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Mechanism of maltose transport in *Escherichia coli*: Transmembrane signaling by periplasmic binding proteins

(ATPase/uncoupling)

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ABSTRACT **Maltose transport across the cytoplasmic membrane of *Escherichia coli* is dependent on the presence of a periplasmic maltose-binding protein (MBP), the product of the *malE* gene. The products of the *malF*, *malG*, and *malK* genes form a membrane-associated complex that catalyzes the hydrolysis of ATP to provide energy for the transport event. Previously, mutants were isolated that had gained the ability to grow on maltose in the absence of MBP. After reconstitution of the transport complex into proteoliposomes, measurement of the ATPase activity of wild-type and mutant complexes in the presence and absence of MBP revealed that the wild-type complex hydrolyzed ATP rapidly only when MBP and maltose were both present. In contrast, the mutant complexes have gained the ability to hydrolyze ATP in the absence of maltose and MBP. The basal rate of hydrolysis by the different mutant complexes was directly proportional to the growth rate of that strain on maltose, a result indicating that the constitutive ATP hydrolysis and presumably the resultant cyclic conformational changes of the complex produce maltose transport in the absence of MBP. These results also suggest that ATP hydrolysis is not directly coupled to ligand transport even in wild-type cells and that one important function of MBP is to transmit a transmembrane signal, through the membrane-spanning MalF and MalG proteins, to the MalK protein on the other side of the membrane, so that ATP hydrolysis can occur.**

Active transport of maltose across the cytoplasmic membrane of *Escherichia coli* is mediated by one of a class of binding protein-dependent transport systems. Four proteins are required for this process (see ref. 1 for review). The maltose-binding protein (MBP), the product of the *malE* gene, is a soluble protein located in the periplasm. The remaining proteins, the products of the *malF*, *malG*, and *malK* genes, are associated with the cytoplasmic membrane. The MalF and MalG proteins are hydrophobic and span the membrane (2, 3), while the MalK protein is more hydrophilic and is presumed to be peripherally associated with the inner face of the cytoplasmic membrane (4). We have recently purified the membrane-associated components and reconstituted maltose transport activity from these components in proteoliposomes (5). The experiments demonstrated that one MalF, one MalG, and two MalK molecules form a complex in the membrane. Furthermore, maltose transport by the proteoliposomes is accompanied by hydrolysis of ATP, presumably catalyzed by the MalK protein, which contains an ATP-binding site (6).

Mutant strains of *E. coli* have been isolated that no longer require MBP for maltose transport (7, 8). The mutations mapped in either the *malF* or *malG* genes. These strains transported maltose actively and specifically with a much higher K_m (≈2 mM) than the wild type (1 μM). These results imply that the membrane proteins must possess a binding site for maltose. We have now undertaken a study of these MBP-independent mutant transport complexes by using reconstituted proteoliposomes. These experiments demonstrate that mutant complexes have gained the ability to hydrolyze ATP constitutively in the absence of MBP and suggest that in wild-type cells an indispensable function of the liganded MBP is to initiate a transmembrane signaling process that stimulates ATP hydrolysis, thus allowing transport to occur.

MATERIALS AND METHODS

Strains and Plasmids. HN741 (*E. coli* K-12 *argH his rpsL1 malT*^{c} Δ*malB13* Δ*uncBC ilv*::Tn*10*/F′ *lacI*^{q} Tn*5*) was always used as the host. This strain was made by transferring the F′ factor to strain HN594 (9) by conjugation and contains a deletion of both the *malB* region, encompassing the *malE*, *malF*, *malG*, and *malK* genes (10), and the genes for F_0F_1 ATPase (Δ*uncB-C*), the latter mutation being necessary for decreasing the basal rate of ATP hydrolysis in reconstituted proteoliposomes.

In all experiments, the MalF, MalG, and MalK proteins were overproduced by placing the respective genes under the control of the *trc* promoter. Because the *malB* genes are organized into two divergent transcription units, we always used HN741 containing two compatible plasmids, one [pMR11 (11)] containing the *malK* gene and another containing the *malF* and *malG* genes [for example, pFG23 (9), containing *malF*$^+$ and *malG*$^+$ alleles]. A plasmid (pLH33) containing *malF*$^+$ and *malG511* alleles was constructed in the laboratory of one of us (H.A.S.). Plasmids containing other mutant alleles behind the *malB* promoter were made at Columbia University. The *Eco*RI/*Sma* I fragment containing the *malB* promoter and 5′ end of the *malF* gene was excised from each of these plasmids and was replaced with the corresponding fragment from pFG23 containing the *trc* promoter and 5′ end of the *malF* gene. For brevity, these plasmids are denoted by the allele designations of the *malF* and *malG* genes present.

Freshly transformed cells were grown in "terrific broth" (12) and induced for overexpression as described (5). Cell envelope fractions, prepared as described (5), were stored at −70°C until use.

Preparation of Maltose-Free MBP. MBP was purified by affinity chromatography (13) and was dialyzed extensively under conditions calculated to remove most of the bound maltose (14). For some experiments, MBP was denatured in 6 M guanidine hydrochloride (Gdn·HCl) and either dialyzed against 6 M Gdn·HCl (15) or passed through a Sephadex G25

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Abbreviation: MBP, maltose-binding protein.

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column equilibrated with 6 M Gdn·HCl before renaturation by dialysis against 10 mM KP_i (pH 7.0). UV-difference spectroscopy (16) showed that the bulk of the MBP was free of maltose.

Preparation of Proteoliposome Vesicles. Membrane proteins solubilized in the detergent octyl β-D-glucopyranoside were reconstituted into lipid vesicles by a detergent dilution method (9). In some experiments, solubilized proteins and purified *E. coli* phospholipids were mixed with 0.1 mM purified MBP and/or 200 mM maltose before dilution in order to trap these compounds inside the vesicles. In these experiments, the weight ratio of *E. coli* phospholipid/MBP/solubilized protein was maintained at 50:25:1. Under these conditions, trapped MBP was present in excess of the other components of the transport system (Fig. 1, lane 2). Samples were diluted 1:25 into buffer containing 20 mM KP_i (pH 6.2) and 1 mM dithiothreitol. After centrifugation, the proteoliposome vesicles were resuspended in a solution containing 20 mM KP_i (pH 6.2), 3 mM $MgCl_2$, and, if maltose was included in the preparation, 10 μM maltose.

Assay of ATP Hydrolysis. Proteoliposome vesicles were incubated at room temperature with 0.1 mM [γ-^{32}P]ATP (50 mCi/mmol; 1 Ci = 37 GBq) and 25-μl portions were removed at the specified times to 175 μl of 1 M perchloric acid/1 mM KP_i. The release of radioactive P_i was assayed as described by Lill *et al.* (17).

Other Methods. SDS/PAGE was performed as described (5). Gels were scanned with a Quick Scan from Helena Laboratories. Protein concentrations were determined as described (5).

RESULTS

Measurement of ATP Hydrolysis by the Maltose Transport Complex. In the past, we have assayed maltose transport activity by measuring the accumulation of [^{14}C]maltose by proteoliposome vesicles containing the MalF, MalG, and MalK proteins (9). The proteoliposomes were formed by incubating sonicated *E. coli* phospholipids with membrane proteins solubilized in octyl β-D-glucopyranoside and diluting the mixture into buffer. Under these conditions, lipid vesicles form, incorporating hydrophobic protein complexes presumably in both possible orientations into the lipid bilayer. Accumulation of maltose was dependent on the presence of ATP inside the vesicles and MBP outside, and ATP was hydrolyzed during transport (5, 9). For the following experiments, we modified the reconstitution procedure by trapping MBP and maltose inside the proteoliposomes and measured the hydrolysis of ATP added to the external medium, presumably catalyzed by protein complexes inserted in the reverse orientation. Under these conditions, it was possible to manipulate conditions so that only a small percentage of the total ATP was hydrolyzed during the assay.

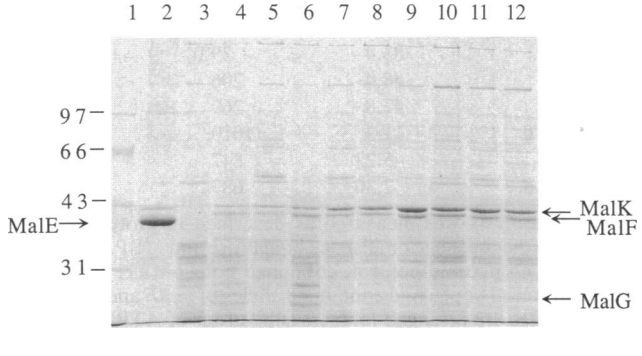

FIG. 1. Composition of proteoliposome vesicles. Proteoliposomes were prepared from strain HN741 (no plasmid) or HN741 overexpressing *malF*, *malG*, and *malK* alleles on plasmids under the control of the *trc* promoter. Samples, each containing 5 μg of protein, were loaded on the gel. The gel was stained with Coomassie blue. Lanes: 1, molecular weight markers (× 10^{-3}); 2, *mal*$^+$ prepared in the presence of MBP; 3, Δ*malB13* (chromosomal); 4, *mal*$^+$; 5, *malG510*; 6, *malG511*; 7, *malF500*; 8, *malF502*; 9, *malF506*; 10, *malF* p; 11, *malF502* d; 12, *malF506* d. For p and d alleles, see Table 1 legend.

In Fig. 2, where proteoliposomes were prepared from a strain overexpressing the wild-type *malF*, *malG*, and *malK* alleles, little or no hydrolysis of ATP was seen in the absence of MBP. When MBP and maltose were present inside the proteoliposomes, ATP was hydrolyzed at a rate of 390 nmol per min per mg of protein. This rate greatly exceeds both the rate of ATP hydrolysis (≈20 nmol per min per mg of protein) and the rate of maltose accumulation (6 nmol per min per mg of protein), found in previous experiments with proteoliposomes (5, 9).

Fig. 2 also shows that the addition of MBP in the absence of maltose can stimulate ATP hydrolysis, albeit at a lower rate. The possibility that this activity resulted from contamination of the MBP by maltose is unlikely for two reasons. First, the MBP used in this experiment had been dialyzed for a period sufficient for removing most of the bound maltose (14), and maltose-free MBP prepared by denaturation in 6 M Gdn·HCl yielded similar results. Second, if any maltose is bound to the MBP trapped inside the vesicles, it should be rapidly transported to the outside upon the addition of ATP. Even if the MBP were saturated with maltose, the amount of MBP and therefore the amount of maltose trapped (10–25 pmol per mg of protein) is far too low to account for the ATP hydrolysis rate found in this experiment (36 nmol per min per mg of protein). The observation that MBP, even in the absence of the transport event, can stimulate ATP hydrolysis raises the intriguing possibility that one previously unrecognized function of the MBP in maltose transport is to stimulate ATP hydrolysis by the membrane components. Support for this hypothesis was gained by studying the mutant proteins as described below.

ATP Hydrolysis by the MalG511 Complex. The *malG511* allele enables the cell to transport maltose in the absence of MBP (8). ATP hydrolysis was measured in proteoliposomes prepared from a strain overexpressing *malK*$^+$, *malF*$^+$, and *malG511* alleles. Fig. 3 shows that, in stark contrast with the wild-type complex, the MalG511 complex was able to hydrolyze ATP at a rate of 70 nmol·min^{-1}·mg^{-1} over a period of 2 hr in the complete absence of maltose and of MBP. The MalG511 complex, but not the wild-type complex, was also

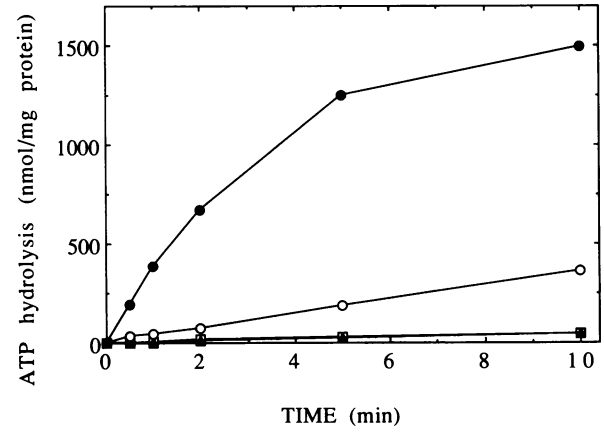

FIG. 2. ATP hydrolysis by the wild-type complex. ATP hydrolysis was measured in proteoliposome preparations as described. Proteoliposomes were prepared as described from HN741 harboring pMR11 as well as pFG23, which carried the *malF*$^+$ and *malG*$^+$ alleles, in the presence of maltose and MBP (●), MBP (○), maltose (□), or no addition (▲).

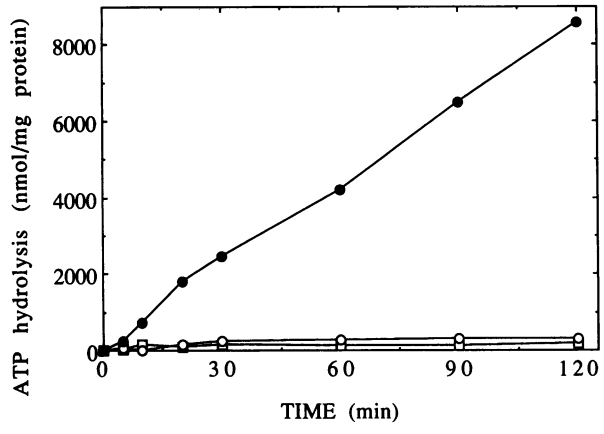

FIG. 3. Comparison of ATP hydrolysis by the transport complex from *mal*$^+$ and *malG511* strains. ATP hydrolysis was measured in proteoliposomes as described. Proteoliposomes were prepared in the absence of MBP and maltose from strain HN741 not containing any plasmids (Δ*malB*) (○), with pMR11 and pFG23 (*mal*$^+$) (□), and with pMR11 and pLH33 (*malG511*) (●).

able to hydrolyze ATP at a rate of 50 nmol·min^{-1}·mg^{-1} when present in detergent solution before reconstitution.

Effect of MBP on ATPase Activity of the MalG511 Complex. In Fig. 4, maltose and/or MBP were trapped inside proteoliposomes containing the MalG511 complex. The presence of maltose alone inside did not affect the basal rate of ATP hydrolysis. However, just as with the wild-type complex, the presence of MBP, and especially the combination of maltose and MBP stimulated ATP hydrolysis by the MalG511 complex.

It is clear from Fig. 4 that the *malG511* mutation does not cause full expression of ATPase activity in the absence of MBP. This result may explain why a Δ*malE*, *malG511* strain grows more slowly on maltose than a *mal*$^+$ strain (8). We therefore examined several other alleles that permit MBP-independent transport to determine whether (*i*) the enhanced ability to hydrolyze ATP is characteristic of all mutations, and (*ii*) there is any correlation between the basal rate of hydrolysis in the absence of MBP and the growth rate in maltose minimal medium.

Measurement of ATP Hydrolysis Activity in Other MBP-Independent Mutants. To compare hydrolysis rates exhibited by different *malF* and *malG* alleles, all alleles were placed on plasmids under the control of the *trc* promoter. Fig. 1 shows

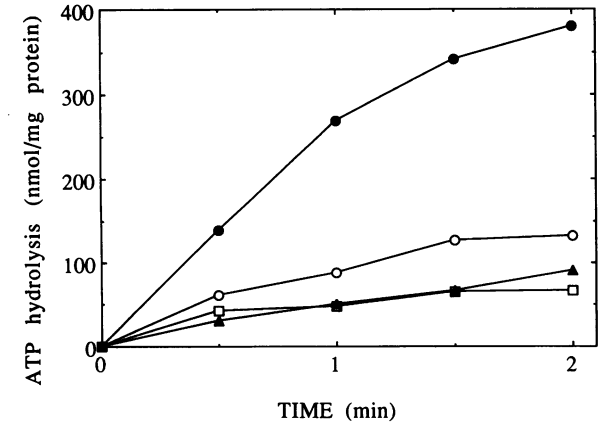

FIG. 4. ATP hydrolysis by the MalG511 complex. ATP hydrolysis was measured in proteoliposome preparations as described. Proteoliposomes were prepared from strain HN741 with pMR11 and pLH33 in the presence of maltose and MBP (●), MBP alone (○), maltose alone (□), or no addition (▲).

the protein composition of proteoliposome vesicles prepared from strains carrying different *mal* alleles separated by SDS/PAGE. Scanning of this gel showed that the degree of overproduction of transport proteins varied <2-fold between strains, with the MalF, MalG, and MalK proteins making up from 17% to 31% of the total vesicular protein. Like the MalG511 complex, each of the mutant complexes was able to hydrolyze ATP in the absence of MBP (Table 1). There is a clear correlation between the rate of ATP hydrolysis in the absence of MBP and the growth rate in the maltose minimal medium of a strain without MBP containing the corresponding *malF* or *malG* allele on the chromosome (Table 1). A strain carrying the *malF500* allele grew as fast in the absence of MBP as the wild type did in the presence of MBP. The MalF500 complex hydrolyzed ATP at a rate of almost 1 μmol per min per mg of protein and this rate may reflect complete uncoupling of ATPase activity. An analysis of the kinetics of ATP hydrolysis yielded a K_m value of 0.18 mM and a V_{max} of 2.3 μmol per min per mg of protein. These kinetic constants do not differ greatly from those obtained with the wild-type complex in the presence of liganded MBP, with a K_m of 0.074 mM and a V_{max} of 0.86 μmol per min per mg of protein. Assuming that the MalF500 complex (M_r 170,000) makes up 25% of the total protein in the proteoliposome (see Fig. 1), the turnover number for ATP hydrolysis by the transport complex is 25 per sec. Given that only 50% of the complex may be oriented in the membrane with the ATP-binding sites facing the medium, this rate compares favorably with the value of 20–200 per sec calculated based on the observations that there are from 100 to 1000 copies of MalF per cell (18) and that the V_{max} for transport in whole cells is 2 nmol per min per 10^8 cells (19).

Sequencing of the alleles responsible for binding protein-independent transport revealed that each allele had two separate mutations within the same gene (H.A.S., unpublished data). Strains carrying only one of these two mutations (indicated as p and d in Table 1) were unable to grow in maltose minimal medium. The ATPase activity of these

Table 1. ATP hydrolysis by mutant transport complexes

Allele on malFG plasmid	ATP hydrolyzed, nmol per min per mg of protein: No addition	ATP hydrolyzed, nmol per min per mg of protein: + MBP, + maltose	Growth rate constant, hr^{-1}
No plasmid	<0.5	<0.5	<0.02
mal$^+$	1.06	341	0.55*
malF500	988	452	0.55
malF502	156	228	0.46
malG510	83.4	39.0	0.36
malF506	48.4	206	<0.21
malG511	47.8	269	0.17
malF500 d	11.3	1010	<0.02
malF p	5.23	847	<0.02
malF506 d	2.30	687	<0.02

ATP hydrolysis was measured as described in proteoliposomes prepared in the presence or absence of maltose and MBP. All strains, except the no plasmid control, contained both pMR11 (coding for MalK) and another plasmid containing alleles of *malF* and *malG*. The mutant alleles are arranged in decreasing order of ATPase activity, so the correlation between the activity and the growth rate is clearly shown. Allele *malF* p contains only the common promoter-proximal mutation that is found in all of the *malF* mutant alleles, whereas alleles denoted by d contain the promoter-distal mutations out of the pair of mutations present in the *malF* alleles indicated (H.A.S., unpublished data). The growth rate constants in maltose minimal medium are for strains containing the corresponding *malF* or *malG* allele on the chromosome, in a Δ*malE* background. The growth data are from ref. 8, except for the p and d alleles.
*This value is for a strain containing a *malE*$^+$ allele.

strains was increased only marginally over the wild type (Table 1).

The effect of wild-type MBP and maltose on hydrolysis rates by the mutant complexes was variable. As shown in Table 1, the MalF502 and MalF506 complexes, like the MalG511 complex, appear to be able to interact productively at least under certain conditions (see *Discussion*). ATPase activity was inhibited by MBP in complexes containing either MalG510 or MalF500 proteins. These results suggest that the inhibitory effect of wild-type MBP on maltose transport in whole cells carrying these alleles (20) is due to an inhibition of the spontaneous ATPase activity. Whereas the stimulatory effect of the MBP seen with the wild-type and MalG511 complexes was enhanced in the presence of maltose, we found that the inhibitory effect that MBP exerted on the MalF500 complex was independent of maltose.

DISCUSSION

The reconstitution technique has proven to be very versatile for the study of the maltose transport system because it appears that the membrane-associated complex is reincorporated into the proteoliposome vesicles in both orientations. The asymmetric addition of MBP, maltose, and ATP across the membrane has permitted the assay of complexes in one orientation while those present in the opposite orientation remain functionally silent. In this fashion, we have measured both maltose accumulation by vesicles (9), and, in this study, ATP hydrolysis by the transport complex. Comparisons of ATP hydrolysis in the presence and absence of maltose and MBP have revealed that mutant transport complexes have gained the ability to hydrolyze ATP constitutively. These results have important implications regarding the role of MBP in maltose transport.

The periplasmic binding proteins are often present in high concentrations and bind their ligands with a K_d of 1–10 μM. Thus, even when the extracellular concentration of maltose is in the micromolar range, the periplasmic concentration of maltose and MBP will be close to 1 mM, undoubtedly facilitating the efficient, uphill transport of maltose. At least in the maltose system, however, the MBP is not a mere facilitator but is absolutely required for transport to occur in wild-type cells (7). Beyond this point, however, there is currently no consensus on the precise function of the periplasmic binding proteins in transport. The liganded MBP must interact with the periplasmic face of the membrane complex, but the direct consequences of this interaction have not been elucidated.

The isolation of mutations in *malF* and *malG* that bypass the requirement for MBP has contributed significantly to the current model for transport. It is hypothesized that the wild-type MalF/MalG channel normally exists in a closed conformation and that the contact with the liganded MBP opens up the channel, exposing the ligand-binding sites within the channel (20). In the MBP-independent mutants, some channels are in an open conformation, so that the MBP is not required for transport. However, it has not been possible to specify at what stage ATP hydrolysis is involved.

The results presented in this article allow us to refine this model in a significant way (Fig. 5). We found that the MBP-independent transport complexes can hydrolyze ATP in the absence of maltose and MBP. Furthermore, the rates of ATP hydrolysis exhibited by the different mutant complexes were strongly correlated with the growth rates of these MBP-deleted strains on maltose and thus, presumably, with the rates of maltose transport in the absence of MBP. These results indicate that it is the ability of these mutant complexes to hydrolyze ATP constitutively that accounts for their ability to accumulate maltose in the absence of MBP. They further suggest that in wild-type cells, ATP hydrolysis is controlled

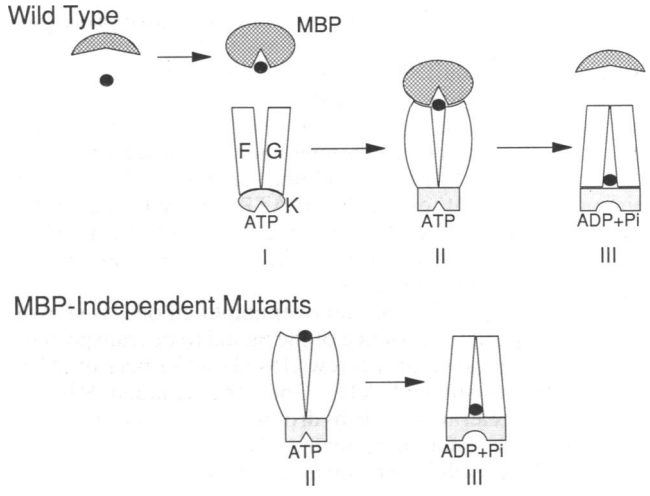

FIG. 5. Model for maltose transport. In the wild type, the MBP in its open conformation binds maltose and is converted into a closed form. This interacts with the MalFGK complex embedded in the membrane, which is in conformation I with the MalK ATPase in its inactive form. The binding of the liganded MBP changes affects the conformation of the transmembrane proteins F and G, finally resulting in activation of MalK ATPase (conformer II). Hydrolysis of ATP is thought to push the ligand through the channel (conformer III). (Although the model shows conformer I contains an open channel accessible to ligand, at this stage the channel may be closed, resembling conformer III.) In the MBP-independent mutants, the MalFGK complex is usually in its activated conformation (conformer II). Note that in this conformation, the periplasmic faces of F and G form a complementary surface for the possible binding of liganded MBP.

by the interaction of MBP with the periplasmic surface of the membrane transport complex. The wild-type complex indeed did not hydrolyze ATP by itself. Furthermore, its hydrolytic activity was stimulated by the addition of even ligand-free MBP, a result showing that the presence of MBP, rather than the presence of transported ligand molecules, is sufficient for causing ATP hydrolysis. All these results are thus best explained by assuming that one of the important functions of MBP, and presumably of binding proteins in general, is to interact with the periplasmic surface of the membrane-associated complex and thereby transmit a signal across the membrane, stimulating ATP hydrolysis by the MalK protein on the other side of the membrane (Fig. 5). In this respect, the maltose transport complex functions in a manner analogous to several other membrane-associated receptor proteins, including the maltose chemotaxis receptor (21) and the growth factor receptor tyrosine kinase (22), which modify the activities of their cytoplasmic domains in response to extracellular signals.

The MBP undergoes a conformational change upon binding the ligand, from an open unliganded form to a closed liganded form (23). ATP hydrolysis by the wild-type complex was maximally stimulated only when the liganded MBP was added (Fig. 2), and thus we assume in Fig. 5 that the membrane complex interacts with the closed conformers of the MBP (23). The modest stimulation caused by the unliganded MBP (Fig. 2) could be due to the small fraction of closed but unliganded conformers of MBP that could exist in equilibrium with the open conformers.

Most previous models of binding protein-dependent transport assumed that ATP hydrolysis is obligatorily coupled to the translocation of the ligand. In contrast, we propose that these processes are totally uncoupled in some mutants and are probably only indirectly coupled even in the wild type. This type of model will accommodate previous results obtained in membrane vesicles and in reconstituted systems in

which the ratio of ATP hydrolysis to maltose transported was generally >1 (9, 24). Although the most active mutant complex containing MalF500 hydrolyzed ATP rapidly (Table 1), we can calculate that a cell expressing this allele from the chromosome will be wasting 2500–25,000 molecules of ATP per sec in medium not containing maltose. However, an *E. coli* cell growing with a doubling time of 1 hr will be generating $\approx 1 \times 10^7$ molecules of ATP per sec based on the accepted value of 10 g of growth yield per mol of ATP (25), and thus we can expect that this futile ATP hydrolysis will not slow down growth detectably.

Our current model, albeit an oversimplified one, is shown in Fig. 5. Thus, in the absence of the ligand to be transported, the presence of no or only a few closed conformers of MBP prevents the wasting of ATP. Once the liganded MBP is formed, it accelerates ATP hydrolysis by functioning as a transmembrane signaling molecule. The ATP hydrolysis may in turn produce cyclic conformational changes in the channel, which result in transport as well as in release of the unliganded MBP from the external surface of the MalFGK complex. In MBP-independent mutants, mutations in MalF and MalG presumably alter the conformation of the membrane complex so that the MalK ATPase remains constantly activated. Examples of point mutations in membrane receptors inducing conformational changes resulting in constitutive activation have been found in several membrane receptors, including the chemotaxis receptor (26), an adrenergic receptor (27), and an oncogenic growth factor receptor (28).

With some of the mutant complexes, the addition of liganded MBP stimulated ATP hydrolysis (Table 1). At first sight this seems to be inconsistent with the finding that the presence of MBP inhibited transport in cells carrying the mutant *malF* or *malG* alleles (20). However, maltose transport into MalG511 membrane vesicles was indeed shown to be stimulated by low concentrations of liganded MBP, whereas strong inhibition was observed when its concentrations were raised to approach that in the periplasm (D. A. Dean, L. Hor, H.A.S., and H.N., unpublished data). It is therefore not surprising to see stimulation of ATP hydrolysis under certain conditions. In fact, these results reinforce our model. We view the membrane-associated complex as an allosteric complex (Fig. 5). According to the induced fit model (29), the binding of the liganded MBP shifts the conformation of the membrane-associated wild-type complex (from I to II in Fig. 5). In the mutant, most of the complexes already exist in the activated conformation (II) even in the absence of MBP. Since this conformer has a binding site providing a good fit to the liganded MBP, one would expect that they show a higher affinity for maltose MBP than the wild-type complex. Indeed this was found to be the case (D. A. Dean, L. Hor, H.A.S., and H.N., unpublished data). With this higher affinity, it is understandable that high concentrations of liganded MBP inhibit the mutant transport complex more strongly than the wild-type complex, perhaps in a manner similar to substrate inhibition of enzymes. There are several examples in which constitutive activation of allosteric proteins by mutation have resulted in increased affinity for the activating ligand, including the α_1-adrenergic receptor (27) and the MalT transcriptional activator (30).

Note. During review of this manuscript, a report appeared showing, in another binding protein-dependent transport system, mutations in the membrane-associated complex also displayed an increased ATPase activity (31). A striking difference between this system and ours is that whereas all of the mutations in the histidine system are located in the ATPase protein HisP, those isolated in our system are located exclusively in the presumed channel proteins MalF and MalG.

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1. Boos, W. & Hengge, R. (1983) *Biochim. Biophys. Acta* **737**, 443–478.
2. Dassa, E. & Hofnung, M. (1985) *EMBO J.* **4**, 2287–2293.
3. Froshauer, S. & Beckwith, J. (1984) *J. Biol. Chem.* **259**, 10896–10903.
4. Shuman, H. A. & Silhavy, T. J. (1981) *J. Biol. Chem.* **256**, 560–562.
5. Davidson, A. L. & Nikaido, H. (1991) *J. Biol. Chem.* **266**, 8946–8951.
6. Higgins, C. F., Hiles, I. D., Walley, K. & Jamieson, D. J. (1985) *EMBO J.* **4**, 1033–1040.
7. Shuman, H. A. (1982) *J. Biol. Chem.* **257**, 5455–5461.
8. Treptow, N. A. & Shuman, H. A. (1985) *J. Bacteriol.* **163**, 654–660.
9. Davidson, A. L. & Nikaido, H. (1990) *J. Biol. Chem.* **265**, 4254–4260.
10. Hofnung, M. (1974) *Genetics* **76**, 169–184.
11. Reyes, M. & Shuman, H. A. (1988) *J. Bacteriol.* **170**, 4598–4602.
12. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed., Vol. 3, p. A.2.
13. Ferenci, T. & Klotz, U. (1978) *FEBS Lett.* **94**, 213–217.
14. Silhavy, T. J., Szmelcman, S., Boos, W. & Schwartz, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2120–2124.
15. Miller, D. M., Newcomer, M. E. & Quiocho, F. A. (1979) *J. Biol. Chem.* **254**, 7521–7528.
16. Gehring, K. (1988) Ph.D. thesis (Univ. of California, Berkeley).
17. Lill, R., Cunningham, K., Brundate, L. A., Ito, K., Oliver, D. & Wickner, W. (1989) *EMBO J.* **8**, 961–966.
18. Shuman, H. A., Silhavy, T. J. & Beckwith, J. R. (1980) *J. Biol. Chem.* **255**, 168–174.
19. Szmelcman, S. & Schwartz, M. (1976) *Eur. J. Biochem.* **65**, 13–19.
20. Treptow, N. A. & Shuman, H. A. (1988) *J. Mol. Biol.* **202**, 809–822.
21. Macnab, R. M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington), Vol. 1, pp. 732–759.
22. Ulrich, A. & Schlessinger, J. (1990) *Cell* **61**, 203–212.
23. Quiocho, F. A. (1990) *Philos. Trans. R. Soc. London Ser. B* **326**, 341–351.
24. Dean, D. A., Davidson, A. L. & Nikaido, H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9134–9138.
25. Tempest, D. M. & Neijssel, O. M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington), Vol. 1, pp. 797–806.
26. Simon, M. I., Mutoh, N. & Oosawa, K. (1986) *J. Bacteriol.* **167**, 992–998.
27. Cotecchia, S., Exum, S., Caron, M. G. & Lefkowitz, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2896–2900.
28. Bargmann, C. I., Hung, M. C. & Weinberg, R. A. (1986) *Cell* **45**, 649–657.
29. Koshland, D. E., Jr. (1959) in *The Enzymes*, eds. Boyer, P. D., Lardy, H. & Myrbäck, K. (Academic, New York), 2nd Ed., Vol. 1, pp. 305–346.
30. Dardonville, B. & Raibaud, O. (1990) *J. Bacteriol.* **172**, 1846–1852.
31. Petronilli, V. & Ames, G. F.-L. (1991) *J. Biol. Chem.* **266**, 16293–16296.