UAG readthrough in mammalian cells: Effect of upstream and downstream stop codon contexts reveal different signals

ABSTRACT

We conclude that, in mouse cells, the upstream and downstream stop codon contexts affect readthrough via different mechanisms, suggesting that complex interactions take place between the mRNA and the various components of the translation termination machinery. Comparison of our results with those previously obtained in plant cells and in yeast, strongly suggests that the mechanisms involved in stop codon recognition are conserved among eukaryotes.

INTRODUCTION

Background Translation termination is a crucial step in the process of information decoding. Its accuracy reaches about 10-4 and ensures that only very few abnormal products are synthesised under normal conditions. Conversely, translation termination is widely used by both animal and plant viruses as a mean of controling expression, through recoding events. Readthrough is the process by which a stop codon is misread as sense by the translational apparatus, allowing the synthesis of an extended polypeptide which carries novel activities. Up to now, no specific gene products have been implicated in the control of translational readthrough, strongly suggesting that only normal interactions between the mRNA and components of the translational machinery are involved. This differs from the mechanism of selenocysteine incorporation, which requires a whole set of specific partners, including a specific tRNA and a particular elongation factor. Readthrough can therefore be seen as "programmed translational errors" occurring at specific sequences on the mRNA. Several studies, either in vivo or "in silico", have analyzed the termination context in mammalian cells. In particular, it has been demonstrated that the nucleotide following the stop codon (defined as +4) is non random, with purines over-represented for the three stop codons. This + 4 nucleotide in fact plays a key role in termination efficiency, leading to the proposition that termination is directed by a "four base signal". Clearly, some programmed readthrough events are based on alteration of these interactions. This is the case in Sindbis virus where the UGAC sequence, which is a very poorly used stop context in mammals, is sufficient to drive an efficient readthrough (2-5%). More generally, it has been demonstrated in numerous experimental systems that the nucleotide at position +4 plays an important role in suppression efficiency. Other nucleotide biases are found around stop signals, non-randomness being observed at up to eight positions downstream of the stop codon and three positions upstream. Such biases possibly reflect the existence of interactions between the mRNA and other components of the ribosomal machinery, which are important for termination efficiency. Elucidating the mechanisms at play during readthrough may help understand normal translation termination. To achieve this goal, we have analyzed the rules governing readthrough in mouse cells in culture. We focused our studies on the sequence CAA UAG CAA UUA, derived from the plant Tobbaco Mosaic Virus (TMV). This sequence was previously shown to drive a high level readthrough both in in vitro systems and in vivo in plant cells and yeast. This sequence is also functional in mammalian cells in culture. We used a highly sensitive luciferase reporter to study the role of amino acid identity, codon frequency and nucleotide context, at the 5' and 3' triplets flanking the stop codon.

CONCLUSION

Conclusions Altogether, our results indicate that different signals are involved in the interaction between the translational machinery and the upstream and downstream stop codon contexts. In addition, they also suggest that the mechanisms involved in readthrough are common to eukaryotes, pointing to either an ancient origin of the translation termination machinery, or to very strong structural constraints at the level of the eukaryotic ribosome. Another implication of these observations is that the readthrough sequence analyzed here, although first described in a plant virus, might be used as a recoding signal also in mammals and yeast. Analyzing DNA sequence data bases should help in identifying such putative physiological recoding events.