(W-Ⅳ-2) :

MATRIX ATTACHMENT REGIONS (MARs) AS A TRANSFORMATION BOOSTER IN RECALCITRANT PLANT SPECIES

Kyung-Hwan HAN

Kumho Life and Environmental Science Laboratory, 572 Ssangamdong, Kwangsangu, Kwangju, 506-712, Korea. Phone (062)970-2638, Fax (062)972-5085, E-mail-hanky@camd2. kkpcr.re.kr

Genetic engineering technique provides a means for inserting genes that confer traits not readily available in traditional gene pool. However, practical application of such technique has been limited to only a few crop species such as cotton, potato, and tomato. Many economically important species remain recalcitrant to gene transfer manipulations. Also, many transgenes including selectable marker genes vary widely in their level of expression, and complete silencing is not uncommon. The standard agronomic practice of seedling transplantation can cause complete loss of transgene expression. According to a recent survey, nearly all of the 30 plant biotechnology companies polled experienced undesired silencing of transgenes. Moreover, silencing of selectable marker genes shortly after their incorporation into plant chromosomes may result in low recovery of transgenic tissues from selection. Therefore, management of transgene expression is important not only for maintaining transgenic phenotypes but also for enhancing survival rate of the transformed cells during selection. Matrix attachment regions (MARs) are DNA sequences that bind to the cell's proteinaceous nuclear matrix to form DNA loop domains. MARs have been shown to increase transgene expression in tobacco cells, and reduce position in mature transgenic plants. Flanking selectable marker gene(s) with MARs could therefore enhance transformation rate by increasing number of transformed cells surviving the selection. Use of MARs may help achieve the desired phenotypes in transgenic plants by increasing and stabilizing the expression of transgenes.

(W-Ⅳ-3) :

YEAST TWO HYBRID ASSAY AND ITS PRACTICAL APPLICATION

Seong-Whan PARK

Korea Research Institute of Bioscience and Biotechnology

Yeast two hybrid assay is a useful and powerful tool to investigate protein-protein interaction. It is based on the fact that many eukaryotic transcriptional regulators are composed of DNA binding domain (BD) and activation domain (AD) which are functionally independent and physically seperable. When they are physically seperated by recombinant DNA technology, two domains do not interact with each other and thus cannot activate the transcription of the responsive genes. However, if two domains can be brought into close proximity in the prometer region the transcriptional activation will be restored.

In two-hybrid system, two different cloning vectors are used to generate fusions of two domains to proteins that potentially interacts with each other, and recombinant hybrid proteins are coexpressed in yeast cells. An interaction between a target protein (fused to BD) and a library encoded protein (fused to AD) creates a novel transcriptional activator. This factor then activates the reporter genes containing upstream promoters which has the binding affinity for BD, and this makes the protein-protein interaction phenotypically detectable. If two proteins do not interact with each other, the reporter genes will not be transcribed. In practice, using LexA two hybrid system, we identified several cDNAs whose encoded proteins interact with the kinase domain of PRK1 (pollen-expressed receptor-like kinase).

(W-Ⅳ-4) :
DYNAMICS OF HISTONE ACETYLATION DURING A CELL CYCLE IN BARLEY.

Toshiyuki WAKO, T., R., SHIMOGAWARA-FURUSHIMA, B.M. TURNER, and K. FUKUI
Laboratory of Rice Genetic Engineering, Hokuriku National Agricultural Experiment Station, Joetsu 943-0193, Japan

One of the nucleosomal protein, histone H4 is subjected to reversible acetylation at N-terminus lysine residues. By antibodies recognizing defined acetylated lysine of H4, paraformaldehyde-fixed barley mitotic cells were analyzed.

The antibody against acetylated histone H4 at lysine 5 (H4Ac5) labeled the entire chromosomes and nucleus, although acetylated regions were different depending on the mitotic stages. In the prophase chromosomes, centromeric regions were acetylated higher than the other chromosomal regions. Nucleolar organizing regions (NORs) become hyperacetylated at the prometaphase and the centromeric regions were deacetylated to the same level that of interstitial or telomeric regions. Hyperacetylation at NORs were kept to the anaphase, and then were suddenly deacetylated at the telophase. The centromeric regions were re-acetylated in the lysine 5 at the telophase.

The antibody recognizing acetylated histone H4 at lysine 16 (H4Ac16) also labeled the entire chromosomes but the pattern was different from that of the lysine 5. Through the mitotic M-phases, the telomeric regions were labeled and the NORs could not be distinguished from the centromeric or the interstitial regions by the level of acetylation.

Both the antibodies labeled the fixed interphase nuclei unevenly and the patterns were similar each other; the one side of the nucleus was stronger than other side. When unfixed nuclei were used for immunostaining with anti-H4Ac5 or anti-H4Ac16, the nuclei appeared some clustered and dot-like highly acetylated regions close to the surface of the nuclei. Although the patterns of acetylation in the interphase were very similar to both the antibodies, those at the prophase and the telophase were different. It suggests dynamic changes in the acetylation at lysine 5 and lysine 16 when entering and exiting the M-phase.

These observations clearly show that the defined chromosomal regions may be subjected to the dynamical changes in the histone acetylation during mitotic cell cycle and also suggest that the acetylation in the different lysine residue may have specific roles for the chromatin conformation and functional significance.