Maternal control of seed development mediated by the flavonoid biosynthesis pathway

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Many plants exhibit post-zygotic barriers to hybridization, in both interploidy crosses within species and interspecific crosses between related species. For example, crosses between diploid (2x) and tetraploid (4x) plants cause a triploid block where severe endosperm under- or over-proliferation kills the developing triploid embryo. While most ecotypes of the model species Arabidopsis thaliana tolerate 2x X 4x crosses to produce large viable seed, one ecotype, Columbia (Col-0), exhibits a triploid block when the paternal parent is tetraploid. Recently, loss of function mutants in the flavonoid biosynthesis pathway (FBP) that operate in the seed coat have been identified as powerful maternal suppressors of the paternal Col4x-mediated triploid block. The present hypothesis is that a maternal messenger responsible for regulating the correct timing of endosperm cellularisation is attenuated or blocked by a functional FBP; hence mutations in the FBP promote cellularisation and reduce seed lethality by removing the signalling block. We are attempting to identify the cellularisation factor, understand the role of the FBP in its regulation and to explore further the effect that different ecotypes have on the severity of the triploid block and the underlying mechanism behind this effect.
The role of ROS1a in rice endosperm development

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DNA methylation is a major epigenetic mark that contributes many biological aspects including development, stress responses and genome stability. In plants, cytosine DNA methylation can occur in the CG, CHG and CHH sequence contexts and maintained by the balance of methylation and demethylation. Cytosine methylation is mediated by DNA methyltransferases and can be passively lost after DNA replication. However, plants can also actively demethylate DNA via the action of DNA glycosylases. In many organisms, reproductive phase is a key developmental stage for epigenetic phenomena. In Arabidopsis, the largest gene for DNA glycosylases, DEMETER (DME), is predominantly expressed in the central cell, the progenitor of endosperm, of the female gametophyte, and promotes maternal allele-specific global hypomethylation and the expression of imprinted genes in the endosperm. To explore the specific roles of DNA demethylation in rice, we disrupted ROS1a gene, the longest of six putative DNA glycosylase genes in the rice genome, using homologous recombination-promoted gene targeting. We found that even in the presence of the wild-type paternal ROS1a allele, the maternal mutant allele caused failure of endosperm development with irregular but viable embryos. Detailed morphological analysis revealed that the cellularization, a key event in endosperm development, was not observed at seven days after pollination with maternal mutant allele. To understand its potential contribution to controlling gene expression during rice seed development, we are now studying the dynamics of gene expression pattern in mutant endosperm.
Overcoming interspecific hybridization barrier in rice endosperm by the ploidy manipulation

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In angiosperms, endosperm is a nutritive tissue supporting embryo development during seed formation and seedling growth after germination. The major event of early endosperm development, endosperm cellularization, is a crucial developmental transition from the syncytial phase to cellularized phase, which in case of failure causes embryo arrest. In interspecific cross between cultivated rice, Oryza sativa (2x), and related wild rice, O. longistaminata (2x), results in arrested hybrid embryo due to delayed endosperm cellularization. Similarly, endosperm defect has been also observed in crosses between plants with different ploidy levels, especially, crossing between a maternal tetraploid plant (4x) and a paternal diploid plant (2x) causes a precocious endosperm cellularization. This interploidy cross shows contrary phenotypes to interspecific cross between O. sativa and O. longistaminata. To investigation of the effect to endosperm cellularization by increased maternal ploidy levels in interspecific hybridization, we performed a cross between maternal tetraploid O. sativa (4x) and O. longistaminata (2x). Although this crosses resulted in a slightly precocious of cellularization, the viability of the hybrid seeds is fully restored. This result indicates that the hybridization barrier in interspecific can be overcame by ploidy manipulation, and suggesting endosperm barriers in interspecific and interploidy crosses are mediated by common mechanisms.
Identification of temporal gene regulatory networks in early maize endosperm development

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Endosperm is a product of double fertilization and functions as a nutritive tissue in the angiosperm seed to support the growth of the embryo or the germinating seedling. In cereal grains, endosperm comprises a large proportion of the mature seed and contains large amounts of carbohydrates and proteins. To understand the gene regulatory processes of the earliest stages of endosperm development, we used laser-capture microdissection to isolate and profile mRNA populations of a developmental series of the endosperm from 0 to 4 days after pollination (DAP) in maize inbred B73. This time series comprised the unfertilized haploid central cell (0 DAP), the transition to a rapidly proliferating endosperm coenocyte (1 and 2 DAP), the coenocyte-to-cellular transition stage (3 DAP), and the fully cellularized endosperm that showed an overall polarity and indications of early cellular differentiation (4 DAP). Using computational tools, we identified distinct temporal co-expression modules during this period of development, including a subset activated upon fertilization. Analysis of the co-expressed transcription-factor genes and the associated cis-regulatory elements allowed us to hypothesize gene regulatory networks involved in early endosperm proliferation in maize.
Miniature seed 6 encodes a chloroplast-targeted pentatricopeptide repeat protein that is required for seed development in maize

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The miniature kernel mutant mn6 was isolated in the F2 generation of a mutagation induced by EMS in B73 inbred line. The mutation was backcrossed to normal B73 over three generations to remove inessential mutagenic locus. Macroscopically, the mn6 mutants could be clearly distinguished from mature wild-type sibling kernels because of the smaller size. The reduction in kernel size involved both the endosperm and embryo. Germination test showed that less than 20% emerged primary root and shoot, and the seedlings were short and stunted after germination. All mn6 seedlings exhibited aberrant development and died three weeks after germination. mn6 kernels were not only smaller than normal sibling kernels, but also exhibited a development delay.

mn6 was out-crossed with Mo17 to create a population for mapping, and the mutant was narrowed into 1.6 Mb apart on chromosome 9. Sequencing the candidate genes in this region and a mutation site in gene GRMZM2G345128 was found. Evidence that disruption of mn6 caused the embryo lethal phenotype was provided by complementation of mn6 by a MN6 cDNA transgene. MN6 encoded a PPR protein of the P family and mainly expressed in ear and developing kernels. Transient expression showed that MN6 localized to plasmid, and rps16 splicing was strongly compromised. The loss of plasmid transcription is the likely cause for the embryo-lethal and seedling-lethal phenotype.
What are the functional roles of imprinted genes in A. thaliana?

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Imprinting has been hypothesized to evolve for several reasons including balancing gene expression and as a result of parental conflict. To formally test these hypotheses and to identify key forces driving the evolution of imprinting, we need an understanding of the functions of imprinted genes. In A. thaliana, mutants of many genes known to be imprinted show no gross phenotypes. We are using a series of assays to analyze seed development and the seed metabolome and test for more subtle phenotypes in imprinted gene mutants. Data from a handful of genes that have served as a testbed for the optimization of these assays will be presented.
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Analysis of the Opaque-2 gene regulatory network in maize endosperm

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The endosperm of cereal grains contains large amounts of carbohydrates and proteins, and is an important source of human food, animal feed, and industrial feedstock. However, our understanding of the gene regulatory mechanisms that control storage product deposition is limited. To begin to uncover the underlying gene regulatory networks in maize (Zea mays), we mapped the in vivo binding sites of the maize bZIP transcription factor Opaque-2 (O2) using ChIP-Seq performed with endosperm dissected from the B73 inbred line with comparison to an o2 mutant (B73o2), and also identified genes differentially expressed in B73 and B73o2 endosperm using RNA-Seq. The analysis identified 1,863 O2-modulated genes, including 186 putative direct targets and 1,677 indirect targets, and revealed a broad role of O2 in the regulation of storage product deposition in maize endosperm. Analysis of the O2-bound genomic regions associated with direct O2 targets detected significantly enriched DNA sequence motifs that likely contain cis-regulatory sequences bound by O2 or transcription factors that co-regulate O2 target genes. Furthermore, examination of the dynamic expression patterns of O2 target genes in developing endosperm of B73 vs. B73o2 revealed at least two distinct modes of O2-mediated gene activation. Together, these results provide significant insights into the high complexity of the regulation of endosperm storage program by O2 and its nuclear partners.