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REVIEW

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The motor domains of ABC-transporters

What can structures tell us?

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Abstract The transport of substrates across a cellular membrane is a vitally important biological function essential for cell survival. ATP-binding cassette (ABC) transporters constitute one of the largest subfamilies of membrane proteins, accomplishing this task. Mutations in genes encoding for ABC transporters cause different diseases, for example, Adrenoleukodystrophy, Stargardt disease or Cystic Fibrosis. Furthermore, some ABC transporters are responsible for multidrug resistance, presenting a major obstacle in modern cancer chemotherapy. In order to translocate the enormous variety of substrates, ranging from ions, nutrients, small peptides to large toxins, different ABC-transporters utilize the energy gained from ATP binding and hydrolysis. The ATP binding cassette, also called the motor domain of ABC transporters, is highly conserved among all ABC transporters. The ability to purify this domain rather easily presents a perfect possibility to investigate the mechanism of ATP hydrolysis, thus providing us with a detailed picture of this process. Recently, many crystal structures of the ATP-binding domain and the full-length structures of two ABC transporters have been solved. Combining these structural data, we have now the opportunity to analyze the hydrolysis event on a molecular level. This review provides an overview of the structural investigations of the ATP-binding domains, highlighting molecular changes upon ATP binding and hydrolysis.

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Abbreviations ABC: ATP-binding cassette · ASA: Solvent-accessible surface area · ICD: Intracellular domain · NBD: Nucleotide binding domain · rmsd: Root mean square deviation · SBP: Substrate binding protein · SDR: Structurally diverse region · TMD: Transmembrane domain

Introduction

ATP binding cassette (ABC) systems form one of the largest families of transmembrane proteins. As reflected in the name, they are characterized according to their property to bind and hydrolyze ATP. The energy derived from these events is utilized to accomplish different cellular processes. One subclass of ABC proteins is involved in DNA repair and RNA translation. A second subclass can be found in cellular membranes and constitutes the transporter family (ABC-transporters). Substrates which are translocated by such transporters are sometimes referred to as allocrites (Blight and Holland 1990). With the help of this nomenclature it is easy to distinguish between the two necessary substrates of an ABC transporter; ATP, which fuels translocation, and the substrate which is actually translocated by the transporter, here referred to as the allocrite. The allocrites display a huge diversity and exhibit extremely different chemical characteristics. An example of ion transport is the cystic fibrosis transmembrane conductance regulator (CFTR). This ATP transporter is a ligand-gated chloride channel (Riordan 2005; Riordan et al. 1989). Lipids and hydrophobic compounds are allocrites for P-glycoprotein, an important factor in multidrug resistance arising during chemotherapy (Gottesman and Pastan 1993). In bacteria, many ABC transporters are found that import nutrients, such as maltose (Bavoil et al. 1980) or glucose (Albers et al. 1999) import systems. The transporter associated with antigen processing (TAP)

transports peptides from the cytosol to the ER lumen (Schmitt and Tampe 2000). Even very large toxins are transported by ABC-transporters. For example, in the type I secretion system, the 107 kDa toxin HlyA is transported via a tunnel-like system which consists of the ABC transporter, haemolysin B (HlyB), HlyD and TolC (Holland et al. 2005). The largest allocrite known today is an S-layer protein, secreted by the ABC-dependent, Type I pathway, with a molecular weight of 900 kDa (Hinsa et al. 2003).

Based on sequence analysis, ABC proteins are divided into three classes (Dassa and Bouige 2001). Class 1 and 3 comprise ABC transporters that, in addition to the nucleotide binding (and hydrolyzing) domain (NBD), also contain a transmembrane domain (TMD). Members of Class 1 have the NBD fused to the TMD, while for Class 3, each domain is encoded as a separate polypeptide chain and thus the transmembrane domain is strictly speaking a distinct transmembrane subunit. However, for clarity we shall retain the name TMD in this review. Members of Class 2 are composed of a dimer of NBDs, sometimes with additional domains, but no membrane spanning domain.

Despite the divergence into these three classes, with one class apparently having no transmembrane transport function, it is rather striking that the NBD subunit remains highly conserved, reflecting a common mechanism of ATP hydrolysis, inherent to this domain. In contrast, the obvious sequence and structural variation in the membrane domain of the Class 1 and 3 proteins is to be expected from the extreme variety of allocrites. As the recent crystal structures of full-length ABC-transporters have proved (Chang and Roth 2001; Locher et al. 2002), the transmembrane spanning domain is built up from α-helices. Usually, there are 6 α-helices per transmembrane spanning domain (called the core domain), although some transporters exhibit more, e.g. MRP1 or the BtuCD transporter (Locher et al. 2002), and some less (Khwaja et al. 2005).

Functional ABC transporters are dimers with two TMD and NBD subdomains. This results in multiple possibilities for the genetic origin of the subdomains. In some, the four subunits are encoded as separate proteins. When one NBD and TMD are fused together in one polypeptide (TMD-NBD), these are called `half size transporters`. This leaves two possibilities for the assembly of the full transporter; it can be a homodimer $(TMD\text{-}NBD)_2$ or a heterodimer (TMD-NBD)(TMD-NBD)*. In contrast to the half size transporters, full size transporters have all 4 subunits encoded by one gene (TMD-NBD-TMD*-NBD*). Other variations are fused NBDs such as RbsA, (Zaitseva et al. 1996), fused TMDs [FhuC,B, (Groeger and Koster 1998)] or NBDs with an additional domain (such as OpuAA) (Horn et al. 2003).

Although ABC transporters can function as importers or exporters, the former are only found in bacteria and archea. Importers exhibit a conserved domain organization with each of the four subdomains (2TMDs and 2NBDs) encoded by separate genes. Furthermore, these systems require an extra protein to bind the allocrite before delivering it to the TMD. This so called substrate binding protein (SBP) is present in the periplasmic space in Gram-negative bacteria, whereas in Gram-positive bacteria and archea, the SBP is inserted into the membrane via a lipid anchor.

The human genome encodes 48 ABC transporters (Dean et al. 2001). These are divided into five different subfamilies:ABCA, ABCB, ABCC, ABCD and ABCG. Two further subfamilies, ABCE and ABCF, lack transmembrane domains and thus are not membrane transporters. One representative of the first subfamiliy is ABCA4 (formally known as ABCR), that apparently transports retinol derivatives and is exclusively found in photoreceptors. Patients with mutations in the corresponding gene may suffer from multiple eye disorders, such as Stargardt disease (Allikmets 2000), resulting in macular dystrophy. The first human ABC transporter cloned and characterized (Schurr et al. 1989) belongs to the ABCB subfamiliy. Cancer cells displaying multidrug resistance exhibit an elevated expression level of P-glycoprotein (P-gp), also known as ABCB1 or MDR1 (Gottesman and Pastan 1993). Here, the allocrites are a wide variety of drugs, e.g. colchicine, adriamycin and vinblastine, and hydrophobic compounds in general including lipids are transported by this protein. The most common genetically inherited disease among Caucasians is linked to CFTR (ABCC7). Cystic fibrosis is caused by a mutation in the CFTR gene and often leads to death during childhood or of young adults. A deletion of residue F508 in NBD1 is the most common (Riordan et al. 1989). The Dubin-Johnson syndrome is conferred by mutations in the ABCC2 gene, leading to a deficiency in canalicular multispecific organic anion transport and bilirubin accumulation in liver (Wada et al. 1998). Long chain fatty acids are transported by ABCD1, and Adrenoleukodystrophy (ADL) is a genetically inherited disease, caused by mutations in the ABCD1 gene (Mosser et al. 1993). Finally, the ABCG2 transporter is also overexpressed in human cancer cells, resulting in multidrug resistance independent of the classic multidrug transporters MDR1 and MRP1 (Robey et al. 2001).

Recently, in addition to the structures of the two full length ABC transporters (Chang 2003; Chang and Roth 2001; Locher et al. 2002; Reyes and Chang 2005), several high resolution crystal structures of ATP-binding domains from all three classes of ABC proteins have been solved. Details of the origin, resolution and PDB access code for all these structures is shown in Table 1. In this review, we shall not discuss full length ABC transporter structures in detail, since these are, still to some extent, controversially discussed elsewhere (Jones and George 2004). The rather high sequence conservation in ABC-NBDs suggests a common mechanism for ATP binding and hydrolysis. The new, recent insights into the three-dimensional arrangement of this domain, brings us much closer to answering the key question of how ATP hydrolysis is accomplished

Table 1 Summary of the crystal structures of ABC-nucleotide binding domains

Name	Organism	Substrate	Resolution	PDB-code	Reference
HisP	*Salmonella typhimurium*	ATP	1.5 Å	1B0U	(Hung et al. 1998)
Rad50cd*	*Pyrococcus furiosus*	ATP Mg*AMP-PNP nucleotide-free	2.6 Å 2.1 Å 1.6 Å	1F2U 1F2T	(Hopfner et al. 2000)
MalK	*Thermococcus litoralis*	Pyrophosphate	1.9 Å	1G29	(Diederichs et al. 2000)
MJ0796	*Methanococcus jannaschii*	Mg*ADP ATP (E171Q)	2.7 Å 1.9 Å	1F3O 1L2T	(Smith et al. 2002; Yuan et al. 2001)
MJ1267	*Methanococcus jannaschii*	Mg*ADP nucleotide-free	1.6 Å 2.5 Å	1G6H 1GAJ	(Karpowich et al. 2001b)
TAP1	Human	Mg*ADP	2.4 Å	1JJ7	(Gaudet and Wiley 2001)
GlcV	*Sulfolobus solfataricus*	Nucleotide-free (A) Nucleotide-free (B) Mg*ADP Mg*AMP-PNP Mg*ATP (G144A)	1.65 Å 2.1 Å 2.1 Å 1.95 Å 1.45 Å	1OXS 1OXT 1OXU 1OXV 1OXX	(Verdon et al. 2003a) (Verdon et al. 2003b)
HlyB	*Escherichia coli*	Nucleotide-free Mg*ATP (H662A)	2.6 Å 2.5Å	1MT0 1XEF	(Schmitt et al. 2003) (Zaitseva et al. 2005a)
MalK	*Escherichia coli*	Nucleotide-free open Nucleotide-free semi-open ATP	2.9 Å 2.8 Å 2.6Å	1Q1E 1Q1B 1Q12	(Chen et al. 2003)
CFTR	Mouse, human	AMP-PNP ATP Nucleotide-free ATP ADP ATP ATP (F508A) ATP (ÄF508)	2.5 Å 2.35 Å 2.2 Å 2.2 Å 2.55 Å 3.0 Å 2.25 Å 2.3 Å	1Q3H 1R0Z 1R0W 1R0X 1R0Y 1R10 1XMI 1XMJ	(Lewis et al. 2004) (Lewis et al. 2005)
CysA	*Alicyclobacillus acidocaldarius*	Nucleotide free	2.0 Å	1Z47	(Scheffel et al. 2005)
MsbA	*Escherichia coli Vibrio cholerae Salmonella typhimurium*	Nucleotide free Nucleotide free ADP*vanadate	4.5 Å 3.8Å 4.2 Å	1JSQ 1PF4 1Z2R	(Chang and Roth 2001) (Chang 2003; Reyes and Chang 2005)
BtuCD	*Escherichia coli*	Cyclovanadate	3.2 Å	1L7V	(Locher et al. 2002)
MalK	*Pyrococcus horikoshii*	Nucleotide free ATP	2.3 Å	1V43 1VCI	(Ose et al. 2004)
MutS*	*Thermus aquaticus Escherichia coli*	Nucleotide free Complex with DNA ADP and DNA	3.2 Å 2.2 Å 2.2 Å	1EWR 1EWQ 1E3M	(Obmolova et al. 2000) (Lamers et al. 2000)
RLI*	*Pyrococcus furiosus*	Mg*ADP	1.9 Å	1YQT	(Karcher et al. 2005)
LmrA	*Lactococcus lactis*	ADP and ATP	3.1 Å	1MV5	Unpublished
transporter	*Thermotoga maritima*	ATP	2.0 Å	1JI0	Unpublished

*Those examples of ABC-proteins which do not belong to the ABC-membrane transport subfamily. A further analysis of these crystal structures would extend the scope of this review

and, in the case of the membrane transporters, how this event might be transmitted to the TMDs. In this review, we shall focus on the available structures of the NBDs of ABC transporters (Table 1), compare them and deduce what we can learn concerning details of the catalytic cycle.

Characteristic features derived from the sequence

As sequence analysis reveals, ABC transporter NBDs are highly conserved, as exemplified by some selected NBDs (Fig. 1). The color codes used in the alignment will be retained throughout this review. Basically, there are three characteristic motifs found in all ABC-ATPases. The Walker A motif (blue) consists of the sequence GXXGXGKS/T where X represents any amino acid (Walker et al. 1982). Together with the Walker B motif (red) (ΦΦΦΦD, where Φ is any hydrophobic residue), this motif forms the nucleotide binding fold of the P-loop ATPase family (Vetter and Wittinghofer 1999). However, unique to ABC-proteins is the C-loop (grey) (LSGGQ), also called the signature motif (Schmitt and Tampé 2002). In addition to these sequences, some characteristic motifs in NBDs contain only one highly conserved residue, the Q-loop (green) and the Pro-loop (orange) are named according to their near invariable glutamine and proline, respectively (Schmitt et al. 2003). These latter are flanked by a stretch of sequence, that consists of α-helices (Schmitt et al. 2003), forming the helical domain (Ames and Lecar 1992). Notably, a few residues after the Walker B, many NBDs carry a SALD motif (black) (D-loop), also quite diagnostic but largely ignored so far. The exact location of these motifs in the three-dimensional structure and what is their function has had to wait for the high resolution structure of nucleotide binding domains that will be described below.

NBD monomer and dimer arrangement

Just before the turn of the millennium it was a common belief that a detailed description of NBDs would greatly promote ABC protein research. Therefore, a major focus was placed upon the structural determination of these

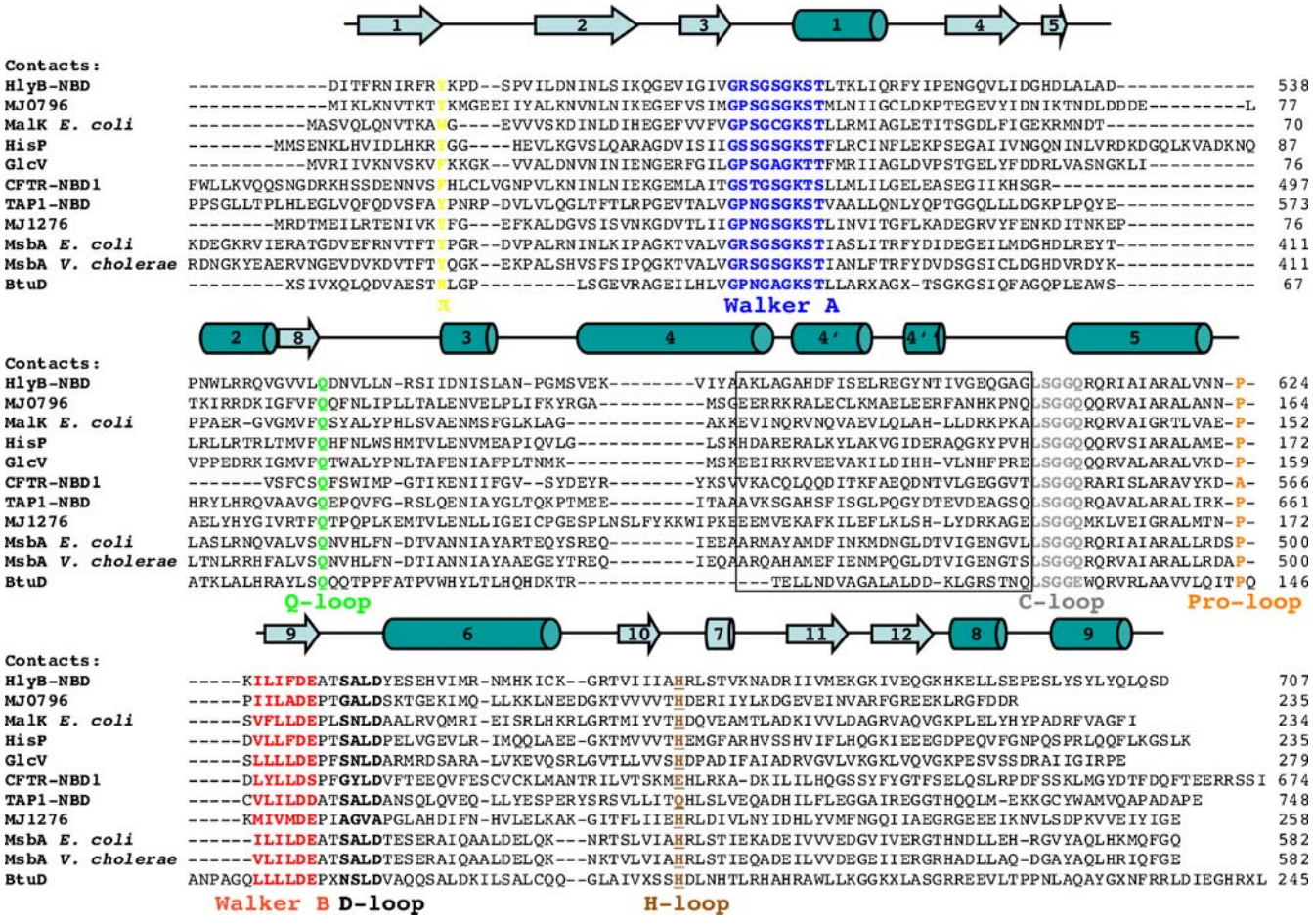

Fig. 1 Sequence alignment of selected ABC transporter NBDs of known structure. Above the sequences the according secondary structure elements of HlyB-NBD are displayed. The Walker A and B motifs are colored in blue and red, respectively. The highly conserved C-loop is displayed in grey and the D-loop following the Walker B is colored in black. The Q-loop Gln is colored in green, the Pro-loop Pro in orange and the H-loop His is depicted in brown. The residue interacting with the adenosine moiety is shown in yellow. The structurally diverse region (SDR) is highlighted by a *black frame*

domains The first NBD 'structure' described, in 1998, was the N-terminal half of the *Escherichia coli* RbsA protein (Armstrong et al. 1998). The ribose transport system (RBS) is a bacterial ABC transporter consisting of the ABC transporter components plus a periplasmic binding protein, which initially binds the allocrite (Barroga et al. 1996). However, this structure of RbsA was never deposited and thus further analysis was not possible.

Conformation of the monomer

In parallel with the analysis of RbsA, the groups of Kim and Ames determined the structure of the ABC-NBD HisP with bound ATP but no Mg^{2+} (Hung et al. 1998). In vivo, in complex with the membrane embedded domains HisQ and HisM, HisP constitutes the periplasmic histidine permease of *Salmonella typhimurium*, $HisQMP_2$. At a resolution of 1.5 Å this hallmark structure not only gave the first overall view of a NBD monomer, but also provided us with a detailed insight into the binding of ATP to the protein. To achieve this, the protein was crystallized in a magnesium free state, thereby avoiding hydrolysis.

The overall fold of the HisP-monomer is depicted in Fig. 2a and shows a stubby L-shaped form which can be divided into arm I and arm II. Arm I (pale blue) consists of β-sheets flanked by α-helices. Arm II (pale cyan) is built up from α-helices, resulting in the name "helical domain". ATP (magenta) is found in the binding pocket, located within arm I. This subdomain is called the catalytic or α/β-domain. Interestingly, this subdomain shows similarities to the structure of RecA (Story and Steitz 1992) and parts of the bovine F_1-ATPase (Abrahams et al. 1994). Therefore, the terms "RecA-like"-domain or "F1-like"-domain are also found in the literature, to describe this part of the ABC-NBD.

Nucleotide binding is accomplished by different molecular contacts, and a more detailed picture of the ATP binding site is given in Fig. 2b. The P-loop or Walker A motif (depicted in blue in Fig. 2a,b) wraps around the phosphate groups of the nucleotide, thus facilitating hydrogen bonding between the main chain nitrogen atoms and the phosphate groups. Additionally, a residue of the Walker A motif also interacts with the γ-phosphate of the substrate. The acidic residues at the end of the Walker B motif (red in Fig. 2a,b) make further contacts with the γ-phosphate, but only via a water molecule. Since the HisP protein was crystallized in a Mg^{2+}-free environment we can only speculate about the position of the cofactor. However, one of the waters mediating the interaction between the Walker B motif and the γ-phosphate is found in a similar position to the magnesium ion in the structure of Ras-GMP-PNP-Mg^{2+} (Pai et al. 1990) and thus suggests a possible position for the cofactor in HisP. Two more water mediated interactions are noteworthy. The Gln of the Q-loop (green in Fig. 2a,b) contacts the γ-phosphate as does the His residue, located in the so-called H-loop (brown in Fig. 2a,b). The importance of these two contacts will be discussed later. Upon

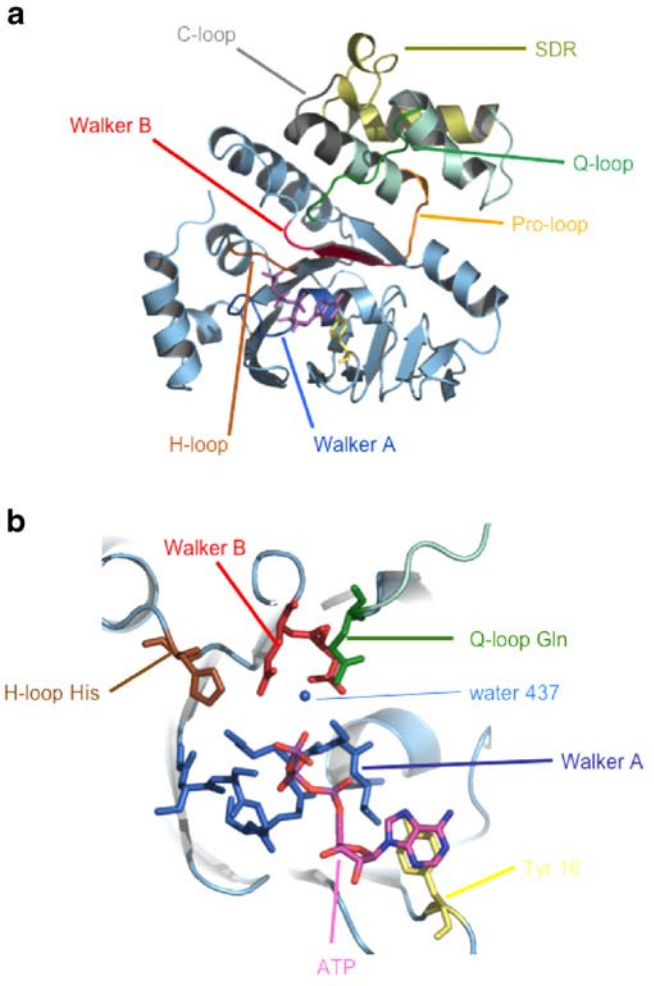

Fig. 2 Structure of the *Salmonella typhimurium* HisP monomer with bound ATP (PDB-code: 1B0U). The catalytic domain is depicted in light blue, the helical domain in pale cyan. The structurally diverse region (SDR) is shown in olive. The same color code as in text and Fig. 1 is used to highlight certain sequences. **a** Represents an overall presentation of the monomer, whereas **b** shows the ATP (magenta) in its binding site

hydrolysis, we envisage that a water molecule attacks the γ-phosphate and that the P-O bond, which connects the γ- with the β-phosphate, is broken. One water molecule (437 blue sphere in Fig. 2b) is situated in a position parallel to the bond in question. This water forms hydrogen bonds to the Q-loop Gln and the Walker B Glu and could possibly be the 'attacking water' for hydrolysis. Furthermore, π-stacking interactions between Tyr16 (yellow in Fig. 2a,b) and the adenine moiety of ATP can be detected in the HisP structure and leads to the idea that this aromatic residue may position the nucleotide in such way that the interactions indicated above are possible. This relatively non-specific π-stacking interaction with the adenine moiety explains the intrinsic promiscuity of ABC-NBDs for other nucleotides.

Subsequent studies of several more transporter NBD structures led to the discovery of other important features. The Pro-loop (Schmitt et al. 2003) (orange in Fig. 2a) and the Q-loop both link the helical domain with the α/β-

domain. We also identified a structurally diverse region (SDR, pale olive in Fig. 2a) of 30-40 residues within the helical domain unique to each NBD (Schmitt et al. 2003). This region is sandwiched between the Q-loop and the signature motif (grey in Fig. 2a) of the NBD. Therefore, we proposed that the SDR controls targeting of the NBDs to their TMDs and possibly signal transmission between the NBD and the cognate TMD. Noteworthy, also, is the finding, unique to HlyB so far, that the nucleotide free form exhibits a 3_{10}-helix, containing the last three residues of the Walker A motif, which normally form the N-terminal section of a regular α-helix (Schmitt et al. 2003).

Investigations of NBD structures of ABC proteins, which are not involved in membrane transport, interestingly confirm general features of the nucleotide binding site. This can be observed in two structures of the DNA mismatch repair enzyme MutS (Lamers et al. 2000; Obmolova et al. 2000) as well as in the structure of the RNase-L inhibitor, a key enzyme in ribosome biogenesis, formation of translation preinitiation complexes and assembly of HIV capsids (Karcher et al. 2005).

Curiously, the structure of the ATP-bound HisP monomer, gave no hint of the function of the motif classifying ATPases as ABC-proteins, the signature motif or C-loop. The highly conserved signature motif is essential for the functional integrity of the ABC domain and we had to wait for the analysis of the dimer conformation of ABC-NBDs until the important function of the C-loop was revealed.

Dimeric arrangement - problems and solutions

Functional ABC transporters require the formation of dimers, either homo- or heteromeric, thus emphasizing the importance of detecting the dimeric arrangement of the NBD in crystal structures.

The structure of HisP was in fact first reported to be a dimer and described as a “back-to-back” formation. Here, the ATP molecules are facing away from each other and occupy a position, which is rather solvent exposed, leaving their ‘backs’ to make contacts. Thus, the HisP monomers are arranged in such way that the only intermolecular contacts are mediated by hydrophobic interactions between the antiparallel β-sheets of arm I of each monomer, explaining the ‘nickname’ of this particular formation (Hung et al. 1998). The size of the dimer is around 60 Å × 40 Å × 90 Å. However, the dimeric assembly presented for HisP leaves a number of questions unanswered. Two energetically unfavorable aspartates are contributing two negative charges to a hydrophobic interface and a compensating charge is not identified. Also, as analyzed subsequently by Yuan et al. (2001), the solvent-accessible surface area (ASA), that is buried in the dimer interface, lies in the range of crystal packing contacts rather than in the range for physiological oligomer formation (Janin 1997). One has to conclude therefore that the HisP dimer is of crystallographic nature, i.e. that the symmetry intrinsic to the crystal lattice accounts for the ‘dimer’ arrangement. The absence of the physiological dimer in the HisP structure left many features of the NBD completely unclear, including the role of the conserved signature motif.

In 2000, additional structures of ABC type ATPases were determined, including structures addressing the key issue of dimer formation. The structures of the catalytic domain of *Pyrococcus furiosus* Rad50 (Rad50cd) in complex with ATP (2.6 Å) and AMP-PNP/Mg^{2+} (2.1 Å), a non-hydrolysable ATP analog, revealed a radically different dimer conformation (Hopfner et al. 2000). Furthermore, a nucleotide free Rad50cd structure (1.6 Å) was determined, providing insights into structural changes upon ATP binding. Rad50cd does not belong to the group of ABC membrane transporters, but is a member of the ABC-protein family, in this case binding DNA and involved in DNA repair mechanisms. Nevertheless, the catalytic domain, formed from the N-terminal and C-terminal Rad50, without the intervening coiled-coil domain, shows strong sequence similarity with NBDs of ABC transporters (Aravind et al. 1999). Thus, the Rad50cd structure is a valid model for ABC-NBD dimer formation and ATP hydrolysis.

The Rad50cd-NBD ‘monomer’ resembles the HisP monomer, showing an L-shaped structure with two arms connected via a central β-sheet. Arm I contains the Walker A motif with the ATP binding site situated near the interface of both arms, thus generating the possibility of transmission of the ATP hydrolysis event from one arm to the other. However, arm II bears relatively little resemblance to the HisP arm II (and as shown later also with respect to arm II from other ABC transporters). Although the α-helical part (arm II) is not as pronounced as it is in HisPit still contains the signature motif. The `dimer´ is arranged in such way that arm I from one `monomer´ contacts arm II of the second `monomer´ and vice versa. The two ATPs are sandwiched between the monomers in such a fashion that the C-loop of one monomer contacts the γ-phosphate of the ATP of the opposing monomer. Thus, residues from one monomer appear to complete the ATP binding site of the second. This arrangement is called “head to tail” formation. Interestingly, this kind of arrangement had already been predicted by Jones and George (1999) the preceding year, based on molecular modeling. The structure of Rad50cd with Mg*AMP-PNP showed no significant difference to the one with ATP, but both showed differences with respect to the nucleotide free state. Thus, upon ATP binding arm II rotates roughly 30° inwards relative to arm I. This leads to a displacement of the signature motif, a crucial prerequisite for dimer formation (Hopfner et al. 2000).

Surprisingly, the structure of the nucleotide binding domain of *Thermococcus litoralis* MalK presented a third variety of dimer arrangement (Diederichs et al. 2000). MalK, together with the membrane embedded domains MalF/MalG and the periplasmic binding protein MalE, constitutes a functional maltose importer in Gram-negative bacteria. The MalK structure exhibits an additional regulatory domain consisting of the C-terminal 149 residues. Although crystallized with ADP, only pyrophosphate was detectable in the binding site. The same bilobal

fold, as found in HisP and Rad50cd, was observed in the MalK structure. However, comparison of the rmsd, of the three structures showed a higher similarity to HisP than to Rad50cd. This raised the question whether Rad50cd could indeed be regarded as a model for NBDs of ABC transporters.

For the elucidation of the MalK dimer in the crystal lattice, the authors analyzed the largest buried solvent-accessible surface area (ASA) and decided to choose the dimer burying the largest interface as the native one. This choice was further supported by the analysis of biochemical data. In this dimer, slight structural rearrangements placed the Q-loop in a position, which matched the biochemical data derived from *S. typhimurium* MalK crosslinking studies (Hunke et al. 2000). The dimer interface of the MalK structure is formed by apolar residues (22), many of them being aromatic, as well as by polar residues (29). However, direct intermolecular hydrogen bonds are missing. This overall arrangement is called the "interlocking stage" and differs from the earlier presented HisP and Rad50cd dimers.

Although, as a result of these studies, three NBD-dimer structures were obtained, no common dimer interface could be proposed that might represent the physiological state. Thus, much hope was placed on the determination of the first full length ABC transporter structure and, in 2001, Chang and Roth (2001) published the structure of the ABC transporter MsbA, the lipid A flippase from *E. coli*. The structure of MsbA exhibited intermembraneous loops, intracellular domain 1 and 2 (ICD1 and ICD2), forming an interface between the transmembrane domains and the NBDs, and, based on genetic analysis, a so far unrecognized intracellular domain 3 (ICD3) (Chang and Roth 2001). Unfortunately, the question of the true NBD dimer could not be answered, since the NBD monomers did not form any contacts at all in this full length structure. In fact, with a resolution of only 4.5 Å, essentially only the protein backbone was visible in the MsbA structure.

Also in 2001, the structure of MJ0796, an NBD of the LolD transporter family from *Methanococcus jannaschii*, was determined in complex with Mg*ADP by Yuan et al. (Yuan et al. 2001). The authors presented a monomer structure of MJ0967 with the usual bilobal configuration. However, an extensive structural analysis was performed to address dimer formation. Special attention was placed on the solvent-accessible surface area (ASA), buried in the dimer interface of HisP, MalK, Rad50cd and the potential dimers of MJ0796 and of MJ1267 (Karpowich et al. 2001a). The analysis revealed that, with the exception of Rad50cd and MalK, all the ASAs were equivalent to those commonly found for crystal packing contacts, rather than for oligomeric complexes (Janin 1997). To elucidate further which of the two dimers, Rad50cd or MalK, might present a physiologically correct conformation, van der Waals (vdW) contacts and hydrogen bonds in the interfaces of these dimers were analyzed. MalK exhibited only 12 H-bonds and 112 vdW contacts, substantially less interactions than Rad50 (20 and 148, respectively). In addition, the monomers in the MalK dimer were not related via a 180° rotation, a finding quite inconsistent with the behavior of a symmetric dimer as found in the Rad50cd structure. Support for the relevance of the Rad50cd structure came from biochemical studies of mutations in the LSGGQ motif which reduced ATPase activity (Schmees et al. 1999). The effect of these mutations can be nicely explained by the fact that this motif interacts with the nucleotide bound to the opposing Walker A motif, as shown in the Rad50cd structure. Other biochemical data are also in agreement with a Rad50cd like dimer (Mourez et al. 1997). Additional evidence for the correctness of the Rad50cd dimer came from experiments performed by Fetch and Davidson (2002). Here, ortho-vanadate, which is trapped at the position of the γ-phosphate after ATP hydrolysis, catalyzes a photocleavage reaction and the cleavage sites were found to lie within the Walker A and the LSGGQ motif. This provided strong evidence that the LSGGQ motif is normally in close apposition to the γ-phosphate in the functional dimer.

The Rad50cd-like dimer was convincingly shown to be physiologically correct when Hunt and coworkers presented their structure of the MJ0796 dimer with ATP bound as substrate (Smith et al. 2002). In order to obtain such a structure, the authors crystallized a mutant of the NBD (E171Q), which shows no detectable ATP hydrolysis of the bound ATP and thus produced a stable dimer. Satisfyingly, the signature motifs complete the ATP binding site of each opposing monomer by capping the substrate. The dimer exhibits a buried solvent-accessible surface area of 1,100 $Å^2$, which generally meets the expectations for physiological dimers. The overall conformation is very similar to the Rad50cd dimer, however, regarding the detailed stereochemistry, there are some differences. These are mainly restricted to the conformation and the binding of the monomer to the ribose and the adenine moiety. Furthermore, due to different sequential spacing of the signature and the Walker A motifs, there is a displacement of the binding cores of the respective second monomer when one monomer of Rad50cd is aligned with the corresponding one of the MJ0796 dimer.

In 2002, Locher et al. (2002) published the second complete ABC transporter structure. BtuCD is a bacterial importer, which together with its periplasmic binding protein BtuF, forms a functional vitamin B12 importer in *E. coli*. Although there is no substrate, but orthovanadate bound in the binding pocket, the NBD (BtuD) dimer interface in this structure in general resembles the one found in Rad50cd. However, the buried solvent-accessible Surface (are 420$Å^2$) of BtuD is again not in the range expected for physiologically relevant oligomers.

In the following years, two more structures of NBD dimers were published. The *E. coli* MalK protein (Chen et al. 2003) proved to be a good system to study dimer formation. As in *Thermococcus litoralis*,the *E. coli* protein contains an additional C-terminal regulatory domain. However, in comparison to the earlier published MalK structure, the *E. coli* MalK dimer arrangement (complexed with ATP) is in agreement with that of the Rad50cd. In contrast to the published structure from *T. litoralis*, the

regulatory domains interact with each other and stabilize a dimer. Interestingly, this form of NBD-dimer was already detected when the possible dimer formation of *T. litoralis* was analyzed, but was neglected in favor of the higher amount of buried ASA, which resulted in the "interlocking stage" choice of an NBD dimer. In the *E. coli* MalK, the regulatory domains form the same contacts regardless of the presence of nucleotide. The opening of the canonical NBD domain, however, is in agreement with the "monomer" formation in the absence of ATP, i.e. no contacts between Walker A and C-loop motifs of the opposing monomers are detectable. Thus, the motion of the whole molecule upon ATP binding is compared to that of tweezers.

The determination of the dimer of the HlyB-NBD in presence of ATP was made possible by mutation of the H-loop histidine (Zaitseva et al. 2005a). This choice followed from studies of the histidine and maltose importers as well as the HlyB-NBD that revealed a near complete loss of ATPase activity when this histidine was mutated (Davidson and Sharma 1997; Nikaido and Ames 1999; Zaitseva et al. 2005b). This deficiency in hydrolysis permits retention of the dimer arrangement, thus making its crystallization in a stable form possible. In addition to the role of the highly conserved H-loop histidine, which will be described further in the catalytic cycle section, an important finding from the Rad50cd-like dimer is that the D-loop residues from one monomer interact with the opposing monomer, for example with the Walker A serine. A detailed picture of ATP binding in the dimer of HlyB is displayed in the schematic presentation in Fig. 3. Here, the adenosine moiety interacts with Tyr and Ile as shown. The triphosphate group forms major interactions with the Walker A and B motifs. The C-loop of the opposing monomer mediates interactions with the other side of the ATP, thus completing the binding site.

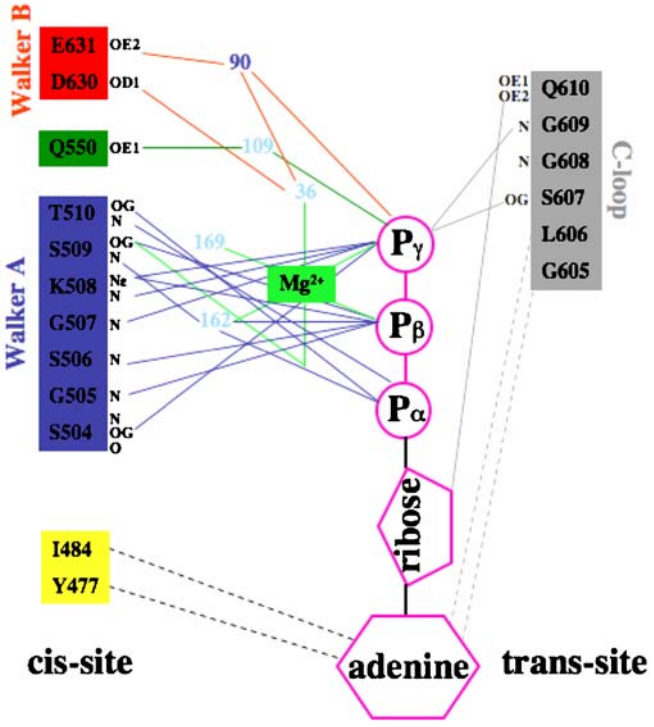

Fig. 3 Schematic representation of ATP binding interactions in the HlyB-NBD dimer. Color coding is the same as in text and Figs. 1 and 2. From the cis-site, the following interactions are evident: Walker A maintains interactions with the phosphate groups of the ATP. The Walker B motif and the Q-loop Gln interact with the γ-phosphate. Residues interacting with the adenosine moiety are Tyr 477 and Ile 484. The C-loop residues of the second monomer (trans-site) make contacts mainly with the γ-phosphate of the nucleotide

Apo, ATP- and ADP bound monomers

With the emergence of NBD structures in various nucleotide bound states, we now have sufficient data in hand to draw comparisons and thus clarify our ideas concerning events happening upon ATP binding and ADP release, from a structural point of view. However, some caution should be taken when analysing the structures with AMP-PNP bound. When Verdon et al. (2003a,b) crystallized GlcV with Mg*AMP-PNP they obtained a monomer which shows high similarities to their Mg*ADP bound form (rmsd< 0.51 Å for 223 Cα atoms). Additionally, Moody et al. (2002) studied the dimerization of the MJ0796 and MJ1267 in the presence of non-hydrolyzable substrates, ATPγS and AMP-PNP, in gel filtration experiments. They concluded that these substrate analogous cannot reflect their natural analogues as dimer formation was either abolished or, in the case of E171Q and E179Q mutations for MJ0796 and MJ1267, respectively, was severely hampered. Rad50cd was determined in complex with ATP and also with the non-hydrolysable analogue, AMP-PNP (Hopfner et al. 2000). The authors stated that the two structures were identical and both showed dimer formation. Unfortunately, the AMP-PNP bound form was not deposited in the protein data bank, so detailed analysis cannot be performed. However, as Rad50cd is not a membrane transporter NBD, it is possible that the AMP-PNP bound form perfectly reflects the ATP bound form.

The intrinsic flexibility of the apo NBD forms

In crystal structure analysis, B-factors are used to determine intrinsic mobility. They represent the crystallographic displacement parameter. Elevated B factors are thus indicative of high flexibility. One striking aspect when looking at the data of the TAP1 monomer NBD structure, are elevated B-factors (Gaudet and Wiley 2001). Interestingly, in the TAP1 structure, where the NBD is complexed with Mg*ADP, two regions of high mobility can be identified. These are the Q-loop and the C-loop, which both form potential interaction sites for the γ-phosphate. This suggests that, upon ATP binding, these regions might be stabilized and possibly also take on another position in the ATP bound state. Furthermore, Karpowich et al. (2001b) extensively analyzed the B-factors in the different nucleotide bound forms of MJ1267. Here the nucleotide-free structure shows higher overall B-factors compared to the

Mg*ADP bound form. Additionally, however, the structure also has especially elevated regions corresponding to the nucleotide binding regions, Walker A, D-loop and H-loop (Karpowich et al. 2001b). This clearly implies that upon nucleotide binding the protein conformation is fixed, especially in the area generating the nucleotide-binding site.

Additionally, when crystallizing GlcV, Verdon et al. (2003a) obtained two different crystal forms of the nucleotide free protein which also show different structural conformations. These alternative structures are due to rotation of the helical domain with respect to the catalytic domain and due to a change in flexibility of the Q-loop. This intrinsic mobility of the nucleotide-free form of NBDs is further reflected in the crystal structures of *E. coli* MalK (Chen et al. 2003). MalK contains an additional regulatory domain that enhances 'dimer' formation (Samanta et al. 2003), thus producing 'dimers' of the apo form of MalK. Here, the authors present two different dimer conformations, accounting for different intermediate forms of the nucleotide-free protein. In the so called open form, the LSGGQ motif and the opposing Walker A are 18 Å apart; this distance decreases to 9 Å in the semi-open form. Since CysA, the NBD domain of the *Alicyclobacillus acidocaldarius* putative sulphate transporter CysTWA, also contains a regulatory domain, one should regard the structures of the protein in this context, in order to make comparisons with the *E. coli* MalK structure (Scheffel et al. 2005). CysA shows two different dimers in the asymmetric unit CysA-1 and CysA-2. Hereby CysA-1 is most likely influenced by crystal contacts (Scheffel et al. 2005).

The induced fit effect and the rotational movement of the helical domain

The analysis of the MJ1267 structures in the nucleotide free and Mg*ADP bound forms, in comparison with the ATP bound form of HisP resulted in the postulation of an induced fit mechanism (Karpowich et al. 2001b). This described the structural changes at the binding site as the consequence of ATP binding. Comparing the apo-with the Mg*ADP bound form the authors recognized large changes in the backbone dihedral angles of the H-loop (211–216). In addition, side chain movements of certain residues are also involved (Arg212). Furthermore, the D-loop undergoes a rigid body movement displacing it 3 Å outward from the binding site in the nucleotide free form compared to the Mg*ADP bound form. As already stated above, these changes are closely linked to a decrease of thermal motion as evidenced by the B-factors. Combining these facts suggests that the molecule undergoes an induced fit and thus becomes fixed upon binding of nucleotide. An additional movement of the Q-loop glutamine, seen in the MJ0796 and MJ1267 Mg*ADP structures, compared with the ATP form of HisP, indicates that this induced fit effect upon ATP binding also involves this region. Here, the Gln swings in towards the binding site, making either direct or water mediated contacts to the γ-phosphate of the nucleotide, resulting in a displacement of the side chain by 5 Å (Yuan et al. 2001). This pulls the whole helical domain towards the binding site, illustrating another important change in the structural arrangement upon nucleotide binding. Mutations in the corresponding glutamine residue in CFTR (Gln493 in NBD1) resulted in reduced channel opening, thus emphasizing the critical role of this residue in the functional activity of this ABC-protein (Berger et al. 2002). This underlines the idea that the γ-phosphate is essential for the stabilization of the NBD. ADP cannot stabilize the NBD as efficiently as ATP, reflecting the greater overall flexibility of the NBD in the presence of ADP. From the structure of the dimer of MJ0796 (E171Q), the side chains of the mutated residue and of the H-loop histidine seem to perform an induced fit, resulting in contacts with the hydrolytic water and the γ-phosphate, respectively. The Q-loop glutamine is also displaced and contacts the Na^{+}-ion, which substitutes the cofactor Mg^{2+}. Finally, in the E171Q dimer, the helical domain undergoes the predicted rigid body rotation of 17° (Smith et al. 2002).

Other available structures now confirm the findings derived from the MJ structures. *E. coli* MalK was crystallized in two different apo-forms as well as in the ATP bound form. In addition to the interdomain rotation of the NBD relative to the regulatory domain, MalK also exhibits a second kind of rotation within the canonical NBD, namely a rotation of the helical domain relative to the catalytic domain. In this case, compared to the ATP bound form, the helical domain rotates 5° and 25° in the semi-open and open form, respectively (Chen et al. 2003). As indicated above, Rad50cd shows an outward rotation of 30° in the nucleotide free form compared to the nucleotide bound form. This is coupled to the displacement of the Q-loop glutamine from the position where it can interact with the γ-phosphate of the ligand. However, the crystal structures of GlcV, the NBD of the glucose importer of the hyperthermoacidophilic *Sulfolobus solfataricus* (Verdon et al. 2003a), in various states lack most of these features. When comparing the nucleotide free state with the Mg*ADP bound state one sees that the Q-loop glutamine is flipped inwards to make a water mediated interaction with the Mg^{2+}-ion, whereas in the MJ structures with Mg*ADP the equivalent glutamine is flipped away from the binding site. The GlcV AMP-PNP bound form exhibits a direct interaction with the Mg^{2+}-ion in one molecule of the asymmetric unit, and water mediated contacts, as in the ADP bound form, in the other two molecules of the asymmetric unit. Furthermore, the H-loop does not contact the γ-phosphate of the ATP, and the overall structure resembles much more the ADP bound form of the MJ monomers rather than the dimer of the ATP bound EQ MJ0796. These findings can be explained by the limited capacity of AMP-PNP to mimic the natural substrate ATP. Indeed, a significant displacement of the Q-loop residues in the nucleotide bound forms can be detected in comparison with the apo forms of the HlyB-NBD, as expected from the MJ model. Thus, comparison of the apo form of HlyB NBD with the ATP bound structure, indicates the following

changes (Zaitseva et al. 2005a). Upon ATP binding, the helical domain rotates about 18° towards the catalytic domain and the signature motif is displaced inwards to contact the γ-phosphate of the nucleotide. The stereo representation in Fig. 4 illustrates the changes in the HlyB-NBD upon ATP binding. The helical domain of the apo monomer (catalytic domain shown in red, helical domain in orange) rotates towards the substrate binding site as shown in the ATP-bound NBD (catalytic domain coloured blue, helical domain green, and bound ATP in white). After hydrolysis, the rotation of the helical domain is almost totally reversed (–17°), resulting in an ADP bound conformation similar to the apo form. However, the interactions of the Walker A and the Q-loop with ADP remain the same as those found with ATP. Interestingly, the C-terminal residues of the Walker A motif, that form a 3_{10}-helix in the apo form, adopt a canonical α-helix in the ATP bound form. This leads to the idea that this molecular switch may operate in some way to control ATP binding in vivo (Schmitt et al. 2003).

In opposition to these complementary findings between HlyB and the MJ structures stands the structure of the mouse NBD1 of CFTR. Although having analyzed all variations of the monomeric form, complexed with different nucleotides or nucleotide free, phosphorylated or not, the authors did not observe any relevant structural differences (rmsd 0.2–0.3 Å) (Lewis et al. 2004) apparently ruling out direct intramolecular movement upon nucleotide binding in this case.

The catalytic cycle

Due to the high conservation of the NBDs of ABC transporters it is very likely that the mechanism of hydrolysis is similar in all ABC transporters. However, this high conservation is not exhibited by the membrane spanning domains, thus probably reflecting a different allocrite translocation pathway for different ABC transporters. In this section, we concentrate on possible mechanisms for ATP hydrolysis with minimal consideration of the coupling of allocrite translocation to ATP hydrolysis.

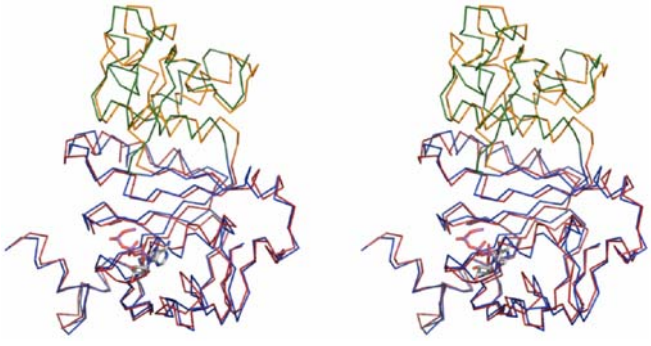

Fig. 4 Stereoview of the nucleotide free HlyB-NBD (catalytic domain in red, helical domain in orange) and the ATP-bound HlyB-NBD (catalytic domain in blue, helical domain in green, and bound ATP in white) structure. ATP binding results in an induced fit and a rotational movement of the helical domain towards the substrate-binding site

Purely biochemical data or structural data alone are clearly insufficient to permit the drawing of definitive conclusions concerning enzyme mechanism. However, when the data from the two approaches are combined, we have a powerful tool to address the question of how ATP is hydrolyzed, and further, how this process can be linked to the translocation of the allocrite.

Structural aspects

Regarding the structural data derived by X-ray analysis of ABC transporter NBDs, the following conclusions can be drawn.

1. Different oligomeric states are encountered during the ATP hydrolysis cycle. Without any nucleotide the NBD rests in a monomeric form. ATP binding induces alterations of the surface organization of the monomer, thus building a platform for dimerization. After hydrolysis, the dimer falls apart and with the release of ADP the monomer finally switches back into its resting state. A rather simple mechanistic cycle for ATP hydrolysis based on these findings is depicted in Fig. 5.
2. The NDB undergoes intrinsic changes during catalysis. The binding of ATP to the monomer results in an induced fit effect (Karpowich et al. 2001a). The intrinsic flexibility of the protein is greatly reduced and the helical domain is rotated towards the catalytic domain. After ATP hydrolysis and phosphate release, the NBD regains flexibility and the rotation is reversed. When ADP is released the molecule relaxes back to the open state.
3. Certain NBDs, containing a regulatory domain, proceed through different stages of dimer formation, the open and the semi-open forms, thus probably reflecting different pre-hydrolysis states.
4. Even though the NBD is structurally highly conserved, the structurally diverse region (SDR) could present one possibility of how ATP binding and/or hydrolysis can be differentially signaled to the TMD.

Processive clamp mechanism vs. alternating catalytic site mechanism

Structural data alone cannot explain the actual mechanism of ATP hydrolysis. To understand this, many biochemical studies were carried out based on trapping experiments. In this method, ATP is hydrolyzed and the resulting ADP is captured in situ, via interaction with beryllium-, aluminium-fluoride or vanadate, that mimic different stages in hydrolysis, the pre hydrolysis state (BeF_x) and the transition state (AlF_x and VO_4) (Smith and Rayment 1996). The radioactively labeled nucleotides are either

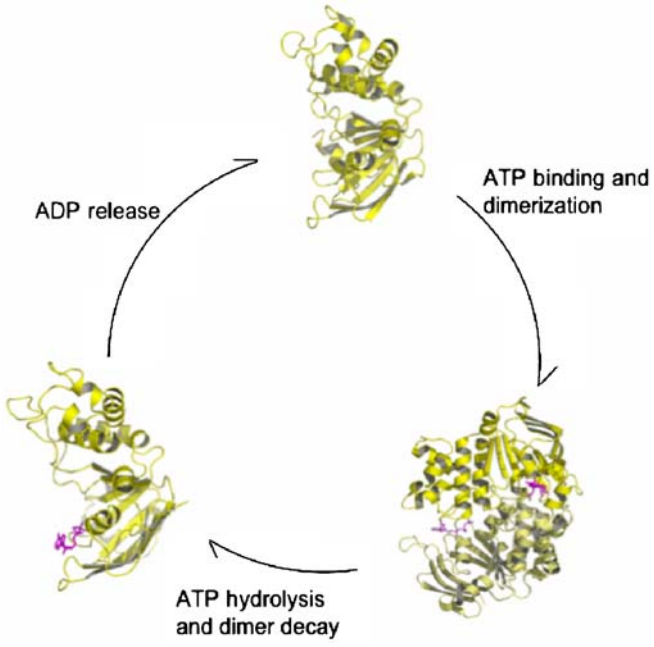

Fig. 5 Catalytic cycle of the nucleotide binding domains (NBD) of HlyB. In the nucleotide free form, the NBD exhibits an open, monomeric conformation. Upon ATP binding, the surface properties are altered in such way that dimerization is allowed. After the hydrolysis event, the dimer falls apart and ADP can leave the monomer. The NBD relaxes back to the open conformation

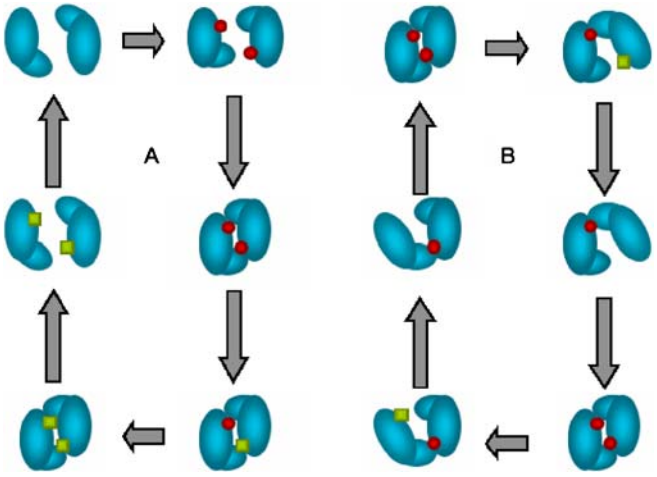

Fig. 6 Mechanistic models of dimer formation coupled to ATP hydrolysis. ATP and ADP are depicted in red cycles and green squares, respectively. The nucleotide binding domain (NBD) monomer (blue) is subdivided into two domains (catalytic and helical domain). **a** Processive clamp model: after binding of ATP to the open NBD monomer a dimer can form. Then ATP is hydrolysed sequentially in the dimer. After the hydrolysis of both nucleotides the dimer falls apart and the ADPs dissociate from the monomers. **b** Alternating catalytic site model: in an ATP bound dimer only one nucleotide is hydrolyzed before the respective monomer opens up. ADP is exchanged for ATP and a dimer is reformed. After hydrolysis and nucleotide exchange in one of the monomers, the ATP in the second monomer can be hydrolysed. With the exchange of the hydrolyzed ADP for ATP the cycle is completed

[α-^{32}P]ATP or [γ-^{32}P]ATP. After trapping, the nucleotide to protein ratio is detected by different means. Supported by the trapping experiments, different models of ATP hydrolysis have been proposed.

For example, in the processive clamp mechanism (Fig. 6a) the two ATPs in the dimer are hydrolyzed sequentially, the dimer dissociates and ADP molecules are exchanged for ATP molecules (Janas et al. 2003; Van der Does and Tampe 2004). In contrast to this stands the alternating catalytic site model (Fig. 6b) (Senior et al. 1995). Here, one nucleotide is hydrolyzed in one monomer of the dimer and this is followed by the 'opening' of this monomer and ADP release. The empty monomer is then ready to be reloaded with another ATP molecule. To this point, the second monomer of the dimer with bound ATP remains in the 'closed' state. However, after reloading monomer 1 the hydrolysis of the hitherto untouched ATP is in some way triggered.

The processive clamp mechanism (Fig. 6a) is based on two observations. First, the relevant crystal structures of dimers apparently show symmetric binding site occupation (Chen et al. 2003; Smith et al. 2002; Zaitseva et al. 2005a). Second, experiments with the Mdl1p-NBD always indicated two nucleotides bound to the dimer. These were either two ATP molecules or one ATP plus one ADP, suggesting that one phosphate can leave the dimer without disrupting it (Janas et al. 2003). The state with two ADPs bound to the dimer could only be isolated if the system was stabilized by BeF_x-trapping.

The alternating catalytic site (Fig. 6b) model for the ATP hydrolysis by P-glycoprotein was already proposed in 1995 by Senior and co-workers (Senior et al. 1995). This was based on studies in which only one nucleotide was detected per dimer, after trapping with vanadate. Even though only one site was trapped, further ATPase activity was fully inhibited (Urbatsch et al. 1995). Trapping experiments with Pgp mutants, in which the Ser in the signature motif, as well as the Glu at the end of Walker B, were substituted, confirmed these findings (Tombline et al. 2004a,c). Vanadate trapping experiments with the maltose transport complex also revealed that only one ADP was bound per fully assembled complex (Sharma and Davidson 2000).

General base versus substrate assisted catalysis

So far, we have discussed the analysis of ATP hydrolysis only with respect to the coupling of ATP hydrolysis to dimer formation and disassembly. The details of the ATP hydrolysis mechanism have actually not been discussed. However, recently, some interesting findings have contributed enormously to this field. First, Moody et al. (2002) proposed that the Glu residue at the end of the Walker B sequence is the general or catalytic base of the NBD. This idea is based on the lack of measured ATPase activity when the Glu in question is mutated to Gln in MJ0796 and MJ1267 (Moody et al. 2002). Similar results were obtained for BmrA (Orelle et al. 2003). However, these findings stand in opposition to data available from other ABC transporters. Mutations of this Glu residue in mouse Pgp-NBDs (E552/E1197) still allow drug stimulated vanadate

trapping, suggesting that at least a single turnover is occurring (Urbatsch et al. 2000). Mutating only one of the Glu residues in the two NBDs actually results in the retention of measurable ATPase activity (Tombline et al. 2004b). Moreover, for similar mutations in human Pgp, efficient vanadate trapping could also be obtained (Sauna et al. 2002). In contrast to these findings concerning the Glu residue, mutation of the H-loop His resulted in a complete loss of ATPase activity and, in some cases, of transport (Davidson and Sharma 1997; Nikaido and Ames 1999; Shyamala et al. 1991; Zaitseva et al. 2005b). The analysis of the function of this His led us to investigate further the hydrolysis event in the HlyB NBD (Zaitseva et al. 2005a). First, ATPase activity experiments in media of different viscosity, i.e. different sucrose concentration, were performed. We argued that a diffusion controlled reaction would decrease in rate with increasing viscosity (Martin and Hergenrother 1999). Since these results showed that ATPase activity was unaltered at different viscosities, the rate limiting step must be a chemical reaction. In this respect, two different reactions are possible, a general base catalysis (GBC) (Fersht 1997) or a substrate assisted catalysis (SAC) (Dall'acqua and Carter 2000). In a general base catalysis the rate limiting step is the abstraction of the proton mediated by the catalytic base. In contrast, in a SAC proton abstraction is not rate limiting. To distinguish between these two possible mechanisms, the isotope effect was utilized (Schowen and Schowen 1982). Here, we observed that ATPase activity measurements in D_2O did not demonstrate any dependency on D_2O, implying that proton abstraction cannot be the rate-limiting step. Other evidence for a SAC mechanism was provided by the analysis of ATPase activity as a function of pH. If Glu is the catalytic base, a mutation to Gln, a poorer base, should change the pH dependence of the ATPase activity. This proved not to be the case, as the E/Q mutation and the wild type showed the same profile of pH dependence, thus further arguing for a SAC rather than a GBC. Finally, ATPase activity was measured after changing the cofactor Mg^{2+} to other bivalent ions, representing different strong Lewis acids. Upon binding of a strong Lewis acid to the ATP molecule, electrons are withdrawn from the γ-phosphate towards the bivalent ion. In a GBC mechanism this would not influence the ATPase activity, as nucleophilic attack is not the rate-limiting step. Thus, alterations in activity should not occur. However, in our experiments, changes in the cofactor did affect the ATPase activity, providing more proof for a SAC mechanism. In conclusion, we proposed that instead of a general base mechanism, rather substrate assisted catalysis, in which the H-loop His interacts with the γ-phosphate of the ATP, underlies ATP hydrolysis by ABC-ATPases. Due to the interactions of the histidine, the transition state of hydrolysis is stabilized, thus explaining the loss of ATPase activity in a mutant lacking this His residue. Furthermore, the His residue interacts via the D-loop with the opposing ATP binding site, thus acting as a "linchpin" for the hydrolysis cycle (Zaitseva et al. 2005a).

Coupling of ATPase activity to changes in the transmembrane domain

Having analyzed changes in NBD arrangements when ATP is hydrolyzed, as well as the precise chemical mechanism of ATP hydrolysis, another important question is still left unanswered. How is ATP hydrolysis coupled to the translocation of the allocrite? One generally accepted idea is that allocrite transport through the membrane initially involves a high affinity binding site, accounting for allocrite binding, and finally a low affinity binding site, allowing the substrate to leave the transmembrane domain. However, how this process is facilitated at the molecular level is still one of the many mysteries of ABC transporters. A recent review by Davidson and Chen (Davidson and Chen 2004) gives an excellent insight into this field.

In the literature, different models of the coupling ATPase activity to transport can be encountered. The alternating two-site model or "two-cylinder engine", derived from studies on LmrA (Van Veen et al. 2000), exhibits two allocrite binding sites, one on each TMD. While one binding site faces inwards, the high affinity site (for an exporter), the other, the low affinity site, faces outwards. The exchange of allocrite between the two binding sites of one TMD half is coupled to the hydrolysis of ATP. Thus, an alternating ATP hydrolysis cycle in the NBDs results in one TMD exhibiting the high affinity and the other exhibiting the low affinity binding site. On the other hand, the alternating catalytic site model presented by Senior et al. (1995) involves only one allocrite binding site, constituted from the two TMDs. In this model, the hydrolysis cycle in the NBD drives the interchange from high affinity to low affinity binding site (Urbatsch et al. 2003). A third model suggests two coexisting allocrite binding sites, a low and a high affinity site (Sauna and Ambudkar 2000). In this mechanism, the allocrite is physically transferred from the high affinity to the low affinity binding site, coupled to binding and hydrolysis of one ATP molecule. The energy derived from the second ATP molecule is used to restore the initial state of the system.

The ATP-switch model proposed by Higgins and Linton (2004) gives a more precise insight into the communication between NBD and TMD. The model is composed of four major steps. First, the allocrite binds to the TMD and initiates the transport cycle. As the binding interaction in the TMD is signaled to the NBD the affinity of the NBD for ATP is increased. The second step is the power stroke, which is not facilitated by ATP hydrolysis, but by the binding of ATP and subsequent dimer formation. Low affinity for the allocrite during this step leads to the suggestion that the transport substrate is released. In a third step, hydrolysis of ATP follows, finally resulting in dimer decay (step 4). Then the release of ADP and P_i resets the transporter to its initial state.

At this stage of knowledge, it is extremely challenging to judge the presented models that couple ATP hydrolysis to allocrite transport. One major question is also whether translocation of the allocrite is accomplished by a single

mechanism for all ABC transporters, i.e. importers and exporters, or if the process of transmembrane translocation is specific to different allocrites. Further biochemical data on different ABC transporters is surely a prerequisite to address this question appropriately. Combining detailed biochemical data of allocrite transport with the corresponding structures of the ABC transporter would certainly be beneficial for learning how ABC transporters accomplish their tasks.

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