Cloning and mapping of a gene for translational initiation factor IF2 in *Escherichia coli*

(cosmid library/*infB*/immunoblotting/recombinant λ phage)

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ABSTRACT   A novel method, not relying on genetic complementation of a mutation, was used to clone a gene for translational initiation factor IF2. Two clones from a cosmid library of total *Escherichia coli* DNA were isolated for their ability to overproduce IF2 *in vivo* as determined by quantitative immunoblotting. "Maxicell" analysis of cosmid-encoded proteins and specific immune precipitation of the labeled proteins showed that the structural gene for IF2 (*infB*) had been cloned. Subcloning fragments from the original cosmids located the *infB* gene to a 4.8-kilobase pair HindIII/BamHI fragment. This fragment has been inserted into an integration-deficient recombinant λ phage that lysogenizes by homology. By mapping the point of lysogenization on the *E. coli* chromosome, *infB* has been located at 68 min, very close to *argG*, *nusA*, *rpsO*, and *pnp*. Because the gene for initiation factor IF3 is located at 38 min on the chromosome, the genes for translational initiation factors are not grouped together.

Initiation of protein synthesis in bacteria is promoted by three initiation factors, called IF1 ($M_r = 8,068$), IF2 ($M_r = 115,000$), and IF3 ($M_r = 20,068$). The IF have been purified from *Escherichia coli* and their functions studied *in vitro* (for reviews, see refs. 1–3). The levels of these proteins in crude cell lysates have been measured by immunochemical techniques (4, 5), but little is known about the regulation of these levels or how the initiation factors function *in vivo*. In contrast, a wealth of knowledge has accumulated in the last few years on the synthesis and regulation of other components of the translational apparatus—namely, ribosomal RNA, ribosomal proteins, and elongation factors (for a review, see ref. 6).

To study the regulation of the expression of IF it is desirable to identify, map, and clone the genes. In addition, cloning may allow construction of overproducing strains and permit the introduction of mutations useful for studying structure–function relationships. No spontaneous mutants that affect the IF have been characterized. One thermosensitive mutation with an altered IF3 was isolated after nitrosoguanidine treatment of cells and then *in vitro* screening of mutants that were affected in translational initiation (7). This permitted the isolation of a λ-transducing phage carrying the region of the *E. coli* chromosome around the IF3 gene (*infC*) and hence, the mapping of *infC* to 38 min on the *E. coli* genome (8). However, no such mutations have been identified for IF1 and IF2.

To avoid the laborious process of *in vitro* screening of possible mutations in the translational initiation process, we adopted a method for cloning IF genes that does not rely on a mutation. We presumed that the presence of an IF gene on a multicopy plasmid would cause some overproduction of the factor in the host bacteria. Justification for this premise is that IF3 is considerably overproduced under such conditions (9, 10). We have recently developed a sensitive quantitative immunoblotting technique that can readily distinguish a 2-fold increase in the level of an IF in a crude cell lysate (4). By using this method to analyze the lysates of only a few hundred clones carrying large portions of the *E. coli* genome in cosmid vehicles, we were able to identify strains that overproduced either IF2 or IF3. We describe here the isolation and mapping of the *infB* gene for IF2.

MATERIALS AND METHODS

Strains and General Methods. The *E. coli* and bacteriophage λ strains and plasmids used in this work are listed in Table 1. General genetic methods are described elsewhere (7, 13). Bacterial and phage growth conditions, plasmid and phage DNA preparations, DNA digestions, agarose gel electrophoresis, and cloning methods are as described (9).

Preparation of Cosmid Library. A cosmid library of total *E. coli* DNA was made by inserting Sau3A-cleaved fragments into the BamHI site of pHC79 (20). *E. coli* DNA was purified from N99 (11), partially digested with Sau3A, sized on a sucrose gradient taking fractions greater than 40 kilobase pairs (kb), and ligated with BamHI-digested pHC79 DNA. The ligated mixture was packaged into λ heads as described (22) and used to infect IBPC5032 selecting for ampicillin-resistant colonies at 30°C. Nine hundred clones were purified on LB plates (13) that contained ampicillin at 100 μg/ml prior to storage in LB medium/7% dimethyl sulfoxide at −70°C. LB medium contains 10 g of Bacto-Tryptone, 5 g of Bacto-Yeast Extract (Difco), and 10 g of NaCl per liter.

Screening of Cosmid Library. Single colonies from the cosmid library were grown in 3 ml of LB medium that contained ampicillin at 500 μg/ml at 30°C until late exponential or early stationary phase. Cells were collected by centrifugation in Eppendorf tubes and frozen at −20°C. Aliquots were removed before harvesting for determination of optical density and cosmid retention. The presence of the cosmid in the cultures at the time of harvest was tested by plating diluted aliquots on LB plates and LB plates that contained ampicillin at 100 μg/ml at 30°C. A plating efficiency of 50% or more on ampicillin was considered sufficient. Segregation of cosmids can be a major problem (20), particularly for this type of library in which ampicillin is the selected antibiotic. The secretion of β-lactamase by the resistant bacteria rapidly destroys the ampicillin present in the growth medium, allowing growth of bacteria no longer retaining the cosmid. The cells were removed from the freezer just before analysis and lysed by the addition of 2× concentrated NaDodSO₄ sample buffer (23), and the mixture was

Abbreviations: IF, initiation factor(s); kDal, kilodalton(s); kb, kilobase pair(s).

Table 1. *E. coli* and bacteriophage λ strains and plasmids used

Strain or plasmid	Relevant genotype	Source or reference
E. coli		
N99	F⁻, *galK2, rpsL,* su°	(11)
IBPC5032	F⁻, *argE3, thi-1, pheS5, pheA97, rpsL*	This work; multistep derivative of AB1360 (8)
CSR603	F⁻, *recA1, uvrA6, phr-1, thr-1, leuB6, proA2, argE3, thi-1, ara-14, lacY1, galK2, xyl-5, mtl-1, rpsL31, tsx-33,* λ⁻, *supE44*	(12)
LE392	F⁻, *rK⁻, mK⁺, supF, supE44, thy, (leu, thi, thr)*(?)	R. Davis
LE329(λNM540)	λNM540 lysogen of LE392	
IBPC5061	F⁻, *purE*(?), *argG, metA, trp, ilv, his, pro, ara, lacY, gal, xyl, mtl, rpoB, rpsL*	TonA⁺ (φ 80ˢ), Mal⁺ (λˢ), and pro⁻ derivative of CSH57 (13) Strᴿ Rifᴿ
KL226	Hfr, *rel-1, tonA22,* T2ᴿ, λ⁻	(14)
KL14	Hfr, *thi-1, rel-1,* λ⁻	(14)
JC15531	F⁻, *nalA, argG6, metB1, his-1, leu, recA, mtl-2, xyl-7, malA1, gal-6, lacY1, rpsL104, tonA2, tsx-1,* λᴿ, λ⁻, *supE44*	Nalᴿ derivative of JC1553 (15)
K95	F⁻, *nusA1, rpsL, galK2*	(16)
K450	F⁻, *nusB5, rpsL, galK2*	(17)
JC357	F⁻, *argG6, metB1, his-1, leu-6, mtl-2, xyl-7, malA1, gal-6, lacY1, tonA2, tsx-1, supE44, rpsL, pnp*: :Tn5, λᴿ, λ⁻, *recA1*	(15)
Bacteriophage λ		
λNM540	*imm*²¹ *ninR*	(18)
λSEW	λgt.λC, *Wam403, Eam1100, Sam100, CI857, nin5*	(19)
λGJ2-28	*infB* recombinant	This work
λ⁺		P. Kourilsky
Plasmids		
pHC79	Amᴿ Tcᴿ	(20)
pACYC184	Cmᴿ Tcᴿ	(21)
p1-81, 1-93	*infB*-expressing clones of cosmid library	This work
p1-64	*infC*-expressing clone of cosmid library	This work
pHA11, pHA5, pHA34	*Hind*III-deleted derivatives of p1-93	This work
pA2-1	*infB*-expressing fragment of p1-81 cloned in pACYC184	This work

heated at 100°C for 3 min. After heating, the mixture was vortexed and a volume equivalent to 0.1 A_{650} unit was applied to a 10–18% gradient NaDodSO₄/polyacrylamide gel (100 × 140 × 1.5 mm). After electrophoresis the gel was blotted onto nitrocellulose paper and treated with antisera to IF1, IF2, and IF3, followed by incubation with ¹²⁵I-labeled *Staphylococcus aureus* protein A, as described (4).

RESULTS

Selection of Cosmids Overproducing IF2. A cosmid library containing random *E. coli* genomic inserts of about 40 kb was constructed by the procedure of Hohn and Collins (20) as described. Of the total 900 clones in the library, about a third were screened in this study. The screening process, which took advantage of an immunoblot procedure already described (4), entailed growing each individual clone, harvesting and lysing the cells, and analyzing the proteins by electrophoresis on NaDodSO₄/polyacrylamide gels. The gels were electrophoretically blotted onto nitrocellulose paper and the paper was incubated with specific IF antibodies, followed by incubation with ¹²⁵I-labeled *S. aureus* protein A.

An example of an autoradiogram is shown in Fig. 1. The ability of the immunoblot procedure to accurately measure levels of factors (4) and the specificity of the factor antibodies (24) allows accurate measurement of changes in factor levels. This was important, because in our screening procedure we were looking for increased levels of IF in those cosmids that expressed factor genes. We assumed that if a factor gene was located on a cosmid and the cosmid copy number was of the order of five, there would be a detectable increase in the factor level above cell background. This has already been shown for IF3 (10). Ratios between IF2 and IF3 were used to measure level changes, instead of the overall concentration of either factor, because we did not know exactly how much protein had been applied to

each gel lane. Random cosmids as controls for the background level of IF2 and IF3 are shown in lanes a, c, and e of Fig. 1. In the lanes of cosmids p1-81 (b) and p1-93 (d), the IF2/IF3 ratio
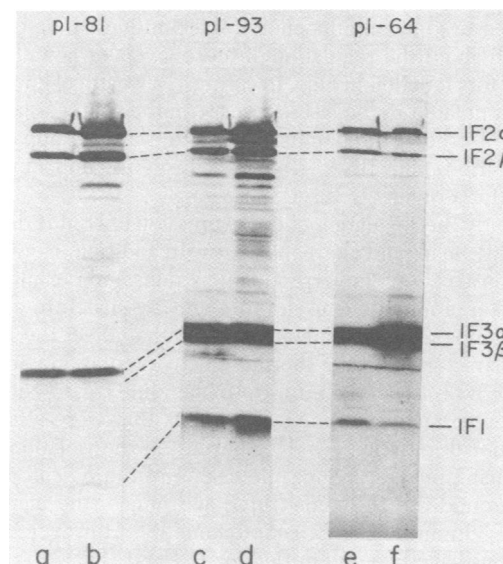

FIG. 1. Immunoblot analysis of cosmid clones. Cell lysates of cosmid-containing bacteria were electrophoresed, blotted onto nitrocellulose paper, and incubated with anti-IF2 and anti-IF3 together (upper two-thirds of paper) or with anti-IF1 alone (the lower third) (4). After subsequent incubation with ¹²⁵I-labeled *S. aureus* protein A, the nitrocellulose papers were dried and autoradiographed for 6 hr. The three cosmids causing overproduction of IF—p1-81, p1-93, and p1-64—are analyzed as indicated in lanes b, d, and f, respectively. Lanes a, c, and e are random adjacent cosmid clones typical of non-overproducers. Locations of IF are shown in the margin.

is greater than that of the control, and in cosmid p1-64 (f), the IF2/IF3 ratio is smaller. This shows an increase of IF2 in p1-81 and p1-93 and an increase of IF3 in p1-64. These were the only cosmids of 330 analyzed to overproduce any of the IF. This is about the number predicted for a single-copy gene on the *E. coli* genome (20). We were unable to identify any IF1 overproducing cosmid strains because of considerable variation from lane to lane in the IF1 levels. We did single out several to be analyzed; however, none synthesized IF1 as analyzed by the "maxicell" system (see below).

Evidence for the Cloning of the Structural Gene for IF2. Overproduction of IF2 in strains carrying p1-81 and p1-93 suggests, but does not prove, that these cosmids carry the *infB* gene. For a more definitive test, we employed the maxicell system of Sancar *et al.* (12), as described in the legend of Fig. 2. This technique allows the specific labeling of plasmid-coded proteins. A fluorogram of a crude cell lysate of maxicells of cosmid p1-93 (Fig. 2, lane 3) shows a number of bands, which is consistent with a cosmid *E. coli* DNA insert of 40 kb. Two minor bands correspond in molecular weight to purified IF2α and IF2β. Analysis of material immunoprecipitated with antiserum specific for IF2 (lane 4) shows only three bands, corresponding to IF2α, IF2β, and an additional protein that is not present in the maxicell extract and presumably is a product of IF2 degradation. Similar results were obtained with maxicells containing p1-81 (results not shown). We therefore conclude that p1-81 and p1-93 encode the gene for IF2.

Restriction Enzyme Analysis of IF2-Overproducing Cosmid. Restriction enzyme digests of cosmids p1-81 and p1-93 were carried out by using enzymes BamHI, EcoRI, and HindIII and allowed the construction of the maps shown in Fig. 3. The proximity of the unique EcoRI and HindIII sites in pHC79 (20) allowed us to position the cosmid vector as shown on the map. The cosmids p1-81 and p1-93 were found to have almost identical restriction maps but they differ at the junctions between *E. coli* and pHC79 DNA. This is strong evidence that the two cosmids are the result of independent *in vitro* ligation events between *E. coli* and pHC79. Thus, the cloned DNA fragment is as it appears on the *E. coli* chromosome and is not the result of *in vitro* ligation between two distant regions. Because the

library was constructed by inserting Sau3A (4-base pair recognition) fragments into the BamHI (6-base pair recognition) site of pHC79, the BamHI site is not necessarily reformed. Of the four junctions between pHC79 and *E. coli* DNA in the two cosmids p1-81 and p1-93, only one is defined by a BamHI site—that to the right of p1-81 as drawn in Fig. 3.

Localization of the IF2 Structural Gene Within the Cosmid. Based on the size of IF2α protein, an estimate of the size of the IF2 structural gene is 3 kb. Because the cosmid *E. coli* DNA insert is 40 kb, it was necessary to reduce the *E. coli* DNA insert to a more stable and manageable size. Inspection of the restriction maps of Fig. 3 showed that HindIII gave the best distribution of sites. As a first attempt to locate the gene for IF2 within the 40-kb insert of *E. coli* DNA, the DNA of cosmid p1-93 was digested with HindIII, religated, and used to transform CSR603. Several hundred ampicillin-resistant transformants were obtained and plasmid DNA was extracted from 20. As expected, all plasmids were found to have the vector-containing 16.4-kb fragment (Fig. 3), alone or in combination with one or more of the other HindIII fragments. A representative selection of the clones was analyzed in maxicells which allowed the assignment of certain protein bands to the different HindIII fragments. In particular, protein bands identified by immune precipitation as IF2α and IF2β are expressed by plasmids containing the 16.4-kb and 5.6-kb fragments of p1-93 (pHA5 and pHA34; Fig. 2, lanes 7–10). The 16.4-kb fragment alone (pHA11; Fig. 2, lanes 5 and 6) does not express IF2. This assigns the IF2 gene to the 5.6-kb HindIII fragment.

The 5.6-kb HindIII fragment of cosmid p1-93 contains about 0.4 kb of cosmid vector pHC79 DNA including the promoter for the gene conferring resistance to tetracycline. This fragment of plasmid DNA is undesirable in subsequent analysis of *infB* gene expression. However, the slightly smaller but corresponding HindIII fragment of p1-81 (5.2 kb) contains a reconstructed BamHI site. The 4.8-kb HindIII/BamHI fragment of p1-81 is thus pure *E. coli* DNA. It was recloned into the vector pACYC184 (21) to give plasmid pA2-1 (Fig. 4). By maxicell analysis and immune precipitation this plasmid was shown to express IF2 (Fig. 2, lanes 11 and 12). This plasmid was used as an enriched source of the 4.8-kb BamHI/HindIII fragment.
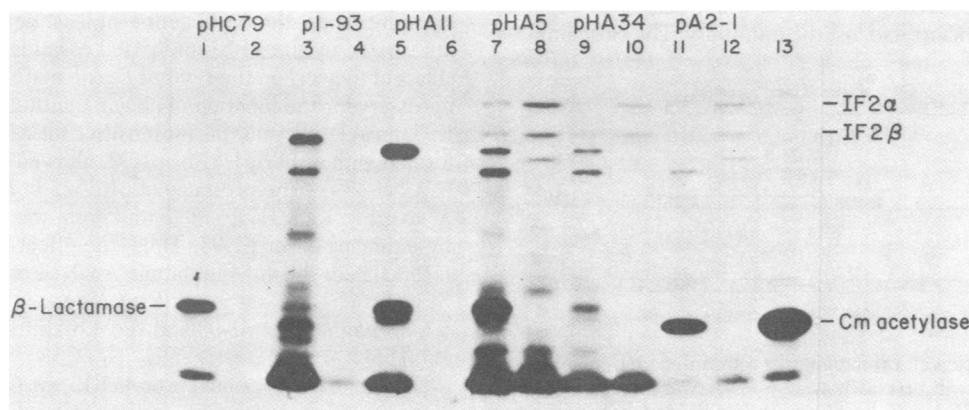

FIG. 2. Immunoprecipitation of labeled maxicell proteins that carry different plasmids. Cultures of CSR603 that carry various plasmids were grown in minimal medium plus Casamino acids containing ampicillin at 200 μg/ml for pHC79, p1-93, pHA11, pHA5, and pHA34 and chloramphenicol at 25 μg/ml for pACYC184 and pA2-1. The cells were irradiated with UV and labeled with [^{35}S]methionine at 10 μCi/ml (1 Ci = 3.7 × 10^{10} becquerels) as described (12). After labeling, the cells were spun down, washed, and then lysed by boiling 3 min in NaDodSO$_4$ sample buffer (23). Immunoprecipitation was carried out as described previously (25) with bovine serum albumin at 0.5 mg/ml as carrier protein in a total volume of 400 μl. Five microliters of anti-IF2 immune sera and 20 mg of protein A-Sepharose (Pharmacia) were used per sample. After elution from the protein A-Sepharose, the supernatants containing antigen–antibody complex were analyzed on a 2-mm thick 10–18% gradient NaDodSO$_4$/polyacrylamide gel. After electrophoresis the proteins were fixed with 12% trichloroacetic acid and then treated for fluorography (26). The gel was exposed to the film for 21 days. Lanes 1, 3, 5, 7, 9, 11, and 13 are total maxicell-labeled extracts (9,000–18,000 cpm per lane). Lanes 2, 4, 6, 8, 10, and 12 are immune precipitations from 10× the material shown in the corresponding total extract lane. Lanes 1 and 2, pHC79; lanes 3 and 4, p1-93; lanes 5 and 6, pHA11; lanes 7 and 8, pHA5; lanes 9 and 10, pHA34; lanes 11 and 12, pA2-1; lane 13, pACYC184. The positions of IF2α and IF2β, the vector plasmid-encoded β-lactamase, and chloramphenicol (Cm) acetylase are also indicated.
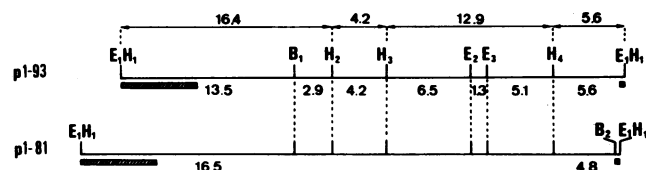
FIG. 3. Restriction endonuclease maps of p1-93 and p1-81. Restriction endonuclease sites are designated as: *Eco*RI, E; *Hin*dIII, H; and *Bam*HI, B. The unique *Eco*RI and *Hin*dIII sites of pHC79, separated by 29 base pairs, are shown as E1H1 and allowed the positioning of the vector DNA (hatched areas) on the maps. The numbers are the distances (kb) between sites. Common sites within both cosmids are indicated by vertical dotted lines. The four *Hin*dIII fragments of p1-93 are indicated.

Construction of a λ Transducing Phage Carrying *infB*.

The method adopted to map *infB* was to clone a fragment of cosmid DNA containing the gene into an integration-deficient λ phage. Forming lysogens by homology introduces a scoreable phenotype (the λ immunity) at the site of lysogenization. The 4.8-kb *Hin*dIII/*Bam*HI fragment of pA2-1 was inserted between the left arm of λNM540 (18) terminating in a *Hin*dIII site and the right arm of λSEW (19) terminating in a *Bam*HI site, as described in Fig. 4. The resulting recombinant phages with the CI857 and S100 characteristics of λSEW were screened for ability to express IF2 during lytic development. Phages expressing IF2 (e.g., λGJ2-28) could be easily identified by a variation of the original immunoblotting technique, as described in Fig. 5. Analysis of the DNA of λGJ2-28 confirmed the presence of the 4.8-kb *Hin*dIII/*Bam*HI fragment.

Mapping of *infB* on the *E. coli* Chromosome.

Because the λGJ2-28 phage is deleted for the region *att*, the only method this phage has for forming a lysogen is by *recA*-dependent recombination. The multiply marked strain IBPC5061 [a derivative of CSH57 (13)] was lysogenized with λGJ2-28. Lysogens were obtained at a frequency of about 10^{-4}.

The initial mapping was done by Hfr × F⁻ crosses by using KL226 and KL14 (14) as donors and a λGJ2-28 lysogen as receptor. With KL226 (which injects its DNA clockwise starting at about 12 min) as donor, 92% (88/96) of the Arg⁺ recombinants had lost the λ immunity. With KL14 (which injects its DNA anti-clockwise starting at about 69 min), 100% (288/288) of the Arg⁺ recombinants had lost the immunity. The close link-
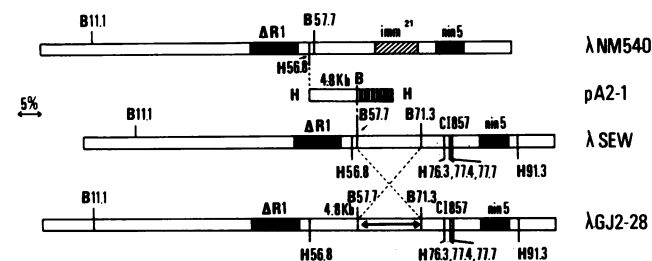

FIG. 4. Construction of λ recombinants containing *infB*. λNM540 DNA was digested with *Hin*dIII, λSEW DNA was digested with *Bam*HI, and pA2-1 DNA was digested with *Hin*dIII and *Bam*HI. The ligated mixture, containing 2 μg of each λ DNA, was used to transfect LE392(λNM540). The λNM540 lysogenic strain was used to eliminate recovery of imm²¹ phages. A total of 30 plaques was obtained, purified, and screened for CI857 and S7 characteristics. Phages synthesizing IF2 under lytic conditions were detected by immunoblotting (see Fig. 5). DNA, extracted from potentially good recombinants, was analyzed by restriction enzyme digestions. Restriction sites are indicated as: *Bam*HI, B, and *Hin*dIII, H. The number after each site is its position expressed as percent of the total length of λ DNA (taken from ref. 27). ΔRI is an *in vitro*-constructed deletion between *Eco*RI sites at 44.5% and 54.3%. ■, Deleted DNA; ▨, imm²¹ substitution; ■, pACYC184 DNA. The *Bam*HI fragment between 57.7% and 71.3% is inverted in λGJ2-28.
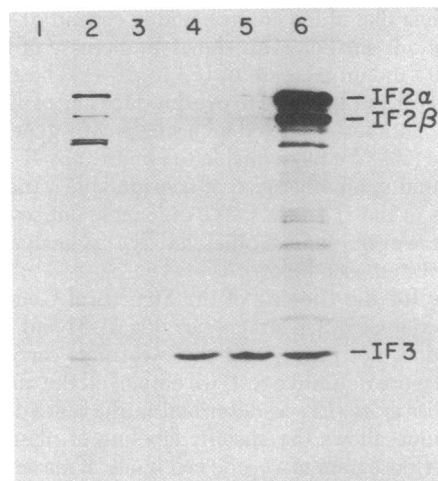

FIG. 5. Immunoblotting screening of recombinant phages. Small aliquots of recombinant and parental phages were placed on a lawn of LE392 on an RMM plate [R plate (11) containing 10 mM MgSO₄ and 0.2% maltose (8); (R medium contains 10 g of Bacto-Tryptone, 2 g of Bacto-Yeast Extract, 8 g of NaCl per liter.)] and incubated at 37°C. After 12 hr a small amount of material (about 1 mm²) from the regions of lysis or the lawn of bacteria was mixed with NaDodSO₄ sample buffer, heated for 3 min at 100°C, and analyzed by gel electrophoresis and immunoblotting, as in Fig. 1, but using anti-IF2 and anti-IF3 only. Positions of IF2 and IF3 on the autoradiogram are marked. Lane 1, random recombinant phage; lane 2, λGJ2-28; lane 3, λSEW; lane 4, λNM540; lane 5, LE392 lawn; lane 6, IBPC5032 p1-81 culture.

age to *argG* was confirmed by P1 transduction. P1vir, grown on bacteria wild type for the *argG* region, was used to transduce IBPC5061 and IBPC5061 (λGJ2-28) to ArgG⁺. With IBPC5061 the frequency of ArgG⁺ transductants was around 10^{-6}, whereas with IBPC5061 (λGJ2-28) it was only 10^{-7}, consistent with a region of nonhomology (the λ DNA) in the *argG* region. Of the IBPC5061 (λGJ2-28)/ArgG⁺ transductants, 86% (116/135) had lost the immunity, again confirming the close linkage to *argG*. These results indicate that *infB* maps at 68 min on the *E. coli* map (28).

The cosmids p1-81 and p1-93 do not express the *argG* gene because they do not convert an *argG* strain, JC15531, to prototrophy. Thus, the *argG* gene—at least not in its entirety—is not carried by the p1-81 and p1-93 cosmids. However, placed adjacent to *argG* on the revised *E. coli* map (28) is *nusA*, a gene necessary for the function of phage λ antitermination factor N. K95 (*nusA1*), a strain thermosensitive for λ⁺ growth (16) transformed with either p1-81 or p1-93, allowed the normal growth of λ⁺ at 42°C. The presence of pBR322 in K95 did not permit λ⁺ to grow. K450 (17), a *nusB* mutation, was not complemented by either p1-81 or p1-93. Thus, the effect of p1-81 and p1-93 is specific for the *nusA* mutation. Analysis of subclones from p1-81 and p1-93 showed that the *nusA* gene is carried on both the 5.6-kb fragment of p1-93 and the 4.8-kb fragment of p1-81, as is *infB*.

The gene for ribosomal protein S15, *rpsO*, is placed adjacent to *nusA* anticlockwise from both *argG* and *nusA* on the *E. coli* map (27). Two-dimensional gel analysis of ribosomal proteins (29) extracted from labeled maxicells of p1-81 showed a protein running in the position of S15. The next gene, going anticlockwise, is *pnp*, the structural gene for the large subunit of polynucleotide phosphorylase (15). JC357, a strain carrying a transposon, Tn5, in the *pnp* gene (15), was transformed with the cosmid p1-93 and the *Hin*dIII-deleted plasmids derived from it. Polynucleotide phosphorylase activity [measured by the UDP–P_i exchange assay (30)] was expressed by p1-93. Analysis of the *Hin*dIII-deleted plasmids derived from p1-93 showed that the 12.9-kb *Hin*dIII fragment carried the *pnp* gene.

DISCUSSION

The cloning of the gene for IF2 was facilitated by the use of a new method (immunoblotting) to screen a cosmid library of the *E. coli* genome. We assumed that an increased copy number of the *infB* gene would result in the overproduction of IF2. The immunoblotting technique was sensitive enough to detect increased levels of IF2 and IF3 and allowed us to screen about 300 clones from the random cosmid library over several weeks. Because we were able to find clones that overproduce IF2 and IF3, this technique should be applicable to the isolation of a wide variety of structural genes for which there are no mutations and to which antisera are available. Successful application of the technique also requires that the protein be overproduced and not extensively degraded and that higher protein levels not be deleterious to cell growth. The failure to detect IF1 production may not reflect a significant limitation because in this case the anti-IF1 titers were very low, causing variable blotting results, and the gel system was chosen to optimize for detection of IF2, not IF1 which migrated very near the dye front.

IF2 has been purified as two forms, IF2α [115 kilodaltons (kDal)] and IF2β (90 kDal), both of which are found in crude cell lysates (24). We find that both forms are synthesized from the 4.8-kb fragment. However, this fragment is not large enough to code for two proteins of this size and therefore the two forms must be derived from the same gene. Whether the smaller form of IF2 is the result of *in vivo* degradation or due to differential transcription or translation of the gene has yet to be determined.

In addition, another protein of about 70 kDal is labeled in maxicells carrying the cloned 4.8-kb fragment (Fig. 2, lane 11). The gene for this protein must be very close to *infB*, as 4.8 kb can barely code for two proteins of this size. The complementation data would strongly suggest that this protein is the *nusA* gene product of 69 kDal (31).

Genetically, *infB* is very closely linked to *argG* (at 68 min on the *E. coli* map). The absence of *argG* from the cosmids p1-81 and p1-93 and the presence of *infB* at one end of the cosmids 40 kb in length would suggest that the *argG* gene is located to the right of the maps drawn in Fig. 3. The genes *nusA*, *rpsO*, and *pnp* are carried by the cosmids. As *infB* and *nusA* are expressed from the same 4.8-kb fragment and *pnp* from the adjacent 12.9-kb *Hind*III fragment, this strongly suggests that *infB* is located somewhere between *argG* and *pnp*, immediately adjacent to *nusA*. Comparison with the standard *E. coli* map (28) would imply that the order is *pnp*, *rpsO*, (*nusA*, *infB*), *argG*, in which *nusA* and *infB* have not been orientated.

The fact that *infB* has been located at 68 min means that the IF2 gene is well separated from the IF3 gene (*infC*) at 38 min. Moreover, *infB* is in a region of the genome devoid of other identified translational components except for ribosomal protein S15 (*rpsO*). The *infA* gene for IF1 also appears to map elsewhere, because it is absent from the plasmids carrying *infB* and *infC*. The three IF are maintained in cells at approximately equimolar levels (4, 5), but the mechanism of the coordinate expression of the three unlinked genes remains unexplained. The fact that IF2 is overproduced in the cosmid-bearing strains shows that, like IF3 (10), its cellular level is influenced by gene dosage and implies that both IF2 and IF3 may not be under autogenous control as found for some ribosomal proteins.

The other genes located near *infB* cover a variety of cellular functions. The *nusA* gene perhaps deserves special mention. A pleiotropic protein, it seems to be involved primarily with transcription termination (32). Its extremely close position with respect to *infB* might signal some common regulation of their expression and recalls the situation of the cotranscription of ribosomal proteins with RNA polymerase subunits and translational elongation factors.

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