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MALTOSE TRANSPORT THROUGH THE INNER MEMBRANE OF *E. COLI*

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1. ABSTRACT

The maltose transport complex of *E.coli* is one of the most well-characterized members of the ATP-Binding Cassette (ABC) protein superfamily. ABC proteins represent the largest superfamily of transmembrane proteins in prokaryotes and eukaryotes, performing diverse functions from ion transport by the cystic fibrosis transmembrane regulator to multiple drug efflux by the P-glycoprotein transporter and sugar transport by the maltose transporter. Characterization of the mechanism of transport for ABC transporters is currently being investigated both biochemically and structurally, however some uncertainty remains as to how the individual subunits of these multisubunit transporters interact. This review discusses the current knowledge of the mechanism of maltose transport, as it relates to the ABC superfamily of transporters as a whole.

2. INTRODUCTION

ATP-binding cassette (ABC) transporters make up one of the largest superfamilies of proteins. They are present in a wide array of organisms from prokaryotes to man, performing a wide array of functions, including import of sugars, amino acids, peptides and vitamins, and export of multiple drugs, ions, proteins, and lipids (1,2). The complete genome sequence of *Escherichia coli* (*E. coli*) K-12 reports more than 80 ABC transporters (3), many of which are importers. Bacterial importers, set in the cytoplasmic membrane, generally require a periplasmic binding protein to deliver the substrate to the transporter. A typical ABC transporter is made up of four domains or subunits, two transmembrane (TM) domains and two cytoplasmic ATP-binding domains. In the maltose transporter complex $MalFGK_2$, the MalF and MalG proteins span the membrane and two copies of MalK bind ATP (4,5). This subunit composition is typical of prokaryotic importers, where each domain is generally encoded as a separate gene, while in some prokaryotic and the majority of eukaryotic transporters, two or more of the four domains are encoded by a single gene (figure 1). Examples of this diversity are illustrated by the recent structures of the vitamin B_{12} transporter $(BtuC)_2(BtuD)_2$ (6) and the homodimer of a half ABC-transporter, $(MsbA)_2$ from *E. coli*, where each monomer contains a transmembrane domain fused to a nucleotide-binding domain (7).

3. BINDING PROTEIN

Binding protein-dependent transporters are present in bacteria and archeon, but have not been observed in eukaryotes. The binding proteins are responsible, in part, for the substrate specificity of transporters, having K_d values between 0.01-10 micromolar (8). Not all substrates that are bound by the binding protein are transported. The maltose binding protein (MBP) from *E. coli* can bind longer maltodextrins, however only oligomers up to maltoheptaose can be transported (9). Some sugars such as beta-cyclodextrins bind in an inactive mode that may prevent a conformational change necessary for transport to occur, resulting in the inability of the binding protein to interact productively with the transport complex (10).

The structure of MBP has been determined (11). It has two separate lobes or domains connected by a hinge, that are predicted to interact with MalF and MalG (12). The two lobes exist in either an open or a closed conformation, with substrate bound in the cleft between them. The binding protein is usually open when substrate is not bound and closed when substrate is bound. MBP with cysteines introduced at amino acid positions G69 and S337 forms an interdomain disulfide bridge which is thought to

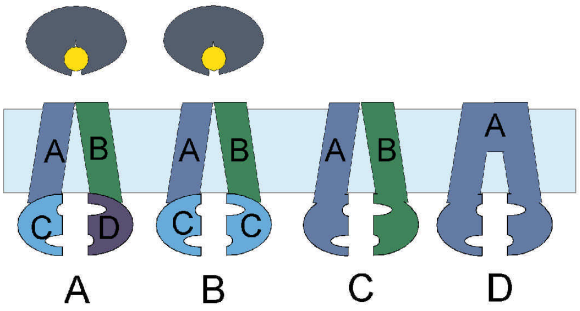

Figure 1. Organization of domains. Four examples of the diversity of domain organization in ABC transporters. A) All four domains are encoded by separate genes, which is common to bacterial importers, additionally requiring a binding protein. The oligopeptide permease is an example of this kind of transporter. B) The transmembrane domains are encoded by separate genes while the nucleotide-binding domains are present in two copies, encoded by the same gene. Examples of transporters with this configuration are the maltose and the histidine transporters which are also bacterial importers requiring a binding protein. C) The transmembrane domain and the nucleotide binding domain are encoded by the same gene and are fused, the complex is a dimer of two such proteins. TAP1/TAP2 is an example of this kind of transporter. D) All four domains are encoded by the same gene and are fused, common to eukaryotic transporters. MDR and CFTR are examples of this configuration of transporter.

mimic the closed conformation of the binding protein (13). These mutants confer a dominant negative phenotype for transport by competing with wild type MBP for the transport complex, suggesting that MBP can interact with the transporter complex when in the closed conformation. The presence of reducing agent partially reinstates transport activity.

4. TRANSMEMBRANE SUBUNITS

MalF and MalG are the transmembrane (TM) subunits for the maltose transport complex. MalF is predicted to contain eight TM alpha-helices, and MalG is predicted to contain six. The canonical number of α-helices for an ABC transporter is twelve, as was observed in the crystal structure of MsbA (7). The histidine permease is thought to be the smallest, having only 10 TM helices (14). 20 TM helices were found in the crystal structure of the vitamin B_{12} importer (BtuCD) (6). The transmembrane protein, FhuB, of the ferrichrome-transporting protein (FhuDCB) has been predicted to contain 20 transmembrane helices as well (6,15). The variety in the number of helices could be a reflection of the low level of conservation between transporters in this region. The larger number of helices could also be necessary to transport larger substrates, as in BtuCD.

The organization of the transmembrane helices of MalF and MalG were modeled by comparison of eight maltose and maltodextrin transporters from different organisms, before any structural information was available for ABC proteins (8). Several of the MalF proteins used in this analysis had six rather than eight TM helices and all of the MalG proteins had six TMs. The helices postulated to line the translocation pathway were TMs 4,5,6, and 7 in *E. coli* MalF and TMs 2, 3, 4, and 5 in MalG. Hydrophilic patches were observed in TM 4 of MalG and TMs 4 and 6 of MalF that might be involved in substrate translocation. It is suggested that the substrate could bind at residue G169 of TM 4 in MalF and then be passed to other hydrophilic residues of TMs 4 and 5 of MalF and MalG that line the channel (8). From the structure of BtuCD (6), it appears that residues outside the TMs may also contribute to substrate binding in the channel.

Mutants of MalF and MalG were isolated that are binding protein independent (BPI). These mutants still transport maltose, although with a greatly reduced affinity (16) and are inhibited by *in vivo* concentrations of wild type MBP (17). They have gained the ability to hydrolyze ATP in the absence of added MBP (18l) leading to the suggestion that MBP is responsible for stimulating the ATPase activity of the wild-type transporter. In fact, MBP stimulates ATPase activity even in the absence of maltose (18). The BPI MalF mutant, MalF500, has been well characterized and shown to be functionally similar to wild type, maintaining similar positive cooperativity in ATP hydrolysis and nucleotide specificity, as well as the ability to be inhibited by vanadate (19,20). These BPI mutants will be useful in determining the mechanism of ATP driven transport of substrate, as they may represent intermediates in the transport pathway (21).

Interaction of the transmembrane subunits with the ATPase subunits is thought to be mediated at least in part by the EAA loop, consisting of EAA-X_3-G-X_9-I-X-LP (22) where X is any residue, found in many bacterial importers. This cytoplasmic loop is usually located between two TM domains near the C- terminus of the protein. This EAA loop is presumed to be represented by the 'L-loop' in BtuCD and the fourth intracellular loop of CFTR (IC4), as well as to the first cytoplasmic loop in drug exporters (6) and is believed to be a conserved mechanism of communication between the transmembrane domains and the ATPase domains. Mutations in this region in both MalF and MalG cause both a decrease in the degree of membrane association of MalK and in maltose transport activity (23). The role of this loop in subunit interaction was supported by the finding that these mutations in MalF and MalG can be suppressed by mutations in MalK (23).

5. NUCLEOTIDE-BINDING SUBUNITS

The nucleotide-binding subunit of the maltose transport complex, MalK, can be separated into two structurally distinct entitites, an N-terminal region responsible for maltose transport, and a C-terminal region responsible for regulatory activities, defined by mutation and insertion studies (24,25). Most other ABC proteins do not contain a C-terminal regulatory domain, thus structure and function comparisons are based mainly on the N-terminal region of MalK that is responsible for transport. The C-terminal domain and its function in regulation of the *malK* gene is further discussed below.

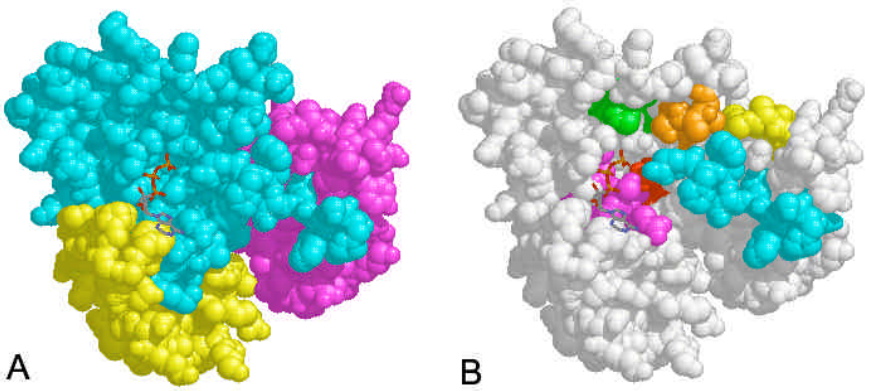

Figure 2. A monomer of HisP. A) The three subdomains of the nucleotide-binding domain. The F1-ATPase domain is cyan. The α-helical domain is magenta. The β−sheet domain is yellow. B) ABC protein motifs. The Walker A is highlighted in magenta. The Q-loop is colored cyan. The LSGGQ signature motif is colored yellow. The Walker B is red. The D-loop is highlighted in orange. The H-loop is colored green.

There have been numerous crystal structures of the nucleotide binding subunits of ABC transporters, including those of HisP (14), MalK (26), Tap1 (27), MJ0796 (28), and MJ1267 (29), as well as the structures of MsbA (7) and BtuCD (6) that include the transmembrane regions. Also of note is the structure of the catalytic domain of Rad50, an ABC protein though not a transporter (30). These structures have helped to define three subdomains common to ATP-binding subunits (figure 2A) (29). The first is the F1-type ATP binding core subdomain (31), made up of a mostly parallel alpha β sheet and its flanking alpha-helices. This domain carries the Walker A and Walker B motifs. The second subdomain consists of an antiparallel beta sheet that lies between the Walker A and the Walker B motifs in the linear sequence, and is involved in binding the ribose and base of the nucleotide. The third subdomain is αlpha-helical and consists of a bundle of three phylogentically conserved αlpha-helices which contain the ABC-family signature sequence, LSGGQ (29). The LSGGQ caps the amino terminus of the third alpha-helix of this subdomain. There are a number of motifs used to characterize the nucleotide binding domains of an ABC transporter. These were identified either from sequence alignments (figure 3) or from structures (figure 2B). All ABC proteins contain the classical consensus Walker motifs that are involved in nucleotide binding, including the Walker A, or the phosphate binding loop (P-loop), consisting of G-X-X-X-X-G-K(T), and Walker B, H-H-H-H-D (32), where H is any hydrophobic residue. The Walker A coordinates the phosphates of ATP and the aspartate in the Walker B coordinates magnesium for hydrolysis. In ABC transporters, the glutamate (E171 of MJ0796 or E159 of MalK) immediately following the aspartate in the Walker B has been suggested to be the catalytic base, coordinating the putative hydrolytic H_2O in the nucleotide-binding site (14,28). Five residues, beginning two residues downstream of the Walker B, have been designated the D-loop, and in the structures of the prevailing model for dimerization of these ABC subunits, residue 5, an aspartate, contacts the opposing subunit near its nucleotide-binding site (6,30,33). The gamma phosphate linker (also known as the Q-loop) (30), extends from a conserved glutamine (Q88 in MalK) to a conserved asparagine approximately 12 residues downstream (33), and is located in the αlpha-helical region between the Walker A and Walker B. It is thought to play a signaling role in coupling the binding of ATP to movement of the alpha-helical subdomain. In the structure of HisP, this glutamine is in contact with the gamma-phosphate of ATP. Comparison of the structures of ADP bound MJ1267 and ATP bound HisP suggests that the loss of contact between this glutamine and ATP following hydrolysis is coupled to rotation of the αlpha-helical subdomain (29). In CFTR, Berger et al have observed that mutation of this glutamine (Q493 in NBD1) reduced the frequency of channel opening, which suggests a role in coupling ATP binding and/or hydrolysis to channel opening (34). However, mutation of this glutamine in MJ0796 did not lead to a total loss of function, so it is suggested that this domain rotation is not the power stroke for transport (33). The LSGGQ motif, or family signature sequence is located in the alpha-helical region downstream of the Q-loop. Mutation of this motif causes a reduction in the ATPase activity of ABC transporters (35,36), however it does not contact nucleotide within a nucleotide-binding monomer. It has been suggested that this motif might function in intersubunit signaling, or might contact nucleotide across the nucleotide binding interface (6,30,37). H211 of HisP (H192 in MalK), downstream of the D-loop, is highly conserved and forms an H-bond with the gamma-phosphate of ATP (14). Mutation of this histidine residue to arginine in either MalK or HisP greatly reduces transport and hydrolysis, although ATP can still bind. Interestingly a heterodimer of HisP consisting of wild type and the H211R mutant retains 66% of wild type ATPase activity, while the same heterodimer in MalK retains only 10% (38,39).

5.1. Mode of interaction between nucleotide-binding subunits

There are several conflicting models describing how two nucleotide-binding subunits interact, based on X-ray crystallography experiments, which have delayed assignment of a specific function to the LSGGQ motif. The first nucleotide cassette from an ABC

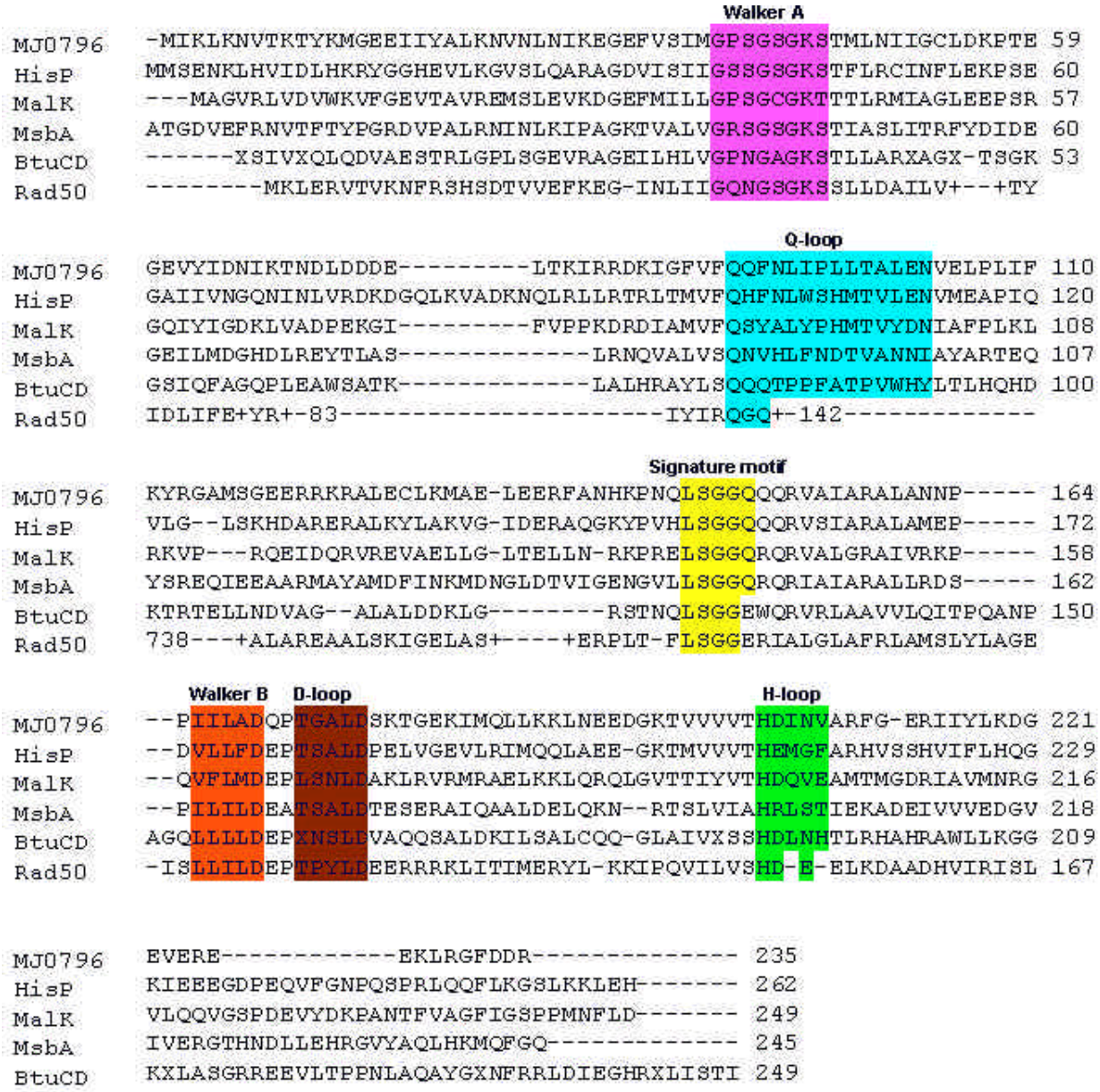

Figure 3. Sequence alignment of six ABC proteins.

transporter to be crystallized, HisP, was from the bacterial histidine permease (figure 4) (14). In the HisP dimer structure the nucleotide-binding sites face away from the dimer interface, and both the nucleotide-binding sites and the LSGGQ motifs are surface exposed (figure 3, 4). Jones and George proposed an alternate model for dimerization of HisP, suggesting that the LSGGQ of one subunit could be a part of the nucleotide binding site of the second subunit, with the hydroxyl of the serine contacting the catalytic magnesium and the backbone amide of the second glycine contacting a gamma-phosphate oxygen (40). Similar contacts to magnesium and the gamma-phosphate were made by a contributing hydroxyl ligand and glycine in myosin (41). This same kind of dimer interface was later observed in the crystal structure of the catalytic domain of Rad50 (figure 4) (30), where the hydroxyl of the serine and the main chain nitrogen of the second glycine of the signature motif bind to the oxygens of the ? amma-phosphate of ATP. Rad50 is an ABC protein that functions in human DNA break repair and maintenance rather than transport. The F_1-ATP binding core subdomain and most of the antiparallel beta sheet subdomain of Rad50 are very similar to that of ABC transporters, and the LSGGQ and invariant glutamine of the gamma? phosphate linker are likewise conserved (30). However, the rest of the gamma? phosphate linker and the alpha-helical subdomain as well as the nucleotide interaction loop of the antiparallel beta ?sheet subdomain bear little resemblance to those in true ABC transporters (28). These differences were substantial enough to suggest that Rad50 may not be an appropriate model for subunit interaction. A structure for the MalK dimer of the archaeon *Thermococcus litoralis*

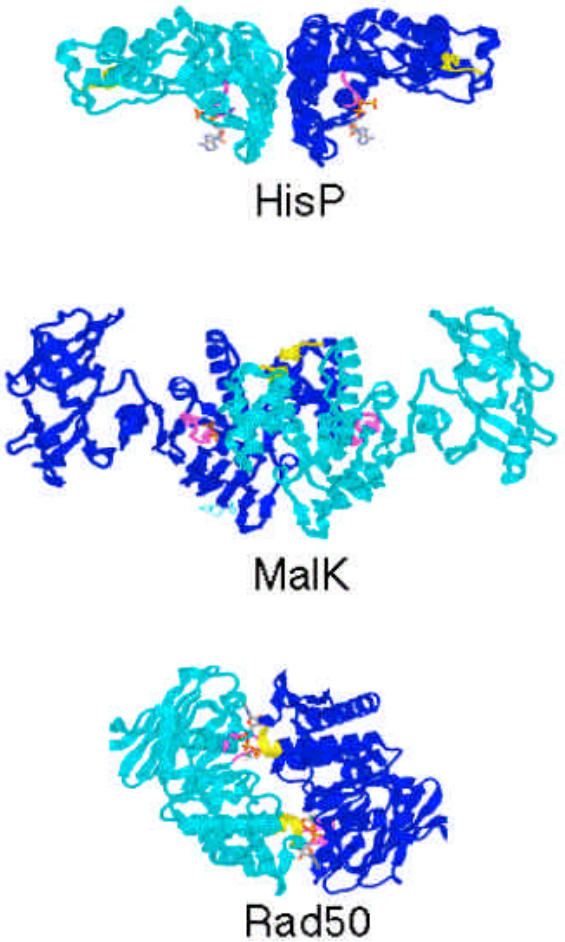

Figure 4. The nucleotide dimer interface of HisP (14), MalK (47), and Rad50 (30). The Walker A is highlighted in magenta, the LSGGQ is highlighted in yellow.

was published later that year (figure 4) (26). In this structure, monomers were aligned such that the nucleotide-binding sites faced the dimer interface, but the LSGGQ motif had no involvment in nucleotide-binding. Based on the observation that residues at a dimer interface are more highly conserved than those at other protein surfaces (42), Kerr et al studied the crystal structures of the dimers of the NBDs of MalK, HisP and Rad50 (43). He found that the Rad50 dimer likely represented a true protein:protein interface while the MalK dimer interface was considered ambiguous and the HisP dimer interface was considered unlikely to represent a true dimer interface. While this study indicates that the Rad50 dimer interface represents a true physiological interface, it does not resolve the controversy over whether or not Rad50 is representative of the entire ABC family structure. The structure of MsbA, the first ABC transporter to be solved in its entirety, did little to address the controversy since the two nucleotide-binding domains did not contact each other and were oriented such that the two nucleotide-binding domains could not interact in the manner suggested by Rad50 and Jones and George (7). The recent crystal structure of the vitamin B_{12} transporter of *E. coli* reported a dimer interface that more closely resembled that of Rad50 (6,30). Recent biochemical data from our lab strongly support a Rad50-like dimer interface for MalK. Vanadate trapped in the position of the gamma-phosphate of ATP catalyzes the photochemical cleavage of MalK at both the Walker A of one subunit and the LSGGQ of the opposite subunit (37), indicating that both the Walker A and the LSGGQ are in close proximity to ATP. Furthermore, mutation of E171 immediately downstream of the Walker B to glutamine in MJ0796 (E159 in MalK) was found to stablize the dimer form of the isolated nucleotide-binding subunit in the presence of NaATP (44). The structure of this dimer also mimics Rad50 (33), hence the structure of Rad50 does appear to be representative of a true ABC transporter.

This orientation of subunits, where residues from both subunits are involved in ATP binding explains many features of ABC transporters that have been known for years. All ABC transporters contain two nucleotide-binding domains, and it has been found that two functional MalK proteins are required for transport to occur (38). The MalK proteins interact with positive cooperativity for ATP hydrolysis (19). Positive cooperativity likely results from a

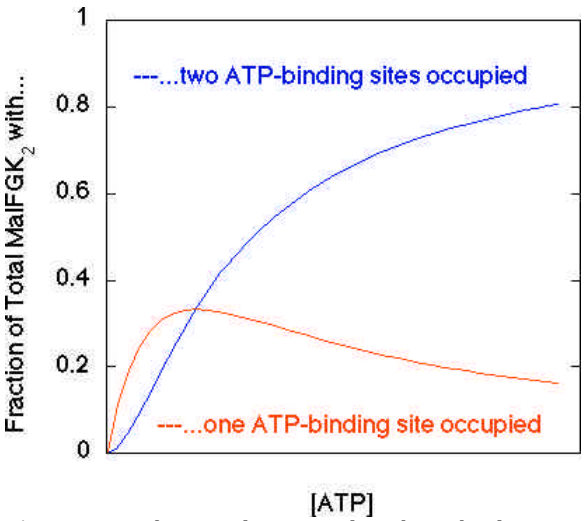

Figure 5. Theoretical curves describing binding site occupancy. The fraction of enzyme with one (EL1) or two (EL2) sites occupied are plotted as a function of ATP concentration where K1=K2. The equations describing the concentration of enzyme with one (EL1) or two ligands bound (EL2), as a function of the total enzyme concentration are as follows:

$$\frac{[EL_1]}{E_T} = \frac{L \cdot K_2}{K_1 \cdot K_2 + L \cdot K_2 + L^2}$$

$$\frac{[EL_2]}{E_T} = \frac{L^2}{K_1 \cdot K_2 + L \cdot K_2 + L^2}$$

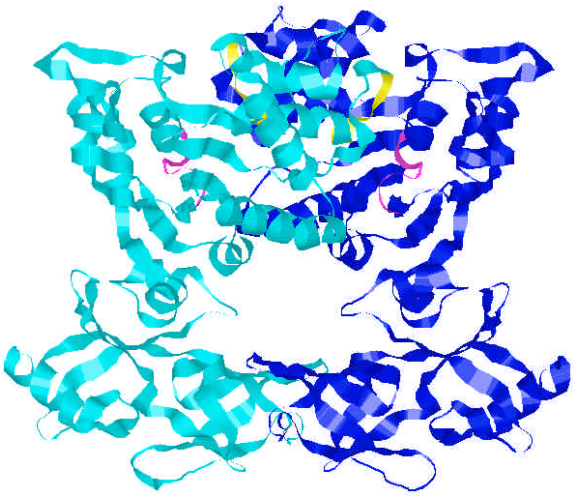

Figure 6. Realignment of MalK (see figure 4), from T. litoralis onto the structure of Rad50 results in contact of the C-terminal subunits with each other.

requirement that both sites be occupied with nucleotide before hydrolysis can occur. This can be illustrated graphically, as seen in figure 5. Equations were derived that describe the filling of the first and second sites as a function of ATP concentration by assuming that ATP, or ligand (L), binds the two sites independently with two distinct equilibrium constants K_1 and K_2. The equation describing EL_2, the concentration of enzyme with two ligands bound, plotted as a function of ligand concentration is sigmoidal and can be fit to the Hill equation with n=1.5. Thus, the rate of ATP hydrolysis by the maltose transport complex may parallel the filling of the second nucleotide-binding site. A similar phenomenon occurs in MDR, where it has been suggested that binding to the second site may induce catalysis at the first, and the sites alternate in catalysis (45). The requirement that both sites must be occupied before hydrolysis can occur can be explained by the nature of the interface between the nucleotide-binding subunits. The dimer conformation may only be stable when ATP is bound to both nucleotide binding sites, as suggested by the structure of MJ0796, where the points of contact between subunits in the dimer are dominated by protein-ATP as opposed to protein-protein interactions (33).

5.2. C-terminal regulatory domain of MALK

The transport and regulatory functions of MalK can be separated by missense mutations (24) and insertion mutations (25). Most regulatory mutations mapped to the C-terminal domain, while those affecting transport were mostly in the N-terminal domain. Regulation is mediated through interaction of MalK with two different proteins. MalT is the transcriptional activator for the maltose operon. MalT activity is induced by maltotriose (a by-product of maltodextrin metabolism) and ATP (46), whereas MalT is negatively regulated by MalK. Schmees and Schneider have shown that expressing only the C-terminal 106 residues of MalK (residues 263 to 369) is sufficient to obtain half maximal regulatory activity (47). Transport activity is also regulated by inducer exclusion. When glucose is present, $EIIA^{glc}$ binds to MalK inhibiting transport and limiting the amount of inducer that enters the cell. It is believed that MalK has separate binding sites for MalT and $EIIA^{glc}$, though it is not known if both can be bound at the same time (48,49). Realignment of the MalK subunits from *T. litoralis* to match the Rad50 dimer interface places the C-terminal domains in contact with each other raising the possilbility that regulation could be mediated by subunit interaction (figure 6).

6. MECHANISM OF TRANSPORT

A model for how maltose transport through the inner membrane occurs can be seen in figure 7. Maltose transport involves a series of conformational changes starting with the binding protein and including the nucleotide-binding domains and the transmembrane domains. MBP closes upon binding of maltose and binds to the transport complex. Binding of maltose bound MBP to the transport complex could provide the stimulus for dimerization of the two nucleotide-binding domains, providing a mechanism by which MBP can stimulate ATP hydrolysis. In fact, if vanadate is used to trap the transition state for ATP hydrolysis, MBP becomes tightly bound to the transporter complex, suggesting that MBP stimulates ATP hydrolysis by stabilizing the transition state (50). This stimulus would have to occur through a transmembrane signal, mediated by MalF and MalG, as the binding protein does not contact the nucleotide-binding subunits. The dimerization could only occur when ATP is bound at both

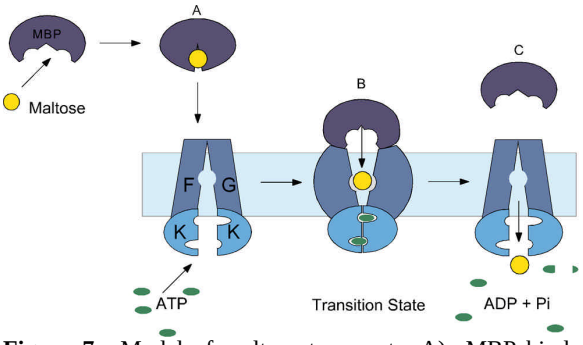

Figure 7. Model of maltose transport. A) MBP binds maltose then binds to the transmembrane domains of the maltose tranport complex. B) The transition state. Binding of MBP stimulates dimerization of the nucleotide-binding domains and ATP hydrolysis, the binding protein becomes tightly bound to the transport complex and has opened, losing its high affinity for the sugar. C) Release of nucleotide and phosphate by the nucleotide-binding domains, maltose has been transported and MBP dissociates.

sites (33). Evidence of a conformational change of the nucleotide-binding domains during ATP binding and hydrolysis comes from fluorescense studies done by Mannering et al (21). Using a vanadate trapped transporter complex, a reduction in the solvent accessibility of a residue in the nucleotide-binding site was seen, as compared to the non-trapped (ground state) complex (21). In addition, a comparison of the structure of nucleotide-free BtuCD and nucleotide-bound Rad50 suggests the nature of a conformational change that might occur upon dimerization of the nucleotide bound subunits. Although the two nucleotide-binding BtuD subunits are in contact in the BtuCD structure, the Walker A of one subunit is ~4 Å farther away from the ABC signature motif in the opposing subunit than in Rad50 (6). Dimerization of the nucleotide-binding subunits is likely to affect their interaction with the transmembrane subunits which could cause a conformational change in these subunits as well. Hence transport could be powered by the MBP-induced dimerization of the nucleotide-binding subunits during ATP binding (6,33).

In Ehrmann's model (8), hydrophilic residues may not be available to the substrate through the full length of the translocation pathway without a conformational change, supporting the theory that a conformational change stimulated by the nucleotide-binding domains takes place as maltose is delivered to the transmembrane domains. In the structure of BtuCD, the putative transmembrane channel is closed at the cytoplasmic surface of the membrane and it is similarly suggested that dimerization of the nucleotide-binding domains would open the channel (6). In figure 7, we suggest that the low-affinity sugar binding site in the transmembrane region is initially accessible from the cytoplasm and that its accessibility changes to receive maltose as it is released from MBP in the catalytic transition state. It is also possible that a channel that spans the entire length of the membrane opens during ATP hydrolysis, since MBP is tightly bound to prevent release into the periplasm. Maltose is absent from the vanadate trapped intermediate (50).

In conclusion, much can be learned about the molecular mechanism of maltose transport by studying the structure and function of the transporter stabilized in different conformational states. The use of vanadate is one method to stabilize an intermediate (20,50). Mutations that lead to binding-protein-independent transport may stabilize the transporter in another conformation that the transporter passes through upon binding MBP but before the transition state is reached. BPI mutants have an increased affinity for MBP, relative to the wild-type transporter (21,51) and the conformation of the nucleotide-binding sites may more closely resemble that of the catalytic transition state than the ground state (21).

7. ACKNOWLEDGEMENTS

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