PLANT GENETIC ENGINEERING

APPROPRIATE BEAN GENE EXPRESSION REPORTED

MADISON, Wisconsin—Genetic engineers are beginning to demonstrate control over the behavior of genes transferred from one plant to another.

John D. Kemp and Timothy C. Hall, researchers at the Agrigenetics facility here, say that the phaseolin gene they have transferred to tobacco is being expressed in the seed of transformed tobacco plants as if the gene were native to it and under normal developmental control. Information necessary for the gene's regulation is contained within a segment of DNA that includes the genomic coding sequence and the gene's promoter, they also say.

Phaseolin is the major storage protein in the bean seed. Last summer the Agrigenetics team announced that a phaseolin gene transferred to tobacco was being expressed by regenerated plantlets at low levels in all tissues. During the last year the group has pursued control of the location, timing, and level of the gene's expression, and the fate of the foreign gene product within its host's tissues.

In the original experiments, phaseolin's low-level non-specific expression in tobacco plantlets was very different from its expression in the mature bean seed, where it accounts for 25 to 50 percent of the total protein. The group, however, grew the transformed tobacco to maturity, self-pollinated the plants, and derived seed. They then grew the progeny of these seeds (F_1) to produce seeds, and assayed the resulting plants for phaseolin.

They report that the phaseolin gene was transmitted through tobacco seed as a Mendelian dominant: three-quarters of the F_1 generation expressed phaseolin, one-quarter did not. Phaseolin was present in the leaves and stems of these plants in small amounts (as it was in the original plantlets). But it was also present in its stable glycosylated form in tobacco seed, where it constituted one to three percent of the seeds' total protein—an amount five orders of magnitude higher than that in other tissues.

According to Kemp and Hall, the phaseolin level in the seed is exactly what would be expected of a developmentally turned-on gene coding for a plant storage protein. The researchers also note that even in the bean plant phaseolin is expressed at very low levels in other differentiated tissues. There are between five and ten phaseolin genes in the bean plants, each accounting for a few percent of the seeds' total protein. Their combined effect results in the large amount of phaseolin normally stored in the bean seed.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The phaseolin gene in this transformed tobacco seed appears to be under normal developmental control, Agrigenetics researchers say.

The researchers also reported indirect evidence for a gene-dose effect in tobacco. Approximately half the tobacco seeds contained two to four percent phaseolin. Another quarter contained about 50 percent less. That is what would be expected of a gene that segregated in a ratio of 1:2:1 (25 percent of the progeny receive one copy; 50 percent receive two; 25 percent receive none).

The researchers plan to proceed in two directions. They will study the gene's promoter in order to characterize the signal or signals responsible for developmental regulation. The group also plans to add multiple copies of the gene to tobacco in order to study the gene-dose effect on expression levels.

Another example of a plant gene that is regulated by a promoter next to the gene's coding sequence was reported recently by Nam-Hai Chua's group at Rockefeller University, which collaborated with Robert Fraley, Stephen Rogers, and Robert Horsch of Monsanto (St. Louis, MO). These researchers transferred to petunia some pea DNA containing the gene encoding the light-regulated small subunit of ribulose-1,5-biphosphate carboxylase. In petunia calli, this protein subunit was expressed in the light, but not in the dark, just as it is in the pea plant. It was also processed correctly and localized in the chloroplasts, where it functioned properly.

Giorgio Morelli, a member of Chua's group, has constructed several deletions in the gene's promoter. He has found one region of the promoter, between 500 to 350 base pairs upstream from the coding sequence, that affects transcription efficiency. When he deletes this region, expression levels fall by about 70 percent. He has found evidence that a signal controlling light regulation is located in a region 35 base pairs from the start of the coding sequence (between the CAAT box and the coding sequence).

These results are a further step toward the practical engineering of traits in plants, the researchers say. They agree, however, that the regulatory patterns they have discovered must be sought in other plant systems before they can be generalized. But increasingly, the picture resembles the one identified in animal systems.

—William J. Netzer