

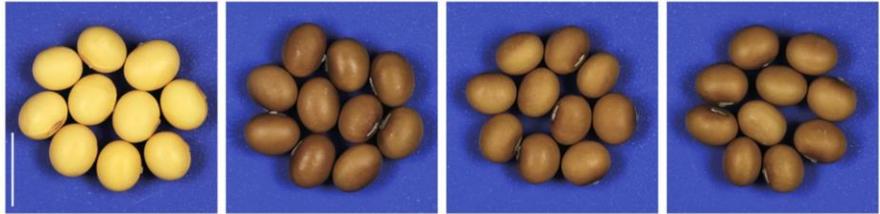
IN BRIEF

Slice and Dice: DCL2 Mediates the Production of 22-nt siRNAs That Influence Trait Variation in Soybean

In plants, small interfering RNAs (siRNAs) serve as key regulators of gene expression. While 24-nucleotide (nt) siRNAs are produced by DCL3 and mediate transcriptional silencing of transposons and pericentromeric chromatin through RdDM (Borges and Martienssen, 2015), 22-nt siRNAs are processed by DCL2 and participate in transgene silencing and viral defense (Wang et al., 2018; Parent et al., 2015). However, since 22-nt siRNAs are relatively rare in wild type *Arabidopsis* (Henderson et al., 2006), and the loss of DCL2 doesn't produce obvious development defects (Henderson et al., 2006; Wang et al., 2018), their role is still largely unknown. By contrast, crops such as soybean (*Glycine max*) exhibit abundant 22-nt siRNAs and are ideal systems to study their biogenesis, targeting, and functions in genome activity.

In a new study, Jinbu Jia and co-authors combine genome editing, sRNA-seq, and transcriptomics to investigate the functions of DCL2-dependent 22-nt siRNAs in soybean (Jia et al., 2020). The authors used CRISPR-Cas9 genome editing to simultaneously generate loss-of-function mutants of the two *GmDCL2* genes in soybean, and performed sRNA-seq on a selection of tissues. The analysis revealed a substantial decrease in 22-nt siRNAs abundance throughout the genome in *Gmdcl2*, but also a drastic reduction of secondary 21-nt siRNAs derived from the 22-nt siRNAs. By investigating the distribution of these DCL2-dependent siRNAs in wild-type soybean, the authors identify that a large majority of 22-nt siRNAs are overlapping with transposable elements (TEs), suggesting a possible role in silencing. In the *Gmdcl2* mutant, the production of such TE-associated 22-nt siRNAs is drastically reduced, but this does not correlate with local reduction in DNA methylation, nor accumulation of TE transcripts. Next, the authors examined the structural features of *GmDCL2* substrates. The transcriptomic approach reveals that DCL2 preferentially processes PolIII-transcribed long inverted repeats to generate 22-nt siRNAs.

The most dramatic developmental phenotype resulting from *GmDCL2* loss-of-function is a darkening of the seed coat, from yellow in wild-type soybean to dark brown in *Gmdcl2s* (Figure). Seed color in soybean is



Loss-of-Function Mutations in *GmDCL2* Results in Darkening of the Seed Coat. CRISPR-Cas9-engineered frame-shift mutations in *GmDCL2a* and *GmDCL2b* triggers overaccumulation of *CHS* transcripts and darkening of the seeds coat in the Tianlong1 cultivars (Adapted from Jia et al. [2020], Figure 1.)

dictated by the activity of Chalcone Synthase (*CHS*)-encoding genes that enable flavonoid biosynthesis that results in dark brown seed coat. The yellow seed coat phenotype is associated to the abundant production of siRNAs from a particular *CHS* gene cluster, with the siRNAs targeting and silencing other *CHS* genes *in trans*. With a higher genomic resolution provided by a *de novo* assembly of the *CHS* cluster, Jinbu Jia and colleagues propose that the *CHS* cluster generating siRNAs can form a long inverted repeat transcript that is possibly processed by DCL2. This hypothesis is further supported by the accumulation of DCL2-dependent 22-nt siRNAs at this *CHS* cluster, which disappear in *Gmdcl2s*. In the absence of *CHS* trans-targeting siRNAs, other *CHS* genes accumulate transcripts at higher level compared to wild type, and flavonoid accumulation results in dark seed coat.

Further from facilitating trait diversification, DCL2-dependent 22-nt siRNAs are also involved in translational repression and stress adaptation (Wu et al., 2020). The high abundance of 22-nt siRNAs in crops suggests more unexplored functions in regulating genome activity in plants. This study revealed that *GmDCL2s* play important roles in the regulation of natural traits by generating endogenous 22-nt siRNAs and provided a new strategy for improving the appearance quality of soybean seeds.

Matthias Benoit

Howard Hughes Medical Institute

Cold Spring Harbor Laboratory

benoit@cshl.edu

ORCID: 0000-0002-3958-3173

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Matthias Benoit

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