Glutamate and cyclic AMP regulate the expression of galactokinase in *Mycobacterium smegmatis*

Santanu Raychaudhuri, Madhumita Basu and Nitai C. Mandal

Author for correspondence: Nitai C. Mandal. Tel: +91 33 337 9544. Fax: +91 33 334 3886.
e-mail: ncmdal@boseinst.ernet.in

It was found that *Mycobacterium smegmatis* is unable to utilize galactose as the sole carbon source because the sugar alone cannot induce galactokinase. However, galactokinase was induced by glutamate alone, and was further stimulated by galactose. Rifampicin completely inhibited the glutamate-mediated expression of *galK* in both the absence and presence of galactose. Extracellular cAMP stimulated the expression of the enzyme only in the presence of glutamate plus galactose. The *galK* gene from *M. smegmatis*, including its upstream promoter region, was cloned in a plasmid in *Escherichia coli*. The expression of kinase from these clones in *E. coli* was dependent on cAMP and its receptor protein (CRP). The expression of UDP-galactose 4-epimerase was constitutive. This and other evidence suggests that the *galK* gene is not linked to *galT* and *galE* in the mycobacterial genome. In a glutamate-independent galactose-utilizing mutant (*gin-T* mutant) of *M. smegmatis*, *galK* was expressed in the absence of both galactose and glutamate, while in the presence of galactose this expression was increased twofold in the absence of glutamate and fourfold in its presence. Extracellularly added cAMP reduced the expression of the enzyme in the presence of galactose plus glutamate nearly to the basal level. It is proposed that in *M. smegmatis* the *galK* gene is expressed from two different promoters; the expression from one promoter is dependent on glutamate but not on galactose and cAMP, while that from the other requires all three components. The role of galactose is possibly to derepress the latter promoter.

**Keywords:** *Mycobacterium smegmatis*, gene regulation, galactokinase induction, glutamate, *galK* operon

INTRODUCTION

Although mycobacteria are among the earliest identified human pathogens, their genetics and gene regulation are poorly understood (Dale & Patki, 1990; Grange, 1983; Greenberg & Woodley, 1985; Jacobs, 1992). The main reasons for this lack of information are the slow growth rate of the organism, poorly developed gene transfer systems and very little understanding of the genetic background of these bacteria. However, efforts are now being made to explore the mechanisms of gene regulation in mycobacteria. It has been shown that mycobacterial promoters function poorly in *Escherichia coli* (Bashyam et al., 1996; Clark-Curtiss et al., 1985; Das Gupta et al., 1993; Kieser et al., 1986). Although a number of mycobacterial metabolic genes have been cloned by complementation of the mutational defects in the corresponding genes of *E. coli* (Anderson et al., 1993; Garbe et al., 1990; Jacobs et al., 1986; Stelandre et al., 1992) and mycobacteria (Hinshelwood & Stoker, 1992), very little is known about the regulatory mechanisms involved. More studies are needed to understand the basic features of the regulation of mycobacterial gene expression.

Galactose is a major component of the mycobacterial cell wall, where it exists as arabinogalactan mycolate covalently linked to peptidoglycan (Kasuka et al., 1981; Minnikin et al., 1985; Segal & Edwards, 1985). D-Galactose induces L-arabinose permease and L-arabinose isomerase in *Mycobacterium smegmatis*. Whereas the former enzyme is capable of transporting galactose into the bacterial cell, the latter cannot use this sugar as
a metabolic substrate (Izumori et al., 1976, 1978). We observed that M. smegmatis could not use galactose as the sole carbon source unless glutamate was also present in the medium. Although glutamate has routinely been used as a component of mycobacterial growth media (Lyon et al., 1967), nothing is known about its involvement in gene regulation in these bacteria. In no other micro-organism is glutamate known to regulate the catabolic utilization of any sugar. Given this situation, we were interested to study the expression of gal genes in M. smegmatis in the hope of shedding new light on the mechanism of gene regulation in this bacterium at the molecular level. In this paper we show that in M. smegmatis galactose alone cannot induce galactokinase, while glutamate alone can do so to a certain level, which is increased further when galactose is also present in the growth medium. The expression of UDP-galactose 4-epimerase is constitutive. Results of the experiments described here suggest that in this mycobacterium, galactokinase is expressed from two different promoters.

METHODS

Materials. Bacterial strains and plasmids are described in Table 1. The restriction and other enzymes and [14C]galactose were purchased from Amersham. All other chemicals were obtained from Sigma.

<table>
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<th>Strain/plasmid</th>
<th>Alternative designation</th>
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<td>mc6 (wild-type)</td>
<td>mc6</td>
</tr>
<tr>
<td></td>
<td>mc6 gin-1</td>
<td>Snapper et al. (1988)</td>
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<tr>
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<td>galE</td>
<td>S. Adhya</td>
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<td>SA2285 (galT Str' his relA1)</td>
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<tr>
<td>DJ189 (thr leu supE thi r&quot;m lac Str' recA galK srl::Tn10)</td>
<td>galK</td>
<td>D. Chattoraj</td>
</tr>
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<td>MCA4100 [araD139 Δ(lac)U169 rpsL thi]</td>
<td>galK*</td>
<td>H. Aiba</td>
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<tr>
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<td>Δ(cya) galK*</td>
<td>H. Aiba</td>
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<tr>
<td>IT1376 [MC4100 Δ(crp :: kan)]</td>
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<tr>
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<td>pGKgin-1</td>
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*S. Adhya and D. Chattoraj, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD, USA; H. Aiba, Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Japan; T. Atlung, Department of Microbiology, Technical University of Denmark.

Media and solutions. Middlebrook 7H9 broth ('7H9 broth'), a supplemented minimal salt medium, and 7H9 hard agar have been described by Chaudhuri et al. (1993). The basal 7H9 broth was prepared by omitting carbon sources such as citrate, glutamate and sugar from 7H9 broth. The basal hard agar contained 1.5% (w/v) agar in basal 7H9 broth. The M9 medium was as described by Miller (1972). Unless otherwise mentioned, the percentage concentrations of galactose and glutamate are defined as w/v and of glycerol as v/v.

Growth of bacteria. Mycobacterial cultures were routinely grown with shaking at 37 °C in the desired medium containing 0.05% (v/v) Tween 80. Growth was monitored by measuring OD600 in a spectrophotometer (Beckman DU-6). For the transport and enzyme assays, cells were processed at 0-4 °C.

Galactose uptake. M. smegmatis was grown to around OD600 1 in basal 7H9 broth with or without supplementation(s). The cells were then processed and the galactose uptake activity was assayed as described by Prasad et al. (1976) except that 14C-labelled amino acid was replaced by [14C]galactose (10⁵ c.p.m. ml⁻¹; 50 c.p.m. nmol⁻¹) and aliquots of the cells containing transported radioactivity were washed with 10 ml chilled solution of 0.2% nonradioactive galactose on a Millipore filter.

Galactokinase and UDP-galactose 4-epimerase assays. The bacteria were grown under the desired conditions and the cells were harvested, washed with M9 salt (1 x) solution and suspended in the same solution. This cell suspension was divided into two equal portions. To one portion was added
RESULTS

Glutamate is essential for galactose-dependent growth and induction of galactokinase in *M. smegmatis*

Fig. 1(a) shows the kinetics of growth and galactokinase expression in *M. smegmatis*. The data reveal that the bacterium could not grow on galactose as the carbon source unless glutamate was also present at a low concentration (0.05%). Also, the growth of the bacteria was much faster in the presence of galactose plus glutamate than it was in the presence of glutamate alone. Stationary-phase cultures of mc6 growing separately on galactose and glutamate resumed steady growth when glutamate and galactose, respectively, were added. The kinase reaction is a key step in the metabolic utilization of galactose (Kalckar, 1958). Surprisingly, the results in Fig. 1(a) show that a significant amount of kinase was produced in the glutamate-grown cells but none at all in the galactose-grown cells. The level of this enzyme in the cells grown on galactose in the presence of glutamate was about 3.5-fold higher than that in the glutamate-grown cells (the enzyme levels at their peaks at 72 h in Fig. 1a were compared). We conclude that mc6 is unable to grow on galactose in the absence of glutamate because this amino acid is absolutely necessary for the induction of kinase.

Glutamate plays a key role in the biosynthesis of other amino acids (Umbarger, 1978). The primary role of glutamate may thus be to reduce the lag phase during growth of mc6 by providing carbon and nitrogen and generating an intracellular pool of other amino acids. However, when the bacteria were first grown to early exponential phase on glycerol without any amino acid and then the galactokinase induction was studied, even then galactose alone failed to induce the enzyme, while glutamate alone did so at low level which was enhanced further by galactose or fucose (Fig. 1b). This suggests that glutamate possibly plays a specific regulatory role in *galK* expression rather than in promoting bacterial growth.

To determine the effect of varying the concentration of glutamate on *galK* expression in mc6, in both the presence and absence of galactose, an experiment was done as described in Fig. 2. The results reveal that with the increase of glutamate concentration in the medium, the expression of kinase was increased in the absence of galactose (Fig. 2a) and decreased in its presence (Fig. 2b). However, growth was not at all inhibited under the latter condition (data not shown). These results suggest that glutamate at high concentration (0.2%) is quite effective for a high level of *galK* expression in the absence of galactose, but is inhibitory in the presence of this sugar.

When the effect of other amino acids on galactokinase induction in *M. smegmatis* was examined, it was observed that (i) tyrosine, serine, valine, isoleucine, glycine and methionine had practically no effect; (ii) two glutamate-generating amino acids, proline and histidine,
Fig. 1. (a) Effect of glutamate on galactose-dependent growth and galactokinase induction in \(M. \) *smegmatis* mc\(^2\)6. Cultures were grown in basal 7H9 medium for 24 h for glutamate starvation and then inoculated into the same medium in different flasks with or without supplement(s) (galactose, 0.2%; glutamate, 0.05%). At different times two aliquots were taken from each culture; one was used to monitor growth, while the other was used to assay galactokinase. At 120 h, aliquots from cultures containing only glutamate (\(\triangle\)) and only galactose (\(\bigcirc\)) were taken; to these were added 0.2% galactose and 0.05% glutamate, respectively, and the growth was monitored for 196 h (indicated by a broken line). For further experimental details, see Methods. Open symbols, growth; solid symbols, kinase activity. \(\bigcirc\), Basal; \(\bigtriangleup\), basal+galactose; \(\bullet\), basal+glutamate; \(\bigtriangledown\), basal+galactose+glutamate. (b) Effect of glutamate on galactokinase induction in mc\(^2\)6 growing in the presence of glycerol. Cultures were grown in basal 7H9 medium for 24 h for glutamate starvation, after which glycerol was added to 0.2%. The culture was grown to around OD\(_{600}\) 0.2 (early exponential phase) and then divided into five equal portions. One portion received no supplement, the other four received galactose (0.2%), glutamate (0.05%) and fucose (2 mM) in different combinations. Aliquots were taken out at different times and galactokinase activity was assayed. For further details, see Methods. \(\bigcirc\), Basal; \(\bigtriangleup\), basal+galactose; \(\bullet\), basal+glutamate; \(\bigtriangledown\), basal+fucose+glutamate. The basal 7H9 medium contained 0.2% glycerol in all five portions.

Fig. 2. Galactokinase expression in mc\(^2\)6 as a function of glutamate concentration. The experiment was done as described in Fig. 1 (a) with different concentrations of glutamate in the absence and presence of galactose. Aliquots were taken at different times and galactokinase activity was assayed. For other details, see Methods. (a) No galactose present. (b) 0.2% (fixed) galactose present. Glutamate concentration was as follows: \(\square\), 0.02%; \(\bigcirc\), 0.05%; \(\bigodot\), 0.1%; \(\triangle\), 0.2%.

could induce the enzyme to the same maximum level as achieved by glutamate but with relatively slower kinetics; (iii) all other amino acids were effective, with efficiencies varying from 10 to 59% of that shown by glutamate; and (iv) the glutamate-derived compounds such as 2-oxoglutarate and \(\gamma\)-aminobutyrate did not show any effect in this regard (data not shown). This suggests that the induction of galactokinase is specifically responsive to glutamate, and that the other amino acids which show a positive effect possibly do so by generating an intracellular pool of glutamate at varying levels.

**Expression of UDP-galactose 4-epimerase is constitutive in \(M. \) smegmatis**

In *E. coli* the three *gal* enzymes, epimerase, transferase and kinase, are organized in one operon and all are inducible by galactose or fucose (Adhya, 1987). Fig. 3 shows that in *M. smegmatis* the level of epimerase expressed in the presence of glutamate plus galactose was maintained almost at a constant level throughout the induction period starting from zero time, while the expression of kinase followed a typical induction profile under identical conditions. It is also seen that nearly the
Fig. 3. UDP-galactose 4-epimerase activity in mc²6. The experiment was done as described in Fig. 1(b) except that fucose supplementation was omitted. Kinase and epimerase were assayed from the same aliquots of the cultures. Inset bar diagrams: kinase (K) and epimerase (E) were determined after 25 h of growth following supplementations. Gly, 0.2 % glycerol; Glu, 0.05 % glutamate + 0.2 % glycerol; Gal, 0.2 % galactose + 0.2 % glycerol. The glutamate and galactose cultures also contained 0.2 % glycerol. For further details see Methods. ○, Kinase; △, epimerase.

Fig. 4. Expression of galactose uptake activity in mc²6. Bacteria were grown in basal medium for 24 h for glutamate starvation and then divided into five equal portions which received glycerol (0.2 %), glutamate (0.05 %), galactose (0.2 %) either singly or in different combinations. These were then grown to around OD₆₀₀ 1 and galactose uptake activity was determined. For further details, see Methods. △, Basal; ○, basal + glycerol; □, basal + glutamate; ●, basal + galactose; ■, basal + galactose + glutamate.

same amounts of epimerase were present in the cells grown separately on glycerol, galactose or glutamate (see inset bar diagrams in Fig. 3). We conclude that the expression of epimerase is constitutive and is not at all influenced by glutamate and/or galactose.

Galactose and glutamate independently induce galactose transport activity in M. smegmatis

To clarify if glutamate regulates the expression of galK in M. smegmatis by controlling the expression of galactose uptake activity, an experiment was done as described in Fig. 4. The data indicate that each of the glutamate-grown and galactose-grown mc²6 cells had higher levels of galactose uptake activity than the glycerol-grown cells. In the cells grown on galactose plus glutamate, the above uptake activity appears to be

Fig. 5. (a) Effect of rifampicin on galactokinase induction in mc²6. Cultures were grown in basal 7H9 broth for 24 h, and then glycerol was added to 0.2 % and grown further to around OD₆₀₀ 0.2. The culture was then divided into two equal portions. Glutamate (0.05 %) and galactose (0.2 %) were added to one, while only glutamate (0.05 %) was added to the other. Each of the two portions was then subdivided into two; rifampicin at 25 μg ml⁻¹ was added to one from each portion, with the other remaining as a control. Cultures were grown and aliquots were taken at different times for galactokinase assay. For further details, see Methods. ○, Glutamate; ○, glutamate + galactose; △, glutamate + galactose + Rif; □, glutamate + galactose + Rif. After glutamate starvation, 0.2 % glycerol was added to all portions. (b) Northern blot analysis of RNA isolated from M. smegmatis grown under different conditions. Experimental methods were as for (a) except that rifampicin was omitted; the four portions of cultures contained basal medium only (lane 1), 0.2 % galactose (lane 2), 0.2 % galactose + 0.05 % glutamate (lane 3), and 0.05 % glutamate (lane 4). All the cultures contained 0.2 % glycerol. Total RNA was isolated and Northern blot analysis was done as described in Methods. The positions of dsDNA markers in kb and galK mRNA are indicated.
the sum of those in the cells grown separately on these two metabolites. However, glutamate did not compete with galactose for its transport into the cells grown on galactose plus glutamate (results not shown). This suggests that *M. smegmatis* does not use the glutamate uptake pathway for galactose transport. The additive effect seen in the presence of both glutamate and galactose is possibly due to the induction of the uptake activity separately by these two compounds. It is interesting to note that although the galactose-grown cells showed galactose uptake activity nearly 1.5-fold higher than the glutamate-grown cells (Fig. 4), galactokinase was induced in the latter cells but not in the former (Fig. 1). It is not clear whether the galactose-induced uptake activity was due to L-arabinose permease that is induced by galactose (Izumori et al., 1976, 1978). However, what is clear is that the induction of galactose uptake activity by glutamate is not a deciding factor in the induction of kinase.

Glutamate regulates the expression of *galK* in *M. smegmatis* at the transcriptional level

The question now arises as to whether the expression of galactokinase is regulated by glutamate at the transcriptional level. It is known that rifampicin is a potent inhibitor of transcription in bacteria (McCleure & Cech, 1978). The results in Fig. 5(a) show that the induction of kinase by glutamate in both the presence and absence of galactose was significantly inhibited by rifampicin. The results of the Northern hybridization experiment in Fig. 5(b) show that the *galK*-specific mRNA was detectable in the bacteria after 20 h growth in the presence of both galactose and glutamate (lane 3) but not in those grown on galactose (lane 2) or even glutamate (lane 4) alone under identical conditions. We conclude that the presence of both galactose and glutamate is essential for efficient transcription of the *galK* gene in *M. smegmatis*.

Exogenous cAMP stimulates *galK* expression in *M. smegmatis* only in the presence of both glutamate and galactose

Transcription of the *gal* operon in *E. coli* is activated by cAMP and CRP (Adhya, 1987). The results in Fig. 6(a) demonstrate that in wild-type mc<sup>6</sup>, the expression of *galK* in the presence of glutamate plus galactose was stimulated 1.7-fold by exogenously added cAMP over that in its absence. However, cAMP could neither stimulate the glutamate-regulated expression of the enzyme nor activate the process in the presence of galactose alone. This suggests that cAMP positively regulates the expression of kinase in *M. smegmatis* in the presence of both glutamate and galactose but does not act in the process regulated by glutamate alone. cAMP needs a receptor protein, CRP, for the activation of gene transcription in bacteria (Kolb et al., 1993). It is known that wild-type *M. smegmatis* produces intracellular cAMP (Padh & Venkatsubramanian, 1978) as well as CRP (Lee, 1979). A 1.7-fold stimulation of kinase expression in wild-type mc<sup>6</sup> by exogenous cAMP over that in the absence of the above nucleotide therefore suggests that possibly the wild-type cells growing under the conditions of the experiment do not maintain the optimum level of cAMP that is needed for maximum stimulation of *galK* expression.

Expression of *galK* in a glutamate-independent galactose-utilizing mutant of *M. smegmatis* occurs in the absence of glutamate and is stimulated further in the presence of glutamate plus galactose

To understand the role of glutamate in *galK* expression, mutants of mc<sup>6</sup> capable of growing on galactose in the absence of any amino acid were isolated by the procedure described in Methods. These were called mc<sup>6</sup> gin (glutamate independent) mutants. These mutants could grow slowly on galactose as the only carbon source (results not shown). The expression of *galK* in one of these mutants, gin-1, was studied under different conditions. The data in Fig. 6(b) reveal that in basal 7H9 broth, the gin-1 mutant could express kinase at a significant level, which was increased twofold in the presence of galactose alone and fourfold in the presence of both glutamate and galactose, while the amino acid alone had no effect. The data in Fig. 6(b) also show that in this mutant, cAMP had no effect on the expression of kinase either in basal 7H9 broth or in the same medium containing glutamate alone, while it reduced expression to the basal level in the presence of galactose alone and to around 45% of that expressed under glutamate plus galactose.
Galactokinase induction in *M. smegmatis*

Expression of mycobacterial galK in *E. coli* is dependent on cAMP and CRP

By screening a Sau3AI library of *M. smegmatis* genomic DNA in pBend2 (see Methods and Fig. 7) four plasmid clones containing DNA inserts of sizes ranging from 1.4 to 4 kb carrying the galK gene were obtained. These plasmids, called pGK1, pGK3, pGK4 and pGK6, could all complement an *E. coli* galK mutant but not galE and galT mutants for galactose utilization (results not shown). The galactokinase level produced in a galK strain of *E. coli* by one of these plasmids, pGK6, carrying the smallest insert, is shown in Table 2. As pBend2 does not carry any promoter within or around the multiple cloning sites (Zweib et al., 1989), the above results suggest that these pGK plasmids carry the galK gene with its upstream promoter which is expressed in *E. coli* and that the galE and galT genes are either absent or that if any of them is present, especially in the 4 kb insert, that it is not expressed. This implies that possibly the latter two gal genes are not members of the galK operon in *M. smegmatis*. It may be noted that although the mycobacterial promoters studied so far are not expressed in *E. coli* (Bashyam et al., 1996; Clark-Curtiss et al., 1985; Das Gupta et al., 1993; Kiesser et al., 1986), the above galK promoter is expressed. While this may appear very unusual, it is not surprising because it is known that the 16S rRNA promoter of *M. leprae* is expressed in *E. coli* (Sela & Clark-Curtiss, 1991).

It was observed further that in the crp and cya deletion mutants of *E. coli*, galactokinase was expressed from the pGK plasmids at drastically reduced levels. The galactokinase data with pGK6 are shown in Table 2. In the *E. coli* cya deletion mutant, the expression of kinase from this plasmid was increased about 36-fold in the presence of exogenously added cAMP over that in its absence. We conclude that an efficient expression of mycobacterial galK from its own promoter requires cAMP, and that *E. coli* CRP can substitute the mycobacterial protein for the activation of mycobacterial galK promoter by cAMP. The galK gene with its upstream regulatory region from the gin-1 mutant was cloned in *E. coli* to give plasmid pGKgin-1 (see Methods and Fig. 7). A comparison of the data on the expression of galactokinase (Table 2) from pGK6 and pGKgin-1 in *E. coli* and *E. coli* crp deletion mutants under identical conditions, suggests that the expression of galK from these two plasmids responds to the *E. coli* regulatory signals exactly in an identical manner. This implies that the gin-1 mutation has not affected any of the upstream positive regulatory sites of galK.

**DISCUSSION**

In this paper we show that the expression of galactokinase in *M. smegmatis* is regulated by L-glutamate and cAMP. Glutamate also induces galactose uptake activity. From the data on the expression of galK in wild-type mc6 and its gin mutant in the absence and presence of galactose, glutamate and cAMP, either alone or in different combinations, we suggest a model of galK regulation that has the following features. (i) The expression of galK occurs in two distinct ways from two different promoters which are differently regulated; (ii) galactose alone is totally ineffective in inducing the enzyme from both the promoters; (iii) galK expression from one promoter, say galP1, is dependent on glutamate only, while that from the second, say galP2, requires both galactose and glutamate; (iv) cAMP activates the latter promoter but not the former.

In *E. coli*, the gal genes galE, galT and galK are clustered...
Table 2. Expression of galactokinase from pGK6 and pGKgin-1 plasmids in cya and crp deletion mutants of E. coli

Bacteria were grown in M9 medium supplemented with Casamino acids and containing 2 mM fucose. Wherever needed, cAMP was used at 5 mM. Galactokinase data from cells containing plasmids were corrected for the values obtained with corresponding control cultures (no plasmid). In all the cases, means of the data from three independent experiments are presented, ± SD. For other details, see Methods and text.

<table>
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<tr>
<th>E. coli genotype</th>
<th>Galactokinase activity (U per OD_{590} unit) in cells containing plasmid:</th>
</tr>
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<tr>
<td></td>
<td>None (control)</td>
</tr>
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<td>galK*</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td>galK**</td>
<td>17.11 ± 0.62</td>
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<tr>
<td>del(cya)galK*</td>
<td>3.92 ± 0.32</td>
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<td>del(cya)galK* + cAMP</td>
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</tr>
<tr>
<td>del(crp)galK*</td>
<td>0.71 ± 0.32</td>
</tr>
</tbody>
</table>

*Both these strains (galK and galK*) of E. coli were cya* crp*.

in one operon under the control of two different promoters, P1 and P2, located 5 bp apart (Adhya, 1987). These promoters are repressed independently by two different repressors, GalR (Majumdar et al., 1987) and GalS (Weickert & Adhya, 1992), and are both derepressed by galactose. The cAMP–CRP complex activates P1 and inhibits P2 (Adhya, 1987). We have shown that in M. smegmatis, while the expression of galK is inductive and is regulated by glutamate and cAMP (see Results), that of galE is constitutive and is not influenced by any of the components which regulate galK expression. These suggest that in this bacterium, the expression of galK and galE is possibly controlled by different promoters. Agrobacterium tumefaciens expresses epimerase constitutively, while it does not code for kinase (Uttar et al., 1993). In Streptomyces lividans, the gal genes are expressed from two different promoters; while galE and galK are transcribed constitutively from galP2, galP1 is transcribed from galP1 only under inducing conditions (Fornwald et al., 1987; Mattern et al., 1993). It appears, therefore, that the organization and control of expression of gal genes are different in different micro-organisms.

What is the role of glutamate in galK expression and what is the identity of the actual inducer of the galK operon in M. smegmatis? The regulation of a gene by its promoter may be negative as well as positive. According to the general model of a negatively regulated operon (Jacob & Monod, 1961), the mycobacterial galK operon should be negatively controlled by its specific gal repressor. Studies with the E. coli gal operon in vivo (Adhya, 1987) and its gal repressor in vitro (Majumdar & Adhya, 1984) indicate that galactose is the inducer of the gal operon in E. coli. If it is assumed that in M. smegmatis galactose is also an inducer which binds the gal repressor specifically and inactivates it, and that galactose does not bind the repressor, then how does glutamate help activate galP1 in the absence of galactose? It may be that galP1 is not repressed by the repressor, while galP2 is. Under these conditions the transcription from galP1 may be initiated only in the presence of glutamate acting as a positive regulator. For the expression of galK from galP2, derepression by galactose is essential but is not sufficient for activation by cAMP unless glutamate is also present.

The data on galactokinase expression in the gin-1 mutant (Fig. 6b) may be explained in the light of the above assumptions as follows. The gin-1 mutant expresses galK in the absence of glutamate and galactose. In the presence of galactose, this expression of kinase was increased twofold in the absence of glutamate and fourfold in its presence, while the amino acid alone had no effect. cAMP reduced the expression in the presence of galactose plus glutamate nearly to the basal level. This implies that in the gin-1 mutant, expression from galP1 can occur without derepression by galactose, while that from galP2 requires both derepression by galactose and activation by glutamate. In the absence of any apparent defect in the positive regulatory sites in the gin-1 mutant, as suggested by the galactokinase data with pGKgin-1 plasmid in E. coli (Table 2), the other possibility is that the mutation has affected a transacting regulatory component, which is likely to be a glutamate-binding protein, and that the mutant version of this protein does not require glutamate for the transcription from galP1. Therefore a twofold increase of galK expression in this mutant after derepression by galactose in the absence of glutamate (Fig. 6b) over the basal level may be viewed as being due to an activation of galP2 by endogenous cAMP which does not require the amino acid in the presence of glutamate-independent binding protein (in wild-type cells, cAMP cannot activate galP2 without glutamate). A further twofold increase of kinase activity by glutamate in the galactose-derepressed cells (a total of a fourfold increase over the basal level) is possibly achieved through activation by...
both glutamate and intracellular cAMP (Fig. 6b). This suggests that the mutant glutamate-binding protein can respond to glutamate in further activating galP2 in the presence of cAMP but not in its absence. However, in the presence of excess cAMP (exogenously supplied), the expression from galP2 is possibly totally inhibited without affecting glutamate-independent, galP1-dependent basal expression in this mutant.

In E. coli, a leucine-responsive global regulatory protein, Lrp, regulates the expression of certain operons positively and others negatively, both at the transcriptional level, and binding of leucine with Lrp affects such regulation (Ernsting et al., 1992; Willins et al., 1991). It is not clear from the present data whether glutamate and cAMP bind to the same protein or two different proteins for activation of galP2, and whether the same glutamate-binding protein regulates both galP1 and galP2. The fact that the expression of mycobacterial galactokinase from both pGK6 and pGKgin-1 in E. coli does not require glutamate (M. Basu & N. C. Mandal, unpublished results) suggests that it is possibly the difference between the cAMP-binding protein molecules of M. smegmatis and E. coli that makes the former bacterium glutamate-dependent for galK expression. Further studies will identify and characterize the two presumptive promoters, galP1 and galP2, and clarify the mechanism(s) of their regulation by glutamate and cAMP in M. smegmatis at the molecular level.

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