Effect of Base Sequence on In Vitro Protein-chain Termination*

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It has been proposed that the sequences surrounding nonsense codons determine the efficiency of protein-chain termination. To test this hypothesis, the termination factor, RF-1, was purified to near homogeneity and was used to examine the specificity of in vitro prokaryotic termination as a function of the nature and number of bases adjacent to UAA. Oligomers with different nucleotide sequences surrounding UAA were synthesized and their conformation was analyzed by NMR spectroscopy. The activity of these oligomers in RF-1-dependent termination was assayed by the release of analogues of peptides, N-acetyl or N-formyl-methionine, that were bound to ribosomes as N-acetyl or N-formyl-Met-tRNA Met with either AUG or AUC covalently linked to another oligoribonucleotide. In the former case, a second oligomer was added to stimulate release.

When added to the AUG-bound intermediate, UAAUAA was 5-fold less effective in stimulating release of N-acetyl-Met by RF-1 than were UAA, UAAN (where N is any base), UAAUGA, or UAAUAGG. Oligomers AUGUAA, AUGUUAA, and AUG(U) m UAA 18-25 (where m = 1-5) stimulated release by RF-1, whereas AUGCUA, AUGCUAA, and this hypothesis, the termination factor; met, formyl-methionine; RF, release factor; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

mRNAs appear to conform to a consensus sequence composed of a termination codon and a preferred 3'-sequence (10). This interpretation also explains the observation that several termination signals are leaky, leading to either termination or synthesis of read-through proteins from the same mRNA (7 and references therein). Thus, the sequences neighboring a nonsense codon could determine whether one or more proteins are designated by these genes.

In this paper, we report that in vitro termination, mediated by the release protein RF-1, is affected by the conformation of bases neighboring the UAA codon.

RESULTS AND DISCUSSION

We report that the ability of UAA to signal in vitro release depends upon the nature of nucleotides adjacent to this triplet.

To assay for termination, fMet-tRNA Met (an analogue of peptidyl-tRNA) was bound to ribosomes with AUG and one of the nonsense codons was added, together with protein RF-1, which responds to UAA and UAG (2). Under these conditions, N-formyl-Met is hydrolyzed from fMet-tRNA Met. Using this assay, we report a simple purification scheme for RF-1 from Escherichia coli cells which yields a nearly homogeneous preparation of this protein as assessed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (Fig. 1).

Fig. 2 shows that UAA(N) oligomers, where N is A,G,C, or U, stimulate release of methionine from ribosome-bound fMet-tRNA Met to the same extent as the reference codon UAA. In contrast, UAAUAA is at least 5-fold less effective than either UAA, UAAUGA, or UAAUAG (Fig. 3). Mixing experiments with each polymer and UAA failed to reveal inhibitors. The restricted termination by UAAUAA or by AUGUAA(A), (11 and Table 1) suggested that the conformation adopted by UAA in the oligomer may be important in the termination reaction. Inspection of sequences neighboring in vitro termination signals supports this suggestion (10) as does the observation that purine-rich regions as well as sequences CUC or CUG, 3' to nonsense codons, promote suppression of termination (5-7). We propose that highly base-stacked sequences neighboring a nonsense codon would restrict termination of protein synthesis (5-7). To study this problem, polymers with different nucleotide sequences around UAA were synthesized.

1 Portions of this paper (including "Experimental Procedures," Tables 1 and 2, Figs. 1-3, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3484, cite the authors, and include a check or money order for $4.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

The abbreviations used are: DTT, dithiothreitol; EF, elongation factor; fMet, formyl-methionine; RF, release factor; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

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and studied by variable-temperature NMR analysis to determine their conformation.3

Based on this analysis, it was predicted that, if the nucleotide conformation were maintained on the ribosomes and affected termination, then AUGUUA would terminate more effectively than AUGUAA or AUGCUUA because of the greater stacking ability of adenosine and cytidine residues relative to uridine residues (12). The intervening uridines decrease base stacking in AUGUUAA (compared to AUGUAA), which may result in the looping out of one or both intervening uridine residues as previously documented (13). Substitution of one of these uridine residues for a cytidine would increase the base stacking of the oligomer, a situation which would not allow the looping out of the uridine residue in the fifth position (U(5)). We propose that the amount of base stacking exhibited by the oligomer partly governs its ability to stimulate release of fMet (see Table 2 and Fig. 4).

The data summarized in Table 2 show that oligomers with different 3' sequences bind fMet-tRNA<sup>3M</sup> to ribosomes with approximately equal efficiency. AUGUU, AUGGUC, AUGUCU, and AUGGCU (Table 2) or longer polymers lacking nonsense codons (Table 1) do not promote RF-dependent release. In contrast, AUGUUA stimulates the release factor-mediated hydrolysis of fMet-tRNA<sup>3M</sup>. As predicted by the conformation model (Fig. 4 and Table 2), AUGUUA did not appreciably stimulate the reaction, and release by AUGUUA was generally half that observed with AUGUUAA.

The possibility that another codon-specific protein stimulates the release of fMet-tRNA<sup>3M</sup> from ribosomes was examined. If present, such a protein would have to occur as a 1% contaminant of the purest release factor preparation (Fig. 1). Also, if another protein were responsible for the observed reactions it would have to respond identically to RF-1 for various denaturing conditions tested, i.e., heat denaturation, inactivation by sulphydryl reagents and several peptidases, as well as sensitivity to either sparsomycin or gogourtin (2). Therefore, it is unlikely that the codon-dependent hydrolysis we observe is due to a hydrolase (14) that responds to a specific sequence in the ribosome-bound mRNA.

We conclude that the release factor reaction is partly controlled by the structure of the mRNA bearing a nonsense codon. Competition of the release factor and suppressor tRNAs could thus depend on the secondary structure of the mRNA surrounding a nonsense codon and not necessarily on recognition by suppressor tRNAs of sequences longer than a triplet (5, 6).

This model allows occasional misreading of out-of-phase nonsense codons. Tate et al. (15) have reported that release factors can recognize discontinuous nonsense codons formed by binding AUG(U)<sub>1-4</sub> to ribosomes and adding ApA or ApG. Recognition of such out-of-phase termination codons must be excluded in vivo to prevent premature chain termination. We expect that this could occur, in part, by the structure of the longer mRNA. We also find that recognition of the out-of-phase UAA in AUGUUAA is drastically reduced in crude extracts or in purified systems by aminoacyl-tRNAs and elongation factors Tu and Ts.4

There is a case of overlapping genes in bacteriophage MS2, where translation of the lysis protein message requires recognition of an out-of-phase nonsense codon in the coat message two triplets before the initiation codon of the lysis gene. It is possible that lysis-gene translation requires recognition of this nonsense codon by the release factor. The existence of mRNAs of multiple termination signals which depend on base context may allow protein synthesis to utilize controllable stop signals. This would enable a measure of fine tuning at the termination level of gene expression as evidenced by lysis-gene expression (16).

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REFERENCES

Additional references are found on p. 14104.

4 M. C. Ganoza, K. Buckingham, P. Hader, and T. Neilson, unpublished data, available upon request.

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Effect of Bases 5' and 3' to the Termination Codon UAA

**Fig. 4.** A model, based on NMR spectroscopy, depicting the effect of various nucleotide sequences on the conformation of UAA. — denotes the P site, and — represents the A site on the ribosome.
Effect of Bases 5' and 3' to the Termination Codon UAA

Supplemental Material For

*Effect of Base Sequence in Initiation Protein Chain Termination*

by

M. Clhei Danne, Kane Hatakan, Paul Hgler and Thomas Nutter

EXPERIMENTAL PROCEDURES

Rat tissues were purchased from Tissue Bank United, Edmonton, B.C., and non-tumor-bearing human tissues were obtained from the University of Califomia. The liver, heart, brain, and skeletal muscles were stored in a 25°C solution of 2.0 M Tris, 0.5 M NaCl, 0.5 M KCl, 0.5 M MgCl2, and 0.1 M NaOH. The liver was homogenized with a Polytron homogenizer and centrifuged at 10,000 rpm for 30 minutes. The supernatant was used for the experiments.

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Table 1. Release Reaction with Affinity of Formula 6900(1)(4A)(1)

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>r(3)[UAA] bound</th>
<th>r(3)[UAA] released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nova-1</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-2</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-3</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-4</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-5</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-6</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-7</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
<tr>
<td>Nova-8</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
</tbody>
</table>

Polymers of composition 6900(1)(4A)(1) were synthesized as described in Experimental Procedure. Induction samples for synthesis and termination reactions were performed as described in Experimental Procedure using 30 nM r(3)[UAA] bound and 200 pmol of each polymer. The numbers in parentheses represent the number of doublets in each polymer. Values are average of duplicate samples which were within 1% of each other. Values of rate of different duplications were also within 1%. Samples were not corrected for nonspecific degradation.

Table 2. Binding and Release with Various 410-Containing Oligomers

<table>
<thead>
<tr>
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<tr>
<td>Nova-3</td>
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</tr>
<tr>
<td>Nova-8</td>
<td>36.7(2)</td>
<td>2.8(3)</td>
</tr>
</tbody>
</table>

Initiation samples for synthesis and termination reactions with purified RNA were prepared as described in Experimental Procedure.

REFERENCES