JOURNAL OF BACTERIOLOGY, Aug. 1977, p. 685-688

Vol. 131, No. 2
Printed in U.S.A.

Secondary Promoter of the Guanine Operon of *Escherichia coli* K-12

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Evidence of a secondary promoter for the *guaA* gene within the *guaB* gene was obtained by using λp*guaA* transducing phage. The technique is generally applicable to distinguish a promoter present within a bacterial deoxyribonucleic acid segment, which has replaced the lambda *b*2 region of transducing phage, from the phage *p*I promoter.

In *Escherichia coli* K-12, the biosynthesis of guanosine 5'-monophosphate (GMP) from inosine 5'-monophosphate (IMP) via xanthosine 5'-monophosphate (XMP) is controlled by two genes, *guaA* and *guaB*, as follows (genetic symbols are those used by Bachmann et al. for *E. coli* [1] and by Szybalski and Herskowitz for λ [10]):

$$\text{IMP} \xrightarrow[guaB]{\text{IMP dehydrogenase}} \cdot\text{XMP} \xrightarrow[guaA]{\text{XMP aminase}} \text{GMP}$$

These two genes are closely linked on the *E. coli* chromosome (1), and the guanine gene cluster is an operon arranged in the sequence "operator-*guaB*-*guaA*" (4, 7). On the basis of this information, the observation that abnormal lysogens, carrying a prophage within the *guaB* gene, can grow on minimal medium supplemented with xanthine (7) appears paradoxical. To explain this paradox, we proposed the following two possibilities (7): (i) the expression of the *guaA* gene is due to the phage *p*I promoter (6); or (ii) there is a secondary promoter for the *guaA* gene between the *guaB*-λ junction and the *guaA* gene (Fig. 1). In this work, we obtained genetic evidence that a secondary promoter for the *guaA* gene is present within the *guaB* gene. The method used is generally applicable to distinguish a promoter within a bacterial deoxyribonucleic acid segment, which has replaced the lambda *b*2 region of a transducing phage, from the phage *p*I promoter (6).

We used λp*guaA* transducing phage isolated from a lysogen carrying λ within the *guaB* gene (7), as shown in Fig. 1, and *E. coli* K-12 strain KS825, which is a *recA* derivative of a strain carrying a cryptic prophage within the leucine operon (Table 1). The cryptic λ prophage of KS825 contains a deletion of the region *int* through A of the λ phage genome and carries POΔ', where O and Δ' represent sequences within the leucine operon (2, 9). The relevant genetic structure of this strain is shown schematically in Fig. 2b. Strain KS825 was lysogenized with λp*guaA* phage (Fig. 2a, b) by Int- and Xis-promoted site-specific recombination, ΔOP' × POΔ' (Fig. 2c). After heat induction of this lysogen, we picked up several Spi$^-$ phages, as described previously (7). One of these Spi$^-$ phages, carrying the *guaA*, *leuC*, and *leuD* genes, was named λSpi$^-$ *guaA*·*leuD* (Fig. 2d). Isolation of this type of transducing phage confirmed that the genetic structure of the KS825 (λp*guaA*) lysogen was as shown in Fig. 2c. We lysogenized *E. coli* K-12 strain KS1616, which carries a deletion of the *gal-att*BOB'-*bio* region and of a region of the guanine operon (Table 1), with λSpi$^-$*guaA*·*leuD*. In these lysogens, the λSpi$^-$*guaA*·*leuD* genome should be integrated within the leucine operon by the host Rec function, as shown in Fig. 2f, since we could isolate λ phages carrying the complete leucine operon from heat-induced lysates of these lysogens. We purified 10 lysogens isolated independently in this way and found that all of them could grow on glucose minimal agar supplemented with 20 μg of xanthine per ml. However, none of them could grow on glucose minimal agar without xanthine. These results indicated that the *guaA* gene functions in these lysogens. The likely genetic structure of the lysogen is shown in Fig. 2f. Thus, a role of a leucine promoter or a promoter within the prophage genome in *guaA* gene expression can be eliminated. We propose the following two possibilities for the origin of transcription of a *guaA* gene in these lysogens (Fig. 2f): (i) the presence of a secondary promoter for the *guaA* gene between the *guaB-leu* junction and the *guaA* gene (see Fig. 2f [*p*G]); or (ii) transcriptional read-through from a promoter adjacent to the *guaA* (*guaB*) gene, as shown in Fig. 2f (*p*X1 or *p*X2).

To test these possibilities, we lysogenized strain KS825 with a λp*trpB* transducing phage (8). This phage carries the complete *trpA* and

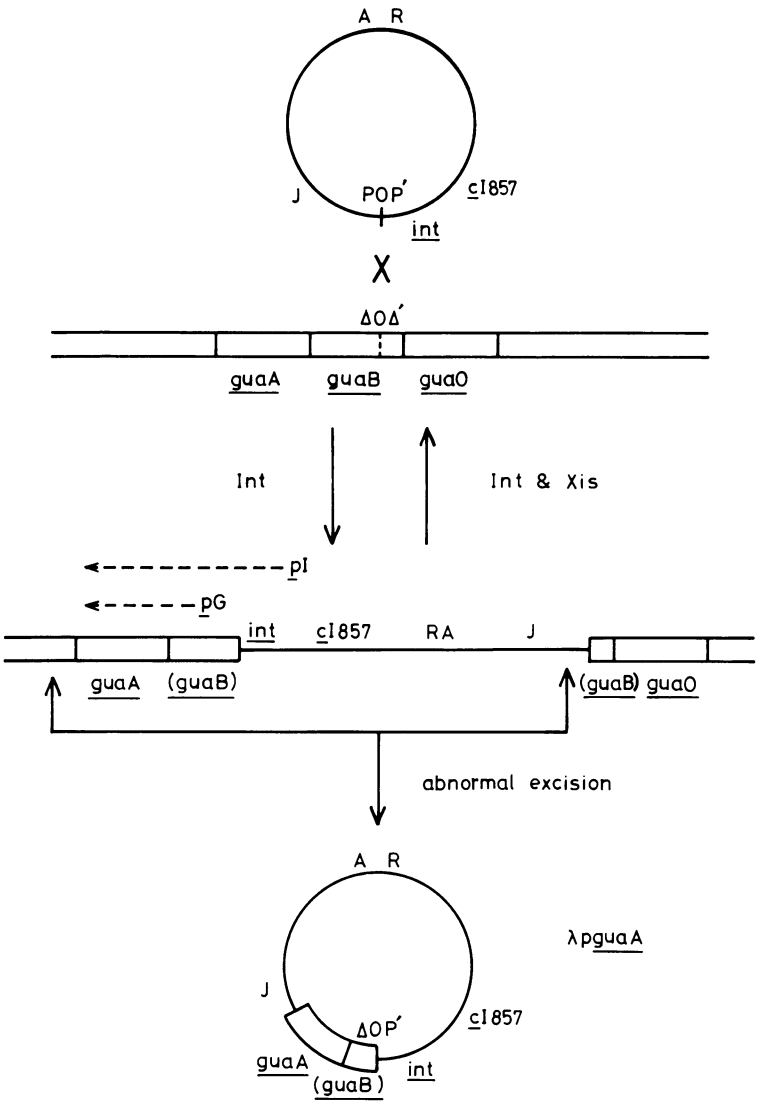

FIG. 1. *Formation of λpguaA transducing phage. Thin lines represent λ phage genome, and double lines denote bacterial chromosome. A, J, int, cI857, R, and POP' are phage markers; guaA, guaB, (guaB), and guaO are bacterial markers, and (guaB) represents a part of the guaB gene. guaO, Promoter-operator of the guanine operon. ΔOΔ', Secondary attachment site for λ phage within the guanine operon (7). Int and Xis, Integrase and excisionase, respectively, specified by the λ phage. pI, Constitutive promoter present within λ phage (6). pG, Secondary promoter for the guaA gene present within the guaB gene. Dashed arrows indicate possible origins and directions of guaA gene transcription in lysogen carrying a prophage in guaB.*

TABLE 1. *Bacterial strains*

Strain	Relevant genotype[a]	Source or reference
KS1616	HfrH(*guaA-guaB*)^{del} (*gal-att*BOB'-*bio*)^{del}	Shimada et al. (7)
KS825	HfrH *recA* (a part of *leuA*)^{del} *att*POΔ'(*gal-att*BOB'-*bio*)^{del}	Shimada et al. (9)
KS648	HfrH(*trpA,B,C,D,E*)^{del} (*gal-att*BOB'-*bio*)^{del}	Shimada et al. (8)
YF38	KS1616 (lysogenic for λSpi$^-$*guaA·leuD*, integrated in *leu*)	This work
PL1072	W3110 *guaB*52	Lambden and Drabble (4)
KS302	HfrH(*gal-att*BOB'-*bio*)^{del}	Shimada et al. (8)

[a] Gene symbols are as described by Bachmann et al. (1).

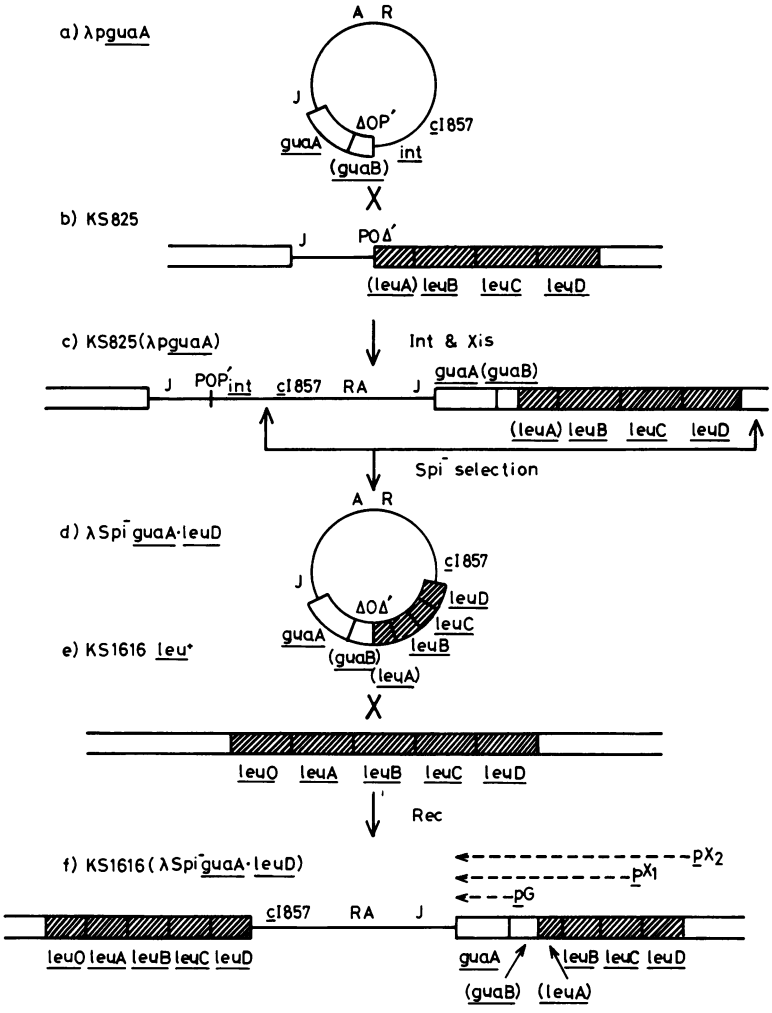

FIG. 2. *Genetic approach to detection of a secondary promoter for the guaA gene. (a) λpguaA phage carries ΔOP' (see Fig. 1); (b) E. coli K-12 KS825 is a recA, attBOB' deletion mutant and carries POΔ' within the leucine operon (9); (c) KS825 was lysogenized with λpguaA phage by ΔOP' × POΔ' site-specific recombination; (d) λSpi⁻ phage carrying guaA and leuD genes, named λSpi⁻ guaA·leuD, was isolated from heat-induced lysates of the KS825 (λpguaA) lysogen by Spi⁻ phage selection (7); (e) KS1616 carries an intact leucine operon; and (f) λSpi⁻ guaA·leuD was used to lysogenize KS1616, which carries a deletion of attBOB' and of the guanine operon. Squares with oblique lines represent the leucine operon; leuO, leuA, leuB, leuC, and leuD are leucine genes, and (leuA) is a part of leuA gene. ΔOΔ' is a secondary attachment site for λ in the guanine or leucine operon. Dashed arrows indicate possible origins and directions of guaA gene transcription in KS1616 (λSpi⁻ guaA·leuD) lysogen; pX1 and pX2, promoters within the leucine gene fragment and adjacent bacterial chromosome, respectively; Rec, recombination protein specified by the E. coli recA gene. Other symbols are as in Fig. 1.*

trpB genes and a part of the *trpC* gene (6). It does not carry either the normal promoter-operator region of the tryptophan operon or a secondary promoter for *trpCBA* genes present within the *trpD* gene (3, 5). After heat induction of these lysogens, the λSpi⁻ phage carrying *trpB* and *leuD* gene, λSpi⁻*trpB·leuD*, was isolated and used to lysogenize (by host Rec function) the strain KS648, which contains a deletion of *att*BOB' and the tryptophan operon (Table 1). Expression of the *trpB* gene in these lysogens requires transcriptional read-through from a promoter adjacent of the *trp*B gene. Five independently isolated KS648 (λSpi⁻*trpB·leuD*) lysogens were streaked onto glucose minimal agar supplemented with 20 μg

of indole per ml, but none of them formed colonies on these plates, indicating that they did not contain a functioning *trpB* gene. We demonstrated the presence of the λSpi⁻*trpB*·*leuD* genome in these lysogens by detecting λ phages carrying the *trpB* and *leuD* genes after heat induction of these five lysogens. All these results eliminated possibility (ii) and supported possibility (i), that expression of the *guaA* gene carried by the λSpi⁻*guaA*·*leuD* phage is due to transcription originating from a secondary promoter present between the *guaB-leu* gene junction and the *guaA* gene (Fig. 2f [*p*G]).

To substantiate this conclusion by biochemical analysis, we measured the *guaA* enzyme activities in one of the λSpi⁻*guaA*·*leuD* lysogens of KS1616, YF38, and in *guaB* point mutant strain PL1072 (4). Cells were grown to the exponential phase in minimal medium containing a low or high concentration of guanine, and then their *guaA* enzyme activities were assayed as described previously (7). The results in Table 2 indicate that YF38 contained a low level of constitutive *guaA* enzyme activity and that this low level was similar to that in an extract of PL1072 grown with a high concentration of guanine. These data indicate that a secondary promoter for the *guaA* gene of low efficiency exists between the *guaB-leu* gene junction and the *guaA* gene (Fig. 2f [*p*G]), i.e., between the *guaB*-λ junction and the *guaA* gene (Fig. 1). Secondary promoters of low efficiency have been detected within the tryptophan operon (3, 5) and for the *int* gene of λ (*p*I promoter) (6). Comparisons of the structures of these secondary promoters may provide valuable information on the physiological functions of secondary promoters and on the evolution of promoters.

TABLE 2. *guaA enzyme activity under repressed and derepressed conditions*

Strain	Relative *guaA* enzyme activity[a]	
	4[b]	40[b]
YF38 =KS1616 (λSpi⁻*guaA*·*leuD* in *leu*)	0.8	0.9
PL1072 =*guaB*52	2.5	0.8

[a] The specific *guaA* enzyme activity of the wild-type strain (=KS302) (7) grown in medium lacking guanine was 0.43×10^{-2} μmol of GMP/min per mg of protein: this value was set at 1.0 to facilitate comparison of activities.

[b] Amount of guanine in medium in micrograms per milliliter.

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