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Autogenous Regulation of the Synthesis of Glutamine Synthetase in *Klebsiella aerogenes*

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We isolated an F′ episome of *Escherichia coli* carrying the *glnA*$^+$ gene from *K. aerogenes* and an F′ eipsome of *E. coli* carrying the *glnA4* allele from *K. aerogenes* responsible for the constitutive synthesis of glutamine synthetase. Complementation tests with these episomes showed that the *glnA4* mutation (leading to the constitutive synthesis of active glutamine synthetase) was in the gene identified by mutations *glnA20*, *glnA51*, and *glnA5* as the structural gene for glutamine synthetase. By using these merodiploid strains we were able to show that the *glnA51* mutation led to the synthesis of a glutamine synthetase that lacked enzymatic activity but fully retained its regulatory properties. Finally, we discuss a model that explains the several phenotypes associated with mutations such as *glnA4* located within the structural gene for glutamine synthetase leading to constitutive synthesis of active glutamine synthetase.

In *Klebsiella aerogenes*, mutations in *glnA*, the structural gene for glutamine synthetase (GS) (5), lead to several different phenotypes. These phenotypes are summarized in Table 1 and are described in detail in preceding papers (3, 9, 12, 14). We have suggested that the existence of mutations in *glnA* leading to the constitutive synthesis of GS (GlnC phenotype) implies that GS is an element of its own regulation (6, 14). To confirm that mutations leading to the GlnC phenotype are indeed alleles of the *glnA* gene, we isolated an F′ episome carrying the *glnA4* allele from *K. aerogenes* (resulting in the GlnC phenotype) and another F′ episome carrying the *glnA*$^+$ allele of *K. aerogenes*. These episomes made it possible to study complementation and dominance patterns with various *glnA* chromosomal mutations in *K. aerogenes*.

Previous work from this laboratory has shown that the introduction into *K. aerogenes* of F′14, an F′ episome from *E. coli* carrying the *glnA*$^+$ gene from *E. coli*, restored GS activity to Gln$^-$ mutants of *K. aerogenes* and also restored the regulation of the synthesis of histidase and glutamate dehydrogenase to these mutants (15). This work also showed that the *glnA*$^+$ allele from *E. coli* was fully dominant to the *glnA4* allele of *K. aerogenes* with respect to the regulation of histidase and glutamate dehydrogenase formation, and that the *glnA*$^+$ allele from *E. coli* was largely dominant to the *glnA4* allele of *K. aerogenes* with respect to the regulation of GS formation. In the merodiploid F′*glnA*$^+$/*glnA4* (in which the *glnA*$^+$ was from *E. coli*), the regulation of histidase and glutamate dehydrogenase formation was exactly like that seen in the *glnA*$^+$ *K. aerogenes* haploid strain and different from that seen in the *glnA4* haploid strain. In addition, the GS of this merodiploid was repressible by ammonia, though not quite so severely as the GS of the *glnA*$^+$ haploid strain. These findings are consistent with the hypothesis of autogenous regulation of GS (6, 14).

The earlier merodiploid studies were limited to complementation using the F′*glnA*$^+$ episome, since no *glnA* mutations leading to the GlnC phenotype have as yet been reported in *E. coli*. Since the complementation was heterologous (episomal *glnA* from *E. coli*, chromosomal *glnA* from *K. aerogenes*), it was necessary to be cautious in interpreting the complementation results, and it was difficult to set the assay conditions for the GS activity since there are slight physical and biochemical differences between the GS of *E. coli* and the GS of *K. aerogenes* (2; R. A. Bender and S. L. Streicher, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K144, p. 160). The work presented here uses a homologous complementation in which both the episomal and the chromosomal *glnA* genes are derived from *K. aerogenes*. Thus, these data confirm and extend the previous work. In particular, these data and the data presented in the preceding paper (3) allow us to explain the regulatory defect that leads to the Gln(AC)$^-$

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phenotype in strains carrying the *glnA51* mutation. Finally, although we are unable to determine the precise molecular mechanism of autoregulation of GS, we are able to put forth a hypothesis to explain the nature of the defect in the GlnC strains that leads to the loss of repressibility of the GS.

TABLE 1. *Phenotypes associated with glnA alleles*

Phenotype	*glnA product is*
Gln^+	Active and repressible
Gln^-	Absent
GlnC	Active but not repressible
$Gln(AC)^-$	Inactive and repressible, but not by ammonia
Phenotype	*Histidase formation is*
Cn^+	Repressible by ammonia
Cn^r	Always derepressed
Cn^s	Always repressed
Allele	*Resulting phenotype*
glnA$^+$	Gln^+ Cn^+
glnA4	GlnC Cn^r
glnA5	Gln^- Cn^s
glnA6	Gln^- Cn^s
glnA20	Gln^- Cn^s
glnA51	$Gln(AC)^-$ Cn^r

MATERIALS AND METHODS

Bacterial and phage strains. *K. aerogenes* strains used here are all derivatives of *K. aerogenes* W-70 (8) made sensitive to the transducing phage P1 (7). *E. coli* strains are derivatives of *E. coli* K-12. All bacterial strains described in this paper are listed in Table 2. Phage P1*clr*100*Km* (7) was used for all transductions and is referred to here as P1.

Growth and harvesting of cultures. Minimal medium (W) and complex medium (LB) have been described previously (2). Abbreviations used here are: G, 0.4% glucose; N, 0.2% ammonium sulfate; gln, 0.2% L-glutamine; H, 0.4% L-histidine. The glutamine was from Calbiochem; it was prepared fresh and sterilized by filtration immediately before each experiment. Cultures were grown at 30°C with vigorous aeration to a density of 100 Klett units (about 5×10^8 cells/ml) from an inoculum of 2×10^7 to 5×10^7 cells/ml, and growth was terminated by the addition of 100 μg of hexadecyltrimethylammonium bromide per ml to preserve the adenylylation state of GS, as described previously (2). Cells were washed once with 1% KCl and resuspended in one-tenth the original volume of 1% KCl.

Genetic techniques. P1-mediated transduction was performed as described previously (14). For intergeneric transduction, the multiplicity of phage was increased to 5 plaque-forming units per cell, and the mixture of phage and cells was concentrated (after adsorption) by centrifugation to a density of

TABLE 2. *List of strains*

Strain	Relevant genotype[a]	Source (reference)
Escherichia coli		
AB1206	F'14/*his pro thi*	B. Bachmann
LS519	*metB rha*	L. Soll
CB100	F'*glnA102*$_c$/*his pro thi*	This paper
CB109	F'*glnA*^+_K/*his pro thi*	This paper
CB112	F'*glnA4*$_K$/*his pro thi*	This paper
Klebsiella aerogenes		
MK9011	*glnA6 ilvA1*	(14)
MK9028	*glnA4 ilvA1*	(3)
MK9040	*glnA4 glnB3*	P1-sensitive strain of MK94 (7, 12)
MK9052	*glnA5 metB4*	(14)
MK9118	*metB4 ilvA3*	(3)
MK9509	*glnA51 ilvA3*	P1·MK9267 × MK9118 (14)[b]
MK9510	Prototroph	P1·MK9000 × MK9118 (14)
MK9515	*glnA20 ilvA3*	P1·MK9282 × MK9118 (14)
MK9528	*glnA4 ilvA3*	P1·MK9028 × MK9118
KB200	F'*glnA*^+_K/*glnA20*	CB109 → MK9515[c]
KB201	F'*glnA4*$_K$/*glnA20*	CB112 → MK9515
KB202	F'*glnA4*$_K$/*glnA5*	CB112 → MK9052
KB203	F'*glnA*^+_K/*glnA4*	CB109 → MK9028
KB204	F'*glnA4*$_K$/*glnA*$^+$	CB112 → MK9118
KB205	F'*glnA*^+_K/*glnA51*	CB109 → MK9509
KB206	F'*glnA4*$_K$/*glnA51*	CB112 → MK9509

[a] All *K. aerogenes* strains carry the *hutC515* mutation and can therefore produce histidase in the absence of inducer. The subscript "C" or "K" after a *glnA* allele number indicates that allele is derived from *E. coli* (C) or from *K. aerogenes* (K). Thus, *glnA102*$_c$ indicates that this is a mutation in the *E. coli glnA* gene, whereas *glnA4*$_K$ indicates that this is a mutation in the *K. aerogenes glnA* gene. Where no subscript is given, the *glnA* gene corresponds to the cytoplasm.

[b] P1 transduction as described in Materials and Methods.

[c] Mating was performed as described in Materials and Methods.

about 10^{11} cells/ml (17). Mating was done by mixing spots of donors and recipients on selective media lacking isoleucine and methionine (14). In all experiments in which the exconjugants were purified or subcultured, segregation of the episome was demonstrated by growing the strain overnight in LB-gln medium (14). Segregants regaining all the markers of the haploid parental strain were obtained at a frequency of 10 to 15% after such treatment. In 2 out of about 20 such segregation experiments, no segregants were observed. This strain was discarded and the mating was repeated. Segregants were obtained from this second mating at the normal frequency.

Assays. Enzyme assays and protein determinations were performed as described previously (2, 3).

RESULTS

Isolation of an F′ episome carrying the *glnA* region from *K. aerogenes*. The genetic manipulability of *K. aerogenes* is as yet quite limited, particularly with respect to conjugal crosses: *K. aerogenes* strains will accept F′ episomes from *E. coli* and will maintain and express these episomes, but they will not serve as efficient donors for such episomes to either *E. coli* or *K. aerogenes* recipients. Therefore, it was necessary to isolate an *E. coli* strain carrying the *glnA* gene from *K. aerogenes* on an F′ episome for use as donor in these experiments. Strain AB1206, carrying F′14 (including *glnA* (10, 14)) and a chromosomal deletion corresponding to all or most of F′14 (11), was chosen as the starting strain.

After mutagenesis with ethyl methane sulfonate and penicillin enrichment (14), a stable Gln^- derivative of strain AB1206, strain CB100, was isolated. Both strains CB100 and AB1206 donate an episome to *K. aerogenes* complementing *ilvA* and *metB* mutations; the episome from strain AB1206 also complements *glnA* mutations, whereas that from strain CB100 does not. To confirm that the mutation in strain CB100 is at *glnA*, the mutation was mapped by P1 transduction. Phage grown on strain LS519 (*glnA*$^+$ *rha metB*) were used to transduce strain CB100 to Gln^+ in the presence of excess methionine. The transductants were purified by streaking for single colonies and were then tested for the Rha and Met characters. Of 192 Gln^+ colonies analyzed, 38 (20%) were Rha^- and 5 (3%) were Met^-. These linkages are consistent with the known position of the *glnA* gene in *E. coli* (14). Strain CB100 (*glnA102*) has never been observed to revert to Gln^+ spontaneously (less than 10^{-11}).

The *glnA* region from *K. aerogenes* was then intergenerically transduced (17) into strain CB100, selecting for growth in the absence of glutamine. Phage P1 grown on strains MK9000 (*glnA*$^+$) and MK9040 (*glnA4*) were used to transduce strain CB100, and glutamine-independent transductants were obtained at frequencies of about 3×10^{-9} and 1×10^{-10}, respectively. One transductant from each cross, strain CB109 (*glnA*$^+$) and strain CB112 (*glnA4*), was purified and mated with *K. aerogenes* MK9011 (*ilvA1 glnA6*). Both strains CB109 and CB112 donated episomes capable of complementing the *ilvA1* and *glnA6* mutations. The exconjugant from the mating with strain CB112 was scored as GlnC by the tryptophan plate test (12) and by assay (see below), whereas the exconjugant from the mating with strain CB109 was scored as Gln^+. Thus, the transduction resulted in faithful phenotypic expression of the *glnA4* and *glnA*$^+$ alleles in the MK9011 cytoplasm. In all the experiments reported here, strain CB109 was used as the donor of the F′*glnA*$^+$ episome, and strain CB112 was used as the donor of the F′*glnA4* episome.

In the experiments described in the following sections, two approaches were used to study the expression of GS in merodiploid cells of *K. aerogenes*. The results, summarized in Table 3, were obtained using merodiploid strains purified by two single-colony isolations. The presence of the episome was inferred by the ability of each strain to segregate haploids that had recovered all the original phenotypes of the haploid parent after one cycle of nonselective growth in rich broth (LB-gln). Because F′14 appeared to be somewhat unstable upon re-

TABLE 3. *Repression of GS in merodiploid strains*[a]

Expt	Strain	Relevant genotype	GS activity[b]	
			G-gln medium	GN-gln medium
1	MK9510	+	1.5	0.2
2	MK9028	*glnA4*	1.0	0.9
3	KB200	F′*glnA*$^+$/*glnA20*	1.6	0.3
4	KB201	F′*glnA4*/*glnA20*	1.3	1.4
5	KB202	F′*glnA4*/*glnA5*	1.1	1.3
6	KB203	F′*glnA*$^+$/*glnA4*	2.9	1.2
7	KB204	F′*glnA4*/*glnA*$^+$	2.2	1.1
8	KB205	F′*glnA*$^+$/*glnA51*	2.2	0.2
9	KB206	F′*glnA4*/*glnA51*	1.6	0.7

[a] Merodiploids were selected as described in Materials and Methods and purified by streaking for single colonies twice: once on selective medium and once on nonselective medium. Diploidy was confirmed by the ability to recover haploid segregants with the original phenotype of the F^- parent.

[b] γ-Glutamyl transferase activity, which measures the total amount of GS polypeptide present (2), was measured in whole-cell suspensions as described in Materials and Methods. It is given as micromoles of product per minute per milligram of protein.

peated selective subculture and because of the possibility of homogenotization (see Materials and Methods), experiments with bulk exconjugants, not purified except by growth in the mating spot itself, were also carried out. The results of these experiments are summarized in Table 4. In this case, we have to consider the possibility that the mixture derived from the growth of the exconjugants contains stable as well as unstable clones of merodiploid cells. Nevertheless, the internal consistency of critical results (compare experiments 7 and 9 of Table 3 with experiments 5 and 6 of Table 4) appears to validate our procedures.

Complementation tests with Gln⁻ and GlnC mutations. These experiments were designed to answer the question whether mutations in the *glnA* region leading to Gln⁻ or to GlnC phenotypes are located in the same cistron or in separate adjacent cistrons. The wild-type response of GS to regulation is shown in experiment 1 of Table 3 and in experiment 1 of Table 4: compared with the level of GS in cells grown on G-gln, the level in cells grown on GN-gln is 8-fold lower, and in cells grown on HN-gln it is at least 50-fold lower.

The response of GS to regulation in a strain carrying the *glnA4* mutation responsible for the GlnC phenotype is shown in experiment 2 of Table 3 and in experiment 2 of Table 4: the level of GS in cells grown on GN-gln is approximately 90%, and in cells grown on HN-gln it is approximately 75% of the level of GS in cells grown on G-gln. In merodiploids, the presence of the *glnA20*, *glnA5*, or *glnA6* alleles, which in haploids are responsible for the Gln⁻ phenotype, does not alter the expression of GS determined by the episomal *glnA*⁺ allele (experiment 3, Tables 3 and 4), or the episomal *glnA4* allele (experiment 4 and 5, Table 3, and experiment 4, Table 4). On the other hand, the presence of the *glnA*⁺ allele restores, at least partially, the regulation of GS in merodiploid cells carrying the *glnA4* allele (experiments 6 and 7, Table 3, and experiment 5, Table 4).

We can conclude from these experiments that the mutations *glnA5*, *glnA6*, and *glnA20* result in the simultaneous loss of GS activity and

TABLE 4. *Merodiploid analysis of regulatory mutants in glnA*

Expt	Strain[a]	Assay	Enzyme activities (U/mg of protein)[b]		
			G-gln	GN-gln	HN-gln
1	+	γGT	1.6	0.18	<0.03
	(MK9510)	Forward[c]	0.4	0.05	<0.01
		Histidase[d]	450	120	580
2	C	γGT	1.5	1.4	1.1
	(MK9528)	Forward	0.05	0.06	0.01
		Histidase	380	330	400
3	+/A6	γGT	2.0	0.20	0.06
	(CB109 → MK9011)	Forward	0.47	0.08	<0.01
		Histidase	360	110	525
4	C/A6	γGT	1.4	1.4	1.1
	(CB112 → MK9011)	Forward	0.04	0.04	<0.01
		Histidase	300	250	500
5	C/+	γGT	3.1	1.5	0.53
	(CB112 → MK9118	Forward	0.6	0.05	<0.01
		Histidase	350	100	500
6	C/A51	γGT	1.5	0.64	0.21
	(CB112 → MK9509	Forward	0.45	0.04	0.01
		Histidase	260	60	440

[a] Merodiploids were selected by spot mating on selective plates (14). Without further purification, these merodiploids were scraped with a sterile applicator stick into fresh selective medium (lacking isoleucine and methionine) at a density of 2×10^7 to 5×10^7 cells/ml. Cultures were grown to a density of about 5×10^8 cells/ml, harvested, and assayed as described previously (2, 3). Similar cultures showed no significant difference in viable count whether on selective or nonselective medium. Strain names reflect the origin of the strain. "C" indicates the *glnA4* allele; "+", the *glnA*⁺; A6, the *glnA6*; etc. The episomal allele is given first. Thus (C/+) indicates that the relevant genotype is F'*glnA4*/*glnA*⁺.

[b] Enzyme activities were measured in suspensions of whole cells as described in Materials and Methods. For histidase, the specific activity is given as nanomoles of product per minute per milligram of protein.

[c] Forward reaction assay correlates with the biosynthetic activity of GS (2). For fully active (nonadenylylated) GS, the activity of GS in the forward reaction is about one-half the activity of the γGT reaction. For fully inactive (adenylylated) GS, the activity of the GS in the forward reaction approaches zero (2).

[d] Histidase activity in the HN-gln medium reflects activation of *hut* by cyclic 3',5'-adenosine monophosphate (13), and not by GS.

of an element capable of restoring the regulation of GS formation lost by the *glnA4* mutation. The mutation *glnA4*, which leads to the GlnC phenotype, appears, therefore, to be located in the same cistron as the mutations leading to the Gln$^-$ phenotype. This cistron has been previously identified as the structural gene for GS (5).

It is of interest that in contrast to the other Gln$^-$ alleles, the *glnA51* allele partially restores regulation of GS in merodiploids carrying the *glnA4* allele on the episome (experiment 9, Table 3, and experiment 6, Table 4). We have shown previously that the *glnA51* mutation results in the production of enzymatically inactive GS (3, 5), which apparently is capable of restoring the regulation of GS formation lost by the *glnA4* mutation.

Regulation of GS and by GS in merodiploids. The *glnA4* mutation not only affects the level of GS but also the adenylylation of GS and the level of histidase. When strains MK9510 (*glnA*$^+$) and MK9528 (*glnA4*) are grown on G-gln medium their total GS levels, measured by the transferase assay, are equally high (Table 4); however, the use of the "forward assay" shows that, in strain MK9510, but not in strain MK9528, a considerable portion of GS is in the non-adenylylated form. On GN-gln medium, the levels of GS and of histidase are low in strain MK9510 and high in strain MK9528 (compare experiments 2 and 1). These patterns of regulation are not altered when the *glnA*$^+$ and the *glnA4* genes are present in merodiploids carrying the chromosomal *glnA6* mutation, which is responsible for the Gln$^-$ phenotype of the haploid (experiments 3 and 4).

The presence of the *glnA*$^+$ or the *glnA51* allele on the chromosome has a profound effect in merodiploids with an episomal *glnA4* gene (experiments 5 and 6). When these merodiploid cells are grown on G-gln, a large portion of their GS is present in the non-adenylylated form as revealed by the forward assay; when they are grown on GN-gln medium, their histidase levels are as low as those of a correspondingly grown *glnA*$^+$ haploid strain, though their levels of GS are considerably higher. In these merodiploid strains, cultivation on HN-gln results in GS levels considerably lower than those found in cells grown on G-gln, but higher than those found in the *glnA*$^+$ haploid strain grown on HN-gln.

DISCUSSION

Genetic experiments reported earlier (14) showed that mutations leading to the GlnC phenotype are located interspersed among mutations leading to the loss of GS (Gln$^-$) in *glnA*, the structural gene for GS (5). We have confirmed this finding with a complementation test that uses the GlnC type mutation (*glnA4*) and the Gln$^-$ type mutation (*glnA20*), which are farthest apart on our map (14). If the complementation test is to be valid, two minimal conditions must be met: (i) the mutations under study cannot be *trans*-dominant, and (ii) the mutations must affect only the gene in which they are located; strongly polar mutations and large deletions can lead to spurious non-complementation results. The data presented here and by Streicher et al. (15) show that all Gln$^-$ mutations tested, including *glnA20*, are fully recessive to a *glnA*$^+$ allele from either *E. coli* or *K. aerogenes* with respect to GS activity and the regulation of GS and histidase formation. The GlnC mutation (*glnA4*) is not fully recessive to either *E. coli* or *K. aerogenes glnA*$^+$; however, it is not fully dominant either. What is critical for the validity of the complementation test is that the (C/+) merodiploid can be distinguished from the (C) haploid strain. In other words, if a wild-type allele for control is present, its effect can be seen in the presence of a GlnC allele.

The *glnA20* mutation is a point mutation, since its 10^{-8} reversion frequency can be increased by three orders of magnitude through mutagenesis with ethyl methane sulfonate. As for polarity, we cannot be certain that *glnA20* is not polar on a hypothetical "*glnC*" gene, but since the same non-complementation is obtained with the *glnA5* allele, which has been characterized as a missense mutation, the lack of complementation between GlnC mutations and Gln$^-$ mutations cannot be attributed to polarity effects. Therefore, if there were two genes, *glnA* and *glnC*, then we would expect a diploid of the type *glnA*$^+$ *glnC*$^-$/*glnA*$^-$ *glnC*$^+$ to score as repressible. If there were only one gene, then GlnC/Gln$^-$ diploids should be identical to the haploid GlnC strain, since the chromosome would provide no product. In fact, the diploid GlnC/Gln$^-$ is fully constitutive, showing no sign of a GlnC$^+$ allele. Thus, this complementation test confirms that *glnA4* and *glnA20* are in the same gene.

In the preceding paper (3), we proposed that there are two conformations for GS, normally associated with the two states of modification (adenylylated and non-adenylylated). We proposed that, in GlnC strains, the GS is frozen into the conformation incapable of repressing GS formation, whatever its state of adenylylation. We have used these merodiploid strains to study the nature of the regulation of GS. The

diploid (C/A51) (F'*glnA4*/*glnA51*) yielded much information. The repression pattern of the GS in (C/A51) is exactly the same as is seen in (C/+) (F'*glnA4*/*glnA*$^+$), but the GS levels are those characteristic of haploid strains. There is no evidence that the *glnA51*-coded subunits of GS regain any GS activity in the presence of the *glnA4* allele. Thus, the repression seen in (C/A51) is a repression of the *glnA4*-coded subunits. Since the *glnA4* allele is completely unregulated in haploids, either on the chromosome or on the episome, the regulatory ability in (C/A51) is being provided in *trans* by the chromosomal *glnA51* gene. What is more, this regulatory ability is provided to the same degree in (C/A51) as it is in (C/+), showing that the *glnA51* is as effective as *glnA*$^+$ in restoring repressibility to *glnA4*. The lower total GS activity in (C/A51) presumably reflects the fact that half the GS polypeptide in (C/A51) is inactive due to the *glnA51* mutation.

Since the GS level in HN-gln cells is much lower in the diploid (C/A51) than in the haploid GlnC strain, we have further evidence that the *glnA51* product acts in *trans* to restore regulation to the *glnA4* product. That is, *glnA4* is not a *cis*-dominant constitutive mutation. It is interesting that both the *glnA4* product and the *glnA51* product are fully resistant to repression by ammonia, yet the mixed diploid (C/A51) is repressible by ammonia. In the preceding paper we have shown that the *glnA51* product is resistant to repression by ammonia because strains carrying *glnA51* cannot metabolize ammonia to the true repressing signal. The episomal *glnA4* gene provides an active GS to correct this inability, and the *glnA51* responds exactly as the wild type despite its lack of GS activity.

Since GS is a dodecamer composed of 12 identical subunits (18), subunit mixing in mixed diploids is not only possible, it is likely. This would explain the co-dominance of *glnA4* and *glnA*$^+$ (or *glnA51*) with respect to repression. In fact, there is suggestive evidence that such subunit mixing does occur. GS from GlnC strains is more susceptible to adenylylation in G-gln medium than is the wild type, presumably reflecting an altered conformation. In the mixed diploids (C/+) and (C/A51), this defect is corrected, and the GS of these strains is significantly non-adenylylated on G-gln medium. This should reflect a conformation change in the dodecamer. Since the *glnA51* product is inactive in our assay, we are seeing a *trans* effect on the conformation assumed by the *glnA4*-coded subunits, most easily explained by subunit mixing.

Further support for the model of subunit mixing and two conformations comes from an analysis of the control of histidase formation in the GlnC haploid strain and the (C/+) diploid grown on GN-gln. Both have the same amount of GS on GN-gln medium, and in both cases the GS is highly adenylylated. Yet in the GlnC haploid strain, the GS is able to activate histidase formation, whereas in the (C/+) diploid, the GS is not able to activate histidase formation. Since the quantity and degree of adenylylation are equivalent, there should be a conformational difference. In the wild type, non-adenylylated GS can activate *hut* transcription, but adenylylated GS cannot do it even in vitro (16). Thus, the GS from the GlnC strain must be in the conformation corresponding to non-adenylylated wild-type GS, despite its high degree of adenylylation, and subunit mixing with *glnA*$^+$ (or *glnA51*)-coded subunits restores the ability to assume the configuration that does not activate *hut* transcription.

The results presented here provide convincing evidence for regulation of GS synthesis by GS. However, they do not tell us whether GS is an activator of GS synthesis, a repressor, or both. Beckwith and Rossow (1) have pointed out that diploid analysis can be used to distinguish negative and positive control of enzyme synthesis only when strains with deletions in the regulatory gene are analyzed. In an autogenously regulated system, such as *glnA*, deletion of the regulatory gene eliminates the regulated product. Fusion of the control region of the *glnA* gene to another structural gene (4) should provide strains that can be used to decide whether control of GS synthesis by GS is negative or positive.

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