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Atmodjo, Melani A.; Sakuragi, Yumiko; Zhu, Xiang; Burrell, Amy J.; Atwood III, James A.; Orlando, Ron; Scheller, Henrik Vibe; Mohnen, Debra

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H. Scheller

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Characterization of the GAUT1- and GAUT7-containing Arabidopsis Pectin Biosynthetic Enzyme Complex

Melani A. Atmodjo, Yumiko Sakuragi, Xiang Zhu, Amy J. Burrell,
James A. Atwood III, Ron Orlando, Henrik V. Scheller, and Debra Mohnen

Complex Carbohydrate Research Center & Dept. of Biochemistry and Molecular Biology,
The University of Georgia, Athens, GA, 30602

Galacturonosyltransferase 1 (GAUT1) is an Arabidopsis pectin biosynthetic alpha-1,4-galacturonosyltransferase (GalAT) that transfers galacturonic acid (GalA) from uridine diphosphate-GalA (UDP-GalA) onto exogenous acceptors of homogalacturonan (HG) (1). Multiple lines of evidence, including co-immunoprecipitation using rabbit polyclonal anti-GAUT1 or anti-GAUT7 antibodies and Bimolecular Fluorescent Complementation experiments, show that GAUT1 exists in a protein complex with GAUT7, another member of the GAUT gene family. To further characterize this pectin biosynthetic enzyme complex, we used GAUT1- and GAUT7-specific IgG conjugated to magnetic beads to affinity-purify the protein complex. The immunoabsorbed proteins were eluted from the beads, separated on SDS-PAGE, and analyzed by in-gel trypsin digestion and subsequent liquid chromatography – tandem mass spectrometry (LC-MS/MS) to identify the components of the complex. The results verified the presence of GAUT1 and GAUT7 in the immunoabsorbed protein mixtures, and identified several other proteins, including putative methyltransferases, a couple of members of the oligosaccharyltransferase complex, and an unknown protein similar to N-acetyltransferase from other organisms, as candidate members of the GAUT1:GAUT7 enzyme complex. This evidence of a novel, multi-subunit wall biosynthetic enzyme complex may shed light to elucidating the mechanism of pectin synthesis. A model for the GAUT1:GAUT7 core complex is also presented. This research is funded by NRI, CSREES, USDA Awards 2003-35318-15377 and 2006-35318-17301.

(1) Sterling, J.D., Atmodjo, M.A., Inwood, S.E., Kumar Kolli V.S., Quigley, H.F., Hahn, M.G., and Mohnen, D. (2006) PNAS 103(13):5236-41.