Final Progress Report

DOE grant DE-FG02-95ER20186

Characterization of Arabidopsis genes involved in gene silencing

Enhancer of gene silencing 1 (egs1) is an Arabidopsis mutant that enhances post-transcriptional gene silencing of the *rolB* gene introduced by genetic engineering (transgene). The goal of our proposal was cloning *EGS1* based on its map position. Although we screened more than 2000 chromosomes for recombination, we were unable to get closer than 2 cM to the gene. We experienced an unexpected tendency of the post-transcriptionally silenced transgene to switch to a more stable silenced state. This made it impossible to select *egs1* homozygotes for map based cloning. This forced us to reconsider our cloning strategy. One possibility would have been to use a different transgene as the target of gene silencing. We tested two other transgenes. Both encoded proteins unrelated to the first but they were all expressed from the same type of promoter and they all had a similar tendency to become post-transcriptionally silenced. After screening over 80 F2 segregants from each cross between our *egs1* mutant and Arabidopsis of the same ecotype homozygous for the new transgene, we were disappointed to find that the *egs1* mutation did not enhance post-transcription silencing of the two new genes. In 80 plants we expected to have between 4 and 6 plants that were homozygous for the transgene and for the mutant *egs1* allele. If *egs1* mutations could enhance gene silencing of the new transgene, these plants would not express it. However all the double homozygotes still expressed the transgene. Therefore, we could not change the target transgene for mapping. This was the state of the cloning at the time for renewal of the grant in 1999. Because the selection of new meaningful recombinant plants had become extremely inefficient using the original *rolB* transgene, we abandoned the attempt at map based cloning and did not apply for further funding.

Publications:

Grant S. R. (1999) Dissecting the mechanisms of posttranscriptional gene silencing: divide and conquer. Cell 96, 303-306.

Personnel other than the PI supported by this grant and their current positions:

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DOE Patent Clearance Granted

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