Embryogenesis in Arabidopsis thaliana: Mutant library construction and embryo mutant identification and characterization

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Abstract

Embryogenesis plays a central role in the plant life cycle. It starts after fertilization. A single zygote divides asymmetrically, giving rise a small apical cell and a larger basal cell. The small apical cell undergoes precise cell divisions, passes through 2, 4 and 8 cell stages, followed by the protoderm, globular, heart, torpedo and cotyledon stage, in the process of forming a mature embryo. In embryo development a large number of genes are estimated to be involved and interact with each other during embryogenesis. We demonstrated that the *RSH* gene was required for normal embryo development of *Arabidopsis*. Its essential role was determined by disrupting expression of the gene by the *Ac*/ *DsE* two-element transposon system, which caused the embryo mutation. The abnormal phenotype was traced to the first asymmetrical division of the zygote and the embryo development lost its precise programmed cell division pattern. The *rsh* mutant showed both apical-basal and radial pattern defects. The *RSH* gene mapped to chromosome I. The gene was cloned and sequenced. It encoded a HRGP-type protein of predicted size, 49 k Dalton. The pre-protein contained a signal peptide and 13 almost identical repeats. Each repeat had 28 amino acids. The rescue of homozygous *rsh* mutants by complementation with the wild-type *RSH* gene demonstrated that the *rsh* mutation is the consequence of the *DsE* insertion. The gene was found to be expressed through out the developing embryo. It was expressed in a tissue specific manner after embryo germination. The *RSH* gene expression pattern was first profiled using the GUS reporter gene assay. The Northern and RT-PCR confirmed the results. To localize the *RSH* protein at the cellular level, an *EGFP* gene was linked to the C-terminus of the *RSH* gene and expressed in wild-type *Arabidopsis* . Confocal microscopy showed that the fusion protein was

localized to the cell wall. Wild type transformants expressing RSH-EGFP showed mutant phenotypes. All these results support the conclusion that the RSH protein plays an important role in cell division during embryogenesis. ^

Subject Area

Biology, Molecular|Biology, Botany|Biology, Genetics

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