Overview

ABC transporters: physiology, structure and mechanism – an overview

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Received 26 January 2001; accepted 12 February 2001

Abstract – ABC transporters form one of the largest of all protein families with a diversity of physiological functions. In *Escherichia coli* almost 5% of the genome is occupied by genes encoding components of these transporters, and there are examples in all species from microbes to man. In this overview, the importance of studies on bacteria in elucidating many basic principles pertaining to ABC transporters is emphasised. The family is described and a general overview of the structure and function of these transporters is presented. © 2001 Éditions scientifiques et médicales Elsevier SAS

ABC transporters / mechanisms / structure / binding proteins

1. Historical background

ABC (ATP binding cassette) transporters comprise one of the largest of all paralogous protein families. Almost 5% of the entire *Escherichia coli* genome encodes components of ABC transporters [27]. ABC transporters are found in all species from the lowliest microbe to man, play a wide variety of physiological roles, and are of very considerable medical and economic importance. In microorganisms ABC transporters are central to antibiotic and antifungal resistance, and in man many are associated with genetic diseases including cystic fibrosis, Tangier disease, obstetric cholestases, and drug resistance of cancers. Although ABC transporters are now ‘high profile’, it is frequently forgotten that they were first identified and characterised in model bacteria where the majority of fundamental principles relating to structure and function were developed.

The physiology of transport systems mediating the uptake of nutrients by bacteria (particularly *E. coli* and *Salmonella typhimurium*) was studied in detail in the 1970s. It soon became apparent that bacteria had multiple systems for the uptake of most nutrients and that these systems fell into a small number of classes: the phosphotransferase systems; secondary, shock-insensitive transporters energised by the electrochemical gradient; and primary, shock-sensitive systems energised directly by the hydrolysis of ATP [4]. This latter class of transporter was sensitive to osmotic shock due to the loss of a substrate-binding protein from the periplasm (hence the term periplasmic binding protein-dependent transport systems).

In 1982 the first complete sequence of the genes encoding one of these periplasmic transporters, the histidine transporter of *S. typhimurium*, was published [15]. In addition to the periplasmic substrate-binding protein (HisJ), the transporter has three membrane-associated components (HisQMP). Shortly thereafter, the sequence of a component of the *E. coli* maltose transporter (MalK) was determined and its sequence similarity to HisP led to the suggestion that such transporters might have evolved from a common ancestor [13]. When the sequence of a third homologue, OppD from the oligopeptide transporter of *S. typhimurium*, was obtained it was noted that these proteins included a consensus nucleotide-binding motif [16], similar to that previously identified in ATP synthase, myosin and adenylate kinase [38]. This led to the suggestion that these domains couple ATP hydrolysis to the transport process. Experimental evidence that these domains bind ATP [16, 20] and that ATP hydrolysis is coupled to transport [5, 29] soon followed.

In 1986, it was recognised that the ATP binding subunits from bacterial transporters defined a large superfamily family of proteins and the core organisation of four domains (see below) was proposed [17]. Although the majority of these domains were clearly...
components of active transport systems, it was noted that some were ‘orphans’ and had probably been recruited to couple ATPase activity to other biological processes such as DNA repair [11, 17]. 1986 also saw identification of the first eukaryotic example of this family of transporter, the human multidrug resistance P-glycoprotein [7, 12, 14]. The name ABC (ATP binding cassette) transporter was coined in 1990 [24], cementing a general recognition of the importance of this evolutionarily related but functionally diverse family of proteins.

2. Diversity of substrate specificity and physiological roles

The number of ABC transporters differs widely between species. Organisms such as E. coli, which live in diverse environments and need to adapt to a great array of external conditions, have many; around 70 ABC transporters are encoded by the E. coli chromosome [27]. In contrast, some other species have far fewer examples, perhaps reflecting their restrictive lifestyles (Dassa, this issue).

Although, in general, each ABC transporter is relatively specific for its own particular substrate(s), it is remarkable that there is an ABC transporter for essentially every type of molecule that must cross a cellular membrane. ABC transporters have been characterised with specificity for small molecules, large molecules, highly charged molecules and highly hydrophobic molecules—systems are known with specificity for inorganic ions, sugars, amino acids, proteins, and complex polysaccharides. Although most exhibit relatively tight substrate specificity, some are multispecific such as the oligopeptide transporter which handles essentially all di- and tripeptides [35], and some have an extremely broad specificity for hydrophobic compounds, such as the multidrug transporter LmrA from Lactococcus lactis [36]. It is intriguing how these related transport proteins can accommodate such diversity.

The diversity in substrate specificity is reflected in the diversity of physiological roles played by ABC transporters in the cell. Although the ABC transporters first characterised were nutrient uptake systems, it is now clear that many ABC transporters are exporters. Many of these play roles in elimination of waste products or toxins from the cell such as the LmrA protein of Lactococcus lactis (van Veen et al., this issue) and ABC transporters in fungi which confer resistance to antifungal agents (Wolfer et al., this issue). Other ABC transporters are essential for the export of cellular components which function outside the plama membrane including cell wall polysaccharides (Silver et al., this issue), in cytochrome c biogenesis (Goldman and Kranz, this issue), and antibiotic production (Mendez and Salas, this issue). Many polypeptides required outside the cell, such as cellulases, proteinases, or toxins such as haemolysin, are also exported via dedicated ABC transporters rather than the general signal peptide-dependent protein secretory pathway.

Finally, it should be emphasised that a few ‘apparent’ ABC transporters appear to have diversified in function (reviewed in [18]). In the mammalian world, the cystic fibrosis protein, which to all intents and purposes looks like a typical ABC transporter, is not a transporter; it is a chloride channel. Potentially, some microbial ABC transporters may also be channels rather than transporters. In addition, some ABC ‘transporters’ serve a regulatory rather than transport function. The best characterised example is the mammalian sulphonyl urea receptor (SUR) which has no known transport activity but imposes nucleotide regulation on a heterologous potassium channel [1]. Such a regulatory role is best exemplified in the bacterial world by the Sap/Trk system of S. typhimurium/E. coli which regulates a potassium channel independently of any transport activity it may or may not have [30, 34]. The mechanisms, though likely involving protein-protein interactions within the membrane, are obscure.

3. Organisation and structure

The basic unit of an ABC transporter consists of four core domains [17, 24]. Frequently, each of the four core domains is encoded as a separate polypeptide (e.g., the oligopeptide transporter; [19], although in other transporters the domains can be fused in any one of a number of ways into multidomain polypeptides (figure 1). In cases in which one of the four domains appears to be absent, one of the remaining domains functions as a homodimer to maintain the full complement [9, 26].

The two transmembrane domains (TMDs) span the membrane multiple times via putative α-helices. Typically, there are six predicted membrane-spanning
Figure 1. Organisation of ABC transporters. The typical ABC transporter has four domains, two membrane-associated domains (TMDs) are depicted by shaded squares. The two ATP-binding domains (NBDs or ABC domains) are depicted by ovals at the intracellular face of the membrane. The domains can be fused in any one of a number of ways: (A) they can be encoded as four separate polypeptides (as for the oligopeptide transporter OppBCDF); (B) fused NBDs (as for the ribose transporter RbsA); (C) fused TMDs (as for the iron-chelate transporter FhuCB); (D) one ABC fused to one NBD, with the hybrid protein functioning as a homodimer (as for LmrA); (E) one TMD fused to one NBD, with the other TMD and NBD as separate polypeptides (as for YhiGHI of E. coli); (F) all four domains fused into a single polypeptide, often found in eukaryotic ABC transporters. From Linton and Higgins, Molecular Microbiology 28 (1998) 5–13.

α-helices per domain (a total of twelve per transporter) although there is some variation on this formula. Some of the predicted membrane-spanning α-helices may not be crucial to the core function of the transporter but may serve auxiliary functions such as membrane insertion or regulation. The TMDs form the pathway through which solute crosses the membrane and determine the specificity of the transporter through substrate-binding sites.

The other two domains, the ATP or nucleotide-binding domains (NBDs), are hydrophilic and peripherally associated with the cytoplasmic face of the membrane. These domains consist of the core 215 or so amino acids of the ABC domain by which these transporters are defined. It is important to emphasise that it is the conservation of this entire domain which is important in defining and delimiting the family [17]; other ATP-binding proteins which are not ABC transporters can include the Walker A and Walker B motifs. Although it has been suggested that the NBDs may span the membrane [3], more recent data now suggest this is unlikely and that they are not exposed extracellularly [6]. The crystal structures of several isolated ABC domains have now been determined and the structures of HisP, MalK, RbsA and the eukaryotic Rad50 published [2, 10, 21, 22], all of which (not unexpectedly) show very similar folds. However, the various structures differ significantly in the dimer interface such that, even though it seems likely that the ABC domains do interact, the residues or faces of the domains involved in such interactions are unknown. Similarly, it remains unclear which faces of the NBDs interact with the transmembrane domains. Although some data pertaining to the residues involved in domain-domain interactions have been obtained (Schneider, this issue), the precise nature of these interactions remains to be determined.

In many ABC transporters auxiliary domains have been recruited for specific functions. The periplasmic binding proteins (PBPs) bind substrate external to the cell and deliver it to the membrane-associated transport complex. In Gram-positive bacteria, which lack an outer membrane (and hence periplasm), the equivalent substrate binding proteins are anchored to the outside of the cell via lipid groups [31]. It appears that PBPs may have two distinct but related functions.

(a) The first is to impart high affinity and specificity. An initial distinction between PBP-dependent and other transporters was that the PBP transporters showed remarkably high affinity. Similarly, most ABC transporters which lack a PBP (eg drug transporters) have rather broad specificity while those with a PBP can be highly specific. It is unclear why a PBP is apparently important for conferring high specificity and affinity – perhaps flexibility in the TMDs necessary for their transport function (see below) precludes the ‘lock and key’ fit required.

(b) The second is to confer directionality. There is a 100% correlation, at least for those systems analysed in detail, between the presence of a PBP and solute uptake, and between the absence of a PBP and solute export. Although this does not prove that the PBP determines directionality, the fact that interaction of the PBP with the transporter at the outside of the cell can trigger ATP hydrolysis at the cytoplasmic face of the membrane strongly implies such a role [8].

Other ABC transporters require outer membrane proteins to facilitate solute entry into the periplasm (eg transporters for iron chelates), while Gram-negative ABC transporters which mediate protein export require additional outer membrane proteins to facilitate transport across the periplasm and outer membrane (eg the HlyD and TolC proteins required for export of haemolysin [25]. No doubt other ABC trans-
Figure 2. Structure of ABC transporters. The overall structure of an ABC transporter is shown diagramatically, based on the structure of the mammalian P-glycoprotein ([32] and Rosenberg et al., submitted for publication). The protein consists of an aqueous pore, formed by the TMDs, with a large opening at the extracellular face of the membrane. The NBDs (light blue) are at the cytoplasmic face of the membrane in close apposition to the TMDs and possibly partly buried in the lipid bilayer though not spanning the membrane. Diagram courtesy of Mark Rosenberg and Bob Ford.

porters with peripheral or auxiliary proteins will be identified.

Although the structures of several ABC domains have now been elucidated, the overall structure of an intact ABC transporter has been very difficult to obtain. Here the microbial field can learn from the eukaryotic field. The structure of the mammalian multidrug resistance P-glycoprotein has been determined to 25 Å by single particle imaging [32], and to close to 10 Å by 2-D cryoelectron microscopy (Rosenberg et al., submitted for publication). Briefly (figure 2), the TMDs form a large ring-like chamber in the membrane, with an opening to the extracellular milieu and closed at the cytoplasmic face of the membrane. The NBDs are located at the cytoplasmic face of the membrane in tight apposition to the membrane domains. This structure is consistent with biochemical and genetic data for other ABC transporters and it seems likely it reflects a general architecture for ABC transporters. The structure differs from other membrane translocating ATPases, such as the Ca²⁺-ATPase and F₁F₀ ATPase in two important ways. First, the TMDs form a large pore in the membrane as opposed to the putative α-helices being tightly packed; this is, however, consistent with the fact that some ABC transporters can transport very large substrates, an observation difficult to reconcile with tightly packed α-helices. Second, in ABC transporters the NBDs are in close association with the TMDs, rather than being separated by ‘stalks’ as in the F₁F₀ ATPase or Ca²⁺ ATPase. The significance of this is unknown. Although these structural data have implications for the mechanisms of transport (see below), the absence of a high resolution structure of the TMDs limits our understanding of how ABC transporters work.

4. Mechanisms of transport

The detailed mechanisms by which ABC transporters move solute across the cell membrane are still far from clear. However, an overall picture is now emerging based upon studies of both prokaryotic and mammalian systems, making the not unreasonable assumption that there are mechanistic similarities. In summary:

The transport cycle is initiated by the interaction of substrate with the TMDs from the intracellular face of the membrane (or inner leaflet of the lipid bilayer). For importers the substrate can be considered as the PBP-substrate complex interacting at the extracellular face of the membrane. The substrate is then released from the PBP to interact with the TMDs. The number of substrate-binding sites on the TMDs is still unclear, although is likely to be two [36]. Substrate binding induces a conformational change in the TMDs which is transmitted to the NBDs to initiate ATP hydrolysis. It is clear that both NBDs are required, and both must hydrolyse ATP. However, they do so by an ‘alternating catalytic cycle’ mechanism [33] in which only one NBD hydrolyses ATP at a time. It is unclear how many ATP molecules are hydrolysed per molecule of substrate transported, and this is crucial to complete elucidation of the transport cycle. However, it appears to be 1 to 2 [29], consistent with a stoichiometric, closed cycle. Estimates of a greater number of ATP molecules hydrolysed per transport cycle, based on purified components, probably reflect ‘uncoupling’ of hydrolysis from transport. ATP hydrolysis induces further conformational changes in the NBDs (e.g., [23, 28]) which is transduced to the TMDs (Rosenberg et al., submitted for publication).

How do these conformational changes lead to solute translocation? It now appears that transport in-
volves more-or-less conventional enzyme-like mechanisms. For exporters, a high-affinity substrate binding site at the inside of the membrane is reoriented to be exposed to the outside of the membrane; simultaneously its affinity is reduced, resulting in extracellular release of the substrate (presumably for importers the PBP induces a reversal of this process). Finally, the transporter must be reset and the binding site reoriented back to the inside of the membrane and affinity restored. It has been proposed that the transporters have two binding sites, one high affinity and intracellular and the other low affinity and extracellular: the two alternate in affinity and sidedness in a ‘two-cylinder engine’ model ([37] and van Veen et al., this issue). It should be emphasised that the reorientation of a binding site does not necessarily mean a large ‘movement’ of the site across the bilayer. More probably the ‘site’ remains at the intracellular face of the membrane and the conformational changes simply serve to alter its exposure to the intracellular milieu or the aqueous pore (extracellular) of the transporter.

In conclusion, it now seems clear that both the ATP hydrolytic by the NBDs and reorientation and changes in affinity of substrate binding sites (on the TMDs the transport cycle) are conventional enzyme-like processes. Precisely how the two are coupled, and the nature of the conformational changes involved, remain to be elucidated.

5. Conclusions

The importance of ABC transporters in cell physiology and medicine cannot be underestimated, and ABC transporters are now recognised amongst the most important of all protein families. The study of ABC transporters, in all their guises, has now become a minor industry. This is a far cry from ‘orphan’ beginnings, and provides a wonderful example of scientific serendipity, how fundamental studies of obscure model microbial processes, pretty much for their intrinsic interest with no obvious commercial or medical implications, can unexpectedly have a significant impact in unimagined arenas of biology. As with many areas of research, studies on ‘high profile’ eukaryotic systems could not have proceeded apace without the fundamental advances derived from studies on microorganisms.

References


