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Regulation of Enzyme Synthesis by the Glutamine Synthetase of *Salmonella typhimurium*: a Factor in Addition to Glutamine Synthetase Is Required for Activation of Enzyme Formation

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In *Klebsiella aerogenes* but not in *Salmonella typhimurium* glutamine synthetase can function during nitrogen-limited growth to increase the rate of synthesis of histidase from the *hut* genes of *S. typhimurium* 15-59 (*hut*S.15-59). Formation of proline oxidase is also not increased in nitrogen-limited cultures of *S. typhimurium*. However, in hybrid strains of *Escherichia coli* or *K. aerogenes*, the glutamine synthetase of *S. typhimurium* activates synthesis of histidase from the *hut*S.15-59 genes. Apparently, glutamine synthetase is necessary but not sufficient for activation of transcription of the *hut* genes; another factor must also be present. This factor is active in both *K. aerogenes* and *E. coli* but is missing or altered in *S. typhimurium*.

The amino acid histidine can be degraded to glutamate, ammonia, and formamide in the enteric bacteria, *Salmonella typhimurium* 15-59 and *Klebsiella aerogenes*, through the action of four enzymes, products of the genes of the two *hut* operons (9, 20). In each organism, expression of these *hut* genes is regulated by specific and general control elements. Specific control is mediated by the *hutC* product, a repressor (10). In the absence of this repressor, synthesis of the Hut enzymes is subject to other more general control mechanisms.

When HutC$^-$ cells of *S. typhimurium* 15-59 or *K. aerogenes* are grown in a medium containing a good source of nitrogen such as ammonia, the rate of *hut* gene expression is inversely proportional to the available supply of energy. In the case of the *hut* genes, as with other catabolite-sensitive operons, physiological (18) and biochemical (28) experiments have indicated that transcription requires the presence of a positive effector; a complex between cyclic adenosine 3',5'-monophosphate and a cyclic adenosine 3',5'-monophosphate-binding protein can serve this function. It is generally thought that a reduction in the intracellular level of cyclic adenosine 3',5'-monophosphate is responsible for catabolite repression.

However, if cells of *K. aerogenes* are grown in a carbon-rich medium containing a poor source of nitrogen, a condition of severe catabolite repression, the Hut enzymes are synthesized at a high rate. This "escape" from catabolite repression is even observed in strains of *K. aerogenes* unable to synthesize cyclic adenosine 3',5'-monophosphate (18). Recently, genetic (17, 23), physiological (4, 6, 23) and biochemical (28) studies have indicated that the regulatory element activating *hut* gene expression under conditions of nitrogen limitation is glutamine synthetase (GS), a key enzyme in nitrogen metabolism. Cells of *K. aerogenes* grown under these conditions contain high levels of a biosynthetically active form of GS. When these cells are grown in medium containing glucose and high levels of ammonia, where catabolite repression is observed, they synthesize less GS, which is primarily in a biosynthetically inactive form caused by the covalent attachment of an adenylyl group (18, 20). Studies with a purified transcription system indicated that only the unmodified form of the GS protein effectively increased *hut* gene transcription (28).

GS is also implicated in the regulation of synthesis of the proline degrading enzymes (*put*), urease, several enzymes involved in arginine degradation, tryptophan transaminase and asparaginase in *K. aerogenes* (15), and nitrogenase in *Klebsiella pneumoniae* (24). GS also appears to inhibit the synthesis of glutamate dehydrogenase in *K. aerogenes* and *K. pneumoniae*.

In contrast to the situation in *K. aerogenes*, when the cells of *S. typhimurium* 15-59 are grown under conditions of nitrogen limitation,

hut gene expression does not escape from catabolite repression. However, Prival and Magasanik (18) have shown that histidase is synthesized from the *hut* genes of *S. typhimurium* 15-59 (*hut*S.15-59) genes at a high rate during nitrogen-limited growth when these genes are present in the cytoplasm of *K. aerogenes*. In addition, the GS of *K. aerogenes* will activate transcription of the *hut*S.15-59 genes in a purified transcription system (28). The simplest explanation for these observations is that the GS molecule native to *S. typhimurium* 15-59 cannot function to activate transcription at *hut* promoters. No studies on the GS of *S. typhimurium* 15-59 have been published.

Recently, the GS of a different strain of *S. typhimurium*, LT-2, has been examined (3, 13, 26). It was reported that in *S. typhimurium* LT-2, GS was subject to adenylylation and its synthesis increased as the availability of the nitrogen source decreased. These findings are very similar to those for *K. aerogenes* (17, 23) and *Escherichia coli* W (19, 21). However, *hut* gene expression has not been studied extensively in *S. typhimurium* LT-2, since this strain normally carries a promoter mutation that effectively eliminates expression of one *hut* operon (16).

It was, therefore, of interest to examine the regulation of GS in *S. typhimurium* 15-59 and *hut* gene expression in *S. typhimurium* LT-2. A common transducing phage P376 has been used to transfer the *hut* genes from *S. typhimurium* 15-59 to *S. typhimurium* LT-2. Intergeneric transduction with phage P1 has also allowed the isolation of strains of *K. aerogenes* and *E. coli* K-12 containing the *hut* genes of *S. typhimurium* 15-59 and $glnA_s$, the structural gene for the GS of *S. typhimurium* LT-2, individually or in combination. In this paper, we report the results of studies on *hut* gene expression in these hybrid bacteria.

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MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are described in Table 1. The intergeneric transductions were performed by the procedure described previously (29). The presence of *S. typhimurium* GS in the hybrid strains of *K. aerogenes* was confirmed using sodium dodecyl sulfate-polyacrylamide gels, since its mobility is different from that of the *K. aerogenes* enzyme (S. L. Streicher, manuscript in preparation). Mutagenesis and the isolation of glutamine auxotrophs were described previously (22). Episomal transfer was accomplished by the plate-mating procedure previously described (22).

Media. The growth media used were described previously (17). The glucose minimal medium was W salts containing 0.4% glucose and the indicated nitrogen source at a final concentration of 0.2%, unless otherwise noted in the tables.

Cultivation of bacteria. The conditions used for bacterial growth were as described previously (23). Similarly, crude extracts were prepared by sonic oscillation (17).

Enzyme assays. GS was assayed for γ-glutamyl transferase (γGT) activity according to a previous method (1, 3). GS biosynthetic activity was determined using the forward assay described by Bender et al. (1). Histidase, proline oxidase, and β-galactosidase were assayed by the procedures of Prival and Magasanik (18). Protein concentrations were determined by the method of Lowry et al. with crystalline bovine serum albumin as the standard (14).

RESULTS

Measurement of GS protein in *S. typhimurium* and *E. coli* K-12. The level of total GS protein in the enteric bacteria has traditionally been measured with the γGT assay (21, 26), in which both the adenylylated and nonadenylylated forms of the enzyme are active. With GS from *E. coli* W or *K. aerogenes*, the enzymatic activity of the two forms of the GS protein have different pH profiles in this reaction; therefore, the "transferase activity" must be assayed at the pH where each form of the enzyme has equal activity (isoactivity point) in order to obtain a valid comparison of the relative levels of total GS protein in cells containing different amounts of adenylylated and nonadenylylated enzyme. The isoactivity points for GS from *E. coli* W (21) and *K. aerogenes* (1) are reported as pH 7.15 and pH 7.55, respectively. No information on the isoactivity point of GS from *S. typhimurium* 15-59 or *E. coli* K-12 has been reported, although it appears that the GS from each of these organisms is subject to adenylylation (26; Streicher, unpublished data).

To determine the isoactivity point of GS from these enteric bacteria, we grew a culture of each organism under conditions of nitrogen limitation (where nonadenylylated GS is the predominant form) and, when the cell density reached about 4×10^8 cells/ml, split the culture into two equal parts and added excess ammonium sulfate to one portion to stimulate adenylylation. The cells were harvested 5 min later, with conditions which have been shown to minimize changes in the adenylylation state of the GS from *K. aerogenes* (1). We then examined the enzymatic activity of the GS in these cells by the γGT assay at a series of pH values. In all

TABLE 1. *Bacterial strains*

Strain	Origin[a]			Relevant genotype	Source
	Cyto-plasm	*hut*	*glnA*		
Klebsiella aerogenes					
MK9000	K	K	K	*hutC515*	B. Magasanik
MK9011	K	K	–[b]	*hutC515 glnA6*	B. Magasanik
KT2401	K	S.15–59	S.LT-2	*F'gal hutC7 bio/[gal hut bio]ΔglnA,*$^+$	This laboratory
KT2601	K	S.15–59	–	*F'gal hutC7 bio/[gal hut bio]ΔglnA6*	This laboratory
KT2001	K	K	S.LT-2	*hutC515 glnA,*$^+$	This laboratory
Salmonella typhimurium					
NE7	S.15–59	S.15–59	S.15–59	*hutC7*	B. Magasanik
NV1	S.LT-2	S.15–59	S.LT-2	*hutC7*	B. Magasanik
AR65	S.15–59	S.15–59	S.15–59	*putC3*	W. Brill
Escherichia coli K-12					
ET2201	E	S.15–59	–	*hutC7 glnA202*	This laboratory
ET2202	E	S.15–59	S.LT-2	*hutC7 glnA,*$^+$	This laboratory

[a] Abbreviations: K = *K. aerogenes*; S.15–59 = *S. typhimurium* 15–59; S.LT-2 = *S. typhimurium* LT-2; E = *E. coli* K-12.
[b] –,

cases (Fig. 1), the pH activity profile was very different with enzyme from cells grown only under conditions of nitrogen limitation as well as from cells exposed to "ammonia shock." We observed both a dramatic decrease in the GS biosynthetic activity in the cultures subjected to ammonia shock and an increased inhibition by Mg^{2+} of the γGT activity (data not shown). These results are similar to those obtained in experiments with *E. coli* W (11) and *K. aerogenes* (1) and, therefore, analogously extend the evidence that the enzymatic activity of the GS in cells of *E. coli* K-12 and *S. typhimurium* is subject to regulation by adenylylation. From these experiments we conclude that for adenylylated and nonadenylylated GS of *E. coli* K-12, *S. typhimurium* LT-2, and *S. typhimurium* 15-59 the isoactivity point in the γGT assay occurs at pH 7.3.

Formation of GS and histidase in *S. typhimurium* under conditions of nitrogen limitation. We next examined in both *S. typhimurium* 15-59 and *S. typhimurium* LT-2 the regulation of synthesis of GS and histidase. The data in Table 2 show the levels of these enzymes in cultures, where the rate of growth was limited by the only available nitrogen source, glutamate, and in cultures grown for many generations in the presence of excess ammonia. It is immediately apparent that cells of *S. typhimurium* 15-59 are capable of regulating the synthesis of the GS protein in response to the availability of nitrogen; in nitrogen-limited cultures, the level of GS transferase activity is almost 11-fold greater than in similar culture grown with excess ammonia in the medium. Nevertheless, no derepression of histidase was

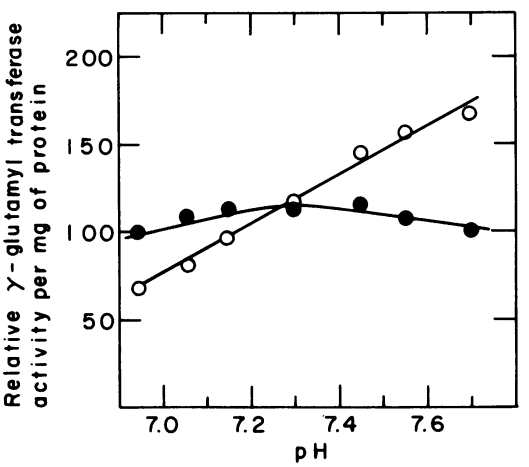

FIG. 1. *Determination of the isoactivity pH for the GS of Salmonella typhimurium 15-59. A 10-ml culture of strain NE7 was grown at 30°C, with shaking, in glucose minimal medium containing glutamine as the sole source of nitrogen. When the cell density reached about 4 × 10⁸ cells/ml (100 Klett units), 5 ml of culture was removed and added to a prewarmed flask containing ammonium sulfate to give a final concentration of 0.2%. After 5 min of shaking at 30°C, hexadecyltrimethylammonium bromide (CTAB) was added to both cultures, and the cells were harvested and assayed as described by Bender et al. (1). Symbols: ○, glucose-glutamine culture; ●, ammonia "shocked" culture. Similar data were obtained with cultures from strains of E. coli K-12 and S. typhimurium LT-2.*

observed. Similar results were obtained in experiments with *S. typhimurium* LT-2. Although the level of GS protein in this strain was almost sevenfold higher in nitrogen-lim-

ited cultures then in those grown in the presence of excess ammonia, the amount of histidase synthesized from the *hut*S.15-59 genes was actually lower in the cells grown with glutamate as the sole source of nitrogen.

Biosynthetic activity of *S. typhimurium* GS. Since activation of *hut* gene transcription by the GS of *K. aerogenes* normally requires the biosynthetically active form of the enzyme (which corresponds to low states of adenylylation in *K. aerogenes*; 28), it seems possible that the low rate of *hut* gene expression in nitrogen-limited cultures of *S. typhimurium* might result from insufficient levels of biosynthetically active GS in these cells. However, we found that both the γGT activity and biosynthetic capacity of the GS in nitrogen-limited cultures of *S. typhimurium* LT-2 and *S. typhimurium* 15-59 are comparable to these activities in cultures of *K. aerogenes* grown under the same conditions (Table 3, compare lines 2, 4, 6, 8, and 10). Similarly, no significant differences were detected, by either assay, in the GS activity of cultures of each of these organisms grown for many generations in the presence of excess ammonia (Table 3, compare lines 1, 3, 5, 7, and 9).

Regulatory activity of *S. typhimurium* GS in *K. aerogenes* and *E. coli* K-12. The results presented above suggest that the difference in the rate of *hut* gene expression in nitrogen-limited cultures of *S. typhimurium* and *K. aerogenes* is not caused by a quantitive difference in the level of nonadenylylated GS in these cells. The lack of GS-mediated *hut* gene expression in *S. typhimurium* cannot be attributed to a defect in the *hut*S.15-59 promoters, since the

TABLE 2. *Effect of nitrogen source on the level of histidase and GS in S. typhimurium strains*

Strain	Origin[a]			Nitrogen source[b]	Enzyme activity[c]	
	Cytoplasm	*hut*	*glnA*		Histidase (μmol/min per mg of protein	GS (μmol/min per mg of protein)
1. NE7	S.15-59	S.15-59	S.15-59	N Glt	21	0.09
2.				Glt	17	0.98
3. NV1	S.LT-2	S.15-59	S.LT-2	N Glt	23	0.17
4.				Glt	11	1.16

[a] Abbreviations: S.15-59 is *S. typhimurium* 15-59; S.LT-2 is *S. typhimurium* LT-2.

[b] Strains were grown at 37°C in glucose minimal medium containing the indicated nitrogen sources (N, ammonium sulfate; Glt, glutamate), harvested when the cell density reached 100 Klett units, concentrated 10-fold, and assayed as described in Materials and Methods.

[c] Enzyme activity is expressed as units per milligram. Glutamine synthetase activity was measured by the γGT assay.

TABLE 3. *Effect of the nitrogen source on GS transferase and biosynthetic activity in strains of S. typhimurium and K. aerogenes*[a]

Strain	Origin[b]			Nitrogen source	GS	
	Cytoplasm	*hut*	*glnA*		Transferase activity (μmol/min per mg of protein	Biosynthetic activity (μmol/min per mg of protein)
1. MK9000	K	K	K	N Gln	0.22	0.07
2.				Gln	1.29	0.47
3. NE7	S.15-59	S.15-59	S.15-59	NGln	0.10	0.05
4.				Gln	1.45	0.31
5.				N Glt	0.09	0.07
6.				Glt	1.73	0.50
7. NV1	S.LT-2	S.15-59	S.LT-2	N Gln	0.08	0.10
8.				Gln	1.61	0.55
9.				N Glt	0.10	0.11
10.				Glt	1.54	0.42

[a] Strains were grown at 30°C in glucose minimal medium containing the indicated nitrogen source at a final concentration of 0.2%. N, Ammonium sulfate; Gln, glutamine; Glt, glutamate. Cells were harvested and assayed by the procedures of Bender et al. (1).

[b] Abbreviations: K = *K. aerogenes*; S.15-59 = *S. typhimurium* 15-59; S.LT-2 = *S. typhimurium* LT-2.

GS of *K. aerogenes* does activate transcription of these genes. Therefore, it seemed likely that a qualitative difference in the structure of the GS native to *S. typhimurium* and *K. aerogenes* was responsible for the difference in GS-mediated transcription in these two organisms. To investigate this possibility we examined the physiology of strains of *K. aerogenes* and *E. coli* that contained the $glnA_s$ gene of *S. typhimurium* LT-2.

Clearly, from the data presented in Table 4, when a hybrid strain of *K. aerogenes*, KT2001, carrying the $glnA_s$ gene, was grown under conditions of nitrogen limitation, the levels of histidase increased to the same extent as in a wild-type strain of *K. aerogenes*, MK9000. In addition (data not shown), the synthesis of glutamate dehydrogenase was repressed in strain KT2001 under conditions of nitrogen limitation. In the glutamine auxotroph (MK9011), from which strain KT2001 was derived, histidase synthesis was not activated, nor was synthesis of glutamate dehydrogenase repressed under conditions of nitrogen-limited growth.

This experiment suggests that the GS of *S. typhimurium* can regulate transcription. However, the *S. typhimurium* GS may only activate transcription from certain promoters. We examined this possibility by transferring into *K. aerogenes* and *E. coli* K-12 both the $glnA_s$ of *S. typhimurium* LT-2 and the *hut* genes of *S. typhimurium* 15-59. It can be seen in Table 5 that in these hybrid bacteria, *K. aerogenes* strain KT2401, and in *E. coli* strain ET2202, histidase levels increased when the cells were nitrogen limited. β-Galactosidase, an enzyme not under GS control, was not derepressed in strains ET2201 and ET2202 under conditions of nitrogen limitation. The strains devoid of GS KT2601 and ET2201 did not have increased

TABLE 4. *Regulation of K. aerogenes hut by S. typhimurium GS*

Strain	Origin[a]			Nitrogen source[b]	Enzyme activity	
	Cytoplasm	*hut*	*glnA*		Histidase (μmol/min per mg of protein)	GS (μmol/min per mg of protein)
1. MK9000	K	K	K	N Gln	30	0.20
2.				Gln	300	1.00
3. KT2001	K	K	S.LT-2	N Gln	65	0.10
4.				Gln	391	0.98

[a] Abbreviations: K = *K. aerogenes;* S.LT-2 = *S. typhimurium* LT-2.

[b] Strains were grown at 30°C in glucose minimal medium containing the indicated nitrogen sources (N, ammonium sulfate; Gln, glutamine), harvested when the cell density reached 100 Klett units, and assayed as described in Materials and Methods.

TABLE 5. *Regulation of S. typhimurium hut by S. typhimurium GS in the cytoplasm of K. aerogenes and E. coli*

Strain	Origin[a]			Nitrogen source[b]	Enzyme activity		
	Cytoplasm	*hut*	*glnA*		Histidase (μmol/min per mg of protein)	GS (μmol/min per mg of protein)	β-Galactosidase (μmol/min per mg of protein)
1. KT2401	K	S.15-59	S.LT-2	N Glt	6	0.07	–[c]
2.				Glt	153	0.83	–
3. KT2601	K	S.15-59	–[d]	N Gln	10	0.01	–
4.				Gln	12	<0.01	–
5. ET2202	E	S.15-59	S.LT-2	N Gln	21	0.11	4.7
6.				Gln	77	0.82	2.6
7. ET2201	E	S.15-59	–[d]	N Gln	8	<0.01	1.9
8.				Gln	8	<0.01	1.5

[a] Abbreviations: K = *K. aerogenes;* S.15-59 = *S. typhimurium* 15-59; S.LT-2 = *S. typhimurium* LT-2; E = *E. coli* K-12.

[b] Strains were grown at 30°C in glucose minimal medium containing the indicated nitrogen source (N, ammonium sulfate; Glt, glutamate; Gln, glutamine), harvested when the cell density reached 100 Klett units, and assayed as described in Materials and Methods.

[c] –, Not done.

[d] Strain KT2601 contains the *glnA6* mutation. Strain ET2201 contains the *glnA202* mutation.

levels of histidase.

The *glnA* and *hut* regions of *S. typhimurium* were not altered by their passage through a foreign cytoplasm. The *hut* operons of *S. typhimurium*, which were activated by GS in *K. aerogenes* (strain KT2401), were transduced into a *S. typhimurium* LT-2 background (strain ST1003). In the resulting Hut$^+$ transductant (strain ST1006), histidase synthesis was no longer increased by nitrogen limitation, although synthesis of GS was derepressed eightfold. The *glnA* gene of *S. typhimurium*, which activated histidase synthesis in *E. coli* strain ET2202, was also transferred into *S. typhimurium* strain ST2500 (*glnA20*). In the resulting *glnA*$^+$ strain (ST2502), histidase synthesis was not activated under nitrogen-limiting conditions, although GS was derepressed 11-fold.

It is clear that the GS of *S. typhimurium* can activate the expression of the *hut* genes of *S. typhimurium* as well as those of *K. aerogenes*. In *S. typhimurium*, however (Table 2), the same combination of *glnA* and *hut* genes did not result in derepression of histidase during nitrogen-limited growth. Thus, we conclude from these results that GS is necessary but not sufficient for activation of transcription of the *hut*S.15-59 genes. Some additional factor is required for GS to function as a positive control element. This factor is functional in *K. aerogenes* and *E. coli* K-12, but it is altered or absent in *S. typhimurium*.

Regulation of synthesis of proline oxidase in *S. typhimurium*. Although *hut* transcription in *S. typhimurium* cannot be activated by GS, it is possible that other genes are transcribed at higher rates during nitrogen-limited growth. This might imply that the factor required along with GS for transcription is present, but in an altered form, in *S. typhimurium*. In *K. aerogenes*, the synthesis of proline oxidase, like that of histidase, can be activated by GS (15, 18). We, therefore, examined the regulation of proline oxidase synthesis in a strain of *S. typhimurium* 15-59 that synthesizes this enzyme constitutively. We found that nitrogen limitation did not lead to increased synthesis of proline oxidase in *S. typhimurium*, although the level of GS protein increased over 10-fold. The level of proline oxidase increased only when cells were grown in a medium containing excess ammonia and a poor source of energy, such as succinate, a condition known to relieve catabolite repression.

DISCUSSION

In the experiments described in this paper we have investigated the molecular basis for the difference, reported long ago, in the regulation of Hut enzyme production in nitrogen-limited cultures of *K. aerogenes* and *S. typhimurium* 15-59. From recent experiments it was clear that this difference could not be attributed solely to alterations in the structure of the promoters of the *hut* operons in the two organisms; consequently, it seemed likely that the GS of these organisms might differ in their regulatory ability. In this case, one might expect these two strains to differ in their ability to synthesize large amounts of GS, since some evidence indicates that transcription of the *glnA* gene is subject to autogenous regulation (6) and may require GS as a positive control factor (30). In addition, experiments with a purified transcription system have demonstrated that only preparations of nonadenylylated GS from *K. aerogenes* effectively stimulated transcription from the promoters of the *hut*S.15-59 genes. Therefore we examined whether the absence of GS-mediated *hut* gene transcription in *S. typhimurium* 15-59 could be due to insufficient levels of nonadenylylated GS protein. We also investigated whether activation of *hut* transcription by GS might occur in other isolates of *S. typhimurium*. Such differences have been reported in the regulatory properties of an enzyme in the *arg* biosynthetic pathway for two isolates of *E. coli*, B and K-12 (11, 12), and variations exist in the regulatory capacity of a protein in the *trp* biosynthetic system of two isolates of *S. typhimurium*, LT-2 and LT-7 (25).

Our data indicate that nitrogen-limited cultures of *S. typhimurium* 15-59, *S. typhimurium* LT-2, and *K. aerogenes* all contain comparable levels of nonadenylylated GS. Nevertheless, in contrast to the situation with *K. aerogenes*, in neither of the isolates of *S. typhimurium* is the synthesis of histidase derepressed under this growth condition.

This result suggested that the GS of *S. typhimurium* might be capable of activating transcription from some promoters, but not from others. Therefore, we constructed hybrid strains of *K. aerogenes* and *E. coli* containing the structural gene for the GS native to *S. typhimurium* LT-2 and the *hut* genes of *K. aerogenes* and *S. typhimurium* 15-59. Our results show that in the genetic background of *K. aerogenes* and *E. coli* the GS of *S. typhimurium* can activate transcription, not only from the promoters of the *hut* genes of *K. aerogenes* but also from the promoters of the *hut* genes of *S. typhimurium* 15-59.

The simplest explanation for these observations is that GS activation of *hut* transcription requires some additional factor active in cells of

K. aerogenes and *E. coli* but missing or altered in cells of *S. typhimurium*.

It is of interest to consider whether *S. typhimurium* in fact contains this GS transcription factor. We find no clear evidence for GS-activated transcription of either the *hut* or *put* operons in this organism. Our results show that cultures of *S. typhimurium* utilizing glucose as a source of carbon contain essentially the same level of the Hut and Put enzymes regardless of the availability of nitrogen. It is possible that variations in the amount of catabolite repression could mask a low level of GS-stimulated *hut* transcription. However, when we examined the regulation of histidase and GS formation in a strain of *S. typhimurium* lacking adenyl cyclase (*cya*$^-$) but containing the *hut*S.15-59 genes, we observed no increase in histidase levels in nitrogen-limited cultures (F. R. Bloom, unpublished data), although GS was derepressed ninefold. Thus our data do not show any stimulation of *hut* or *put* expression by GS in strains of *S. typhimurium*.

These results might argue that the transcription factor is absent in *S. typhimurium*. However, there is some evidence that could be interpreted to suggest the presence of such a factor acting on a limited number of promoters. We have shown that in cultures of *S. typhimurium* grown under different conditions the expression of *glnA*, as reflected in levels of GS, is quite similar to that in *K. aerogenes*, where it has been suggested that GS regulates *glnA* expression. In addition, it has been reported (5, 16a) that the *nif* genes of *K. pneumoniae*, believed to be regulated only by GS (24, 27), are expressed and normally regulated in *S. typhimurium* LT-7. It has also been suggested that small variations in the activity of a glutamine transport system in *S. typhimurium* LT-2 may reflect GS activation of transcription of the structural gene for a glutamine permease (2). It is, however, possible that these are examples of GS-mediated regulation that do not require the transcription factor. Thus, at this time, no evidence clearly favors the presence, or absence, of a GS transcription factor in *S. typhimurium*. However, further experiments examining the molecular function of this GS transcription factor should help to resolve this question.

An obvious candidate for the GS transcription factor is ribonucleic acid polymerase. This model would suggest that the interaction of the GS of *S. typhimurium* with the *hut* deoxyribonucleic acid causes only a partial melting of the promoter region, not sufficient for the *S. typhimurium* ribonucleic acid polymerase to move into the open complex with the deoxyribonucleic acid, which is necessary for transcription, but sufficient for the *K. aerogenes* or *E. coli* enzyme. Alternatively, this factor may be a protein that facilitates the interaction of GS with deoxyribonucleic acid. In this case, the factor need not necessarily exist in *S. typhimurium*, since it may not be required for GS activation of transcription from all promoters.

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