Maize RNA Polymerase III Subunit NRPC2: New Kid on the Kernel Development Block

Seed (kernel) size affects evolutionary fitness and crop yield. In angiosperms, seed size is largely determined by the endosperm and the embryo, the products of double fertilization. Particularly in grass crop species (i.e. cereals), endosperm accounts for ~90% of kernel size and weight and is a major determinant of human nutrition (Li et al., 2017; Zhan et al., 2017). Genetic and molecular insight into endosperm development in relation to other seed-specific developmental processes has greatly been advanced by kernel size mutations in maize (*Zea mays*; Sabelli and Larkins, 2009). However, such mutations have pleiotropic effects in processes including embryo development, storage reserve filling, and seedling development. Therefore, the identified kernel-size genes are not good candidates for maize breeding programs. Identifying novel genes that control endosperm development might enrich our understanding of the regulatory mechanisms behind kernel development and could ultimately contribute to breeding crops with increased grain yield and quality. In this issue of *Plant Physiology*, Zhao et al. (2020) describe a mechanism that links altered endosperm cell size and number to the maize kernel size, without confounding pleiotropic effects. This process includes the activity of an RNA polymerase III (RNAPIII; also known as Pol III) subunit that uncouples endosperm cell proliferation and storage reserve accumulation.

Maize endosperm, a paradigm for grasses, differentiates to constitute five recognizable cell types: the aleurone layer, central starchy endosperm cells, conducting zone, basal endosperm transfer layers, and embryo-surrounding region, with each cell type performing specific functions (Sabelli and Larkins, 2009). Concurrent with cell differentiation is an increased rate of cell division and endoreduplication of the central starchy endosperm and aleurone layer cells, which together determine endosperm cell number and size and eventually lead to the final kernel size (Sabelli and Larkins, 2009).

Mutation of the maize *FLOURY3* (*FL3*) gene, encoding one of 17 subunits forming the RNAPIII complex that regulates gene expression involved in storage protein synthesis, results in small and floury endosperm (Li et al., 2017). Eukaryotic RNAPIII transcribes transfer RNAs, ribosomal 5S rRNA, and other small noncoding RNAs required for protein synthesis to control cell division and proliferation events essential for organ/tissue development (Li et al., 2017; Wang et al., 2019). In maize, the interaction of FL3 with other subunits of RNAPIII is critical for the proper functionality of RNAPIII transcription events and normal endosperm development. Despite this finding, other factors and mechanisms of the RNAPIII complex potentially regulating cell differentiation and cell cycle events during endosperm development remain to be determined.

Zhao et al. (2020) screened for ethylmethane sulfonate-induced mutations that resulted in visible *small kernel* (*smk*) phenotypes and isolated the *smk7* mutant. The *smk7* kernels had normal embryos but exhibited abnormalities in cell proliferation and cell size and number in basal endosperm transfer layer, aleurone layer, conducting zone, and starchy endosperms. The authors mapped the *smk7* mutation to a gene encoding the second-largest subunit of RNAPIII, NRPC2 (Nuclear RNA Polymerase C2), using map-based sequencing and confirmed that the mutation leads to a defective NRPC2 protein in *smk7*. The *smk7* kernels showed compromised biogenesis of small noncoding RNAs such as transfer RNAs and 5S rRNAs, confirming that NRPC2 is part of the RNAPIII machinery. To further verify the function of the gene, lines with reduced NRPC2 function were generated using CRIPSR-Cas9 mutagenesis, which phenocopied the mutant.

Unlike *fl3*, *smk7* did not appear to affect the nutritional value of maize. The *fl3* mutation disrupts the accumulation of starch and storage proteins (e.g. zein and nonzein proteins), leading to a soft and opaque endosperm. By contrast, storage product accumulation is normal in *smk7* kernels. While endosperm quality traits are genetically uncoupled in *fl3* and *smk7* mutants, the small-kernel traits appeared to be additive. The latter interpretation also found support from a compensatory increase in *NRPC2* by the *fl3* mutation and the physical interaction of NRPC2 with FL3.

The authors performed RNA sequencing of single and double *smk7* and *fl3* mutants relative to wild-type kernels. In gene sets with altered expression in *smk7* or *fl3*, enriched genes were consistent with their smaller and floury kernels, respectively. In particular, the set of genes expressed at higher levels in *smk7* was enriched for the terms DNA-dependent DNA replication, DNA replication, Cell cycle process, etc. Individual genes associated with the *smk7* mutation are implicated in cell proliferation and endoreduplication: the *mini-chromosome maintenance2-7* gene family, the proliferating cell nuclear antigen subunit of DNA polymerase δ, and retinoblastoma-related proteins and cyclins. Up-regulation of a subset of these genes positively regulates endoreduplication and cell size during maize endosperm development (Sabelli et al., 2009, 2013), a process also occurring in the *smk7* endosperms. Similarly, the set of genes preferentially expressed in *fl3* is

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enriched for Fatty acid biosynthesis and metabolism. Individual genes affected by the *fl3* mutation include several well-characterized opaque endosperm genes such as *Opaque*, *Oxalyl-CoA decarboxylase1*, and *Naked endosperm1*. These results suggest a major role for *NRPC2* in kernel development but not in kernel quality.

This study identifies a novel gene in the control of seed size, maize *NRPC2*, that is functionally unique. Because *NRPC2* specifically targets endosperm-specific cell differentiation events, it largely eliminates pleiotropic effects caused by other gene regulatory mutants functioning during early kernel development. Experimental and circumstantial evidence repeatedly highlight the interplay of transcriptional, hormonal, and nutrient signals underlying cell fate specification and the differentiation of endosperm cell types as well as the communication between intrakernel tissue types during kernel development (Sabelli and Larkins, 2009; Doll et al., 2017; Li et al., 2019). Whether ZmNRPC2 may be involved in such interplay would be worthwhile to investigate, with the potential also to decipher the functional differences between two maize RNAPIII subunits during maize kernel development. Understanding such hierarchical regulation of these networks would help build robust maize breeding programs for improved seed quality.

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