# Plant mitochondria and chloroplast tRNAs$^{Trp}$ do not suppress the UGA stop codon

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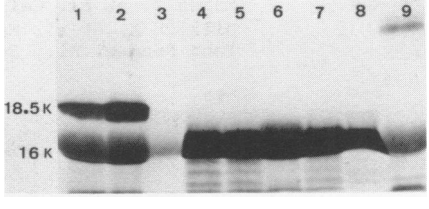
Several tRNAs$^{Trp}$ have been shown to promote the read-through of the UGA stop codon . For instance, in E. coli , the wild type ($su^-$) tRNA$^{Trp}$ (anticodon CCA) promotes the read-through of the UGA codon in vivo with a very low efficiency. The efficiency is enhanced in a $su^+$ E. coli strain whose tRNA$^{Trp}$ only differs from $su^-$ tRNA$^{Trp}$ by one nucleotide, leading to a $U_{11}$-$A_{24}$ base-pair instead of a $U_{11}$-$G_{24}$ base-pair, which suggests that this $U_{11}$-$A_{24}$ 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 $C_mCA$) are very similar (97% homology), that they have a high homology with E. coli tRNAs$^{Trp}$ (about 70%), and possess a $U_{11}$-$A_{24}$ 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 $U_{11}$-$A_{24}$ 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 (BRL) and 35 µCi of $^{35}$S-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.

1 - Buckingham, R.H. and Grosjean, H. (1986), in "Accuracy in molecular processes: its control and relevance to living systems" (Galas, D.J.,Kirkwood, T.B.L. and Rosenberger, R.F., eds) pp.83-126, Chapman and Hall Ltd, London.
2 - Marechal, L., Guillemaut, P., Grienenberger, J.M., Jeannin, G. and Weil, J.H. (1985), Nucl.Acids Res. 13, 4411-4416.
3 - Martin, R., Sibler, A.P., Dirheimer, G., de Henau, S. and Grosjean, H. (1981) , Nature 293, 235-237.