Signal transduction

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Tobacco ERF3 was Identified to Associate with Ubiquitin Conjugating Enzyme

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Plants produce a number of PR (pathogenesis-related) proteins in response to pathogen attack. The crucial roles of tobacco ERFs (ethylene-responsive transcription factors), binding proteins to GCC-box in the promoter region of basic PR genes, are postulated in the regulation of basic PR genes.

To isolate proteins involved in the post-translational modification of ERF3, we screened cDNA library prepared from cultured tobacco cells, using ERF3 as a bait in yeast two-hybrid system. In this screen we identified a ubiquitin conjugating enzyme (UBC) as an ERF3 interacting protein. UBC conjugates a poly-ubiquitin chain to the substrate protein and target it to the proteasome. Association of ERF3 with UBC suggests that the stability of ERF3 may be regulated by the ubiquitin-proteasome pathway.

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PROMOTER ANALYSIS OF THE DNA DAMAGE RESPONSIVE GENE FROM *ARABIDOPSIS*.

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AtRAD51 gene encodes a *E.coli* RecA homologue. Although it has been shown that the expression level is regulated by DNA damage and cell cycle, mechanisms and signal transduction pathway involved in *AtRAD51* gene expression are largely unknown. In order to characterize regulatory mechanisms of DNA damage induced gene expression, we carried out promoter analysis of *AtRAD51* gene. 5'-deletion series of *AtRAD51* promoter fragments were fused to the firefly luciferase reporter gene and introduced into tobacco BY-2 cells by microprojectile bombardment. Induction of relative luciferase activity by bleomycin treatment indicated that the 44-bp sequence located in the proximal region of *AtRAD51* gene is sufficient to mediate induction of *AtRAD51* expression by DNA damage.

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ANALYSIS OF DOMINANT-NEGATIVE ATHK1 IN *ARABIDOPSIS*

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To analyze roles of two-component systems in plant signal transduction, we have so far cloned a cDNA encoding a hybrid-type histidine kinase ATHK1 from *Arabidopsis*. We demonstrated that ATHK1 has the potential ability to act as an osmosensor by analyzing both sensing (input) and catalytic (output) activities with yeast osmosensing-defective mutants.

In order to examine the function of ATHK1 in planta, we attempted to generate *Arabidopsis* plants transformed with mutated ATHK1 cDNAs. We initially found that ATHK1 forms a homodimer through each cytoplasmic region by yeast two-hybrid interaction analysis. We then constructed a cDNA libraly of mutated ATHK1 using PCR-based random mutagenesis and co-transformed a yeast SLN1 deletion mutant with a wild-type ATHK1 cDNA. We screened dominant-negative ATHK1 mutants that inhibited the activity of the wild-type ATHK1, which in turn suppresses the yeast SLN1 deletion mutant, and isolated six candidates (*ATHK1-1* to *6*). Sequence analysis revealed that *ATHK1-2* has an N-terminal deletion and *ATHK1-6* has a nucleotide substitution at a putative ATP binding site. We are currently analyzing transgenic *Arabidopsis* plants overexpressing the dominant-negative ATHK1 cDNAs.

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Expression analysis of *Arabidopsis erd1* gene induced by senescence and dehydration

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The *erd1* gene encoding a protein with sequence homology to the ATP-binding subunit, ClpA is strongly induced by dehydration stress but not by heat, cold or heavy-metal stress. The *erd1* gene is up-regulated by senescence as well as by water stress. To analyze the regulatory mechanisms of the *erd1* gene by water stress and senescence, we constructed a chimeric gene with the promoter region (-723 ~+165) of the *erd1* gene fused to the *LUC* (luciferase) reporter gene and then tobacco or *Arabidopsis* plants were transformed with their constructs. Expression analysis of the *LUC* gene in the transgenic plants incubated in the dark for 12 days revealed that 69-bp (-202~-134) region of the *erd1* promoter may contain *cis*-acting elements that are involved in senescence-recognized expression of the *erd1* gene. The 69-bp region contains the ABRE-like sequences (ABA Responsive Element). We are analyzing *cis*-acting elements involved in senescence and dehydration induced expression using deletion and base substituted DNA fragments of the *erd1* promoter fused *LUC* genes.