Plant mitochondria and chloroplast tRNAs Trp do not suppress the UGA stop codon

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Several tRNAs Trp have been shown to promote the read-through of the UGA stop codon. For instance, in E. coli, the wild type (su+) tRNA Trp (anticodon CCA) promotes the read-through of the UGA codon in vivo with a very low efficiency. The efficiency is enhanced in a su E. coli strain whose tRNA Trp only differs from su- tRNA Trp by one nucleotide, leading to a U11-A24 base-pair instead of a U11-G24 base-pair, which suggests that this U11-A24 base-pair plays an important role in suppression. Both su+ and su- tRNAs Trp suppress in vitro the stop codon UGA of rabbit β-globin mRNA (for a review, see 1). Recently we have shown that bean mitochondria (mt) and chloroplast (cp) tRNAs Trp (anticodon CCA) are very similar (97% homology), that they have a high homology with E. coli tRNAs Trp (about 70%), and possess a U11-A24 base-pair (as the su+ tRNA Trp) (2). In view of these similarities, we have investigated whether bean mt and cp tRNAs Trp are able to read the UGA codon in the rabbit reticulocyte lysate protein synthesizing system. It can be seen on the autoradiogram that, in contrast to yeast mt tRNA Trp (a gift from R. Martin) which suppresses UGA termination (3), leading to the production of a β-globin-related readthrough protein of 18,500 daltons, both bean mt and cp tRNAs Trp lead only to the production of the normal β-globin protein of 16,000 daltons, and thus, do not appear to be able to suppress the UGA codon used as termination codon in plant cp and mt mRNAs. This shows that the presence of a U11-A24 base-pair is not sufficient to promote suppressor activity.

Protein synthesis assays (12.5 µl) in rabbit reticulocyte lysate system (NEN) have been done in the presence of 0.4 µg of purified globin mRNA (SRL) and 35 µCi of [35S]methionine. TCA precipitable radioactivity has been analysed by electrophoresis on a 17% polyacrylamide SDS-gel and by fluorography. Assays have been supplemented with Trp-tRNA Trp obtained by aminoacylation of various tRNAs Trp with tryptophan using E. coli enzyme.

1) 28 µg of total yeast mt tRNA; 2) 2 µg of yeast mt tRNA Trp; 3) 28 µg of total bean cp tRNA; 4) 1 µg of bean cp tRNA Trp; 5) 2 µg of bean cp tRNA Trp; 6) 32 µg of total bean mt tRNA; 7) 2 µg of bean mt tRNA Trp; 8) and 9) control assays (no tRNA added) with and without globin mRNA respectively.