METALLOTHIONEIN GENE EXPERTS DETAIL EXPRESSION, REGULATION

The Third Annual Congress for Recombinant DNA Research yielded few research findings for the serious biotechnologist. However, two papers on metallothionein gene expression and regulation provided a useful summary for researchers interested in the practical applications of this unusual system. The work presented showed the versatility of the metallothionein promoter/regulator sequence in getting foreign genes to function in yeast, cultured animal cells, and whole animals.

Ubiquitous among animals and plants, metallothioneins (MT) are low molecular weight, cysteine-rich proteins that bind potentially toxic divalent heavy metal cations, such as those of zinc, cadmium and copper. In every species studied so far, MT gene expression is induced in a straightforward manner by the heavy metal ions that bind to the proteins.

MTs have generated excitement over the past few months because of the work of Ralph Brinster, at the University of Pennsylvania, and Richard Palmiter, at the Howard Hughes Medical Center. At the Philadelphia meeting, Brinster described how they have successfully used an MT gene promoter/regulator (P/R) sequence to control the expression of other structural genes. The researchers linked a mouse MT P/R sequence to the structural gene of mouse growth hormone, inserting this into a pBR322 plasmid and then injecting it into the male pronucleus of fertilized mouse embryos. When the resulting mice were fed high zinc diets, they grew to nearly twice their normal size, indicating that the cloned growth hormone MT P/R gene was expressed. Tissue assays showed that the genes functioned in tissues such as liver and kidneys, where MTs are normally produced. The stability of this new gene construct was shown when the progeny of these mice also expressed the gene.

Incorporation of the gene is increased if the pBR322 plasmid is linearized with the Bg11 restriction enzyme prior to its injection into the mouse embryo, said Brinster. The male pronucleus was chosen to receive the chimeric gene because it is bigger and closer to the surface than the female pronucleus.

Gene incorporation did not increase when mouse DNA BAM III restriction fragments were added to the plasmid, indicating that the incorporation is likely to be random and non-homologous. However, once incorporated into the mouse genome, the gene construct appears to stay in one place.

Also speaking at the conference was Dean Hamer of the National Cancer Institute, who detailed his group's investigation into the mechanism of MT gene regulation. To assess which parts of the MT gene are required for expression and induction by heavy metals, the NCI researchers prepared several SV40 vectors containing 5′ and 3′ deletions linked to the SV40 galactokinase. The results of the 3′ deletion studies conclusively showed that the MT structural gene was not required for proper expression. In fact, 3′ deletions up to −18 still showed normal expression and induction.

The 5′ deletion studies showed that a region 60 base pairs 5′ to the structural gene was necessary for proper induction of gene expression by heavy metals, although overall expression was reduced by deletions that extended out as far as 350 base pairs 5′ to the start of the structural gene. Hamer said the function of the extensive P/R sequence was not clear. The NCI group also compared the sequence homology between several species' MT P/R sequences, including those of the human genes, and found them to be very similar. Hamer said this was good news for those who wanted to use the MT P/R sequence to obtain cDNA gene expression in a variety of animal cells.

Hamer also addressed a controversial aspect of MT gene regulation—whether it is repressor or activator controlled. In either instance the regulator protein is presumably affected by the presence of heavy metal ions. In repressor control the protein disengages the gene when bound to heavy metals, while in activator control the regulatory protein binds to the gene when heavy metals are present.

Hamer added extra regulatory sequences to cells in which the MT-containing SV40 vector was incorporated. If the MT gene was under repressor control, extra P/R DNA would attract some of the repressor protein, thus activating some of the hybrid genes' P/R sequences. The result would be the production of galactokinase by the cells. Since huge excesses of P/R DNA did not produce galactokinase, Hamer concluded, in this case the P/R sequence must operate under activator control.

—Joseph Alper

DNA TRANSFER

SHENK, LEVINE, WIGLER CLONE PROMISING VIRAL GENES

PHILADELPHIA—Viruses have provided genetic engineers with much of the genetic material for their vectors and plasmids. At the Third Annual Recombinant DNA Congress last month, Tom Shenk of SUNY-Stony Brook described an adenovirus gene that may be useful for increasing the production of specific proteins by mammalian cells.

The VA I gene produces a small segment of RNA in huge amounts in the late (post-DNA replication) period of adenovirus infection. Although the specific function of VA I RNA is unknown, it dramatically increases the translation of viral mRNAs, probably via interaction with 5′ leader sequences on the mRNAs and the protein synthesizing machinery of the cell.

A single viral gene product can alter the phenotype of a normal cell to a transformed or tumor cell. Several groups recently showed that the p21^{ras} protein derived from human