Maltose/Maltodextrin System of *Escherichia coli*: Transport, Metabolism, and Regulation

WINFRIED BOOS1* AND HOWARD SHUMAN2

Department of Biology, University of Konstanz, D-78457 Konstanz, Germany, 1 and Department of Microbiology, Columbia University, College of Physicians & Surgeons, New York, New York 100322

INTRODUCTION AND SCOPE .................................................................................................................................204
MALTOSE GENES AND THEIR PRODUCTS ..............................................................................................................205
POSITIVE TRANSCRIPTIONAL ACTIVATOR, MalT ..................................................................................................205
    MalT Box ..................................................................................................................................................209
MALTOSE/MALTODEXTRIN TRANSPORT SYSTEM ..........................................................................................210
    Periplasmic Substrate Recognition Site and Its Interaction with Membrane Components ..........................210
    Membrane-Spanning Subunits MalF and MalG of the Transport System ....................................................212
MalK, the Energy-Coupling Protein of the Transport System .............................................................................213
Role of the Lambda Receptor (Maltporin) in the Diffusion of Maltose and Maltodextrins through the Outer Membrane ........................................................................................................................................214
ENZYMES OF THE MALTOSE SYSTEM ................................................................................................................215
    Amylolamalase ..............................................................................................................................................215
    Maltodextrin Phosphorylase ..........................................................................................................................216
    Maltodextrin Glucosidase, an Enzyme of Unclear Function .......................................................................217
    Role of Glucokinase and Phosphoglucomutase in Maltose/Maltodextrin Metabolism ..............................217
    Periplasmic α-Amylase ...................................................................................................................................217
    Maltose Utilization in Other Bacteria ............................................................................................................218
NONCLASSICAL REGULATORY PHENOMENA ......................................................................................................218
    MalK, the ATP-Hydrolyzing Subunit, as a Sensor for mal Expression .........................................................218
    MalY as a mal Repressor ...............................................................................................................................219
    Aes as a mal Repressor ..................................................................................................................................220
    Effect of Phosphorylated PocP on mal Expression .......................................................................................220
ENDOGENOUS INDUCER OF THE MALTOSE SYSTEM .......................................................................................220
    Glycogen as a Source of Maltotriose ............................................................................................................220
    Second Pathway for Maltotriose Formation ..................................................................................................221
    Elevated Expression of the mal Genes during Glucose Starvation ..............................................................221
    Role of Glucose in Internal Induction of the Maltose System .......................................................................221
    Osmoregulation of the Maltose System .......................................................................................................221
INTERCONNECTION BETWEEN THE MALTOSE AND TREHALOSE SYSTEMS ..............................................222
    Trehalose as an Inducer of the Maltose System ............................................................................................222
    Transport of Maltose and Trehalose in the Archaeon Thermococcus litoralis .............................................223
FINAL CONSIDERATIONS ......................................................................................................................................223
ACKNOWLEDGMENTS .............................................................................................................................................223
REFERENCES .........................................................................................................................................................223

* Corresponding author. Mailing address: Department of Biology, University of Konstanz, Universitätstrasse 10, D-78457 Konstanz, Germany. Phone: 49 7531 882658. Fax: 49 7531 883356. E-mail: winfried.boos@uni-konstanz.de.

INTRODUCTION AND SCOPE

Historically, some of the earliest work on *Escherichia coli* genetics and physiology in the Jacob and Monod groups concentrated on the maltose system, but it was rapidly overshadowed by the spectacular success in understanding the lactose operon. Fortunately, interest in the maltose system persisted as a result of its being an example of a “positively regulated” group of genes and the mysterious connection between maltose metabolism and the susceptibility of *E. coli* to phage λ infection.

The maltose system is responsible for the uptake and efficient catabolism of α(1→4)-linked glucose polymers (maltodextrins) up to 7 to 8 glucose units. This system has turned out to be a far richer source of interesting molecules and regulatory phenomena than anyone might have anticipated 30 years ago when the malA and malB regions were mapped on the *E. coli* chromosome (239). For example, in the course of studying the receptor activity of *E. coli* responsible for λ attachment, Randall and Schwartz discovered the λ receptor (LamB) (207) and, in collaboration with Hofnung, Szmelcman, and one of us (W.B.), showed that this outer membrane protein constituted the channel for the passage of sugars, especially maltodextrins, across the outer membrane (263, 265). This information focused attention on the possibility of porins as specific channels and culminated in the solution of the three-dimensional structure of the maltoporin channel associated with malto-oligosaccharide ligands (231). Together with a large body of information about the functional sites within LamB, the structure represents one of the most complete descriptions of a membrane transport protein.
From the earliest time that the maltose system was studied, transcriptional control was a major focus because of the interest in a positively controlled system. The MalT protein, which is the activator at all mal promoters (112, 206, 214), and the sites at which it binds (MalT boxes) have provided an important alternative view to the lac operon about how transcription is activated. Although the precise mechanism of how MalT interacts with and stimulates RNA polymerase is still unknown, there is considerable information about the structural requirements for MalT binding to DNA as well as its ability to form a nucleoprotein complex with the catabolite activator protein (CAP).

The periplasmic binding protein-dependent ATP binding cassette (ABC) transporter of the maltose system has been studied to understand the mechanism of transport and the mechanism of protein localization to different cellular compartments (e.g., inner membrane, outer membrane, and periplasm) (21, 156, 173, 250), as well as the determinants of membrane protein topology. The periplasmic maltose binding protein (MBP) has been characterized structurally (257), and a variety of functional sites within its structure for interacting with the chemosensory apparatus and the ABC transporter have been identified. The actual membrane transporter (MalFGK), made up of two integral membrane proteins (MalF and MalG) and two copies of the ATP-hydrolyzing subunit (MalK), has been characterized biochemically, and there is some information about functional sites that are important for substrate recognition, ATP binding, and subunit contacts and interactions with MBP. The ability to manipulate the transporter genetically offers a rare opportunity to dissect the mechanism of transport by a combined approach of biochemistry and genetics. The direct participation of the transporter in transcriptional regulation indicates that gene regulation in bacteria may depend on the rates of substrate entry in addition to the physical presence of the substrate itself.

Reviews on the maltose system of E. coli have appeared (22, 241). In this review, we attempt to summarize what is currently known about this system. We have focused on three areas: (i) transport of maltodextrins into the cell via an outer membrane porin and a periplasmic binding protein-dependent ABC transporter, (ii) metabolism of the internalized sugars, and (iii) transcriptional regulation of the mal genes. We attempt to clarify what we believe are significant functional relationships between transport activity and transcriptional control. Also, we discuss the interconnections between the maltose-degrading enzymes and those of glucose catabolism and gluconeogenesis from the perspective of how endogenous inducers of the mal system are produced. Because the initial breakdown of maltodextrins results in both glucose and glucose-1-phosphate, these enzyme activities must be coordinated with those for glycogen synthesis and breakdown. Finally, other less obvious connections with phosphate metabolism and trehalose metabolism are also discussed. We will not consider aspects of secretion (169), folding (272), or assembly (151, 223) of the components of the maltose system.

MALTOSE GENES AND THEIR PRODUCTS

Table 1 summarizes all known mal genes in E. coli. Their definition is based on the function of MalT. Thus, all genes regulated by MalT belong to the maltose regulon, while the expression of malT itself is independent of MalT. malP and malQ encode essential enzymes for maltose and maltodextrin metabolism, whereas malS and malZ encode nonessential maltodextrin-metabolizing enzymes. malEFG and malK lamB encode the binding protein-dependent ABC transporter. Some mal genes are organized in clusters. The malA region at 76.5 min contains two divergently oriented operons, with malT transcribed clockwise and malPQ transcribed counterclockwise (65, 126, 200). Likewise, all maltose transport genes are clustered at 91.4 min in the malB region with two divergently organized operons: malEFG in the counterclockwise orientation and malK lamB malM in the clockwise orientation (203, 254).

Other gram-negative enteric bacteria have additional genes, not present in E. coli, that are under the control of MalT. For instance, Klebsiella pneumoniae harbors a battery of malT-controlled pul genes that are necessary for the biosynthesis and the secretion of pullulanase (71), an extracellular enzyme that degrades pullulan to maltotriose (14, 285). Pullulan, first isolated from Pullularia pullulans (13), consists of α(1→6)-glucosidically linked maltotriose units. The MalT-dependent pulA gene, encoding the lipoprotein pullulanase (163), is positioned divergently from a series of genes, also dependent on MalT, that are necessary for the export of pullulanase through the outer membrane (193, 195). Likewise, Klebsiella oxytoca harbors a series of cym genes encoding proteins for the uptake and metabolism of cyclic dextrins (89). The expression of these genes may also directly or indirectly depend on malT. The expression of the maltose regulon in Vibrio cholerae affects the virulence of this organism, and mutations in malQ and malF render it less virulent (149). The products of all the MalT-dependent E. coli genes have been identified, but the function of one, the periplasmic MalM protein (104, 222), is still unclear. The observation that MalM is also present in Salmonella typhimurium may suggest that this periplasmic protein has an important function (234).

Table 1 contains many more genes that do not belong to the maltose regulon but whose encoded proteins affect the metabolism of maltodextrins or the regulation of mal gene expression at different levels. Dextrin-metabolizing or synthesizing enzymes affect the level of internal maltotriose, the inducer of the system, which is recognized by MalT; cyclic AMP (cAMP)/CAP, as well as the product of the mle gene, controls the expression of malT itself; and the levels of MalK, the ATP-hydrolyzing subunit of the transport system, as well as of MalY (a β-C-S lyase) and of Aes (an esterase with homology to lipases) affect the expression of the maltose transport genes. There are some more genes which, when mutated, have a subtle effect on mal gene expression. Among them is asuE, encoding a tRNA-modifying enzyme, and treR, encoding a repressor for the trehalose-utilizing system in E. coli. These aspects will be discussed in detail below.

POSITIVE TRANSCRIPTIONAL ACTIVATOR, MalT

**malT**, Its Product, and Regulation of Its Expression

An early genetic approach identified malT as a gene that appeared essential for the expression of all maltose-inducible functions (112, 113, 126). Since all maltose-inducible operons require MalT and since malT fails to repress the operons in the absence of inducer (67, 126), it was clear that the protein encoded by malT is a purely positive regulator that is activated by an inducer and stimulates transcription by activating RNA polymerase (48). The *malT* gene was cloned (204) and sequenced (40), and its product, MalT, was purified and characterized (214). The protein appears monomeric in dilute solution, contains 901 amino acids, and exhibits a molecular weight of 103,000. It binds ATP (*K*<sub>AP</sub> 0.4 μM) (215) and maltotriose (*K*<sub>D</sub> about 20 μM) (49), both of which are necessary for transcriptional activation, even though ATP hydrolysis is not re-
<table>
<thead>
<tr>
<th>Gene</th>
<th>Position on chromosome (min)</th>
<th>Gene product and function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mal genes and their main regulator</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>malT</em></td>
<td>76.5</td>
<td>Transcriptional activator, essential for transcription of all <em>mal</em> genes except the <em>malI/X/Y</em> gene cluster. Binds ATP and maltotriose as inducer.</td>
<td>34, 40, 66, 206, 214, 215, 280</td>
</tr>
<tr>
<td><em>malE</em></td>
<td>91.4</td>
<td>Periplasmic MBP; binds maltose/maltodextrins with micromolar affinity.</td>
<td>37, 76, 107–109, 128, 138, 244, 248, 257, 264, 265</td>
</tr>
<tr>
<td><em>malF</em></td>
<td>91.4</td>
<td>Intrinsic membrane protein of the transport system. In association with MalG and MalK, it forms the MalFGK2 translocation complex.</td>
<td>41, 80, 82, 93, 252, 273</td>
</tr>
<tr>
<td><em>malG</em></td>
<td>91.4</td>
<td>Intrinsic membrane protein of the transport system. In association with MalF and MalK, it forms the MalFGK2 translocation complex.</td>
<td>25, 50–52, 254, 273</td>
</tr>
<tr>
<td><em>malK</em></td>
<td>91.5</td>
<td>Transport ATPase, responsible for energization of transport. In association with MalF and MalG, it forms the MalFGK2 translocation complex. Target of inducer exclusion by unphosphorylated EIIAGlc of the PTS. In the absence of inducer, it interacts with MalT to cause repression.</td>
<td>7, 44, 53, 55, 60, 103, 115, 147, 151, 166, 167, 184, 210, 251</td>
</tr>
<tr>
<td><em>lamB</em></td>
<td>91.5</td>
<td>Receptor for phage λ and specific pore for maltodextrins (maltoporin, glycoporin).</td>
<td>16, 39, 85, 92, 124, 136, 207, 231, 263, 265</td>
</tr>
<tr>
<td><em>malM</em></td>
<td>91.5</td>
<td>Periplasmic protein of unknown function, partially associated with the outer membrane. Contains an Ala-Pro linker also found in OmpA.</td>
<td>104, 222, 234</td>
</tr>
<tr>
<td><em>malP</em></td>
<td>76.5</td>
<td>Maltodextrin phosphorylase. Substrates are maltopentaose and larger maltooligosaccharides. <em>malP</em> mutants still grow on maltose but accumulate large amount of maltodextrins under these conditions.</td>
<td>177, 181, 229, 242, 290</td>
</tr>
<tr>
<td><em>malQ</em></td>
<td>76.4</td>
<td>Amylomaltase. Maltodextrinyltransferase with maltotriose as the smallest substrate. <em>malQ</em> mutants cannot grow on maltose, are sensitive to maltose, and are constitutive for <em>mal</em> gene expression.</td>
<td>69, 165, 182, 194, 294, 295</td>
</tr>
<tr>
<td><em>malS</em></td>
<td>80.5</td>
<td>Periplasmic α-amylase, cleaves preferentially maltotetraose from the nonreducing end of maltodextrins.</td>
<td>91, 92, 235, 256</td>
</tr>
<tr>
<td><em>malZ</em></td>
<td>9.1</td>
<td>Maltodextrin glucosidase and γ-cyclodextrinase, cleaves glucose sequentially from the reducing end of maltodextrins. Maltotriose is the smallest substrate. It linearizes γ-cyclodextrin but not α- and β-cyclodextrin.</td>
<td>185, 211, 267</td>
</tr>
</tbody>
</table>

**Genes whose products control *mal* gene expression**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position on chromosome (min)</th>
<th>Gene product and function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cya</em></td>
<td>85.9</td>
<td>Adenylate cyclase. Production of cAMP, involvement in catabolite repression.</td>
<td>75, 134, 187</td>
</tr>
<tr>
<td><em>crp</em></td>
<td>75.1</td>
<td>cAMP-binding protein, needed for the transcription of <em>malT</em> and the transport gene cluster.</td>
<td>35, 135, 143, 206</td>
</tr>
<tr>
<td><em>malI</em></td>
<td>36.6</td>
<td>Repressor for <em>malI/X</em>, not dependent on MalT, inducer unknown.</td>
<td>209</td>
</tr>
</tbody>
</table>

Continued on following page
<table>
<thead>
<tr>
<th>Gene</th>
<th>Position on chromosome (min)</th>
<th>Gene product and function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>malX</td>
<td>36.6</td>
<td>Enzyme II of the PTS, transports and phosphorylates glucose, can transport maltose by diffusion.</td>
<td>208</td>
</tr>
<tr>
<td>malY</td>
<td>36.6</td>
<td>βC-S lyase (cystathionase). Overproduction reduces mal gene expression by interaction with MalT and its inactivation.</td>
<td>208, 301</td>
</tr>
<tr>
<td>aes (ybaC, orf203)</td>
<td>10.8</td>
<td>Esterase. Overproduction reduces mal gene expression, presumably by interaction with MalT and its inactivation.</td>
<td>164, 186</td>
</tr>
<tr>
<td>mlc</td>
<td>35.9</td>
<td>Gene regulator, represses the expression of malT and manXYZ.</td>
<td>70, 131</td>
</tr>
<tr>
<td>maa (mac, F183a)</td>
<td>10.32</td>
<td>Glucose/maltose transacetylase, not MalT dependent, responsible for exit of maltose and glucose in their acetylated forms.</td>
<td>20, 26</td>
</tr>
</tbody>
</table>

**Genes whose products affect endogenous synthesis of inducer**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position on chromosome (min)</th>
<th>Gene product and function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glgA</td>
<td>75.4</td>
<td>Glycogen synthase. ADP-dependent synthesis of glycogen. Degradation of glycogen yields maltotriose, which, in malQ mutants, leads to constitutivity of the maltose system. glgA mutants have a lower level on uninduced mal gene expression than glgA+ strains.</td>
<td>148, 176</td>
</tr>
<tr>
<td>glgC</td>
<td>75.4</td>
<td>ADP-glucose-pyrophosphorylase. Synthesis of ADP-glucose, needed for constitutive mal gene expression in malQ mutants. glgC mutants have a lower uninduced mal gene expression than glgC+ strains.</td>
<td>4, 176</td>
</tr>
<tr>
<td>glgP</td>
<td>75.4</td>
<td>Glycogen phosphorylase. Glycogen degradation and formation of glucose-1-phosphate. Possibly involved in synthesis of endogenous inducer.</td>
<td>300</td>
</tr>
<tr>
<td>glgB</td>
<td>75.4</td>
<td>Branching enzyme</td>
<td>5, 176</td>
</tr>
<tr>
<td>glgX</td>
<td>75.4</td>
<td>Amylase-like enzyme, role in glycogen degradation unclear.</td>
<td>221</td>
</tr>
<tr>
<td>amyA</td>
<td>43.2</td>
<td>Cytoplasmic α-amylase, not MalT dependent, no apparent role in glycogen degradation.</td>
<td>198</td>
</tr>
<tr>
<td>galU</td>
<td>27.8</td>
<td>UDP-glucose pyrophosphorylase. Possible origin of cytoplasmic unphosphorylated glucose.</td>
<td>292</td>
</tr>
<tr>
<td>glgS</td>
<td>68.7</td>
<td>Short polypeptide, involved in RpoS-dependent glycogen synthesis.</td>
<td>11, 118</td>
</tr>
<tr>
<td>glk</td>
<td>54.0</td>
<td>Glucokinase. Reduces level of internal glucose which can form endogenous inducer, responsible for mal gene repression at high osmolarity.</td>
<td>43, 95, 161</td>
</tr>
<tr>
<td>treR</td>
<td>96.2</td>
<td>Repressor for treB and treC. treR mutants allow transport of maltose via the treB-encoded transport system and induce treC, whose product is involved in inducer synthesis.</td>
<td>129</td>
</tr>
<tr>
<td>treB</td>
<td>96.1</td>
<td>Enzyme II for trehalose of the PTS, allows transport of maltose.</td>
<td>142</td>
</tr>
<tr>
<td>treC</td>
<td>96.1</td>
<td>Trehalose-6-phosphate hydrolase, involved in inducer synthesis.</td>
<td>21</td>
</tr>
</tbody>
</table>

*Continued on following page*
TABLE 1—Continued

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position on chromosome (min)</th>
<th>Gene product and function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pgm</td>
<td>15.4</td>
<td>Phosphoglucomutase. Needed for the synthesis of endogenous inducer. A pgm mutant can still grow on maltose but only in the presence of MalZ.</td>
<td>1, 69, 153, 220</td>
</tr>
</tbody>
</table>

Genes that affect mal gene expression by an unknown mechanism when mutated

<table>
<thead>
<tr>
<th>Gene</th>
<th>25.6</th>
<th>tRNA-modifying enzyme. An astE mutant increases mal gene expression at high osmolarity.</th>
<th>262</th>
</tr>
</thead>
<tbody>
<tr>
<td>yjeA (genX)</td>
<td>94.4</td>
<td>Homolog to lysyl-tRNA synthases LysS and LysU. A genX mutant interferes with the ability of a malQ malZ92 pgm strain to grow on maltose.</td>
<td>146, 170</td>
</tr>
<tr>
<td>envZ</td>
<td>76.1</td>
<td>Sensor kinase of the two-component osmoregulatory system. Certain envZ mutants that lead to the overphosphorylation of OmpR show reduced malT expression.</td>
<td>33</td>
</tr>
<tr>
<td>phoP phoQ</td>
<td>25.7</td>
<td>Two-component system responding to Mg(^{2+}) starvation. Overexpression of the response regulator leads to mal gene repression.</td>
<td>278, 283</td>
</tr>
</tbody>
</table>

quired, since the binding of ADP or nonhydrolyzable ATP analogs also stimulates transcription (215).

Mutations in malT (malT(Con)), resulting in the constitutive expression of all mal genes, have been isolated (49, 67). These mutations center around amino acids 243 and 358 of the polypeptide chain. Whereas in an in vitro assay the wild-type MalT protein is inactive in the absence of maltotriose, the MalT(Con) proteins are active in the absence of maltotriose but still bind maltotriose with an increased affinity. All mutant proteins can still be stimulated further in their transcriptional activity by maltotriose (49). The last C-terminal 95 amino acids of MalT constitute the DNA-binding domain. In this area, MalT exhibits homology to a number of prokaryotic transcriptional activators (280) of the UhpA-LuxR family of regulatory proteins (260). Truncated forms of MalT lacking its C-terminal DNA binding domain are negatively dominant over the function of the wild-type protein, indicating that the protein interacts with itself when binding to DNA (40).

The expression of malT is not autoregulated by MalT but is subject to catabolite repression and therefore requires the presence of the cAMP/CAP complex (35, 36, 65). According to the current view, the mechanism of catabolite repression is based mainly on the regulation of intracellular cAMP levels. This, in turn, is a function of adenylate cyclase, controlled by the enzyme IIA\(^{C}_{GC}\) of the phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase system (PTS) in its phosphorylated (activating) and dephosphorylated (inactivating) states (191). Indeed, mutants lacking adenylate cyclase or CAP are unable to grow on maltose. It is rather informative to monitor mal gene expression in a wild-type strain during growth in rich media such as Luria-Bertani broth or tryptone broth, in the absence of external inducer. After inoculation (1:20 dilution) from an overnight culture, expression of a malK-lacZ fusion or maltose transport activity is high. It dramatically decreases (indicating a lack of new synthesis) as growth commences and reaches its lowest level during mid-log-phase growth. Since this phenomenon coincides with a cessation of cell division, it was once erroneously concluded that the synthesis of maltose binding protein (74) as well as galactose binding protein (246) might be connected to cell division. However, this phenomenon most probably reflects the concentration of internal cAMP (15), pointing once more to the importance of cAMP in catabolite repression.

Mutations in the control region of the malT gene (Fig. 1) have shown that its expression in the wild type is limited at both the transcriptional and translational levels. Mutations with increased malT expression have been isolated (malT\(_{P1}\), malT\(_{P7}\)) (Fig. 1) (34). The use of deletions introduced upstream of the malT promoter revealed that the 120 bp upstream of the transcriptional start site, encompassing the binding sites for the polymerase and the cAMP/CAP complex, are sufficient for malT expression. However, deletion of DNA further upstream increased the expression of malT, indicating that a binding site for a protein reducing malT expression had been removed (200, 205). In addition, a Tn10 insertion (malT\(_{P418::Tn10}\) (Fig. 1) that increases malT expression has been isolated. The position of this insertion is still upstream of the DNA region which, when deleted, increases expression (Fig. 1). Whether or not these sites are involved in the dominant negative effect of certain mutations in envZ (105, 286, 289), resulting in an overphosphorylated OmpR protein (2, 224, 291), is still unclear. Interestingly, the DNA upstream of the malT promoter whose deletion results in an increase in malT expression contains sequences that are similar to sequences identified as binding sites for phosphorylated OmpR (133, 155) (Fig. 1). Although ompR null mutants do not exhibit an increase in malT expression, mutations in envZ leading to uncontrolled phosphorylation of OmpR reduce the transcription of malT (33).

Expression of malT is controlled by a repressor called Mlc. This was detected by isolating a chromosomal insertion that increased malT expression. The insertion was found to be in mlc, a known gene whose product, when overproduced, impairs the utilization of glucose (131). The increase in malT
expression could still be observed in mutants carrying the deletions as well as the insertion upstream of the malT promoter, excluding these regions as binding sites for Mlc. Mlc shows homology to NagC, a gene regulator functioning as a repressor for the divergent nag operons (encoding proteins for the uptake and degradation of N-acetyl-D-glucosamine) and as an activator as well as a repressor for the glmUS operon encoding enzymes for the biosynthesis of N-acetyl-D-glucosamine (188).

By footprint analysis, it was shown that Mlc binds to malT DNA at a position 1 to 23 bp from the start of the malT transcript (Fig. 1). In mutants lacking Mlc, the expression of malT is increased by a factor of 2 to 3 when grown in glycerol. On the other hand, overexpression of Mlc from a multicopy plasmid strongly reduces malT expression (68). The identity of the effector for Mlc is still unclear. Internal free glucose or glucose derived from the metabolism of disaccharides such as trehalose (141) or even maltose (28) slightly induces malT expression in a Mlc-dependent fashion. However, free glucose, even 100 mM, does not prevent DNA binding of Mlc during footprint or band shift assays. Therefore, it is likely that a metabolic product of glucose could be the controlling compound for Mlc. Mlc not only regulates malT expression but also represses the man operon (259) when overproduced (189). Mlc may thus represent a new global regulator for sugar-metabolizing systems.

### MalT Box

The first feature recognized for at least three MalT-dependent promoters was the lack of the usual $-35$ region, whereas the $-10$ region corresponds to those of constitutive promoters (47). Instead, the asymmetric hexanucleotide sequence $5'-GG(A/G)GA-3'$, the so-called MalT box, was identified centered at bp $-37.5$ or at $-38.5$ upstream of the transcriptional start point (9, 10, 106, 201). In addition, DNA upstream of the $-38$ region was found to be essential for mal gene expression (106, 201), whereas the 30-bp sequence preceding the transcriptional start point contains only a few positions that are essential for promoter activity (64).

The second structural feature found to be essential for MalT-dependent mal gene expression was two additional MalT boxes in a direct repeat upstream of the $-38$ region (206, 279, 281). The analysis of these MalT boxes also led to an extension in the consensus sequence, which is now defined as $5'-GAGGG$
abolish the expression of MalT. Removal of the two distal MalT boxes reduces but does not require the full expression of both operons. Sequential three cAMP/CAP binding sites. The entire control region is two MalT boxes, the repeat, in front of malE (three MalT boxes, including the repeat, in front of malE) separated by three cAMP/CAP binding sites. The entire control region is required for the full expression of both operons. Sequential removal of the two distal MalT boxes reduces but does not abolish the expression of malE, whereas the removal of any MalT box abolishes the expression of the malK operon (206). This suggested that multiple copies of MalT and cAMP/CAP form a unique nucleoprotein structure at this regulatory region that is required for maximal activity of both promoters. The observation that the function of these sites is sensitive to the phase of the DNA helix has led to a model in which the DNA is wrapped around the complex composed of MalT and cAMP/CAP (199, 206). The state of supercoiling also appears to be important for the effectiveness by which the two operons are transcribed. Not only is relaxed DNA less efficient in transcription initiation, but also the sites occupied by the activator complex are shifted in supercoiled relative to relaxed DNA (216).

By a series of elegant experiments, it was found that binding of cAMP/CAP in the intergenic regulatory region between malE and malK results in a repositioning of MalT binding. In the absence of cAMP/CAP, MalT binds with high affinity to the three MalT boxes upstream of the malK transcriptional start site (identified previously). In the presence of cAMP/CAP, MalT binding is shifted by three nucleotides toward the Pribnow box of the malK promoter. The cAMP/CAP effect requires the malKp-distal MalT binding sites (218). The repositioning is caused by DNA bending, which can be mimicked by replacing cAMP/CAP with the integration host factor (217). The role of the CAP and MalT binding sites in transcriptional regulation of the two divergently oriented promoters continues to be the subject of extensive studies (213).

Recently, it has been observed that Lrp, the leucine-responsive protein (32), also affects the transcription of malT as well as of some malT-dependent genes (268). By using operon fusions to malT, malE, and malK in the genetic background of strain MC4100, this Lrp dependence could not be observed in the laboratory of W.B. It has also been reported that the expression of the malK lamB malM and malEFG operons, but not of the malT gene, is controlled by the pH of the medium, being elevated at high pH (121). The mediator of this pH-dependent control is unknown.

MALTOSE/MALTODEXTRIN TRANSPORT SYSTEM

Periplasmic Substrate Recognition Site and Its Interaction with Membrane Components

The maltose/maltodextrin transport system is a member of the family of multicomponent and periplasmic binding protein...
dependent ABC high-affinity transport systems of gram-negative enteric bacteria (21, 57, 116, 173, 249, 250). The substrate recognition site of the system is determined primarily by the soluble binding protein with a high affinity for maltose and maltodextrins ($K_D$ around 1 μM) (138, 265) that is located in the periplasm in high concentration (around 1 mM and in 30- to 50-fold molar excess over the intrinsically membrane-bound proteins of the system) (74). This maltose binding protein (MalE protein or MBP) consists of two nearly symmetrical lobes between which the binding site is formed (197, 257). Substrate-loaded and substrate-free forms of MBP, as well as of other substrate-binding proteins, differ dramatically in their conformations, so that substrate bound to the protein no longer has access to bulk solvent. In the substrate-free form, the lobes are open and the substrate-binding site becomes accessible to the bulk solvent. Several physical, spectroscopic, and thermodynamic properties of MBP change when ligand is bound to it (100, 101, 109, 175, 244, 247, 265). Structural data on the open (244) and closed substrate-loaded (243, 244, 257) forms of the protein are available (Fig. 3A). The two lobes not only perform a bending movement relative to each other but also perform a twisting movement (Fig. 3B). In addition, the structures of a MBP with a dominant negative mutation (248) as well as of MBPs harboring a foreign epitope (226) or deletions and insertions (245) have been solved. Both the substrate-loaded and the substrate-free forms of MBP have access to the membrane components MalF and MalG (17, 158), even though it is unclear whether the interacting substrate-free form is of the open or the closed configuration.

MBP has been subjected to a detailed mutational analysis, and regions affecting transport (78, 264) or chemotaxis (77) have been defined. Also, intensive studies on the renaturation process of denatured MBP (37, 96) and on the biosynthesis and secretion of the protein (37, 73, 90, 261, 272) have been published. A recent report even indicates that periplasmic binding proteins, MBP among them, exhibit properties of molecular chaperonins in the periplasm (212).

The interaction of MBP with MalF and MalG has been studied by the genetic approach of mutant and suppressor analysis (274). This study, in combination with the knowledge of the crystal structure of MBP, has led to the conclusion that one lobe of MBP interacts with MalF and the other lobe interacts with MalG (128). The starting point for this genetic analysis was the isolation of mutants (actually, two point mutations were always required in either malF or malG) that transported maltose in the absence of MBP. Surprisingly, some of these MBP-independent mutations became maltose negative in the presence of wild-type MBP, a situation that allowed isolation of mutants with suppressor mutations in malE (274). Most of these suppressor mutations in MBP were dominant negative in combination with a wild-type MalFGK complex, indicating a higher affinity towards the membrane components when in the non-ATP hydrolysis-triggering mode (128, 248). Similarly, after the introduction of two cysteines (G69C and S337C) by site-directed mutagenesis into each domain of MBP, the formation of an interdomain disulfide cross-link that holds the protein in a closed conformation could be observed. This mutant MBP confers a dominant negative phenotype for growth on maltose, for maltose transport, and for maltose chemotaxis (303). The observation that wild-type MBP interferes with the transport activity of the MalFGK complex of the MBP-independent mutant permitted further studies on the interaction of MBP with the MalFGK complex. Transport studies with vesicles revealed that the wild-type binding protein inhibited transport only at high concentrations while it actually stimulated transport at low concentration (59). This analysis,
together with the properties of dominant negative mutations in MBP, has led to the proposal that the MalFGK2 complex must be able to attain at least two different conformations in its interaction with MBP, only one of which is able to trigger ATP hydrolysis by the MalK subunit (21, 248).

Even though the major recognition entity of the maltose transport system is the MBP, it is clear that not all substrates that are bound by the binding protein are transported. This is particularly obvious with cyclodextrins and the p-nitrophenyl derivatives of maltooligosaccharides that are bound very well by MBP but are not transported by the intact system (87, 150, 269). This is due to the modes of substrate recognition by MBP. Substrates that can be attached to MBP at the reducing end are transported; those which are bound within the dextrinyl chain (such as cyclodextrins) are not (107, 108, 173). MBP also functions as the substrate recognition site for maltose chemotaxis (114). The sites in MBP that are essential for the interaction with the maltose transport machinery and the chemotaxis machinery are distinct but partially overlapping (77, 97, 98, 302, 303). The α helix 7 of MBP appears to be exclusively involved in the interaction with the membrane components of the transport system. Mutations in this α helix belonging to the C-lobe of MBP affect transport without affecting the binding of substrate or the interaction with the chemotactic machinery (264).

Membrane-Spanning Subunits MalF and MalG of the Transport System

The application of the phoA fusion technique allowed determination of the two-dimensional topology of both MalF (24, 82, 94) and MalG (25, 52). Accordingly, MalF consists of eight membrane-spanning α-helical segments (MSS) with both termini of the polypeptide chain protruding into the cytoplasm. MalG, as judged by the same criteria, consists of six MSS, and again both termini extend into the cytoplasm (Fig. 4). Near their C termini, both proteins carry a sequence between MSS 6 and 7 (MalF) and MSS 4 and 5 (MalG), respectively, that is very similar to the consensus sequence EAA-X$_3$-G-X$_9$-I-X-LP conserved in all intrinsically membrane-bound subunits of binding protein-dependent ABC systems (227, 228). Substitutions at the same positions in MalF and MalG cause different phenotypes, and mutations in malG or malF that slightly affect or do not affect transport by themselves cause a completely defective phenotype when present together. This indicates that MalF and MalG are acting together asymmetrically in the

![FIG. 4. Two-dimensional structure of MalF and MalG. A topological model of MalF and MalG based on the analysis of malF:phoA and malG:phoA fusions is shown. MBP-independent mutants always require two mutations, one in the p (proximal) region and one in the d (distal) region. The G338R mutation in MalF alone causes MBP-dependent lactose transport. The EAAXLG consensus motif is located in the cytoplasmic loop between MSS 6 and 7 of MalF and MSS 4 and 5 of MalG. Reprinted from reference 41 with permission of the publisher.](http://mmbr.asm.org)
translocation step. Suppressor mutations to transport negative mutations in \textit{malF} or \textit{malG} were found in \textit{malK}, indicating that the EAA-harboring domain interacts with MalK (167).

Studies with mutations in \textit{malF} and their intragenic suppressors represent the first attempts to determine the positions of the different MSS relative to each other. Thus, mutations in MSS 7 were suppressed by mutations in MSS 6 or 8, perhaps an indication that these helices are next neighbors. In addition, mutations in MSS 6 leading to altered substrate specificity occur only on one side of the helical wheel, pointing to a participation of this surface in the substrate-specific transport channel (80).

The MalF subunit of the maltose transport system of \textit{E. coli} and other gram-negative enteric bacteria (44) represents something of an exception among the membrane-bound components of binding protein-dependent ABC transporters, since these MalF proteins contain a large periplasmic loop between MSS 3 and 4. This loop may not mediate the interaction with the binding protein, since all pairs of mutations in \textit{malF} leading to an MBP-independent phenotype (and which can be suppressed by mutations in MBP for the dominant negative phenotype caused by wild-type MBP) are positioned outside the loop. It may be involved in the docking of MBP to the membrane components. Consistent with this view is the observation that in none of the MBP-independent mutants is docking of MBP prevented. Also, no periplasmic loop is found in the MalF homologs of binding protein-dependent maltose transport systems in gram-positive bacteria (196, 225, 276) or even in the \textit{Archaea} (299), whose members contain lipid-anchored high-affinity binding proteins for maltose on the outside of the cytoplasmic membrane. In the latter case, docking is no problem because close vicinity to the membrane components is based on the lipid anchorage.

Even though it represents the major substrate recognition site, MBP cannot be the only substrate binding site of the transport system. MBP-independent mutants with mutations in MalF or MalG that retain specificity for maltose have been isolated, indicating the presence of a latent substrate binding site in the MalFGK\textsubscript{2} membrane complex (273). The \(T_{\text{max}}\) of these mutants for maltose transport can sometimes be similar to the wild-type value, while the apparent \(K_{\text{m}}\) of uptake is always increased by a factor of about 10\(^3\). Transport in these mutants is still active, i.e., against the concentration gradient, and dependent on the MalK-mediated hydrolysis of ATP. Characteristically, these MBP-independent mutants with mutations in MalF always carry two mutations, one in MSS 5 near the periplasmic surface, in the p (proximal) region, and the other one deep within the cytoplasmic membrane, in either MSS 6, 7, or 8, in the d (distal) region (41). It is interesting that substrate specificity mutations have been isolated in MSS 6 and that nearest-neighbor analysis predicts that MSS 6, 7, and 8 are close to each other (80) and at the same time form the d region. The attractive concept that mutations in the p region affect binding-protein recognition and mutations in the d region affect the coupling to the ATPase activity mediated by the associated MalK subunit apparently does not hold true, however. Both mutations are required for the uncoupled MalK-ATPase phenotype.

The screening of mutations in \textit{malF} and \textit{malG} for the ability to transport other sugars revealed that one mutation, \textit{malF}515, which changes Leu334 to Trp (L334W) on the periplasmic side of MSS 5, permitted the transport of lactose in addition to maltose. MalK as well as MalG is required for lactose transport, as is MBP. The requirement for MBP is particularly intriguing because MBP clearly does not bind lactose. Therefore, these results are consistent with the idea that the unliganded form of MBP interacts with the membrane complex (160). The position for such a “specificity” mutation is surprising since it is on the periplasmic side of MalF, which is not expected to form the substrate recognition site. This may indicate that substrate specificity for lactose is already present in MalF or MalG but needs the proper opening of the gate for the substrate to enter. In a different construct, large portions of the MalF protein have been deleted (beginning from the C terminus up to MSS 1). These deletion mutants are able to transport lactose in a MalG-, MalK-, and MBP-dependent manner without an additional mutation (159). This may indicate that the substrate recognition site of the membrane complex for lactose resides in MalG.

**MalK, the Energy-Coupling Protein of the Transport System**

The MalK subunit contains a classical consensus sequence found in all ATP-hydrolyzing proteins (284). It consists of two subsites, the A and B domains. These two motifs, as well as the sequence surrounding these sites, are conserved in the equivalent subunits of all binding protein-dependent transport systems (123). In addition, they are found in many proteins of prokaryotic and eukaryotic origin whose function is in the transport of molecules, including polysaccharides, peptides, and proteins (83, 122). MalK and equivalent proteins of other binding protein-dependent transport systems have been characterized not only as ATP-binding proteins but also as enzymes that hydrolyze ATP. When MalK is embedded in the membrane as a complex with MalF and MalG, maximal rates of ATP hydrolysis are achieved only in the presence of substrate-loaded MBP (54, 57-59, 184). The ATPase activity of the detergent-solubilized MalFGK\textsubscript{2} complex of the MBP-independent mutant F500 has also been measured in solution (53). The active complex consists of two MalK subunits connected by one molecule each of MalF and MalG (55). When this complex is reconstituted in liposomes, ATP-dependent active transport of maltose into the liposomes can be measured, demonstrating the function of the MalK dimer as an energy module driving the active transport of maltose. In agreement with the dimeric structure of MalK in the translocation complex is the observation that the ATP binding sites of both subunits are essential (56) and that the kinetics of ATP hydrolysis show cooperativity (53). Using the technique of Hu (132) to probe proteins for their ability to form dimers by fusing them to a truncated L repressor (lacking the dimerization domain but retaining the operator binding), we were able to show that wild-type MalK has the ability to form dimers in vivo (68).

Little is known about the mechanism of energy coupling of ATP hydrolysis to the accumulation of maltose. Since neither the substrate nor any of the proteins involved have been seen to become phosphorylated during transport, it has become commonplace to interpret energy coupling as the transfer of the energy gained through ATP hydrolysis to protein conformational energy in MalF and MalG, followed by its subsequent binding protein-triggered release, resulting in the unidirectional translocation of the substrate through the membrane. Mutants with mutations in \textit{malF} or \textit{malG} that no longer require the triggering by substrate-loaded MBP have been isolated. It is noteworthy that the MalFGK\textsubscript{2} complex of these mutants no longer requires the presence of substrate-loaded MBP to stimulate the ATPase activity of MalK (57). ATPase activity has become uncoupled in these mutants, similar to the uncoupled ATPase activity of the purified wild-type MalK subunit (166). This has led to the notion that the transport system is a signal transduction pathway that begins in the periplasm.
with the recognition of maltose by the binding protein and ends with the control of MalK-ATPase activity in the cytoplasm (41, 57). The helical domain of the ATPase in MalK is likely to be involved in the signal transduction between MalF and MalG (167).

No quantitative studies on the synthesis of MalK in comparison to its partners in the membrane, MalF and MalG, are available. Even though the stoichiometric composition of the biochemically active complex has been determined as MalFGK$_{\text{a}}$ (55), it is unclear whether MalK is synthesized in excess or, depending on the regulatory conditions, in varying amounts. The latter possibility is not unlikely since malF and malG are located in a different operon from malK and since substantial amounts of MalK have been found, in contrast to MalF and MalG (7, 251). As discussed below, MalK plays an important role in the MalT-dependent control of mal gene expression. It is only natural to see MalK not only as an energy module to drive transport but also as a control unit of transport activity. Obviously, the availability of ATP should influence transport activity. However, since the $K_{\text{m}}$ of MalK for ATP is in the micromolar range (58) and the physiological concentration of ATP is in the millimolar range, the cells would presumably have to become quite exhauste of ATP before they stopped transporting maltose.

We would like to postulate an additional level of control for the function of MalK in transport, which works by regulating the affinity of MalK for its partners, MalF and MalG. We observed that transport of glycerol-3-phosphate by the binding protein-dependent Ugp transport system is inhibited by internal P$_i$ (29). The Ugp system exhibits a surprising degree of sequence similarity to the maltose system in all subunits in spite of the differences in substrate specificity and regulation (178). In an attempt to find whether UgpC, the MalK analog of the Ugp system, was the target of P$_i$ inhibition, we exchanged MalK with UgpC (115) and tested a possible inhibition of maltose transport by P$_i$. Although P$_i$ did not inhibit the UgpC-mediated uptake of maltose, it also failed to inhibit the uptake of glycerol-3-phosphate via the Ugp system when UgpC was overproduced. Apparently, the reduced affinity of UgpC for its cognate membrane partners in the presence of P$_i$ can be compensated for by increasing the UgpC concentration. It suggests the possibility that the degree of association of the ATP-hydrolyzing subunit with the membrane components may control transport activity. An equivalent finding in the histidine transport system in Salmonella typhimurium has been interpreted in the same way. High concentrations of internal histidine were able to inhibit transport of histidine in trans (152). These observations may indicate that the transport activity of ABC transporters is controlled from the inside via the ATP-hydrolyzing subunit by binding the accumulated substrate.

The MalK subunit is usually pictured as being peripherally membrane associated through binding to the intrinsic membrane proteins MalF and MalG. This membrane association of MalK may in fact be more intimate (296). Studies with the functionally related binding protein-dependent histidine transport system of Salmonella typhimurium have shown that HisP, the MalK analog of this system, might actually be accessible from the periplasm (6), even though the interaction of the cognate periplasmic binding protein, HisJ, takes place with the intrinsic membrane proteins of the system and not with HisP (192). Similar findings of accessibility of MalK on the periplasmic side of the membrane have been reported for MalK in S. typhimurium (236). Models of the transport mechanism mediated by binding protein-dependent ABC systems picture the input of energy by ATP hydrolysis as conservation of a strained protein structural conformation in MalF and MalG that is released by triggering with substrate-loaded binding protein (21, 59, 250). The analogy of the repetitive movement of SecA through the membrane that has been postulated as crucial for Sec-dependent protein secretion (140, 293) to a possible repetitive movement of a subdomain of MalK through the membrane (236) is less convincing. The transported molecule would be far smaller than the moving machinery, and the moving part of MalK should then exhibit substrate specificity for the transported molecule, which has never been observed. In contrast, UgpC, the ATP-hydrolyzing subunit of the glycerol phosphate transport system, can complement malK mutants to transport maltose (115), indicating that the ATPase subunit does not carry substrate specificity for the transported molecule.

**Role of the Lambda Receptor (Maltoporin) in the Diffusion of Maltose and Maltodextrins through the Outer Membrane**

Efficient uptake of maltose and, particularly, longer maltodextrins at low concentrations requires the presence of $\lambda$-receptor (maltoporin), the specific diffusion pore for maltodextrins and other carbohydrates, in the outer membrane (62, 63, 92, 141, 263, 265). It is interesting that lamB, the gene for the $\lambda$-receptor, is, together with malK, located in a different operon from the remaining genes of the transport system (malE, malF, and malG). Since MalK is involved in the regulation of the system, and since the $\lambda$-receptor is also required for the diffusion of carbohydrates other than maltodextrins (63, 141), a differential regulation of these two genes (in comparison to the malEFG operon) should be considered. In addition, translational control of lamB (72, 110) may be evoked when considering the need of carbohydrate uptake under starvation conditions (62, 174).

A number of studies have analyzed the function of LamB (maltoporin) in the diffusion of maltodextrins (16, 88, 92, 136, 154, 171). All these studies demonstrate the presence of a maltodextrin binding site that is essential for the facilitated diffusion process of maltodextrins. A major contribution to understanding the molecular basis of transport through porin channels has come from determination of the three-dimensional structure of LamB, which was done in the presence of a series of maltooligosaccharides. These studies showed that each subunit of the trimeric protein contains a wide channel formed by an 18-stranded (230), antiparallel $\beta$-barrel. Three inwardly folded loops contribute to a constriction about halfway through the channel (231) (Fig. 5). The crystal structures of maltoporin complexed with maltose, maltotriose, or maltotetraose reveal an extended binding site within the channel. The maltooligosaccharides are in apolar van der Waals contact with the "greasy slide," a hydrophobic path that is composed of aromatic residues and is located at the channel lining (79, 287). Interestingly, the LamB protein contains two of four sequences (235) that are conserved in amylases and form the maltodextrin binding sites there (282). One of them, FYQRHD, at positions 106 to 111 of LamB, is actually part of the sugar binding site as determined by X-ray crystallography (79). A similar structure for the maltoporin from S. typhimurium, including the prominent greasy slide for maltodextrins, has been reported (162).

Calculations show that the diffusion of maltose through LamB and the following uptake by the ABC transporter are closely matched at maltose concentrations in the micromolar range. The $V_{\text{max}}$ for the disaccharide maltose in a fully induced strain has been measured to be 20 nmol per min per 10$^9$ cells (265). The diffusion of maltose through the outer membrane, mediated by about 10,000 trimeric LamB porin molecules per...
ENZYMES OF THE MALTOSE SYSTEM

Incoming maltose and maltodextrins of up to seven glucose moieties are metabolized to glucose and glucose-1-phosphate by the combined action of three cytoplasmic enzymes, amylomaltase (MalQ), maltodextrin phosphorylase (MalP), and maltodextrin glucosidase (MalZ) (Fig. 6). Since maltodextrins larger than six glucose moieties are not very well transported by the ABC transporter, they are reduced in size by a periplasmic amylase, the MalS protein.

Amylomaltase

Amylomaltase (165, 194, 294, 295), encoded by malQ, is a dextrinyl transferase that can transfer maltosyl and longer dextrinyl residues onto glucose, maltooligosaccharides, and maltose (265). In the presence of an alternative carbon source, mutations arise that are not lethal. Under these conditions, they accumulate large amounts of free maltose inside the cell (265). In the presence of an alternative carbon source, mutations arise that are found preferentially in malT or in malK. Why no mutations in other genes arise whose products are essential in transport remains a mystery (241). Even in the absence of malT, malQ mutants contain significant concentrations of free glucose, maltose, maltotriose, and larger maltodextrins, provided that the strain contains the glycogen-synthesizing enzymes (69, 81). Therefore, within the cell, glycogen must continually be degraded to maltodextrins, which are produced by the combined action of three cytoplasmic enzymes: amylopullulanase, maltodextrin glucosidase (MalZ) (Fig. 6). Since maltodextrins larger than six glucose moieties are not very well transported by the ABC transporter, they are reduced in size by a periplasmic amylase, the MalS protein.

Maltose/Maltodextrin System of E. coli

Vol. 62, 1998

FIG. 5. Crystal structure of LamB. A schematic drawing of the λ-receptor (maltoporin) monomer is shown. The cell exterior is at the top, and the periplasmic space at the bottom. The area of the subunit in trimer contacts is facing the viewer. The 18 antiparallel β strands of the barrel are represented by arrows. Strands are connected to their nearest neighbors by loops or regular turns. Loops L1 (blue), L3 (red), and L6 (green) fold inward toward the barrel. L3 is the major determinant of the constriction site. The yellow bond symbolizes the disulfide bridge Cys22-Cys38 within loop 1. Loop 2, facing the viewer, latches onto an adjacent subunit in the trimer. Loops L4 to L6 and L9 form a large protrusion. The horizontal lines delineate the boundaries of the hydrophobic core of the membrane as inferred from the hydrophobic area found on the molecular surface. Reprinted from reference 231 with permission of the publisher.

cell, is apparently not yet limiting at micromolar maltose concentrations. The Km of the transport system (27). We calculated the diffusion of maltose for different external concentrations through these 30,000 pores with an approximate diameter of 1 nm, using a diffusion constant of 10^{-5} cm^2/sec and an outer membrane thickness of 6 nm. We found that a rate of diffusion of 20 nmol per min per 10^9 cells, the V_{max} of the fully induced ABC transporter, is reached at 0.1 μM. Considering that not all maltose molecules that reach the external face of the lambda receptor will actually enter the pore, this concentration is most probably a lower limit, and in reality the concentration might be closer to 1 μM. Therefore, one can conclude that the overall uptake of maltose at its Km is close to the maximal rate of diffusion through the outer membrane. The validity of this conclusion has been experimentally tested with mutants containing reduced amounts of lambda receptor (27). In other words, a transport system that requires a V_{max} of about 20 nmol per min per 10^9 cells and is equipped with at least equal amounts of a specific diffusion pore to those in the fully induced lambda receptor cannot exhibit an apparent Km considerably lower than 1 μM, in spite of a periplasmic binding protein with a considerably lower K_{D}, of binding. In contrast, transport systems with an inherently low V_{max}, such as binding protein-dependent amino acid transport systems, may very well approach in their Km of transport the K_{D} of the corresponding binding protein.
Maltodextrin Phosphorylase

Maltodextrin phosphorylase, encoded by \textit{malP}, forms glucose-1-phosphate by sequential phosphorolysis of the nonreducing end glucose moieties of larger dextrins. As discussed above, amylomaltase does not split the glycosidic bond of maltose, but the net products of the action of MalP on maltose are glucose and maltodextrins. Since glucose will be removed in vivo by glucokinase to form glucose-6-phosphate, maltodextrins would accumulate. Indeed, \textit{malP} \textit{malQ} mutants can grow on maltose. Under these conditions, they become very large, are filled with long, linear dextrins (238, 239), and stain blue with iodine (1). Surprisingly, they do not transform these dextrins into glycogen, even though the \textit{glgB}-encoded branching enzyme is present. Maltodextrins do not accumulate in the \textit{malP} wild-type strain. Maltodextrin phosphorylase (8, 181, 242) recognizes maltopentaose and longer linear maltodextrins and forms \( \alpha \)-glucose-1-phosphate by phosphorolysis from the nonreducing end of the maltodextrin. The enzyme consists of a dimer with 796 amino acids per polypeptide. The three-dimensional structure of the enzyme has been solved recently (177, 290).

Obviously, it is important that maltodextrin phosphorylase does not attack maltotetraose and maltotriose, since dextrins of a minimum size are required for full activity of amylomaltase. Notably, glycogen phosphorylase, the \textit{glgP}-encoded enzyme, supposedly involved in the degradation of glycogen and linear dextrins (300), cannot replace maltodextrin phosphorylase in the utilization of maltose and maltodextrins as a carbon source.

The reaction catalyzed by maltodextrin phosphorylase is, of course, reversible. The rate in the phosphorolysis direction will be stimulated by increasing concentrations of cytoplasmic Pi. In turn, the concentration of internal Pi will vary depending on the availability of external Pi and phosphorus-containing organic compounds, as well as the state of induction of the \textit{phoB}-dependent \textit{pho} regulon (298). There are other connections between the \textit{mal} and the \textit{pho} regulon. Aside from the sequence similarity between the proteins of the maltose- and \textit{phoB}-dependent Ugp transport systems for glycerol-3-phosphate (178) and the functional exchangeability between their ATP binding subunits, MalK and UgpC (115), there is a common response (i.e., repression) of the two systems to dominant mutations in \textit{envZ}, resulting in overphosphorylation of OmpR, the response regulator of the two-component regulatory EnvZ-OmpR system involved in osmoregulation (33).

In addition, the utilization of a fermentable carbon source such as glucose, trehalose, or maltose at Pi concentrations below 1 mM requires the derepression of the \textit{pho} regulon. \textit{phoB} mutants do not turn deep red on MacConkey indicator plates (0.3 mM Pi) unless 5 mM Pi is added. Also, whereas strains that turn red on these plates derepress the \textit{pho} regulon, nonfermenters that appear pale remain repressed for the \textit{pho}\textit{B} regulon.
regulon. Apparently, the utilization of a fermentable carbon source requires higher cellular P_{i} concentrations than does that of a noncarbohydrate carbon source present in MacConkey plates (111).

**Maltodextrin Glucosidase, an Enzyme of Unclear Function**

MalZ was discovered in malF or malG mutants that transport maltose independently of MBP. In contrast to the wild type, these mutants also transport p-nitrophenyl α-maltoside (NPG2) and are able to hydrolyze this compound in the cytoplasm (211). Since amyloylase is unable to hydrolyze NPG2 and since the observed NPG2-hydrolyzing activity was maltose inducible and MalT dependent, it was clear from the start that the novel enzyme must be a member of the maltose regulon. The cloning and sequencing of the malZ gene and the isolation and biochemical characterization of the encoded protein revealed an enzyme that hydrolyzed maltodeptaose and smaller maltodextrins to glucose and maltose. The smallest substrate is maltotriose; maltose is not a substrate. In contrast to other glucosidases, the MalZ enzyme preferentially removes glucose (and to some extent maltose) consecutively from the reducing end of the maltodextrin chain (267). The deduced amino acid sequence of MalZ reveals homology to cyclodextrinyl transferases (190). We found that the enzyme does not hydrolyze α-cyclodextrin or pullulan. However, γ-cyclodextrin (and, to a much smaller extent, β-cyclodextrin) is an excellent substrate, forming maltose and glucose. The significance of this finding is unclear (185). γ-Cyclodextrin is not a carbon source for *E. coli*. The compound cannot diffuse through the outer membrane or be taken up by the maltose transport system. *malZ* mutants grow normally on maltose and maltodextrins. Only in combination with a *pgm* mutation do *malZ* mutants have a Mal− phenotype.

*malQ* mutants cannot grow on maltose or maltotriose. This is somewhat surprising since MalZ should release glucose (which could be used as a carbon source) from maltotriose. Most probably, the simultaneous accumulation of maltose resulting from the MalZ-dependent hydrolysis of maltotriose in the *malQ* mutant is toxic. By selecting *malQ* mutants to grow on maltose, a mutation in *malZ* (*malZ292*) was obtained. Surprisingly, the purified mutant MalZ enzyme is not able to hydrolyze purified maltose and shows the same maltodextrin-hydrolyzing activity as the wild-type enzyme. However, by using [14C]glucose in the presence of unlabeled maltotriose or maltotetraose, the mutant enzyme, unlike the wild-type enzyme, is able to transfer maltodextrins onto the labeled glucose. The mutant MalZ enzyme has acquired a quality which is similar to that of amyloylase. However, amyloylase is strictly a transferase, which is not true for the mutant MalZ enzyme. The latter also remains a hydrolyase, allowing the net hydrolysis of maltose to glucose in the presence of maltodextrins (185). As in the case of amyloylase, the utilization of maltose by the mutant MalZ enzyme in vivo requires the endogenous formation of maltodextrins that can be used as primers. The first step in the catalysis performed by amyloylase and the MalZ enzyme is identical, namely, release of glucose. However, whereas in the case of amyloylase the maltodextrinyl residue can be transferred only onto the C-4 carbon hydroxyl group of the nonreducing glucose residue of the acceptor molecule, in the case of MalZ it can also be transferred to water.

The *malZ* gene has been found and characterized as a typical *mal* gene that is dependent on MalT (235, 267). Recently, we found that *malZ* expression can also occur in the absence of MalT, in particular under conditions of high medium osmolarity. Using the method of primer extension with reverse transcriptase, we found that besides the MalT-dependent transcript described first (235), *malZ* is also transcribed into a second mRNA, originating upstream of the start site of the main promoter (145). The significance of this phenomenon is unclear.

**Role of Glucokinase and Phosphoglucomutase in Maltose/Maltodextrin Metabolism**

The final products of the combined action of amyloylase, maltodextrin phosphorylase, and maltodextrin glucosidase are glucose and α-glucose-1-phosphate. Therefore, to funnel these end products of the specific maltose enzymes into general metabolism, the cells rely on glucokinase (encoded by *glk*) for the phosphorylation of glucose to glucose-6-phosphate and on phosphoglucomutase (encoded by *pgm*) for the transformation of α-glucose-1-phosphate to glucose-6-phosphate, which enters into glycolysis. Mutants unable to phosphorylate glucose due to the lack of glucokinase, enzyme II<sub>α</sub> (encoded by *ptsG*) and enzyme II<sub>β</sub>MalI (ptsM) of the PTS-mediated phosphorylation, are unable to grow on maltose (30). Similarly, they are unable to grow on trehalose, which is degraded by an enzyme that produces internal glucose (219). Therefore, the utilization of internally produced glucose appears to be essential for growth. It is unclear whether the inability to grow on these sugars in the absence of glucose phosphorylation is due to a possible toxic effect of accumulating internal glucose (requiring extrusion) or the insufficient flow of carbon and energy via the phosphoglucomutase pathway alone. The drain of glucose-1-phosphate for the biosynthesis of polysaccharides might be another limiting factor.

*pgm* mutants lacking phosphoglucomutase activity are able to grow on maltose, even though seemingly only one half of the maltose molecule can be used as a carbon and energy source. These strains exhibit a maltose blue phenotype (1, 220) caused by the massive production of dextrins due to the accumulation of glucose-1-phosphate and the reversal of the maltodextrin phosphorylase reaction. The problem created by the accumulation of maltodextrins is eased by the action of maltodextrin glucosidase, the MalZ enzyme, which produces glucose from the maltodextrins, followed by glucokinase-dependent phosphorylation to glucose-6-phosphate. Indeed, *pgm malZ* double mutants are unable to grow on maltose. Similarly, *pgm* mutants can grow on galactose (1) but only when all the enzymes of the maltose system, including MalZ, are present (69). *pgm* mutants, even null mutations created by insertion elements (153), still show residual Pgm-like activity when the enzyme activity is assayed by coupling the formation of glucose-6-phosphate from glucose-1-phosphate to the NADP<sup>+</sup>-dependent oxidation by glucose-6-phosphate dehydrogenase. This activity is caused by a sugar-phosphate transferase which is able not only to hydrolyze sugar phosphates but also to transfer the phosphate moiety to another sugar molecule (258). Thus, in the presence of free glucose, glucose-1-phosphate can be transformed to glucose-6-phosphate, mimicking phosphoglucomutase activity.

**Periplasmic α-Amylase**

The *malS* gene (235) was discovered as a maltose-inducible and MalT-dependent gene by the lacZ fusion technique (91). MalS is a periplasmic α-amylase with weak homology to the cytoplasmic MalZ enzyme but not to amyloylase. MalS cleaves maltodextrins except maltose. Its preferred product released from larger dextrins is maltohexaose. The enzyme needs Ca<sup>2+</sup> for activity and DsbA (157) for proper folding in
the periplasm. The four cysteine residues of MalS form intramolecular disulfide bonds. The disulfide bond at Cys40-Cys58 is located in an N-terminal extension of about 160 amino acids which has no homology to other amylases but to the proposed peptide-binding domain of GroEL, the Hsp60 of E. coli. The N-terminal extension is linked to the C-terminal amylase domain via disulfide bond Cys104-Cys520. Reduction of the disulfide bonds by dithiothreitol treatment led to aggregation, suggesting that the N terminus of MalS may represent an internal chaperone domain (256). MalS mutants have no recognizable maltose phenotype. The function of the enzyme is most probably the degradation of longer dextrans that enter the periplasm to shorter dextrans that can be transported by the binding protein-dependent maltose/maltodextrin transport system (92). Even though MBP, the recognition site of the transport system, binds all maltodextrins from maltose to amylose, only dextrans up to the size of maltohexaose can be transported across the membrane (84, 86).

Figure 6 summarizes the pathway by which, according to our view, maltose is degraded into glucose and α-glucose-1-phosphate by the maltose-specific enzymes.

**Maltose Utilization in Other Bacteria**

Maltose uptake and utilization in other bacteria, notably in gram-positive bacteria, usually proceeds differently. In Bacillus subtilis, maltose is probably taken up by a proton motive force-dependent transport system and split internally into glucose and glucose-1-phosphate by a maltose-specific phosphorylase (266). The observation that Lactobacillus sanfrancisco takes up maltose and excretes half of it as glucose points to the same mechanism (172). In addition, maltose may also be taken up in lactobacilli by a PEP-dependent sugar PTS system and is internally hydrolyzed to glucose and glucose-6-phosphate (270). The isolation of a maltose-6-phosphate hydrolase and the vicinity of its gene, malH, to malB, encoding a putative IIB-like enzyme of the PTS, points to the same maltose degradative pathway in Fusobacterium mortiferum (23). This is quite in analogy to the uptake and degradation of trehalose in E. coli (142, 219).

**NONCLASSICAL REGULATORY PHENOMENA**

**MalK, the ATP-Hydrolyzing Subunit, as a Sensor for mal Expression**

It has been known for a long time that mutations in the transport system, later identified as malK mutations, lead to elevated expression of the remaining mal genes (31, 125). This can be conveniently observed by using malK-lacZ fusions with a nonfunctional MalK, which exhibit high and constitutive β-galactosidase activity. An intact MalT activator is required for repression. MalK mutants that are unable to repress mal gene expression no longer show a tendency to form dimers in vivo (68). This may indicate that the dimeric form of MalK is the one that interacts with MalT.

As discussed above, MalK forms dimers in vivo, as indicated by fusions to a truncated α repressor (which is unable to dimerize). MalK mutants that are unable to repress mal gene expression no longer show a tendency to form dimers in vivo (68). This may indicate that the dimeric form of MalK is the one that interacts with MalT.

Figure 7 shows a model of how MalK could mediate repression. MalK is pictured as a protein that is able to shift between a state of association with MalFG and a state of association with MalT. In the presence of substrate to be transported, MalK would shift to the transport conformation allowing MalT to function as mal gene activator. In the absence of substrate to be transported, MalK would be free to interact with MalT and cause repression. The model is reminiscent of findings with the proline utilization system, where PurA, the proline dehydrogenase, functions as a membrane-bound enzyme in the presence of proline and as a cytoplasmic repressor in its absence (168). Two observations support such a MalK cycle. First, there is a particular mutation in malK (malK941) (147) which leads to a superrepressor MalK protein. This mutation is near the ATP binding site. Labelling of the mutant protein with radioactive 8-azido-ATP is still possible; therefore, binding of ATP, but no ATP hydrolysis, probably also takes place. This would mean that the absence or presence of ATP hydrolysis determines the quality of MalK as a repressor and that ATP bound to MalK is required for repression. Second, mutations in malT and malG that result in high constitutive levels of MalK ATPase activity (41, 57) show a high level of mal gene expression in the absence of inducer, presumably because the ATP-hydrolyzing form of MalK is mimicking transport activity and is unable to repress MalT activity (183).

The MalK protein is unusually long in comparison to other ATP-hydrolyzing subunits of binding protein-dependent ABC transport systems; the difference is the presence of a C-termi-
The MalK subunit is also the target of PTS-mediated inducer exclusion that is brought about by the interaction of nonphosphorylated EIIC\[^{\text{Glc}}\] with MalK (60, 147, 275). This interaction leads to a reduction in \(V_{\text{max}}\) without changing the \(K_{\text{m}}\) of the transport system (60). This is reminiscent of the effect of the UgpC-mediated inhibition of the Ugp transport system by internal P\(_i\) (29, 298), which we explain by the dissociation of UgpC from its protein partners in the membrane. Thus, it appears that the state of MalK (membrane bound or cytosolic) is important for its function in transport and regulation.

MBP-dependent ABC transport systems have also been found in gram-positive bacteria (102, 196), in thermophilic bacteria (120, 225), and in a hyperthermophilic archaeon (130, 299). The canonical composition of these transport systems is the same as in gram-negative bacteria, even though the respective binding proteins are anchored in the membrane by a diacylated glycerol linked via a thioether to the N-terminal cysteine of the binding protein. Surprisingly, in many of these systems that have been analyzed by sequencing, the genes encoding the MalEFG analogs are clustered in the same orientation as in the \(E.\ coli\) maltose system whereas the gene encoding the corresponding ATP-hydrolyzing enzyme is localized elsewhere on the chromosome or has not been found yet (225). In \(Streptomyces\), the same ATP-hydrolyzing subunit appears to serve at least two different transport systems (233). These observations, together with the finding that ATP-hydrolyzing subunits can (under certain conditions) be functionally exchanged among heterologous systems, indicate that this component may have been the first one to appear during evolution.

On the other hand, UgpC, the corresponding subunit of the Ugp system, exhibits the same C-terminal extension as MalK and shows sequence similarity to MalK except at its C-terminal extension. MalK and UgpC can be exchanged between the two systems and can complement the transport defect of the heterologous system, but only in the absence of the cognate subunit (115), indicating a lower affinity in forming the heterologous complex. A similar exchange of MalK from \(S.\ typhimurium\) by LacK, the ATP-hydrolyzing subunit of a binding protein-dependent ABC transporter for lactose in \(Agrobacterium\ radiobacter\), has been reported (296). Similarly, CymD, the ATP-hydrolyzing subunit of the cycloextrin transport system of \(K.\ oxytoca\), can substitute for MalK in \(E.\ coli\) (179).

Considering the additional segment at the C terminus of MalK, where all the regulation-negative mutations have been found (147), it was of interest to find whether UgpC, which carries the same C-terminal extension as MalK, would also be active as a repressor. While the overproduction of UgpC had no repressing effect on mal gene expression, it did reduce the expression of the remaining ugp genes (271). Thus, repression exerted by an ATP-hydrolyzing subunit of a binding protein-dependent transport system is not a peculiarity of MalK but may represent a novel form of regulation occurring in this type of transport system.

**MalY as a mal Repressor**

The finding that \(malK-lacZ\) fusions lacking MalK function are expressed at high levels has been used in a genetic screen (looking for white colonies on \(\beta\)-galactosidase indicator plates) with the intention of selecting mutations that prohibit the synthesis of an internal inducer. A chromosomal Tn\(10\) insertion which abolished the constitutivity of the \(malK-lacZ\) fusion was isolated. The gene was mapped at 36 min on the chromosome, outside any known mal operon. It was named \(malI\) to indicate the expected function of the gene product in the synthesis of the endogenous \(mal\) gene inducer (81). \(malI\) was cloned and sequenced. The deduced amino acid sequence of the \(mal\) gene product revealed that it is highly similar to the classical repressor proteins, such as LacI or GalR (209) and must represent a repressor itself. An operon containing two genes, \(malX\) and \(malY\), is located next to and transcribed divergently from \(malI\). This operon, as well as \(malI\) itself, is repressed by the MalI protein (208). Therefore, MalI is not involved in the synthesis of the endogenous inducer of the \(mal\) system, as had been expected, but mutations in \(malI\) lead to the constitutive expression of the \(malX\) \(malY\) operon, which in turn represses the \(mal\) system. Sequencing revealed that \(malX\) encodes a protein exhibiting homology to the enzyme IIC\[^{\text{B}}\]\[^{\text{Glc}}\] of the PTS, encoded by \(ptsG\) and catalyzing the transport of glucose. Indeed, the expression of \(malX\) could complement \(ptsG\) mutants for growth on glucose. However, the expression of \(malX\) does not cause repression of the \(mal\) genes. It is \(malY\), the distal gene in the operon, that causes repression when overexpressed, either in a \(mal\) mutant or when cloned under \(tac\) promoter control (208).

The sequence of \(malY\) indicates weak homology to aminotrans-
ferases, including the consensus sequence of a pyridoxal phosphate binding site, but the purified enzyme had no detectable aminotransferase activity with glutamate as a substrate. Instead, we found βCS lyase activity (cleavage of a βCS bond in amino acids) connected to the protein, similar to cystathionase activity (301). The latter enzyme, encoded by metC, is essential in methionine biosynthesis and cleaves cystathionine to homocysteine, ammonia, and pyruvate (12). Indeed, metC mutations can be complemented for growth in the absence of methionine by malT mutants or by multicy copy plasmids harboring malY, even though MalY and MetC do not show significant sequence homology, and the quaternary structures (38) of the two proteins are different; MetC is a tetramer and MalY is a monomer.

The βCS lyase activity of MalY is not the cause of the repression of the mal genes. A mutation in the pyridoxal phosphate binding site of MalY was constructed that resulted in the loss of βCS-lyase activity, even though the protein was still active as a mal gene repressor. In addition, overproduction of MetC had no effect on the regulation of the mal genes (301). As in the case of MalK, mutants with mutations in MalY that still exhibit nearly unchanged enzymatic activity but have lost their ability to repress the maltose system can be isolated. The clustering of these mutations favors a mechanism of protein-protein interaction with MalT as causing repression. There are several indications to suggest that MalY acts by the same mechanism as MalK: malT(Con) mutants are largely insensitive to the effect of either MalK or MalY. Also, when maltose enters the cell via a constitutive transport system for trehalose (see below), an inducer that renders MalT insensitive to overproduction of MalK as well as MalY can be formed. On the other hand, the inhibiting effect of MalY can still be observed in mlc mutants that have elevated levels of MalT. Therefore, the effect of MalY and MalK on the activity of MalT depends on the amount of MalT and on the presence of inducer. A high inducer concentration (or the malT(Con) mutation) is more effective than an increase in the concentration of MalT.

**Aes as a Mal Repressor**

Another enzyme with similar regulatory functions on mal gene expression as MalY and MalK is Aes, an enzyme with acetyl esterase activity, aes, the gene encoding Aes, is identical to a previously described open reading frame (orf203) of unknown function whose deduced amino acid sequence showed homology to lipases (164). Overproduction of Aes strongly and specifically represses mal gene expression. Again, the effect of Aes on mal gene expression is similar to the effect of MalK and MalY. Thus, malT(Con) strains, which are resistant to the overproduction of MalK and MalY, are also resistant to the overproduction of Aes. Also, the insertion in mlc resulting in elevated malT expression reduces the effect of Aes (186). It is our working hypothesis that both MalY and Aes act in the same way as MalK by interacting with MalT to shift the equilibriums to its inactive state. Even though they do not exhibit sequence similarity, all three proteins must contain a similar structural motif by which they recognize MalT.

**Effect of Phosphorylated PhoP on mal Expression**

PhoQ-PhoP represents a two-component regulatory system that responds to low Mg$^{2+}$ concentrations in the medium (277, 278, 283). By using a mutant with the sensor kinase PhoQ deleted and with pyruvate as the carbon source (to increase the phosphorylation capacity by introducing a high internal concentration of acetyl phosphate), malK as well as malE but not malT expression was reduced by a factor of 10. The same reduction in malEK expression was obtained by introducing phoP on a multicopy plasmid and after growth on glycerol. At present, it is not clear whether the effect of PhoP on mal gene expression is a direct one or is mediated by one of the many PhoQ- and PhoP-regulated proteins (18).

**ENDOGENOUS INDUCER OF THE MALTOSE SYSTEM**

The relatively high “uninduced level” of mal expression in *E. coli* when the cells are grown without maltodextrins in the medium (i.e., with glycerol) points to the endogenous synthesis of the inducer. Several lines of evidence support this conclusion: first, malK-lacZ fusions (lacking MalK function) display a much higher β-galactosidase activity than does the corresponding malK$^{+}$ merodiploid strain. This shows not only that MalK acts as a repressor but also that MalT is activated by internal sources. Second, as outlined above, maltose metabolism initiated by amylomaltase needs a maltodextrin primer with the minimal chain length of maltotriose. Thus, in the absence of endogenous maltodextrins, *E. coli* could not grow on maltose. Third, mutants with mutations in enzyme II′ Glc (pgm), enzyme II′ Man (ptsM), or glucokinase (glk) can be induced by glucose entering the cell in its unphosphorylated form via the galactose permease. This indicates that the inducer is formed from glucose. The induction by glucose does not occur in a mutant lacking phospoglucomutase (pgm). Such a strain exhibits a very low level of mal gene expression (in comparison to its pgm$^{+}$ parent) as measured by transport activity (69). Since the induction-preventing mutation is in pgm, it is clear that the synthesis of inducer can arise from gluconeogenesis. The only inducer established so far to be able to activate MalT in vitro is maltotriose (202). Therefore, the cell must have the ability to synthesize maltotriose or another sugar with inducer capabilities. There are at least two ways in which maltotriose can be synthesized endogenously.

**Glycogen as a Source of Maltotriose**

MalQ mutants are constitutive for the maltose transport system and other malT-dependent mal gene products. This constitutivity is dependent on glycogen or, more correctly, on the presence of the glycogen-synthesizing enzymes (69). MalQ mutants that carry an additional mutation in glgA (encoding glycogen synthetase) or glgC (encoding ADP-glucose pyrophosphorylase) are no longer constitutive but normally inducible by maltose. Thus, the constitutivity in MalQ mutants must be caused by the production of endogenous inducer from glycogen, and the function of amylomaltase is to remove the inducer. With the knowledge of the activity of amylomaltase, it is clear that maltotriose is removed and kept at low concentrations by the formation of larger maltodextrins and glucose. Indeed, extracts of MalQ mutants contain glucose, maltose, maltotriose, and larger maltodextrins that are absent or found in much lower concentrations in the corresponding malQ$^{+}$ strain (81). Whereas the removal of glycogen-derived endogenous inducer is clearly a function of amylomaltase, it is less clear how maltotriose is derived from glycogen. MalQ MalZ or MalQ amyE double mutants are still constitutive in the expression of the mal genes, indicating that maltodextrin glucosidase (267) or a cytoplasmic amylase (198) is not involved in producing maltotriose from glycogen (69). A likely candidate for maltotriose production is glgX, a gene, found in the glycogen gene cluster, whose deduced amino acid sequence shows homology to amylases (221).
Second Pathway for Maltotriose Formation

Even in the absence of glycogen, i.e., in glgA and glgC mutants defective in the synthesis of glycogen, the mal genes can be induced in the absence of external maltodextrins but under conditions that generate glucose and α-glucose-1-phosphate (or glucose-6-phosphate) inside the cell. The best example is the metabolism of trehalose, which induces the mal genes (in the absence of glycogen) to about 30% of the fully induced or constitutive level of expression (141). The immediate products of trehalose metabolism are glucose and glucose-6-phosphate (219). Similarly, when glucose is transported into the cell without phosphorylation (via galactose permeases in a ptsG ptsM glk mutant), the maltose genes become induced. This glucose-independent induction needs glucose-1-phosphate, since pgm mutants which lack phosphoglucomutase and hence are unable to deliver glucose-1-phosphate by gluconeogenesis are not induced by glucose. By using exogenous [14C]glucose, the glucose-1-phosphate-dependent formation of internal maltose and maltotriose can be observed in such a mutant (69). One has to stress, however, that mal gene induction by the external addition of glucose and the formation of maltotriose from exogenous glucose may simply be coincidental events. In addition, it is clear that ptsG ptsM glk and even ptsG ptsM glk pgm mutants do transform [14C]glucose into other, still unidentified, products (69).

It appears that even in the absence of trehalose metabolism or when the cells are growing on glycerol and no glucose is taken up from the medium, the mal genes are endogenously induced by the formation of small amounts of maltotriose (or another unidentified inducer). In wild-type strains, the basal expression of the mal genes is low, curbed by the action of the MalK protein, but it becomes significant in malK-lacZ fusion strains lacking MalK function. Since this constitutivity is also seen in strains defective in glycogen synthesis, it is obvious that the endogenous inducer can also be derived from glucanogen synthesis without bringing in glucose from the medium. These observations have led us to formulate a scheme for the synthesis of the internal inducer that is based on the function of an as yet unidentified maltose/maltotriose phosphorylase. This enzyme should reversibly form maltose (and maltotriose) from glucose (or maltose) and glucose-1-phosphate (22). The formation of these maltodextrins and therefore the postulated enzyme(s) for their synthesis are independent of any maltose enzyme, since maltotriose is created in malT mutants as well (69). The glucanogenetic origin of glucose-1-phosphate seems clear; less clear is the origin of free internal glucose. There are several possibilities. The first is the hydrolytic function of a phosphoglucotransferase, an enzyme that was purified some time ago but whose gene has not yet been identified. This enzyme transfers the phosphate moiety from glucose-phosphate not only to another sugar but also to water, thus forming free glucose (258). The other possibility is the release of free glucose from UDP-glucose by analogy to the release of galactose from UDP-galactose (297). Indeed, galU mutants lacking UDP-glucose pyrophosphorylase are somewhat impaired in the constitutivity of a malK-lacZ fusion.

If the model of inducer synthesis is correct, pgm mutants should be strongly reduced in their basal expression of the maltose genes and uninducible by trehalose. This is exactly what is observed when measuring maltose transport of pgm mutants grown in glycerol or, even more dramatically, grown on trehalose. However, malK-lacZ (Mal−) fusions are only weakly (two- to threefold) reduced in their constitutive expression when tested in pgm mutants. Similarly, the induction of a malK-lacZ fusion (be it Mal+ or Mal−) by trehalose is only weakly reduced in pgm mutants. At present, there is no reasonable explanation for the difference in these two tests for mal gene expression.

Elevated Expression of the mal Genes during Glucose Starvation

When E. coli is grown in the chemostat with glucose as the limiting carbon source and at micromolar concentrations, the maltose system is expressed at elevated levels (62, 63, 174). The authors concluded that this was due to the increased synthesis of endogenous maltotriose as inducer of the system. Most probably, glucose enters the cell via the Mgl transport system (61) that also recognizes this sugar with high affinity. The Mgl system may really be more of a glucose transporter than a galactose transporter since it not only transports glucose with high affinity but also is induced by internal glucose (99). Here again, the strong correlation between the appearance of endogenous free glucose and the induction of the maltose transport genes can be seen.

This is in sharp contrast to the situation for cells growing logarithmically in batch cultures at high glucose concentrations. Under these conditions, transport of glucose via the PTS exerts strong catabolite repression, blocking the transcription of malT and the malT-dependent genes of the malK lamB malM and malE malF malG operons. In addition, transport of maltose is diminished by inducer exclusion. These effects are caused by the interaction of unphosphorylated enzyme IIA\textsubscript{Glc} of the PTS with MalK (60, 147, 275).

Role of Glucose in Internal Induction of the Maltose System

There are several facts that emphasize the importance of free internal glucose in the induction of the maltose system: (i) trehalose whose metabolism yields internal glucose induces the maltose system; (ii) the transport of free glucose into the cell induces the system; (iii) maltose and maltodextrin metabolism itself results in the formation of free internal glucose; and (iv) maltose and [14C]glucose can endogenously form maltotriose whose reducing glucose moiety is 15C labelled. This obvious association of unphosphorylated internal glucose with the induction process is further stressed when the concentration of internal glucose is manipulated by phosphorylation via over-produced glucokinase. Raising the amount of glucokinase 10-fold over the chromosomally encoded level by plasmid-carried glk reduces mal gene expression. This repression occurs only when the gene expression is due to endogenous induction either during growth under “uninduced conditions” (with glyceral as the carbon source) in a wild-type strain or in a constitutive malK-lacZ fusion lacking MalK function. The effect of glucokinase overproduction cannot be observed when constitutive mal gene expression is due to the formation of maltotriose from glycogen in malQ mutants (where free glucose is not involved) or in malT(Con) mutants, which are largely independent of inducer (161).

Osmoregulation of the Maltose System

A malK-lacZ fusion that lacks any MalK activity is expressed constitutively due to the presence of endogenous inducer. With increasing osmolarity of the growth medium, the expression of the fusion is abolished (31). Using a malK-lacZ fusion that is merodiploid for malK (and can therefore grow on maltose), the fusion is expressed at low levels when grown on glycerol but can be induced by maltose, thus reflecting the normal induction pattern by maltose in a wild-type strain. High osmolarity
still leads to repression of the uninduced levels but not when induced with maltose (31), consistent with the observation that high osmolarity does not affect growth on maltose. This osmo-dependence of mal gene regulation does not affect malT expression (31), and it is not dependent on the envZ/ompR (255) regulatory circuit or the σ70-dependent (117) control system. However, during the attempt to isolate mutations that counteract the effects of high osmolarity, an insertion was isolated upstream of the promoter of malT (malT-P418::Tn10 [Fig. 1]). This insertion does not abolish osmoregulation but simply elevates expression of malT. Another mutation found in this selection (144) was an insertion (null mutation) in assuE, a gene known to encode a lysine tRNA-modifying enzyme (262). This mutation does not affect malT expression and only dampens the effect of high osmolarity on malK and malE expression. The mechanism by which AssuE is involved in the osmoregulation of mal gene expression is unclear.

Since only the uninduced level of mal gene expression is affected by osmolarity, one may argue that it is the synthesis of endogenous inducer that is inhibited under conditions of high osmolarity. Most recently, we found that the presence of glucokinase is necessary for the effective repression of uninduced gene expression. Since the expression of glk at high osmolarity remains unchanged (as measured by a translational glk-lacZ fusion), the most likely explanation is a stimulation of glucokinase activity at high osmolarity. In strains carrying ptsM and ptsG mutations in addition to the glk mutation, no osmoregulation of mal gene expression is observed. This demonstrates that osmoregulation (i.e., repression) of mal gene expression is caused by the removal of internal free glucose.

INTERCONNECTION BETWEEN THE MALTOSE AND TREHALOSE SYSTEMS

Maltose and trehalose are disaccharides composed of two α-glucosidically linked glucose residues, but their structures are quite different. Whereas maltose contains an α(1→4)-glucosidic linkage and exhibits a reducing end glucose, trehalose contains two α(1→4)-glucosidic linkages and no reducing sugar residue (Fig. 8). Due to the particular symmetrical and backfolded structure of this nonreducing sugar, trehalose, in contrast to maltose, has properties that make it an excellent agent to preserve protein and membrane structures (42). It has been observed that trehalose is synthesized as a protecting agent or osmolyte in many cell types including E. coli under conditions of dehydration as well as high osmolarity (137). Thus, it is not surprising that E. coli has developed two entirely different systems for the metabolism of maltose and trehalose, both of which are excellent carbon sources for this organism. Unlike maltose, trehalose uses a PTS-dependent enzyme II^Ttre (encoded by treB) for its uptake as trehalose-6-phosphate (142) followed by its internal hydrolysis by trehalose-6-phosphate hydrolase (encoded by treC) to glucose and glucose-6-phosphate (219). treB and treC form an operon that is controlled by the adjacent treR gene, encoding a repressor (129). The internally acting inducer of the system is trehalose-6-phosphate, which inactivates TreR. There is one complicating fact: in addition to its degradative abilities, E. coli is able to synthesize internal trehalose at high osmolarity. The two necessary enzymes are trehalose-6-phosphate synthase (encoded by otsA), catalyzing the transfer of glucose from UDP-glucose to glucose-6-phosphate, thus forming trehalose-6-phosphate, and trehalose-6-phosphate phosphatase (encoded by otsB), catalyzing the hydrolysis of trehalose-6-phosphate to free trehalose (137). otsB and otsA form an operon that is induced at high osmolarity in an RpoS-dependent manner (119). The expression of otsB otsA and of treB treC is mutually exclusive in that high osmolarity prevents the induction of the treB treC operon. Despite these entirely different pathways of maltose and trehalose uptake and metabolism, there are curious links between the two systems.

Trehalose as an Inducer of the Maltose System

It has been observed that the presence of trehalose in the growth medium induces the maltose system (240, 253). Trehalose is not transported by the maltose system, nor does trehalose or trehalose-6-phosphate stimulate MalT; also, MalT does not regulate any of the genes involved in the degradation of trehalose, since malT mutants grow well on trehalose. Nonetheless, there is a subtle dependence of trehalose utilization on MalT function. When malT mutants are grown on trehalose, they exhibit an apparent K_{m} of trehalose uptake that is in the

FIG. 8. Structures of maltose and trehalose. (A) Maltose (α-D-glucopyranosyl-1→4-D-glucopyranose) in its predominant α enantiomeric form. (B) Trehalose, shown as the naturally occurring α-D-glucopyranosyl-1→1-α-D-glucopyranoside. The structures were calculated by minimizing the free energy of the axial and equatorial hydroxyl positions. The structures are shown in their chair conformation, with all substituents (except the anomeric oxygens) in the equatorial configuration.
order of millimolar, while \textit{maltT}\^{+} strains exhibit a \( K_m \) of 16 \( \mu \)M (19). The explanation for this difference is that the MalT-dependent \( \lambda \) receptor is needed for the diffusion of trehalose at concentrations below mM (141). This is not only true for the utilization of trehalose. Apparently, the \( \lambda \) receptor also functions as a “glycoporin” for the uptake of other sugars, even monosaccharides, at low external concentrations. \textit{mal} gene expression at limiting glucose concentration may provide this increase in glycoporin activity (63). The ability of trehalose to induce the maltose system depends on the presence of TreC, the enzyme that degrades trehalose-6-phosphate to glucose and glucose-6-phosphate (141). We have interpreted this finding as an indication that glucose and glucose-6-phosphate (and, subsequently, glucose-1-phosphate via Pgln) are the precursors of the synthesis of maltose and maltotriose. However, in \textit{treR} mutants, even in the absence of trehalose, the \textit{mal} genes are expressed at an elevated level, indicating that TreC not only provides the precursors for maltotriose synthesis from trehalose-6-phosphate hydrolysis but also is itself involved in inducer synthesis (68). Whether the product of TreC activity in the absence of trehalose-6-phosphate is maltotriose or another inducing compound is unclear.

\textbf{Transport of Maltose and Trehalose in the Archaeon \textit{Thermococcus litoralis}}

Recently, we observed that the hyperthermophilic archaeon \textit{Thermococcus litoralis} has high transport activity at 85°C for maltose and trehalose, with \( K_m \) values of 15 to 20 mM (299). Both substrates are transported by the same system, since they are transported by the same system that is also connected to the cell’s chemotactic machinery, it appears that the maltose system is geared for the scavenging of low levels of maltose and maltodextrins. Even before the arrival of the true substrate, the system is becoming prepared by endogenous induction, particularly when fasting for glucose or other carbohydrate carbon sources. However, only in the presence of the true substrate is full-fledged induction developed. It makes perfect sense that the function of holding back full induction should be connected to a component of the transport system itself. In this way, the system responds not only to the presence of substrate by induction but also to loss of substrate by repression. This could in principle also be achieved by a gene regulator that is an activator in the presence of the inducer and a repressor in the absence of the inducer (the ara system is the classical example [232]), but apparently, a finer tuning results from the regulation seen in the maltose system. The transport step itself is connected to regulation is not unprecedented. We find this in the regulation of the \textit{pho} regulon by the Pho transport system (288); in the Bgl system, where an antiterminator is controlled by its cognate phosphotransferase system (3); and in the regulation of the Fec system by the transport of ferric citrate through its cognate outer membrane receptor FecA (139).

Aside from these two main streams (endogenous induction and MalK-dependent repression), there appear to be several additional regulatory interconnections such as the existence of a repressor for the activator or the inhibition of the activator by seemingly unrelated enzymes (MalY or Aes). The characteristic feature of these effectors is that they have hardly any recognizable effect under normal physiological conditions but may become important under extreme growth conditions. It is possible that the maltose system is nothing special in this respect, and several more “classical” systems that show this kind of network regulation may be discovered.

\textbf{ACKNOWLEDGMENTS}

We are indebted to Armin Geyer (Department of Chemistry, University of Konstanz), who produced Fig. 8. We thank Sherry Mowbray, Tilman Schirmer, and Florante Quiocho for their help in preparing Fig. 3 and 5. We thank Erika Oberer-Bley for her help in preparing the manuscript. Work done in the laboratory of W. Boos was supported by grants from the Deutsche Forschungsgemeinschaft (SFB156 and Schwerpunkt Netzwerkregulation in Bakterien). Work in the Shuman laboratory was supported by NIH grant GM51118. The collaboration of the two laboratories was supported by the Max-Planck-Forschungspreis 1991.

\textbf{REFERENCES}


Trehalose synthesis genes are controlled by the putative sigma-factor encoded by psf and are involved in stationary-phase thermotolerance in Escherichia coli. J. Bacteriol. 173:7918–7924.


Pugsley, A. P., and C. Dubreuil. 
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.
Peist, R., C. Schneider-Fresenius, and W. Boos.

274. Boos and Shuman 2017-11-18