Identification of First RG-I GalA transferase RGGAT1: Opening the Door to Studies of Plant Cell Architecture, Cell:Cell Adhesion and Biomass Recalcitrance

Background

- Pectin, a family of cell wall glycans with homogalacturonan (HG) and rhamnogalacturonan (RG-I) backbones, has roles in biomass yield, recalcitrance, and cell growth. Pectin has recognized functions in cell wall structure, cell adhesion, and cell expansion, which are difficult to understand due to structural complexity and because the identities of several key biosynthetic enzymes have not been determined. Prior to this study, the enzyme that catalyzes the addition of galacturonic acid (GalA) into the RG-I backbone was unknown.

Approach

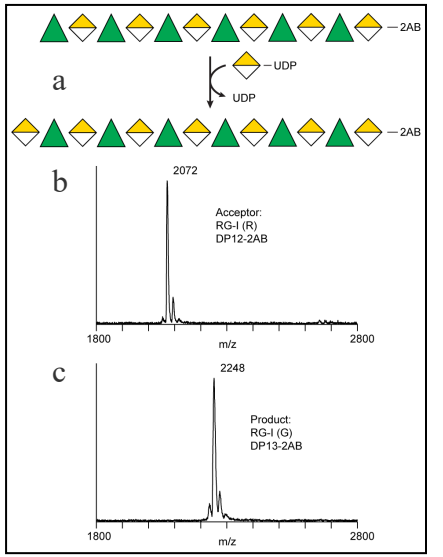
- Arabidopsis gene *MUCI70* was selected as a candidate RG-I biosynthetic enzyme due to high expression in Arabidopsis RG-I biosynthetic seed mucilage cells. Enzyme activity of purified MUCI70 protein obtained following expression of MUCI70 in human kidney cells was tested using RG-I oligosaccharide acceptors in the presence of UDP-GalA donor substrate. Polymerization of RG-I was tested by incubation of MUCI70 with the rhamnosyltransferase RRT4. Deep-learning-based methods and AlphaFold2 were used to predict the glycosyltransferase structure and aspects of the catalytic mechanism.

Results

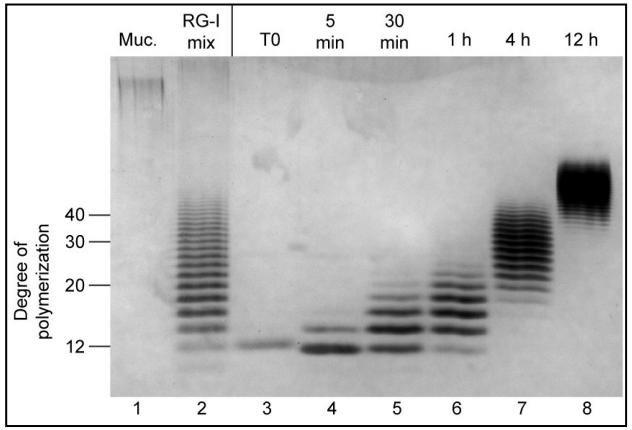
- The putative glycosyltransferase At1g28240/MUCI70 was shown to be an RG-I galacturonosyltransferase. The name RGGAT1 was proposed for this enzyme. The combined activities of RGGAT1 and RRT4 catalyzed *in vitro* elongation of RG-I oligosaccharide acceptors into polymeric RG-I. Because RGGAT1 was found to be phylogenetically distinct from the GAUTs, which synthesize HG, RGGAT1 was named the founding member of a new glycosyltransferase family (GT116). RGGAT1 was predicted to have a GT-A fold structure and employ a metal-independent catalytic mechanism that is rare among glycosyltransferases.

Significance

- The identification of RGGAT1 and the new 8-member Arabidopsis GT116 family provides a new avenue for studying the mechanism of RG-I synthesis, the function of RG-I in plants, and roles of RG-I in biomass yield and recalcitrance.



a, Scheme of RG-I:GalAT activity. Rha, green triangles; GalA, yellow divided diamond. RGGAT1 is a GalA transferase that elongates RG-I acceptors. **b**, MALDI-TOF-MS spectrum of the DP12-2AB oligosaccharide acceptor (predicted mass 2,072 Da). **c**, MALDI-TOF-MS spectrum of product after GalA transfer. Mass increase of 176 Da is consistent with addition of a single GalA.



RGGAT1 and RRT4 were combined with UDP-GalA, UDP-Rha, and an RG-I oligosaccharide acceptor. Polymerization of RG-I polysaccharides of DP >40 was detected.

Amos, R.A. et al. Nat. Plants 8:1289-1303. doi: 10.1038/s41477-022-01270-3.