ABC transporters, mechanisms and biology: an overview

I. Barry Holland¹

Université Paris-Sud, Institut de Génétique et Microbiologie, UMR CNRS 8621, Bâtiment 409, 91405 Orsay Cedex, France

Abstract

This chapter concentrates mainly on structural and mechanistic aspects of ABC (ATP-binding cassette) transporters and, as an example of the physiological significance of these proteins, on lipid transport, vitally important for human health. The chapter considers those aspects of ABC transporter function that appear reasonably well established, those that remain controversial and what appear to be emerging themes. Although we have seen dramatic progress in ABC protein studies in the last 20 years, we are still far from a detailed molecular understanding of function. Nevertheless two critical steps – capture and release of allocrites (transport substrates) involving a binding cavity in the membrane domain, and hydrolysis of ATP by the NBD (nucleotide-binding domain) dimer – are now described by persuasive and testable models: alternating access, and sequential firing of catalysis sites respectively. However, these need to be tested rigorously by more structural and biochemical studies. Other aspects considered include the level at which ATP binding and dimer activation are controlled, the nature of the power stroke delivering mechanical energy for transport, and some unexpected and intriguing differences between importers and exporters. The chapter also emphasizes that some ABC transporters, although important for elimination of toxic compounds

¹email barry.holland@igmors.u-psud.fr
(xenobiotics), are also increasingly seen to play crucial roles in homeostatic regulation of membrane biogenesis and function through translocation of endogenous allocating such as cholesterol. Another emerging theme is the identification of accessory domains and partners for ABC proteins, resulting in a corresponding widening of the range of activities. Finally, what are the prospects for translational research and ABC transporters?

**Introduction**

I shall try to review briefly in this introductory chapter which basic aspects of ABC (ATP-binding cassette) transporter function appear reasonably well established, which aspects remain controversial and what I consider are some of the emerging growth points. However, there are now more than 23 000 entries for ABC transporters in PubMed, 1600 in the first half of 2010 already, and therefore the field is much too big to cover everything in an overview. Consequently, I chose to concentrate mainly on structural and mechanistic aspects and, as an example of the physiological significance of these proteins, on lipid transport, which is vitally important for human health. Although we have made enormous progress in identifying and analysing ABC proteins in the last 20 years, our understanding of most areas is still far from detailed and therefore I also chose, where possible, to identify questions arising from what we know, rather than attempting too many solutions.

**Why do ABC proteins never cease to be fascinating?**

ABC proteins have fascinated me since the 1980s with the first realization that the *Escherichia coli* proteins HisP (histidine uptake), UvrA (DNA repair), HlyB (Type I protein export) and the newly identified human MRP (multidrug-resistance protein) Pgp (P-glycoprotein) (ABCB1) were all members of the same protein family [1]. These proteins evidently contained conserved sequence motifs in the ATPase domain, so obvious that they could be spotted with a single rapid glance. ABC proteins continue to be no less fascinating, since new ones are constantly being discovered, and, despite the obvious conservation of the ABC ATPase, the more we learn, the more we find both subtle and possibly major differences (in some cases) in the way they actually work. In addition, the atomic-level structures of the dimers of the NBDs (nucleotide-binding domains), in particular, have revealed exquisite inter- and intra-molecular communication or signalling mechanisms that I certainly find surprising and unexpected. Why ABC proteins are still so appealing to study, however, is not least from our growing understanding of their physiological functions, which are essential to human wellbeing. Following some earlier initial reticence in the field, human ABC transporters are now perceived as even more important in the movement of endogenous compounds, rather than simply cleaning machines expelling toxic molecules.
Basic properties

An ABC protein is defined by the presence of a P-type ATPase (the ABC or NBD). This is composed of a RecA-like, mostly β-strand, subdomain containing the highly conserved Walker A and B motifs, connected by the Q-loop to the α-helical or signalling domain, which contains other conserved motifs, the C-loop (signature motif, LSGGQ), H-loop (switch region) and the D-loop (consensus motif, SALD). However, ABC proteins have essentially evolved in two forms, one as a tandem cassette, containing insertions of variable nature and size that interrupt both cassettes (e.g. UvrA [2]). These insertions must be ‘removed’ by conformational reorganization in order to permit close association of the two subdomains to form a classical ABC-catalytic site. Finally, together with appropriate partners, these ABC proteins, like UvrA, bind to DNA to repair lesions. Indeed, the great majority of these ABC ATPases appear to function with nucleic acids to promote recombination/repair or condensation and cohesion of DNA or, for example, mRNA translation into polypeptides (see Chapter 2). These are the cytoplasmic ABC ATPases (sometimes erroneously called ‘soluble’ ATPases), whereas the second and much larger form are the ABC transporters, the major subject of this Essays in Biochemistry volume, where the core ABC associates with a TMD (transmembrane domain). These constitute a large superfamily present in all three kingdoms of life, which in total promote the transport of a staggering variety of ions and molecules across membranes. Indeed, to define the range of transport substrates, it would probably be simpler to indicate which molecules are not translocated by ABC transporters.

The ABC transporters in turn are divided into two distinct functional types, ‘importers’, apparently restricted so far to prokaryotes, and ‘exporters’, present in all forms of life. The importers are characterized by having the TMD and ABC (NBD) on separate polypeptides and a requirement for an SBP (solute-binding protein) that delivers the transport substrate to the TMDs for translocation. The TMD, reflecting the component that is involved in the primary specificity for allocrites in both importers and exporters, is, unlike the ABC domain, poorly conserved. Since the true substrate of these enzymes is, of course, ATP, for the molecules that are transported, I prefer to use the word we coined, allocrite in preference to (transport) substrate [3].

A functional transporter is composed of two TMDs and two NBDs, although either of these can be identical or different, i.e. encoded by different genes. Both the TMD and ABC domains form dimers in the transporter. Thus ATPase activity of an ABC transporter requires ‘head-to-tail’ NBD dimers, in which the active catalytic entities are actually ‘composite sites’ formed from residues contributed from both monomers, including the association of the Walker A motif of one subunit with the signature motif of the other (see [4,5] for general reviews). Exporters in prokaryotes occur as half-transporters with a single polypeptide containing one TMD and one ABC, with the formation of homo- or, in some cases, hetero-dimers, whereas in eukaryotes, many...
ABC transporters have non-identical halves fused in the single molecule. Surprisingly, several classes of eukaryote transporters contain NBD dimers with one consensus composite site (catalytically active) and one degenerate (largely inactive) composite site, thus strongly suggesting that a stoichiometry of two ATPs per molecule transported is not obligatory for ABC transporters.

**Major advances in structures of transporters: alternating access model**

Over the last 8 years, we have seen dramatic advances in our understanding of the fundamental mechanism of allocrite translocation with the publication of several crystal structures of intact importers and exporters, including one mouse homologue (Abcb1a) of Pgp (ABCB1). These structures (reviewed in [6]), even from a very limited number of examples so far available (five importers and three exporters), have revealed a TMD structure unique to exporters and, in addition, two distinctive folds for two subclasses of importers. These are Class 1 (usually 12–14 TMDs, one allocrite-binding site and a type 1 SBP) and Class 2 (usually 20 TMDs, accommodating larger allocrites, plus a type 2 SBP) ([6] and see Chapter 5). Notwithstanding the limited number of intact transporter proteins so far analysed (eight different proteins represented by very few of the different possible intermediates in the transport or catalytic cycles), two major features have emerged that are likely to prove of universal significance. Thus all of the structures reveal a significant cavity formed between the NBDs and the outermost regions of the TMDs, apparently constituting an allocrite-binding chamber. Moreover, the structures have provided two alternative configurations of this region formed by the TMDs, outward-facing (i.e. open to the exterior) or inward-facing (open to the cytoplasm). This now forms the basis of the proposed ‘alternating access model’ for a conserved transport mechanism for all types of ABC transporter [6,7]. The transport substrate or allocrite is first strongly bound within the TMD cavity. Then, as the topography of the TMDs changes, dependent upon ATP binding/hydrolysis, a reduced binding affinity allows release of the allocrite from the rearranged cavity, which now faces the external or the cytoplasmic side of the membrane, for exporters or importers respectively. This proposed mechanism is both pleasing and plausible, confirming the much earlier generalized prediction for membrane translocation by Jardetzky [8]. Thus the alternating access model represents an ‘airlock’-type mechanism, rather than a ‘transport pathway’, for shuttling a variety of molecules across membranes. In simple terms, for an importer, the model says that, when the NBDs close (dimerize), the outward-facing cavity is inverted and the allocrite is translocated. Nevertheless, a note of caution is advised before uncritically extrapolating this model to cover all ABC transporters. Thus there are still relatively few structures of intact complexes available and, of these, only one, the importer MalFGK₂ complex, is represented by the stages of the transport cycle, with or without nucleotide [9,10]. Moreover, for exporters, the model
of alternating access is based primarily on the comparison of the Sav1866 drug exporter plus ATP with the ModABC importer without ATP [11]. Finally, it is also important to note that importers crystallized in the absence of nucleotide can nevertheless be captured with the TMDs in the outward- or inward-facing mode. This provides some indication of the ‘plasticity’ of the ABC transporter molecule and possible difficulties in retaining true intermediates in crystal structures.

Interestingly, the results from structural studies already indicate clear differences between importers and exporters. Thus, in contrast with importers, long helices extend from the TMD well beyond the membrane bilayer in exporters. As a further distinguishing property of exporters, two of these helices, compared with only one in importers, contact the signalling domain of the NBDs through specific ICLs (intracytoplasmic or intracellular loops), the so-called coupling helices, at the ‘transmission surface’ between the TMDs and NBDs. In addition, a surprising feature of the exporters is ‘domain swapping’, whereby each TMD, in the three-dimensional structure in the three examples analysed so far, is formed from the intertwining of helices contributed by both subunits, with the two ICL helices from each TMD contacting the NBD of the opposing subunit. In addition to these important features that distinguish importers and exporters, I think that we can anticipate further differences to emerge with additional novel folds in the TMDs as more examples of both types are crystallized. In particular, it is reasonable to expect ABC exporters with extended numbers of TMDs, such as ABCA1, or those involving allocrites at the extremes of the size range, such as small Cl⁻ ions [CFTR (cystic fibrosis transmembrane conductance regulator) channels] and large polypeptides (e.g. HlyB [12]) or polysaccharides [13], to provide additional structural folds. Intriguingly, in the latter two cases, regions on the NBDs themselves have been shown to bind the allocrite at some stage [13,14], suggesting possible major variations here in the execution of the translocation mechanism.

Towards a full understanding of the mechanism of action of ABC transporters

It is useful to consider what questions we must ask in order to obtain a full accounting of the mechanism of action of ABC transporters at the atomic level, with respect to both the catalytic and transport cycles and the intramolecular signalling that is involved.

What is the ground or resting state of transporters?

First, we still need to know the nature of the ‘ground or resting state’ of the transporter in vivo, i.e. lacking any allocrite. This presumably has the TMDs in a state ready to accept an allocrite, facing outward or inward for importers and exporters respectively, but what about the NBDs? On the basis of the structure of the Mal-complex from E. coli and the molybdate/tungstate transporter from Archaeoglobus fulgidus (AfModBC-A),
Oldham et al. [6] concluded that, in the ground state, the NBD monomers, restrained by the conformation of the TMDs, are positioned in close apposition, but still separated, forming a so-called open dimer. This is also indicated in all the known structures for other importers as well as the exporter Sav1886. In contrast, surprisingly and, to me, puzzlingly, the crystal structures of the exporters MsbA and Abcb1a (without nucleotide) are captured as a form in which the NBDs are separated by up to 30 Å (1 Å=0.1 nm) [15,16]. Moreover, these structures are supported by the analysis of two-dimensional crystals [17] and by some biochemical evidence (cross-linking and molecular probes [18]). However, perhaps these structures arise inadvertently due to the highly plastic nature of ABC transporters, especially exporters with long helices extending more than 15 Å beyond the membrane.

Importantly, the finding of an open dimer with an apparently water-filled interface, in all crystal structures in the absence of nucleotide, leaves open the possibility (if not the probability) that the high concentration of cellular ATP ensures that monomers with a consensus binding site are always loaded, but unable to form the (productive) ATP sandwich dimer that would otherwise immediately trigger catalysis. This has the merit that the ATP-monomer ground state would avoid non-productive or futile ATP hydrolysis. Importantly, in summary, we have to consider that, under physiological conditions, transporters in the ground state (absence of allocrite, if not ATP) might maintain a structural conformation of monomers, conferring a low affinity for ATP. Alternatively, if ATP is bound in the ground state, the helical lobes might be locked in a form unable to form productive dimers, or the loaded monomers are simply physically held apart.

**Signalling the presence of allocrite to the NBDs**

The next question is does the allocrite activate the catalytic cycle and, if so, how is this accomplished? Most models would favour the idea that availability of the specific allocrite dictates the activation of the catalytic cycle through its binding to the TMDs. From the current data, we would expect that this process would be initiated through conformational changes in the TMDs, but how are these conveyed to the NBDs? A reasonable presumption is that such movement of the TMDs would be transmitted, via the coupling helices (ICLs) present at the cytoplasmic termini of specific helices in each TMD. These ICLs form close contacts with the helical domain in a groove between the two lobes of the NBD. This region of interaction involves in particular the residues from the Q-loop extending to the adjacent downstream X-loop, overlapping the short (30 residues) SDR (structurally diverse region), which may present a unique structure in many different types of ABC transporter [19]. In the importers, a single coupling helix has been identified, carrying the conserved EAA motif located in the final TM helix, whereas, in contrast, in exporters, the two ICL helices do not contain conserved motifs.
What is the mechanism of ‘activation’ of the NBD dimer?
In crystal structures of isolated NBDs, the Q-loop forms part of a network of contacts involving the ATP γ-phosphate and is thought to be an important sensor of Mg²⁺-ATP (reviewed in [20,21]). Stimulated by binding of allocrite, which changes the disposition of the ICLs, the consequent movement of the strategically placed Q-loop could, in theory, result in alternative outcomes to promote the formation of active dimers (and catalysis), depending upon the nature of the resting state of the NBDs discussed above. Thus the Q-loop would click into a position that (i) increases the affinity of an unloaded NBD for ATP and therefore dimerization, or (ii) promotes the conversion of a loaded, but inactive, dimer into a functional dimer. These scenarios are compatible with the reported structures of importers and Sav1866 with ‘open’ dimers. In contrast, does it appear conceivable that modulation of the ICL–Q-loop interface would be sufficient to bring the reported widely separated NBD monomers in the structures of MsbA and AbcB1a (mouse Pgp) into close enough proximity to form catalytic dimers? It is a tough task, but it remains for the structuralists to convince us that these structures of splayed NBDs are authentic intermediates, and/or that the implied energy requirements for closure are feasible.

How is chemical energy converted into mechanical movement?
Logically, the next obvious, but far from simple, question is what are the consequences of binding and/or hydrolysis of ATP in ABC transporters? More specifically, what is the nature of the actual power stroke that converts chemical into mechanical energy, i.e. ATP action, leading to reversible reorganization of the TMDs and allocrite translocation? Structural analysis of isolated NBDs at high resolution (1.5–2.6 Å) has shown that ATP binding apparently induces a large (up to 20 Å) movement, in which the Q-loop is pivotal, of the helical lobe relative to the RecA-like domain (i.e. ‘inwards’ when ATP binds). This so-called RBM (rigid-body movement) was shown for at least one isolated ABC-NBD to involve hinges at the beginning of the Q-loop and at the terminus of the SDR region, just upstream of the LSGGQ motif [20]. This movement is essential for the formation of the ATP sandwich dimer interface of the opposing head-to-tail monomers and consequent catalysis [22]. Recalling that monomers in cells may always be bound to ATP (i.e. pre-loaded), we would have to conclude in that case that RBM would already have occurred before allocrite binding and therefore would not be available to generate a power stroke for transport. In this case, we must consider other possible power strokes, for example, generated in some way by dimerization itself. Alternatively, underlining the point made previously, if pre-loaded monomers in vivo are not only held apart, but also in a pre-RBM state (constrained by the conformation of an empty TMD chamber), then binding of allocrite could still generate an RBM-dependent power stroke. Similarly, fixation of allocrite could trigger ATP binding and RBM, if NBDs
are normally held in a ground state of low affinity for ATP. We require
in vivo experiments to test the possibility that such non-productive forms of
NBDs in resting state exist in reality. Interestingly, possible candidates for
inactive or latent forms may have been captured in the reported structures
of isolated HlyB and GlcV NBDs [19,23]. Finally, in my view, the question
relating to the nature of the initial conformational changes, i.e. responding
to allocrite binding, for the moment remains unresolved. However, we
can envisage with more confidence that the next step, ATP hydrolysis,
results in separation of the NBDs or at least opens up one ATP-composite
site (see below). Moreover, at this stage, the RBM should be available to
drive the helical lobe outwards to provide the power stroke to return (reset)
the low-affinity binding cavity in the TMD to the ground state, where the
high-affinity binding site would again be exposed to incoming allocrites, for
further import or export accordingly.

Still further puzzles to solve
Does that account for everything we need to know and explain? Certainly
not! The established view of, albeit a limited number of structural studies, as
described above, indicates that specific helices from the intertwined bundles
of helices that make up each TMD in exporters, interact with both NBDs.
However, the functional significance of this domain-swapping and cross-over
to opposing NBDs, which is evidently not necessary for importers, is not
clear and remains to be investigated. Similarly, the question of simultaneous
compared with alternate site hydrolysis of ATP, or more specifically, a
preferred ATP hydrolysis site in those ABC transporters such as TAP
(transporter associated with antigen processing) and CFTR, where one site
is degenerate, still remains not fully resolved. In fact, the alternating site
hydrolysis model, proposed by Alan Senior in 1995 [24] is supported by most
of the available biochemical evidence. This includes the recent important result
that in Pgp (ABCB1), where both sites are active, the two sites can nevertheless
display widely different $K_d$ values for ATP binding [25]. In addition, the fact
that alternate site hydrolysis necessitates structural asymmetry within the NBD
dimer, is indeed supported by recent evidence that even homodimeric NBDs
do display some detectable structural asymmetry [20,26,27]. Nevertheless,
no completely convincing view has emerged as yet to explain the functional
significance or advantage of the alternating site mechanism and what positive
role, if any, the degenerate site plays in transporters such as TAP or CFTR.
Other questions, which I personally feel still require detailed investigation,
include the precise sequence of events (simultaneous or sequential) leading to
phosphate, then ADP release and finally dimer half-openings or full separation
These events, involving the motifs such as the D-loop, which is as diagnostic
for ABC proteins as LSGGQ (but much less investigated), are certain to be
further exciting examples of inter- and intra-molecular signalling and associated
conformational changes at the atomic level. As far as I am aware, these have
only been investigated and described carefully in structures representing all conformational stages of the catalytic cycle of the isolated NBD of HlyB, which is required for export of large polypeptides. These structures revealed the possible use of hinges, bending residues and energy storage–release devices linked to conformational changes [20]. Finally, the basic mechanism of ATP hydrolysis itself remains controversial, with evidence for substrate-assisted catalysis, emphasizing the role for the histidine ‘switch residue’, or general base catalysis where the Walker B glutamate residue is prominent, or do indeed different ABC transporters use different mechanisms?

**Structural analysis: some perspectives**

With respect to further progress towards a consensus on mechanistic details, I note that Siarheyeva et al. [25] provide a very sharp reminder of the necessity, when doing biochemical or structural studies, of working with absolutely authentic (physiological) intermediates or ground states, if extrapolations to reality are to be achieved. This paper, as well as providing important new evidence for sequential catalysis, nicely illustrates the state of the field and its limitations. While X-ray crystallographic analysis remains an essential and informative tool, and clearly many more high-resolution structures at different stages of the mechanistic cycles will be needed, it is going to be very difficult always to ensure that an active transporter, in a clearly established valid intermediate stage, is being analysed. This is, of course, not only a problem in the ABC field. One possibility is to engineer mutants for analysis that might be predicted, from the large amount of available knowledge, to be blocked, for example, at critical points in the signalling pathway from allocrite binding to closing of the NBD dimer, and at different stages in the ATP-hydrolysis cycle. Obviously, in addition, in work concerning structural and functional analyses of any kind, the presence of detergent should be avoided if at all possible. This clearly invites some novel approaches to at least some aspects of structural analysis and a timely example of this has been recently provided by Alvarez et al. [28], using lipid bilayer nanodiscs for a functional analysis of the maltose transporter by EPR spectroscopy.

**ABC transporters: biological importance**

In contrast with the emphasis on structure and the basic underlying transport mechanisms considered so far, I wish now to shift to equally dramatic recent developments towards the understanding of the physiological importance of ABC transporters, in particular the transport of endogenous molecules and their relevance to human health.

**Specificity of ABC transporters: secretion of many endogenous allocrites**

Earlier studies of mammalian ABC transporters tended to focus on the role of ABC exporters as drug-resistance mediators, on the basis of the pioneering work of Victor Ling and colleagues (see [29]) concerning drug resistance in tumour cells and its linkage to increased expression of Pgp (ABCB1). This approach
was further developed by Piet Borst and Susan Cole and their co-workers with
the ABCC subfamily (MRPs) of multidrug transporters (for reviews, see [30]
and Chapter 10). These studies demonstrated transport of anti-tumour drugs
and analysed the underlying mechanism of drug extrusion in different systems,
including cell lines and vesicle preparations. However, subsequently, a now
rather-well-established theme is that ABC transporters export a wide variety
of endogenous allocrites associated with normal physiology. These include
important metabolites used as intercellular signalling molecules, illustrated by
the export of leukotrienes and prostaglandins by the ABCC subfamily (reviewed
in Chapter 10). Moreover, many mammalian ABC transporters are involved
in building membranes as well as cleaning them, while also acting as key
homoeostatic regulators of the structure and therefore function of the membrane
itself. Thus ABC transporters from classes A (see below), B (Pgp, ABCB1), C
(MRPs) and G (for reviews, see [31] and Chapter 14) have all been implicated
in lipid transport; cholesterol as well as phospholipids. Moreover, such transporters
can affect the disposition of lipids in membranes, involving possible flippase,
floppase and scramblase activities.

It is an interesting question how these different activities, apparently
displayed by the same transporter in some cases, plus an apparent ability to
totally expel drug molecules, can be consistent with the proposed alternate
access model in its simplest form. So far, concerning specificity for allocrite
binding to the TMDs, there is evidence of various kinds for multiple binding
sites, but otherwise we know little beyond the fact that the importers, with a
more restricted allocrite range per transporter, have, from the known structures,
smaller binding cavities than exporters with their more varied range of all-
ocrites. Similarly, we know relatively little about the entry points for allocrites
into the binding cavity, although lateral access points from the bilayer have been
proposed on the basis of structural studies. In fact, as excellently reviewed by
Nagao et al. [31] (and see Chapter 14), an ABC exporter may in reality encounter
different molecules (determined at least in part by their hydrophobicity/
hydrophilicity) in either the inner or outer leaflet for subsequent transport to
other destinations in the bilayer, or indeed secreted directly from cytosol to the
exterior. This, in short, suggests the presence of different entry/exit points into
the TMD cavity, for even the same transporter. To understand how this might
be achieved, we need to know precisely how the architecture of the TMD cav-
ity in crystal structures fits into the bilayer. It is also possible to envisage that
the availability of multiple apertures for entry/exit could depend upon potential
auxiliary or partner proteins, and protein interaction studies with given ABC
transporters would be needed to confirm these ideas.

Cholesterol transport and homeostasis
Lipid transport, and transport of cholesterol in particular, as described in
the compelling review by Quazi and Molday (Chapter 14), is now emerging
as one of the most important functions of human ABC proteins whose
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Dysfunction leads to disease. Although this process is proving extremely complex, it serves here as a good example of the importance of at least one vital ABC function related to lipid transport and how knowledge is advancing. Delivery of cholesterol from intracellular sites in the cytosol to the outer leaflet is crucial to the function of membranes, where cholesterol rafts in particular have a major influence on the activities of many membrane proteins, including ABC transporters themselves. Regulation of the membrane content of cholesterol is subject, as would be expected, to homoeostatic control and an excess of intracellular cholesterol in the absence of functional ABCA1 leads to Tangier disease. Illustrating the variability in design of ABC transporters, ABCA1 forms a specific complex, via its extracellular domain [32], with apoA1 (apolipoprotein AI) [a lipid-poor precursor of HDL (high-density lipoprotein)]. This allows excess cholesterol to be transferred out of cells and then, in a series of steps, transported to the liver, although the precise sequence of events is still controversial [31]. It apparently remains similarly unclear, however, whether transfer to apoA1 is coupled directly to export from the cytoplasm or from cholesterol-rich regions of the membrane.

Interestingly, a second ABC transporter, ABCG1, appears to work in tandem downstream of ABCA1, transferring in this case cholesterol to the 'good cholesterol' acceptor HDL itself [33]. Additionally, a third transporter, ABCB4, also secretes cholesterol, but in this case has evolved to transfer it to another acceptor: bile. These overall processes underline the complexities of cholesterol transport, but also illustrate the importance of the additional functions conveyed on ABC transporters by accessory domains: ABCA1 contains another accessory domain at the C-terminal implicated in autoregulation of expression. This addition to the list of accessory domains in ABC proteins with novel functions illustrates the growing interest in this area. These cholesterol studies also point out the necessity to transfer very hydrophobic compounds from the membrane by an ABC protein to a suitable acceptor, while also raising an interesting broader question that could be relevant to other ABC transporters. Thus if cholesterol, like drug molecules, is normally flipped to the outer leaflet by ABCA1 (as shown, for example, for the Pgp homologue MsbA [34]), competition for binding to apoA1 could determine whether the lipid transported by ABCA1 is sequestered into the membrane or is removed completely to the exterior. By analogy, it would therefore be tempting to think that drug removal from cells, following flipping by ABC transporters, would be rendered much more efficient if these drugs were likewise siphoned off from the outer leaflet by binding to dedicated proteins for delivery to the liver. This would avoid the rapid return of hydrophobic drug molecules to the membrane; however, there is no evidence so far for such a mechanism in drug transport that I am aware of.

Since it now seems clear that a major function of ABC transporters can involve biogenesis and homoeostatic control of the membrane itself, this raises a potentially serious complication in identifying the specific physiological role
of a given transporter. Loss of function (experimentally or by naturally occurring mutations) of lipid transporters will inevitably lead to complex pleiotropic effects, some of which will be directly due to the loss or reduction in secreted levels of specific allocrites, or indirect effects resulting from changes in the lipid content of a membrane. Just to make matters more complicated, a recent study with Pgp indicated that the stimulation of ATPase activity with some drugs was dependent on membrane levels of another ABC allocrite, cholesterol [35].

**Summing up**

I started out writing this introductory chapter with the view that, although the principles of the mechanistic aspects of ABC transporter function were reasonably well established, my perception was that lipid transport was a little dull. However, after seeing the excellent accompanying reviews in this volume of *Essays in Biochemistry* and my extensive reading around the whole field, I have come to the view that much still remains to be done on mechanism, and that lipid transport is vitally important and exciting, although much also needs to be discovered. Moreover, recent reports that ABC transporters are involved in development in embryogenesis in *Drosophila* [36], in lung development [37], and haemopoietic stem cell proliferation in mice [33], are examples of why the biology and related physiological roles of ABC transporters are so exciting. In fact, while the study by Ricardo and Lehmann [36] apparently identifies the specific allocrite secreted by the ABC transporter Ste6P involved in promoting the developmental changes they observe, the other studies are probably examples of the indirect effects – no less interesting – of membrane changes resulting from loss of lipid transport dependent on ABCA3 and Abcc/Abcg1 respectively. Furthermore, considering the important physiological roles of ABC transporters, including multidrug-resistance transporters, I would make the comment, underlined in Chapter 10, that, for many mammalian transporters, evidence for most allocrites assigned to a given transporter rests on *in vitro* assays, from which extrapolation of the efficiency of transport rates to the *in vivo* context is difficult. *In vivo* measurements are ultimately more convincing, especially where this is confirmed, if possible by mouse knockout experiments. Finally, in relation to the cell biology of ABC transporters, I find intriguing the differential expression of different ABC transporters in different tissues, as illustrated by the multiple levels of regulation for Pgp at the blood–brain barrier [38]. Generally, neither the mechanism nor the logic behind this tissue regulation is well understood.

In relation to mechanistic considerations, although alternating access and alternating site hydrolysis models look plausible and are likely to be correct in principle, much of the atomic detail (which is sure to be fascinating) is still mysterious. Moreover, most of the intermediate steps between allocrite access and ATP catalysis are overall still not clear. Thus, for example, whether ATP is always bound to the ABC monomer *in vivo*, or whether ATP binding or its actual hydrolysis triggers translocation initiation, is
also unclear. The best available data and, in my view, the most comprehensive accompanying model so far to describe how ABC transporters work [10] come from the MalFGK2 import system, but this still does not explain everything. My personal view is that any model must minimally provide a plausible (and testable) explanation of how the presence of a bound allocrite is transmitted to and activates the catalytic cycle, how the latter then leads to (i) conformational inversion of the allocrite chamber, and (ii) restoration of the orientation of the TMD cavity to the ground state, while, in addition, discriminating between which part of the cycle, from ATP binding to hydrolysis at one or both sites, triggers these two steps.

Converting fundamental knowledge into practical use

The ABC transporter field has advanced greatly since the 1980s, as indicated in the excellent reviews in this volume of *Essays in Biochemistry* on human ABC transporters such as TAP, CFTR, Pgp (ABCB1) and ABCCs (MRPs), the lipid transporters in the A, B, C and G subfamilies, and transporters from bacteria, fungi, plants and human parasites. However, there is still much to be understood at the mechanistic level and, in terms of relevance to human health, much still remains to be discovered as well as understood. The recent application of gene therapy in humans to correct a defect in the ABC transporter ABCD1 [39] is extremely encouraging. On the other hand, the careful evaluation by Tamaki et al. (Chapter 11) of attempts so far to develop anticancer therapies, involving the use of inhibitors of ABC transporters, provides all too good a cautionary tale (at least in retrospect) of trying to apply fundamental knowledge to clinical practice prematurely. This underlines the fact that translational research in general is not easy, and we should not raise expectations too high too quickly. Nevertheless, we should place, in my view, a higher priority and greater efforts towards the application of basic research. In the ABC field, certainly such efforts should greatly benefit from multidisciplinary approaches by collaborations involving clinicians, cell biologists, molecular biologists, physicists and mathematicians (modellers) as appropriate, to advance both general fundamental knowledge and its application to healthcare and agriculture.

Note added in proof (received 4 July 2011)

Two very recent publications highlight significant advances concerning key questions raised in this chapter: a precise cellular mechanism to block ATP binding to the NBD when cholesterol export is not required [40] and a much sought after crystal structure of a translocation intermediate [41], which illuminates the allosteric communication process linking allocrite (substrate) binding to a conformational change in the NBD, involving the Walker A and histidine switch residues, necessary for complete dimer closure.
Summary

• The alternate access model nicely explains how allocrites (transport substrates), are ‘carried’ across membranes as a result of conformational reorganization of the TMDs, that simultaneously convert initial high-affinity into low-affinity binding sites. However, we need more evidence from more structures with bound allocrites, and especially structures of mutant translocators forming authentic translocation intermediates.

• A few structures have already revealed multiple protein folds for the TMD region. This, plus more and more biochemical evidence that the nature of the energy coupling steps and associated conformational changes differ between importers and exporters, and perhaps as a function of allocrite type, indicates that there will be many variations in the details of the mechanism of action of different ABC transporters.

• Growing evidence now supports the alternating catalytic site model for ATP hydrolysis within NBD dimers, but we need more structural and biochemical evidence for the required underlying structural asymmetry and how this determines sequential firing. Evidence from ABC dimers with one degenerate catalytic site indicates that, in principle, one ATP molecule hydrolysed is sufficient for translocation of one allocrite molecule, but why do degenerate sites persist; what is their role?

• The helical domain of isolated NBDs undergoes substantial movement relative to the RecA-like domain (RBM) accompanying ATP binding. This probably represents the ‘power stroke’ leading to dimer formation, then translocation of allocrites, ATP hydrolysis and resetting the system. However, in the intact transporter, the restraining action of the TMDs in the absence of allocrite probably impedes the NBD dimerization step until allocrite binds.

• Intramolecular signalling and associated conformational changes at the atomic level between membrane and NBDs or between NBD monomers, and not least between monomer subdomains, remains for me one of the most fascinating, but as yet poorly understood, aspects of ABC protein action. Tracing the succession of short- and long-range conformational changes triggered by allocrite binding through to activation of catalysis, the resulting release of allocrite, and finally sequential release of phosphate and ADP, followed by dimer opening, and resetting the allocrite-binding domain is an exciting prospect.

• The biochemical basis of multi-allocrite binding to the ‘translocation chambers’ of a given transporter still remains to be established and, although highly speculative, the possibility of specific entry and exit points according to allocrite type is an intriguing possibility.
• Human ABC transporters are well known for the elimination of exogenous toxic molecules. However, as a group, they are probably more important for the export of endogenous allocrites across membranes, including signalling molecules, and, perhaps most important of all, ABC transporters are vital for their role in assembling components of membranes themselves.

• The accumulating evidence of ABC transporter ‘accessory’ domains and accessory partners that widen the range of activities and regulation of these transporters is an intriguing emerging theme.

• Some critical issues still to be settled include the precise nature of the resting or ground state of the ABC transporter in vivo; the significance of ‘domain swapping’ in exporter structures; the mechanism which determines sequential hydrolysis of ATP and what advantage this confers; the role, if any, of degenerate catalytic sites and the related question of ATP stoichiometry; the mechanism of action of ABC-modulators and inhibitors (not considered here); and the physiological significance of varying levels of a given ABC transporter in different tissues.

• Effective translational research involving ABC transporters is vitally important and requires integrated multidisciplinary approaches, but, as with other systems, the difficulties must not be underestimated.

I am delighted to acknowledge the longstanding rich and fruitful discussions with Lutz Schmitt and now his critical reading of this chapter. I am also extremely grateful to Simone Séror for careful and critical reading of this chapter. Finally, I am happy to acknowledge the continued support of the Université Paris-Sud and the Institut de Génétique et Microbiologie.

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