Workshop 3. Plant transcription factor studies in the post-genome era

W24(W3-01)

Functional analysis of transcription factors, DREBs, involved in drought- and cold-inducible gene expression

Kazuko YAMAGUCHI-SHINOZAKI[1], Kazuo SHINOZAKI[2]
[1]Biological Resources Division, Japan Int. Res. Cent. Agri. Sci. (JIRCAS); [2]Lab. Plant Mol. Bio., RIKEN Tsukuba Institute,

A *cis*-acting element, DRE, plays an important role in regulating gene expression in response to drought, salt loading, and freezing stresses in *Arabidopsis*. Five cDNA clones that encode DRE binding proteins, DREB1 and DREB2, were isolated by using the yeast one-hybrid screening. Both proteins specifically bind and activate transcription of genes containing the DRE sequence in *Arabidopsis*. Overexpression of the cDNA encoding DREB1A in transgenic *Arabidopsis* plants activated the expression of the target stress-inducible genes under normal growing conditions and resulted in improved tolerance to drought, salt loading, and freezing. Using full-length cDNA microarray we identified 12 target stress-inducible genes of DREB1A, and 6 of them were novel. On the other hand, use of the strong constitutive 35S cauliflower mosaic virus (CaMV) promoter for overexpression of DREB1A resulted in severe growth retardation under normal growing conditions. In contrast, expression of DREB1A from the stress inducible promoter had minimal effects on plant growth and provided greater tolerance to stress conditions than did expression of the gene from the CaMV promoter.

W25(W3-02)

PLANT MYB GENES INVOLVED IN STRESS RESPONSES

Kazuhiko SUGIMOTO, Shin TAKEDA, Hirohiko HIROCHIKA
Dept. of Molecular Genetics, Natl. Inst. of Agrobiol. Resources, Tsukuba 305-8602, JAPAN

The transcriptional activation of the tobacco retrotransposon *Tto1*, by stresses such as wounding and elicitor treatment depends on the enhancer/promoter region located in long terminal repeats (LTRs). The 13-bp repeated motif in the LTR has been identified as a *cis*-regulatory element involved in the activation by these stresses. 13-bp motif contains the L-box which is one of *cis*-elements involved in the regulation of defence-related genes such as *PAL*. NtMyb2 is one of positive regulators of 13-bp motif upon wounding and elicitor treatment. Analysis of the NtMyb2 promoter showed that AG-motif (AGATCC) is a sufficient *cis*-element *in vitro* and *in vivo*. The analysis of AG-motif binding protein named LBP1 suggests that LBP1 may be regulated by modification such as phosphorylation. Epitope-tagging suggests that LBP1 is phosphorylated by MAPKs according to wounding. Now we try to understand the effect of phosphorylation on LBP1 activity.

Recently gene analysis using genome-post genome approaches such as mutant panel and micro-array is progressed in rice. Therefore we started to isolate the orthologou of NtMyb2 from rice. The cDNAs encoding 13-bp/L box binding protein were cloned by yeast one-hybrid system from the cDNA library prepared from wounded rice leaves. Multiple arraignment analysis and expression patterns show that two Myb genes named OsMybS1 and S3 are orthologous to NtMyb2. By using PCR screening of rice mutant panel, we obtained a insertion mutant of OsMybS1 whose insertion point is 3'UTR. Analysis of insertion mutants of wound-response related genes is under going.

W26(W3-03)

ARABIDOPSIS MAPK PATHWAYS AND TRANSCRIPTION FACTORS RELATED TO PATHOGEN RESISTANCE

Tsuneaki ASAI, Guillaume TENA, Frederick M. AUSUBEL, Jen SHEEN; Dept. Genet., Harvard Med. Sch., and Dept. Mol. Biol., MGH, Boston, MA 02114, USA.

Plants can detect invasion by microbial pathogens and protect themselves by mounting a variety of defense responses. One of the early responses is the induction of various transcription factors, which, in turn, regulate the expression of many defense-related genes. It has been suggested that WRKY transcription factors, a family of plant-specific zinc-finger-type proteins, play an important role in pathogen resistance. However, signaling pathways leading from pathogen attack to the induction of WRKY proteins remain unclear. Mitogen-activated protein kinase (MAPK) pathways are involved in the transduction of extracellular signals to intracellular targets in all eukaryotes. Recent evidence suggests that defense signaling in plants also employs MAPKs. To study a potential role of MAPK pathways in WRKY induction, we have developed an Arabidopsis protoplast model system. We have found that a peptide elicitor, called flg22, derived from the most conserved region of bacterial flagellin activates MAPKs and WRKY factors in Arabidopsis protoplasts. We will present the signaling components that mediate flg22-induced WRKY expression and discuss possible roles of WRKY proteins in pathogen resistance.

W27(W3-04)

ZINC FINGER TRANSCRIPTION FACTORS PLAY ESSENTIAL ROLES IN POLLEN DEVELOPMENT IN PETUNIA

Sanjay KAPOOR, Hiroshi TAKATSUJI; Dept. Plant Physiol. Natl Inst. Agrobiol. Resources (NIAR) Tsukuba 305-8602

Here we describe functional characterization of four zinc finger-containing genes, *ZPT2-5, 3-1, 4-1* and *3-2*, which are essential for male fertility in petunia. Expression of *ZPT 2-5, 3-1* and *4-1* is localized in microspores. Cosuppression of these genes in transgenic plants causes severe defects in meiosis which in turn results in ~90% reduction in the number of mature pollen grains. Pollen that reach maturity show aberrant morphology and considerably reduced germination rate. *ZPT3-2*, on the other hand, expresses mainly in the tapetal layer. Cosuppression of this gene results in poor development of tapetal layer, which starts to degenerate much earlier than in wild type anthers, i.e. before the dissolution of callose wall. In these plants, pollen development stages up to tetrad formation are similar to that of the wild type plants. However, after their release from tetrads, microspores start to degenerate presumably due to lack of flavanols and other nutrient material which are otherwise provided by the tapetum for the development of microspores. Efforts are under way to search for the orthologues of these genes in arabidopsis and exploit the vast resources generated by the genome sequencing program to search for the target genes of these transcription factors.