Structures and functions of mitochondrial ABC transporters

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Abstract

A small number of physiologically important ATP-binding cassette (ABC) transporters are found in mitochondria. Most are half transporters of the B group forming homodimers and their topology suggests they function as exporters. The results of mutant studies point towards involvement in iron cofactor biosynthesis. In particular, ABC subfamily B member 7 (ABCB7) and its homologues in yeast and plants are required for iron-sulfur (Fe-S) cluster biosynthesis outside of the mitochondria, whereas ABCB10 is involved in haem biosynthesis. They also play a role in preventing oxidative stress. Mutations in ABCB6 and ABCB7 have been linked to human disease. Recent crystal structures of yeast Atm1 and human ABCB10 have been key to identifying substrate-binding sites and transport mechanisms. Combined with *in vitro* and *in vivo* studies, progress is being made to find the physiological substrates of the different mitochondrial ABC transporters.

Sequence analysis of mitochondrial ABC transporters

Mitochondria of most eukaryote species harbour 2–4 different ABC transporters that belong to the B subfamily of half transporters. The proteins contain an N-terminal transmembrane domain (TMD) and a C-terminal nucleotide-binding domain (NBD), which form homodimers for full functionality [1]. Plant and algal genomes additionally encode a unique ABC transporter, termed cytochrome *c* maturation (Ccm)AB, in which the TMD and NBD are encoded by separate genes. CcmAB is placed in the ABCI subfamily, see section 'CcmAB in plant mitochondria'.

Phylogenetic relationship of mitochondrial transporters of the ABCB subfamily

As a starting point for the discussion of recent crystal structures and functional data, we have reanalysed the phylogenetic relationships of B-type mitochondrial ABC transporters and their closest prokaryotic homologues (Figure 1). The CcmAB proteins were excluded from the phylogenetic analysis due to their large evolutionary distance from the other mitochondrial ABC transporters.

Key words: ATP-binding cassette (ABC) transporter, glutathione, haem, iron, mitochondria, oxidative stress.

Abbreviations: ABC, ATP-binding cassette; Aft, Activator of ferrous transport; ATM, ABC transporter of the mitochondria; Ccm, cytochrome *c* maturation; dALA, δ-amino levulinic acid; IRP1, iron regulatory protein 1; Mdl, multi-drug resistance-like; Mfrn, mitoferrin; NBD, nt-binding domain; PPIX, protoporphyrin IX; TMD, transmembrane domain; TMH, transmembrane helix.

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The ABCB7 group, which includes the ABC transporters of the mitochondria Atm1 in yeast and ATM3 in *Arabidopsis*, can be found in virtually all eukaryotic species. Human ABCB7 and *Arabidopsis* ATM3 can largely rescue the phenotypes of yeast *atm1* mutants [2–4], providing experimental support that members of this subfamily may have the same function. Human ABCB6 can also partially complement a yeast *atm1* mutant [5] but differences in the intracellular localization of ABCB6 and Atm1 may point to distinct physiological roles.

ABCB8 and ABCB10 fall into distinct phylogenetic groups and are only present in metazoa. The fungal Mdl (multi-drug resistance-like) proteins are closely related to metazoan ABCB10 proteins, although they form a separate branch. Alignment of 80 ABCB10 paralogues identified conserved amino acids on the inner surface of transmembrane helices (TMHs) that could be part of the substrate-binding site [6]. These residues are conserved in yeast Mdl1 and Mdl2 (Figure 2), suggesting that they are the yeast homologues of ABCB10. It would be interesting to test if indeed mammalian ABCB10 can complement the yeast *mdl1*Δ *mdl2*Δ mutant (see section 'Mdl1 and Mdl2 in yeast').

Bacterial ABC transporters involved in heavy metal transport, such as *Cupriavidus metallidurans* AtmA [7] and *Novosphingobium aromaticivorans* Atm1 [8], share overall sequence similarity (40% identical and conserved residues) with mitochondrial ABC transporters of the B6 and B7 groups. The closest homologue of mitochondrial ABC transporters in *Rickettsia* is located in the ABCB7 clade, corroborating the close evolutionary relationship between this α-proteobacterium and mitochondria.

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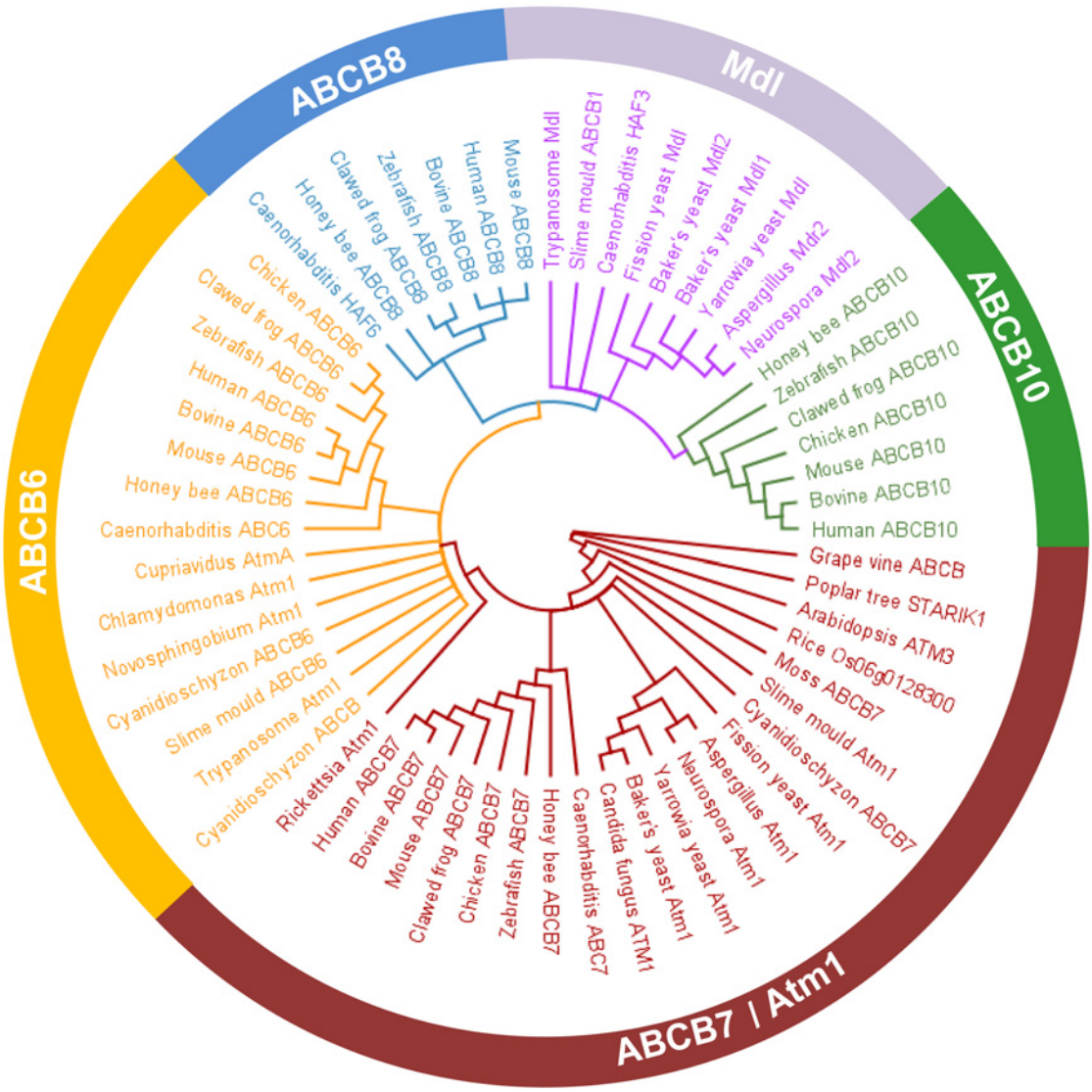
Figure 1 | Phylogenetic analysis of mitochondrial ABC transporters

The tree was constructed with homologues from model organisms across the evolutionary spectrum, using the Geneious 6.1.6 server. Sequences were aligned with ClustalW based on PAM matrix, taking evolutionary developments into account. CcmAB is not included due to its larger evolutionary distance and hence poor alignment, from other mitochondrial ABC transporters.



Targeting of ABCB proteins to the mitochondria

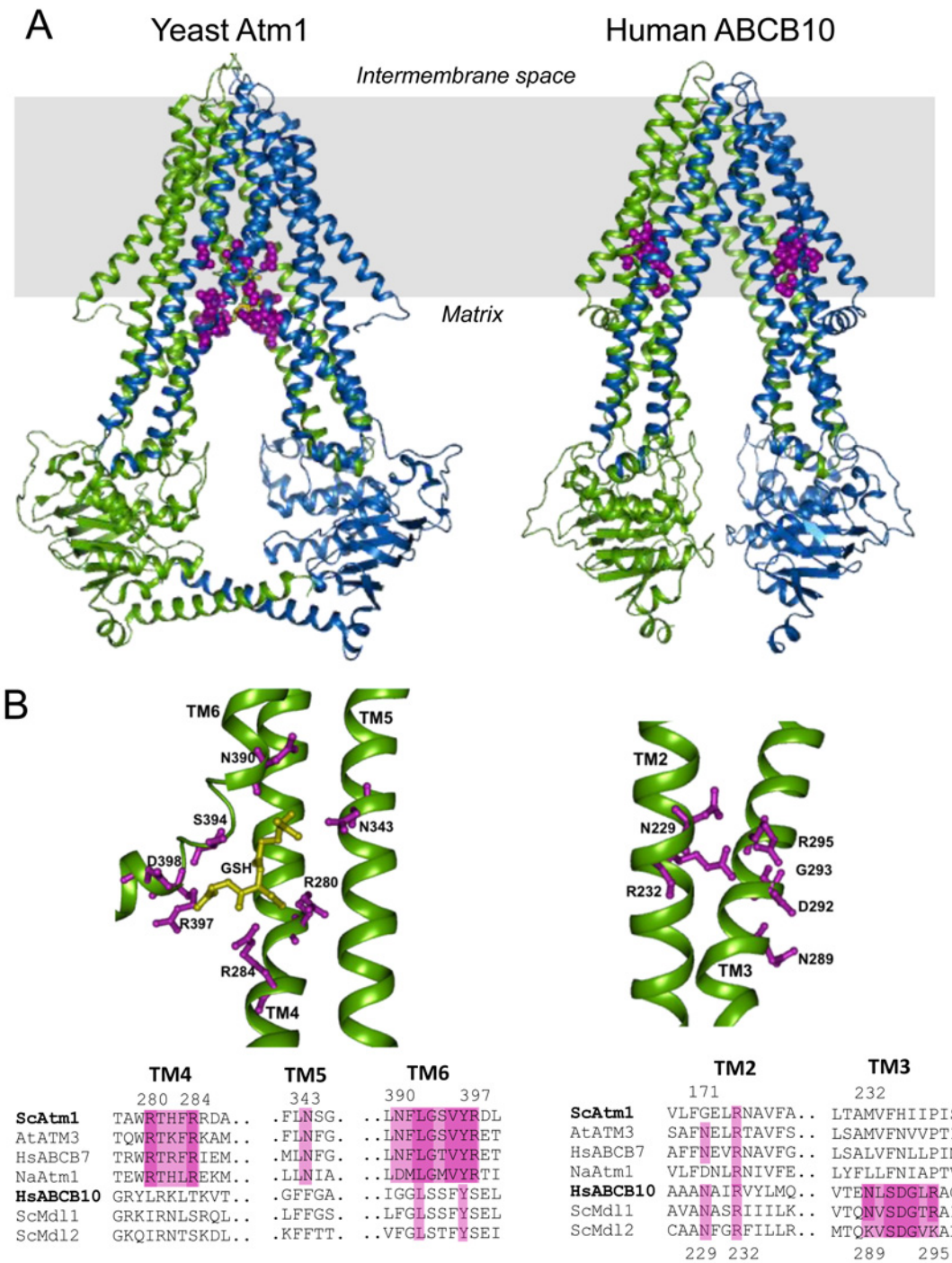
All mitochondrial ABC transporters, except CcmB, are encoded on the nuclear genome and imported into the mitochondria via the translocase of the outer membrane complex (TOM complex) [9]. The ORFs of ABCB7, ABCB8 and ABCB10 contain N-terminal mitochondrial targeting sequences that are exceptionally long compared with the usual length of 10–60 amino acids. The cleavage position of the mouse ABCB10 targeting sequence was determined to be at amino acid 105 [10] and that of yeast Mdl1 at amino acid 59 [11]. The targeting peptide of mouse ABCB10 contains three α-helical sub-domains. The first domain (amino acids 1–35) is essential for correct insertion of ABCB10 into the inner mitochondrial membrane. The second domain includes amino acids 36–70 and is critical for mitochondrial targeting, whereas the role of the third domain (amino acids 71–105) was not fully elucidated. Yeast Atm1 expressed in *Escherichia coli* was truncated at 93 ± 2 amino acids [12] and we found that removal of the first 58 N-terminal amino acids is required for ATPase activity [13]. However, in the case of both Atm1 and ATM3, the lengths of the actual native targeting sequences are unknown.

Structures of Atm1 and ABCB10

The structures of yeast Atm1 and human ABCB10 have recently been determined by X-ray crystallography [6,14]. Both proteins have a highly conserved ABC transporter fold

Figure 2 | Comparison of crystal structures of yeast Atm1 and human ABCB10

(**A**) Overall structure of Atm1 (left, Protein Data Bank 4MYH) and ABCB10 (right, Protein Data Bank 3ZDQ). The half transporter subunits are illustrated in green and blue. Residues involved in GSH binding in Atm1 and residues of a highly conserved signature motif in ABCB10 are presented as spheres in magenta for both transporters. GSH is in yellow. (**B**) Close-up of GSH-binding site in Atm1 (left) and ABCB10 signature motif (right) and sections of sequence alignment to show the conservation of these amino acid residues in related ABC transporters. GSH is in yellow and GSH-binding residues in magenta (left), both in ball-stick representation. ABCB10 signature residues are in magenta ball-stick presentation (right). Sequences used for the alignment were yeast Atm1 (ScAtm1, UniProtKB P40416), *Arabidopsis* ATM3 (AtATM3, Q9LVM1), human ABCB7 (hABCB7, O75027), the bacterium *N. aromaticivorans* Atm1 (NaAtm1, Q2G506), human ABCB10 (hABCB10, Q9NRK6), yeast Mdl1 (ScMdl1, P33310) and yeast Mdl2 (ScMdl2, P33311). Amino acid numbers for ScAtm1 are given above the alignment and those for HsABCB10 below.



seen in eukaryotic ABC transporters and some bacterial exporters, such as the multi-drug resistance transporter Sav1866 [15], P-glycoprotein from mouse [16] and its homologue in *Caenorhabditis elegans* [17], as well as the bacterial lipid flippase MsbA (multicopy suppressor of htrB, gene A) [18]. The two half transporter peptides of Atm1 and ABCB10 form a homodimer via a domain-swap with TMH4 and TMH5 of one monomer interconnecting with TMH1–3 and TMH6 of the opposing one (Figure 2A), thus creating a substrate-binding cavity at their interface [19]. The TMD protrudes through the lipid bilayer into the mitochondrial matrix. The NBDs are N-terminally attached to the TMDs via a linker and adapt a classical NBD fold with a recombination gene A-like R-like core subdomain and an α-helical subdomain important for ATP binding and hydrolysis [6,14].

Crystal structures of yeast Atm1 with glutathione and fully resolved C-terminal α-helices

Yeast Atm1 was expressed in *E. coli* and purified by affinity and size-exclusion chromatography. Two crystal structures were determined, both in a nucleotide-free 'open-inward' state, one without and one with bound glutathione (GSH; Figure 2A) [14]. Atm1 forms a symmetrical dimer with a large, positively charged cavity that is open to the mitochondrial matrix. The cavity contained a molecule of GSH, mainly interacting with residues Arg^{280}, Arg^{284} and Asp^{398}, indicating that this is a stable ligand formed during expression in *E.coli* and maintained during purification at low temperature. The GSH co-ordinating residues are conserved in Atm1-like proteins including human ABCB7 and bacterial homologues (Figure 2B).

It is interesting to compare the crystal structure of yeast Atm1 to the Atm1-homologue from the bacterium *N. aromaticivorans* which was determined in an inward-facing conformation [8]. Bacterial Atm1 has a GSH-binding site in a similar location as yeast. In addition, the bacterial transporter was crystallized with oxidized GSSG bound at two different sites. The primary site is accessible from the cytoplasm and mainly formed by residues of TMH5 and TMH6. The secondary, lower occupancy site is formed by Arg^{206}, Arg^{210} and Arg^{323} but its functional role is not known. Notably, the location of the GSH-binding site is substantially different from that of the hydrophobic inhibitors in the well-studied mammalian ABC exporter P-glycoprotein [16], despite the similar size and shape of the cavities in both structures. Clearly, the substrate-binding sites and properties have to be defined on an individual basis for each transporter.

In the structure of yeast Atm1, the C-terminus was fully resolved and showed a 24-residue long α-helix, a structural feature that has not been observed previously in other ABC exporter crystal structures. The helix from one monomer forms tight interactions with the other helix from the second monomer, keeping the NBDs in contact as well as stabilizing the dimer in the 'open-inward' conformation. The Atm1 with a truncated C-terminal α-helix was fully functional, although the protein expression levels were substantially lower in both yeast and *E. coli* [14]. The C-terminal α-helix may be a common stabilizing structural feature of many, if not all, ABC exporters since all exporters contain amino acid sequences at the C-terminus that could potentially form an α-helix.

Crystal structures of human ABCB10 in apo- and nucleotide-bound states

In an effort to determine the structure of all human ABC transporters, ABCB10 was one of the first to go successfully through a pipeline using insect cells for protein expression. The ABCB10 structure was solved both without nucleotide (Figure 2A, right) and as a complex with two non-hydrolysable ATP analogues in different crystal forms [6]. Rather unexpectedly, ABCB10 adopts an inward-facing conformation, similar to the state seen for P-glycoprotein, regardless of whether or not there is nucleotide bound to the NBDs. The NBDs are separated leaving access to a large substrate-binding site in the core of the TMD, facing the mitochondrial matrix. According to the alternating-access cycle model [20], ATP-binding and hydrolysis in the NBDs drives conformational changes in the TMDs allowing alternating access of the substrate to both sides of the membrane. Substrate binding to the inward-facing state might be required for full dimerization of the two NBDs in ABCB10, sandwiching ATP for hydrolysis and triggering conversion into the outward-facing state.

Although the substrate for ABCB10 is as yet unknown, analysis of the sequence conservation for homologues of ABCB10 from yeast to mouse revealed a highly conserved patch in the centre of TMH2 (N/IxxR) and the N-terminal end of TMH3 (NxxDGxR; Figure 2B). This ABCB10 signature motif is located at the inner concave surface in the core of the TMD pointing to a potential role in substrate binding. No conservation of the external surface was found, challenging the suggestion for ABCB10 as a binding partner for mitoferrin (Mfrn)-1 or ferrochelatase [21,22].

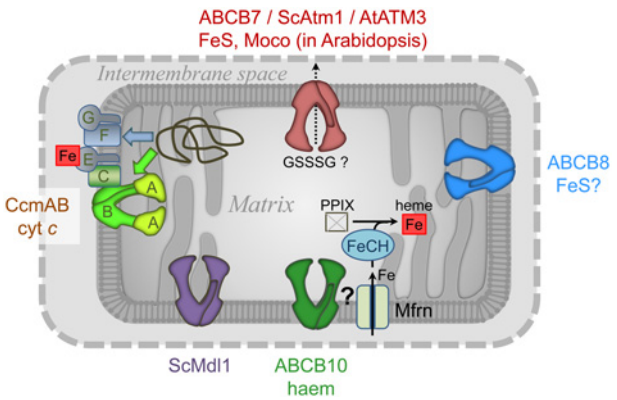
Biological function and substrates

A wealth of functional studies has pointed to the biological processes that mitochondrial ABC transporters are involved in. The transporters have often been linked to oxidative stress [23–26], which may be a consequence of their role in the maturation of iron (Fe) cofactors. Topology studies have shown that all five groups of ABC transporters in the inner mitochondrial membrane function as homodimers as the minimal functional unit, with the NBDs facing the matrix (Figure 3).

Although *in vivo* studies provide clues about the substrates of ABC transporters, *in vitro* studies are necessary to test for actual transport, as initiated for yeast and plant ABCB7 homologues. For meaningful results, the use of mutant proteins (e.g. catalytic lysine in Walker A motif or glutamate adjacent to the Walker B motif) is required to control for contaminating ATPase activities. A combination of *in vivo* and *in vitro* approaches, related to structural studies, is

Figure 3 | Schematic representation of ABC transporters found in mitochondria

See main text for details. All proteins are encoded in the nucleus, except for the CcmB subunit found in plants, for which the gene is on the mitochondrial DNA. Only part of the Ccm system is depicted. Mammalian ABCB6 is not included because its localization is uncertain.



indispensable for a full understanding of the biological functions of ABC transporters.

ABCB6 is localized in different membranes

Initial studies showed that human ABCB6 (then named MTABC3) could complement a yeast *atm1* point mutant and rescue phenotypes such as iron accumulation and respiratory dysfunction [5]. Overexpressed FLAG-tagged ABCB6 was shown to be localized to mitochondria by immunohistochemistry [5]. However, subsequent studies suggested that ABCB6 was targeted to the outer mitochondrial membrane with the NBDs facing the cytosol [27,28] and not in the inner membrane with inwards facing NBDs, like in yeast Atm1 [29]. ABCB6 was also found in the plasma membrane in N-glycosylated form [27,28] and in the Golgi apparatus [30]. Recently, evidence has been presented for an endolysosomal localization of ABCB6 [31–33], whereas ABCB7, ABCB8 and ABCB10 were found exclusively in mitochondria [31].

Apart from controversial localization data, the function of ABCB6 is also still unclear. Previous studies suggested a role of ABCB6 in transport of porphyrins for haem biosynthesis [27,28], but these findings could not be repeated in another study [31]. Recently, three different point mutations have been associated with a dominant skin pigmentation disorder called dyschromatosis universalis hereditaria [33]. The exact cause of disease symptoms is as yet unknown but it could be shown that mutant ABCB6 is retained in the Golgi apparatus [33]. Taking together, both the localization and the function of ABCB6 are still debated, but the link to disease could help unravel its precise role.

ABCB7 and its homologues in different eukaryotes

Proteins of the ABCB7 group have been studied in various organisms regarding their biological function. Mutants have pleiotropic phenotypes, which has made it difficult to unravel primary and secondary physiological effects. Deletion of the *ATM1* gene in Baker's yeast leads to very slow growth, loss of mitochondrial DNA and loss of cytochromes [23,29]. However, the most striking phenotype is perhaps the dramatic accumulation of iron in mitochondria of yeast *atm1* mutants, which was suggested to indirectly cause the loss of mitochondrial DNA and also oxidative stress in the knockout mutant [26]. Careful dissection of yet another phenotype, leucine auxotrophy, pointed to a deficiency in the assembly of Fe-S clusters in the cytosol. Kispal et al. [34] found that *atm1* mutants lacked activity of isopropyl malate isomerase (Leu1), the second step in leucine biosynthesis, which depends on a Fe-S cluster as cofactor. Many follow-up studies in yeast have since confirmed the requirement of Atm1 for cytosolic and nuclear Fe-S proteins, whereas mitochondrial Fe-S proteins are not dependent on Atm1 [35]. The functional specificity would also explain the role of Atm1 in iron homoeostasis: The assembly of a Fe-S cluster is a signal for the Fe-regulated transcription factors, named activation of ferrous transport Aft1 and Aft2, that there is sufficient Fe in the cell ([36] and references therein). Glutaredoxins Grx3 and Grx4 bind a Fe-S cluster in complex with the Fe repressor of activation protein Fra2, anchoring Aft1/2 in the cytosol. Lack of cytosolic Fe-S clusters in the *atm1* mutant activates (or de-represses) Aft1 and Aft2, which travel to the nucleus and constitutively up-regulate genes involved in Fe uptake, regardless of the Fe status of the cell.

Mutants in the mouse *ABCB7* and *Arabidopsis ATM3* (*ABCB25*) likewise exhibit strongly decreased levels and activities of cytosolic/nuclear Fe-S proteins without any effect on mitochondrial Fe-S enzymes [37,38]. In mammals, Fe sensing depends on dis/assembly of a Fe-S cluster in the iron regulatory protein 1 (IRP1) and indeed ABCB7 mouse models had disrupted Fe homoeostasis and iron accumulation in or surrounding mitochondria [38,39]. Pathogenic mutations in human ABCB7 also lead to Fe homoeostasis defects, such as in the disease X-linked sideroblastic anaemia [39]. In contrast, Fe accumulation is not found in *atm3* mutants in plants [37], which do not have Aft1 or IRP1 and are thought to have a different Fe sensing mechanism. Detailed analysis in plants revealed another phenotype in *atm3* mutants, namely a defect in molybdenum cofactor assembly and accumulation of the cyclic pyranopterin monophosphate precursor [40].

Taken together, there is evidence from different model organisms that ABCB7 and its homologues are required for cytosolic Fe-S clusters. The most simple and direct explanation would be that the protein transports a 'precursor' of Fe-S clusters. In more recent literature, a molecule containing persulfide 'X-S' has been favoured (see [41,42] and discussions therein). However, the idea of a glutathione-conjugated [2Fe-2S] cluster as a candidate substrate is also being tested *in vitro* [43,44].

Recent transport studies of yeast Atm1 and plant ATM3 using a *Lactococcus* expression system [13] demonstrated transport of GSSG and GSSSG. GSH was virtually not

transported, similar to a strong preference for GSSG over GSH of bacterial Atm1 [8]. The extra sulfur contained in GSSSG is in the form of persulfide, which is chemically available for Fe-S cluster assembly [45]. The persulfide in GSSSG is protected from nucleophilic attack and more stable than GSSH, which did not stimulate ATPase activity [13]. To provide physiological evidence that a glutathione persulfide may be transported by ATM3 in the model plant *Arabidopsis*, *atm3* mutations were combined with a non-lethal mutant allele of *ETHE1*, encoding a matrix-located sulfur dioxygenase. The ethylmalonic encephalopathy protein ETHE1 in humans and plants ETHE1 detoxifies persulfide using glutathione as a cofactor [46,47]. Most of the double *atm3 ethe1* mutants died during embryogenesis, indicating that toxic persulfide accumulates in the mitochondrial matrix when ATM3 is not functional [13].

In further investigations, it would be interesting to test if GSSSG is a substrate of mammalian ABCB7 and how K_m values compare for GSSSG and GSSG. It also remains to be investigated how GSSSG is formed in the mitochondrial matrix and metabolized for cofactor biosynthesis after transport. Another potential substrate to be tested on plant ATM3 and mammalian ABCB7 is the pterin precursor of molybdenum cofactor [40].

ABCB8 in mouse

ABCB8 was first described as M-ABC1 [48] and later mABC1 [49] and was confirmed to localize to mitochondria as predicted by its N-terminal targeting sequence. Induced genetic deletion of *ABCB8* in mouse hearts resulted in severe cardiomyopathy [50], indicating that ABCB8 has a unique function different from ABCB7 and ABCB10, which were expressed normally. Isolated heart mitochondria in the mutant mice had a ∼30% increase in non-haem iron and similar results were obtained upon down-regulation of *ABCB8* by siRNA in neonatal rat cardiomyocytes. Xanthine oxidase enzyme activity and glycerol-3-phosphate acyltransferase (GPAT) protein levels, two markers of cytosolic Fe-S protein biogenesis, were decreased in both heart and cardiomyocytes depleted of ABCB8. Cytosolic aconitase activity was ∼20% lower in ABCB8$^{\Delta/\Delta}$ hearts. The activity of the mitochondrial Fe-S enzyme succinate dehydrogenase was also decreased, but not significantly [49]. The extent to which cytosolic Fe-S proteins were affected by ABCB8 depletion is relatively mild compared to other studies. For example, deletion of ABCB7 in liver resulted in 80% less cytosolic aconitase activity [39]. Possibly, the observed Fe accumulation and lower Fe-S enzyme activities upon ABCB8 depletion may be secondary phenotypes.

ABCB10 in mammals

The exact role of ABCB10 in mitochondria is unknown, although there is considerable evidence to suggest it is involved in the formation of haem and/or protection of mitochondria against oxidative stress. The differentiation of red blood cells, erythropoiesis, has been a useful developmental system to identify proteins involved in mitochondrial iron transport and haem biosynthesis. Searching for highly induced transcripts, Shirihai et al. [51] identified an ABC transporter [ABC-me (ABC mitochondrial erythroid)], now renamed ABCB10, which was induced by the erythropoiesis transcription factor GATA-1 (recognizing the consensus sequence [A/T]GATA[A/G]). ABCB10 is expressed in red blood cells, in bone marrow, liver and heart. ABCB10 homozygous knockout mice die *in utero* at 12 days. These embryos are white and produce very little haem [52]. Mice with one ABCB10 gene removed survived to become adults and were apparently normal, but their hearts were more susceptible to damage by oxidative stress during ischaemia and reperfusion [25]. A recent study using conditional knockouts of the *ABCB10* gene in haematopoietic cells showed a dramatic accumulation of protoporphyrin IX (PPIX) in erythrocytes and accumulation of iron inside the mitochondria [53].

The process of haem synthesis starts in mitochondria, with production and export of δ-amino levulinic acid (dALA). This is then converted into PPIX in the cytoplasm. PPIX and iron have to enter the mitochondria, where they are combined to form haem catalysed by ferrochelatase, followed by export of haem from the mitochondria. It has been suggested that ABCB10 transport a component of the haem biosynthesis pathway, such as dALA [54]. However, the accumulation of PPIX suggests that the haem biosynthesis pathway is intact, including transport of dALA to the cytosol. ABCB10 has been reported to co-immunoprecipitate with FLAG-tagged Mfrn1 (SLC25A37), a mitochondrial transporter mediating Fe import into the mitochondria [22]. In the absence of ABCB10, Mfrn1 (but not Mfrn2) is destabilized and Fe import is decreased. The ABCB10–Mfrn complex also interacts with ferrochelatase, but this interaction is weaker [21] and is not required for ferrochelatase stability [53]. The suggestion that the main role for ABCB10 is to stabilize Mfrn1 is challenged by the observation that there are no conserved regions on the external surface of ABCB10 [6]. Therefore, the exact mechanism by which loss of ABCB10 causes changes in Fe and PPIX levels remains unclear, including what the substrate may be.

Mdl1 and Mdl2 in yeast

Sequence homology and conserved amino acid residues in the putative substrate-binding pocket suggest that the fungal Mdl proteins are homologues of mammalian ABCB10 [6] (Figure 2B). There are as yet no functional data to support this, therefore the Mdl proteins are discussed separately here.

Yeast Mdl1 and Mdl2 were first studied as candidates for peptide transporters, in analogy with the mammalian ABC transporters TAP1 and TAP2 involved in antigen presentation. Young et al. [55] found that deletion of Mdl1, but not the closely related Mdl2, resulted in a 40% decrease in the release of radiolabelled peptides, 6–20 amino acids in length, from the mitochondria. However, later studies failed to confirm that generic peptides were a substrate as no stimulation of ATPase activity or transport was observed in reconstituted Mdl1 [56].

MDL1 was independently identified in a genetic screen for genes that suppress the phenotypes of *atm1*Δ cells [11,24]. These results suggest that Mdl1 and Atm1 might have overlapping substrate specificities. The *mdl1*Δ mutant has no growth phenotype, but deletion of the related *MDL2* gene resulted in poor growth on non-fermentable carbon sources. The function of *MDL2* remains to be studied, alone and as an *mdl1*Δ *mdl2*Δ double mutant. Other fungi have only one copy of *MDL* (Figure 1) and could be useful model organisms to reveal the biological roles of this mitochondrial ABC transporter.

CcmAB in plant mitochondria

An ABC transporter involved in Ccm is found in the mitochondria of plants and red algae [57]. The Ccm pathway is required for the covalent attachment of haem to c-type cytochromes at two conserved cysteines (CxxCH). This process takes place on the outside of the inner membrane (Figure 3). Classified as system I, the Ccm pathway is conserved in α and γ bacteria and is more elaborate than system II found in chloroplasts and Gram-positive bacteria. Yeast and animals have evolved a much more simplified and mechanistically unrelated maturation pathway known as system III. The plant ABC transporter of the Ccm pathway has retained its prokaryotic structure in that the TMD and NBD domains are encoded by separate genes. Interestingly, the *ccmB* gene (*ABCI2*) encoding the TMD is located on the mitochondrial genome, whereas the *CCMA* gene (*ABCI1*) for the NBD is encoded in the nucleus of higher plants [58]. The *Arabidopsis* CCMA protein was shown to face the matrix side of mitochondrial membranes and interacted with CcmB in yeast two-hybrid studies. Recombinant CCMA protein had ATPase activity [58]. From these data it can be assumed that the plant mitochondrial CcmAB is analogous to the better characterized homologue in *E. coli*, although the substrate of the transporter is not yet known. It was initially thought that CcmAB transport haem into the periplasm, but several pieces of evidence argue against this. Mutation of the catalytic lysine in CcmA (K40D) does not prevent haem transfer to the other side of the membrane, but haem does get trapped on CcmE, a chaperone that binds haem on the periplasmic side in complex with CcmC [59,60]. Recent engineering of the Ccm pathway showed that CcmFH constitute the functional synthetase [61] and a similar strategy may also help to unravel the precise function of CcmAB.

Conclusions

Functional studies on the mitochondrial ABC transporters, mostly in yeast and mammals, have revealed their biological importance. Data on the pleiotropic phenotypes, genetic interactions and putative protein binding partners may at first seem confusing, but careful dissection of the information should help find the substrates of each transporter. Recent structures of yeast Atm1 and human ABCB10 have identified conserved amino acid residues involved in substrate binding. Additionally, candidate substrates can be tested *in vitro*, with systems now available for a number of mitochondrial transporters, either in proteoliposomes or in lactococcal membrane vesicles. An alternative approach will be to screen extracts of mitochondrial matrix for compounds that are transported, but the precise experimental set up remains to be developed.

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