The properties of a peptide encoded by a nested alternative open reading frame in the matryoshka gene determine the level of maternal mRNA accumulation

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Until recently, our understanding of protein-encoding genes was based on (a) the assumption that one open reading frame (ORF) encodes one protein and (b) the minimal length of the translated ORF. However, due to the discovery of translated ORFs upstream and downstream of the main ORF and of alternative ORFs within annotated ORFs, it became clear that these concepts were incorrect. By studying plant responses to stress, we identified the gene that encodes the Kunitz peptidase inhibitor-like protein (KPILP) in plants of the Solanaceae family, the mRNA content of which dramatically increased after abiotic and biotic stress. To reveal the mechanism underlying the regulation of KPILP mRNA, we identified a nested alternative open reading frame (aORF) encoding a 53-aa-peptide (MGRPQVCLRFRNMKMSMRYSIVQELQGVLLFAPDCCVGILVFHLQLMDRGVWL), which is predicted to form a transmembrane domain (residues 27-45 are underlined). We previously found that aORF translation determines the efficiency of "maternal" KPILP mRNA accumulation in the leaf. Here, we identified the features of the 53-aa peptide amino acid sequence that are responsible for the observed effects. For this purpose, we generated a series of genetic constructs encoding KPILP with substitutions that affect the properties of the 53-aa peptide. We found that mutations leading to a loss of the transmembrane domain (TMD) of the 53-aa peptide and its ability to bind to membranes resulted in the enhanced accumulation of "maternal" mRNA. The replacement of cysteine residues with alanine in the 53-aa peptide leads to the same result. We concluded that the presence of the TMD as well as intermolecular -S-S- bonds in the 53-aa peptide are responsible for the ability of the translated aORF to control the expression of the "maternal" KPILP gene. This study was performed with the financial support of the Russian Foundation for Basic Research (project No. 17-29-08012).