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Glutamine Synthetase-Constitutive Mutation Affecting the *glnALG* Upstream Promoter of *Escherichia coli*

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The spontaneous *gln-76* mutation of *Escherichia coli* (Osorio et al., Mol. Gen. Genet. 194:114–123, 1984) was previously shown to be responsible for the *cis*-dominant constitutive expression of the *glnA* gene in the absence of a *glnG-glnF* activator system. Nucleotide sequence analysis has now revealed that *gln-76* is a single transversion T·A to A·T, an up-promoter mutation affecting the −10 region of *glnApl*, the upstream promoter of the *glnALG* control region. Both, wild-type and *gln-76* DNA control regions were cloned into the promoter-probe plasmid pKO1. Galactokinase activity determinations of cells carrying the fused plasmids showed 10-fold more effective expression mediated by *gln-76* than by the *glnA* wild-type control region. Primer extension experiments with RNA from strains carrying the *gln-76* control region indicated that the transcription initiation sites were the same in both the *gln-76* mutant and the wild type.

The structural gene for glutamine synthetase, *glnA*, is a part of the complex *glnALG* operon located at 86 min on the *Escherichia coli* chromosome. It is transcribed counterclockwise from *glnA* to *glnG* (13, 16, 19, 31, 37). The products of other genes of the operon, *glnL* and *glnG*, as well as the product of the unlinked gene, *glnF*, are regulatory proteins (21, 26). Genetic and physiological studies have shown that these proteins control the expression of the *glnALG* operon both positively and negatively (2, 12, 17, 20, 24, 32). Furthermore, these gene products are required to activate the expression of a number of genes or operons involved in the transport or utilization of various nitrogenous compounds, the Ntr phenotype (21).

Recently, it has been shown that the *glnA* gene of *E. coli* is transcribed from two tandem promoters (34; A. Garciarrubio et al., submitted for publication). Therefore, the *glnALG* operon can be transcribed from three promoters: two preceding *glnA* (*glnApl* and *glnAp2*) and a third one preceding *glnL* (*glnLp*) (31, 40). According to these results, the view of how the *glnALG* operon is regulated has been slightly modified. Under carbon excess and nitrogen limitation all transcription from the *glnALG* operon starts at the downstream promoter *glnAp2*. Expression from this promoter requires the *glnG* as well as the *glnF* products. Under these conditions, the upstream promoter, *glnApl*, and *glnLp* are repressed by the *glnG* product (33). Under conditions of carbon limitation and nitrogen excess, the activation of *glnAp2* is reduced through the action of the *glnL* product, presumably in combination with the P_{II} protein (21), and the repression of *glnApl* and *glnLp* exerted by the *glnG* product is partially relieved. Compared with conditions of nitrogen limitation, this results in an increase of the *glnA* transcripts originated from *glnApl* and a decrease of those initiated at *glnAp2*.

To better understand the function of the two *glnA* promoters, mutations in this region of the DNA are clearly needed. *cis*-Dominant mutations for glutamine synthetase expression have been isolated in *Klebsiella aerogenes* (36) and *Salmonella typhimurium* (23). Osorio et al. (29) reported the genetic characterization of a *cis*-dominant mutation (*gln-76*) in *E. coli* which leads to high levels of expression of glutamine synthetase in the absence of a functional *glnG* product (under both nitrogen limitation and excess). When all regulatory molecules are present, the effect of this mutation becomes evident only under nitrogen excess conditions, leading to an increase in the glutamine synthetase specific activity. A careful analysis of the phenotypic characteristics of the *gln-76* allele in different genetic backgrounds suggested the presence of an up-promoter mutation.

In this paper we report the molecular characterization of the *gln-76* mutation. The alteration associated with this mutation is a single transversion, T·A to A·T, resulting in an up-promoter mutation affecting *glnApl*. The *glnA* transcripts in this mutant start at the same sites as those in the wild-type strain.

MATERIALS AND METHODS

Bacterial strains and phage. All strains used were derivatives of *E. coli* K-12 (Table 1). P1 *virA* was used for transduction experiments.

Culture media. The NN minimal medium used has been described (9). Additions to this medium in final concentrations were 0.2% glucose as the carbon and energy source and 15 mM NH_4Cl for N-excess medium and either 0.5 mM NH_4Cl or 1 mg of L-glutamine per ml for N-limiting medium. Tests for resistance to 80 μM L-methionine-DL-sulfoximine were made on N-excess medium (29). The presence of transposon Tn*5* was scored by resistance to 30 μg of kanamycin per ml. Concentrations for other nutritional requirements used ranged from 0.5 to 2 mM. The M56 minimal medium was used as described elsewhere (1). This medium was supplemented with 0.2% glucose as a carbon source, 15 mM NH_4Cl, 0.2% Casamino Acids, and 100 μg of ampicillin per ml.

Plasmids. All plasmids used are shown in Table 1. Plasmid DNA was purified by the method of Betlach et al. (4). The constructed recombinant plasmids were derivatives of either pBR327 (39) or pACR1 (9). pKO*glnA* and pKO*gln-76* were

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TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Description	Source or reference
E. coli		
RR1	*hsdS20* (*hsdR hsdM*) *recA13 ara-14 proA2 leuB6 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44*	(9)
MX614	Δ(*pro-lac*) *galE ilv-680 thi-1*	(3)
MX727	Δ(*pro-lac*) Δ(*gal att*$^{\lambda}$ *bio*) *ilv-680 thi-1 glnA71*::Tn*5*	(3)
MX794	RR1 *glnG74*::Tn*5*	Laboratory collection
MX914	Δ(*pro-lac*) Δ(*gal att*$^{\lambda}$ *bio*) *supF ilv-680 thi-1 lamB* Δ(*glnALG-rha*)	(29)
MX919	MX914 *recA56*	This work
MX922	MX919 (pACR1)	This work
MX924	MX914 (pACR1)	This work
MX929	MX614 *gln-76*	(29)
MX966	MX914 (pACR71)	This work
MX990	MX919 (pACR76)	This work
JC5088	Hfr *thr ilv thi rpsE recA56*	(6)
JM101	Δ(*pro-lac*) *supE thi-1*/F′ *traD36 proAB*$^{+}$ *lacI*^{q}Δ*lacZM15*	(27)
Plasmids		
pBR327	Ap^{r} Tc^{r}	(39)
pACR1	ColE1^{imm} *glnA*$^{+}$ *glnL*$^{+}$ *glnG*$^{+}$	(9)
pACR71	pACR1 *glnG74*::Tn*5*	This work
pACR76	pACR1 *gln-76*	This work
pKO1	Ap^{r} *galK*[a]	(25)
pKO1Δ	Ap^{r} *galK*, deletion of the 311-bp *Eco*RI-*Sma*I fragment of pKO1	This work
pKO*glnA*	pKO1-Δ containing the wild-type *glnA* control region	This work
pKO*gln-76*	pKO1-Δ containing the *gln-76* control region	This work
pACR101	Ap^{r} Tc^{s}, pBR327 derivative containing the wild-type *glnA* control region	This work
pACR761	Ap^{r} Tc^{s}, pBR327 derivative containing the *gln-76* control region	This work

[a] The *galK* gene in these plasmids is not expressed since it lacks a promoter region.

hybrid plasmids derived from pKO1 (25); these plasmids contain a 524-base-pair (bp) *Hae*III-*Sma*I fragment carrying the wild-type *glnA* and the *gln-76* regulatory region, respectively, just upstream the *galK* gene. DNA manipulations were by the method of Maniatis et al. (22).

Genetic procedures. The preparation of P1 lysates and the protocol for transductions were as described by Miller (28). To construct *recA* strain derivatives, *thyA* mutants were obtained by trimethroprim selection (28). These were used as recipients in crosses with Hfr strain JC5088 (*recA56*) (Table 1). Rec^{-} derivatives among the Thy^{+} recombinants were recognized by their sensitivity to 2 μg of nitrofurantoin per ml (15).

E. coli cells were prepared for transformation as described by Cohen et al. (7).

Enzyme activities. Glutamine synthetase activity was determined by the γ-glutamyl transferase assay as previously described (9). Specific activities are given as nanomoles of γ-glutamyl hydroxamate formed per minute per milligram of protein at 37°C. Galactokinase activity was assayed as described by Duester et al. (11). Galactokinase specific activities are expressed as nanomoles of galactose phosphorylated per minute per milligram of protein at 30°C.

Protein was determined by the method of Lowry et al. (18) with bovine serum albumin as the standard.

DNA sequence analysis. All DNA sequences were determined by the method of Sanger et al. (38) with deoxy([α-^{35}S]thio)ATP (410 Ci/mmol) and a buffer-linear gradient polyacrylamide gel (5). The DNA fragments to be sequenced were previously cloned into M13mp8 (27) or pBR327 vehicles, and the universal sequencing primer (17-mer) (27) or the *Eco*RI primer (16-mer) (41) was used for the polymerization reactions. The recombinant clones were screened by plaque and colony hybridization as described by Maniatis et al. (22).

Primer extension procedure. A modification of a protocol kindly provided by John Rossi (Beckman Institute of the City of Hope, Duarte, Calif.) was followed by using a synthetic oligonucleotide as primer for the synthesis of cDNA, which is homologous to a sequence adjacent to the *Eco*RI site in pACR101 and pACR761 (41). A ^{32}P-5′ end-labeled primer (0.1 pmol) was mixed with 50 μg of total RNA obtained from an RR1 strain carrying either pACR101 or pACR761 plasmid and grown in N-excess medium. The mixture was denatured at 94°C for 5 min in 8.7 mM Tris hydrochloride (pH 8.3)–0.35 mM EDTA and immediately chilled on dry ice. Primer-RNA hybridizations were incubated at 43°C for 3 h. The reverse transcription reaction was carried out in 52 mM Tris hydrochloride (pH 8.3), 10.4 mM $MgCl_2$, 4.2 mM dithiothreitol, 1 mM each dGTP, dATP, dCTP, and dTTP, and 34 U of reverse transcriptase in a final volume of 25 μl and incubated for 30 min at 43°C. The RNA was degraded with 2 μl of an RNase A solution (1 mg/ml) for 1 h at 37°C. After phenol extraction and ethanol precipitation, the cDNA was suspended in 3 μl of water, mixed with 5 μl of stop dye (95% formamide, 0.02% xylene cyanol, and 0.02% bromophenol blue), and electrophoresed in a 6% acrylamide–7 M urea gel.

Reagents. Enzyme and dideoxyribonucleotides were obtained from P-L Biochemicals, Inc.; amino acids, vitamins, deoxyribonucleotides, and L-methionine-DL-sulfoximine were from Sigma Chemical Co. Radiochemicals were from Amersham International. All other reagents used were of analytical grade.

RESULTS

Cloning of *gln-76* by P1 transduction. The *glnA71*::Tn*5* insertion was first cloned in the ColE1 hybrid plasmid

TABLE 2. Glutamine synthetase levels in extracts of wild-type and *gln-76* strains

Strain	Relevant genotype	Glutamine synthetase sp act[a]	
		N-limiting medium	N-excess medium
MX614	Wild type	1,990	240
MX929	*gln-76*	1,970	530
MX922	Δ(*glnA-glnG*) (pACR1)	3,000	250
MX990	Δ(*glnA-glnG*) (pACR76)	3,120	898
MX966	Δ(*glnA-glnG*) (pACR71)	10[b]	ND[c]

[a] Nanomoles of γ-glutamyl hydroxamate formed per minute per milligram of protein at 37°C. Cultures were grown in minimal medium containing 0.2% glucose and 0.5 mM NH_4Cl (N-limiting medium) or 0.2% glucose and 15 mM NH_4Cl (N-excess medium).

[b] In the case of MX966 the N-limiting medium contained 0.2% glucose and 1 mg of glutamine per ml.

[c] ND, Not determined.

pACR1 (9). P1 phage propagated on MX727 was used to transduce strain MX924; selection was done for Km^r, scoring for glutamine auxotrophy. One selected transductant, MX966, carried pACR1 plasmid harboring the *glnA71*::Tn*5* insertion (which was termed pACR71).

Plasmid pACR76 was then constructed by transducing strain MX966 with P1 propagated on MX929. Transductants were selected for Gln^+, MS^r, and $ColE1^{imm}$, and counterselection was done for kanamycin sensitivity. Plasmid DNA was purified and used to transform MX919 to obtain strains carrying plasmids in *recA56* backgrounds. The glutamine synthetase specific activities of the strains thus obtained are given in Table 2.

DNA sequence of the *gln-76* control region. To obtain the complete nucleotide sequence of the *glnA* control region carrying the *gln-76* mutation, two different strategies were used (Fig. 1). First, a 625-bp *Hae*III fragment from pACR76 was subcloned into the *SmaI* site of M13mp8 phage and sequenced; since this *Hae*III fragment does not contain the entire *glnA* control region, the remainder was obtained by sequencing an *Eco*RI-*Hae*III fragment from the pACR761 (Fig. 1). In every sequencing gel, a parallel lane with an equivalent clone containing the wild-type control region was run as an internal control. The only difference observed in the nucleotide sequence of the promoter-control region carrying the *gln-76* mutation as compared with that of the *glnA* wild type control region was a transversion from T·A to A·T localized at position −127 bp (Fig. 2). This change is located in the proposed −10 region of the upstream *glnA* promoter, *glnApl* (Fig. 3).

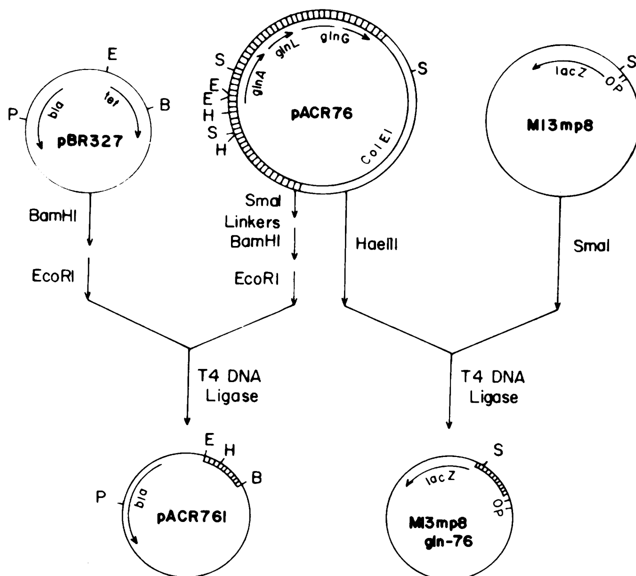

FIG. 1. Schematic representation of the construction of the pACR761 and M13mp8 *gln-76*. Plasmid pACR76 was digested with *Sma*I, and *Bam*HI linkers were added. After cleavage by *Eco*RI and electrophoresis, the 731-bp fragment was eluted from a low-melting-point agarose gel and ligated with pBR327 digested with *Bam*HI and *Eco*RI to obtain pACR761. To clone in M13mp8, pACR76 was digested with *Hae*III, and the 625-bp fragment was purified from a low-melting-point agarose gel and ligated with the M13mp8 (RF) digested wtih *Sma*I. A similar strategy was followed to construct equivalent plasmids carrying the *glnA* wild-type control region (pACR101 and M13mp8-*glnA*, respectively). The restriction enzyme sites relevant in these constructions are shown as B (*Bam*HI), E (*Eco*RI), H (*Hae*III), P (*Pst*I), and S (*Sma*I). The dashed bar indicates the chromosomal DNA, and the open bar indicates ColE1 DNA. The arrows inside the circles show the direction of transcription.

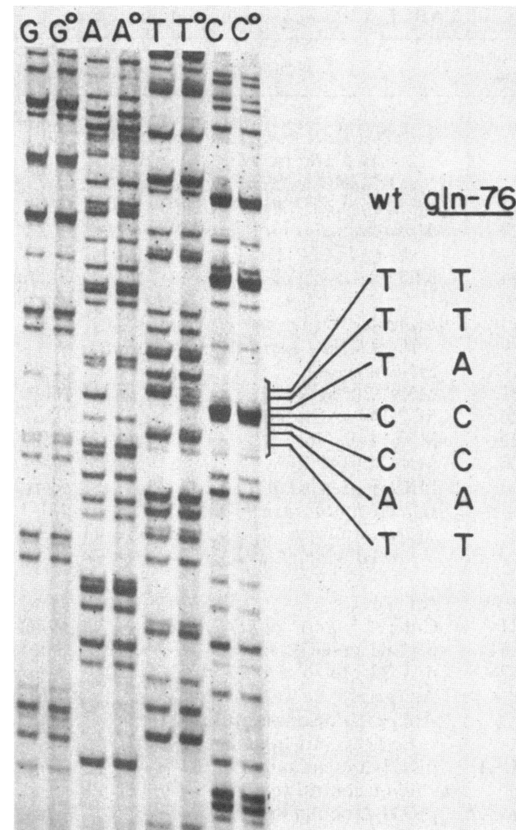

FIG. 2. Autoradiograph showing the nucleotide sequence of the *glnA* (*gln-76*) promoter-control region with the M13 vector. The complete nucleotide sequence of a 814-bp fragment containing the wild-type and the *gln-76* promoter-control region was obtained. A difference in only one nucleotide was detected. This figure shows the region where this alteration was found. Lanes G, A, T, and C correspond to the *gln-76* mutant sequence, whereas lanes G^0, A^0, T^0, and C^0 correspond to the wild-type strain. The single transversion found is indicated at the right of the figure, where wt indicates the wild-type sequence.

Transcription initiation sites in the *glnA-76* control region. To explore whether the transcription initiation start sites of the *glnA* gene were the same in a strain carrying the *gln-76* mutation and in the wild-type strain, we carried out primer extension experiments. Total RNA was prepared from the RR1 strain carrying either pACR761 or pACR101 grown in N-excess medium since the effect of the *gln-76* mutation, in an otherwise wild type background, is best observed under these conditions (Table 2). As a primer we used the synthetic oligonucleotide employed in sequencing plasmids with *Eco*RI inserts (41); it hybridized specifically with the *glnA*-pBR327 fusion transcripts synthesized from either pACR761 or pACR101 (Fig. 4). Two main extended primers can be seen from each RNA preparation. Extended primers detected from RNA isolated from the strain containing pACR761 were identical in length to those obtained when RNA was purified from the strain carrying pACR101 (Fig. 4). The transcription initiation sites were determined by following the sizes for the extended primers.

The fact that the larger transcript was more intense in the RNA prepared from RR1(pACR761) than that from

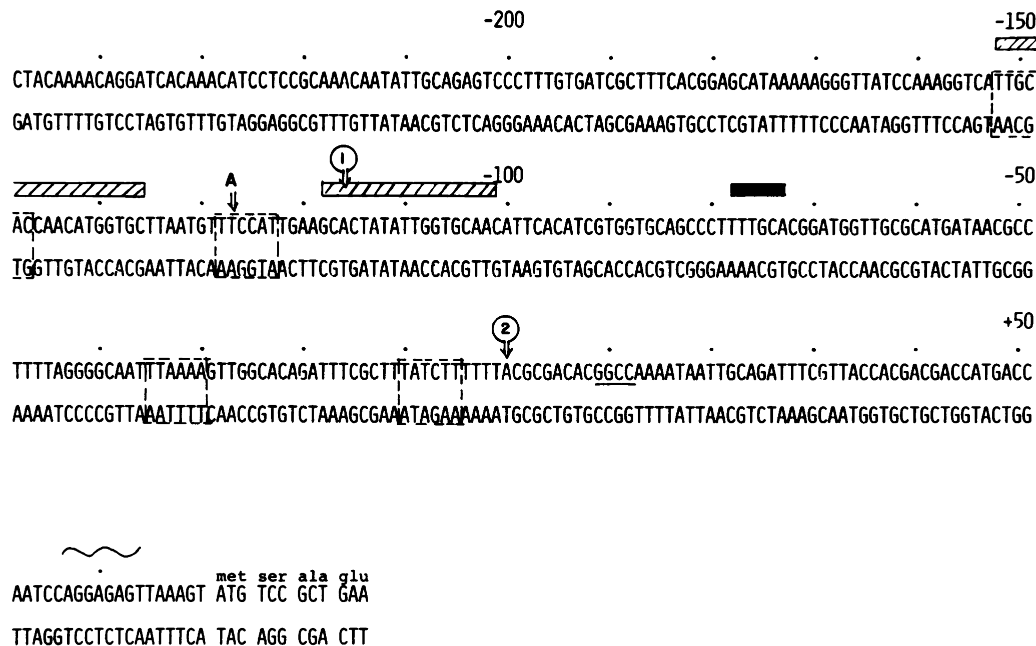

FIG. 3. Nucleotide sequence of the promoter-regulatory region of the *glnA* gene. The sequence of the wild-type promoter-regulatory region of the *glnA* gene presented here is the one previously reported by Covarrubias and Bastarrachea (8), with some corrections: the GC at positions −150 and −149 was a CG; between the C and A at positions −125 and −124 a G has been eliminated. The wavy line denotes the presumed ribosomal binding site. The locations of the two transcription initiation sites are shown by the circled numbers. The presumptive −10 and −35 regions for the *glnA* promoters (*glnApl* and *glnAp2*) are squared. Dashed bars show DNA regions with high homology with the one present in the *glnL* control region and which is protected from DNase digestion by the *glnG* product (35, 40). An *Hae*III site between coordinates +9 and +14 is underlined. The sequence overlined with a black bar corresponds to the sequences proposed by Ow et al. (30) as a possible *glnG* product binding site. The T·A to A·T transversion found in the strain carrying *gln-76* is indicated at position −127.

RR1(pACR101) suggests that *glnApl* bearing the *gln-76* mutation is more active than the *glnApl* wild type. No apparent effect was observed on the synthesis of the smaller transcript.

Galactokinase synthesis from fused plasmids. To determine the transcription efficiency of *glnApl* (*gln-76*), we fused this promoter to the galactokinase structural gene (*galK*). The *galK* system used is that of McKenney et al. (25). The transcriptional probing plasmid vector pKO1 was constructed in such a way that *galK* expression reflects transcriptional signals inserted upstream. *Hae*III-*Bam*HI 524-bp fragments from either pACR101 or pACR761, containing either the wild-type or the mutated *glnApl* promoter, were inserted into plasmid pKO1 to construct plasmids pKO*glnA* and pKO*gln-76*, respectively. The ligation mixtures were used to transform strain RR1 (Table 1), selecting Gal^+ transformants on McConkey-galactose medium. Cultures were grown at 37°C in M56 minimal medium supplemented with 0.2% glucose, 0.2% Casamino Acids, 15 mM NH_4Cl, and 100 μg of ampicillin per ml. The galactokinase activities (nanomoles of [^{14}C]galactose phosphorylated per minute per milligram of protein) were as follows: RR1(pKO1-Δ), 0.8; RR1(pKO*glnA*), 3.4; RR1(pKO*gln-76*), 22.6; MX794(pKO1-Δ), 0.8; MX794(pKO*glnA*), 2.4; MX794(pKO*gln-76*), 25.2. It appears that, under these conditions, transcription initiated from pKO*gln-76* is about six to seven times more efficient than that originating from the pKO*glnA* plasmid. It should be noted, however, that the cloned *Hae*III-*Bam*HI fragment contains the two regulated promoter sequences involved in the transcription of the *glnA* gene. Thus, the galactokinase activities measured under these conditions must be the result of transcription initiated at promoter *glnApl* plus that arising from *glnAp2*. To measure more precisely the strength of the *glnApl* promoter, we determined galactokinase activities in *glnG*::Tn*5* strains harboring pKO*glnA* or pKO*gln-76*. In the absence of the *glnG* product, no activation at *glnAp2* or repression at *glnApl* was expected (10, 32, 34; Garciarrubio et al., submitted for publication). *galK* expression from MX794(pKO*gln-76*) was about 10-fold higher than that from MX794(pKO*glnA*).

DISCUSSION

The *gln-76* mutation is thus far the only *E. coli* *cis*-dominant mutation linked to *glnA* that promotes high levels of glutamine synthetase in the absence of the *glnG-glnF* activator system (29). From the genetic and biochemical characterization of strains carrying this mutation it was proposed that the *gln-76* mutation increases the strength of an existing *glnA* promoter, because *glnA* transcription in strains carrying this mutation remains sensitive to repression. It has been recently found that the *glnA* gene of *E. coli* and that of *Klebsiella pneumoniae* are transcribed from two promoters (10, 34; Garciarrubio et al., submitted for publication). In *E. coli*, expression from the downstream promoter, *glnAp2*, requires the *glnG* and *glnF* products. According to Reitzer and Magasanik (34), transcription from the upstream promoter, *glnApl*, requires the catabolite-activating protein. This promoter is also subject to repression by the *glnG* product.

The molecular characterization of the *gln-76* mutation presented in this work shows that the only alteration associated with *gln-76* is a transversion T·A to A·T localized in the −10 region of the upstream *glnA* promoter, *glnApl* (Fig. 2). The *gln-76* mutation increases the homology of this region to the consensus promoter sequence (14) by introducing one of the most conserved bases in the −10 region (Table

3). Studies by different groups (reviewed by Hawley and McClure [14]) have suggested that the most highly conserved base pairs in the promoter are the main determinants of promoter strength. According to this proposal, the *gln-76* mutation should behave as an up-promoter mutation. This possibility is supported by the fact that the most characteristic phenotype of a strain carrying the *gln-76* allele is an increase in the synthesis of glutamine synthetase under ammonium excess conditions, when this promoter is preferentially used (29) (Table 2). Galactokinase activity synthesized by *glnG* strains carrying plasmid pKO*gln-76* was about 10-fold higher than that synthesized from the wild-type *glnApl* promoter in pKO*glnA* (see above). Even though the fragments fused to *galK* contained both *glnA* promoters, the activity of galactokinase must reflect the strength of *gln-76pl*, since MX794 cells carrying the pKO*gln-76* plasmid were devoid of functional *glnG* product. In the absence of the *glnG* product, both activation of transcription originating from *glnAp2* and repression of *glnApl* are impaired (34; Garciarrubio et al. submitted for publication). As shown above, no difference in the galactokinase activities was observed between the wild type (RR1) and the *glnG* (MX794) strains containing the promoter-probe with either the wild-type or the *gln-76* control region. A plausible explanation for this is that the *glnG* product is being titrated by the high copy number of the plasmids.

The higher transcription efficiency from the *gln-76* promoter is also supported by the results from the primer extension experiment shown in Fig. 4, where the larger transcript is synthesized in higher amount from the mutated promoter than from the wild-type promoter. Apart from *gln-76*, no other mutation which results in an up-promoter has been reported where a change from T·A to A·T has occurred at the −12 position (14).

Location of *gln-76* mutation at the −10 region of the upstream promoter proposed agrees well with the location of one of the *glnA* transcription initiation sites at position −116 (Garciarrubio et al., submitted for publication); this, together with the fact that this mutation generates a stronger promoter, supports the conclusion that the *glnA*p1 promoter (Fig. 3) is physiologically functional. Recently, McCarter et al. (23) have reported the characterization of a mutation that lies in the promoter-regulatory region of the *glnA* gene of *S. thyphimurium*. Some of these mutations present characteristics similar to *gln-76*, since they appear to increase the *glnApl* efficiency without eliminating the repression control

TABLE 3. Sequence of *E. coli glnA* promoters[a]

Promoter	−35 region	−10 region	Reference or source
Consensus promoter	TTGACA	TATAAT	(14)
glnApl	TTGCAC	TTCCAT	Garciarrubio et al., submitted for publication
gln-76pl	TTGCAC	TACCAT	This paper
glnAp2	TTAAAA	TATCTT	Garciarrubio et al., submitted for publication

[a] Comparison of the *glnA* and *gln-76* promoter sequences with the consensus. The location of *glnAp2* is assumed on the basis of the transcriptional start site and by homology with the consensus sequence of a typical *E. coli* promoter. It has been suggested that the *glnF* product functions as a sigma-like factor (10), implying that *glnAp2* belongs to a family of promoters which are recognized by a different RNA polymerase.

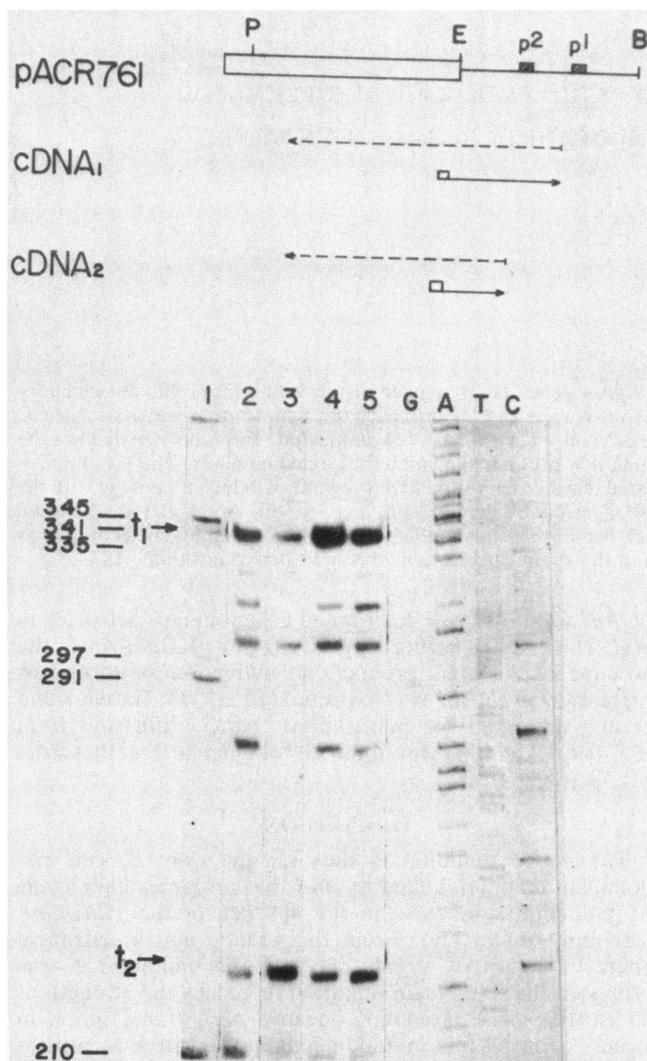

FIG. 4. Localization of the transcription initiations sites in the *glnA* (*gln-76*) promoter-control region. The primer extension experiments were carried out as described in Materials and Methods. The upper part of the figure shows a schematic representation of this experiment. The primer used hybridized with a region adjacent to the *Eco*RI site corresponding sequence in the fused transcripts (discontinuous arrows) coming from either *glnApl* or *glnAp2*. The small open bars indicate the 5′ end-labeled *Eco*RI primer from which the cDNAs were extended up to the 5′ end of the transcripts (continuous arrows). The large open bar indicates the DNA region corresponding to the cloning vector pBR327. p1 and p2 indicate the *glnApl* and *glnAp2* promoters, respectively. Some relevant restriction sites are shown as B (*Bam*HI), E (*Eco*RI), and P (*Pst*I). The lower part of the figure shows the autoradiograph of the extended primers. Two main bands are seen with sizes of 340 bases (t1) and 224 bases (t2). The experiment was carried out with total RNA purified from the RR1 strain bearing pACR761 (lanes 2, 4, and 5) or pACR101 (lane 3). Lanes 2 and 5 correspond to 1:3 and 1:2 dilutions, respectively, from the sample in lane 4. The fainter bands could be due to pauses during reverse transcription. The molecular weight markers used are ϕX174 (RF) DNA digested with *Hin*cII enzyme (lane 1) and G, A, T, and C ladders of a known dideoxy-sequencing reaction. The sizes of the ϕX174 (RF)-*Hin*cII fragments are indicated in bases at the left part of the figure.

by the *glnG* product. McCarter et al. (23) suggest that these mutations lie in the *glnA* upstream promoter.

Whether the regulation of *glnApl* by the *glnG* product in *gln-76* cells is exactly the same as in wild-type cells cannot be concluded at present. It has been proposed that a sequence that is conserved in the *glnA* as well as in the *glnL* control regions corresponds to the recognition site for the *glnG* product (34, 35; Garciarrubio et al., submitted for publication). In the *E. coli glnA* control region this 19-bp sequence has been found twice as a palindromic sequence and overlapped with the upstream promoter (Fig. 3). The *gln-76* mutation does not lie within either of the two presumptive recognition sites for the *glnG* product; rather, the mutation occurred in between these two (Fig. 3). Even if the definition of the operator at *glnApl* deserves further attention, several data indicate that the repression at *glnApl* by the *glnG* product in a strain carrying the *gln-76* mutation is qualitatively normal. Osorio et al. (29) have shown that the *glnG* product in *gln-76* cells is still able to exert its positive as well as its negative effects on *glnA* expression. This is in agreement with data from Northern analysis which shows that in *gln-76* cells, as in wild-type cells, transcription from *glnApl* is more strongly repressed under nitrogen limitation than under nitrogen excess. Furthermore, from this analysis it can be concluded that the repression by the *glnG* product on *gln-76pl* is still very efficient (Garciarrubio et al., submitted for publication).

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