# Understand Plant Membrane Trafficking Using Cellulose Synthase Complex As A Cargo

In the late 1800s, the endomembrane system was first discovered by scientist Camillo Golgi, which was the start point of this special type of transport in cell. Endomembrane traffic in eukaryotic cells have many functions within cell growth and plant development, partially as a means of communication and balancing membrane system. (Serge Feyder et al. 2015) Thanks to Randy Schekman and Peter Novick's work, the secretory pathway in yeast was the first identified trafficking pathway, taking advantage of eukaryotic intracellular organization and gene tractability. Through analyzing the invertase synthesis, the isolated yeast sec mutants revealed the accumulation of secretory vesicles, suggesting that Sec represented as a key effector in the transport of secretory proteins. Their thermosensitive defects result also indicated that at least 23 gene products were involved in secretory machinery. (Peter Novick et al. 1980)

Nowadays, it is clear that endoplasmic reticulum (ER) functions in synthesizing, processing, and sorting proteins, and similar with ER, Golgi apparatus is a vital organelle in secretory pathway, receiving newly synthesized proteins from ER and directing them to cell surfaces or vacuoles. The membranes of the secretory pathway, involving ER, Golgi apparatus, multivesicular body and the nuclear envelop makes up the principal membrane system which ensures the delivery of molecules between different compartments. During the anterograde and retrograde traffic, COP II and COP I act as carriers delivering newly synthesized molecules to their destination and recycling to their sites of origin respectively. COP II vesicles, bud from ER and transport cargo molecules to cis- Golgi cisternae, while COP I is divided into two types vesicles, COPIa recycles escaped ER proteins and COPIb transports cisternae membrane proteins in a retrograde direction (Federica Brandizzi et al. 2018). From Golgi apparatus to plasma membrane, unconventional protein secretion process involving soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) and small GTPase family proteins facilitates the vesicles carrying cargo proteins to fuse with the cell surface and transports newly synthesized proteins outside of the cell (Adnan M. 2019). To function properly, plant cell must develop the necessary machinery to sort and direct thousands of different proteins to the correct subcellular location, and this is the reason why we attach much importance to protein trafficking process. Therefore, more evidences are needed to make the molecules transportation between Golgi and plasma membrane clear.

Prior to SNARE-mediated fusion, there is an octameric protein complex, the exocyst, which helps the secretory vesicles to tether with the fusion site on plasma membrane. This exocyst vesicle-tethering complex consists of 8 subunits: sec3, sec5, sec6, sec8, sec10, sec15, Exo70, and Exo84, which might be direct targets of a number of small GTPases and kinases (Novick et al. 1980; TerBush and Novick, 1995). Such as, Sec15 of the yeast exocyst interacted the Rab protein Sec4 which is putative to mediate the recruitment of the exocyst to secretory vesicles (Guo et al. 1999a); Exo84 in budding yeast can be phosphorylated by Cdk1 kinase during mitosis, which provides molecular evidences for pathogen filamentous growth.(Luo et al. 2013; Caballero-Lima and Sudbery 2014) Therefore, the exocyst complex plays a significant role in membrane trafficking, which provides us with more evidences for the molecular machinery of signaling pathway.

Previously, it is already identified that a small molecule, Endosidin2 (ES2), targets EXO70 to inhibit exocytosis in a dosage-dependent manner. With the treatment of ES2, Arabidopsis seedlings have shorter roots and roots hair and are less sensitive to gravity stimulation. After ES2 short time treatment, lower fluorescence intensity of total plasma membrane-localized PIN2:GFP was observed while the vacuoles have an increased amount of GFP fluorescence, . While in the western blot detection, EXO70A1 showed a strong interaction with ES2 compared with another analog. Besides, one peptide of EXO70G2 that share 24% amino acid sequence identity with EXO70A1 also appeared in the active matrix which induced PIN2 aggregations, suggesting that G2 might be a potential target for ES2 (Chunhua Zhang et al. 2015). Therefore, whether ES2 targets EXO70A1 specifically or generally targets all the members of EXO70 family protein will guide us to get more extra evidence for understanding the mechanism of exocyst complex regulation.

Among the secretory proteins during exocytosis, cellulose synthase complex (CSC) is a large protein complex which is assembled at the Golgi and then transported to the plasma membrane for the catalytic activities. that catalyzes the biosynthesis of cellulose.

CSC is composed of multiple units of cellulose synthase (CesA) proteins and is transported through exocytic pathways as a large protein complex (at least 2 MDa) (Polko, Joanna K., 2019). It is not known how exocytic trafficking machineries operate the regulated transportation of large protein complexes like CSC in a spatiotemporal manner. We aim to use chemical genetic approach to understand the mechanisms of plant membrane trafficking using CSC as a cargo. Chemical genetics is an efficient method which uses phenotypic screen probing the entire molecular signaling pathway in order to get the drug-sensitive node in an unbiased manner. Compared with traditional genetics target identification way which relies on a predefined and poorly validated target, chemical genetics has huge potential to establish a more specific target identification mechanism, in that it allows temporal analysis of biological consequence and minimizes the complication of compensatory mechanisms with overcoming gene redundancy (Stockwell, Brent R, 2000).

Arabidopsis has 23 members of EXO70 gene family, which can be classified in to 8 clusters on the phylogenetic tree, A to H. According to GUS-based expression analysis and knockout analysis, all the family gene of EXO70 were cell-type specific expression in potential exocytosis-active cells, among them, EXO70A1 and EXO70G2 were expressed in developing xylem elements during secondary cell wall thickening, meanwhile high expression of A1 and G2 were observed in root cells (Li, S., 2010). According to the gene expression of EXO70 family in root cells, we chose seven homologous genes to test the relation between them and ES2 with DARTS and Differential scanning fluorimetry (DSF) method, which are approaches to assessment of the relationship between small molecules and target protein. With all the method involved, we expect to gain more evidence about the role of ES2 in exocytosis process.

Following the main point of chemical genetics method, we developed our own screening strategy for ES20 hypersensitive mutants. In our case, with the treatment of the small molecule, Endosidin 20, the root of Arabidopsis became shorter and swollen, which gave us a hint that the ligand impacts the cellulose synthesis leading to unhealthy root growth. With this in mind, we first selected plants which had shorter and swollen roots on low dosage inhibitor (ES20) medium from EMS treatment seeds (M2 generation). Then, we chose the ones showing phenotype, with shorter and swollen roots, and transferred selected plants to growth media ['without inhibitor for recovery. After that, the recovered ones were the candidates for our next step, which were

growing in soil for M3 generation. With M3 generation seeds, we sowed them on ½ MS and low dosage ES20 plates respectively to observe the difference of root growth in order to verify the hypersensitivity. After the confirmation test on growth media and drug media, we have our candidate hypersensitive mutants, for which we can conduct genetic analysis to reveal the gene involved in this signaling pathway. We already screened 37 pools for M2 generation, from which we got 470 lines of M3 candidates and 158 lines of them were tested for the hypersensitivity. For the future work, keeping screening all the 47 and cloning the mutant gene by whole genome sequencing and crossing candidate mutants with fluorescence-tagged CesA to investigate CSC trafficking in the hypersensitive mutants.

Based on our previous screening work, we screened about 450,000 seeds and got 26 individual lines that are less sensitive to ES20. After high-throughput sequencing combined with wide-seq sequencing, eventually 12 independent mutant alleles were identified to be less sensitive to ES20. Among the 12 lines of Arabidopsis, we isolated the mutated CesA gene from Arabidopsis which shows the most resistance to ES20. Because of one amino acid changing from Glutamic acid to lysine, this line showed strongest resistant to ES20, which gave us a board perspective in practical agriculture development — if crops are able to carry the CesA gene that resistant to drug ES20, we can develop ES20 into a novel kind of herbicide since that weeds can be killed and transgenic crops will survive under the application of ES20. For this promising development in agriculture, we conducted gene transformation project. We transformed Arabidopsis CESA6 carrying mis-sense mutation at the catalytic site into tomato to get transgenic tomato plants using argobacterium-mediated transformation. So far, we already got the transgenic callus and shoots for next step.

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