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Efficient isolation of genes by using antibody probes

(λ vector/recombinant DNA/high-frequency lysogeny/hybrid protein expression/protein stability)

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ABSTRACT **A sensitive and general technique has been devised for the dual purposes of cloning genes by using antibodies as probes and isolating unknown proteins encoded by cloned DNA. The method uses an expression vector, λgt11 (*lac5 nin5* cI857 S100), that permits insertion of foreign DNA into the β-galactosidase structural gene *lacZ* and promotes synthesis of hybrid proteins. Efficient screening of antigen-producing clones in λgt11 recombinant cDNA libraries is achieved through lysogeny of the phage library in *hflA* (high-frequency lysogeny) mutant cells of *Escherichia coli*; lysogens produce detectable quantities of antigen on induction, even when plated at high cell densities. The vector is also designed to facilitate the isolation of proteins specified by previously cloned gene sequences. Hybrid proteins encoded by recombinant phage accumulate in strains defective in protein degradation (*lon* mutants) in amounts amenable to large-scale purification. Antibodies produced against the portion of the hybrid encoded by foreign DNA could in turn be used to isolate the native polypeptide from eukaryotic cells.**

The isolation of protein-encoding genes from large recombinant DNA libraries can be achieved, in principle, by using antibodies to detect antigen produced by specific recombinants. Various methods have been developed to detect antigens produced by individual bacterial colonies or phage plaques (1–7); some of these have been used to identify limited numbers of individual colonies that express foreign DNA inserted in plasmid vectors (5–7). This report details the development of a λ vector and a method that allow the construction of cDNA libraries of 10^5–10^7 recombinants from which individual antigen-producing clones can be isolated efficiently.

A useful recombinant expression vector should have properties that permit the construction and maintenance of large cDNA libraries. The recombinant should be propagated in its host cell as a single-copy genomic insert to enhance its stability and to facilitate repression of foreign genetic information. The expression vector should also respond to induction with a rapid increase in copy number and high-level transcription of the foreign DNA. Because the ability to detect antigen will depend on its stability, the expression vector and its host should include features that minimize the degradation of the foreign eukaryotic protein.

These properties occur in the phage expression vector λgt11. The general ability of λ lysogens to produce large quantities of phage products on induction has been exploited in λgt11 to enhance the sensitivity and efficiency of antigen screening. The construction of a library of recombinant DNA-containing lysogens with λgt11 permits the growth, induction, and lysis of antigen-producing cells directly on nitrocellulose filters.

Because bacteria rapidly degrade most unusual polypeptides (8–10), eukaryotic proteins, and especially portions thereof, are potentially unstable in prokaryotic cells. Fusion of the eukaryotic moiety with all but a small portion of the prokaryotic protein β-galactosidase, encoded by λgt11, has been shown to enhance the stability of a somatostatin hybrid protein (11). The use of host cells defective in protein degradation pathways (12) may also increase the lifetime of novel proteins produced from the induced λgt11 lysogens.

In summary, a method is presented for the isolation of gene sequences by using antibody probes. A λ phage expression vector, λgt11, has been constructed whose properties permit insertion of foreign DNA to produce a recombinant DNA library, high-frequency lysogeny in particular *Escherichia coli* strains, induced synthesis of β-galactosidase fused to protein specified by the foreign DNA, and reproducible detection of antigen in populations of up to 10^6 lysogens per 82-mm nitrocellulose filter. Other features and potential uses of the vector are described.

MATERIALS AND METHODS

Enzymes. T4 DNA ligase and *E. coli* DNA polymerase I were gifts of S. Scherer (California Institute of Technology). DNA restriction endonucleases were from New England BioLabs.

Bacterial Strains. Strains are listed in Table 1. BNN98 results from spontaneous loss of the F′ episome in BNN94. BNN100, BNN101, BNN102, and BNN103 were constructed by P1 transduction of the relevant alleles (*hflA150* from BNN92, *supF* from BNN99) into BNN93, BNN96, or BNN98. The medium used was LB (pH 7.5), unless otherwise specified. All media and strain construction were as described by Miller (13). λ phage were from our collection and have been described by Williams and Blattner (14).

Construction of λgt11 (*lac5 nin5* cI857 S100). λgt11 is essentially a derivative of λgt7-*lac*5 (*b522 nin5*) and λgt4 (cI857 S100 *nin5*). To construct this phage, λgt7-*lac5* and λ540 (Δ*B imm21 nin5*) were cleaved with *Hin*dIII and the fragments were pooled and then ligated with T4 DNA ligase. The desired phage recombinant produced turbid (*imm21*) blue (*lac5*) plaques when the DNA was transfected into *E. coli* BNN93 and cells were plated on medium containing the chromogenic indicator 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal). The λgt7-*lac5*–λ540 hybrid was then crossed with λgt4 (cI857 S100 *nin5*) and recombinants, grown at 42°C, were scored for the formation of clear (cI857) blue plaques on X-Gal plates. The presence of the amber mutation S100 was confirmed by examining relative plating efficiency on hosts that contained or lacked the amber suppressor *supF* (BNN45 or BNN93, respectively). Finally, the *lac5* cI857 S100 phage were mapped for *Eco*RI cleavage sites. λgt11 contained a single *Eco*RI cleavage site and was mapped in detail with other enzymes (see Fig. 1).

Preparation of Antibodies. Rabbit antiserum made against the pancreatic α-amylase of C57BL/6J mice was a gift of M. Miesler (University of Michigan). Rabbit antiserum made against

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Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; kb, kilobase pair(s).

Table 1. Bacterial strains

Strain	Alias	Genotype	Source
BNN45	LE392	*hsdR⁻hsdM⁺ supE44 supF thi met lacY*	This laboratory
BNN91	MA150	*ΔlacZ hflA150 strA*	A. Hoyt (Berkeley)
BNN92	MA156	MA150[chr::Tn*10*]	A. Hoyt (Berkeley)
BNN93	C600	*hsdR⁻hsdM⁺ supE thr leu thi lacY1 tonA21*	This laboratory
BNN94	CSH41	F′ *lacI lacP proA⁺ proB⁺*/Δ(*pro-lac*) *galE thi*	M. Howe (Wisconsin)
BNN95	AB1899	*thr-1 leuB6 thi-1 argE3 his-4 proA2 lon-1 lacY1 galK2 mtl-1 xyl-5 ara-14 strA31 tsx-33 supE44*	B. Bachman (Yale)
BNN96	SG1041	Δ(*lac*IPOZYA)U169 *proA⁺ Δlon araD139 strA thi*	S. Gottesman, D. Court (National Institutes of Health)
BNN97	Y1004	BNN93(λgt11)	This work
BNN98	Y1048	F⁻ Δ(*pro-lac*) *galE thi-1*	This work
BNN99	Y1059	F⁺ *supE57 supF58 mel-1* [*trpC22*::Tn*10*](λ)	This work
BNN100	Y1068	F⁻ Δ(*pro-lac*) *galE thi-1 supF58*	This work
BNN101	Y1070	F⁻ Δ(*pro-lac*) *galE thi-1 supF58 hflA150* [chr::Tn*10*]	This work
BNN102	Y1073	BNN93 *hflA150* [chr::Tn*10*]	This work
BNN103	Y1083	BNN96 *hflA150* [chr::Tn*10*]	This work

chr::Tn*10*, chromosomal insertion of Tn*10* that exhibits 65% cotransduction with *hflA150*.

chicken ovalbumin was provided by M. Wickens (Medical Research Council, Cambridge). One microliter of either serum precipitated 0.5–1 μg of pure antigen. IgG was purified from the sera as described by Broome and Gilbert (4) and stored at 5–10 mg/ml.

Examination of Lysogens for Antigen Production by Using Antibody Probes. BNN91 or BNN103 is grown to stationary phase in LB medium, pH 7.5/0.1% maltose, Cells are infected at a multiplicity of infection of 1.0 for 30 min at 32°C in 0.1 ml of 10 mM Tris·HCl, pH 7.5/10 mM $MgSO_4$. The infected cells are diluted with 0.5 ml of LB medium, and the suspension is poured carefully onto an 82-mm Schleicher & Schuell BA85 nitrocellulose filter previously placed on an LB plate. The liquid culture is spread evenly over the filter and permitted to soak through into the plate (relatively dry plates are preferable). The plate is incubated at 32°C for 8 hr, and a replica is made in the following manner. The master filter is removed from its plate and excess liquid is blotted from its underside. Another filter is wetted on an LB plate, blotted, placed over the master, pressed evenly against it and marked with a needle. Both master and replica filters are then replated and incubated at 32°C for 1 hr. Then, one of the plates is incubated at 42°C for 2 hr, and the other is refrigerated. The induced cells are lysed on the filter by inverting the plate over a small vessel of chloroform for 15 min to create a chloroform-saturated atmosphere. The nitrocelluose filter is removed from the plate and immersed in 3 ml of buffer A (0.17 M NaCl/0.01 M Tris·HCl, pH 7.5/0.1 mM phenylmethylsulfonyl fluoride) together with 0.01% $NaDodSO_4$ for 1 hr (this and all subsequent steps are carried out at 24°C). The solution becomes viscous at this stage and DNA, if not removed, appears to reduce antigen availability. Therefore, the filter is rinsed with 3 ml of buffer A, incubated with 3 ml of buffer A containing DNase I at 2 μg/ml for 10 min, and then rinsed again with buffer A. To reduce nonspecific protein binding to the nitrocellulose, filters are incubated in 3 ml of buffer A/3% bovine serum albumin for 1 hr. IgG is generally diluted to 50 μg/ml in buffer B (buffer A/0.1% $NaDodSO_4$/0.1% Triton X-100/1 mM EDTA), and the filter is gently agitated in this solution for 3 hr. The filter is washed for two 10-min periods with buffer B, and the bound antibody is then allowed to react with $\approx 5 \times 10^6$ cpm of ^{125}I-labeled (1 to 2×10^6 cm/μg) (*Staphylococcus aureus*) protein A in 3 ml of buffer B for 1 hr. Finally, the filter is washed for five 15-min periods with 5-ml portions of buffer B. Good autoradiographic signals are usually obtained overnight in a screen of 10^6 colonies per filter. Note that labeled anti-idiotypic antibodies could be used to detect antibody classes of the type that do not bind protein A.

Preparation of Lysates from Induced Recombinant Lysogens. Lysogens were grown at 32°C to a cell density of 2×10^8, incubated at 42°C for 15 min, and then well aerated by shaking at 38°C for 2 hr. (Induced lysogens containing some recombinants will lyse after 2 hr at 38°C, presumably because of the detrimental effects of the high levels of some hybrid proteins.) Cells were pelleted, quickly suspended in gel sample buffer (50 mM Tris·HCl, pH 6.8/1.5% $NaDodSO_4$/50 mM dithiothreitol/ 4 M urea) at 3% the original volume, and mixed well by passaging several times through a 21-gauge needle. The solution was then heated to 70°C for 2 min and insoluble material was removed by centrifugation for 3 min in a Microfuge.

RESULTS

The expression vector λgt11 (*lac5 nin5 cI857 S100*) has been constructed (Fig. 1). The site used for insertion of foreign DNA is a unique *Eco*RI cleavage site located within *lacZ*, 53 base pairs upstream of the β-galactosidase termination codon (15, 16). Phage containing inserts generate an inactive β-galactosidase fusion protein; these phage can be distinguished from nonrecombinant phage by their inability to produce blue plaques on a *lacZ⁻* host on X-Gal plates. The vector can accommodate up to 8.3 kilobases (kb) of insert DNA, assuming a maximum packageable phage DNA length of 52 kb. λgt11 cDNA libraries containing 10^5–10^7 recombinant phage (in which recombinants account for 4–30% of total phage) have been constructed using 1 μg of polyadenylylated RNA isolated from *Saccharomyces cerevisiae* strain X2180 (unpublished data), *Caenorhabditis elegans* strain CB1490, and rat preputial gland and human placenta (B. Meyer, T. Chappell, and D. Pauza, personal communication) using procedures described in ref. 17.

The ability to form lysogens from the λ *c*I857 S100 expression vector can be exploited to maximize the yield of protein synthesized from transcripts of the foreign DNA. The phage vector produces a temperature-sensitive repressor (*c*I857), inactive at 42°C, and contains an amber mutation (S100) that renders it lysis defective (18, 19); consequently, lysogens can be induced by temperature shift to accumulate large quantities of phage products in the absence of lysis. To obtain efficient lysogeny, strains containing the high-frequency-lysogeny mutation *hflA150* (20, 21) have been used. Essentially every *hflA* mutant cell is lysogenized when infected with λ at 32°C, yet *c*I857 prophage in-

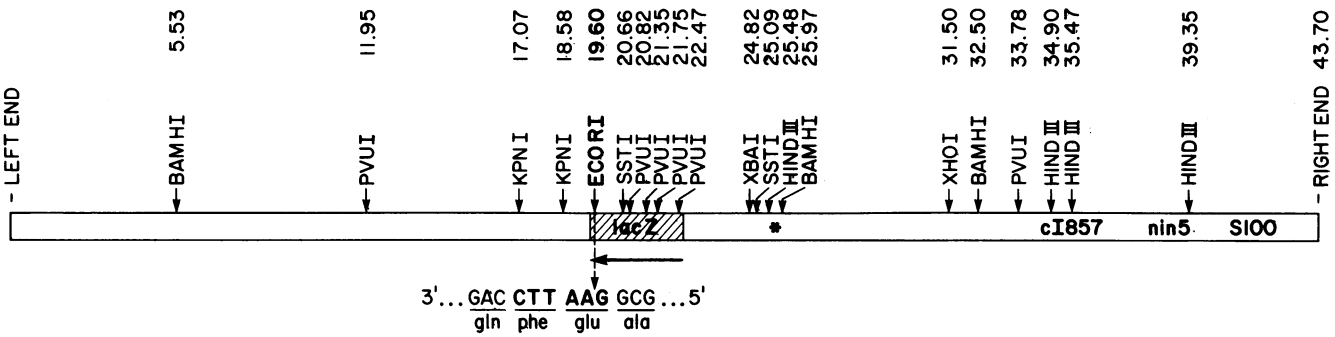

FIG. 1. Features of λgt11. Restriction endonuclease cleavage sites are designated in kb from the left end. *, λ attachment site. The transcriptional orientation of *lacZ* (ZZZ) is given by the horizontal arrow. The sequence of the unique *Eco*RI site (boldface letters), the nucleotides that immediately surround it, and the amino acids encoded are shown below the phage map.

duction at 42°C remains unhindered. *hflA* strains can be lysogenized efficiently with λgt11 recombinant DNA libraries and these lysogens can be induced to produce normal phage yields.

Construction of Model Recombinants. To test the ability of λgt11 to express foreign DNA as a fusion product and as a detectable antigen, model recombinants were constructed by inserting mouse α-amylase and chicken ovalbumin cDNAs into the *Eco*RI site of the vector. Since the DNA sequence surrounding the *lacZ Eco*RI site and the amino acid sequence of β-galactosidase are known (refs. 15 and 16; Fig. 1), the foreign DNA insertion could be engineered to obtain a continuous (or noncontinuous) coding frame from β-galactosidase into α-amylase or ovalbumin. The origin of the DNA used in these recombinants and the portion of eukaryotic protein they encode are given in Table 2.

Detection of Eukaryotic Antigens. Relative levels of antigen were examined from induced lysogens containing λgt11 recombinant phage whose inserts vary in orientation and fusion frame. Purified IgG was used to detect antigen produced by 2×10^6 lysogenized cells in 4-mm dots on nitrocellulose filters. The results are shown in Fig. 2. Good signals were obtained with both α-amylase and ovalbumin IgG. All lysogens containing inserts in the proper transcriptional orientation (αP3, T81, T104, and P82) produce detectable antigen; in contrast, αP2 and T83, which have DNA inserts in the opposite orientation, yield signals comparable with those in control spots. T104, containing an out-of-frame ovalbumin cDNA insert, produced approximately one-eighth the signal obtained with the lysogen containing the fused polypeptide, T81 (see *Discussion*). This experiment indicates that antigenic detection is dependent on proper orientation of the insert DNA with respect to the β-galactosidase transcription unit and that the relative signal strength is greatest when the reading frames of *lacZ* and insert DNA coincide.

Table 2. Model recombinants

Name	cDNA	Fragment	Orientation	Frame	Hybrid size, daltons
αP2	Amylase	1.5-kb *Eco*RI	Reversed		
αP3	Amylase	1.5-kb *Eco*RI	Proper	In	142,000
T81	Ovalbumin	2.0-kb *Taq* I	Proper	In	153,000
T83	Ovalbumin	2.0-kb *Taq* I	Reversed		
T104	Ovalbumin	2.0-kb *Taq* I	Proper	Out	
P82	Ovalbumin	3.2-kb *Pvu* II	Proper	In	140,000

Model recombinants were constructed according to methods described in refs. 17 and 22. α-Amylase cDNA was isolated from pMPa21 (23, 24) and ovalbumin cDNA was isolated from pOv230 (25). Transcriptional orientation of the cDNA and its coding frame are relative to that of *lacZ*. The predicted size of the hybrid protein produced by recombinants whose coding frames coincide is also given. The β-galactosidase portion of the hybrid accounts for 114,000 daltons (16).

Screening λgt11 Recombinant DNA Libraries with Antibody Probes. The following reconstruction experiment illustrates the screening procedure. Approximately 20 BNN91 cells were lysogenized with a recombinant DNA phage (either α-amylase or ovalbumin) and then added to 10^5, 10^6, or 5×10^6 λgt11 lysogens of BNN91. Each culture was plated immediately on 82-mm nitrocellulose filters, grown for 8 hr at 32°C, replica plated, induced, and probed with antiserum. The results of probing the α-amylase recombinant αP3 among 10^6 and 5×10^6 λgt11 lysogens are shown in Fig. 3. Antigen produced by cells containing recombinant phage was detected even at the highest cell density tested (5×10^6). However, replica filters retained fewer signals with greater cell density: approximately 5%, 15%, and 60% of the signals obtained from the master filter were lost at cell densities of 10^5, 10^6, and 5×10^6 per plate, respectively. Thus, satisfactory signal reproducibility, at least for the antigen–antibody interaction examined here, is obtained at a plating density of 10^6 cells per filter.

Production of Fusion Polypeptides. The amount of hybrid protein that accumulates in BNN91 cells containing the λgt11 recombinants αP3, T81, and P82 was investigated by subjecting lysates of three lysogens to $NaDodSO_4$/polyacrylamide gel electrophoresis. Bands exhibiting the mobilities predicted for the hybrid proteins were observed when the gels were stained with silver (26) but not when they were stained with the less sensitive Coomassie brilliant blue, suggesting that very small amounts of the novel proteins accumulate. To improve the yield of these proteins, the recombinant phage were lysogenized in the *lonΔ* mutant strain BNN103. Since *lon* mutants increase the stability of β-galactosidase peptide fragments (12, 27), BNN103 lysogens might be expected to accumulate larger quantities of the unstable β-galactosidase hybrids. Indeed, we found that BNN103 lysogen lysates contain proteins of the predicted size for both α-amylase (αP3) and ovalbumin (P82) hybrids (Fig. 4).

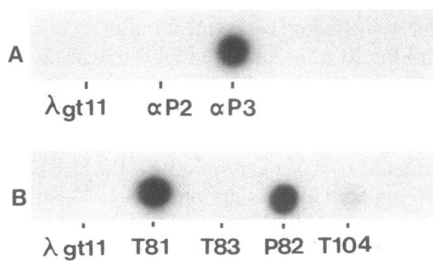

FIG. 2. Relative antigen detection from lysogens containing various λgt11 recombinants. Dots (4 mm) containing 2×10^6 BNN91 (λgt11) recombinant lysogens were induced, lysed, and probed with either α-amylase (*A*) or ovalbumin (*B*) IgG. Kodak XR-5 films were exposed for 5–8 hr with a Cronex Lightning Plus intensifying screen at −70°C.

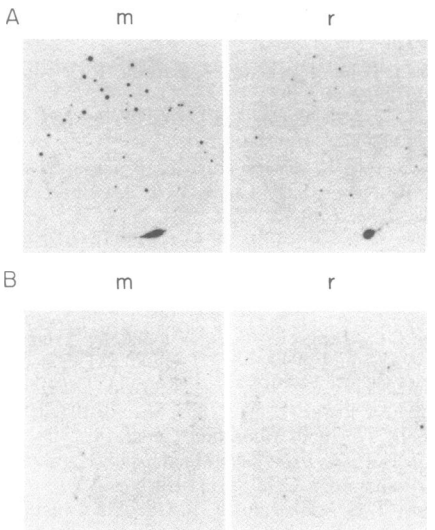

FIG. 3. High-cell-density lysogen screening. Filters containing 10^6 (*A*) or 5×10^6 (*B*) BNN91 (λgt11) lysogens interspersed with ≈20 BNN91 (αP3) lysogens were grown at 32°C and then induced, lysed, and probed with α-amylase IgG (m, master; r, replica). Film exposure was 15 hr with an intensifying screen.

Similar results were obtained using another *lon* mutant, BNN95. These proteins have not yet been purified to confirm their identity as fusion polypeptides absolutely. The predicted fusion protein produced by the T81 recombinant phage, with a molecular weight of 153,000, could not be resolved from the RNA polymerase β subunit.

To determine whether the enhanced yield of the presumptive full-length fusion polypeptide in *lon* mutants increases the level of detectable antigen, 4-mm dots of BNN91 and BNN103 lysogens of αP3 (4×10^5 cells per dot) were probed. The BNN103 *lon*Δ lysogen generated about 3 times as much signal as the BNN91 lysogen, indicating that BNN103 may be useful for enhancing signal/noise ratios in high-cell-density screening of lysogen libraries.

DISCUSSION

High-Density-Lysogen Screening. λgt11 is a general recombinant DNA expression vector capable of producing polypeptides specified by inserted DNA. Antibodies can be used to detect reproducibly the antigen produced in single colonies of induced λgt11 lysogens when up to 10^6 lysogen colonies are examined on an 82-mm nitrocellulose filter.

Proper expression of foreign DNA in λgt11 recombinant lysogens will depend on the orientation and reading frame of the insert DNA with respect to those of *lacZ*. Thus, one-sixth of the λgt11 recombinants containing a specific cDNA will produce β-galactosidase fused to the protein of interest. cDNA quality will further affect the ability to detect antigen produced by the recombinant; nearly full-length cDNAs specify more potential antigenic determinants than shorter cDNAs made against the same mRNA.

Antibody quality plays an important role in a successful screen. The α-amylase and ovalbumin IgG used here produced good signal/noise ratios without additional purification. However, antibodies directed against coliform proteins are found in many preparations and must be removed from the specific probe. In fact, the specific signal obtained with ovalbumin IgG could be improved by prior incubation with a λgt11 lysogen (BNN97) lysate bound to nitrocellulose and others have successfully used column-bound lysates with similar results (6). The best specific reaction with antigens produced by λgt11 recombinants should be obtained with affinity-purified antibodies. Affinity-purified anti-κ light chain immunoglobulin antibody has been used successfully to detect κ-producing λgt11 plaques at densities of up to 10^5 per 90-mm plate (T. St. John, personal communication).

Several lines of evidence indicate that complete hybrid protein as well as other forms of the antigen contribute to antibody binding. The strength of the signal observed when probing the α-amylase recombinant BNN91 lysogen at high cell density was

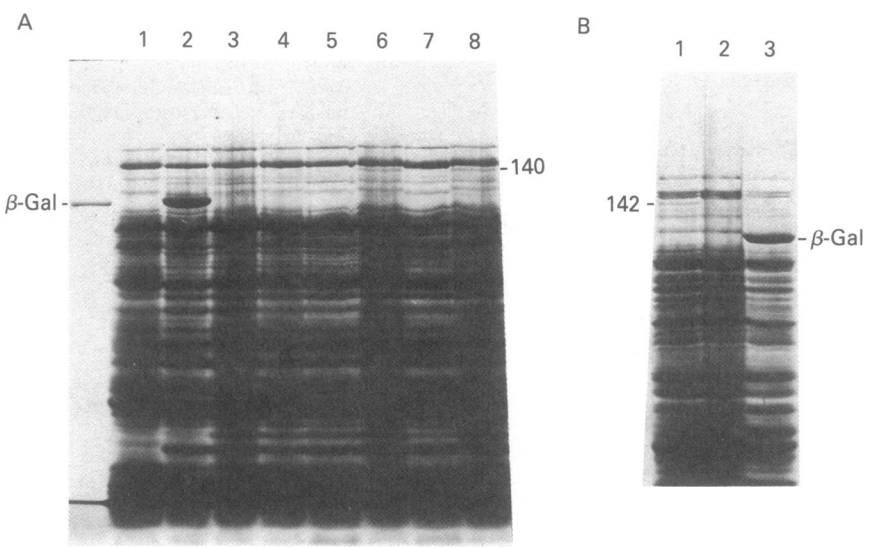

FIG. 4. $NaDodSO_4$/polyacrylamide gel analysis of hybrid protein accumulation. Proteins accumulating in induced *lon*$^+$ and *lon*$^-$ lysogens containing αP3, T81, and P82 were compared. Lysate samples (20 μl) were loaded on 7.5% running/4% stacking polyacrylamide gels, subjected to electrophoresis, and stained with Coomassie brilliant blue according to Laemmli (28). 140 and 142, predicted positions of the αP3 and P82 hybrid proteins (αP3, M_r = 142,000; T81, M_r = 153,000; P82, M_r = 140,000). The RNA polymerase subunits β and β′ migrate just behind the 140 marker. (*A*) $NaDodSO_4$/polyacrylamide gel showing the entire cell lysate. Lanes: 1, 0.25 μg of β-galactosidase; 2, BNN91 (λgt11); 3, BNN91 (αP3); 4, BNN91 (T81); 5, BNN91 (P82); 6, BNN103 (αP3); 7, BNN103 (T81); 8, BNN103 (P82). (*B*) Lysates from *lon*$^+$ and *lon*$^-$ lysogens of αP3 were subjected to electrophoresis of greater duration than in *A* to better resolve the region of the gel containing hybrid polypeptides. Lanes: 1, BNN103 (αP3); 2, BNN91 (αP3); 3, BNN91 (λgt11). Lysates from uninfected BNN91 and BNN103 appear identical, as do those from BNN91 (λgt11) and BNN103 (λgt11) (data not shown).

greater than might be expected given the amount of hybrid protein accumulation judged by gels. Kemp and Cowman (6) were similarly unable to observe *trpD* fusion polypeptides in polyacrylamide gels after staining with Coomassie brilliant blue but detected both full-length hybrid protein and peptide fragments in protein blots examined with antibody probes. The ability to detect antigen produced by recombinants whose coding sequences are inserted out of frame (Fig. 2 and ref. 6) suggests that anomalous translation initiation can also contribute to the pool of available antigen.

Isolation of Unknown Native Proteins. λgt11 can facilitate the identification and isolation of proteins that are specified by previously cloned DNA. The hybrid protein produced by cells containing the recombinant DNA can be purified and used to obtain antibodies. Antibodies produced against these proteins should include activity against the eukaryotic portion of the protein fusion; this antibody could be used as a tool to locate the native protein and to isolate it from the organism of interest. Synthetic polypeptide antigens have been used to produce antibodies for similar purposes (29). One advantage of the approach described here is that it circumvents the DNA sequence information required to produce synthetic polypeptides.

The hybrid proteins examined here accumulate in *lon* mutant strains to amounts amenable to purification. For example, a BNN103 lysogen of αP3 contains ≈1 fg of α-amylase hybrid protein (≈0.5% of total protein), as estimated by comparison with known amounts of β-galactosidase on NaDodSO$_4$/polyacrylamide gels. Thus, 1 mg of this protein could be obtained from 10^{12} cells or about 2 liters of induced culture. The hybrid protein can be purified by standard techniques, taking advantage of its size (using, for example, ammonium sulfate fractionation and gel filtration) and the charge properties of its β-galactosidase moiety (binds DEAE tightly) (13, 16). Alternatively, fusion proteins can be purified directly by preparative polyacrylamide gel electrophoresis.

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1. Skalka, A. & Shapiro, L. (1976) *Gene* **1**, 65–79.
2. Sanzey, B., Mercereau, O., Ternynck, T. & Kourilski, P. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3394–3397.
3. Erlich, H. A., Cohen, S. N. & McDevitt, H. O. (1978) *Cell* **13**, 681–689.
4. Broome, S. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2746–2749.
5. Hitzeman, R. A., Clarke, L. & Carbon, J. (1980) *J. Biol. Chem.* **255**, 12073–12080.
6. Kemp, D. J. & Cowman, A. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4520–4524.
7. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. L. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
8. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803.
9. Charnay, P., Gervais, M., Louise, A., Galibert, F. & Tiollais, P. (1980) *Nature (London)* **286**, 893–895.
10. Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M. & Rutter, W. J. (1981) *Nature (London)* **291**, 503–506.
11. Itakura, K., Hirose, T., Crea, R., Riggs, A. D., Heyneker, H. L., Bolivar, F. & Boyer, H. W. (1977) *Science* **198**, 1056–1063.
12. Mount, D. W. (1980) *Annu. Rev. Genet.* **14**, 279–319.
13. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Williams, B. G. & Blattner, F. R. (1980) in *Genetic Engineering*, eds. Setlow, J. K. & Mullander, A. (Plenum, New York), Vol. 2, p. 201.
15. Galas, D. J., Calos, M. P. & Miller, J. H. (1980) *J. Mol. Biol.* **144**, 19–41.
16. Zabin, I. & Fowler, A. V. (1978) in *The Operon*, eds. Miller, J. H. & Reznikoff, W. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 89–121.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
18. Neubauer, Z. & Calef, E. (1970) *J. Mol. Biol.* **51**, 1–13.
19. Goldberg, A. R. & Howe, M. (1969) *Virology* **38**, 200–202.
20. Belfort, M. & Wulff, D. L. (1973) *Virology* **55**, 183–192.
21. Hoyt, M. A., Knight, D. M., Das, A., Miller, H. I. & Echols, H. (1982) *Cell* **31**, 565–573.
22. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
23. Schibler, U., Tosi, M., Pittet, A.-C. & Wellauer, P. K. (1980) *J. Mol. Biol.* **142**, 93–116.
24. Hagenbuchle, O., Bovey, R. & Young, R. A. (1980) *Cell* **21**, 179–187.
25. McReynolds, L. A., O'Malley, B. W., Nisbet, A. D., Fothergill, J. E., Givol, D., Fields, S., Robertson, M. & Brownlee, G. G. (1978) *Nature (London)* **273**, 723–728.
26. Merril, C. R., Switzer, R. C. & Van Keuren, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4335–4339.
27. Bukhari, A. I. & Zipser, D. (1973) *Nature (London) New Biol.* **243**, 235–241.
28. Laemmli, U. K. (1970) *Nature (London)* **227**, 630–685.
29. Lerner, R. A. (1982) *Nature (London)* **299**, 592–596.