Nature of the Ribosomal mRNA Track: Analysis of Ribosome-Binding Sites Containing Different Sequences and Secondary Structures†

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Received June 9, 1993; Revised Manuscript Received July 2, 1993•

ABSTRACT: The ribosomal mRNA track was investigated by toeprinting 30S ribosomes, in the presence or absence of tRNA, using a variety of different ribosome-binding sites. We found that: (1) the ribosome, by itself, recognizes the mRNA translational initiation site; (2) the ribosomal mRNA track makes extensive contact with mRNA independent of tRNA and the start codon; (3) ribosome–mRNA complexes are less stable than complexes containing tRNA; and (4) toeprinting can be used to analyze the contour of the ribosomal mRNA track, yielding information on its "height" as well as its "length" dimension. Examination of several ribosome-binding sites, including those containing very stable secondary structure, indicated that the "height" of the mRNA track is quite roomy, while the nucleotide distance between the site of Shine–Dalgarno annealing, the P site, and the 3′-edge of the mRNA track is fixed. The data suggest a mechanism for tethering regulatory elements to the ribosome during translation.

Translational initiation is a multistep process which involves at least two RNA: RNA interactions: annealing of the 3′-terminal nucleotides of the 16S rRNA to the Shine–Dalgarno (SD)[1] sequence and pairing of the initiator tRNA anticodon to the mRNA start codon (Calogero et al., 1988; Gualerzi et al., 1987). Initiation requires as well a variety of essential protein factors and mRNA regulatory elements that function during initiator tRNA selection and decoding of the start codon and contribute to the regulation of the level of translational expression [for recent reviews, see Rudd and Schneider (1992), Gualerzi and Pon (1990), McCarthy and Gualerzi (1990), and Gold (1988)]. Typically, the regulatory mRNA elements are contained within the ribosome-binding site (RBS), often comprising an unstructured region (Dreyfus, 1988) of about 35 nucleotides containing a purine-rich sequence 5–11 nucleotides upstream of the start codon (Stormo et al., 1982). The regulatory effect of naturally occurring start codons, SD sequences, the spacing between them, and second codons on translational yield has been studied (Ringquist et al., 1992), as has the effect of stable secondary structure at the RBS (de Smit & Van Duin, 1990; Ringquist et al., 1992).

Toeprinting, the inhibition of cDNA synthesis by the complex formed between mRNA, tRNA, and a ribosome, has provided an important technique for investigating the process of translational initiation (Hartz et al., 1989). A typical toeprint signal occurs 15 nucleotides 3′ of the first nucleotide of the codon in the ribosomal P site and probably corresponds to the 3′-edge of the mRNA track (Hartz et al., 1988, 1989; Kang & Cantor, 1985). Selection of the RBS by 30S particles (Hartz et al., 1989), decoding of initiator tRNA and the start codon by the interaction between protein initiation factors and 16S rRNA (Ringquist et al., 1993; Hartz et al., 1989, 1990, 1991a), and the effect of RBS elements on translational yield (Ringquist et al., 1992; Hartz et al., 1991a; Lang et al., 1989; Spedding et al., 1993) have been examined. Briefly, initiator tRNA and the start codon are recognized by the 30S ribosome with the aid of the translational initiation factor IF3 (Hartz et al., 1989, 1990; Risuleo et al., 1976) and rRNA residues within the decoding site (Ringquist et al., 1993). Formylation of the formylmethioninyl-tRNA is proofread by IF2 (Hartz et al., 1989). Surprisingly, toeprint experiments in the absence of initiation factors and tRNA (binary complexes) have also identified a site-bound ribosome–mRNA complex, indicating that the 30S particle, by itself, identifies the translational initiation region (Hartz et al., 1991b). However, binary complexes gave toeprints immediately downstream from the SD region (Hartz et al., 1991b) and (we thought) not at the position of the ternary toeprint, suggesting a role of tRNA in stabilizing additional contacts between the ribosomal mRNA track and the message.

The present work reinvestigates the interaction between ribosome and message in order to assess the role of the ribosomal mRNA track during translation. In contrast to our previous experiments, toeprint data in the absence of tRNA clearly show extensive ribosome–mRNA interactions 19–23 nucleotides 3′ to the SD sequence, as though the message occupies the full track of the 30S particle. In addition, the data also suggest a mechanism for the action of mRNA-regulatory elements during translation. We studied two such cis-acting regulatory elements engaged during translational recoding in *Escherichia coli* (Gesteland et al., 1992). One of these, the selenocysteine insertion sequence (SECIS) from the *E. coli fdhF* mRNA, directs cotranslational insertion of selenocysteine at an adjacent UGA codon (Zinoni et al., 1990), while the other constitutes the translational coding gap in the bacteriophage T4 gene 60 mRNA (Huang et al., 1988). These mRNA elements exhibit unusual interactions in the translational initiation complex, suggesting that very stable secondary structures can exist within the ribosomal–mRNA track

† This work was funded by a research grant from the National Institutes of Health to L.G. (GM-28685).

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• Abstract published in *Advance ACS Abstracts*, September 1, 1993.

[1] Abbreviations: SD, Shine–Dalgarno; RBS, ribosome-binding site; RT, reverse transcriptase; SECIS, selenocysteine insertion sequence; UIVS, unspliced intervening sequence.

0006-2960/93/0432-10254$04.00/0

Table I: DNA Oligos Used in This Study

oligo[a,b]	sequence[c]
A	5′-CCCGTCGACAAAGCTGTTTAGCTACTGCAGTTCGTTAATAGCATT GCTCGTTGCTCACA$(T)_6$TCCTCCTTA$(T)_8$AGATCTCAGCATGCTCG ACAGGCATCTCCCTATAGTGAGTCGTATTAAGCTTCGG-3′
B	5′-CCCGTCGACAAAGCTGTTTAGCTACTGCAGTTCGTTAATAGCATT GCTCGTTGCTCACA$(T)_{10}$TCCTCCTTA$(T)_8$AGATCTCAGCATGCTCG ACAGGCATCTCCCTATAGTGAGTCGTATTAAGCTTCGG-3′
C	5′-CCCGTCGACAAAGCTGTTTAGCTACTGCAGTTCGTTAATAGCATT CTTTATTTTAGT$(CA)_5$ATCCTCCTTA$(T)_8$AGATCTCAGCATGCTCG ACAGGCATCTCCCTATAGTGAGTCGTATTAAGCTTCGG-3′
D	5′-CCCGTCGACAAAGCTGTTTAGCTACTGCAGTTCGTTAATAGCATT GCTCGTTGCGCCATTACCGACCGATTGGTGCAGACCTGCAACCG ATGGGCCGTGTCACA$(T)_6$TCCTCCTTA$(T)_8$AGATCTCAGCATGCTC GACAGGCATCTCCCTATAGTGAGTCGTATTAAGCTTCGG-3′
E	5′-CGCCAGGGTCTTCCCAGAGTGCTCGTTGTAGGACGACGGGATCCT GTGATCATAATCCAATAATCTCTTAATTATGAGGTATTTCTATAG ATAGCCCGAAGGCTACATAAGATCTTCCTCCTTAAGCTTGGC-3′
5G3 (5′ PCR primer)	5′-CCGAAGCTTAATACGACTCACTATAGGGAGATGCCTGTCGAGCATGCTG-3′
3G3 (3′ PCR primer)	5′-CCCGTCGACAAAGCTGTTTAGCTAC-3′
5g60 (5′ PCR primer)	5′-CCCGAATTCTAATACGACTCACTATAGCCAAGCTTAAGGGAGGAAGATCTT-3′
3g60 (3′ PCR primer)	5′-CGCCAGGGTCTTCCCAGAGTGCTGCTTGTA-3′

[a] DNA oligos 5G3 and 3G3 are PCR primers for oligos A–D. [b] DNA oligos 5g60 and 3g60 are PCR primers for oligo E. [c] The symbol $(T)_n$ or $(CA)_n$ represents the number of times either T or the dinucleotide CA is repeated.

and may form part of the translational recoding signal for these mRNAs.

EXPERIMENTAL PROCEDURE

Materials. M-MLV reverse transcriptase was purchased from Bethesda Research Laboratories; AMV reverse transcriptase was from Life Sciences, Inc.; T7 RNA polymerase was from US Biochemicals Corporation; Taq polymerase was from Promega; and T4 polynucleotide kinase was from New England Biolabs. 30S ribosomes were a gift from R. Traut. *E. coli* tRNAs were purchased from Subriden (Rolling Bay, WA). Other chemicals were reagent quality.

Nucleic Acids. RNAs were generated *in vitro* by transcription of PCR-amplified DNA oligos using T7 RNA polymerase. DNA oligos (Table I) were synthesized on an Applied Biosystems Model 394 DNA/RNA synthesizer and purified by 6% preparative polyacrylamide gel electrophoresis. PCR amplification was performed in 50 mM KCl, 10 mM Tris-HCl, pH 8.6, 2.5 mM $MgCl_2$, and 1.7 mg/mL BSA. dNTPs were present at 1 mM each, and Taq polymerase was used at 1 unit per 0.1 mL reaction mixture. The 5′ and 3′ primers were present at 1 μM each. Transcription was performed in 8% PEG 8000, 5 mM DTT, 40 mM Tris-HCl, pH 8.0, 12 mM $MgCl_2$, 1 mM spermidine, and 0.002% Triton X-100, and reaction mixtures contained T7 RNA polymerase at a final concentration of 1 unit/μL. The reaction mixture was incubated at 37 °C for 2 h and then treated with 20 units of DNAse I (RNAse free) for an additional 5 min at 37 °C. The reaction was stopped by the addition of an equal volume of loading buffer (72% formamide and 120 mM EDTA, pH 8.0), and the mixture was heated to 60 °C for 3 min prior to electrophoresis on a 6% polyacrylamide, 8 M urea denaturing gel. The RNA transcript was visualized by UV shadowing, and the RNA band was excised from the gel and eluted into dH_2O. RNA was then ethanol precipitated, dried under vacuum, and redissolved in dH_2O. The concentration of the RNA was determined, assuming one A_{260} unit is equal to 40 μg/mL.

Ternary Toeprints. Extension inhibition (toeprinting) was performed as described by Hartz et al. (1988) using M-MLV reverse transcriptase between 160 and 0.02 unit per reaction, as indicated in the Results. The ^{32}P-labeled primer 3G3, which is complementary to each of the RNA transcripts, was annealed as described in Hartz et al. (1988). Toeprint reaction mixtures were prepared in standard buffer (10 mM Tris–acetate, pH 7.4, 60 mM NH_4Cl, 10 mM magnesium acetate, and 6 mM β-mercaptoethanol) and contained 7 nM mRNA along with 30S ribosomes and tRNAs in a volume of 10 μL, as specified in the Results. The reaction components were combined on ice and preincubated at 37 °C for 15 min before addition of reverse transcriptase and incubation for an additional 15 min.

Binary Toeprints. Binary complexes were toeprinted in the same manner as ternary complexes except that tRNA was absent and the concentration of M-MLV reverse transcriptase was less, as indicated in the Results. Toeprint results were quantitated by measuring the amount of ^{32}P radioactivity in the full-length message band and in the toeprint band with an AMBIS Radioanalytic Imaging System (AMBIS Systems, Inc.) and expressed as relative toeprint, calculated as described by Hartz et al. (1989).

RESULTS

Toeprinting experiments with binary and ternary complexes were performed using five different RBSs (Figure 1) in order to map the distance between the site of SD annealing, the P site, and the 3′-edge of the track. RBSs containing potentially stable secondary structure were investigated to assess the "height" of the mRNA track ("height" refers to the amount of room for extra RNA structures or other materials around the location of bound mRNA; in the extreme the track would be fully accessible to solvent). RBSs shared the SD sequence, UAAGGAGG, but otherwise differed in sequence and secondary structure potential.

Mapping the distance between the Site of SD Annealing, the P Site, and the 3′-Edge of the Ribosome. Toeprint experiments were used to map the position of landmarks along the ribosomal mRNA track, such as the site of SD annealing and the P site, relative to the toeprint. RNAs A and B, which differed in the number of adenine residues between the SD and the start codon, were examined to determine if the position of the binary toeprint was sensitive to the location of the SD or the start codon. Binary complexes toeprinted RNA A 19 nucleotides from the SD sequence and RNA B 19–23 nucleotides from the SD (Figures 2 and 3), indicating that the binary toeprint covered a range of sizes and that there must

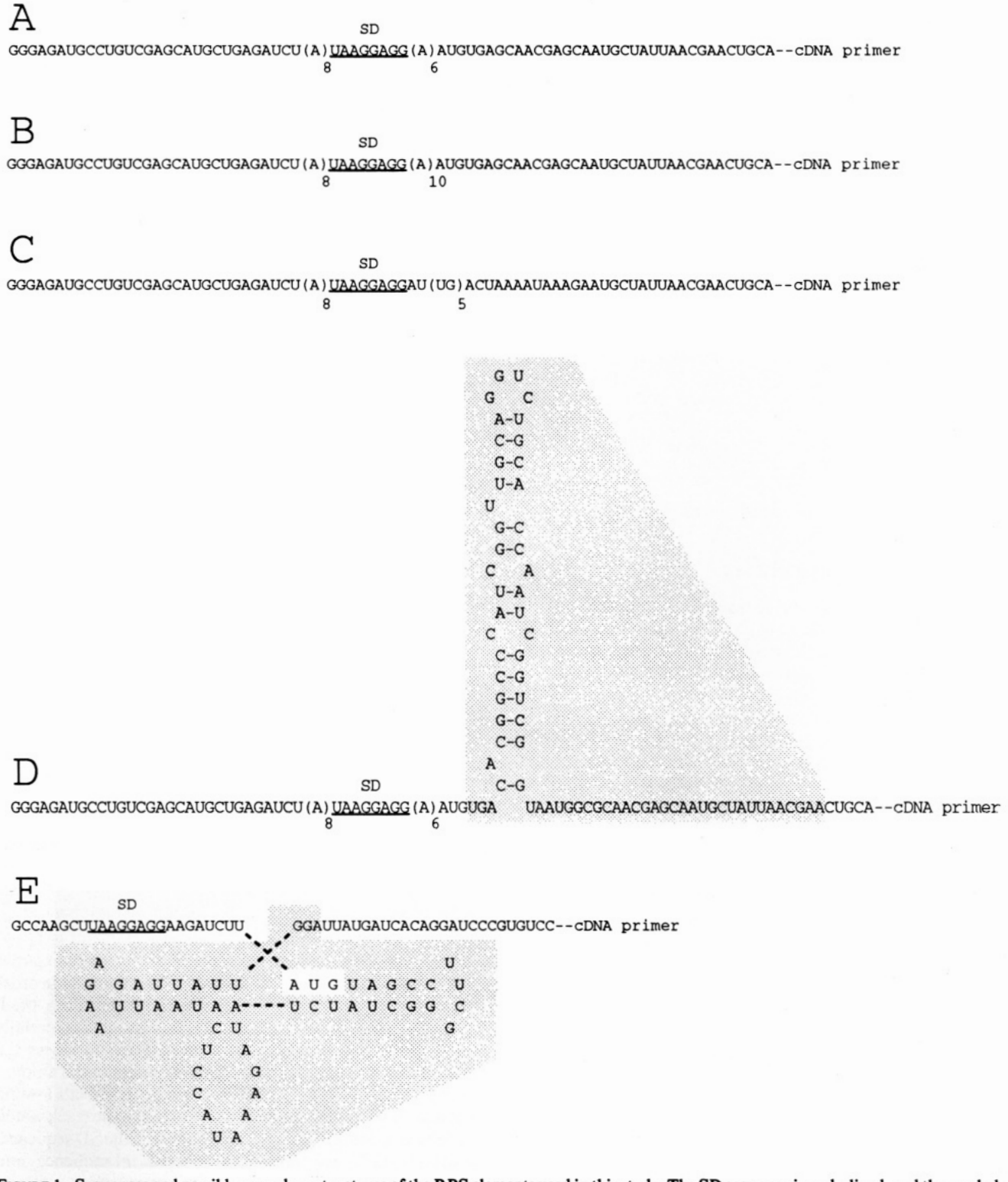

FIGURE 1: Sequences and possible secondary structures of the RBS elements used in this study. The SD sequence is underlined, and the symbols $(A)_n$ and $(UG)_n$ indicate the number of times these nucleotides were repeated. Shaded regions indicate naturally occurring nucleotides and the potential secondary structure of the *E. coli fdhF* and bacteriophage T4 g60 mRNAs (Zinoni et al., 1990; Huang et al., 1988; Burke-Agüero & Hearst, 1990).

be heterogeneity in the structure of the binary complexes. The data also indicate that the position of the binary toeprint was sensitive to the presence of the SD but independent of the start codon. The binary toeprints were sensitive to the concentration of reverse transcriptase (RT), with the maximum relative toeprint signal occurring below 0.2 unit of RT per reaction when RNA A was examined (Figure 2). Above this concentration the signal decreased, probably representing the fast off-rate of the ribosome–mRNA complex in the absence of tRNA. In contrast, the 30S–mRNA–tRNA ternary complex toeprinted both RNAs over the entire range of RT concentrations, but the position of the toeprint shifted when less RT was used. The concentration dependence may represent the probability of cDNA synthesis reinitiating at the toeprint site and indicates that RT can "drive" into the complex. Moreover, when these RBSs were examined in the presence of tRNA, the position of the ternary toeprint was dependent solely on the start codon, in agreement with previous

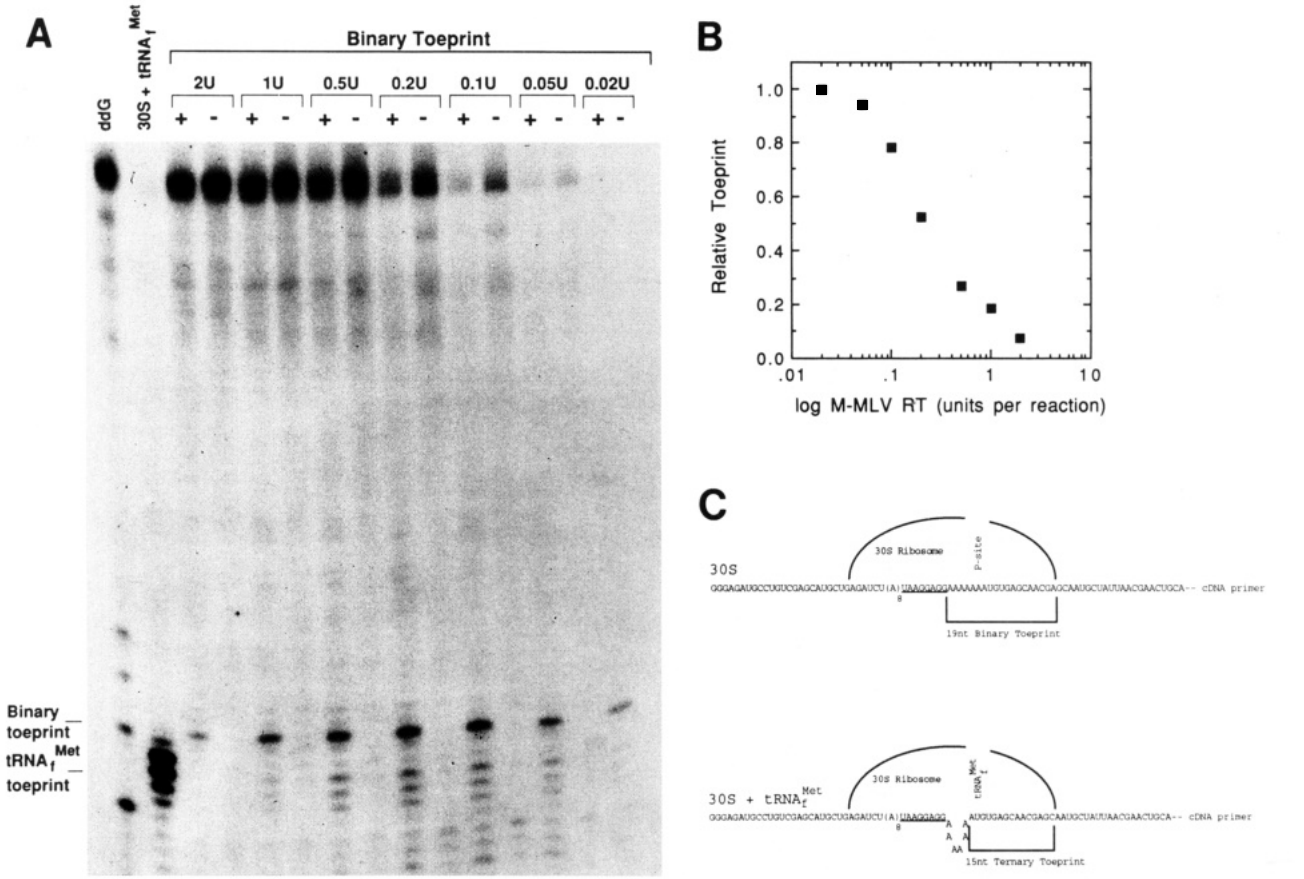

FIGURE 2: Toeprinting of the ribosome–mRNA binary complex is sensitive to the concentration of reverse transcriptase. (A) 30S ribosomes were toeprinted in the presence (lane 2) or the absence of tRNA^{fMet} (lanes 3–16). The concentration of M-MLV reverse transcriptase is indicated and was varied between 2 and 0.02 unit. The symbols (+) and (–) indicate whether 30S ribosomes were present or absent, respectively. The ddG sequence of RNA A is shown in lane 1. The concentrations of tRNA^{fMet}, 30S ribosomes, and mRNA were 500, 100, and 7 nM, respectively. (B) Plot of the relative toeprint signal from the binary complex as a function of M-MLV RT concentration. (C) Illustrations of the binary and ternary complexes and the sites of toeprinting.

results (Hartz et al., 1988, 1989), and occurred about 15 nucleotides away.

RNA C, an RNA containing multiple start codons, was used to examine whether the site of toeprinting could occur at the same position for binary and ternary complexes, indicating the absence of a conformational change upon tRNA–codon pairing. Examination of ternary complexes with RNA C, which contained the standard SD sequence and four overlapping GUG and UGU triplets, was performed to measure the smallest spacing between the SD and the start codon that allowed ternary complex formation (Figure 4, panel A). Toeprint analysis of the ternary complex formed with tRNA^{fMet} or tRNA^{Cys} revealed that there must be at least five nucleotides between the SD sequence and the P site, consistent with previous results (Hartz et al., 1991a,b; Ringquist et al., 1992). However the two tRNAs behaved differently: tRNA^{Cys} toeprinted at two codons, while tRNA^{fMet} toeprinted at a single codon under the same experimental conditions and may represent a specialized feature of the initiator tRNA during start site selection.

RNA C was then used to investigate whether binary complexes would toeprint at the same position as the tRNA^{fMet}-containing ternary complexes (Figure 4, panel B). Both complexes toeprinted RNA C 21 nucleotides from the SD sequence when low RT was used, implying the absence of a conformational change during anticodon–codon pairing. The data suggest that the ribosomal mRNA track lies at the base of the P site, regardless of the position of the start codon, since the binary toeprint band did not shift upon addition of tRNA.

The Ribosomal mRNA Track Can Accommodate Large Stem–Loop Structures. Toeprint experiments were performed with RBSs containing the potential to form stable secondary structure to determine if structured RNA could coexist within the ribosomal mRNA track. RBSs containing natural sequences associated with translational recoding were used (Figures 5 and 6). RNA D contained the 38-nucleotide hairpin associated with the selenocysteine insertion sequence (SECIS) from the *E. coli* selenoprotein-encoding mRNA *fdhF* (Zinoni et al., 1990), while RNA E contained the 50-nucleotide unspliced intron from bacteriophage T4 g60 (Huang et al., 1988).

The 30S–mRNA–tRNA^{fMet} complex toeprinted RNA D at two sites, +16 and +52 nucleotides from a single start codon (Figure 5). The extended-toeprint signal at +52 may represent the contribution of the folded mRNA. The intensity of the extended toeprint increased when the RT concentration decreased and when the temperature was lowered from 37 to 23 °C, indicating that the extended toeprint was sensitive to RT activity (Figure 5). Interestingly, the extended toeprint would have been 14 nucleotides from the start codon if the predicted secondary structure were deleted, as though the height of the mRNA track is large enough to accommodate the intact, proposed stem–loop. In the Discussion we consider the idea of an unbounded mRNA track, a situation in which the mRNA is exposed to solvent.

A similar result was observed when the unusual, unspliced intervening sequence (UIVS) from bacteriophage T4 (Haung et al., 1988; Weiss et al., 1990) was examined (Figure 6).

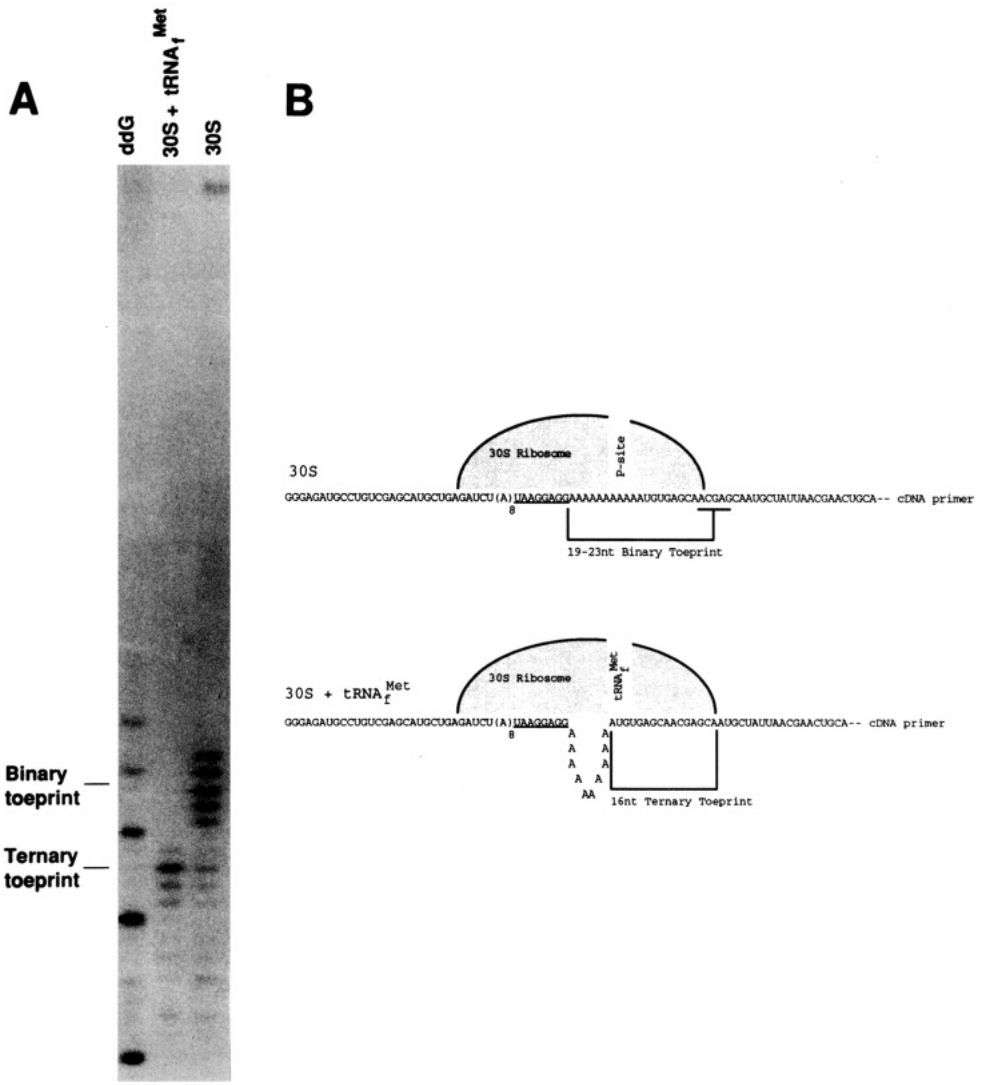

FIGURE 3: Toeprinting by the binary complex is independent of the position of the start codon. (A) Lane 1 is the ddG sequence of RNA B; lane 2, the ternary toeprint when tRNA^{fMet} and 30S ribosome were present; lane 3, the binary toeprint. The concentrations of tRNA^{fMet}, 30S ribosomes, and RNA B were 500, 100, and 7 nM, respectively. M-MLV RT was present at 0.02 unit per reaction. (B) Illustrations of the binary and ternary complexes and the sites of toeprinting.

Toeprinting in the presence of tRNA^{fMet} identified two AUG codons interacting with the ribosomal P site, but not the extended toeprint observed with RNA D. Surprisingly, these codons were 8 and 63 nucleotides from the SD sequence. tRNA^{Gly} also caused the ribosome to toeprint at a single site, but the glycine codon, GGA, was 58 nucleotides from the SD. *In vivo*, the glycine codon defines the 3′-edge of the UIVS (Huang et al., 1988) and has been proposed to interact with the ribosomal P/A site(s) during recoding (Burke-Agüero & Hearst, 1990). *In vitro*, the UIVS from g60 may fold into a compact structure which bridges the site of SD annealing and the P site, consistent with the branch migration model proposed by Burke-Agüero and Hearst (1990) for efficient translation of this message.

DISCUSSION

Results. The toeprint results are intriguing. They illustrate that the ribosome binds the RBS tightly, even without tRNA, and that there is room for a large folded mRNA structure to exist within the complex. The dimensions of the mRNA track, as measured by toeprinting, indicate that the 3′-edge of the ribosome is 19–23 nucleotides from the SD sequence and 15–16 nucleotides from the P site, consistent with comparative analysis of *E. coli* RBSs as well as "bind and chew" experiments in the presence of tRNA [reviewed by Gold (1988) and Gold et al. (1982)]. The data indicate that the binary toeprint covers a range of sizes but always includes the size that will be the ternary toeprint. This suggests that the alignment of the P site by the ribosome may not be exact, although heterogeneity from the cDNA synthesis reaction may account for at least some of the extra toeprint sites. The results suggest that there must be considerable flexibility in identifying what will be the cognate codon that is selected for use in the P site. Moreover, the data also indicate that toeprinting can measure the contour length of the mRNA track. The overlapping toeprints from binary and ternary complexes when RNA C was examined suggest that the mRNA track positions the message at the bottom of the P site independent of the start codon or tRNA. Surprisingly, this distance correlates to the natural distances observed between SD sequences and start codons in *E. coli* (Stormo et al., 1982) and suggests that the ribosomal mRNA track holds the message in proper orientation for annealing to the anticodon only when the optimum spacing between the SD sequence and the start codon is used. Interestingly, cross-linking experiments between rRNA and 4-thiouridine-substituted mRNA also exhibited a pattern of cross-linking that was independent of the presence of tRNA (Rinke-Appel et al., 1991; Bhangu & Wollenzien, 1992). The

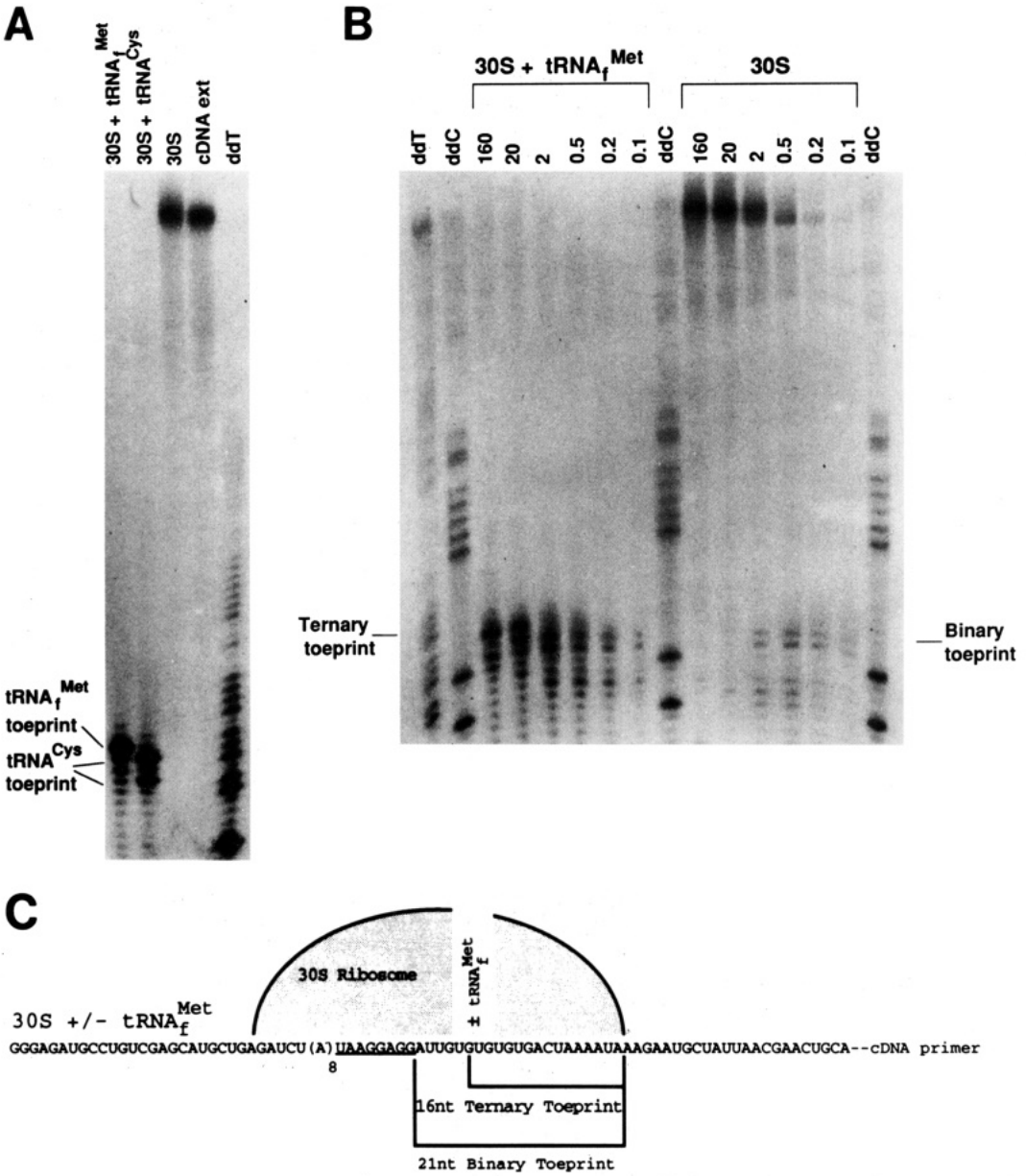

FIGURE 4: Ternary complex requires a minimum spacing of five nucleotides between the SD and the start codon. (A) 30S ribosomes were toeprinted in the presence of tRNA^{fMet} (lane 1) or tRNA^{Cys} (lane 2). Lane 3 is the 30S ribosome alone, while lane 4 is the cDNA extension control. Lane 5 is a cDNA size standard. The concentrations of tRNA, 30S ribosomes, and mRNA were 500, 10, and 7 nM, respectively. RT was present at 160 units per reaction. (B) 30S ribosomes were toeprinted in the presence (lanes 3–8) or the absence of tRNA^{fMet} (lanes 10–15). Lanes 2, 9, and 16 are the ddC sequence, and lane 1 is the ddT sequence of RNA C. The concentration of M-MLV reverse transcriptase was varied between 160 and 0.1 unit as indicated. The concentrations of tRNA, 30S ribosomes, and mRNA were 500, 10, and 7 nM, respectively. (C) The sequence of RNA C along with a summary of the toeprint results.

binary toeprint data are consistent with the report by Hartz et al. (1991b) in which a single binary toeprint three nucleotides beyond the SD was reported. These authors also observed a toeprint 19–23 nucleotides from the SD sequence but interpreted their data as a separate RBS. On the basis of the present experiments, that signal was probably identical to the binary signal reported here (D. Hartz, personal communication).

These experiments address several characteristics of RBS recognition as well as decoding of the start codon. The results clear up the discrepancy between the observed spacing of five nucleotides from SD to start codon measured previously with RNA C (Hartz et al., 1991a,b) and the optimum spacing of eight nucleotides measured for translational initiation *in vivo* and *in vitro* when a unique start codon was used (Ringquist et al., 1992; Hartz et al., 1991a). The unusually small spacing observed within the RBS of RNA C is probably related to the strong tendency of RT to reinitiate cDNA synthesis at the toeprint site; thus RT drives the message out of the ribosomal mRNA track. RNA C toeprinted at a spacing of five nucleotides, and not at the optimum of seven to nine nucleotides, because the anticodon–codon pair slipped as a result of cDNA synthesis. Slipped mispairing has been observed during replication of a repeating tetranucleotide (Farabaugh & Miller, 1978) as well as at runs of a single nucleotide (Pribnow et al., 1981; Shinedling et al., 1987) or a repeating dinucleotide (Miller, 1985), suggesting that slippage at a repeating GU is a possible event for a bound tRNA that is held rigidly in the ribosomal P site. When the ribosome–tRNA complex is presented with a choice of codons, the toeprint assay probably selects against the longer spacing observed when a single start codon is examined, and in a way that does not reflect upon the optimal spacing for translation (Hartz et al., 1991a; Ringquist et al., 1992).

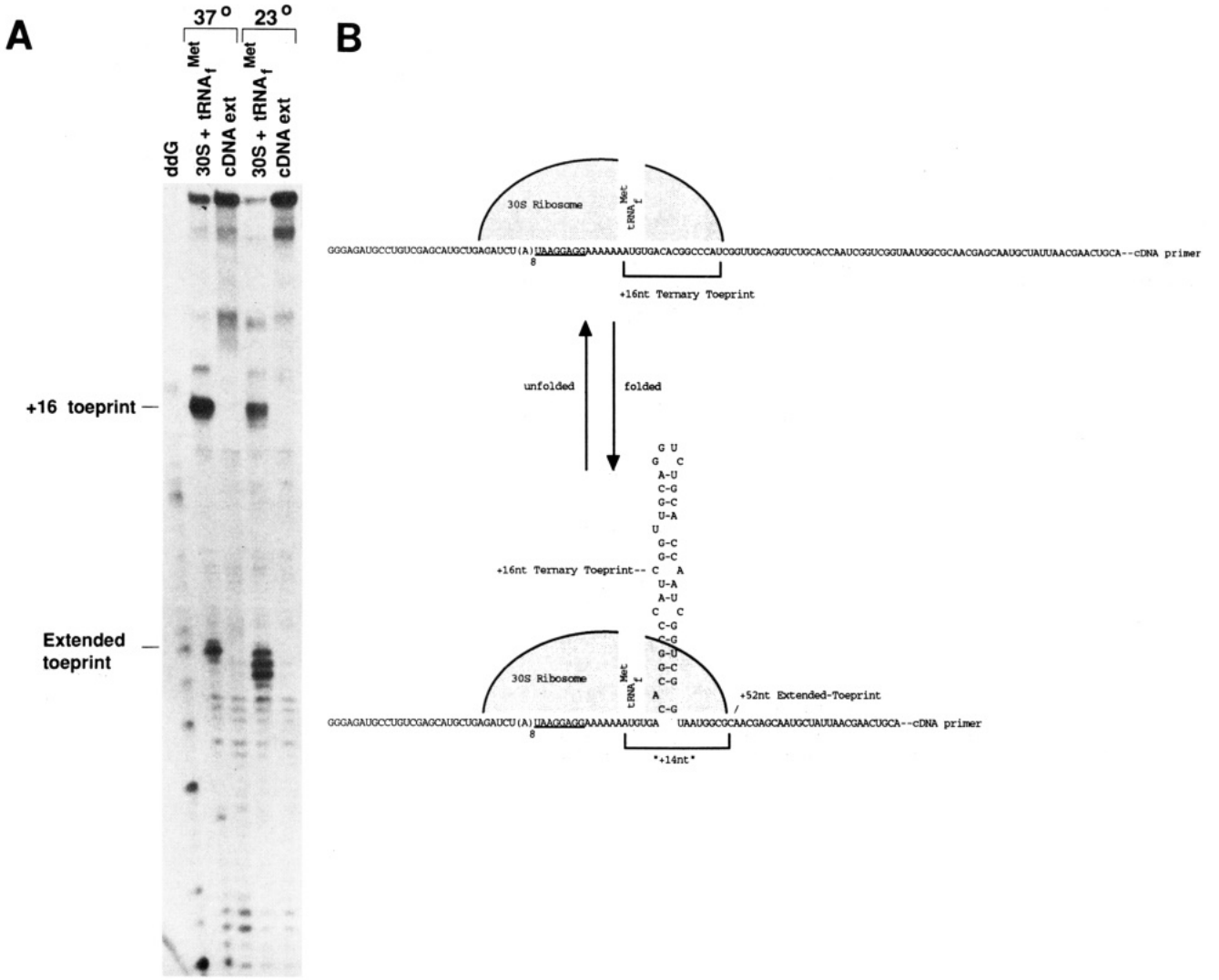

FIGURE 5: The SECIS-containing message RNA D was toeprinted at +16 and +52 nucleotides from a single start codon. (A) Lanes 2 and 4 are the ternary toeprint at either 37 or 23 °C as indicated. Lanes 3 and 5 are the extension controls, while lane 1 is the ddG sequence of the message. The concentrations of tRNA^{fMet}, 30S ribosomes, and mRNA were 500, 10, and 7 nM, respectively. The sequence of RNA D and the sites of toeprinting are summarized in panel B. The SECIS is folded as proposed by Zinoni et al. (1990).

Interestingly, the observation that tRNA^{fMet}-containing complexes toeprinted at a single site while tRNA^{Cys} complexes toeprinted at two sites may indicate a unique property of the initiation complex (Ringquist et al., 1993). Perhaps the different toeprint patterns reflect an important aspect of start codon selection: that initiator tRNA must proofread near-cognate as well as cognate start codons. The initiation complex probably "scans" several potential start codons during translational initiation (Ringquist et al., 1992) and may be aided by an enhanced off-rate for anticodon–mRNA pairing. During toeprinting, RT might take advantage of a fast off-rate for the anticodon–codon pairing to drive the initiation complex toward the minimum SD and start codon spacing. High concentrations of RT also altered the equilibrium between the two tRNA^{Cys}-containing complexes, converting them into a single complex with a spacing of six nucleotides between the SD and the P site (data not shown).

Possible Role of mRNA Elements during Translation Recoding. The toeprint results illustrate that mRNA elements associated with translational recoding fold so that nucleotides far from the SD interact with the ribosomal mRNA track. Structured sequences can occur on either side of the P site. For instance, the track accommodated up to 63 nucleotides between the SD and the P site when the g60 UIVS was examined and at least 52 nucleotides between the P site and 3′-edge of the ribosome in the case of the SECIS (see Figures 5 and 6). This was consistent with the data of Hartz et al. (1991) in which it was shown that the bacteriophage T4 gene 38 mRNA contained a small stable structure between the SD and the P site. These data also suggest that the unstructured RNAs can loop out of the mRNA track when ternary complexes are formed at RBSs containing SD to start codon spacing greater than optimal. Of course, it is also possible that the position of the SD region of the ribosome is flexible and can account for at least some of the natural variability observed within the SD to P site distance (Stormo et al., 1982; Rudd & Schneider, 1992).

The accommodation of stable, structured mRNA within the ribosome suggests that the track is unbounded. This may allow structured mRNA-regulatory elements to extend into the solvent and bind protein factors (Gold, 1988). For instance, the specialized translational elongation factor SELB binds the loop region of the *fdhF* message (S. Ringquist, unpublished experiments; Baron et al., 1993), and mRNA-bound SELB may be essential during recoding of a UGA stop codon from nonsense to selenocysteine. Tethering of regulatory elements to the translation complex via mRNA structure may turn out to be a common feature of translational recoding. Elucidation

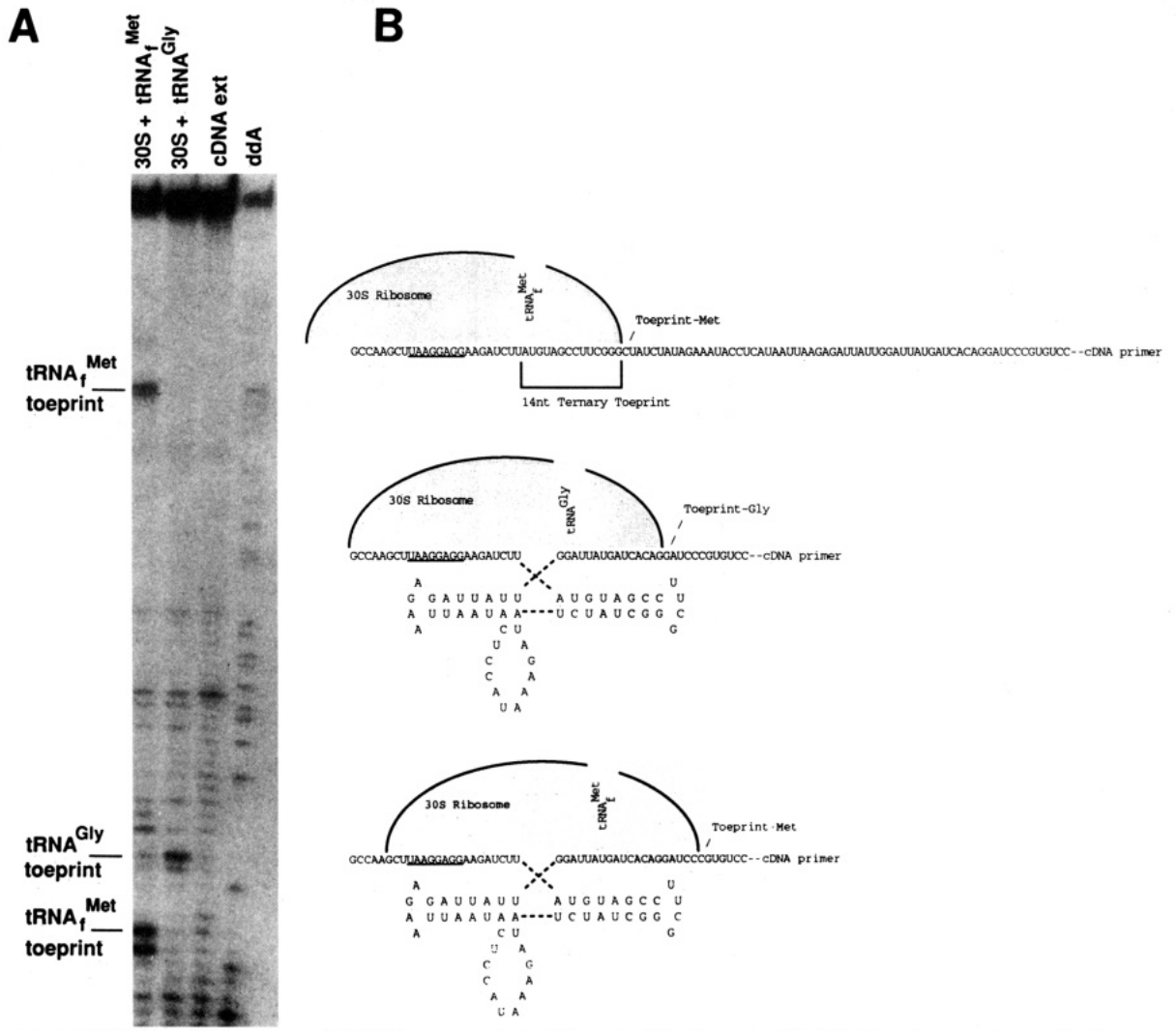

FIGURE 6: The UIVS-containing message RNA E was toeprinted 78 nucleotides from the SD. (A) Toeprint experiments in the presence of either tRNA^{fMet} or tRNA^{Gly}. Lane 1 contained tRNA^{fMet}, lane 2 contained tRNA^{Gly}, and lane 3 illustrates the cDNA extension control. Lane 4 is the ddA sequence of RNA E. Reaction mixtures were incubated for 60 min at 37 °C prior to cDNA synthesis for 15 min at 37 °C. The concentrations of tRNA, 30S ribosomes, and mRNA were 1 μM, 10 μM, and 7 nM, respectively. (B) Sequence of the g60 message along with a summary of the toeprint results. The intron is folded as proposed by Burke-Agüero and Hearst

of these elements and their mechanism of action during translation will lead to a fresh appreciation of the versatility of the genetic code.

ACKNOWLEDGMENT

We thank Don Burke-Agüero and August Böck's laboratory for helpful discussions. We are grateful to Lori Weyant for preparation of the manuscript. We also thank the W. M. Keck Foundation for their generous support of RNA science on the Boulder campus.

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