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# A plasmid cloning vehicle allowing regulated expression of eukaryotic DNA in bacteria

(restriction enzyme/lactose operon/eukaryotic gene expression)

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**ABSTRACT** **We have constructed a plasmid cloning vehicle in which transcription of inserted heterologous DNA fragments can be regulated by a defined bacterial operator and promoter. The lambda *plac* 5 *Eco*RI DNA fragment containing the operator, promoter, and β-galactosidase gene of the lactose operon was linked to the ColE1 derivative plasmid pSF2124, creating a plasmid designated pBGP100. pBGP100 contains one *Eco*RI site at the *lac* DNA/pSF2124 DNA junction and another at the lambda DNA/pSF2124 DNA junction. We deleted the latter *Eco*RI site to generate a plasmid (pBGP120) retaining a single *Eco*RI site at the *lac* DNA/pSF2124 DNA junction.**

**To determine whether DNA introduced at the *Eco*RI site of pBGP120 was expressed under lactose control, we inserted the *Eco*RI fragment containing 28S ribosomal DNA of *Xenopus laevis*, creating the hybrid plasmid pBGP123. RNA·DNA hybridization of pulse-labeled RNA from cells containing pBGP123 showed that induction of the *lac* operon increases the percentage of labeled RNA complementary to *Xenopus* 28S DNA about 9-fold. This vehicle may be of use for production of eukaryotic gene products in bacteria.**

Although several eukaryotic DNA sequences have been introduced into bacterial plasmids (1–6) and coliphage lambda (7, 8), it has not been demonstrated that eukaryotic promoter sequences are correctly recognized by bacterial RNA polymerase *in vivo*. Indeed, transcription initiated in cloned mouse mitochondrial DNA occurs predominantly from the L-strand in *Escherichia coli* rather than from the H-strand, as occurs in mouse cells (4), indicating that bacterial RNA polymerase recognizes different sequences from those recognized in the mitochondrion. Even if recognition occurs with fidelity, expression may be limited by the probability that the cloned fragment contains a functional promoter. Thus, it would be advantageous to place the transcription of cloned eukaryotic DNA under the control of a defined bacterial promoter. This paper describes the introduction of the control elements of the *E. coli* lactose operon into a plasmid such that the transcription of subsequently inserted heterologous DNA is under lactose control. This plasmid not only allows for control of the transcription of the inserted DNA, but also permits induction of high levels of these transcripts.

## MATERIALS AND METHODS

**Bacterial Strains.** All bacterial strains are derivatives of *E. coli* K12. W3101 ($\lambda_{T11}$)/1,5 (9) was provided by Dr. J. Carbon. *E. coli* lysogenic for $\lambda CI_{857}S_7$ was provided by Dr. H. Boyer. pSF2124/C600 ($Thr^-$, $Leu^-$, $Thi^-$) harbors a ColE1 ampicillin-resistant recombinant plasmid (10) and was provided by Dr. S. Falkow. W3140, an Hfr strain containing a *lacZ* deletion (11), was obtained from Dr. B. Bachmann (*E. coli* Genetic Stock Center, Yale University). RR101 (*hsdS1, proA2, leu-6, ara-14, galK2, lacY1, xyl-5, mtl-1, str-20, thi-1, supE44*, $F^-$, $lambda^-$) is a *recA*$^+$ derivative of HB101 (12) and was provided by Dr. R. Rodriguez. DG72 is a *lac*$^+$ transductant (13) of RR101. DG73 is a *pro*$^+$, *lac* Z39 exconjugant obtained after mating (13) DG72 and W3140. A derivative of W3110 that is *trpR*$^-$, *trpE*$^-$, *trpA*$^-$, *lacZ*$^-$, *val*$^r$ (14) was generously provided by Dr. C. Yanofsky.

**Media.** L broth contained 10 g of Tryptone (Difco), 5 g of yeast extract (Difco), and 5 g of NaCl per liter. Minimal medium E (15) was supplemented with 10 μg/ml of thiamine, 0.2–0.5% appropriate carbon source, and 100 μg/ml of required amino acids or 0.25% Norit-treated vitamin-free casamino acids (Difco). Drug selective medium contained 20–30 μg/ml of ampicillin (Bristol Laboratories). Plates were solidified with 1.5% agar (Difco).

**Enzymes.** Lambda 5′-exonuclease was purified by slight modification of the published procedures (9, 16). Fraction VI (α) had a final specific activity of about 140,000 units/mg when assayed with T7 [$^3$H]DNA (17). Isolated one-cut linear pBGP100 DNA (12 μg/ml) was treated with 1.8 μg of lambda 5′-exonuclease for 60 min at 0° in a total volume of 700 μl containing 67 mM glycine-KOH, pH 9.4, and 4 mM $MgCl_2$ (18). The enzyme/DNA ratio represented a 200-fold molar excess of enzyme to DNA 5′-termini. The length of digestion at 0° corresponded to the time required to remove approximately 25 nucleotides from each 5′-end.

Restriction endonuclease *Eco*RI, purified and assayed according to Greene *et al.* (19), was generously provided by Dr. R. Meagher or L. DeGennaro. Digestions with restriction endonuclease *Bam*HI (New England Biolabs) were assayed in 7 mM Tris·HCl, pH 7.4, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 60 mM NaCl. Digestions with combinations of two enzymes were initiated with the enzyme that required low salt concentration followed by addition of NaCl and/or Tris·HCl and the enzyme that required high salt concentration. Lambda *plac5* DNA was generously provided by Dr. A. Riggs, and plasmid CD4 DNA (2) was provided by G. Bell. Lambda $CI_{857}S_7$ DNA was purified as described (13). *In vivo* labeling, amplification, and purification of covalently closed circular DNA were essentially as described (20).

**Other Methods.** DNA ligation (21), agarose slab gel electrophoresis (22), transformation of bacteria (21), and colicin production tests (23) were essentially as described. A rapid screening procedure for covalently closed circular DNA in individual transformed colonies was suggested by R. Tait (personal communication). Preparative electrophoresis and elution of partially digested DNA fragments were essentially as described (24).

**Safety Procedures.** These experiments were conducted in a P2 facility using precautions outlined in a draft of the "NIH Guidelines for Research Involving Recombinant DNA Mole-

Abbreviation: $M_r$, molecular weight.

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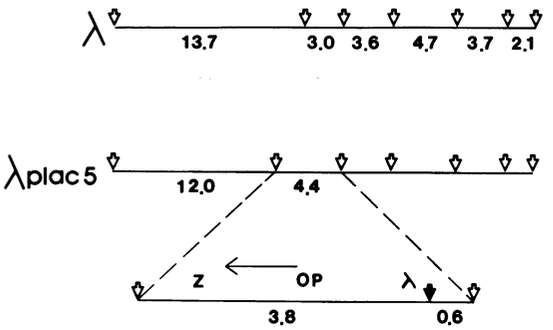


FIG. 1. Schematic diagram of the lambda and lambda *plac5* genomes. The open vertical arrows indicate the *Eco*RI endonuclease cleavage sites and the solid vertical arrow indicates the *Hin*dIII endonuclease site within the lambda *plac5* *Eco*RI fragment that contains the lactose operator (O), promoter (P), and part of the β-galactosidase gene (Z) of *E. coli*. The horizontal arrow indicates the direction of transcription within the lactose operon. The numbers indicate the $M_r$ ($\times 10^{-6}$) of the fragments produced by *Eco*RI and *Hin*dIII endonuclease digestion.

cules" (25). Liquid cultures were treated with Wescodyne (West Chemical Products, Inc.) and DNA solutions with bleach prior to autoclaving and disposal. Agar plates containing transformed bacteria were autoclaved in polypropylene "Disposa-Bags" (Dolby Scientific).

## RESULTS

### Cloning *lac* operon elements from lambda *plac5*

Lambda *plac5* is a derivative of lambda in which elements of the *lac* operon have been substituted for viral DNA (26). Comparison of fragment patterns of wild-type lambda and lambda *plac5* DNA cleaved with *Eco*RI endonuclease prompted Helling *et al.* (22) to suggest that an *Eco*RI cleavage site existed in the β-galactosidase gene of the *lac* operon. These workers reported that a *plac5* *Eco*RI fragment of molecular weight ($M_r$) $4.4 \times 10^6$ contained the *lac* operator. The orientation of the *lac* operon within this fragment is shown in Fig. 1.

To clone the *lac* elements, we covalently ligated *Eco*RI-cleaved *plac5* DNA fragments to *Eco*RI-cleaved pSF2124 DNA with T4 DNA ligase. The ligated DNA was used to transform DG73, an ampicillin-sensitive *E. coli* host that has a large deletion in the β-galactosidase gene. Ampicillin-resistant ($ap^r$) transformants capable of metabolizing lactose ($lac^+$) were selected. *Eco*RI fragment patterns of plasmid DNA isolated from independent $ap^r$ $lac^+$ clones were analyzed by agarose gel electrophoresis (Fig. 2). Plasmids from all $ap^r$ $lac^+$ clones examined contained the fragment from *plac5* DNA of $M_r$ $4.4 \times 10^6$ in addition to the pSF2124 DNA of $M_r$ $7.4 \times 10^6$. Plasmids from some selected clones contained additional *plac5* DNA fragments. Hybrid $ap^r$ transformants that did not contain the lambda *plac5* fragment of $M_r$ $4.4 \times 10^6$ were phenotypically $lac^-$. From these results we conclude that the DNA fragment of $M_r$ $4.4 \times 10^6$ carries information sufficient to complement the deletion present in DG73. In subsequent experiments we used a hybrid plasmid (pBGP100) containing only the fragment of $M_r$ $4.4 \times 10^6$ in addition to pSF2124 DNA.

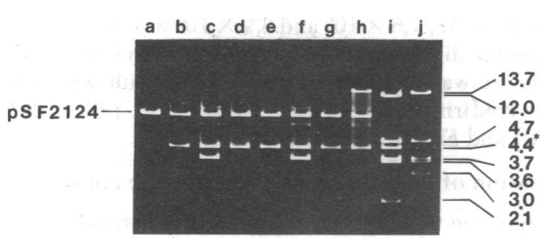


FIG. 2. Agarose gel electrophoresis of *Eco*RI endonuclease-digested plasmid DNA isolated from independent $ap^r$ $lac^+$ transformants of DG73. (a) pSF2124, (b–h) independent $ap^r$ $lac^+$ transformants of DG73, (i) lambda *plac5*, and (j) lambda. The numbers to the right indicate the $M_r$ ($\times 10^{-6}$) of the *Eco*RI fragments. (*) Lambda *plac5* *Eco*RI fragment containing the operator, promoter, and β-galactosidase gene of *E. coli*. See *Materials and Methods* for isolation of plasmid DNA, endonuclease digestion, and agarose gel electrophoresis.

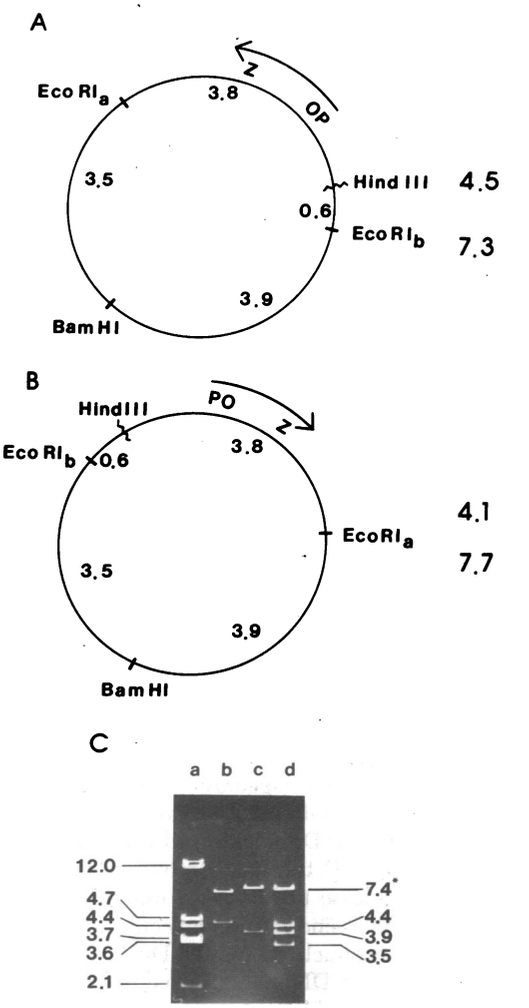


FIG. 3. (A and B) Schematic diagram of the two possible orientations of the *lac* fragment of $M_r$ $4.4 \times 10^6$ within pBGP100. The numbers within the circular molecule indicate the $M_r$ ($\times 10^{-6}$) of the fragments expected after combined digestion with *Eco*RI, *Hin*dIII, and *Bam*HI endonucleases. The numbers to the right of the diagram indicate the $M_r$ ($\times 10^{-6}$) of the fragments produced by a combined *Bam*HI and *Hin*dIII digestion. (C) Agarose gel electrophoresis of (a) lambda *plac5* digested with *Eco*RI, (b) pBGP100 digested with *Bam*HI and *Hin*dIII, (c) pBGP101 digested with *Bam*HI and *Hin*dIII, and (d) mixture of pSF2124(*) digested with *Eco*RI and pBGP100 digested with *Bam*HI and *Eco*RI. See *Materials and Methods* for isolation of plasmid DNA, endonuclease digestion and agarose gel electrophoresis.

### Orientation of the *lac* fragment in pBGP100

pBGP100 contains two *Eco*RI sites (Fig. 3). One of these sites ($Eco RI_a$) is at the *lac* DNA/pSF2124 DNA junction ("downstream" from the *lac* promoter), while the other site ($Eco RI_b$) is located at the lambda DNA/pSF2124 DNA junction. Inser-

tion of heterologous DNA at the "downstream" site creates the possibility of controlling expression of the insert by modulating initiation of transcription at the *lac* promoter. We assumed that expression of an insertion at the *Eco*RI$_b$ site would be independent of *lac* control. Since we intended to remove the *Eco*RI$_b$ site it was critical to determine the orientation of the *lac* insertion relative to the *Bam*HI site in pSF2124 (F. Heffron, personal communication). The two possible orientations of the *lac* insert relative to the *Bam*HI site are shown in Fig. 3. These possibilities could be distinguished because of the existence of a *Hin*dIII endonuclease site located in the lambda DNA region of the *lac* insert (27). Combined digestion of pBGP100 with both *Hin*dIII and *Bam*HI endonucleases lead to different predicted limit fragments depending on the orientation of the *lac* fragment. If the orientation were as shown in Fig. 3A, two fragments of $M_r$ $7.3 \times 10^6$ and $4.5 \times 10^6$ result from the double digestion. In the other orientation (Fig. 3B) fragments of $M_r$ $7.7 \times 10^6$ and $4.1 \times 10^6$ were predicted. The results (Fig. 3C, lane b) indicated unambiguously that the *lac* fragment was inserted as shown in Fig. 3A. Therefore, the *Eco*RI site we wanted to delete was located $3.9 \times 10^6$ daltons from the *Bam*HI site in pSF2124. Subsequently, we isolated pBGP101, another ap$^r$ *lac*$^+$ plasmid which contains the lambda *plac5 lac* fragment inserted in the opposite orientation to pBGP100 (Fig. 3C, lane c).

## Deletion of the *Eco*RI site in BGP100

We used a procedure developed by Carbon *et al.* (28) to delete the *Eco*RI$_b$ site in pBGP100. This procedure involves three steps: (*i*) closed circular DNA is cleaved with *Eco*RI to generate a linear molecule, (*ii*) the linear molecule is digested with lambda 5′-exonuclease to remove a limited number of nucleotides from the 5′-ends of the molecule, and (*iii*) transformation with the exonuclease-treated DNA. Within the transformed cell the linear DNA is converted to a covalently closed circular form presumably by base pairing between the short, single-stranded 3′-ends, removal of unpaired DNA, and repair and ligation of the resulting gapped molecule (28).

In order to remove the *Eco*RI$_b$ site in pBGP100 it was necessary to use pBGP100 molecules that had sustained cleavage at only one *Eco*RI site as substrates for exonuclease digestion. To obtain these one-cut linear DNA molecules, we partially digested pBGP100 with *Eco*RI. DNA fragments, produced as a function of time of digestion, were analyzed by agarose gel electrophoresis. A band with the mobility expected for a one-cut linear DNA molecule ($M_r$ $11.8 \times 10^6$) was readily identifiable (not shown). At 37°, under conditions of enzyme excess, a 10-min digestion of 200 μg of pBGP100 with *Eco*RI resulted in optimal production of the one-cut linear DNA—approximately 30% of the total DNA mass migrated as a one-cut linear molecule under these conditions. The remaining DNA mass was distributed mainly into the two limit linear fragments and open circles. Partially digested pBGP100 DNA fragments were separated on a preparative 0.8% agarose gel (22). The region containing the one-cut linear band was removed and the DNA was extracted from the gel (24). This DNA was digested with lambda 5′-exonuclease under conditions sufficient to remove approximately 25 nucleotides from each 5′-end. Six micrograms of the exonuclease-treated DNA were used to transform RR101. Eighty ap$^r$ transformants were selected and the *Eco*RI digestion pattern of plasmid DNA obtained from each clone was determined by agarose gel electrophoresis. A single clone contained a plasmid that had one *Eco*RI site (Fig. 4A). As expected, the supercoiled form of this plasmid migrated slightly faster in agarose gels than the parent pBGP100 (Fig. 4B). The plasmid

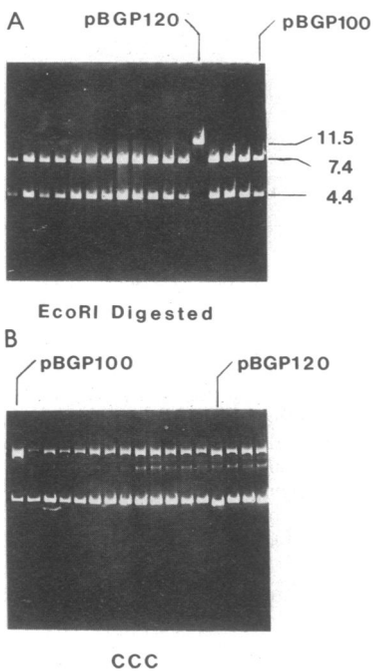


FIG. 4. (A) Agarose gel electrophoresis of *Eco*RI-cleaved plasmid DNA isolated from independent ap$^r$ RR101 clones obtained after transformation with linear, lambda 5′-exonuclease-treated pBGP100 DNA. The numbers to the right indicate the $M_r$ ($\times 10^{-6}$) of the *Eco*RI fragments. (B) Agarose gel electrophoresis of covalently closed circular (CCC) plasmid DNA from the clones run in gel (A). See *Materials and Methods* for isolation of plasmid DNA, lambda 5′-exonuclease digestion, *Eco*RI digestion, and agarose gel electrophoresis.

derived from pBGP100 containing one *Eco*RI site was designated pBGP120.

The remaining 79 screened transformants contained plasmids identical in size to pBGP100, and each retained two *Eco*RI sites. It is likely that these 79 transformants resulted from uncleaved supercoils. Since supercoils transform with $10^5$ greater efficiency than exonuclease-treated linear molecules (unpublished experiments), low level supercoil contamination of one-cut linear molecules would be greatly magnified among the transformants.

## Location of the *Eco*RI site in pBGP120

The two possible locations for the remaining *Eco*RI site in pBGP120 were distinguished by a combined digestion of the plasmid with *Bam*HI and *Eco*RI endonucleases. If the *Eco*RI$_a$ site remained (Fig. 5A), fragments of $M_r$ $8.0 \times 10^6$ and $3.5 \times 10^6$ were predicted. Alternatively, if the *Eco*RI$_b$ site remained, fragments of $M_r$ $7.6 \times 10^6$ and $3.9 \times 10^6$ were expected (Fig. 5B). Double digestion of pBGP120 clearly indicated that the *Eco*RI$_a$ site was retained (Fig. 5C). This result was independently confirmed by double digestion of pBGP120 with *Hin*dIII and *Eco*RI (not shown).

## Expression of eukaryotic DNA under *lac* control

To determine whether transcription of a heterologous DNA fragment inserted at the *Eco*RI site in pBGP120 was under *lac* control, we inserted an *Eco*RI fragment that contained 28S ribosomal DNA sequences of *Xenopus laevis*. This plasmid has been designated pBGP123. The *Xenopus* DNA fragment ($M_r$ $3.0 \times 10^6$) was isolated from a pSC101-*Xenopus* DNA hybrid plasmid (CD 4) previously cloned by Morrow *et al.* (2). If transcription of *Xenopus* DNA in pBGP123 is under *lac* control, cells induced with cyclic AMP and isopropylthiogalactoside

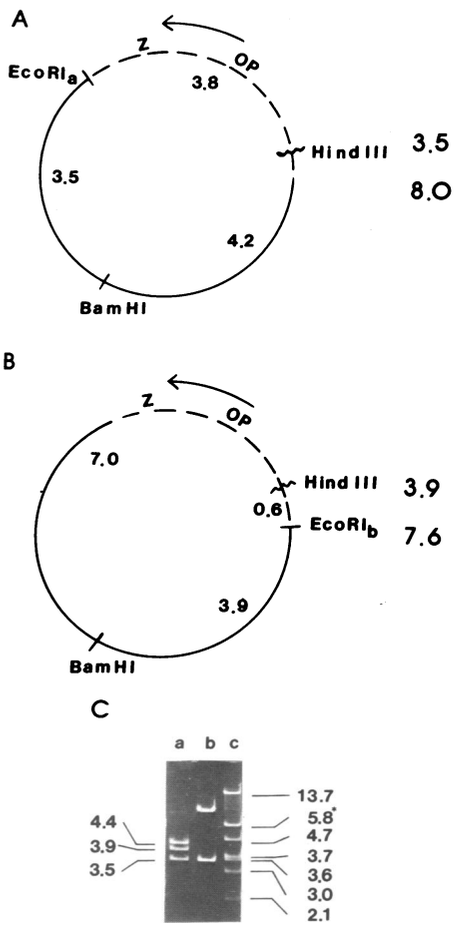


FIG. 5. (A and B) Schematic diagram of pBGP120 showing the two possible locations of the remaining *Eco*RI site. The numbers within the circular molecule indicate the $M_r$ ($\times 10^{-6}$) of the fragments expected after combined digestion with *Eco*RI, *Hin*dIII, and *Bam*HI endonucleases. The numbers to the right of the diagram indicate the $M_r$ ($\times 10^{-6}$) of the fragments produced by a combined *Bam*HI and *Eco*RI digestion. (C) Agarose gel electrophoresis of (a) *Bam*HI and *Eco*RI digestion of pBGP100 (see Fig. 3A), (b) *Bam*HI and *Eco*RI digestion of pBGP120, and (c) mixture of *Eco*RI-digested lambda and *Eco*RI-digested pSC101(*). See *Materials and Methods* for isolation of plasmid DNA, endonuclease digestion, and agarose gel electrophoresis.

should contain a higher percentage of pulse-labeled RNA complementary to the *Xenopus* DNA fragment than uninduced cells. RNA·DNA hybridization experiments were performed with RNA extracted from induced and uninduced cultures of pBGP123 pulse-labeled for 2 min with [$^3$H]uridine. Labeled RNA was hybridized to filters containing excess pSF2124, pBGP120, or *Xenopus* 28S DNA. The results (Table 1a–c) show that induction of the *lac* operon increased the percentage of pulse-labeled RNA complementary to *Xenopus* 28S DNA about 9-fold. *lac*-specific RNA increased about 80-fold upon induction.

To show that the increase in *Xenopus* DNA transcription in induced cells containing pBGP123 was due to initiation at the *lac* promoter, we constructed pBGP23, a plasmid identical to pBGP123 except that it does not contain the lambda *plac5 lac* DNA fragment. Pulse-labeled [$^3$H]RNA from induced cells containing pBGP23 was not enriched for *Xenopus* RNA sequences (Table 1d and e). Pulse-labeled RNA from uninduced or induced W3110 cells did not hybridize significantly to *Xenopus* 28S, *lac*, or pSF2124 DNA (Table 1g and h).

Table 1. DNA·RNA hybridization of [$^3$H]RNA from W3110 and W3110 containing pBGP123 or pBGP23

| | | % cpm hybridized to | | |
|---|---|---|---|---|
| Strain | Induction | *lac* DNA | 28S *X. laevis* DNA | pSF2124 DNA |
| W3110/pBGP123 | (a) − | 0.08 | 0.56 | 1.36 |
| | (b) + | 6.77 | 4.82 | 0.93 |
| | (c) +/− | 79.6 | 8.61 | 0.68 |
| W3110/pBGP23 | (d) − | 0.00 | 0.53 | 0.63 |
| | (e) + | 0.00 | 0.47 | 0.56 |
| | (f) +/− | | 0.89 | 0.88 |
| W3110 | (g) − | 0.02 | 0.02 | 0.11 |
| | (h) + | 0.03 | 0.03 | 0.09 |

Cells were grown at 30° in M9 minimal medium supplemented with tryptophan (100 μg/ml), anthranilate (10 μg/ml), vitamin B1 (1 μg/ml), and glucose (0.4%). At an $OD_{660}$ of 0.25 the cultures were divided into two 5-ml parts. One part (+) received isopropylthiogalactoside ($5 \times 10^{-4}$ M) and 3′:5′-cyclic AMP (20 mM). The other part (−) was untreated. After 10 min of vigorous shaking, both cultures were labeled with [$^3$H]uridine (50 μCi/ml; 22 Ci/mM) for 2 min. Cells were then immediately poured onto iced medium containing sodium azide (20 mM) and centrifuged. RNA was extracted as described by Varmus *et al.* (29) and hybridized to nitrocellulose filters containing excess pSF2124, pBGP100, or *Xenopus* 28S ribosomal DNA. The *Xenopus* fragment was isolated by velocity sedimentation in sucrose gradients after *Eco*RI digestion of a derivative of pBGP120 that contained the 28S fragment. The purified *Xenopus* DNA fragment was uncontaminated with pSF2124 or *lac* DNAs. All filters contained 1 μg of DNA, except for filters containing *Xenopus* 28S DNA, which contained 0.1 μg. Hybridization reactions were in 0.60 M NaCl, 0.060 M trisodium citrate at 68° for 16 hr. Filters were then treated with pancreatic RNase (20 μg/ml, 30 min, 25°) and dried; radioactivity was determined. *lac*-specific cpm were determined by subtracting cpm bound to filters containing pSF2124 from cpm bound to filters containing pBGP100. Hybridization of all [$^3$H]RNA preparations to filters containing excess *Drosophila* DNA was approximately 0.04%, and this background was subtracted from the data presented. Lines (c) and (f) are ratios of b/a and e/d, respectively. Input cpm to each filter were: (a) 148,300; (b) 115,700; (d) 119,000; (e) 151,600; (g) 183,200; and (h) 131,300.

## DISCUSSION

This paper describes the construction of a plasmid vehicle that contains the *lac* operator, promoter, and most of the structural gene for β-galactosidase. This plasmid, pBGP120, has been constructed such that heterologous DNA fragments can be inserted into the lactose operon and expressed by readthrough transcription initiated at the lactose promoter. We have shown that transcription of 28S ribosomal DNA of *Xenopus laevis* in pBGP123 is increased by isopropylthiogalactoside and cyclic AMP, molecules that specifically increase transcription from the *lac* promoter.

Approximately 5% of the pulse-labeled RNA from induced cells containing pBGP123 hybridizes to filters containing *Xenopus* 28S DNA (Table 1b). Considering that the efficiency of hybridization is about 20%‡ (unpublished experiments), we estimate that approximately 25% of the messenger RNA syn-

‡ This efficiency was determined by hybridization of pulse-labeled RNA from an uninduced culture of W3110 to excess W3110 DNA on filters. If the efficiency of the hybridization shown in Table 1b were substantially higher than 20%, due to the increased concentration of hybridizable RNA species in induced cells, then the estimate of mRNA synthetic capacity involved in synthesis of the *lac-Xenopus* RNA would have to be lowered proportionately.

thetic capacity in induced cells is directed towards production of the *lac-Xenopus* RNA sequences. Data in Table 1a and d indicate that transcription of the *Xenopus* 28S DNA fragment is readily detectable in the absence of *lac*-specific transcription. Such transcription could result from initiation within the 28S DNA fragment or from readthrough transcription initiated at promoters in the pSF2124 region of pBGP123 or pBGP23.

The *Eco*RI site in the *lac* operon appears to be in the structural gene for β-galactosidase near the COOH-terminus. This is suggested by the fact that the β-galactosidase produced in DG73 containing pBGP120 has low enzymatic activity *in vitro* and is distinguishable from wild-type enzyme on two-dimensional acrylamide gels (P. O'Farrell and D. H. G., unpublished experiments). These observations have two implications. First, they suggest that the genetic complementation of DG73 to a *lac*$^+$ phenotype by pBGP100 could have resulted from omega complementation (30) since DG73 contains the COOH-terminal region of the β-galactosidase gene but is deleted for a region proximal to the $NH_2$-terminus. Second, if the normal translational stop signals for β-galactosidase are missing in pBGP120, in-phase translational readthrough into adjacent inserted sequences might occur, resulting in a significant increase in the size of the β-galactosidase polypeptide subunit. In fact, we have recently observed that induced cultures of pBGP123 contain elevated levels of β-galactosidase of higher subunit molecular weight than wild-type enzyme (P. O'Farrell, unpublished experiments). We believe this increase results from translation of *Xenopus* RNA sequences covalently linked to mRNA for β-galactosidase, resulting in a fused polypeptide. This translation *in vivo* confirms and obviously extends the results of RNA·DNA hybridization experiments performed with [$^3$H]RNA from cells containing pBGP123.

The extent of readthrough transcription of an inserted sequence depends on both its orientation and sequence. Insertion of the 28S fragment into pBGP120 in the opposite orientation from that of pBGP123 results in less extensive readthrough transcription than is observed in pBGP123 (B.P., unpublished experiments). We have also inserted a *Xenopus* DNA fragment of $M_r$ $4.4 \times 10^6$, containing spacer and 18S ribosomal sequences (2), into pBGP120. To date we have been unable to detect inducible readthrough transcription of this fragment in either orientation. We are currently investigating whether the extent of readthrough transcription of 18S and 28S fragments is affected by altering the transcriptional termination factor rho (14, 31) of the host.

It would be interesting to examine the expression of a normally translated eukaryotic sequence in pBGP120. If an inserted sequence contains a ribosome binding site that can be utilized in bacteria, production of high levels of a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide. In the absence of an independent ribosome binding site, the eukaryotic sequence would be translated to yield a peptide covalently linked to β-galactosidase. The extent of readthrough translation under *lac* control will depend on the number of translatable codons between the *Eco*RI site and the first in-phase nonsense codon in the inserted sequence.

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