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A Role for a Highly Conserved Protein of Unknown Function in Regulation of *Bacillus subtilis purA* by the Purine Repressor

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Regulation of the purine biosynthetic gene *purA* was examined by using a transcriptional fusion to a luciferase reporter gene. Transcription was repressed about 10-fold by the addition of adenine and increased approximately 4.5-fold by the addition of guanosine. This regulation is mediated by a purine repressor (PurR). In a *purR* mutant, basal expression was increased 10-fold, and there was no further stimulation by guanosine or repression by adenine. An open reading frame, *yabJ*, immediately downstream from *purR* was found to have a role in the repression of *purA* by adenine. Repression by adenine was perturbed in a *purR*$^+$ *yabJ* mutant, although guanosine regulation was retained. Mutations in the PurR PRPP binding motif abolished guanosine regulation in the *yabJ* mutant. Thus, PRPP appears to be required for upregulation by guanosine. The amino acid sequence of YabJ is homologous to the YER057c/YjgF protein family of unknown function.

There is an 11-step pathway for the de novo synthesis of IMP in *Bacillus subtilis* (22) and *Escherichia coli* (23). IMP is a branch point for the synthesis of AMP in two steps and the synthesis of GMP in two steps. A 12-gene *pur* operon encodes the enzymes required for the synthesis of IMP in *B. subtilis* (1). Two genes, *purA* and *purB*, are required to convert IMP into AMP. The *purA* gene is unlinked to the *pur* operon, while *purB* is in the operon. The *purA* gene encodes adenylosuccinate synthetase, and adenylosuccinate lyase is the product of *purB*. Purine biosynthesis is feedback regulated by end products of the pathway, and production of adenine and guanine nucleotides is balanced by regulation of the AMP and GMP branches. Expression of the *pur* operon is subject to dual regulation of transcription initiation and termination (1). The addition of adenine to cells results in the repression of transcription initiation, and the addition of guanosine promotes premature transcription termination in an mRNA leader region preceding the first structural gene. A purine repressor (PurR) mediates the regulation of transcription initiation. *purR* was cloned and overexpressed (20), and the protein was purified (19). The present studies were undertaken to assess the regulation of the branch from IMP to AMP. Earlier it was reported that adenine decreased the adenylosuccinate synthetase activity in *B. subtilis* and that guanosine increased the enzyme activity (17). Here we present evidence that adenine mediates a PurR-dependent repression of *purA* transcription and that guanosine leads to an upregulation of transcription. The repression by PurR is dependent upon a second protein, YabJ, which is homologous to a group of proteins of unknown function. On the other hand, the activation by guanosine is dependent upon an interaction of PRPP with PurR but not upon YabJ.

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MATERIALS AND METHODS

Bacterial strains and vectors. The bacterial strains and vectors used in this study are listed in Table 1. Since the *purA* promoter is lethal to *E. coli* in high-copy-number plasmids (12), strain KE94 was used for the propagation of plasmids pPAL1 and pPAL3. Plasmid pPAL4 contained an inactivated *purA* promoter and could therefore be amplified in DH5α F′. Strain XL2 Blue was used to propagate vectors containing the *purR* gene. The *B. subtilis* transformants were selected on either Penassay broth agar (Difco) or Luria-Bertani agar plates containing either chloroamphenicol or neomycin at 5 μg/ml.

Construction and integration of a *purA′-lucGR* fusion. A *purA′-lucGR* fusion in plasmid pPAL1 was constructed in two steps. First, *lacZ* in pCATZ1 (2) was excised and replaced with the click beetle *lucGR* gene (21) from plasmid pCSS962 (9). Second, a fragment containing the 5′ end of *purA* from nucleotides −419 to +66 (relative to the start of transcription at +1) (12) was amplified by PCR from *B. subtilis* DE1 chromosomal DNA and inserted into the polylinker sequence immediately upstream of the *lucGR* gene. In this construction, codon 7 of *purA* is followed by 15 nucleotides of plasmid polylinker. Translation of *purA* terminates at a TGA in the polylinker. Translation of *lucGR* is expected to start at the initiator ATG which overlaps the *purA* TGA stop as follows: ATGA. The nucleotide sequence of the *purA′-lucGR* junction was verified. The resulting plasmid with the *purA′-lucGR* transcriptional fusion was named pPAL1. Plasmid pPAL1 was integrated into the chromosome of *B. subtilis* DE1 by homologous recombination as shown in Fig. 1.

Construction and integration of mutations in the *purA* control site and in the *purR* locus. The *purA* gene contains an *Eco*RV site at nucleotide −174 (12). An *Mlu*I site at −55 was introduced into the *purA* control region in pPAL1 by site-directed mutagenesis. The resulting plasmid was digested with *Eco*RV and *Mlu*I, the 5′ cohesive end was filled by Klenow fragment, and a *Sma*I Nm^r cartridge from pBEST 501 (6) was added to produce pPAL3. Plasmid pPAL3 thus contains a −174 to −55 *cis*-control-site deletion upstream of the *purA′-lucGR* fusion. pPAL3 was integrated into the chromosome of *B. subtilis* PAL1 by homologous recombination.

A mutation at the −10 site of *purA* was introduced into pPAL1 by a PCR method (10), resulting in a change of the −10 promoter element from TAAACT to TGCACT. The resulting plasmid, pPAL4, was integrated into the chromosome of *B. subtilis* DE1 in the same manner as pPAL1.

Disruption of *purR* was done by integration of plasmid pMW11, which contains a *purR*::*neo* disruption (20), into the chromosome of PAL1.

Mutations were constructed in the PurR PRPP binding site. Plasmid pR6H is a *purR*$^+$ derivative in which six histidine codons are fused onto the 3′ end of *purR* (19). PurR mutations D203A and D204A were constructed by site-directed mutagenesis by using the method of Kunkel et al. (8). The His-tagged *purR6H* gene was excised from pR6H and transferred into M13mp18 for the mutagenesis. The mutations were verified by DNA sequencing. After mutagenesis, the mutant genes were returned to the pT7-7-derived vector to yield pR6H3A(D203A) and pR6H4A(D204A).

A series of integration vectors for the *purR* locus were constructed. First, the Nm^r gene from pBEST 501 was inserted into the *Sma*I site of pGEM-7Zf(+) (Promega). Next, an *Hpa*I-*Hin*dIII fragment of *purR6H*, *purR6H*(D203A), or *purR6H*(D204A), was inserted into the *Ecl*136II and *Hin*dIII sites. Finally, a

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Strains		
B. subtilis		
DE1	Prototrophic revertant of W168 *trpC2*	1
PAL1	*purA′-lucGR* integrant	This study
PAL3	*purA′-lucGR* integrant with a *cis*-control-site (−174 to −55) deletion	This study
PAL4	*purA′-lucGR* integrant with a −10 promoter mutation	This study
NMW	*purA′-lucGR* integrant with *purR*::*neo* disruption	This study
N6H	*purR6H* Nm^r *′yabJ* integrant of PAL1	This study
N3A	*purR6H*/D203A Nm^r *′yabJ* integrant of PAL1	This study
N4A	*purR6H*/D204A Nm^r *′yabJ* integrant of PAL1	This study
NPR	$purR^+$ Nm^r *′yabJ* integrant of PAL1	This study
E. coli		
DH5α F′	F′ ϕ80 *dlacZΔM15* Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdr17*(r_K^- m_K^+) *supE44* λ^- *thi-1 gyrA96 relA1*	5
XL2 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB* $lacI^qZ\Delta M15$ Tn*10* (Tet^r) Amy Cam^r]	Stratagene
KE94	F′ *hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK12 rpsL20* (*str*) *xyl-5 mtl-1 supE44* λ^- *pcnB80* *zad*::Tn*10*; used to maintain low plasmid copy number	11
Plasmids		
pPAL1	Integration vector with transcriptional *purA′-lucGR* fusion	This study
pPAL3	pPAL1 derivative with −174 to −55 *cis* control site replaced by Nm^r gene	This study
pMW11	pT7-7 derivative containing *purR*::*neo* disruption	20
pPAL4	pPAL1 derivative with −10 promoter mutation	This study
pR6H	*purR6H* derivative of pT7-7	19
pR6H3A	*purR* D203A mutation in pR6H	This study
pR6H4A	*purR* D204A mutation in pR6H	This study
pN6H2	*′purR6H* Nm^r *′yabJ′* derivative of pGEM-7Zf(+)	This study
pN3A2	*′purR6H*/D203A Nm^r *′yabJ′* derivative of pGEM-7Zf(+)	This study
pN4A2	*′purR6H*/D204A Nm^r *′yabJ′* derivative of pGEM-7Zf(+)	This study
pNPR1	*′purR* Nm^r *′yabJ′* derivative of pGEM-7Zf(+)	This study

fragment from codon 39 to codon 122 of *yabJ* downstream from *purR* was amplified by PCR and inserted into *Xho*I-*Sph*I sites downstream from the Nm^r gene to obtain plasmids pN6H2, pN3A2, and pN4A2, respectively. pNPR1 was prepared in the same way as pN6H2, pN3A2, and pN4A2, except that a wild-type *Hpa*I-*Hin*dIII fragment from pMW10 (20) was used instead of His-tagged *purR6H*. pN6H2, pN3A2, pN4A2, and pNPR1 were integrated into the chromosome of PAL1 by a double crossover type of homologous recombination. These strains contain disrupted *yabJ*. The gene replacement was verified by Southern analysis and PCR.

PRPP inhibition of PurR binding to DNA. Plasmid pR6H was used for overexpression and hyperproduction of PurR containing a C-terminal His tag (19). The two PurR PRPP binding site mutants, D203A and D204A, were hyperproduced from plasmids pR6H3A and pR6H4A, respectively, by the same procedure as for the wild type. The proteins were purified by using an Ni^{2+} affinity resin as described previously (19). Binding of the wild type and the two mutants to a DNA fragment containing the *purA* control region (−163 to +47) was determined as described previously (19). The 20-μl binding mixture contained 10 mM HEPES (pH 7.6), 100 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 10 fmol of ^{32}P-labelled DNA fragment, 5 ng of purified protein, and varied concentrations of PRPP. Free DNA and PurR-DNA complexes were separated by electrophoresis on agarose gels and quantitated by counting radioactivity with a Packard instant imager.

Primer extension mapping. The *B. subtilis* DE1 cells were grown in 200 ml of minimal medium (1) supplemented with 1 mM guanosine to an optical density of 650 nm (OD_{650}) of 0.7. The cells were poured onto ice chilled to −20°C and collected by centrifugation for 10 min at 3,000 × *g* at 2°C. The total RNA was isolated by the chromosomal DNA isolation method for gram-positive bacteria (15) with slight modifications and additions as necessary. The cells were lysed with lysozyme (0.5 mg/ml) in the presence of 30 U of RNasin/ml (Promega) for 30 min, followed by proteinase K treatment in 1% sodium dodecyl sulfate at 55°C for 2 h. RNA was extracted once with phenol-chloroform and once with chloroform and precipitated with isopropanol. RNA pellet was dissolved in water containing 30 U of RNasin/ml. The RNA solution was treated with RNase-free DNase and extracted with phenol-chloroform. RNA was precipitated with isopropanol and dissolved in water containing 30 U of RNasin/ml. When possible, all solutions were treated with diethylpyrocarbonate. The yield of RNA was approximately 5 mg, with an A_{260}/A_{280} ratio of 1.7.

The primer for primer extension was the same one that was used previously for mapping the transcription initiation site of *B. subtilis purA* (12). Approximately 450 ng of the fluorescent-labelled primer was annealed to 55 μg of total RNA in 15 μl of solution containing 10 mM Tris-HCl (pH 8.3), 150 mM KCl, and 1 mM EDTA, by maintaining the mixture at 65°C for 90 min and then letting it cool slowly to room temperature. Thirty microliters of solution containing 30 mM Tris-HCl (pH 8.3), 15 mM MgCl, 8 mM dithiothreitol, a mixture of the four deoxynucleoside triphosphates (1 mM each), 38 U of RNasin, and 18 U of avian myeloblastosis virus reverse transcriptase was added, and incubation was performed for 60 min at 42°C. The cDNA products were precipitated with ethanol and analyzed by the automatic ABI 377 sequencer by using GeneScan software (Perkin-Elmer). The length of the cDNA product was determined by comparing the retention time of the primer extension product with those of the products from the dideoxy sequencing reaction of pGEM-3Zf(+) (Promega) by using M13 reverse dye primer (Applied Biosystems) and dideoxy GTPs.

Luciferase assay. Expression of *purA* was determined by measuring luciferase activity in strains with *purA-lucGR* integrated into the chromosomal *purA* gene. The cultures for luciferase assay were grown in minimal medium (1) supplemented either with 1 mM adenine, 1 mM guanosine, or both adenine and guanosine (1 mM each) or with no added purine compounds. The medium contained either chloramphenicol (strains PAL1 and PAL4) or neomycin (other integrants) at 5 μg/ml. The 5-ml overnight cultures were centrifuged, and the pellets were suspended in 50 ml of minimal medium containing the respective supplements. The cultures were grown to an OD_{650} of 0.5. From each culture, a 1-ml sample was taken, centrifuged, and resuspended in 5 ml of minimal medium containing the respective supplements. The 5-ml cultures were grown for 2 h. Samples of 1 ml were taken, and 100 μl of solution A (1 M K_2HPO_4 and 20 mM EDTA [pH 7.8]) was added to each sample. The final samples were frozen at −70°C. For the measurement of luciferase activity, the samples were thawed and centrifuged, and the supernatant was carefully removed. The cells were resuspended in 50 μl of the supernatant solution. One volume of cell lysis buffer 1 (Bio-Orbit) containing 2 mg of lysozyme/ml and 2 mg of bovine serum albumin (BSA)/ml was added, and the mixture was incubated at room temperature for 5 min. The lysate was centrifuged, and a 50-μl aliquot of the supernatant was sampled. One hundred microliters of Luciferin reagent and 100 μl of ATP reagent of GenGlow-100 kit (Bio-Orbit) were added to the extract, and the maximum light output was measured by using an LKB 1250 luminometer. The values were compared with a standard curve made by assaying known amounts of firefly luciferase standard in dilution buffer (1 mg of BSA/ml, 0.5× cell lysis buffer 1, 0.45× minimal medium, and 0.05× solution A). When necessary, the

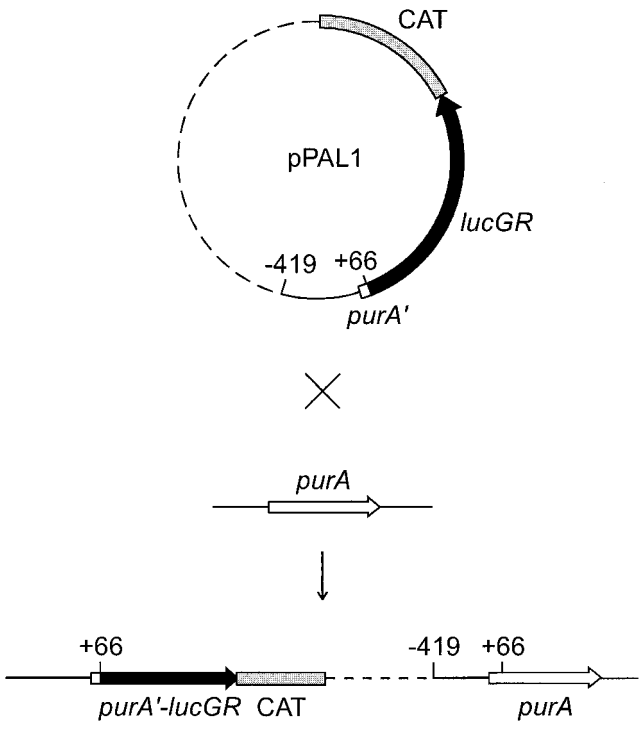

FIG. 1. Integration of pPAL1 into the *B. subtilis* chromosome. Vector sequences are shown by a dashed line, and *purA* upstream sequence is shown by a solid line.

samples were diluted with dilution buffer. The results are expressed as attomoles of luciferase per 10^8 CFU.

RESULTS

Integration of a *purA'-lucGR* fusion and *purR* locus mutations into the *B. subtilis* chromosome. A gene encoding luciferase was used as a reporter to monitor *purA* expression. To obtain a single-copy *purA'-lucGR* integrant, plasmid pPAL1 was recombined into the *B. subtilis* chromosome in strain DE1, as diagrammed in Fig. 1, to give strain PAL1. Strain PAL1 is *purA*$^+$. Two *purA'-lucGR* derivatives, one with a promoter mutation (PAL4) and another with a *cis*-control-site deletion (PAL3), were constructed in a like manner. The integrations were verified by PCR amplification and DNA sequencing. Like strain PAL1, the two *purA'-lucGR* derivatives are *purA*$^+$.

In order to evaluate regulation by *purR*, a series of mutations was constructed in the *purR* region. The *purR* locus in these strains, obtained by recombining *purR* plasmids into PAL1 (*purA'-lucGR*), is shown in Fig. 2. NMW contains a *purR*::*neo* disruption. Strain N6H (*purR*$^+$) contains a functional repressor with a C-terminal His tag. In strain N6H (*purR6H*, *'yabJ*), the downstream *yabJ* gene was disrupted. Mutations of Asp 203 or Asp 204 were incorporated into *purR6H* in N3A or N4A.

Regulation of *purA*. The level of *purA* expression was determined by using the *lucGR* gene as a reporter. In the *purA'-lucGR* transcriptional fusion, a DNA fragment containing nucleotides −419 to +66 of the *purA* operon was ligated immediately upstream from the *lucGR* gene. A basal luciferase activity of 498 amol per 10^8 CFU was obtained from strain PAL1 (*purA'-lucGR*) grown in medium without added purines (Table 2). For a luciferase control, a −10 promoter mutation was incorporated into *purA'-lucGR* in strain PAL4. The −10 promoter mutation abolished luciferase activity (data not shown), indicating that all of the activity was derived from *purA'-lucGR* expression. To examine regulation by PurR, *purR* and O_{purA} operator mutations were incorporated into the *purA'-lucGR* reporter strain. The levels of luciferase activity in these strains are given in Table 2. In the *purR*$^+$$O^+_{purA}$ wild type, there was 10-fold repression of *purA* expression by adenine and a 4.5-fold activation by guanosine. When adenine and guanosine were combined, repression by adenine overrode the activation by guanosine.

In the strains with a *purR* disruption or an operator deletion (nucleotides −174 to −55), regulation of *purA* was lost. In the regulatory mutants, basal expression was about 10-fold higher than in the *purR*$^+$$O^+_{purA}$ wild type, reflecting release from repression by the endogenous pool of adenine or adenine nucleotides. Basal expression was not repressed by the addition of adenine to cells or upregulated by the addition of guanosine in these mutants. These results support the view that both repression and upregulation are a consequence of the PurR interaction with the *purA* control site. Repression of PurA (adenylosuccinate synthetase) by adenine and upregulation by guanosine were reported previously, although the regulatory elements were not identified (17).

Primer extension mapping. To rule out the possibility that activation by guanosine in the wild-type cells is due to a shift of the transcription initiation site, the 5′ end of the *purA* transcript in DE1 cells grown with excess guanosine was determined by primer extension mapping by using the same primer previously used for mapping the 5′ end of *purA* mRNA (12). The length of primer extension product was 117 nucleotides (data not shown), the same length as that previously determined with the cells grown in the absence of purines.

Role of *yabJ* in the regulation of *purA*. To test the significance of the *yabJ* gene downstream from *purR* for regulation of *purA*, *yabJ* was disrupted to obtain strain N6H. The data in Table 3 show that regulation by *purR6H* in strain N6H was perturbed and is not similar to that by *purR* in PAL1 (Table 2). Repression of basal expression by adenine was abolished in N6H (*purR6H 'yabJ*), although the upregulation by guanosine was retained.

In order to verify that the loss of *purA* repression in N6H (*purR6H 'yabJ purA'-lucGR*) was due to the disruption of *yabJ* rather than to the addition of six His codons in *purR*, an

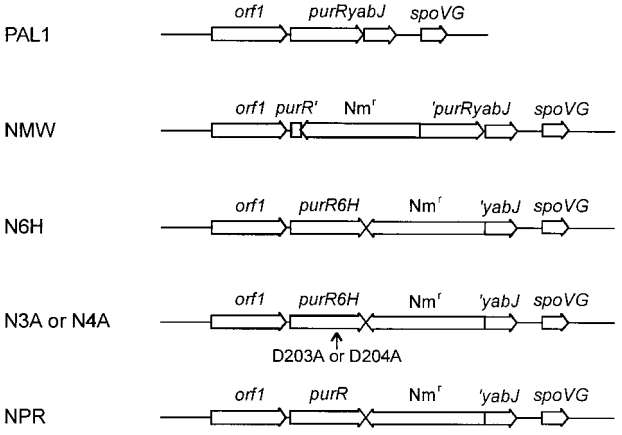

FIG. 2. Gene arrangement of wild-type (PAL1) and various *purR* integrants. *purR6H* is a *purR*$^+$ derivative with six His codons at the 3′ end, followed by two translation stop codons. Truncation of the *yabJ* or the *purR yabJ* operon is marked by an apostrophe. The site of mutations D203A and D204A is marked by an arrow.

TABLE 2. Effect of *purR* disruption or deletion of *purA* 5′-flanking region on transcription of *purA*

B. subtilis strain	Operator[b]	*purR*	Mean luciferase activity ± SD for culture grown as indicated[a]			
			No purines	Ade	Guo	Ade + Guo
PAL1	+	+	498 ± 153	48 ± 5.0	2,278 ± 428	34 ± 1.4
NMW	+	−	5,382 ± 817	7,514 ± 1,405	5,870 ± 2,114	7,611 ± 1,128
PAL3	Δ	+	5,525 ± 915	8,852 ± 1,328	5,667 ± 548	8,275 ± 1,190

[a] Values were obtained from three independent experiments. Ade, adenine; Guo, guanosine.
[b] The *cis* control region is either wild type (+) or deleted (Δ). Bacteria were grown as described in Materials and Methods.

integrant with a disrupted *yabJ* but an intact *purR* was constructed. This strain, NPR, is diagrammed in Fig. 2. Data in Table 3 show that regulation in strain NPR was similar to that in N6H, the *purR6H 'yabJ* integrant. Although repression by adenine was lost, high-level constitutive expression, as in strain NMW, was not seen, and upregulation by guanosine was retained. This result thus points to a role for *yabJ* in PurR repression by adenine.

PRPP inhibition of PurR binding to DNA. A K_d of 7.9 μM was determined previously for the interaction of PurR with *purA*-control-site DNA (19). Given that PRPP inhibits the binding of PurR to the *pur* operon control site (20), a similar inhibition by PRPP was expected for PurR binding to *purA*. The data in Fig. 3 show PRPP inhibition of the PurR-*purA* operator DNA interaction. For 50% inhibition of binding, approximately 30 μM PRPP was required, but complete inhibition was not attained even at 2.5 mM PRPP (data not shown).

PurR contains a PRPP binding sequence motif that is conserved in all type I phosphoribosyltransferases (20). Mutations were introduced into the two conserved aspartates of the PRPP binding motif to further evaluate PRPP's role as an effector that modulates PurR binding to DNA. The corresponding aspartate side chains in glutamine PRPP amidotransferase have essential interactions with the C2 and C3 hydroxyl groups of PRPP (7). The two mutant proteins PurR D203A and PurR D204A each bound to *purA* operator DNA with an affinity similar to that of the wild type (data not shown). However, the binding of both PurR mutants was relatively insensitive to inhibition by PRPP (Fig. 3). Binding of the D204A repressor to DNA was more resistant to inhibition by PRPP than the D203A PurR. Loss of PRPP effector function likely results from defective binding to PurR, although PRPP binding was not determined directly.

Regulation of *purA* by PurR PRPP binding mutants. The PurR D203A and D204A mutations were incorporated into the chromosome of strain PAL1 (*purA′-lucGR*) in order to determine the in vivo role of PRPP as a PurR effector. The two Asp mutants *purR6H*/D203A and *purR6H*/D204A, encoding repressors having a C-terminal His tag, were integrated into PAL1 to give *yabJ* mutant strains N3A (*purR6H*/D203A *purA′-lucGR*) and N4A (*purR6H*/D204A *purA′-lucGR*) (Fig. 2). The *purR6H* gene encodes a repressor with a His tag identical to that used for the in vitro PurR-*purA* operator DNA binding experiments (19) (Fig. 3). The results in Table 3 show that in the two PurR *′yabJ* Asp mutants, strains N3A and N4A, repression by adenine was lost, as in the *yabJ* mutants with His-tagged or wild-type *purR*. In addition, the upregulation of guanosine was completely lost in both of the PurR PRPP binding mutants. It thus appears that the two regulatory events, repression by adenine and upregulation by guanosine, were separated in strain N6H. YabJ was required for repression by adenine but not for upregulation by guanosine. The PRPP effector site was necessary for upregulation by guanosine.

DISCUSSION

It is not surprising that regulation of *purA* expression should contribute to controlling de novo AMP synthesis and to balancing the production of AMP with GMP. The data in Table 2 establish that *purA* expression, monitored by a luciferase reporter gene, is repressed by the addition of adenine to cells and is upregulated by added guanosine. Mutant analysis indicates that both repression and upregulation are dependent upon the interaction of PurR with the *purA* control region. Direct in vitro evidence for this binding has been reported previously (19). The data are consistent with the view that transcriptional regulation of *purA* by PurR contributes to controlling the production of AMP and to maintaining the balanced synthesis of adenine and guanine nucleotides.

The data reported in Table 3 have brought to light an important new aspect of PurR function. Repression of *purA* by PurR depends upon *yabJ*, an overlapping downstream gene. This gene encodes a member of a protein family with unknown function. Data in Tables 2 and 3 show that $yabJ^+$ is needed for PurR-mediated *purA* repression by adenine although not for upregulation by guanosine. The *yabJ* mutation thus appears to uncouple the repression and activation functions of PurR.

How can PurR mediate repression by adenine and upregulation by guanosine, and what role might *yabJ* have? A working model shown in Fig. 4 explains these results. First, we consider three states of the *purA* control region as follows: (i) little or no PurR bound, giving maximal *purA* expression; (ii) partial occupation by PurR, giving basal expression; and (iii) saturation by PurR, resulting in full repression. *purA* expression in strains

TABLE 3. Regulation of *purA* in the PRPP binding mutants

B. subtilis strain	*purR*	*yabJ*	Mean luciferase activity ± SD for culture grown as indicated[a]			
			No purines	Ade	Guo	Ade + Guo
NPR	*purR*	−	1,186 ± 98	822 ± 134	3,415 ± 464	1,041 ± 196
N6H	*purR6H*	−	1,106 ± 221	1,357 ± 145	5,334 ± 628	2,173 ± 370
N3A	*purR6H*/D203A	−	726 ± 148	984 ± 211	932 ± 154	1,063 ± 461
N4A	*purR6H*/D204A	−	399 ± 74	799 ± 146	810 ± 59	820 ± 35

[a] Values were obtained from three independent experiments. Ade, adenine; Guo, guanosine.

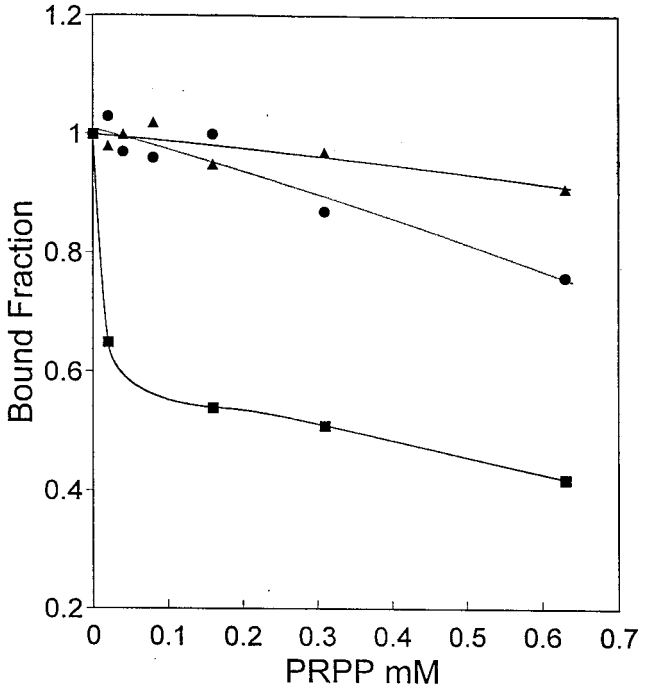

FIG. 3. PRPP inhibition of PurR binding to the *purA* control region DNA. The effect of PRPP on binding is shown for wild-type PurR (■) and two mutants, PurR D203A (●) and D204A (▲). The fraction of DNA bound to PurR in the absence of PRPP (0.82 for wild type, 0.81 for D203A, and 0.89 for D204A) was normalized to 1.0. The apparent K_d values for binding to operator DNA for the wild type and the mutants were similar.

NMW (*purR*::*neo purA'-lucGR*) and PAL3 ($O_{purA\Delta1}$ *purA'-lucGR*) (Table 2) reflects the unoccupied state I control region that leads to high constitutive expression. In these mutants, there is no binding of PurR to the control region. State II, partial occupancy by PurR, giving basal expression, is seen in the wild-type grown without purines (Table 2). Partial occupancy of the *purA* control region results from the interplay between the endogenous purine compounds and the PRPP pool. PRPP, which inhibits PurR binding to the *purA* control region (Fig. 3), has been proposed to be the key regulatory molecule for de novo purine nucleotide synthesis in *B. subtilis* based on its exclusive ability to influence PurR-DNA binding in vitro (20). Adenine lowers the PRPP concentration in *B. subtilis* (17), thus allowing PurR to bind to the control region and shift the I⇆II equilibrium toward II. Guanosine, on the other hand, increases the cellular PRPP pool (17), promoting a shift toward state I. According to this model, YabJ is needed for the state II-to-state III conversion, in which additional PurR is bound and the control site is saturated with PurR. This YabJ-dependent step may correspond to the high PurR/control site binding stoichiometry detected in vitro at an elevated PurR concentration (19). Alternatively, YabJ could inhibit the ability of endogenous adenine to lower the intracellular PRPP level. In contrast to the state II-state III equilibrium, YabJ is not required for the state II-to-state I shift promoted by guanosine.

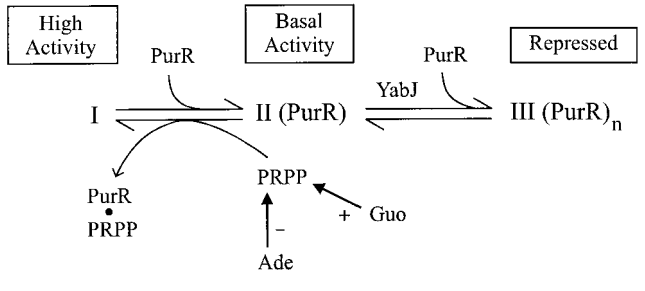

FIG. 4. Hypothetical model explaining roles of YabJ and PRPP. PRPP is required to inhibit binding of PurR, leading to state I. The PRPP concentration in the cell is increased by Guo and decreased by Ade. Basal activity results from partial occupancy of the control region with PurR. Repression requires the binding of additional molecules of PurR (state III). YabJ is essential for this additional PurR binding, perhaps as a PurR-YabJ complex. The possible association of YabJ with PurR in state III is not addressed.

This model explains the loss of repression by adenine in the *yabJ* mutants N6H and NPR (Table 3). These strains exhibit basal expression when grown with excess adenine. Guanosine, however, increases the PRPP pool and shifts the equilibrium toward high state I activity. A mutation in the PurR PRPP site abolishes this PRPP-mediated shift in the *yabJ* mutant strains N3A and N4A (Table 3).

In an earlier report from one of our laboratories (20), Weng et al. stated that a *yabJ* disruption had no effect on the expression or regulation of *purR* or the *pur* operon. Unfortunately, this mutant was lost, and the result cannot be replicated. We assume that the earlier result was incorrect and that *yabJ* has a similar role in the regulation of *purA*, the *pur* operon, and *purR*.

What is YabJ and how does it work? The deduced amino acid sequence of YabJ (125 residues) is homologous to a group of 35 other proteins of unknown function from archaea, procaryotes, and eucaryotes. Some organisms (e.g., *E. coli* and yeast) encode several YabJ paralogs, whereas some have only one YabJ ortholog. In pairwise comparisons of these 35 sequences with YabJ, there is an identity of 21 to 53% over a span of 117 to 125 amino acids. A multiple alignment of all the sequences does not reveal any invariant residues, although approximately 10 conserved amino acids can be identified. YabJ belongs to the YER057c/YjgF protein family of unknown function (PROSITE accession no. PS01094). The family is defined by a conserved signature motif located at the C terminus of these proteins, consisting of the following amino acids: P-[AT]-R-[SA]-X-[LIVMY]-X_2-[AK]-X-L-P-X_4-[LIVM]-E. The consensus pattern is between amino acids 100 and 117 in YabJ. The YER057c/YjgF motif is conserved in all 36 YabJ homologs presently in sequence databases, although the degree of conservation varies to some extent.

The homologs from rat and human have 45 and 44% identity with YabJ, respectively. These proteins have been shown to inhibit cell-free protein synthesis at a high concentration (14, 18). Samuel et al. (16) found that the YabJ homolog Hrp12 from mouse had some similarity to heat shock proteins Hsp70 and Hsp90. They also noticed that the purified mouse Hrp12 could be phosphorylated in vitro with protein kinase C. Melloni et al. (13) reported that the bovine and goat YabJ homologs had the capacity to activate calpains. An *aldR* gene product from *Lactococcus lactis* (4), which is 52% identical to *B. subtilis* YabJ, has been suggested to interfere with branched-chain amino acid synthesis, although *aldR* does not encode an isoleucine biosynthetic enzyme. Mutations that allow thiamine synthesis in the absence of both PurF (glutamine phosphoribosylpyrophosphate amidotransferase) and the pentose phosphate pathway in *Salmonella typhimurium* have been localized in a gene encoding a YabJ homolog, YjgF. Moreover, in these mutants the isoleucine biosynthetic pathway seems to be affected as in *L. lactis* (3).

A common molecular function for YabJ and its 35 homologs

is implied by their high degree of sequence identity and similar sizes. However, no certain biological function or common molecular function has emerged from studies of YabJ homologs. In some cases, as in *L. lactis aldR* and in the present study, the target seems to be related to the operon containing the gene for the YabJ homolog. Our work adds a new dimension to the question of the function of a YabJ homolog, which for the first time, can be located to a specific target. The effect of the disruption of YabJ on the regulation of transcription has been established by using a promoter-reporter system. Given the complex PurR-DNA interaction that has been studied in vitro (19), several possibilities exist for YabJ function, all of which require adenine dependence and guanosine independence in vivo. One possibility is that YabJ associates with PurR and promotes an interaction with DNA that is more stable than the interaction that is possible with PurR alone. A stoichiometry of two or six PurR dimers per *pur* operon was reported in studies with a DNA control-site fragment (19). Perhaps interaction of PurR and YabJ favors the higher binding stoichiometry and a more stable PurR-DNA interaction. Another possibility is that YabJ may stabilize PurR and increase its half-life. It has been speculated that some YabJ homologs may have a chaperone-like function (13). To test this hypothesis, it will be necessary to determine the intracellular level of PurR in *yabJ*$^+$ and *yabJ* strains.

The results presented here provide important new information on the YER057c/YjgF protein family of unknown function. We have shown by using a promoter-reporter system that *B. subtilis* YabJ, a member of this family, affects the purine repressor-mediated regulation of *purA*. The established promoter-reporter system will be valuable for future in vivo studies of YabJ. In addition, a collaborative study of the three-dimensional structure of YabJ (19a) provides important clues about the function of YER057c/YjgF family members.

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