Analytical study on the peptide sequence-dependent regulatory upstream open reading frame of a tomato homologue of the *Arabidopsis ANAC096* gene

（シロイヌナズナ *ANAC096* 遺伝子のトマトホモログの発現をペプチド配列依存的に制御する上流 ORF の研究）

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ABSTRACT

Many eukaryotic mRNAs contain one or more upstream open reading frames (uORFs) in their 5′ untranslated regions (5′-UTRs). Some uORFs encode regulatory peptides that repress translation of the main ORF. To comprehensively search for uORFs encoding regulatory peptides, uORFs with evolutionarily conserved amino acid sequences, referred to as conserved peptide uORFs (CPuORFs), have been identified using bioinfomatic approaches.

The *Arabidopsis thaliana ANAC096* gene is one of the CPuORF-containing genes; however, the *ANAC096* CPuORF exerts only little peptide sequence-dependent effect on expression of the main ORF. This project focused on the effect of the CPuORF sequence of a tomato *ANAC096* homologue, *LOC101264451*, on expression of the main ORF, because it has a more highly conserved amino acid sequence than the *ANAC096* CPuORF.

In this study, to address the importance of the CPuORF amino acid sequence for the regulatory function, mutational analyses on the *LOC101264451* CPuORF sequence were conducted, and the effects on main ORF expression were examined using transient expression assay with protoplasts prepared from tobacco BY-2 cultured cells. Alteration of the CPuORF amino acid sequence by a frameshift (fs) mutation conferred more than two-fold increase in main ORF expression compared with the wild-type (WT). The effect of the fs mutation was abolished in the absence of the CPuORF start codon. This result indicates that translation of the CPuORF is required for the fs mutation to exert its effect, and suggests that the effect of the fs mutation is caused by the amino acid sequence alteration of the CPuORF rather than by the nucleotide sequence change.

Furthermore, to determine the critical amino acid residues of the *LOC101264451* CPuORF peptide responsible for the regulation, alanine scanning analysis was performed. Most of the Ala substitutions introduced into the conserved region showed a significant increase in the reporter activity compared with the WT. By contrast, synonymous codon changes introduced into the similar region showed only a slight increase in the reporter activity. These observations suggest that the peptide encoded by the *LOC101264451* CPuORF is involved in the repression of main ORF expression.

The *ANAC096* gene encodes a NAC (NAM, ATAF1,2 and CUC2) domain-containing transcription factor. The expression of this gene is induced at the mRNA level in response to dehydration and osmotic stress in *A. thaliana*. Since the *LOC101264451* main ORF is orthologous to *ANAC096*, the expression of the main ORF may be induced at the post-transcriptional level in response to similar stresses, and the CPuORF may be involved in the regulation. To address the effect of the *LOC101264451* CPuORF on main ORF expression under stress conditions, the reporter plasmid carrying the CPuORF upstream of a luciferase gene was transfected into BY-2 protoplasts, and the protoplasts were incubated with several different concentration of mannitol. The inhibitory effect of the CPuORF on main ORF expression was dependent on mannitol concentration. This result suggests that the *LOC101264451* CPuORF is involved in post-transcriptional regulation in response to osmotic stress.