Chemically-Regulated Cucumber Mosaic Virus Amplicon for the Expression of Foreign Genes/Proteins in Plants and Plant Cell Cultures

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Plant viruses have been recognized as a very useful means for high level, rapid expression of foreign proteins in plants. The major advantage is that virus replication increases the copy number of the desired gene, giving greater heterologous protein production per cell. Recently, virus based expression has been used for the production of vaccines, antibodies and other human therapeutic proteins in whole plants and similar approaches may be useful for heterologous protein production in plant cell cultures in bioreactors. One approach is to genetically engineer the host plant cell so that replication-competent recombinant virus can be produced intracellularly, under the control of a chemically-inducible promoter.

We have constructed a *Cucumber mosaic virus* (CMV) based amplicon, utilizing infectious cDNA clones of the three CMV genomic RNAs (RNAs-1, 2, and 3), for the expression of foreign genes/proteins in plants. The cDNA of RNA-1, without the 5'-most 58 nt, was fused to an operator sequence for expression mediated by a glucocorticoid receptor-based transactivator. A binary vector, carrying transactivator-tagged RNA-1 and 35S promoter driven cDNA copies of CMV RNA-2 and RNA-3, was agroinfiltrated into *Nicotiana benthamiana* leaves. Estradiol treatment of agroinfiltrated leaves expressed coat protein (CP) to levels comparable to those found in CMV-infected pumpkin. Agroinfiltration with another binary vector containing the beta-glucuronidase (GUS) gene in place of CP in RNA-3, followed by estradiol application allowed GUS expression. We have also engineered mGFP5ER (green fluorescent protein gene fused to an endoplasmic reticulum targeting signal peptide) and SP-AAT (a human alpha-1-antitrypsin gene with a secretory signal peptide) genes into this virus amplicon system. We have transformed *N. benthamiana* plants to generate cell lines for the analysis of inducible expression of SP-AAT, and the two reporter genes, GUS and mGFP5ER.