Analysis of the Complex *BIO3 / BIO1* Locus of *Arabidopsis*

Rosanna Muralla[1], Colleen Sweeney[1], Basil Nikolau[2], and David Meinke[1]

[1] Department of Botany, Oklahoma State University, Stillwater, OK 74078
[2] Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011

The biosynthesis and utilization of biotin in plants has been elucidated in part through the analysis of two auxotrophic mutants of *Arabidopsis* (*bio1* and *bio2*) that we first identified 15-20 years ago using a forward genetic screen for embryo-defective mutants that could be rescued by growth of arrested embryos on enriched media. We demonstrate here through reverse genetics that the *bio3* mutant, which is disrupted in a region predicted to encode an enzyme that functions in an intermediate step in the pathway, has a phenotype similar to that of the *bio1* and *bio2* mutants. The surprising discovery is that the *BIO3* and *BIO1* loci are positioned adjacent to each other on the chromosome, in the same orientation as found in many bacterial and fungal species, and that differential splicing results in the production of two types of transcripts, one with the potential to encode two separate proteins, and the other capable of producing a fusion protein that catalyzes two different steps in the pathway. The existence of the fusion protein in plant extracts is being tested using antibodies directed against each of the monocistronic gene products produced in *E. coli*. The results obtained to date have provided important clues to the genomic organization of biotin biosynthetic genes in *Arabidopsis*, the intracellular localization of biotin synthesis, and the evolutionary remnants of a prokaryotic operon in a flowering plant.