Efficient isolation of genes by using antibody probes

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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, Agt11 (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 Agt11 recombinant cDNA libraries is achieved through lysogeny of the phage library in hflA (high-frequency lysogenic) 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 plaque 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 10^9 to 10^10 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 phase expression vector Agt11. The general ability of λ lysogens to produce large quantities of phage products on induction has been exploited in Agt11 to enhance the sensitivity and efficiency of antigen screening. The construction of a library of recombinant DNA-containing lysogens with Agt11 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 Agt11, 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 Agt11 lysogens.

In summary, a method is presented for the isolation of gene sequences by using antibody probes. A λ phage expression vector, Agt11, has been constructed whose properties permit insertion of foreign DNA to produce a recombinant DNA library, high-frequency lysogenic 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 92-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 Agt11 (lac5 nin5 cI857 S100). Agt11 is essentially a derivative of Agt7-lac5 (b522 nin5) and Agt4 (cI857 S100 nin5). To construct this phage, Agt7-lac5 and A540 (ΔB imm21 nin5) were cleaved with HindIII 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 Agt7-lac5-A540 hybrid was then crossed with Agt4 (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 (BN45 or BN93, respectively). Finally, the lac5 cI857 S100 phage were mapped for EcoRI cleavage sites. Agt11 contained a single EcoRI 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

Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-d-galactoside; kb, kilobase pair(s).
chicken ovalbumin was provided by M. Wickens (Medical Research Council, Cambridge). One microfiltr 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 MgSO4. 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 nitrocellulose 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% NaN3 or 0.1°C 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. The filter is then washed and immersed in 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% NaN3 or 0.1°C for 10 min). The filter is then rinsed with 20-ml portions of buffer B, and the bound antibody is then allowed to react with ~5 × 10^6 cm²/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°C 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 × 10⁹, 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 35°C, presumably because of the detrimental effects of the high levels of some hybrid proteins.) Cells were pelleted, quickly suspended in gel sample buffer (250 mM Tris-HCl, pH 6.8/1.5% NaDodSO4/50 mM dithiothreitol/4 M urea) at 3°C the original volume, and mixed well by passing 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 pAgt11 (lac5 min5 c1857 S100) has been constructed (Fig. 1). The site used for insertion of foreign DNA is a unique EcoRI 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 package phage DNA length of 52 kb. pAg11 cDNA libraries containing 10²–10⁶ recombinant phage (in which recombinants account for 4–30% of total phage) have been constructed using 1 μg of polyadenylated 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 *A. clavatus* S100 expression vector can be exploited to maximize the yield of protein synthesized from the transcripts of the foreign DNA. The phage vector produces a temperature-sensitive repressor (c1857), 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 h/A150 (20, 21) have been used. Essentially every h/A150 mutant cell is lysogenized when infected with *A. clavatus* at 32°C, yet c1857 phage in-

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**Table 1. Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Alias</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNN45</td>
<td>L392</td>
<td>hsdR hsdM supE44 supF thi met lacY</td>
<td>This laboratory</td>
</tr>
<tr>
<td>BNN91</td>
<td>MA150</td>
<td>ΔlacZ hF/A150 strA</td>
<td>A. Hoyt (Berkeley)</td>
</tr>
<tr>
<td>BNN92</td>
<td>MA156</td>
<td>MA150(chr::Tn10)</td>
<td>A. Hoyt (Berkeley)</td>
</tr>
<tr>
<td>BNN93</td>
<td>C600</td>
<td>hsdR hsdM supE6 thr leu thi lacY1 tonA21</td>
<td>This laboratory</td>
</tr>
<tr>
<td>BNN94</td>
<td>CSH41</td>
<td>F' lacI857 (3) proA' Δ(pro-lac)-(4) lacI857 (3) proA2 lacI244 (0.17 M tris-HCl, pH 7.5/0.1 mM MgSO4)</td>
<td>M. Howe (Wisconsin)</td>
</tr>
<tr>
<td>BNN95</td>
<td>AB1899</td>
<td>thr-1 leuB th-1 argB3 his-4 proA2 ton-1 lacY1</td>
<td>B. Bachman (Yale)</td>
</tr>
<tr>
<td>BNN96</td>
<td>SG1041</td>
<td>Δ(lacIPOZYA)U690 proA' Δlon araD139 str thi</td>
<td>S. Gottesman, D. Court (National Institutes of Health)</td>
</tr>
<tr>
<td>BNN97</td>
<td>Y1004</td>
<td>BNN93(agr11)</td>
<td>This work</td>
</tr>
<tr>
<td>BNN98</td>
<td>Y1048</td>
<td>F' Δ(pro-lac) galE thi-1</td>
<td>This work</td>
</tr>
<tr>
<td>BNN99</td>
<td>Y1059</td>
<td>F' supE57 supF58 mel-1 (trpC22::Tn10)(λ)</td>
<td>This work</td>
</tr>
<tr>
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<td>Y1068</td>
<td>F' Δ(pro-lac) galE thi-1 supF58</td>
<td>This work</td>
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<td>BNN101</td>
<td>Y1070</td>
<td>F' Δ(pro-lac) galE thi-1 supF58 hF/A150(chr::Tn10)</td>
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<td>BNN102</td>
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<td>BNN93 hF/A150(chr::Tn10)</td>
<td>This work</td>
</tr>
<tr>
<td>BNN103</td>
<td>Y1083</td>
<td>BNN96 hF/A150(chr::Tn10)</td>
<td>This work</td>
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chr::Tn10, chromosomal insertion of Tn10 that exhibits 65% cotransduction with hF/A150.
duction at 42°C remains unhindered. hflA strains can be lysogenized efficiently with Agt11 recombinant DNA libraries and these lysogens can be induced to produce normal phage yields.

Construction of Model Recombinants. To test the ability of Agt11 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 EcoRI site of the vector. Since the DNA sequence surrounding the lacZ EcoRI 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 Agt11 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 to 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

<table>
<thead>
<tr>
<th>Name</th>
<th>cDNA</th>
<th>Fragments</th>
<th>Orientation</th>
<th>Frame</th>
<th>Hybrid size, daltons</th>
</tr>
</thead>
<tbody>
<tr>
<td>αP2</td>
<td>Amylase</td>
<td>1.5-kb EcoRI</td>
<td>Reversed</td>
<td></td>
<td>142,000</td>
</tr>
<tr>
<td>αP3</td>
<td>Amylase</td>
<td>1.5-kb EcoRI</td>
<td>Proper</td>
<td>In</td>
<td>153,000</td>
</tr>
<tr>
<td>T81</td>
<td>Ovalbumin</td>
<td>2.0-kb Taq I</td>
<td>Proper</td>
<td>In</td>
<td>140,000</td>
</tr>
<tr>
<td>T83</td>
<td>Ovalbumin</td>
<td>2.0-kb Taq I</td>
<td>Reversed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T104</td>
<td>Ovalbumin</td>
<td>2.0-kb Taq I</td>
<td>Proper</td>
<td>Out</td>
<td></td>
</tr>
<tr>
<td>P82</td>
<td>Ovalbumin</td>
<td>3.2-kb Pst II</td>
<td>Proper</td>
<td>In</td>
<td></td>
</tr>
</tbody>
</table>

Model recombinants were constructed according to methods described in refs. 17 and 22. α-Amylase cDNA was isolated from pMI221 (23, 24) and ovalbumin cDNA was isolated from pOv220 (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 Agt11 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 Agt11 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 10^5 and 5 × 10^6 Agt11 lysogens are shown in Fig. 3. Antigens produced by cells containing recombinant phage was detected even at the highest cell density (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 Agt11 recombinants αP3, T81, and P82 was investigated by subjecting lysates of three lysogens to NaDodSO4/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 Cooamassie 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).
These proteins weight of merase, B as tity results

Similar (A) lonA a-amylase IgG an level full-length BNN91 (aP3) 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.

FIG. 3. High-cell-density lysogen screening. Filters containing 10⁶ (A) or 5 × 10⁷ (B) BNN91 (Agt11) 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.

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 Agt11 lysogen (BNN97) ly- sate bound to nitrocellulose and others have successfully used column-bound lysates with similar results (6). The best specific reaction with antigens produced by Agt11 recombinants should be obtained with affinity-purified antibodies. Affinity-purified anti-κ light chain immunoglobulin antibody has been used successfully to detect κ-producing Agt11 plaques at densities of up to 10² 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

**DISCUSSION**

**High-Density-Lysogen Screening.** Agt11 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 Agt11 lysogens when up to 10⁶ lysogen colonies are examined on an 82-mm nitrocellulose filter.

Proper expression of foreign DNA in Agt11 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 Agt11 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.

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**Fig. 4.** NaDodSO₄/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). Lanes 1, 2, 3, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3). Lanes 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (Agt11); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3); 1, 2, 3, 4, 5, 6, 7, 8, BNN91 (aP3).
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. Act111 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 pG3 contains 1 fg of a-amylase hybrid protein (~0.5% of total protein), as estimated by comparison with known amounts of β-galactosidase on NaDodSO4/polyacrylamide gels. Thus, 1 mg of this protein could be obtained from 1012 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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