(W-Ⅲ-3) :

T-DNA INSERTIONAL MUTAGENESIS

Deok-Hoon PARK, Sang-Yeb LEE, Hong-Gil NAM
Department of Life Science, Pohang University of Science and Technology, Pohang, 790-784, Korea

Nowadays different transformation systems for higher plants are available, of which the most efficient is probably the T-DNA vector system derived from the Ti-plasmid of *Agrobacterim* tumefaciens. The T-DNA is a unique insertional element that is integrated into the plant nuclear genome after transfer from agrabacteria. All the results available indicate that these insertions are random and stable. Thus, T-DNA border sequence serves as a mutator via interruption of the plant genome and as a tag for isolation of the interrupted sequences by insertion sequences linked to the flanking genome DNA.

However, many insertions will not cause gene inactivation, because of the large proportion of repetitive DNA in most higher plant species. Since *Arabidopsis*, due to its small genome size and excellent genetics, has become a model for plant molecular biology, the possible application of gene tagging techniques to this plant was explored.

To detect and isolate T-DNA insertions in plant genes, gene fusion techniques were developed. We constructed a Ti-plasmid-derived vector containing a GUS reporter gene devoid a promoter(pOST 2002). The expression of this reporter depends on the fromation, by integration, of an adequate fusion with a plant gene.

Here we describe the isolation via T-DNA tagging and the characterization of the isolated gene and a new vector for the gene tagging. New improved construct(pOST 2005) carrying two plant selectable marker genes and a bacterial selectable marker gene. a bacterial replicon and promoterless reporter gene linked to the right integration site of the T-DNA will be used to induce and identify transcriptional or translational plant gene fusions and to rescue mutated plant genesin *E.coli*. Now we are constructing transgenic lines with this vector in *Arabidopsis*.

(W-Ⅲ-4) :

SEARCH FOR PLANT HOST FACTORS INTERACTING WITH CUCUMBER MOSAIC VIRUS PROTEINS USING YEAST TWO-HYBRID SYSTEM

Jin-Sam YOU
Graduate School of Biotechnology, Korea University

This study focused on searching for plant host factors interacting with CMV proteins using yeast two hybrid system. Protein-protein interaction mechanism is regarded as an important factor in almost all of the biological phenomena and yeast two-hybrid system has a merit in finding the interacting proteins

at gene level. We first introduced CMV encoded proteins into GAL4 DNA binding domain vector, pAS2-1 and GAL 4 transcriptional activation domain vector, pACT2. Yeast cell was transformed with two hybrids simultaneously and beta-galactosidase reporter gene transcription activation was monitored. In the yeast cell, none of viral protein pair was proved interacting each other except 2a and 2b functioning as a transcriptional activator of the GAL4 system. We colud not find any host factor(s) interacts with CMV MP or CP using the GAL4-based yeast two-hybrid system. However, pASΔ98, C-terminal deletion mutant of 2b, turned out to interact with LytB gene homolog, and pACT2a interacted with calmodulin, calcum binding protein. In the CMV infected tobacco plant, expression level of calmodulin was greatly increased, but LytB homolog was not.

(W-Ⅳ-1) :

EXPRESSION OF RECOMBINANT PROTEINS IN VARIOUS EXPRESSION SYSTEMS

Chung-Mo PARK

Kumho Life & Environmental Science Laboratory, Kwangju, Korea

Since the introduction of recombinant DNA technology, a number of recombinant gene expression systems and purification strategies have been developed for structural and functional studies of proteins derived from the rapidly growing new gene sequences. Each expression system has its own intrinsic properties. Bacterial system is the most commonly used one for large-scale production. Although some proteins tend to precipitate in the inclusion body as it is intracelluarly expressed, this problem can be minimized by managing such proteins expressed in periplasm or secreted into medium. Yeast and Pichia systems have a combined advantage of prokaryotic and eukaryotic organisms. Many eukaryotic proteins have been successfully expressed to a high level using P. pastoris cells. Other expression systems used in some cases include baculovirus/insect cells. Hansenula polymorpha, tobacco cell suspension culture, etc. Many affinity fusion strategies have been developed for rapid and efficient purification of recombinant proteins. These are based on different types of interactions, such as enzyme-substrate, receptor-ligand, antibody-antigen, and streptavidin-biotin. These gene fusion systems enable the purification possible by even a single-step chromatography.

We have extensively tested *E. coli* and P. pastoris cells to overexpress some recombinant proteins, plant and prokaryotic phytochromes and plant GTP-binding proteins. Several strategies are employed to get sufficient amount of proteins for structural and functional analyses. In addition to the full-size constructs, several partial-size constructs with different functional and structural motifs are expressed. Strep-, GST-and His-based tags are attached to the N-or C-termini for efficient isolation. Expression host, induction mode, protein configuration, and purification strategies will be discussed.