Escherichia coli gal Operon Proteins Made After Phage Lambda Induction

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Expression of the Escherichia coli gal operon under the control of the prophage lambda promoter pL leads to gross discoordination of gal expression. Expression of the most promoter-distal cistron galK is much greater than expression of the promoter-proximal cistron galE. We had previously shown that transcription of the gal operon is coordinate after prophage induction. A survey of protein synthesized after prophage induction indicated that lack of expression of galE is due to a failure of translation of the galE sequence in the pL-gal transcript. This failure of translation of the galE sequence may be due to extensive dyad symmetry present in the vicinity of the gal promoter region of the pL-gal transcript. This symmetry could result in a ribonucleic acid stem-loop structure, blocking the attachment of ribosomes at the Shine-Dalgarno sequence of galE. To test this model, strains bearing the IS1 or IS2 insertion, deletion, or new promoter mutation within the symmetrical region were constructed. The restoration of some galE expression after such disruptions of the symmetrical region indicated that the ribonucleic acid stem-loop structure did play a role in the discoordinate expression of gal from pL. However, failure to obtain galE expression coordinated with high levels of galK expression suggested that other components were involved, perhaps other symmetries between galE and the pL transcript.

The induction of lambdoid prophages results in the phage-controlled expression of neighboring bacterial genes (2, 10, 14). Under conditions in which prophage replication is blocked, this "escape synthesis" is due exclusively to transcription initiating at phage promoters and extending into the bacterial chromosome. The lambda N gene product, or its analog in related phages, permits phage-initiated transcription to proceed through transcription termination sites on the phage or bacterial DNA. Aside from gene N and a site on the prophage chromosome, nutL, which is necessary for the action of the N product, no other phage gene is required for N-mediated escape synthesis (2, 8, 9, 26).

We have studied extensively the expression of the Escherichia coli gal operon under the control of the prophage lambda promoter pL. We reported previously that phage-controlled expression of the enzymes of the gal operon is abnormal (18). Induction of the gal operon by D-galactose or D-fucose results in the coordinate synthesis of the gal enzymes uridine diphosphate galactose 4-epimerase (epimerase), galactose 1-phosphate uridyltransferase (transferase), and galactokinase (kinase), the products of the galE, galT, and galK cistrons, respectively (5).

Induction of prophage lambda, however, leads to gross discoordination of gal expression, with extensive synthesis of kinase and little or no synthesis of epimerase (18). Thus, the expression of the most promoter-distal cistron galK is much greater than the expression of the promoter-proximal cistron galE. Since the rate of transcription of the cistrons is coordinate after prophage induction, the discoordination must result from a failure to translate properly the galE sequences in the pL-gal transcript (18). It is possible that the galE sequences are not translated at all. Alternatively, the sequences may be translated incorrectly, with the synthesis of an abnormal, inactive polypeptide. For example, translation may initiate upstream of galE on the pL-gal transcript; two unutilized AUG codons are present in frame and immediately upstream from galE (22, 27).

We have attempted to distinguish between these possibilities by examining by two-dimensional gel electrophoresis the proteins synthesized after lambda induction. Our results are consistent with the first model, viz., that translation of galE does not take place from the pL-gal transcript. Analysis of insertion and deletion mutants which partially restore epimerase syn-
thesis after prophage induction suggests that a region of symmetry in the pL-gal transcript may occult the ribosome attachment site for galE.

MATERIALS AND METHODS

Radioisotope 1C (as mixed 14C-amino acids with specific activities ranging from 175 to 516 Ci/mol) was obtained from New England Nuclear Corp., Boston, Mass.

Materials for electrophoresis. Ampholytes for isoelectric focusing (pH range, 3 to 10) were obtained from Bio-Rad Laboratories, Richmond, Calif. Sodium dodecyl sulfate was from BOH Biochemicals Ltd., Poole, England. Urea (ultrapure) was from Schwarz/Mann, Orangeburg, N.Y., and protein molecular weight markers were from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

In vivo labeling. E. coli cells were grown in M56 minimal medium supplemented with 0.5 ml of 1% histidine, 0.47 ml of 1% valine, 0.047 ml of 1% biotin, and 0.5 ml of 40% glucose per 100 ml of medium. The cells were grown to a density of 1 x 10^9 to 3 x 10^9/ml. Induction of the gal operon was performed for 15 min with 0.3% D-fucose or D-galactose or, in the case of escape synthesis with A. cibir7 lysogens, a temperature shift from 34 to 41°C. After induction, 25 μCi of a mixture of 14C-amino acids (New England Nuclear; NEC-445) was added to 2 ml of each culture. In experiments in which the position of kinase was determined by antibody precipitation, E. coli proteins were labeled with 25 μCi of 35S]methionine (New England Nuclear; NEG-009). At the end of a 5-min labeling period, 0.2 ml of 10% Casamino Acids and 20 μl of 2 M sodium azide were added, and the culture was chilled in an ice bath. The culture was centrifuged for 2 min in an Eppendorf centrifuge (1.5-ml tubes) at 12,500 x g. The supernatant was discarded, and the pellet was suspended in an equal volume of two-dimensional lysis buffer (20) and heated to 95° for 5 min. The tubes were then chilled in ice and again centrifuged at 12,500 x g for 2 min. The supernatant extract was saved and stored at −70°C until use. Antibody precipitation was performed by double-antibody technique described previously (19). Antikinase antibody was a gift of David Wilson. The precipitation was performed with 50 μl of E. coli cell extract prepared as described above but with an additional 20-min period of centrifugation at 90,000 x g (Airfuge; Beckman Instruments, Inc., Fullerton, Calif.). Kinase specific antibody (1.5 μl; gift of D. Wilson) was added with 20 μl of a 10% solution of Nonidet P-40 (BRL, Gaithersburg, Md.) and 180 μl of a solution containing 20 mM Tris-hydrochloride (pH 7.8), 20 mM KCl, 0.5 mM dithiothreitol, 6 mM MgCl₂, and 0.1 mM EDTA. The mixture was incubated overnight at 4°C. The following day, 15 μl of antigoat rabbit antibody (code no. 65-006; Miles-Yeda, Ltd.) was added, and incubation was continued for an additional 12 h. The mixture was then centrifuged at 12,500 x g for 2 min, and the pellet was washed with phosphate-buffered saline containing 1% Nonidet P-40. It was then suspended in 30 ml of a solution containing 2% sodium dodecyl sulfate, 5% mercaptoethanol, 20% glycerol, and 2% Nonidet P-40 (BRL). This suspension was heated at 85°C for 5 min and then cooled to room temperature. Nonradioactive E. coli cell extract proteins were then added (15 μl), and the solution was made 9 M with urea. Two-dimensional electrophoresis was then performed.

Two-dimensional gel analysis. Two-dimensional electrophoresis was performed by the method of O’Farrell (24), with 3/10 Biolyte in the first dimension and a 10% acrylamide uniform gel in the second dimension. Isoelectric focusing was at 500 V for 2 h; slab gels were run at 20 mA/gel. The methods for gel staining and autoradiography have been described previously (20).

Enzyme assays. Kinase and epimerase were assayed as described before (18).

Strain constructions. Constructions are indicated in Table 1.

RESULTS

Identification of kinase, transferase, and epimerase by two-dimensional gel electrophoresis. The location of the gal enzymes was determined by comparing the gel patterns of the 14C-labeled polypeptides of two strains, N5408 and N5409. N5409 carries plasmid pBR313; N5409 bears pSB101, a pBR313 derivative into which the gal operon was cloned (see Materials and Methods). After being labeled with 14C-amino acids, cultures of the two strains were lysed, and the polypeptides were denatured and subjected to two-dimensional gel electrophoreses (24). Separation in the first dimension was based on isoelectric focusing; separation in the second was based on molecular weight. The gels were then dried and autoradiographed for 24 to 48 h. The resultant pattern of polypeptides is displayed in Fig. 1A and B. Three very dense spots with approximately equal intensities appeared in the gels derived from N5409 (panel A) which were missing from parental strain N5408 (panel B). The spots appeared, although in smaller amounts, after D-galactose induction of a gal + strain, N4903, not carrying a plasmid (panel C). The gal-deleted strain S165, grown in the presence of D-galactose (panel D), or N4903, grown in the absence of D-galactose (panel E), did not synthesize these polypeptides.

These results indicate that the three labeled polypeptides were derived from the gal operon. The molecular weights of the SB101 specific polypeptides seen in Fig. 1 were 40,000, 38,000, and 34,000. These molecular weights are consistent with the apparent subunit molecular weights of the three gal enzymes, viz., kinase (40,000), transferase (39,000), and epimerase (32,000), as determined by sodium dodecyl sulfate-gel electrophoresis (D. Wilson, personal communication). On the basis of their molecular weights, we indicated the location of the kinase transferase and epimerase subunits on our two-dimensional gels (Fig. 1A).
The identification of the kinase subunit was supported by the following analysis. ¹⁴C-labeled cellular proteins were immunoprecipitated with purified antibody to kinase. The precipitate was then washed and subjected to two-dimensional gel electrophoresis. An autoradiograph of such a gel showed a single radioactive spot against a background of total *E. coli* proteins stained with Coomassie blue (Fig. 2). Additional evidence for the identification of the transferase and epimerase subunits is presented below.

### Polypeptides synthesized after prophage lambda induction

We next examined the polypeptides made after induction of a lambda lysogenic strain, N4830. N4830 carries a prophage, λ c1857 ΔBAM ΔH1, which is deleted for all
known phage cistrons except $N$, $rex$, and $cl$ ($\lambda$ repressor). The $cI857$ repressor is thermolabile; prophage is induced by shifting a culture of lysogenic cells from 32 to 41°C. The patterns of polypeptides synthesized after induction of strains N4830, N4831 (an $N^-$ control), and N5179 (an $N^+ nut_{1,3}$ derivative of N4830) are displayed in Fig. 3. Three labeled polypeptides appeared after induction of the $N^+$ strain N4830 which were absent from the control strains N4831 ($N^-$) and N5179 ($N^+ nut_{1,3}$) (Fig. 3). These were transferase, kinase, and an unidentified spot, referred to here as "X." No spot appeared in the location of epimerase.
Kinase, transferase, and X must have been synthesized from the λ pL transcript since they were absent in induced cultures of N5179. Recall that the nut₃ mutation prevents the extension of transcription from pL beyond transcription termination signals, but does not inhibit the expression of the N gene. We assume that X, like kinase and transferase, is encoded by bacterial DNA to the left of the λ attachment site.

The absence of phage-induced polypeptides in extracts of N5179 indicated that the synthesis of no major polypeptides was stimulated in trans by the N product. The observation that the expression of the sigma subunit of RNA polymerase is enhanced by the N product (23) could not be confirmed or eliminated by these data; sigma is a relatively minor polypeptide which we were not able to locate unambiguously on our gels.

The polypeptide product of gene N was not visible under our conditions; the N protein is of low molecular weight (14,000) and is extremely unstable, with a half-life of 5 min (12, 13, 16).

We next asked whether X was encoded by sequences within the gal operon. The X polypeptide did not appear in gels of extracts from a strain carrying pSB101 or in gal⁺ strains after d-fucose induction (Fig. 1); it was, therefore, not expressed from the gal promoter. X also appeared in extracts of induced lysogens with mutations in galT and galE (Fig. 4). The X polypeptide was seen after prophage induction of NG315, which carries N102, an IS1 insertion in galT (15). N5383 bears the 482 deletion, which removes a portion of galT, all of galE, and the bacterial DNA between galT and gene N. It also expresses X after prophage induction. X cannot, therefore, be encoded by galE sequences. Our results are best explained if X were encoded by bacterial DNA to the left of the gal operon.

In Fig. 5 we present the gel analysis of polypeptides synthesized after d-fucose or prophage induction in strains bearing amber mutations in galE or galK (4). D-Fucose induction of N5141 [galE57(Am)] yielded no gal-specific polypeptides; the E57(Am) mutation was polar on galT and galK. The appearance of epimerase and transferase after d-fucose induction of N5151 [galK150(Am)] can be seen clearly. Neither extract contained the X polypeptide.

Prophage induction of N5141 resulted in the appearance of transferase, kinase, and X; the polarity of the galE57(Am) mutation was suppressed by the N function. N5151, when thermally induced, synthesized transferase and X. These data are consistent with those presented above: (i) expression of galE did not occur after prophage induction; (ii) galE operon nonsense mutations did not eliminate the synthesis of the X polypeptide.

**gal discoordination is not caused by the N gene product.** The formation of the pN-gal transcript normally requires the antitermination activity of the prophage N gene product. It has been suggested that the N product may act at the ribosomal level and, thus, might influence the translation of specific proteins (1, 3, D. I. Friedman, A. T. Schauer, M. R. Baumann, L. S. Baron, and S. L. Adhya, Proc. Natl. Acad. Sci. U.S.A., in press). To determine whether pN
interfered with the translation of galE, we constructed a rho-15 (7) derivative of N4831, N4837. gal escape synthesis occurs in N4837, despite the fact that it is mutant for N, because the termination signals between pL and gal in this strain are Rho dependent (12).
FIG. 4. Lambda-induced gal polypeptides in gal mutant strains. $^{14}$C-labeled polypeptides from strains (A) N4830, (B) N5383, and (C) N5383.
We observed the same discoordinacy with the N⁻ strain as with an N⁺ lysogen; there was an extensive synthesis of kinase without a corresponding increase in epimerase (Table 2). The relatively high levels of epimerase in rho-15 strains at 42°C is due to constitutive expression of the gal promoter in Rho-defective cells (7). We concluded that the failure to translate galE from the pL-gal transcript was a property of the transcript itself and not the result of the action of pN.

**Dyad symmetry in the gal control region affects discoordinacy.** A distinctive feature of the gal operon is extensive DNA symmetry in the operator-promotor region (27). This symmetry is not normally included in gal mRNA, since the transcription start site lies within the symmetrical region (Fig. 6). The expression of gal from pL, however, does yield a transcript which includes the symmetry and has the potential of forming the duplex structure shown in Fig. 6. The stem of this structure contains 20 base pairs and is stable, with a calculated free energy loss of −86.7 kJ mol⁻¹ (28). The ribosome-binding site of galE mRNA is included in this stem and would presumably be inaccessible for translation.

To assess the contribution of this symmetry to the discoordinate expression of gal, we constructed a series of mutants with alterations in the symmetrical region.

(i) Mutation gal-128 was an 800-base pair IS1 insertion in gal DNA between positions +3 and +4, corresponding to the gal mRNA start site (15, 17). This insertion interfered with stem formation and, thus, exposed the occluded galE mRNA ribosome-binding site.

(ii) The kinase and epimerase values for strains N4830 and N5503, a gal-128 derivative of N4830, are given in Table 3. Both strains showed high kinase levels after prophage induction. Strain N5503, in contrast to N4830, also displayed significant synthesis of epimerase at 42°C. The relief of discoordinacy by the IS1 insertion was only partial. The ratio of epimerase activity to kinase activity (E/K) after D-galactose induction was about 2.4. The E/K ratio for 5503 was about 0.1. If discoordinacy were entirely eliminated, we would observe induced cultures of

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**TABLE 2.** **Discoordinate gal expression in lambda N⁻ lysogens**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Promoter induced</th>
<th>N</th>
<th>rho</th>
<th>Epimerase (U)</th>
<th>Kinase (U)</th>
<th>E/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>N4830</td>
<td>Pgal</td>
<td>+</td>
<td>+</td>
<td>29.8</td>
<td>11</td>
<td>2.7</td>
</tr>
<tr>
<td>N4845</td>
<td>Pgal</td>
<td>-</td>
<td>rho-15</td>
<td>28.7</td>
<td>12</td>
<td>2.4</td>
</tr>
<tr>
<td>N4830</td>
<td>PL</td>
<td>+</td>
<td>+</td>
<td>5.9</td>
<td>81</td>
<td>0.07</td>
</tr>
<tr>
<td>N4831</td>
<td>PL</td>
<td>-</td>
<td>+</td>
<td>4.1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>N4845</td>
<td>PL</td>
<td>+</td>
<td>rho-15</td>
<td>8.4</td>
<td>84</td>
<td>0.10</td>
</tr>
<tr>
<td>N4837</td>
<td>PL</td>
<td>-</td>
<td>rho-15</td>
<td>6.3</td>
<td>51</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*gal-* strains lysogenic for λ cI857 ΔBAM ΔH1 were induced for 50 min in Luria broth with 0.3% D-galactose at 32°C or at 41°C in the absence of D-galactose. At 32°C without D-galactose, epimerase and kinase basal levels were 3 and 2 U, respectively. Units (U) of kinase are nanomoles of [¹⁴C]galactose-1-phosphate formed per minute by 10⁸ cells. Units (U) of epimerase are nanomoles of UDP-galactose converted to UDP-glucose per minute by 10⁸ cells.

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**FIG. 5.** ¹⁴C-labeled polypeptides synthesized in gal Kam150 or gal Eam57 lysogens. N5151 (gal Kam150) or N5141 (gal Eam57) was grown at 32°C with D-fucose (right-hand panels) or at 41°C (left-hand panels).
DNA

\[ 3' \ldots \text{GAAAAGCGTAGAACAATACTC\ldots} \quad 5' \]
\[ 5' \ldots \text{CTTTTCGATCTTTGTTATGATTTGGTATTCGGATTACCTCGCTTAATAC\ldots} \]

TRANSCRIPTION FROM GALACTOSE OPERON PROMOTER

mRNA

\[ 5' \ldots \text{AUACCAUAAAGCCUA} \quad 3' \]
\[ 3' \ldots \text{GAATAATATTCCGATTACCTCGCTTAAATAC} \quad 5' \]
\[ 5' \ldots \text{CTTTTGCATCTTTGTTATGATTTGGTATTCGGATTACCTCGCTTAATAC\ldots} \]

mRNA

\[ 3' \ldots \text{GAAAAGCGTAGAACAATACTC\ldots} \quad 5' \]
\[ 5' \ldots \text{CTTTTGCATCTTTGTTATGATTTGGTATTCGGATTACCTCGCTTAATAC\ldots} \]

TRANSCRIPTION FROM λ QL PROMOTER

mRNA

\[ 5' \ldots \text{CUUUUCGCAUCUUUGUUAUGAUAUGGUUAUUUCAUA} \quad 3' \]
\[ 3' \ldots \text{GAAAAGCGTAGAACAATACTC\ldots} \quad 5' \]
\[ 5' \ldots \text{CTTTTGCATCTTTGTTATGATTTGGTATTCGGATTACCTCGCTTAATAC\ldots} \]

Fig. 6. DNA sequence of the gal operon and of the gal mRNA initiated at the gal or lambda pL promoter. The stem-loop structure of the pL-gal transcript proposed by Steitz (28) is shown at the bottom.

N5503 about 700 U of epimerase instead of 30 U.

Because the dyad symmetry remained in N5503, albeit displaced by 800 base pairs, the possibility of RNA duplex formation was not eliminated. To prevent definitively the formation of a gal operator-promoter duplex, we constructed strains N5575-1 and N5575-3, which were deleted for one arm of the symmetrical sequence. These strains were derived from N5572, a gal-128 lysogen of λ cl857 ΔH1. Thermal induction of N5572 led to cell death owing to the expression of the prophage kil gene. Surviving cells were likely to carry a continuous deletion from the prophage-proximal end of the IS1 insertion, through an arm of the stem structure, the gal promoter, and into or beyond kil. Strains N5575-1 and N5575-3 represented two survivors of thermal induction of N5572 which did not revert to gal⁺ and were, therefore, deleted for the gal promoter (Table 2). The presence of a continuous deletion in N5575-1 was verified by crossing the mutation into λ pgal and analyzing the DNA of the mutant by restriction analysis (data not shown).

The kinase and epimerase values of induced
cultures of these strains are shown in Table 2, lines 6 to 8. N5572, like the IS1 insertion strain N5503, displayed partial discoordination. There was little or no further increase in epimerase levels when one arm of the symmetry was deleted, as in N5575-1 or N5575-3. Since these strains still displayed gross discoordination, we must conclude that additional factors play a role in the efficiency of translation of the galE sequence in the pL transcript.

(ii) Analysis of strains carrying IS2 insertions in gal also suggested that the gal discoordination could only be partially accounted for by symmetry in the gal operator-promoter region (Table 4). Strain N4927 carried galEop490, a 1,400-base pair IS2 insertion between nucleotides −1 and +1 of gal (R. Musso, personal communication). The 490 insertion, which lay within the gal symmetry, was expected to interfere with mRNA duplex formation. In contrast to its gal+ parent, N4830, prophage induction in N4927 led to significant accumulation of epimerase and an E/K of 0.53. After prophage induction, the E/K ratio of 4830 was 0.07; d-galactose induction gave an E/K of 2.7. Thus, the IS2 insertion, like IS1, gave incomplete suppression of discoordination.

Also shown in Table 4 are two control strains, N5678 and N5679, as well as their parent, N5705. The former carry an IS2 insertion, int+, in the λ prophage, approximately 2,000 base pairs upstream from galE. N5705 is int+. Thermal induction of the three strains revealed no significant synthesis of epimerase. This demonstrated that, whereas an IS2 insertion in the gal symmetrical region partially alleviates discoordination, IS2 insertions located elsewhere in the pL transcript do not.

To eliminate any possibility of RNA duplex formation in the gal operator-promoter region, we studied two gal+ revertants of N4927 (Table 5). N4927-1 was a true revertant, with complete loss of the IS2 element. It showed full discoordination after prophage induction. A second revertant, N4927-12, was constitutively gal+ and reverted frequently to gal−. Pseudorevertants of this type have been demonstrated to carry new promoters within the IS2 insertion (A. Ahmed, K. Bidwell, and R. Musso, Cold Spring Harbor Symp. Quant. Biol., in press). Transcripts initiating at the IS2 promoter will not contain the gal symmetry. gal expression in N4927-12 was still partially discoordinate. Thus, in every case studied, the translation of galE from a transcript initiated upstream from the gal promoter, whether or not it contained the symmetrical region, was always reduced.

**DISCUSSION**

We have extended our observation that the expression of a bacterial cistron can depend upon the promoter from which it is transcribed. In the *E. coli* gal operon, expression of galE (epimerase) did not occur when the cistron was transcribed from the prophage lambda pL promoter located upstream from the gal promoter. Expression of the promoter-distal cistrons, galT (transferase) and galK (kinase), can be directed efficiently from λ pL (18). After surveying the proteins synthesized after lambda induction by two-dimensional gel electrophoresis, we have come to the conclusion that the absence of epimerase activity results from failure to translate the galE sequences in the pL-gal transcript.

The gal promoter region contains an extensive dyad symmetry which flanks the normal transcription start site. A transcript originating at pL would include this symmetry. An RNA stem-
TABLE 4. Effect of gal IS2 mutations on discoordination

<table>
<thead>
<tr>
<th>Expt</th>
<th>Strain</th>
<th>Genotype structure</th>
<th>Epimerase (U)</th>
<th>Kinase (U)</th>
<th>E/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N4830</td>
<td>gal*</td>
<td>4.4</td>
<td>65</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>N4927</td>
<td>gal IS2</td>
<td>34.6</td>
<td>66</td>
<td>0.53</td>
</tr>
<tr>
<td>B</td>
<td>N4830</td>
<td>gal*</td>
<td>6.7</td>
<td>184</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>N5705</td>
<td>gal* xis*</td>
<td>5.5</td>
<td>277</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>N5678</td>
<td>gal* xis IS2</td>
<td>6.0</td>
<td>174</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>N5679</td>
<td>gal* xis IS2</td>
<td>5.2</td>
<td>158</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Induction was at 42°C for 50 min. Uninduced levels of kinase were 1 to 2 U; uninduced levels of epimerase were 2.5 to 3.2 U. In experiment A, D-galactose induction of strain 4830 at 32°C gave 11 U of kinase and 29.8 U of epimerase (E/K = 2.7), as shown in Table 3.

Genetic map of the gal-λ region showing the positions of the IS2 insertions.

TABLE 5. gal operon expression from an IS2 promoter

<table>
<thead>
<tr>
<th>Strain</th>
<th>32°C</th>
<th>32°C + D-galactose</th>
<th>41°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kinase (U)</td>
<td>Epimerase (U)</td>
<td>Kinase (U)</td>
</tr>
<tr>
<td>N4927-1</td>
<td>2</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>N4927-12</td>
<td>84</td>
<td>104</td>
<td>65</td>
</tr>
</tbody>
</table>

* N4927-1 is a true revertant of the IS2 strain N4927; N4927-12 is a pseudorevertant with the structure indicated below. Induction time was for 50 min.

Genetic map of the true (N4927-1) and the pseudorevertant (N4927-12) of the gal IS2 strain (N4927).

Loop structure with considerable stability (~86.7 J/mol) could form within the pL-gal transcript, blocking the attachment of ribosomes to the translation initiation region galE (28) (Fig. 6). Inhibition of ribosome attachment owing to RNA secondary structure has previously been demonstrated for phage f2 (28). To assess the contribution of this symmetry to the failure of galE expression, we constructed a variety of pL-gal fusion strains in which the formation of the stem-loop structure should have been impaired or prevented. These strains included those bearing an IS1 or IS2 insertion, deletion, or new promoter within the symmetrical region. In each of these cases, some expression of galE was observed after prophage induction, although in no case was the level of epimerase as high as when galE was expressed from its cognate promoter.

These data suggest that the stem-loop structure of Fig. 6 does play a role in the discoordinate expression of gal from pL, but that other components are also involved, perhaps other symmetries between galE and the pL transcript. In
the case of lambda cro-lac fusions, the efficiency of lac translation is influenced, in an apparently unpredictable way, by the sequences upstream from the lacZ translation initiation region (25). We cannot exclude alternative possibilities: (i) that ribosomes attached to sequences preceding galE might occlude the translation initiation region of galE; (ii) that the galE sequence of the $p_l$ transcript might be uniquely unstable (6).

The induced prophage in these experiments was $\lambda$ c1857 $\Delta$BAM $\Delta$H1, which carries only $N$, rex, and a thermosensitive (c1857) derivative of the repressor gene. When the polypeptides made after induction were analyzed by two-dimensional gel electrophoresis, we found that the synthesis of only three was stimulated. These were kinase, transferase, and a third polypeptide, $X$, not yet identified, but which was not a product of the gal operon. All three were translated from the $p_l$ transcript, since the introduction of the $nut, l_3$ mutation into the prophage blocked their expression; $nut, l_3$ caused the $p_l$ transcript to terminate within the prophage (26).

It has been reported that the synthesis of the sigma subunit of RNA polymerase is stimulated, in trans, by the $N$ gene product. Our results neither confirm nor contradict this finding, since we have not been able to locate unequivocally sigma, a relatively minor polypeptide, on our gels.

The identification of kinase and transferase was aided immeasurably by the analysis of a strain carrying a high-copy-number plasmid into which the gal operon had been cloned. The three products of the gal operon were readily apparent in two-dimensional gels of labeled extracts made from this strain, without $\beta$-galactose induction or prior purification. This approach, using cloned genomic fragments, should prove generally useful in identifying gene products.

These experiments also indicate that the discordant expression of gal is not dependent upon the $N$ gene function. Expression of gal from $p_l$ in the absence of the $N$ product occurs in rho mutant strains. Under these conditions, the characteristic high levels of kinase and low levels of epimerase are observed.

The finding that galK can be expressed when galE is not also shows that the translation of a promoter-distal cistron does not depend upon the translation of the promoter-proximal cistron in the $p_l$ transcript. This notion is different from the conclusion derived from the study of bacterial promoters that showed equimolar translation of each cistron in an operon (21, 29).

LITERATURE CITED


