JOURNAL OF BACTERIOLOGY, June 1986, p. 884–891
0021-9193/86/060884-08$02.00/0

Vol. 166, No. 3

Osmoregulation of the Maltose Regulon in *Escherichia coli*

BERND BUKAU, MICHAEL EHRMANN, AND WINFRIED BOOS*

Department of Biology, University of Konstanz, D-7750 Konstanz, Federal Republic of Germany

Received 6 December 1985/Accepted 7 March 1986

The maltose regulon consists of four operons that direct the synthesis of proteins required for the transport and metabolism of maltose and maltodextrins. Expression of the *mal* genes is induced by maltose and maltodextrins and is dependent on a specific positive regulator, the MalT protein, as well as on the cyclic AMP-catabolite gene activator protein complex. In the absence of an exogenous inducer, expression of the *mal* regulon was greatly reduced when the osmolarity of the growth medium was high; maltose-induced expression was not affected, and *malT*^{c}-dependent expression was only weakly affected. Mutants lacking MalK, a cytoplasmic membrane protein required for maltose transport, expressed the remaining *mal* genes at a high level, presumably because an internal inducer of the *mal* system accumulated; this expression was also strongly repressed at high osmolarity. The repression of *mal* regulon expression at high osmolarity was not caused by reduced expression of the *malT*, *envZ*, or *crp* gene or by changes in cellular cyclic AMP levels. In strains carrying mutations in genes encoding amylomaltase (*malQ*), maltodextrin phosphorylase (*malP*), amylase (*malS*), or glycogen (*glg*), *malK* mutations still led to elevated expression at low osmolarity. The repression at high osmolarity no longer occurred in *malQ* mutants, however, provided that glycogen was present.

In their natural habitat, enteric bacteria such as *Escherichia coli* are exposed to environments with widely varying osmolarities and have evolved strategies to adapt to osmotic stress (for review, see references 13, 25, 27). *E. coli* and *Salmonella typhimurium* respond to changes in the osmolarity of the medium in a number of ways, but the mechanisms of control are unclear. At low external osmolarity, osmotic balance across the inner membrane is believed to be maintained by the synthesis of membrane-derived oligosaccharides (24). In response to high medium osmolarity, transport systems for the uptake of K^+ (13) and betaine (6, 17, 29) are induced. The accumulation of these and other (25) compounds in the cytoplasm maintains turgor pressure across the inner membrane.

The significance of other cell responses to changes in external osmolarity is poorly understood. Relative amounts of the outer membrane porins OmpF and OmpC are osmoregulated (39). This regulation is at the transcriptional level and is partly mediated by the *ompR* and *envZ* genes (18–20, 33, 35, 40), which compose the *ompB* operon. In addition, certain *envZ* point mutations exhibit a strong negative pleiotropic effect on the synthesis of outer membrane porins OmpF and PhoE, alkaline phosphatase, and the proteins of the *mal* regulon (41, 42). However, in cells with these mutations, osmoregulation of OmpC is unaltered (33).

In search of *lacZ* fusions to genes whose expression is turned off at high osmolarity and turned on at low osmolarity, we found, to our surprise, fusions in the *malK* gene. This result suggested that the *mal* regulon is also osmoregulated.

The maltose system mediates maltose and maltodextrin uptake and metabolism (for review, see reference 21; M. Schwartz, *in* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, ed., *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*, in press). The proteins required for active transport of maltose and maltodextrins are encoded by genes of two divergent operons in the *malB* region, located at 91 min on the genetic map. In one operon, the *malE* gene encodes the periplasmic maltose-binding protein, and the *malF* and *malG* genes encode transport components located in the cytoplasmic membrane. In the other operon, the *malK* gene encodes a protein bound to the inner membrane, possibly through interaction with the *malG* product (38), and the *lamB* gene encodes the outer membrane protein maltoporin. MalK has been proposed to participate in energization of maltose transport.

Metabolism of maltose and maltodextrins involves three enzymes, amylomaltase, maltodextrin phosphorylase, and amylase, which are encoded by the *malQ*, *malP*, and *malS* genes, respectively. The *malP* and *malQ* genes constitute an operon within the *malA* region at 75 min. The *malS* gene is located at 80 min in an operon containing an unknown number of genes (14a). The MalP and MalQ proteins are cytoplasmic; MalS is periplasmic.

Transcription of all four operons is increased 10- to 30-fold by exogenous maltose and maltodexrins. Induction is mediated by the product of the positive regulator gene *malT*, located in the *malA* region and transcribed divergently from *malPQ*. In addition, transcription of all *mal* genes, with the exception of *malPQ*, is directly dependent on the binding of the cyclic AMP (cAMP)-catabolite gene activator protein (CAP) complex to their promotors. Expression of *malPQ* depends on the cAMP-CAP complex only indirectly via the cAMP-CAP dependence of the *malT* gene (9).

malT^c mutations lead to expression of the maltose genes at induced levels even in the absence of an exogenous inducer (12). For an unknown reason, so do mutations in the *malK* gene (23, 36). The latter observation suggests that there might be an endogenous inducer for the maltose regulon which accumulates in *malK* mutants (32; Schwartz, in press).

Here we report that the uninduced expression of the structural genes of the maltose regulon was further reduced when the osmolarity of the medium was high, while in maltose-induced or *malT*^{c} cells expression remained largely osmoindependent. In *malK* mutants, elevated expression was abolished at high osmolarity. In *malK malQ* double mutants, however, gene expression remained elevated even

* Corresponding author.

at high osmolarity. In *malK malQ glg* triple mutants, which can not synthesize glycogen, elevated expression was again abolished at high osmolarity.

MATERIALS AND METHODS

Bacterial strains and phages. Bacterial strains and phages used are listed in Table 1.

Media and growth conditions. Double yeast tryptone (DYT) medium and minimal medium A (MMA) have been described previously (28). Samples of precultures (10 to 25 μl each) grown in DYT at 37°C (or at 30°C for strains carrying Mu dl phages) were added to 5 ml of sixfold-diluted MMA supplemented with NaCl and 0.2% carbon source(s) as indicated. These cultures were grown at 37°C (or 30°C) to mid- to late exponential phase, harvested by centrifugation, and kept on ice until used for β-galactosidase assays. For induction of the maltose regulon in *malPQ* strains, cells were grown overnight in the appropriate minimal medium plus 0.2% glycerol until late exponential phase, diluted 1:6 into fresh minimal medium containing 0.2% glycerol plus 0.2% maltose, and grown for a further 3 to 4 h until used in the β-galactosidase assays.

Genetic manipulations. P1 transductions were done by the method of Miller (28). Deletions in the *malB* region were introduced into *malPQ* mutants via P1-mediated transduction, selecting for a nearby Tn*10* insertion and assaying for maltose transport. For the construction of strains BB491 and ME260, we first introduced a Δ*crp* allele into strains BRE1162 and BRE1219 (which carry *malK-lacZ* fusions). Because of a lack of CAP, these strains can not use lactose. Transductants receiving the *crp* allele of strain CA8404 that encodes a cAMP-independent CAP (*crp** [34]) were selected on minimal plates containing 0.2% lactose as the sole carbon source. Mutations in the *glg* genes were introduced into strains carrying a *malK-lacZ* gene fusion (BB496, BB709, ME252, ME262) by P1-mediated cotransduction with the nearby *asd* and *glpD* genes. Since the *glg*, *glpD*, and *asd* genes all map within 1 min on the *E. coli* chromosome, these markers were scored after each transduction. Cells were screened for the Glg phenotype by staining colonies for glycogen with an iodine solution, by the method of Govons et al. (16). The *glpD* marker was scored on minimal-glycerol plates. Tn*10* insertions in the *malP* or *malQ* gene were then introduced by P1 transduction, selecting for tetracycline resistance and screening for glycogen.

β-Galactosidase assay. β-Galactosidase assays were done by the method of Miller (28). Specific activities were expressed as micromoles of ortho-nitrophenyl-galactoside hydrolyzed per minute per milligram of protein at room temperature.

RESULTS

Expression of the maltose regulon at low osmolarity in various maltose mutants. To study the expression of the maltose regulon, we used fusions of *lacZ* to various *mal* genes. Except for one early *lamB-lacZ* protein fusion, we used operon fusions that produced wild-type β-galactosidase, although the transcription of *lacZ* was under the control of the promoter of the fused gene. Upon insertion of a *lacZ* fusion phage, the target gene was destroyed, creating a stable lysogen. In strains in which a phage carrying a *mal-lacZ* fusion was integrated by *mal* homology into a target gene, a second, intact copy of the target gene was still present, creating a merodiploid.

Null mutations in the *malK* gene result in an elevated expression of the maltose regulon in the absence of an exogenous inducer (23, 36; Schwartz, in press). Before determining the effects of medium osmolarity on the expression of the *mal* genes, we tested whether lesions in any *mal* genes would affect the induced and uninduced levels of *mal* regulon expression. We measured the β-galactosidase activity of *lacZ* fusions to *malK*, *lamB*, *malPQ*, and *malS* genes (Table 2). In strains lacking the *malK* gene, whether through deletion of the *malB* region or the presence of a stable lysogen in *malK*, β-galactosidase activity was expressed at high levels (Table 2).

In strains carrying Tn*10* insertions in the *malF* or *malG* gene, the expression of the other genes of the maltose regulon was 3 to 4 times higher than in the corresponding *malB*$^+$ strain (Table 2). This was also true for the expression of the *malEFG* operon itself; a strain with a stable *lacZ* lysogen in the *malE* gene that is polar on the *malF* and *malG* genes had somewhat elevated β-galactosidase levels (Table 2). However, a nonpolar deletion in the *malE* gene and Tn*10* insertions in, or *lacZ* fusions to, the *lamB*, *malPQ*, or *malS* gene did not lead to an alteration of wild-type gene expression (Table 2). We conclude that the lack of MalK function caused constitutivity. Polar mutations in *malE* or *malF* and *malG* mutations could have exerted their effect by reducing the amount of MalG that was available to bind MalK to the membrane.

In contrast, as has been reported previously (11), the expression of *malT* is not influenced by the absence of *malK* function. The expression of a stable *lacZ* lysogen in *malT* (strain BRE1161), which shuts off all structural genes of the maltose regulon including *malK*, was the same as that in strain BRE1217, which carries the same *malT-lacZ* fusion in a merodiploid state and thus allows the expression of *malK* and *malG*.

Effect of osmolarity of the growth medium on maltose gene expression. The osmotic strength of the medium was adjusted with NaCl, which is known to be an osmotically active substance in *E. coli* (1, 26). NaCl concentrations of up to 375 mM had little influence on generation time (Fig. 1).

The expression of all *mal* genes (with the exception of the regulator gene *malT*) was repressed by high osmolarity in the absence of an inducer but not when expression was induced with maltose. The most dramatic osmodependent change in gene expression was observed with a stable *malK-lacZ* lysogen; β-galactosidase activity decreased from its maximal level at around 50 mM NaCl to essentially zero at 375 mM NaCl (Fig. 2A). With a *malK-lacZ* fusion in the merodiploid state, uninduced *malK* expression at low osmolarity was much lower than that in a *malK-lacZ* stable lysogen, but still was repressed about 10-fold at 375 mM NaCl (Fig. 2B). However, when this strain was induced with maltose, β-galactosidase activity remained unaltered at high osmolarity (Fig. 2A). When the effect of high osmolarity on expression of the stable *malK-lacZ* lysogen was tested in a *malT*^{c} background, repression at high osmolarity still occurred, but to a much lesser extent than in the corresponding *malT*$^+$ strain.

Expression of merodiploid fusions to *malE* and stable as well as merodiploid fusions to *lamB* and *malS* showed the same response to changes in medium osmolarity as did expression of the merodiploid *malK-lacZ* fusion. The effect of osmolarity on expression of the *malE-lacZ* fusion is illustrated in Fig. 3. These experiments demonstrated that osmoregulation of the *mal* regulon is a phenomenon that not only affects *malK*-dependent constitutivity but also occurs in uninduced wild-type cells.

TABLE 1. Strians and phages

Strain or phage	Known markers[a]	Construction	Source(s) (reference)
BB453	MC4100 Φ(*malK-lacZ*)*1113* (λ *plac* Mu 50) *malT*^{c}*1*	P1 on BRE1162 into strain JB3018-2 (*malT*^{c}*1*); select Lac^{+}, screen Mal^{-}	This study, 3
BB454	BRE1162 *malPQ*::Tn*10*	P1 on SH203 (*malPQ*::Tn*10*) into strain BRE1162; select Tet^{r}	This study, H. Schweizer
BB461	BRE1219 *malG*::Tn*10*	P1 on TST2 (*malG*::Tn*10*) into strain BRE1219; select Tet^{r}, screen Mal^{-} Lac^{+}	This study, T. Silhavy
BB463	BRE1219 *lamB*::Tn*10*	P1 on MM144 (*lamB*::Tn*10*) into strain BRE1219; select Tet^{r}, screen Lac^{+}, λ^{r}	This study M. Manson
BB475	SF1708 Δ*malE444 zja-742*::Tn*10*	P1 on BB106 (Δ*malE444 zja-742*::Tn*10*) into strain SF1708; select Tet^{r}, screen Mal^{-} by maltose transport assay	This study, 37
BB477	SF1708 *malG*::Tn*10*	P1 on TST2 (*malG*::Tn*10*) into strain SF1708; select Tet^{r}	This study, T. Silhavy
BB478	BRE100 *malG*::Tn*10*	P1 on TST2 (*malG*::Tn*10*) into BRE100; select Tet^{r}, screen Mal^{-}, immunity to λ *cI h80*, Lac^{+}	This study, T. Silvavy
BB491	BRE1162 *crp**	See Material and Methods	This study, 34
BB496	BRE1162 *glcC glgA*^{b} *malP*::Tn*10*	See Material and Methods; *glg* mutations from strain G127, *malP*::Tn*10* from strain pop4063	This study
BB706	BRE1162 *malQ*::Tn*10*	P1 on pop4064 (*malQ*::Tn*10*) into strain BRE1162; select Tet^{r}	This study, C. Guttierrez
BB709	BRE1162 *glgA*^{c} *malQ*::Tn*10*	See Material and Methods; *glgA* mutation from strain G192, *malQ*::Tn*10* from strain pop4064	This study
Bof143	MC4100 [λ Φ(*crp-lacZ*)]		P. Cossart (10)
BRE100	MC4100 Φ(*lamB-lacZ*)*100*(Hyb) [λ p1(209)]		E. Bremer (2,7)
BRE1120	MC4100 (λ p*mal-1163-1*)		E. Bremer
BRE1161	MC4100 Φ(*malT-lacZ*)*1110* (λ *plac* Mu50)		E. Bremer (5)
BRE1162	MC4100 Φ(*malK-lacZ*)*1113* (λ *plac* Mu50)		E. Bremer (5)
BRE1163	MC4100 Φ(*malE-lacZ*)*1163* (λ *plac* Mu50)		E. Bremer (5)
BRE1217	MC4100 (λ p*mal-1161-1*)		E. Bremer (5)
BRE1219	MC4100 (λ p*mal-1162-1*)		E. Bremer (5)
G127	HfrG6 *glgC glgA*^{b}		J. Cattaneo
G192	HfrG6 *glgA*^{c}		J. Cattaneo
MC4100	F^{-} *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rpsR flbB301*		Casadaban (7)
ME250	BRE1162 *malP*::Tn*10*	P1 on strain pop4063 (*malP*::Tn*10*) into strain BRE1162; select Tet^{r}	This study, C. Guttierrez
ME251	MC4100 Φ(*malK-lacZ*)*1113* (λ *plac* Mu50) Δ*malP518*	P1 on BRE1162 into strain pop2158 (MC4100 Δ*malP518*); select Lac^{+}, screen Mal^{-}	This study, 31

Continued on following page

TABLE 1—*Continued*

Strain or phage	Known markers[a]	Construction	Source(s) (reference)
ME252	BRE1162 *glgA*[c] *malP*::Tn*10*	See Material and Methods; *glgA* mutation from strain G192, *malP*::Tn*10* from strain pop4063	This study
ME253	BRE1219 *malF*::Tn*10*	P1 on TST7 (*malF*::Tn*10*) into strain BRE1219; select Tet^r, screen Mal$^-$ Lac$^+$	This study, T. Silhavy
ME254	BRE1219 *malPQ*::Tn*10*	P1 on SH203 (*malPQ*::Tn*10*) into strain BRE1219; select Tet^r, screen Mal$^-$	This study, H. Schweizer
ME255	BRE100 *malF*::Tn*10*	P1 on TST7 (*malF*::Tn*10*) into BRE100; select Tet^r, screen Mal$^-$, immunity to λ *cI h80*, Lac$^+$	This study, T. Silvavy
ME256	SF1708 *lamB*::Tn*10*	P1 on MM144 (*lamB*::Tn*10*) into strain SF1708; select Tet^r	This study
ME257	SF1708 *malF*::Tn*10*	P1 on TST7 (*malF*::Tn*10*) into strain SF1708; select Tet^r	This study, T. Silhavy
ME258	SF1708 Δ*malB112 zjb-729*::Tn*10*	P1 on MM154 (Δ*malB112 zjb-729*::Tn*10*) into strain SF1708; select Tet^r, screen Mal$^-$ by maltose transport assay	This study, 4
ME259	MC4100 Φ(*malK-lacZ*)*1113* (λ *plac* Mu50) *envZ22*	P1 on BRE1162 into strain SG477 (*envZ22*); select Lac$^+$, screen Mal$^-$	This study, 15
ME260	BRE1219 *crp**	See Material and Methods	This study, 34
ME261	SF1707 Δ*malB112*	P1 on MM154 (Δ*malB112 zjb-729*::Tn*10*) into strain SF1707; select Tet^r, screen Mal$^-$	This study, 4
ME262	BRE1162 *glgA*	See Material and Methods; *glgA* mutation from strain G192	This study
SF1707	MC4100 Φ(*malS-lacZ*) Mu dl(Ap *lac*)		8, 14a
SF1708	MC4100 Φ(*malPQ-lacZ*) Mu dl(Ap *lac*)		S. Freundlieb
Phage			
λ p*mal* BRE1161-1	Lac$^+$-transducing phage from BRE1161		5
λ p*mal* BRE1162-1	Lac$^+$-transducing phage from BRE1162		5
λ p*mal* BRE1163-1	Lac$^+$-transducing phage from BRE1163		E. Bremer

[a] Φ, *lacZ* fusion present; Hyb, gene fusion encodes a hybrid protein.
[b] This *glgA* mutation led to low glycogen synthase activity.
[c] This *glgA* mutation led to absence of glycogen synthase activity.

However, in contrast to all other stable *lacZ* fusions to genes in the *malB* or *malS* region, the expression of a stable *malPQ-lacZ* fusion either in the presence (Fig. 4) or absence (data not shown) of the MalK function did not decrease with increasing osmolarity, when cells were grown either with glycerol alone or with glycerol plus maltose. As will be explained below, the reason for the insensitivity of stable *malPQ-lacZ* fusions to high osmolarity was the absence of the MalQ function. The expression of an operon fusion to the activator gene *malT* remained constant at all osmolarities tested, both as a stable lysogen and in the merodiploid state and with either glycerol or maltose as the carbon source (Fig. 5). This indicates that the osmodependency of *malB* and *malS* gene expression was not caused by alterations in the expression of *malT*, the gene encoding the positive activator of the *mal* regulon.

Evidence for an internal inducer in *malK* mutants. Amylomaltase and maltodextrin phosphorylase were not required for production, at low osmolarity, of the internal inducer in a *malK* mutant, but amylomaltase was required for a decrease in expression at high osmolarity. We introduced *malP* and *malQ* Tn*10* insertions and a nonpolar *malP* deletion into strain BRE1162, which carries a stable *malK-lacZ* fusion. The expression of *malK* remained elevated in both Tn*10* insertion strains but not in the *malP* deletion strain (Table 3), indicating that repression at high osmolarity depended on amylomaltase but not on maltodextrin phosphorylase.

The substrates and products of amylomaltase are involved in maltodextrin and glycogen metabolism in *E. coli* (Schwartz, in press). We therefore wanted to test whether maltodextrin and glycogen metabolites function as internal inducers of the maltose regulon and whether the intracellular level of these putative metabolites is osmodependent. To this end, into a strain carrying a stable *malK-lacZ* fusion we introduced a null mutation in *glgC* (the structural gene for ADP-glucose-pyrophosphorylase) or *glgA* (the structural gene for glycogen synthase); both genes encode enzymes of glycogen biosynthesis (for review, see reference 30). We also introduced Tn*10* insertions in *malP* or *malQ* into this strain. High-level expression of the *malK-lacZ* fusion no longer occurred at high osmolarity (Table 3). Therefore, the internal inducer which was responsible for elevated expression at high osmolarity was derived from glycogen (or was a product from glycogen-synthesizing enzymes) and was a substrate of amylomaltase.

TABLE 2. Effect of mutations in different *mal* genes on the expression of *lacZ* fusions to *malK*, *lamB*, *malPQ*, and *malS*

Type of fusion and strain	Mutated gene	Expression of gene fusion[a]	
		Uninduced	Induced
Φ(*malK-lacZ*)			
Merodiploid			
BRE1219	*mal*$^+$; wild-type	1 (0.35)	12.9
BB463	*lamB*::Tn*10*	0.9	
ME253	*malF*::Tn*10*	3	
BB461	*malG*::Tn*10*	2.9	
ME254	*malPQ*::Tn*10*	1.4	12.7
Stable lysogen			
BRE1162	*malK-lacZ* fusion	12.1	
BB454	*malK-lacZ* fusion; *malPQ*::Tn*10*	16.2	
Φ(*lamB-lacZ*)(Hyb)			
Stable lysogen			
BRE100	*lamB-lacZ* fusion	1 (0.85)	12.5
ME255	*lamB-lacZ* fusion; *malF*::Tn*10*	4.1	
BB478	*lamB-lacZ* fusion; *malG*::Tn*10*	4.3	
Φ(*malPQ-lacZ*)			
Stable lysogen			
SF1708	*malPQ-lacZ* fusion	1 (0.048)	27.1
ME256	*malPQ-lacZ* fusion; *lamB*::Tn*10*	2.5	36.5
BB475	*malPQ-lacZ* fusion; Δ*malE*, nonpolar	1.4	
ME257	*malPQ-lacZ* fusion; *malF*::Tn*10*	3.8	
BB477	*malPQ-lacZ* fusion; *malG*::Tn*10*	4.4	
ME258	*malPQ-lacZ* fusion; Δ*malB112*	25.6	
Φ(*malS-lacZ*)			
Stable lysogen			
SF1707	*malS-lacZ* fusion	1 (0.02)	55
ME261	*malS-lacZ* fusion; Δ*malB112*	17	

[a] Cells were grown in sixfold-diluted MMA supplemented with 25 mM NaCl and 0.2% glycerol (uninduced) or 0.2% glycerol plus maltose (induced). Uninduced β-galactosidase activity of the strains carrying *mal-lacZ* fusions without additional mutations were set equal to 1. The specific β-galactosidase activity is given in parentheses as micromoles of ortho-nitrophenyl-galactoside hydrolyzed per minute per milligram of protein.

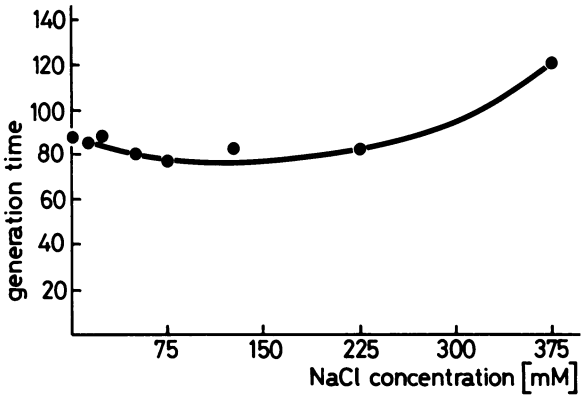

FIG. 1. Influence of the osmolarity of the growth medium on generation time. Portions (50 μl each) of an overnight culture of strain BRE1217 grown in DYT were inoculated into 300-ml volumes of sixfold-diluted MMA containing 0.2% glycerol and different NaCl concentrations. The cultures were grown at 37°C under vigorous aeration, and growth was monitored by observing the optical density at 578 nm.

Under low osmolarity the *mal* genes were expressed at high levels in *malK* mutants despite the absence of glycogen and amylomaltase. Therefore, the internal inducer acting at low osmolarity must have been different from the glycogen-derived inducer acting at high osmolarity.

cAMP, *crp*, and *envZ* are not involved in the osmoregulation of *malK*. Osmoregulation of the maltose regulon could be a consequence of osmodependent alterations in cellular cAMP levels. Such alterations could lead to changes in catabolite repression of the *mal* genes or of genes involved in the synthesis of the unknown inducer molecule. To test this possibility, we introduced the *crp** mutation (34) into strains BRE1162 and BRE1219. Expression of the *malK-lacZ* fusion was not affected by the presence of *crp** (strains BB491 and ME260). Expression of a *crp-lacZ* gene fusion in a *crp*$^+$ background was also not osmoregulated (strain Bof143). Therefore, unless *crp** recognized other nucleotides such as cGMP, the synthesis of which might have been osmoregulated, the CAP system did not appear to play a role in the osmoregulation of the maltose regulon. Furthermore, a *malK-lacZ* fusion strain carrying an *envZ*(Am) mutation (strain ME259) was not altered in its osmoregulation.

DISCUSSION

Expression of the maltose regulon in *E. coli* was found to be osmoregulated in the uninduced state but not in the induced state. With the exception of *malT*, uninduced expression of all maltose genes in *mal*$^+$ cells was turned off when the osmolarity of the growth medium was high. This indicates that the uninduced level of expression of the *mal* regulon was determined by the presence of an internal inducer, the concentration of which was dependent on the osmolarity of the medium. The osmodependent decrease in gene expression was most dramatic when the expression of *malK* was measured by using the β-galactosidase activity of

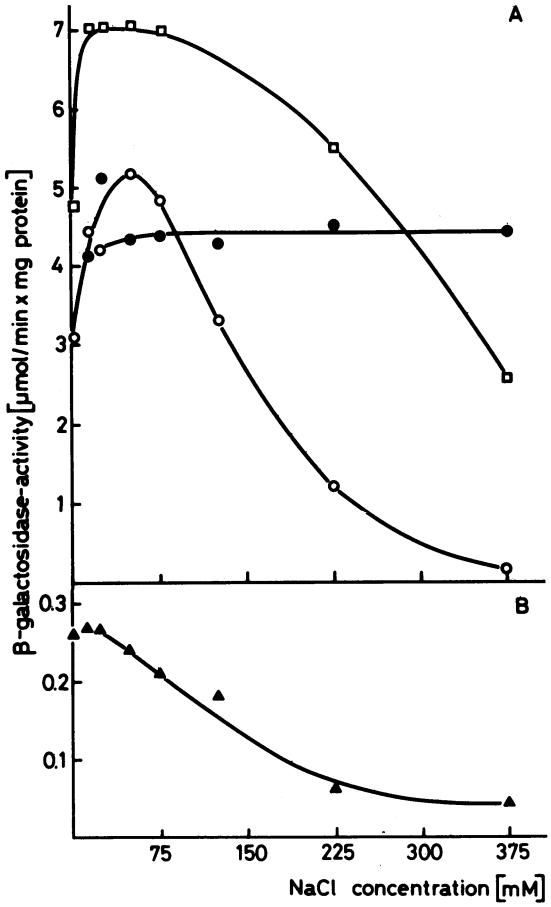

FIG. 2. Expression of *malK-lacZ* fusions after growth in different salt concentrations. (A) ●, Mal$^+$ strain BRE1219 grown with maltose; ○, Mal$^-$ strain BRE1162 grown with glycerol; □, *malT*^{c} (Mal$^-$) strain BB453 grown with glycerol. (B) ▲, Mal$^+$ strain BRE1219 grown with glycerol.

a *malK-lacZ* fusion integrated as a stable lysogen. This fusion led to a transport-negative phenotype and to elevated expression of the *mal* regulon. Expression of the *malK-lacZ* fusion was turned off completely at high osmolarity. The *malK*-dependent constitutivity was most likely due to the accumulation of an internal inducer of the maltose system. Expression of the *malK-lacZ* fusion was still repressed by two to threefold in a *malT*^{c} background at high osmolarity. This mutant produced a MalT protein that did not need exogenous maltose for activation. If internal inducers exist, it is possible that the *malT*^{c} gene product is not independent of inducer but rather binds internal inducer more tightly than the *malT*$^+$ gene product does.

A *malQ* mutation allowed the expression of a stable *malK-lacZ* fusion to remain elevated even at high osmolarity. In contrast, *malK* gene expression in a *malQ glg* mutant that is unable to synthesize maltodextrins or glycogen decreased strongly at high osmolarity, as in the *malQ*$^+$ *glg*$^+$ strain. The fact that mutations in the gene encoding amylomaltase, a maltodextrin-synthesizing enzyme, abolished the osmoregulation of the *mal* regulon suggests that osmoregulation was mediated not at the transcriptional level via a regulatory protein but indirectly, by regulating the amount of an as yet unidentified internal inducer. Osmoregulation of the *lamB* gene has been reported (17), but the regulatory mechanism has not been discussed. To explain our findings we can envision two different molecules serving as endogenous inducers. The high-osmolarity inducer could be a linear maltodextrin derived from glycogen via the yet unknown but postulated glycogen-debranching enzyme. It might also be a product of glycogen-synthesizing enzymes that produce dextrins smaller than glycogen when the osmolarity is high. The second class of inducer molecule, possibly present only at low osmolarity, may also be a maltodextrin, but it could not be derived from glycogen nor by amylomaltase action, nor could it be a substrate for amylomaltase.

The key to understanding endogenous induction is the membrane-bound MalK protein. The presence of this protein reduced endogenous induction of the *mal* system. At least three functions of MalK could explain this phenomenon. (i) MalK could act at the transcriptional level as a repressor of the *mal* regulon. Upon binding maltose (or an internal inducer), MalK could be inactivated as a repressor. The absence of the repressor in *MalK* mutants thus could cause elevated *mal* gene expression. The fact that this elevated expression was abolished at high osmolarity and could be restored by *malQ* mutations complicates this hypothesis. In addition, it is difficult to imagine that MalK, in addition to its function in maltose transport and as the target molecule of inducer exclusion (Schwartz, in press), should also act as a DNA-binding protein. (ii) MalK could mediate

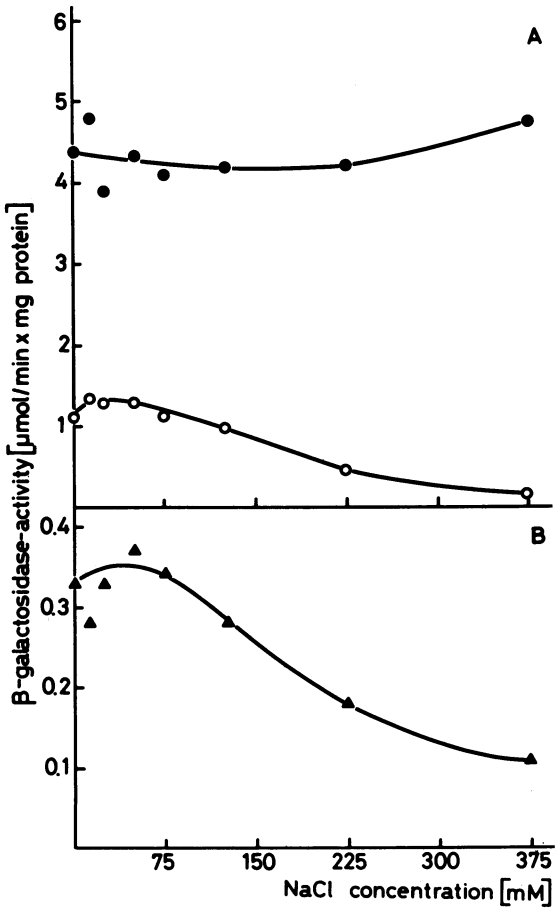

FIG. 3. Expression of *malE-lacZ* fusions after growth in different salt concentrations. (A) ●, Mal$^+$ strain BRE1120 grown with maltose; ○, Mal$^-$ strain BRE1163 grown with glycerol. (B) ▲, Mal$^+$ strain BRE1120 grown with glycerol.

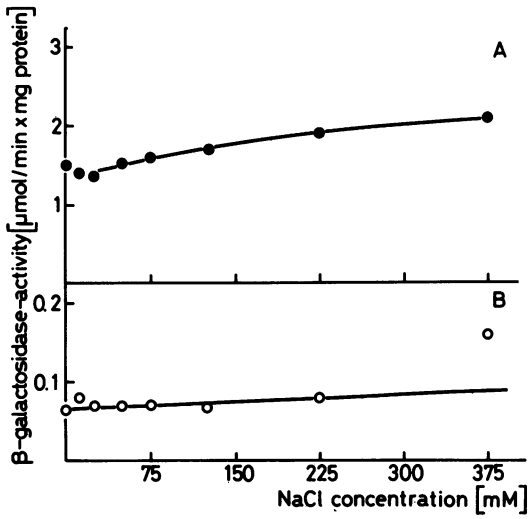

FIG. 4. Expression of the *malPQ-lacZ* fusion of strain SF1708 after growth in different salt concentrations. (A) Expression with glycerol and maltose as the carbon sources. (B) Expression with glycerol alone as the carbon source.

the exit of the internal inducer, thus preventing cytoplasmic accumulation of high, possibly toxic concentrations of these substances. In this case, *malF* or *malG* mutants should express the *mal* genes to the same extent as do *malK* mutants. This was not the case. The somewhat elevated expression observed in *malG* and polar *malF* mutants is more likely explained by the fact that MalK becomes cytoplasmic and therefore less active in mutants lacking MalG (38). The exit hypothesis makes sense only when the exit of internal inducer molecules occurs exclusively via MalK, independent of MalF and MalG. Also, one would have to postulate that the internal inducer could not reenter the cell via the maltose-binding-protein-dependent machinery. On the other hand, it is attractive to speculate that MalK-mediated exit is regulated by ATP binding to the MalK protein. Such a binding site has been found (22). Its function, however, is always interpreted in terms of energy coupling rather than regulation of exit. One could imagine that a high-energy charge would increase exit and that a low energy

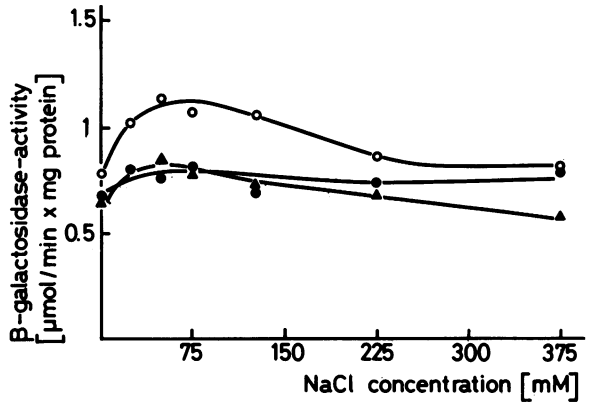

FIG. 5. Expression of *malT-lacZ* fusions after growth in different salt concentrations. Symbols: ○, Mal⁻ strain BRE1161 grown with glycerol; ●, Mal⁺ strain BRE1217 grown with maltose; ▲, Mal⁺ strain BRE1217 grown with glycerol.

TABLE 3. Effect of mutations in *malPQ* and *glg* on osmoregulation of the expression of a stable *malK-lacZ* fusion

Strain	Additional mutation	β-Galactosidase activity in medium with NaCl concn[a]:			Ratio of low/high concn
		Low (50 mM)	Medium (225 mM)	High (375 mM)	
BRE1162	None	4.4	1.1	0.23	19
BB453	*malT*^{c}	7.2	5.6	2.6	2.8
ME250	*malP*::Tn*10*	4.6	4	1.64	2.8
BB706	*malQ*::Tn*10*	4.1	2.6	2	2.1
ME251	Δ*malP*, nonpolar	4.5	1.1	0.28	16
BB496	*malP*::Tn*10 glgA glgC*	4.4	0.3	0.2	22
ME252	*malP*::Tn*10 glgA*	4	0.5	0.3	13.3
BB709	*malQ*::Tn*10 glgA*	4.9	0.6	0.34	14.4
ME262	*glgA*	5.1	0.35	0.2	26

[a] β-Galactosidase activity is expressed in micromoles of ortho-nitrophenyl-galactoside hydrolyzed per minute per milligram of protein. Strains containing *malK-lacZ* stable lysogens and the additional mutations were grown in sixfold-diluted MMA supplemented with 0.2% glycerol and NaCl as indicated.

charge would prevent it. Indeed, maltose exit is prevented by energy uncoupling (14). (iii) MalK could act as an enzyme that either polymerizes or degrades the internal inducer into a form which can no longer be recognized by MalT. The idea of degradation of the internal inducer via MalK becomes interesting in view of the role of MalK in the energy coupling of the maltose transport system.

ACKNOWLEDGMENTS

The writing of this paper and the interpretation of the complex regulatory processes described here were possible only through the thoughtful help and constructive criticism of M. D. Manson. Johannes Brass and Sabine Freundlieb gave many helpful suggestions. We thank E. Bremer, J. Cattaneo, P. Cossart, S. Freundlieb, and other colleagues for generous gifts of bacterial strains.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 156) and the Fond der Chemischen Industrie. B.B. was supported by a fellowship from the Friedrich-Ebert-Stiftung.

LITERATURE CITED

1. **Alemohammad, M. M., and C. J. Knowles.** 1974. Osmotically induced volume and turbidity changes of *Escherichia coli* due to salts, sucrose and glycerol, with particular reference to the rapid permeation of glycerol into the cell. J. Gen. Microbiol. **82:** 125–142.
2. **Benson, S. A., E. Bremer, and T. J. Silhavy.** 1984. Intragenic regions required for LamB export. Proc. Natl. Acad. Sci. USA **81:**3830–3834.
3. **Brass, J. M., U. Ehmann, and B. Bukau.** 1983. Reconstitution of maltose transport in *Escherichia coli*: conditions affecting import of maltose-binding protein into the periplasm of calcium-treated cells. J. Bacteriol. **155:**97–106.
4. **Brass, J. M., M. D. Manson, and T. J. Larson.** 1984. Transposon Tn*10*-dependent expression of the *lamB* gene in *Escherichia coli*. J. Bacteriol. **159:**93–99.
5. **Bremer, E., T. J. Silhavy, and G. M. Weinstock.** 1985. Transposable λ *placMu* bacteriophages for creating *lacZ* operon fusions and kanamycin resistance insertions in *Escherichia coli*. J. Bacteriol. **162:**1092–1099.
6. **Cairney, J., I. R. Booth, and C. F. Higgins.** 1985. Osmoregulation of gene expression in *Salmonella thyphimurium*: *proU* encodes an osmotically induced betaine transport system. J. Bacteriol. **164:**1224–1232.
7. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:**541–555.

8. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
9. **Chapon, C.** 1982. Role of catabolite activator protein in the maltose regulon of *Escherichia coli*. J. Bacteriol. **150**:722–729.
10. **Cossart, P., and B. Gicquel-Sanzey.** 1985. Regulation of expression of the *crp* gene of *Escherichia coli* K-12: in vivo study. J. Bacteriol. **161**:454–457.
11. **Débarbouillé, M., and M. Schwartz.** 1979. The use of gene fusions to study the expression of *malT*, the positive regulator gene of the maltose regulon. J. Mol. Biol. **132**:521–534.
12. **Débarbouillé, M., H. A. Shuman, T. J. Silhavy, and M. Schwartz.** 1978. Dominant constitutive mutations in *malT*, the positive regulator gene of the maltose regulon in *Escherichia coli*. J. Mol. Biol. **124**:359–371.
13. **Epstein, W.** 1985. The Kdp system: a bacterial K^+ transport ATPase. Curr. Top. Membr. Transp. **23**:153–175.
14. **Ferenci, T., W. Boos, M. Schwartz, and S. Szmelcman.** 1977. Energy-coupling of the transport system of *Escherichia coli* dependent on maltose-binding protein. Eur. J. Biochem. **75**:187–193.

14a. **Freundlieb, S., and W. Boos.** 1986. Alpha amylase of *Escherichia coli*: mapping and cloning of the structural gene, *malS*, and identification of its product as a periplasmic protein. J. Biol. Chem. **261**:2946–2954.

15. **Garrett, S., R. K. Taylor, and T. J. Silhavy.** 1983. Isolation and characterization of chain-terminating nonsense mutations in a porin regulator gene, *envZ*. J. Bacteriol. **156**:62–69.
16. **Govons, S., R. Vinopal, J. Ingraham, and J. Preiss.** 1969. Isolation of mutants of *Escherichia coli* B altered in their ability to synthesize glycogen. J. Bacteriol. **97**:970–972.
17. **Gowrishankar, J.** 1985. Identification of osmoresponsive genes in *Escherichia coli*: evidence for participation of potassium and proline transport systems in osmoregulation. J. Bacteriol. **164**:434–445.
18. **Hall, M. N., and T. J. Silhavy.** 1979. Transcriptional regulation of *Escherichia coli* K-12 major outer membrane protein 1b. J. Bacteriol. **140**:342–350.
19. **Hall, M. N., and T. J. Silhavy.** 1981. Genetic analysis of the *ompB* locus in *Escherichia coli* K12. J. Mol. Biol. **151**:1–15.
20. **Hall, M. N., and T. J. Silhavy.** 1981. The *ompB* locus and the regulation of the major outer membrane porin proteins of *Escherichia coli* K12. J. Mol. Biol. **146**:23–43.
21. **Hengge, R., and W. Boos.** 1983. Maltose and lactose transport in *Escherichia coli*. Biochim. Biophys. Acta **737**:443–478.
22. **Higgins, C. F., I. D. Hiles, K. Whalley, and D. J. Jamieson.** 1985. Nucleotide binding by membrane components of bacterial periplasmic binding protein-dependent transport systems. EMBO J. **4**:1033–1040.
23. **Hofnung, M., D. Hatfield, and M. Schwartz.** 1974. *malB* region in *Escherichia coli* K-12: characterization of new mutations. J. Bacteriol. **117**:40–47.
24. **Kennedy, E. P.** 1982. Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **79**:1092–1095.
25. **Le Rudulier, D., A. R. Strom, A. M. Dandekar, L. T. Smith, and R. C. Valentine.** 1984. Molecular biology of osmoregulation. Science **224**:1064–1068.
26. **Mager, J., M. Kuczynski, G. Schatzberg, and Y. Avi-Dor.** 1956. Turbidity changes in bacterial suspensions in relation to osmotic pressure. J. Gen. Microbiol. **14**:69–75.
27. **Measures, J. C.** 1975. Role of amino acids in osmoregulation of non-halophilic bacteria. Nature (London) **257**:398–400.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Perroud, B., and D. Le Rudulier.** 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. J. Bacteriol. **161**:393–401.
30. **Preiss, J.** 1984. Bacterial glycogen synthesis and its regulation. Annu. Rev. Microbiol. **38**:419–458.
31. **Raibaud, O., M. Débarbouillé, and M. Schwartz.** 1983. Use of deletions created *in vitro* to map transcriptional regulatory signals in the *malA* region of *Escherichia coli*. J. Mol. Biol. **163**:395–408.
32. **Raibaud, O., M. Roa, C. Braun-Breton, and M. Schwartz.** 1979. Structure of the *malB* region in *Escherichia coli* K-12. I. Genetic map of the *malK-lamB* operon. Mol. Gen. Genet. **174**:241–248.
33. **Ramakrishnan, G., K. Ikenaka, and M. Inouye.** 1985. Uncoupling of osmoregulation of the *Escherichia coli* K-12 *ompF* gene from *ompB*-dependent transcription. J. Bacteriol. **163**:82–87.
34. **Sabourin, D., and J. Beckwith.** 1975. Deletion of the *Escherichia coli crp* gene. J. Bacteriol. **122**:338–340.
35. **Sarma, V., and P. Reeves.** 1977. Genetic locus (*ompB*) affecting a major outer-membrane protein in *Escherichia coli* K-12. J. Bacteriol. **132**:23–27.
36. **Schwartz, M.** 1966. Location of the maltose A and B loci on the genetic map of *Escherichia coli*. J. Bacteriol. **92**:1083–1089.
37. **Shuman, H. A.** 1982. Active transport of maltose in *Escherichia coli* K12. Role of the periplasmic maltose binding protein and evidence for a substrate recognition site in the cytoplasmic membrane. J. Biol. Chem. **257**:5455–5461.
38. **Shuman, H. A., and T. J. Silhavy.** 1981. Identification of the *malK* gene product: a peripheral membrane component of the *Escherichia coli* maltose transport system. J. Biol. Chem. **256**: 560–562.
39. **van Alphen, W., and B. Lugtenberg.** 1977. Influence of osmolarity of the growth medium on the outer membrane protein pattern of *Escherichia coli*. J. Bacteriol. **131**:623–630.
40. **Villarejo, M., and C. C. Case.** 1984. *envZ* mediates transcriptional control by local anesthetics but is not required for osmoregulation in *Escherichia coli*. J. Bacteriol. **159**:883–887.
41. **Wandersman, C., F. Moreno, and M. Schwartz.** 1980. Pleiotropic mutations rendering *Escherichia coli* K-12 resistant to bacteriophage TP1. J. Bacteriol. **143**:1374–1383.
42. **Wanner, B. L., A. Sarthy, and J. Beckwith.** 1979. *Escherichia coli* pleiotropic mutant that reduces amounts of several periplasmic and outer membrane proteins. J. Bacteriol. **140**:229–239.