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Translation Initiation Factor IF2 of the Myxobacterium *Stigmatella aurantiaca*: Presence of a Single Species with an Unusual N-Terminal Sequence

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The structural gene for translation initiation factor IF2 (*infB*) was isolated from the myxobacterium *Stigmatella aurantiaca* on a 5.18-kb *Bam*HI genomic restriction fragment. The *infB* gene (ca. 3.16 kb) encodes a 1,054-residue polypeptide with extensive homology within its G domain and C terminus with the equivalent regions of IF2s from *Escherichia coli*, *Bacillus subtilis*, *Bacillus stearothermophilus*, and *Streptococcus faecium*. The N-terminal region does not display any significant homology to other known proteins. The *S. aurantiaca infB* gene encodes a single protein which cross-reacted with antiserum to *E. coli* IF2 and was able to complement an *E. coli infB* mutant. The *S. aurantiaca* IF2 is distinguished from all other IF2s by a sequence of 160 residues near the N terminus that has an unusual composition, made up essentially of alanine, proline, valine, and glutamic acid. Within this sequence, the pattern PXXXAP is repeated nine times. Complete deletion of this sequence did not affect the factor's function in initiation of translation and even increased its capacity to complement the *E. coli infB* mutant.

Myxobacteria are gram-negative, rod-shaped soil bacteria which, in response to starvation, undergo a developmental cycle leading to multicellular structures termed fruiting bodies. These specialized structures enclose dormant cells, the myxospores (41). Multicellular development of myxobacteria is regulated by intercellular signals, with some of them presumably transduced by GTP-binding proteins as in eukaryotes (8, 25), and by cell-cell interactions (10). The expression of specific sets of genes is coordinated via various signalling compounds, thus triggering the different stages of multicellular development (24). For another sporulating bacterium, *Bacillus subtilis*, Tipper et al. (45) suggested that some components of the translational machinery, namely ribosomal proteins and elongation factor G, could play a specific role during the spore formation process. In agreement with this idea, Cheng et al. (6) identified the lesions in a *Myxococcus xanthus* development-deficient strain displaying a mutation in *dsg*, encoding a protein similar to translation initiation factor IF3. Compared to IF3s from other species, the *Myxococcus* IF3 is much larger and bears a C-terminal extension which was shown to be necessary for developmental functions but not for viability, even though during vegetative growth, colonies were shown to be more compact and to be a darker tan color (19).

In addition to IF3, at least two other factors, IF1 and IF2, are required for the initiation of mRNA translation in prokaryotes. The genes for all three factors, namely, *infA* (IF1), *infB* (IF2), and *infC* (IF3), were first cloned in *Escherichia coli* (30, 36, 43). These three proteins play essential roles in several steps of translation initiation, from the selection of the initiator tRNA (15) to the control of the entry into the elongation cycle (26).

The *infB* gene, encoding IF2, is located on the *E. coli* chromosome at 69 min and is part of a complex polycistronic operon, the *nusA-infB* operon (22, 30, 31, 37). IF2 is an essential protein in *E. coli* (23) and is present in the cell in two major cellular forms, α (97.3 kDa) and β (79.7 kDa) (27, 28, 33). It was also shown that IF2β comprises two barely distinguishable forms (β1 and β2) arising from two internal in-frame initiation codons, GUG and AUG, respectively, separated by only 18 nucleotides (34). So far, the occurrence of two IF2 forms has also been established for *B. subtilis* (39), but this has not been investigated with other bacteria, such as *Bacillus stearothermophilus*, *Streptococcus faecium*, and *Streptomyces aureofaciens*, for which this factor has also been studied (4, 12, 18). IF2, the largest initiation factor, belongs to the class of GTP-binding proteins (9). The homology among known IF2 factors is very extensive over the C-terminal three-quarters of the protein and is greatest near the central region which binds GTP, the G domain.

In this work, we report the molecular cloning and the in vivo characterization of the *infB* gene of the myxobacterium *Stigmatella aurantiaca*. Taking advantage of the strong homologies present in the central part of the protein, the *S. aurantiaca infB* G domain was amplified by PCR, providing a DNA fragment that was used as a homologous probe to clone the entire *infB* gene and the flanking regions from the *S. aurantiaca* genome (2). Compared to other known IF2s, the IF2 from *S. aurantiaca* has a much longer N-terminal region. The expression of this newly described *infB* gene, the second reported for gram-negative bacteria, was analyzed, and the occurrence of several forms of the factor was investigated.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Tables 1 and 2. *S. aurantiaca* DW4 (ATCC 33878) was grown to late exponential phase in 1% Bacto Casitone (Difco) with 8 mM $MgSO_4$ at 30°C and harvested at ~4×10^8 cells/ml. *E. coli* strains were propagated at 30, 37, 40, or 42°C in Luria-Bertani (LB) broth or on LB agar plates (1.5%, wt/vol)

TABLE 1. *E. coli* strains

Strain	Genotype	Source or reference
DH5α	*supE44* Δ*lacU169* (ϕ80*lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Bethesda Research Laboratories
IBPC5321	F$^-$ *thi-1 argG6 argE3 his-4 xyl-5 tsx-29 rpsL* Δ*lacX74*	29
Y1090	*supF hsdR araD139* Δ*lon* Δ*lacU169 rpsL proA*$^+$ *strA trpC*22::Tn*10*(pMC9)	35
SL598R	F$^-$ *thi-1 argG6 argE3 his-4 xyl-5 tsx-29 rpsL* Δ*lacX74* Δ*infB*::*cat-2 recA1* (λGJ9-2)	23
LBYC5	DH5α(pLBYC5)	This study
LBYC5-1	DH5α(pLBYC5-1)	This study
LBYC5-2	DH5α(pLBYC5-2)	This study
SLB18-1	SL598R(pB18-1)	This study
SLB7	SL598R(pLBYC7)	This study
SLB7Δ690	SL598R(pLBYC7Δ690)	This study
SLB8	SL598R(pLBYC8)	This study

(35). If required, ampicillin (100 μg ml^{-1}), chloramphenicol (10 μg ml^{-1}), IPTG (isopropyl-β-D-thiogalactopyranoside) (50 μg ml^{-1}), and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (50 μg ml^{-1}) were added. Strain Y1090 was grown in the presence of 0.4% maltose. Growth of liquid cultures was monitored by measuring the optical density at 650 nm (OD_{650}).

DNA manipulations, cloning, and transformation. Total genomic DNA was isolated from *S. aurantiaca* DW4 by the method of Starich and Zissler (44). Plasmids from *E. coli* were extracted and purified as described by Sambrook et al. (35). Plasmid and genomic DNAs were digested with restriction enzymes (Gibco-BRL) according to the supplier's recommendations. Analytical and preparative agarose gel electrophoreses in Tris-borate–EDTA (pH 8.3) were performed as described by Sambrook et al. (35). DNA restriction fragments were purified from agarose gels by using the Prep-a-Gene kit (Bio-Rad Laboratories, Richmond, Calif.). Ligation was obtained by using T4 DNA ligase (Gibco-BRL) according to the manufacturer's recommendations. Competent *E. coli* cells were prepared and transformed as described by Hanahan (14).

Screening of an *S. aurantiaca* λgt11 library. The λgt11 library was kindly provided by H. U. Schairer (Heidelberg, Germany). This library was constructed by partially digesting *S. aurantiaca* genomic DNA with *Hpa*II and selecting fragments ranging from 2 to 6 kb. These fragments were appended with *Hpa*II-*Eco*RI adapters and cloned into the *Eco*RI site of λgt11. DNA products amplified by PCR with oligonucleotides specific to the G domain (2) were labeled with [α-^{32}P]dCTP (ICN Biomedicals) by random priming (11) and used to screen 15,000 previously plated clones (35).

Construction of a restricted genomic library. Five micrograms of *S. aurantiaca* genomic DNA was digested with *Bam*HI. The digest was run on a 0.8% agarose gel, transferred to a nitrocellulose membrane, and hybridized with a radiolabeled probe. Fragments ranging from 4.5 to 5.5 kb were excised and purified with the Prep-a-Gene kit. The DNA was recovered by ethanol precipitation and used directly in ligation reactions with pBluescript II SK+ treated with *Bam*HI and bacterial alkaline phosphatase. Transformant colonies were screened by colony lift for the presence of plasmids bearing the genomic fragment hybridizing to the PCR-synthesized probe.

Hybridization. Plasmid and chromosomal DNA fragments, digested with the appropriate restriction endonucleases and analyzed by agarose gel electrophoresis, were transferred onto nylon membranes (Hybond-N$^+$; Amersham) as described by Southern (42) and treated according to the supplier's instructions. The membranes were prehybridized for at least 1 h at 65°C and then hybridized overnight with the labeled probe at 65°C. After being washed, the membranes were autoradiographed with X-ray films.

DNA sequencing and computer analysis. The 5.18-kb *Bam*HI insert from pLBYC5 was digested with *Pst*I, giving two fragments of 4.1 and 1.1 kb which were subcloned in pBluescript II SK+ (see below and Fig. 1) (pLBYC5-1 and pLBYC5-2). Random nested deletions were then created by using the Erase-a-Base system (Promega Corporation) to generate plasmids for sequencing. DNA sequencing was performed by the dideoxy chain termination method (38) with an automated sequencer (A.L.F.; Pharmacia). Sequencing was carried out with fluorescent universal and reverse primers and T7 DNA polymerase (Autoread sequencing kit; Pharmacia). Sequence analysis was performed with the program of the Genetics Computer Group (Madison, Wis.) sequence analysis software package.

Maxicell analysis. Maxicell analysis of plasmid-encoded proteins was performed as described previously (35a).

Curing of λ *infB* transducing phage from strain SL598R. *E. coli* SL598R (23) was transformed with plasmids carrying the *infB* gene of *S. aurantiaca* or *E. coli* (as a control). Cells were grown overnight in LB broth supplemented with ampicillin (100 μg ml^{-1}) and chloramphenicol (10 μg ml^{-1}), diluted into fresh medium, and grown at 30°C. When the *infB* expression was dependent on the Trc promoter, induction was performed at an A_{650} of 1 by using 0.5 mM IPTG for 1 h (pLBYC8 carrying the *E. coli* gene) or 1 mM IPTG for 2 h (pLBYC7 and pLBYC7Δ690 carrying *S. aurantiaca* genes). After dilution of the cell suspension to an A_{650} of 0.3, aliquots (500 μl) were transferred to 5 ml of prewarmed LB broth at 42°C, shaken vigorously for 5 min, and then immediately chilled on ice. Aliquots (1.5 ml) were concentrated by centrifugation at 5,000 × *g* and 4°C, plated on LB plates containing ampicillin and chloramphenicol, and incubated overnight at 42°C. Survivors were purified several times on the same plates at 42°C, and the loss of λ was verified by their sensitivity to λ*cI*$^-$ phage. Southern blotting of restricted DNA of the strains surviving at 42°C was performed to verify that no rearrangements of the relevant parts of the plasmid clones had been selected during the 42°C treatment and subsequent purifications (data not shown), as already demonstrated by Laalami et al. (23).

Blotting and immunodetection of the IF2 protein. The presence of IF2 from *E. coli* and *S. aurantiaca* and also of the *S. aurantiaca* IF2 truncated derivative was examined in cell extracts from *S. aurantiaca* and *E. coli* strains by the quantitative immunoblotting procedure (^{125}I-labeled protein A reacting with an *E. coli* anti-IF2 antibody) described by Howe and Hershey (17). The relative amounts of anti-IF2-reacting proteins in the different cell extracts were evaluated by excising the corresponding band from the blot, measuring the radioactivity present in it, and comparing the amount to that for IF2α and IF2β in a similar amount of the wild-type *E. coli* strain IBPC5321.

TABLE 2. Plasmids

Plasmid	Vector	Relevant genotype	Source or reference
pBluescript II SK+		*bla 'lacZ*	Stratagene
pUC18		*bla 'lacZ*	Pharmacia
pTrc99A		*bla 'lacZ lacI*^{q}	Pharmacia
pBR322		*bla tet*	1
pB18-1	pBR322	*bla*, *E. coli nusA*, *E. coli infB*	31
pLBYC5	pBluescript II SK+	*bla*, *S. aurantiaca infB*, *S. aurantiaca* ORF3, *S. aurantiaca rbfA*	This study
pLBYC5-1	pBluescript II SK+	*bla*, *S. aurantiaca infB*	This study
pLBYC5-2	pBluescript II SK+	*bla*, *S. aurantiaca* ORF3, *S. aurantiaca rbfA*	This study
pLBYC6	pUC18	*bla*, *S. aurantiaca infB*	This study
pLBYC7	pTrc99A	*bla*, *S. aurantiaca infB*	This study
pLBYC7Δ690	pTrc99A	*bla*, *S. aurantiaca infB*Δ*690*	This study
pLBYC8	pTrc99A	*E. coli infB*	This study

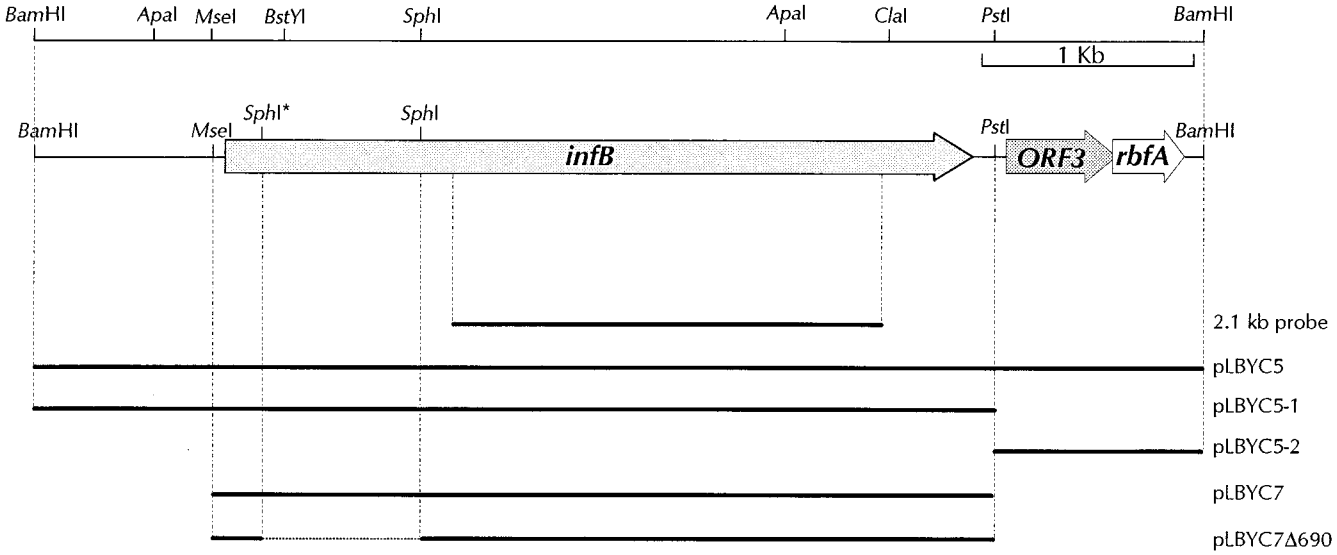

FIG. 1. Schematic overview of the genetic organization around the *S. aurantiaca infB* gene. The diagram gives the locations and orientations of the *infB* gene and two putative ORFs (ORF3 and *rbfA*). The upper part of the diagram displays a partial restriction map of the 5.18-kb *Bam*HI fragment from pLBYC5. The restriction fragments cloned within the pBluescript II SK+ plasmid (for pLBYC5, pLBYC5-1, and pLBYC5-2) and plasmid pTrc99A (for pLBYC7Δ690 and pLBYC7) and the 2.1-kb *infB* probe are indicated by thick horizontal bars. The 2.1-kb probe was originally isolated by *Eco*RI restriction from λLBYC2. *Sph*I* denotes the restriction site created by site-directed mutagenesis (see Materials and Methods for details).

Construction of a shortened form of IF2. The 1.6-kb *Bam*HI-*Sph*I fragment of pLBYC5-1, carrying the 5′ end of the *infB* gene of *S. aurantiaca*, was cloned into M13mp18. Oligonucleotide mutagenesis (21) was used to replace a T by an A (boldface) in the sequence 5′GTCACCGAGC**A**TGCGGGGCTG3′, thus creating an *Sph*I site at the 29th codon of IF2. The mutation was confirmed by DNA sequencing. The 0.94-kb *Bam*HI-*Sph*I DNA fragment created by mutation was excised from the M13 replicative form and cloned into pLBYC7 (see Fig. 1) to replace the wild-type 1.63-kb *Bam*HI-*Sph*I DNA fragment which had been previously excised. This allowed a 690-bp *Sph*I deletion (corresponding to 230 amino acids in the N region of the protein), to yield the plasmid pLBYC7Δ690.

Nucleotide sequence accession number. The overall nucleotide sequence for the 5.18-kb *Bam*HI fragment from pLBYC5 appears in the EMBL data bank under accession number X87940.

RESULTS

Cloning and identification of the *infB* locus. A PCR fragment comprising part of the G domain of the *S. aurantiaca infB* gene (2) was used as a probe to screen a λgt11 library of *S. aurantiaca* DW4. Only one clone (λLBYC2) was detected; it carried a 2.1-kb *Eco*RI insert which was isolated and sequenced. It was found to carry only part of the putative *infB* gene of *S. aurantiaca* (Fig. 1).

Therefore, the complete *infB* gene was cloned by construction of a limited *Bam*HI genomic library in the pBluescript II SK+ vector, as described in Materials and Methods. Approximately 3,000 transformant colonies were screened, by colony lift, against the 2.1-kb fragment. A single colony was found, which harbored plasmid pLBYC5 (Table 2), consisting of pBluescript II SK+ bearing a 5.18-kb *Bam*HI insert complementary to the 2.1-kb probe (Fig. 1). The 5.18-kb *Bam*HI fragment isolated from pLBYC5 was subcloned in the pBluescript II SK+ vector (pLBYC5-1 and pLBYC5-2), and both strands were sequenced. This sequence (average G+C content of close to 69%) showed the presence of three open reading frames (ORFs), including the *infB* gene. The first ORF is preceded by 847 bp showing no significant homology to any known sequence. The first ORF, corresponding to the *infB* gene, is composed of 3,162 bp and codes for a protein of 1,054 amino acids with extensive homologies to other IF2 proteins (see below). The putative AUG initiation codon (positions 848 to 850) is preceded by an A-A-G-C-A Shine-Dalgarno sequence (positions 838 to 842), and the ORF terminates with a UAA stop codon (positions 4010 to 4012). The probability of the third base of a codon being G or C is 90.5% over the entire gene, which is an essential characteristic of myxobacterial genes and confirms the myxobacterial origin of this gene. Downstream from the UAA stop codon we found a sequence (positions 4048 to 4077) that can form a stable hairpin structure ($\Delta G = -29.2$ kcal). Moreover, this hairpin structure is followed by a U-rich region (U-U-U-U-U-U-C-A-U-G-U-U, positions 4085 to 4096) and thus resembles documented *rho*-independent transcription terminators in *E. coli* and other gram-negative bacteria (32), although the stretch of Us is separated from the bottom of the stem by seven nucleotides. The efficiency of this transcriptional termination or pausing site remains to be investigated.

The structural *infB* gene is followed by an ORF (585 bp) potentially encoding a 21,450-M_r polypeptide, starting with an AUG codon (positions 4092 to 4094) and ending at a UGA codon (positions 4677 to 4679). The N-terminal portion of this putative protein shows 71% similarity with the product of ORF3, which is located immediately downstream of *infB* in the *nusA-infB* operon of *B. subtilis* (40) but which is absent from this operon in *E. coli*. Considering its location and the homology with *B. subtilis* ORF3, we have named it ORF3 in *S. aurantiaca* even though it is twice as large. Compared to the that of *B. subtilis* ORF3, the *S. aurantiaca* ORF3 product bears a C-terminal extension rich in alanine, proline, and glutamic acid (Table 3), reminiscent of the C-terminal extension found in *M. xanthus* IF3 (6) and the N-terminal segment of *S. aurantiaca* IF2 (see below and Discussion). In this region of the ORF3 product, glycine, hydrophobic residues like leucine, and basic amino acids like arginine are abundant. ORF3 is followed by a 119-codon ORF potentially encoding a 13,090-M_r polypeptide displaying 66 and 75% similarity with the *rbfA* genes of *E. coli* and *B. subtilis*, respectively. The *rbfA* gene (7), previously called *p15B*, is located downstream of *infB* in both *E. coli* and *B. subtilis*, but in the latter organism it is separated

TABLE 3. Compositions of the APE sequences[a]

Protein	Position of APE region	Length of APE region (amino acids)	%				
			A	P	E	V	A + P + E
IF2	61–221	160	27	19	11	11	57
ORF3 product	76–195	119	12	10	18	2.5	40
IF3	181–247	66	34	11	14	1.5	59

[a] The *M. xanthus infC* EMBL accession number is UO4438.

from *infB* by ORF3. The initiation codon of this putative *rbfA* gene (AUG, positions 4676 to 4678) overlaps the UAG termination codon of ORF3 (positions 5033 to 5035). The initiation codons of these two putative genes are not preceded by any obvious ribosome-binding site, thus raising the question of whether they are translated efficiently.

Protein sequence of IF2. As deduced from the DNA sequence data, the *S. aurantiaca infB* gene encodes a protein of 1,054 amino acids with an M_r of 116,000. The size of the gene product was verified by expression of the *S. aurantiaca infB* gene in *E. coli* maxicells (data not shown). A protein of the expected size, putatively IF2, was produced only by the clone containing pLBYC6 (data not shown). Thus, the myxobacterial IF2 is longer than its *E. coli*, *B. subtilis*, *B. stearothermophilus*, and *S. faecium* homologs by 164, 338, 312, and 269 additional amino acids, respectively. The *S. aurantiaca* IF2 displays extensive homology with the C-terminal two-thirds of other IF2s, and there is a striking primary-sequence homology (70% identical residues) between their central regions, corresponding to the GTP-binding domains (residues 554 to 703 of *S. aurantiaca* IF2) (Fig. 2). Relatively little homology between the N-terminal sequences is observed. Moreover, the IF2 protein of *S. aurantiaca* has an N-terminal region which is 164 amino acids longer than that in *E. coli* and even longer than those in the gram-positive bacteria. However, by using a hydrophobic-cluster algorithm (13) designed to reveal common conformational homologies among distantly related proteins, the first 50-residue region of *S. aurantiaca* IF2 reveals a structural feature typical of a highly hydrophobic N terminus (data not shown), as previously described for other IF2s (39). This observation correlates with a limited amount of amino acid similarity in this region of IF2s from *S. aurantiaca* and the gram-positive bacteria. The adjacent region (residues 61 to 221) has an abnormal amino acid composition, made up primarily (66%) of four different amino acids, i.e., 43 alanine, 29 proline, 17 valine, and 17 glutamic acid residues (Table 3). The presence of regions rich in alanine, proline, and glutamic acid in the C-terminal portions of two other myxobacterial proteins, i.e., *M. xanthus* IF3 and the *S. aurantiaca* ORF3 protein (see above), leads us to propose the name APE sequences for these regions. Remarkably, within the APE sequence of *S. aurantiaca*, valine is also overrepresented, and the pattern PXXXAP is repeated nine times, of which the majority is PXXEAP, which is repeated six times (Fig. 2). The high level of proline (Table 3) suggests that this repeated domain is nonstructured. The remainder of the N-terminal part of *S. aurantiaca* IF2 (residues 222 to 553) does not display any particular structural features.

Cross-reaction of *S. aurantiaca* protein with an antibody raised against *E. coli* IF2. Phylogenetically, *S. aurantiaca* belongs to the δ subgroup of proteobacteria, while *E. coli* belongs to the γ subgroup. Nevertheless, ribosomes and their associated proteins are largely conserved among eubacterial species. *S. aurantiaca* DW4 cellular extracts were found to contain only one protein which cross-reacted with *E. coli* anti-IF2 antibodies when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (Fig. 3A, lane 2); a control experiment using a crude protein extract from *E. coli* was also performed (Fig. 3A, lane 1). The anti-IF2 serum recognized two bands in the *E. coli* extracts, corresponding to IF2α and IF2β, respectively.

In vivo complementation of an *E. coli infB* null mutation. The 3.3-kb *Mse*I/*Pst*I fragment of pLBYC5-1 was subcloned in pTrc99A downstream of the IPTG-inducible Trc promoter. The subcloning removed the 5′ untranslated region of the *infB* gene. To demonstrate the expression of the *S. aurantiaca infB* gene in *E. coli*, we tested our construction pLBYC7 (Fig. 1), which synthesizes only *S. aurantiaca* IF2, for its ability to replace the *E. coli* chromosomal *infB* gene and to satisfy the cellular requirement for IF2. We used *E. coli* SL598R, which was constructed to test the viability of any *infB* allele (23). In this strain, the resident chromosomal copy of *infB* is deleted and replaced by a chloramphenicol resistance cassette. A functional wild-type copy of *infB* is supplied in *trans* by a thermosensitive lysogenic λ phage integrated at *att*λ. After heat induction at 42°C, it is possible to cure this λ *infB* phage without killing the bacteria, but only provided that a viable *infB* allele is supplied in *trans.*

Plasmid pLBYC7, as well as the controls pLBYC8 (carrying the *E. coli* IF2 gene cloned in pTrc99A), pB18-1, and pTrc99A, was used to transform SL598R. Plasmid pB18-1 is a pBR322 derivative multicopy plasmid (Table 2) that carries an *E. coli nusA-infB* insert lacking the main promoters of the *nusA-infB* operon. The amount of IF2 produced by this multicopy plasmid is quite similar to that produced by the chromosomal copy of the *nusA-infB* operon (28). The transformants were selected on LB-chloramphenicol-ampicillin plates at 30°C. SL598R transformed with each one of our plasmids was subjected to heat curing of λ *infB* (see Materials and Methods for details). Survivors at 42°C were detected at similar frequencies with pB18-1, pLBYC7, and pLBYC8. No colonies were obtained with the negative control (pTrc99a) after thermal induction of the phage. The cured strains were shown to be stable through several purifications at 42 and 30°C.

Surviving strains were named according to the plasmid they carry, by replacing the designation pLBYC or pB by SLB (e.g., SLB7 is the cured strain derived from pLBYC7-transformed SL598R). Western blots of crude extracts from several independent complemented clones demonstrated that only the *S. aurantiaca* IF2 protein was expressed in SLB7 (Fig. 3B, lane 5). This result suggests that the *S. aurantiaca infB* gene is transcribed and translated in *E. coli* and that it is expressed in only one large form, in agreement with the observation that anti-IF2 antibodies detect only a single band of 116,000 M_r in *S. aurantiaca* extracts and with maxicell analysis. The complemented cells are able to grow in the total absence of *E. coli* IF2, with the sole source of cellular IF2 activity being provided in *trans* by plasmid-encoded *S. aurantiaca* IF2. A representative number of survivors from SLB7, SLB8, and SLB18-1 cells cured of the λ *infB* gene were purified. We tested the cured strains for growth at various temperatures. Growth on LB

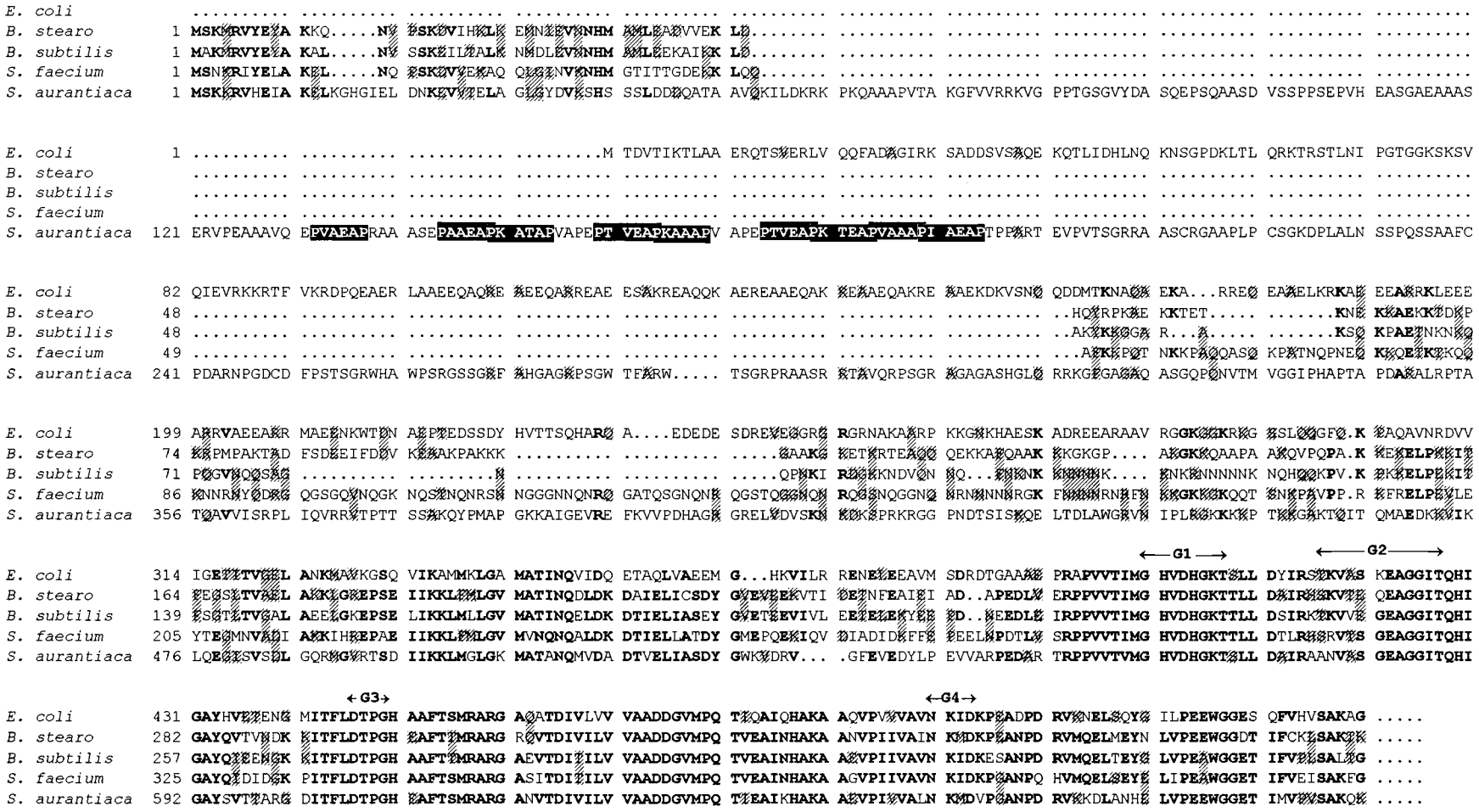

FIG. 2. Comparison of amino acid sequences in the N-terminal and G-domain regions of IF2s from five bacterial species. PXXEAP (or PXXXAP) motifs present in the APE region (see text) appear as white characters on a dark background. Shaded and boldface characters identify identical amino acids at the same position in two or at least three IF2 species, respectively. Consensus sequences G1 to G4 found in the G domains of GTP-binding proteins are also indicated. *B. stearo*, *B. stearothermophilus.*

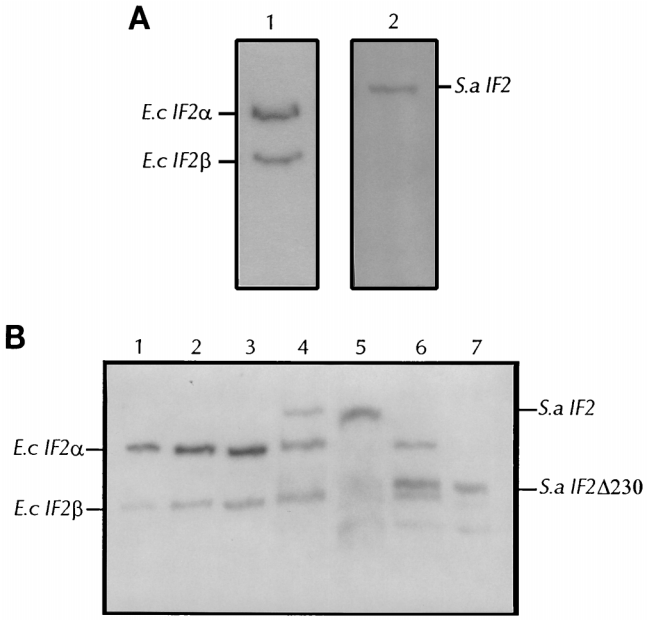

FIG. 3. Immunodetection of IF2 proteins. (A) Crude cell extracts of *S. aurantiaca* DW4 (*S.a*) and *E. coli* DH5α (*E.c*) were fractionated by SDS–7.5% PAGE, blotted onto nitrocellulose, and probed with an antiserum raised against *E. coli* IF2 and ^{125}I-protein A (17). Lanes: 1, *E. coli* DH5α (IF2α and IF2β); 2, *S. aurantiaca* DW4. (B) Western blot analysis of the cured strains. Cultures were grown in LB medium at 37°C and harvested during late exponential growth (A_{650}, ~1). Cells were lysed by boiling in SDS sample buffer. Proteins were separated by SDS–6% PAGE, transferred to a nitrocellulose membrane, and treated with anti-IF2 antibodies and ^{125}I-protein A as described previously (17). Lanes: 1, *E. coli* SL598R carrying pTRC99A at 30°C (before curing); 2, SL598R carrying pLBYC8 at 30°C (before curing); 3, SLB8 at 37°C; 4, SL598R carrying pLBYC7 at 30°C (before curing); 5, SLB7 at 37°C; 6, SL598R carrying pLBYC7Δ690 at 30°C (before curing); 7, SLB7Δ690 at 37°C.

plates is summarized in Table 4. Strains carrying the *S. aurantiaca* IF2 are slightly retarded for growth at 30 and 37°C compared to SLB8 or SLB18-1, the reference strains. In liquid medium (LB) at 37°C, the cured strain carrying pLBYC8 (SLB8) and the cured strain carrying pB18-1 (SLB18-1) show an initial doubling time of 30 min and exhibit normal exponential growth, whereas the growth of SLB7 is slower, with a doubling time of 50 min. After 16 h of liquid culture at 37°C, SLB8 was in the stationary phase with an OD_{650} of 4, whereas the OD of SLB7 did not exceed 3. It is interesting that an *E. coli* strain expressing only one form of IF2 also exhibits cold-sensitive growth compared with a strain expressing both IF2α and IF2β (34).

Function of the N-terminal insertion of *S. aurantiaca* IF2. Although the amino acid sequence of *S. aurantiaca* IF2 is 50% homologous with those of the other sequenced IF2s for the carboxy-terminal three-quarters of the protein, there is little or no homology in the N-terminal region, which is considerably larger than that of any of the other IF2s and, in particular, has an unusual sequence of 160 amino acids very rich in Pro, Ala, Glu, and Val: the APE region. To determine whether this N-terminal portion is essential for cell viability, we constructed an *S. aurantiaca* IF2 mutant in which part of the N-terminal region, including all of the APE sequences, had been deleted. The ability of this truncated *infB* gene to replace the *E. coli* chromosomal *infB* gene and satisfy the cellular requirement for IF2 was tested.

This deleted form of *S. aurantiaca* IF2 was constructed in vitro as described in Materials and Methods. By site-specific mutagenesis, we created an *Sph*I restriction site, located at the 29th codon of IF2, immediately upstream of the APE region. Another *Sph*I site is present downstream at amino acid 258. An in-frame deletion between the two *Sph*I sites eliminates 690 bp corresponding to 230 amino acids in the N-terminal region of the protein in front of the G domain. The gene carrying the deletion of the N-terminal region encoding the 160-amino-acid APE repeated motif was recloned into pTrc99A to give plasmid pLBYC7Δ690 (Fig. 1). This plasmid produces a 91,000-M_r protein, in which the entire G domain and C-terminal part were conserved. Western blot analysis of the protein encoded by pLBYC7Δ690 shows that the construct expresses a protein of the expected size, detected by anti-IF2 antibodies, and that the full-size *S. aurantiaca* IF2 is eliminated (Fig. 3B, lane 6). A faint band, attributed to a degradation product with an M_r of ca. 60,000, was consistently visible in the constructs expressing the truncated form of *S. aurantiaca* IF2.

The plasmid pLBYC7Δ690 was introduced into SL598R to test for its ability to permit survival of bacteria after heat curing of the λ *infB* transducing phage (Table 4). Survivors were detected at similar frequencies in strains containing pLBYC7Δ690 and pLBYC8. Survivors carrying pLBYC7Δ690 were called SLB7Δ690. Western blotting of the cured strain confirmed the exclusive expression of the truncated form of *S. aurantiaca* IF2 (Fig. 3B, lane 7). These clones produced colonies which grow significantly better than SLB7 (Table 4). They were of uniform size and stable throughout subsequent purifications. In liquid medium (LB) cultures at 37°C, the exponential growth rate of the truncated-IF2 strain was only slightly lower (doubling time, 35 min) than that of SLB18-1 and SLB8. This result indicates, as expected, that the additional N-terminal repeated motif of *S. aurantiaca* IF2 is not essential for growth of *E. coli* but that, on the contrary, in the latter it is somewhat detrimental to its activity.

TABLE 4. Analysis of the cured strains derived from SL598R

Plasmid	Frequency of cured strains[a]	Resulting strain	Doubling time (min) at 37°C	Growth on plates[b] at:			
				42°C	40°C	37°C	30°C
pB18-1	10^{-4}	SLB18-1	30	++++	++++	++++	+++
pLBYC8	9×10^{-5}	SLB8	30	++++	++++	++++	+++
pLBYC7	3×10^{-5}	SLB7	50	++++	+++	++	+
pLBYC7Δ690	5×10^{-5}	SLB7Δ690	35	++++	+++	+++	+++
pTrc99A	—[c]						

[a] Number of survivors at 42°C divided by number of bacteria exposed to 42°C.
[b] ++++, very good growth after 18 h; +++, good growth but smaller colonies after 18 h; ++, growth producing small colonies after 24 h; +, growth visible only after 24 h.
[c] —, no survivors.

DISCUSSION

The *infB* structural gene from the myxobacterium *S. aurantiaca* has been identified on a 5.18-kb chromosomal fragment and sequenced. It encodes a protein which has amino acid sequence homology with IF2s from *E. coli* and several gram-positive bacteria. In *E. coli*, the genetic organization around *infB* is quite complex. The *infB* gene belongs to the polycistronic *nusA-infB* operon, encoding, in order, a minor form of initiator tRNA (*metY*), the gene for a putative protein p15KA, the gene for a protein involved in transcriptional termination (*nusA*), *infB*, the gene for a ribosome binding factor (*rbfA*), and the gene for another protein of unknown function (p35K). A similar but not identical arrangement of genes is found in *B. subtilis*. The *B. subtilis infB* operon comprises seven ORFs (four of which are homologous to genes present in the *E. coli nusA-infB* operon): *Bsp15A*, *nusA*, two genes encoding proteins of unknown function (ORF1 and ORF2), *infB*, and then a 92-codon open reading frame called ORF3 which is immediately followed by *Bsp15B*, which has homology with *rbfA* of *E. coli* (40). The analysis of the *S. aurantiaca* 5.18-kb fragment revealed the presence of two putative genes downstream of *infB*. The first ORF is predicted to encode a protein with significant homology to the *B. subtilis* ORF3 product (40), which is absent from *E. coli*. The second ORF is homologous to *rbfA*, found both in *E. coli* and *B. subtilis*. This conservation of genes in the *infB* operon of these different organisms is remarkable, but the intergenic sequences and distances are apparently not conserved. The conservation of the same genetic organization between the two distantly related organisms *S. aurantiaca* and *B. subtilis* is surprising. They do have one common property: both organisms form spores upon starvation. Whether a specific function is provided by the ORF3 gene during sporulation remains to be established.

We have sequenced 800 bp upstream from *infB*, and surprisingly, the gene encoding NusA, flanking *infB* in *E. coli*, was not found in *S. aurantiaca*. Neither did we find any homology to ORF2, which overlaps both the 3′ end of *nusA* and the beginning of *infB* in *B. subtilis*. However, we found two interesting features in the 800-bp segment of *S. aurantiaca*: first, a stem-and-loop structure (between nucleotides 89 and 127; the stem and the loop are 15 and 9 bp long, respectively) that might act as a transcription termination or pausing site (calculated free energy, $\Delta G_0 = -31.5$ kcal mol^{-1}); and second, the end of an ORF from the beginning of the sequence to nucleotide 420, displaying no homology to any known protein. This ORF nevertheless has a typically high GC content in its DNA. The coding sequences of myxobacterial DNAs share a particular feature, namely, a strong preference (above 90%) for GC at the third codon position, a lower preference for GC at the first position, and a preference of below 50% at the second position. In the 3′ region of the putative ORF, the GC contents are 91, 71, and 48% at the third, first, and second codon positions, respectively.

The observation of a potential terminator upstream of *infB* raises the question of the presence of promoter sequences downstream from the above-mentioned putative termination site. Taking into account the consensus sequences TTGCNN (−35) and AANGCT (−10) determined for another myxobacterium, *M. xanthus* (20), or the boxes that we have previously identified in the tRNA-*tufB* operon of *S. aurantiaca* (3) (TTT CCA and AGGGCG at −35 and −10, respectively), no obvious promoter can be identified. However, promoter sites are not sufficiently well documented in myxobacteria, and transcriptional analysis of the *infB* gene within *S. aurantiaca* itself is necessary to give a definite answer to this question.

Several *S. aurantiaca infB* constructs were expressed in *E. coli* devoid of its chromosomal copy of the IF2-encoding gene (23). Plasmids which retained the upstream 800-bp sequence produced only a few transformants, and those clones grew poorly, whereas plasmids expressing *S. aurantiaca infB* in the absence of the upstream sequence allowed almost normal growth of *E. coli*. At least in *E. coli*, this might imply that the above-mentioned secondary structure can stop any transcription coming from the vector promoter.

Immunodetection experiments showed that only one IF2 form (ca. 120 kDa) was clearly visible in *S. aurantiaca* cells, whereas two or even three different forms of the factor had been described for *E. coli* and *B. subtilis* (16, 34, 39).

The role of the N-terminal region of IF2 is still unclear, since, in *E. coli*, it does not appear to be essential for the initiation of protein synthesis under many circumstances (5, 23). In *S. aurantiaca* IF2, this region is longer than any other identified to date (see Results). It was consequently tempting to delete it and test whether truncated forms could still complement *E. coli* cells devoid of their endogenous IF2. After site-directed mutagenesis, a deletion mutant of *S. aurantiaca infB* was created, yielding a protein shortened by 230 amino acids at the N terminus. This did not alter the viability of the transformant clones, which grew as well as the control strain. We measured the relative amounts of IF2 in the different cured strains grown at 37°C, using a quantitative immunoblotting technique (see Materials and Methods). The expression of *S. aurantiaca* IF2 in strain SLB7 is only about twofold higher than that of the *E. coli* chromosomally derived IF2 ($\alpha+\beta$) in the reference strain IBPC5321. The decrease in doubling time (from 30 to 50 min) was thus not due to a lack of IF2 factor in strain SLB7. Furthermore, SLB7Δ690 cells (corresponding to IF2 of *S. aurantiaca* lacking 230 amino acids in the N-terminal region of the protein) express only 80% of the total amount of *E. coli* IF2 in the wild-type strain and grow much better at 37°C than SLB7 cells do. These data show that the absence of the 230-amino-acid segment in the N-terminal region of the IF2 protein of *S. aurantiaca* results in better growth of *E. coli* cells. This is very likely due to the fact that the deleted form of *S. aurantiaca* IF2 has a structure closer to that of its *E. coli* counterpart, and the slow growth of SLB7 probably reflects an inhibitory effect of the very atypical N-terminal region of the protein.

The lack of importance of this region of *S. aurantiaca* IF2 in *E. coli* leaves open the possibility of another role, perhaps related to the multicellular development in myxobacteria. A striking observation comes from the comparison of the amino acid compositions of the IF3 C-terminal extension, the ORF3 C-terminal extension, and the IF2 N-terminal additional region in *S. aurantiaca* described here. These regions of all three proteins are extremely rich in three amino acids, i.e., alanine, proline, and glutamic acid, and we propose the term APE sequence to describe them (Table 3). In *M. xanthus* IF3, the C-terminal APE extension is shorter than that in *S. aurantiaca* IF2 (66 compared to 160 amino acids), with alanine (34%), proline (11%), and glutamic acid (14%) representing together 59% of the overall composition. As already mentioned, the same amino acids make up 40% of the 119-amino-acid C-terminal extension of the ORF3 product. Within the additional 160 amino acids of IF2, alanine, proline, and glutamic acid are overrepresented (57% total [27, 19, and 11%, respectively]). In addition, valine is very abundant in IF2 (11%) but not in IF3 or the ORF3 product. Moreover, *M. xanthus* IF3 and the *S. aurantiaca* ORF3 product do not carry the repeated motif PXXEAP found in IF2. However a 50% similarity appears upon continuous alignment of the amino acid sequences found

in both IF3 and IF2 from myxobacteria. The fact that a developmental mutation in *M. xanthus* has been mapped to this region in IF3 (6) makes it very tempting to speculate that these two regions could play a common but as yet unknown role in development, possibly also involving the ORF3 C-terminal extension. Experimental data are now needed to prove that N-terminally deleted forms of IF2 lead to developmental mutants in myxobacteria.

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REFERENCES

1. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterisation of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.
2. **Bremaud, L., B. Derijard, and Y. Cenatiempo.** 1993. Selective amplification of DNA fragments coding for the G-domain of factors IF2 and EF-Tu, two G proteins from the myxobacterium *Stigmatella aurantiaca*. PCR Methods Appl. **3:**195–199.
3. **Bremaud, L., C. Fremaux, S. Laalami, and Y. Cenatiempo.** 1995. Genetic and molecular analysis of the *tRNA-tufB* operon of the myxobacterium *Stigmatella aurantiaca*. Nucleic Acids Res. **23:**1737–1743.
4. **Brombach, M., C. O. Gualerzi, Y. Nakamura, and C. L. Pon.** 1986. Molecular cloning and sequence of the *Bacillus staerothermophilus* translational initiation factor IF2 gene. Mol. Gen. Genet. **206:**97–102.
5. **Cenatiempo, Y., F. Deville, J. Dondon, M. Grunberg-Manago, C. Sacerdot, J. W. B. Hershey, H. F. Hansen, H. U. Petersen, B. F. C. Clark, M. Kjeldgaard, T. F. M. la Cour, K. K. Mortensen, and J. Nyborg.** 1987. The protein synthesis initiation factor 2 G-domain. Study of a functionally active C-terminal 65-kilodalton fragment of IF2 from *Escherichia coli*. Biochemistry **26:**5070–5076.
6. **Cheng, Y. L., L. V. Kalman, and D. Kaiser.** 1994. The *dsg* gene of *Myxococcus xanthus* encodes a protein similar to translational initiation factor IF3. J. Bacteriol. **176:**1427–1433.
7. **Dammel, C. S., and H. F. Noller.** 1995. Suppression of a cold sensitive mutation in 16S rRNA by overexpression of a novel ribosome-binding factor, RbfA. Genes Dev. **9:**626–637.
8. **Derijard, B., M. Ben Aïssa, B. Lubochinsky, and Y. Cenatiempo.** 1989. Biochem. Biophys. Res. Commun. **158:**562–568.
9. **Dever, T. E., M. J. Glynias, and W. C. Merrick.** 1987. GTP-binding domain: three consensus sequence elements with distinct spacing. Proc. Natl. Acad. Sci. USA **84:**1814–1818.
10. **Dworkin, M.** 1996. Recent advances in social developmental biology of the myxobacteria. Microbiol. Rev. **60:**70–102.
11. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
12. **Friedrich, K., M. Brombach, and C. L. Pon.** 1988. Identification, cloning, and sequence of the *Streptococcus faecium infB* (translational initiation factor IF2) gene. Mol. Gen. Genet. **214:**595–600.
13. **Gaboriaud, C., V. Bissery, T. Benchetrit, and J. P. Mornon.** 1987. Hydrophobic cluster analysis: an efficient new way to compare and analyse amino acid sequences. FEBS Lett. **224:**149–155.
14. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmid DNA. Mol. Microbiol. **166:**557–580.
15. **Hartz, D., D. S. McPheeters, and L. Gold.** 1989. Selection of the initiator tRNA by *Escherichia coli* initiation factors. Genes Dev. **3:**1899–1912.
16. **Howe, J. G., and J. W. B. Hershey.** 1984. The rate of evolutionary divergence of initiation factors IF2 and IF3 in various bacterial species determined quantitatively by immunoblotting. Arch. Microbiol. **140:**187–192.
17. **Howe, J. G., and J. W. B. Hershey.** 1981. A sensitive immunoblotting method for measuring protein synthesis initiation factor levels in lysates of *Escherichia coli*. J. Biol. Chem. **256:**12836–12839.
18. **Janata, J., and K. Mikulik.** 1995. Translation initiation factors of a tetracycline-producing strain of *Streptomyces aureofaciens*. Biochem. Biophys. Res. Commun. **208:**569–575.
19. **Kalman, L. V., Y. L. Cheng, and D. Kaiser.** 1994. The *Myxococcus xanthus dsg* gene product performs function of translational initiation factor IF3 in vivo. J. Bacteriol. **176:**1434–1442.
20. **Komano, T., T. Franscheschini, and S. Inouye.** 1987. Identification of a vegetative promoter in *Myxococcus xanthus*: a protein that has homology in histones. J. Mol. Biol. **196:**517–524.
21. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
22. **Kurihara, T., and Y. Nakamura.** 1983. Cloning of the *nusA* gene of *Escherichia coli*. Mol. Gen. Genet. **190:**189–195.
23. **Laalami, S., H. Putzer, J. Plumbridge, and M. Grunberg-Manago.** 1991. A severely truncated form of translational initiation factor 2 supports growth of *Escherichia coli*. J. Mol. Biol. **220:**335–349.
24. **Lee, K., and L. J. Shimkets.** 1996. Suppression of a signaling defect during *Myxococcus xanthus* development. J. Bacteriol. **178:**977–984.
25. **March, P. E.** 1992. Membrane-associated GTPases in bacteria. Mol. Microbiol. **6:**1253–1257.
26. **McCarthy, J. E. G., and C. O. Gualerzi.** 1990. Translational control of prokaryotic gene expression. Trends Genet. **6:**78–85.
27. **Morel-Deville, F., G. Vachon, C. Sacerdot, A. Cozzone, M. Grunberg-Manago, and Y. Cenatiempo.** 1990. Characterisation of the translational start site for IF2β, a short form of *Escherichia coli* initiation factor IF2. Eur. J. Biochem. **188:**605–614.
28. **Plumbridge, J. A., F. Deville, C. Sacerdot, P. Petersen, Y. Cenatiempo, A. Cozzone, M. Grunberg-Manago, and J. W. B. Hershey.** 1985. Two translational initiation sites in the *infB* gene are used to express initiation factor IF2α and IF2β in *Escherichia coli*. EMBO J. **4:**223–229.
29. **Plumbridge, J. A., J. Dondon, Y. Nakamura, and M. Grunberg-Manago.** 1985. Effect of NusA protein on expression of the *nusA*, *infB* operon in *E. coli*. Nucleic Acids Res. **13:**3371–3388.
30. **Plumbridge, J. A., J. G. Howe, M. Springer, D. Touati-Schwartz, J. W. B. Hershey, and M. Grunberg-Manago.** 1982. Cloning and mapping of a gene for translational initiation factor IF2 in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **79:**5033–5037.
31. **Plumbridge, J. A., and M. Springer.** 1983. Organisation of the *Escherichia coli* chromosome around the genes for translation initiation factor IF2 (*infB*) and a transcription termination factor (*nusA*). J. Mol. Biol. **167:**227–243.
32. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of transcription. Annu. Rev. Genet. **13:**319–353.
33. **Sacerdot, C., P. Dessen, J. W. B. Hershey, J. A. Plumbridge, and M. Grunberg-Manago.** 1984. Sequence of the initiation factor IF2 gene: unusual protein features and homologies with elongation factors. Proc. Natl. Acad. Sci. USA **81:**7787–7791.
34. **Sacerdot, C., G. Vachon, S. Laalami, F. Morel-Deville, Y. Cenatiempo, and M. Grunberg-Manago.** 1992. Both forms of translational initiation factor 2 (α and β) are required for maximal growth of *Escherichia coli*. Evidence for two translational initiation codons for IF2β J. Mol. Biol. **225:**67–80.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

35a. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for the identification of plasmid coded proteins. J. Bacteriol. **137:**692–693.

36. **Sands, J. F., H. S. Cummings, C. Sacerdot, L. Dondon, M. Grunberg-Manago, and J. W. B. Hershey.** 1987. Cloning and mapping of *infA*, the gene for protein synthesis initiation factor IF1. Nucleic Acids Res. **16:**5157–5168.
37. **Sands, J. F., P. Regnier, H. S. Cummings, M. Grunberg-Manago, and J. W. B. Hershey.** 1988. The existence of two genes between *infB* and *rpsO* in the *Escherichia coli* genome: DNA sequencing and S1 nuclease mapping. Nucleic Acids Res. **16:**10803–10816.
38. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
39. **Shazand, K., J. Tucker, R. Chiang, K. Stansmore, H. U. Sperling-Petersen, M. Grunberg-Manago, J. C. Rabinowitz, and T. Leighton.** 1990. Isolation and molecular genetic characterization of the *Bacillus subtilis* gene (*infB*) encoding protein synthesis initiation factor IF2. J. Bacteriol. **172:**2675–2687.
40. **Shazand, K., J. Tucker, M. Grunberg-Manago, J. C. Rabinowitz, and T. Leighton.** 1993. Similar organization of the *nusA-infB* operon in *Bacillus subtilis* and *Escherichia coli*. J. Bacteriol. **175:**2880–2887.
41. **Shimkets, L. J.** 1990. Social and developmental biology of the myxobacteria. Microbiol. Rev. **54:**473–501.
42. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments selected by gel electrophoresis. J. Mol. Biol. **98:**503–517.
43. **Springer, M., M. Graffe, and H. Hennecke.** 1977. Specialised transducing phage for the initiation factor 3 gene in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **74:**3970–3974.
44. **Starich, T., and J. Zissler.** 1989. Movement of multiple DNA units between *Myxococcus xanthus* cells. J. Bacteriol. **171:**2323–2336.
45. **Tipper, D. J., C. W. Johnsson, C. L. Ginther, T. Leighton, and H. G. Wittman.** 1977. Erythromycin-resistant mutations in *Bacillus subtilis* causes temperature sensitive sporulation. Mol. Gen. Genet. **150:**147–159.