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Map-based cloning of *Arabidopsis* gametophytic mutant genes: *gemini pollen3* and *sidecar pollen*

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Objectives

The aim of this study is to isolate genes being involved in pollen mitosis in order to elucidate the mechanisms of cell polarization and signaling in the pathway of pollen development and function.

Materials and Methods

1. Materials

Arabidopsis male-specific gametophytic mutant: *gemini pollen3* (*gem3*, genetic background: Landsberg)

Arabidopsis gametophytic mutant: *sidecar pollen* (*scp*, genetic background: Nossen)

Plants heterozygous for *gem3* and *scp* was crossed to plants of wild-type Columbia(Col). F1 plants heterozygous for mutations, identified by screening DAPI-stained pollen, were allowed to self-fertilize.

2. Methods.

DNA was isolated from leaves of the *gem3* X Col and *scp* X Col F2 population and used for PCR-based mapping using SSLP markers which show useful polymorphism between two ecotypes.

Results and Discussion

Pollen mitosis I (PMI) produces two unequal daughter cells, the vegetative and generative cells (GC), which have different structures and developmental fates. The larger vegetative cell (VC) produces the pollen tube, whereas the smaller generative cell divides to produce two sperm cells. Thus, the asymmetric division at PMI results in the establishment of differential cell fates through gametophytic cytokinesis which can be defined as an unusual process forming a hemispherical cell plate. Control of gametophytic cytokinesis is therefore a critical process in pollen cell fate determination, which results in the asymmetric distribution of cellular components that presumably include cell fate determinants.

We have taken a genetic approach using two gametophytic mutants of *Arabidopsis* to identify key genes expressed gametophytically for the completion of microgametogenesis and focused on genes which control cell division and cell fate determination. We have identified two gametophytic mutants through EMS mutagenesis including *gem3* affecting microspore polarity and asymmetric division at PMI and *scp* required for the normal cell division pattern during pollen development (Development, 122:3243-3253, 1996). Map-based cloning of these genes is in progress. Genetic data including the fine mapping, the cytological observation of the pathway, and processes involved in male gametogenesis in the mutants will be presented.

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