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Review

The ABC transporter structure and mechanism: perspectives on recent research

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Abstract. ATP-binding cassette (ABC) transporters are multidomain integral membrane proteins that utilise the energy of ATP hydrolysis to translocate solutes across cellular membranes in all phyla. ABC transporters form one of the largest of all protein families and are central to many important biomedical phenomena, including resistance of cancers and pathogenic microbes to drugs. Elucidation of the structure and mechanism of ABC transporters is essential to the rational design of agents to control their function. While a wealth of high-resolution structures of ABC proteins have been produced in recent years, many fundamental questions regarding the protein's mechanism remain unanswered. In this review, we examine the recent structural data concerning ABC transporters and related proteins in the light of other experimental and theoretical data, and discuss these data in relation to current ideas concerning the transporters' molecular mechanism.

Key words. ABC transporter; ABC-ATPase; membrane transport; nucleotide-binding domain; ATP hydrolysis; mechanism; P-glycoprotein.

Introducing the ABC superfamily: what are they? where are they?

ABC transporters are integral membrane proteins that typically utilise cellular energy to translocate solutes across cellular membranes in all phyla. Through the transport of molecules such as ions, sugars, amino acids, vitamins, peptides, polysaccharides, hormones, lipids and xenobiotics, ABC transporters are involved in diverse cellular processes such as maintenance of osmotic homeostasis, nutrient uptake, resistance to xenotoxins, antigen processing, cell division, bacterial immunity, pathogenesis and sporulation, cholesterol and lipid trafficking, and developmental stem cell biology [1–6].

ABC transporters have been conserved across the three kingdoms of archaea, eubacteria and eukarya [1, 2]. Their ubiquitous distribution and primordial origin reflect the fundamental requirement of cellular homeostasis to import and concentrate essential nutrients, and expel toxins acquired from the environment or produced as metabolic by-products [7]. Prokaryotes and eukaryotes also contain an additional large group of ABC proteins – nontransporters – located in the cytosol and employed mostly for maintenance and repair of DNA and for gene regulation [8]. These two major families of ABC proteins are known collectively as the ABC-ATPase superfamily [8, 9]. In a 1997 report of the complete genome sequence of *Escherichia coli* K-12, at least 80 ABC proteins – ~5% of the genome – were identified [10]. ABC transporters make up one of the four major gene families in humans [11]; and it is now believed that ABC proteins may be found in all cells of all species.

The signposting of ABC transporters as a superfamily with a core structure of four domains was made in 1986 [12] and was followed in 1992 by an encyclopaedic review on ABC transporters [2]. Despite the plenitude of

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solute types and processes with which they are involved, ABC transporters comprise a conserved core structure of two transmembrane domains (TMDs) and two cytosolic ABCs, also commonly known as nucleotide-binding domains (NBDs). The acronym 'ABC' derives from ATP-binding cassette [13]. The TMDs contain multiple hydrophobic segments, which span the membrane and form the transmembrane (TM) channel. The primary sequences of ABC transporter TMDs are markedly variable compared with those of the NBDs, which contain the highly conserved Walker A and B consensus motifs for nucleotide binding [14] and the 'LSGGQ' motif [15], the diagnostic signature sequence of ABC proteins. While the TMDs form the TM channel and are thought to contain the substrate binding sites, the NBDs are molecular motors that transform the chemical potential energy of ATP into protein conformational changes. Prokaryote importers or permeases generally deploy four separate subunits in the core configuration together with accessory periplasmic-binding proteins, which collect and present solute to the membrane-bound transporters. Eukaryotes only have export ABC transporters, which commonly have a single polypeptide for the core structure with each NBD being C-terminal to each TMD. There are exceptions to this scheme, with the most usual of these being half-transporters found in both prokaryotes and eukaryotes, in which each TMD is fused covalently to a C-terminal NBD, or with an N-terminal NBD followed by a TMD. The complete core structures for half-transporters can be homo- or heterodimers.

Some ABC transporters are involved in multidrug resistance in bacteria, fungi, yeasts, parasites and mammals [16–19], with resistance to anticancer agents being a major concern in humans and, in particular, that elicited by the human multidrug transporter P-glycoprotein (P-gp; MDR1; or ABCB1). Human P-gp is the archetypal ABC transporter and has earned its reputation by being the first discovered, the most important medically, the most studied and the one having arguably the broadest portfolio of substrates and reversing agents [20–27]. MDR1 is a single polypeptide of 1280 amino acids (170 kDa) and belongs to a small family of nine closely related genes whose isoforms have greater than 70% sequence identity [24].

Though research interest in the ABC superfamily is widespread, the stimulus for much of this research may be traced to P-gp, discovered by Victor Ling's group nearly 30 years ago as an integral plasma membrane glycoprotein [28]. This group [29] also established the experimental basis of P-gp's role in multidrug resistance in cancer cells; that it was a near perfect tandem duplication of the haemolysin transporter from *E. coli*; and its similarity with bacterial permease subunits HisP, MalK and OppD. They also made the prescient predictions that these proteins arose from a common ancestor; that transport was coupled to energy production; that P-gp could bind and export structurally diverse drugs; that its chief physiological role might be to protect cells from lipophilic toxins; and that its multidrug resistance property simply reflects gene amplification or overexpression.

A hydropathy analysis of the TMDs of P-gp [30] is the original source of the so-called 6 + 6 TM channel structure in which the TMDs are proposed to contain six TM helices each, arranged in two arcs surrounding a central pore. Alternative topologies of the TM helices have also been proposed (reviewed in [19]), and we have presented a radically different model in which each TMD forms a transmembrane β barrel connected to an intracytoplasmic helix bundle, which is proposed to interact with the NBDs and also to contain the substrate binding sites. In this model, the complete transporter thus consists of two TM β barrels and two substrate binding sites [31, 32]; but it remains to be tested experimentally.

Mutations in certain human ABC transporter genes cause a number of genetic diseases of which cystic fibrosis is the most well known [33]; but no fewer than 15 human ABC genes have been associated with a disease phenotype [34]. There is a Web site (http://www.nutrigene.4t.com/humanabc.htm) for the 48 known mammalian ABC transporters, classified into seven subfamilies with designations ABCA to ABCG. Some journals have devoted entire issues to reviews on ABC transporters [35–38]; and the first complete book of ABC proteins was published last year [19]. Finally, there is a rich collection of excellent inventorial reviews on the phylogeny of the ABC superfamily [19, 39–42].

On the eve of the new millennium, it was widely considered that significant further progress in ABC transporter research would require high-resolution structural data. Since that time, a wealth of structural information has been generated concerning ABC transporters and related proteins, beginning in 1998 with the landmark crystal structure of HisP, the NBD subunit of the well-characterised bacterial histidine permease complex [43]; a structure of the remotely related ABC ATPase DNA repair enzyme, the Rad50 catalytic domain (Rad50cd) [44]; through to recent crystal structures of complete ABC transporters [45, 46]. One of the most significant questions answered by these structural studies concerns the role of the ABC signature sequence and the mode of interaction of the two NBDs. The dimeric structure of the Rad50cd showed the signature sequence completing the active site of the opposite monomer in an NBD dimer [44], as we had first predicted for ABC transporters [47]. Since then two crystal structures of ABC transporter NBDs [46, 48] together with a number of experimental studies [49–53] have confirmed this mode of interaction of the NBDs. Notwithstanding these advances, however, many fundamental questions remain unanswered. In particular, the signal routes and mechanisms by which the TMDs control ATPase activity in the NBD in response to

substrate binding, and the way in which the NBDs harness the energy of ATP to drive conformational changes in the TMDs that drive substrate translocation, remain to be elucidated. In this review, we examine the recent structural data concerning ABC transporters and related proteins in the light of other recent experimental and theoretical data, and discuss these data in relation to current ideas concerning the transporters' molecular mechanism.

Transmembrane domains: is it crystal clear?

The first crystal structure of a complete ABC transporter appeared in 2001, the lipid A transporter from *E. coli*, Eco-MsbA [45]; it was followed in 2002 by the *E. coli* vitamin B_{12} transporter, BtuCD [46], and in 2003 by a structure of the *Vibrio cholera* MsbA transporter (VC-MsbA) [54]. MsbA is a homodimer of two fused TMD-NBD halves and is a close homologue of human P-gp. As originally predicted for P-gp on the basis of hydropathy analysis of the primary sequences, and later substantially corroborated by experimental approaches, the TMDs of the MsbA monomer form an arc of six α helices, which in dimers could form a TM channel (fig. 1A). Indeed, such a channel-like configuration was observed in the recent VC-MsbA dimer structure [54]. In view of the close homology between P-gp and MsbA, the crystal structures of MsbA should provide a fruitful template for interpreting the extensive experimental data concerning P-gp and related ABC transporters, but this has not proven to be the case. Beneath the happy coincidence of predicted and experimental structure lies a Pandora's box of puzzling anomalies and intriguing conundrums, many of which have not been discussed in the literature.

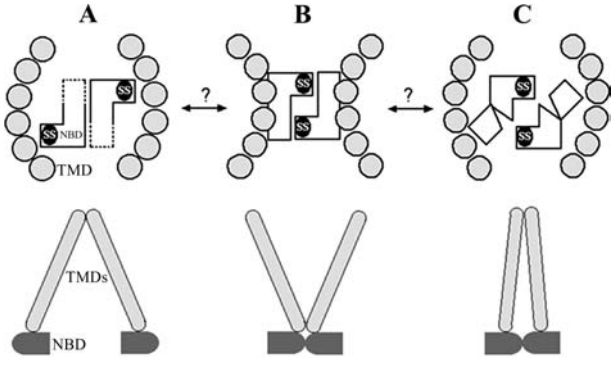

Figure 1. Schematic representations of MsbA dimer configurations viewed from the extracellular side, along the bilayer normal (upper panel), with the corresponding view within the plane of the membrane (lower panel). In the upper panels the NBDs are depicted as 'L'-shaped boxes with the signature sequences indicated by the letters 'SS' and the TM helices depicted as grey circles. In the lower panel, the TMDs are depicted as light-grey lozenges and the NBDs as dark-grey 'bullets', with the curved surface indicating the relative orientation of the NBD. (*A*) Eco-MsbA crystallographic dimer [45] with two arcs of six helices closed at the extracellular surface. The NBDs are more than 50 Å apart, with the SS facing away from the opposite monomer. The first 100 residues in each NBD were disordered in the crystal structure, indicated by the dashed lines in each NBD. (*B*) Eco-MsbA homology modelled to the Rad50cd NBD dimer [56–58]. In this configuration, the TMD arcs are open at the extracellular surface and are now inverted relative to the arcs in (*A*). (*C*) VC-MsbA dimer [54]. The NBDs are approximately in a Rad50cd dimer configuration, and the TMDs form a channel-like structure. The 120 degrees rotation of the two NBD halves relative to the consensus fold is depicted as a split in the L-shaped block.

Perhaps the most puzzling feature of the two MsbA structures is that in neither case can the monomers be oriented in such a way that they form a TM channel and a Rad50cd-like NBD dimer at the same time. In the case of the Eco-MsbA structure, while the N-terminal regions of the NBDs are disordered, their approximate locations can readily be predicted and indeed have been homology modelled by ourselves [55] and others [56–58]. When this exercise is performed and two Eco-MsbA monomers are arranged such that their NBDs form a Rad50cd-like dimer, the two TM arcs are inverted back to back relative to their orientation in the Eco-MsbA crystal dimer, and cannot form a channel without rotating 180 degrees about the bilayer normal relative to the NBD, or radical rearrangement of the putative TM helices (fig. 1B). Most astonishingly, the putative substrate sites within the interior of the TM channel would now be on the outside.

The TM structure of the VC-MsbA monomer is very close to that of Eco-MsbA, with homologous residues forming the concave surface of the 6 TM arc, and thus the interior of the putative TM channel formed by the VC-MsbA dimer (fig. 1C). While the NBDs are fully resolved, in comparing the VC-MsbA NBD to the consensus NBD fold, it appears that about one-half of the NBD is rotated about 120 degrees relative to the rest of the NBD. The two halves of the NBDs have approximately preserved their structure in this rotation, based on sequence and structural comparisons between VC-MsbA and other ABC transporters for which structures are known. The relative rotation of the two VC-MsbA NBD halves was likened to the rotation of a helical subdomain observed among the various NBD crystal structures [54]. However, whilst the NBD helical subdomain rotates about flexible loops at its N- and C-termini, the rotation of the VC-MsbA NBD halves breaks the structurally conserved central β sheet of the NBD core, rupturing the catalytic site and moving highly conserved active site residues, including the Walker B motif, over 25 Å from the Walker A nucleotide-binding region (fig. 1C). This configuration has not been observed in any other related nucleotide-binding protein and, rather than representing a natural mechanistic transition, may be an artefact of the crystallisation process. Nonetheless, if the N-terminal half of each VC-MsbA NBD is rotated 120 degrees to form a consensus NBD fold, the NBD dimer thus formed indeed approximates the mode of interaction observed in

Rad50 and other ABC transporter structures. Incredibly, however, in this configuration, each complete NBD is rotated 180 degrees relative to the TMDs when compared with the orientation observed in the Eco-MsbA monomer structure (fig. 1A, C; top panels), and the NBD interacts with the TMD in a completely different way in the two structures. Rather than representing a mechanistic transition, which to our knowledge is without precedent, this may indicate that either or both MsbA structures are artefactual to some extent.

In striking contrast to the MsbA structures, the two TMDs in the BtuCD structure comprise 10 helices each, arranged as discrete, tightly packed bundles, with the NBDs forming a Rad50-like dimer. This is a significant deviation from the canonical 6 + 6 TM channel structure and yet is in good agreement with the topological mapping study of the related FhuB protein [59]. Another notable difference between BtuCD and MsbA, is that regions equivalent to the three large 'intracytoplasmic loops' that in the MsbA structures interact with the NBDs are largely absent in the BtuCD structure. The radical difference between the Eco-MsbA and BtuCD structures, and the inability to fit either structure to low-resolution EM structures of P-gp, has led to speculation that the TMDs of different ABC transporter subfamilies may be structurally and/or evolutionarily unrelated [60]. This is indeed a radical idea. While it is true that the ABC ATPase has been harnessed through evolution to power diverse processes, the diversity of its cognate oligomeric partners is reflected in its structure. However, both sequence analysis and structural comparisons [41, 42] indicate that ABC transporter NBDs are evolutionarily closely related, and share a common structure and mechanism, implying that they interact with evolutionarily, mechanistically and structurally related TMDs. Also running counter to ideas of unusual structural divergence in the TMDs of ABC transporters engendered by the MsbA and BtuCD crystal structures [60, 61], a screening project of 24 human membrane transporter genes, including distinct ABC transporter subfamilies, found that amino acid diversity in TMDs was significantly lower than in loop domains. This was especially striking in the ABC superfamily, in which there was little variation in the TMDs, even in evolutionarily nonconserved residues. These data were taken to suggest that there are functional constraints on the TMDs of ABC transporters such that TMD structural variations do not parallel the functional diversity of these proteins.

A homology-modelling study found that an Eco-MsbA-based structural model of P-gp [58] could not explain extensive and detailed data concerning the proximity of putative TM segments of P-gp that have been obtained by studies using cysteine mutagenesis [62]. Even proposed alternative dimer configurations were not able to accommodate these data. This homology study also revealed that most of the residues that are known from mutagenesis and labelling experiments to be associated with the drug binding site are distinct from those inside the chamber of the modelled P-gp. Inconsistencies such as these between experimental data and the 6 + 6 TM model [32] have led to ideas that the TM segments undergo large conformational changes in the catalytic cycle [58, 60, 62]. However, even between the open and closed conformations of MsbA, proposed to reflect different stages of the catalytic cycle [54], the TM structure is essentially unchanged. Moreover, it has been argued for TM proteins in general that large conformational rearrangements of TM helices are unlikely, due to the energetic cost of altering the extensive packing interfaces [63].

The rapid inactivation of membrane proteins in detergent solution is a recognised problem in structure determination, and this inactivation has been found to be due to conformational alterations, caused by exposure of previously protected regions of the protein or by increased flexibility of the protein chain, allowing the formation of stable, non native conformations [63]. Both MsbAs and BtuCD were crystallised in detergent in the absence of lipid, and functional activities were not demonstrated. Indeed, it has been shown that the ATPase activity of some ABC transporters, including MDR1, has an absolute requirement for the presence of membrane lipids [64, 65]. In view of such considerations, leading ABC transporter researchers have cautioned against literal acceptance of the X-ray structures of complete ABC transporters in the absence of corroborative experimental evidence [19]. Thus, it is notable that the total α-helical content of the MsbA and BtuCD crystal structures is 65–70%, far higher than estimates obtained for the functionally reconstituted MsbA homologues P-gp and LmrA, whose α-helical contents were measured by spectroscopic methods to be only 35% [66, 67].

Substrate sites: where and how many?

What is the architecture of the substrate sites in ABC transporters? What does a multidrug site look like? How many sites are there per transporter? If most ABC transporters deploy the canonical 6 + 6 TM configuration, how are so many different types of molecules bound and translocated? The answers to these questions should be clearer now that we have the resolved structures of two complete ABC transporters, albeit not multidrug transporters and crystallised without bound substrates.

Bacterial ABC permeases use periplasmic binding proteins (PBPs) to capture substrate and present it at the intake vestibules of the membrane translocator units. The betaine ABC transporter OpuA in *Lactococcus lactis* [68, 69] has a PBP fused to each TMD, suggesting that there are two substrate transfer sites at the PBP-TMD inter-

faces. A database search of ABC operons in published genome sequences [69] discovered that chimeric PBP-TMD ABC proteins are widespread among several families of Gram-positive bacteria, making this a more common arrangement than thought previously. Despite these examples, the stoichiometry for nonchimeric PBPs might still be a single PBP per transporter, as inferred for the crystallized BtuF PBP, which forms the complex $BtuC_2D_2F$ in vitro [70]. Do bacterial permeases utilise one or two PBPs per transport complex? The answer will require a crystal structure of an ABC permease complete with bound PBP(s) and substrate.

ABC multidrug exporters in bacteria and eukarya present a more complex picture than that of bacterial importers. How does one reconcile the enormous differences in polarity, size and shape of substrates for multisubstrate ABC transporters that have virtually identical TMD channel configurations? The 'flippase' translocation model [71] proposes that the substrate 'sites' are within the TMDs [72, 73], and that the main criterion for efflux is the partitioning of largely hydrophobic drugs into the lipid bilayer and thence to the polar phase within the TM channel. Yet, the publication of several solution structures of transcriptional regulators gives support to a 'hydrophobic basket' model of multidrug binding [74, 75] that might have relevance to ABC transporters, since the multidrug portfolios are similar. Transcriptional regulators BmrR [76], MarR [77], TipA [78] and QacR [79, 80] all bind drugs in hydrophobic pockets. For BmrR and QacR, modelling and structural studies have shown that ligands penetrate a deep hydrophobic pocket. QacR has been crystallised with six different drugs and is presently the benchmark for multidrug binding models [79, 80]. In the QacR-rhodamine 6G and QacR-ethidium bromide complexes, the drug molecules are much smaller than the binding pocket, whose unoccupied space is filled with water molecules. Other larger substrates are capable of straddling the binding footprints of smaller drugs, with spaces filled in by water molecules.

It is tempting to postulate that ABC transporter multidrug binding sites might have similar architectures to QacR and BmrR, and that these sites may be sculptured from the loops at either end of the TMDs. ABC transporters are crowned with large intra- and extracytosolic loop domains, and it is difficult to reconcile their complete omission from substrate site construction and/or filtering; as recognised in other classes of membrane proteins with gated channels. A recent study [81] used MRP1-specific antibodies and glutathione azido-derivatives to locate binding sites in MRP1 [82]. The three regions identified were the large intracytoplasmic domain separating TMD1 and TMD2, the linker domain separating TMD2 and TMD3, and the last two TM segments. Another example comes from a recent study of the resistance nodulation division-type (RND) MexB and MexY *pmf* transporters from *Pseudomonas aeruginosa*, which export β-lactams and aminoglycosides, respectively [83]. MexB and MexY each have two large periplasmic loops that extend out of the TMDs in a manner not unlike that of ABC transporters. When the MexY loops were replaced by those from MexB, the hybrid now exhibited MexB-like β-lactam selectivity, and failed to recognise aminoglycoside. When the TM segments of MexB were replaced one by one with TM segments from MexY for all 12 segments, all of these hybrids still showed aminoglycoside selectivity. These data suggested that RND transporters select and efflux drugs within the large periplasmic domains and that the TMDs are unlikely to participate in substrate selectivity [83].

A screening project of the exons and flanking intronic regions of 24 human membrane transporter genes found that amino acid diversity in TMDs was significantly lower than in the loop domains, suggesting that TMDs have special functional constraints [84]. This difference was especially striking in the ABC superfamily in which there was little variation in the TMDs, even in evolutionarily nonconserved residues. The restricted variation in TMDs relative to loops is supported by a previous phylogenic comparison of 93 integral membrane proteins with multiple TMDs [85]. These data suggest that functional constraints on the TMDs of ABC transporters do not parallel the functional diversity of these proteins, and that this diversity might more likely be reflected in the loop domains, as we and others have argued for proposed substrate binding sites at one side of, or either side of, the TMDs [32, 86].

The P-loop ATPase family

ABC transporter NBDs contain the Walker A and Walker B consensus sequences for nucleotide binding [14]. These sequence motifs have been identified in a range of proteins engaged in diverse cellular processes such as: receptor signalling, phosphoryl transfer reactions, motility, ATP synthesis/proton efflux, membrane transport, DNA translation and DNA maintenance/repair. The Walker A motif (G-X-X-G-X-G-K-S/T) embodies a structure known as the phosphate-binding loop or P-loop, a glycine-rich loop followed by an uncapped α helix (fig. 2). This structure functions to bind the nucleotide through electrostatic interactions with the triphosphate moiety. The Walker B motif is Φ-Φ-Φ-Φ-D, where 'Φ' is a hydrophobic residue. In P-loop ATPases, this sequence constitutes a buried β strand within the core of the nucleotide-binding fold [87]. The Walker B aspartate hydrogen bonds to coordinating ligands of the catalytic Mg^{2+} ion, thereby assisting in establishing and maintaining the geometry of the active site.

The core fold of P-loop ATPases is characterised by a central, mostly parallel β sheet flanked by α helices [87].

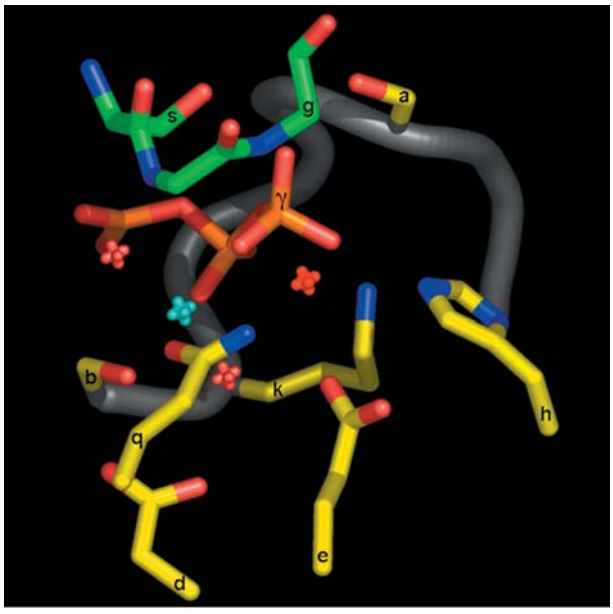

Figure 2. ABC-ATPase catalytic site. Representative ABC transporter active site derived from the MJ0796 E171Q dimer (1L2T) [48] showing deployment of conserved residues, water and catalytic magnesium ion in relation to nucleotide triphosphate moiety. The P-loop of one monomer is shown in a 'worm' representation and is coloured grey. Residue side chains and the nucleotide triphosphate group are shown in stick form with oxygen atoms coloured light red, nitrogen atoms blue and phosphorous orange. Carbon atoms of the monomer containing the P-loop are coloured yellow, while those of the (L)SGG of the opposite monomer are coloured green. The catalytic magnesium is cyan, and its two coordinating water molecules are light red. The nucleophilic water is situated at the centre of the figure and is dark red. Residues depicted are: a, Walker A 3 (serine); b, Walker A 8 (serine); d, Walker B aspartate; e, conserved glutamate at C-terminus of β-strand 6; g, C-motif second glycine; h, conserved histidine at C-terminus of β-strand 7; k, Walker A lysine; q, conserved glutamine at C-terminus of β-strand 5; s, C-motif serine; γ, nucleotide γ-phosphate. This figure and figs. 3, 5 and 7 were rendered with PyMOL (http://pymol.sourceforge.net/).

ABC transporter NBDs belong to a broad subclass of topologically homologous P-loop ATPases which includes RecA, F_1-ATP synthase, UvrB, helicases and a number of more closely related proteins involved in DNA maintenance and repair such as Rad50, MutS and SMC. These latter proteins form a superfamily together with ABC transporter NBDs, known as ABC ATPases [88, 89]. The RecA-like ATPase subclass is distinguished from other P-loop ATPases by the pattern of interdigitation of the parallel β strands, which form the backbone β sheet of the nucleotide-binding fold [90]. In *ras*-like proteins, adenylate kinase and myosin, the Walker B β strand is located immediately adjacent to the β strand preceding the P-loop, while in the F_1-ATPase, RecA and ABC ATPases, a third strand, derived from the peptide C-terminal to the Walker B, is inserted between the P-loop β strand and the Walker B β strand [47].

The ABC ATPase: the adaptable engine

Notwithstanding the striking functional divergence of the transmembrane transport ABC ATPases and the DNA maintenance/repair ABC ATPases, it is becoming increasingly clear that these multisubunit enzymes are unified through a conserved global architecture and conformational mechanism of the ATPase domains [89]. The ABC ATPase consists of two lobes or arms, lobe I constituting a characteristic conserved ATP-binding subdomain and lobe II comprising a more structurally diverse 'helical' subdomain. The central functional unit of ABC proteins consists of a heterotetramer comprising one NBD dimer and one substrate/function-specific dimer, this latter corresponding to the two TM domains in ABC transporters. X-ray crystal analysis reveals that the orientation of the substrate-specific domains with respect to the NBD dimer, and the regions of the NBDs which form the oligomeric interfaces, are analogous in all ABC proteins. In addition to this conserved quaternary structure, the region of the NBDs implicated in the transmission of conformational changes to the substrate-specific dimer, although structurally divergent, maps to the equivalent lobe II subdomain of the NBD. Further evidence of a conserved global mechanism amongst ABC proteins comes from biochemical data indicating that the catalytic domains hydrolyse ATP alternately [91, 92], and this appears to be coupled to an alternating mechanism in the substrate-specific dimer [86, 92, 93]. Thus, important insights into ABC transporter function are to be gained from the mechanistic and architectural parallels between the ABC ATPases.

The ABC transporter NBD: a bilobal design

The NBDs of ABC transporters can be divided into three structurally and functionally distinct subdomains, and these are colour coded in figure 3A, which depicts a generic topology and numbering system for the secondary structural elements of the NBD, and figure 3B, which depicts a ribbon diagram of HisP [43]. The central, mostly parallel β sheet forming the binding site for the nucleotide phosphates, and the α helices flanking and joining these β strands, are referred to collectively as the ABC core subdomain [90]. The core subdomain contains the Walker A and B consensus motifs. The antiparallel β sheet which functions in binding the ribose and adenine moieties of the bound nucleotide is designated the ABCβ antiparallel subdomain [90] or β subdomain. The β-subdomain β sheet is characteristic of ABC ATPases and is deployed approximately at right angles to the core subdomain β sheet, with helix 1 situated in the angle thus formed, held in place by extensive hydrophobic contacts which thus constitute the interior of lobe I. Together, these two β sheets, comprising β-strands 1, 2, 3, 4, 6, 7, 8

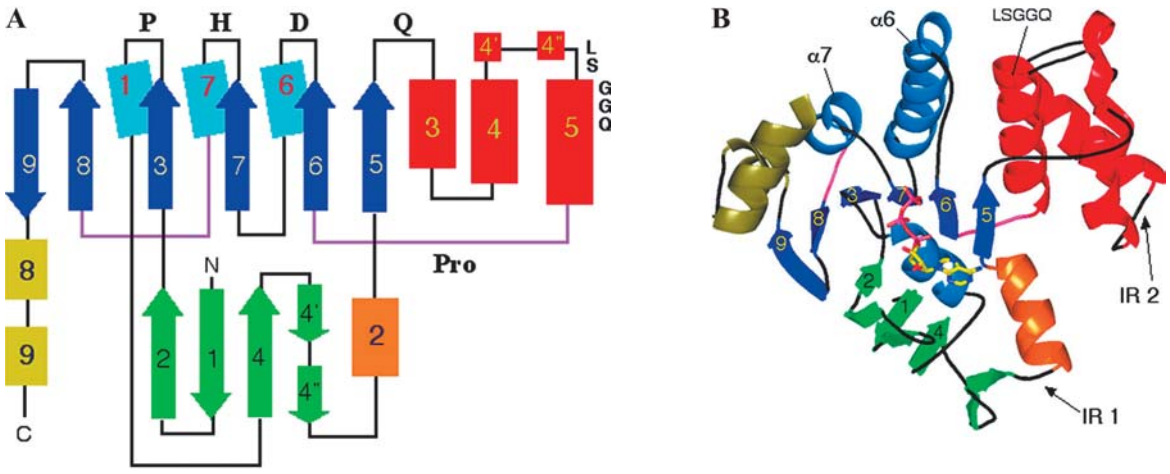

Figure 3. (*A*) Topology diagram of the consensus ABC transporter NBD fold. β strands are depicted as arrows and α helices as rectangles. Loops connecting secondary structural elements are depicted as thick lines. The ATP-binding core subdomain is shown in dark blue (β strands) and light blue (α helices), the antiparallel β subdomain (ABCβ) in green and the α subdomain (lobe II) in red. Loop regions are coloured black or magenta to indicate continuity. Key loop regions discussed in the text are labelled with the appropriate letter(s). A numbering system is introduced in which the variable β strands which occur immediately C-terminal to β-strand 4 and N-terminal to α-helix 2 are numbered $\beta 4'$, $\beta 4''$ etc. In addition, whilst consensus α-helix 2 does not occur in MalK, the first helix of the α subdomain is always referred to in the text as α-helix 3. (*B*) Three-dimensional structure of ABC transporter NBD. Ribbon diagram of HisP (1B0U) [43] colour coded and numbered as in (*A*). β strands are numbered and depicted as arrows and α helices as coiled ribbons. ATP is shown in stick form with carbon (yellow), nitrogen (blue), oxygen (orange) and phosphorous (magenta). Regions proposed to form interfaces with the TMDs, as discussed in the text, are labelled IR1 and IR2.

and 9, and P-loop helix 1 form a structurally rigid core which has a root mean square (r.m.s.) deviation between the ABC transporter NBD structures of less than 1.3 Å. The core subdomain and the ABCβ subdomain together constitute lobe/arm I of the ABC ATPase.

The ABC transporter NBD also contains a third subdomain with a structurally conserved core comprising a bundle of three α helices (fig. 3 A, B), variously known as arm/lobe II, the helical domain [94], the ABCα subdomain or α subdomain [90]. The N-terminal halves of helix 3 and helix 5 within lobe II comprise of residues which are, for the most part, highly conserved amongst ABC transporters [55]. The (L)SGGQ signature sequence forms the N-terminus of helix 5 within the bundle, with the serine side chain capping the helix. Helix 4 and the two loops at its N- and C-termini, which join the two conserved regions in the α subdomain, are highly variable in sequence, length and structure, and the loop regions are the sites of transporter-specific insertions (fig. 4) [55, 95]. Some nontransporter ABC ATPases, such as Rad50, also contain the LSGG sequence, while others which do not, such as MutS, nevertheless contain structural homologues of the capped helix structure.

The ABC dimer

Amongst the crystal structures of ABC ATPases, several different conformations of the ATPase dimer have been observed [43, 48, 96]. However, only dimers in which the LSGG sequence, or the structurally homologous region, completes the ATP binding site in the opposite monomer have been observed more than once, namely in Rad50cd [44], MutS [97, 98], BtuCD [46] and MJ0796 E171Q [48]; and these are all akin to our earlier modelled HisP dimer [47]. This dimer is also most consistent with biochemical and sequence data [47, 49–53]. The MJ0796 E171Q dimer structure (fig. 5) represents the only atomic level structure of the complete ABC transporter NBD active site with bound nucleotide – the BtuD dimer contains cyclo-vanadate and the details of the BtuD dimer interface are unlikely to represent the native structure. Although the catalytic sites in the Rad50cd and MJ0796 E171Q dimers are closely similar and likely represent the natural interaction of the LSGGQ with the ATP-bound active site, it is important to note that neither structure represents the wild-type protein and cannot therefore be taken to prove that the observed ‘dimer’, with two bound ATP or ATP-analogue molecules, represents a natural physiological state of the NBDs.

The big questions in ABC NBD research

In general, in P-loop ATPases, loop regions immediately C-terminal to the Walker B and other core β strands make contacts with the γ-phosphate of the bound nucleotide, and constitute important switch regions, which undergo conformational changes during the catalytic cycle. In ABC ATPases, three highly conserved active site residues

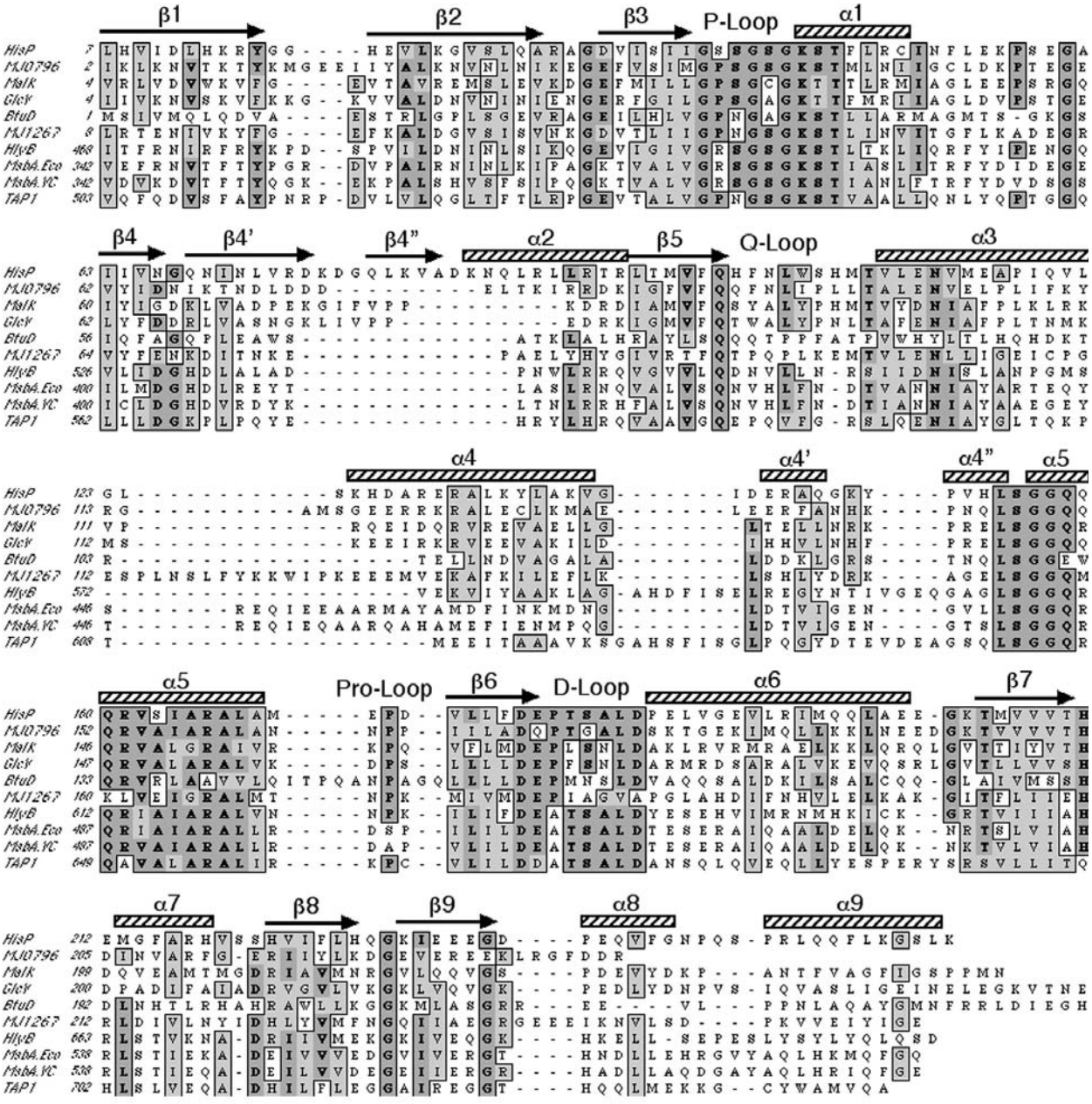

Figure 4. ABC sequence alignment. Manually adjusted structure-based ClustalW [140] sequence alignment of ABC transporter NBDs for which atomic structures are available. *Salmonella typhimurium* histidine permease HisP (1B0U) [43]; *Methanococcus jannaschii* putative ABC importer protein MJ0796 (1F30) [104], and E171Q ATP-bound dimer (1L2T) [48]; *Thermococcus litoralis* maltose permease MalK (1G29) [96]; *Sulfolobus solfataricus* glucose permease GlcV (1OXS, 1OXT, 1OXU, 1OXV) [116]; *Escherichia coli* vitamin B_{12} permease BtuD (1L7V, complete ABC transporter) [46]; *Methanococcus jannaschii* branched-chain amino acid permease MJ1267 MgADP bound (1G6H) and nucleotide-free (1GAJ) [90]; *Escherichia coli* haemolysin A exporter HlyB NBD (1MT0) [95]; *Escherichia coli* MsbA lipid exporter complete ABC transporter, for which only part of the NBD is resolved in the crystal structure (1JSQ) [45]; *Vibrio cholera* MsbA lipid exporter complete ABC transporter (1PF4) [54] and human antigen exporter TAP1 NBD with MgADP bound (1JJ7) [105]. Secondary structural elements are numbered as in figure 3 A, and their extent in HisP (1B0U) delineated by arrows (β strands) and stripped rectangles (α helices). Key loop regions discussed in the text are also labelled.

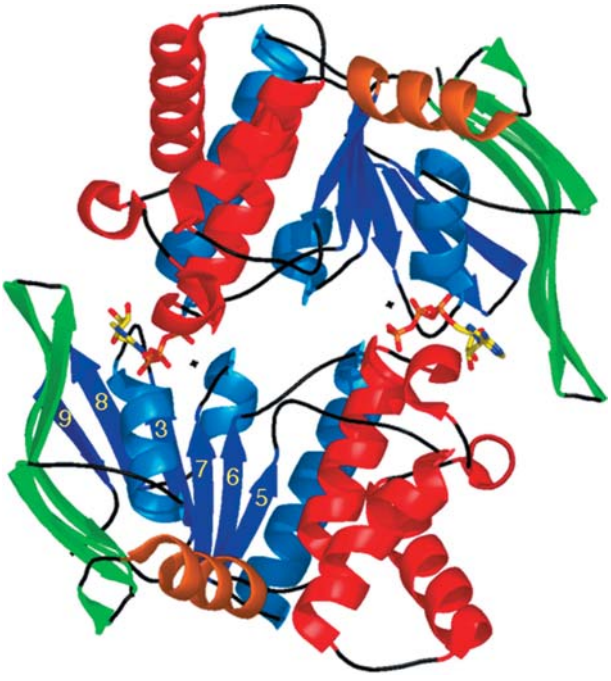

Figure 5. ABC transporter NBD dimer. Ribbon diagram of the MJ0796 E171Q dimer colour coded and numbered as in figure 3 A. β strands are depicted as arrows and α helices as coiled ribbons. Two ATP molecules are shown in stick form and colour coded as in figure 3 B.

(fig. 2) have been observed to make contact with the γ-phosphate in various crystal structures. These conserved residues are situated on loops C-terminal to β-strands 5, 6 and 7 (fig. 3 A, B), which are known as the Q-, D- and H-loops, respectively. The role of these active site residues and their associated loops in the mechanism of the NBD is a key question in current research into ABC transporters. Other important questions include: what are the communication mechanisms and interfaces between the TMDs and NBDs? How is ATP binding and hydrolysis in the NBDs coordinated and controlled by the TMDs? How is the free energy of ATP harnessed to produce conformational changes that result in substrate translocation? How is ATP hydrolysed – processively or alternately? In the catalytic cycle, do the NBDs come together to form a dimer and subsequently separate or do they remain in direct contact? Herein we discuss current ideas regarding these and related questions.

ATP: central organising force of the NBD

Binding of ATP by the P-loop mediates NBD:NBD interactions by altering the surface of the monomer, and this is achieved in two ways. First, ATP is sandwiched between the monomers in the NBD dimer and forms a significant part of the dimer interface (fig. 5), acting as the 'glue' between the monomers, with NBD residues making direct contacts with the bound nucleotide rather than with residues from the opposite monomer. Second, comparative analysis of ABC NBDs suggests that ATP binding, and in particular the γ-phosphate, produces an 'induced fit' effect, altering the conformation of the Q-, D-, H- and P-loops in the vicinity of the γ-phosphate, thereby altering or ordering the surface of the monomer [90]. Since the P-, H- and D-loops form part of the NBD-NBD interface, as shown in the MJ0796 E171Q and Rad50cd dimer structures, sharing the binding of the γ-phosphate with the LSGG region of the opposite monomer, this nucleotide binding-induced fit effect is also likely to be important for NBD 'dimer' formation [90].

Rotation of the α subdomain: an allosteric mechanism of substrate-stimulated ATPase activity?

The α subdomain has long been thought to be involved in transmitting the energy released by ATP hydrolysis to the TMDs to effect substrate translocation [13]. Strong conservation in the packing core, but not the surface of the α subdomain is consistent with the idea that this region contacts the cognate TM domains [90]. Indeed, experimental studies of the maltose permease using protein fusions [99, 100], chemical cross-linking [101], trypsin proteolysis [102] and mutagenesis [103] indicated that the α subdomain of the maltose permease NBD, MalK interacts directly with the TMDs and, through this interaction, effects conformational changes in the TMDs in response to ATP binding and hydrolysis.

Superposition of all available ABC transporter NBD structures by r.m.s. fit of the structurally conserved core of the fold (β-strands 1, 2, 3, 4, 6 and 7, and P-loop helix 1) reveals that while the core domains can be overlayed with an r.m.s. deviation of less than 1.3 Å, the α subdomain shows much greater positional variation in relation to the ATP-binding core, and appears to rotate about loops at its N- and C-termini [59, 104]. The rotation of the α subdomain is perhaps best illustrated by comparison of the wild-type MJ0796 ADP-bound monomer structure with the monomer structure from the ATP-bound MJ0796 E171Q dimer. Figure 6 shows a superimposition of these two structures using r.m.s. fit of the structurally rigid core of lobe I. This reveals that in the ADP-bound structure, the Cα atom of the conserved serine of the signature sequence is nearly 8 Å away from its position in the ATP-bound dimer structure. Comparison of the two structures shows that the α subdomain has essentially preserved its structure in this movement. The rigid body rotation of the α subdomain has been effected largely by changes in the backbone conformation of loops at its N- and C-termini [55].

Although it is possible that the rotation of lobe II (α subdomain) with respect to lobe I may be a crystallographic artefact, due to lack of oligomeric stabilisation of this re-

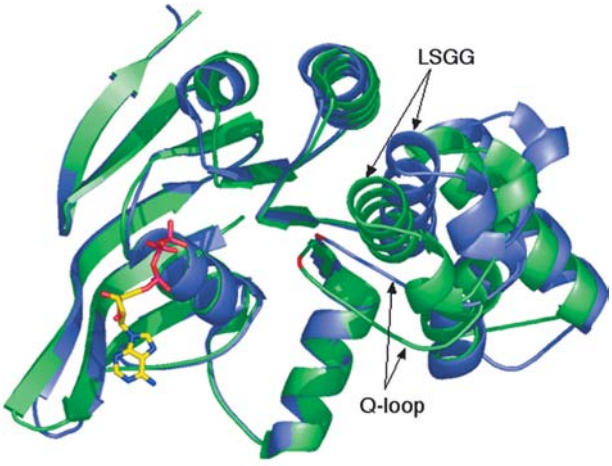

Figure 6. α subdomain rotation-superimposition of ADP-bound MJ0796 monomer (1F30, coloured lavender) with the ATP-bound monomer from the dimer structure (1L2T, coloured green) illustrating the rotation of lobe II (α subdomain, at right of picture) in relation to lobe I. Structures were aligned by r.m.s. fit of the Cα atoms of residues in β-strands 1, 2, 3, 4, 6, 7 and helix 1. The Q-loop and the LSGG region are indicated in both structures. ATP is shown in stick form and colour coded as in figure 3B. The backbones of the conserved glutamines (Q90) are coloured red, illustrating the movement of this residue in relation to the γ-phosphate.

gion in the monomer-only structures [46], there is growing evidence that the observed rotation indeed represents an important aspect of the NBD mechanism. Our MD simulations of the HisP monomer show that the residues in HisP equivalent to those identified by structural analysis as effecting the α-subdomain rotation exhibit high torsion angle fluctuations, consistent with our proposal that these residues act as hinges of a conformational transition [55]. The importance of the loop at the C-terminus of the α domain, known as the Pro-loop [95], to the functioning of the transporter has been revealed by studies of transporters that have mutations at this point. Mutations in this loop in CFTR (cystic fibrosis transmembrane regulator) are known to cause cystic fibrosis [104], and TAP1 mutation R659Q, which occurs on the equivalent loop, reduces peptide transport 50% [105]. The TAP1 mutation was proposed to affect the coupling of hydrolysis to peptide transport by TAP, due to its likely ability to affect the flexibility of the α subdomain [105]. Mutation P172L in the HisP Pro-loop has been found to release HisP from the regulatory control of the TM subunits, resulting in constitutive ATPase activity [43]. Thus, these data are consistent with our idea that the rotational flexibility of the α subdomain is integral to the control of ATP hydrolysis by the TMDs [55]. Szakács et al. [106] have suggested that control of the rotation of the α subdomain by the TMDs may constitute an allosteric mechanism by which substrate binding stimulates ATP hydrolysis. Consistent with this idea, recent experiments suggest that the TMDs control ATPase activity in response to substrate binding by mediating the interaction of the LSGGQ, located in the α subdomain, with the active site of the opposite NBD in an NBD dimer. Data from studies of P-gp indicated that substitutions in the LSGGQ sequence resulted in miscommunication between the TMDs and the NBDs [106]. Fluoroscopic measurements from experiments with the maltose transport complex indicated differences in catalytic site solvent accessibility between wild-type and mutant transporters in which ATP hydrolysis was independent of substrate binding to a periplasmic maltose binding protein, also consistent with the notion that substrate binding controls engagement of the LSGGQ with the active site [107].

Another recent study of the maltose permease complex [108] investigated the functional effects of a monoclonal antibody whose epitope overlapped residues in the α subdomain, which are proximal to the opposite monomer in a Rad50-like MalK NBD dimer [109]. Interestingly, the presence of ATP diminished the accessibility of the epitope in soluble MalK, but did not affect its accessibility in the full transporter complex. Since NBD dimer formation is likely to reduce the accessibility of the region encompassing the epitope, we observe that these data are consistent with the notion that interaction of the LSGGQ with ATP bound in the opposite NBD monomer is retarded in the full maltose transporter complex in the absence of substrate.

A recent investigation of P-gp used covalent derivitisation of P-gp N508C, a mutation which also maps to a region of the α subdomain proximal to the NBD:NBD interface [110]. Derivitisation significantly reduced the maximal velocity of drug-stimulated ATP hydrolysis while not affecting nucleotide binding or altering the substrate specificity profile of drug-stimulated ATPase activity. This indicates that the covalent modification does not perturb the signal route by which substrate binding in the TMDs stimulates ATP hydrolysis, but rather a step subsequent to nucleotide binding [110]. Significantly, vanadate trapping, but not nucleotide binding, reduced the accessibility of N508C. Together with the structural data, this finding is consistent with the idea that the region of P-gp N508, and hence, in view of its apparent internal structural rigidity, the α subdomain, undergoes a conformational change in the catalytic cycle involving engagement of the LSGG with the opposite monomer, and that this represents the endpoint of the signal path by which the TMDs stimulate ATP hydrolysis.

Finally, recent cysteine cross-linking experiments with P-gp indicate that the proximity of the LSGGQ region varies with respect to the P-loop of the opposite monomer during the catalytic cycle, and that substrates that stimulate ATP hydrolysis also increase chemical cross-linking between residues in these two regions [111]. These data strongly support a model in which the TMDs control conformational transitions of the α subdomain in response to substrate binding. This may be achieved by controlling the rotation of the α subdomain, since only the inwardly

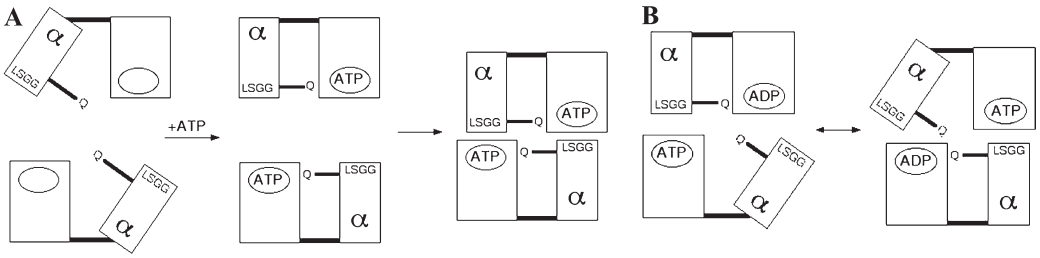

Figure 7. Models of NBD dimer interactions. Each NBD monomer is represented by two rectangular boxes, corresponding to lobes I and II connected by a thick bar. Lobe II is indicated by the symbol 'α' and also contains the letters 'LSGG' to indicate the signature sequence. Lobe I contains an oval representing the catalytic site; the letters 'ATP' within this oval indicate bound trinucleotide. The letter 'Q' indicates the conserved glutamine and the associated thick bar represents the Q-loop. The rotation of the α subdomain (lobe II) is represented by the two different orientations of the lobe II box; the closed conformation is indicated by the closer proximity of the 'Q' to the bound ATP. (*A*) Schematic representation illustrating a model in which binding of ATP to the NBD induces NBD dimer formation. (i) two nucleotide-free NBD monomers with their respective α subdomains rotated outward from the catalytic site; (ii) ATP has bound to each NBD monomer, and interaction of the conserved glutamine with the γ-phosphate has induced the inward rotation of their respective α subdomains; (iii) formation of NBD 'sandwich' dimer with two ATP molecules. (*B*) Schematic illustration of an alternating ATP hydrolysis mechanism of the NBD dimer. Nucleotide exchange occurs when the α subdomain is outwardly rotated. ATP hydrolysis occurs when the LSGGQ contacts ATP in the inwardly rotated conformation of the α subdomain. The two NBD monomers remain in contact, with hydrolysis and rotation of the α subdomains occurring alternately in each monomer.

rotated 'closed' conformation is correctly oriented to enable engagement of the LSGG with ATP bound in the opposite active site in the NBD dimer [55, 104].

A conserved glutamine: the signaller?

A phylogenetically invariant glutamine residue (Q100 in HisP) is located at the C-terminus of β strand 5 (fig. 4). This residue is followed by a flexible loop known as the Q-loop [44] or γ phosphate linker [104], which joins the ATP-binding core (lobe I) to the α subdomain (lobe II). Comparative analysis of the NBD crystal structures suggests that the conserved glutamine 'switches' in and out of the active site during the catalytic cycle, engaging the Mg^{2+}ATP-bound active site, and disengaging and moving away subsequent to ATP hydrolysis [55, 104]. This idea is consistent with the mechanism of other P-loop proteins, such as G proteins and the F_1-ATPase, in which the Mg^{2+} coordinating protein ligand equivalent to the conserved glutamine is situated on a switch region that mediates oligomeric interactions in response to nucleotide binding and hydrolysis [112, 113].

Our MD simulations of HisP [55] suggest that the Q-loop may undergo conformational switching transitions, and this idea is supported by the structural variability of this region amongst the ABC crystal structures. The structural data also indicate that the Q-loop forms part of the NBD:TMD interface, making contacts with the ICDs in both the Eco-MsbA and BtuCD structures [55, 58]. The high sequence variability amongst ABC transporters of the N- and C-termini of the Q-loop (fig. 4) is consistent with our notion that these segments are involved in subunit-subunit interactions [55], and there is also biochemical evidence that the conserved glutamine and the Q-loop are involved in interdomain communication [101, 114, 115]. In summary, it appears that through its interactions with the nucleotide and catalytic Mg^{2+}, the conserved glutamine may signal between the active site and the TMDs, possibly by moderating conformational transitions of the Q-loop, and thereby TMD:NBD interactions [55].

Yuan et al. [104] have suggested that interaction of the conserved glutamine with the γ-phosphate of the bound nucleotide mediates the rotation of the α subdomain into the 'closed' conformation, in which its LSGGQ is correctly oriented to engage the opposite catalytic site within the dimer. Since it seems clear that both the conserved glutamine and the LSGGQ must engage an ATP-bound catalytic site, these ideas suggest that ATP binding may mediate formation of a dimer such as that observed for MJ0796 [48] and Rad50cd [44], with ATP bound in both active sites. This idea is illustrated schematically in figure 7A. Interestingly, a recent crystal structure of the GlcV monomer with bound ATP analogue [116] shows that the conserved glutamine can engage the catalytic Mg^{2+} when the α subdomain is rotated outward, and it was suggested that the glutamine may not mediate α subdomain rotation [116]. The GlcV structure suggests to us that the flexibility of the Q-loop may function, at least in part, to accommodate the outward rotation of the α subdomain while the glutamine remains engaged in the active site. Thus, the nature of the relationship between rotation of the α subdomain and Q-loop transitions is uncertain at present.

ATP hydrolysis: processive or alternating?

In order to mediate the active translocation of substrates across cellular membranes, ABC transporters must couple conformational changes in the NBDs, powered by the

free energy released by ATP hydrolysis, to conformational changes in the TMDs. However, the manner in which the energy of ATP hydrolysis is coupled to substrate transport is as yet unclear. Recently a model for this process was proposed in which the energy of ATP binding drives the formation of an NBD 'sandwich' dimer with two ATP molecules bound at the dimer interface, similar to that observed in the MJ0796 E171Q dimer structure [44, 48, 117, 118], thus representing the 'power-stroke' of the transport cycle. In this scheme, hydrolysis of ATP and release of products generates electrostatic and conformational changes that drive the monomers apart [48, 118]. Both dimer formation and separation processes are proposed to provide opportunities to couple free-energy changes to solute transport [48, 118].

Recent crystal structures have further supported the existence of an NBD sandwich dimer and the notion that the NBDs dimerise and subsequently move apart in the catalytic cycle. Crystal structures of nucleotide-free and ATP-bound forms of the maltose permease NBD MalK suggest a tweezers-like movement of the NBDs in which ATP induces formation of a double ATP-bound sandwich dimer and nucleotide hydrolysis and product release results in separation of the NBDs [119]. An NBD sandwich dimer containing two ATP molecules, structurally similar to the MJ0796 E171Q dimer, has also recently been observed in a crystallographic analysis of the GlcV E166Q mutant [120], in which the conserved glutamate following the Walker B aspartate, the putative catalytic base, was also changed to glutamine (equivalent to MJ0796 E171Q).

An interesting consequence of the sandwich dimer mechanism, however, lies with respect to the ATP hydrolysis cycle of the transporter. Either the NBD monomers must move apart subsequent to ATP hydrolysis in one site only, thus wasting the binding energy of the nonhydrolysed ATP, or there is a processive, asymmetric pattern of ATP hydrolysis, in which the first ATP is hydrolysed with ATP in the second site, and the second ATP is hydrolysed while the first active site either contains hydrolysis products or is empty. Indeed, a processive 'clamp' model for the ATPase cycle was recently proposed, based on biochemical data from the isolated NDB of MDl1p, a mitochondrial TAP-like half-transporter from *Saccaromyces cerevisiae* [121]. Incubation of the wild-type MDl1p NBD with orthovanadate or beryllium fluoride and MgATP induced the formation of stable dimers, which contained two ADP molecules, shown to be produced exclusively by ATP hydrolysis in the NBD active site. MDl1p NBD mutant E599Q, in which the conserved glutamate following the Walker B aspartate was changed to glutamine, similarly to MJ0796 E171Q formed stable dimers containing two ATP molecules in the presence of MgATP. Interestingly, the E599Q mutant slowly hydrolysed ATP at 30 °C, and a stable dimer containing one ADP and one ATP molecule was identified under limiting concentrations of MgATP. Together these data were interpreted as indicating that binding of ATP to two NBDs induces NBD dimer formation and that ATP hydrolysis occurs in a sequential processive mode.

We believe, however, that the central role that the γ-phosphate appears to have in determining the global conformation of the NBD [90], and its likely influence on the activity of the opposite catalytic site, argues against the notion of an asymmetric sequential hydrolysis mode, and thus the processive clamp model of NBD dimer formation and ATP hydrolysis. In addition, we note that the NBD sandwich dimer has only been observed for mutant or incomplete NBDs, or with wild-type NBDs in presence of transition-state analogues, or in the case of MalK [119], in the absence of magnesium. Significantly, ATP analogues such as AMPPNP and ATPγS do not induce NBD dimer formation [118, 121]. Whilst it has been suggested that these analogues may distort the dimer interface, thus prohibiting dimer formation [118], the crystal structure of the Rad50cd dimer, which has a dimer interface identical in its essential elements to that of the ABC transporter NBD dimer, contains AMPPNP [44]. Thus, we suggest, the physiological existence of the double ATP-bound NBD sandwich dimer is as yet uncertain.

An alternative scenario is that the NBDs remain in close proximity and ATP hydrolysis occurs in a continuous alternating cycle in which ATP is hydrolysed when the opposite site contains products or is empty. This idea is illustrated in figure 7B. There is biochemical evidence that ATP is bound in one active site while ADP is bound in the opposite site for P-gp [122, 123], Mdl1p [121] and MutS [124]. Moreover, in P-gp, one active site is empty in the vanadate-trapped transition state [117, 125], consistent, we believe, with the idea that products are expelled from the active site upon hydrolysis in the opposite site. In the alternating mechanism, nucleotide exchange could occur upon outward rotation of the α subdomain and disengagement of the LSGGQ with the active site, as suggested by Yuan et al. [104]. This leaves the problem of how the NBD dimer could be stabilised with only one nucleotide bound, although it is possible that this could occur through interactions with the TMDs [104]. This alternating mode also appears to better accommodate the expected negative cooperativity between the NBDs, since in this mechanism there is always an asymmetry in the conformation of the two monomers in the NBD dimer (fig. 7B).

Energy transduction: conformational gearing to active site geometry?

In examining the coupling of ATP hydrolysis to productive conformational changes in ABC transporters, it is relevant to consider data which show that the vanadate-

trapped state of the transporter is in a global conformation that differs significantly from either the ATP-bound state or the ADP + Pi bound state. The vanadate-trapped species is presumed to mimic the pentacoordinate transition state of ATP-hydrolysis, which differs from the ground state only in that atoms of the γ-phosphate and the attacking nucleophile have moved by 1–2 Å or less from their positions in the ground state [126]. A number of studies of vanadate-trapped ABC transporters have indicated that in going from the ground state to the transition state, the transporter undergoes a global change resulting in a unique 'transition state' conformation in which, among other changes, the accessibility of the active site(s) and/or the substrate binding site(s) is significantly altered [86, 110, 127–129]. We have observed that, together, these data are consistent with the idea that significant global conformational changes in the protein are directly 'geared' to the relatively small changes in the geometry of the active site that occur upon formation and collapse of the transition state of ATP hydrolysis [55].

We have suggested previously [47] that in ABC transporters, the transduction of the chemical free energy of ATP may be achieved in a manner related to that proposed to occur in myosin [130]. A variety of transition state analogues are known to bind to the active site of myosin, and crystallographic evidence suggests that each differs slightly in its geometry [131], corresponding to different intermediate stages of the hydrolysis reaction [132]. Park et al. [130] found a direct correlation between the nature of the transition-state analogue trapped in the myosin active site and the closure of a cleft in the myosin S1 subfragment, at the bottom of which is found the active site. It was suggested that coupling between active site geometry and the S1 cleft closure was mediated by direct interactions between a highly conserved active site glycine residue and the γ-phosphate. Significantly, studies of P-gp using various transition state analogues also indicate differential effects upon the global conformation of the transporter [133, 134].

With these ideas in mind, we have proposed an alternative model for the transduction of the free energy of ATP in ABC transporters [47]. In this model, the LSGG mediates the coupling or 'gearing' of active site geometry to global conformational changes which effect substrate translocation. On the basis of MD simulations and analysis of X-ray structures [55], we further proposed that direct interactions between either or both of the LSGG serine and second glycine with the γ-phosphate and attacking nucleophile produce, upon formation and collapse of the transition state, conformational changes in these residues that alter, in turn, the conformation of the upstream $\alpha 4''$ region. These conformational changes in the $\alpha 4''$ region are thence propagated to other regions of the protein, ultimately resulting in substrate translocation.

What is the NBD : TMD interface?

Comparative analysis of the ABC transporter NBD structures reveals two regions of heightened structural variability that occur on one edge of the monomer (fig. 3B) and which correspond to regions of high sequence variability (α-helix 1 to α-helix 5; fig. 4). The heightened variability of these regions suggests that they may be involved in interactions with the TM domains, which similarly display higher sequence variability, and indeed there is evidence that this is the case. The first of these putative TMD:NBD interfaces encompasses the C-terminus of β-strand 4 and the N-terminus of β strand 5 (fig. 3A, B). In the BtuCD structure, residues in the equivalent regions make contact with residues from the TM domains, and similar interactions are predicted in Eco-MsbA based on modelling of the missing N-terminal NBD residues [55–58]. Notably, in the structures of the more remotely related MutS [97, 98], the equivalent region forms an extensive interface with a structurally and functionally distinct subdomain involved in DNA binding. Instability of the equivalent region in our MD simulations of HisP suggested that it may require interaction with other parts of the protein in order to adopt a stable conformation [55]. Significantly, mutations in the equivalent region in HisP have been found to disrupt signalling between the TMDs and the NBDs in the histidine permease [43].

The second putative NBD:TMD interfacial region includes the loop joining helices 3 and 4 within the α subdomain, which is one of the most structurally diverse in ABC transporter NBDs (fig. 4) [55]. The equivalent loop makes direct contacts with the TMDs in the Eco- and VC-MsbA structures and also in the BtuCD structure, although details of these interactions differ markedly between these structures. Biochemical evidence from experiments with the maltose permease suggests that this loop interacts with the TMDs and also undergoes conformational changes upon ATP binding and/or hydrolysis [102]. High backbone dihedral angle transitions and positional fluctuations of residues within this loop were found during our MD simulations of HisP**,** consistent with a role in intersubunit contacts, and we have suggested that this region may transmit conformational changes generated by ATP hydrolysis to the TMDs to effect substrate translocation [55]. In relation to this idea, it is interesting to note from a recent crystal structure of Rad50 [135] that the large antiparallel coiled coil that binds DNA in Rad50, and is crucial to oligomeric interactions and ternary complex formation, appears, in evolutionary terms, to be an extension or development of the region equivalent to the variable loop joining the helices 3 and 4 in the α subdomain of ABC transporters.

The D-loop: central switch of the NBD mechanism?

β-strand 6 forms part of the core subdomain β sheet (fig. 3) and corresponds to the Walker B consensus motif [87]. A highly conserved aspartate residue at its C-terminus is involved in the coordination of the catalytic magnesium ion (fig. 2). In ABC ATPases, the Walker B aspartate is followed immediately by a highly conserved glutamic acid residue (figs 2, 4), postulated to act as the catalytic base of the hydrolysis reaction [43], and thence by a conserved six-residue loop known as the D-loop [44]. While the conserved glutamate at the N-terminus of the D-loop interacts with the active site within the monomer, a backbone oxygen atom near the C-terminus of the D-loop interacts with the putative nucleophilic water in the active site of the opposite monomer in both the Rad50cd and MJ0796 E171Q dimers.

The D-loop corresponds, in terms of its position in the protein fold, to an important switch region in other P-loop ATPases such as myosin and G proteins [87, 136]. Like the corresponding switch regions in other P-loop ATPases, the ABC D-loop shows significant structural variation among the crystal structures, and this implied hyperflexibility is supported by MD simulations, which revealed heightened torsion angle and positional fluctuations for residues within this region in HisP [55]. In view of its potential ability to influence hydrolysis in both catalytic sites of the dimer, we and others have suggested that the D-loop may be involved in communication between the catalytic sites [44, 47, 92]. Changes in the conformation of the D-loop are able to affect the orientation of key residues in the catalytic sites, particularly the putative catalytic base, thereby enabling and/or preventing hydrolysis in each active site [92], and we thus suggest that the D-loop appears well suited to mediate negative allostery between the ABC NBD monomers.

What is the role of the conserved active site histidine?

In all ABC transporter NBD crystal structures, with the exception of TAP1, a conserved histidine residue occurs at the C-terminus of β strand 7, and this residue is followed by a short, approximately six-residue α helix (figs 3, 4). This region is known collectively as the H-loop [44] and corresponds approximately to a region earlier referred to as the switch region [7]. Comparison of the X-ray structures of ABC NBDs reveals that the position of the histidine, and of the immediately downstream helix 7, is quite variable with respect to the central β sheet of the nucleotide-binding core, with this region appearing to pivot about Cα atoms at its N- and C-termini, a notion supported by our MD simulations of HisP, which reveal hinges at these points [55]. The function of the conserved histidine is not known. Mutation of this residue to arginine in both HisP [137] and in MalK [138] resulted in complete loss of ATPase activity of the purified mutant NBD subunits, and loss of transport function in the complete transporter. The location of the histidine within the catalytic site, its high conservation and the effects of its mutation indicate clearly that it has a crucial role in transporter function. Indeed, we observe that in the catalytic site of the MJ0796 E171Q dimer, the histidine is well positioned to shield the attacking nucleophile from the electronegative charge of the γ-phosphate oxygen atoms (fig. 2), possibly indicating a role in catalysis for this residue.

Summary and overview: the big picture

The γ-phosphate of the bound nucleotide is the central focus of the ABC transporter NBD both structurally and functionally. The γ-phosphate plays a crucial organising role, altering and stabilising the conformation of the key P- Q-, D- and H-loop regions and thereby influencing subunit-subunit interactions within the transporter complex. While their exact roles are unknown, the Q-, D- and H-loops appear to be important switch regions of the NBD mechanism, with the Q-loop likely to mediate signalling between the TMDs and the NBD active sites, and the D-loop to influence the catalytic activity and intercommunication of the active sites. The α subdomain (lobe II) appears to undergo a mechanistically important rotational movement in relation to lobe I, and this rotation may influence ATP hydrolysis by correctly orienting the LSGGQ signature sequence to enable NBD 'dimer formation'. The engagement of the LSGGQ within the α subdomain with the ATP-bound catalytic site in the opposite monomer is essential for ATP hydrolysis and appears to be determined by the TMDs, thus representing a possible allosteric mechanism by which the TMDs control ATP hydrolysis. It has been suggested that the conserved glutamine and the Q-loop mediate the rotation of the α subdomain in response to ATP binding, but the exact nature of Q-loop transitions and their relationship to lobe II rotation is unclear.

Two models exist for the transduction of the free energy of ATP to conformational work in ABC transporters. In the first of these, ATP binding in both NBDs promotes dimer formation, and the energy of ATP binding ultimately drives substrate translocation. In the second model [55], the NBDs remain pre-oriented with one or both catalytic sites being engaged and completed by the LSGGQ of the opposite monomer at all times. The α subdomains alternately rotate during cycles of engagement/disengagement with the catalytic site and concomitant ATP hydrolysis, in response to signals from the TMDs. The free energy of ATP is harnessed by conformational gearing to active site geometry during the formation and collapse of the transition state of ATP hydrolysis.

Although the ABC transporter NBD appears to contain a complex array of switches, hinges and levers, we suggest that these are likely to be integrated into patterns of concerted conformational switching between a small number of global states. The rotational symmetry of the NBD dimer and the nature of the interactions between the two NBDs in the dimer, in which the N-termini of the helices immediately downstream of the D-loop and of the conserved histidine interact across the dimer interface, suggest that as one monomer switches in one direction the other switches in the opposite direction. This idea is consistent with a functional relationship known as 'half-of-the-sites reactivity' [139], in which each monomer of a dimer operates 180 degrees out of phase with the other; when active, one monomer suppresses the activity of the other. Half-of-the-sites-reactivity has been suggested for P-gp on the basis of kinetic data [93] and may be an appropriate conceptual framework with which to approach the understanding of ABC transporter function.

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