Structure and mechanism of ABC transporters

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ATP-binding cassette (ABC) transporters are central to many physiological processes, including the uptake of nutrients, the non-classical secretion of signaling molecules and toxins, multidrug resistance and the development of human disease. As one might expect from this spectrum of translocation events, these ubiquitous, ATP-dependent pumps or channels are capable of transporting an enormous variety of substrates, ranging from small ions to large proteins. Recently determined structures of full-length ABC transporters and isolated ABC domains have increased our understanding of the functional mechanism of these proteins.

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Current Opinion in Structural Biology 2002, 12:754–760

0959-440X/02/$ – see front matter

Abbreviations

ABC	ATP-binding cassette
CFTR	cystic fibrosis transmembrane conductance regulator
ICD	intracellular domain
MDR	multidrug resistance protein
MHC	major histocompatibility complex
NBD	nucleotide-binding domain
PDB	Protein Data Bank
SUR	sulfonylurea receptor
TAP	transporter associated with antigen processing
TMD	transmembrane domain

Introduction

In 1982, the laboratory of Giovanna Ames [1] cloned and sequenced the first ATP-binding cassette (ABC) transporter, the histidine permease. From this point on, the family of ABC transporters has steadily grown and now represents one of the largest families of paralogous transmembrane proteins in many organisms [2]. For example, the genome of *Escherichia coli* contains 80 ABC transporters, corresponding to *2%* of the genome [3], the genome of *Saccharomyces cerevisiae* contains 31 [4] and the human genome contains 48 (see http://www.humanabc.org).

Despite their large number and overwhelming substrate diversity, ABC transporters share a basic blueprint. These ubiquitous, ATP-dependent pumps or channels possess a modular architecture. Two nucleotide-binding domains (NBDs), or ABC domains, and two transmembrane domains (TMDs) form a functional transporter. All four domains can be arranged in any possible fashion. In archaea and eubacteria, four separate subunits usually provide the four domains, whereas in higher organisms, these domains are normally fused together.

ABC transporters play an important role in many human diseases and pathophysiological processes, such as adrenoleukodystrophy, Stargardt macular dystrophy, X-linked sideroblastic anemia and ataxia, Dubin–Johnson syndrome, bare lymphocyte syndrome, virus persistence and many more [5,6]. For example, the ABC transporter multidrug resistance protein 1 (MDR1) is responsible for the resistance of tumor cells to chemotherapy, and mutations in the ABC transporter cystic fibrosis transmembrane conductance regulator (CFTR) lead to cystic fibrosis, the most frequently occurring deadly inherited disease.

In this review, we summarize recent achievements in the field of ABC transporters with an emphasis on their structure and mechanism of action.

Structure of ABC domains – the motor domains

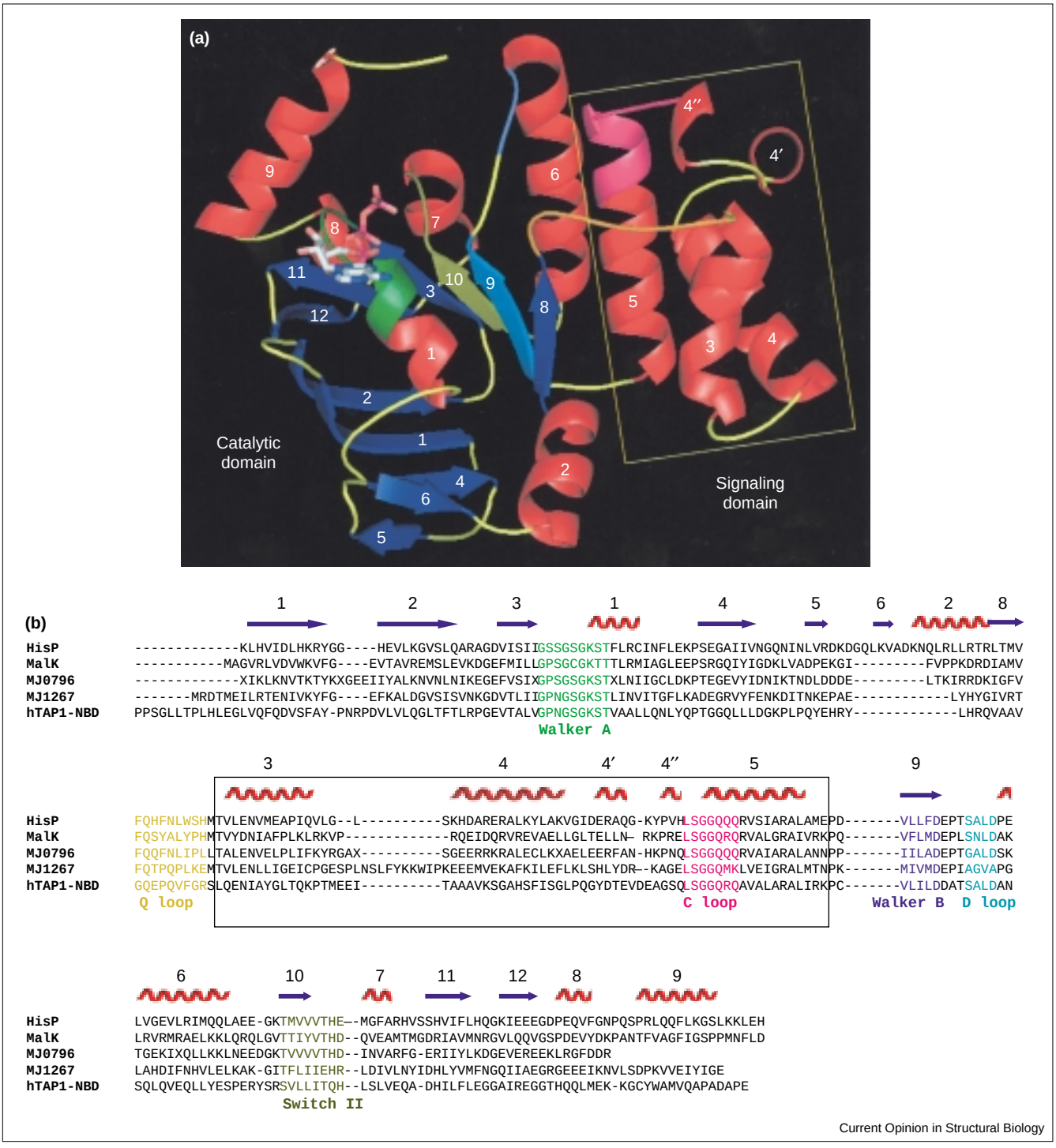
In 1998, the first high-resolution structure of an ABC domain — HisP, the NBD of histidine permease — was reported [7]. HisP showed a novel, two-domain architecture (Figure 1). Domain I, the catalytic domain, has an α/β structure and contains the nucleotide-binding site, which is formed by a Walker A motif [8] that precedes helix 1 plus the first three residues of this helix. In addition to this frequently encountered sequence (which is observed in many ATPases and GTPases), arm I contains the Walker B motif [9], which is located in strand 9, and a conserved histidine, which acts as a γ-phosphate sensor, in the so-called 'switch II' region between strand 10 and helix 7. A sequence alignment of the ABC domains for which structures have been solved is shown in Figure 1.

The interaction of the phosphate moieties of ATP occurs mainly in a canonical mode with residues of the Walker A motif. The major contact between the aromatic ring system of ATP and the protein occurs through π–π interactions with a conserved tyrosine or phenylalanine residue. This explains in structural terms why ABC domains and ABC transporters have no pronounced preference for individual nucleotide triphosphates. Domain II, the so-called signaling domain, is composed entirely of α helices and contains the C-loop or signature motif, which precedes and continues into helix 5. This domain is thought to interact with the TMDs (see below). The three-dimensional structure of domain I is reminiscent of RecA [10] and F_1-ATPase [11], whereas no structural homologue of domain II has been observed.

Dimer organization of ABC domains

The cooperativity in ATP hydrolysis observed for HisP suggested the presence of dimers of the ABC domain [12]. In the crystal structure of HisP, a dimer was observed with a 'back-to-back' orientation (Figure 2a). The dimer interface was formed by the outer β strand of domain I.

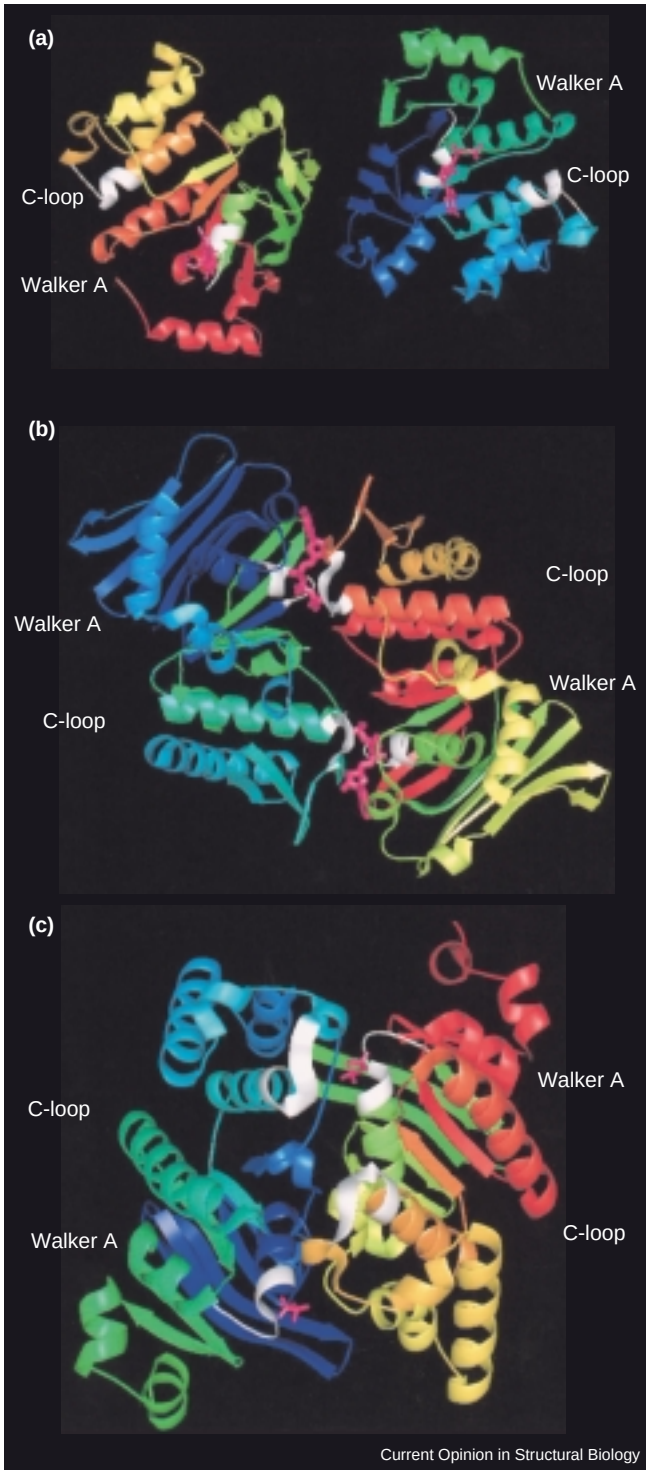
Figure 1



ABC domains: structure-sequence conservation. (a) Structure of HisP (PDB code 1BUO). Helices are shown in red, strands are shown in blue and ATP is shown in ball-and-stick representation. Important secondary structure elements are labeled according to [7]. (b) Sequence alignments of all reported X-ray structures of NBDs. Conserved motifs are colored and labeled. Secondary structure elements indicated above the alignment are from HisP. The box indicates the position of arm II, which is also highlighted by a box in the (a). All figures were prepared using MOLSCRIPT [46] and PYMOL (http://pymol.sourceforge.net/).

Consequently, the ATP-binding sites were exposed and the signature motifs, which were believed to sense ATP, were too far away to interact with the bound nucleotide. Subsequently, the X-ray structure of Rad50, a DNA repair enzyme, was reported [13]. Rad50 belongs to a family of helicases that are related to ABC transporters. The overall

Figure 2



Different dimer interfaces that have been proposed for ABC domains. (a) 'Back-to-back' dimer of HisP. (b) 'Head-to-tail' dimer of Rad50 (PDB code 1F2U). (c) 'Head-to-head' dimer of MalK (PDB code 1G29). For simplicity, the regulatory domain of MalK has been omitted. Walker A and C-loop sequences are highlighted in white. Ligands are shown in ball-and-stick representation.

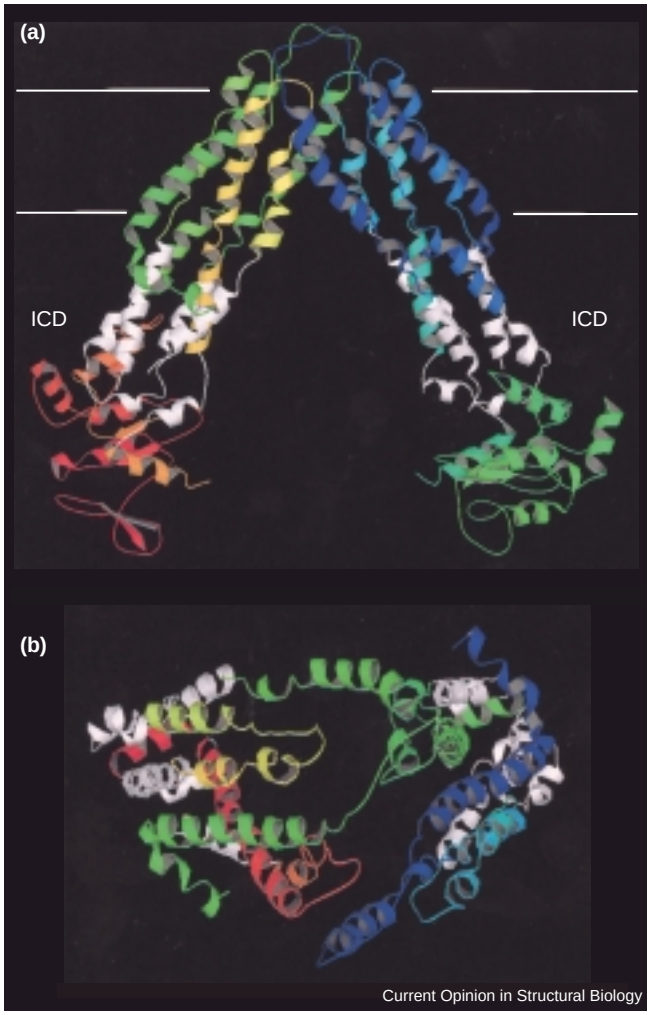
structures of Rad50 and HisP were quite similar, with the exception of domain II; however, Rad50 displayed a strikingly different dimer interface (Figure 2b). In Rad50, the nucleotide-binding site was located in the dimer interface and formed by the Walker A motif in monomer 1 and the C-loop of monomer 2, which resulted in a 'head-to-tail' orientation. Jones and George [14] used this structure in combination with other ABC domains, derived from fungal ABC transporters, to analyze the dimer interface of HisP; they proposed that other ABC domains actually adopt the interface that is observed in Rad50. Finally, the crystal structure of MalK from *Thermoccocus litoralis*, the ABC domain of the maltose importer, was solved [15] (Figure 2c). Here, the interface adopted a 'head-to-head' orientation, with the largest amount of buried surface area. For thermodynamic reasons, Diederichs *et al.* [15] proposed that the interface of the MalK dimer corresponded to the arrangement generally observed in ABC domains.

Over the years, the X-ray structures of three other ABC domains have been reported [16–18], but the discussions about the nature of the dimer interface continued. Only recently, Hunt and co-workers [19•] published the structure of a putative ABC domain from *Methanoccocus janaschii* (open reading frame 1267). This ABC domain contains a mutation of a glutamate residue to a glutamine, which is immediately C-terminal to the Walker B motif. This mutation induces the formation of stable dimers on ATP binding, but the protein was unable to hydrolyze ATP [20]. In the crystal structure, the mutated ABC domain adopted a dimer interface that is identical to the one in Rad50 (Figure 2b). This structure is supported by biochemical data obtained for MalK [21•]. Thus, it seems to be established that ATP hydrolysis is a cooperative process, in which key residues of each monomer participate in ATP binding and sensing, thereby forming a dimer interface with a head-to-tail orientation.

Structure of ABC transport machines

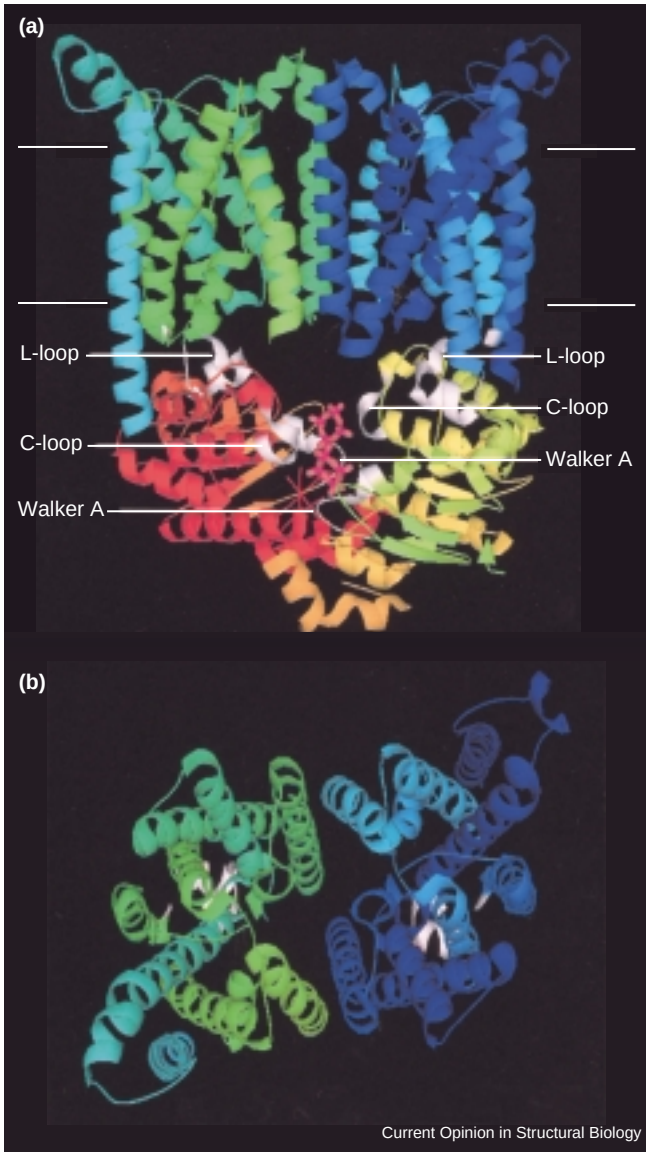
In 2001, Chang and Roth [22••] reported the first structure of a full-length ABC transporter, the lipid A flippase MsbA from *E. coli*. In an impressive *tour de force*, the structure was solved in the absence of substrate and nucleotide at a resolution of 4.5 Å (Figure 3a). The arrangement of MsbA within the crystal is consistent with a homodimeric state of the protein. The transmembrane region is composed of six tilted α helices per monomer and displayed contacts between helices from different monomers (Figure 3b). The tilt angle of the helices varied between 30° and 40° relative to the membrane normal. A large chamber within the TMDs was observed that has a large orifice facing the cytoplasm. On the basis of the size and architecture of this chamber, the authors speculated that this region contains the substrate-binding site. Based on the charge and polarity distribution within the TMDs and the presence of a chamber in the putative location of the inner leaflet of the membrane bilayer, a model for substrate transport was postulated that involves large conformational rearrangements of the helices. In addition to the canonical domain organization with two TMDs and two NBDs, a third

Figure 3



The lipid A flippase of *E. coli*. (a) Structure of MsbA (PDB code 1JSQ). The ICD is highlighted in white and the white lines indicate the putative position and dimension of the lipid bilayer. (b) View from the top of MsbA. For simplicity, the NBD has been omitted to highlight the arrangement of the transmembrane helices.

Figure 4



The vitamin B_{12} importer of *E. coli*. (a) Structure of BtuCD (PDB code 1I7V). Walker A and C-loop sequences are highlighted in white and labeled. The white lines indicate the putative position and dimension of the lipid bilayer. The ligand, cyclotetravanadate, is shown in ball-and-stick representation. (b) View from the top of BtuCD. For simplicity, the NBD (BtuD) has been omitted.

domain was identified. This so-called intracellular domain (ICD) is composed of α helices and is located between the NBD and TMD (Figure 3a). This arrangement led Chang and Roth [22••] to assume that the ICD acts as a transducer unit, which shuttles signals arising from nucleotide or substrate binding between the NBD and TMD.

Very recently, Locher *et al.* [23••] reported the second structure of an ABC transporter, the vitamin B_{12} importer BtuCD from *E. coli*. Again, this structure was solved in the absence of substrate and nucleotides, and a dimer was located in the asymmetric unit (Figure 4). However, each TMD contains ten α helices instead of six — the core number of helices normally found in ABC transporters. The packing of the TMD helices is fundamentally different from that observed in MsbA. Thus, it is very intriguing to speculate that the number and three-dimensional architecture of these helices may vary from transporter to transporter, and that the helices may represent basic building blocks, which are put together in a modular and highly flexible fashion. Although no substrate was present, a giant water-filled channel crosses the flat face of the transporter. The channel is closed on the cytoplasmic side of the transporter but open at the periplasmic entrance. Thus, the transporter resembles the shape of an inverted portal (Figure 4b). In contrast to MsbA, no ICD was identified in BtuCD and the NBDs are in direct contact with the TMDs. The TMDs contact the NBDs via a cytoplasmic

loop, which is located between transmembrane helices 6 and 7. This loop folds into two short α helices, which adopt the shape of an 'L' (Figure 4a). The sequence of this L-loop coincides with the 'EAA' motif of many bacterial ABC transporters [24], which has been shown to be involved in communication between the TMD and NBD. Residues of the Q-loop, which connects domains I and II within the ABC domain, and residues of the first two helices of domain II form the contact sites with the TMD. This architecture is in agreement with results from mutational studies, which have indicated that domain II acts as a signaling domain that transmits information between the TMD and the catalytic domain of the NBD [25]. Interestingly, many mutations in human ABC transporters such as CFTR or the transporter associated with antigen processing (TAP), which are related to diseases, are located in the region of the ABC domain that interacts with the L-loop. The NBDs form a 'head-to-tail' dimer.

The nucleotide-binding site is composed of residues from the Walker A motif in one monomer and residues from the C-loop of the other monomer. The architecture is identical to the mutated ABC domain of MJ1267 and reminiscent of Rad50, although the ligand is cyclotetravanadate. Under the crystallization conditions, orthovanadate is predominantly in the form of cyclotetravanadate. Two of the four vanadates superimpose nicely with the α and β positions of nucleotides in other ABC domain structures. Notably, the interface buries a relatively small surface area, which might explain why other interfaces had been observed in the past.

From the two structures of full-length ABC transporters, it is already evident that the structure and packing of the TMD will vary from ABC transporter to transporter, and that not only substrate specificity but also the chemical nature of the transported substrate (e.g. hydrophobic or hydrophilic) will impose certain constraints on the TMD and might influence their arrangement. Whether or not one of the two modes of TMD–NBD interaction is the general one for ABC transporters cannot be determined yet. It is possible that all ABC transporters with an import function share the L-loop architecture and that ABC transporters with an export function use the ICD to communicate between TMDs and NBDs. One can also imagine that new structural strategies of domain communication will be determined in other ABC transporters.

One should keep in mind, however, that some ABC transporters are a central part of supermolecular complexes. For example, haemolysin B, an ABC transporter from *E. coli*, is involved in a type I secretion process that shuttles the toxin haemolysin A in a one-step process across both membranes in concert with haemolysin D and TolC [26]. Also, TAP is part of a so-called 'MHC peptide loading complex', comprising TAP, MHC class I molecules and tapasin [27]. Sulfonylurea receptors (SUR1 or SUR2), which form ATP-dependent potassium channels, constitute a third example of a supermolecular complex. These complexes are composed of SUR and potassium inward rectifiers with a 4:4 stoichiometry [28]. There are more known examples of such arrangements and it is very likely that more will be discovered. Thus, structural and functional analysis of ABC transporters will not stop at the level of the isolated protein, but rather this will be the starting point for a further understanding of the complex and processive mode of translocation across biological membranes, including quality control and regulatory steps.

Mechanism of ABC transporters – two cylinders, one machine?

All ABC transporters catalyze vectorial transport across biological membranes, but their substrate diversity is enormous. It ranges from small inorganic ions such as chloride, amino acids, sugars, peptides and anticancer drugs to large proteins. Regardless of the nature of the substrate, the transport process is fuelled by ATP hydrolysis in all these systems. Analysis of the stoichiometry of ATP hydrolysis per molecule of substrate indicated that roughly one molecule of ATP is consumed in the case of MDR1 [29] and the maltose importer [30]. But it is not known at which stage of the transport cycle ATP is hydrolyzed or how the chemical energy is converted into the 'power stroke', which finally shuttles the substrate across the membrane; in other words, is the binding of ATP, its hydrolysis or the dissociation of inorganic phosphate the triggering step?

Another puzzling question concerns the necessity of two ABC domains. All ABC transporters contain two NBDs and can bind, *a priori*, two ATP molecules. In vanadate inhibition studies of the maltose importer, only one of the two ATP-binding sites became occupied by vanadate [31]. The same observation has been made in MDR1 [32], and it seems that MDR1 [33,34] and LmrA [35] — the bacterial homologue of MDR1 [36] — act in an alternating fashion like a two-cylinder engine. How this sequential mechanism fits together with the symmetric dimer structure containing two bound ATP molecules [19•] remains to be clarified. Thus, it is not entirely clear how the site for ATP binding and hydrolysis is selected. This issue is even more fascinating in ABC transporters that contain two identical copies of the NBD, such as the maltose transporter or LmrA. In the BtuCD structure [23••], the two NBDs are facing each other, which implies that there is some kind of communication between the two domains because the protein was crystallized in the absence of ATP. In the nucleotide-free structure of MsbA [22••], however, the NBDs are more than 50 Å apart. On the other hand, some fungal ABC transporters, including the yeast ABC transporter Pdr5, as well as the human ABC transporters CFTR and TAP, contain a degenerated C-loop sequence in one of the two NBDs. This observation implies that only one of the two NBDs is functional and the other one might have regulatory functions. The recently published low-resolution structure of MDR1 [37•] suggests that ATP binding induces major conformational changes within the TMDs. Overall, however, it is still unknown how the nonequivalency of the NBDs arises,

how the two domains communicate with each other and at which stage substrate transport takes place.

Another key question is the location and nature of the substrate-binding site. None of the crystal structures was obtained in the presence of ligand. Some ABC transporters, such as the maltose importer or the histidine permease, are rather specific for certain substrates. In these so-called 'traffic ATPases', however, the specificity arises from the substrate-binding protein, which, by definition, does not belong to the ABC transporter but is required for efficient translocation. On the other hand, ABC transporters such as MDR1 or TAP are promiscuous. MDR1 is able to expel nearly every known anticancer drug from the inner leaflet of the plasma membrane into the extracellular space. This process confers tumor cells with resistance to chemotherapeutic drugs, which is one of the largest problems in modern cancer therapy. TAP is able to transport peptides ranging from 8 to 40 amino acids from the cytosol into the lumen of the endoplasmic reticulum (for a recent review, see [38]). As for MDR1, TAP recognizes a large diversity of substrates. This substrate diversity of TAP can be literally superimposed on the peptide binding principle of MHC class I molecules [39]. Although the substrate-binding sites of MDR1 and TAP have been mapped by cross-linking and other biochemical approaches, it is still a mystery how a single protein can deal with a myriad of ligands without loosing affinity, specificity or efficiency.

Even the mechanism of substrate transport remains controversial. In the case of MDR1, it is assumed that two ligand-binding sites, one with high affinity and one or more with low affinity, exist within the TMD. Both recently proposed models assume that the NBDs act in an alternating manner. In one model [33], drug transport from the high-affinity to the low-affinity binding site occurs on dissociation of inorganic phosphate from one of the NBDs. The dissociation represents a relaxation of the NBD from a high-energy to a low-energy level. The other model proposes that two drugs bind simultaneously and that ATP hydrolysis provides the energy necessary for drug dissociation [34]. The laboratory of Ambudkar [40] has proposed a modified model in which ATP sites are recruited randomly. After ATP binding to one site, the affinity of the other ATP-binding site is reduced so that only one NBD acts at a time. ATP hydrolysis moves the substrate from the high-affinity to the low-affinity binding site. After ADP dissociation, which restores high affinity to the other ATP-binding site, ATP binds to the other NBD. Hydrolysis of this second ATP is used to restore the ground state of the transporter. It has been demonstrated that substrate binding and ATP hydrolysis are tightly coupled [41]; however, the alternative model [40] implies that two ATP molecules are hydrolyzed per molecule of transported drug. Which of the proposed models of the catalytic cycle of MDR1 and other ABC transporters is correct remains a subject of intensive research.

Recently, modulators and inhibitors have been recognized as useful tools to study the mechanism of ABC transporters. In the case of human TAP, viral proteins such as ICP47 from the herpes simplex virus [42,43] or US6 from the cytomegalovirus [44,45] target this ABC transporter and inhibit its key function in cellular immunity, leading to immune evasion of infected cells. With these inhibitors in hand, many aspects of function and/or structure can be addressed, not only for TAP but also for several other ABC transporters.

Conclusions

This review was intended to summarize recent breakthroughs in the field of ABC transporters. Two decades of research in this area have passed, with tremendous achievements contributed from all disciplines of life science. From the structure of the ABC domains and the two full-length ABC transporters, we are beginning to understand the molecular principles of ABC transporters. However, it is necessary to determine further structures with and without substrate, and in different functional states of the ABC domain to understand structure/function relationships and to extract mechanistic principles on a molecular level. Nevertheless, the appearance of structural information in such a short period of time is very promising and we are likely to see exciting three-dimensional structures of full-length transporters and their ABC domains in the near future. Many aspects of the function of ABC transporters are still a mystery, but these issues are currently being addressed and their resolution in the future will contribute much to our understanding of the principles governing the structure and function of ABC transporters.

Acknowledgements

The Deutsche Forschungsgemeinschaft DFG (Emmy-Noether Program to L Schmitt and SFB 286 to R Tampé) has supported our work.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Higgins CF, Haag PD, Nikaido K, Ardeshir F, Garcia G, Ames GF: Complete nucleotide sequence and identification of membrane components of the histidine transport operon of ***S. typhimurium***. *Nature* 1982, 298:723-727.

2. Higgins CF: ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* 1992, 8:67-113.

3. Linton KJ, Higgins CF: The ***Escherichia coli*** ATP-binding cassette (ABC) proteins. *Mol Microbiol* 1998, 28:5-13.

4. Bauer BE, Wolfger H, Kuchler K: Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. *Biochim Biophys Acta* 1999, 1461:217-236.

5. Klein I, Sarkadi B, Varadi A: An inventory of the human ABC proteins. *Biochim Biophys Acta* 1999, 1461:237-262.

6. Abele R, Tampé R: Function of the transport complex TAP in cellular immune recognition. *Biochim Biophys Acta* 1999, 1461:405-419.

7. Hung LW, Wang IXY, Nikaido K, Liu PQ, Ames GFL, Kim SH: Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature* 1998, 396:703-707.

8. Schulz GE: Binding of nucleotide by proteins. *Curr Opin Struct Biol* 1992, 2:61-67.

9. Walker JE, Saraste M, Runswick MJ, Gray NJ: Distantly related sequences in the **α**- and **β**-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J* 1982, 1:945-951.

10. Story RM, Steitz TA: Structure of the RecA protein–ADP complex. *Nature* 1992, 355:374-376.

11. Abrahams JP, Leslie AG, Lutter R, Walker JE: Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. *Nature* 1994, 370:621-628.

12. Liu P-Q, Ames GF-L: ***In vitro*** disassembly and reassembly of an ABC transporter, the histidine permease. *Proc Natl Acad Sci USA* 1998, 95:3495-3500.

13. Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, Tainer JA: Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 2000, 101:789-800.

14. Jones PM, George AM: Subunit interactions in ABC transporters: towards a functional architecture. *FEMS Microbiol Lett* 1999, 179:187-202.

15. Diederichs K, Diez J, Greller G, Muller C, Breed J, Schnell C, Vonrhein C, Boos W, Welte W: Crystal structure of MalK, the ATPase subunit of the trehalose/maltose ABC transporter of the archaeon ***Thermococcus litoralis***. *EMBO J* 2000, 19:5951-5961.

16. Karpowich N, Martsinkevich O, Millen L, Yuan YR, Dai PL, MacVey K, Thomas PJ, Hunt JF: Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter. *Structure* 2001, 9:571-586.

17. Yuan YR, Blecker S, Martsinkevich O, Millen L, Thomas PJ, Hunt JF: The crystal structure of the MJ0796 ATP-binding cassette. Implications for the structural consequences of ATP hydrolysis in the active site of an ABC transporter. *J Biol Chem* 2001, 276:32313-32321.

18. Gaudet R, Wiley DC: Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing. *EMBO J* 2001, 20:4964-4972.

19. • Smith PC, Karpowich N, Millen L, Moody JE, Rosen J, Thomas PJ, Hunt JF: ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. *Mol Cell* 2002, 10:139-149.
The authors reported a dimer interface that is similar to the one in Rad50 and, for the first time, provide experimental evidence that the dimer interfaces of ABC domains and helicases such as Rad50 are identical.

20. Moody JE, Millen L, Binns D, Hunt JF, Thomas PJ: Cooperative, ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters. *J Biol Chem* 2002, 277:21111-21124.

21. • Fetch EE, Davidson AL: Vanadate-catalyzed photocleavage of the signature motif of an ATP-binding cassette (ABC) transporter. *Proc Natl Acad Sci USA* 2002, 99:9685-9690.
The authors presented biochemical evidence that the ABC dimer interface in solution is similar to the one described by Hunt and co-workers [19•].

22. •• Chang G, Roth CB: Structure of MsbA from ***E. coli***: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science* 2001, 293:1793-1800.
The first structure of a full-length ABC transporter. The authors cloned, purified and crystallized various ABC transporters from different organisms. To obtain crystals suitable for X-ray diffraction, a breath-taking number of 96 000 crystallization trials were analyzed.

23. •• Locher KP, Lee AT, Rees DC: The ***E. coli*** BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science* 2002, 296:1091-1098.
The second structure of an ABC transporter. Again, various ABC transporters were tested for their ability to form ordered three-dimensional crystals. The resolution of 3.2 Å is sufficient to obtain a first understanding of how the vitamin B_{12} importer might act on a molecular level.

24. Mourez M, Hofnung M, Dassa E: Subunit interactions in ABC transporters: a conserved sequence in hydrophobic membrane proteins of periplasmic permeases defines an important site of interaction with the ATPase subunits. *EMBO J* 1997, 16:3066-3077.

25. Schmees G, Stein A, Hunke S, Landmesser H, Schneider E: Functional consequences of mutations in the conserved 'signature sequence' of the ATP-binding-cassette protein MalK. *Eur J Biochem* 1999, 266:420-430.

26. Buchanan SK: Type I secretion and multidrug efflux: transport through the TolC channel-tunnel. *Trends Biochem Sci* 2001, 26:3-6.

27. Ortmann B, Copeman J, Lehner PJ, Sadasivan B, Herberg JA, Grandea AG, Riddell SR, Tampe R, Spies T, Trowsdale J *et al.*: A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. *Science* 1997, 277:1306-1309.

28. Bryan J, Aguilar-Bryan L: Sulfonylurea receptors: ABC transporters that regulate ATP-sensitive K^+ channels. *Biochim Biophys Acta* 1999, 1461:285-303.

29. Shapiro AB, Ling V: Transport of LDS-751 from the cytoplasmic leaflet of the plasma membrane by rhodamine-123-selective site of P-glycoprotein. *Eur J Biochem* 1998, 254:181-188.

30. Muir M, Williams L, Ferenci T: Influence of transport energization on the growth yield of ***Escherichia coli.*** *J Bacteriol* 1985, 163:1237-1242.

31. Chen J, Sharma S, Quiocho FA, Davidson AL: Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerted mechanism of maltose transport. *Proc Natl Acad Sci USA* 2001, 98:1525-1530.

32. Senior AE, al-Shawi MK, Urbatsch IL: The catalytic cycle of P-glycoprotein. *FEBS Lett* 1995, 377:285-289.

33. Senior AE: Catalytic mechanism of P-glycoprotein. *Acta Physiol Scand* 1998, 163:213-218.

34. Shapiro AB, Ling V: The mechanism of ATP-dependent multidrug transport by P-glycoprotein. *Acta Physiol Scand* 1998, 643:227-234.

35. van Veen HW, Margolles A, Muller M, Higgins CF, Konings WN: The homodimeric ATP-binding cassette transporter LmrA mediates multidrug transport by an alternating two-site (two-cylinder engine) mechanism. *EMBO J* 2000, 19:2503-2514.

36. van Veen HW, Callaghan R, Soceneantu L, Sardini A, Konings WN, Higgins CF: A bacterial antibiotic-resistance gene that complements the human multidrug-resistance P-glycoprotein gene. *Nature* 1998, 391:291-295.

37. • Rosenberg MF, Velarde G, Ford RC, Martin C, Berridge G, Kerr ID, Callaghan R, Schmidlin A, Wooding C, Linton KJ *et al.*: Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle. *EMBO J* 2001, 20:5615-5625.
The authors present low-resolution electron microscopy data derived from two-dimensional crystals of MDR1, which indicate that large conformational changes occur in the TMD during substrate transport.

38. Schmitt L, Tampé R: Affinity, specificity, diversity: a challenge for the ABC transporter TAP in cellular immunity. *Chem Biochem* 2000, 1:16-35.

39. Uebel S, Kraas W, Kienle S, Wiesmüller K-H, Jung G, Tampé R: Recognition principle of the TAP-transporter disclosed by combinatorial peptide libraries. *Proc Natl Acad Sci USA* 1997, 94:8976-8981.

40. Sauna ZE, Ambudkar SV: Characterization of the catalytic cycle of ATP hydrolysis by human P-glycoprotein. The two ATP hydrolysis events in a single catalytic cycle are kinetically similar but affect different functional outcomes. *J Biol Chem* 2001, 276:11653-11661.

41. Gorbulev S, Abele R, Tampe R: Allosteric crosstalk between peptide-binding, transport, and ATP hydrolysis of the ABC transporter TAP. *Proc Natl Acad Sci USA* 2001, 98:3732-3737.

42. Ahn K, Meyer TH, Uebel S, Sempe P, Djaballah H, Yang Y, Peterson PA, Fruh K, Tampe R: Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus ICP47. *EMBO J* 1996, 15:3247-3255.

43. Neumann L, Tampé R: Kinetic analysis of peptide binding of the TAP transporter complex: Evidence for structural rearrangements induced by substrate binding. *J Mol Biol* 1999, 294:1203-1213.

44. Hewitt EW, Gupta SS, Lehner PJ: The human ***cytomegalovirus*** gene product US6 inhibits ATP binding by TAP. *EMBO J* 2001, 20:387-396.

45. Kyritsis C, Gorbulev S, Hutschenreiter S, Pawlitschko K, Abele R, Tampe R: Molecular mechanism and structural aspects of transporter associated with antigen processing inhibition by the cytomegalovirus protein US6. *J Biol Chem* 2001, 276:48031-48039.

46. Kraulis PJ: MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J Appl Crystallogr* 1991, 24:946-950.