A New Mechanism of Upstream Open Reading Frame of SAMDC Gene: Transcriptional and Translational Regulation

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Synthesis of S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.4.50), a key regulated enzyme in the pathway of polyamine biosynthesis. Carnation SAMDC genes have an upstream open reading frame (uORF) of 52 or 54 amino acids in 5 -leader sequences. In this study, we have provided the direct evidence of peptide synthesis from the SAMDC uORF using an in vitro translation system. It was determined that uORF protein has a role as a translational inhibitor of main ORF using point-mutated constructs in uORF sequences. When we measured mRNA expression in those mutations, the steady-state mRNA levels were also changed. Moreover, modulation of mRNA decay constitutes an important mean of regulating gene expression. And the most mechanism on action of uORF has been explained by hypotheses such as reinitiation inhibition or ribosomal stalling. To determine how these uORF control translation, we used to a primer extension inhibition assay by mapping the positions of ribosomes translating mRNA. Also, we treated cycloheximide to identify the first initiation events occurring on an mRNA and initiation events occurring during steady-state translation. Also, the phosphorylation of SAMDC uORF might involve in its regulation, because there are detected immature mRNAs in directed-mutation constructs of SAMDC uORF by primer extension experiments, Therefore, these results imply the new mechanism of uORF action in vitro system.