Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1979–1983, April 1985
Biochemistry

Expression of *glnA* in *Escherichia coli* is regulated at tandem promoters

(glutamine synthetase/nitrogen metabolism/*glnALG* operon/transcript mapping/*glnL* product)

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ABSTRACT **We have determined that the *glnA* gene of the complex *glnALG* operon of *Escherichia coli* is transcribed from tandem promoters. Expression from the upstream promoter, *glnAp1*, requires the catabolite activating protein, is repressed by nitrogen regulator I (NR_I), the product of *glnG*, and produces a transcript with an untranslated leader of 187 nucleotides. Expression from the downstream promoter, *glnAp2*, requires NR_I as well as the *glnF* product; full expression also requires growth in a nitrogen-limited environment. The downstream transcript has an untranslated leader of 73 nucleotides. We also provide evidence that the function of the *glnL* product is to mediate the interconversion of NR_I between a form capable of activating *glnAp2* and an inactive form in response to changes in the intracellular concentration of ammonia. The function of the two minor promoters of the *glnALG* operon, *glnAp1* and *glnLp*, is to maintain the products of *glnA*, glutamine synthetase, an essential enzyme, and of *glnG*, NR_I, an activator of nitrogen-controlled genes, during carbon-limited growth.**

Escherichia coli and other enteric bacteria respond to nitrogen deprivation by increasing the intracellular concentration of glutamine synthetase and of a number of enzymes essential for the degradation of nitrogen-containing compounds (1). Activation of the expression of *glnA*, the structural gene for glutamine synthetase, and of other nitrogen-regulated genes requires the products of both *glnG* (*ntrC*) and *glnF* (*ntrB*) (2–5). The former gene is a member of the complex *glnALG* operon, which has promoter operators at both *glnA* and *glnL* (*ntrA*) (6–8). We have previously identified the transcriptional start site at *glnLp* and have shown that nitrogen regulator I (NR_I), the product of the *glnG* gene, can prevent the initiation of this transcript by binding to this site (9, 10). The products of the *glnL*, *glnB*, and *glnD* genes also affect the expression of *glnA*. The *glnB* and *glnD* products are part of a system that modifies the catalytic activity of glutamine synthetase by assessing the ratio of 2-ketoglutarate to glutamine, a measure of the intracellular ammonia concentration (11). The *glnB* and *glnD* products also transmit information to the *glnL* product, which in turn acts through NR_I to affect the expression of *glnA* (1).

The experiments described in this paper were directed to the elucidation of the mechanisms responsible for the regulation of *glnA* transcription. We extracted RNA from the wild-type *E. coli* K-12 strain and from strains with mutations affecting *glnA* expression grown under conditions of nitrogen excess and deficiency. We used this RNA to protect a portion of an appropriate probe from digestion by S1 nuclease. Our results reveal the existence of tandem *glnA* promoters and identify the nucleotide sequences involved in the regulation of the expression of *glnA* in response to changing environmental conditions.

MATERIALS AND METHODS

All strains used were derivatives of the *E. coli* K-12 strain YMC9, and all plasmids were derivatives of pBR322 (Table 1). Growth conditions for cells, the minimal medium (W salts) (14), and L broth medium (15) have been described. Minimal medium derepressing for *glnALG* contained 0.4% D-glucose and 0.2% L-glutamine (Ggln); medium repressing for *glnALG* also contained 0.2% $(NH_4)_2SO_4$ (GNgln). L broth medium contained 0.2% L-glutamine (LBgln) and sometimes 0.4% D-glucose (GLBgln), as indicated.

All manipulations involving nucleic acids have been described in Maniatis *et al.* (16) unless otherwise indicated. DNA was labeled at the 5′ end using T4 polynucleotide kinase (IBI) (17, 18), and the strands were separated (16) as described. RNA was extracted from cells and quantified as described (16, 19). The 5′ ends of transcripts were determined as described (20, 21). The DNA–RNA hybridization was performed with at least 100 μg of RNA. Yeast tRNA was added to adjust the RNA to this total. Digestion with 3000 units of S1 nuclease (Boehringer Mannheim) was for 30 min at 37°C. The products of the S1 nuclease digestion were subjected to electrophoresis in 7 M urea/5% polyacrylamide gel (29:1, acrylamide/bisacrylamide) until the xylene cyanol had run 10 cm. The probe was a 740-base *Eco*RI/*Kpn* I fragment from pLR1 labeled at the *Eco*RI end unless otherwise indicated. The *Eco*RI site was known to be 129 bases into the *glnA* structural gene (22).

RESULTS

Transcription of *glnA* in Wild-Type *E. coli*. We determined the 5′ end of *glnA* mRNA extracted from a wild-type *E. coli* strain and found evidence for two transcriptional starts. RNA from cells grown in glucose-containing minimal medium protected 201 bases of a 740-base single-stranded probe, regardless of the source of nitrogen (see t_2 in Fig. 1, lane 2 and 3). RNA from cells grown in nitrogen-limiting medium (Ggln) protected more of the probe than RNA from cells grown in medium with plentiful nitrogen (GNgln) (compare lane 2 and lane 3). This result is consistent with the increase of glutamine synthetase during nitrogen-limited growth. Evidence for a small amount of a second longer transcript, which protected 315 bases of the probe, can also be seen (t_1 in lane 3). Only the longer transcript was found in cells grown in either LBgln (lane 4) or in a carbon-limited minimal medium (histidine as the sole carbon source) (data not shown). The addition of glucose to broth substantially reduced the level of the longer transcript (lane 5). RNA from a strain with a deletion of the *glnALG* operon did not protect the probe (lane 1).

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Abbreviations: NR_I, nitrogen regulator I; GNgln, glucose/ammonia/glutamine; Ggln, glucose/glutamine; LBgln, L broth/glutamine; GLBgln, glucose/L broth/glutamine.

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Table 1. Bacterial strains and plasmids

	Relevant genotype	Source or derivation
Strain		
YMC9	Wild type	Ref. 12
YMC10	Wild type	Ref. 12
YMC11	Δ(*glnA-G*)*2000*	Ref. 12
YMC12	*glnG10*::Tn*5*	Ref. 12
YMC15	*glnL302*	Ref. 13
YMC18	*glnF208*::Tn*10*	Ref. 7
YMC21	Δ(*glnA-G*)*2000*	Ref. 12
YMC26	*glnD99*::Tn*10*	T. Hunt
TH9	*glnB7*	T. Hunt
TH4101	*glnA2501*::Mud1	T. Hunt
GP2	Δ*crp39*	G. Pahel
GP3	Wild type	G. Pahel
GP7	Δ(*glnLG*)*2100* Δ*crp39*	G. Pahel
GP8	Δ(*glnLG*)*2100*	G. Pahel
Plasmids and phage		
pgln6	*glnA*	Ref. 12
pgln26	*glnA* promoter region	*glnA* DNA from λgln101, Ref. 12 cloned into pBR322 (K. Backman)
pLR1	*glnA* (as pgln6)*	This work
pTH814	*glnA* (as pgln6)†	T. Hunt
λgln101	*glnA'–'lacZ*	Ref. 12
λgln103	Wild-type *glnALG* in λ	Ref. 12
λgln104	*glnA*⁺ Δ*glnL2001* *glnG*⁺ in λ	Ref. 12

*The *Sma* I site outside of *glnA* was converted to a *Kpn* I site.
†The *Mst* I site outside of *glnA* was converted to an *Xho* I site.

The pattern of *glnA* transcription was different when *glnA* was borne on a plasmid with a single copy of *glnG* on the chromosome. The shorter transcript was found in cells grown on Ggln, but only the longer transcript was found in cells grown on GNgln (Fig. 1, lanes 6 and 7).

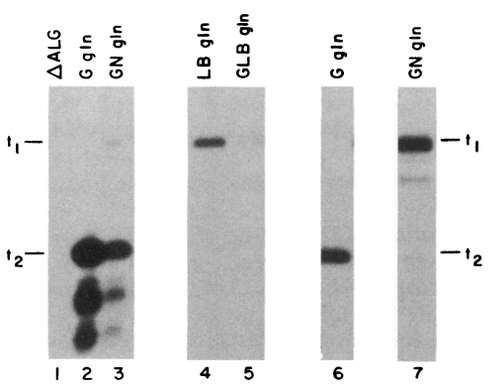

FIG. 1. Transcript mapping of *glnA* mRNA from wild-type *E. coli* K-12. The 5' end of the *glnA* transcripts was determined by the S1 nuclease technique. RNA was from the following sources: lane 1, 60 μg from strain YMC11 grown in GNgln; lanes 2–5, 20 μg, 20 μg, 100 μg, and 100 μg from strain YMC10 grown in Ggln, GNgln, LBgln, and GLBgln, respectively; lanes 6 and 7, 20 μg from strain YMC10/pgln6 grown in Ggln and GNgln, respectively. The fragments of DNA <200 bases, which were seen only when *glnAp2* was fully activated (lane 2) are probably DNA hybridizing to partially degraded RNA. Backman *et al.* (12) showed that all sequences necessary for activating *glnA* expression are upstream from an *Hae* III site, 13 bases downstream from the base coding for the start of t_2 and 188 bases from the 5' end of the DNA probe. These shorter DNAs were <188 bases.

These results identify two *glnA* promoters. We designate the upstream promoter *glnAp1* and the downstream promoter *glnAp2* and their corresponding transcripts t_1 and t_2, respectively. The upstream and downstream transcripts start from bases at positions −114 and +1, respectively (Figs. 2 and 3).

Transcription of *glnA* in Mutant Strains of *E. coli*. Strains with insertions in *glnF*, *glnG*, or *glnD* do not fully increase glutamine synthetase activity when grown in nitrogen-limiting medium (1). Strains with insertions in *glnG* or *glnF* produced only t_1, regardless of the quality of the nitrogen source (Fig. 4, lanes 1, 2, 5, and 6). Strains with an insertion in *glnD* produced t_1 and t_2 when grown in ammonia-containing medium and produced only t_2 when grown in nitrogen-limiting medium (lanes 7 and 8). These results indicate that the products of *glnF* and *glnG*, but not of *glnD*, are absolutely required for activation of transcription from *glnAp2*.

Mutations in *glnL* and *glnB* suppress the effect of the *glnD* mutation on the regulation of glutamine synthetase formation (1, 13). Strains with either the *glnL302* mutation or a deletion of *glnB* have high levels of glutamine synthetase when grown in ammonia-containing or nitrogen-limiting medium. Transcription of *glnA* originated from *glnAp2* in these strains (Fig. 4, lanes 3, 4, 9, and 10). Strains with a deletion of *glnL* also suppress the effect of the *glnD* mutation, but glutamine synthetase is almost normally regulated (13). In nitrogen-limiting growth medium, *glnA* was transcribed from *glnAp2*, as in the wild type (Fig. 5, compare lane 6 to lane 2). In medium with succinate as the carbon source and ammonia and glutamine as nitrogen sources, the wild-type strain produced both t_1 and t_2, while the strain with a *glnL* deletion produced t_2 only (lanes 10 and 14).

A strain with a *crp* deletion did not produce t_1 (Fig. 4, lanes 11–14), consistent with the observation that a *glnF* strain, which produces only t_1, is a glutamine auxotroph when grown with glucose, but not when grown with a poor carbon source (24). In the strain with the *crp* deletion, transcription from *glnAp2* was not impaired when it was grown in sufficiently nitrogen-limited medium (unpublished observations).

Nutritional Shifts and the *glnL* Product. When ammonium sulfate is added to cells of a wild-type strain of *E. coli* growing in nitrogen-limited medium, accumulation of glutamine synthetase ceases immediately (ref. 25; unpublished observations). Five minutes after the shock, transcription from *glnAp2* has essentially ceased (Fig. 5, lanes 2–5). However, in a strain with a *glnL* deletion, the addition of ammonia did not eliminate transcription from *glnAp2* after 20 min (lanes 6–9). When the wild-type strain was grown in carbon-limited medium and shifted to nitrogen-limited medium, there was no appreciable lag in the synthesis of *glnA* mRNA from *glnAp2* (lanes 10–13); however, the strain with a *glnL* deletion had not activated transcription from *glnAp2* significantly within 40 min (lanes 14–17).

DISCUSSION

The upstream *glnA* promoter, *glnAp1*, produces a transcript that has a nontranslated leader of 187 nucleotides and starts at position −114 (Fig. 3). The best −10 RNA polymerase contact site, T-T-C-C-A-T, is 50% homologous to the T-A-T-A-A-T consensus sequence. Transcription is initiated from the DNA 5 bases downstream from this sequence. The best −35 RNA polymerase contact site is T-T-G-C-A-C, which is also 50% homologous to the T-T-G-A-C-A consensus. It is 18 bases from the −10 region, which is one more than optimal (26). Transcription from the upstream *glnA* promoter has an absolute requirement for catabolite activating protein. The closest fit to the 10-base consensus

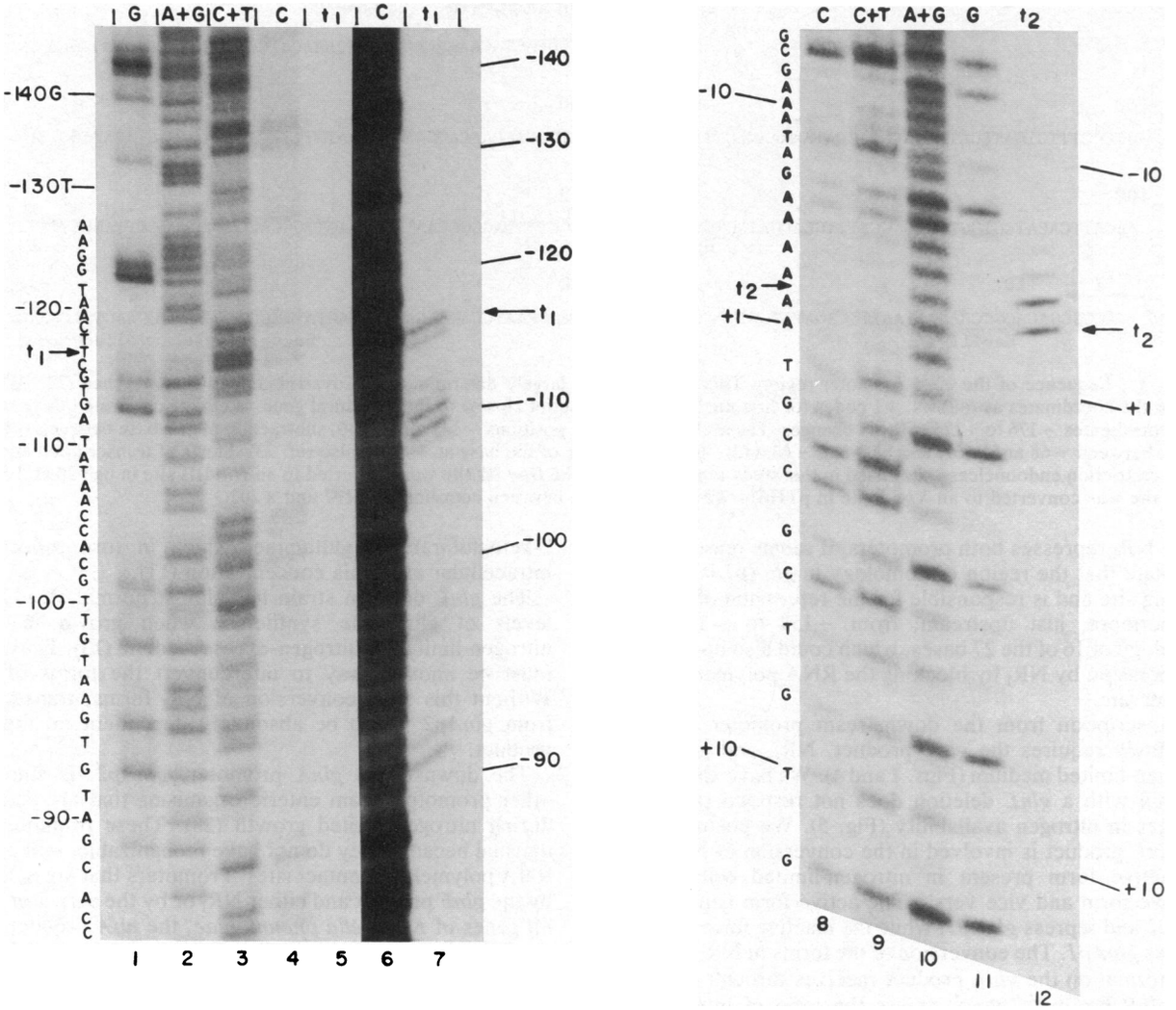

FIG. 2. Determination of the 5′ end of the two *glnA* transcripts. (*Left*) Exact start of upstream transcript t_1 was determined by subjecting the products of S1 nuclease digestion to electrophoresis next to the chemical cleavage sequencing reactions. DNA used for sequencing was the 470-base-pair double-stranded *Xho* I/*Sma* I fragment from pTH814 labeled at the *Xho* I end. A 20-μg portion of RNA, extracted from cells of strain TH4101/pTH814 grown in GNgln medium, was hybridized to the single-stranded *Xho* I/*Sma* I fragment of pTH814. Lanes 1–4, products of G, A+G, C+T, and C sequencing reactions, respectively, electrophoresed into an 8% sequencing gel. Lane 5 shows products of S1 nuclease digestion. Lanes 6 and 7, longer exposure of lanes 4 and 5, respectively. Multiple bands shorter than t_1 seen after S1 nuclease digestion in lanes 5 and 7 result from degradation of RNA when RNA was produced from this particular plasmid. These degradation products were not seen when RNA was from the chromosome or other plasmids (Fig. 1). (*Right*) Start of downstream transcript t_2 was determined by using a 270-base fragment from pgln26. A purified 630-base-pair fragment from pgln26 was end-labeled and digested with *Rsa* I. The largest fragment, 270 bases, was purified, sequenced, and used as a probe for transcript mapping after separating the strands. Products of sequencing reactions and S1 nuclease digestion were subjected to electrophoresis into a 20% sequencing gel. RNA was extracted from strain YMC9/λgln101. Lanes 8–11 show products of C, C+T, A+G, and G sequencing reactions, respectively; lane 12 shows product of S1 nuclease digestion. The assignment of the first nucleotide of t_2 was made as marked because a longer S1 nuclease digestion gave only two bands, of which the more intense band was chosen as correct (unpublished observation).

catabolite activating protein recognition sequence 5′-A-A-N_1-T-G-T-G-A-N_2-T-N_4-C-A-3′ is 5′-C-T-N_1-T-G-T-G-A-N_2-G-N_4-C-A-3′ located between positions −196 and −180 (27).

The upstream *glnAp1* is repressed by NR_I. This conclusion is based on the levels of glutamine synthetase in strains with mutations in *glnG* and/or *glnF*. Strains with genotypes $glnF^-glnG^+$, $glnF^-glnG^-$ and $glnF^+glnG^-$ have 10, 50, and 50 units of glutamine synthetase, respectively, when they are grown in GNgln medium (5). In other words, transcription from *glnAp* was higher without NR_I irrespective of the *glnF* product. The fact that NR_I represses *glnAp1* allowed us to understand the altered origin of transcription from plasmid *glnA* compared to chromosomal *glnA* for cells in GNgln medium: the repressor is titrated out.

We have previously characterized the NR_I binding site at the *glnL* promoter (10). Purified NR_I protected from DNase I digestion a 27-base-pair sequence extending from 12 bases before to 15 bases after the base coding for the start of transcription. A mutation in this region abolished NR_I binding *in vivo* and *in vitro*. We find a striking sequence homology between this NR_I-binding site of *glnLp* and a region from 8 bases preceding to 19 bases after the base coding for the start of the upstream transcript (−122 to −96 of Fig. 3). The homology with 20 of 27 matches is shown below:

```
glnLp 5'   C-T-A-T-A-A-T-G-C-A-C-T-A-A-A-A-T-G-G-T-G-C-A-A-C-C-T 3'
glnAp1     C-A-T-T-G-A-A-G-C-A-C-T-A-T-A-T-T-G-G-T-G-C-A-A-C-A-T
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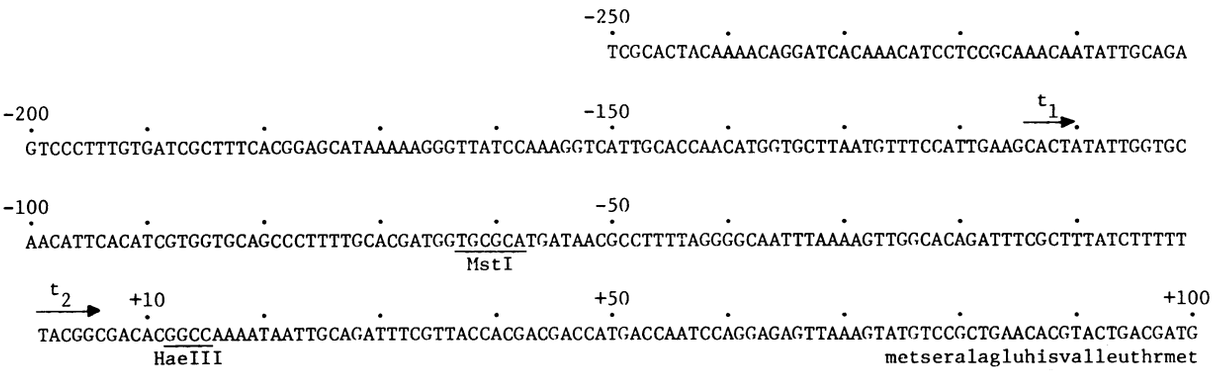

FIG. 3. Sequence of the *glnA* promoter region. This sequence was largely determined by Covarrubias and Bastarrachea (22). We have altered the coordinates as follows: +1 codes for first nucleotide of transcript closest to the structural gene. We have confirmed this sequence from coordinates −176 to +175 with six changes. These changes involve positions −147 and −146, subtraction of one base between −122 and −121, between −68 and −67, and between −63 and −62, and addition of the base at +4 (see also ref. 23). Starts of transcription and some of the restriction endonuclease sites used in this study are designated. The *Hae* III site was converted to an *Hin*dIII site in pgln26 (12) and the *Mst* I site was converted to an *Xho* I site in pTH814. The *Eco*RI site is between coordinates +197 and +202.

Since NR_I represses both promoters, it seems reasonable to postulate that the region of homology in *glnAp1* is the NR_I binding site and is responsible for the repression of *glnAp1*. Furthermore, just upstream, from −154 to −128, is a homology of 16 of the 27 bases, which could also be involved in repression by NR_I by blocking the RNA polymerase −35 contact site.

Transcription from the downstream promoter, *glnAp2*, absolutely requires the *glnF* product, NR_I, and growth in nitrogen-limited medium (Figs. 1 and 4). We have shown that a strain with a *glnL* deletion does not respond rapidly to changes in nitrogen availability (Fig. 5). We postulate that the *glnL* product is involved in the conversion of NR_I from the active form present in nitrogen-limited cells to the inactive form and vice versa. The active form can activate *glnAp2* and repress *glnAp1*, while the inactive form can only repress *glnAp1*. The conversion of the forms of NR_I depends on information the *glnL* product receives through the *glnD* and *glnB* products; these assess the ratio of intracellular 2-ketoglutarate to glutamine, which in turn reflects the intracellular ammonia concentration (11).

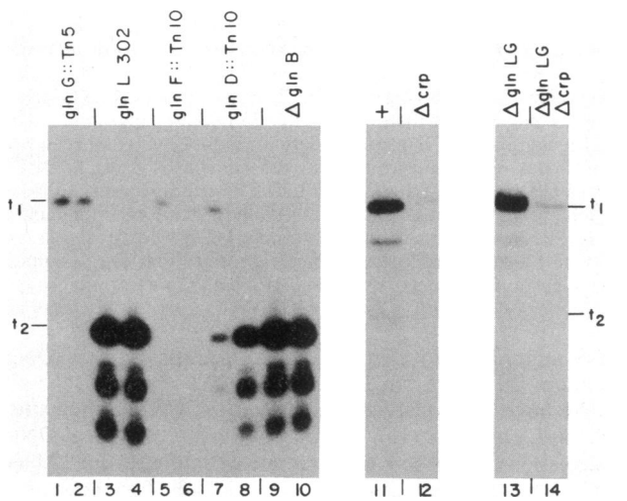

FIG. 4. Transcript mapping of *glnA* mRNA from strains of *E. coli* K-12 with mutations affecting regulation of glutamine synthetase. RNA was from the following strains grown in the indicated medium: lane 1, YMC12 (GNgln); lane 2, YMC12 (Ggln); lane 3, YMC15 (GNgln); lane 4, YMC15 (Ggln); lane 5, YMC18 (GNgln); lane 6, YMC18 (Ggln); lane 7, YMC26 (GNgln); lane 8, YMC26 (Ggln); lane 9, TH9 (GNgln); lane 10, TH9 (Ggln); lane 11, GP3 (LBgln); lane 12, GP2 (LBgln); lane 13, GP8 (GNgln); lane 14, GP7 (GNgln). Experiments represented in lanes 1–10 were done with 20 μg of RNA; in lanes 11–14, they were done with 100 μg of RNA.

The *glnL* deletion strain has almost normal steady-state levels of glutamine synthetase when grown in either nitrogen-limited or nitrogen-excess medium (13). Thus, there must be another way to interconvert the forms of NR_I. Without this slow conversion of NR_I forms, transcription from *glnAp2* would be absolutely dependent on the *glnL* product.

The downstream *glnA* promoter, *glnAp2*, is similar to other promoters from enteric organisms that are activated during nitrogen-limited growth (28). These promoters are unusual because they do not have recognizable −10 or −35 RNA polymerase contact sites. Promoters that are activated by the *glnF* product and either NR_I or by the activator of the *nif* genes of *Klebsiella pneumoniae*, the *nifA* product, have the following consensus: C-T-G-G-Y-A-Y-R-N_4-T-T-G-C-A-N_{6-11}- +1. The sequence 10 bases upstream from the adenine at position +1, which codes for the first base of t_2, conforms well to this consensus: T-T-G-G-C-A-C-A-N_4-T-C-G-C-T-N_{10}- +1 (Figs. 2 and 3). The *nifA* product can replace and activate transcription from *glnAp2* (unpublished observations). The consensus sequence contains an important element of the NR_I binding site, T-G-C-A, which appears many times in the sequence between −300 and +1. Furthermore, the region of DNA between *glnAp1* and *glnAp2* contain many elements with larger stretches of sequence homology with the known NR_I binding site, especially the sequence T-G-G-T-G-C-A. These sequences may allow fine-tuning of the regulation of *glnA* expression. However, the sequence most closely resembling the consensus sequence is located between −27 and −11 (Fig. 3).

The data presented in this paper, together, with previous experiments, allow us to determine when the three promoters of the *glnALG* operon, *glnAp1*, *glnAp2*, and *glnLp*, are used and the function of each promoter (10). For wild-type *E. coli*, *glnAp2* is the predominant promoter for transcription of all three genes whenever the cells are grown in glucose-containing minimal medium. The quality of the nitrogen sources determines the level of expression from *glnAp2*. During growth in carbon-limited minimal medium or in broth without glucose, *glnAp1* is the promoter for *glnA*, and *glnLp* is the promoter for *glnL* and *glnG*: the operon divides into two separate units of transcription. Therefore, the function of these two minor NR_I-repressible promoters is to maintain basal levels of the three products of the operon during carbon-limited growth. The presence of *glnLp* in addition to *glnAp* is not redundant despite parallel expression and a similar mechanism of repression because of a transcription

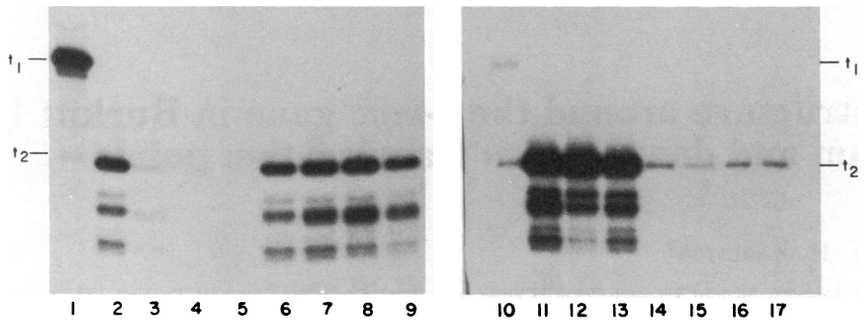

FIG. 5. Transcript mapping of *glnA* mRNA from a wild-type strain of *E. coli* K-12 and a strain with a deletion in *glnL*. (*Left*) Cells were growing in a nitrogen-limiting medium (Ggln) and shifted to a nitrogen-excess medium by addition of $(NH_4)_2SO_4$ to 0.2% when cell density had reached $\approx 4 \times 10^8$ cells per ml. RNA was extracted from cells of strain YMC21/λgln103 (lanes 2–5) and YMC21/λgln104 (lanes 6–9), which had been frozen −5, +5, +11, and +20 min after addition of $(NH_4)_2SO_4$. For lane 1, the RNA was extracted from YMC10/pgln6 grown in GNgln medium to show where t_1 would migrate. For lanes 2–9, 4 μg of RNA was used. (*Right*) Cells growing in a carbon-limiting nitrogen-excess medium [0.4% succinate/0.2% $(NH_4)_2SO_4$/0.2% glutamine] were shifted to a nitrogen-limiting minimal medium (0.4% glucose/0.2% aspartate) when the culture had reached a density of $\approx 4 \times 10^8$ cells per ml. Shift was achieved by centrifugation, washing the cell pellet in 0.85% NaCl, and resuspending it in prewarmed 0.4% D-glucose/0.2% L-aspartate medium. There was no lag in growth rate after the shift. RNA was extracted from cells of strain YMC21/λgln103 (lanes 10–13) and YMC21/λgln104 (lanes 14–17), which had been frozen −2, +7, +20, +40 min, respectively, after the shift; 20 μg of RNA was used.

terminator in the *glnA–glnL* intercistronic region (10). We have previously shown that the ratio of glutamine synthetase to NR_I polypeptides is 80:1 and that there are ≈5 molecules of NR_I dimer per cell when the wild-type *E. coli* strain is grown on GNgln (9). When this strain is grown in carbon-limited medium or broth, the glutamine synthetase level is reduced by a factor of 2–6. If the 80:1 ratio were maintained, then the number of NR_I molecules would become <1 per cell. Therefore, another promoter, *glnLp*, is required to maintain a level of NR_I that enables the cell to respond to alterations in the nitrogen content of the growth medium.

Some features of the regulation of *glnA* in *Anabaena* may be similar to those described here for *E. coli* (29). The *Anabaena glnA* gene is transcribed from a downstream promoter in cells grown in nitrogen-limited medium, and from two or more upstream promoters when they are grown in ammonia-containing medium.

A report showing two promoters of the *glnA* gene of *Klebsiella pneumoniae* appeared after this paper was written (30). We agree with the location and regulation of the downstream promoter. However, the author identified two co-regulated upstream transcripts, RNA2 and RNA3, neither of which corresponds to the *E. coli* upstream transcript t_1. Repression of RNA2 and RNA3 required both the *glnL* (*ntrB*) and *glnG* (*ntrC*) products; we show unequivocally that the *glnL* product is not required for repression of the chromosomal *glnAp1* (Fig. 5, lanes 10 and 14). The upstream *K. pneumoniae glnA* transcripts may in fact start where the author postulates, but because the RNA used for S1 nuclease mapping of the upstream transcripts was from a plasmid, which was expressed in *E. coli* and does not extend to what we identify as the catabolite activating protein binding site, it is possible that both RNA2 and RNA3 were artifacts of the plasmid construction. No evidence for the existence of an upstream promoter for the chromosomal *glnA* gene of *K. pneumoniae* was obtained.

This research was supported by Research Grants GM-07446 from the National Institute of General Medical Sciences and AM-13894 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases and by Grant PCM78-08576 from the National Science Foundation.

1. Magasanik, B. (1982) *Annu. Rev. Genet.* **16,** 135–168.
2. Garcia, E., Bancroft, S., Rhee, S. G. & Kustu, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1662–1666.
3. Gaillardin, C. M. & Magasanik, B. (1978) *J. Bacteriol.* **133,** 1329–1338.
4. Kustu, S., Burton, D., Garcia, E., McCarter, L. & McFarland, N. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4576–4580.
5. Pahel, G. & Tyler, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4544–4548.
6. Pahel, G., Rothstein, D. M. & Magasanik, B. (1982) *J. Bacteriol.* **150,** 202–213.
7. Ueno-Nishio, S., Backman, K. C. & Magasanik, B. (1983) *J. Bacteriol.* **153,** 1247–1251.
8. Krajewska-Grynkiewicz, K. & Kustu, S. (1984) *Mol. Gen. Genet.* **193,** 135–142.
9. Reitzer, L. J. & Magasanik, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5554–5558.
10. Ueno-Nishio, S., Mango, S., Reitzer, L. J. & Magasanik, B. (1984) *J. Bacteriol.* **160,** 379–384.
11. Ginsburg, A. & Stadtman, E. R. (1973) in *The Enzymes of Glutamine Metabolism*, eds. Prusiner, S. & Stadtman, E. R. (Academic, New York), pp. 9–44.
12. Backman, K., Chen, Y.-M. & Magasanik, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3743–3747.
13. Chen, Y.-M., Backman, K. & Magasanik, B. (1982) *J. Bacteriol.* **150,** 214–220.
14. Smith, G. R., Halpern, Y. S. & Magasanik, B. (1971) *J. Biol. Chem.* **246,** 3320–3329.
15. Prival, M. J. & Magasanik, B. (1971) *J. Biol. Chem.* **246,** 6288–6296.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Chaconas, G. & Van de Sande, J. H. (1980) *Methods Enzymol.* **65,** 75–85.
18. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
19. Aiba, H., Adhya, S. & deCrombrugghe, B. (1981) *J. Biol. Chem.* **256,** 11905–11910.
20. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
21. Hansen, U., Tenen, D. G., Livingston, D. M. & Sharp, P. A. (1981) *Cell* **27,** 603–612.
22. Covarrubias, A. A. & Bastarrachea, F. (1983) *Mol. Gen. Genet.* **190,** 171–175.
23. Ow, D. W., Sundaresan, V., Rothstein, D. M., Brown, S. E. & Ausubel, F. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2524–2528.
24. Pahel, G. (1980) Dissertation (Massachusetts Institute of Technology, Cambridge, MA).
25. Friedrich, B. & Magasanik, B. (1977) *J. Bacteriol.* **131,** 446–452.
26. Hawley, D. K. & McClure, W. R. (1983) *Nucleic Acids Res.* **11,** 2237–2255.
27. Ebright, R. H. (1982) in *Molecular Structure and Biological Activity*, eds. Griffins, J. F. & Duax, W. L. (Elsevier, New York), pp. 91–99.
28. Ausubel, F. M. (1984) *Cell* **37,** 5–6.
29. Tumer, N. E., Robinson, S. J. & Haselkorn, R. (1983) *Nature (London)* **306,** 337–342.
30. Dixon, R. (1984) *Nucleic Acids Res.* **12,** 7811–7830.