The active transport of maltose in *Escherichia coli* requires the products of five genes. These include a water-soluble periplasmic maltose-binding protein, three cytoplasmic membrane proteins, and an outer membrane protein. In order to evaluate the role of the maltose-binding protein in active transport, a nonpolar internal deletion of the structural gene for the maltose-binding protein was constructed. A strain which contains this deletion is unable to grow on maltose at an external concentration of 25 mM, even when the remaining components of the transport system are synthesized constitutively. This demonstrates that the periplasmic maltose-binding protein is essential for detectable translocation of maltose across the cytoplasmic membrane.

Mal⁺ revertants of the deletion strain were obtained. In one of these strains, the remaining components of the maltose transport system gained the ability to translocate maltose across the membrane independently of the periplasmic binding protein. Maltose transport in this revertant strain is specific for maltose; it is not inhibited by other α and β glucosides and galactosides. In contrast to the wild type, transport activity in the Mal⁺ revertant strain is retained by spheroplasts.

The cytoplasmic membrane components of the maltose transport system in the revertant appear to form a substrate recognition site. It is likely that this site exists in wild type cells but is available only to substrate molecules that are bound to the maltose-binding protein. A model for the operation of the transport system is presented. In this model, the substrate recognition site in the cytoplasmic membrane is exposed to alternate sides of the membrane.

The energy-dependent accumulation of solutes by cells can occur by a variety of mechanisms. These can be generally classified into two groups. In the first of these, solutes are cotransported across the membrane with an ion; the energy of the ion gradient provides the driving force for the accumulation of the solute. In the other group, solute translocation is driven by the energy derived from the breaking of a high energy chemical bond.

Many active transport systems have been studied in *Escherichia coli*. Perhaps the most extensively characterized system is the β-galactoside or lactose transport system. This system corresponds to the first group described above. The electrochemical gradient of protons, established by the respiratory chain, provides the energy for lactose accumulation. Lactose-proton co-transport has been demonstrated and quantitated under a variety of conditions (1-3). While many systems have been identified which are mechanistically similar to the lactose system, there are some which differ substantially from the ion co-transport mechanism. This distinction was first made in *E. coli* as a result of the elegant studies of Berger (4, 5). Berger showed that the electrochemical gradient of protons was sufficient for the energization of some systems, while other systems were independent of the proton gradient but required ATP or some form of energy derived from glycolysis. Berger was able to establish that in general, systems which depend on phosphate energy have a water-soluble periplasmic protein which binds substrate. In contrast, systems which depend on the proton gradient have no such soluble binding protein. Although there is some understanding of how the ion co-transport systems function, the operation of the systems which involve a soluble periplasmic binding protein remains almost totally obscure.

Most of the effort in studying these systems has concentrated on the physical properties of the binding proteins themselves. A number of these have been purified and sequenced (6, 7) and the crystal structure has been resolved for one of these to a resolution of 2.4 Å (8). In contrast, very little is known about the plasma membrane components of these transport systems. It is only recently that any of these have been identified (9-12). These proteins seem to consist of both integral and peripheral membrane proteins which are present in smaller amounts than the corresponding periplasmic component. While it has not been possible to directly demonstrate an interaction of the membrane components with the periplasmic binding protein in *vitro*, there is genetic evidence in the histidine transport system of *Salmonella typhimurium* that some interaction occurs (13). There is other genetic evidence for the β-methyl galactoside transport system which suggests that the membrane components are able to translocate substrate across the membrane independently of the binding protein (14).

The accumulation of maltose and longer α(1 → 4)-linked maltodextrin oligosaccharides by *E. coli* requires the products of five genes, including a periplasmic maltose-binding protein coded for by the malE gene (15). The remaining components of this system consist of an outer membrane protein and three plasma membrane proteins. The outer membrane protein which also serves as the receptor for bacteriophage λ is coded for by the lamB gene and is necessary for the passage of maltodextrins across the outer membrane (16, 17). The three plasma membrane proteins are coded by the malF, malG, and malK genes.

These three plasma membrane components mediate the
energy-dependent accumulation of maltose and maltodextrins in the cytoplasm. The role of the periplasmic binding protein in this process has not been clear. The results described below demonstrate that in wild-type cells the binding protein is essential for substrate translocation across the plasma membrane. In addition, a mutant is described in which the transport system has been altered so that the membrane components can function independently of the binding protein. These results are discussed in terms of a model for the operation of binding protein-dependent transport systems.

**MATERIALS AND METHODS**

**Bacterial Strains**—Bacterial strains are listed in Table I.

**Media and Genetic Techniques**—Rich media, minimal media, and indicator have been described previously (21). Standard genetic techniques were performed as described by Miller (22). The techniques for mapping the malEFG operon have been described in detail (21).

In order to select for the presence of the Tn5 transposon, kanamycin (30 μg/ml) was included in media, when appropriate.

**Isolation of an Internal Nonpolar Deletion of the malE Gene**—In order to evaluate the role of the maltose-binding protein it was necessary to construct a strain containing a deletion of the structural gene for the binding protein (malE) which did not interfere with the production of the remaining components of the transport system. The organization of the genes coding for the maltose transport system is shown in Fig. 1. In order to select for strains in which part of the malE gene is deleted, a copy of the Tn5 transposon was inserted in the malE gene. This insertion inactivates the malE gene and also prevents expression of the malF and malG genes from Pmal. By selecting for derivatives of the Tn5-containing strain in which expression of the malF and malG genes was restored, it was possible to isolate strains in which the Tn5 transposon and adjacent portions of the malE gene were deleted.

In order to facilitate selection for restoration of malF expression, a strain was used in which the genes for lactose

**Table I**

**Bacterial strains**

Genetic nomenclature is from Bachmann and Low (15). The symbol (Φ) designates a gene fusion which is described in parentheses. The hy+ designation refers to the fact that a hybrid protein is produced; the 1 - 1 is the number assigned to the particular fusion; : indicates an insertion within the preceding gene.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSM7</td>
<td>F' araD139 Δlac U169 rpsL thi malE::Tn5-7</td>
<td>T. J. Silhavy</td>
</tr>
<tr>
<td>H56010</td>
<td>F' araD139 Δlac U169 rpsL thi malE::Tn5-7 Φ(malF-lacZ) hy+1-1</td>
<td>This study</td>
</tr>
<tr>
<td>H53050</td>
<td>F' araD139 Δlac U169 rpsL thi smalE444 Φ(malF-lacZ) hy+1-1</td>
<td>This study</td>
</tr>
<tr>
<td>H53110</td>
<td>F' KL10 metB* malE* F'*G'/ H50905</td>
<td>This study</td>
</tr>
<tr>
<td>H56000</td>
<td>F' araD139 Δlac U169 rpsL thi Φ(malF-lacZ) hy+1-1</td>
<td>Silhavy et al. (19)</td>
</tr>
<tr>
<td>H56700</td>
<td>F' KL10 metB* malE* F'*G'/ H56000</td>
<td>This study</td>
</tr>
<tr>
<td>H52019</td>
<td>F' araD139 Δlac U169 rpsL thi smalE444</td>
<td>This study</td>
</tr>
<tr>
<td>H53018</td>
<td>F' araD139 Δlac U169 rpsL thi malT*1 smalE444</td>
<td>This study</td>
</tr>
<tr>
<td>H53025</td>
<td>F' araD139 Δlac U169 rpsL thi malT*2 smalE444</td>
<td>Mal* revertant of H52019</td>
</tr>
<tr>
<td>H53045</td>
<td>F' araD139 Δlac U169 rpsL thi malG<em>1 malF</em>1</td>
<td>MalE revertant of H52019</td>
</tr>
<tr>
<td>H53069</td>
<td>F' KL10 metB* Φ(malF-lacZ) hy+1-1 H53025 recA-1</td>
<td>This study</td>
</tr>
<tr>
<td>pop301</td>
<td>F' araD139 Δlac U169 rpsL thi Φ(malF-lacZ)</td>
<td>Debarbouillé et al. (20)</td>
</tr>
<tr>
<td>H53013</td>
<td>F' araD139 Δlac U169 rpsL thi smalE101</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Fig. 1. Structure of the malB region of the Escherichia coli chromosome.** A, the wild type region. The malEFG operon and the malK lamB operons are transcribed in opposite directions. All five genes are necessary for normal transport function. Pmal indicates the location of the promoter(s) and control regions for the two operons (21, 24). B, steps leading to the isolation of an internal nonpolar deletion of the malE gene. i, the structure of a malF-lacZ fusion gene. Expression of the malF-lacZ fused gene and the lacY gene is now under the control of Pmal. ii, insertion of a Tn5 element in the malE gene results in a polar block so that the malF-lacZ and lacY genes cannot be expressed. The strain containing this structure is Lac-. iii, one class of Lac+ revertants results from deletion of Tn5 element and surrounding malE DNA. In the malE deletion can be recombined from the fusion strain to another strain which does not contain the gene fusion.
Active Transport of Maltose in E. coli K12

Table II

<table>
<thead>
<tr>
<th>Levels of malF-lacZ hybrid β-galactosidase in malE+ and ΔmalE strains</th>
<th>β-Galactosidase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Uninduced</td>
</tr>
<tr>
<td>HS6790</td>
<td>1.5</td>
</tr>
<tr>
<td>F' malE-G'</td>
<td>3.0</td>
</tr>
<tr>
<td>F' malE-lacZ111-1</td>
<td></td>
</tr>
<tr>
<td>HS3110</td>
<td></td>
</tr>
<tr>
<td>F' malE-G'</td>
<td></td>
</tr>
<tr>
<td>F' ΔmalE4444 [malF-lacZ]111-1</td>
<td></td>
</tr>
</tbody>
</table>

*1 unit of β-galactosidase is that amount of enzyme that hydrolyzes 1 nmol of o-nitrophenyl-β-D-galactoside per min at 30 °C. Cells were grown overnight in M63 containing either glycerol (uninduced) or maltose (induced). The cultures were diluted 1:20 with fresh media grown to an optical density of 0.5 at 600 nm. Assays for β-galactosidase were done in triplicate, and the numbers refer to units per mg of protein.

Utilization, lacZ and lacY were fused to the malF gene. In the absence of the Tn5 element, the expression of the hybrid malF-lacZ gene and the lacY gene is under the control of Pmal (Fig. 1). A strain (HS6010), which contains the malF-lacZ fusion and the Tn5 insertion in malE is, therefore, unable to grow on lactose. It is possible to select derivatives of strain HS6010 which are able to grow on lactose. In some of these strains, expression of the malF-lacZ and lacY genes from Pmal has been restored. These can arise either by excision of the Tn5 element or by the formation of deletions within the malE gene surrounding the Tn5 insertion that do not result in premature termination codons.

Seventy-five spontaneous Lac+ derivatives were obtained from eight independent cultures of HS6010. Fourteen kanamycin-sensitive clones were chosen for further study. The amount of malE genetic material remaining in each of these strains was determined by mapping procedures that are described elsewhere. One of the 14 strains contained only the first three and last two genetic markers in the malE gene.

The strain which contains this deletion (HS3050) was chosen for further study. If the formation of the deletion in this strain did not generate a termination codon then expression of the malF-lacZ hybrid protein ought to be identical in the malE- strain (HS6000) and the ΔmalE deletion strain (HS3050). The β-galactosidase activity in malE- strains was determined with the aid of mapping procedures (see Table II). These results show that the malE deletion in strain HS3050 does not affect expression of the malF gene.

The malE deletion was then transferred from the fusion strain, HS3050, to a strain containing an otherwise normal maltose genetic region. This was accomplished using a λatched malF-lacZ transducing phage as described previously (25). The resulting strain, HS2019, could be complemented to Mal' by different episomes carrying mutations in malF malG and malK but not by those carrying mutations in the malE gene. In addition, mapping of the malE gene in HS2019 yielded results identical with those obtained with strain HS3050. A strain of HS2019 that expresses the maltose regulon constitutively was then constructed (HS3018). This was accomplished with the aid of a specialized transducing phage that carries a copy of the malT*-malF-allele. This allele of the malT gene codes for a mutant positive regulatory protein that results in constitutive expression of the maltose regulon at approximately 80% of the fully induced level (20).

The Maltose-binding Protein Is Required for Substrate Translocation across the Plasma Membrane—In order to evaluate the necessity of MBP for substrate translocation across the plasma membrane, the ability of strain HS3018 to grow on maltose was examined. The ability of intact cells to metabolize a substrate can be a convenient measure of the entry of the substrate into the cell when the rate of metabolism is not rate limiting (26). It has been previously shown that the rate of substrate entry, rather than the level of amyloyl maltase, is rate limiting for growth on maltose (27). A strain which contains the complete transport system grows in M63 media containing 5.5 mM maltose with a doubling time of 60 min. The isogenic strain containing the deletion of the malE gene described above does not show detectable growth on maltose under the same conditions (i.e., t1/2 > 600 min). Neither strain HS2019 nor HS3018 shows appreciable growth on maltose (Table II).

The inability of these strains to grow on maltose is not due to the inability of maltose (M = 342) to penetrate the outer membrane since the porin proteins allow hydrophilic compounds less than 700 molecular weight access to the periplasm and the plasma membrane (28). In addition, it has been shown elsewhere that maltose can reach the plasma membrane in strains which lack all components of the transport system (29). These results demonstrate that maltose-binding protein is essential for substrate translocation across the plasma membrane. Furthermore, Brass et al. have demonstrated that maltose uptake does not occur in strain HS3018 but that exogenous MBP can restore maltose transport activity in cells which have been treated with lambda phage to allow MBP access to the plasma membrane (30).

Isolation of Mutants in Which Maltose Transport Occurs in the Absence of the Maltose-binding Protein—Since the malE mutation in strain HS3019 is a deletion, it is not possible to isolate Mal+ revertants which have regained wild type maltose-binding protein. However, after mutagenesis with UV light (3 J/m2, for 1 min), 60 derivatives of strain HS2019 were obtained that were able to grow on maltose as sole carbon source. These mutants were obtained at a frequency of 10⁻⁷. Three possible explanations were considered for these mutants. The first is that another transport system has gained the ability to recognize maltose. This type of mutant has been previously known to occur (29,31). The second possibility was that another periplasmic binding protein had gained the ability to replace the function of the maltose-binding protein. The third possibility was that the remaining components of the maltose transport system had gained the ability to function in the absence of the binding protein.

In order to distinguish these possibilities it was determined if the mutation which resulted in the Mal+ phenotype mapped near the genes of the maltose transport system. If the mutation had altered another transport system or binding protein then it would be unlikely to map near the malB genes. If, however, the mutation had altered the maltose transport system itself, then it should be closely linked to the remaining malB genes. Six of the Mal+ derivatives of HS2019 were transduced to kanamycin resistance with P1 phage grown on a derivative of HS2019 which had Tn5 inserted in the lamB gene (see Fig. 1). In five of the mutants, all of the kanamycin-resistant A-resistant transductants (100/100 for each) retained the Mal+ phenotype. In one of the mutant strains, HS3025, a large fraction of the kanamycin-resistant A-resistant transductants (80/100) had become Mal-. In addition, when this strain (HS3025) was transduced to Lac+ with P1 phage grown on a malF-lacZ fusion strain, all of the Lac+ transductants (48/48) were Mal+. These results are consistent with the idea that the Mal+ phenotype is due to mutation in a gene that is closely
linked to the lamB and malF genes.

Maltose Transport in Strain HS3025 is Maltose Inducible—If maltose transport in strain HS3025 is mediated by the remaining components of the maltose transport system, then transport activity ought to be maltose inducible. That is, transport activity should be greater in cells grown on maltose than in cells grown on another carbon source. Fig. 2 shows that when strain HS3025 is grown on maltose, the rate of maltose uptake is much greater than when it is grown on glycerol. This demonstrates that maltose transport activity observed in strain HS3025 is mediated by proteins that are part of the maltose regulon. It is possible that transport activity in HS3025 is due to a periplasmic binding protein that is not part of the maltose regulon and coded for by a gene very close to the malB region that is expressed constitutively. Evidence presented later demonstrates that this is not likely and, therefore, only components coded for by the remaining malB genes are responsible for maltose transport in strain HS3025.

Kinetic Characteristics of Maltose Transport in Strain HS3025—In order to measure maltose active transport in strain HS3025, a derivative was constructed that is unable to metabolize maltose. In this strain, HS3046, lacks both amylo-maltase and maltodextrin phosphorylase and is constitutive for expression of the maltose regulon.

The initial rate of maltose entry into HS3045 cells was measured as a function of the external concentration of maltose. The data are depicted in Fig. 3. Half-maximal velocity of transport occurred at an external maltose concentration of 1.7 mM. The maximum velocity of maltose entry, determined from the double reciprocal plot of the data, is 0.07 nmol/min/10^9 cells. These values are quite different from those obtained with cells having the wild type transport system. In these cells the apparent K_m is 1 μM; V_max is 2 nmol/min/10^9 cells (17).

Fig. 4 shows the time course of maltose entry into HS3045 cells at an external substrate concentration of 0.58 mM. It can be seen that these cells are able to accumulate maltose and maintain an internal concentration that is approximately 10-fold greater than the concentration in the medium. Formation of acetylmaltose (a nonphysiological compound found in cells containing high internal concentrations of maltose) under these conditions amounted to less than 20% of the total internal radioactivity (data not shown). This is a much smaller gradient than the 10^4:1 gradient maintained by cells containing the wild type system. These data indicate that although the transport system in strains HS3025 and HS3045 is much less efficient than the wild type system it can still actively transport substrate against a gradient.

Effect on Arsenate Treatment on Maltose Transport—It is well documented that arsenate treatment depletes bacterial cells of ATP without affecting the proton electrochemical gradient across the membrane (32). Many of the transport systems which involve a periplasmic binding protein have been shown to be sensitive to inhibition by arsenate (4). In order to examine the effect of decreasing the ATP concentration on the activity of the altered maltose transport system, transport assays were done on cells that had been pretreated with sodium arsenate in the absence of phosphate. It was found that treatment of strain HS3045 with 10 mM arsenate for 10 min totally abolished maltose transport activity (data not shown). These results indicate that the uptake of radio-
Active maltose represents the active energy-dependent accumulation of maltose.

Replacement of Maltose-binding Protein in Strain HS3025—The wild type maltose transport system enables cells to utilize polymers of glucose in α(1 → 4) linkage which are longer than maltose (maltodextrins). Strain HS3025, however, is unable to utilize longer dextrins as a carbon source. In order to study the strain HS3025 genetically it was necessary to first determine the effect of replacing the maltose-binding protein on the growth properties of this strain. This was accomplished by introducing an episome which carries the malE gene into strain HS3025. The phenotype of the malE derivative of strain HS3025 (HS3059) was Mal+Dex− and indistinguishable from that of HS3025 on maltose indicator media. This result suggests that the maltose-binding protein does not interact productively with the remaining components of the altered transport system in the mutant strain. In order to examine this point more carefully, the apparent KM and Vmax of maltose uptake were determined in strain HS3059. The value for KM was 3.0 mM, and the Vmax was 0.1 nmol/min/10^8 cells. These values are not significantly different than those obtained with HS3045.

If the ability of strain HS3025 to grow on maltose was due to a change in some other system, one would expect that replacement of MBP would restore the wild type Mal+Dex+ phenotype. Since this does not occur, it can be concluded that the revertant phenotype is due to a change in some component of the maltose transport system.

Maltose Transport in Strain HS3045 Occurs in Spheroplasts—If the maltose transport system in strain HS3045 operates independently of a periplasmic binding protein, then it should function in cells which have been converted to spheroplasts. Maltose transport was measured via the wild type and the mutant systems in whole cells and cells which had been converted to spheroplasts by treatment with lysozyme and EDTA. The results of this experiment are shown in Fig. 5.

It can be seen in Fig. 5A that although greater than 85% of the mutant cells were converted to spheroplasts, the rate of maltose entry is not substantially different than in whole cells. Fig. 5B shows that when 85% of wild type cells are converted to spheroplasts there is a dramatic decrease in the rate of maltose entry. The small residual transport activity in the wild type spheroplast preparation can be accounted for by cells which had not been converted to spheroplasts. In these cells the steady state level of transport is attained within 3 min. The 10-fold difference in the scales of Fig. 5, A and B, is due to the difference in the kinetic properties of the mutant and wild type transport systems (see above). These results confirm that the mutant transport system can operate independently of any periplasmic component. In addition it has been observed that HS3045 cells retain transport activity after the cold osmotic shock treatment of Neu and Heppel (33) (data not shown).

Specificity of the Revertant Transport System—In order to examine the specificity of the revertant transport system, various carbohydrates were tested for their ability to inhibit maltose transport in strain HS3045. These tests were performed with spheroplasts so that the ability of a given substance to penetrate the outer membrane would not affect its capability to interact with the plasma membrane components. Table III contains a summary of the results of these tests. The ability of a and β glucosides and galactosides to inhibit maltose transport suggests that the revertant transport system has the same specificity as the wild type system. Although maltodextrins are not transported by the revertant system, it is clear from their ability to inhibit maltose transport that they are capable of interacting with the plasma membrane components.

Preliminary Genetic Characterization of Strain HS3025—Initial attempts to determine which of the malB genes had been altered in strain HS3025 by classical complementation yielded ambiguous results (data not shown). Subsequent efforts to study this strain by marker rescue with λmalB transducing phages have shown that two mutations give rise to the Mal+Dex+ MBP-independent phenotype. These are in the malF and malG genes. This was established by first separating the two mutations by lysogenization of HS3025 with a λmalE+ malF-lacZ hybrid transducing phage (21). The mutation in malF is transferred to the malF-lacZ hybrid gene by recombination. As a result the lysogen becomes Mal− since the second mutation alone results in a Mal− phenotype. When this lysogen is cured of the phage it remains Mal−. Classical complementation tests revealed that this Mal− strain is Mal+ but malG (data not shown). The original λmalE+ malF-lacZ transducing phage is unable to transduce this Mal+ strain to Mal− since the phage does not carry any malG sequences (21). Only λmalF-lacZ phages from the Mal− lysogen of HS3025 are able to transduce this strain to Mal+Dex+.

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
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<tbody>
<tr>
<td><strong>Inhibition of maltose transport in spheroplasts</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Addition</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Maltose (5 mM)</td>
</tr>
<tr>
<td>Maltotriose (3.7 mM)</td>
</tr>
<tr>
<td>&quot;Dextrin&quot;*</td>
</tr>
<tr>
<td>Glucose (11 mM)</td>
</tr>
<tr>
<td>Lactose (5 mM)</td>
</tr>
<tr>
<td>Melibiose (5 mM)</td>
</tr>
<tr>
<td>Cellodextrin (5.8 mM)</td>
</tr>
<tr>
<td>Sucrose (5.8 mM)</td>
</tr>
</tbody>
</table>

*100% activity ranged from 1.08 to 1.33 nmol/10 min/1.4 × 10^10 cells. Transport assays were performed as described in the legend to Fig. 5 using a 0.275 mm radioactive maltose.
upon integration and transfer of the putative malF mutation from the hybrid malF-lacZ gene to the chromosomal malF gene. More careful mapping studies are in progress.

It is also possible to show that the malF function is required for the Mal"Dex" phenotype of strain HS3025. The malEFG operon of HS3025 was transferred to KLF 10, an episome that carries the entire malEFG operon but not the malK lamb operon. When this KLF 10 derivative was transferred into an malK"::malE::Tn5 strain (TSM 7) the resulting merodiploid was Mal"Dex". However, when the episome was transferred into a strain that carries a deletion of the entire malB region (HS2013), the resulting merodiploid strain was completely Mal+. These results confirm that the active transport of maltose in strain HS3025 is due to the altered components of the maltose transport system.

**DISCUSSION**

The maltose transport system is genetically well characterized and is the only transport system present in wild type cells that is able to transport maltose and maltotriose. In order to ask if the periplasmic binding protein is required for transport under various conditions, a well defined extensive deletion of the structural gene for the binding protein was isolated. It has been shown that this deletion does not interfere with the expression of the remaining mal genes.

The strain containing this deletion is unable to grow on maltose at external concentrations of maltose up to 25 mM. In addition, an isogenic derivative of the deletion-containing strain, which expresses all of the genes of the maltose regulon constitutively, was similarly unable to grow on maltose as a sole carbon source. Since in this strain any maltose that entered the cell would be rapidly catabolized, the inability of this strain to grow on maltose argues strongly that in the absence of binding protein translocation of maltose across the membrane does not take place at a detectable rate. Therefore, the maltose-binding protein appears to be necessary for translocation of maltose across the membrane via the membrane components of the system.

Since the deletion mutation cannot revert to wild type, it was possible to look for mutant strains in which maltose transport could occur independently of the maltose-binding protein. Most of the Mal" mutants which were obtained did not alter the remaining components of the wild type maltose system. These probably represent instances in which other transport systems have acquired the ability to transport maltose (29, 31). The mutation in one of the Mal" derivatives was shown, by P1 transduction, to be linked to the malB locus. The phenotype of this mutant is Mal"Dex", that is maltose (and maltotriose) can be transported and used as carbon sources but longer polymers of glucose cannot. This phenotype is unaltered when a wild type copy of the binding protein structural gene is introduced to the strain. The revertant strain is able to concentrate maltose against a gradient but to a much lesser extent than the wild type system. The maltose concentration which results in half-maximal rate of transport in the mutant strain (1 mM) is much higher than the corresponding value of the wild type system (1 μM). Arsenate abolishes the accumulation of maltose in the revertant strain as it does in wild type bacteria. This implies that ATP, or a derivative of ATP, is essential for active transport. A novel feature of maltose accumulation in the mutant strain is that transport activity is retained by spheroplasts. This is in contrast to wild type cells in which maltose transport is exquisitely sensitive to removal of binding protein from the periplasmic space. It is possible that transport activity will be retained when the spheroplasts are lysed and converted into membrane vesicles. This will provide an opportunity to determine the chemical nature of the energy source for transport.

It will be possible to perform chemical modification experiments to determine which amino acid residues are involved in substrate transport or energy coupling.

A detailed genetic analysis of the mutation in the revertant strain, HS3025, will be presented in a subsequent publication. Evidence presented above demonstrates that there is a specific binding site for maltose and maltodextrins in the strain that transports maltose independently of the binding protein. Maltose transport in this mutant is stereospecific; its inhibited only by oligomers of glucose. Carbohydrates of similar size and composition such as melibiose, lactose, and cellobiose do not inhibit transport activity.

There are two explanations to account for this recognition site that is present in the mutant strain. The first of these is that this recognition site does not exist in wild type cells but has been "created" as a result of mutation. The other explanation is that this site does exist in wild type cells but is accessible only to substrates that are bound to the periplasmic binding protein. In the absence of the periplasmic binding protein the wild type recognition site is not accessible to free substrate molecules. As a result of mutations in the malF and malG genes this "hidden" site has been made accessible to free substrate molecules. While it is difficult to imagine how a specific recognition site could be formed by mutation, it is somewhat easier to picture how the arrangement of a pre-existing recognition site could be altered by mutation. It should be noted that the maltose-binding protein does not participate in transport via the mutant transport system. Since the binding protein is essential for detectable substrate translocation via the wild type system it must interact with some cytoplasmic membrane component(s) of the wild type system. It is possible that the inability of the binding protein to interact properly with the mutant membrane components is in some way related to their ability to transport independently of the binding protein. The proposed substrate recognition site in the membrane might overlap with the site at which maltose-binding protein interacts with the membrane components. In order to examine this question it will be necessary to study the properties of different mutant strains that are similar to strain HS3025.

Wandersman et al. have isolated mutants that have a Mal"Dex" phenotype. Some of their mutants appear to map in the malG gene. These mutants could be defective in the proposed substrate recognition site such that only maltose is recognized (34).

It is possible to formulate a working model for the operation of the maltose transport system that is based on the data presented here and the properties of the malF, malG, and malK gene products. This model is shown in Fig. 6. Three important points are to be taken into consideration. (i) The malF gene product is a 42,000 molecular weight integral membrane protein (10). (ii) The 40,000 molecular weight malK protein is located on the inner aspect of the cytoplasmic membrane via an interaction with the malG protein (12). (iii) In wild type cells the maltose-binding protein is essential for substrate translocation across the membrane. According to the model depicted in Fig. 6, accumulation of substrate occurs as a result of a cycle of conformational changes of the malF and malG proteins that proceeds in the clockwise direction as drawn. The substrate recognition site formed by some combination of the malF and malG proteins can exist in two states, exposed on the inner surface of the membrane (T1) or exposed to the outside surface of the membrane (T2). The requirement of the binding protein for transport and the properties of the binding protein-independent mutant can be explained in terms of this model. If the T2 and T1 confor-
mations of the system are in equilibrium so that the amount of $T_1$ is very small and if the binding protein interacts only with the $T_1$ conformation than in the absence of binding protein there will be very little of the $T_1$ conformation. Consequently only a small number of substrate recognition sites will be exposed on the external side of the membrane. In the binding protein-independent mutant the substrate recognition site is exposed on the external side of the membrane without the involvement of the binding protein. This could be due to a change in the equilibrium between the $T_2$ and $T_1$ conformations or to a structural change that allows the substrate recognition site to be exposed in both conformations.

In this model energy derived from the binding and hydrolysis of ATP (or some other compound that contains a high energy phosphoester) is used to drive the conformational changes that result in the exposure of the substrate recognition site to alternate sides of the membrane. Evidence for this type of scheme is available for many systems that are energized by ATP (for reviews, see Refs. 35-37).

Although this model is only hypothetical it should be useful as a framework to design experiments. It will be possible to test various aspects of this model and to determine if other "shock-sensitive" transport systems exhibit properties which are similar to or different from those described here.

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H A Shuman


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