JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6705–6713 Vol. 179, No. 21
0021-9193/97/$04.00+0

IF3-Mediated Suppression of a GUA Initiation Codon Mutation in the *recJ* Gene of *Escherichia coli*

TIMOTHY J. HAGGERTY† AND SUSAN T. LOVETT*

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254-9110

Received 5 June 1997/Accepted 30 August 1997

A mutational change of the initiation codon to GUA was found to reduce, but not abolish, expression of the *recJ* gene of *Escherichia coli*. Specific mutations in translational initiation factor IF3 have been isolated as second-site suppressors of this GUA initiation codon mutation. One of these, *infC135*, with an arginine-to-proline change at amino acid 131, completely restores a wild-type phenotype to *recJ* GUA initiation codon mutants and acts in a semidominant fashion. The *infC135* mutation increased expression of RecJ from the GUA mutant but had no effect on the normal GUG start. The *infC135* mutation also abolished autoregulation of IF3 in *cis* and in *trans*. The behavior of this IF3 mutant suggests that it has specifically lost its ability to abort initiation from poor initiation codons such as GUA of *recJ* and the AUU of *infC*. Because of the impact of IF3 on *recJ*, a recombination and repair gene, this role of IF3 must be general and not restricted to translation genes. The dominance of *infC135* suggests that the other functions of IF3, for instance its ability to bind to 30S ribosomes, must remain intact. Although the ability to discriminate among initiation codons has been lost in the *infC135* mutant, translational initiation was still restricted to the normal initiation site in *recJ*, even in the presence of a closely juxtaposed alternative initiation codon. Because the *recJ* gene lacks a canonical Shine-Dalgarno sequence, other unknown features of the mRNA must serve to specify the initiation site.

Prokaryotic translation initiation requires several protein factors (23, 24, 27). The translation initiation factor IF3 is believed to be essential for selection of the initiation codon by monitoring the pairing between the initiator fMet-tRNA and the initiation codon (26, 28). If this pairing is good, as in an AUG start codon, IF3 stabilizes the translation initiation complex and translation initiation proceeds. If the codon is suboptimal, IF3 destabilizes this complex and translation initiation is disfavored (4, 22, 43). This function is dependent on the ratio of IF3 to ribosomes in vivo (31). If the ratio is high, IF3 promotes discrimination between initiation codons. If the ratio falls so that IF3 is limiting, translational initiation from non-AUG codons will be increased. This phenomenon allows IF3 to autoregulate its own translation through use of its unique AUU initiation codon (7–9). IF3 therefore controls translation fidelity by preventing inappropriate initiation.

IF3 serves several other functions in translation initiation. IF3 favors the dissociated state of ribosomes by binding to the 30S subunit and preventing association with the 50S subunit until a stable translation initiation complex is formed (20, 51). IF3 may also recruit IF1 and IF2 to the 30S subunit and destabilize the binding of noninitiator tRNAs (23).

We have previously shown that certain mutations in IF3 could genetically suppress two particular alleles of the *recJ* gene of *Escherichia coli* (25). One such suppressor was initially designated as *srjA5*, for suppressor of *recJ*; the allele has been renamed *infC135*. The *recJ* gene encodes a 5′-to-3′ single-strand DNA-specific exonuclease (35) involved in *recBC*-independent pathways of recombination (33, 37) and pathways for methyl-directed mismatch repair (13) and base excision repair (16). The suppressible *recJ* alleles *recJ153* and *recJ154* confer a phenotype which is somewhat less extreme than a *recJ* insertion allele, *recJ284*::Tn*10*, which we presume to be a null mutation (34). RecJ protein is normally poorly expressed in *E. coli* cells, with as little as five molecules per cell (35). Expression of *recJ* is low compared to that of the upstream gene of the same operon, *dsbC* (which encodes a disulfide bond isomerase [39]), and is, in part, limited by a poor ribosome binding site for the gene. Introduction of a consensus ribosome binding site upstream of *recJ* increases its expression 15-fold (35).

This investigation seeks to clarify the mechanism by which mutations in IF3 such as *infC135* suppress *recJ153* and *recJ154*. We have determined that both *recJ153* and *recJ154* mutations involve a change of the GUG initiation codon of *recJ* to GUA. This mutation decreases, although it does not abolish, expression of functional RecJ protein. The *infC135* suppressor mutation increases the levels of *recJ* expression from the GUA initiation codon approximately threefold but has little effect on initiation from GUG. This effect is consistent with the hypothesis that the *infC135* mutant has lost its ability to abort translational initiation from suboptimal codons such as GUA. In this paper, we discuss the location of this class of suppressor alleles within the recently published structure of IF3 domains. The pleiotropic genetic effects of the *infC135* mutant are also discussed.

MATERIALS AND METHODS

Strains and media. The *E. coli* strains used in this study and their derivations are listed in Table 1. Routine cultures were done with Luria-Bertani (LB) medium (56), with plate media containing 2% agar. Minimal medium was based on 56/2 salts medium (56) supplemented with 0.2% glucose, 1 mg of thiamine per ml, and 50 μg of the appropriate required amino acids per ml. The antibiotics ampicillin, tetracycline, kanamycin, and chloramphenicol were used at concentrations of 100, 15, 60, and 30 μg/ml, respectively. The UV resistance of strains was determined by exposure of plated cells to UV light as previously described (37). Growth rates were determined by growth of cells at 37°C in LB medium and measurement of the absorbance of the culture at regular intervals with a Klett meter.

* Corresponding author. Mailing address: Rosenstiel Basic Medical Sciences Center, MS029, Brandeis University, Waltham, MA 02254-9110. Phone: (617) 736-2497. Fax: (617) 736-2405. E-mail: lovett@hydra.rose.brandeis.edu.

† Present address: Division of Rheumatology, Johns Hopkins University, Baltimore, MD 21205.

TABLE 1. *E. coli* K-12 strains used in this study

Strain	Genotype	Source or reference
AB1157 and derivatives		
AB1157	F$^-$ *thi-1 hisG4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 rfbD1 ara-1 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31 rac*	3
JC10287	+ Δ(*srlR-recA*)304	15
JC12123	+ *recJ284*::Tn*10*	34
JC13021	+ *recB21 recC22 sbcA23 recJ153*	34
JC13024	+ *recB21 recC22 sbcA23 recJ154* (=*recJ153*)	34
JC13075	+ *recB21 recC22 sbcA23 recJ153 infC135*	25
JC14223	+ *infC135 zdh*::Tn*10*	25
JC14225	+*recB21 recC22 sbcA23 infC135 zdh*::Tn*10*	25
JC8679	+ *recB21 recC22 sbcA23*	19
RDK1656	+ *recB21 recC22 sbcA23 recJ284*::Tn*10*	36
RDK1899	+ *sbcB15*	R. Kolodner
STL1025	+ lacZU118 lacI3098::Tn*10-kan*	Km^r Lac$^-$ transductant P1.CAG18420 × AB1157
STL1057	+ *infC135 zdh*::Tn*10 lacZU118 lacI3098*::Tn*10-kan*	Km^r Lac$^-$ transductant P1.CAG18420 × JC14223
STL1058	+ *recB21 recC22 sbcA23 lacZU118 lacI3098*::Tn*10-kan*	Km^r Lac$^-$ transductant P1.CAG18420 × JC8679
STL1059	+ *recB21 recC22 sbcA23 infC135 zdh*::Tn*10 lacZU118 lacI3098*::Tn*10-kan*	Km^r Lac$^-$ transductant P1.CAG18420 × JC14225
STL1273	+ *recJ284*::Tn*10 sbcB15*	Tc^r transductant P1.JC12123 × RDK1889
STL1455	+ *cysC3152*::Tn*10-kan infC135 zdh*::Tn*10*	Km^r Cys$^-$ transductant P1 CAG12182 × JC14223
STL1607	+ Δ(*xseA-guaB*) *zff3139*::Tn*10-kan sbcB15 recJ284*::Tn*10*	Km^r Gua$^-$ transductant P1.STL1606 × STL1273
STL1670	+Δ(*srlR-recA*)304 *infC135 zdh*::Tn*10*	Cys$^+$ Rec$^-$ transductant P1 JC10287 × STL1455
STL2359	+ *lacZU118 lacI3098*::Tn*10-kan* λBSX3[a]	λBSX3 lysogen of STL1025
STL2360	+ *infC135 zdh*::Tn*10 lacZU118 lacI3098*:Tn*10-kan* λBSX3	λBSX3 lysogen of STL1057
STL2361	+ *recB21 recC22 sbcA23 lacZU118 lacI3098*::Tn*10-kan* λBSX3	λBSX3 lysogen of STL1058
STL2362	+ *recB21 recC22 sbcA23 infC135 zdh*::Tn*10 lacZU118 lacI3098*::Tn*10-kan* λBSX3	λBSX3 lysogen of STL1059
Other		
CAG12182	F$^-$ *cysC3152*::Tn*10-kan*	C. Gross
CAG18420	F$^-$ *lacZU118 lacI3098*::Tn*10-kan*	C. Gross

[a] λBSX3 is a *c*I857 *nin5 infC-lacZ* protein fusion (8).

DNA techniques. The plasmids used in this study and their descriptions are listed in Table 2. Chromosomal DNA was isolated by a lysozyme and proteinase K lysis procedure (2). Restriction enzyme digestion and ligation were performed as recommended by the supplier (New England Biolabs, Inc.). DNA fragments were resolved by agarose gel electrophoresis, and the appropriate size class was purified by glass bead extraction (with a kit from U.S. Biochemical-Amersham). Colony hybridizations were performed with a digoxigenin-labeled probe (Boehringer Mannheim) consisting of the *Eco*RI-*Sal*I *recJ*$^+$ fragment from plasmid pTJH51. Plasmids were transformed into cells by electroporation (17) or the polyethylene glycol-dimethyl sulfoxide transformation procedure (11).

For DNA sequencing, single-stranded DNA was prepared from plasmids carrying the f1 origin by infection of transformants with helper phage M13 K07 (U.S. Biochemical-Amersham). Dideoxy sequencing with the appropriate primers and Sequenase 2.0 was then carried out as recommended by the manufacturer (U.S. Biochemical-Amersham). Sequence changes were determined by direct comparison to *recJ*$^+$ from reactions run in parallel. Site-directed mutants

TABLE 2. Plasmid constructions used in this study

Plasmid	Derivation
pBluescript KS+ derived (Stratagene, Inc.; high copy number, Ap^r)	
pTJH51	*Eco*RI-*Sal*I *recJ*$^+$ fragment from pJC765 (32) subcloned into *Eco*RI-*Xho*I-digested pBluescript KS+
pTJH52	*Eco*RI-*Sal*I cloned from JC13024 (*recJ154*) into *Eco*RI-*Sal*I-digested pBluescript KS+
pTJH53	*Eco*RI-*Sal*I cloned from JC13021 (*recJ153*) into *Eco*RI-*Sal*I-digested pBluescript KS+
pTJH55	*Sac*II-*Eco*RI *dsbC*$^+$ fragment from pJC763 subcloned into pTJH51
pTJH56	*Sac*II-*Eco*RI *dsbC*$^+$ fragment from pJC763 subcloned into pTJH53
pTJH61	(Δ*dsbC* Δ2T *recJ*$^+$) site-directed mutant of pTJH51
pTJH65	(*dsbC*$^+$ Δ2T *recJ*$^+$) site-directed mutant of pTJH55
pTJH63	(Δ*dsbC* Δ2T *recJ153*) site-directed mutant of pTJH53
pTJH66	(*dsbC*$^+$ Δ2T *recJ153*) site-directed mutant of pTJH56
pTJH73	[*dsbC*$^+$ Δ2T *recJ2060*(TGA)] site-directed mutant of pTJH66
pBR322 derived (medium copy number, Ap^r)	
pTJH10	(*infC135* = *srjA5*) (25)
pTJH16	(*infC*$^+$) (25)
pGP1-2 derived (*c*I857-controlled T7 RNA polymerase gene)	
pGP1-2	Km^r (54)
pTJH30	*Bsa*BI-*Xmn*I *cat* fragment from pACYC184 (10) inserted into *Sma*I site of partially digested pGP1-2; Cm^r

were constructed with single-strand DNA templates and an in vitro mutagenesis kit supplied by U.S. Biochemical. The primers used were 5′ GGTAAATAACG CGTGAAACAACAG (*recJ*$^+$) and 5′ GGTAAATAACGCGTAAAACAACAG (*recJ153*). Mutants were initially screened for acquisition of an *Mlu*I restriction enzyme cleavage site, and the mutations were then confirmed by sequencing. The primer used for site-directed mutagenesis to create a UGA stop codon at the GUA was 5′ GGTAAATAACGCTGAAAACAACAG. The mutant was initially identified by loss of the *Mlu*I restriction site, and the mutation was then confirmed by sequencing.

Protein analysis and expression. RecJ protein expression was induced from the T7 ϕ10 promoter by a two-plasmid system in which the T7 RNA polymerase gene on plasmid pGP1-2 or pTJH30 is inducible by temperature shift (54). Transformant cultures carrying the overexpression plasmids were grown to an optical density at 600 nm of 0.6 at 32°C in LB medium-ampicillin-kanamycin for pGP1-2 or LB medium-ampicillin-chloramphenicol for pTJH30. The cells were then collected by centrifugation and resuspended in 56/2 minimal medium. After growth for 1 h, cells were shifted to 42°C for 20 min. Rifampin (Sigma) was added to a final concentration of 50 μg/ml, and growth at 42°C was continued for 20 min. Half of the cells at this point were collected by centrifugation and resuspended in 1/10 volume of TS buffer (10 mM Tris-HCl [pH 7.5], 10% sucrose) for cell extracts for nuclease assays. The rest of the cells were then shifted to 30°C and labeled with 11 μCi of [^{35}S]methionine (800 Ci/mmol; Dupont-NEN) for 5 min. Cells were collected by centrifugation and resuspended in 1/10 volume of sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (30).

Proteins were resolved by SDS-PAGE (30). The amounts of [^{35}S]methionine-labeled proteins were quantitated with a National Diagnostics PhosphorImager. Amounts of RecJ are expressed relative to the amount of the DsbC protein, expressed upstream in the *recJ* operon. Amounts of IF3 were normalized to the amount of plasmid-encoded β-lactamase.

Crude cell extracts for nuclease assays were prepared with lysozyme as previously described (35). Nuclease activity was measured by the conversion of heat-denatured ^{3}H-labeled T7 phage DNA into acid-soluble fragments as previously described (35). One unit corresponds to conversion of 1 nmol of acid-soluble product in a 20-min reaction at 37°C. Protein concentrations were measured by the method of Bradford (6) with the standard reagent purchased from Bio-Rad with bovine serum albumin as a standard.

Expression of *infC* was measured from λBSX3 lysogens (8) carrying an *infC-lacZ* gene fusion, which was kindly provided by M. Springer. β-Galactosidase activity was assayed with *o*-nitrophenyl-β-D-galactopyranoside substrate (ICN Biochemicals) and is expressed in Miller units (38).

RESULTS

Identification and characterization of an initiation codon mutation causing reduced RecJ expression. Previous genetic studies identified two mutant alleles of *recJ*, *recJ153* and *recJ154*, which caused UV-sensitive and recombination-deficient phenotypes somewhat less than null insertion alleles of *recJ* did (29, 34). This weaker phenotype than that of the presumed null alleles indicates that some residual function is retained in *recJ153* and *recJ154* mutants. Suppressor mutations mapping to the gene for translation IF3, *infC*, reverse the defective phenotypes conferred by *recJ153* and *recJ154* but no other *recJ* allele (25). We determined the DNA sequences of both *recJ153* and *recJ154*. Both contain single mutations in the third base of the *recJ* gene putative initiation codon, changing a GUG to a GUA (Fig. 1). Both mutants were initially isolated from a single mutant screen and may be derived from a single parent (29). For this reason and to simplify the discussion, we will refer hereafter to both alleles as *recJ153*.

The *recJ* gene is in a transcriptional unit (Fig. 1) downstream of *dsbC* (previously denoted *xprA*), which encodes a periplasmic disulfide bond isomerase (32, 39, 49). The putative GUG initiation codon for *recJ* is five nucleotides downstream of the termination codon for *dsbC*. The *recJ* gene lacks a consensus ribosome binding (Shine-Dalgarno [SD]) sequence (36), and the RecJ protein is expressed normally at extremely low levels in the cell (35, 36). The location of the GUG codon immediately adjacent to the upstream gene places it in a favorable position for translational coupling of RecJ protein with the upstream DsbC peptide. In the absence of a good ribosome binding site, translation of *recJ* may occur by reinitiation, if the ribosome does not dissociate after termination of *dsb* translation. Addition of a synthetic ribosome binding site seven nucleotides upstream of this GUG increases RecJ protein expression dramatically and gives a protein with a size and activity indistinguishable from those of wild-type RecJ.

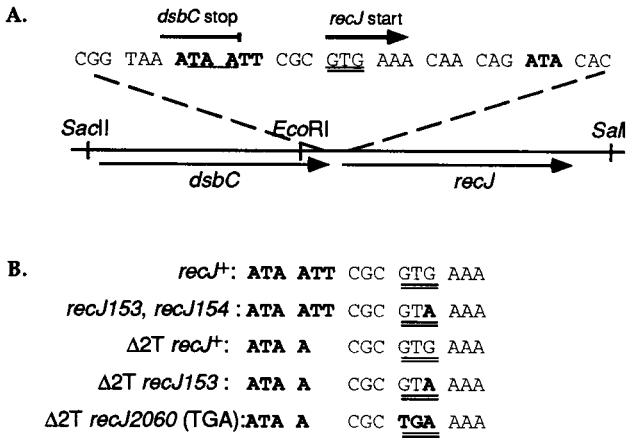

FIG. 1. *recJ* initiation region. (A) Diagram of *recJ* translational initiation region. The stop codon of the previous open reading frame, *dsbC*, and the normal start codon for *recJ* are underlined. Shown in boldface are other potential initiation codons. (B) Sequence changes in several mutant derivatives.

Several plasmids were constructed to express the wild-type *recJ* gene and the *recJ153* allele from the strong T7 ϕ10 promoter (53). Derivatives with and without the upstream *dsbC* gene were made. Expression of the T7 RNA polymerase gene from a second plasmid, pGP1-2, or its derivative, pTJH30, is controlled by the lambda repressor *c*I*ts*857 (54). The natural *recJ* promoters are weak (36), and these plasmids allow us to induce higher levels of mRNA than are normally expressed. In addition, we can regulate transcription of the *recJ*$^+$ and *recJ153* genes, with repression at low temperatures and induction at elevated temperatures. Such induction leads to at least a 20-fold increase in RecJ protein expression (35). In the presence of rifampin, an inhibitor of *E. coli* RNA polymerase, the proteins expressed from the T7 transcripts can be specifically labeled.

We examined the amount of RecJ protein and single-strand DNA-specific nuclease activity expressed from the wild-type *recJ*$^+$ gene and the *recJ153* mutant allele when high levels of transcription were induced from T7 promoter plasmid constructs. This should reveal differences in translation efficiency between the *recJ*$^+$ and *recJ153* genes. Similar quantitation with the natural promoter of *recJ* is not possible because of the very low level of expression of the *recJ* gene.

Expression was induced from the *recJ*$^+$ and *recJ153* genes from the T7 ϕ10 promoter by growth at high temperature, and proteins were labeled with [^{35}S]methionine in the presence of rifampin. The *recJ153* allele encodes a protein with a size identical to that encoded by *recJ*$^+$, albeit at greatly reduced levels (Fig. 2 [also see Fig. 4]). Expression of the upstream *dsbC* gene product was the same for both the *recJ*$^+$ and *recJ153* plasmid-carrying strains. Quantitation of the labeled protein bands by a PhosphorImager reveals that expression of *recJ*$^+$ is at least 16-fold higher than that of *recJ153*.

In addition, we measured RecJ expression by assaying single-strand DNA-specific nuclease activity. The extracts from cells expressing the *recJ153* plasmid gene have nuclease activity consistently 5- to 26-fold lower than those from cells expressing *recJ*$^+$ (Table 3). In addition, we observed that the presence of the upstream *dsbC* gene increases expression of *recJ*$^+$ nuclease

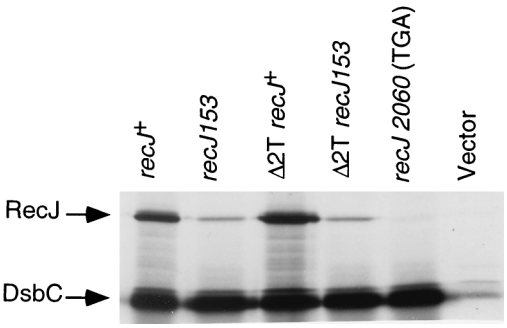

FIG. 2. Effect of mutations within or near the initiation codon on RecJ expression in *infC*$^+$ cells. An SDS-PAGE autoradiogram of [^{35}S]methionine-labeled proteins expressed from various plasmids is shown. RecJ expression was driven by the strong T7 ϕ10 promoter in the presence of rifampin at 42°C and a coresident plasmid, pGP1-2. The plasmid-expressed allele is indicated and includes *recJ*$^+$ (pTJH55), *recJ153* (pTJH56), Δ2T *recJ*$^+$ (pTJH65), Δ2T *recJ153* (pTJH66), Δ2T *recJ2060* (TGA) (pTJH73), and vector (pBluescript KS+) in the RDK1656 genetic background.

activity about fourfold, as measured in this assay. It is possible that this is the result of increased transcription from the *dsbC*-containing constructs or improved mRNA stability. Because no promoters for *recJ* were found in this region (36), we believe it is more likely that the increase represents improved translation of *recJ* in the presence of translation of the upstream *dsbC* gene. The spacing of the two reading frames may be close enough to allow translational reinitiation (50).

If the defect in the *recJ153* mutants lies in inefficient translation initiation, then elevation of mRNA levels artificially should relieve the mutant phenotype. An insertion mutation of *recJ* on the chromosome confers UV sensitivity in a strain also deficient for exonucleases I and VII (55). At 30°C, when transcription from the T7 promoter was repressed, *recJ153* plasmids showed little ability to complement the *recJ* mutation on the chromosome (Fig. 3A). Inclusion of the *dsbC* gene upstream of *recJ153* promoted higher levels of resistance than that of the constructs containing only the *recJ153* gene itself. However, after induction at 42°C, all of the *recJ153*-containing strains exhibited enhanced levels of UV resistance (Fig. 3B) and thus complemented the *recJ* insertion allele.

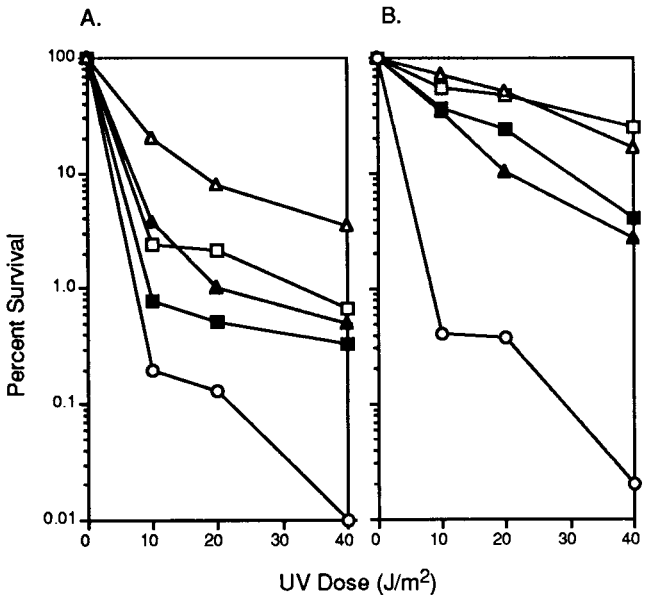

FIG. 3. Ability of *recJ153* to complement *recJ284*::Tn*10*, with and without expression from a strong exogenous promoter, determined with UV survival assays. Expression of various plasmid-encoded *recJ* alleles from the T7 ϕ10 promoter was controlled by heat induction of the T7 RNA polymerase from the coresident plasmid, pTJH30. Plasmid transformants of strain STL1607 (*recJ284*::Tn*10*) were grown to an optical density at 600 nm of 0.6 in selective medium. Cultures were then split and grown for 1 h at the indicated temperature. (A) UV survival of uninduced cells at 30°C. (B) UV survival of induced cells grown in parallel at 42°C. ■, pTJH53 (Δ*dsbC recJ153*); □, pTJH63 (Δ*dsbC* Δ2T *recJ153*); ▲, pTJH56 (*dsbC*$^+$ *recJ153*); △, pTJH66 (*dsbC*$^+$ Δ2T *recJ153*); and ○, pBluescript KS+ (vector control).

TABLE 3. Single-strand DNA-specific nuclease activity

Plasmid[a]	Expressed genes	Sp act of single-strand nuclease in cells carrying allele[b]:	
		infC$^+$	*infC135*
recJ$^+$ vs *recJ153*, with and without *dsbC*			
pTJH55	*dsbC*$^+$ *recJ*$^+$	4.8	4.8
pTJH51	Δ*dsbC recJ*$^+$	1.2	1.1
pTJH56	*dsbC*$^+$ *recJ153*	0.18	0.37
pTJH53	Δ*dsbC recJ153*	0.26	0.29
With Δ2T mutation			
pTJH65	*dsbC*$^+$ Δ*2T recJ*$^+$	5.7	3.5
pTJH61	Δ*dsbC* Δ*2T recJ*$^+$	2.2	1.4
pTJH66	*dsbC*$^+$ Δ*2T recJ153*	0.14	1.2
pTJH63	Δ*dsbC* Δ*2T recJ153*	0.12	0.32

[a] Plasmid constructs are derived from pBluescript KS+.

[b] Nuclease activity was measured for crude extracts of cells expressing various *recJ* alleles driven from a plasmid T7 ϕ10 promoter controlled by heat induction of the T7 RNA polymerase from the coresident plasmid pGP1-2. Induction was carried out by shifting the cells to 42°C for 1 h in the presence of rifampin. Specific activity is given in units of single-strand-specific exonuclease per microgram of protein. A unit of single-strand exonuclease is measured as nanomoles of radiolabeled T7 [^{3}H]DNA released at 37°C in 20 min. The value of the vector has been subtracted from each point as background. Data represent the average of three experiments. The transformant strains were JC13024 and JC13075, carrying *infC*$^+$ and *infC135*, respectively.

We conclude from the previous analysis of the *recJ153* mutation that changing the translation initiation codon from GUG to GUA reduces the translational efficiency of *recJ* approximately 20-fold. Surprisingly, a low level of RecJ protein expression from the *recJ153* mutant gene was detected. Genetic experiments also suggest that there must be either residual expression of *recJ* from the GUA codon or use of another poor initiation codon, because the phenotype of *recJ153* is not as severe as that of presumed null mutants and increased transcription of *recJ153* relieves its mutant phenotype.

Use of GUA as an initiation codon. Although GUA has not been reported to function as an initiation codon, we observed a substantial amount of protein expressed from *recJ153* plasmids, of a size indistinguishable from that of wild-type *recJ*. However, three other possible weak initiation codons occur within the vicinity of the GUA codon (Fig. 1). Two of these are an AUU and AUA, one and two codons upstream of GUA, respectively. Translational initiation at these codons is poor but has been observed in certain cases (44, 47) and is enhanced by mutations of IF3 (52). To investigate the possibility that translation initiates at these upstream codons instead of GUA, we constructed site-directed mutations that destroyed one of the possible starts and placed the other out-of-frame to the RecJ open reading frame. This was accomplished by removal of the two thymines immediately downstream of the *dsbC* stop

codon (Fig. 1), a mutation which we refer to as "Δ2T." The Δ2T mutation was introduced into the T7 promoter plasmids expressing *recJ*$^+$ and those expressing the mutant *recJ153* gene, both with and without the upstream *dsbC* gene present.

Nuclease activity measured in cell extracts from Δ2T-expressing strains (Table 3) was not grossly altered from that seen previously for the comparable constructions. This was true for both *recJ*$^+$ and *recJ153* plasmid-encoded alleles. If anything, the Δ2T mutation led to higher levels of RecJ expression from the *recJ*$^+$ plasmids. Because this mutation is in the region of the mRNA that normally interacts with the rRNA during translation, it may fortuitously increase the efficiency of ribosome binding. Or, because the deletion of two nucleotides places the GUG initiation codon of *recJ* closer to the last codon of *dsbC*, it may improve the translational coupling between the two genes.

The effect of the site-directed Δ2T mutation on RecJ complementation was also investigated with the UV survival assay. The Δ2T mutants of the *recJ153* plasmids were slightly more UV resistant than their *recJ153* counterparts at both 30 and 42°C (Fig. 3). (Deletion of the two nucleotides did not have a detectable effect in a control *recJ*$^+$ construct [data not shown].) Therefore, the residual genetic function retained by the *recJ153* allele does not require the use of the putative AUA and AUU codons immediately upstream of the GUA mutations.

Three codons downstream of the GUA codon is an additional AUA codon, a potential initiation site (Fig. 1A). To assay the contribution of this potential initiation codon, a mutation converting the potential GUA initiation codon to a UGA stop codon, *recJ2060*, was introduced (Fig. 1B). This construct also carried the 2ΔT mutation to eliminate the possibility that the upstream AUA and AUU codons could be used for initiation. Labeling of proteins expressed from this construct showed that the UGA mutation reduces RecJ expression to undetectable amounts (Fig. 2). Therefore, the downstream AUA does not contribute significantly to RecJ expression. Translation initiation occurs, rather, at the GUA codon in the *recJ153* mutant gene.

Increased expression of *recJ153* in *infC135* strains. RecJ protein levels from the *recJ*$^+$ gene with a GUG initiation codon and the *recJ153* gene with a GUA were compared in an *infC*$^+$ and *infC135* background. Little to no effect of *infC135* was seen on the expression of wild-type RecJ (Fig. 4). In contrast, the presence of *infC135* increased RecJ expression from the GUA initiation codon mutant threefold (Fig. 4). That *infC135* is increasing expression from the GUA initiation codon itself is confirmed by labeling of site-directed mutants (Fig. 5). Although RecJ protein expression was detected for the Δ2T *recJ153* mutants in which the two potential upstream initiation codons have been shifted out of frame, no detectable expression of RecJ was observed for the Δ2T *recJ2060* (TGA) construct with an additional mutation of the GUA initiation codon to UGA.

A similar effect of *infC135* on *recJ* expression was also seen in nuclease assays of crude cell extracts. Our results indicated a two- to eightfold increase in nuclease activity in *infC135* cells expressing *recJ153* compared to that in those that were *infC*$^+$ (Table 3). One exception was the Δ*dsbC recJ153* construct, which expressed the smallest amount of RecJ and showed little effect of *infC*; low levels of nuclease close to background may have obscured the *infC135* effect. No significant difference was seen in nuclease activity between *infC135* or *infC*$^+$ cells expressing *recJ*$^+$ (Table 3).

Defective IF3 autoregulation in *infC135* mutants. IF3 is known to autoregulate its own expression by way of the unique initiation codon AUU (7, 8, 21, 46). The *infC135* allele was investigated for a potential effect on initiation at the AUU of *infC* and IF3 autoregulation.

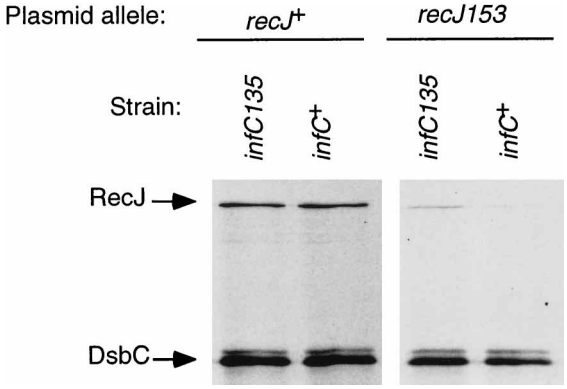

FIG. 4. Effect of *infC135* on RecJ protein expression. Results of SDS-PAGE of [^{35}S]methionine-labeled proteins (20% acrylamide gel) are shown. RecJ expression was driven by the strong T7 ϕ10 promoter and controlled by heat induction of the T7 RNA polymerase from the coresident plasmid pGP1-2 in the presence of rifampin. Extracts were prepared from strains containing pTJH55 (*dsbC*$^+$ *recJ*$^+$) (lanes 1 and 2) or from strains containing pTJH56 (*dsbC*$^+$ *recJ153*) (lanes 3 and 4). The chromosomal genetic background was JC13024 (*infC*$^+$) (lanes 1 and 3) or JC13075 (*infC135*) (lanes 2 and 4). The positions of RecJ and DsbC are indicated. With a PhosphorImager, the amount of labeled RecJ was quantitated relative to the amount of DsbC: 0.179 ± 0.046 for the *recJ*$^+$ allele in an *infC*$^+$ strain, 0.223 ± 0.101 in an *infC135* mutant; 0.011 ± 0.005 for the *recJ153* allele in an *infC*$^+$ background, and 0.034 ± 0.017 in an *infC135* background (average from 12 experiments).

The levels of IF3 labeled from a plasmid containing *infC135* are two- to fivefold greater than those of a plasmid encoding wild-type *infC* (Fig. 6). IF3 was expressed from either pTJH10 (*infC135*) or pTJH16 (*infC*$^+$). The levels of plasmid-encoded IF3 relative to β-lactamase were 0.40 ± 0.091 from an *infC135* plasmid and 0.19 ± 0.066 from a *infC*$^+$ plasmid (average from four experiments) in a strain carrying *infC*$^+$ on the chromosome (JC10287). The relative levels of IF3 were 0.59 ± 0.082 from an *infC135* plasmid and 0.11 ± 0.068 from an *infC*$^+$

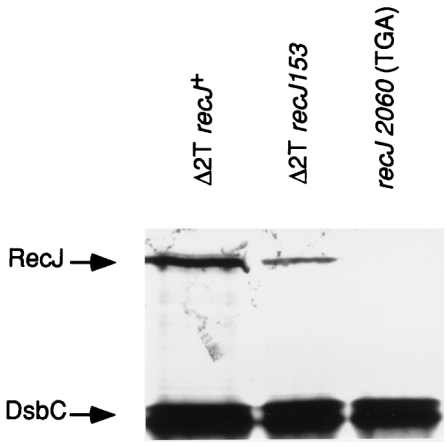

FIG. 5. Effect of site-directed mutations in potential initiation codons on RecJ expression in *infC135* mutant cells. SDS-PAGE autoradiogram of [^{35}S]methionine-labeled proteins expressed from various plasmids. RecJ expression was driven by the strong T7 ϕ10 promoter in the presence of rifampin at 42°C and a coresident plasmid, pGP1-2. The plasmid-expressed allele is indicated and includes Δ2T *recJ*$^+$ (pTJH65), Δ2T *recJ153* (pTJH66), and Δ2T *recJ2060* (TGA) (pTJH73) in the JC13075 (*infC135*) genetic background.

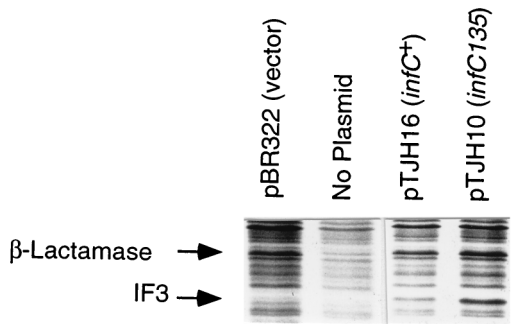

FIG. 6. Increased IF3 expression in *infC135* strains. An autoradiogram of proteins labeled with [^{35}S]methionine resolved on a 20% polyacrylamide gel is shown. Extracts were made from the *recA* strain JC10287 carrying a pBR322-derived plasmid and the *infC* allele indicated. Relative positions of β-lactamase and IF3 are indicated by arrows.

plasmid (average from three experiments) in the strain carrying *infC135* on the chromosome (STL1670).

This result supports the idea that IF3 encoded by the *infC135* mutant is defective in autoregulation (at least in *cis*) and is therefore expressed at higher constitutive levels. The proteins encoded by both *infC* alleles had similar stabilities (data not shown). Autoregulation by IF3 in *trans* was investigated with an *infC*::*lacZ* fusion which initiates with an AUU codon. The levels of β-galactosidase expressed by this gene fusion were threefold higher in strains that also carried *infC135* than that in strains that carried *infC*$^+$ (Table 4). This increase in utilization of an AUU initiation codon was seen in two genetic backgrounds: a *rec*$^+$ strain and a *recBC sbcA* mutant strain in which the *infC135* mutation was originally isolated. The *infC135* mutant protein therefore acts both in *cis* and in *trans* to increase IF3 expression.

Phenotypic properties of *infC135*. Because the *infC135* mutation appears to affect translational initiation in at least two contexts, *recJ* and the *infC* gene itself, we suspected that this mutation may have global effects on translation initiation and cell physiology. We therefore examined the growth phenotype conferred by this mutation.

The growth rates of *infC135* strains in rich medium were compared to those of *infC*$^+$ isogenic strains. The *infC135* allele extended doubling times somewhat compared to those of wild-type strains. The doubling time of wild-type strain AB1157 was 39 min, while that of the isogenic *infC135* strain JC14223 was 50 min. In a strain that is deficient in recombination (*recBC sbcA*), the doubling time was 44 min, while the isogenic *infC135* strain doubled every 50 min. Although *infC135* strains are viable in rich medium at 37 and 42°C and in minimal medium at 37°C, we observed that the *infC135*-carrying strains are conditionally inviable on minimal media at 42°C (Fig. 7).

TABLE 4. Expression from *infC*::*lacZ* fusions

Strain	Genotype[a]	β-Galactosidase activity [Miller units] (no. of expts)[b]	Fold difference
STL2359	*infC*$^+$	960 ± 190 (5)	1
STL2360	*infC135*	3,400 ± 560 (5)	3.5
STL2361	*recB21 recC22 sbcA23 infC*$^+$	650 ± 130 (6)	1
STL2362	*recB21 recC22 sbcA23 infC135*	1,560 ± 510 (6)	2.4

[a] All strains are derivatives of AB1157 (λBSX3) with additional markers as noted.

[b] Activity was measured for exponential-growth-phase cells grown in minimal media (38). Values are means ± standard deviations.

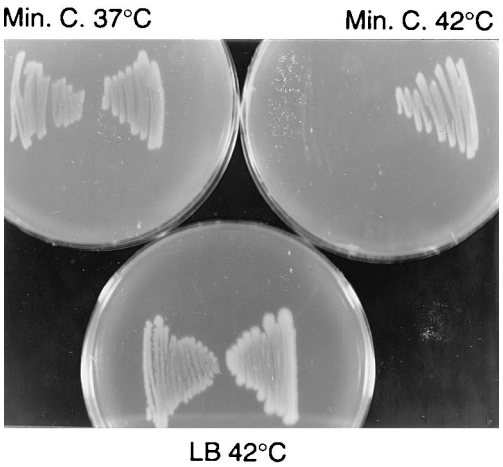

FIG. 7. Conditional growth of the *infC135* mutant. The two upper plates contain minimal synthetic medium. The plate on the upper left was incubated at 37°C, and the one on the upper right was incubated at 42°C. The lower plate contains LB medium and was incubated at 42°C. AB1157 (*infC*$^+$) and JC14223 (*infC135*) were streaked on the left and right halves of each plate, respectively.

DISCUSSION

GUA initiation codon mutants of *recJ*. The change of the initiation codon of *recJ* from GUG to GUA, as in the alleles *recJ153* and *recJ154*, results in a strong recombination- and repair-deficient phenotype in the appropriate strain backgrounds (29, 34). We have demonstrated here that the GUA mutation results in 15-fold reduction in RecJ expression relative to that of the wild-type GUG-initiating gene. Some expression, however, was detected, in agreement with the genetic observations that these mutants do not exhibit phenotypes as severe as those of null insertion mutants. That these mutations reduce but do not eliminate expression is also supported by our finding that increased transcription of the mutant allele suppressed its mutant phenotype. The GUA mutant allele encoded a protein with a size, stability, and activity indistinguishable from those of the wild-type gene.

Three specific mutations in IF3 at two positions, R99H, R99L, and R131P, were previously isolated as suppressors of GUA initiation codon mutations. One of these, *infC135* with an R131P change, exhibited full suppression and acted in a semidominant fashion. Here, we show that this mutation improved RecJ expression from the GUA mutant gene threefold but had no effect on the normal GUG construct. The ability of this mutation to increase translation from suboptimal initiation codons therefore provides a basis for its suppressive effect in vivo. Although we did not observe complete restoration of the wild-type expression levels, a threefold elevation may be enough to ensure that almost every cell has one RecJ molecule. (RecJ protein is normally poorly expressed, with an estimated 5 to 10 molecules per cell [30].)

Confirmation of GUA as an initiation codon. Translation initiation codons fit into three classes (52). Class 1 codons, including AUG, GUG, and UUG, are used efficiently. About 90% of genes initiate with AUG, and 8% initiate with GUG (45, 48). The second class of codons, CUG, AUU, AUC, AUA, and ACG, are inefficient as initiators, giving 1 to 3% of the levels of the AUG initiation codons. IF3 mutations, similar to the *infC135* mutation described here, have been reported to

increase the translation of the second class three- to fivefold, but have little to no effect on the first class (52). The third class of codons, including AGG and AAG, are not used. This hierarchy of initiation codons agrees with the wobble hypothesis (14). Eukaryotic initiation codons and initiation factors also follow similar rules (1, 12).

The use of GUA as an initiation codon has not been previously documented. Change of the initiation codon of *lacZ* to GUA causes protein expression to drop drastically (40). In contrast, for the *recJ* gene, there is a surprisingly high residual level of expression from the GUA initiation codon mutant, at approximately 7% of wild-type levels.

Several other class 2 initiation codons occur in the vicinity of the normal initiation site. Introduction of a two-nucleotide deletion upstream of *recJ*, which disallows initiation from upstream AUA and AUU codons, did not reduce RecJ expression in several assays. Further alteration of the putative GUA initiation codon to a UGA stop codon, however, lowered RecJ expression below the levels of detection in our assays. This, and the fact that no change in the size or stability of RecJ is detected for the GUA initiation mutant, argues strongly for the use of GUA as the initiation codon in this particular instance.

We suggest that GUA falls into the second class of initiation codons. Our observed effect of IF3 mutations on GUA, similar to that seen for other class 2 initiation codons (52), also supports its placement into this class. The relatively efficient utilization of the GUA in *recJ* may be the result of several, yet unknown, features of this initiation site.

A general role for IF3 in initiation codon selection. Autoregulation of IF3 protein is achieved by virtue of its unique initiator codon, AUU (7, 8, 21, 46, 47). In the presence of high levels of IF3, initiation from suboptimal codons such as AUU is aborted. When levels of IF3 fall, initiation of IF3 translation via the AUU becomes more efficient. We report here that *infC135*, isolated as a suppressor to the *recJ* GUA initiation codon mutation, also affects IF3 autoregulation, both in *cis* and in *trans*. The *infC135* mutants have two- to threefold higher levels of IF3 than those of the wild type, similar in magnitude to other mutations of this type (46, 52). This supports the role of IF3 in vivo for the discrimination of the initiation codon.

Our results also suggest the role of IF3 in selection of initiation codon is a general phenomenon and is not restricted to specific genes involved in translation, such as *infC* itself. A previous study has shown this to be the case for genes such as *thrS* and *rpsO* (46). However, in this study, initiation at the *thrS* and *rpsO* genes was not affected as strongly as that at *infC*, suggesting that some feature of the mRNA may govern the effects of IF3. The translation of *recJ* is unique among genes for which the effects of IF3 have been noted. The *recJ* gene is poorly expressed and possesses no SD sequence to stabilize interaction of the mRNA with the 16S rRNA (36). In addition, translation initiation at *recJ* may occur by reinitiation without dissociation after translation of its upstream gene, *dsbC*. This translational coupling of *recJ* with *dsbC* may be required to facilitate initiation in the absence of a good SD sequence.

Other features that specify the initiation site. One striking observation was that translation initiation was still restricted to the normal site on *recJ*, even when the specificity of initiation was relaxed by the *infC135* mutation. Although an alternative AUA initiation site exists four codons downstream from the GUA initiator, it was not used at sufficient levels to express RecJ. (Although it is possible that this initiation site is used but the resultant peptide is not detected because of its instability, we think this is unlikely, since RecJ is an extremely stable protein in vivo and other N-terminal truncations are similarly stable.) Moreover, gross changes in the pattern of peptides expressed from the *recJ* gene were not seen in the *infC135* mutant. Other features of the initiation region, in addition to the IF3-fMet-tRNA-initiation codon interaction, must serve to select the initiation site. The SD sequence cannot play this role in *recJ*, and we presume that several factors, including the second codon (AAA, as in *recJ*, is preferred [42]), proximity to the upstream *dsbC* open reading frame, and potential unknown factors, must specify the initiation site.

Initiation codon suppressor mutations and the structure of IF3. The *infC135* mutant exerts genetically a semidominant effect in suppression of suboptimal initiation codons (25). Semidominance or incomplete dominance refers to the fact that *infC135* confers a phenotypic effect even in the presence of $infC^+$; however, this effect is somewhat less extreme than that of *infC135* homozygous strains. Semidominance may result from competition of the wild-type protein with the mutant form. The dominant nature of *infC135* means that the mutant must retain the other functions of IF3 that are essential for initiation and be specifically impaired in the discrimination of initiation codons. These other essential functions could include other proposed roles of IF3 in translation, such as binding to the 30S ribosome and recruitment of initiation factors IF1 and IF3. Mutations such as *infC135* may therefore define a domain that interacts with mRNA at the initiation codon and the anticodon of fMet-tRNA.

Mutations at three sites of IF3, our *recJ* suppressor mutations *infC133* (R99H), *infC134* (R99L), and *infC135* (R131P) (25) and mutation *infC19* (E134K) (46) cause the loss of initiation codon discrimination. These mutations map in the carboxy-terminal domain of IF3 (IF3-C), the structure of which has been determined by nuclear magnetic resonance spectroscopy (18) and by X-ray crystallography (5). These residues are not absolutely conserved among species and correspond to residues S91, K121, and A126 in the *Bacillus stearothermophilus* crystal structure (5). All three residues map in the turn regions adjacent to two β-strands in the structure. A fourth residue of this type, *infC362* at Y75 (52), is in the helix of the N-terminal portion that is believed to connect the two IF3-N and IF3-C domains. However, this mutation is reported to be recessive and may have other defects as well as an effect on initiation codon selection.

The phenotype of IF3 nondiscrimination mutants. The phenotype of the *infC135* mutant was surprisingly mild. We found a modest reduction in growth rate in the *infC135* mutants relative to that of the $infC^+$ strains. Mutants with reduced IF3 expression have been reported to be more seriously impaired in growth (41). One might expect that loss of initiation codon discrimination causes gross changes in peptide expression with concomitant growth defects, but this was not observed. As discussed above, translational initiation in *recJ* was largely still restricted to the normal initiator region. Therefore, other features must assist in the specification of initiation site.

Two curious aspects of the pleiotropic phenotype of *infC135* mutants were the inability to grow on defined minimal medium at elevated temperatures, shown here, and the previously reported increase in recombination and repair capacity of the cell (25). It was the latter property that led us to propose that IF3 might regulate recombination genes such as *recJ* normally. We no longer believe this to be the case, because *infC135* has no effect on wild-type *recJ* expression. Rather, we suspect that some low level of abnormal translation may induce a physiological state in which repair capabilities may be enhanced.

ACKNOWLEDGMENTS

We thank M. Springer for helpful discussions and for providing the *infC*::*lacZ*λ transducing phage.

This work was supported by Public Health Service grant T32, training grant GM07122 (to T.J.H.), and grant RO1 GM43889.

REFERENCES

1. **Altmann, M., and H. Trachsel.** 1994. The yeast *Saccharomyces cerevisiae* system: a powerful tool to study the mechanism of protein synthesis initiation in eukaryotes. Biochimie **76:**853–861.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Short protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
3. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:**525–557.
4. **Berkhout, B., C. J. van der Laken, and P. H. van Knippenberg.** 1986. Formylmethionyl-tRNA binding to 30 S ribosomes programmed with homopolynucleotides and the effect of translational initiation factor 3. Biochim. Biophys. Acta **866:**144–153.
5. **Biou, V., F. Shu, and V. Ramakrishnan.** 1995. X-ray crystallography shows that translational initiation factor IF3 consists of two compact α/β domains linked by an α-helix. EMBO J. **14:**4056–4064.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
7. **Brombach, M., and C. L. Pon.** 1987. The unusual translation initiation codon AUU limits the expression of *infC* (initiation factor IF3) gene of *Escherichia coli*. Mol. Gen. Genet. **208:**94–100.
8. **Butler, J. S., M. Springer, J. Dondon, M. Graffe, and M. Grunberg-Manago.** 1986. *Escherichia coli* protein synthesis initiation factor IF3 controls its own gene expression at the translational level *in vivo*. J. Mol. Biol. **192:**767–780.
9. **Butler, J. S., M. Springer, and M. Grunberg-Manago.** 1987. AUU-to-AUG mutation in the initiator codon of the translation initiation factor IF3 abolishes translational autocontrol of its own gene (*infC*) *in vivo*. Proc. Natl. Acad. Sci. USA **84:**4022–4025.
10. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141–1156.
11. **Chung, C. T., S. L. Niemela, and R. H. Miller.** 1989. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA **86:**2172–2175.
12. **Clements, J. M., T. M. Laz, and F. Sherman.** 1988. Efficiency of translation initiation by non-AUG codons in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4533–4536.
13. **Cooper, D. L., R. S. Lahue, and P. Modrich.** 1993. Methyl-directed mismatch repair is bidirectional. J. Biol. Chem. **268:**11823–11829.
14. **Crick, F. H. C.** 1966. Codon-anticodon pairing: the wobble hypothesis. J. Mol. Biol. **19:**548–555.
15. **Csonka, L., and A. J. Clark.** 1979. Deletions generated by the transposon Tn*10* in the *srl recA* region of the *Escherichia coli* K-12 chromosome. Genetics **93:**321–343.
16. **Dianov, G., B. Sedgwick, G. Daly, M. Olsson, S. Lovett, and T. Lindahl.** 1994. Release of 5′-terminal deoxyribose-phosphate residues from incised abasic sites in DNA by the *Escherichia coli* RecJ protein. Nucleic Acids Res. **22:**993–998.
17. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6145.
18. **Garcia, C., P.-L. Fortier, S. Blanquet, J.-Y. Lallemand, and F. Dardel.** 1995. Solution structure of the ribosome-binding domain of *E. coli* translation initiation factor IF3. Homology with the U1A protein of the eukaryotic spliceosome. J. Mol. Biol. **254:**247–259.
19. **Gillen, J. R., D. K. Willis, and A. J. Clark.** 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145:**521–532.
20. **Godefroy-Colburn, T., A. D. Wolfe, J. Dondon, and M. Grunberg-Manago.** 1975. Light-scattering studies showing the effect of initiation factors on the reversible dissociation of *Escherichia coli* ribosomes. J. Mol. Biol. **94:**461–478.
21. **Gold, L., G. Stormo, and R. Saunders.** 1984. *Escherichia coli* translational initiation factor IF3: a unique case of translational regulation. Proc. Natl. Acad. Sci. USA **81:**7061–7065.
22. **Gualerzi, C., G. Risuleo, and C. Pon.** 1979. Mechanism of the spontaneous and initiation factor 3-induced dissociation of 30S aminoacyl-tRNA polynucleotide ternary complexes. J. Biol. Chem. **254:**44–49.
23. **Gualerzi, C. O., and C. L. Pon.** 1990. Initiation of mRNA translation in prokaryotes. Biochemistry **29:**5881–5889.
24. **Gualerzi, C. O., A. La Teana, R. Spurio, M. A. Canonaco, M. Severini, and C. L. Pon.** 1990. Initiation of protein biosynthesis in procaryotes: recognition of mRNA by ribosomes and molecular basis for the function of initiation factors, p. 281–291. *In* W. E. Hill, A. Dahlberg, R. A. Garrett, P. B. Moore, D. Schlessinger, and J. R. Warner (ed.), The ribosome: structure, function and evolution. American Society for Microbiology, Washington, D.C.
25. **Haggerty, T. J., and S. T. Lovett.** 1993. Suppression of *recJ* mutations of *Escherichia coli* by mutations in translation initiation factor IF3. J. Bacteriol. **175:**6118–6125.
26. **Hartz, D., J. Binkley, T. Hollingsworth, and L. Gold.** 1990. Domains of initiator tRNA, an initiation codon crucial for initiator tRNA selection by *Escherichia coli* IF3. Genes Dev. **4:**1790–1800.
27. **Hartz, D., D. S. McPheeters, and L. Gold.** 1990. From polynucleotide to natural mRNA translation initiation: function of *Escherichia coli* initiation factors, p. 275–280. *In* W. E. Hill, A. Dahlberg, R. A. Garrett, P. B. Moore, D. Schlessinger, and J. R. Warner (ed.), The ribosome: structure, function and evolution. American Society for Microbiology, Washington, D.C.
28. **Hartz, D., D. S. McPheeters, and L. Gold.** 1989. Selection of the initiator tRNA by *Escherichia coli* initiation factors. Genes Dev. **3:**1899–1912.
29. **Horii, Z.-I., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80:**327–344.
30. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
31. **La Teana, A., C. L. Pon, and C. O. Gualerzi.** 1993. Translation of mRNAs with degenerate initiation triplet AUU displays high initiation factor 2 dependence and is subject to initiation factor 3 repression. Proc. Natl. Acad. Sci. USA **90:**4161–4165.
32. **Lovett, S. T., and A. J. Clark.** 1985. Cloning of the *Escherichia coli recJ* chromosomal region and identification of its encoded proteins. J. Bacteriol. **162:**280–285.
33. **Lovett, S. T., and A. J. Clark.** 1983. Genetic analysis of regulation of the RecF pathway of recombination in *Escherichia coli* K-12. J. Bacteriol. **153:** 1471–1478.
34. **Lovett, S. T., and A. J. Clark.** 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J. Bacteriol. **157:**190–196.
35. **Lovett, S. T., and R. D. Kolodner.** 1989. Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86:**2627–2631.
36. **Lovett, S. T., and R. D. Kolodner.** 1991. Nucleotide sequence of the *Escherichia coli recJ* chromosomal region and construction of RecJ-overexpression plasmids. J. Bacteriol. **173:**353–364.
37. **Lovett, S. T., C. Luisi-Deluca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics **120:**37–45.
38. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Missiakas, D., C. Georgopoulos, and S. Raina.** 1994. The *Escherichia coli dsbC* (*xprA*) gene encodes a periplasmic protein involved in disulfide bond formation. EMBO J. **13:**2013–2020.
40. **Munson, L. M., G. D. Stormo, R. L. Niece, and W. S. Reznikoff.** 1984. *lacZ* translation initiation mutations. J. Mol. Biol. **177:**663–683.
41. **Olsson, C. L., M. Graffe, M. Springer, and J. W. B. Hershey.** 1996. Physiological effect of translation initiation factor IF3 and ribosomal protein L20 limitation in *Escherichia coli*. Mol. Gen. Genet. **250:**705–714.
42. **Ringquist, S., S. Shinedling, D. Barrick, L. Green, J. Binkley, G. D. Stormo, and L. Gold.** 1992. Translation initiation in *Escherichia coli*: sequences within the ribosome-binding site. Mol. Microbiol. **6:**1219–1229.
43. **Risuleo, G., C. Gualerzi, and C. Pon.** 1976. Specificity and properties of the destablization induced by initiation factor IF-3, of ternary complexes of the 30-S ribosomal subunit, aminoacyl-tRNA and polynucleotides. Eur. J. Biochem. **67:**603–613.
44. **Romero, A., and P. Garcia.** 1991. Initiation of translation at AUC, AUA, and AUU codons in *Escherichia coli*. FEMS Microbiol. Lett. **68:**325–330.
45. **Rudd, K. E., and T. D. Schneider.** 1992. Compilation of *E. coli* ribosomal binding sites, p. 17.19–17.45. *In* J. H. Miller (ed.), A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
46. **Sacerdot, C., C. Chiaruttini, K. Engst, M. Graffe, M. Milet, N. Mathy, J. Dondon, and M. Springer.** 1996. The role of the AUU initiation codon in the negative feedback regulation of the gene for translation initiation factor IF3 in *E. coli*. Mol. Microbiol. **21:**331–346.
47. **Sacerdot, C., G. Fayat, P. Dessen, M. Springer, J. A. Plumbridge, M. Grunberg-Manago, and S. Blanquet.** 1982. Sequence of a 1.26-kb DNA fragment containing the structural gene for *E. coli* initiation factor IF3: presence of an AUU initiator codon. EMBO J. **1:**311–315.
48. **Schneider, T. D., G. D. Stormo, L. Gold, and A. Ehrenfeucht.** 1986. Information content of binding sites on nucleotide sequences. J. Mol. Biol. **188:** 415–431.
49. **Shevchik, V. E., G. Condemine, and J. Robert-Baudouy.** 1994. Characterization of DsbC, a periplasmic protein of *Erwinia chrysanthemi* and *Escherichia coli* with disulfide isomerase activity. EMBO J. **13:**2007–2012.
50. **Springer, M.** 1996. Translational control of gene expression in *E. coli* and bacteriophage, p. 85–126. *In* E. C. C. Lin and A. S. Lynch (ed.), Regulation of gene expression in *Escherichia coli*. R. G. Landes Company, Austin, Tex.
51. **Subramanian, A. R., and B. D. Davis.** 1970. Activity of initiation factor F_3 in dissociating *Escherichia coli* ribosomes. Nature **288:**1273–1275.
52. **Sussman, J. K., E. L. Simons, and R. W. Simons.** 1996. *Escherichia coli* translation initiation factor 3 discriminates the initiation codon *in vivo*. Mol. Microbiol. **21:**347–360.

53. **Tabor, S.** 1990. Expression using the T7 RNA polymerase/promoter system, p. 16.2.1–16.2.11. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
54. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
55. **Viswanathan, M., and S. T. Lovett.** Unpublished data.
56. **Willets, N. S., A. J. Clark, and B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97:**244–249.