JOURNAL OF BACTERIOLOGY, Jan. 1994, p. 198–205
0021-9193/94/$04.00+0

Vol. 176, No. 1

Translation Initiation Factor IF1 Is Essential for Cell Viability in *Escherichia coli*

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Received 22 June 1993/Accepted 30 October 1993

Translation initiation factor IF1 is a highly conserved element of the prokaryotic translational apparatus. It has been demonstrated earlier that the factor stimulates in vitro the initiation phase of protein synthesis. However, no mutation in its gene, *infA*, has been identified, and a role for IF1 in translation has not been demonstrated in vivo. To elucidate the function of IF1 and determine if the protein is essential for cell growth, the chromosomal copy of *infA* was disrupted. Cell viability is maintained only when *infA* is expressed in *trans* from a plasmid, thereby demonstrating that IF1 is essential for cell growth in *Escherichia coli*. Cells depleted of IF1 exhibit few polysomes, suggesting that IF1 functions in the initiation phase of protein synthesis.

Initiation of protein synthesis in prokaryotes involves formation of a 30S preinitiation complex in which fMet-tRNA interacts with the initiation codon of an mRNA bound to the surface of the 30S ribosomal subunit. The efficiency and fidelity of formation of the 30S preinitiation complex is promoted by three initiation factors: IF1, IF2, and IF3. The properties and mechanism of action of the initiation factors have been reviewed recently (13). All three initiation factors bind to the 30S ribosomal subunit in the absence of other translational components. IF2 binds GTP and fMet-tRNA$_f$, thereby ensuring that the tRNA bound to the 30S subunit is the initiator tRNA, fMet-tRNA$_f$. IF3 plays at least two roles: it acts as an antiassociation factor to maintain the ribosomal subunits in a dissociated state, and it promotes the selection of the initiator fMet-tRNA by monitoring the tRNA anticodon stem-loop region and the correctness of the anticodon-initiator codon interaction on the ribosome. The function of IF1 is less clear, since no specific role has yet been assigned to this protein.

IF1 is the smallest of the initiation factors (M_r, 8,100) and consists of 71 amino acid residues. The gene for IF1, *infA*, has been cloned, sequenced, and mapped to about 20 min on the *Escherichia coli* chromosome (25). The *infA* gene is transcribed by two promoters to yield two sizes of monocistronic mRNAs both ending at the same terminator (8). Recently, homologous genes have been identified in *Bacillus subtilis* and in the chloroplasts of several plants (4, 23, 28). The high degree of homology indicates that IF1 is a conserved protein and suggests that it plays an important role in the cell. However, our knowledge of the function of IF1 comes exclusively from a number of in vitro assays for translation. IF1 enhances the rates of 70S ribosome dissociation and subunit association but does not change the equilibrium position (10). It alone binds poorly to 30S ribosomal subunits, but when added with IF2 both factor-binding affinities are increased (32). IF1 stimulates IF2-dependent fMet-tRNA$_f$ binding to 30S or 70S ribosomes in the presence of mRNA. However, the best demonstration that IF1 truly functions in protein synthesis comes from the observation that β-galactosidase synthesis in a DNA-linked, highly purified transcription/translation system is stimulated eightfold by IF1 (17).

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Since in vitro assays for IF1 may give misleading information, it is desirable to determine in vivo whether IF1 is essential for cell growth. The prior cloning of the gene *infA* enables us to approach this question by using recombinant DNA techniques. We report here the construction of a strain with a disruption in the chromosomal copy of *infA* and demonstrate a cessation of cell growth correlated with depletion of IF1. Our results show that IF1 is essential for the viability of *E. coli*.

MATERIALS AND METHODS

Bacteriological methods and growth conditions. The genotypes and sources of all *E. coli* strains, M13 bacteriophage, and plasmids used in this work are described in Table 1. Solid media were either Luria broth (LB), nutrient agar plates (Difco), or minimal M9 medium supplemented with the appropriate amino acids (40 μg/ml) as required. Physiological growth rate experiments were conducted in LB medium or minimal MOPS (morpholinepropanesulfonic acid) medium (22) containing 0.4% glucose and a total amino acid mix (each at 40 μg/ml). The liquid media or plates were supplemented with the relevant antibiotics: ampicillin (Amp), 50 μg/ml; chloramphenicol (Cm), 20 μg/ml; tetracycline (Tet), 15 μg/ml; and kanamycin (Kan), 30 μg/ml. Two concentrations of spectinomycin (Spc), 100 or 50 μg/ml, and streptomycin (Sm), 30 or 15 μg/ml, were used depending on whether the resistance marker was carried in a multicopy plasmid or as a single-copy gene, respectively. For induction of the *tac* promoter, isopropylthio-β-galactoside (IPTG) was routinely added to plates and liquid cultures at a concentration of 0.1 mM. For some physiological studies, an IPTG concentration of 0.5 mM was used. Growth was at 37°C for all strains except HSC994 and HSC1986, which were grown routinely at 30°C or as indicated. P1*vir* transductions were performed according to the method of Miller (20).

Plasmid and bacteriophage constructions. A schematic diagram of the construction of the chromosomal *infA* disruption is shown in Fig. 1. The plasmid pHP45Ω (9, 24) was digested with *Sma*I, and the omega (Ω) fragment, approximately 2 kb, was purified from an agarose gel by utilizing GeneClean (Stratagene). The Ω fragment carries an aminoglycoside adenylyl-transferase gene (*aad*) and thus mediates resistance to both Spc and Sm (2, 31). The fragment was ligated into pTH2 (25), which had been partially digested with *Msc*I. The insertion and orientation of the Ω fragment into the second codon

TABLE 1. Bacterial strains, bacteriophage, and plasmids

Type and designation	Relevant characteristics and comments	Reference or source
Bacteria		
JM101	*supE thi* Δ(*lac-proAB*) F′ [*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	18
KL208	CGSC strain 4314; Hfr; suppressor-free strain	B. Bachmann[a]
CAG18478	MG1655 *zbj-1230*::Tn*10*	29
NO3427	*polA lys lacZ*::Tn*9G8 lacI*^{q} Cm^r	7
UB947	*polA*(Ts) *his*	A. Björnsson
HSC920	KL208 carrying pHSC92; Kan^r	This work
HSC1940	HSC920 with bacteriophage M13mp11Cat-*infA*::Ω integrated into the chromosome by homologous recombination; Kan^r Cm^r Spc^r Sm^r	This work
HSC1941	*infA* segregant derived from HSC1940; carries *infA*::Ω disruption; Kan^r Spc^r Sm^r	This work
HSC1945	*infA infA*::Ω *zbj-1230*::Tn*10*, constructed by P1 transduction of HSC1941 to Tet^r from CAG18478; Kan^r Spc^r Sm^r	This work
HSC934	NO3427 carrying pHSC92; Kan^r	This work
HSC1984	*polA lacI*^{q} *infA*::Ω *zbj-1230*::Tn*10* Kan^r; constructed by P1 transduction of Spc^r Sm^r Tet^r from HSC1945 to HSC934	This work
HSC994	UB947 carrying pCC-1; Amp^r	This work
HSC1986	*polA*(Ts) *infA*::Ω *zbj-1230*::Tn*10* Amp^r; constructed by P1 transduction of Spc^r Sm^r Tet^r from HSC1984 to HSC994	This work
Bacteriophages		
M13mp11Cat	I^{am} II^{am} *lacZ*α *cat* Derivative of M13mp11 (19); contains the chloramphenicol transacetylase gene	21
M13mp11Cat-*infA*::Ω	Derivative of M13mp11Cat carrying *infA*::Ω; contains an *Eco*RI-*Pst*I fragment from pTHC2Ω inserted into *Eco*RI-*Pst*I-digested M13mp11Cat	This work
Plasmids		
pHP45Ω	Carries Ω fragment which confers Sm and Spc resistance; Amp^r	24
pTH2	Carries 3.1 kb of genomic DNA surrounding *infA*; Amp^r	25
pTHC2Ω	Derivative of pTH2 with the Ω fragment inserted into the second codon of *infA*; contains a *Sma*I fragment from pHP45Ω inserted into partially *Msc*I-digested pTH2	This work
pLG339	Cloning vector with pSC101 origin of replication; confers Kan and Tet resistance	30
pCC-1	Carries *infA* structural gene under the control of the inducible *tac* promoter; ColE1 origin of replication; Amp^r	6
pHSC92	Derivative of pLG339 with the *infA* structural gene under the control of the inducible *tac* promoter; contains a *Bam*HI fragment from pCC-1 inserted into *Bam*HI-cut pLG339; Kan^r	This work

[a] *E. coli* Genetics Stock Center, Yale University, New Haven, Conn.

of *infA* were determined by restriction analysis. The resulting plasmid, pTHC2Ω (Fig. 1B), was digested with *Eco*RI and *Pst*I, and the 3.2-kb fragment containing *infA*::Ω was isolated and subcloned into M13mp11Cat (21) by using JM101 as the host strain to yield M13mp11Cat-*infA*::Ω.

Strain constructions. Selection of M13mp11Cat-*infA*::Ω lysogens was basically as described elsewhere (3, 21). The recipient strain HSC920 (Table 1) was grown to mid-log phase at 37°C. Bacteriophage M13mp11Cat-*infA*::Ω was heated at 70°C for 20 min to kill host bacteria prior to incubation with the recipient cells at multiplicities of infection of 10^{-2}, 10^{-3}, and 10^{-4}. Phage and recipient cells were mixed and incubated at 37°C for 60 min with gentle shaking, and lysogens were selected by spreading ca. 1×10^7 cells on LB plates containing IPTG and either Cm or Spc.

In order to select for cells that had lost the prophage by excision, single-colony isolates of an M13mp11Cat-*infA*::Ω lysogen (HSC1940) were spread onto nutrient agar plates containing IPTG (100 μM), deoxycholate (DOC; 0.3% [wt/vol]), and sodium chloride (0.5% [wt/vol]) (21) with or without Spc. The number of segregants observed per plate varied depending upon selection with or without Spc (see Table 2). Several hundred Spc-resistant colonies were then screened for Cm sensitivity by replica plating onto LB plates with Cm.

To move the disrupted *infA* gene into other backgrounds, a selectable marker, *zbj-1230*::Tn*10*, was introduced near *infA*::Ω by P1 transduction of HSC1941 to Tet resistance with P1 phage grown on strain CAG18478 (kindly provided by M. Rydén-Aulin, University of Stockholm). The resulting strain, HSC1945, was used as the donor for subsequent P1 transductions to introduce the *infA*::Ω and Tn*10* alleles into HSC935 and HSC994, giving HSC1984 and HSC1986, respectively.

RESULTS

Disruption of the chromosomal copy of *infA*. To determine whether the *E. coli* initiation factor IF1 is essential for cell viability and growth, we sought a method of constructing a null mutant strain lacking a functional *infA* gene. We chose the strategy of disrupting the chromosomal gene with a selectable marker by using a strain carrying the wild-type gene in *trans* on a plasmid. The selectable marker used was the Ω fragment (24), which consists of 2 kb of DNA carrying the Spc and Sm resistance gene, *aad*. This fragment contains its own transcriptional promoter and is flanked by transcription and translation termination signals in both orientations to prevent translational readthrough into the disrupted gene. Since *infA* is expressed as a monocistronic transcript (Fig. 1A) (8), its disruption is not expected to affect the expression of neighboring genes since these are driven by their own promoters. The Ω fragment was inserted into the *infA* structural gene after the fourth nucleotide (Fig. 1B), thus causing a disruption in the

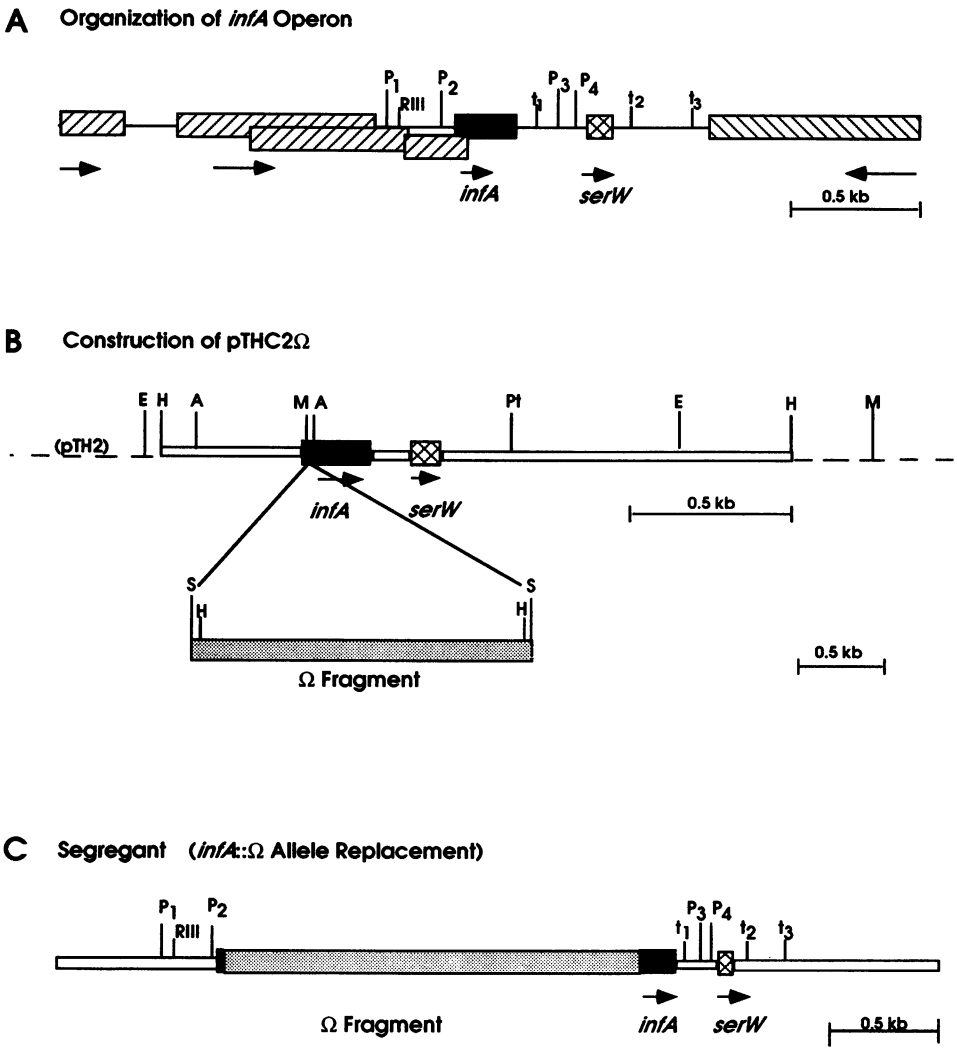

FIG. 1. Plasmid and strain constructions. *infA* and *serW* are represented as a solid box and a crosshatched box, respectively. Other open reading frames for uncharacterized or putative proteins (panel A) are depicted by boxes with diagonal lines slanted upwards in the direction of transcription. Transcriptional signals: P, promoter; t, terminator; RIII, RNaseIII cleavage site. (A) A schematic diagram of the organization of a 3.1-kb region of DNA surrounding *infA*. (B) The construction of pTHC2Ω from pTH2 by the insertion of the Ω fragment (stippled box) within *infA* is described in Materials and Methods. From pTHC2Ω, the *Eco*RI-*Pst*I fragment containing *infA*::Ω was cloned into M13mp11Cat as described in Materials and Methods. Relevant restriction sites used in the plasmid and bacteriophage constructions or used in Southern blot analyses are indicated: E, *Eco*RI; H, *Hin*dIII; Pt, *Pst*I; M, *Msc*I; A, *Asp* 718; S, *Sma*I. (C) The segregant illustrates the final chromosomal arrangement following the allele exchange whereby the chromosomal copy of *infA* has been replaced by *infA*::Ω (strains HSC1941, HSC1945, HSC1984, and HSC1986).

second codon (see Materials and Methods for details of plasmid and strain constructions). The resulting plasmid, pTHC2Ω, was digested with *Eco*RI and *Pst*I to generate a fragment containing *infA*::Ω flanked by 440 and 740 bp of chromosomal DNA 5′ and 3′ to *infA*, respectively. This fragment was inserted into phage M13mp11Cat (21) to generate M13mp11Cat-*infA*::Ω.

In order to move the disrupted *infA* gene into the chromosome, an allele exchange system was employed on the basis of homologous recombination of the circular recombinant M13 phage to form a lysogen and of subsequent excision and loss of the phage vector. M13mp11Cat-*infA*::Ω carries a nonsense mutation in gene II which is necessary for autonomous replication of the phage (19). When the phage infects a strain lacking tRNA nonsense suppressors, phage DNA persists only by integrating into the host chromosome. The phage markers for Cm, Spc, and Sm resistance allow ready selection of lysogens. Subsequent excision and loss of the phage is selected by growing the lysogens on plates containing bile salts. M13mp prophages confer sensitivity to bile salts (1, 3); therefore growth on such plates scores for cells that have lost the prophage. Further selection for the Spc-Sm markers allows identification of cells in which the *infA* region has been replaced by the disrupted gene. Because *infA* may be required for cell growth, a wild-type copy of *infA* is provided in *trans* on a plasmid.

E. coli HSC920 (Table 1) was infected with M13 mp11Cat-*infA*::Ω, and cells resistant to Cm or Spc were selected (see Materials and Methods). Strain HSC920 carries the plasmid pHSC92, which is present in about six to eight copies per cell and contains the *infA* structural gene (219 bp, plus 33 bp of flanking region) driven by the inducible *tac*

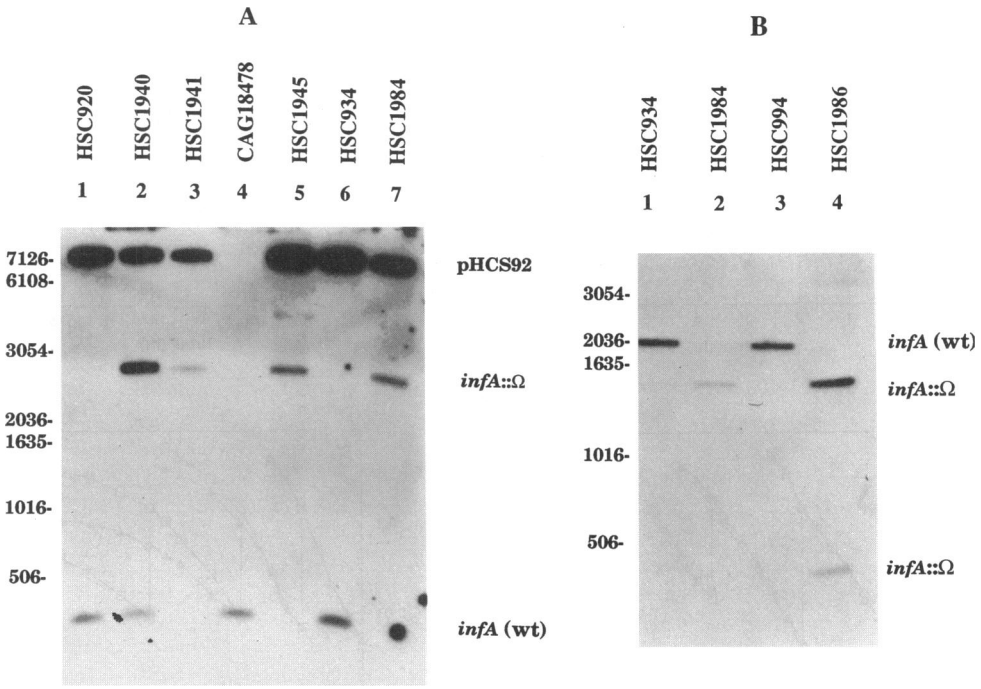

FIG. 2. Southern blot analysis of chromosomal DNA. Chromosomal DNA was prepared from the parental and *infA*::Ω strains used in this work (Table 1). The DNA was digested with *Asp* 718 (A) or *Hin*dIII (B), separated by 0.7% agarose gel electrophoresis, and transferred to Hybond-N membranes (Amersham). The identities of bands hybridizing to the *infA* probe (pTH2) are shown on the right; molecular weight markers are shown on the left. (A) Lane 1, HSC920, the parental strain; lane 2, HSC1940, the lysogenic strain containing the M13mp11Cat-*infA*::Ω integration; lane 3, HSC1941, the *infA*::Ω-disrupted strain resulting from segregation of the prophage; lane 4, CAG18478, a donor strain; lane 5, HSC1945, the same as HSC1941 but with the insertion of Tn*10* near 20 min; lane 6, HSC934, a donor strain; lane 7, HSC1984, derived from P1 transduction of *infA*::Ω and Tn*10* from HSC1945 to HSC934. The predicted band sizes (based on sequence information) for wild-type *infA* and for the disrupted *infA*::Ω are 0.37 and 2.34 kb, respectively. (B) Lanes 1 and 3 are the parental strains HSC934 and HSC994, and lanes 2 and 4 are the *infA*::Ω-disrupted strains HSC1984 and HSC1986, respectively. The top portion of the gel was removed prior to blotting to avoid hybridization of the probe to pHSC92 or pCC-1 (as seen in panel A). Predicted band sizes are 2.0 kb for wild-type *infA* and 1.55 and 0.38 kb for *infA*::Ω. The two bands labeled *infA*::Ω are due to the presence of *Hin*dIII restriction sites within the Ω cassette (see Fig. 1B).

promoter. Because the plasmid has only short regions of sequence identity with M13mp11Cat-*infA*::Ω, homologous recombination of the M13 phage is expected to occur mainly with the chromosome, not the plasmid. Three different multiplicities of infection were tested, and a multiplicity of infection of 10^{-2} generated an optimal number of isolated colonies (ca. 500 per plate). Equal numbers of lysogens were observed on both Cm and Spc selection plates. Because the phage and strain carry both *infA* and *lac* operon sequences, the desired insertion of the M13 phage into the chromosomal *infA* region was confirmed by Southern blot analysis of restricted DNA from one of the lysogens, called strain HSC1940 (Fig. 2A, lane 2).

To obtain cells that had lost the prophage and retained the disrupted *infA* gene, lysogens were spread on DOC and DOC plus Spc plates (both with IPTG), and the numbers of viable cells were recorded (Table 2). There is a difference of about two orders of magnitude in the number of cells that grow on DOC versus DOC plus Spc, indicating that excision events that leave the disrupted gene are unexpectedly rare. Of 104 randomly chosen segregants tested, when isolated by selection on DOC alone, none possessed a disrupted *infA* gene (Table 2). However, when DOC and Spc were used to select segregants, most carried the disrupted gene (Table 2). This demonstrates the difficulty in obtaining the desired second recombination event and provides a rationale for using a selection scheme rather than screening to identify excisions that leave the desired allele. A colony growing on DOC plus Spc was purified and named HSC1941. Replacement of *infA* with the chromosomal *infA*::Ω disruption in HSC1941 was confirmed by Southern blot analysis (Fig. 2A, lane 3).

Demonstration that IF1 is essential. Two approaches were tried to deplete IF1 in the *infA*-disrupted strain: either repressing the *infA* gene by growing the cells in the absence of the inducer of the *tac* promoter, IPTG, or curing the strain of the plasmid carrying *infA*. In liquid cultures, growth of strain HSC1941 is not influenced by IPTG levels; the disrupted strain grows well in the absence of IPTG. This presumably is due to

TABLE 2. Selection of M13mp11Cat-*infA*::Ω integration and *infA*::Ω segregants

Lysogen selection method	Segregant selection		No. of segregants tested	No. of segregants of indicated resistance type growing on antibiotics		
	Method	No. obtained		Cm^s	Spc^r	Sm^r
Spc	DOC	$>10^4$	52	0	0	0
Spc	DOC-Spc	ca. 400	166	55	166	165
Cm	DOC	$>10^4$	52	0	0	0
Cm	DOC-Spc	ca. 400	208	18	208	189

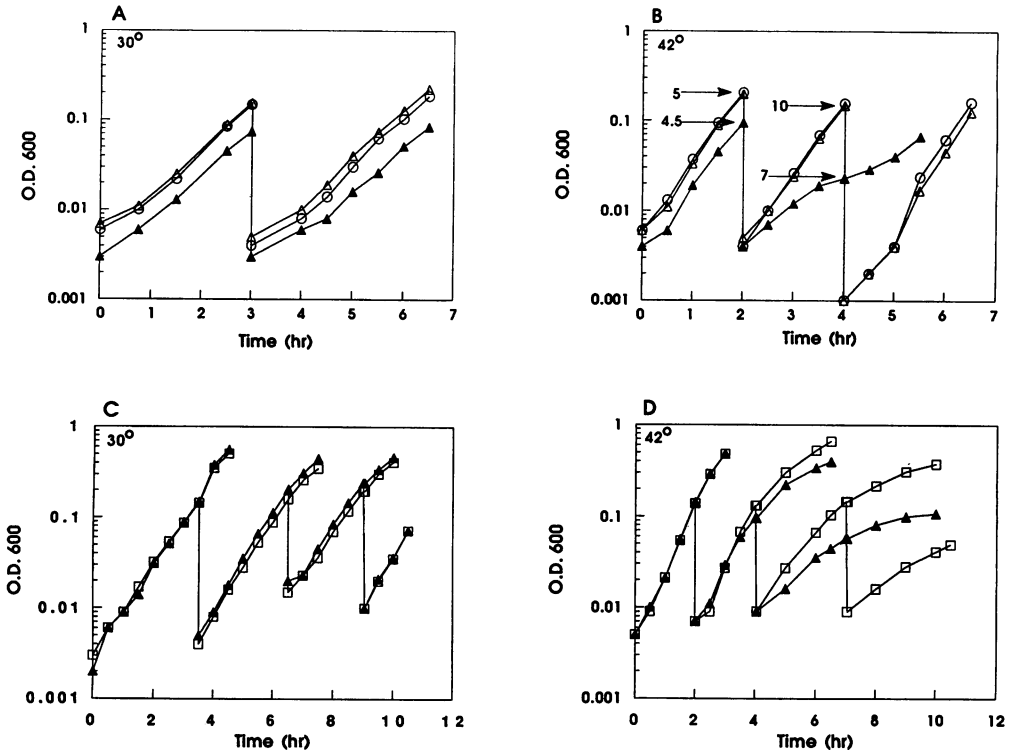

FIG. 3. Growth rate dependence on IF1. Cultures were grown in LB medium in the absence of antibiotics at 30°C (A and C) or 42°C (B and D) as described in Materials and Methods. In all panels, the vertical lines indicate the times at which dilutions of the growing cultures were made into fresh, prewarmed LB medium. Horizontal arrows indicate the number of generations of growth and correspond to the times of harvest for Western blot analyses of initiation factor levels. (A and B) UB947 (○), HSC994 (△) and HSC1986 (▲); (C and D) HSC1986 with (□) or without (▲) IPTG (0.5 mM).

sufficient expression of the plasmid-borne *infA* gene, due to the gene copy number (six to eight) and insufficient repression by the *lac* repressor. However, IPTG-dependent growth effects on HSC1941 are easily observed on plates; the colonies grow at a much lower rate in the absence of IPTG. Even when the *infA*::Ω disruption was moved into a strain carrying *lacI*^{q} and pHSC92, IF1 levels in the absence of IPTG were only reduced to 50% of wild type and no effect on growth rate was observed (data not shown). Therefore we chose the approach of curing the plasmid.

To cure the plasmid carrying wild-type *infA*, we used the strategy of employing a plasmid whose replication is dependent on the *polA* gene and an *infA*-disrupted strain that is *polA*(Ts). The *polA*(Ts) strain UB947 was transformed with pCC-1 (6), a plasmid with the ColE1 origin of replication (dependent on the *polA* gene product) and the identical P_{tac}-*infA* construct as pHSC92. The transformed strain, called HSC994, can be cured of pCC-1, as verified by its inability to grow at 42°C on Amp plates. The *infA* chromosomal region in HSC994 was replaced by the *infA*::Ω disruption by transducing to Spc and Tet resistance with P1 phage and plating at 30°C to yield HSC1986. The transducing P1 phages had been grown on HSC1984, an *infA*::Ω strain with a closely linked Tn*10* transposon (see Materials and Methods). The genomic structure of the disrupted *infA* region in the resulting strain, HSC1986, was confirmed by Southern blot analysis (Fig. 2B, lane 4).

Cultures of HSC1986 (disruptant) and two controls not carrying the *infA*::Ω disruption (UB947 and HSC994) were grown at 30°C and 42°C as described in Materials and Methods and in the legend to Fig. 3. At 30°C, HSC1986 and the controls grew at the same rate for at least 10 generations (Fig. 3A). The IF1 level determined by Western blotting (immunoblotting) (Fig. 4) was substantial after 11 generations (lane 2). At 42°C, the two control cultures grew exponentially at the same rate for at least 10 generations. However, the growth rate for HSC1986 diverged after about five generations and began to decline (Fig. 3B). By seven generations, the growth rate was about 25% of the control strains. The level of IF1 in cells also decreased dramatically from 4.5 generations (Fig. 4, lane 4) to 7 generations (lane 7), when it was barely detectable. Subsequently, cell lysis occurred as the growth rate of the culture approached stationary phase. These results are consistent with the view that IF1 is essential for cell viability and growth.

The lag observed in growth rate reduction is consistent with the rate of plasmid loss and dilution of IF1 levels by cell division. It follows that if the IF1 level in HSC1986 is raised by derepressing the *tac* promoter with IPTG, cells should show a longer delay in growth retardation at 42°C. HSC1986 was grown at 30°C and 42°C in the presence and absence of IPTG (Fig. 3C and D). The presence of IPTG had no effect on the growth rate of HSC1984 at 30°C (Fig. 3C). However, HSC1986 at 42°C was affected by the presence of IPTG, with the wild-type growth rate maintained for nearly eight generations before the onset of a more moderate decline of growth rate (Fig. 3D). These results reinforce the view that reduced levels of IF1 are responsible for the slowing of cell growth.

Although cell growth slows substantially at 42°C (Fig. 3B and D), it actually never ceases entirely, and the growth rate increases after long incubations at 42°C. This most likely is due to reversion of *polA*(Ts) or to mutations in *infA*, allowing

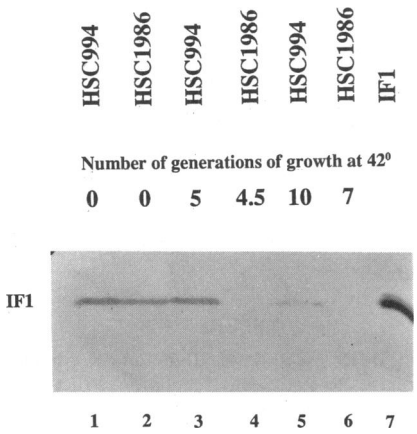

FIG. 4. Western blot analysis of initiation factor proteins. Protein samples were prepared from HSC1986 growing at 30°C or 42°C (Fig. 3A and B) and harvested at the times indicated below. Crude lysates were prepared, and samples obtained from equal numbers of cells were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoblotting. A duplicate set of Coomassie-stained gel profiles was obtained to verify that equal concentrations of total protein were present in the gels (data not shown). Protein samples, molecular weight markers, and purified initiation factors were electrophoresed on a 3-part tricine-SDS-polyacrylamide gel composed of a 4% acrylamide stacking gel, a 10% acrylamide spacer gel, and a 16.5% acrylamide separating gel (26). Following electrophoresis, protein in the gel was electrotransferred to Immobilon-P (Millipore). The blot was probed with polyclonal rabbit antibodies (16) raised against initiation factor IF1. The antigen-antibody complex was detected by using an alkaline phosphatase conjugate system (Sigma). Lanes 1 and 2, HSC994 and HSC1986, respectively, grown at 30°C to mid-log phase and harvested just prior to dilutions to begin the physiological studies at 42°C (shown in Fig. 3); lane 3, HSC994 grown for 5 generations; lane 4, HSC1986 grown for 4.5 generations; lane 5, HSC994 grown for 10 generations; lane 6, HSC1986 grown for 7 generations; lane 7, purified IF1.

expression of IF1. When a fresh culture of HSC1986 is grown at 30°C and then is spread on Spc plates and incubated at 42°C, large colonies appear within 24 h at a frequency of about 10^{-3}. These few colonies represent undefined phenotypic revertants. In growth curve experiments similar to those shown in Fig. 3B and D, viable cells were counted by plating equal optical densities at 600 nm (0.004; this corresponds to ca. 2,500 viable cells when grown at 30°C) at various times of incubation at 42°C. When 42°C cells were spread on Spc plates at 30°C, the number of viable cells steadily decreased because of the loss of the plasmid carrying *infA* (Table 3). The colonies differed greatly in size, some being very small, presumably because of a slow recovery rate. When 42°C cells were plated at 42°C, very few colonies were seen, but these colonies were uniformly large. The numbers of such colonies increased substantially from 5 to 8 h of liquid growth at 42°C, from 5 to 100 (Table 3). These colonies most likely represent the phenotypic revertants that contribute to the resumption of growth at 42°C seen in Fig. 3. Since some of the phenotypic revertants may represent altered forms of IF1 or bypass mutants, they will be characterized in greater detail in later experiments. Although complicated by the apparent mutations, it is clear that growth ceases for the bulk of the cells when IF1 levels approach zero.

Demonstration that IF1 is an initiation factor. Polysome profile analyses (7, 11) were performed on HSC994 and HSC1986 grown at 37°C in liquid cultures and harvested after 12 and 9 generations of growth, respectively (Fig. 5). The *polA*

TABLE 3. Viable cell counts during IF1 depletion[a]

Time (h)	Viable cell count for colony		
	Plated at 30°C		Plated at 42°C; +IPTG
	−IPTG	+IPTG	
0	~2,500	~2,500	
2	155	~1,500	
4	100	600	0
5	95	405	5
6	90	295	9
8.5	52	203	100

[a] Strain HSC1986 was grown at 30°C with (+) or without (−) IPTG as described in the legend to Fig. 3 and then shifted to 42°C for the times indicated in the table. Aliquots at an optical density at 600 nm of 0.004 (ca. 2,500 cells) were plated onto Spc-IPTG plates, and colonies were counted after 48 h.

phenotype of UB946 is intermediate at 37°C. This temperature was chosen to avoid alterations of protein synthesis due to heat shock. The apparent rate of loss of the plasmid pCC-1 is lower at 37°C, as made evident by the fact that the growth rate does not begin to decrease until about eight generations of growth (data not shown) compared with a decrease at about five generations of growth at 42°C. The polysome profiles of the parental strain, HSC994 (Fig. 5A), and the *infA*-disrupted strain, HSC1986 (Fig. 5B), were analyzed for differences in the height of the 30S, 50S, and 70S peaks and in the amplitude and size of the polysomes. As seen in Fig. 5A, most of the ribosomes in HSC994 are present in polysomes, indicating active protein synthesis. In contrast, the HCS1986 lysate shows an increase in the 70S peak and virtually no polysomes (Fig. 5B). The HSC1986 profiles indicate a decrease in the rate of initiation of protein synthesis upon IF1 depletion. The results strongly suggest that IF1 plays a stimulatory role in the initiation phase of translation.

DISCUSSION

It is generally agreed that three initiation factors, IF1, IF2, and IF3, are involved in the initiation phase of protein

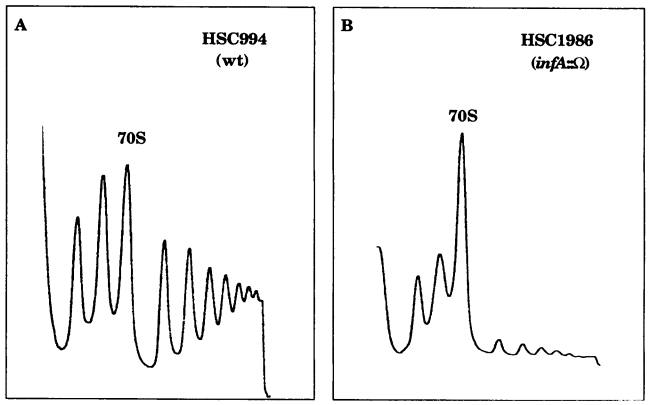

FIG. 5. Sucrose density gradient centrifugation analysis of polysomes in normal and IF1-depleted cells. HSC994 (parental strain, panel A) and HSC1986 (IF1-depletion strain, panel B) were grown in LB medium and harvested after 12 and 9 generations of growth at 37°C, respectively. The cells were pelleted by centrifugation, lysed, and prepared for sucrose density gradient analysis (15 to 40%) as described elsewhere (7, 12). Sedimentation was from left to right. The peak corresponding to 70S ribosomes is labeled 70S.

synthesis in prokaryotes. The three factors were originally identified by their stimulation of in vitro assays for initiation, such as the mRNA-dependent binding of fMet-tRNA$_f$ to 30S or 70S ribosomes (for reviews, see references 13 and 14). Each factor stimulates such assays either marginally or up to 10- to 20-fold, depending on the specific assay and the conditions employed. Dependence on IF1 tends to be the weakest of the factors, and there has been some doubt that IF1 is truly involved in translation. Reliance exclusively on in vitro reactions for identification and characterization of the initiation factors carries the risk that an observed stimulation might be an artifact of the nonphysiological assay system. It is therefore highly desirable to demonstrate in intact cells the requirement of each of the factors for protein synthesis. Even though all three initiation factors are present in equimolar amounts in *E. coli* and their levels are coordinately regulated with ribosomes as a function of growth rate (15), such circumstantial evidence for their involvement in translation is not conclusive.

Conditional mutations in *infB* and *infC*, coding for IF2 and IF3, respectively, have been isolated and characterized (5, 27). Bacterial growth greatly diminishes or ceases at nonpermissive temperatures, thereby indicating that these proteins are essential. Furthermore, an *E. coli* strain in which the IF2 level can be regulated by the concentration of the *lac* operon inducer IPTG was constructed (7). At low IPTG concentrations, the IF2 level is reduced to less than 25% of wild type, growth rate and protein synthesis diminish, and polysomes become smaller, thus demonstrating in vivo that IF2 is involved in the initiation phase of protein synthesis. Similar results with IF3 have been obtained recently (23a). In contrast, no mutations in *infA* encoding IF1 are known and proof of its requirement for translation has been lacking.

The cloning and characterization of the *infA* gene (8, 25) provide the means for establishing whether the protein is essential for cell growth. We report here the construction of a strain in which the chromosomal copy of *infA* is disrupted by a gene providing a selectable marker and a wild-type *infA* gene is supplied on a plasmid whose replication is dependent on *polA*. By using a temperature-sensitive *polA* strain, plasmid replication can be blocked at the nonpermissive temperature (42°C), thereby leading to the loss of *infA* and subsequent depletion of IF1. When IF1 levels fall, polysomes become smaller and cell growth decreases. The results demonstrate that IF1 is essential for cell growth and suggest that it is involved in the initiation phase of protein synthesis. However, because the cells being depleted of IF1 are a mixture of cells which have lost the IF1-expressing plasmid at different times following the temperature shift, it is impossible to demonstrate the effects of a partial loss of IF1 in a homogeneous population of cells. Further experiments are needed to construct and characterize a strain in which *infA* can be regulated precisely at different levels of expression. The results reported here with *infA* expressed from the *tac* promoter indicate that IF1 levels are not limiting in wild-type cells, since depletion in strain HSC1986 (grown in the absence of IPTG) to 50% of normal does not effect the growth rate. A similar result was obtained with IF2 (7). More severe depletion of IF1 will be required to assess changes in growth, protein synthesis, and polysome profiles.

The availability of a strain with *infA* disrupted allows us to screen for mutations in *infA* carried on the plasmid. Furthermore, the appearance of mutant cells that grow on plates incubated at 42°C may be due to bypass mutations in other genes involved in translation. Future investigations along these lines may provide insight into the functional role of IF1 in the cell.

ACKNOWLEDGMENTS

We are grateful to L. Isaksson for many stimulating discussions and for the generous gifts of strains. We thank A. Björnsson for helpful advice and for kindly providing the strain UB947 and the source of the Ω fragment, pHP45Ω. We thank R. Provost for helpful advice and for providing M13mp11Cat.

This work was supported by grants NP70, GM40082, and INT8612363 from the American Cancer Society, the National Institutes of Health, and the National Science Foundation, respectively. H.S.C. was supported by an NIH Individual National Research Service Award, GM10914.

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