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# Regulation of *Escherichia coli glnB*, *prsA*, and *speA* by the Purine Repressor†

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**A strategy was devised to identify *Escherichia coli* genes subject to coregulation by *purR*. From a data base search, similarities to the *pur* regulon *cis*-acting control site were found in 26 *E. coli* genes. Of five genes examined in which the putative *pur* operator is upstream of the coding sequence, *glnB*, *prsA*, and *speA* bound purified purine repressor in vitro. Binding of the repressor to a *pur* operator in these genes was dependent upon a corepressor. The *pur* operator in *glnB* is located between two major transcription start sites that were located by primer extension mapping. The effect of *purR* on expression of *glnB*, *prsA*, and *speA* was examined by using a *lacZ* reporter. The results indicated two- to threefold repression of these genes by *purR*. Coregulation by *purR* provides evidence that expands the *pur* regulon to include *glnB*, *prsA*, and *speA*. These genes have functions related to nucleotide metabolism.**

Genes for purine nucleotide synthesis in *Escherichia coli* are organized in nine operons, eight of which are coregulated by *purR* (14, 25). The mechanism for regulation of one gene, *purA* (49), has not been reported. *purR* encodes an aporepressor that combines with purine corepressors (26, 34), resulting in an increased affinity for a 16-bp palindromic operator (34) in the eight operons of the regulon. At least four other genes that supply intermediates for purine nucleotide biosynthesis or are involved in synthesis or salvage of pyrimidine nucleotides are part of the *purR*-regulated regulon. These genes include *glyA* (40), *codA* (22), *pyrC* (7, 48), and *pyrD* (48). In addition, *purR* is autoregulated (25, 35).

To determine whether there are additional genes that may be coregulated by *purR*, we searched a nucleotide sequence data base for PurR-binding sites. Here we report the results of this search and the characterization of three additional genes, *prsA*, *speA*, and *glnB*, which each contain a site to which PurR binds in vitro and which are coregulated in vivo by *purR*.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** Strains and plasmids are listed in Table 1. LB and 2xYT were used as rich media (27). Minimal growth medium has been described (14).

**Data base search for *PurR*-binding sites.** *E. coli* nucleotide sequences in the GenBank and EMBL data bases (release no. 71), consisting of $2.8 \times 10^6$ nucleotides and 1,727 entries, were screened by using the sequence analysis program FIND in the Wisconsin Genetics Computer Group software package. The search pattern for PurR-binding sites was 5′-G(A,T,C)AA(A,T,C)CG(T,G)(T,G)T(A,T,C,G)C. Alternative bases, allowed in nonconserved positions based on the operators aligned in Fig. 1, are given in parentheses. No mismatches were allowed in conserved positions in this search pattern.

**Plasmid constructions.** All plasmids used in this work are listed in Table 1. In all cases nucleotide sequences are numbered relative to the start of translation. To clone the *glnB* control region, a segment of *glnB* from nucleotide −140 to nucleotide +203 (39), containing the PurR-binding site, was amplified from chromosomal DNA of *E. coli* W3110 by the polymerase chain reaction (PCR) (29). The amplified fragment containing *Eco*RI and *Bam*HI adapters was ligated into the *Eco*RI and *Bam*HI sites of pUC118, and the resulting plasmid was named pBH500. The same DNA fragment was also inserted into the *Eco*RI and *Bam*HI sites of pRS415 to yield a *glnB′-lacZ* transcriptional fusion in plasmid pBH550.

The 5′ flanking region of *prsA* from *Salmonella typhimurium* containing the control site was isolated from plasmid pBS111R (4). First, pBS111R was digested with *Eco*RI and a 128-bp fragment from nucleotide −500 to nucleotide −373 was isolated. This fragment, containing the *prsA* promoter and *purR* control site, was treated with the *Pol*I Klenow fragment to make blunt ends and was then ligated into the *Hin*cII site of pUC118, yielding plasmid pPRSA1. Plasmid pPRSA1 was the source of the *purR* control site in *prsA* that was used for interaction with PurR. For construction of a *prsA′-′lacZ* translational fusion, an 873-bp *Rsa*I fragment of pBS111R extending from 559 nucleotides upstream of the GTG translation start site to position 70 downstream of the GTG was isolated and ligated into the *Hin*cII site of pUC118 to give plasmid pPRSA2. Next, a 643-bp *Bam*HI fragment that included the *pur* operator, *prsA* promoter, the first 23 codons of *prsA*, and the adjoining polylinker was isolated from pPRSA2, made blunt with *Pol*I Klenow enzyme, and inserted into the *Sma*I site of pMLB1034 to yield pKY100 (see Fig. 2D). In this *prsA′-′lacZ* translational fusion, the first 23 codons of *prsA* followed by 7 triplets from the polylinker are joined to codon 8 of *′lacZ*.

To clone the putative *speA* control region, a segment of *speA* from nucleotide −426 to nucleotide 120 (28) containing the PurR-binding site was amplified from *E. coli* chromosomal DNA by PCR (29). The amplified fragment containing *Eco*RI and *Bam*HI adaptors was ligated into the *Eco*RI and *Bam*HI sites of pRS415 to yield a *speA′-lacZ* transcriptional fusion in plasmid pBH600 (see Fig. 2C). The *lacZ* reporter constructs were all crossed into λRZ5 for insertion into the chromosome (32).

**Enzyme assay.** Cells were grown to mid-log phase in minimal medium with 100 μg of adenine per ml. Strains

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TABLE 1. Strains and plasmids

| Strain or plasmid | Genotype or description | Source or reference |
|---|---|---|
| MC4100 | Δ(*argF-lac*)*U169* | 32 |
| R320 | MC4100 *purR300* | 32 |
| W3110 | Wild type | Laboratory stock |
| BW9653 | *rpoN208*::Tn*10 phoR68* Δ(*argF-lac*)*U169* | R. Somerville |
| KYC4100 | MC4100 *pyrB482*::*kan* | 7 |
| KRC320 | R320 *pyrB482*::*kan* | 7 |
| BH401 | MC4100(λBH550), *glnB'-lacZ* transcriptional fusion, $Lac^+$ $Ap^r$ | This work |
| BH501 | R320(λBH550), *glnB'-lacZ* transcriptional fusion, $Lac^+$ $Ap^r$ | This work |
| BH601 | MC4100(λBH600), *speA'-lacZ* transcriptional fusion, $Lac^+$ $Ap^r$ | This work |
| BH701 | R320(λBH600), *speA'-lacZ* transcriptional fusion, $Lac^+$ $Ap^r$ | This work |
| KYC4201 | KYC4100(λPKY100), *prsA'-'lacZ* translational fusion, $Lac^+$ $Ap^r$ | This work |
| KRC4201 | KRC320(λPKY100), *prsA'-'lacZ* translational fusion, $Lac^+$ $Ap^r$ | This work |
| pUC118 | Phagemid cloning vector, $Ap^r$ | 15 |
| pRS415 | *lacZ* transcriptional fusion vector, $Ap^r$ | 38 |
| pMLB1034 | *'lacZ* translational fusion vector, $Ap^r$ | 15 |
| pBS111R | 1.7-kb *Bam*HI fragment of *prsA* in *Bam*HI site of pUC13 | 4 |
| pPRSA1 | *prsA* nucleotides −500 to −373 (4) in pUC118 | This work |
| pPRSA2 | 873-bp *Rsa*I *prsA* fragment of pBS111R containing 5′ flanking region in *Hin*cII site of pUC118 | This work |
| pPKY100 | *prsA'-'lacZ* translational fusion in pMLB1034 (*prsA* nucleotides −559 to 70) | This work |
| pBH500 | 344-bp PCR fragment of *glnB* nucleotides −140 to 203 (39) in *Eco*RI-*Bam*HI sites of pUC118 | This work |
| pBH550 | Transcriptional fusion of *glnβ* nucleotides −140 to 203 (39) to *lacZ* in *Eco*RI-*Bam*HI sites of pRS415 | This work |
| pHB600 | Transcriptional fusion of *speA* nucleotides −426 to 120 to *lacZ* in *Eco*RI-*Bam*HI sites of pRS415 | This work |

KYC4201 (*prsA'-'lacZ pyrB purR*$^+$) and KRC4201 (*prsA'-'lacZ pyrB purR*) were grown in the same medium, but the pyrimidine auxotrophs were starved for pyrimidines. The medium contained 0.24 mM UMP as a limiting source of pyrimidines (7). β-Galactosidase activity was determined in permeabilized cells by the Miller assay (27).

**Repressor-operator binding.** Corepressor-dependent binding of PurR to operator DNA was assayed by gel retardation as previously described (34). DNA fragments of *glnB* from nucleotide −140 to nucleotide +203, *speA* from −426 to +120, *cytR* from −296 to −29 (44), and *dskA* from −426 to −40 (21) were cloned by PCR from *E. coli* W3110. These fragments, containing *Eco*RI and *Bam*HI adaptors, were end labeled at either the *Bam*HI or *Eco*RI sites by using [γ-$^{32}$P]ATP and T4 polynucleotide kinase. A 158-bp *Bam*HI-*Hin*dIII fragment of *prsA* from plasmid pBS111R was also end labeled with $^{32}$P. Incubations contained 10 fmol of $^{32}$P-labeled DNA fragment, homogeneous PurR, 50 μM guanine corepressor, and buffer II (34) in 20 μl. The DNase I footprinting assay conditions have been described (33).

**Primer extension mapping.** *E. coli* R320 (*purR*) either with or without plasmid pBH550 was grown in 50 ml of minimal medium. Cells in mid-log phase were poured onto ice chilled to −20°C and were collected by centrifugation. Cells were suspended in cold TE buffer containing 10 mM Tris-HCl (pH 7.6)–0.1 mM EDTA, and RNA was isolated by hot phenol extraction (15). The RNA was dissolved in TE buffer and stored at −70°C. Two 19-base oligomeric primers complementary to *glnB* mRNA from nucleotides 29 to 47 and 80 to 98 were labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase and annealed to 50 μg of RNA. The conditions for primer extension have been described (33).

## RESULTS

**Identification of putative target sites for PurR protein.** A computer search of known *E. coli* nucleotide sequences was conducted to identify genes which may be regulated by *purR*. A target sequence, G(A,T,C)AA(A,T,C)CG(T,G)(T,G)T(A,T,C,G)C, was derived from the known *pur* regulon operator sites shown in Fig. 1. The search found 39 sequences that are closely related to the purine repressor control site target. Of the 39 matches, 26 were newly identified and 13 were previously reported operator sites shown in Fig. 1. The 26 newly identified candidates for a *cis*-acting control site are placed in three groups in Table 2 based on location in the 5′ flanking region, coding sequence,

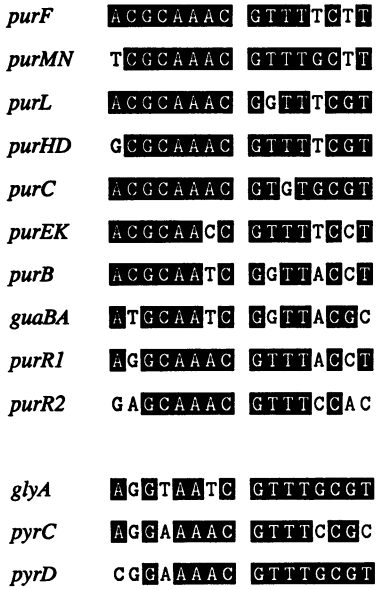


FIG. 1. Alignment of *pur* regulon operators. Bases that match the perfect palindrome are highlighted.

TABLE 2. Sequences related to PurR-binding sites in genes not directly related to purine nucleotide synthesis

| Region and gene | Gene product or function | Nucleotide sequence[a] | Reference |
|---|---|---|---|
| 5′ flanking | | | |
| *glnB* | PII protein, nitrogen regulation | ATGCAAAC GATTTCAA | 39 |
| *cytR* | CytR repressor | CTGTAACC GTTTTCAC | 44 |
| *dksA* | DnaK suppressor protein | GTGAAATC GGTTACGT | 21 |
| *speA* | Arginine decarboxylase | AAGAAACC GGTTGCGC | 28 |
| *ascF* | Phosphotransferase system | GTGAAACC GGTTTCTT | 11 |
| *gsh*II | Glutathione synthetase | TCGTAACC GGGTGCCG | 10 |
| *ppiA* | Peptidyl-prolyl *cis-trans*-isomerase | TTGCAATC GGTTGCTA | 13 |
| *gltS* | $Na^+$/glutamate symport carrier | GCGCAACC GGTTGCGC | 9 |
| *gltS* | $Na^+$/glutamate symport carrier | AAGCAAAC GTTTGCCA | 9 |
| *prsA* | PRPP synthetase | AAGAAAAC GTTTTCGC | 4 |
| Coding | | | |
| *kdpD* | Regulatory protein of *kdp* operon | CGGGAAAC GTTTGCTG | 46 |
| *tdcR* | Regulatory protein of *tdc* operon | ATGCAATC GTTTTCCA | 36 |
| *LT-IIa* | Heat-labile enterotoxin type IIa | CAGCAAAC GATTTCTT | 31 |
| *rimI* | Enzyme acetylating ribosomal protein S18 | ATGAACAC GATTTCTT | 50 |
| *metH* | $B_{12}$-dependent methionine synthase | TTGAAACC GTTTTCGA | 2 |
| *terC3* | Replication terminus gene | CTGAACCC GGTTTCGA | 17 |
| *rrnB* | 23S rRNA | CTGAAACC GTGTACGT | 6 |
| *rrnA* | 23S rRNA | CCGAAACC GTGTACCG | 37 |
| *gltA* | Citrate synthase | AGGCAAAC GGGTTCGC | 19 |
| *nirB* | NADH-dependent nitrite reductase | TGGCAACC GGTTCCTA | 3 |
| *gap* | Glyceraldehyde-3-phosphate dehydrogenase | ACGAAACC GGTTACTC | 5 |
| 3′ flanking | | | |
| *recQ* | Recombination | CCGTAAAC GTTTCCCC | 20 |
| *rshA* | DNA duplication | TTGAACCC GATTTCTG | 45 |
| *fimA* | Type 1 fimbrial subunit | CAGTAACC GGTTTCAT | 23 |
| *metK* | *S*-adenosylmethionine synthetase | CTGAAACC GATTACAC | 24 |
| *aacC2* | Aminoglycoside-(3)-*N*-acetyltransferase | AGGAAATC GGGTTCGT | 43 |

[a] Positions that match the perfect palindromic *pur* regulon operator shown in Table 1 are in boldface type.

or 3′ flanking region. Of the genes listed in Table 2, eight have functions related to nucleotide metabolism or DNA replication. *prsA* encodes phosphoribosylpyrophosphate (PRPP) synthetase (4), which supplies PRPP for nucleotide and amino acid synthesis. *cytR* encodes a protein that regulates genes involved in nucleoside and deoxynucleoside salvage (44). *gltS* and *glnB* encode proteins that function in the uptake of glutamate and conversion of glutamate to glutamine, respectively. The amide of glutamine provides three of the purine ring nitrogen atoms. *speA* and *metH* are involved in the synthesis of polyamines, which have been implicated in the synthesis of RNA and DNA (41). *terC3* and *dksA* are thought to have roles in DNA replication (17, 21). We have focused on a subset of the genes with a possible relationship to nucleotide metabolism in which the putative *purR* control site is in the 5′ flanking region. Our strategy was to isolate fragments of the 5′ flanking region of relevant genes and determine whether PurR binds in vitro and confers regulation in vivo.

**Binding of PurR to control sites.** DNA fragments containing the putative *purR* control site from *glnB*, *speA*, *cytR*, and *dksA* were amplified by PCR and cloned from *E. coli* chromosomal DNA. The *prsA* 5′ flanking region was isolated from the cloned *S. typhimurium* gene (4). This site is identical in *E. coli* and *S. typhimurium prsA* and in each case is situated 4 bp upstream of the −35 promoter element (4). DNA fragments were end labeled with $^{32}P$, and binding of PurR was evaluated by gel retardation assays. The results presented below indicate that PurR bound to sites in *glnB*, *prsA*, and *speA* but not to the putative sites in *cytR* and *dksA*. Thus, the sites in *glnB*, *prsA*, and *speA* were examined further.

Interaction of PurR with *glnB* was determined with a DNA fragment corresponding to nucleotides −140 to +203 relative to the translation start (39). The nucleotide sequence of the *glnB* 5′ region is shown in Fig. 2A. Binding of PurR to the *glnB* DNA fragment is shown in Fig. 3A. Binding of PurR was quantitated by counting the radioactivity in gel slices containing free DNA and protein-DNA complexes. The concentration dependence for PurR binding is plotted in Fig. 3B and is compared with the binding to the *purF* operator (16). Approximately 0.2 μM PurR was required for 50% binding to *glnB* compared with 6 nM for comparable binding to *purF*. Therefore, the affinity of PurR for the *purF* control site was approximately 33-fold higher than for the site in *glnB*. Binding of PurR to the *glnB* site was dependent upon a purine corepressor (Fig. 3B).

Gel retardation assays described above provided evidence for corepressor-dependent binding of PurR to a *glnB* fragment containing the 5′ flanking region. DNase I footprinting was performed to define the site of protein-DNA interaction. A DNase I footprint for the PurR-*glnB* interaction is shown in Fig. 4A. A region from −63 to −85 that includes the inferred *pur* operator site was protected against DNase I cleavage. Thus, PurR binds specifically to a *pur* gene operator in *glnB*.

Interaction of PurR with *prsA* and *speA* was examined by an approach similar to that used for *glnB*. Gel retardation assays were conducted with a *prsA* fragment corresponding to nucleotides −500 to −373 relative to the start of translation and with a 386-bp *speA* DNA fragment, nucleotides −426 to −41 relative to the translation initiation site. The level of binding of PurR to *speA* is shown in Fig. 3C, and that to *prsA* is shown in Fig. 3D. The concentrations of PurR

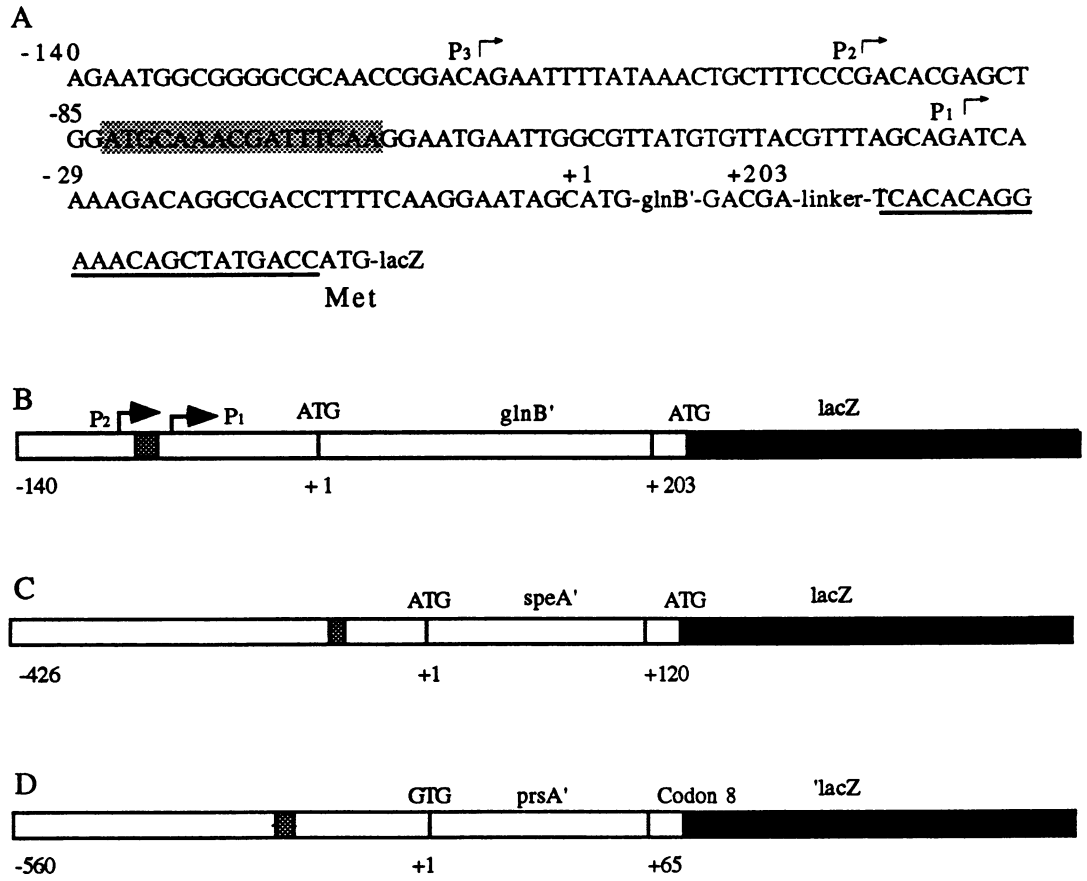


FIG. 2. (A) DNA sequence of *glnB'-lacZ* fusion. Numbers indicate positions relative to the translational start site of *glnB*. The PurR-binding site is shaded. Arrows represent transcriptional start sites. The 5′ flanking sequence of *lacZ* preceding the translation start is underlined. (B) Schematic representation of *glnB'-lacZ* fusion in plasmid pBH550. The minor promoter $P_3$ has been omitted. (C) Schematic representation of *speA'-lacZ* fusion in plasmid pBH600. (D) Schematic representation of *prsA'-'lacZ* fusion in plasmid pPKY100. In all cases, numbers indicate the nucleotide positions relative to the translation start, and arrows indicate transcriptional start sites. The shaded boxes indicate the PurR-binding sites. Open boxes in front of *lacZ* indicate either a plasmid polylinker or *lacZ* 5′ flanking region. Black bars indicate *lacZ* coding regions.

required for 50% binding were 0.15 μM for *prsA* and 0.045 μM for *speA*. Binding affinities for PurR were thus *purF*>*speA*>*prsA*>*glnB*. In each case repressor binding was dependent upon a corepressor. DNase I footprinting established that PurR bound to the predicted control site in *speA* and *prsA* (Fig. 4C and E).

***glnB* promoter.** Based on the identification of a *pur* gene operator in the 5′ flanking region of *glnB*, it was of interest to localize the promoter. In all of the *pur* genes except *purB* and *purR* the operator overlaps or is contiguous with the promoter (14, 35). Although *glnB* has been cloned and sequenced (39), the promoter was not previously identified. A transcriptional fusion plasmid was constructed to detect *glnB* promoter activity. A DNA fragment corresponding to *glnB* nucleotides −140 to +203 was ligated to a promoterless *lacZ* gene to yield plasmid pBH550 (Fig. 2B). This plasmid was transformed into strain R320 (*purR*), and β-galactosidase activity was measured. A β-galactosidase activity of 16,500 Miller units was obtained from the multicopy *glnB'-lacZ* fusion, indicating that the *glnB* fragment has promoter activity. The β-galactosidase control with the vector alone gave 15 U of β-galactosidase activity.

To determine the physical relationship of the PurR-binding site relative to the *glnB* promoter, the transcription start site was mapped by primer extension using reverse transcriptase. With a primer complementary to *glnB* nucleotides 29 to 47, two major cDNA bands were obtained and provisionally aligned to start sites at nucleotides −33 and −95 (Fig. 2A and 5). This experiment was conducted with RNA isolated from strain R320 carrying the *glnB'-lacZ* fusion plasmid pBH550. The same mRNA 5′ ends were obtained with a second primer that annealed to nucleotides 80 to 98 (data not shown). The 5′ end of the *glnB* mRNA was also mapped by using RNA transcribed from the chromosomal gene in plasmid-free strain R320 (Fig. 5, lane 3). Although smearing occurred, two bands were obtained that correspond to species seen with RNA from the multicopy *glnB'-lacZ* plasmid. In addition a minor band was detected corresponding to a 5′ end at approximately nucleotide −118. Although it is not possible to distinguish between cDNA species arising from premature termination by reverse transcriptase and those resulting from authentic multiple transcription start sites, the data are consistent with transcription from two major promoters, designated P1 and P2 in Fig. 2A, and a weaker promoter, P3. The PurR-binding site is located between P1 and P2.

There are potential −10 sites for a σ70 promoter upstream of P1 and P2, but these positions lack appropriately spaced

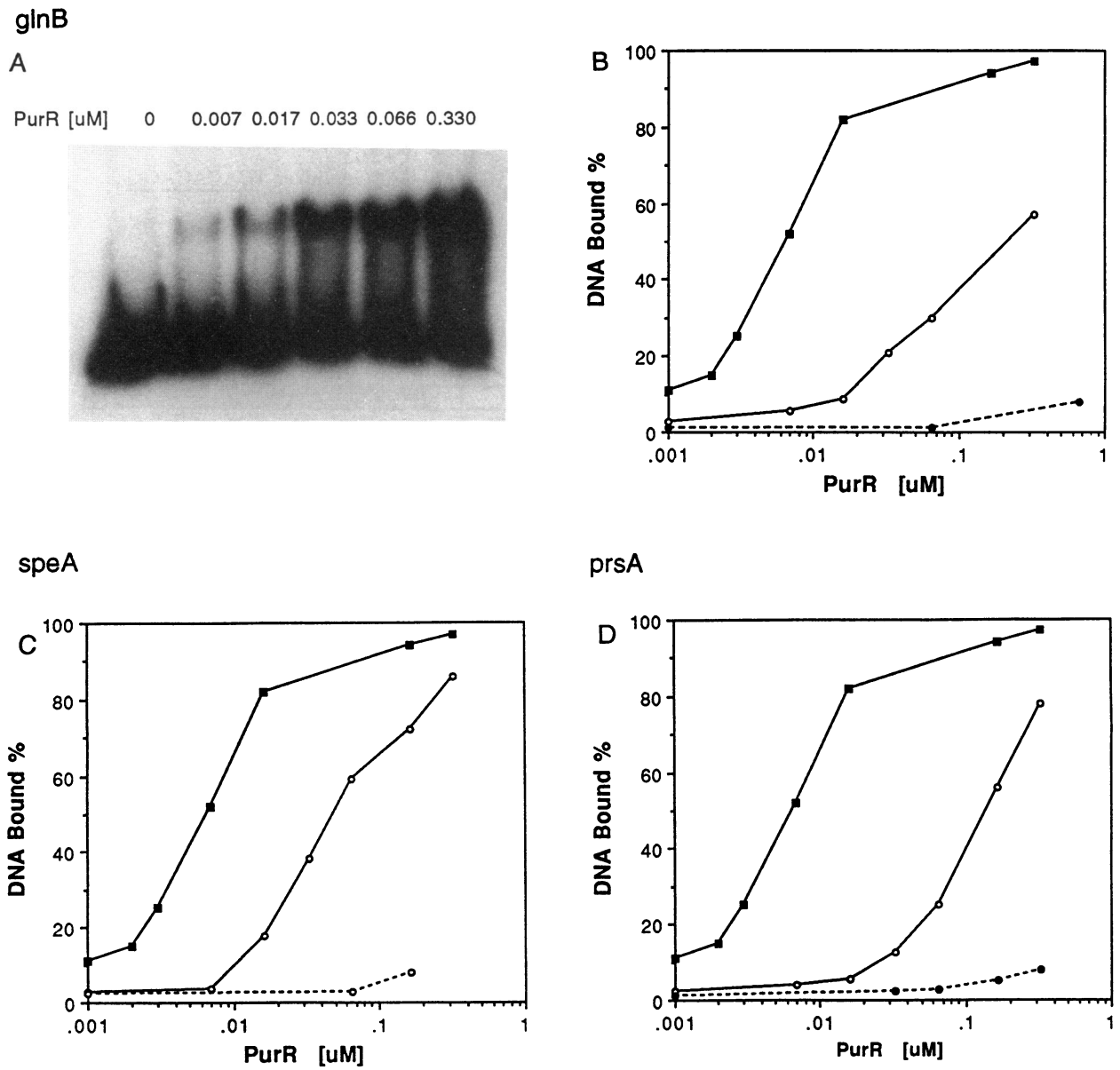


FIG. 3. Binding of PurR to operators in *glnB*, *speA*, and *prsA*. Gel retardation assays were carried out as described in Materials and Methods. Bound and unbound DNA fragments were counted for radioactivity, and the percent bound was plotted as a function of PurR concentration. Guanine-dependent binding of PurR to *glnB*, *speA*, and *prsA* was compared with that to *purF* (16). (A) Gel retardation assay for binding of PurR to *glnB*; (B) binding curves for *glnB*; (C) binding curves for *speA*; (D) binding curves for *prsA*. Symbols: ○, binding with guanine; ●, binding without guanine; ■, binding with guanine to purF.

matches to a consensus −35 element (12). In vitro transcripts were not obtained when RNA polymerase was used with σ70 and a linear *glnB* template containing nucleotides −140 to 230, although in a control experiment correct transcription initiation was obtained from linear *purF* template DNA. Therefore, we cannot distinguish whether *glnB* promoter function in vivo requires additional factors, a different sigma factor, or specialized template DNA topology or whether the in vitro conditions were inappropriate. To determine whether the *glnB* promoter is nitrogen regulated and dependent upon σ54, plasmid pBH550 was transformed into strain BW9653 with a disruption of the *rpoN* gene encoding σ54. β-Galactosidase levels were essentially unchanged in the *glnF* mutant compared with those in the wild type, suggesting that transcription of *glnB* does not require σ54 (data not shown).

**In vivo regulation by *purR*.** Fusions to a *lacZ* reporter were used to examine regulation of *glnB*, *prsA*, and *speA* by *purR*. For *glnB* and *speA*, *purR*$^+$ and *purR* strains were grown with excess adenine. Data in Table 3 show about twofold repression of *glnB* and *speA* by purR$^+$. Under similar growth conditions there was nearly complete repression of *prsA* by endogenous pyrimidines in a prototrophic strain (data not shown). Accordingly, a *pyrB* strain was limited for pyrimidines. Under this growth condition expression of *prsA* was readily detectable (Table 3). The low level of pyrimi-

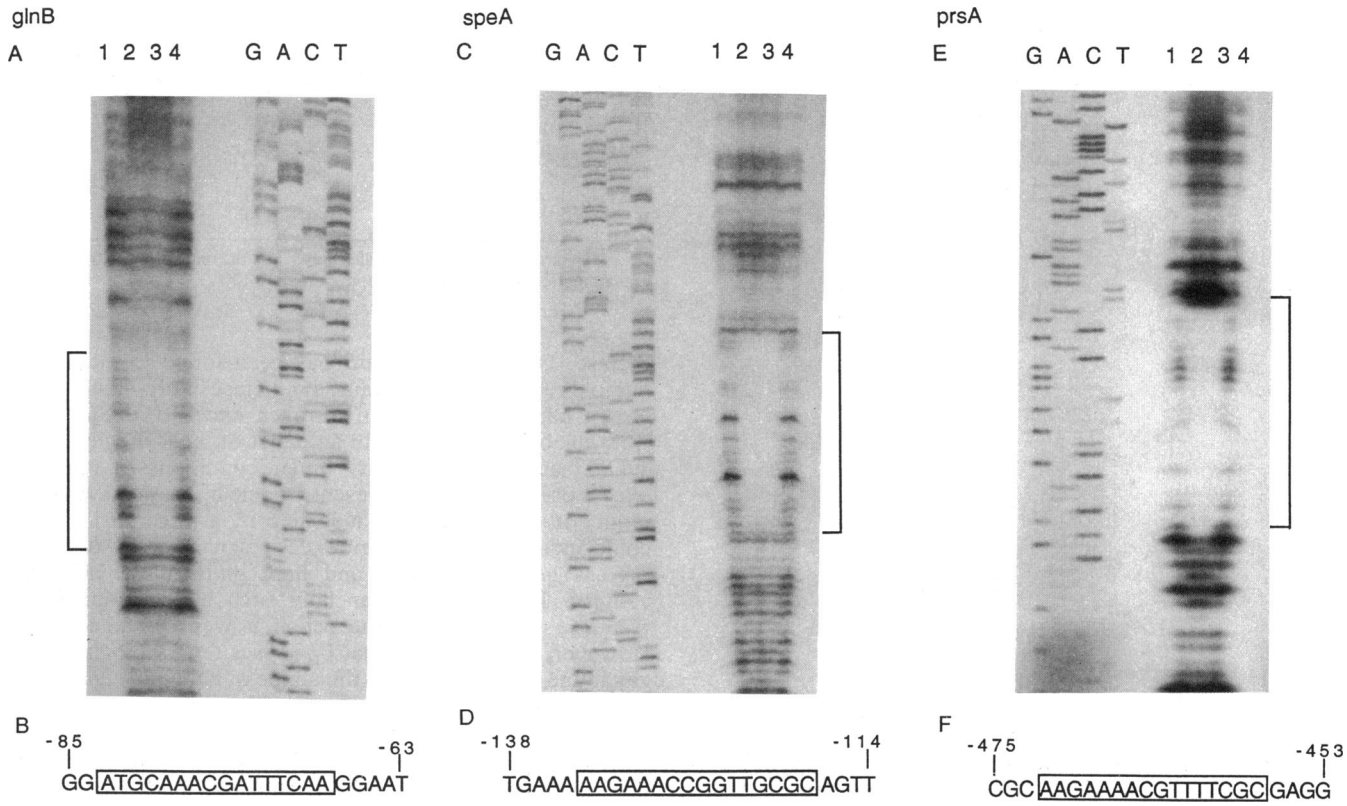


FIG. 4. DNase I footprinting for interaction of PurR with *glnB*, *speA*, and *prsA* control sites. (A) *glnB*. Lanes 1 and 4, 343-bp *Eco*RI-*Bam*HI fragment of *glnB* labeled at the *Eco*RI site; lanes 2 and 3, the same DNA plus the purine repressor. G, A, C, and T, sequencing ladder for size standards. (B) Nucleotide sequence of *glnB* protected by the repressor. The *pur* operator is boxed. (C) *speA*. Lanes 1 and 4, 547-bp *Eco*RI-*Bam*HI fragment of *speA* labeled at the *Bam*HI site; lanes 2 and 3, the same DNA plus the purine repressor. G, A, C, and T, sequencing ladder size standards. (D) Nucleotide sequence of *speA* protected by the repressor. The *purR* site is boxed. (E) *prsA*. Lanes 1 and 4, 158-bp *Bam*HI-*Hin*dIII fragment of *prsA* labeled at the *Hin*dIII site; lanes 2 and 3, the same DNA plus the purine repressor. G, A, C, and T, sequencing ladder size standards. (F) Nucleotide sequence of *prsA* protected by the repressor.

dine-regulated *prsA* expression may result from regulatory elements in the long ~416-nucleotide 5′ untranslated region (4). Nevertheless, *prsA* expression was repressed about threefold by *purR* (Table 3).

Since *glnB* has a role in regulating the expression of *glnA* (see reference 1) and *glnB*-encoded $P_{II}$ functions to modulate the activity of glutamine synthetase (39), we looked for an effect of *purR* on glutamine synthetase level and activity. An effect of *purR* on the level or activity of glutamine synthetase was not seen under conditions of nitrogen excess or limitation (data not shown).

## DISCUSSION

Previous studies have shown that *purR* regulates not only eight of the nine operons for de novo purine nucleotide synthesis (14, 25) but also *glyA* (40), *codA* (22), *pyrC* (7, 48), and *pyrD* (48), in addition to autoregulation of *purR* itself (25, 35). These genes thus constitute a *pur* regulon having functions associated with purine and pyrimidine nucleotide synthesis. Here we have provided evidence that expands the *pur* regulon to include *prsA*, *glnB*, and *speA*. *prsA* and presumably *glnB* function to generate metabolites that are used for synthesis of purine nucleotides. Control of *speA*, on the other hand, suggests some type of coordination between the pool of purines and polyamines. Three criteria were applied to the assignment of these genes to the *pur* regulon. (i) Each gene contains a 16-bp nucleotide sequence that is similar to the *pur* operator consensus. For this study we restricted our analysis to genes with putative operator sites located upstream of the coding sequence. It is known, however, that operator sites in *purB* (15) and in *purR* (33) are within the coding region. (ii) Each putative operator bound the purine repressor in vitro, and binding was dependent upon a guanine corepressor. It is, however, possible that in some cases additional factors such as cyclic AMP and cyclic AMP receptor protein (30) may be required for binding of the repressor to the operator. Absence of an accessory factor could account for lack of binding in vitro. (iii) In each case the putative control site conferred regulation by *purR* to a *lacZ* reporter gene in vivo. Considering criteria i and ii, it is possible that additional genes listed in Table 2 may be subject to control by *purR*. In addition, it is important to note that our data base search may have missed some biologically relevant *pur* operator sites since mismatches were not allowed in the search pattern at conserved positions. An evaluation of each conserved position in the *pur* operator has not been conducted.

It is necessary to consider whether a two- to threefold *purR*-mediated effect on expression has regulatory significance or is simply adventitious. There are two aspects to this issue. First, is a two- to threefold effect on expression large

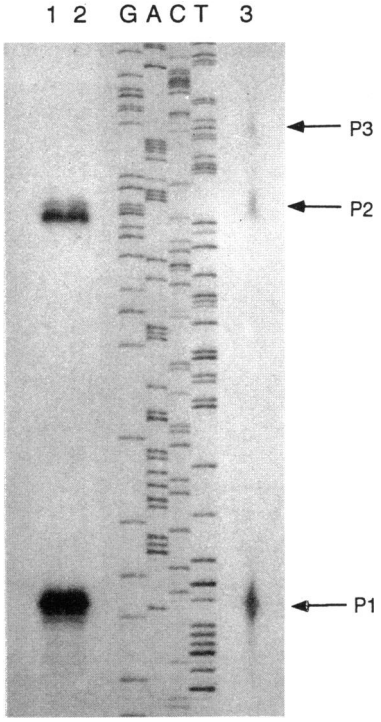


FIG. 5. Primer extension mapping of 5′ ends of *glnB* mRNA. RNA was isolated from strains R320 and R320(pBH550). An oligonucleotide primer was used for the 5′ end mapping of mRNA and for generating a sequencing ladder (lanes G, A, C, and T) from a phagemid clone. The sequence is of the coding strand, complementary to the mRNA. Lanes 1 and 2, primer-extended cDNAs from strain R320(pBH550). Start sites P1 and P2 were assigned from a lighter exposure of the autoradiogram in which the bands were sharper. Lane 3, primer-extended cDNA from strain R320. cDNA bands are indicated by arrows.

enough to be significant? Second, what is the likelihood that a twofold difference in *glnB* expression actually modulates glutamine production? With regard to the first consideration, we believe that a two- to threefold modulation of gene expression can be significant. To begin with, two bona fide *pur* regulon genes, *purR* (35) and *purB* (15), are regulated two- to threefold. In these cases the 16-bp palindromic PurR-binding sites are constrained by their location in protein-coding sequences and yet have been retained during a billion years of evolution. Neither third-position codon changes nor amino acid replacements in a nonconserved region of *purB* (15) have obliterated the 16-bp palindromic sequence, consistent with the view that there has been selection for this operator site that is involved in two- to threefold regulation. Furthermore, regulation by *purR* of *glyA* (40) is 1.5- to 3-fold, while *pyrC* (7, 48) and *pyrD* (48) are regulated approximately twofold. There is thus precedence for two- to threefold coregulation of functionally related genes.

The question of whether a twofold modulation of *glnB* by *purR* could influence glutamine production is more difficult. *glnB* encodes a $P_{II}$ protein that is considered to be a central element of nitrogen regulation in bacteria (see for example reference 1). $P_{II}$ participates in transcriptional control of nitrogen-regulated genes, including *glnA*, as well as in controlling the adenylylation and activity of glutamine synthetase. Operationally, *glnB* has been found to mediate a fivefold reduction of glutamine synthetase expression in cells grown under conditions of nitrogen excess (1). Since we were unable to demonstrate an effect of *purR* on glutamine synthetase level or activity under conditions of nitrogen excess or limitation, it is presently unclear whether a twofold reduction in $P_{II}$ synthesis mediated by a large purine pool could influence the nitrogen excess signal which serves to repress *glnA* expression and limit glutamine synthetase activity. It is also unclear how such a purine-mediated response would benefit the cell if it were to take place. However, given the fact that $P_{II}$ senses the α-ketoglutarate/glutamine ratio in cells and the amide of glutamine is used in three steps in the purine nucleotide biosynthetic pathway, a regulatory link may exist.

TABLE 3. Regulation of *prsA*, *speA*, and *glnB* by *purR*

| Strain | Gene | *purR* | β-Galactosidase[a] | Repression (fold) |
|---|---|---|---|---|
| BH401 | *glnB* | + | 510 | 2.1 |
| BH501 | *glnB* | − | 1,022 | |
| BH601 | *speA* | + | 42 | 2.3 |
| BH701 | *speA* | − | 97 | |
| KYC4201 | *prsA* | + | 10 | 2.9 |
| KRY4201 | *prsA* | − | 29 | |

[a] β-galactosidase values in Miller units are the means of two or three experiments, with a variation of approximately 10%.

It is interesting that PurR failed to bind to the putative *pur* regulon sites in *cytR* and *dksA*. Comparison of these sites with purine operator sequences revealed that nucleotides which are absolutely conserved in purine operators were also conserved in the putative sites in *cytR* and *dksA*. Previous work (7, 16, 32, 35) indicated that mutations in highly conserved nucleotides abolished binding of PurR whereas mutations in nonconserved nucleotides did not. Therefore, at least two possibilities exist: (i) bases in nonconserved positions may contribute to PurR binding, or (ii) binding of PurR to *cytR* and *dksA* requires additional *trans*-acting factors. We did not investigate this issue further.

The two- to threefold regulation of *glnB*, *speA*, and *prsA* relative to the 17- to 28-fold regulation of *purF* (14, 32) can be explained by reduced repressor-operator affinity. Data in Fig. 3 show that the affinity of PurR for binding to the *purF* operator is 8- to 33-fold greater than that for binding to *speA*, *prsA*, and *glnB*. However, the three- to fourfold greater affinity of PurR for *speA* than for *prsA* and *glnB* was not reflected in comparably greater in vivo regulation of *speA* by *purR*. Thus, within these three genes, the *pur* operator position relative to the promoter may be important for regulation (8). In *glnB* the *pur* operator site is located between two major transcription start sites, P1 and P2, as shown in Fig. 2A. In *prsA* (4) and *speA* (28) the *pur* operator is situated near the inferred promoter sites.

There is only limited information available that would allow comparison of *purR* coregulation with pathway-specific regulation of *glnB*, *prsA*, and *speA*. We are unaware of previous reports about *glnB* regulation. *prsA* from *S. typhimurium* was reported to undergo 3- to 10-fold derepression during pyrimidine starvation (47), whereas a twofold regulation was seen in *E. coli* (18). Putrescine and spermidine were reported to repress *speA*-encoded arginine decarboxylase approximately three- to fourfold (42). These results suggest that the magnitude of corepression by *purR* compares favorably with pathway-specific regulation of these genes.

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