Identifying novel regulators of exocytosis through a chemical genetic screen in Arabidopsis

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Exocytosis is a process necessary for the efficient delivery of new membrane, membrane proteins and extracellular matrix components to the plasma membrane (PM) and cell wall. The process encompasses packaging of cargo into secretory vesicles at the trans-Golgi network as well as their transport, tethering, docking, and fusion with the PM. The exocyst complex facilitates the tethering of secretory vesicles to the PM. A small molecule, Endosidin2 (ES2), serves as an exocyst inhibitor and binds to the EXO70A1 subunit of the exocyst complex.

To identify additional exocytosis regulators, we carried out a forward genetic screen using ethyl methanesulfonate (EMS)-mutagenized Arabidopsis lines expressing the PIN2-GFP marker. During this study, we developed a screening pipeline for identification of drug-hypersensitive mutants and a computational biology pipeline for mutant mapping, which can be easily translated to other hypersensitive mutant screens.

Mutants hypersensitive to ES2 were named *es2s*. One such mutant, *es2s-15*, had a strong ES2 hypersensitive phenotype for root growth and showed defects in the polar trafficking of PIN2 in root epidermal cells. We mapped the causal mutation to *argj* and identified a second allele with similar phenotypes. Surprisingly, ArgJ is an enzyme in the arginine biosynthesis pathway. Supplementation with arginine partially rescued the ES2 hypersensitive phenotype of both *argj* alleles. Taken together, our results indicate that impaired arginine biosynthesis leads to exocytosis defects. Successful completion of this work will provide new insights about the regulation of secretion by uncovering links between amino acid metabolism, signaling and plant cell growth.