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

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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.