How are the ABC transporters energized?

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ATP-binding cassette (ABC) transporters are probably the most common as well as the most wide-spread active transport systems. This family includes many bacterial transporters that either export complex molecules (lipids, polysaccharides, or proteins), or import small nutrients in cooperation with the soluble solute-binding proteins (e.g., the maltose and histidine transport systems of *Escherichia coli*), as well as such human proteins as the cystic fibrosis transmembrane conductance regulator and the P-glycoprotein that pumps out many anticancer agents. Each transport complex usually contains four domains or subunits, two transmembrane domains each typically containing six transmembrane helices, and two ATPase domains (Fig. 1). The sequence of ABC or ATPase domains always contains the characteristic Walker A and B motifs that are involved in ATP binding (1), but the intervening section between these two motifs is usually longer here than in other ATP-binding enzymes. It ends in a unique motif called LSGGQ motif, C motif, or "signature motif," because it is present in all ABC subunits, but usually not in other ATPases. Although ATP hydrolysis is clearly the driving force for active transport by ABC transporters, it was not clear how it was coupled to the transmembrane transport of solutes. In this issue of PNAS, Fetsch and Davidson (2) report results that indicate how the two ATPase domains collaborate in ATP hydrolysis, and suggest how this is likely to be coupled to solute transport.

It is not immediately obvious why two ATPase domains are present in one transporter complex. Yet, in most cases, inactivation of one of the ATPase domains results in the inactivation of transport, in both human and bacterial transporters (reviewed in ref. 3). Furthermore, in the *E. coli* maltose and histidine transporters, the ATP hydrolysis shows positive cooperativity, suggesting the tight interaction of two domains during catalysis (4, 5).

An important achievement in this area was the determination of the crystal structure of ATPase subunit, HisP, of the bacterial histidine transport system (6). HisP was shown to be an L-shaped molecule with two arms, one including the ATP-binding domain containing the Walker A and B motifs, and the other including the signature motif. The protein appeared to have crystallized as monomers. Nevertheless, among possible combinations of pairs of neighboring monomers in the crystal, a crystallographic dimer could be chosen (6), in which the signature motifs of the two monomers were facing outward (Fig. 2 *Left*). Subsequently, the crystal structure of the ATPase MalK of a thermophilic archaeon, a part of an ABC maltose transporter complex, was reported (7). In this case, the asymmetric unit contained two monomers, although other dimer structures could be proposed between other pairs of monomers in the crystal. The authors chose the structure that buried the largest surface area at the interface between monomers (Fig. 2 *Center*). Finally, an ATPase domain of a DNA-repair protein, Rad50 (8), which contains the signature motif, was crystallized in the absence and presence of a nonhydrolyzable ATP analog. This study showed that the ATP analog bound to the interface between the two monomers, causing their dimerization in a head-to-tail manner (Fig. 2 *Right*). Furthermore, the binding of ATP was shown to involve not only Walker motifs from one monomer but also the LSGGQ motif from the other monomer, the serine side chain oxygen of the latter motif interacting with the γ-phosphorus of ATP.

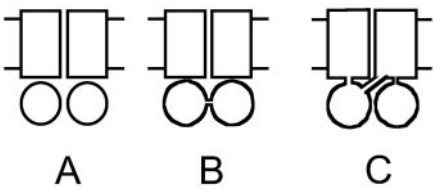

Fig. 1. Schematic structures of some ABC transporters. (*A*) In bacteria, many transporters are composed of two transmembrane subunits (rectangles) and two ATPase subunits (circles). If it is an importer, a fifth subunit, the periplasmic-binding protein (not shown) is also required. (*B*) In some bacterial transporters, the two ATPase domains are fused into a single protein. (*C*) In most fungal and animal transporters, all of the domains are fused into a single polypeptide.

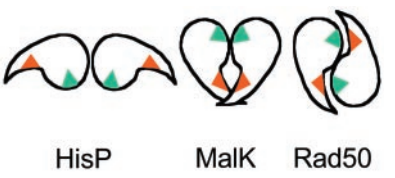

Fig. 2. Proposed arrangement of ATPase dimers in HisP (6), MalK (7), and Rad50 ATPase domain (8). Red section corresponds to the Walker A motif, and the green section corresponds to the LSGGQ motif. Monomers are represented in a highly schematic manner, and the large regulatory domain in MalK is not shown.

Fetsch and Davidson (2) tested the dimeric interaction between MalK ATPase subunits in an elegant manner. The Davidson laboratory, in collaboration with the laboratory of Quiocho, has shown (9) that vanadate binds to one of the ATPase domains in the transition state right after ATP hydrolysis, fixing the entire transport complex at one step during the transport cycle. If the two ATPase domains cooperate in ATP hydrolysis as in Rad50, they must be in contact with each other at this step, with the LSGGQ motif of one monomer in contact with the Walker A motif of another monomer. Fetsch and Davidson used the ability of vanadate to catalyze photocleavage of the residues that are nearby. Photocleavage of maltose transporter complex under these conditions resulted in the cleavage of either the Walker A motif or the LSGGQ motif in a given monomer, unequivocally establishing that the two ATPase domains of ABC transporters form Rad50-like dimers during ATP hydrolysis, with the LSGGQ motif of one monomer facing the Walker motifs of the other. The fact that the catalytic pocket for ATP hydrolysis is produced only by cooperation between two monomers explains why two ABC subunits are needed in each transporter (Fig. 1).

In retrospect, it is remarkable that this mode of dimer formation was predicted on the basis of comparative sequence analysis and modeling by Jones and George (10) soon after the appearance of the HisP structure. These scientists felt that the published structure of HisP dimer could not explain the expected strong

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functional interaction between the monomers and made a survey of Walker A/B and signature motif sequences in ABC transporters. They found that in some fungal ABC transporters, in which all domains occur as parts of a single polypeptide (Fig. 1*C*), or in some bacterial transporters in which the two ATPase domains are fused into a single polypeptide (Fig. 1*B*), the N-terminal ATPase domain contains apparently nonfunctional, much altered Walker A/B sequences yet a canonical signature motif, whereas the C-terminal ATPase domain contains canonical Walker sequences yet a drastically altered signature motif. This arrangement can be explained only with the Rad50-like dimer structure (Fig. 2) that creates at least one functional active site. The Rad50 model also was supported by a different, more recent theoretical analysis (11).

Recently, the structures of two complete ABC transporters, the *E. coli* BtuCD transporter that takes up vitamin B_{12} from outside (12) and the *E. coli* MsbA transporter that exports intermediates of lipopolysaccharide biosynthesis (13), have been reported. In the BtuCD structure that appeared around the time of the submission of the Fetsch-Davidson paper (2), the two ATPase domains are arranged in the Rad50 manner. Thus, the structural data (12) are in agreement with the biochemical data obtained with the functional transporter complex (2). The BtuCD study showed that the interface buried between the two ATPase subunits was surprisingly small. This finding explains why the isolated ATPase units do not dimerize easily *in vitro* in the absence of constraints imposed by the transmembrane subunits. Although cases of dimerization were reported in the presence of ATP (14, 15), in agreement with the Rad50 model, the required conditions are strict and often unexpected, such as the absence of Mg^{2+} (14, 15) and even K^+ (15), both abundant cations in the cytoplasm.

The BtuCD structure is also interesting in other ways. First, it shows that a channel-like cavity exists in between the two transmembrane subunits, BtuC, and that a gate-like structure separates this channel from cytoplasm. Second, the transmembrane subunits are in tight interaction with the ATPase subunits, partly via the conserved "EAA loop" in the former. Finally, these arrangements suggest the possible mechanism of transport (12). Thus, in BtuCD, the Walker A motif of one ABC subunit and the LSGGQ signature motif of the other subunit are farther away (by 4 Å) than in the Rad50 structure with its bound ATP analog. Possibly the two ATPase subunits will approach each other more in the presence of ATP, and this will then move the transmembrane helices, resulting in the opening of the channel gate.

There are, however, many questions that await further study, and only a few of them will be listed. (*i*) The ATPase dimer can *a priori* bind two ATP molecules; several reviews mention, rather carelessly, that the simultaneous hydrolysis of two ATP molecules is needed for transport. However, there is much evidence supporting transport driven by the hydrolysis of a single ATP. For example, in those transporters analyzed by Jones and George (10), one set of ATP-binding sites seems to be inactive, yet the transport occurs efficiently. A high-precision analysis of maltose transporter conclusively showed that one solute is imported by the hydrolysis of one ATP molecule (16). In the vanadate inhibition studies of maltose transporter, only one of the two sites becomes occupied by vanadate (9). Even so, it is not entirely clear how the ATP-hydrolyzing site is selected from the two possible sites. In P-glycoprotein, hydrolysis seems to occur in alternating sites (17), although there is controversy on this topic (18). (*ii*) In the BtuCD structure, the two ATPase domains are fairly close to each other, although the protein was crystallized in the absence of ATP (12). In contrast, in MsbA, which was also crystallized without ATP, the two ATPase domains are very far away from each other, with a distance of about 50 Å (13). Do these represent two separate classes of ABC transporters with different structures? A recent low-resolution structure of P-glycoprotein suggests that the two ATPase domains are fairly close to each other in the absence of ATP (19). (*iii*) At what stage does the solute transport occur? The P-glycoprotein study suggests that the largest change in the structure of transmembrane domains occurs upon the binding of an ATP analog (19), a somewhat surprising finding in view of the low affinity of ATP binding in ABC transporters. In contrast, in a bacterial transporter, the vanadate-trapped complex, corresponding to the transition state just after ATP hydrolysis, is tightly bound to maltose-binding protein, and therefore could correspond to the stage after a large conformational change (9).

In the future, we will certainly see more structures of ABC transporters; hopefully, some will be at various steps of the ATP hydrolysis cycle. No doubt these will be extremely instructive, but we also need biochemical studies of functioning transporters such as the one by Fetsch and Davidson, because ultimately it is studies of this latter type that will give us the last word on how the transporter works in living cells.

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