

Gene therapy for soybean diseases (Primary Investigator: Les Domier)

The Domier lab team has worked in molecular plant virology for 19 years and have constructed and modified infectious copies of virus genomes to study gene function and express foreign genes. In a recent paper from my lab, Moon et al. (2001) used infectious clones of *Barley yellow dwarf virus* (BYDV) to study transcription initiation of virus RNAs. In a second manuscript, (Domier and McCoppin, 2003) we developed a system for the *in vivo* transcription and translation of cloned RNA virus sequences in cultured cells by modifying an insect virus to express bacteriophage T7 RNA polymerase. We used the system to study the activity of the internal ribosome entry sequences of a virus that infects aphids. In a related project, my lab is currently studying the synergistic interaction between *Bean pod mottle virus* (BPMV) and SMV. As part of that project my lab has produced transgenic soybean plants expressing the SMV gene that is responsible for enhancement of BPMV symptoms.

The goals of this project are to develop gene delivery systems to integrate DNA segments to germ-line cells without the need for specialized *in vitro* cell-culture techniques by taking advantage of the unique embryo-invading abilities of proteins expressed by different plant pathogens. *Soybean mosaic virus* (SMV) and *Tobacco ringspot virus* (TRSV) are transmitted very efficiently through soybean seed by virtue of their abilities to circumvent barriers that normally exclude viruses from embryonic tissues. Depending on the soybean genotype and the virus isolate, the incidence of transmission through seed of SMV and TRSV can be as high as 75% (Hill, 1999; Stace-Smith, 1985). For this reason, SMV and TRSV have been chosen as candidates for the construction of gene delivery vectors. Since the genomes of SMV and TRSV are composed of RNA, but DNA molecules are needed for transformation, the reverse transcriptase (RT) gene and origin of replication (ORI) from *Cauliflower mosaic virus* (CaMV) will be added to the vector. To aid in the nuclear importation and integration of the resulting single-stranded (ss) DNA, the *VirD2* and *VirE2* genes, whose products protect and direct the nuclear localization of ssDNA, respectively (Ziemienowicz et al., 2001), and left and right border sequences from the *Agrobacterium* C58 TI plasmid will be added to the vectors.

The proposed studies will focus first on developing vectors from TRSV because it has a segmented genome that is composed of two ssRNA molecules. The larger RNA (RNA1) contains the genes for RNA replication. The smaller RNA (RNA2) contains the movement and coat protein genes. Hence, it will be possible to insert genes into RNA2 without disrupting the ability of the virus to replicate. Biologically active DNA copies of both TRSV RNAs will be synthesized and cloned between the CaMV 35S promoter and nopaline synthase (NOS) terminator by standard techniques (Moon et al., 2001). The infectivity of the cloned viral genomes will be assessed by biolistically inoculating soybean seedlings and assaying inoculated plants and resulting seedlings for TRSV antigen (Clark, 1977; Gal-On et al., 1997).

Once infectious clones have been produced, large-capacity expression vectors will be constructed from RNA2 by inserting translational control and restriction enzyme recognition sequences downstream of the capsid protein gene. Two types of control sequences will be assessed. In one set of clones, DNA encoding amino acid sequences that are cleaved by virus-encoded proteases will be inserted between the capsid protein and the exogenous genes (von Bodman et al., 1995). In a second set of clones, virus internal ribosome entry sequences that facilitate the translation of multiple genes from a single RNA will be inserted upstream of the introduced gene(s) (Domier and McCoppin, 2003). The ability of the modified virus to express

exogenous genes will be tested using a green fluorescent protein (GFP) gene (Haseloff et al., 1997). Once the placement of control signals has been optimized, the CaMV RT and ORI (provided by J.E. Schoelz, U. Missouri, Columbia) will be added to the vector. The ability of the chimeric virus to specifically synthesize DNA copies of TRSV RNA2 containing the CaMV ORI will be tested using a DNA-dependent polymerase chain reaction assay. Next, the *Agrobacterium VirD2* and *VirE2* genes (provided by S.K. Farrand) will be added to the vector. The abilities of *VirD2* and *VirE2* to localize to and transport ssDNA into the nuclei of infected cells will be determined by *in situ* hybridization and immunolocalization assays (Nass et al., 1998). The integration of TRSV RNA2 sequences into genomic DNA of soybean seedlings will be assayed by Southern-blot analysis.

Once stable vectors are constructed, vector capacity and transgene copy number and expression levels will need to be optimized. To alleviate the constraint of size and number of genes that can be inserted into RNA2, we will attempt to produce a synthetic RNA3 that contains the 5'- and 3'-noncoding regions of RNAs 1 and 2 and the movement protein gene transferred from RNA2. The synthetic RNA3 would add approximately 5 kb of capacity to the TRSV-based vector. In addition to size, SMV and TRSV differ greatly in their susceptibility to posttranscriptional-antiviral defenses of soybean. While TRSV is very susceptible to posttranscriptional RNA silencing, SMV, like other potyviruses, actively suppresses this defense response (Carrington et al., 2001). These differences may be important in defining the number of integration events per cell and the expression level of the integrated genes. In a separate project (see below), we have produced soybean lines that express the SMV gene responsible for suppression of posttranscriptional antiviral defenses. We will use these plants to investigate combining the unique features of TRSV with the antiviral defense of SMV in transgene copy number and expression level.