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Regulation of Glutamine Synthetase in *Streptomyces coelicolor*

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Glutamine synthetase (GS) in *Streptomyces coelicolor* was shown to be regulated at two levels. First, the *S. coelicolor* GS protein is subject to a posttranslational covalent modification which is likely to involve adenylylation. Adenylylation is important in regulating GS activity both after sudden changes in ammonium availability and during steady-state growth. Since higher levels of adenylylated GS were seen in *S. coelicolor* mutants deficient in glutamate synthase than in wild-type cells, glutamine or a metabolite derived from glutamine is likely to be involved in the metabolic signal that regulates GS adenylylation. Second, the GS structural gene (*glnA*) is transcriptionally regulated in response to nitrogen availability during steady-state growth. Transcription of the *glnA* gene occurred from the same promoter during vegetative growth, stationary phase, and sporulation. The nucleotide sequence of this promoter has significant homology with the −10, but not the −35, region of the consensus sequence of *Streptomyces* vegetative promoters. The *glnA* gene is transcribed as a monocistronic mRNA.

Members of the genus *Streptomyces* are gram-positive, filamentous, sporeforming soil bacteria that synthesize a number of medically important antibiotics and other chemotherapeutic agents (1, 6). Synthesis of these compounds is regulated by metabolic parameters that are poorly understood (1, 6). To increase our understanding of the physiology of growth and antibiotic production in these bacteria, we have begun to investigate nitrogen metabolism in *Streptomyces coelicolor*. This *Streptomyces* species is well suited for genetic analysis because a variety of vectors for DNA cloning and methods for plasmid-mediated transfer of chromosomal DNA have been developed for it (13).

Bacterial glutamine synthetase (GS) is an enzyme composed of 12 identical subunits (7, 19, 21). This enzyme is responsible for the ATP-dependent synthesis of glutamine from ammonium (NH_4^+) and glutamate. Since the amide moiety of glutamine serves as the nitrogen donor for the synthesis of many metabolites, GS occupies a crucial position in nitrogen metabolism. In enteric bacteria such as *Escherichia coli*, the ability to synthesize glutamine is tightly regulated in response to NH_4^+ availability (21). The enzymatic activity of preformed GS can be reduced by covalent attachment of AMP groups to the GS protein (adenylylation), and the GS structural gene (*glnA*) is transcriptionally regulated. Both GS activity and *glnA* expression are controlled by the Ntr (nitrogen regulated) regulatory system, which responds to the intracellular ratio of glutamine to 2-ketoglutarate (21). The Ntr system also regulates the expression of degradative enzymes and transport systems for a number of other nitrogen-containing compounds that are catabolized to ammonium (21).

The regulation of nitrogen metabolism in gram-positive bacteria has not been as well characterized. In *Bacillus subtilis*, *glnA* transcription is regulated by nitrogen availability (9), but there is no evidence that the GS protein is covalently modified (7, 10) or that a global nitrogen regulatory system is present (25). In contrast, GS activity is regulated by adenylylation in *S. cattleya* (26). The addition of high levels of NH_4^+ to *S. clavuligerus* cells resulted in a rapid decline in GS activity, but the mechanism responsible for this inactivation was not identified (4). GS synthesis is regulated in response to NH_4^+ availability in *S. cattleya* (19, 26), *S. clavuligerus* (4), and *S. noursei* (11) but not in *S. venezuelae* (24). In this communication, the physiology and regulation of glutamine synthesis are described for *S. coelicolor*.

MATERIALS AND METHODS

Bacterial strains. *S. coelicolor* 547 (*glt-5*) (8), J1508 (*hisA1 uraA1 strA1 pgl*) (14), and S11 (*glt-11 hisA1 uraA1 strA1 pgl*) (8) were ultimately derived from *S. coelicolor* A3(2) (13). A3(2) is the parent of the glutamate synthase-deficient (Glt^-) mutant 547; J1508 is the parent of the Glt^- mutant S11.

Media and culture techniques. Cells were grown in SMS minimal medium (8) containing 5% polyethylene glycol 8000 (Sigma Chemical Co., St. Louis, Mo.) and 0.2 mM $CaCl_2 \cdot 2H_2O$. Carbon and nitrogen sources were as described previously (8). The 17-amino-acid medium contained (in milligrams per liter of basal SMS solution) cystine (40), arginine (400), isoleucine (200), leucine (200), valine (200), glutamate (800), lysine (100), phenylalanine (100), proline (100), threonine (100), L-aspartate (665), L-alanine (445), glycine (375), serine (525), tryptophan (150), and methionine (160); 2% glucose, 0.02% $MgSO_4 \cdot 7H_2O$, 0.2% NH_4Cl, and 0.4% glutamine were added immediately before use. YEME (13), a complex medium containing yeast extract and Bacto-Peptone (Difco Laboratories, Detroit, Mich.), was modified by addition of 0.1 M MOPS (morpholinepropanesulfonic acid; pH 7). Nutritional supplements were added to liquid cultures at twice the recommended amounts (13). Reasonably dispersed growth in liquid culture was obtained by using previously described inoculation and culture techniques (8).

Enzyme assays. Extracts for GS assays were prepared from cells grown to mid-logarithmic growth phase (Klett reading of 70 to 90). Harvested cells were washed once with buffer A (50 mM imidazole [pH 7.5], 150 mM NaCl, 1 mM $MnCl_2$, 0.5 mM dithiothreitol) and stored at −20°C. Thawed cells were suspended in the same buffer and disrupted by brief sonication at 4°C; the cell debris was removed by centrifugation for 15 min at 4°C in an Eppendorf microfuge. GS was assayed as described for *S. cattleya* GS (26).

Extracts to be treated with snake venom phosphodiester-

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TABLE 1. GS activity in extracts of *S. coelicolor* J1508 cells grown with various nitrogen sources[a]

Nitrogen source[b]	GS activity[c]		% Adenylylated GS
	−SVPE	+SVPE	
YEME + NH_4^+ + Gln	27 ± 13 (8)	29 ± 12 (5)	7
17 amino acids + Gln + NH_4^+	70 ± 31 (3)	70 ± 40 (3)	0
17 amino acids + Gln + NH_4^+ + Pur-Pyr	58	60	3
Gln + NH_4^+ + Asp	77 ± 13 (5)	167 ± 14 (3)	54
Gln + NH_4^+ + Asp + Pur-Pyr	80 ± 2 (3)	177 ± 40 (3)	55
Gln + Asp	77 ± 23 (3)	170	55
NH_4^+	118 ± 21 (6)	171 ± 34 (4)	31
Gln	112 ± 30 (4)	178 ± 22 (3)	38
Urea	123 ± 14 (2)	175	30
Asparagine	129	175	27
Aspartate	169 ± 11 (2)	181 ± 13 (2)	7
Nitrate	180 ± 11 (2)	180 ± 11 (2)	0
Histidine	182 ± 39 (3)	190 ± 39 (3)	4
Arginine	186 ± 15 (3)	187 ± 16 (3)	0.5

[a] *S. coelicolor* J1508 cells were grown in SMS minimal medium with glucose as the carbon source and the indicated nitrogen sources.

[b] Abbreviations: Gln, glutamine; Asp, aspartate; Pur-Pyr, purine-pyrimidine (adenine, uracil, guanine, and cytosine added at 20 μg/ml).

[c] Expressed as nanomoles per minute per milligram of protein. Numbers in parentheses are numbers of independent determinations. Values are averages of all measurements; error is the standard deviation. −SVPE, no SVPE treatment; +SVPE, SVPE treatment of extracts before measurement of GS activity.

ase (SVPE; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) were diluted twofold with buffer B (0.2 M Tris hydrochloride [pH 8.9], 200 mM NaCl, 2 mM $MgCl_2$) and incubated at 37°C after addition of 50 μg of SVPE per ml. Aliquots were removed at various times, and GS activity was assayed. The level of GS modification was calculated from the ratio of GS activity in extracts incubated with and without SVPE. Typically, SVPE treatment was complete after 45 min.

Protein concentrations were determined by the method of Lowry et al. (16), with bovine serum albumin as the standard.

Northern (RNA) blots and RNA isolation. Northern analyses were performed as described by Maniatis et al. (18), using a 1% agarose formaldehyde gel. Hybridization and washing steps were performed at 60°C. A 20-μg sample of RNA was loaded in each gel slot. Molecular weight markers were linear DNA fragments. Nick-translated *glnA* DNA probes were prepared as described by Maniatis et al. (18).

S. coelicolor RNA for primer extension experiments and Northern blots was isolated by using guanidine thiocyanate and CsCl centrifugation. A 40-ml sample of a culture at Klett 70 to 90 was poured over 0.2 volume of frozen 100 mM $NaPO_4$ buffer (pH 7.5), cells were harvested by centrifugation in an SS34 rotor at 12,000 rpm for 3 to 4 min, and the cell pellet was immediately transferred to −70°C. Frozen cells were thawed in 5 to 7 ml of disruption buffer (30 g of guanidine thiocyanate, 0.25 g of sarcosyl dissolved in 50 ml of DEPC (diethyl pyrocarbonate)-treated [3] 25 mM sodium citrate [pH 7.0], 0.7% β-mercaptoethanol), and disrupted in a French pressure cell at 10,000 to 12,000 lb/in^2. The resulting cell extract was layered over a 2.5-ml cushion of 5.7 M CsCl in 0.1 M EDTA (pH 7) and spun in an SW50.1 rotor for 16 h at 36,000 rpm and 25°C. The RNA pellet at the bottom of the tube was dissolved in 0.4 ml of DEPC-treated water, and 20 μl of 5 M NaCl and 2.5 volumes of ethanol were added; the precipitate was collected after incubation for 1 h at 4°C. RNA was reprecipitated in 70% ethanol–0.3 M sodium acetate and stored at −20°C until use. Sporulating cells from R2 plates were harvested by scraping cells into partially thawed $NaPO_4$ buffer.

Primer extensions. The two synthetic oligonucleotides used in primer extension experiments were an 18-base oligonucleotide (5′-GAACTTCTTGACGTCGTC) that is complementary to nucleotides 16 to 33 of the *glnA* coding region and a 26-base oligonucleotide (5′-CGACGAACT TGACGTCCTCGTCCGCG) complementary to nucleotides 36 to 61 of the *glnA* coding region (28). The two oligonucleotides were used in separate experiments to identify primer-specific artifacts. A 5-ng amount of terminally ^{32}P labeled synthetic oligonucleotide (18) and 2 to 5 μg of *S. coelicolor* RNA were dissolved in 20 mM Tris (pH 8.3)–200 mM KCl, denatured by heating to 80°C for 5 min, and annealed by incubation at 42°C for 3 h. The oligonucleotide primer was extended by using avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.) for 30 min at 42°C in a reaction mixture containing 100 mM Tris (pH 8.3), 100 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM dATP, dCTP, dGTP, and dTTP, 50 μg of actinomycin D per μl, and 1 U of RNasin (Promega Biotec) per μl. Nucleic acids were precipitated with ethanol, suspended in formamide, denatured by heating at 95°C for 2 min, and analyzed on 6% polyacrylamide–6 M urea gels. DNA-sequencing size markers were prepared by using the same oligonucleotide as a primer for dideoxy-sequencing reactions (22).

RESULTS

Adenylylation of GS in whole cells. GS levels were elevated in crude extracts of *S. coelicolor* J1508 cells grown in minimal medium containing arginine as the nitrogen source (Table 1). To determine whether the enzymatic activity of the GS protein was regulated by posttranslational modification, NH_4^+ was added to an exponential-phase culture of *S. coelicolor* J1508 cells growing with arginine as the nitrogen

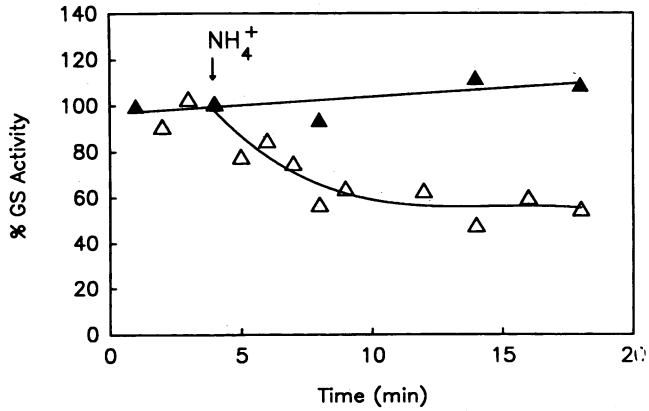

FIG. 1. Kinetics of GS inactivation after NH_4^+ shock in whole cells. A culture of *S. coelicolor* J1508 cells growing in minimal glucose-arginine medium was split into two cultures at time zero, and GS transferase activity was assayed by adding culture samples to assay mixtures containing CTAB at a final concentration of 100 μg/ml. The mixtures were immediately vortexed for 1 min and incubated at 30°C. Ammonium chloride was added to 0.2% to one of the cultures at 4 min, and the sampling continued. Symbols: △, GS activity in NH_4^+-shocked cells; ▲, GS activity in control cells.

TABLE 2. GS activity in extracts of *S. coelicolor* A3(2) cells grown with various nitrogen sources[a]

Nitrogen source[b]	GS activity[c]		% Adenylylated GS
	−SVPE	+SVPE	
YEME + Gln + NH_4^+	16.5 ± 10.5 (3)	29 ± 11.5 (3)	45
Gln + Asp + NH_4^+	44 ± 16 (2)	89 ± 16 (2)	51
Gln	53 (2)	74	29
Urea	79 ± 4 (2)	117 ± 6 (2)	33
Histidine	160 ± 4 (2)	160 ± 4 (2)	0

[a] *S. coelicolor* A3(2) cells were grown in SMS minimal medium with glucose as the carbon source and the indicated nitrogen sources.
[b] Abbreviations: Gln, glutamine; Asp, aspartate.
[c] See footnote *c*, Table 1.

source, and GS activity was determined. At 10 min after NH_4^+ addition, GS levels were twofold lower in NH_4^+-shocked cells than in control cells (Fig. 1). GS levels in crude extracts of the NH_4^+-shocked cells were 85 nmol/min per mg of protein, twofold lower than the GS levels seen in control extracts (186 nmol/min per mg of protein). Incubation of NH_4^+-shocked extracts with SVPE, which is known to cleave the AMP moiety from the adenylylated *E. coli* GS (23), restored GS activity in NH_4^+-shocked cells to 90% of the level seen in control extracts (data not shown). There was no alteration in GS levels in the control extract incubated with SVPE or in the NH_4^+-shocked extract incubated in the absence of SVPE. Therefore, it appears that the enzymatic activity of *S. coelicolor* GS can be inactivated in vivo by covalent modification of the GS protein after sudden changes in NH_4^+ availability. This modification most likely involves adenylylation, since SVPE treatment restores enzymatic activity to the modified protein and since GS in both *S. cattleya* (26) and *E. coli* (21) is also modified by adenylylation.

Nitrogen regulation of GS expression. To examine the regulation of GS synthesis in *S. coelicolor*, GS was assayed in crude extracts of J1508 cells grown with various nitrogen sources (Table 1). These extracts were also treated with SVPE to determine whether adenylylated and enzymatically inactive GS was present and thus determine the total amount of GS protein in the extract. GS activity varied sixfold. The highest levels of GS activity were seen in extracts of cells grown with arginine, histidine, or nitrate as the nitrogen source. Compared with the levels seen in nitrate-grown cells, the level of GS activity was reduced 2- to 2.5-fold in extracts of cells grown with a good nitrogen source, such as glutamine, NH_4^+, urea, or a mixture of glutamine and aspartate. Surprisingly, adenylylation of GS was responsible for this 2- to 2.5-fold reduction in GS activity, because after SVPE treatment, GS activity was restored to the level seen in nitrate-grown cells (Table 1). This result indicated that similar amounts of GS protein were present in extracts of cells grown with nitrate, glutamine, NH_4^+, urea, or a mixture of glutamine and aspartate as the nitrogen source.

TABLE 3. GS activity in wild-type and Glt$^-$ strains[a]

Strain	Relevant genotype	Nitrogen source	GS activity[b]		% Adenylylated GS
			−SVPE	+SVPE	
A3(2)	*glt*$^+$	Y	11.5 ± 3.5 (2)	25 ± 3.5 (2)	54
547	*glt-5*	Y	5.2 ± 2 (2)	21.6	76
A3(2)	*glt*$^+$	GAN	44 ± 16 (2)	89 ± 16 (2)	51
547	*glt-5*	GAN	12 ± 3.5 (2)	74 ± 26 (2)	84
J1508	*glt*$^+$	GAN	78 ± 11 (2)	163 ± 04 (2)	52
S11	*glt-11*	GAN	40 ± 10 (2)	140 ± 22 (2)	72
J1508	*glt*$^+$	NH_4^+	124 ± 11 (2)	165 ± 23 (2)	25
S11	*glt-11*	NH_4^+	70	164	58

[a] Cells were grown in SMS with glucose as the carbon source and the indicated nitrogen sources. Abbreviations: Y, YEME, glutamine, and NH_4^+; GAN, glutamine, aspartate, and NH_4^+.
[b] See footnote *c*, Table 1.

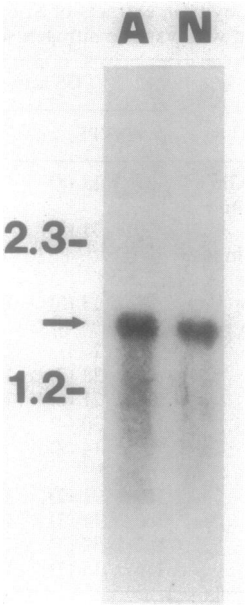

FIG. 2. Northern blot analysis of *S. coelicolor glnA* RNA. RNA extraction and Northern blot procedure are described in Materials and Methods. The *S. coelicolor* RNA blots were probed with nick-translated *glnA* DNA. RNA was isolated from J1508 cells. Lanes: A, RNA source (cells grown with arginine as the nitrogen source); N, cells grown with NH_4^+ as the nitrogen source. Arrow indicates full-length *glnA* mRNA.

To rule out the possibility that GS was adenylylated during harvesting because of changes in intracellular pools, J1508 cells grown with glutamine, NH_4^+, and aspartate as the nitrogen source were permeabilized before harvesting by the addition of 10 μg of cetyltrimethylammonium bromide (CTAB) per ml. CTAB treatment fixes the adenylylation state of GS in *E. coli* cells because the cells are killed by permeabilization and the enzyme responsible for the addition and removal of adenylyl groups from GS, adenylyltransferase, is inactivated (2). Identical levels of active and adenylylated GS were present in extracts of the CTAB-treated and untreated *S. coelicolor* J1508 cells (data not shown). Thus, it appears that adenylylation is involved in regulating GS activity in vivo both during steady-state growth and in response to sudden changes in NH_4^+ availability in *S. coelicolor*.

Not all regulation of *S. coelicolor* GS expression is mediated by adenylylation. Extracts of J1508 cells grown in minimal medium containing 17 amino acids, glutamine, and NH_4^+ or in a rich medium, YEME, supplemented with glutamine and NH_4^+ had 2.5- and sixfold-lower levels of GS activity, respectively, than did nitrate-grown cells (Table 1). GS activity in extracts of cells grown in amino acid-containing medium or YEME did not increase after SVPE treatment. Therefore, the total amount of GS protein present

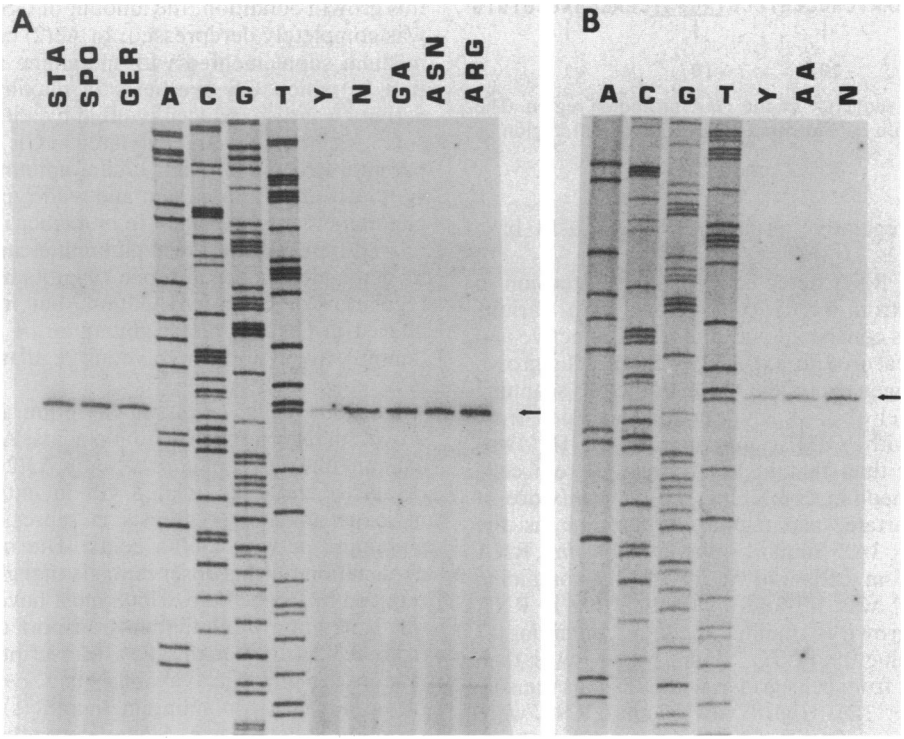

FIG. 3. Identification of the start point of *glnA* transcription. Primer extension analysis was performed with a 26-mer synthetic oligonucleotide as described in Materials and Methods. RNA was isolated from J1508 cells. Lanes: STA, RNA source (cells grown on asparagine as the nitrogen source and harvested 24 h after the end of logarithmic growth); SPO, sporulating cells that had synthesized the pigmented antibiotic actinorhodin and produced aerial mycelium and some spores after 6 days of incubation on R2 (14) solid medium; GER, heat-shocked spores incubated in germination medium (8) for 5 h; Y, cells grown in YEME, glutamine, and NH_4^+ as the nitrogen source; N, cells grown with NH_4^+ as the nitrogen source; GA, cells grown with aspartate and glutamine as the nitrogen source; ASN, cells grown with asparagine as the nitrogen source; ARG, cells grown with arginine as the nitrogen source; AA, cells grown with 17 amino acids, glutamine, and NH_4^+ as the nitrogen source. The sequencing ladders, derived from the same primer, are shown with the A, C, G, and T reactions from left to right. Arrows indicate products of the primer extensions. Panels A and B show separate experiments with independently isolated RNA preparations.

in these extracts was lower than that in extracts of nitrate-grown cells.

Since reduced levels of GS protein were present only in extracts of J1508 cells grown in medium containing amino acids, it would appear that GS synthesis in J1508 cells was repressed only during growth in amino acid-containing medium. To determine whether this was true for all *S. coelicolor* strains, GS expression was also examined in A3(2) cells. A3(2) is the strain from which J1508 was ultimately derived. The pattern of expression of GS in extracts of A3(2) grown with various nitrogen sources was similar to that seen with J1508 (Tables 1 and 2). After SVPE treatment, however, the level of GS activity in extracts of A3(2) cells grown with glutamine or with a mixture of glutamine, aspartate, and NH_4^+ was about twofold lower than in extracts of A3(2) cells grown with histidine (Table 2). Thus, reduced levels of the GS protein were found in extracts of A3(2) cells grown in medium containing either glutamine or amino acids.

GS synthesis in *S. coelicolor* was not regulated by changes in either purine, pyrimidine, or carbon availability. Addition of purines and pyrimidines to cells growing in medium containing either a mixture of glutamine, aspartate, and NH_4^+ or 18 amino acids and NH_4^+ as the nitrogen source caused no alterations in GS levels (Table 1). When ammonium was the nitrogen source, nearly identical levels of active and adenylylated GS were seen in extracts of J1508 cells grown with either a good (glucose) or a poor (glycerol) carbon source (data not shown).

GS regulation in Glt$^-$ mutants. GS levels were twofold lower in extracts of two Glt$^-$ mutants than in wild-type cells (Table 3). SVPE treatment showed that the reduced level of GS activity in these Glt$^-$ mutants was due to increased adenylylation of the GS protein.

***glnA* mRNA.** The size of *S. coelicolor glnA* transcript was determined by Northern blot analysis, using RNA extracted from *S. coelicolor* J1508 cells grown on different nitrogen sources (Fig. 2). The *S. coelicolor glnA* gene was transcribed in vivo as a monocistronic mRNA, since only a 1.6-kilobase band hybridized with the *glnA* probe in these experiments. A 1.6-kilobase RNA molecular would be sufficient to code for the *glnA* protein, whose molecular weight is predicted from its DNA sequence to be 52,568 (28).

Determination of the *glnA* transcriptional start site. The start site of *glnA* transcription was determined by primer extension, using synthetic oligonucleotide primers that hybridize to *glnA* RNA immediately downstream of the *glnA* start codon. The resulting autoradiograms (Fig. 3) showed that transcription of the *glnA* gene started 73 nucleotides upstream of the adenine nucleotide in the *glnA* initiation codon. Since no new *glnA* start sites were seen with RNA isolated from stationary-phase, sporulating, or germinating cells (Fig. 3A), *glnA* transcription occurred from only one promoter during all growth phases. Figure 4 shows the nucleotide sequence upstream of the *glnA* start site. The nucleotide sequence at the −10 position, but not at the −35 position, was similar to the consensus sequence proposed

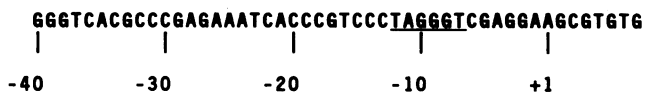

FIG. 4. Nucleotide sequence of the *glnA* promoter region. The transcriptional start site is indicated as +1; the −10 region is underlined.

for *Streptomyces* vegetative promoters, TTGaca-18 base pairs-tAGgaT (12).

The level of *glnA* RNA detected by primer extension in the RNA isolated from J1508 cells grown with various nitrogen sources was consistent with the levels of active and adenylylated GS measured in extracts of these cells grown on the same nitrogen sources. Densitometry scanning showed that the level of *glnA* RNA in RNA isolated from J1508 cells grown with YEME, glutamine, and NH_4^+ was 3.5- to 5-fold lower than that in RNA isolated from cells grown in minimal medium containing NH_4^+, a mixture of glutamine and aspartate, asparagine, or arginine as the nitrogen source (Fig. 3A). Similarly, the level of *glnA* RNA in RNA isolated from cells grown in YEME containing glutamine and NH_4^+ was 1.3-fold lower than that in RNA isolated from cells grown in minimal medium containing 17 amino acids, glutamine, and NH_4^+ and 2.5-fold lower than that in RNA isolated from cells grown with NH_4^+ as the sole nitrogen source (Fig. 3B). High levels of *glnA* RNA were found in RNA isolated from sporulating and germinating cells. Since cells in these sporulating and germinating populations were probably heterogeneous, it is not possible to draw any firm conclusions about the level of *glnA* transcription during these growth phases.

DISCUSSION

The life cycle and ecological niche of *S. coelicolor*, a gram-positive, filamentous sporeforming soil bacterium (6), is quite distinct from that of enteric bacteria such as *E. coli*. Nonetheless, analogous mechanisms regulate GS synthesis and activity in the two types of bacteria. First, GS enzymatic activity can be inactivated in both bacteria by covalent, posttranslational modification of the GS protein. This modification most likely involves adenylylation in *S. coelicolor*, as it does in *E. coli*. Kustu et al. (15) have shown that in *Salmonella typhimurium*, adenylylation of GS is important in preventing metabolic imbalances during shifts from ammonium-poor to ammonium-rich medium. Since GS is modified after ammonium shock in *S. coelicolor* cells, regulation of GS activity by adenylylation may play a similar protective role in *S. coelicolor*.

Second, transcription of *glnA* is regulated in response to nitrogen availability in both enteric bacteria and *S. coelicolor*. In enteric bacteria, both GS adenylylation and *glnA* transcription are regulated by the Ntr system in response to the intracellular ratio of glutamine to 2-ketoglutarate (21). Thus, the degree of GS adenylylation parallels the level of GS synthesis in these bacteria. That is, little or no adenylylation of GS is seen in cells with completely derepressed GS levels, whereas GS is highly adenylylated in cells with completely repressed GS levels (17). A different pattern of GS adenylylation is seen during steady-state growth in *S. coelicolor*. GS can be modified to the same extent in *S. coelicolor* cells regardless of the level of GS synthesis. For example, a twofold reduction in GS levels, ascribable to GS adenylylation, was seen in J1508 cells grown with glutamine, aspartate, and NH_4^+ as the nitrogen source (Table 1). Under this growth condition, the amount of GS protein synthesized was completely derepressed. In A3(2) cells grown in YEME medium supplemented with glutamine and NH_4^+, a condition in which GS synthesis is maximally repressed, GS activity was also reduced twofold by adenylylation (Table 2).

GS is more highly adenylylated in Glt^- mutants than in the parental strains, although similar amounts of GS protein are synthesized in both mutant and wild-type cells. Intracellular glutamine pools are likely to be higher in Glt^- mutants than in wild-type cells because glutamine cannot be metabolized to glutamate in the absence of glutamate synthase. Since regulation of GS adenylylation, but not GS synthesis, is altered in Glt^- mutants, glutamine or a metabolite of glutamine is likely to be involved in regulation of adenylylation in *S. coelicolor*.

In J1508 cells, *glnA* transcription is reduced only by growth in the presence of amino acids. Although we have not examined the level of *glnA* RNA in A3(2) cells, measurement of active and adenylylated GS in extracts of A3(2) cells indicates that GS synthesis is repressed by growth with glutamine or with amino acids. The most straightforward explanation for this observation is that glutamine transport is reduced in J1508. If so, J1508 must have acquired a unidentified mutation in glutamine transport during its derivation from A3(2). Although *glnA* transcription is regulated in response to nitrogen availability in *S. coelicolor*, the identity of the nitrogen-containing metabolite(s) that regulates transcription is not known. Since GS synthesis is not repressed in Glt^- mutants, which should accumulate glutamine, glutamine may not participate directly in transcriptional regulation.

A different pattern of GS regulation is seen in *S. cattleya*, in which GS expression is more strongly regulated by nitrogen-containing compounds. GS levels were six- to eightfold higher in extracts of *S. cattleya* cells grown with glutamate or nitrate as the nitrogen source than in cells grown with NH_4^+ or glutamine (19). Adenylylation of GS after NH_4^+ shock of *S. cattleya* cells caused GS levels to decline sevenfold (26). However, adenylylation did not play a large role in regulating GS during steady-state growth in *S. cattleya*, since the highest level of adenylylation found in growing cells was 16% (19).

The *S. coelicolor glnA* gene is transcribed from a single promoter during all growth phases and during growth on all nitrogen sources. Analysis of the *S. coelicolor glnA* promoter region did not reveal the presence of any sequences similar to the conserved sequences used by the enteric Ntr regulatory system (21, 28). This finding suggests that a regulatory system distinct from the enteric Ntr system controls *glnA* transcription in *S. coelicolor*. Comparison of the *glnA* promoter sequence with the *S. coelicolor* vegetative promoter consensus sequence (12) indicates that a vegetative −10 sequence, but no apparent −35 sequence, is present in the *glnA* promoter. Many *E. coli* promoters with poor homology to the −35 region consensus sequence are activated by a positive regulatory protein (20). This may also be true for the *S. coelicolor glnA* promoter. However, since multiple forms of RNA polymerase have been detected in *S. coelicolor* (5, 27), it is possible that the *glnA* promoter is transcribed in vivo by a different form of RNA polymerase.

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