G215

Construction of Ethanol-inducible Expression Vector and its Expression Analysis in *Arabidopsis* Protoplast

Hee-Jung Yuck[1], Sung-Jin Kim[P], Youn-Hee Choi[1], Ji-Seon Baek[1], Kwang-Woong Lee[C]

School of Biological Sciences, Seoul National University, Seoul 151-747

In order to establish ethanol-inducible gene expression system in plant, alcoholic fermentable gene ADH2 (Alcohol Dehydrogenase 2) promoter of yeast (Saccharomyces cerevisiae) and ADR1 transcription factor were isolated. *ADH2* gene is expressed by the transcription factor, ADR1 to use alcohol as carbon source. In this experiment, The transcription factor *ADR1*was introduced into pBI121 vector and *ADH2* promoter into pCAMBIA1303 vector. Finally, p35S-*ADR1*-tnos region of pBI121 vector transferred modified pCAMBIA1303 vector and ethanol-inducible expression vector was constructed. To examine the reaction of ethanol-inducible expression vector in *Arabidopsis* protoplast, expression level of β-glucuronidase (GUS) gene and green fluorescence protein (GFP) was monitored after 2% ethanol was treated to transgenic protoplast. All of the three ethanol-inducible plant expression vectors showed the GFP expression after 2% ethanol treatment. Among them, *pADH2*:p35S fused plant expression vector of those showed the highest GFP expression level. The results suggest that the ethanol-inducible plant expression vector works under ethanol treatment.

G216

Molecular Genetic Analysis of the Brassinosteroids Signaling Mutants

Indeok Hwang[P], Hyeonsook Cheong[C]

Department of the genetic engineering, Chosun university, Gwangju 501-759

Brassinosteroids (BRs) are a class of steroid hormones essential for normal plant growth and development. In order to expand our knowledge of the molecular mechanisms of plant steroid signaling, we performed a genetic screening, in which activation-tagged Columbia lines were examined on the medium containing Brassinasole (Brz) in dark. Mutants resistant to a BRs biosynthetic inhibitor, Brassinazole, were shown to have longer hypocotyls than the ones with the wild type background. We identified 3 mutants that have longer hypocotyls and recovered T-DNA flanking sequences from the activation-tagged brassinazole insensitive (*abz*) mutants. They were designated as *abz126, abz314, and abz453*. These mutants were analyzed for the genetic mapping and characterization. *abz126* is a loss-of-function mutant of Gigantea (*GI*) gene by T-DNA insertion that delayed flowering under long days and displayed longer hypocotyls on the medium containing Brz. This phenotype was similar to the gi-1 and gi-2 in aspect of the late flowering and the longer hypocotyls on the medium containing brz in the dark. Genetic analysis of *abz314* indicated that the T-DNA was inserted in the centromere region. *abz314* mutant showed both the longer hypocotyls on the medium containing Brz in dark and the phenotype including longer petiole and accelerated senescence. Further functional repression of the centromere in plants might be elucidated by the mutants analysis.. The *abz453* is knock-out mutant of the 24-sterol C-methyltransferase gene (*24-SMF*) in BAC F14O10. RT-PCR result also showed that FCP serine phosphatase gene(*FSP*), neighboring gene of *24-SMF*, was over-expressed by the four enhancers.

G217

Molecular Genetic Analysis of the Bril-suppressor Mutants *(bsu)*

Lyoungmae Kim[P], Hyeonsook Cheong[C], Indeok Hwang[1]

Department of the genetic engineering, Chosun University, Gwangju 501-759

Genetic screens were performed for the identification of brassinosteroid (BRs) signal components using activation tagging bri1-5 in Arabidopsis. We selected based on the bri1 suppressed phenotype in brassinazole (Brz) which BRs biosynthesis inhibitor to block specifically at steps from campestanol to teasterone catalyzed cytochrome P450. bri1 suppressor mutants *bsu2* and *bsu7* have reduced sensitivity to Brz and restored dwarfism with longer hypocotyls and having elongated petioles such as a nearly wild type phenotype. *bsu2* and *bsu7* were semidominant and have the longer pertioles and swirling leaves. It has been revealed that *BSU2* encodes a cell division related protein kinase cdc2 and *BSU7* encode upstream region of cytochrome P450 CYP90. Further genetic analysis of these bri1 suppressor mutants and cloning of the tagged genes will elucidate their functions in BRs signaling

G601

Control of XIST Expression by MeCP2 and HDAC

Jee-Hye Choi[P], Yoon Sung Kang[1], Jin-Sook Son[1], Na-Young Min[1], Young-Hoon Park[1], Kwang-Ho Lee[C]

Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 156-756

The XIST expression is regulated by differential methylation of CpG sites in the promoter region of the XIST gene. The proteins that bind to the differentially methylated XIST promoter and mediate transcriptional silencing have not been identified, although a family of proteins containing a methyl-CpG-binding domain is obvious candidate. Previously, we reported that MeCP2 (methyl-CpG-binding protein 2) is recruited to the methylated promoter of the XIST on Xa (active X) and can repress the expression of the XIST in a methylation-dependent manner. In this study, we observed re-expression of the XIST on Xa by treatment with TSA (trichostatin A), an HDAC (histone deacetylase complex) inhibitor. The result indicates that HDAC is required for transcriptional repression of the XIST and probably acts as a mediator, which links some regulating proteins including MeCP2 for transcriptional repression. In addition, to ascertain whether the XIST is re-expressed by dysfunction of MeCP2, we analysed the expression pattern of transcriptionally inert XIST on Xa through a functional blocking of MeCP2 using antisense RNA in male lymphoblastoid cells. These results imply that MeCP2 and HDAC participate in XIST silencing through chromatin remodeling by methylation of CpG sites and acetylation of histone.