Temperature-Sensitive Catabolite Activator Protein in Escherichia coli BUG6

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BUG6 is a temperature-sensitive cell division mutant which forms filaments at the nonpermissive temperature. Synthesis of the maltose- and galactose-binding protein-dependent transport systems is also temperature sensitive in BUG6. Using operon and protein fusions of the maltose transport genes to lacZ, we observed that the temperature-sensitive control of the maltose transport system in BUG6 occurs at the transcriptional level. By P1-mediated transductions, we found that BUG6 contains two independent temperature-sensitive mutations. One map between 2 and 3 min on the Escherichia coli linkage map, in close proximity to the fts-envA region. This mutation is responsible for temperature-sensitive cell division. The other mutation maps at 73 min in crp, the structural gene of the catabolite activator protein. The latter could be complemented by a hybrid plasmid carrying the wild-type crp as the only gene on a 0.9-kilobase HindIII-AluI restriction fragment. This mutation in crp alone was found to be responsible for the temperature-sensitive synthesis of the maltose transport system. Although it causes a complete block of transcription of the maltose transport genes at 41°C, this mutation had only a marginal effect on the transcription of the lac operon.

Several years ago, we observed that synthesis of the periplasmic binding protein-dependent transport systems for galactose (mgl dependent) (27) and maltose (14) is connected to events occurring during the cell cycle of Escherichia coli. Similarly, syntheses of the corresponding periplasmic binding proteins were found to follow the cell cycle pattern. This effect was observed by measuring transport activity as well as by radioactive pulse labeling of the respective binding proteins in synchronized cells. Synchronization was achieved by the method of Cutler and Evans (13) that is based on repeated dilution of stationary cultures into fresh medium. To corroborate our conclusion of a connection between cell division and the synthesis of these transport systems, we studied their expression in BUG6, a mutant of E. coli K-12 that is temperature sensitive for cell division. The defect in this mutant results in a lack of septum formation at the nonpermissive temperature (23). Indeed, we found that synthesis of the transport activity for maltose and galactose as well as synthesis of the corresponding periplasmic binding proteins does not occur at the nonpermissive temperature of 41°C, thus supporting a correlation between cell division and synthesis of the maltose and galactose transport systems. Ryter et al. (24) came to a similar conclusion with respect to the integration of the λ receptor (λamB gene product) into the outer membrane. Like the maltose-binding protein (malE gene product), the λ receptor belongs to the malB-encoded proteins. Both proteins are secretory proteins and are synthesized with an amino-terminal signal sequence that is cleaved during secretion, suggesting that the secretion process might be coupled to cell division. Therefore, we wanted to determine the level, transcription, translation, or secretion, on which the temperature-sensitive control in BUG6 is exerted on the mal regulon.

The malB region contains all five structural genes for the transport system. They are organized in two divergent operons: malK and lamB (clockwise) and malE, malF, and malG (counterclockwise) at 91 min on the E. coli linkage map (21, 29). Both operons are under positive control of the maltose-activated malT gene product. The latter is part of the mal regulon, which is also organized divergently: malT (clockwise) and malP and malQ (counterclockwise) at 75 min on the linkage map (22). Transcription of both malB operons as well as of the malT gene are under positive control by cyclic AMP (cAMP)-catabolite activator protein (CAP). The malP, malQ genes which code for the maltose- and maltodextrin-degradative enzymes are not directly under the control of the cAMP-CAP complex. Since the malT product in complex with maltose acts as a positive regulator for the expression of malP, malQ, and since malT is under control by cAMP-CAP, the malP, malQ genes are also ultimately under cAMP-CAP control (8, 9).

To determine the level of control in BUG6, we used fusions of lacZ to various malB genes. Consequently, we constructed derivatives of BUG6 that carry deletions in lac and are constitutive for the mal regulon (malF'). We then lysogenized these derivatives with λ phages that carry protein and operon fusions of lacZ to different genes of the mal regulon. β-Galactosidase activity of all fusions ceased to increase after the growth temperature was shifted from the permissive to the nonpermissive temperature. This demonstrated that the temperature-dependent regulation of the mal regulon occurs on the transcriptional level. However, genetic analysis of the mutant phenotype revealed that BUG6 contains at least two temperature-sensitive mutations. This paper describes that the effect on the mal regulon is caused by an altered crp gene, resulting in a temperature-sensitive CAP, and is independent of a second temperature-sensitive mutation, which causes filamentous growth at the nonpermissive temperature. Therefore, the temperature-sensitive regulation of the maltose transport system, as well as other temperature-sensitive phenomena reported in BUG6 (3, 6, 11), can no longer be used as evidence for their relationship to cell division.

MATERIALS AND METHODS

Bacterial strains, λ phages, and plasmids. The bacterial strains, λ phages, and plasmids used in this study are listed in Table 1.
Medium and conditions of growth. Overnight cultures were grown at 28°C in minimal medium A (MMA) containing 0.2% glycerol and 0.1% Casamino Acids. The cells were subcultured into fresh medium of the same composition with an initial optical density at 578 nm of 0.01 to 0.02. The cultures were grown with aeration at 32°C. At an optical density of 0.1, the cultures were divided into two parts, one held at 32°C, and the other shifted to the nonpermissive temperature (41°C).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Known genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUG6</td>
<td>NTG-EMS* treated AB1157 F' thr leu thi arg proA his gal xyl ara mel lac rpsL</td>
<td>Reeve et al. (3)</td>
</tr>
<tr>
<td>LA5613</td>
<td>BUG6, lac* phoR malT*</td>
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</tr>
<tr>
<td>LA5628</td>
<td>BUG6, phoR malT* Δ(argF-lac)U169</td>
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<tr>
<td>LA5659</td>
<td>LA5628 (kp72b-12 srl::Tn10 recA)</td>
<td>This work</td>
</tr>
<tr>
<td>LA5660</td>
<td>LA5628 recA srl::Tn10</td>
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<tr>
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<td>LA5660 (kpSH350)</td>
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<td>LA5694</td>
<td>LA5628, zab::Tn10; loss of filamentous growth at 41°C</td>
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<td>JB3018.2, zhd-732::Tn10 crp*buuos</td>
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<td>MC4100, malT*</td>
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<td>LA5709</td>
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<td>H. G. Heine (unpublished data)</td>
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<td>LA5709, zab::Tnl5::Tn10 Δcyrp-96</td>
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Plasmids

| pBR322   | Tetr Amp* | Bolivar et al. (4) |
| pHG14    | pBR322-hybrid mgl (Salmonella) tet* | H. G. Heine (unpublished data) |
| pBD1     | Tetr Amp* crp* with crp promoter | This work |
| pBD2     | Tetr Amp* crp* without crp promoter | This work |

Genetic manipulations. P1 transductions, Hfr crosses, and λ lysogenizations were done by the method of Miller (19). P1 transduction into the recA strain LA5709 was done with the help of the Amp' recA* cosmid pLCB19. The cosmid was subsequently removed by growing the transductants in the absence of ampicillin (10). DNA manipulations were performed as described by Maniatis et al. (18). For elution of DNA from agarose gels, the method of Silhavy et al. (28) was used. Plasmid transformation into different recipient strains was performed by the method of Lederberg and Cohen (16).

For selection of plasmids containing the crp gene, we used the crp mutant strain BD20. Due to the lack of CAP, this strain has lost the ability to utilize maltose. Clones which gained an intact crp gene after transformation could be detected on MacConkey maltose indicator plates as deep-red colonies.

β-Galactosidase assay. At different time intervals, 1-ml samples were taken from the growing culture and put on ice after the addition of 10 μl of chloramphenicol (2 mg/ml). The assay for β-galactosidase was performed as described by Miller (19). The total β-galactosidase activity was calculated in units (micromoles per minute) contained in 1 ml.

Assay of alkaline phosphatase. Samples (2 ml) were taken from the growing culture and put on ice after 20 μl of chloramphenicol (2 mg/ml) had been added. The cells were suspended in 5 ml of 1 M Tris-hydrochloride (pH 8.2). The enzymatic reaction was started by the addition of 0.1 ml of 50 mM p-nitrophenyl phosphate and was stopped by the addition of excess phosphate from 0.5 ml of 10× MMA (19). The suspension was centrifuged, and the optical density at 405 nm was determined. The activity of alkaline phosphatase was calculated in units (micromoles per minute) contained in 1 ml of culture.

Maltose transport assay. To 0.5 ml of culture [14C]maltose (604 mCi/mmole) was added to a final concentration of 0.2 μM. Twenty seconds after the addition of the labeled maltose, the total volume was filtered through a membrane filter (pore size, 0.45 μm) and washed with 10 ml of MMA (19). After drying the filters at 90°C for 5 min and adding a toluene-based scintillation cocktail, the accumulated radioactivity was determined by scintillation counting.

RESULTS

Temperature-sensitive repression of the Mal regulon in BUG6. We introduced Δlac and malT into BUG6 and lysogenized this derivative with different λ phages carrying fusions to malE, lamB, or malP. The fusion phages were of both types, operon and protein fusions. Figure 1 shows the results obtained with strain LA5659 that was lysogenized with λp72b-12 carrying a malE-lacZ operon fusion. At 32°C, transport of maltose and β-galactosidase activity increased with growth. However, after shifting the culture to 41°C, transport of maltose and β-galactosidase activity no longer increased, even though the optical density continued to increase exponentially (Fig. 1A). Similarly, at 41°C the culture exhibited a constant level of maltose transport and β-galactosidase activity, both of which increased again after the culture was returned to 32°C (Fig. 1B). Essentially, all malE-lacZ fusions that we tested in various derivatives of BUG6 behaved identically. One noteworthy exception was a phage carrying a protein fusion in which the fusion point was early in lamB (Δ61-4). The hybrid protein produced by this fusion was itself either temperature sensitive or degraded at 41°C. After shifting a culture lysogenized with this phage to 41°C and preventing protein synthesis with chlorampheni-
col, β-galactosidase activity declined, with a half-life of 30 min. This did not occur at 32°C. The observation that the expression of both protein fusions and operon fusions to the different mal genes was turned off at 41°C clearly demonstrates that this temperature-dependent regulation is exerted at the level of transcription.

As a non-temperature-sensitive control, a derivative of strain MC4100 carrying the same Δlac and malT markers (JB3018.2) was lysogenized with phage Ap72b-12 carrying a malE-lacZ operon fusion. Both maltose transport and β-galactosidase activity increased nearly identically in cultures growing at 32 and 41°C (data not shown).

Mapping of the mutation causing temperature-sensitive septum formation in BUG6. Several genes involved in cell division are clustered between 2 and 3 min of the E. coli linkage map (17). To test whether the mutation causing filamentous growth at 41°C in BUG6 is located in this area, we used a tetracycline resistance transposon (zab::Tn10) located next to leu (at 2 min on the linkage map) as a selective marker. In P1-mediated crosses with strain LA5628 (an appropriate BUG6 derivative) as recipient, 80% of the tetracycline-resistant (Tc') transductants lost the mutation that causes filamentous growth at the nonpermissive temperature. Using the same Tn10 insertion, we could cotransduce the mutation with a frequency of 50 to 70% from BUG6 into strain MC4100. However, transductants of MC4100 that had become temperature sensitive for cell division no longer exhibited the temperature-dependent regulation of the maltose (or galactose) transport system. In addition, transductants of BUG6 that no longer exhibited temperature-sensitive septum formation still turned off synthesis of the maltose transport system at 41°C. Therefore, BUG6 must contain at least two mutations exhibiting temperature-sensitive phenotypes. One is responsible for temperature-sensitive cell division and is located between 2 and 3 min, and the other causes the transcriptional turnoff at 41°C of the maltose transport system. The two mutations are independent of each other.

Transcription of ugp. A phoR Δlac derivative of BUG6 was lysogenized with a phage carrying a ugp-lacZ operon fusion (strain LA5671). The ugp genes code for a pho regulon-dependent transport system for sn-glycerol-3-phosphate that is mediated by a periplasmic binding protein. In contrast to the malB- and mgl-encoded transport systems, the ugp system is not subject to catabolite repression and is thus fully active in cultures grown on glucose (2). When the ugp-lacZ fusion in the BUG6 derivative LA5671 was tested for β-galactosidase activity in a culture growing on glycerol at 32°C, enzyme activity continued to increase after shifting to 41°C, with a rate similar to that of the culture growing at 32°C. Likewise, the activity of alkaline phosphatase, another periplasmic member of the pho regulon, increased in the same fashion as β-galactosidase fused to ugp (data not shown).

Alteration in BUG6 of the crp gene coding for CAP. From the above finding, it appeared likely that a protein involved in catabolite repression was temperature sensitive in BUG6. To test whether the crp gene was affected, we first introduced a Δcrp mutation into BUG6 via P1-mediated transduction with the help of a nearby Tn10 insertion (zhd-732::Tn10). Δcrp transductants were recognized by their inability to grow on maltose. Then, a wild-type crp gene (obtained from strain DL47) was introduced by P1 transduction and selection for growth on maltose at 32°C. All Mal+
transductants had lost the temperature-sensitive synthesis of the maltose transport system. In addition, by using the zhd-732::Tn10 insertion, we could transduce the mutation from BUG6 into a crp + wild-type strain. The Tc' transductants that gained the crp region from BUG6 were recognized by their low level of maltose transport activity in cultures grown at 41°C. This made it likely that the temperature-sensitive expression of the maltose transport system in BUG6 was caused by a mutation within or near the crp gene.

**Complementation of the BUG6 phenotype by the wild-type crp gene.** Starting with the transducing λ phage YF1079 (S. Adhya, personal communication) that carries the crp gene on a 3.6-kilobase (kb) BamHI insert in the *kint* region, we cloned this fragment into pBR322, yielding pBD1 (cf. references 1 and 12).

To exclude the possibility that a gene other than crp on pBD1 would complement the temperature-sensitive phenotype of BUG6, we subcloned the crp-containing 0.9-kb *AluI*-HindIII fragment into the *HpaI*-HindIII sites of pHG14, a derivative of pBR322 carrying *Salmonella mgl* DNA with suitable *HpaI* and HindIII sites (H.-G. Heine, personal communication) (Fig. 2).

The sequenced 0.9-kb *AluI*-HindIII fragment is known to carry only the structural gene of crp without a functional promoter (1). Appropriate cleavage of pHG14 and the insertion of the fragment (yielding pBD2) allow the crp gene to be transcribed by the P1 promoter of the *tet* region in pBR322 (31).

The temperature-sensitive *malB* expression in the appropriate BUG6 derivative (LA5694) is overcome in the presence of pBD2 (Fig. 3A). The same strain carrying the vector still exhibited the typical turnoff of the maltose transport system at the nonpermissive temperature (Fig. 3B). This clearly demonstrates that the temperature-sensitive block of transcription of the *malB* operons in BUG6 is caused by a temperature-sensitive CAP encoded by a mutated crp gene.

**Temperature-sensitive crp mutation has little effect on the CAP-dependent lac system.** The shift of the growth temperature from 32 to 41°C had only a small effect on the increase of β-galactosidase activity in a BUG6 derivative that carries a constitutive lac operon on an F' episome (LA5690). Enzyme activity at 41°C increased after a lag of about 45 min at a rate not much smaller than that at 32°C (data not shown). Using a BUG6 derivative with a chromosomal wild-type lac operon (LA5613) and growing the culture with 0.2 mM isopropyl-β-D-thiogalactopyranoside as inducer, we could not observe any adverse effect on lac expression at the nonpermissive temperature (data not shown).

**DISCUSSION**

BUG6 is a mutant isolated from the K-12 strain AB-1157 by Reeve et al. (23) after *N*-methyl-*N*-nitro-*N*-nitrosoguanidine or ethyl methanesulfonate mutagenesis and enrichment for increased cell size after growth at 42°C. BUG6 exhibits normal bulk DNA, RNA, and protein synthesis but is unable to form septa at the nonpermissive temperature. At 41°C, it forms filaments that can exceed several times the normal length of the cell. BUG6 has been used to relate several phenomena to cell division. We have demonstrated a correlation to the synthesis of the periplasmic galactose- (27) and maltose- (14) binding protein-dependent transport systems. Beck and Park (3) found that the periplasmic carboxypeptidase II is less active at the nonpermissive temperature, and recently Cook et al. (11) reported a dramatic

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**FIG. 2.** Construction of plasmid pBD2 (crp +). pBD1 (pBR322/crp +) was digested with *BamHI* and HindIII. The resulting 2.0-kb fragment (1) was eluted from the agarose gel after electrophoresis. After cleavage by *AluI* and electrophoresis, the 0.9-kb fragment was eluted from the agarose gel and ligated with the 4.6-kb *HpaI*-HindIII fragment of pHG14. pHG14 was chosen because of its blunt-end *HpaI* site, which ensured the integration of the *AluI*-HindIII fragment in the right orientation. The P1 promoter of pBR322 (38) substitutes for the lacking crp promoter. Symbols: , pBR322 DNA; , *Salmonella mgl* DNA; , crp region of *E. coli.*
maltoose- (and galactose-) binding protein-mediated transport system, there is still the phenomenon of the cell cycle-dependent synthesis of both transport systems in wild-type cells observed by us as well as others (14, 20, 24, 27). In our experiments (27) linking the synthesis of the galactose transport system to cell division in synchronized cells, we used the method of repeatedly inoculating a culture from the early stationary phase into fresh medium (13). It is possible that these nutritional shifts caused the cyclic nucleotide levels to fluctuate, causing the seemingly cell division-dependent regulation of the highly CAP-dependent transport systems. However, the relation of cell division to cyclic nucleotide levels may not be only an artifact introduced by the synchronization protocol, as evidenced by the following. (i) BUG6 which was selected for filamentous growth at 42°C not only obtained a mutation causing temperature sensitivity of cell division but also a mutation causing a temperature-sensitive CAP. This suggests that at 42°C a wild-type CAP is of disadvantage to strains carrying the mutation, causing temperature-sensitive cell division. (ii) Utsumi et al. (33, 34) described a temperature-sensitive division mutant of E. coli K-12 (fc [filamentation induced by cAMP]) in which cAMP inhibited septum formation and induced cell filamentation at elevated temperatures. They also found that a membrane protein with a molecular weight of 40,000 (40K protein) was induced concurrently with cell filamentation. In the cpr derivative of the fc mutant, cell filamentation by cAMP was not observed, and the 40K protein was not induced (32). From these findings the authors concluded that the induction of the 40K protein is regulated by the cAMP-CAP complex and is closely related to cell filamentation by cAMP in the fc mutant. Despite this apparent correlation of cyclic nucleotides to cell division, they cannot be essential for this process, since Δcyr and Δcpr strains can, in fact, grow and divide (25).

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LITERATURE CITED

7. Casadaban, M. J. 1976. Transposition and fusion of the lac genes to selected promoters in Escherichia coli using bacterio...