LETTERS TO NATURE

Untranslated T7 Phage mRNA is stabilized in *suA* Host

IT has been shown[1] that relief from the polar effect of nonsense mutations in the tryptophan operon by the *suA* allele[2] is associated with increased survival of mRNA operator distal to the nonsense mutation. Experiments described here confirm the effect of *suA* on mRNA distal to amber mutations and directly demonstrate the complete mRNA molecule only in the presence of the *suA* mutation.

Phage-specific RNA from T7-infected cells has been resolved into multiple discrete species by gel electrophoresis[3] and one of these species has been identified as the transcription product of gene 1 (ref. 4). This gene is required for transcription of the majority of the T7 genome, so the RNA complement is quite simple in cells infected with T7 carrying mutations in this gene[4]. For this reason, the metabolism of this mRNA was examined in su^-, su_{II} and *suA* hosts. The protein product of this gene was also analysed in order to check if *suA* permitted translation past the amber codon (that is, insertion of mis-sense).

Infection of the su^- host (*E. coli* $B_{s-1}/1$) with $T7^+$ and T7 *am* 342 (gene 1, near operator distal end of the gene[5]) resulted in RNA profiles on gel electrophoresis which were similar to those already published[4]. The gene 1 mRNA is not seen as a discrete band on the gel in the case of infection with T7 *am* 342, whereas a sharp peak at about 1.1×10^6 Daltons is characteristic of the complete mRNA species observed after infection with $T7^+$. Similar results were obtained with T7 *am* 23 (operator proximal end of gene[5]) and T7 *am* 94 (middle of gene[5]). The fact that in no case was a discrete band of lower molecular weight observed suggests that this RNA was either not present at all or was terminated in some non-uniform way.

The electrophoretic analysis of T7 RNA made after infection of *E. coli* XA7007 (*suA*) with T7 *am* 342 is shown in Fig. 1 along with the analysis of RNA from the su^- host also infected with T7 *am* 342. Whereas no pool of full length gene 1 mRNA molecules accumulates in the su^- host, a significant amount of complete gene 1 mRNA is present in the *suA* host.

Analysis of the T7 proteins by SDS-gel electrophoresis[6] showed that a short peptide fragment is made in both the su^- and *suA* hosts, which indicated that translation is terminated at the amber codon in the *suA* host as well as in the su^- strain. This implies, then, that the T7 message distal to the amber codon is stabilized in the *suA* strain, but not translated. Similar conclusions were drawn by Morse and Primakoff[1]. It was suggested earlier[1] that the *suA* mutation may affect an endonuclease which normally attacks the untranslated portion of mRNAs. Such an interpretation is compatible with the results presented here. In T7-infected cells the mRNA appears to be stable[7,8]. This stability, however, may reflect a deficiency in RNAase V activity[9] (5′ exonucleolytic activity associated with ribosomes), while the hypothetical endonuclease is still free to make internal cuts in untranslated T7 mRNA as in the case of the amber mutant in an su^- host.

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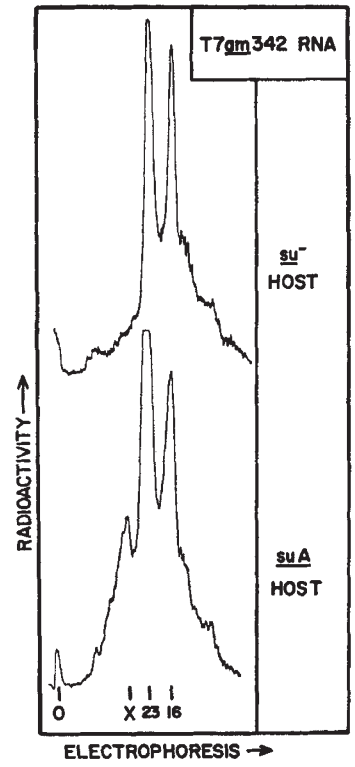

Fig. 1 Gel electrophoresis of ^{14}C-RNA from cells infected with T7 *am* 342. Upper trace shows RNA from su^- cells; lower trace shows RNA from *suA* cells. Microdensitometer tracing is of a contact autoradiograph of a longitudinally sliced, dried 2.5% polyacrylamide–0.5% agarose gel. Electrophoresis, labelling, and sample preparation were carried out as described previously[3,4]. The band marked (×) corresponds to the mRNA for the T7 gene 1 protein. The 23s and 16s host species are indicated.

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