Abstract

Ribosomes translate the genetic information encoded in the mRNA with an extremely high efficiency and accuracy. In this respect, maintenance of the correct reading frame is one of the major tasks achieved by the ribosomes during the protein synthesis process. A spontaneous change in the reading frame generates truncated and usually non-functional proteins resulting in the loss of the genetic information. Normally a spontaneous frame-shift occurs approximately once in 30,000 incorporations of amino acids. However, in the case of the synthesis of the termination factor a frameshift has to occur, since the 26th codon of the RF2 mRNA is the stop codon UGA. At this stop codon a +1 frameshift is required for the RF2 synthesis, and it occurs with an efficiency of up to 100% if the RF2 concentration is low, that is with a frequency that is four orders of magnitude larger than that normally observed during protein synthesis.

The presence of the internal stop codon of the RF2 mRNA is the basis of the translational feed-back regulation of the RF2 synthesis. If the concentration of RF2 is sufficiently high in the cell, RF2 recognizes the stop codon UGA on its own RF2 mRNA at the codon position 26, with the result that an oligopeptide of 25 amino acids is synthesized, released and fast degraded. However, at a low concentration of RF2 a (+1) frameshift occurs that is necessary for a complete synthesis of the RF2 protein.

In this work the mechanism of this extreme frameshift event has been elucidated and resolved. Moreover, evidence is provided that an occupation of the E site with a deacylated tRNA is essential for maintaining the reading frame.

We demonstrate that the internal Shine Dalgarno (SD) sequence in front of the stop codon UGA (26th codon) of the RF2 mRNA plays a critical role for the mechanisms of the RF2 feed-back regulation. When the internal UGA is at the A site, the SD sequence is separated from the peptidyl-tRNA at the P site by an extremely short spacer sequence of only two nucleotides and thus interferes with the first nucleotide of the E site codon. The result is a steric clash between the SD-antiSD of the 16S rRNA and codon-anticodon interaction, and we show that this clash triggers the release of the E-site tRNA. The resulting ribosome with only one tRNA, the peptidyl-tRNA at the P site, is a situation that never exists during elongation, where statistically always two tRNAs are on the ribosome. The short spacer forces this non-elongating ribosome to move (+1) nucleotide downstream displaying the frameshifted codon GAC for Asp at the A site. We demonstrate that the loss of the tRNA at the E site is correlated with the incorporation of Asp. However, when the SD sequence is shifted by two or six nucleotides upstream from the wild type position, the tRNA at the E-site is not released and a frameshift does not occur.

The *in vitro* translation system developed for the analysis of the frameshift at the RF2 mRNA shows for the first time a frameshift frequency near 100% reflecting the known data *in vivo*. We further demonstrate that the E-site tRNA is not removed by a normal RF2 "decoding" of the stop codon UGA. Therefore, the tRNA release from the E site has to occur at a later step of a normal termination process, a fact that is neglected by current models of termination.

Our data demonstrate further for the first time that a cognate tRNA at the E site is instrumental for maintaining the reading frame, and that the loss of the E site tRNA is the trigger for the enormous efficiency of frameshifting during the translational regulation of the RF2.