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Regulation of *guaC* Expression in *Escherichia coli*

ANDREW I. KESSLER AND JOSEPH S. GOTS*

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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The *guaC* gene encodes GMP reductase, which converts GMP to inosine monophosphate. Regulation of *guaC* expression was examined by use of *guaC-lac* fusions created by Mu d1(*lac*). In these strains, beta-galactosidase is induced by guanine derivatives, and this induction is prevented by adenine. Our previous implication that glutamine acts as a negative effector of transcription was confirmed by showing that glutamine analogs (diazo-oxo-norleucine and methionine sulfoximine) can also induce beta-galactosidase. GMP was implicated as a likely candidate for the in vivo inducer by introducing a *gpt* block to prevent the conversion of guanine to GMP and a *deoD* block to prevent the interconversion of guanine and guanosine. Regulatory mutants were isolated by growth on lactose plus adenine. Though these showed high constitutive levels of beta-galactosidase, they were normal for the regulation of GMP reductase when the fusion was corrected by transduction to *guaC*$^+$ or when *guaC*$^+$ was introduced by plasmid complementation. The regulatory mutants were linked to *guaC*.

GMP reductase (NADPH:GMP oxidoreductase; EC 1.6.6.8) catalyzes the irreversible reductive deamination of GMP to inosine monophosphate (IMP), is coded for by the structural gene *guaC* (8, 13), and plays an important role in the interconversions of purine nucleotides, particularly adenine and guanine nucleotides (Fig. 1). GMP reductase is not normally required for the efficient growth of prototrophic *Escherichia coli* since a deficiency of this enzyme leaves the de novo synthesis of both GMP and AMP unimpaired. When the de novo synthesis of IMP is blocked, GMP reductase is now necessary to supply AMP when guanine (or xanthine) or its derivatives are the proferred purine sources. Thus, a purine auxotroph with an additional *guaC* block can grow on adenine or hypoxanthine but not on guanine or xanthine. Previous studies in *Salmonella typhimurium* (1) have shown that the synthesis of GMP reductase appears to be modulated by changes in the intracellular ratio of guanine and adenine nucleotides. The enzyme seems to be induced when the ratio increases and repressed when it decreases. Induction requires transcription and translation, and GMP appears to be the best candidate for the inducer. It has also been shown in *S. typhimurium* that glutamine plays a role in the regulation of GMP reductase, in that it is induced under conditions of glutamine starvation or by competitive analogs of glutamine (4).

In this study, we have used the *lac* fusion technique to study the expression of beta-galactosidase which is placed under the control of the transcriptional elements of the *guaC* gene. The *guaC-lac* fusions were used to study the kinetics of induction by guanine compounds, repression (or antiinduction) by adenine compounds, and the role of glutamine. Selective blocks in the interconversions of guanine derivatives also allowed for a more confident assignment of GMP as the most probable in vivo inducer. The fusions were also used for the isolation and characterization of regulatory deficient mutants.

MATERIALS AND METHODS

Chemicals. 6-Diazo-5-oxo-L-norleucine (DON) was obtained through the courtesy of H. Dion, Parke, Davis & Co. All other compounds were obtained from various commercial sources.

* Corresponding author.

Bacterial strains. The bacterial strains used in this study are derivatives of *E. coli* K-12 and are described in Table 1.

The *deoD* mutation in strain GP311 was spontaneously obtained and detected as a colorless colony on L agar containing guanosine (1 mg/ml) as described by Robertson (16). The (*pro-gpt-lac*) deletion in strain GP310 was obtained by Hfr mating of strain GP303 with NK6051 as donor, selecting for ampicillin and tetracycline (Tn*10*) resistance, and scoring for a proline requirement. The pLC37-40 (ColE1 *guaC*$^+$) plasmid came from the Clarke-Carbon (3) colony bank containing ColE1 *E. coli* hybrid plasmids. It was detected in colony no. 37-40 by complementation of previously isolated *guaC* mutants. It was transferred to various strains in this study (e.g., strain GP313) by F$^+$-mediated conjugation.

Media and culturing. Medium E (21) served as the minimal salts medium and routinely contained glucose (0.2%) and thiamine (1 μg/ml). Additional supplementation was made as indicated in the experiments described. L broth (or agar) served as the rich medium. MacConkey agar base (Difco Laboratories) with 1% lactose was used to check Lac phenotypes. Growth turbidity in liquid media was measured with a Bausch & Lomb Spectronic 88 spectrophotometer at 600 nm.

Phage preparations and transductions. P1 *cm* lysates were prepared by heat induction and used for transduction by the methods described by Rosner (17). The Mu *cts* d1(*lac* Ap^r) phage was prepared by heat induction of strain MAL103 and used for transduction to ampicillin resistance as described by Casadaban and Cohen (2).

Mutagenesis and isolation of *guaC* mutants. Strain TX257 was transduced to ampicillin resistance with the special Mu phage as described previously (2), and the transduction mixture was grown at 30°C in medium E containing glucose, thiamine, casein hydrolysate (0.1%), hypoxanthine (30 μg/ml), and ampicillin (50 μg/ml). Since the usual ampicillin enrichment for auxotrophs could not be used, we used another inhibitor of cell wall synthesis, cycloserine, for the counterselection step. After overnight growth, the bacteria were subcultured to the same medium, containing guanosine (50 μg/ml) instead of hypoxanthine, and when the culture reached mid-log phase, cycloserine (100 μg/ml) was added and followed until lysis. This counterselection was repeated, and the cycloserine lysate was plated on L agar containing

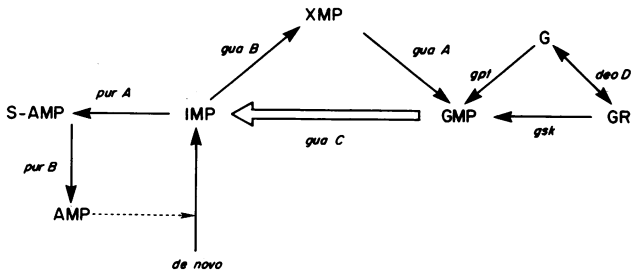

FIG. 1. Salvage and interconversion pathways of purine derivatives. The reactions are identified by their gene designations. Abbreviations: G, guanine; GR, guanosine; S-AMP, adenylosuccinate. The dotted line refers to the histidine pathway whereby AMP can enter the de novo pathway via amino-imidazole carboxamide ribonucleotide.

ampicillin. Colonies were scored for growth on hypoxanthine and guanosine. Those that grew on hypoxanthine but not guanosine were considered to be potential *guaC* mutants.

Enzyme assays. Beta-galactosidase activity was measured in toluenized cells as described by Miller (10). The samples (usually 0.5 ml) were either from mid-log-phase cultures (optical density at 600 nm, ca. 0.5 to 0.8) or drawn at various times during the time course studies. Specific activity is expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of protein.

GMP reductase was assayed in cell extracts from sonicated samples as described by Garber et al. (4). Specific activity was recorded as nanomoles of substrate consumed per minute per milligram of protein.

RESULTS

Properties and beta-galactosidase expression of *guaC-lac* fusions. All of the potential *guaC* mutants obtained could use adenine or hypoxanthine as the purine source, but not guanine, guanosine, or xanthine. They were first shown to lack GMP reductase activity and then characterized for their Lac phenotype on MacConkey-lactose media. Candidates for control of beta-galactosidase by guanosine were those that were strongly Lac$^+$ (bright red) in the presence of guanosine (100 μg/ml) and pink in its absence. Those that were colorless on both media were deemed to be Mu insertions in the opposite orientation and were not further pursued. None of the mutants was strongly Lac$^+$ in the absence of guanosine.

A total of six candidates were tested for beta-galactosidase activity in the presence of various purines during growth. In all cases, guanosine consistently induced the expression of beta-galactosidase four to eight times over that obtained by growth in hypoxanthine or adenine. Induction by guanosine was consistently prevented by adenine. One strain was retained for further study and designated GP303. It was tested for a single insertion by transducing it to *guaC*$^+$ with P1 (TX40). The resulting transductions were all Lac$^-$ and ampicillin sensitive, indicating a single insertion of Mu d1(*lac* Ap^r) into *guaC*. A temperature-resistant, Mu-sensitive derivative was obtained to prevent Mu transpositions to other sites. Repeated monitoring of this strain throughout this study indicated no evidence of Mu transpositions.

A *purE*$^+$ derivative of strain GP303 (strain GP308) was used to study induction kinetics. Figure 2 presents a differential plot of beta-galactosidase induction by guanosine. The constitutive rate in the absence of purines was not altered by the addition of hypoxanthine or adenine. The preliminary demonstration that adenine strongly prevents induction by guanine is also shown. When adenine was added after

TABLE 1. Bacterial strains

Strain	Relevant markers	Source or references
TX40	*ara* Δ*lac*	18
TX257	*ara* Δ*lac purE210*	J. M. Smith
GP303	TX257 *guaC225*::Mu d1(*lac* Ap^r)	This study
GP308	GP303 *purE*$^+$	Transduction P1 (TX40) × GP303
GP310	GP303 Tn*10*::*purE* Δ(*gpt-pro-lac*)	Hfr mating NK6051 × GP303
GP311	GP303 *deoD47*	Spontaneous mutant
GP313	GP303(pLC37–40) (ColE1 *guaC*$^+$)	JA200 (37-40) × GP303
NK6051	HfrH Δ(*gpt-pro-lac*) Tn*10*::*purE*	N. Kleckner
JA200(pLC37–40)	JA200/(ColE1 *guaC*$^+$)	3
MAL103	Mu *cts* d1(*lac* Ap^r) Mu *cts* Δ(*pro-lac*) *rpsL*	2

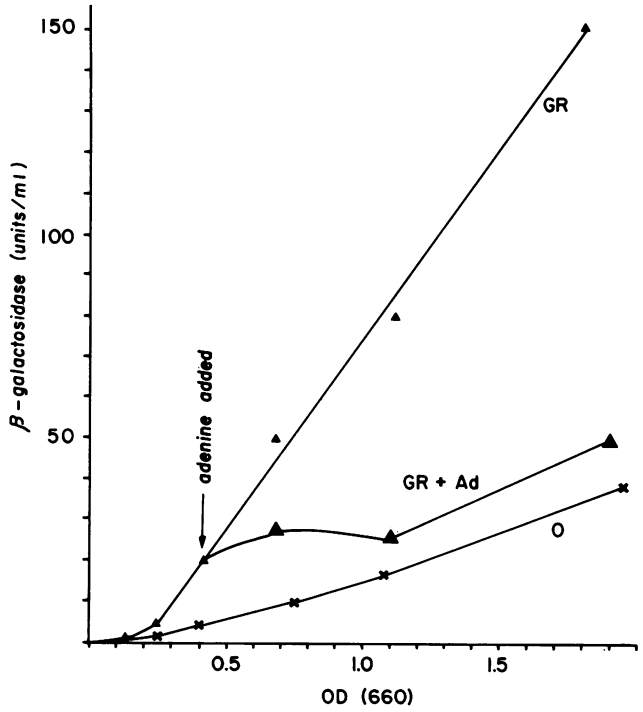

FIG. 2. Effect of purine compounds on the synthesis of beta-galactosidase in strain GP308. Units of beta-galactosidase per milliliter of culture is plotted against growth as measured by absorbance (OD) at 600 nm. An overnight culture growing in minimal salts-glucose medium was inoculated into fresh medium with and without guanosine (GR) (100 μg/ml) to give an initial turbidity of about 0.1. Adenine (Ad) (50 μg/ml) was added to one of two guanosine tubes after about two generations. The presence of adenine or hypoxanthine from the beginning gave curves exactly like that obtained in the absence of any purine (0).

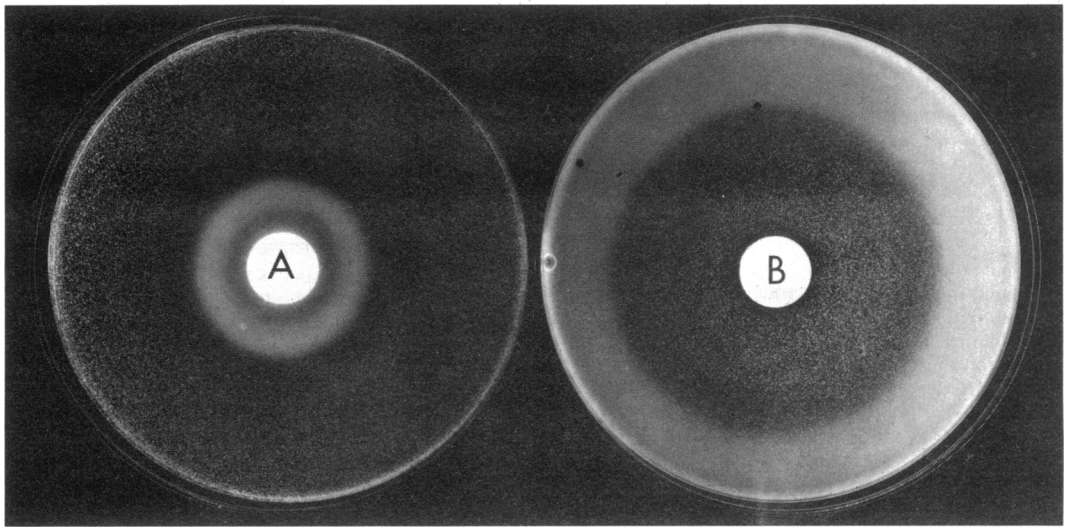

FIG. 3. Effect of purines on the growth of strain GP308 with lactose as the sole carbon source. (A) The medium contains only lactose, and the disk contains guanosine. (B) The medium contains lactose and guanosine (100 μg/ml), and the disk contains adenine. Similar results were obtained with strain GP313.

induction by guanosine was under way, it drastically abolished continued induction and reduced the formation of the enzyme to the constitutive level. This antagonistic effect of adenine on induction by guanosine is shown in Fig. 3. Here, growth of strain GP308 (or GP313) does not occur on lactose as the sole carbon source unless guanosine is added and this growth is strongly inhibited by adenine. Figure 4 shows the dose response with guanosine. Near-maximum effect is obtained with 200 μg/ml.

Nature of the in vivo inducer. To determine the nature of the in vivo inducer, we made use of genetic blocks in the guanine-guanosine-GMP salvage pathway (Fig. 1) by introducing them into strain GP303. The direct conversion of guanine to GMP by guanine phosphoribosyltransferase was blocked by a *gpt* mutation (strain GP310), and the reversible conversion of guanosine to guanine by purine nucleoside phosphorylase (PUP) was prevented by a *deoD* block (strain GP311). Figure 5 compares the induction kinetics in these strains with that in the parent (strain GP303). It is immediately evident that the *gpt* block in strain GP310 drastically reduces the induction by guanine to that of a near-constitutive level. In this strain, guanosine shows a biphasic response whereby the initial induction, presumably via the *gsk* pathway (6), is followed by a reduction to the constitutive level. This is apparently due to the known ability of purine nucleosides to induce PUP (11, 16). When this happens, the guanosine is rapidly lost to the nonrecoverable guanine pool. This biphasic effect of guanosine is also seen on the parent strain (gpt^+) but in an opposite manner. In this

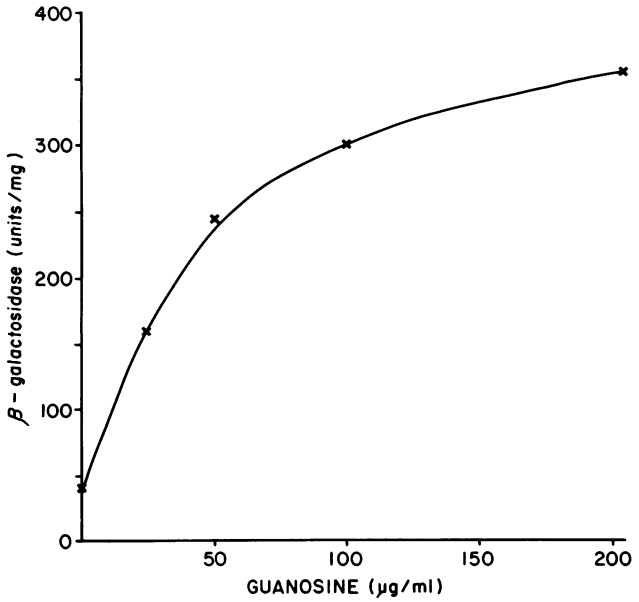

FIG. 4. Guanosine dose-response curve on the induction of beta-galactosidase in strain GP308. Measurements were made on mid-log cultures at an optical density at 600 nm of 0.6 to 0.8.

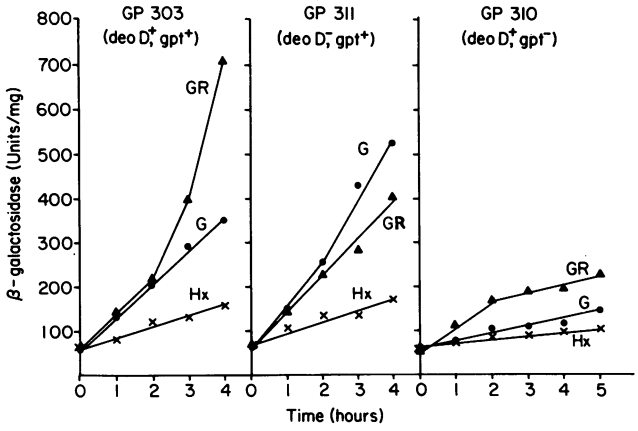

FIG. 5. Effect of *gpt* and *deoD* mutations on the induction of beta-galactosidase by guanosine (GR) and guanine (G). Since these strains were $purE^-$, hypoxanthine (Hx) was present in all media at a concentration of 10 μg/ml to provide growth. Concentrations of guanosine and guanine were 100 and 30 μg/ml, respectively.

TABLE 2. Effect of glutamine and related analogs on beta-galactosidase activity in strain GP303

Addition (μg/ml)	Sp act (nmol/min per mg of protein) of beta-galactosidase[a]	
	−Glutamine	+Glutamine
None	60 (1.0)	63 (1.0)
Guanosine (100)	238 (4.0)	127 (2.0)
DON (10)	192 (3.2)	56 (0.9)
MSO (50)[b]	181 (3.0)	73 (1.2)

[a] The media contained limiting hypoxanthine (10 μg/ml) to permit growth, and beta-galactosidase was measured in mid-log cultures without (−) and with (+) glutamine (500 μg/ml) plus the other additions. Numbers in parentheses refer to fold induction.

[b] MSO, L-Methionine-DL-sulfoximine.

case, the induction of PUP by guanosine would increase the rate of induction by rapidly shunting guanosine into the more efficient *gpt* pathway. This idea is substantiated in the PUP-deficient mutant (strain GP311), through which the biphasic effect of guanosine is abolished and the initial rate continues with the less efficient guanosine kinase. Guanine is more efficient as an inducer in this strain since it is not lost to guanosine in the absence of PUP. These considerations allow us to conclude that the inducer of GMP reductase is a guanine nucleotide, most likely its substrate GMP, though GDP and GTP cannot be ruled out by these experiments.

Effect of glutamine and its analogs. A previous report from our laboratory has implicated glutamine as a negative effector in the regulation of GMP reductase (4). Either glutamine starvation or interference with glutamine utilization by competitive analogs (e.g., DON) markedly increased the activity of GMP reductase. It was not known whether this effect operated at the level of transcription of the *guaC* gene or at a later translational, or even posttranslational, step. The regulation of beta-galactosidase activity by the *guaC-lac* system would provide a more direct assessment of the role of the transcriptional regulatory elements which are proximal to the insertion. Glutamine starvation was obtained with L-methionine-DL-sulfoximine, which inhibits glutamine synthetase and creates an intracellular glutamine deficiency (19). DON is a competitive analog of glutamine at glutamine-utilizing sites such as glutamine amidotransferases (5). Table 2 shows the effects of both L-methionine-DL-sulfoximine and DON on the activity of beta-galactosidase in strain GP303. Both analogs have an inducing effect which is completely neutralized by glutamine. Glutamine also had some effect on the induction by guanosine. Figure 6 depicts a time course experiment in which induction by DON is almost as efficient as that by guanosine. DON had no effect on the activity of beta-galactosidase in other *pur-lac* fusion strains such as *purE-lac* and *purF-lac* (data not shown). These results show that the glutamine effect that was originally described in *S. typhimurium* also occurs in *E. coli* and strongly implies that glutamine is a negative effector in the transcription of *guaC*.

GMP reductase is also a deaminating enzyme, and as such it might belong to the class of nitrogen-metabolizing enzymes that are known to be regulated by nitrogen repression and positively controlled by glutamine synthetase (20). The increase of GMP reductase during glutamine starvation might therefore be a consequence of such control. We therefore examined the levels of GMP reductase in strain TX40 and beta-galactosidase in strain GP308 when grown in media supplied with limiting and excess nitrogen under the conditions described by Pahel et al. (14). Glutamine synthetase was simultaneously assayed in all extracts. The ratio of activities in nitrogen-limiting to those in nitrogen-excess media was 1.1 to 1.4 for both GMP reductase and beta-galactosidase, as compared with 8.5 to 10.2 for glutamine synthetase. Thus, the synthesis of GMP reductase is not altered by the amount of nitrogen in the media or controlled by glutamine synthetase. Similar results were previously reported for *S. typhimurium* (4) in which GMP reductase was induced by glutamine starvation in a *glnA* mutant devoid of glutamine synthetase and not induced by nitrogen starvation. Furthermore, neither *E. coli* nor *S. typhimurium* is able to grow when guanosine is supplied as the sole nitrogen source. We must therefore conclude that the induction of GMP reductase by glutamine limitation is not related to the regulation of other nitrogen-assimilating enzymes.

Regulatory mutants. A search for regulatory mutants was made by isolating colonies that appeared when strain GP303 was plated on media containing adenine and lactose as sole carbon sources. Several were obtained, and their beta-galactosidase activities are shown in Table 3. All mutants showed a marked increase in their constitutive levels, ranging from 5- to 10-fold higher than that of the parent. These levels were higher than the induced level of the parent and were only slightly increased (one- to twofold) by growth in

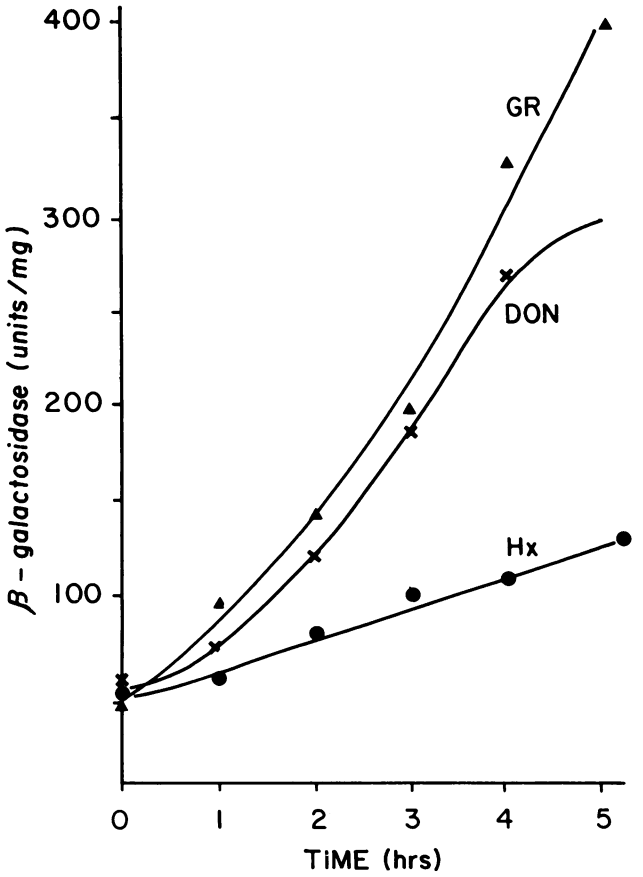

FIG. 6. Effect of diazo-oxo-norleucine on the induction of beta-galactosidase in strain GP303. Conditions were as described in the legend to Fig. 5. Hypoxanthine (Hx) (10 μg/ml), guanosine (GR) (100 μg/ml), and diazo-oxo-norleucine (DON) (10 μg/ml) were present in the medium.

guanosine. To determine the effect of the regulatory defect on the expression of GMP reductase, and at the same time its possible linkage to *guaC*, representative strains were converted to *guaC*$^+$ by transduction. These *guaC*$^+$ transductants became ampicillin sensitive and Lac$^-$, indicating that their constitutive Lac$^+$ property was not due to a secondary insertion at another site. The regulation of GMP reductase activity in these strains was no different than that in the original parent (Table 4). The abolition of the regulatory defect by the *guaC*$^+$ transduction implies that the altered regulatory element is closely linked to the *guaC* locus, i.e., either a *cis*-active operator type or a closely linked repressor type.

Table 4 also shows GMP reductase activities when the *guaC*$^+$ gene was introduced by a plasmid (pLC37-40). The increased activity as compared with the chromosomal *guaC*$^+$ is apparently a plasmid copy number dosage effect. The constitutive activities of the regulatory mutants were slightly higher than that of the parent but did not exceed its induced level as in the case of beta-galactosidase (Table 3). The presence of pLC37-40 did not alter beta-galactosidase activity in these strains (data not shown), thus ruling out a possible closely linked *trans*-active element.

Further confirmation of the close linkage and *cis*-effect of the regulatory mutants was genetically obtained by transducing a Mu lysogen of TX257 to ampicillin resistance with a P1 lysate grown on the regulatory mutants. All Ap^r transductants were *guaC*$^-$ and constitutively Lac$^+$, i.e., the phenotype of the donors. Thus, the regulatory mutants are apparently defective in a *cis*-active, operator-like regulatory element closely linked to *guaC*. As yet, no unlinked *trans*-active mutation has been obtained.

DISCUSSION

Soon after GMP reductase was discovered in *E. coli* (8), it became evident that its activity was increased by growth in the presence of guanine (7, 13). This effect was more accurately analyzed in *S. typhimurium* with the additional implication that induction by guanosine compounds was modulated by adenine derivatives (1). In this study with *guaC-lac* fusions in *E. coli* in which the expression of beta-galactosidase is under control of the regulatory elements of the *guaC* gene, we have provided stronger evidence that the guanine-adenine interplay occurs at the transcriptional level. The use of selective blocks in the interconversion of guanine compounds indicates that a nucleotide, probably GMP, is the inducer. An adenine derivative, probably AMP, acts as an antiinducer (15) in that it not only prevents but also reverses the induction by GMP. Thus, GMP reductase is induced by its substrate only when there is a deficiency of the eventual end product, AMP.

TABLE 3. Beta-galactosidase activities of regulatory mutants of strain GP303

Strain	Sp act (nmol/min per mg of protein) of beta-galactosidase[a]	
	−GR (constitutive)	+GR (induced)
GP303	67 (1.0)	365 (5.5)
R-1	489 (7.3)	1,035 (2.1)
R-3	722 (10.8)	935 (1.3)
R-4	413 (6.2)	675 (1.6)
R-6	546 (8.2)	841 (1.5)
R-7	453 (6.8)	741 (1.6)
R-8	657 (9.9)	674 (1.1)
R-9	383 (5.7)	726 (1.9)
R-10	357 (5.3)	738 (2.1)

[a] The media contained limiting hypoxanthine (10 μg/ml) to permit growth, and beta-galactosidase was measured without (−GR) or with (+GR) the addition of guanosine (100 μg/ml). The numbers in parentheses are relative constitutive levels as compared with that in the parent or fold induction as compared with constitutive levels (+GR/−GR).

TABLE 4. GMP reductase activities of *guaC*$^+$ derivatives of regulatory mutants of strain GP303

Strain	Sp act (nmol/min per mg of protein) of GMP reductase[a]					
	guaC$^+$ Transductants			pLC37-40 (ColE1 *guaC*$^+$)		
	−GR	+GR	Ratio (+GR/−GR)	−GR	+GR	Ratio (+GR/−GR)
GP303	1.1	6.2	5.7	3.6	18.2	5.2
R-1	0.8	3.9	4.9	5.0	13.0	2.6
R-3	1.0	4.8	4.8	4.8	10.9	2.3
R-6	0.9	3.6	4.0	7.6	15.3	2.0

[a] Conditions and abbreviations are as described in Table 3, footnote *a*.

GMP reductase plays a unique role in the IMP-GMP cycle (Fig. 1). The conversion of IMP to GMP requires the two enzymes of the *guaBA* operon, and the return of GMP to IMP is mediated by GMP reductase. The direction in which the cycle runs is regulated by a unique dual-reciprocal control mechanism. As indicated in this study, GMP reductase is most likely induced by GMP, and this is counteracted by an adenine derivative. Conversely, the *guaBA* operon is known to be repressed by a guanine derivative and derepressed in the presence of adenine (9, 12). We have also examined the control of expression of beta-galactosidase in a *guaB-lac* fusion and have found this same type of reciprocal control (unpublished observations). Thus, when the cell is starved for GMP in the presence of adenine, one arm of the cycle (*guaBA*) is induced to provide GMP via IMP. Conversely, when the cell is starved for AMP in the presence of guanine, then the other arm (*guaC*) is induced to allow efficient conversion of GMP to AMP via IMP.

Another complication in understanding the mechanisms which regulate GMP reductase is the apparent role of glutamine as an effector. This may be related to a secondary role of the reductase in providing a nitrogen source via its deaminating activity. Evidence has been presented that the increase in activity during glutamine starvation is apparently not related to this secondary role. The striking effect of DON on the induction of beta-galactosidase in the *guaC-lac* fusion strain confirms the previous results with *S. typhimurium* and in addition strengthens the argument that it functions during transcription rather than translation. The hypothesis that is compatible with the data is that glutamine, or a product of its metabolism, functions as a negative effector in the regulation of GMP reductase in both *S. typhimurium* and *E. coli*. Any intervention that would create a glutamine deficiency at the regulatory site would counteract this negative effect and increase transcription. This could operate at a competing site on a regulatory protein.

The eventual resolution of the exact nature of the regulatory elements that control expression of *guaC* will require additional isolation and characterization of regulatory mutants. The mutants described here were isolated on the basis of their resistance to adenine inhibition in the presence of lactose as the sole carbon source. As would be expected, they were all strongly constitutive for beta-galactosidase.

Their effect on GMP reductase provided by substituted *guaC*$^+$ genes indicates that they are probably due to mutations in a closely linked *cis*-active element, either an operator locus or an attenuation leader sequence. The existence of an unequivocal repressor-type element has not as yet been revealed.

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