RECOMBINANT DNA TECHNOLOGIES AND THEIR PHAMACUTICAL APPLICATIONS IN YEAST SYSTEM

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In recent years genetic engineering has been the focus of attentions throught out the scientific communities in the world. In order to understand the mechanisms how the genes are regulated at the molucular level, numerous studies have been made recently using recently developed recombinant DNA technolodgy. In some cases application of these techniques in pharmaceutical industry has been successful and several phamacutical companies are trying to apply these techniques to improve the productions of certain biologically important substances. However, unless we understand the basic mechanisms how a gene is regulated at the certain physiological conditions, we can not successfully apply these techniques to meet the our desires. Thus, many basic researches are being carried out in the area of regulation of gene expression in either homologous or heterologous system. One system I have been working on to understand better the basic mechanisms of gene expression is allantoin degradation system in yeast. Allantoin, a purine degradation product, can be used as a nitrogen source in yeast. When yeast cells are in rich nitrogen environments, such as asparagine or ammonia containing medium, yeast cells do not use the allantoin as a nitrogen source. However, when cells are in poor nitrogen environments, yeast cells use the allantoin as a nitrogen source by degrading it into ammonia and carbon dioxide. Sets of structural genes (DAL1, DAL2, DAL3, DAL4, DAL5, DUR1, 2, and DUR3, 4) are involved in the degradation of the allantoin. Regulation of expression of these genes are controlled by the two regulatory elements, DAL81 (positive element) and DAL80 (negative element) and the inducer of the genes is the allophanic acid which is the last intermediate of the degradation pathway. Regulation occurs in both negative and positive manner by the two regulatory elenents at the transcriptional level. To elucidate the mechanisms how these genes are regulated by these two regulatory elements, the structural genes have been cloned and their DNA sequences have been determined. By way of DNA sequencing analyses and promoter fusion deletion studies, important DNA sequences necessary for the positive regulation of the expression of the system have been identified. Homologous sequences are present in most of the structural genes in the allantoin degradative pathway. Using synthetic oiligonucleotides, and in vitro mutagenesis, we are in the process of analyzing the sequences more vigorously to narrow down to the minimum necessary sequences which interact with the positive and nagative regulatory elements in order to understand how regulatory elements interact with target sequences they control.

Similar types of researches are being pursued in galactose metabolism system, cytochrome-C system, phosphate metabolism system, general amino acid control system, and many other systems in yeast to understand the basic mechanisms of control of gene expression. Once we understand the basic mechanisms of regulation of a

gene in one cell system, we can extend these knowledges in more practical area. Number of foreign genes (certain mammalian or plant genes) have been cloned in yeast and expression of these foreign genes have been studied using promoter fusion methods and deletion analyses. And the factors involved in the proper expression and regulation of the foreign gene in yeast have been examined by various groups. Proper expression of the foreign gene depends not only on the promoter function but also on the other many different factors. For instance, if the gene produces the secreted protein, it should have proper signal sequences in order to be secreted into the final extracellular membranes. Expression of the human tissue plasminogen activator (t-PA) has been successful using yeast promoter and signal sequences necessary for processing of a secreted protein. Human α1-antitrypsin DNA, immunoglobulin μ heavy chain cDNA, calf prochymosin gene, small peptide hormone genes, and human fibroblast interferon cDNAs have been fused into promoters of the several yeast genes and successful expression of these gene in yeast were observed.

Yeast has been the convenient system to introduce foreign genes and to study their expression in yeast. Some of the genes have phamaceutical importances and now are under more detailed studies for their functions and clinical applications. Using recently developed recombinant DNA technique,in near future some of the gene products can be available for phamacutical and clinical usuage. However, when we apply these techniques to clone a foreign gene and to study its expression in yeast or in any other heterologous system, we have to consider many factors involved in regulated expression of the foreign genes. Still a lot more basic mechanisms how gene regulation occurs have to be understood before we can expect general production of these genetically engineered products. Without basic understranding of mechanisms of gene expression in either homologous or heterologous system, we can not have successful outcomes. In order to apply these recombinant techniques in phamaceutical fields, we have to have careful consideration in basic mechanisms how the gene expression occurs in the different cell systems.