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Review

ATP-binding cassette transporters in *Escherichia coli*

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ATP-binding cassette (ABC) transporters are integral membrane proteins that actively transport molecules across cell membranes. In *Escherichia coli* they consist primarily of import systems that involve in addition to the ABC transporter itself a substrate binding protein and outer membrane receptors or porins, and a number of transporters with varied functions. Recent crystal structures of a number of ATPase domains, substrate binding proteins, and full-length transporters have given new insight in the molecular basis of transport. Bioinformatics approaches allow an approximate identification of all ABC transporters in *E. coli* and their relation to other known transporters. Computational approaches involving modeling and simulation are beginning to yield insight into the dynamics of the transporters. We summarize the function of the known ABC transporters in *E. coli* and mechanistic insights from structural and computational studies.

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1. Introduction

ATP-binding cassette (ABC) transporters are integral membrane proteins that actively transport molecules across the lipid membrane against a concentration gradient, using the energy derived from the hydrolysis of ATP to ADP. This ubiquitous class of transporters is present in virtually all living organisms and accounts for large variety of biological processes. It should be noted that the ABC domain can also be found in proteins that may couple ATP hydrolysis to functions other than transport, for instance in DNA repair [1,2]. However, while such proteins can contribute to our understanding of catalytic processes, the present review will be focused only on the ABC transporter family.

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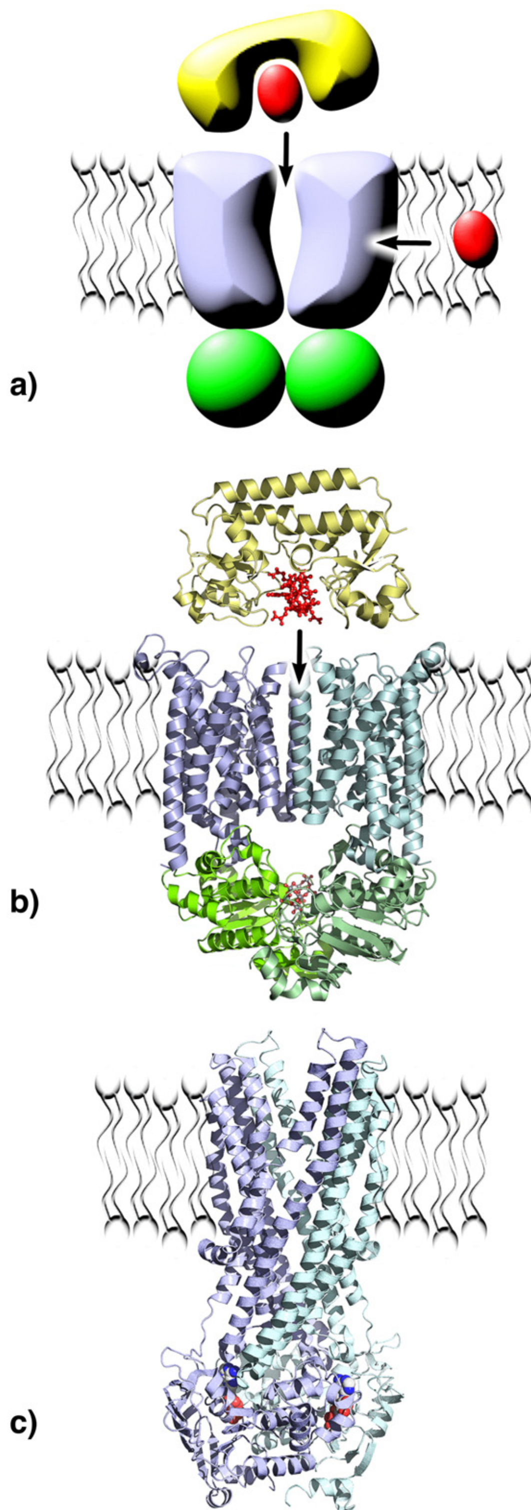

Fig. 1. General ABC transporter architecture (a) comprises two transmembrane domains (TMD, blue) and two nucleotide binding domains (NBD, green). Some transporters receive their substrate (red) from the bilayer, some from the aqueous phase. In case of the latter specialized substrate binding proteins (yellow) can deliver the substrate to the transporter. This is typically found in Gram-negative bacteria. BtuCD (b) consists of four single polypeptide chains and receives its vitamin B12 substrate via the substrate binding protein BtuF. Sav1866 (c) comprises two polypeptide chains and binds its substrate from the bilayer. BtuCD and Sav1866 represent two types of TMD organization with the polypeptide chain crossing the bilayer ten (b) or six times (c). (b) has been adapted from [67].

Specialized ABC transporter types transport a diverse range of substrates, ranging from small molecules such as ions, sugars or amino acids to larger compounds such as antibiotics, drugs, lipids and oligopeptides. ABC transporters take part in the uptake of nutrients or secretion of toxins in bacteria, as well as confer multidrug resistance in cancer or bacterial cells by pumping diverse anti-cancer drugs and antibiotics into the extracellular spaces. The ABC transporters are also medically relevant as some of their mutations have been implicated in genetic disorders such as cystic fibrosis.

These proteins constitute a very ancient family of transporters, believed to date back in evolutionary time more than 3 billion years [3]. Phylogenetic evidence supports the idea that the ABC transporter family diversified before bacteria, archea and eukaryotes diverged on separate evolutionary paths [4], thus many human ABC transporters have bacterial homologues in organisms such as *Escherichia coli* (*E. coli*).

E. coli is a Gram-negative bacillus native to the intestinal flora of many animals, including humans [5]. It is a facultative anaerobe which survives when released to the natural environment and can often be found in soil or as a contaminant in untreated water. The diversity of the natural reservoir and the vectorial transfer of plasmid genes means that *E. coli* as a species is not well delineated, encompassing a wide variety of biotypes or strains. In addition to chromosomal and plasmid genes encoding antibiotic resistance, biochemical adaptations or other virulence factors, individual strains also express various antigenic lipoproteins or glycolipoproteins anchored in the peptidoglycan cell wall. As these antigenic surface proteins vary from strain to strain, differentiation of *E. coli* species is based on antigen expression [6]. The K-12 strain, a non-virulent strain lacking the O and K antigens [5], is the most widely used laboratory strain and is often referred to as the standard *E. coli* culture.

In 1997, the complete genome of *E. coli* K-12 serotype was sequenced [7]. The largest single family of proteins in the *E. coli* K-12 genome is the ABC transporter family, comprising 5% of the total genome together with all transport related components [1]. In a recent book, the classification of ABC transporters in families in Gram-negative bacteria was reviewed [8]. Here we focus on ABC transporters from *E. coli*, their roles, and the available structural data from both experiment and computation. It is expected that the information collected about *E. coli* ABC transporters would provide a good base for study and comparison of structural and functional aspects in the homologous proteins from other organisms.

2. Structure of ABC transporters

2.1. General structure of ABC transporters

As illustrated in Fig. 1a all ABC transporters share a common basic structure regardless of their function as importer or exporter or the substances transported. ABC transporters are composed of two transmembrane domains (TMDs) which are the integral membrane proteins and two nucleotide binding domains (NBDs), water soluble proteins associated with the TMDs on one side of the membrane (Fig. 1a).

The TMD parts of the transporter form the transport channel and consist of several membrane-spanning alpha-helices presenting considerable structural variability among ABC transporters. The number of transmembrane helices also varies between 8–20 for importers and 12 for exporters [9].

In contrast, the NBD parts are highly conserved among the ABC protein family presenting the characteristic Walker A and B motifs found in all ATP-binding proteins as well as a signature motif which is specific to the ABC transporter family. The NBDs are the engines of an ABC transporter as they bind and hydrolyse ATP, powering transport. ATP binding induces conformational changes in NBDs, forcing them into closer contact and forming the characteristic nucleotide sandwich

Table 1
Functional *E. coli* ABC transporters, number of components, biological function and analogous role in other bacteria

Transporter	Components	Substrate	Biological function	Reference
Prokaryotic-like transporters				
Als	AlsB (BP), AlsC (TM), AlsA (NB)	Allose, ribose	Monosaccharide importer	[84]
Ara	AraF (BP), AraH (TM), AraG (NB)	L-arabinose, fructose, xylose	Monosaccharide porter	[85]
Arg	ArgT (BP), HisQ (TMD), HisM (TMD), HisP (NBD)	L-lysine, L-arginine, L-ornithine	Polar amino acid transporter	[86]
ArtIMQP	ArtI (BP), ArtM (TMD), ArtQ (TMD), ArtP (NB)	L-arginine	Polar amino acid transporter	[86,87]
ArtJMQP	ArtJ (BP), ArtM (TMD), ArtQ (TMD), ArtP (NB)	L-arginine	Polar amino acid transporter	[86,87]
Btu	BtuF (BP), BtuC (TMD), BtuD (NB)	Vitamin B12	Vit B12 uptake system	[18]
Cys	CysP (BP), CysU (TMD), CysW (TMD), CysA (NBD)	Thiosulfate	Sulfate/thiosulfate importer	[88]
Ddp	DdpA (BP), DdpF (TMD) DdpD (TMD)	D,d-dipeptide	Dipeptide transporter	[89]
Dpp	DppA (BP), DppB (TMD), DppC (TMD), DppD (NB), DppF (NB)	Dipeptide, 5-aminolevulinic acid (ALA)	Dipeptide transporter	[90]
Fec	FecB (BP), FecC (TMD), FecD (TMD), FecE (NBD)	Ferric citrate	Iron porter	[91]
Fep	FepB (BP), FepD (TMD), FepG (TMD), FepC (NBD)	Ferrientero-bactin	Iron porter	[92]
Fhu	FhuD (BP), FhuB (TMD), FhuC (NBD)	Ferric hydroxamate/ferrichrome	Hydroxamate-dependent iron transport	[93]
Fli/Yec	FliY (BP), YecS (TMD), YecC (NBD)	Unknown	Putative cysteine/diaminopimelic acid transporter	[94]
Gln	GlnH (BP), GlnP (TMD), GlnQ (NBD)	Glutamine	Polar amino acid porter	[95]
Glt	GltI (BP), GltK (TMD), GltJ (TMD), GltL (NBD)	Glutamate/aspartate	Polar amino acid importer	[96]
Gsi {Yli}	GsiB {yliB} (BP), GsiC {yliC} (TMD), GsiD {yliD} (TMD), GsiA {yliA} (NBD)	Glutathione	Oligopeptide transporter	[97]
His	HisJ (BP), HisM (TMD), HisQ (TMD), HisP (NBD)	L-histidine, also arginine, lysine, ornithine	Polar amino acid transporter	[98]
LivFGHJM	LivJ (BP), LivH (TMD), LivM (TMD), LivG (NBD), LivF (NBD)	L-leucine (LivJ), L-isoleucine (LivJ), L-valine (LivJ)	Hydrophobic amino acids and amide importer	[99]
LivFGHKM	LivK (BP), LivH (TMD), LivM (TMD), LivG (NBD), LivF (NBD)	L-leucine (LivK)	Hydrophobic amino acids and amide importer	[99]
Lpt	LptA {yhbN} (BP), ?YrbK (TMD), LptB {yhbG} (NBD)	Lipo-polysaccharide	lipopolysaccharide (LPS) porter	[100]
Lsr	LsrB (BP), LsrC (TMD), LsrD (TMD), Ego (NBD)	AI-2 quorum-sensing signaling molecule	Monosaccharide porter	[101]
Mal	MalE (BP), MalF (TMD), MalG (TMD), MalK (NBD)	Maltose (malto-oligosaccharides prediced)	Disaccharide importer	[102]
Met	MetQ {MetD/YaeC} (BP), MetI {MetD/YaeE} (TMD), MetN {abc} (NBD)	D-methionine	Methionine transporter	[103]
Mgl	MglB (BP), MglC (TMD), MglA (NBD)	β-D-galactose	Monosaccharide porter	[104]
Mod	ModA (BP), ModB {ChlJ} (TMD), ModC {ChlD} (NBD), ModF {ChlD} (NBD)	Molybdate, tungsten	Molybdate transporter	[105,106]
Mpp {Opp}	MppA (BP), OppB (TMD), OppC (TMD), OppD (NBD), OppF (NBD)	Murein tripeptide	Oligopeptide transporter	[107]
Nik {hydC}	NikA (BP), NikB (TMD), NikC (TMD), NikD (NBD), NikE (NBD)	Nickel	Nickel porter	[108]
Opp	OppA (BP), OppB (TMD), OppC (TMD), OppD (NBD), OppF (NBD)	Oligopeptides	Oligopeptide porter	[109]
Phn	PhnD (BP), PhnE (TMD), PhnC (NBD)	Phosphonate, phosphites	Phosphonate importer	[110]
PotABCD	PotD (BP), PotC (TMD), PotB (TMD), PotA (NBD)	Spermidine	Spermidine importer	[111]
PotFGHI	PotF (BP), PotH (TMD), PotI (TMD), PotG (NBD)	Putrescine	Putrescine importer	[112]
Pro	ProX (BP), ProW (TMD), ProV (NBD)	Glycine, betaine, L-proline	Glycine/betaine/proline importer	[113]
Pst	PstS {nmpA} (BP), PstC {phoW} (TMD), PstB {phoT} (NBD)	Phosphate	High-affinity phosphate transport	[114]
Rbs	RbsB (BP), RbsC (TMD), RbsA (NBD)	D-ribose	Monosaccharide importer	[115]
Sap	SapA (BP), SapB (TMD), SapC (TMD), SapD (NBD), SapF (NBD)	Cationic peptide	Probable oligopeptide transporter	[116]
Spb	Sbp (BP), CysU (TMD), CysW (TMD), CysA (NBD)	Sulfate, thiosulfate	Sulfate/thiosulfate porter. TMD/NBD components from the Cys system.	[88]
Ssu	SsuA (BP), SsuC (TMD), SsuB (NBD)	Sulfonate	Aliphatic sulfonate transporter	[117]
Tau	TauA {ssiA} (BP), tauC {tssiC} (TMD), tauB {ssiB} (NBD)	Taurine	Taurine porter	[119]
Tbp Thi	TbpA {thiB} (BP), ThiP {sfuB} (TMD), ThiQ {sfuC} (NBD)	Thiamine thiamin pyrophosphate	Thaimine importer	[120]
Ugp	UgpB (BP), UgpA (TMD), UgpE (TMD), UgpC (NBD)	*sn*-glycerol 3-phosphate	Glycerol-phosphate transport protein	[121]
Xyl	XylF (BP), XylH (TMD), XylG (NBD)	D-xylose	Monosaccharide transporter	[122]
Ycj	YcjN (BP), YcjO (TMD), YcjP (TMD), YcjU (NBD)	Unknown	Putative sugar transporter	[94]
Ydc	YdcS (BP), YdcV (TMD), YdcU (TMD) YdcT (NBD)	Unknown	Putative spermidine/putrescine transporter	[94]
Yeh	YehZ (BP), YehW (YMD), YehY (TMD), YehX (NBD)	Unknown	Putative glycine/betaine/choline transporter	[123]
Ytf/Yjf	YftQ (BP), YtfT (TMD), YjfF (TMD), YtfR (NBD)	Unknown	Putative sugar transporter	[94]

(continued on next page)

Table 1 *(continued)*

Transporter	Components	Substrate	Biological function	Reference
Prokaryotic-like transporters				
Yhd	YhdW (BP), YhdX (TMD), YhdY (TMD), YhdZ (NBD)	Unknown	Putative polar amino acid transporter	[94]
Ynj	YnjB (BP), YnjC (TMD), YnjD (NBD)	Unknown	Putative thiamine transporter	[7]
Yph	YphF (BP), YphD (TMD), YphE (NBD)	Unknown	Putative sugar transporter	[94]
Yrb	YrbD (BP), YrbE (TMD), YrbF (NBD)	Unknown	Putative transporter	[7]
Znu	ZnuA (BP), ZnuC (TMD), ZnuB (NBD)	Zn^{2+}	High-affinity zinc uptake	[124]
Eukaryotic-type transporters				
Ccm {Yej}	CcmC {YejT} (TMD), CcmB {YejW} (TMD), CcmA {YejV} (NBD)	Heme	Putative Heme exporter. ccmC may act separately to ccmAB	[125]
CydBD	CydBC homodimer	Unknown	Periplasmic c-type cytochrome exporter	[126]
Fts	FtsX (TMD), FtsE (NBD)	Unknown	Putative ABC transporter involved in cell division	[127]
Lol	LolC (TMD), LolE (TMD), lolD (NBD)	Lipoproteins	Lipoprotein translocator	[128]
MacAB	MacAB {ybjYZ} homodimer	14- and 15-membered lactones	Macrolide exporter	[129]
MdlAB (ABCC family)	mldAB homodimer	Peptides of 6–21 amino acyl residues	Mitochondrial peptide exporter	[129]
MsbA (ABCB family)	msbA homodimer	Phospholipid, LPS, lipid A, vinblastine, Hoechst 33342	Lipid flippase	[130,131]
Yad	YadH (TMD), YadG (NBD)	Predicted: polyketide drugs, teichoic acid	Putative antibiotic exporter	[94]
Ybb	YbbP (TMD), YbbA (NBD)	Unknown	Putative metal exporter	[94]
Ybh	YbhR (TMD), YbhS (TMD), YbhF (NBD)	Unknown	Putative ABC transporter, unknown function	[94]
YddA	YddA homodimer	Unknown	Putative fatty acid exporter	[129]
YhhJ	YhhJ homodimer	Unknown	Putative drug exporter	[94]
YojI	YojI homodimer	Microcin J25	Drug exporter	[132]

Collated from: http://www.genome.ad.jp/kegg/pathway/eco/eco02010.html; http://www.tcdb.org/tcdb/index.php?tc=3.A.1; http://www.york.ac.uk/res/thomas/searchABC.cfm; http://ecogene.org/index.php.
Non-functional ABC transporters in *E. coli* are not listed.
Putative transporters are components listed in order from substrate BP to NBD's.
Alternative gene name is listed in brackets.

dimer [10]. These changes are transmitted to TMDs, causing a conformational change which opens a conduit between the TMDs to either the inside or outside of the cell.

The exact mechanism of the coupling between NBDs and TMDs is largely unknown. Due to the presence of high variability in the nature of TMDs it was suggested that different TMDs were likely to develop different mechanisms for coupling between both types of domain [11]. Since there is little direct evidence on how NBDs functionally interact with TMDs, two opposing mechanisms for transport have been proposed. One [12] suggests that in the ATP free state when NBDs are open, the TMDs are closed to the outside and open to the cytoplasm. Once the ATP is bound and NBD dimer is formed TMDs change their conformation closing to the inside of the cell and opening to the periplasmic side. After ATP hydrolysis occurs, the structure returns to its initial state. An alternative mechanism [13] proposes opposite motions where TMDs are open to the extracellular space in an ATP free state and closed in an ATP-bound state. Current structural information based on crystallographic studies supports the first mechanism of coupling between TMDs and NBDs.

2.2. Bacterial ABC transporters

All bacteria express two major classes of ABC transporters, prokaryotic-type (PK-type) and eukaryotic-type (EK-type) ABC transporters. In contrast eukaryotes only have genes for EK-type ABC transporters. There are major functional and gene organization differences between the PK- and EK-type ABC transporters [14].

The PK-type ABC transporters are importers which require additional extracellular proteins, called substrate binding proteins (SBPs) or specifically for Gram-negative bacteria periplasmic binding proteins (PBPs), to recruit substrates from the extracellular space and deliver them to the transporter. The presence of SBPs determines the direction of transport. A schematic representation of such importers is shown in Fig. 1a. An example is the *E. coli* vitamin B12 transporter (BtuCD) in Fig. 1b [13]. In contrast, EK-type ABC transporters are exporters, moving substances either from the cytoplasm and out of the cell or from the cytoplasm into the organelles (in particular, the endoplasmic reticulum, mitochondria and peroxisome) [15]. The EK-type ABC transporters expressed in bacteria function as exporters, including bacterial multidrug transporters that confer drug resistance to the cell. Some EK-type transporters, like the human protein P-gp or the bacterial Sav1866 (Fig. 1c), may receive their substrate from the lipid bilayer (Fig. 1a), while others sequester substrates from the aqueous phase.

Some bacterial exporters show a reasonably high degree of sequence identity and structural homology to mammalian transporters, including the mammalian P-gp multidrug transporter. Examples of these bacterial transporters are the lipid flippase MsbA (from *E. coli*), the multidrug exporter LmrA (from *Lactococcus lactis*) [16] and Sav1866 (from *Staphylococcus aureus*) [17].

Typically each component in PK-type ABC transporters is coded as a separate protein, arising from an individual gene in a cluster of genes coding for the complete transporter. Thus, TMDs and NBDs together comprise four separate domains as in BtuCD [13] (Fig. 1b). The two TMD and two NBD domains may or may not be identical in such transporters. In some PK-type ABC transporters the TMD or NBD genes can become fused, so that three separate proteins comprise a full transporter. In these cases the transporter can contain one TMD domain and two identical NBDs, as in the Fhu importer [18], or two identical TMDs and one NBD domain as in the Rbs importer [18].

In EK-type ABC transporters, evolutionary modifications have led to gene fusion between the separate TMD and NBD component genes to produce contiguous TMD-NBD half-transporter proteins that homo- or hetero-dimerize to form full ABC transporters [14] as in Sav1866 [17] (Fig. 1c). It is also possible that all four core-domains may

Table 2
ABC transporter crystal structures

Transporter	PDB identifier	Resolution	Native organism	Wild type or mutant	Nucleotide ligand	Region crystallized	Reference
BtuCD	1L7V	3.2	*Escherichia coli*	WT	CVT	Full transporter	[13]
HI1470/1	2NQ2	2.4	*Haemophilus influenzae*	WT	apo	Full transporter	[25]
ModBCA	2ONK	3.1	*Archaeoglobus fulgidus*	WT	TNG	Full transporter	[24]
Sav1866	2HYD	3	*Staphylococcus aureus*	WT	ADP	Full transporter	[17]
Sav1866	2ONJ	3.4	*Staphylococcus aureus*	WT	ANP	Full transporter	[23]
BtuCDF	2QI9	2.6	*Escherichia coli*	WT	apo	Full transporter	[46]
HlyB	1XEF	2.5	*Escherichia coli*	MT	ATP	Dimer	[28]
$MalFGK_2E$	2R6G	2.8	*Escherichia coli*	MT	ATP	Full transporter	[65]
MsbA	3B5W	5.3	*Escherichia coli*	WT	Apo	Full transporter	[57]
MsbA	3B5X	5.5	*Vibrio cholerae*	WT	Apo	Full transporter	[57]
MsbA	3B5Y	4.5	*Salmonella typhimurium*	WT	AMPPNP	Full transporter	[57]
MsbA	3B5Z	4.2	*Salmonella typhimurium*	WT	ADP	Full transporter	[57]
MsbA	3B60	3.7	*Salmonella typhimurium*	WT	AMPPNP	Full transporter	[57]
HlyB	2FGJ	2.6	*Escherichia coli*	MT	ATP	Dimer	[31]
HlyB	2FGK	2.7	*Escherichia coli*	MT	ATP	Dimer	[31]
MalK	1Q12	2.6	*Escherichia coli*	WT	ATP	Dimer	[12]
MalK	1Q1B	2.8	*Escherichia coli*	WT	apo	Dimer	[12]
MalK	1Q1E	2.9	*Escherichia coli*	WT	apo	Dimer	[12]
MalK	2AWN	2.3	*Escherichia coli*	WT	ADP	Dimer	[48]
MalK	2AWO	2.8	*Escherichia coli*	WT	ADP	Dimer	[48]
MalK	1G29	1.9	*Thermococcus litoralis*	WT	apo	Dimer[a]	[26]
MJ0796	1L2T	1.9	*Methanococcus jannaschii*	WT	ADP	Dimer	[30]
TAP1	2IXE	2	*Rattus norvegicus*	MT	ATP	Dimer	[29]
TAP1	2IXF	2	*Rattus norvegicus*	MT	ATP	Dimer	[29]
SufC	2D3W	2.5	*Escherichia coli*	WT	apo	Dimer	[49]
SufC	2D2E	1.7	*Thermus thermophilus* HB8	WT	apo	Monomer	[133]
SufC	2D2F	1.9	*Thermus thermophilus* HB8	WT	ADP	Monomer	[133]
????	1JI0	2	*Thermotoga maritima*	WT	ATP	Monomer	[134]
????	1SGW	1.7	*Pyrococcus furiosus*	WT	apo	Monomer	[135]
????	2GHI	2.2	*Plasmodium yoelii*	WT	apo	Monomer	[41]
CFTR	1Q3H	2.5	*Mus musculus*	WT	ANP	Monomer	[35]
CFTR	1R0W	2.2	*Mus musculus*	WT	apo	Monomer	[35]
CFTR	1R0X	2.2	*Mus musculus*	WT	ATP	Monomer	[35]
CFTR	1R0Y	2.55	*Mus musculus*	WT	ADP	Monomer	[35]
CFTR	1R0Z	2.35	*Mus musculus*	WT	ATP	Monomer	[35]
CFTR	1R10	3	*Mus musculus*	WT	ATP	Monomer	[35]
CFTR	1XF9	2.7	*Mus musculus*	MT	ATP	Monomer	[40]
CFTR	1XFA	3.1	*Mus musculus*	MT	ATP	Monomer	[40]
CFTR	1XMI	2.25	*Homo sapiens*	MT	ATP	Monomer	[36]
CFTR	1XMJ	2.3	*Homo sapiens*	MT	ATP	Monomer	[36]
CFTR	2BBO	2.5	*Homo sapiens*	MT	ATP	Monomer	[36]
CFTR	2BBS	2	*Homo sapiens*	MT	ATP	Monomer	[36]
CFTR	2BBT	2.3	*Homo sapiens*	MT	ATP	Monomer	[36]
CysA	1Z47	1.9	*Alicyclob. acidocaldarius*	WT	apo	Monomer	[39]
GlcV	1OXS	1.65	*Sulfolobus solfataricus*	WT	apo	Monomer	[42]
GlcV	1OXT	2.1	*Sulfolobus solfataricus*	WT	apo	Monomer	[42]
GlcV	1OXU	2.1	*Sulfolobus solfataricus*	WT	ADP	Monomer	[42]
GlcV	1OXV	1.95	*Sulfolobus solfataricus*	WT	ANP	Monomer	[42]
GlcV	1OXX	1.45	*Sulfolobus solfataricus*	MT	apo	Monomer	[43]
HisP	1B0U	1.5	*Salmonella typhimurium*	WT	ATP	Monomer	[136]
HlyB	1MT0	2.6	*Escherichia coli*	WT	apo	Monomer	[47]
HlyB	2FF7	1.6	*Escherichia coli*	WT	ADP	Monomer	[31]
HlyB	2FFA	1.7	*Escherichia coli*	MT	ADP	Monomer	[31]
HlyB	2FFB	1.9	*Escherichia coli*	MT	ADP	Monomer	[31]
LmrA	1MV5	3.1	*Lactococcus lactis*	WT	ADP ATP	Monomer	[137]
MalK	1V43	2.2	*Pyrococcus horikoshii*	WT	Apo	Monomer	[37]
MalK	1VCI	2.9	*Pyrococcus horikoshii*	WT	ATP	Monomer	[37]
MRP1	2CBZ	1.5	*Homo sapiens*	WT	ATP	Monomer	[38]
MJ0796	1F3O	2.7	*Methanococcus jannaschii*	WT	ADP	Monomer	[44]
MJ1267	1G6H	1.6	*Escherichia coli*	WT	ADP	Monomer	[34]
MJ1267	1G9X	2.6	*Methanococcus jannaschii*	WT	ADP	Monomer	[45]
MJ1267	1GAJ	2.5	*Methanococcus jannaschii*	WT	apo	Monomer	[34]
TAP1	1JJ7	2.4	*Homo sapiens*	WT	ADP	Monomer	[32]
TAP1	2IXG	2.7	*Rattus norvegicus*	MT	ATP	Monomer	[29]
TM0544	1VPL	2.1	*Thermotoga maritima* MSB8	WT	apo	Monomer	[138]

The ABC transporter crystal structures available in the protein data bank at the time of writing.
Abbreviations used: WT, wild type; MT, mutant; CVT, cyclo-tetrametavanadate; TNG, tungstate (VI) ion; ADP, adenosine diphosphate; ATP, adenosine triphosphate; ANP, phosphoaminophosphonic acid-adenlyate ester.

[a] The 1G29 MalK dimer structure has the NBDs in a different orientation than all the other MalK structures and is most likely an artifact.

be fused together, forming one continuous polypeptide chain, as in the human multidrug transporter P-glycoprotein [18].

In bacterial ABC transport systems the differences are not limited to the PK or EK-type of transporter. The presence of some structural components and their interaction with the ABC transporter are influenced by the nature of the bacterial cell wall and vary between Gram-negative and Gram-positive bacteria. In PK-type ABC transporters of Gram-negative bacteria such as *E. coli*, high concentrations of

both substrate-bound and substrate-free SBPs are found in the periplasmic space as free proteins, in ratios far exceeding the concentration of the specific ABC importer to which they bind. It has been proposed that the excess SBPs help sequester any available substrate, undergoing lateral transfer of substrate to a nearby substrate-free SPB until the substrate is delivered to a substrate-free SBP bound to its specific ABC importer or the substrate-bound SPC binds to a free importer [19] (Fig. 1b). In Gram-positive bacteria, the lack of an outer membrane leaves SBPs exposed on the cell surface. In this case the SBPs can either become attached to the cytoplasmic membrane near the transporter via a lipid or protein anchor, or the SBP gene can be fused to that of the TMD, being expressed as a separate domain at the C- or N-terminus of the TMDs [16,18,19].

In case of bacterial exporters Gram-negative bacteria need to ensure interaction of the ABC transporter with an outer membrane protein to facilitate the transport across the periplasm. An example of this is the hemolysin export system in *E. coli*. The hemolysin transporter HlyB interacts with the outer membrane protein TolC, which crosses both the outer membrane and the periplasmic space forming a channel for hemolysin export [16].

3. ABC transporters in *E. coli*

Despite the sequencing of the *E. coli* K-12 genome [7] and characterization of many of the ABC transporter genes encoded, there is still some conflict over the precise number of functional ABC transporters in the *E. coli* K-12 serotype. The Pasteur Institute ABC system database [20] has identified genes for 71 discrete ABC transporter systems in *E. coli* K-12, while others report 69 [1], 80 [7], 52 [21] and 72 (4 of which are non-functional) [22] ABC transporters. Two other serotypes whose genomes have been sequenced, *E. coli* CFT073 and *E. coli* O157:H7 are believed to have 83 and 82 ABC transporter gene systems, respectively [20].

In contrast, 48 ABC transporter gene systems have been identified in humans, while *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Arabidopsis thaliana* possess 28, 56 and 113 ABC transporter gene systems, respectively [20]. With the exception of plants, eukaryotes generally have fewer ABC system genes than prokaryotes. Not surprisingly, the general rule of thumb appears to be the more substances an organism needs to import or export, the greater the number of ABC transporter systems in its genome. Therefore, in eukaryotes, motile organisms such as animals require fewer ABC transporters than non-motile organisms like plants, which rely heavily on the presence of an active transport system for nutrients and metabolites as they diffuse into the local environment.

To aid in the development of a comprehensive list of *E. coli* K-12 ABC transporters, Table 1 presents a collation of the current information, listing both the experimentally verified and putative (based on gene sequence) ABC transporters expressed as functional proteins in the K-12 serotype. Of the 65 ABC transporters listed, 50 are PK-type importers which facilitate the import of substrates into the cell and are characterized by the presence of an extracellular binding protein. The remaining 15 are EK-type exporters.

Many of these transporters can have multiple functions, coded by alternate genes for a particular transporter component which may be expressed or repressed, depending on the environmental conditions. For example, the TMD and NBD components of the prokaryotic-type Arg and His importer systems are identical, consisting of a single copy each of the transcribed HisM and HisQ protein for the TMDs and two copies of the transcribed HisP as the NBD. The functional difference between the two transporter systems arises from the presence of an arginine-specific (ArgT) or histidine-specific (HisJ) BP which delivers the substrate to the transporter complex. This “modular” expression reduces the necessity for entire gene duplication and allows for greater genetic variation in the *E. coli* ABC transporter genome.

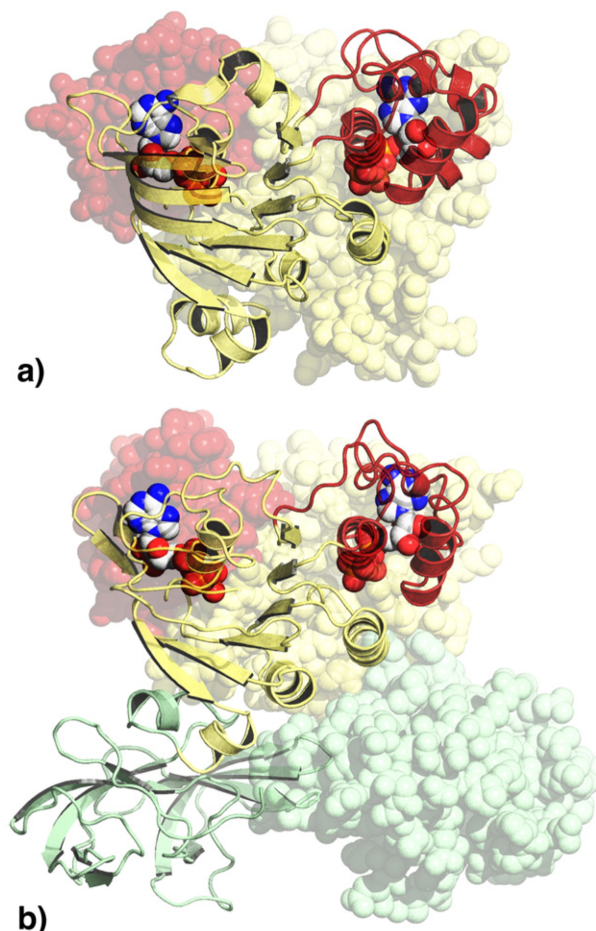

Fig. 2. General NBD architecture comprises up to three sections: a helical domain (red), a catalytical domain (yellow) and a C-terminal regulatory domain (green). While the majority of NBD structures were found to be two-sectioned like HlyB (a) only the GlcV, CysA and MalK structures (b) contain an additional regulatory domain.

4. Structural data on *E. coli* ABC transporters

As of the end of 2007, there were 91 crystal structures of ABC transporters and their components from over 45 distinct ABC transporter systems. They come from a variety of different bacteria, although all were expressed in *E. coli* prior to crystallization. Thirty-five structures are of SBPs; the remaining 56 crystal structures are of NBDs and full transporters from 21 different ABC transporter systems. The latter 56 include five structures of four different full transporters [13,17,23–25] and 51 structures of nucleotide binding domains (Table 2). Of the 51 NBD structures, 13 were resolved as dimers [12,26–31] and 38 as monomers [29,31–45].

From *E. coli* stem four different ABC transporters. For these a total of 16 X-ray structures have been determined so far: two full-length transporter structures of the vitamin B12 importer BtuCD [13,46], eight NBD structures of HlyB [27,28,31,47], five NBD structures of MalK [12,48] and one NBD structure of SufC [49]. HlyB is involved in type I protein secretion [50], MalK is the motor domain of the maltose/maltodextrin importer system MalGFK2-E and SufC is involved in the assembly of iron–sulfur clusters. More recently several additional crystal structures have been determined; clearly the numbers above are moving targets and are given just to give an idea of the state of structural studies of ABC transporters. We discuss additional full complex structures below in more detail.

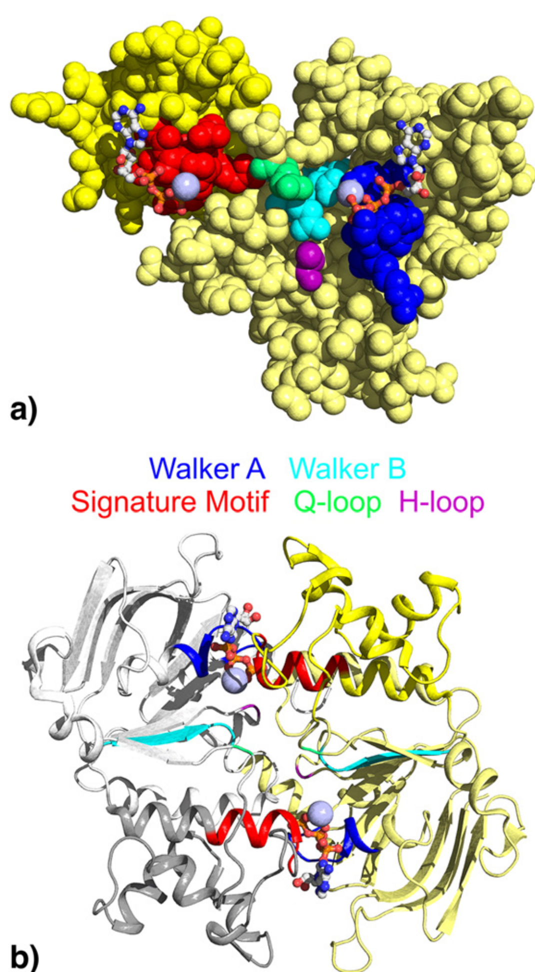

Fig. 3. ABC transporter NBDs contain highly conserved structure and sequence motifs. While Walker A and B are found in almost all ATP-binding proteins, Signature motif, glutamine (Q) and histidine (H) loops are unique to ABC transporters. The structures shown are *E. coli* HlyB in monomeric (a) and dimeric configurations (b). Two Mg-ATP are sandwiched in-between the two NBDs. Helical domains appear in a darker shade of yellow or gray.

4.1. Structure of nucleotide binding domains

Based on the available crystal structures, general NBD architecture comprises up to three sections: a helical domain, a catalytical domain and a regulatory domain (Fig. 2). While the first two are found in all the available NBD structures, only the crystal structures of CysA [39], GlcV [42,43] and MalK [12,26–28,31,37,48] contain an additional regulatory domain. In Fig. 2 the structural organization of a two and three-sectioned NBD dimer is exemplified by the ATP-bound structures of *E. coli* HlyB (Fig. 2a) and MalK (Fig. 2b).

All ABC transporter NBDs contain highly conserved sequence and structural motifs. While the Walker A (GXXGXGKS/T with X representing any residue) and Walker B (ΦΦΦΦD with Φ representing any hydrophobic residue) motifs are found in nearly all ATP-binding proteins [51–53], the signature motif LSGGQ as well as the histidine (H) and glutamine (Q) loop are unique to ABC transporters [10]. Structural organization and interaction of these motifs in *E. coli* HlyB are illustrated in Fig. 3 depicting a close-up view of the binding site of a single monomer (Fig. 3a) and the HlyB dimer [28] with two Mg-ATP sandwiched in-between the two NBDs (Fig. 3b).

ATP binding, hydrolysis and ADP release induce conformational changes in the NBDs whose mechanical energy is transferred to the TMDs to drive transport. The landmark study of MalK dimers in the apo, ATP-bound and ADP-bound states showed that ATP binding was associated with an inwards pincering of the NBDs relative to MalK's regulatory domain [12] and the formation of a nucleotide sandwich dimer. On ATP hydrolysis to ADP, there is an outward movement of the NBDs, producing a slight dissociation of domains and opening of the nucleotide catalytic site which is exaggerated in the nucleotide-free state [12]. The crystal structures of the apo and ATP-bound MalK dimer show that conformational changes can be large [12,48] varying from 14 to 18 Å in distance between signature motif and Walker A (Fig. 4). By analogy, these conformations may also illustrate the general conformational changes arising in other ABC transporter NBDs during the nucleotide catalytic cycle, a theory supported by the highly conserved structural architecture and sequence identity across all NBDs crystallized to date. However, the magnitude of these changes could be attributed to the NBDs studied in isolation from their TMDs. The presence of a regulatory domain might also play a role here to stabilize apo dimer structures. Nevertheless, so far MalK remains the only example of a tripartite NBD dimer resolved in different states of nucleotide binding.

A more subtle conformational change upon ATP binding has become known as the “rotation of the helical domain” and was first observed in 2001 when the Mg-ADP bound structure of *Methanococcus jannaschii* MJ1267 [34] was compared to ATP-bound HisP from *Salmonella typhimurium* [33]. Upon superposition of these highly homologous structures a small closing-like motion of helical and catalytical domain became evident (Fig. 5a) which was later also reported for other NBDs. Interestingly, when elastic network normal mode analysis [54,55] is performed on the same MJ1267 monomer, the first lowest frequency mode describes the same opening closing motion of the two domains (Fig. 5b) but the second lowest frequency mode is a converse twisting rotation of helical and catalytical domains (Fig. 5c) (Kandt et al., unpublished). These dominant motions are also found for *E. coli* NBDs of known structure. However, the details of how a rotation or kinking motion of the helical domain contributes to

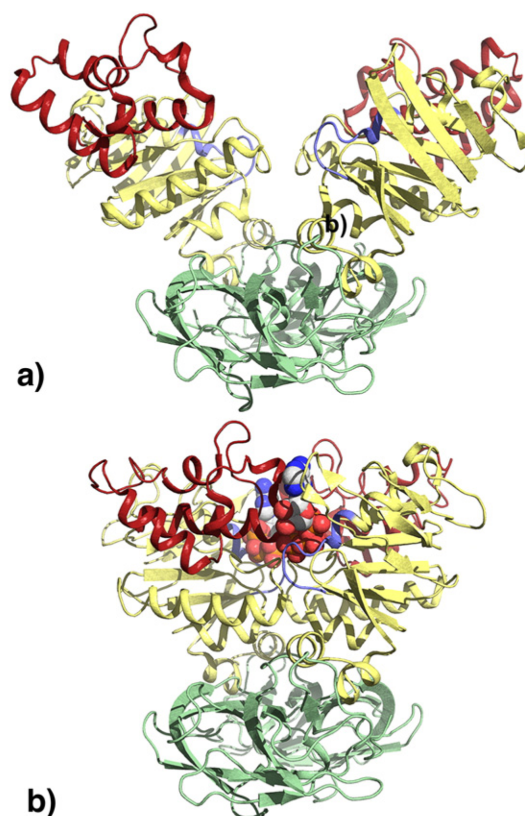

Fig. 4. ATP binding induces a large conformational change in MalK.

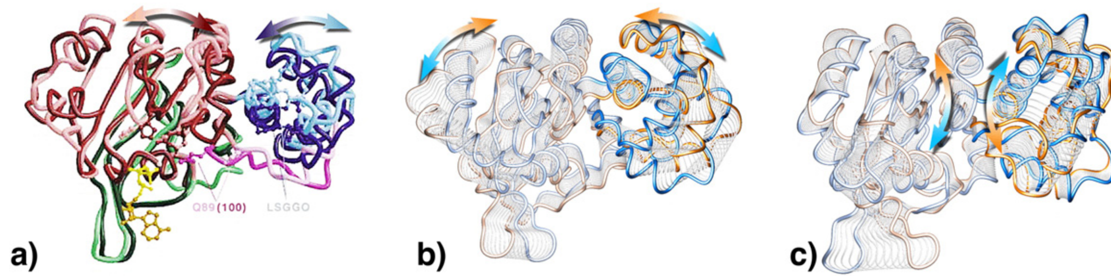

Fig. 5. The so-called rotation of the helical domain upon ATP binding was first observed in 2001 when the Mg-ADP bound structure of *E. coli* MJ1267 (bright) was superimposed on ATP-bound HisP (dark) from *Salmonella typhimurium* (a). The small opening and closing of helical and catalytical domains are motion inherent to the NBD structure as illustrated by the first lowest frequency normal mode of MJ1267 (b). Interestingly, the second lowest frequency normal mode describes a converse twisting-like motion of the two domains (c). Elastic network normal mode analysis of other NBD structures yields the same results. (a) has been reproduced from [34].

signaling and power transmission between NBDs and TMDs are still not understood.

The multiple crystal structures for HlyB and its various mutants in the presence of ATP or ADP have allowed a detailed analysis of the changes occurring throughout the catalytic cycle in the isolated HlyB NBDs. They also provided an opportunity to study key functional residues, hinge regions and novel structures within the NBDs that may act as a means of storing energy [31]. The nucleotide-free structure of the monomeric SufC also showed small but significant deviations from the generalized ABC NBD structure. In SufC, the formation of a salt bridge between Glu171 and Lys152 rotates the D-loop onto the NBD surface. Although dimerization is thought to be possible in the presence of two molecules of ATP, this rotated Q-loop acts to prevent NBD dimerization, possibly regulating ATP hydrolysis [49]. SufC also contains an atypical Q-loop structure that may help form a binding site for SufB or SufD in the assembly of the functional Suf ABC transporter [49].

4.2. Structures of full ABC transporters

The lipid flippase MsbA was the first to be described in the literature, but the first three papers were retracted for reinterpretation of the data [56]. The MsbA structures have recently been published again [57]. The Cα-trace structure still resembles the original structure, but there now is a subunit interconnection provided by two helices of subunit A reaching over to subunit B and vice versa, similar to the structure of Sav1866.

BtuCD was the first ABC transporter whose full-length X-ray structure could be determined [13]. The protein was captured in the presence of two orthovanadate molecules sandwiched in-between the NBDs, most likely resembling an intermediate between apo and ATP-bound forms [24,25]. For the Btu (vitamin *B* *t*welve *u*ptake) system structures of all its key components are known: ABC transporter BtuCD, substrate binding protein BtuF [58,59] and outer membrane protein BtuB [60,61].

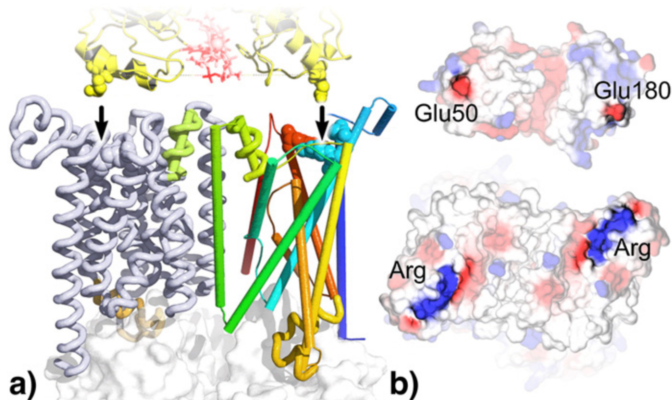

Fig. 6. In BtuCD TMDs the polypeptide chain crosses the membrane ten times (a). For TMD-NBD communication the L-loops (yellow tubes) are important whereas for the docking of the substrate binding protein BtuF (yellow) conserved surface BtuF glutamates 50 and 180 and BtuC arginines 56, 59 and 295 have been suggested as crucial interface residues (b).

Each of the BtuC TMDs spans the membrane ten times with both N and C-termini located on the cytoplasmic side of the bilayer (Fig. 6a). The TMD-NBD interface is characterized by the L-loops, in BtuC a 16 residue long α-helix-coil-α-helix stretch of each TMD polypeptide chain connecting the transmembrane helices 6 and 7. On the periplasmic side each TMD holds a cluster of three conserved arginine residues which have been suggested to interact with a pair of equally conserved glutamates in the substrate binding protein (Fig. 2b) upon formation of the BtuCD-F docking complex [13,58]. The structure of the complete BtuCD(F) transporter system has served as an exceedingly useful prototype ABC transporter system for several molecular dynamics studies of the conformational changes that may occur during substrate translocation [62,63]. It will be discussed in a later section about computational findings. A further landmark was the publication of the structure of the BtuCD-F complex in absence of substrate and nucleotide [46]. BtuF was found in a more open conformation than in its isolated structure, confirming the predictions made in [64]. While the other full-length transporter structures are clearly open to one side of the membrane, apo BtuCD-F is closed towards both periplasm and cytoplasm. Transmembrane helix 5 is found in an tilted orientation resembling the one observed in HI1470/1 [25] which displayed an opposite accessibility compared to BtuCD. The apo BtuCD-F crystal structure is currently interpreted as representing a post-translocation state [46].

Last year's nucleotide-free crystal structure of the putative metal-chelate-type ABC transporter HI1470/1 [25] revealed a remarkable similarity to BtuCD (Fig. 7a). Besides the larger NBDs in HI1470/71 the two transporters mainly differ in their accessibility from the aqueous phase: while HI1470/1 is open towards the cytoplasm and closed towards the periplasm (Fig. 7b), the opposite is found for BtuCD (Fig. 7c). As detailed in Fig. 7d the main contributor to this converse accessibility is a different orientation of transmembrane helix 5 and the immediately adjacent non-TM helix 172–180 in BtuC and 175–185 in HI1470/1.

More subtle differences between the two ABC transporters become evident when the two structures are superimposed. There is a rotational twist of ~10° about an axis close to the molecular two-fold axis between the TMD subunits of both transporters [25]. Also in the superimposed NBD subunits there is a translational difference of ~4.5° along an axis parallel to the TMD-NBD interface. Such translation suggests a screw-like motion of NBD from HI 1470/71 relative to NBDs from BtuCD. This motion arises as the two rotational axes of NBDs and TMDs are coupled to each other suggesting

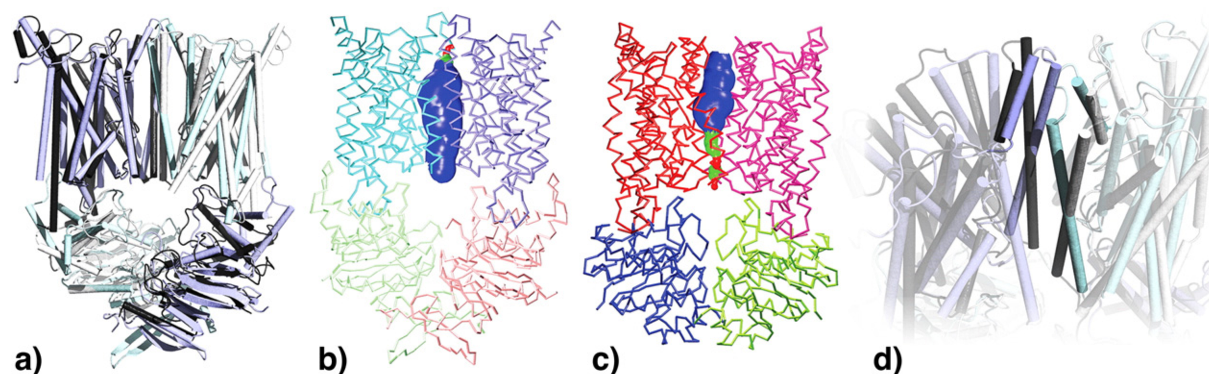

Fig. 7. HI1470/1 (blue and cyan) and BtuCD (black and white) have a highly similar structure (a). The main difference between the two transporters is an opposite accessibility to the water phase. While HI1470/1 is open towards the cytoplasm and closed towards the periplasm (b) the opposite is found in BtuCD (c). The main contributor to this converse accessibility is a different orientation of transmembrane helix 5 and the immediately adjacent non-TM helix 172–180 in BtuC and 175–185 in HI1470/1 (d).

possible mechanism for TMD and NBD subunits coupling where a twist in TMDs position is induced by the change in the position of NBDs.

Recently two new crystallographic structures of full ABC transporters trapped with their PBPs have been published, namely BtuCDF [46] and MalFGK-E [65], with both transporters originating from *E. coli*. These new structures allow us to make a comparison between the three currently known structures of ABC transporters complete with their PBPs: BtuCDF, MalFGK-E and ModABC. All three transporters are importers which differ from each other in the total number of TMHs, with 20 for BtuCDF, 14 for MalFGK-E, and 12 for ModABC. However, the maltose transporter is unique as its TMDs are not symmetrical where one subunit contains 8 TMHs (MalF) and the other subunit contains 6 TMHs (MalG). Each of the three structures has been captured at a different position along the respective translocation pathway representing a different stage of the transport process.

The ModABC and BtuCDF structures show nucleotide-free open conformations. In contrast MalFGK-E contains a mutation that abolishes ATP hydrolysis and increases the affinity of the NBDs for each other. The resulting structure shows the NBDs structure as closed dimer. Despite the similarities in the conformation of NBDs between BtuCDF and ModABC there are significant differences in the conformation of TMDs of both transporters: in the case of ModABC the TMDs are in an inward-facing conformation that is closed to the extracellular space [24] while in the case of BtuCDF TMDs are asymmetrical providing access to neither side of the membrane [46]. While the ModABC structure conforms to previous findings, the BtuCDF conformation appears unusual. The observed conformation of TMDs is due to the orientation of helices TM3 to helix 5a that appear to control the inward–outward conformations based on the comparison between HI1470/1 and BtuCD (Fig. 7d) [13,66]. In BtuCDF these helices from the first TM subunit have similar conformation to BtuCD's outward-facing conformation while the same helices from the second TM subunit are similar to HI1470/1's inward-facing conformation. As a consequence the access is closed to either side. In addition the central cavity appears too small to allocate a B_{12} molecule. This conformation has not been previously seen among ABC transporters. Therefore the novel results obtained from crystal structure were confirmed by additional experiments conducted in lipid membranes. These experiments suggested that this structure is indeed found in native membranes.

The binding proteins BtuF and MalE are free of ligand and their structure correspond to the open state with lobes of PBPs spreading. Interestingly, the absence of ligand correlates with the insertion of periplasmic loops into the binding pocket of PBPs: in the case of BtuCD complex the insertion of loops between TM5 and helix 5a from both BtuC subunits, and in the case of MalFGK-E complex the insertion of the periplasmic loop P3 from MalG subunit [46,65]. It has been suggested that this insertion of periplasmic loops, combined with the spreading of the lobes of the binding protein, is responsible for causing ligand displacement from the binding pocket [46]. While the published BtuCDF complex contains no substrate, maltose is present in the MalFGK-E complex, bound at the interface between the TM subunits. This provides the first structural evidence for the existence of such a binding site. The chemical environment of this maltose binding site is similar to that from the maltose binding protein (PBP). Thus, MalFGK-E structure represents an intermediate state in the translocation pathway when the NBDs are closed and prepared to hydrolyse ATP, the maltose has already been translocated from the binding protein into the TMDs, and the configuration of TMDs remains outward-facing [65]. In contrast, BtuCDF complex represents a post-translocation intermediate, when ATP hydrolysis has already occurred [46].

Another feature of the MalFGK-E structure is the topology of the TMD region where the two subunits are oriented in a crescent-shaped structure with the concave part towards each other and the TMH cross over from on subunit to another [65]. This arrangement is similar to that found in the ModABC complex and different from the one found in BtuCD, BtuCDF and HI1470/1 where TMHs from both subunits are oriented side by side. In the published structures the ModABC NBDs are open and the TMDs are facing inward while for MalK are closed and TMDs are facing outward. Therefore, the ModABC structure represents a conformation at the resting state with a tungstate bound to the binding protein ModA [24], while the MalFG-E structure represents an intermediate state [65].

The structural data for *E. coli* ABC transporter components, while an important source of information in its own right, also allows for comparison of key structural motifs in the analogous proteins from other prokaryotic organisms and homologous proteins in eukaryotes. This allows the development of a general scheme of ABC transporter architecture and how individual variations, such as those identified in SufC [49], might impact on the structural mechanics of an individual ABC transporter. The antiquity of the ABC transporter family [3], suggests that functionally important structural variations in transporters such as SufC might be conserved in its homologues. Small structural variations, such as those of SufC, are ideal candidates for homology modeling studies of homologous NBDs. While any homology model has a very similar overall structure to crystal structure on which it is based, the highly conserved NBD architecture and sequence identity simplify the prediction of hydrogen bonding

Table 3
PSI-BLAST search on crystallized TMDs against *Escherichia coli* K12 (taxid:83333)

Structure name	Homologous region name	Homologous gene name	NCBI accession number	E value	Sequence identity	Subunit transport substrate
BtuC	FecCD	fecD	NP_418708	1e−29	39%	Iron-dicitrate
	FecCD	fecC	NP_418709	4e−28	32%	Iron-dicitrate
	Res 41–292 = FecCD Res 386–660 = FepD	fhuB	NP_414695	3e−23	29%	Fused iron-hydroxamate
	FecCD	fepD	NP_415122	1e−21	38%	Iron-enterobactin
	FepG	fepG	NP_415121	1e−18	31%	Iron-enterobactin
Sav1866	MdlB	mdlB	NP_414983	5e−23	24%	Fused putative multidrug
	MdlB	msbA	NP_415434	2e−22	20%	Fused lipid/multidrug
	MdlB	mdlA	NP_414982	5e−21	20%	Putative multidrug
	CydC	cydC	NP_415406	6e−5	20%	Fused cysteine transporter
ModBC	CysU	cysU	NP_416919	9e−20	28%	Sulfate/thiosulfate
	CysW	cysW	AP_003017	1e−17	28%	Sulfate/thiosulfate
	PotB	potH	NP_415377	8e−12	24%	Spermidine/putrescine
	Res 36–259 = CysU Res 320–506 PotC	thiP	NP_414609	5e−10	25%	Fused thiamin transporter
	PotC	potC	NP_415642	1e−8	29%	Polyamine
	PotB	potB	NP_415643	2e−8	21%	Polyamine
	MalG	malG	NP_418456	1e−6	28%	Maltose
	ProW	proW	NP_417164	5e−6	27%	Glycine/betaine
	PotB	ydcU	NP_415959	6e−6	22%	Putative spermidine/putrescine
HI1471	BtuC	btuC	NP_416226	4e−28	35%	Vitamin B12
	FecCD	fecD	NP_418708	5e−28	32%	Iron-dicitrate
	FecCD	fepD	NP_415122	9e−25	32%	Iron-enterobactin
	FecCD	fecC	NP_418709	1e−23	31%	Iron-dicitrate
	FepD	fhuB	NP_414695	1e−18	27%	Fe3+ siderophore
	Res 41–292 = FecCD Res 386–660 = FepD	fhuB	AAC73264	2e−14	29%	Fused iron-hydroxamate transporter

patterns and salt bridges that may have mechanistic implications for the modeled homologues.

5. Computational findings

Computational techniques like molecular modeling or molecular mechanical simulations have grown into powerful tools that have contributed to increase our understanding of protein structure and function. In particular for membrane proteins, which are difficult to crystallize, often crystallize at low resolution and/or in unidentified functional states, and often involve relatively obscure bacterial homologues of proteins of interest, computational methods may be a useful complement to experimental structures. A detailed review of earlier simulations is available [67]. Here we focus on a number of examples that illustrate how simulations may help in obtaining a molecular mechanism based on experimental structural data.

5.1. Structure prediction

Homology modeling is a useful tool for related proteins, but depends critically on a sufficiently high sequence identity between target and template. NBDs tend to have a high sequence identity, but transmembrane domains vary from a reasonable sequence identity between related transporters (e.g. BtuCD and FhuBC) to little or no sequence identity at all. Homology modeling is also a route towards applying structural knowledge of bacterial proteins to human transporters, but this is a complicated and speculative route given the low degrees of homology involved that requires careful experimental validation and a healthy degree of skepticism about the results. An example of one of the most favorable cases is modeling the human protein P-glycoprotein on the bacterial protein Sav1866 [68], with an application to experimental work [69,118]. Table 3 summarizes the homology between *E. coli* ABC transporters and ABC transporters with known structures.

5.2. Complex modeling

Another level of structure predictive modeling concerns the structure of multimeric protein complexes and resorts to experimentally derived constraints as guiding parameters for model building. Using highly conserved surface residues at the potential ABC transporter — PBP docking interface [58], both manual [63] and steered protein–protein docking was applied to predict the structure of the substrate-bound BtuCD-F complex of the vitamin B12 importer BtuCD and its cognate substrate binding protein BtuF [70]. Two possible docking complexes were found mainly differing by a 180 rotation of BtuF. Both complexes were subsequently further refined via extensive multi-copy molecular dynamics simulations in a realistic lipid/water environment. It will be interesting to see how the resulting models will compare to an experimentally determined structure of holo BtuCD-F (Kandt and Tieleman, in preparation). The later published apo BtuCD-F crystal structure has the periplasmic binding protein in an orientation that is similar to the 1st docking complex. So far no experimental evidence has been published supporting the 2nd BtuF orientation.

5.3. Protein dynamics

When high-quality crystal or NMR structures are available, molecular dynamics and normal mode analysis are powerful methods to study the intrinsic motions of proteins. Both type of calculations have been applied to a number of ABC transporter proteins. We highlight a selection of these calculations to give an idea of the type of information that has been obtained.

5.3.1. Substrate binding proteins

Several molecular dynamics (MD) simulation studies on substrate binding proteins have been reported. Simulations of the glutamine binding protein [71] and the maltose binding protein [72] focused on the transition between open and closed state, whereas a simulation study of ferrochrome binding protein FhuD explored the conformational space accessible to an apo form of the protein created by removing the bound ligand [73]. Extensive simulations of apo and holo forms of the vitamin B12 binding protein BtuF found the protein capable of clear opening and closing motions even though the corresponding crystal structures did not show any conformational differences [64]. Additional elastic network normal mode analysis in

the same study provided further evidence that other BtuF-like SBPs including FhuD may behave in a similar way. This suggests that the SBP Venus flytrap functional mechanism of large opening and closing upon ligand binding and release [74], from which BtuF-like binding proteins appeared to be excluded, may after all apply to all three classes of substrate binding proteins. This hints at one major mechanism of SBP – ABC transporter interaction, recognition and communication.

5.3.2. Nucleotide binding domains

Simulations of MalK are of particular interest, because MalK has been crystallized in several different states and the dynamic paths between these states may be relevant for the mechanism of a complete ABC transporter (in this case, the maltose importer). Oloo et al. successfully reproduced conformational transitions between the three different MalK conformations by adding or removing Mg-ATP [75]. In addition to the ATP-bound closed and nucleotide-free semi-open and open crystal structures two further starting structures were created where Mg-ATP was added to the semi-open and open crystal structures. Each scenario was sampled by five independent 15 ns MD runs. Adding Mg-ATP was found to trigger a closing of the MalK dimer whereas ATP removal induced an opening of the NBDs. Next to showing the potential of unbiased molecular mechanical simulations to connect between and generate new intermediate structures, this work, like the BtuF study mentioned earlier, also demonstrates how effectively the bandwidth of conformational sampling can be increased by comparably simple means such as runs from multiple different starting conditions. For a more detailed review of computational studies of the dynamics of NBDs see these reviews [10,67].

5.3.3. Full-length ABC transporters

Two independent MD studies [76,77] were performed on the since-withdrawn MsbA structures [78]. Both of these studies used the *E. coli* “open state” MsbA structure, which consisted of an α-carbon trace for approximately 75% of the protein and resolved to 4.5 Å. In order to perform atomistic MD studies, the missing residues and side-chain atoms first needed to be rebuilt. Details of this procedure can be found in the original publications [76,77]. The first MD study, conducted by Campbell et al. [76], investigated the stability of both the solvated MsbA monomer and dimer systems embedded in an octane slab. These 2 ns simulations showed large backbone fluctuations of approximately 6 Å in the first nanosecond of unrestrained MD simulations and significant loss of helical secondary structure in the lipid-embedded TMDs, calling in question the correctness of the crystal structure [76]. This was not conclusive, however, given the low resolution of the structure and potential problems with the modeling procedures. The second MD study, conducted in 2006 by Haubertin, et al, consisted of a solvated system containing a reconstructed TMD dimer embedded in a POPE bilayer. NBDs were not included in this simulation [77]. While the timescale of this simulation was much longer (34 ns of unrestrained MD) and physiologically relevant POPE lipids were used, the backbone deviations between the simulated TMDs and the crystal structure increased to ~5 Å [77]. Both studies describe large protein instabilities of approximately the same magnitude, which they attribute to the low resolution of the MsbA crystal structure and the expected intrinsic flexibility of MsbA throughout the catalytic cycle. Although it is well established that MD simulations on low resolution structures have large backbone fluctuations, it is somewhat cautionary that neither study clearly detected the incorrectness of the defunct MsbA structures. In a recent review, Jones and George also point out that the inherent flexibility of MsbA throughout the catalytic cycle does not account for the disruption of transmembrane helices in short simulation periods [79]. Further studies by Stenham et al. [80] challenged the conformation of the *E. coli* MsbA structure, as it did not fit the cross-linking data collected for its eukaryotic homologue, P-gp. These results led them to

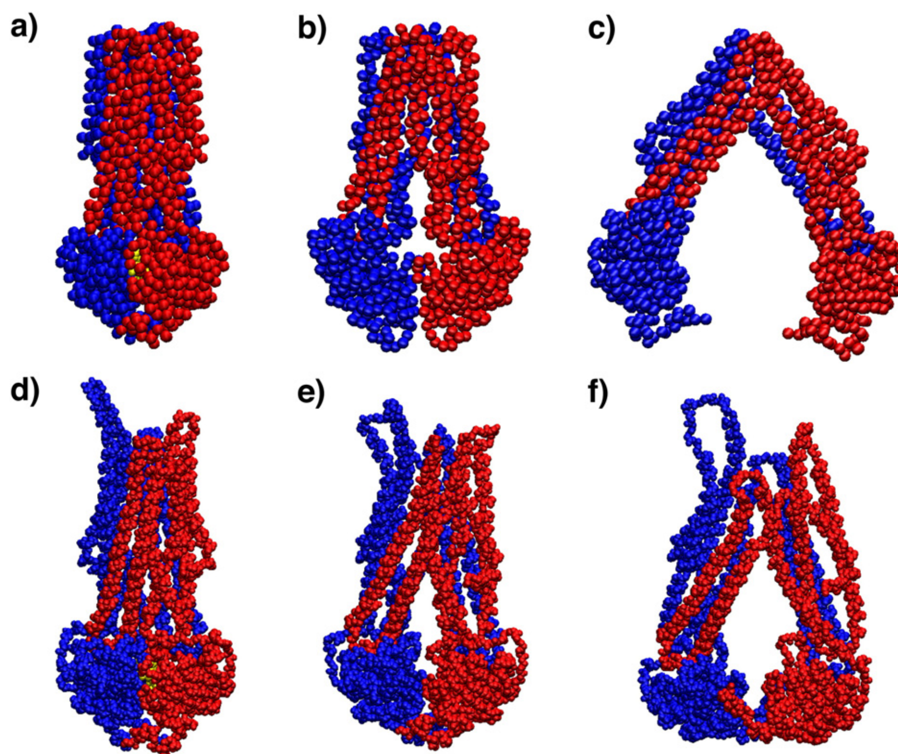

Fig. 8. Conformations of MsbA crystal structures (alpha carbons), which may represent major steps in the nucleotide catalytic cycle, are compared to modeled conformations of P-glycoprotein backbone [68]. In both cases, one NBD–TMD subunit is coloured blue and the second is in red. Nucleotide bound conformation of (a) MsbA (3B60. pdb)";; (b) P-glycoprotein. Nucleotide-free conformations of (c) MsbA (3B5X.pdb)";; (d) semi-open P-glycoprotein; and (e) MsbA (3B5W.pdb)";; (f) open P-glycoprotein.

conclude that MsbA crystal structure was not a good structural template for P-gp and most likely did not reflect a physiological state for the native MsbA [80].

The recent republication of the MsbA crystal structures [57] has again prompted speculations as to whether these large scale domain motions are indeed part of the ABC transport cycle. While the republished structures are of lower resolution than their original counterparts, precluding any meaningful molecular dynamics simulations, the TMD packing and domain interlinking is consistent with that of the Sav1866 structures, allowing a comparison to the projected conformations of the Sav1866- and MalK-based P-gp homology model developed by O'Mara and Tieleman [68]. The MsbA structures show the same qualitative conformation changes in MsbA and the MalK derived P-gp projections (Fig. 8), although the magnitude of NBD dissociation and TMD opening is far greater in the MsbA "open state" structure than evidenced in MalK structures or the P-gp projections. This raises the question of the biological relevance of the low resolution MsbA structures, particularly that of the "open state", all of which were crystallized outside the membrane environment. In the physiological MsbA "open state", the presence of the lipid bilayer may constrain the large scale domain motions exhibited in the crystal structure through inertial damping.

For ABC transporters receiving their substrate from the water phase, the so-called MalK and BtuCD models of functional mechanism propose that ATP binding has opposite conformational effects on the TMDs. Originally stemming from vanadate trapping experiments on the maltose importer MalGFK_2-E [81] as well as crystal structures of BtuCD [13] and isolated MalK NBDs [12], both models agree that ATP binding draws the NBDs closer together, which was also observed in the first 15 ns simulation study of BtuCD [82]. However, the BtuCD model [13] predicts the TMDs adopting an open-towards-cytoplasm conformation and close towards periplasm when the NBDs are drawn together, whereas the MalK model [12] proposes the opposite effect. To gather evidence which transport model is more likely, a combined MD and elastic network normal mode analysis study brought the BtuCD NBDs closer together and further apart testing how the two scenarios influenced the TMDs [62]. Though no clear opening or closing motions were observed – except for BtuCD closing towards the periplasm within 8 ns of unbiased MD simulation – an opening trend on substrate and closing trend on cytoplasmic side was observed when the NBDs were pushed closer together. Pulling them further apart had the opposite effect. In this light and also in regard to the HI1470/1, ModBC-A [24] and the MalGFK_2-E [65] crystal structures, the MalK functional model appears more likely. Multi-copy simulations of our models of the entire vitamin B12-bound BtuCD-F complex also provide further evidence supporting the MalK model (Kandt and Tieleman, in preparation). Regarding the effect of Mg-ATP, the first simulation study modeling the apo BtuCD-F complex [63] makes an interesting observation: whereas simulations of BtuCD [63,75] and isolated Btu [63] and maltose transporter NBDs [75] reported an ATP-driven NBD closure, B12-free BtuCD-F was found to exhibit NBD closing behavior regardless if Mg-ATP was present or not. We make the same observation in our holo BtuCD-F simulations (Kandt and Tieleman, in preparation). If experimental findings confirmed Mg-ATP-induced dimerization effect is indeed weaker in BtuCD-F than in BtuCD, this could suggest an extended model of ABC transporter mechanism, where NBD dimerization is not only dependent on nucleotide but also influenced by the presence of the docked binding protein.

6. Conclusion

We surveyed the ABC transporters found in *E. coli* and reviewed structural data available in the database until the end of 2007 as well as selected newer structures of full ABC transporters. The rapid increase in crystal structures in the past few years is beginning to give detailed insight into how ABC transporters work, although the complexity of coupling ATP hydrolysis, transport, and peripheral proteins continues to challenge our understanding of the exact mechanism. Computer modeling and simulation can play a role in elucidating the details of the transport mechanism, but more biochemical characterization of transporters of known structures is crucial. The very recent publication of a structure of the maltose importer [83], which has been studied by biochemical techniques for a long time, is an important step in this respect, as will be biochemical studies of BtuCD-like importers and of Sav1866-like proteins.

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References

[1] K.J. Linton, C.F. Higgins, The *Escherichia coli* ATP-binding cassette (ABC) proteins, Mol. Microbiol. 28 (1998) 5–13.
[2] R.F. Doolittle, M.S. Johnson, I. Husain, B. Van Houten, D.C. Thomas, A. Sancar, Domainal evolution of a prokaryotic DNA repair protein and its relationship to active-transport proteins, Nature 323 (1986) 451–453.
[3] M.H. Saier, I.T. Paulsen, M.K. Sliwinski, S.S. Pao, R.A. Skurray, H. Nikaido, Evolutionary origins of multidrug and drug-specific efflux pumps in bacteria, FASEB J. 12 (1998) 265–274.
[4] G. Fuellen, M. Spitzer, P. Cullen, S. Lorkowski, Correspondence of function and phylogeny of ABC proteins based on an automated analysis of 20 model protein data sets, PROTEINS: Structure, Function, and Bioinformatics 61 (2005) 888–899.
[5] F.C.e.i.c. Neidhardt, *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular biology, ASM Press, Wachington, DC, 1987.
[6] P. Singleton, Bacteria in Biology, Biotechnology and Medicine, 4th ed.John Wiley and Sons Ltd, Chichester, 1997.
[7] F.R. Blattner, G. Plunkett, C.A. Bloch, N.T. Perna, V. Burland, M. Riley, J. ColladoVides, J.D. Glasner, C.K. Rode, G.F. Mayhew, J. Gregor, N.W. Davis, H.A. Kirkpatrick, M.A. Goeden, D.J. Rose, B. Mau, Y. Shao, The complete genome sequence of *Escherichia coli* K-12, Science 277 (1997) 1453–1457.
[8] M. Ehrmann (Ed.), The Periplasm, ASM Press 2007.
[9] K.P. Locher, Structure and mechanism of ABC transporters, Curr. Opin. Struct. Biol. 14 (2004) 426–431.
[10] I.D. Kerr, Structure and association of ATP-binding cassette transporter nucleotide-binding domains, Biochim. Biophys. Acta 1561 (2002) 47–64.
[11] K.J. Linton, C.F. Higgins, Structure and function of ABC transporters: the ATP switch provides flexible control, Pflugers Arch. 453 (2007) 555–567.
[12] J. Chen, G. Lu, J. Lin, A.L. Davidson, F.A. Quiocho, A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle, Mol. Cell. 12 (2003) 651–661.
[13] K.P. Locher, A.T. Lee, D.C. Rees, The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism, Science 296 (2002) 1091–1098.
[14] Y. Igarashi, K.F. Aoki, H. Mamitsuka, K.-i. Kuma, M. Kanehisa, The evolutionary repertoires of the eukaryotic-type ABC transporters in terms of the phylogeny of ATP-binding domains in eukaryotes and prokaryotes, Mol. Biol. Evol. 21 (2004) 2149–2160.
[15] M. Dean, A. Rzhetsky, R. Allikmets, The human ATP-binding cassette (ABC) transporter superfamily, Genome Res. 11 (2001) 1156–1166.
[16] A.L. Davidson, J. Chen, ATP-binding cassette transporters in bacteria, Annu. Rev. Biochem. 73 (2004) 241–268.
[17] R.J. Dawson, K.P. Locher, Structure of a bacterial multidrug ABC transporter, Nature 443 (2006) 180–185.
[18] E.L. Borths, B. Poolman, R.N. Hvorup, K.P. Locher, D.C. Rees, In vitro functional characterization of BtuCD-F, the *Escherichia coli* ABC transporter for vitamin B12 uptake, Biochemistry 44 (2005) 16301–16309.
[19] T. van der Heide, B. Poolman, ABC transporters: one, two or four extracytoplasmic substrate-binding sites? EMBO Rep. 3 (2002) 938–943.
[20] P. Bouige, D. Laurent, L. Piloyan, E. Dassa, Phylogenetic and functional classification of ATP-binding cassette (ABC) systems, Curr. Protein. Pept. Sci. 3 (2002) 541–559.
[21] M.H. Serres, S. Goswami, M. Riley, GenProtEC: an updated and improved analysis of functions of *Escherichia coli* K-12 proteins, Nucleic Acids Res. 32 (2004) D300–302.
[22] R.V. Misra, R.S. Horler, W. Reindl, I.I. Goryanin, G.H. Thomas, EchoBASE: an integrated post-genomic database for *Escherichia coli*, Nucleic Acids Res. 33 (2005) D329–333.
[23] R.J. Dawson, K.P. Locher, Structure of the multidrug ABC transporter Sav1866 from *Staphylococcus aureus* in complex with AMP-PNP, FEBS Lett. 581 (2007) 935–938.

[24] K. Hollenstein, D.C. Frei, K.P. Locher, Structure of an ABC transporter in complex with its binding protein, Nature 446 (2007) 213–216.
[25] H.P. Pinkett, A.T. Lee, P.L. Lum, K.P., D.C. Rees, An inward-facing conformation of a putative metal-chelate-type ABC transporter, Science 315 (2007) 373–377.
[26] K. Diederichs, J. Diez, G. Greller, C. Muller, J. Breed, C. Schnell, C. Vonrhein, W. Boos, W. Welte, Crystal structure of MalK, the ATPase subunit of the trehalose/maltose ABC transporter of the archaeon *Thermococcus litoralis*, EMBO J. 19 (2000) 5951–5961.
[27] S. Kitaoka, K. Wada, Y. Hasegawa, Y. Minami, K. Fukuyama, Y. Takahashi, Crystal structure of *Escherichia coli* SufC, an ABC-type ATPase component of the SUF iron–sulfur cluster assembly machinery, FEBS Lett. 580 (2006) 137–143.
[28] J. Zaitseva, S. Jenewein, T. Jumpertz, I.B. Holland, L. Schmitt, H662 is the linchpin of ATP hydrolysis in the nucleotide-binding domain of the ABC transporter HlyB, EMBO J. 24 (2005) 1901–1910.
[29] E. Procko, I. Ferrin-O'Connell, S.L. Ng, R. Gaudet, Distinct structural and functional properties of the ATPase sites in an asymmetric ABC transporter, Mol. Cell. 24 (2006) 51–62.
[30] P.C. Smith, N. Karpowich, L. Millen, J.E. Moody, J. Rosen, P.J. Thomas, J.F. Hunt, ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer, Mol. Cell. 10 (2002) 139–149.
[31] J. Zaitseva, C. Oswald, T. Jumpertz, S. Jenewein, A. Wiedenmann, I.B. Holland, L. Schmitt, A structural analysis of asymmetry required for catalytic activity of an ABC-ATPase domain dimer, EMBO J. 25 (2006) 3432–3443.
[32] R. Gaudet, D.C. Wiley, Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing, EMBO J. 20 (2001) 4964–4972.
[33] L.W. Hung, I.X. Wang, K. Nikaido, P.Q. Liu, G.F. Ames, S.H. Kim, Crystal structure of the ATP-binding subunit of an ABC transporter, Nature 396 (1998) 703–707.
[34] N. Karpowich, O. Martsinkevich, L. Millen, Y.R. Yuan, P.L. Dai, K. MacVey, P.J. Thomas, J.F. Hunt, Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter, Structure 9 (2001) 571–586.
[35] H.A. Lewis, S.G. Buchanan, S.K. Burley, K. Conners, M. Dickey, M. Dorwart, R. Fowler, X. Gao, W.B. Guggino, W.A. Hendrickson, J.F. Hunt, M.C. Kearins, D. Lorimer, P.C. Maloney, K.W. Post, K.R. Rajashankar, M.E. Rutter, J.M. Sauder, S. Shriver, P.H. Thibodeau, P.J. Thomas, M. Zhang, X. Zhao, S. Emtage, Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator, EMBO J. 23 (2004) 282–293.
[36] H.A. Lewis, X. Zhao, C. Wang, J.M. Sauder, I. Rooney, B.W. Noland, D. Lorimer, M.C. Kearins, K. Conners, B. Condon, P.C. Maloney, W.B. Guggino, J.F. Hunt, S. Emtage, Impact of the deltaF508 mutation in first nucleotide-binding domain of human cystic fibrosis transmembrane conductance regulator on domain folding and structure, J. Biol. Chem. 280 (2005) 1346–1353.
[37] T. Ose, T. Fujie, M. Yao, N. Watanabe, I. Tanaka, Crystal structure of the ATP-binding cassette of multisugar transporter from *Pyrococcus horikoshii* OT3, Proteins 57 (2004) 635–638.
[38] O. Ramaen, N. Leulliot, C. Sizun, N. Ulryck, O. Pamlard, J.Y. Lallemand, H. Tilbeurgh, E. Jacquet, Structure of the human multidrug resistance protein 1 nucleotide binding domain 1 bound to Mg2+/ATP reveals a non-productive catalytic site, J. Mol. Biol. 359 (2006) 940–949.
[39] F. Scheffel, U. Demmer, E. Warkentin, A. Hulsmann, E. Schneider, U. Ermler, Structure of the ATPase subunit CysA of the putative sulfate ATP-binding cassette (ABC) transporter from *Alicyclobacillus acidocaldarius*, FEBS Lett. 579 (2005) 2953–2958.
[40] P.H. Thibodeau, C.A. Brautigam, M. Machius, P.J. Thomas, Side chain and backbone contributions of Phe508 to CFTR folding, Nat. Struct. Mol. Biol. 12 (2005) 10–16.
[41] M. Vedadi, J. Lew, J. Artz, M. Amani, Y. Zhao, A. Dong, G.A. Wasney, M. Gao, T. Hills, S. Brokx, W. Qiu, S. Sharma, A. Diassiti, Z. Alam, M. Melone, A. Mulichak, A. Wernimont, J. Bray, P. Loppnau, O. Plotnikova, K. Newberry, E. Sundararajan, S. Houston, J. Walker, W. Tempel, A. Bochkarev, I. Kozieradzki, A. Edwards, C. Arrowsmith, D. Roos, K. Kain, R. Hui, Genome-scale protein expression and structural biology of *Plasmodium falciparum* and related Apicomplexan organisms, Mol. Biochem. Parasitol. 151 (2007) 100–110.
[42] G. Verdon, S.V. Albers, B.W. Dijkstra, A.J. Driessen, A.M. Thunnissen, Crystal structures of the ATPase subunit of the glucose ABC transporter from *Sulfolobus solfataricus*: nucleotide-free and nucleotide-bound conformations, J. Mol. Biol. 330 (2003) 343–358.
[43] G. Verdon, S.V. Albers, N. van Oosterwijk, B.W. Dijkstra, A.J. Driessen, A.M. Thunnissen, Formation of the productive ATP-Mg2+-bound dimer of GlcV, an ABC-ATPase from *Sulfolobus solfataricus*, J. Mol. Biol. 334 (2003) 255–267.
[44] Y.R. Yuan, S. Blecker, O. Martsinkevich, L. Millen, P.J. Thomas, J.F. Hunt, 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. 276 (2001) 32313–32321.
[45] Y.R. Yuan, O. Martsinkevich, J.F. Hunt, Structural characterization of an MJ1267 ATP-binding cassette crystal with a complex pattern of twinning caused by promiscuous fiber packing, Acta Crystallogr. D. Biol. Crystallogr. 59 (2003) 225–238.
[46] R.N. Hvorup, B.A. Goetz, M. Niederer, K. Hollenstein, E. Perozo, K.P. Locher, Asymmetry in the structure of the ABC transporter-binding protein complex BtuCD-BtuF, Science 317 (2007) 1387–1390.
[47] L. Schmitt, H. Benabdelhak, M.A. Blight, I.B. Holland, M.T. Stubbs, Crystal structure of the nucleotide-binding domain of the ABC-transporter haemolysin B: identification of a variable region within ABC helical domains, J. Mol. Biol. 330 (2003) 333–342.
[48] G. Lu, J.M. Westbrooks, A.L. Davidson, J. Chen, ATP hydrolysis is required to reset the ATP-binding cassette dimer into the resting-state conformation, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 17969–17974.
[49] S. Kitaoka, K. Wada, Y. Hasegawa, Y. Minami, Y. Takahashi, K. Fukuyama, Crystal Structure of *Escherichia coli* SufC, an ATPase component of the SUF iron–sulfur cluster assembly machinery, FEBS Lett. 580 (2006) 137–143.
[50] I.B. Holland, L. Schmitt, J. Young, Type 1 protein secretion in bacteria, the ABC-transporter dependent pathway, Mol. Membr. Biol. 22 (2005) 29–39.
[51] C. Oswald, I.B. Holland, L. Schmitt, The motor domains of ABC-transporters. What can structures tell us? Naunyn Schmiedebergs Arch. Pharmacol. 372 (2006) 385–399.
[52] I.R. Vetter, A. Wittinghofer, Nucleoside triphosphate-binding proteins: different scaffolds to achieve phosphoryl transfer, Q Rev. Biophys. 32 (1999) 1–56.
[53] J.E. Walker, M. Saraste, M.J. Runswick, N.J. Gay, Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold, EMBO J. 1 (1982) 945–951.
[54] E. Lindahl, C. Azuara, P. Koehl, M. Delarue, NOMAD-Ref: visualization, deformation and refinement of macromolecular structures based on all-atom normal mode analysis, Nucleic Acids Res. 34 (2006) W52–W56.
[55] F. Tama, Y.H. Sanejouand, Conformational change of proteins arising from normal mode calculations, Protein Eng. 14 (2001) 1–6.
[56] G. Chang, Retraction of "Structure of MsbA from *Vibrio cholera*: a multidrug resistance ABC transporter homolog in a closed conformation" [J. Mol. Biol. (2003) 330 419–430], J. Mol. Biol. 369 (2007) 596.
[57] A. Ward, C.L. Reyes, J. Yu, C.B. Roth, G. Chang, Flexibility in the ABC transporter MsbA: alternating access with a twist, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19005–19010.
[58] E.L. Borths, K.P. Locher, A.T. Lee, D.C. Rees, The structure of *Escherichia coli* BtuF and binding to its cognate ATP binding cassette transporter, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16642–16647.
[59] N.K. Karpowich, H.H. Huang, P.C. Smith, J.F. Hunt, Crystal structures of the BtuF periplasmic-binding protein for vitamin B12 suggest a functionally important reduction in protein mobility upon ligand binding, J. Biol. Chem. 278 (2003) 8429–8434.
[60] D.P. Chimento, A.K. Mohanty, R.J. Kadner, M.C. Wiener, Substrate-induced transmembrane signaling in the cobalamin transporter BtuB, Nat. Struct. Biol. 10 (2003) 394–401.
[61] D.D. Shultis, M.D. Purdy, C.N. Banchs, M.C. Wiener, Outer membrane active transport: structure of the BtuB:TonB complex, Science 312 (2006) 1396–1399.
[62] J. Sonne, C. Kandt, G.H. Peters, F.Y. Hansen, M.Ø. Jensen, D.P. Tieleman, Simulation of the coupling between nucleotide binding and transmembrane domains in the ABC transporter BtuCD, Biophys. J. 92 (2007) 2727–2734.
[63] A. Ivetac, J.D. Campbell, M.S. Sansom, Dynamics and function in a bacterial ABC transporter: simulation studies of the BtuCDF system and its components, Biochemistry 46 (2007) 2767–2778.
[64] C. Kandt, Z. Xu, D.P. Tieleman, Opening and closing motions in the periplasmic vitamin B12 binding protein BtuF, Biochemistry 45 (2006) 13284–13292.
[65] M.L. Oldham, D. Khare, F.A. Quiocho, A.L. Davidson, J. Chen, Crystal structure of a catalytic intermediate of the maltose transporter, Nature 450 (2007) 515–521.
[66] H.W. Pinkett, A.T. Lee, P. Lum, K.P. Locher, D.C. Rees, An inward-facing conformation of a putative metal-chelate-type ABC transporter, Science 315 (2007) 373–377.
[67] E.O. Oloo, C. Kandt, M.L. O'Mara, D.P. Tieleman, Computer simulations of ABC transporter components, Biochem. Cell. Biol. 84 (2006) 900–911.
[68] M.L. O'Mara, D.P. Tieleman, P-glycoprotein models of the apo and ATP-bound states based on homology with Sav1866 and MalK, FEBS Lett. 581 (2007) 4217–4222.
[69] J. Storm, M.L. O'Mara, E. Crowley, J. Peall, D.P. Tieleman, I.D. Kerr, R. Callaghan, Residue G346 in transmembrane segment six is involved in inter-domain communication in P-glycoprotein, Biochemistry 46 (2007) 9899–9910.
[70] C. Kandt, E. Oloo, D. Tieleman, Domain coupling in the ABC transporter system BtuCD/BtuF: molecular dynamics simulation, normal mode analysis and protein–protein docking, 21st International Symposium on High Performance Computing Systems and Applications, IEEE Computer Society Press, Saskatoon SK, Canada, 2007, p. 17.
[71] A. Pang, Y. Arinaminpathy, M.S.P. Sansom, P.C. Biggin, Interdomain dynamics and ligand binding: molecular dynamics simulations of glutamine binding protein, FEBS Lett. 550 (2003) 168–174.
[72] T. Stockner, H.J. Vogel, D.P. Tieleman, A salt-bridge motif involved in ligand binding and large-scale domain motions of the maltose-binding protein, Biophys. J. 89 (2005) 3362–3371.
[73] K.D. Krewulak, C.M. Shepherd, H.J. Vogel, Molecular dynamics simulations of the periplasmic ferric-hydroxamate binding protein FhuD, Biometals 18 (2005) 375–386.
[74] J.S. Sack, M.A. Saper, F.A. Quiocho, Periplasmic binding-protein structure and function — refined X-ray structures of the leucine isoleucine valine-binding protein and its complex with leucine, J. Mol. Biol. 206 (1989) 171–191.
[75] E.O. Oloo, E.Y. Fung, D.P. Tieleman, The dynamics of the MgATP-driven closure of MalK, the energy-transducing subunit of the maltose ABC transporter, J. Biol. Chem. 281 (2006) 28397–28407.
[76] J.D. Campbell, P.C. Biggin, M. Baaden, M.S.P. Sansom, Extending the structure of an ABC transporter to atomic resolution: modeling and simulation studies of MsbA, Biochemistry 42 (2003) 3666–3673.
[77] D.Y. Haubertin, H. Madaoui, A. Sanson, R. Guerois, S. Orlowski, Molecular dynamics simulations of *E. coli* MsbA transmembrane domain: formation of a semipore structure, Biophys. J. 91 (2006) 2517–2531.

[78] G. Chang, C.B. Roth, C.L. Reyes, O. Pronillos, Y.-J. Chen, A.P. Chen, Retraction, Science 314 (2006) 1875.
[79] A.M. George, P.M. Jones, Molecular dynamics simulations and analysis of ABC transporters, Curr. Comput. Aided Drug Design 2 (2006) 95–103.
[80] D. Stenham, J.D. Campbell, M.S.P. Sansom, C.F. Higgins, I.D. Kerr, K.J. Linton, An atomic detail model for the human ATP binding cassette transporter P-glycoprotein derived from disulphide cross-linking and homology modeling, FASEB J. 17 (2003) 2287–2289.
[81] J. Chen, S. Sharma, F.A. Quiocho, A.L. Davidson, Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerted mechanism of maltose transport, PNAS 98 (2001) 1525–1530.
[82] E.O. Oloo, D.P. Tieleman, Conformational transitions induced by the binding of MgATP to the vitamin B12 ATP-binding cassette (ABC) transporter BtuCD, J. Biol. Chem. 43 (2004) 45013–45019.
[83] M.L. Oldham, D. Khare, F.A. Quiocho, A.L. Davidson, J. Chen, Crystal structure of a catalytic intermediate of the maltose transporter, Nature 450 (2007) 515–521.
[84] C. Kim, S. Song, C. Park, The D-allose operon of *Escherichia coli* K-12. J. Bacteriol. 179 (1997) 7637–7931.
[85] B.F. Horazdovsky, R.W. Hogg, Genetic reconstitution of the high-affinity L-arabinose transport system, J. Bacteriol. 171 (1989) 3053–3059.
[86] U. Wissenbach, S. Six, J. Bongaerts, D. Ternes, S. Steinwachs, G. Unden, A third periplasmic transport system for L-arginine in *Escherichia coli*: molecular characterization of the artPIQMJ genes, arginine binding and transport. Mol. Microbiol. 17 (1995) 675–686.
[87] U. Wissenbach, B. Keck, G. Unden, Physical map location of the new artPIQMJ genes of *Escherichia coli*, encoding a periplasmic arginine transport system, J. Bacteriol. 175 (1993) 3687–3688.
[88] A. Sirko, M. Zatyka, E. Sadowy, D. Hulanicka, Sulfate and thiosulfate transport in *Escherichia coli* K-12: evidence for a functional overlapping of sulfate- and thiosulfate-binding proteins, J. Bacteriol. 177 (1995) 4134–4136.
[89] I.A. Lessard, S.D. Pratt, D.G. McCafferty, D.E. Bussiere, C. Hutchins, B.L. Wanner, L. Katz, C.T. Walsh, Homologs of the vancomycin resistance D-Ala-D-Ala dipeptidase VanX in *Streptomyces toyocaensis*, *Escherichia coli* and *Synechocystis*: attributes of catalytic efficiency, stereoselectivity and regulation with implications for function, Chem. Biol. 5 (1998) 498–504.
[90] W.N. Abouhamad, M. Manson, M.M. Gibson, C.F. Higgins, Peptide transport and chemotaxis in *Escherichia coli* and *Salmonella typhimurium*: characterization of the dipeptide permease (Dpp) and the dipeptide-binding protein. Mol. Microbiol. 1991 (1991) 1035–1047.
[91] U. Pressler, H. Staudenmaier, L. Zimmermann, V. Braun, Genetics of the iron dicitrate transport system of *Escherichia coli*, J. Bacteriol. 170 (1988) 2716–2724.
[92] D.L. Stephens, M.D. Choe, C.F. Earhart, *Escherichia coli* periplasmic protein FepB binds ferrienterobactin, Microbiology 141 (1995) 1647–1654.
[93] A. Mademidis, W. Köster, Transport activity of FhuA, FhuC, FhuD, and FhuB derivatives in a system free of polar effects, and stoichiometry of components involved in ferrichrome uptake, Mol. Gen. Genet. 258 (1998) 156–165.
[94] D.O. Daley, M. Rapp, E. Granseth, K. Melén, D. Drew, G. von Heijne, Global topology analysis of the *Escherichia coli* inner membrane proteome, Science 308 (2005) 1321–1323.
[95] T. Nohno, T.H. Saito, J.S., Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (glnHPQ), Mol. Gen. Genet. 205 (1986) 260–269.
[96] R.C. Willis, C.E. Furlong, Interactions of a glutamate-aspartate binding protein with the glutamate transport system of *Escherichia coli*, J. Biol. Chem. 250 (1975) 2581–2586.
[97] H. Suzuki, T. Koyanagi, S. Izuka, A. Onishi, H. Kumagai, The yliA, -B, -C, and -D genes of *Escherichia coli* K-12 encode a novel glutathione importer with an ATP-binding cassette, J. Bacteriol. 187 (2005) 5861–5867.
[98] C.E. Liu, G.F. Ames, Characterization of transport through the periplasmic histidine permease using proteoliposomes reconstituted by dialysis, J. Biol. Chem. 272 (1997) 859–866.
[99] D.L. Oxender, J.J. Anderson, C.J. Daniels, R. Landick, R.P. Gunsalus, G. Zurawski, E. Selker, C. Yanofsky, Structural and functional analysis of cloned DNA containing genes responsible for branched-chain amino acid transport in *Escherichia coli*, PNAS 77 (1980) 1412–1416.
[100] P. Sperandeo, R. Cescutti, R. Villa, C. Di Benedetto, D. Candia, G. Dehò, A. Polissi, Characterization of lptA and lptB, two essential genes implicated in lipopolysaccharide transport to the outer membrane of *Escherichia coli*, J. Bacteriol. 189 (2007) 244–253.
[101] K.B. Xavier, B.L. Bassler, Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*, J. Bacteriol. 187 (2005) 238–248.
[102] R. Dippel, W. Boos, The maltodextrin system of *Escherichia coli*: metabolism and transport, J. Bacteriol. 187 (2005) 83222–88331.
[103] C. Merlin, G. Gardiner, S. Durand, M. Masters, The *Escherichia coli* metD locus encodes an ABC transporter which includes Abc (MetN), YaeE (MetI), and YaeC (MetQ), J. Bacteriol. 184 (2002) 5513–5517.
[104] W. Boos, The properties of the galactose-binding protein, the possible chemoreceptor for galactose chemotaxis in *Escherichia coli*, Antibiot. Chemother. 19 (1974) 21–54.
[105] S. Rech, U. Deppenmeier, R.P. Gunsalus, Regulation of the molybdate transport operon, modABCD, of *Escherichia coli* in response to molybdate availability, J. Bacteriol. 177 (1995) 1023–1029.
[106] Y. Hu, S. Rech, R.P. Gunsalus, D.C. Rees, Crystal structure of the molybdate binding protein ModA, Nat. Struct. Biol. 4 (1997) 703–707.
[107] J.T. Park, D. Raychaudhuri, H. Li, S. Normark, D. Mengin-Lecreulx, MppA, a periplasmic binding protein essential for import of the bacterial cell wall peptide L-alanyl-gamma-D-glutamyl-meso-diaminopimelate, J. Bacteriol. 180 (1998) 1215–1223.
[108] L.F. Wu, C.M.-B. Navarro, M.A., The hydC region contains a multi-cistronic operon (nik) involved in nickel transport in *Escherichia coli*, Gene 107 (1991) 37–42.
[109] T.G. Davies, R.E. Hubbard, J.R. Tame, Relating structure to thermodynamics: the crystal structures and binding affinity of eight OppA-peptide complexes, Prot. Sci. 8 (1999) 1432–1444.
[110] K. Makino, S.K. Kim, H. Shinagawa, M. Amemura, A. Nakata, Molecular analysis of the cryptic and functional phn operons for phosphonate use in *Escherichia coli* K-12, J. Bacteriol. 173 (1991) 2665–2672.
[111] K. Kashiwagi, S. Miyamoto, E.K. Nukui, H.K. Igarashi, Functions of potA and potD proteins in spermidine-preferential uptake system in *Escherichia coli*, J. Biol. Chem. 268 (1993) 19358–19363.
[112] R. Pistocchi, K. Kashiwagi, S. Miyamoto, E. Nukui, Y. Sadakata, H. Kobayashi, K. Igarashi, Characteristics of the operon for a putrescine transport system that maps at 19 minutes on the *Escherichia coli* chromosome, J. Biol. Chem. 268 (1993) 146–152.
[113] G. May, E. Faatz, M. Villarejo, E. Bremer, Binding protein dependent transport of glycine betaine and its osmotic regulation in *Escherichia coli* K12, Mol. Gen. Genet. 205 (1986) 225–233.
[114] M. Amemura, K. Makino, H. Shinagawa, A. Kobayashi, A. Nakata, Nucleotide sequence of the genes involved in phosphate transport and regulation of the phosphate regulon in *Escherichia coli*, J. Mol. Biol. 184 (1985) 241–250.
[115] S.L. Mowbray, L.B. Cole, 1.7 A X-ray structure of the periplasmic ribose receptor from *Escherichia coli*, J. Mol. Biol. 225 (1992) 155–175.
[116] C. Harms, Y. Domoto, C. Celik, E. Rahe, S. Stumpe, R. Schmid, T. Nakamura, E.P. Bakker, Identification of the ABC protein SapD as the subunit that confers ATP dependence to the K+-uptake systems Trk(H) and Trk(G) from *Escherichia coli* K-12, Microbiology 147 (2001) 2991–3003.
[117] J.R. van der Ploeg, R. Iwanicka-Nowicka, T. Bykowski, M.M. Hryniewicz, T. Leisinger, The *Escherichia coli* ssuEADCB gene cluster is required for the utilization of sulfur from aliphatic sulfonates and is regulated by the transcriptional activator Cbl, J. Biol. Chem. 274 (1999) 29358–29365.
[118] J. Lawson, M.L. O'Mara, I.D. Kerr, Structure-based interpretation of the mutagenesis database for the nucleotide binding domains of P-glycoprotein, Biochim. Biophys. Acta 1778 (2) (2008) 376–391.
[119] J.R. van der Ploeg, M.A. Weiss, E. Saller, H. Nashimoto, N. Saito, M.A. Kertesz, T. Leisinger, Identification of sulfate starvation-regulated genes in *Escherichia coli*: a gene cluster involved in the utilization of taurine as a sulfur source, J. Bacteriol. 178 (1996) 5438–5446.
[120] E. Webb, K. Claas, D. Downs, thiBPQ encodes an ABC transporter required for transport of thiamine and thiamine pyrophosphate in *Salmonella typhimurium*, J. Biol. Chem. 273 (1998) 8946–8950.
[121] H. Schweizer, W. Boos, Characterization of the ugp region containing the genes for the phoB dependent *sn*-glycerol-3-phosphate transport system of *Escherichia coli*, Mol. Gen. Genet. 197 (1984) 161–168.
[122] M. Sumiya, E.O. Davis, L.C. Packman, T.P. McDonald, P.J. Henderson, Molecular genetics of a receptor protein for D-xylose, encoded by the gene xylF, in *Escherichia coli*, Receptors Channels 3 (1995) 117–128.
[123] C. Checroun, C. Gutierrez, Sigma(s)-dependent regulation of yehZYXW, which encodes a putative osmoprotectant ABC transporter of *Escherichia coli*, FEMS Microbiol. Lett. 236 (2004) 221–226.
[124] S.I. Patzer, K. Hantke, The ZnuABC high-affinity zinc uptake system and its regulator Zur in *Escherichia coli*, Mol. Microbiol. 28 (1999) 1199–1210.
[125] L. Thöny-Meyer, F. Fischer, P. Künzler, D. Ritz, H. Hennecke, *Escherichia coli* genes required for cytochrome *c* maturation, J. Bacteriol. 177 (1995) 4321–4326.
[126] M.S. Pittman, H. Corker, G. Wu, M.B. Binet, A.J. Moir, R.K. Poole, Cysteine is exported from the *Escherichia coli* cytoplasm by CydDC, an ATP-binding cassette-type transporter required for cytochrome assembly, J. Biol. Chem. 277 (2002).
[127] K.L. Schmidt, N.D. Peterson, R.J. Kustusch, M.C. Wissel, B. Graham, G.J. Phillips, D.S. Weiss, A predicted ABC transporter, FtsEX, is needed for cell division in *Escherichia coli*, J. Bacteriol. 186 (2004) 785–793.
[128] T. Yakushi, K. Masuda, S. Narita, S. Matsuyama, H. Tokuda, A new ABC transporter mediating the detachment of lipid-modified proteins from membranes, Nat. Cell. Biol. 2 (2000) 212–218.
[129] N. Kobayashi, K. Nishino, A. Yamaguchi, Novel macrolide-specific ABC-type efflux transporter in *Escherichia coli*, J. Bacteriol. 183 (2001) 5639–5644.
[130] Z. Zhou, K. White, A. Polissi, C. Georgopoulos, C.R. Raetz, Function of *Escherichia coli* MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis, J. Biol. Chem. 273 (1998) 12466–12475.
[131] B. Woebking, G. Reuter, R.A. Shilling, S. Velamakanni, S. Shahi, H. Venter, L. Balakrishnan, H.W. van Veen, Drug-lipid A interactions on the *Escherichia coli* ABC transporter MsbA, J. Bacteriol. 187 (2005) 6363–6369.
[132] M.A. Delgado, P.A. Vincent, R.N. Farías, R.A. Salomón, YojI of *Escherichia coli* functions as a microcin J25 efflux pump, J. Bacteriol. 187 (2005) 3465–3470.
[133] S. Watanabe, A. Kita, K. Miki, Crystal structure of atypical cytoplasmic ABC-ATPase SufC from *Thermus thermophilus* HB8, J. Mol. Biol. 353 (2005) 1043–1054.
[134] R. Zhang, A. Joachimiak, A. Edwards, A. Savchenko, S. Beasley, The 2.0 A crystal structure of ABC transporter from *Thermotoga maritima*, to be published (2008).
[135] Z.J. Liu, W. Tempel, A. Shah, L. Chen, D. Lee, L.-L.C. Kelley, B.D. Dillard, J.P. Rose, J.C. Eneh, R.C. Hopkins, F.E. Jenney Jr., H.S. Lee, F.L. Poole II, C. Shah, F.J. Sugar, M.W.W. Adams, D.C. Richardson, J.S. Richardson, B.C. Wang, Putative ABC transporter

(ATP-binding protein) from *Pyrococcus furiosus* Pfu-867808-001, Southeast Collaboratory for Structural Genomics (2004).
[136] L.W. Hung, I.X.Y. Wang, K. Nikaido, P.Q. Liu, G.F.L. Ames, S.H. Kim, Crystal structure of the ATP-binding subunit of an ABC transporter, Nature 396 (1998) 703–707.
[137] Y. Yuan, H. Chen, D. Patel, Crystal structure of LmrA ATP-binding domain reveals the two-site alternating mechanism at molecular level To be Published (2003).
[138] JCSG, Crystal structure of ABC transporter ATP-binding protein (TM0544) from *Thermotoga maritima* at 2.10 A resolution, To be published (2004).