Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3165–3169, November 1973

Reinitiation of a *lac* Repressor Fragment at a Codon Other Than AUG*

(protein synthesis/antibody/gel electrophoresis/amber mutants/negative complementation/*E. coli*)

DONALD GANEM†, JEFFREY H. MILLER†, JAMES G. FILES‡, TERRY PLATT‡§, AND KLAUS WEBER‡

† Department of Molecular Biology, University of Geneva, Geneva, Switzerland; and ‡ Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Communicated by Charles Yanofsky, May 16, 1973

ABSTRACT **52 Spontaneous nonsense mutants in the *lac i* gene of *Escherichia coli* were isolated and characterized. All mutants located early in the gene show negative complementation *in vivo* with a wild-type *i* gene in a *recA* diploid strain. *In vitro* studies show that those mutants that display negative complementing activity *in vivo* also make *lac* repressor fragments retaining inducer binding and immunological crossreactivity with wild-type repressor. Amino-acid sequence analysis of these fragments shows that they arise by reinitiation at internal sities of the *i* message after chain termination at a prior amber or ochre codon.**

There are at least two different internal reinitiation sites in the first 200 nucleotides of the translated part of the *i* message. The first site corresponds to the first internal in phase AUG codon, which specifies the methionine residue at position 42 of the repressor protein. This site can be activated by an amber codon, 45 nucleotides before the AUG codon. The second site is only 60 nucleotides past the first site and can be activated by an amber mutation derived from residue 60 of the protein. The second initiation codon specifies the amino-acid leucine in the wild-type repressor, but the reinitiated fragment shows an amino-terminal methionine residue at this position. Therefore, the second initiation site seems to involve an *in vivo* ambiguity of the genetic code in that the same codon can be translated into two different amino acids depending on the recognition of this codon during initiation (when methionine is inserted) or elongation of protein synthesis (when leucine is inserted). The possibility that a codon other than AUG can act as an initiation codon *in vivo* is discussed.

One object of our current work is to understand how a cell directs the initiation of translation of messenger RNA (mRNA) to occur at specific nucleotide sequences and also to determine the exact nature of these sequences. One approach to this problem has been the direct isolation and sequence analysis of ribosomal initiation sites (1–4). A second, independent approach makes use of reinitiation of translation after chain termination. Reinitiation at internal sites within cistrons was first described by Sarabhai and Brenner (5) in the *rIIB* cistron of phage T4, and later demonstrated by Zipser (6–8) and Newton (9, 10) and their coworkers in the *z* gene of the *lac* operon of *Escherichia coli*. These studies showed that after chain termination at certain nonsense codons, reinitiation of polypeptide synthesis can occur.

Recently, we have shown that an early spontaneous amber mutation in the *i* gene gives rise to translational reinitiation; reinitiation occurs past the amber block at the first internal AUG codon of the repressor mRNA (11, 12). Here we describe the isolation and characterization of a large collection of spontaneous nonsense mutations in the *i* gene and demonstrate that at least those which map early in the gene generate reinitiation fragments. From protein chemical analysis of these fragments we conclude that there are at least two internal reinitiation sites early in the *i* gene and that these two sites are separated by only 60 nucleotides in the mRNA. Furthermore, we show that the second initiation site involves most likely an *in vivo* ambiguity of the genetic code in that the same codon can be translated into two different amino acids, depending on the recognition of this codon during initiation (when methionine is inserted) or elongation of protein synthesis (when leucine is inserted). We favor the hypothesis that a leucine codon (most probably UUG or CUG) can be recognized by *N*-formylmethionyl-tRNA and thus serve as an initiation signal *in vivo*.

RESULTS

Selection for lac Constitutive Mutants. Wild-type strains of *E. coli* require the *lac* permease (*y*-gene product) for growth on melibiose at high temperature (13). *Lac* promoter mutants that synthesize low amounts of this enzyme grow extremely slowly or not at all on melibiose at 37–42° (14). However, constitutive i^- or o^c derivatives of *lac* promoter mutants make sufficient *y*-gene product to allow normal growth on this sugar at high temperature. This observation provides us with a simple procedure for isolating i^- mutants if we start with a strain carrying a mutation in the *lac* promoter.

All i^- mutants were isolated in a strain deleted for the proB-lac region and carrying an F′*lacproA,B* factor. The F′ factor was transferred to a set of isogenic amber and ochre suppressor strains to test for suppression of the i^- character. We isolated 15,000 independent spontaneous Mel$^+$ revertants at 42° and recovered 5000 high-level *lac* constitutive mutants. From these we isolated 52 i^- nonsense mutants: 22 were identified as carrying amber mutations and 30 as containing ochre mutations (Table 1).

The nonsense mutations can be separated into 13 groups on the basis of deletion mapping (Fig. 1). Further distinctions can be achieved by examining the pattern of suppression. From these data, we can demonstrate that the 52 spontaneous nonsense mutations represent at least 25 different sites in the gene.

In Vivo Detection of Reinitiation Fragments. Reinitiation sites located early in the gene would be expected to yield fragments large enough to retain some properties of the *lac* repressor. Earlier studies of *trp-i* fusion strains showed that repressor fragments missing the amino terminus had lost the ability to repress, but still interacted in a negative way with

* The first paper in this series "Translation Reinitiation" is ref. 12.

§ Present address: Department of Biological Science, Stanford University, Stanford, Calif. 94305.

wild-type repressor (15). We reasoned that if a reinitiation signal was responsible for the generation of such negative complementing fragments, it should be possible to activate it by introducing a nonsense mutation into the gene, just proximal to it. Therefore, we tested each nonsense mutation for negative complementation with a wild-type *i* gene in *recA*$^-$ diploids (Table 2). Of the 52 mutations tested, five gave a positive result (Table 2). All five (970, 100, 971, 919, and 136) are amber mutations, and these map into two groups

TABLE 1. *Pattern of suppression of nonsense mutations*

MUTANT	Su 1	Su 2	Su 3	Su 6	Su B	Su C	Su 5
100	+	+	+	+	NT	±	±
970	+	+	+	+	NT	±	NT
971	+	±	+	+	±	+	±
919	+	-	+	+	-	+	±
136	+	-	+	+	-	+	NT
972	-	-	-	-	+	+	+
953	-	-	-	-	±	+	NT
917	-	-	-	-	+	+	+
973	+	+	+	+	+	+	+
918	-	-	-	-	-	±	-
811	+	+	+	+	+	+	±
706	+	+	+	+	+	+	NT
509	+	+	+	+	+	+	±
212	+	+	+	+	±	+	±
913	+	+	+	+	+	+	+
816	+	+	+	+	±	+	±
978	-	-	-	-	+	+	+
907	-	-	-	-	-	+	NT
78	-	-	-	-	+	+	NT
956	-	-	-	-	-	+	NT
955	-	-	-	-	-	+	NT
954	-	-	-	-	+	+	NT
920	-	-	-	-	-	+	-
912	-	-	-	-	-	+	+
790	-	-	-	-	-	+	NT
604	+	+	+	+	+	+	NT
512	-	-	-	-	-	+	±
449	-	-	-	-	-	+	-
446	-	-	-	-	-	+	±
417	-	-	-	-	-	+	±
301	-	-	-	-	-	+	±
211	-	-	-	-	-	+	-
959	+	+	+	+	-	+	-
535	-	-	-	-	-	+	NT
315	±	-	±	+	-	±	-
258	±	-	±	+	-	±	-
69	-	-	-	-	-	±	±
986	+	+	±	NT	NT	±	NT
984	-	-	-	-	+	+	±
921	-	-	-	-	+	+	NT
550	-	-	-	-	+	+	±
302	+	+	+	NT	NT	+	NT
960	+	+	+	+	±	+	+
987	+	+	+	NT	NT	+	NT
951	+	+	+	+	+	+	NT
942	+	+	+	+	+	+	NT
510	-	-	-	-	NT	+	NT
985	-	-	-	-	±	+	-
914	-	-	-	-	-	+	±
901	-	-	-	-	NT	±	NT
891	-	-	-	-	-	+	NT
259	-	-	-	-	±	±	±

F′ *lacpro* factors carrying different i^- mutations were transferred into a set of isogenic strains carrying the amber suppressors Su1, Su2, Su3, and Su6 and the ochre suppressors SuB, SuC, and Su5. The diploids were examined on Xgal indicator plates for the approximate amount of constitutive β-galactosidase. "+" indicates restoration of i^+ activity, whereas "±" indicates only partial restoration of i^+ activity. "−" indicates failure to restore i^+ activity. Suppressor designations are as in ref. 35. *NT*, not tested.

(Fig. 1). A sixth mutant, 9A, also gave a positive result. This mutation was derived by 2-aminopurine mutagenesis and is an ochre mutation mapping one deletion group before mutants 919, 971, and 136 (Miller & Ganem, manuscript in preparation).

In Vitro Characterization. The amber mutants displaying negative complementation *in vivo* also displayed isopropyl-β-D-thiogalactoside (inducer) binding in partially purified cell extracts (12). There is an approximate 1:10 reduction in total isopropylthiogalactoside binding activity which is a reflection of reduced levels of repressor fragments (at least for i100) rather than of an impaired ability of the repressor fragments to bind isopropylthiogalactoside, since the binding constant for isopropylthiogalactoside is identical to that of the wild-type repressor (12).

Reinitiation fragments from several amber mutants were purified by an ammonium sulfate fractionation step followed by antibody precipitation and Na dodecyl sulfate gel electrophoresis of the antigen–antibody complex. This procedure yields the molecular weight of the repressor fragment as well as sufficient amounts of pure protein needed to obtain an amino-terminal sequence (12).

Fig. 2 shows the results of an analysis of antigen–antibody complexes obtained on 10% Na dodecyl sulfate–polyacrylamide gels (16) for the wild-type repressor and the reinitiation proteins obtained with amber mutants 100 and 136. The reinitiation fragment of i100 (termed the i100 repressor, molecular weight 34,000) is smaller than the wild-type polypeptide chain (molecular weight 38,000). The i136 repressor (molecular weight 30,000–32,000) is in turn smaller than the i100 repressor. Using the antibody–Na dodecyl sulfate gel procedure on a preparative scale (12) we obtained 100 μg of pure reinitiation fragments by elution from Na dodecyl sulfate gels. The recovered proteins were subjected to the micro-dansyl-Edman procedure (17) and the following amino-terminal sequences were obtained: i100, Ala-Glx-Leu-Asx-Tyr-Ile-Pro; i136, Met-Leu-Ile-Gly-Val-Ala-Thr-. The same

TABLE 2. *β-Galactosidase levels in* *recA*$^-$ *$i^+z^+/F'i^-$ z^- diploids*

Mutant	−IPTG	+IPTG (1 mM)
i100 z$^-$	9	600
i136 z$^-$	29	600
i919 z$^-$	29	600
i78 z$^-$	0.9	600

Strains were assayed for β-galactosidase as described (36). Units are as reported (15). Diploids were constructed with the chromosome containing a wild-type *lac* region (i^+z^+) and the F′ factor a z^- derivative of each nonsense mutation (*iQ*, i^-, z^-). The sensitivity of this test was increased by the overproduction of any altered repressor due to the presence of the *iQ* allele (39). The only β-galactosidase synthesized in this strain is directed by the chromosomal *z* gene which is repressed by the chromosomal *i* gene. If any of the nonsense mutations result in synthesis of a repressor fragment capable of partially inactivating the wild-type repressor *in trans*, it would be displayed as an increase in the uninduced level of β-galactosidase. Mutants 970 and 971 gave results similar to 100 and 136, respectively (data not shown). Strain 78 carries a mutation that maps towards the middle of the *i*-gene and that does not display negative complementation. *IPTG*, isopropyl-β-D-thiogalactoside.

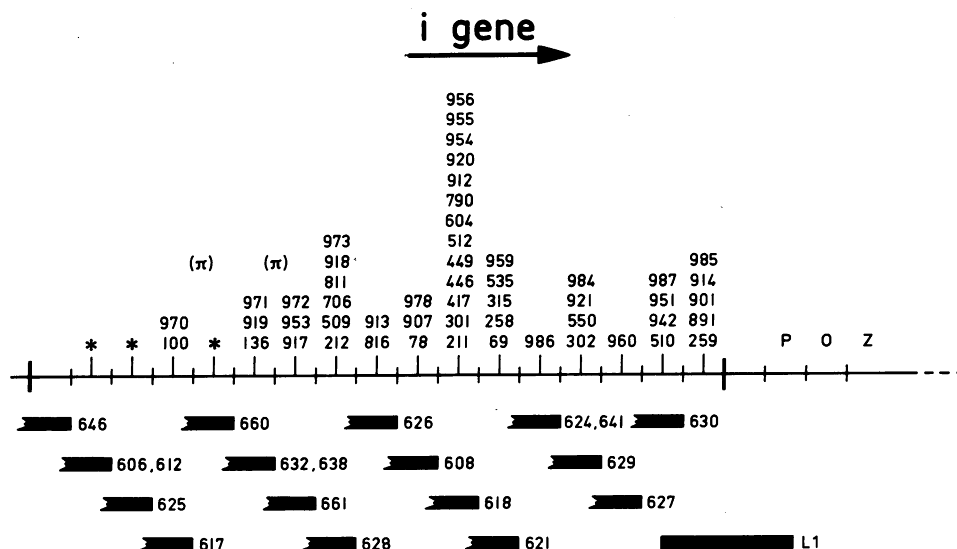

FIG. 1. Nonsense mutations in the *i*-gene. 20 Deletions were used to map 52 nonsense mutations. The five amber mutations that result in negatively complementing material are *i100*, *i970*, *i136*, *i919*, and *i971*. Since the direction of reading of the *i*-gene is from left to right (15, 37), these are the five earliest nonsense mutations on the map. The "π" symbols mark the position of postulated reinitiation points. *Asterisks* mark the position of mutations isolated by D. Gho and J. Miller used to order the early deletions. The relative order of the deletions used above has also been determined independently by M. Pfahl (38).

amino-terminal sequence that was determined for i136 was also found for amber 919 and ochre 9A.

The results clearly demonstrate that the i100 and i136 repressors differ not only in the molecular weights of their polypeptide chains but also in their amino-terminal sequence. We then determined the amino-acid sequence (Fig. 3) for residues 1–82 (12, 19; J. Files & K. Weber, unpublished) and found that the amino-terminal sequence of the i136 protein is identical to that following the leucine at position 62, with the exception of the *N*-terminal amino acid. In the wild-type chain a leucine precedes the Leu-Ile-Gly-Val-Ala- sequence, while in the reinitiation fragment a methionine residue appears in that position (Fig. 3).

Protein Chemical Mapping of the Amber Mutants. A simple procedure for mapping early mutants in the *lac* repressor is based on the rapid release of peptides corresponding to the amino-terminal 80–100 residues of the *lac* repressor after treatment of the protein under native conditions with trypsin (18, 19). Because of its insolubility, the tryptic peptide spanning the sequence between residues 60 and 82 is difficult to isolate by this procedure. This peptide, however, is readily obtained as part of the peptide spanning residues 52–82, if succinylated repressor is digested by trypsin and the resulting peptides separated by gel filtration on Sephadex G-50 followed by ion-exchange chromatography on DEAE-Cellulose (Files & Weber, unpublished). The combination of both procedures was used to determine the amino-acid change resulting from suppression of amber i136. The suppressor used in this experiment was Su3 (originally termed SuYmel), which inserts tyrosine (20). The suppressed derivative was constructed by P1 transduction of the i136 mutant to Su^+ with a lysate grown on an Su^+ strain. Insertion of the suppressor into the i136 strain restores the wild-type repressor activity. Amino-acid sequence analysis of the suppressed i136 derivative showed only one amino-acid change in residues 1–82 (Fig. 4). The glutamine residue in position 60 is changed to tyrosine. This result shows that the *i136* mutation results from a transition to UAG of the CAG triplet coding for glutamine at position 60.

DISCUSSION

We characterized two internal translation reinitiation sites in the early part of the *i* gene and showed that these two sites are distinct from one another. The first of these sites results

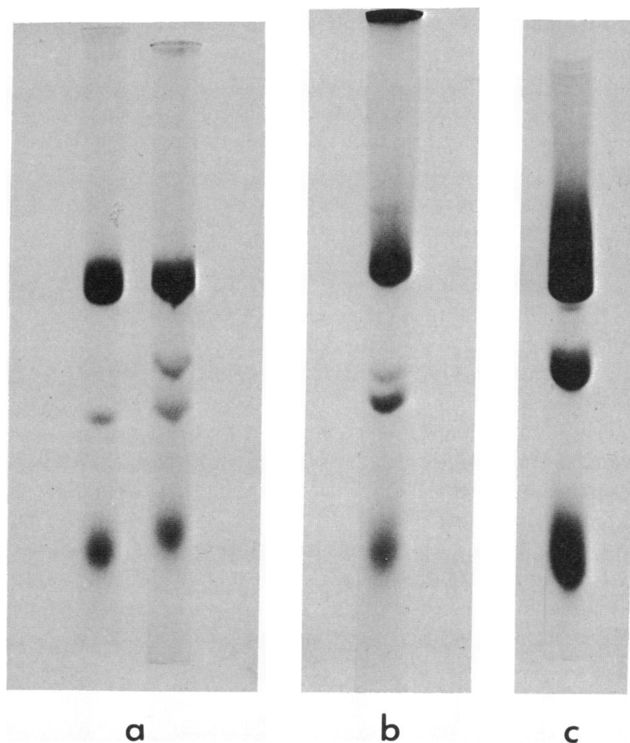

FIG. 2. Nb dodecyl sulfate–polyacrylamide gels of antigen–antibody complexes. (*a*) Analytical gels used for molecular weight determination. In order, from the top of the gel, the bands are: (*left*) heavy IgG (50,000), i^{136} protein, and light IgG (23,000); (*right*) heavy IgG, *lac* repressor (38,000), i^{136} protein, and light IgG. (*b*) Similar anylytical gels showing, in order from top: heavy IgG, *lac* repressor, i^{100} protein, and light IgG. (*c*) Heavily overloaded preparative gel of repressor obtained from a wild-type strain (*center band*). No i^{100} or i^{136} material is visible.

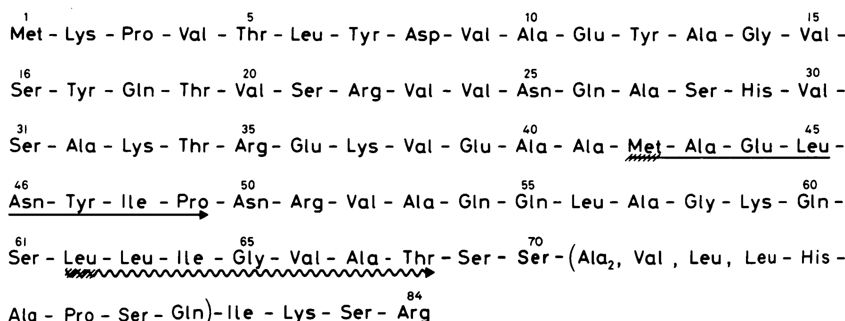

FIG. 3. Amino-terminal sequence of the wild-type repressor* and the internally reinitiated proteins. *i100:* The amino-terminal sequence of the i100 protein (→) corresponds to residues 43–49 and immediately follows the first internal methionine (residue 42) of the wild-type polypeptide chain. *i136:* The amino-terminal sequence of the i136 protein (⇝) corresponds to residues 63–68 and shows an amino-terminal methionine in the position corresponding to a leucine (residue 62) in the wild-type polypeptide chain.

* The amino-acid sequence for residues 1–59 has been reported (12, 19). In order to isolate the peptide spanning residues 52–84 (peptide A), repressor protein was succinylated and digested with trypsin. Peptide A was isolated by gel filtration on Sephadex G-50 followed by chromatography on DEAE–cellulose. The amino-acid sequence for residues 52–70 is based on direct Edman degradation and isolation of fragments obtained by digestion of peptide A with thermolysin, subtilisin, and chymotrypsin. The carboxyl-terminal residues are assigned from the results obtained by carboxypeptidase digestion of peptide A and by isolation of a thermolysin tetrapeptide, Ile-Lys-Ser-Arg. The residues given in the *parentheses* are tentative and were deduced from the difference between the amino-acid composition of peptide A and the amino-acid sequence given. Six of these residues are accounted for by the amino-acid sequence of a thermolysin hexapeptide shown in *parentheses.*

from reinitiation at the first internal in-phase AUG codon of the mRNA of the *i* gene (12). This site is activated by the introduction of an amber mutation in the position corresponding to Gln26 in the amino-acid sequence of the *lac* repressor. Characterization of the second restart, described in this paper, shows that it results from reinitiation at the codon specifying the amino-acid leucine at position 62 in the repressor protein. The position of the amber block that activates this initiation site has been identified as residue 60 of the protein sequence. The localization of the initiation site is based on the comparison of the amino-terminal seven residues of the i136 repressor with the wild-type repressor amino-acid sequence available and, independently, on the polypeptide-chain molecular weight of the i136 protein. For both i100 (12) and i136 proteins, the efficiency of reinitiation is at least 10%, based on the amount of inducer-binding activity and crossreacting material in cell extracts of the mutant strains.

The assumption of a codon specifying the amino-acid leucine as an initiation codon *in vivo* is a novel one. Although it could be argued that initiation occurs at an in-phase AUG codon before position 62, this hypothesis requires a specific proteolytic degradation of the amino terminus and is ruled out by the fact that one of the ambers (i136), which generates the second restart, has been localized in position 60.

The current results also eliminate the hypothesis that reinitiation occurs at a place later than leucine-specifying codon at position 62. Within the amino-terminal 100 residues of the *lac* repressor, only the sequence between residues 63 and 68 is identical to the amino-terminal sequence of the i136 protein and no known methionine-containing peptide of the *lac* repressor contains this amino-acid sequence (19). Also, the experimentally determined molecular weight of the i136 repressor (30,000–32,000 for the monomer) is in excellent agreement with the value (31,000) predicted by assigning the amino terminus of the i136 protein to residue 62.

It could also be argued that initiation at the codon specifying leucine 62 is due not only to introduction of an amber mutation before this site, but simultaneous mutation of the leucine codon by a single base change to an AUG codon. There are two arguments against this possibility. First, we isolated the i136 mutant without the aid of mutagenesis and a second, independently isolated spontaneous amber (i919) mapping in the same deletion group as 136 produces the same reinitiation fragment. Second, the presence of a suppresssor in the i136 strain restores the wild-type polypeptide chain without any alterations other than the anticipated insertion of a tyrosine residue at the amber site in position 60. Specifically, the leucine residue in position 62 is not changed upon suppression (Fig. 4).

There are two plausible explanations for the fact that

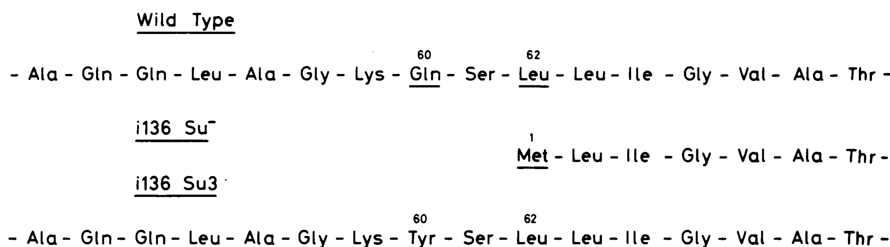

FIG. 4. Localization of the i[136] amber mutation.

initiation can occur at a codon specifying a leucine residue in the wild-type protein, each involving a novel ambiguity in the translation of this codon. In the first model the codon specifying leucine 62 is an AUG codon, which due to the effect of the surrounding sequence on the mRNA can be misread during elongation by a leucine tRNA. This explanation seems only a formal possibility and could be checked if we were to obtain an appropriate frameshift mutation to enable us to deduce the mRNA sequence for this region. The best argument against this model comes from protein chemical studies of the *lac* repressor which indicates only leucine and no methionine for the amino acid in position 62. This result would, therefore, demand a nearly quantitative misreading of the hypothetical AUG codon by a leucyl-tRNA, which we consider unlikely.

In the second model, the codon signaling initiation is a leucine codon that is recognized by an initiator, formylmethionyl-tRNA. We presume that *N*-formylmethionyl-tRNA (21) would be the initiator, although we cannot rule out the possibility that an unidentified methionyl-tRNA would be used. In this model a novel type of recognition by a formylmethionyl-tRNA occurs, and this should be considered in light of the current evidence for initiation at non-AUG codons. Experiments *in vitro* have shown that the binding of *N*-formylmethionyl-tRNA to ribosomes can be stimulated by AUG and also to a variable degree by GUG, UUG, CUG, and certain other triplets (22–32). Since however, Ghosh and coworkers have argued that only AUG and GUG show significant stimulation of *N*-formylmethionine incorporation *in vitro* (22), it has been widely assumed (33) that only these two codons can act as initiator codons. Isolation of the initiation site sequences of RNA phages has yielded only AUG initiation codons, providing an *in vivo* proof for AUG initiation by *N*-formylmethionyl-tRNA (1–4). In addition, Sherman and coworkers have found a system in yeast, namely the iso-cytochrome *c* cistron, in which AUG but not GUG promotes *in vivo* initiation (34). A leucine codon (either UUG or CUG) fails to promote initiation in this system.

Identification of the second initiation site in the *i* message could be easily explained assuming that *N*-formylmethionyl-tRNA is indeed the only transfer RNA capable of initiating and that it can recognize not only AUG and GUG, but also at least one leucine codon, presumably a CUG or UUG leucine codon. This hypothesis would be consistent with the possibility of ambiguity in the first base of the initiation codon during recognition by *N*-formylmethionyl-tRNA, as has been proposed to explain the *in vitro* results (22).

The existence of at least two internal initiation sites (neither apparently active in the absence of nearby termination) within the first 200 nucleotides of the *i*-gene message raises the possibility that internal initiation sites are very common in bacterial messages. Our evidence that a codon other than AUG can be used for internal initiation increases the number of potential internal initiation sites. We do not know whether reinitiation signals contain special nucleotide sequences that act in conjunction with the initiation codon. Clearly, more initiation signals must be elucidated before the minimal requirements for initiation can be deduced.

NOTE ADDED IN PROOF

We have recently found a new reinitiation site at the codon specifying the valine residue 23 of the *lac* repressor polypeptide chain. This leads us to conclude that GUG is also used *in vivo* for initiation of protein synthesis (Files, Weber and Miller, unpublished observation).

We thank Drs. J. Beckwith, A. Garen, and M. van Montagu for supplying us with bacterial strains. J.H.M. was supported by a grant from the Swiss National Foundation (F.N. 3.800.72). K.W. was supported by NIH Grant GM 17662-02.

1. Steitz, J. A. (1969) *Nature* **224**, 957–964.
2. Min Jou, W., Haegeman, G., Ysebaert, M. & Fiers, W. (1972) *Nature* **237**, 82–88.
3. Gupta, S. L., Chen, J., Schaefer, L., Lengyel, P. & Weissman, S. M. (1970) *Biochem. Biophys. Res. Commun.* **39**, 883–888.
4. Hindley, J. & Staples, D. H. (1969) *Nature* **224**, 964–967.
5. Sarabhai, A. & Brenner, S. (1967) *J. Mol. Biol.* **27**, 145–162.
6. Michels, C. A. & Zipser, D. (1969) *J. Mol. Biol.* **41**, 341 347.
7. Zipser, D., Zabell, S., Rothman, J., Grodzicker, T. & Wenk, M. (1970) *J. Mol. Biol.* **49**, 251–254.
8. Grodzicker, T. & Zipser, D. (1968) *J. Mol. Biol.* **38**, 305–314.
9. Newton, A. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 181–187.
10. Newton, A. (1969) *J. Mol. Biol.* **41**, 329–339.
11. Ganem, D. (1972) Honors Thesis, Harvard University.
12. Platt, T., Weber, K., Ganem, D. & Miller, J. H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 897–901.
13. Beckwith, J. R. (1963) *Biochim. Biophys. Acta* **76**, 162–164.
14. Scaife, J. & Beckwith, J. R. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 403–408.
15. Miller, J. H., Beckwith, J. R. & Müller-Hill, B. (1968) *Nature* **220**, 1287–1290.
16. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
17. Weiner, A. M., Platt, T. & Weber, K. (1972) *J. Biol. Chem.* **247**, 3242–3251.
18. Weber, K., Platt, T., Ganem, D. & Miller, J. H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3624–3628.
19. Platt, T., Files, J. G. & Weber, K. (1973) *J. Biol. Chem.* **248**, 110–121.
20. Dennert, G. & Henning, U. (1968) *J. Mol. Biol.* **33**, 327–329.
21. Capecchi, M. R. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 1517–1524.
22. Ghosh, H. P., Söll, D. & Khorana, H. G. (1967) *J. Mol. Biol.* **25**, 275–298.
23. Sundararajan, T. A. & Thach, R. E. (1966) *J. Mol. Biol.* **19**, 74–90.
24. Dube, S. K., Rudland, P. S., Clark, B. F. C. & Marcker, K. A. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 161–166.
25. Clark, B. F. C. & Marker, K. A. (1966) *J. Mol. Biol.* **17**, 394–406.
26. Uhlenbeck, O. C., Baller, J. & Doty, P. (1970) *Nature* **225**, 508–510.
27. Takeishi, K., Ukita, T. & Nishimura, S. (1968) *J. Biol. Chem.* **243**, 5761–5769.
28. Smith, A. E. & Marcker, K. A. (1970) *Nature* **226**, 607–610.
29. Rajbhandary, U. L. & Ghosh, H. P. (1969) *J. Biol. Chem.* **244**, 1104–1113.
30. Brown, J. C. & Smith, A. E. (1970) *Nature* **226**, 610–612.
31. Rajbhandary, U. L. & Kumar, A. (1970) *J. Mol. Biol.* **50**, 707–711.
32. Lengyel, P. & Söll, D. (1969) *Bacteriol. Rev.* **33**, 264–301.
33. Lengyel, P. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 828–841.
34. Stewart, J. W., Sherman, F., Shipman, N. A. & Jackson, M. (1971) *J. Biol. Chem.* **246**, 7429–7445.
35. Gorini, L. (1970) *Annu. Rev. Genet.* **4**, 107–134.
36. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
37. Kumar, S. & Szybalski, W. (1969) *J. Mol. Biol.* **40**, 145–151.
38. Pfahl, M. (1972) *Genetics* **72**, 393–410.
39. Müller-Hill, B., Crapo, L. & Gilbert, W. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 1259–1264.