学位論文内容の要旨

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学位論文題名

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）

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, mutational analyses on the *LOC101264451* CPuORF sequence were conducted, and the effects on main ORF expression were examined to address the importance of the CPuORF amino acid sequence for the regulatory function and to determine the critical amino acid residues responsible for the regulation. Furthermore, the effect of the CPuORF under stress conditions was investigated to elucidate the physiological role of the CPuORF-mediated regulation of *LOC101264451.*

1. Identification of a novel peptide sequence-dependent regulatory uORF

Site directed-mutagenesis on *LOC101264451* CPuORF sequences was performed and the effects of the mutations were analyzed 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.

In alanine scanning analysis, 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.

2. Analysis of the physiological role of the uORF-mediated regulation

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.

In stress response analysis of *LOC101264451* CPuORF, 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 protoplasts incubated in higher concentration of mannitol showed a lower reporter activity. This result suggests that the CPuORF represses expression of *LOC101264451* in response to osmotic stress.