MalK, the ATP-binding Cassette Component of the *Escherichia coli* Maltodextrin Transporter, Inhibits the Transcriptional Activator MalT by Antagonizing Inducer Binding*

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MalK, the ATP-binding cassette component of the *Escherichia coli* maltodextrin transporter, has long been known to control negatively the activity of MalT, a transcriptional activator dedicated to the maltose regulon. By using a biochemical approach and the soluble form of MalK as a model substrate, we demonstrate that MalK alone inhibits transcription activation by MalT in a purified transcription system. The inhibitory effect observed in vitro is relieved by maltotriose and by two malT mutations and one malK mutation known to interfere with MalT repression by MalK in vivo. MalK interacts directly with the activator in the absence of maltotriose but not in the presence of maltotriose. Conversely, MalK inhibits maltotriose binding by MalT. Altogether, these data strongly suggest that MalK and maltotriose compete for MalT binding. Part, if not all, of the MalK-binding site is located on DT1, the N-terminal domain of MalT. All of these features indicate that MalK inhibits MalT by the same mechanism as two other proteins, MalY and Aes, that also act as negative effectors of MalT by antagonizing maltotriose binding by MalT. These results offer new insights into the mechanism by which gene regulation can be accomplished by the ATPase component of a bacterial ATP-binding cassette-type importer.

Recent studies have revealed that bacterial transport systems can be directly involved in signal transduction pathways and play a key role in gene regulation by displaying a regulatory function that is tightly coupled to their transport activity. Based on the few examples of transport-based sensory systems that have been characterized in detail to date, the mechanisms of signal transduction differ widely, depending on the system. For instance, BglF, a phosphotransferase system enzyme II that catalyzes the import and phosphorylation of β-glucosides, phosphorylates and sequesters the BglG antiterminator in the absence of β-glucoside transport, and dephosphorylates and releases it when transport resumes (1). Active sugar transport triggers the recruitment of the Mc repressor by the dephosphorylated glucose-specific phosphotransferase system enzyme II (PisG), thereby derepressing the Mc-controlled genes (for a review, see Ref. 2). In the case of the ferric citrate transport system, substrate binding to the receptor located in the outer membrane triggers the activation of a specific sigma factor via a complex signaling cascade (3).

This paper deals with the roles that ATP-binding cassette transporters play in signaling pathways. The model system is the *Escherichia coli* maltodextrin transporter, whose ATP-binding cassette component, the MalK protein, down-regulates the activity of MalT, a transcriptional activator that controls the expression of the maltose regulon (4). MalT control by MalK was first recognized with the finding that malK null mutants express constitutively the maltose regulon, whereas MalK overproduction blocks mal gene induction (5, 6). The observation that the repression by MalK is relieved both by MalT overexpression and by a class of malT mutations (malT*) that confer constitutive expression of the maltose regulon suggested further that MalT is the target of the negative control exerted by MalK (6).

The maltodextrin transport system is one of the best characterized ATP-binding cassette transporters, and its study has provided a wealth of information about the mechanism of coupling between transport and ATP hydrolysis (7). The physiology of the regulatory function of MalK is also well understood. MalT repression by MalK ensures that the maltose regulon is not induced by endogenous maltotriose, the inducer of the system, in the absence of external maltodextrin (8). The concentration of maltotriose of internal origin is indeed high enough to cause full expression of the regulon in a *malK* strain in minimal medium supplemented with glycerol (8). The uninduced level of expression of the maltose regulon observed in a wild-type strain in the absence of maltose in the growth medium corresponds to the residual MalT activity that escapes repression by MalK. In contrast, the mechanism of MalT inhibition by MalK remains unclear. Some MalT* variants are known to display a higher affinity for maltotriose and to be less sensitive to repression by MalK, which suggests that MalK inhibits MalT by competing with maltotriose (6, 9). However, direct evidence for this model is still lacking. Böhm et al. (10) identified a surface determinant on the C-terminal domain of MalK that is specifically involved in MalT repression and might represent the MalT binding site. Panagiotidis et al. (11) observed a MalT-MalK interaction *in vitro*, but this MalT-MalK complex did not respond to signals known to relieve MalT repression by MalK *in vivo*. Also, how maltodextrin entrance is coupled to derepression remains elusive. On the basis of genetic data, Panagiotidis et al. (11) proposed that only the ATP-bound form of MalK, which is expected to predominate when the transporter is resting, is able to repress MalT.

MalT is the archetype of a family of ~100-kDa transcriptional activators found in prokaryotes (12, 13). Transcription activation by MalT involves MalT self-association, cooperative

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binding to an array of MalT sites located in the target promotors, and stimulation of open complex formation by the RNA polymerase (14–16). A complex interplay of signaling compounds and proteins controls the activity of the MalT protein (4). MalT is active only in the presence of ATP and maltotriose, both of which are required for multimerization (16). In addition to MalK, two other proteins, namely MalY, a β C-S lyase, and Aes, an acyl esterase, down-regulate MalT activity (17, 18). Recent studies have revealed that both MalY and Aes interact directly with MalT and compete with maltotriose for MalT binding (19, 20). Available data suggest that unliganded MalT is in equilibrium between an inactive monomeric form and an active monomeric form prone to self-association. The inactive form would be stabilized by maltotriose. The requirement for active monomeric form prone to self-association. The inactive binding (19, 20). Available data suggest that unliganded MalT is in equilibrium between an inactive monomeric form and an active monomeric form prone to self-association. The inactive form would be stabilized by maltotriose. The requirement for ATP as a positive effector of MalT might reflect a role for the ATPase activity of MalT in the competition between positive and negative effectors. Indeed, both ATP and ADP can promote MalT multimerization or inhibitory complex formation, but ATP is more effective in driving MalT self-association, whereas ADP is more effective in promoting the formation of an inhibitory complex (16, 19, 20).

Structural studies have revealed that MalT is composed of four domains (21). DT1 (residues 1–241) binds and hydrolyzes ADP (21). It also binds MalY and Aes (20, 22) whereas maltotriose is bound by DT3 (residues 437–806) (21, 23). DT3 (residues 807–901), which belongs to the LuxR-type family of DNA-binding domains, contains the DNA-binding site and most likely also carries a surface determinant contacting the RNA polymerase (24). The transition between the inactive and the active form of MalT involves DT1, DT2, and DT3, three domains that are specific to the MalT family of transcriptional activators and are thought to constitute a signal integration module (21).

To gain insight into the mechanism whereby MalK modulates MalT activity, we developed an in vitro system that reproduces MalT inhibition by MalK. The observation that MalK causes a Mal antidote phenotype when overproduced (6) suggested that free MalK, i.e. the MalK form that is not associated with the membrane components of the transporter (MalF and MalG), is able to inhibit MalT. We therefore used the soluble form of MalK as a model substrate. We show that MalK alone impedes transcription activation by MalT in a purified transcription system. Characterization of the repression process revealed that MalK inhibits MalT by forming a complex with the activator and by blocking inducer binding. Part, if not all, of the MalK-binding site is present on DT1.

**EXPERIMENTAL PROCEDURES**

Strains and Plasmids—E. coli BL21(DE3) has been described by Studier et al. (25). BL21(DE3) amPl229 contains a deletion of the entire malT gene (19). Plasmids pOM163 and pOM163-T3SR are pT28c(+) (Novagen) derivatives producing DT1 or DT1-T3SR with a Ser-Glu tag at their C terminus (DT1S) (20). Plasmid pOM174 was obtained by amplifying the MalK-encoding fragment of pKN101 (obtained from K. Nikaide) by PCR using the oligonucleotides IU1001 (5′-GGCGG-CCCAATGTCGCCG-CGATGCGTCC-3′) and KD001 (5′-GGCGG-CAAGGTTCAACGGTTCG-3′), digesting it with NcoI and HindIII, and inserting it between the NcoI and HindIII sites of pT28c(+). Followed by the insertion of a His tag encoding linker, HT-AH1/HT2G2 (HTAG1, 5′-CATGATCATACATCATCAATCT-3′; HTAG2, 5′-CATGATCATGATGATGATG-3′), in the Ncol site of the intermediate construct. The sequence of the construct was verified. The encoded polypeptide has a M(H)6MG extension at the MalK N terminus. The pOM174-T3-488S derivative was obtained by replacing the EcoRI-HindIII fragment of pOM174 with the EcoRI-HindIII fragment of pAB204 (10).

Chemicals—ADP containing <0.2% ATP was purchased from Roche Applied Science. 1(3)C-maltotriose (900 mCi/mmol) was obtained from America Radiolabeled Chemicals.

Proteins—RNA polymerase holoenzyme (E. coli) was from Epicentre. Wild-type MalT, MalT26, and MalT358R proteins were purified in the presence of ATP as described by Danot and Ribaud (15), Schreiber et al. (19) and Joly et al. (20), respectively. ATP-free MalT was prepared by precipitating the purified protein with ammonium sulfate and filtering the resuspended material through a 47-μm Sephadex column according to Schreiber and Richet (16). The concentration of MalT was measured as described (16). ATP-free MalT was used throughout this work.

The MalK protein, with a His tag at its amino terminus, was purified from strain BL21(DE3) harboring pOM174. Bacteria were grown at 37 °C in 1.5 liters of Luria-Bertani medium (5 g of yeast extract, 10 g of tryptone, and 10 g of NaCl, adjusted to pH 7) in the presence of 50 μg/ml kanamycin, induced with 1 mM isopropyl β-D-galactoside at 30 °C and, 2 h later, added 500 μM 11°C (pH 8), 33 mM tripotassium citrate, 10 mM magnesium acetate, 0.1 mM dithiothreitol, and 2.10 μg/ml 2°C (pH 8), and 15 μg/ml 3°C (pH 8), 27 μM tripotassium citrate, 15 μM NaCl, 10 μM magnesium acetate, 20 μM imidazole, 1 mM dithiothreitol, and 20 μM 3°C (pH 8), 15 μM NaCl, and 5 μM MgCl2 to a final 3°C (pH 8), 7 μM Tris-HCl (pH 7.3), 27 μM tripotassium citrate, 15 μM NaCl, 10 mM magnesium acetate, 20 μM imidazole, 1 mM dithiothreitol, and 200 μM 1°C (pH 8), 7 μM Tris-HCl (pH 7.3), 27 μM tripotassium citrate, 17 μM NaCl, 11 μM magnesium acetate, 0.11 μM EDTA, 1.1 μM dithiothreitol, 22 μM imidazole, 230 μM 1°C (pH 8), 7 μM Tris-HCl (pH 7.3), 27 μM tripotassium citrate, 17 μM NaCl, 11 μM magnesium acetate, 0.11 μM EDTA, 1.1 μM dithiothreitol, 22 μM imidazole, 230 μM 1°C (pH 8), 7 μM Tris-HCl (pH 7.3), 27 μM tripotassium citrate, 17 μM NaCl, 11 μM magnesium acetate, 0.11 μM EDTA, 1.1 μM dithiothreitol, 22 μM imidazole, 230 μM ATP-PNP, adenosine 5′-[(β,γ-imino)triphosphate].
producing His-tagged MalK<sup>+</sup> or MalK-G346S were prepared as for MalK purification. A 10-nl cell suspension (ΔmalKΔ-100) was thawed, disrupted in a French press cell (16,000 p.s.i.), and centrifuged (60 min at 20,000 × g) to collect the supernatant. Affinity chromatography was performed at 4 °C in Micro BioSpin® Bio-Rad columns packed with 50 μl of Ni-NTA-agarose (Qiagen). Solutions were passed through the columns by spinning at 5 × g for 30 s in a bench top centrifuge. The columns were equilibrated with buffer A and loaded with 2 ml of soluble extract. The columns were washed with buffer A (5 × 100 μl) and buffer A plus 40 mM imidazole (5 × 100 μl). The MalK-loaded column was then equilibrated with buffer A plus 2 mM ATP and 100 mM maltotriose (2 × 100 μl), and purified MalT (200 μl at 0.5 mg/ml) in buffer A plus 2 mM ATP was allowed to flow through the column. Unbound proteins were washed out with 5 × 100 μl of buffer A plus 40 mM imidazole plus 2 mM ATP (10 mM maltotriose). His-tagged MalK was eluted with 4 × 100 μl of buffer A plus 500 mM imidazole. 100-μl fractions were collected starting from the MalT washing step and analyzed by 12% SDS-PAGE (acylamide/bisacylamide, 37.5:1).

**RESULTS**

**MalK Inhibits Transcription Activation by MalT in Vitro**

We first examined whether purified MalK inhibits MalT in an *in vitro* transcription system. MalT activity was assayed by monitoring its ability to activate open complex formation at malPp, a MalT-dependent promoter, in the presence of RNA polymerase. MalT was incubated with malPp DNA and AMP-PNP in the presence or absence of MalK before adding RNA polymerase and allowing open complex formation. The amount of the open complexes formed was then determined by measuring the rate of abortive product synthesis. The assay was performed in the presence of AMP-PNP instead of ATP to avoid MalK- and MalT-driven ATP hydrolysis, which might interfere with the assay. In addition, given that MalK-dependence on maltotriose is only partial in the presence of AMP-PNP, maltotriose was omitted from the assay. This omission avoided the possibility that maltotriose might impede repression by MalK. Note also that, for a given concentration of MalT, the fraction of the protein that is in the active form, as judged by the promoter response, depends on the concentrations of the positive effectors and on the MalT variant assayed. Therefore, for each combination of effectors and MalT variant used in this work, we determined an individual response curve, i.e. the amount of open complexes formed as a function of MalT concentration. The concentration of MalT that was used to test the effect of MalK was that eliciting half of the maximum response under the chosen conditions. The presence of a limiting concentration of MalT in the assay ensures that MalT inhibition by MalK can be readily detected.

**How MalK Modulates MalT Activity**

As shown in Fig. 1, MalK strongly depressed malPp activation by MalT in the absence of maltotriose; the amount of open complexes formed was reduced by 80% in the presence of 8 μM MalK. Furthermore, the inhibitory effect of MalK disappeared when the assay was performed in the presence of 0.1 mM maltotriose (Fig. 1). The fact that maltotriose antagonizes MalT inhibition by MalK, as was expected based on *in vivo* data, provides evidence that the inhibitory effect observed *in vitro* is physiologically relevant.

**MalT and malK Mutations Relieving MalT Inhibition by MalK in Vivo Also Suppress the Inhibitory Effect of MalK in Vitro**

To confirm that the inhibition caused by MalK *in vitro* is functionally significant, we examined whether two different *malT* mutations that are known to confer resistance to MalK *in vivo* (*malT*26 and *malT*38R) have the same effect *in vitro*. The *malT*26 mutation, which generates the R242P substitution in the DT1-DT2 linker, increases the affinity of MalT for maltotriose by favoring the transition from the inactive state to the active state (9, 21). It also suppresses MalT-sensitivity to MalK *in vivo* (22). The T38R substitution, which is located in DT1, diminishes MalT-sensitivity to MalK *in vivo* (22). As shown in Fig. 2A, neither of these MalT variants was affected by MalK when assayed in the presence of AMP-PNP alone, i.e. under conditions in which wild-type MalT is inhibited by MalK.

We also tested whether the inhibitory effect associated with purified MalK was affected by the *malK-G346S* mutation. The G346S substitution, which is thought to alter the MalT-binding site, specifically decreases the ability of MalK to down-regulate MalT in *vivo* without damaging its ability to catalyze maltose transport both when overproduced or when constitutively expressed from a chromosomal locus (10). As expected, MalT is not inhibited by the MalK-G346S variant in the presence of AMP-PNP.
AMP-PNP alone (Fig. 2B). To rule out the possibility that the lack of effect of MalK-G346S was caused by protein misfolding, we verified that the purified MalK variant hydrolyzes ATP in vitro at exactly the same rate as the wild-type protein (0.32 and 0.33 molecule of ATP are hydrolyzed at 30 °C per minute per protomer of wild-type and mutant protein, respectively). The uncoupled ATPase activity of MalK is a good indicator of correct protein folding, given that ATP hydrolysis by MalK relies on protein dimerization and that dimerization involves both the nucleotide-binding domain and the regulatory domain, as revealed by the x-ray structure of MalK (28, 29).

Repression of MalT Activity by MalK Involves a Direct Interaction between MalK and MalT—Having established an in vitro repression system mimicking in vivo events, we characterized the repression process and tested the simple hypothesis that MalT inhibition by MalK involves formation of a MalK-MalT complex. To do this, we examined whether an His-tagged MalK protein immobilized on a Ni-NTA-agarose column would bind the activator. Purified MalT was run over a MalK-loaded column, and, after extensive washing, MalK was eluted with 500 mM imidazole. Affinity chromatography was performed in the presence of ATP (ATP hydrolysis was not expected to interfere with the assay because it was present at a high concentration). SDS-PAGE analysis of the collected fractions and Coomassie Blue staining of the gel revealed that a significant fraction of the loaded MalT protein was retained on the column and co-eluted with MalK (Fig. 3A). In contrast, MalT was not retained on the MalK-loaded column when affinity chromatography was performed in the presence of ATP and 10 mM maltotriose (Fig. 3B). Likewise, the T38R MalT variant, which is insensitive to repression by MalK, did not bind MalK, nor does wild-type MalT bind MalK-G346S, the MalK variant defective in MalT regulation (Fig. 3, C and D). These data provide compelling evidence that MalT physically interacts with MalK, that the interaction is functionally relevant, and that maltotriose prevents formation of the MalT-MalK complex.

MalK Antagonizes Inducer Binding by MalT—We then tested whether, reciprocally, MalK would inhibit maltotriose binding by MalT. MalT was incubated in the presence of ATP, labeled maltotriose, and MalK, and the amount of bound maltotriose was measured by precipitating the proteins with ammonium sulfate. As shown in Fig. 4, wild-type MalK markedly reduced maltotriose binding by wild-type MalT, whereas it had no significant effect on maltotriose binding by MalTT26 or MalTT38R. The same data were obtained for 10- and 30-min incubation times, which excludes the possibility that the differences between the responses of wild-type MalT and the mutated proteins were due to kinetic effects. We verified that the concentrations of wild-type MalT, MalTT26, and MalTT38R in the assays were limiting, i.e. that the absence of an inhibitory effect was meaningful (data not shown). Furthermore, as predicted, G346S MalK does not prevent maltotriose binding by wild-type MalT (Fig. 4). Based on these results, we conclude that MalK inhibits maltotriose binding by MalT.

MalK Interacts with DT1—The similarity between the action modes of the three negative effectors of MalT suggests that, like MalY and Aes, MalK might interact with DT1. To test this hypothesis, we examined whether immobilized DT1 binds the MalK protein. Purified MalK was chromatographed in the presence of AMP-PNP on a Strep-Tactin®-Sepharose® column preloaded with the C-terminally Strep-tagged DT1 domain (DT1S), as described previously (20). As shown in Fig. 5A, a significant fraction of the wild-type MalK protein was retained on the column and recovered with DT1S upon elution with desthiobiotin. Furthermore, no retention was observed when wild-type MalK was replaced by the G346S variant (Fig. 5B). We therefore infer that MalK interacts specifically with DT1.

Because the T38R substitution is located in DT1, we considered the possibility that this amino acid substitution renders MalT insensitive to MalK by altering the DT1 determinant recognized by MalK. Affinity chromatography experiments performed with immobilized DT1S-T38R unexpectedly revealed that the mutated domain still binds wild-type MalK as well as MalK-G346S (Fig. 5, C and D). These results demonstrate that the T38R substitution does not prevent MalK binding to isolated DT1, although it modifies the DT1 determinant recognized by MalK so that it does not respond any more to the malK-G346S mutation. However, full-length MalT-T38R does not bind MalK (Fig. 3C); the effect of the T38R substitution on the interaction between MalK and full-length MalT must therefore be indirect and probably involves a conformational effect.
DISCUSSION

The work presented here clearly establishes that the regulatory action of MalK on MalT is direct and does not require any additional factor. It also demonstrates that MalK inhibits MalT by interacting with the activator and by antagonizing inducer binding. These conclusions are based on the following observations. (i) Purified MalK is able to prevent open complex formation at the malP promoter in the presence of RNA polymerase in a purified transcription system; (ii) as shown by affinity chromatography, MalK specifically interacts with MalT; and (iii) MalK inhibits maltotriose binding. Most importantly, the inhibitory effect observed in vitro is relieved by two malT mutations (malTc26 and malT-T38R) and one malK mutation (G346S) that are known to suppress MalT repression by MalK in vivo. This observation provides compelling evidence that our in vitro repression system is of physiological relevance. Finally, we have demonstrated that, when isolated, DT1 specifically interacts with MalK, which indicates that part if not all of the MalK binding site is located on DT1, the N-terminal domain of MalT. Yet, the MalT/DT1 interaction is weak, which suggests that other MalT domains might contribute, directly or indirectly, to MalK binding.

It might seem surprising that the MalT/MalK interaction revealed here by the affinity chromatography experiments performed with immobilized MalK responds to maltotriose or to the malTc26 mutation, whereas this was not the case in similar experiments that were performed with immobilized MalT by Panagiotidis et al. (11). However, in their experiments the immobilized MalT protein might be unfolded because of the absence of ATP during the extraction process (30). As a result, the ability of MalT to interact with MalK was possibly unregulated, and the secondary structural motifs involved in MalK recognition were permanently exposed. As discussed below, maltotriose and malT mutations such as malTc26 are indeed
Fig. 5. Affinity chromatography on a DT1-loaded column. Purified MalK (1 ml at 0.25 mg/ml) was chromatographed on Strep-tactin®-Sepharose® micro-columns preloaded with wild-type DT1S or the DT1S-T38R variant in the presence of 2 mM AMP-PNP as described under “Experimental Procedures.” FT, W, and E stand for the flow-through (200 μl), wash fractions (100 μl each), and desthiobiotin-eluted fractions (100 μl each), respectively. A, wild-type DT1S and wild-type MalK. B, wild-type DT1S and MalK-G346S. C, DT1S-T38R and wild-type MalK. D, DT1S-T38R and MalK-G346S. wt, wild-type.

An interesting finding of this work is that MalK inhibits MalT via the same mechanism as MalY and Aes (19, 20). Indeed, in all three cases the negative effector inhibits maltotriose binding and vice versa. In addition, as revealed by the MalT activity assays, a high concentration of maltotriose fully relieves MalT inhibition by MalK (Fig. 1A), MalY, or Aes, thereby suggesting a phenomenon of competitive inhibition. Finally, DT1 is directly involved in the binding of the negative effector in all three cases. Taking all available data into account, the following picture emerges (Fig. 6). MalT is in equilibrium between an inactive and an active form with the negative effector stabilizing the inactive form and maltotriose stabilizing the active form. The competition between maltotriose and the negative effector would be indirect and occur via an interconversion between an active and an inactive form. In the inactive form, the binding sites for the negative effectors, which are made up, at least in part, of determinants provided by DT1, would be available or correctly configured, whereas the maltotriose-binding site would not be accessible. Conversely, the active form would offer a high-affinity binding site for maltotriose because of a proper relationship between DT2 and DT3, whereas the binding sites for the negative effectors would not be exposed or properly configured (21). The interconversion between the inactive and active states involves DT1, DT2, and DT3 based on the observation that the transition toward the active state causes a change in the sensitivity of both the DT1-DT2 and the DT2-DT3 linkers to protease attack (21). Given that the three inhibitory proteins are unrelated at the sequence level and that MalK and MalY do not share any obvious structural similarity (29, 32), the molecular details of the interaction between the inactive form of MalT and each of the negative effectors is likely to be different. Consistent with this idea, the T38R substitution interferes with the DT1/Aes interaction (20) in the DT1 context, whereas it has no effect on the DT1/MalK interaction.

The model proposed is consistent with the observations that mutation malT26 renders MalT insensitive to repression by
MalK, MalY (19), or Aes (20). Mutation malT'26, which corresponds to the R242P substitution in the DT1-DT2 linker, is indeed known to shift the equilibrium toward the active form of MalT. The mutated protein is partially active in the absence of maltotriose and displays a higher affinity for the inducer compared with the wild-type protein (9). Moreover, in the absence of maltotriose a fraction of the MalT'26 variant exists spontaneously under a conformation characteristic of the wild-type maltotriose-bound protein, as revealed by mild proteolysis (21). Hence, although we cannot exclude the possibility that the DT1-DT2 linker might be part of the MalK, MalY, and Aes binding sites, we favor the idea that the malT'26 mutation makes MalT insensitive to the repressor proteins simply by stabilizing the active form of MalT, i.e. via a long range effect.

Finally, it is worth emphasizing that the multiplicity of the signals sensed by MalT makes this activator unique among prokaryotic transcription factors. In this respect, it will be interesting to understand how all of these signals are integrated at the level of the protein and, in particular, to identify the conformational changes that are involved in the transition between the inactive and the active forms and to determine the role that the ATPase activity of MalT plays in the control of this interconversion.

Two important questions remain to be answered. First, the model substrate used here is the soluble form of MalK but, in vivo, MalK associates with MalP and MalG, the two integral membrane components, to form the maltodextrin transporter. Thus, which form of MalK down-regulates MalT in vivo, the MalFGK2 transporter or a soluble MalK form that would cycle between the membrane and the cytoplasm? How does maltodextrin transport trigger derepression of MalT? Panagiotidis et al. (11) observed that a MalK variant that binds but does not hydrolyze ATP has a super-repressor phenotype, whereas MBP-independent transporter variants with high ATPase activity, even in the absence of transportable substrate, show a reduced ability to repress MalT. Based on these observations, the authors inferred that the regulatory activity of MalK would actually be coupled to its ATPase activity and that the repressive form of MalK would be the ATP-bound form, which is expected to predominate when the transport system is resting. Such a model is not supported by the structural data of Chen et al. (29). Instead, the crystal structures of free and ATP-bound MalK dimers suggest that ATP hydrolysis is not accompanied by conformational changes of the C-terminal regulatory domain, where the MalT binding-site is located (10). The structural consequences of ATP hydrolysis might be different, however, in the context of the assembled transporter, and conformational changes of the MalK regulatory region might accompany the ATP hydrolysis cycle when coupled to transport and thus control the ability of MalK to interact with MalT. Furthermore, it seems unlikely that the repressing function of MalK is constitutive and that the induction of the maltose regulon results solely from reversal of the inhibition by MalK due to an increase in the intracellular concentration of maltotriose upon maltose entrance, given that the concentration of MalK, whose structural gene is part of the maltose regulon, increases concomitantly.

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REFERENCES
1. Lopian, L., Nussbaum-Shochat, A., O'Day-Kerstein, K., Wright, A., and Amster-Choder, O. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7099–7104
2. Plumbridge, J. (2002) Curr. Opin. Microbiol. 5, 187–193
3. Braun, V., Mahren, S., and Oegerlein, M. (2003) Curr. Opin. Microbiol. 6, 173–180
4. Boos, W., and Bohn, H. (2000) Trends Genet. 16, 404–409
5. Hofnung, M., Hatfield, D., and Schwartz, M. (1974) J. Bacteriol. 117, 40–47
6. Reyes, M., and Shuman, H. A. (1986) J. Bacteriol. 167, 4598–4602
7. Davidson, A. L. (2002) Science 296, 1038–1040
8. Bakau, B., Ehrmann, M., and Boos, W. (1996) J. Bacteriol. 178, 1846–1852
9. Dardenne, B., and Rubin, O. (1990) J. Bacteriol. 166, 884–891
10. Bohn, A., Diez, J., Diez, J., Welte, W., and Boos, W. (2002) J. Biol. Chem. 277, 3708–3717
11. Panagiotidis, C. H., Boos, W., and Shuman, H. A. (1998) Mol. Microbiol. 30, 535–546
12. De Schrijver, A., and De Mot, R. (1999) Microbiology 145, 1287
13. Valdez, F., Gonzalez-Cerin, G., Kieser, H. M., and Servin-Gonzalez, L. (1999) Microbiology 145, 2363–2374
14. Vidal-Ingiigliardi, D., Richet, E., and Raibaud, O. (1991) J. Mol. Biol. 218, 323–334
15. Danot, O., and Raibaud, O. (1994) Mol. Microbiol. 14, 335–346
16. Schreiber, V., and Richet, E. (1999) J. Biol. Chem. 274, 33220–33226
17. Reidl, J., and Boos, W. (1991) J. Bacteriol. 173, 4862–4876
18. Peist, R., Koch, A., Helix, P., Sewitz, S., Kolbus, T., and Boos, W. (1997) J. Bacteriol. 179, 7679–7686
19. Schreiber, V., Steegborn, C., Clausen, T., Boos, W., and Richet, E. (2000) Mol. Microbiol. 35, 765–776
20. Joly, N., Danot, O., Schlegel, A., Boos, W., and Richet, E. (2002) J. Biol. Chem. 277, 16606–16613
21. Danot, O. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 435–440
22. Schlegel, A., Danot, O., Richet, E., Perenci, T., and Boos, W. (2002) J. Bacteriol. 184, 3069–3077
23. Steegborn, C., Danot, O., Huber, R., and Clausen, T. (2001) Structure 9, 1051–1060
24. Danot, O., Vidal-Ingiigliardi, D., and Raibaud, O. (1996) J. Mol. Biol. 262, 1–11
25. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 69–89
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
27. McClure, W. R. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5634–5638
28. Fetsch, E. E., and Davidson, A. L. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 9685–9690
29. Chen, J., Lu, G., Lin, J., Davidson, A. L., and Quiocho, F. A. (2003) Mol. Cell 12, 651–661
30. Richet, E., and Raibaud, O. (1987) J. Biol. Chem. 262, 12647–12653
31. Saraste, M., Sibbald, P. R., and Wittinghofer, A. (1990) Trends Biochem. Sci. 15, 430–434
32. Clausen, T., Schlegel, A., Peist, R., Schneider, E., Steegborn, C., Chang, Y.-S., Haase, A., Bourenkov, G. P., Bartunik, H. D., and Boos, W. (2000) EMBO J. 19, 831–842
33. Richet, E., and Raibaud, O. (1989) EMBO J. 8, 981–987
MaJK, the ATP-binding Cassette Component of the *Escherichia coli* Maltodextrin Transporter, Inhibits the Transcriptional Activator MalT by Antagonizing Inducer Binding

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Additions and Corrections

Vol. 279 (2004) 33123–33130

MalK, the ATP-binding cassette component of the *Escherichia coli* maltodextrin transporter, inhibits the transcriptional activator MalT by antagonizing inducer binding.

*Nicolas Joly, Alex Böhm, Winfried Boos, and Evelyne Richet*

Page 33124, lines 7 and 8 under “Experimental Procedures”: The name and sequence of the upstream primer used to amplify the *malK* gene, rather than KU001 (5'-GCGGCCCATGGGGACCCACGATCAGGTCGA-3', should be: KU006 (5'-CGCCGCCCATGGGGATGGCGAGCGTACAGCTGC-3').

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Binding specificity of sea anemone toxins to Na, 1.1–1.6 sodium channels. Unexpected contributions from differences in the IV/S3-S4 outer loop.

*Joacir Stolarz Oliveira, Elisa Redaelli, André J. Zaharenko, Rita Restano Cassulini, Katsuhiro Konno, Daniel C. Pimenta, José C. Freitas, Jeffrey J. Clare, and Enzo Wanke*

Page 33323, line 10 of the summary: L36A should be changed to K36A.

Page 33331, next to the last line in the left-hand column: The first Asp-Ile should be changed to Asp-Leu, to read “The Asp-Leu instead of the Asp-Ile motif...”

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
R-cadherin influences cell motility via Rho family GTPases.

Emhonta Johnson, Christopher S. Theisen, Keith R. Johnson, and Margaret J. Wheelock

Page 31046: The wrong Fig. 5 was printed. The correct figure is shown below:

![Fig. 5](image-url)