Exploring exocytosis using chemical genomics

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Genome-sequencing data coupled with the availability of genetic manipulation tools in diverse model organisms has rapidly advanced our understanding of the role of individual molecular players in biological

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processes (1). Classical genetic approaches (using knockout mutants), although highly successful, are often hindered by the functional redundancy due to occurrence of multiple genes with similar functions. Additionally, loss-of-function mutants, which either lead to lethality or severe defects, hinder a proper understanding of the primary role of individual genes in biological processes. Such obstacles prompted the development of small chemical inhibitors to dissect biological pathways in an approach popularly known as chemical genomics (2). Use of chemical genomics overcomes several of the limitations posed by the classical genetic approaches. For example, simultaneous chemical targeting of several closely related genes in a family overcomes the obstacle of functional redundancy. The possibility to modulate the dosage and reversibility of chemical treatments also enables one to elucidate the primary function of indispensable players without causing long-term and severe developmental defects. Moreover, use of small molecules allows the possibility to perform local or tissue-specific applications, thus making it feasible to study specific developmental processes. Furthermore, the use of small-molecule inhibitors coupled with target identification has considerable promise in the area of drug discovery. The group of Natasha Raikhel has been one of the pioneers in the use of chemical genomics in plants and have demonstrated its use in identifying several novel compounds that can be used in analysis of endomembrane trafficking (3). In PNAS, Zhang et al. (4) describe a novel molecule Endosidin 2 (ES2) as a specific inhibitor for EXO70, a key player in exocytosis in both plants and animals.

Exocytosis refers to the process of delivering newly synthesized lipids and proteins to the plasma membrane as well as the secretion of vesicle-enclosed cargo to the extracellular milieu. Exocytosis is not merely a nondirectional general transport mechanism but is thought to be a component in polarity establishment of cells via directional or polarized secretion. Polarized delivery of material helps in asymmetric growth of cells as seen in neurite formation in animals and root hair and pollen tube growth in plants (5). How the specific site for delivery and fusion of exocytic vesicles are selected in such cases is not yet fully understood and is an area of active research. Nevertheless, the exocyst complex that acts as a tethering factor between the plasma membrane and the incoming exocytic vesicle is thought to be an essential component in determining the site of fusion of secretory vesicles at the plasma membrane (6). The exocyst is an octameric protein complex composed of single copies of the proteins Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, EXO70, and EXO84 (7). It is hypothesized that EXO70 and Sec3, by binding to phosphatidylinositol 4,5-bisphosphate at the inner leaflet of the plasma membrane determine the site of vesicle fusion. Subsequently, they interact with other exocyst proteins localized on the arriving secretory vesicle to tether it to that site (8). Although its functions are well documented in cultured animal cells, reports of overall developmental defects associated with EXO70 mutations are rare, presumably due to the severe phenotypes and often embryonic lethality associated with mutations in exocyst components (6).

Dissecting the functions of EXO70 is far more complex in plants due to redundancy. For example, *Arabidopsis* genome codes for a staggering 23 paralogues for EXO70. Although the divergence of expression profiles of these paralogues in different tissues (9) suggests tissue-specific roles in different cellular processes, the genetic redundancy has rendered the analysis of individual components problematic. Intriguingly, protoplast transfection of GFP-tagged versions of EXO70 has revealed that only a subset of these paralogues are recruited to membranes (10).

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Genetic perturbations have provided clues about specialized functions of some EXO70 homologs. For example, EXO70H4 is involved in callose deposition and trichome development (11), EXO70C1 is involved in pollen tube growth (9), and EXO70B1 is involved in autophagy-related vacuolar transport (12). Genetic knockout of EXO70A1, the most abundant paralogue in *Arabidopsis*, causes pleiotropic defects, as may be expected from interfering with a vital cellular process such as exocytosis (13).

ES2 Is a Secretion Inhibitor That Binds to EXO70A1

The strong phenotypes associated with loss of function of EXO70A1 renders the dissection of its specific role in intracellular trafficking and developmental processes difficult, thereby highlighting the usefulness of rapid, reversible, and dose-dependent action of chemical inhibitors in dissection of such processes. ES2 was described in a previous study as an inhibitor of pollen tube growth—a process heavily reliant on polarized secretion (14). Zhang et al. (4) now report ES2 to be a general inhibitor of protein trafficking to the plasma membrane. Short-term treatment with ES2 reduces the plasma membrane levels of the brassinosteroid receptor (BR1) and auxin efflux carriers, PIN2 and PIN1. Taking PIN2 as a candidate, Zhang et al. (4) demonstrate that ES2 treatment reduces the secretory trafficking as well as recycling of PIN2 to the plasma membrane with simultaneous increase in its accumulation at the vacuole presumably targeted for degradation.

Although several chemical modulators of vesicular trafficking have been identified by developmental phenotype and fluorescence imaging-based screens, the targets of the majority of these compounds remain unidentified (15). Indeed, finding the target of a chemical effector remains the most challenging part of a chemical genetics screen. However, in contrast with many earlier studies, Zhang et al. (4) have made a critical breakthrough by identifying EXO70A1 as a target of ES2. Intriguingly, although the overall phenotypic effect of long-term ES2 treatment matches that of EXO70A1 knockout mutants, short-term treatments with ES2 reveals novel facets of EXO70 action. Notably, with short-term treatment of ES2, a significantly greater quantity of PIN2 was found to accumulate in the prevacuolar compartments compared with the loss-of-function mutants described earlier (16). This observation brings forth the unique advantage of transient inhibition by chemical effectors, which is often phenotypically more prominent than the equilibrated cellular conditions of genetic knockouts. Importantly, complete knockout alleles of EXO70A1 (*exo70a1-1* and *exo70a1-2*) were sensitive to ES2, pointing to additional targets of ES2 other than EXO70A1. Thus, ES2 can potentially act as a general antagonist of multiple EXO70 paralogues, thus circumventing the issue of redundancy. However, a stringent screening for finding additional targets will be required to ascertain this possibility.

Dual Regulatory Role of N- and C-Terminal Segments of EXO70A1

Although screening for ES2 resistance in the available EXO70 mutants, Zhang et al. (4) identified an allele, *exo70a1-3*, to be

resistant to ES2. The *exo70a1-3* mutant expressed a truncated N-terminal segment of the EXO70A1 protein (amino acids 1–231). Because the predicted binding site of ES2 does not lie within this region, it is intuitive that the activity of this shorter version will not be affected by ES2. Intriguingly, it was found that heterozygous *exo70a1-3* plants or transgenic plants overexpressing the amino acids 1–231, although normal as seedlings, displayed severe growth defects at later stages of development. Such a phenotype is different from the phenotype of complete knockout mutants of EXO70A1 and points toward the possibility that the N- and C-terminal segments of the EXO70A1 might have distinct roles at different stages of development. Characterizing the exocyst assembly at different stages of growth in wild-type plants in comparison with the *exo70a-3* mutant might provide valuable insight into the stoichiometry of subunits in assembly of the exocyst complex and its role in controlling developmental stage-specific cellular processes.

Activity of ES2 in Mammalian Cells Points to Conservation of Vital Processes Across Diverse Eukaryotic Systems

Interestingly, Zhang et al. (4) found that ES2 was effective in blocking transferrin receptor recycling, a process dependent on EXO70 activity, in HeLa (human cancer) cells, suggesting that ES2 can act across various phyla that diverged long ago during evolution. Comparison of the crystal structure of *Arabidopsis* EXO70A1 and mouse EXO70 protein revealed that, despite their low sequence similarity, the overall structure of the proteins are conserved. This suggests that, during evolution, the crucial amino acids that determine the key protein structure and function are under high selection pressure and therefore conserved across phyla. It will therefore not be unreasonable to extend the compounds identified from chemical genetics screens using plants for dissection of cognate processes in animal systems and even in development of possible therapeutic agents. For example, a number of human diseases are associated with EXO70 function, such as diabetes (17), cancer cell metastasis (18), and bacterial invasion of host cells (19). However, it should be borne in mind that plant assay systems might not be a complete replacement for animal models of human diseases. Despite function similarity, slight deviations in protein structures might render compounds behaving differently in plant versus animal systems. A prominent example of such dichotomy is the drug Brefeldin A (BFA) that acts as an inhibitor of the GTP exchange factor (GEF) for the ARF GTPases in both plants and animals. Although BFA treatment blocks protein secretion in animal cells, evolution of BFA-resistant GEFs rendered protein secretion in *Arabidopsis* root cells resistant to BFA (20).

To summarize, the study by Zhang et al. (4) is a notable success story of chemical genomics in exploration of a key cellular function. The study is exceptional in its meticulous approach in target identification and cross-system validation. Such detailed biochemical characterization coupled with the enormous diversity of chemical libraries will greatly advance the understating of cell biological processes—in plants and animals alike.

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