Final Report
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Programmed Cell Death During Female Gametophyte Development

Summary
During year 1, we carried out a screen for cell death mutants. From that screen, we identified a mutant, *fem111*, in which the female gametophyte's central cell appeared to undergo cell death upon fertilization. During years 2 and 3, we focused our efforts on this mutant and carried out the molecular analysis summarized below. Analysis of *AGL80* led to the identification of *AGL62*. We showed that *AGL62* is a regulator of endosperm cellularization. This work resulted in the publication of three papers in Plant Cell and Plant Physiology.

fem111/agl80 Affects Central Cell Function

Genetic segregation analysis showed that *fem111* affects the female gametophyte but not the male gametophyte.

To determine whether *fem111* female gametophytes are competent to give rise to seeds, we analyzed *fem111* female gametophytes at 18 h after pollination using confocal microscopy. In contrast to wild type, endosperm nuclei were not present and the central cell cavity was instead filled with highly fluorescent material.

This post-fertilization phenotype resembles a degenerating synergid cell, suggesting that fertilization triggers a cell death process in *fem111* central cells. We also showed that degeneration of the central cell does not happen in the absence of pollination, suggesting that it is induced by fertilization.

To determine whether *fem111* affects megagametogenesis, we analyzed female gametophytes at the terminal developmental stage (stage FG7) using confocal microscopy. The egg cell and synergid cells are indistinguishable from those of the wild type. By contrast, the central cell exhibited a subtle phenotype: its vacuole was smaller than that of wild type.

The *fem111* mutant was identified in a screen of T-DNA-mutagenized lines and we used TAIL-PCR to identify the affected gene. The T-DNA in *fem111* is inserted into the *AGL80* gene. Introducing a wild-type copy of the *AGL80* gene into the *fem111* mutant rescued the mutant phenotype, indicating that *AGL80* is is the affected gene in *fem111* mutants.

AGL80 encodes a Type I MADS-domain protein that likely functions as a transcription factor. *AGL80* was the first Type I gene functionally characterized.

To characterize the expression of *FEM111*/*AGL80* during female gametophyte development, we analyzed transgenic *Arabidopsis* plants containing a protein fusion construct (*AGL80-GFP*). During female gametophyte development,

FEM111/AGL80 was expressed specifically in the central cell. During seed development, *FEM111/AGL80* was expressed exclusively in the endosperm, from the 1- to 16-nucleate stage.

MADS-domain-containing proteins function as transcriptional regulators that control many developmental processes in plants. To test this further, we analyzed the expression of several central cell-expressed genes in *fem111* mutants. This analysis showed that *FEM111/AGL80* is required for expression of *DME* and *DD46* but not *FIS2* during central cell development.

In summary, we showed that *AGL80* functions in the female gametophyte's central cell and controls the expression of genes required for development of the central cell into the endosperm.

AGL62 Interacts With AGL80 and Regulates Endosperm Cellularization

MADS proteins function as dimers. Using yeast two hybrid assays and bimolecular fluorescence complementation, we showed that AGL80 interacts with AGL62.

To characterize *AGL62* expression, we analyzed transgenic *Arabidopsis* plants containing a protein fusion construct (*AGL62-GFP*). *AGL62* was not expressed in the central cell but was expressed in the endosperm, from the 1-nucleate stage to just before endosperm cellularization.

To gain insight into *AGL62* function, we analyzed *agl62* mutants obtained from the Arabidopsis stock center. In *agl62* seeds, the endosperm cellularizes prematurely, indicating that *AGL62* is required for suppression of cellularization during early development (i.e., during the syncytial phase).

To identify *AGL62* regulators, we analyzed *AGL62* expression in *fie*, *fis2*, and *mea* seeds, which fail to cellularize. *AGL62* expression failed to decline and continued throughout seed development in these mutants. These data indicate that the *FIS* PcG complex is required for suppression of *AGL62* expression at the end of the syncytial phase

In summary, our data suggest that *AGL62* functions in a pathway that controls the syncytial-cellular transition during endosperm development. According to this model, *AGL62* is expressed during early endosperm development (i.e., during the syncytial phase) and suppresses cellularization during this period. *AGL62* most likely does so by suppressing the expression of genes required for cellularization. At the end of the syncytial phase, the *FIS* PcG complex suppresses *AGL62* expression, which allows expression of the cellularization genes and triggers the initiation of the cellularized phase.

Publications

This grant resulted in the publication of three research papers:

(1) Portereiko, M.F., Lloyd, A., Steffen, J.G., Punwani, J.A., Otsuga, D., and Drews, G.N. (2006) *AGL80* is required for central cell and endosperm development in *Arabidopsis*. Plant Cell 18, 1862-1872.

(2) Sandaklie-Nikolova, L., Palanivelu, R., King, E.J., Copenhaver, G.P., and Drews, G.N. (2007) Synergid cell death in *Arabidopsis* is triggered following direct interaction with the pollen tube. Plant Physiology 144, 1753-1762.

(3) Kang, I.-H., Steffen, J.G., Portereiko, M.F., Lloyd, A., and Drews, G.N. (2008) The AGL62 MADS domain protein regulates cellularization during endosperm development in *Arabidopsis*. Plant Cell 20, 635-647.