhibit tumor growth in vivo and in vitro (Jang et al., 2008a). On the other hand, overexpression of ANT1 induces apoptosis and inhibits tumor growth in vivo (Jang et al., 2008b), and this suggests that the different ANT isoform have different roles in apoptosis. ANT1 and ANT3 function as pro-apoptotic, while ANT2 and ANT4 are anti-apoptotic (Gallerne et al., 2010).

SUPPLEMENTARY REFERENCES

Baines, C.P., Kaiser, R.A., Purcell, N.H., Blair, N.S., Osinska, H., Hambleton, M.A., Brunskill, E.W., Sayen, M.R., Gottlieb, R.A., Dorn, G.W., Robbins, J. and Molkentin, J.D. (2005) Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature 434, 658-662.

Betina, S., Gavurníková, G., Haviernik, P., Sabová, L. and Kolarov J. (1995) Expression of the AAC2 gene encoding the major mitochondrial ADP/ATP carrier in Saccharomyces cerevisiae is controlled at the transcriptional level by oxygen, heme and HAP2 factor. Eur J Biochem 229, 651-657.

Bonod-Bidaud, C., Chevrollier, A., Bourasseau, I., Lachaux, A., Mousson de Camaret, B. and Stepien, G. (2001) Induction of ANT2 gene expression in liver of patients with mitochondrial DNA depletion. Mitochondrion 1, 217-224.

Brower, J.V., Rodic, N., Seki, T., Jorgensen, M., Fliess, N., Yachnis, A.T., McCarrey, J.R., Oh, S.P. and Terada, N. (2007) Evolutionarily conserved mammalian adenine nucleotide translocase 4 is essential for spermatogenesis. J. Biol. Chem. 282, 29658-29666.

Chevrollier, A., Loiseau, D., Chabi, B., Renier, G., Douay, O., Malthièry, Y. and Stepien, G. (2005) ANT2 isoform required for cancer cell glycolysis. *J Bioenergetics Biomembranes* 37: 307-316

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

Drgon, T., Sabová, L., Gavurniková, G., Kolarov, J. (1992) Yeast ADP/ATP carrier (AAC) proteins exhibit similar enzymatic properties but their deletion produces different phenotypes. FEBS Lett 304, 277-280.

Francis, B.R., White, K.H. and Thorsness, P.E. (2007) Mutations in the Atp1p and Atp3p subunits of yeast ATP synthase differentially affect respiration and fermentation in Saccharomyces cerevisiae. J. Bioenerg. Biomembr. 39, 127-144.

Fontanesi, F., Viola, A.M. and Ferrero, I. (2006) Heterologous complementation of the Klaac null mutation of Kluyveromyces lactis by the Saccharomyces cerevisiae AAC3 gene encoding the ADP/ATP carrier. FEMS Yeast Res 6, 414-420.

Gallerne, C., Touat, Z., Chen, Z.X., Martel, C., Mayola, E., Sharaf El Dein, O., Buron, N., Le Bras, M., Jacotot, E., Borgne-Sanchez, A., Lemoine, A., Lemaire, C., Pervaiz, S. and Brenner, C. (2010) The fourth isoform of the adenine nucleotide translocator inhibits mitochondrial apoptosis in cancer cells. Int J Biochem Cell Biol 42, 623-629.

Gavurníková, G., Sabova, L., Kissová, I., Haviernik, P. and Kolarov, J. (1996) Transcription of the AAC1 gene encoding an isoform of mitochondrial ADP/ATP carrier in Saccharomyces cerevisiae is regulated by oxygen in a heme-independent manner. Eur J Biochem 239, 759-763.

Giraud, S., Bonod-Bidaud, C., Wesolowski-Louvel, M. and Stepien, G. (1998) Expression of human ANT2 gene in highly proliferative cells: GRBOX, a new transcriptional element, is involved in the regulation of glycolytic ATP import into mitochondria. J Mol Biol 281, 409-418

Graham, B.H., Waymire, K.G., Cottrell, B., Trounce, I.A., MacGregor, G.R. and Wallace, D.C. (1997) 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.

Jang, J.Y., Choi, Y., Jeon, Y.K. and Kim, C.W. (2008a) Suppression of adenine nucleotide translocase-2 by vector-based siRNA in human breast cancer cells induces apoptosis and inhibits tumor growth in vitro and in vivo. Breast Cancer Res 10, R11.

Jang, J.Y., Choi, Y., Jeon, Y.K., Aung, K.C. and Kim, C.W. (2008b) Over-expression of adenine nucleotide translocase 1 (ANT1) induces apoptosis and tumor regression in vivo. BMC Cancer 8, 160.

Kokoszka, J.E., Waymire, K.G., Levy, S.E., Sligh, J.E., Cai, J., Jones, D.P., MacGregor, G.R. and Wallace, D.C. (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature. 427, 461-465.

Kolarov, J., Kolarova, N. and Nelson, N. (1990) A third ADP/ATP translocator gene in yeast. J Biol Chem 265, 12711-12716.

Lee, J., Schriner, S.E. and Wallace, D.C. (2009) Adenine nucleotide translocator 1 deficiency increases resistance of mouse brain and neurons to excitotoxic insults. Biochim Biophys Acta 1787, 364-370.

Leung, A.W. and Halestrap, A.P. (2008) Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. Biochim Biophys Acta 1777, 946-952.

Levy, S.E., Chen, Y.S., Graham, B.H. and Wallace, D.C. (2000) Expression and sequence analysis of the mouse adenine nucleotide translocase 1 and 2 genes. Gene 254, 57-66.

Loiseau, D., Chevrollier, A., Douay, O., Vavasseur, F., Renier, G., Reynier, P., Malthièry, Y. and Stepien, G. (2002) Oxygen consumption and expression of the adenine nucleotide translocator in cells lacking mitochondrial DNA. Exp Cell Res 278, 12-18.

Mentel, M., Piskur, J., Neuvéglise, C., Rycovská, A., Cellengová, G. and Kolarov, J. (2005) Triplicate genes for mitochondrial ADP/ATP carriers in the aerobic yeast Yarrowia lipolytica are regulated differentially in the absence of oxygen. Mol Genet Genomics 273, 84-91.

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

Nebohácová, M., Mentel, M., Nosek, J. and Kolarov, J. (1999) Isolation and expression of the gene encoding mitochondrial ADP/ATP carrier (AAC) from the pathogenic yeast Candida parapsilosis. Yeast 15, 1237-1242.

Palmieri, L., Alberio, S., Pisano, I., Lodi, T., Meznaric-Petrusa, M., Zidar, J., Santoro, A., Scarcia, P., Fontanesi, F., Lamantea, E., Ferrero, I. and Zeviani, M. (2005) Complete loss-of-function of the heart/muscle-specific adenine nucleotide translocator is associated with mitochondrial myopathy and cardiomyopathy. Hum Mol Genet 14, 3079-3088.

Perales-Clemente, E., Bayona-Bafaluy, M.P., Pérez-Martos, A., Barrientos, A., Fernández-Silva, P. and Enriquez, J.A. (2008) Restoration of electron transport without proton pumping in mammalian mitochondria. Proc Natl Acad Sci USA 105, 18735-18739.

Pereira, C., Camougrand, N., Manon, S., Sousa, M.J. and Côrte-Real, M. (2007) ADP/ATP carrier is required for mitochondrial outer membrane permeabilization and cytochrome c release in yeast apoptosis. Mol Microbiol 66, 571-582.

Sabová, L., Zeman, I., Supek, F. and Kolarov, J. (1993) Transcriptional control of AAC3 gene encoding mitochondrial ADP/ATP translocator in Saccharomyces cerevisiae by oxygen, heme and ROX1 factor. Eur. J. Biochem. 213, 547-553.

Schinzel, A.C., Takeuchi, O., Huang, Z., Fisher, J.K., Zhou, Z., Rubens, J., Hetz, C., Danial, N.N., Moskowitz, M.A. and Korsmeyer, S.J. (2005) Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. Proc Natl Acad Sci USA 102, 12005-12010.

Sokolíková, B., Sabová, L., Kissová, I. and Kolarov, J. (2000) A carbon-source-responsive element is required for regulation of the hypoxic ADP/ATP carrier (AAC3) isoform in Saccharomyces cerevisiae. Biochem J 352, 893-898.

Trézéguet, V., Zeman, I., David, C., Lauquin, G.J. and Kolarov, J. (1999) Expression of the ADP/ATP carrier encoding genes in aerobic yeasts; phenotype of an ADP/ATP carrier deletion mutant of Schizosaccharomyces pombe. Biochim Biophys Acta 1410, 229-236.

Wang, X., Salinas, K., Zuo, X., Kucejova, B. and Chen, XJ. (2008) Dominant membrane uncoupling by mutant adenine nucleotide translocase in mitochondrial diseases. Hum Mol Genet 17, 4036-4044.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Consensus cladogram of eukaryotes. The 5 major eukaryotic groups and taxa belonging to their subgroups are shown. Model organisms mentioned in the main text are in bold. In organisms that lack mitochondria *Hy* indicates that the organism contains hydrogenosomes, *Mt* indicates that it contains mitosomes and *aM* indicates that it contains anaerobic mitochondria. Amitochondriate organisms mentioned in the main text are underlined. They are scattered along the different major groups, and thus mitosomes and hydrogenosomes have probably arisen independently several times during evolution, as a result of movement of different organisms into anaerobic habitats. Adapted from [12].

Figure S2. Generation of a mitochondrial membrane potential in eukaryotic cells. A) Yeast cells growing in respiratory (non-fermentable) carbon sources, normal mammalian cells and procyclic form trypanosomes generate a membrane potential ($\Delta\Psi$) by proton pumping in the electron transport chain (ETC), and synthesize ATP by the F_0F_1 H^+-ATP synthase. Then, the ATP is exported to the cytosol by the ADP/ATP carrier (AAC) in exchange for ADP. **B**) Yeast cells growing in fermentable carbon sources in aerobiosis or anaerobiosis, some tumour cells, mammalian cells growing in anaerobiosis or bloodstream from trypanosomes generate a $\Delta\Psi$ by the electrogenic exchange of cytosolic ATP (with four negative charges) for mitochondrial ADP (with three negative charges) through the AAC. The F_0F_1 H^+-ATP synthase works to hydrolyze ATP, and this is coupled to proton pumping, which also contributes to the generation of $\Delta\Psi$. **C**) In cells lacking mitochondrial DNA such as ρ^0 yeast, ρ^0 mammalian cells, dyskinetoplastic trypanosomes and perhaps also in some apicomplexan parasites with a simplified mitochondrial DNA, the ETC and the F_0 fragment of the F_0F_1 H^+-ATP synthase, partially encoded by the mitochondrial DNA, are not functional and thus, the $\Delta\Psi$ is only generated by the AAC. ATP hydrolysis by the F_1 ATPase is essential to maintain an ADP concentration high enough for a sufficient rate of electrogenic adenine nucleotide translocation in the AAC.

Table S1. ADP/ATP carriers (AACs) in yeast.

Table S2. ADP/ATP carriers (AACs) in mammals.

Table S3. Accession numbers of the listed proteins. Proteins in red do not belong to the mitochondrial carrier family.

Fig S1

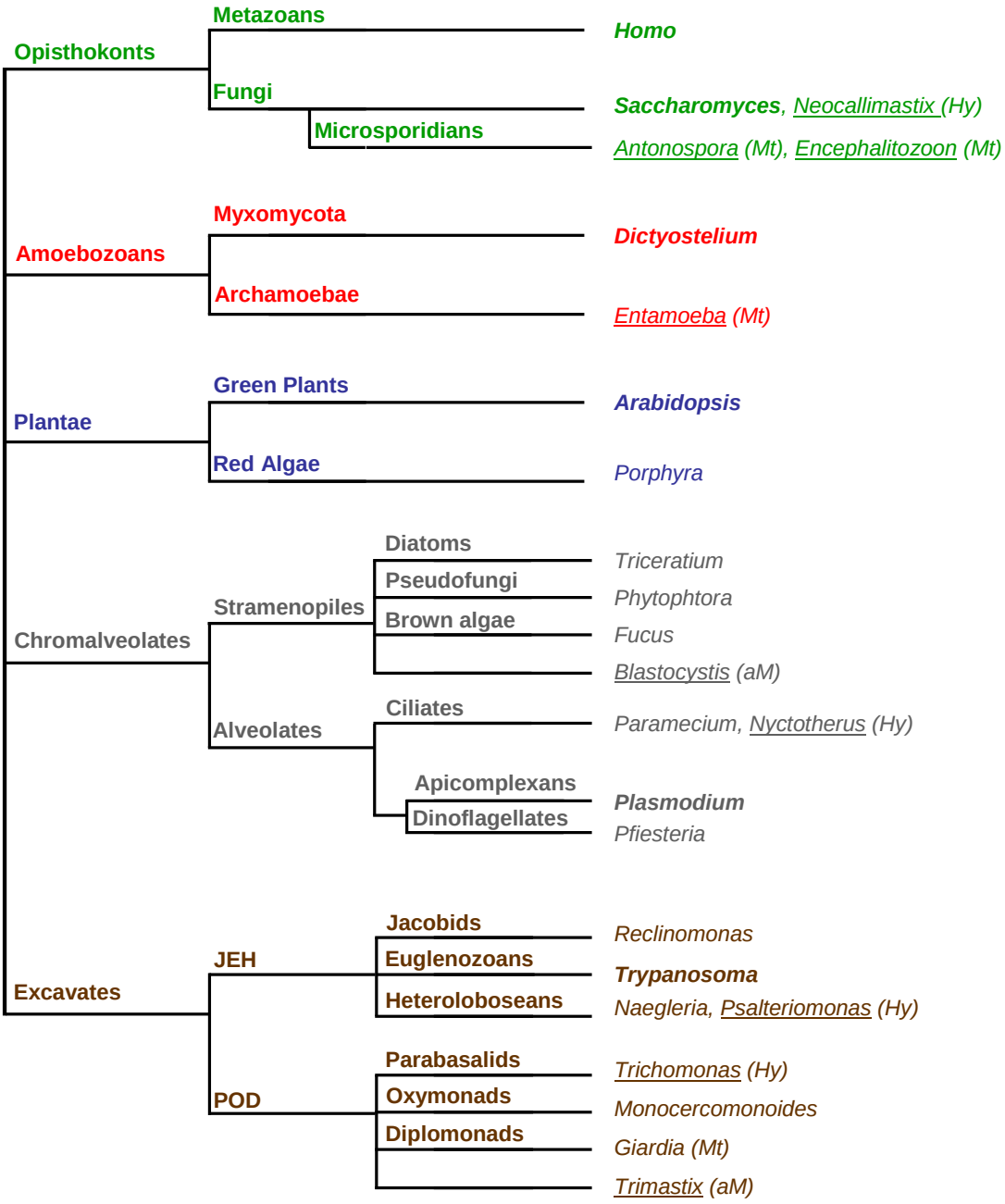
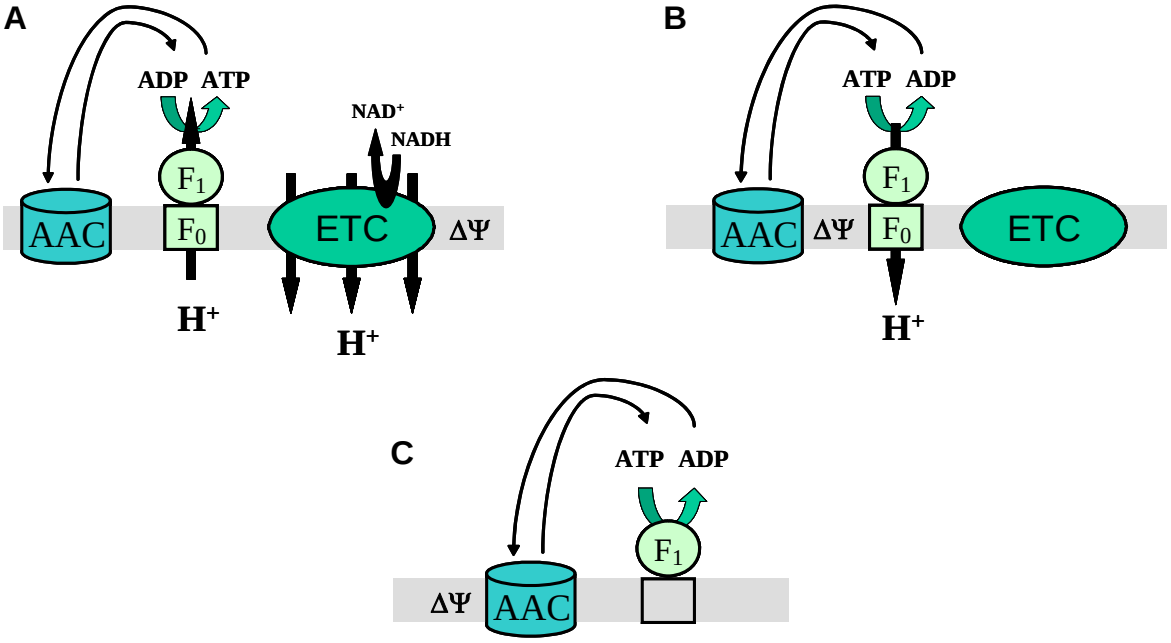

Opisthokonts
Metazoans
Homo
Fungi
Saccharomyces, Neocallimastix (Hy)
Microsporidians
Antonospora (Mt), Encephalitozoon (Mt)
Amoebozoans
Myxomycota
Dictyostelium
Archamoebae
Entamoeba (Mt)
Plantae
Green Plants
Arabidopsis
Red Algae
Porphyra
Chromalveolates
Stramenopiles
Diatoms
Triceratium
Pseudofungi
Phytophtora
Brown algae
Fucus
Blastocystis (aM)
Alveolates
Ciliates
Paramecium, Nyctotherus (Hy)
Apicomplexans
Plasmodium
Dinoflagellates
Pfiesteria
Excavates
JEH
Jacobids
Reclinomonas
Euglenozoans
Trypanosoma
Heteroloboseans
Naegleria, Psalteriomonas (Hy)
POD
Parabasalids
Trichomonas (Hy)
Oxymonads
Monocercomonoides
Diplomonads
Giardia (Mt)
Trimastix (aM)

Fig S2


A
ADP ATP
NAD+
NADH
F1
F0
AAC
ETC
ΔΨ
H+
H+
B
ATP ADP
F1
AAC
ΔΨ
F0
ETC
H+
C
ATP ADP
F1
ΔΨ
AAC

Gene	Expression	Regulation	Disruption mutant	Disruption mutant in combintation with an *aac2* disruption	Proposed function
AAC1	Very low. Undetectable	Positively regulated by oxygen. Independent of carbon source	No phenotype	As the *aac2* mutant	?
AAC2	High. Detectable	Preferentially expressed in aerobiosis and respiratory carbon sources	Unable to grow on respiratory carbon sources. Unable to live without mitochondrial DNA. Able to grow on glucose in aerobiosis and anerobiosis	----------	Forward exchange in respiratory growth. Inverse exchange in fermentative growth and anaerobiosis.
AAC3	Very low and only in anaerobiosis	Preferentially expressed in anaerobiosis and glucose	No phenotype	As the *aac2* mutant, but also unable to grow in anaerobiosis	Reverse exchange in anaerobiosis to generate $\Delta\Psi$

Table S1. ADP/ATP carriers (AACs) in yeast

Gene	Expression in humans	Expression in rodents	Effect in apoptosis	Phenotype of KO mice	Diseases
ANT1	Skeletal muscle and heart	Skeletal muscle and heart	Pro-apoptotic	Myopathy and cardiomyopathy	Gain of function mutations cause adPEO Loss of function mutations cause myopathy and cardiomyopathy
ANT2	Proliferative cells	Ubiquitous	Anti-apoptotic	----------	Overexpressed in tumors and cells with mutations in mitocondrial DNA
ANT3	Ubiquitous	Absent	Pro-apoptotic	----------	----------
ANT4	Testis	Testis	Anti-apoptotic	Infertile males. Reduction in testicular size	----------

Table S2. ADP/ATP carriers (AACs) in mammals

Species	Function	Carrier	Protein name	Acc. Number
H. sapiens	ADP/ATP carrier	SLC25A4	ANT1	NP_001142.2
		SLC25A5	ANT2	NP_001143.2
		SLC25A6	ANT3	NP_001627.2
		SLC25A31	ANT4	NP_112581.1
	ATP-Mg/Pi carrier	SLC25A23	SCaMC-3a (APC-2)	NP_077008.2
		SLC25A24	SCaMC-1	NP_037518.3
			SCaMC-1a (APC-1)	NP_998816.1
		SLC25A25	SCaMC-2a (APC-3)	NP_443133.2
			SCaMC-2b	NP_001006642.1
			SCaMC-2c	NP_001006643.1
			SCaMC-2d	NP_001006644.1
		SLC25A41	SCaMC-3like	NP_775908.2
	CoA carrier	SLC25A16	GDC	NP_689920.1
		SLC25A42		NP_848621.2
	Peroxisomal AMP/ATP carrier	SLC25A17	PMP34	NP_006349.1
	Putative nucleotide uniporter	SLC25A43		NP_660348.2
	Vesicular nucleotide carrier	SLC17A9	VNUT	NP_071365.3

Species	Function	ORF	Protein name	Acc. Number
S. cerevisiae	ADP /ATP carrier	YMR056c	Aac1p	NP_013772.1
		YBL030c	Aac2p	NP_009523.1
		YBR085w	Aac3p	NP_009642.1
	ATP-Mg/Pi carrier	YNL083w	Sal1p	CAY82520.1
	CoA carrier	YHR002w	Leu5p	NP_011865.1
	Unknown	YPR011c	Ypr011c	NP_015336.1
	Peroxisomal AMP/ATP carrier	YPR128c	Ant1p	NP_015453.1

Table S3. Accesion numbers of the listed proteins. Proteins in red do not belong to the mitochondrial carrier family

Species	Function	Locus	Protein name	Acc. Number
A. thaliana	ADP/ATP carrier	At3g08580	AAC1	NP_850541.1
		At5g13490	AAC2	NP_196853.1
		At4g28390	AAC3	NP_194568.1
	ATP-Mg/Pi carrier	At5g61810		NP_568940.1
		At5g07320		NP_196349.1
		At5g51050		NP_199918.1
	CoA carrier	At4g26180		NP_194348.1
		At1g14560		NP_172908.1
	AMP/ATP carrier	At4g01100	ADNT1	NP_192019.1
	Peroxisomal AMP/ATP carrier	At3g05290	PNC1	NP_566251.1
		At5g27520	PNC2	NP_198104.1
	Peroxisomal carrier	At2g39970		NP_181526.1

Species	Function	Gene	Protein name	Acc. Number
D. discoideum	ADP/ATP carrier		AncA	XP_647166.1
	ATP-Mg/Pi carrier		McfC	XP_001733022.1
	Putative adenine nucleotide carrier		McfA	XP_635136.1
	Putative adenine nucleotide carrier		McfV	XP_643352.1
	CoA carrier		McfP	XP_629781.1
			McfR	XP_638883.1
	Ypr011c-like carrier		McfB	XP_638149.1
	Peroxisomal AMP/ATP		McfQ	XP_645160.1

Species	Function	Gene	Name	Acc. Number
T.brucei	ADP/ATP carrier	Tb10.61.1830	MCP5	XP_827960.1
	ATP-Mg/Pi carrier	Tb927.4.1660	MCP6	XP_844316.1
	CoA carrier	Tb10.70.2290	MCP4	XP_822738.1

Species	Function	Gene	Protein name	Acc. Number
P. falciparum	ADP/ATP carrier			CAA58541.1
	ATP-Mg/Pi carrier			XP_001351960.1

Table S3 (continued)

Species	Function	Gene	Protein name	Acc. Number
N. ovalis	Hydrogenosomal ADP/ATP carrier			AAM97609.1
N. frontalis	Hydrogenosomal ADP/ATP carrier			AAN04660.1
B. hominis	Putative hydrogenosomal SLC25A43-like carrier			CBK23107.2
	Putative hydrogenosomal CoA carrier			CBK24530.2
	Putative hydrogenosomal ATP-Mg/Pi carrier			CBK20638.2
T. vaginalis	Hydrogenosomal adenine nucleotide carrier		HMP31	XP_001582728.1
E. histolytica	Mitosomal adenine nucleotide carrier			AAK69775.1
T. pyriformis	Putative hydrogenosomal adenine nucleotide carrier			ABW76101.1
P. lanterna	Putative hydrogenosomal adenine nucleotide carrier			ACZ97597.1
A. locustae	Mitosomal adenine nucleotide carrier			AAY27416.2
E. cuniculi	Plasma membrane ADP/ATP carrier		ecNTT1	ABW20407.1
	Plasma membrane ADP/ATP carrier		ecNTT2	ABW20408.1
	Mitosomal ADP/ATP carrier		ecNTT3	ABW20409.1
	Plasma membrane ADP/ATP carrier		ecNTT4	ABW20410.1

Species	Function	Locus	Protein name	Acc. Number
A. thaliana	Thylakoid ADP/ATP carrier	At5g01500	TAAC	NP_195770.1
	Unknown	At3g51870		NP_190755.2
	Adenine nucleotide uniporter in the plastid envelope	At4g32400	AtBT1	NP_194966.1
	Unknown	At3g20240		NP_188659.1
	ADP/ATP carrier in the plastid envelope	At1g80300	AATP1	NP_178146
		At1g15500	AATP2	CAA64329.1
	Endoplasmic reticulum ADP/ATP carrier	At5g17400	ER-ANT	NP_568345.1
	Unknown	At5g56450		NP_200456.1
Z. mays	ADP-Glucose/ADP carrier in the plastid envelope		ZmBT1-1	ACF78275.1
	Adenine nucleotide uniporter in the plastid envelope		ZmBT1-2	NP_001159268.1

Table S3 (continued)

Species	Function	Gene	Protein name	Acc. Number
P. infestans	Putative CoA carrier			XP_002897719.1
N. gruberi	Ypr011c-like carrier			XP_002674890
P. infestans	Ypr011c-like carrier			XP_002901049.1
N. vectensis	Ypr011c-like carrier			XP_001626526.1
N. gruberi	Putative peroxisomal AMP/ATP carrier			XP_002678224.1
P. infestans	Putative peroxisomal AMP/ATP carrier			XP_002900592.1 XP_002902667.1 XP_002909633.1
P. infestans	SLC25A43-like carrier			XP_002895040.1
N. vectensis	SLC25A43-like carrier			XP_001627437.1
B. floridae	SLC25A43-like carrier			XP_002611712.1

Table S3 (continued)