Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4022–4025, June 1987
Biochemistry

AUU-to-AUG mutation in the initiator codon of the translation initiation factor IF3 abolishes translational autocontrol of its own gene (*infC*) *in vivo*

(protein synthesis control/site-specific mutagenesis)

J. SCOTT BUTLER*, MATHIAS SPRINGER†, AND MARIANNE GRUNBERG-MANAGO†

*Department of Biochemistry, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642; and †Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, Paris 75005, France

Contributed by Marianne Grunberg-Manago, February 20, 1987

ABSTRACT **We previously showed that *Escherichia coli* translation initiation factor IF3 regulates the expression of its own gene *infC* at the translational level *in vivo*. Here we create two alterations in the *infC* gene and test their effects on translational autocontrol of *infC* expression *in vivo* by measuring β-galactosidase activity expressed from *infC-lacZ* gene fusions under conditions of up to 4-fold derepression or 3-fold repression of *infC* expression. Replacement of the *infC* promoter with the *trp* promoter deletes 120 nucleotides of the *infC* mRNA 5′ to the translation initiation site without affecting autogenous translational control. Mutation of the unusual AUU initiator codon of *infC* to the more common AUG initiator codon abolishes translation initiation factor IF3-dependent repression and derepression of *infC* expression *in vivo*. These results establish the AUU initiator codon of *infC* as an essential cis-acting element in autogenous translational control of translation initiation factor IF3 expression *in vivo*.**

The initiation of protein synthesis in *Escherichia coli* requires the activity of three protein factors (IF1, IF2, and IF3) (for reviews, see refs. 1–3). One of these initiation factors, IF3, binds to the 30S ribosomal subunit and shifts the equilibrium between 70S ribosomes and their 50S and 30S ribosomal subunits in favor of the free subunits, thus enhancing the availability of 30S subunits on which protein synthesis initiation begins. In addition, IF3 may play a direct role in the binding of mRNA to the 30S ribosome.

The cellular levels of the protein synthesis initiation factors, like other protein synthesis components, increase with increases in cellular growth rate (4), suggesting a shared system of genetic regulation. However, the genes for each of the factors do not map adjacent to one another, implying that coordinate control of IF gene expression requires separate, but not necessarily independent, control elements. The gene for IF2, *infB*, lies at 69 min on the *E. coli* genetic map, and it appears that the product of the adjacent gene, NusA protein, regulates both *infB* and *nusA* gene expression at the transcriptional level *in vivo* (5, 6). The *infC* gene for IF3 maps at 38 min on the *E. coli* chromosome (7), adjacent to other genes involved in protein synthesis (8). The genetic organization of *infC* is very unusual: (*i*) *infC* is unique in initiating translation at an AUU codon (9), (*ii*) the *infC* AUU initiator codon lies only three nucleotides 3′ to the stop codon of *thrS*, the gene for threonyl-tRNA ligase (10), and (*iii*) transcription of the majority of *infC* mRNAs initiates at a promoter, pO′, inside the *thrS* coding sequence and 182 base pairs upstream of the *infC* initiator AUU (11).

Our recent experiments demonstrated that IF3 regulates expression of its own gene at the translational level *in vivo* (11). First, *infC* mutant strains with elevated expression of structurally altered IF3 do not have corresponding increases in *infC* mRNA synthesis rates. Second, *infC* mutant alleles cause derepression of *infC-lacZ* gene fusions (translation from the translation initiation site of *infC*) but not *infC-lacZ* operon fusions (translation from the translation initiation site of *lacZ*). Third, a cellular excess of IF3 supplied in trans from a multicopy plasmid represses expression of an *infC-lacZ* gene fusion without affecting the rate of synthesis of hybrid *infC-lacZ* mRNA.

In the present work we tested whether the unusual AUU initiator codon plays a role in *infC* translational autoregulation. The results clearly show that a single mutation of AUU-to-AUG abolishes autogenous translational control of *infC* expression. This result is discussed in light of a theoretical analysis of the *infC* system by Gold *et al.* (12) that predicted the *infC* AUU initiator codon to be an essential element in the autoregulatory system.

MATERIALS AND METHODS

The *E. coli* K-12 strains used here were as described (11). Genetic techniques and β-galactosidase measurements were as described by Miller (13). Molecular cloning techniques were as described by Maniatis *et al.* (14). Selection and screening of λ monolysogens were as described (15). Measurements of *infC-lacZ* mRNA levels were made as described (11) with details given in the legend to Table 3.

Construction of M13mp19TSX3. Our first step in *in vitro* mutagenesis of *infC* was to clone the *Hin*dIII–*Sal* I, *trp*PO-containing fragment of pDR720 (16) into the same sites in M13mp19 to yield the Lac$^-$ derivative M13mp19ptrp4. Next, the promoterless *Sal* I–*Sst* II fragment of pUSX15 (11) containing the translation initiation site and the first 54 codons of *infC* was inserted into the same sites between *trp*PO and *lacZ* on M13mp19ptrp4 to make the Lac$^+$ derivative M13mp19TSX3.

Oligonucleotide Directed Site Specific Mutagenesis. We used the deoxyoligonucleotide 5′ TCCGCCTTTCATACCTTA 3′ provided by B. Ehresmann (Institut de Biologie Moléculaire et Cellulaire du Centre National de la Recherche Scientifique, Laboratoire de Biochimie, Strasbourg, France) to mutate the *infC* AUU initiator codon to AUG using the method of Kunkel (17). The nucleotide sequence of the resulting mutant M13mp19TSG2 and the parent M13mp19TSX3 was determined (18) by sequencing across the M13mp19 polylinker at the *infC–lacZ* fusion all the way to the *Eco*RI site just 5′ to *trp*PO. The change of AUU-to-AUG is the only difference between the two sequences.

Transfer of Wild-Type and Mutant *infC–lacZ* Fusions to λ. To allow study of our *infC-lacZ* gene fusions as single copies

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: IF, translational initiation factor.

in vivo, we constructed a λ bacteriophage carrying all of the *lac* operon except *lacI*, the promoter, the translation initiation site, and the first seven amino acids of *lacZ*. The *Eco*RI–*Sst* II *lac* fragment of pNM482 (19) was inserted between the left arm, up to the *Sst* II site of λNM540 (20), and the right arm, from the far right *Eco*RI site of λSCX16 (11). After *in vitro* packaging we characterized by restriction analysis the cI857, Lac⁻ phage λNNS4.

The *Eco*RI fragments containing the *trp*PO-*infC* fusion of M13mp19TSX3 and its mutant derivative M13mp19TSG2 were inserted separately into the *Eco*RI site in front of *lac* in λNNS4, creating an in-frame gene fusion between *infC* and *lacZ*. After *in vitro* packaging and screening of Lac⁺ λ phage, we characterized by restriction analysis the respective derivatives λTSX31 (wild type, AUU) and λTSG25 (mutant, AUG) (Fig. 1).

RESULTS

Replacement of the *infC* Promoter with the *trp* Promoter Does Not Affect Translational Autoregulation of *infC* Expression. We replaced the *infC* promoter pO′ with the controllable *trp* promoter because changing the *infC* initiator AUU to AUG in the presence pO′ resulted in apparent instability of *infC-lacZ* gene fusions cloned in λ (J.S.B., unpublished results), possibly due to lethal levels of hybrid IF3-β-galactosidase expression. The data in Table 1 show that although IF3-β-galactosidase expression decreases from 519 to 93 units when the *infC* pO′ promoter is replaced with the *trp* promoter, the level of derepression from the fusions is exactly the same: the derepression factor is about four in an *infC19* background and about two in an *infC37* background whatever promoter is used to express the *infC-lacZ* gene fusion. Similarly, the data in Table 2 show that an excess of IF3 produced from an *infC*-carrying, multicopy plasmid represses IF3-β-galactosidase expression almost 3-fold with either the *trp* or the *infC* pO′ promoter. We showed previously that such repression of *infC* pO′ gene fusions occurs without changes in the synthesis rate of *infC-lacZ* mRNA (11), and the data in Table 3 show that, using the *trp* promoter, the derepression seen in an *infC19* background occurs without a significant change in the rate of synthesis of *infC-lacZ* mRNA. These results demonstrate that translational autoregulation of *infC* expression occurs independent of the nature of the promoter expressing the gene and thereby confirm our previous experimental results showing autogenous translational control of *infC* expression *in vivo* (11).

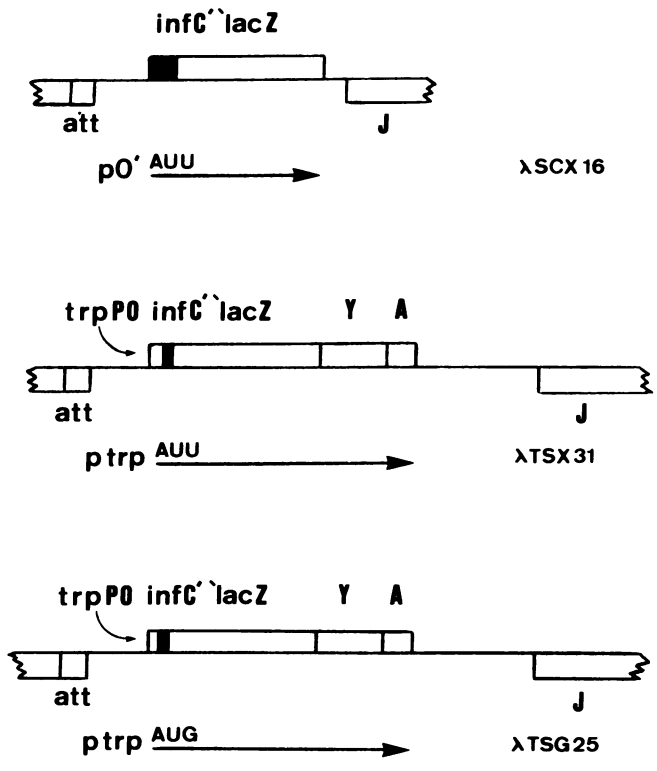

FIG. 1. Physical maps of the λ bacteriophage carrying *infC-lacZ* gene fusions. The λ genomes are abbreviated and drawn in the direction opposite to normal. Boxes above the line represent *E. coli* DNA. Open boxes below the line represent λ DNA. A prime before or after a gene means that it is incomplete on that side. The arrows represent mRNA transcripts and are preceded by a designation of their promoter. The nucleotide triplets represent the translation initiation codons and the approximate positions in DNA and RNA where *infC-lacZ* protein synthesis initiates. Y, *lacY* gene; A, *lacA* gene; and J, *J* gene.

Changing the *infC* AUU Initiator Codon to AUG Abolishes Translational Autoregulation of *infC* Expression *in Vivo*. The unusual usage of an AUU initiator codon in translation of *infC* (9) and the fact that IF3 regulates its own gene expression at the level of translation (11) suggest that the AUU codon may play a role in the autoregulatory system (12). To test this hypothesis we changed the *infC* initiator AUU to the more common initiator AUG and tested the ability of IF3 to control its own gene expression by monitoring the levels of IF3-β-galactosidase and *infC-lacZ* mRNA expressed from *infC-lacZ* gene fusions under conditions normally causing derepression or repression of *infC* expression *in vivo*. Mutation of the AUU initiator codon of *infC* has two interesting effects on *infC* expression *in vivo*. First, initiation of translation with AUG (λTSG25) instead of AUU (λTSX31) increases the expression of an *infC-lacZ* gene fusion 10-fold in a wild-type background (Table 1) without a comparable increase in *infC-lacZ* mRNA levels (Table 3). These results indicate that the lower level of expression from the fusion carried by λTSX31 relative to λTSG25 is due to a translational, instead of transcriptional, phenomenon. Second, and most dramatically, the mutation of AUU to AUG completely abolishes translational autoregulation of *infC* expression. Neither *infC* mutant alleles (Table 1) nor over-production of IF3 in trans (Table 2) affects AUG-initiated translation of the *infC-lacZ* gene fusion carried by λTSG25, whereas its AUU counterpart λTSX31 is normally regulated. We conclude, therefore, that the *infC* AUU initiator codon is a necessary cis-acting element in the translational autoregulation of *infC* expression *in vivo*.

DISCUSSION

The experimental results presented here identify the unusual initiator AUU codon of *infC* as a necessary cis-acting element in autogenous translational control of *infC* expression *in vivo*. Replacement of the major promoter pO′ of *infC* by the *trp* promoter has no effect on autogenous translational control of *infC* expression, confirming our previous conclusion that IF3 controls its own synthesis at the translational level *in vivo* (11). The replacement also deletes the first 120 nucleotides of the leader of the *infC* mRNA without affecting regulation of *infC* expression, indicating that information required for translational autocontrol lies in a region 62 nucleotides 5′ to the initiator AUU. Because each of the gene fusions used here contains the first 54 codons of *infC* fused to *lacZ* we cannot exclude the possibility that control elements lie in the 159 nucleotides 3′ to the AUU codon.

The most important result of our experiments is that a single nucleotide mutation changing the wild-type AUU initiator codon to AUG eliminates autogenous translational control of *infC* expression. This mutation causes the loss of derepression of *infC* expression by *infC* mutant alleles (Table 1) and the loss

Table 1. Effect of *infC* alleles on *infC-lacZ* expression from various λ bacteriophage

Gene fusion	β-Galactosidase expression, units			Derepression factor	
	Wild type	*infC19*	*infC37*	*infC19/wt*	*infC37/wt*
λSCX16 (wild-type *infC* promoter, AUU initiator codon)	519 ± 20	1942 ± 239	1020 ± 20	3.7	1.9
λTSX31 (*trp* promoter, AUU initiator codon)	93 ± 6	358 ± 8	179 ± 2	3.8	1.9
λTSG25 (*trp* promoter, mutant AUG codon)	1037 ± 56	976 ± 27	899 ± 6	0.94	0.87

Measurements were made on monolysogenic, Δ*lac* strains IBPC5311 (wild type), IBPC5231 (*infC19*), and IBPC5251 (*infC37*) (11) growing in exponential phase at 30°C in 4-morpholinepropanesulfonic acid (Mops)-glucose culture media (21) supplemented with arginine at 0.05 mg/ml. The doubling times for the strains are 120 min each. The values are the average of five or six measurements ± SD and are expressed per A650 unit of bacteria as described by Miller (13).

of repression of *infC* expression by increased cellular levels of IF3 (Table 2) without a significant increase in *infC-lacZ* mRNA levels (Table 3). It is not likely that the increased translational efficiency caused by mutation of AUU-to-AUG can, alone, account for loss of translational autocontrol because excess IF3 represses, 2.5-fold, comparable levels of IF3-β-galactosidase expression from the wild-type fusion on λSCX16 (Table 2). Instead, the AUU initiator codon must itself specify recognition by the system governing translational autocontrol of *infC* expression, and its mutation to AUG results, most likely, in a combination of derepression of translation initiation due to loss of autocontrol and an increase in efficiency of translation initiation from AUG instead of AUU.

Our earlier experimental results showing autogenous translational control of *infC* expression (11) and the present results showing its dependency on an AUU initiator codon confirm two predictions of a theoretically based model proposed by Gold *et al.* (12). In their model *infC* mRNA is translated in an IF3-independent manner due to the abnormal AUU initiator codon and other unusual features of the translation initiation site of *infC*. The model predicts that when the IF3 level is low relative to the number of 30S ribosomal subunits, IF3-independent translation of *infC* mRNA will be preferred to IF3-dependent translation of other cellular mRNAs—causing a relative increase in IF3 levels. The Gold *et al.* model also predicts twenty-nucleotide base pairing interactions between the translation initiation site of *infC* mRNA and various domains of the 16S RNA that specify *infC* mRNA–30S ribosomal subunit interaction in the proposed IF3-independent mode. In this respect, it is somewhat surprising that a single mutation of U-to-G, changing only a single base pair, should result in complete loss of translational autoregulation. It is, however, possible that the AUU-to-AUG change is enough to favor translation of the mutated *infC* mRNA in the IF3-dependent mode, which should, if the model is correct, cause loss of autoregulation of IF3 synthesis.

The negative autoregulation of *infC* translation can be explained alternately by a more conventional model whereby IF3 binds to an operator site covering the translation initiation site on the *infC* mRNA. In this case, the third base of the AUU initiator codon should be essential to the binding of IF3 to the translational operator. Chemical crosslinking experiments (22) and assays showing binding of IF3 to the 3′ end of the 16S RNA (23) suggest that IF3 may interact directly with sites on the 16S RNA during translation initiation. Some nucleotide sequence homology exists between these sites and the translation initiation site of *infC* (9, 22, 23)—yet these homologies do not include the *infC* initiator AUU codon. Such nucleotide sequence homologies might participate in secondary structures recognized by IF3 and destabilized by mutation of AUU-to-AUG. However, examination of the nucleotide sequence of the portion of *infC* shown here to specify autocontrol does not reveal significant secondary structure involving the translation initiation site. Lack of apparent homology between the 16S RNA and *infC* mRNA does not rule out the existence of a functional competition between the RNAs for IF3. We see little reason at this time, however, to favor a classical IF3–mRNA interaction model over the more indirect model of Gold *et al.*

A growing number of proteins control their own gene expression at the translational level *in vivo*. These include genes for ribosomal proteins (24, 25), the gene 32 protein (26,

Table 2. Effect of a cellular excess of IF3 on *infC-lacZ* expression from various λ bacteriophage

Gene fusion	β-Galactosidase expression, units		Repression factor pSB1/pBR322
	pBR322 (vector plasmid)	pSB1 (*infC* plasmid)	
λSCX16 (wild-type *infC* promoter, wild-type *infC* AUU initiator codon)	981 ± 86	363 ± 80	0.37
λTSX31 (*trp* promoter, wild-type *infC* AUU initiator codon)	135 ± 10	57 ± 2	0.42
λTSG25 (*trp* promoter, mutant *infC* AUG initiator codon)	790 ± 83	762 ± 65	0.97

The strains and techniques are described in the legend of Table 1 except that 4-morpholinepropanesulfonic (Mops)-glucose culture media was supplemented with all the amino acids (21) except tryptophan; ampicillin was added to 0.1 mg/ml every 2 hr during growth; and the doubling time for the strains was 90 min.

Table 3. Cellular levels of *infC-lacZ* mRNA expressed from *infC-lacZ* gene fusions

Strain	Input RNA, cpm × 10^{-4}	RNA bound, cpm	Percent bound, % × 10^2
IBPC5311λTSX31 (wild-type strain, gene fusion with wild-type AUU initiator codon)	103	91 ± 17	0.9 ± 0.2
IBPC5311λTSG25 (wild-type strain, gene fusion with mutant AUG initiator codon)	58	90 ± 24	1.5 ± 0.4
IBPC5231λTSX31 (*infC19* strain, gene fusion with wild-type AUU initiator codon)	166	170 ± 2	1.0 ± 0.01

The strains were grown as described in the legend of Table 1. The [^{3}H]uridine-labeled RNA was isolated and hybridized to an excess of the *lacZ*-specific probe M13mp8lac14 as described (11). Background hybridization to single-stranded M13mp8 was <25% of the values in column 3 and has been subtracted from them.

27) and the RegA protein (28) of bacteriophage T4, and threonyl-tRNA ligase (15, 29). In all of these examples (RegA being a possible exception) the gene products made by and controlling each gene interact functionally, in a well-defined manner, with nucleic acids. The ability of the protein to control translation is thought to depend upon its binding to a region on its own mRNA that shares some homology with its primary nucleic acid substrate. For example, mutations on the *thrS* mRNA disrupting translational repression by threonyl-tRNA ligase lie in an area of the mRNA adjacent to the ribosome binding site showing striking homologies in sequence and structure to threonine isoacceptor tRNAs (30). IF3 differs from these examples because, although it interacts with a variety of RNAs, it does so without any apparent nucleotide sequence specificity (23, 31, 32). Nevertheless, IF3 may control its own translation simply by binding to its AUU codon, thereby blocking the access of 30S ribosomes. Such a model, together with the results presented here, suggests that IF3 should have a measurable difference in binding affinity for *infC* mRNAs containing an AUU initiator codon or a AUG initiator codon.

Translation initiation of *E. coli* mRNAs occurs at specific sites characterized by a certain number of determinants whose importance depends upon their spatial organization (33). One of these determinants is the translation initiation codon to which initiator fMet-tRNA binds on the 30S ribosomal subunit. The translation initiation codon is generally AUG, but *E. coli* initiator tRNA can also recognize GUG and exceptionally AUA and UUG (33). Surprisingly, IF3, which is essential for efficient translation initiation complex formation *in vitro*, has a unique AUU initiator codon. The results reported here show that this exception is not fortuitous, but is necessary for the controlled synthesis of IF3 itself.

The authors are grateful to Terry Platt, for reading the manuscript and for generously providing laboratory facilities where this work was completed. J.S.B. was supported by the National Institutes of Health–French Centre National de la Recherche Scientifique Program for Scientific Collaboration. This work was supported by grants from Centre National de la Recherche Scientifique, from the Fondation pour la Recherche Medicale, and from E. I. du Pont de Nemours.

1. Grunberg-Manago, M. (1979) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (University Park Press, Baltimore), pp. 445–447.
2. Hershey, J. W. B. (1980) in *Cell Biology: A Comprehensive Treatise*, eds. Prescott, D. M. & Goldstein, L. (Academic, New York), Vol. 4, pp. 1–68.
3. Kozak, M. (1983) *Microbiol. Rev.* **47**, 1–45.
4. Howe, J. G. & Hershey, J. W. B. (1983) *J. Biol. Chem.* **258**, 1954–1959.
5. Plumbridge, J. A., Dondon, J., Nakamura, Y. & Grunberg-Manago, M. (1985) *Nucleic Acids Res.* **13**, 3371–3388.
6. Nakamura, Y., Plumbridge, J. A., Dondon, J. & Grunberg-Manago, M. (1985) *Gene* **36**, 189–193.
7. Springer, M., Graffe, M. & Hennecke, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3970–3974.
8. Plumbridge, J. A., Springer, M., Graffe, M., Goursot, R. & Grunberg-Manago, M. (1980) *Gene* **11**, 33–42.
9. Sacerdot, C., Fayat, G., Dessen, P., Springer, M., Plumbridge, J., Grunberg-Manago, M. & Blanquet, S. (1982) *EMBO J.* **1**, 311–315.
10. Mayaux, J.-F., Fayat, G., Fromant, M., Springer, M., Grunberg-Manago, M. & Blanquet, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6152–6156.
11. Butler, J. S., Springer, M., Dondon, J., Graffe, M. & Grunberg-Manago, M. (1986) *J. Mol. Biol.* **192**, 767–780.
12. Gold, L., Stormo, G. & Saunders, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7061–7065.
13. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
15. Springer, M., Plumbridge, J. A., Butler, J. S., Graffe, M., Dondon, J., Mayaux, J. F., Lestienne, P., Blanquet, S. & Grunberg-Manago, M. (1985) *J. Mol. Biol.* **185**, 93–104.
16. Russel, D. R., Auger, E. A., Vermersch, P. S. & Bennett, G. N. (1984) *Gene* **32**, 337–348.
17. Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
18. Sanger, F., Coulson, A. R., Barrel, B. G., Smith, A. J. H. & Roe, B. (1980) *J. Mol. Biol.* **143**, 161–178.
19. Minton, N. P. (1984) *Gene* **31**, 269–273.
20. Borck, K., Beggs, J. D., Brammar, W. J., Hopkins, A. S. & Murray, N. E. (1976) *Mol. Gen. Genet.* **146**, 199–207.
21. Neidhardt, F. C., Bloch, P. L. & Smith, D. F. (1974) *J. Bacteriol.* **119**, 736–747.
22. Ehresmann, C., Moine, H., Mougel, M., Dondon, J., Grunberg-Manago, M., Ebel, J.-P. & Ehresmann, B. (1986) *Nucleic Acids Res.* **14**, 4803–4821.
23. Wickstrom, E. (1983) *Nucleic Acids Res.* **7**, 2035–2052.
24. Lindahl, L. & Zengel, J. (1986) *Annu. Rev. Genet.* **20**, 297–326.
25. Nomura, M., Gourse, R. & Baughman, G. (1984) *Annu. Rev. Biochem.* **53**, 75–177.
26. Russel, M. L., Gold, L., Morrissett, H. & O'Farrell, P. Z. (1976) *J. Biol. Chem.* **251**, 7263–7270.
27. Lamaire, G., Gold, L. & Yarus, M. (1978) *J. Mol. Biol.* **129**, 66–70.
28. Karam, J., Gold, L., Singer, B. & Dawson, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 75–117.
29. Butler, J. S., Springer, M., Dondon, J. & Grunberg-Manago, M. (1986) *J. Bacteriol.* **165**, 198–203.
30. Springer, M., Graffe, M., Butler, J. S. & Grunberg-Manago, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4384–4388.
31. Schleigh, T., Wickstrom, E., Twombly, K., Schmidt, B. & Tyson, R. W. (1980) *Biochemistry* **19**, 4486–4492.
32. Johnson, B. & Szekely, M. (1977) *Nature (London)* **267**, 550–552.
33. Gold, L., Pribnow, D., Schneider, T., Shinedling, S., Singer, B. S. & Stormo, G. (1981) *Annu. Rev. Microbiol.* **35**, 365–403.