F815 E3 ligase activity of RING finger proteins that interact with Hip-2, a human ubiquitin-conjugating enzyme

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To identify proteins that interact with Hip-2, a ubiquitin-conjugating enzyme, a yeast two-hybrid screen system was used to isolate five positive clones. Sequence analyses showed that, with one exception, all Hip-2-interacting proteins contained the RING finger motifs. The interaction of Hip-2 with RNF2, one of the clones, was further confirmed through in vitro and in vivo experiments. Mutations in the RING domain of RNF2 prevented the clone from binding to Hip-2, an indication that the RING domain is the binding determinant. RNF2 showed a ubiquitin ligase (E3) activity in the presence of Hip-2, suggesting that a subset of RING finger proteins may have roles as E3s.

F816 Molecular-Cytogenetic Analysis of the 1RS in the Translocated New Wheat (1BL/1RS) Olmil

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The short arm of rye chromosome 1R (1RS) has been widely used in wheat breeding programs worldwide. A new 1BL.1RS wheat-rye translocation line was selected from the backcross of F_1 hybrid of wheat cultivar Olmil and rye cultivar Paldanghomil, and both cultivars were released from Korea. For detailed identification of 1RS in the translocated Olmil line, we applied in the translocated metaphase chromosomes by means of fluorescence in situ hybridization as the probe 5S rRNA and 18S-26S rRNA gene families which is known to be located in 1RS. And also In the translocated line, we confirmed rye 5S rRNA sequence and secalin gene sequence which originated 1RS chromosome.

F817 Characterization of the DNA repair genes for XRCC1 and XRCC3 from Arabidopsis

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Many DNA repair genes have identified by current genome sequencing projects. Two of the genes for X-ray cross-complementing group 1 (XRCC1) and X-ray cross-complementing group 3 (XRCC3) were isolated and characterized from Arabidopsis. It was determined that XRCC1 cDNA contains 1062 bp open reading frame (ORF) with 353 amino acids. The predicted amino acid sequence of XRCC1 with molecular mass of 40 kDa contains a consensus BRCT domain and is similar to but significantly shorter than XRCC1 of animal counterparts. XRCC3 gene consists of single exon in chromosome 5 in Arabidopsis. The XRCC3 gene isolated from genomic DNA by PCR contains 915 bp ORF with 304 amino acids. The XRCC3 amino acid sequence contains nuclear localization signal in amino-terminus and characteristic P-loop for ATP/GTP binding site motif A. It is similar to other RecA / RAD51 family sequences and highly homologous to human XRCC3 with 46% similarity. Yeast cells expressing XRCC1 or XRCC3 both showed resistance to methylmethane sulfonate. However XRCC3 can complement a rad51 mutant strain of yeast but XRCC1 cannot. We will use the

DNA repair genes to improve efficiency for gene targeting involved in designed single base change by short oligonucleotides for functional genomics in plants.

F818 Caspase-3-mediated cleavage of the NF-B subunit p65 at the NH2-terminus potentiates naphtoquinone analog-induced apoptosis

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The transcription factor NF-B plays a crucial role in immune and inflammatory response, and protects cells from apoptosis. In this report, we investigate whether the NF-B signaling pathway is blocked during apoptosis induced by 2,3-dichloro-5,8-dihydroxy-1,4-naphtoquinone (NA), an analog of naphtoquinone. It is observed that NA triggers apoptotic cell death in HeLa cells and destroys resistance to apoptosis caused by TNF-. Data presented in this study establish that p65/RelA, a subunit of NF-B, is cleaved at Asp97 by caspase-3 during apoptosis. Caspase-3-cleaved p65 loses transcriptional activity and potentiates NA-induced apoptosis, in contrast to an uncleavable mutant of p65, which protects the cell from apoptosis. Caspase-3, which is responsible for the cleavage of p65, is activated via the cytochrome c/caspase-9 signaling pathway rather than Fas/caspase-8 pathway during NA-induced apoptosis. Our results suggest that NA induces apoptosis by the negative regulation of cell survival through caspase-3-mediated cleavage of p65

F819 Detection of Trinucleotide Repeat Disease Using Micro-Capillary Electrophoresis Chip

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Several major human single gene disorders are attributed to the expansions of highly unstable trinucleotide repeat (TR) sequences. TR numbers are closely related to not only the onset age and severity but also diagnosis and prognosis of disease. Therefore, it is very effective and essential to estimate the accurate TR size for screening or confirmation of trinucleotide repeat disease (TRD). DNA testing such as southern blotting or silver staining has been commonly used in the diagnosis of TRD. However, such methods require laborious steps for the diagnosis and lack accuracy in detection of carriers and estimation of TR numbers. Therefore, we have developed a new method for the detection of TRD using micro-capillary electrophoresis chip (micro-CE chip). Amplified target sequences by polymerase chain reaction using specific primers designed for TR were separated in micro-CE chip and determined the size of TR. We evaluated the method by analysis of samples from normal subjects, 9 HD patients, 13 SBMA patients, 2 DRPLA patients, 6 FX carriers and 3 DM carriers. Southern blotting method was simultaneously performed to confirm our results. The data obtained from the micro-CE chip and southern blotting were highly comparable. The estimated TR number was also confirmed by sequencing. This study suggests that micro-CE chip is very useful for the detection of TRD and the determination of TR numbers. This new method could be very helpful in early diagnosis, carrier testing, and proper genetic counseling.

F820 The distribution of Actinobacillus actinomycetemcomitans, Hemophilus aphrophilus and Hemophilus paraphrophilus in subgingival plaque and saliva from Korean periodontitis patients using PCR