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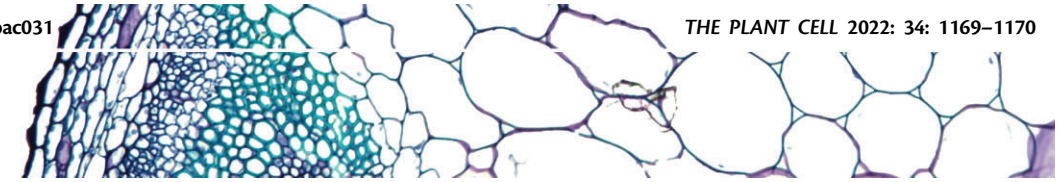

Coat in the act: a mechanistic insight into the transcriptional regulation of seed mucilage biosynthesis

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In Brief

Upon imbibition, some angiosperm seeds secrete a polysaccharide-based, hydrophilic extracellular matrix known as mucilage, which has important biological functions in seed germination. The pectic polysaccharide rhamnogalacturonan I (RG-I), a polymer with an alternating backbone of galacturonic acid and rhamnose, accounts for 90% of mucilage while the remaining 10% is composed of cellulose, hemicellulose, and pectic homogalacturonan. Seed mucilage formation has been used as a model for understanding cell wall assembly in Arabidopsis (Francoz et al., 2015).

The enzymes GALACTURONOSYLTRANSFERASE-LIKE5 (GATL5) and UDP-L-Rhamnose synthase MUCILAGE-MODIFIED4 (MUM4) are essential for RG-I accumulation (Francoz et al., 2015). In parallel, a number of transcription factors (TFs) are required for mucilage formation, including WRKY TRANSPARENT TESTA GLABRA2 (TTG2), the trihelix DE1-binding factor 1 (DF1), and the homeodomain-leucine zipper GLABRA2 (GL2) (Francoz et al., 2015). However, the links between the regulatory and biosynthetic steps of mucilage formation are unclear. Now in *The Plant Cell*, **Yan Xu and colleagues (**Xu et al., 2022**)** provide convincing evidence that DF1 and GL2 physically interact to cooperatively control the transcriptional activation of *MUM4* and *GATL5* for RG-I biosynthesis (see Figure). The authors also provide data that integrate DF1 and GL2 into the existing transcriptional regulatory network controlling mucilage formation.

As shown by high-performance liquid chromatography, mucilage in the *df1* mutant is specifically depleted in rhamnose and galacturonic acid, but not in other monosaccharides such as glucose or xylose, suggesting a specific defect in RG-I biosynthesis in *df1* mutant seeds. Based on the co-expression of DF1 and GL2, the authors tested the interaction of DF1 with GL2 by yeast two hybrid, bimolecular fluorescence complementation, and co-immunoprecipitation assays. Taken altogether, these protein interaction assays strongly indicate that DF1 and GL2 directly interact in the nucleus via specific domains. Interestingly, transcripts required for RG-I biosynthesis were less expressed in mutant *df1* and *gl2* seeds, whereas those related to cellulose were unchanged. Among the RG-I-related genes under-expressed in those mutants, the authors noted that only the *MUM4* promoter contains the DF1-binding motif, the GT3 box (Shibata et al., 2018). By combining chromatin immunoprecipitation and gel shift assays, the authors show that DF1 directly binds the GT3 boxes in the *MUM4* promoter. Likewise, chromatin immunoprecipitation and gel shift assays demonstrate that GL2 binds directly to the *MUM4* and *GATL5* promoters through an L1 DNA box. Based on a series of elegant dual luciferase reporter assays using normal and mutant promoters, the results of Xu and colleagues suggest that DF1 and GL2 are both required to reach the maximum transcriptional outputs of *MUM4* and *GATL5*, although the *GATL5* promoter lacks the DF1 DNA-binding motif. Importantly, DF1 alone is not sufficient to activate *GATL5*, but *GATL5* instead requires both DF1 and GL2 for full transcriptional activation. Therefore, DF1 enhances the GL2-dependent transcriptional activation of *GATL5* through their protein–protein interaction, while

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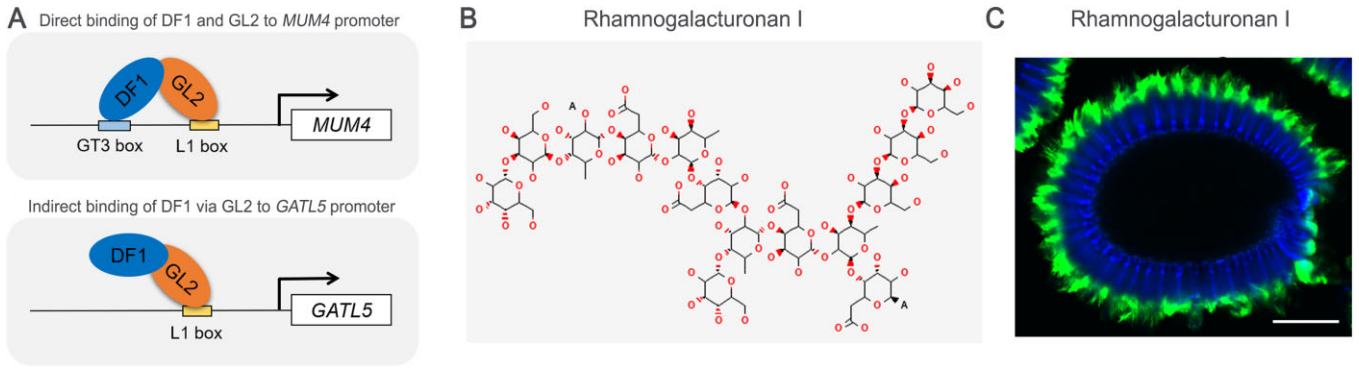

Figure DF1 and GL2 TFs control RG-I biosynthesis for mucilage formation in Arabidopsis seeds. A, DF1 and GL2 physically interact and bind the *MUM4* and *GATL5* promoters to activate transcription. B, MUM4 and GATL5 transcriptional activation enables RG-I biosynthesis. Model of RG-I from soybean from PubChem. C, RG-I (green) and cellulose (blue) form the mucilage. A and C, Adapted from Xu et al. (2022).

DF1 and GL2 both directly binds the promoter of *MUM4* for transcriptional activation (see Figure). Finally, the authors integrate DF1 and GL2 within the pre-existing knowledge on transcriptional regulation of seed mucilage formation. Indeed, they show that TTG2 directly binds the W box of *DF1* and *GL2* promoters to, respectively, repress or activate transcription. The authors further uncover a negative feedback loop between TTG2 and DF1 by showing that DF1 can directly bind the GT3 box of the *TTG2* promoter to repress transcription.

The data presented in this work update an already complex transcriptional regulatory network, but more importantly provide a clear mechanistic point of view of the transcriptional regulation of mucilage formation. In future studies, it would be interesting to identify the precise mechanism responsible for DF1/GL2-dependent transcriptional activation, for example, recruitment of a histone modifier and/or an RNA polymerase. This study makes a leap forward in unraveling the biological complexity of specialized cell wall formation in plants.

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