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Molecular Genetic Traits Influencing Maize Endosperm Development and Value

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Development of the endosperm in cereal grasses entails different phases characterized by cell division, endoreduplication, accumulation of storage metabolites and cell death, which need to be carried out in an orderly fashion. While correct regulation of the cell cycle plays an essential role in endosperm development, the key regulatory factors and how the cell cycle interfaces with other pathways in this developmental context are largely unknown. We investigated the cyclin-dependent kinase (CDK)-retinoblastoma pathway and how it controls the cell cycle and coordinates it with other processes during maize endosperm development. Retinoblastoma-related (RBR) proteins may be inactivated through CDK-mediated phosphorylation, but the identity of the responsible kinase in maize is unknown. We have previously shown that down-regulation of CDKA;1 severely inhibits the endoreduplication cell cycle and suggested that CDK may be an up-stream regulator of the retinoblastoma pathway.

We discovered two types of maize RBR genes, RBR1 and RBR3, which differ in terms of structure, regulation and function. Phylogenetic analyses indicate that these genes may be distinctive features of the Poaceae. We found that RBR3 plays a positive rather than a negative role in DNA replication, cell transformation, and the expression of the minichromosome maintenance (MCM)2-7 family of DNA replication factors. These features are a paradigm shift in RBR gene function and appear to be unique within the RBR gene family. They suggest the existence in maize and related cereal crops of specific RBR/E2F-dependent pathways impinging on the cell cycle and development.

RBR1 was down-regulated in transgenic endosperm using RNAi approaches. This resulted in the de-repression of a number of down-stream E2F targets, including RBR3, the MCM2-7 gene family, DNA methyltransferase (MET)1, CDKB;1, and the recently identified RBR4 gene. It also increased endosperm ploidy levels, stimulated the production of a larger number of cells, reduced the average cell size, and promoted programmed cell death. To test whether CDKA;1 inhibits RBR1 (through phosphorylation) in the pathway that leads to DNA synthesis and endoreduplication, the two CDKA;1 and RBR1 down-regulated mutants were crossed and their progeny analyzed. Our results indicate that CDKA;1 controls endoreduplication through an RBR1-dependent pathway. However, the ability of RBR1 to repress gene expression programs is independent from CDKA1, suggesting the presence of two differently regulated RBR1 activities in developing endosperm. One type of RBR1 activity controls E2F-dependent gene expression and is largely independent from CDKA;1, while another suppresses endoreduplication and can be inhibited by CDKA;1. In addition, RBR1 is part of a regulatory feedback loop that impinges on CDK activity. Together, these results indicate that the CDKA;1-RBR1 pathway integrates and controls different processes associated with endosperm development. Genome-wide analyses of the transcriptome, metabolome, and epigenetic mechanisms to understand how the cell cycle is coordinated with other pathways at a systems biology level are currently underway.

Publications related to the project:

Paolo A. Sabelli and Brian A. Larkins (2009) The development of endosperm in grasses. *Plant Physiology* **149**, 14-26.

Paolo A. Sabelli, George Hoerster, Lucina A. Lizarraga, Sarah W. Brown, William J. Gordon-Kamm and Brian A. Larkins (2009) Positive regulation of MCM gene expression, DNA replication, and cell transformation by a plant retinoblastoma gene. *Proceedings of the National Academy of. Sciences USA* **106**, 4042-4047.

Paolo A. Sabelli and Brian A. Larkins (2009) The contribution of cell cycle regulation to endosperm development. *Sexual Plant Reproduction* **22**, 207-219.

Paolo A. Sabelli and Brian A. Larkins (2009) Regulation and function of retinoblastoma-related plant genes. *Plant Science* **177**, 540-548.

Paolo A. Sabelli, Yan Liu, Ricardo A. Dante, Luciana E. Lizarraga, Hong N. Nguyen, Sara W. Brown, John P. Klinger, Jingjuan Yu, Evan LaBrant, Tracy M. Layton, Max Feldman, and Brian A. Larkins (2012) The retinoblastoma-related pathway is a central hub that controls cell proliferation, endoreduplication, cell size and cell death during maize endosperm development. *Plos Genetics* (submitted).