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Invited Expert Review

The Plant Exocyst

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Abstract

The exocyst is an octameric vesicle tethering complex that functions upstream of SNARE mediated exocytotic vesicle fusion with the plasma membrane. All proteins in the complex have been conserved during evolution, and genes that encode the exocyst subunits are present in the genomes of all plants investigated to date. Although the plant exocyst has not been studied in great detail, it is likely that the basic function of the exocyst in vesicle tethering is conserved. Nevertheless, genomic and genetic studies suggest that the exocyst complex in plants may have more diversified roles than that in budding yeast. In this review, we compare the knowledge about the exocyst in plant cells to the well-studied exocyst in budding yeast, in order to explore similarities and differences in expression and function between these organisms, both of which have walled cells.

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Introduction

Exocytosis is the fusion of Golgi-derived, exocytotic vesicles with the plasma membrane, where the vesicle membrane is inserted into the plasma membrane and the contents of the vesicles are deposited into the extracellular matrix. Exocytosis is a fundamental process in all eukaryotic cells and is vital for cell growth, cell polarity establishment, neurotransmission in animal cells, cell wall formation in plants and fungi, and numerous other processes that require delivery of components to the plasma membrane or to the extracellular environment. In most cell types, if not all, exocytosis is localized; it does not occur equally over the cell surface, but preferentially at specific locations.

In cell-wall forming plant cells, exocytotic vesicles contain cell-wall matrix precursors in their interior and cellulose synthase complexes in their membrane. Upon exocytosis, the matrix materials are deposited into the existing cell wall and cellulose synthase complexes inserted into the plasma membrane (Lindeboom et al. 2008). This makes the Golgi vesicle the unit, the building stones for cell growth. Most plant cells expand by intercalary growth; i.e., some cell wall facets expand more than others, for instance, the longitudinal ones in root epidermal cells. Since the cell wall of rapidly expanding facets does not become thinner and the wall of the slowly expanding facets does not get thicker, this suggests that the exocytosis in intercalary growing cells is polarized. In tip growing cells, e.g., root hairs and pollen tubes, where expansion takes place over a small surface area, the tip, exocytosis clearly is polarized (Miller et al. 1997; Geitmann and Emons 2000; Ketelaar et al. 2003).

During exocytosis, specific SNARE proteins, present on the vesicle (v-SNAREs) and the plasma membrane (t-SNAREs),

are essential for membrane fusion. Tethering proteins are thought to form a connection between the vesicle and target membranes (Sztul and Lupashin 2006). Some of these tethering proteins bind directly to SNAREs and may play a critical role in the spatio-temporal regulation of SNARE complex assembly before membrane fusion (Cai et al. 2007; Kummel and Heinemann 2008). Many different tethering factors have been identified. Some of these factors are conserved over a wide range of organisms; others are specific for single trafficking pathways. Examples of these tethering factors are the exocyst, GARP (Golgi-associated retrograde protein), COG (conserved oligomeric Golgi), and Dsl1 complexes (Songer and Munson 2009).

The exocyst, an evolutionarily conserved protein complex, is required for tethering and fusion of the vesicles and plasma membrane at the sites of polarized exocytosis (Munson and Novick 2006). The exocyst is comprised of eight subunits: SEC3, SEC5, SEC6 SEC8, SEC10, SEC15, EXO70, and EXO84 (Boyd et al. 2004; Hsu et al. 2004; Tsuboi et al. 2005). Homologues of all exocyst subunits have been identified in the genome of *Arabidopsis* (Elias et al. 2003) rice, poplar and the moss *Physcomitrella patens* (Chong et al. 2009). In yeast and mammalian cells, each exocyst subunit is encoded by a single gene (Elias et al. 2003; **Table 1**). In *Arabidopsis*, SEC6 and SEC8 are encoded by a single gene, SEC3, SEC5, SEC10 and SEC15 are encoded by two genes, EXO84 is encoded by 3 genes and EXO70 by 23 genes (**Table 1**). The plant specific expansion of the number of *EXO70* genes suggests either that different *EXO70* genes are expressed during development and/or in different tissues that perform identical functions or that different *EXO70* genes may function in different, most likely plant specific, types of exocytosis.

Although very little is known about the plant exocyst, recent evidence indicates that it is essential for plant survival (Cole et al. 2005; Hála et al. 2008). It is likely that in plants, like in a wide range of other organisms, the exocyst plays a role in targeted exocytosis. However, much work is needed to generate a coherent picture of the function of the plant exocyst. In this review, we will compare the knowledge of the exocyst in the model organism budding yeast, which is a walled, unicellular organism, with results that have been obtained for the plant exocyst, in order to speculate about its functions in plants, multi-cellular walled organisms.

Table 1. The number of genes encoding different exocyst subunits in yeast, human and *Arabidopsis*

	Number of genes		
Exocyst genes	*S.cerevisiae*	*Homo sapiens*	*Arabidopsis*
SEC3	1	1	2
SEC5	1	1	2
SEC6	1	1	1
SEC8	1	1	1
SEC10	1	1	2
SEC15	1	1	2
EXO70	1	1	23
EXO84	1	1	3

The Structure and Function of the Budding Yeast Exocyt

The budding yeast, *Saccharomyces cerevisiae*, serves as a model system for polarized exocytosis, in which many of the components involved in both cell polarity and exocytosis have been well investigated (Brennwald and Rossi 2007). *S. cerevisiae* cells undergo a budding process, which requires polarized exocytosis to deliver newly synthesized materials to the daughter cell (Zhang et al. 2008). In budding yeast, the exocyst is essential for the polarized exocytosis. Loss of function of different exocyst subunits inhibits exocytosis and causes accumulation of secretory vesicles in the cytosol close to the budding region (bud tips and mother-bud necks (Hsu et al. 2004; Munson and Novick 2006; Zhang et al. 2008; Songer and Munson 2009). All of these 8 exocyst subunits accumulate at the sites where the active exocytosis takes place during budding and cell growth: the sites of bud emergence, the tips of the forming daughter cells and the mother-daughter connection (Zhang et al. 2005). The accumulation of the exocyst at the mother-daughter bud neck in *S. cerevisiae* is thought to be related to a function in cytokinesis. Although all the subunits have the same localization on membrane areas where active exocytosis takes place during the final stages of vesicle tethering, they achieve this localization via at least two different mechanisms: most exocyst subunits (SEC5, SEC6, SEC8, SEC10, SEC15 and EXO84) are delivered to the exocytosis sites through their attachment to the secrerory vesicles. This transport is dependent on actin cables which serve as tracks for the vesicle delivery (Hutagalung et al. 2009).

In contrast, SEC3 is localized to the active growing sites independent of actin cables and the ongoing secretion events. Hence, SEC3 is regarded as a spatial landmark for defining of the sites of secretion and for the recruitment of other exocyst subunits to the exocytotic sites. Polarized localization of Exo70 to the plasma membrane is partially actin-dependent (Finger et al. 1998; Wiederkehr et al. 2003; He and Guo 2009; Hutagalung et al. 2009). From the knowledge mentioned above it is expected that, in yeast, disruption of exocyst function will lead to defects in processes that depend on polarized exocytosis.

How does the exocyst recognize a specific membrane domain and bring the vesicles to this location for exocytosis?

In yeast, the recruitment of SEC3 and EXO70 to the plasma membrane is mediated by both binding of these two subunits to inner plasma membrane leaflet phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) (He et al. 2007; Liu et al. 2007; Zhang et al. 2008) and Rho family GTPases. Disruption of the interaction of SEC3 and EXO70 with $PI(4,5)P_2$ (He et al. 2007; Zhang et al. 2008) or reduction of cellular $PI(4,5)P_2$ levels (He et al. 2007) inhibits the assembly of the yeast exocyst at the plasma membrane. It is certain that $PI(4,5)P_2$ binding is not sufficient for polar localization of the yeast exocyst, since $PI(4,5)P_2$ is not polarly distributed in the plasma membrane (He et al. 2007). Most likely the positional information for polar localization of the exocyst to the budding site is provided by Rho family GTPases. Such interactions with $PI(4,5)P_2$ are conserved in mammals (Liu et al. 2007), while in plants, $PI(4,5)P_2$ binding by exocyst subunits has not yet been studied. In addition, SEC3 is a downstream effector of activated Rho family GTPases CDC42 and RHO1. In *cdc42* or *rho1* mutants, the polarity of the exocyst localization at the plasma membrane is abolished (Guo et al. 2001; Zhang et al. 2001). In addition, Exo70 interacts with another Rho family GTPase, activated Rho3 (Robinson et al. 1999); however, since disruption of the interaction by mutations in EXO70 does not cause exocyst mis-localization or malfunction, it is not clear what kind of role this interaction plays (He et al. 2007; Hutagalung et al. 2009). There are indications that CDC42 acts as an upstream activator of EXO70 (Adamo et al. 2001). Taken together, these data clearly show that the regulation of the yeast exocyst by Rho family GTPases is poorly understood (He and Guo 2009). Besides Rho family GTPases, interactions with other GTPases have also been found. The interaction between SEC15 and the Rab GTPase SEC4 is essential for exocyst assembly (Guo et al. 1999). Vesicle tethering by the exocyst is followed by SNARE-mediated vesicle fusion with the plasma membrane. In this review, we will not focus on these interactions, as none of them have been identified in plants yet. We refer the reader to He and Guo (2009) for more information.

To gain insight into the function of the exocyst in exocytosis, knowledge about the exocyst structure and assembly, i.e. the spatial-temporal interaction patterns between the different exocyst subunits, is crucial. Protein-protein interactions within the exocyst complex have been identified in yeast (and animal) cells which were determined using various approaches, including yeast two hybrid experiments, co-immunoprecipitation and pull-down assays (reviewed by Munson and Novick 2006). The results of these interaction analyses are summarized in **Figure 1**.

Besides the above experiments, protein crystallography studies have given insight in the exocyst structure. The crystal structure of the large domains of three yeast exocyst subunits have been determined: nearly full-length budding yeast

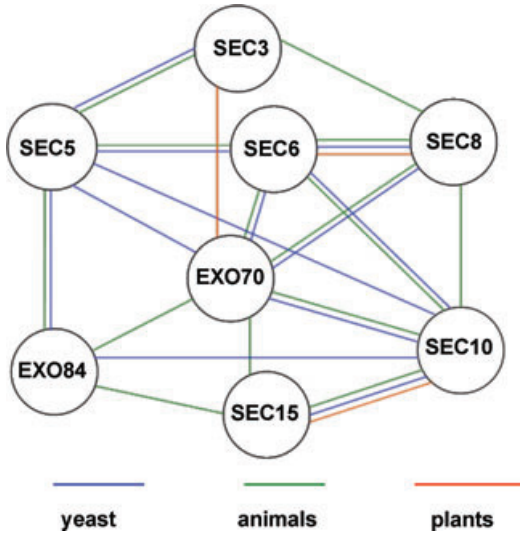

Figure 1. Interactions among different exocyst subunits.

A comparison between yeast, animal and plants (the interactions in yeast and animals are reviewed by Munson and Novick 2006; The plant specific interactions are described in Hála et al. 2008).

EXO70p (Dong et al. 2005; Hamburger et al. 2006), the C-terminal domain of budding yeast EXO84p (Dong et al. 2005) and the C-terminal domain of budding yeast SEC6p (Sivaram et al. 2006). All these proteins contain multiple helical bundles, which are reflected in a similar, long, rod-like shape, with identical topology (Munson and Novick 2006; Sivaram et al. 2006; Songer and Munson 2009). Full length EXO70 consists of four bundles, and the C-terminal domains of EXO84 and SEC6 each consist of two bundles (Munson and Novick 2006). These bundles can pack together in an elongated side-to-side manner to form an elongated rod-like shape (Munson and Novick 2006). Recently, Croteau et al. (2009) employed hidden Markov models combined with secondary structure predictions to examine the sequence of each exocyst subunit. They showed that all exocyst subunits contain helical bundles, even though different exocyst subunits only share less than 10% of similarity at the primary sequence level. Using quick freeze/deep-etch cryo-electron microscopy of the chemically unfixed exocyst complex and exocyst complex prefixed with glutaraldehyde, a similar rod-like shape has been found, suggesting that this is the predominant structure of the exocyst. The unfixed complex appears as a flower with four to six 'petals' (4–6 nm wide $\times$ 10–30 nm long; Hsu et al. 1998). In contrast, the prefixed complex is a 13 nm $\times$ 30 nm structure with a couple of small appendages, which may more closely resemble the native exocyst structure (Munson and Novick 2006). Munson et al. (2006) suggested that that the extended shape of unfixed complexes in the electron microscope might represent the structure of exocyst

complexes bound to Rab, Rho and Ral small GTPases, while connecting the vesicle and plasma membrane when they are relatively distant from each other. The closed structure of the fixed complex may represent the shape of the exocyst during later steps of membrane fusion. Undoubtedly, more evidence is needed to test this hypothesis.

Exocytosis in Plants and the Plant Exocyst

Exocytosis is probably used in a more diverse set of processes in multi-cellular plants, relative to budding yeast. Similar to budding yeast, exocytosis in plants functions during cell growth and cell division. However, numerous other processes also require polarized exocytosis in plants. Examples are the production of nectar by nectar-producing glands in flowers attracting bees (review: Nepi and Stpiczynska 2007), mucilage secretion by root cap cells (Wen et al. 2007), and secretion for secondary cell wall thickening. Besides these processes, polarized exocytosis has also been implicated in the positioning of PIN proteins to certain sides of the plasma membrane, which plays an important role in controlling the direction in which the plant growth regulator auxin leaves the cell (for review see: Vanneste and Friml 2009). PIN proteins are the first class of plant proteins shown to be associated with differential localization at plasma membrane and are the efflux carriers mediating directional transport of auxin. It is likely that many other processes require (polarized) exocytosis in plants. In other multicellular organisms such as insects and mammals, in which also exocytosis for different types of secretion occurs, the exocyst is involved in some, but not all types of polarized exocytosis (for review see: He and Guo 2009). Indeed, alternative tethering factors are available in fungi and animals. In plants, to our knowledge, only the exocyst genes have been proposed as a tethering complex. If the plant exocyst is involved in exocytosis for various types of secretion, the plant specific expansion of the EXO70 family of genes (**Table 1**) may represent a means of regulation of the plant exocyst during exocytosis processes for different purposes. From the evolutionary point of view, this hypothesis is plausible. Phylogenetic analyses suggest that, although the basic exocytosis machinery is established in the common eukaryotic ancestor and conserved among fungi, plants and animals, organisms in different kingdoms have apparently evolved different strategies to cope with trafficking the endomembrane and cellular components (Vernoud et al. 2003; Mouratou et al. 2005; Cole and Fowler 2006; Synek et al. 2006; Dacks et al. 2008; Chong et al. 2009; Zarsky et al. 2009). The use of T-DNA insertions in genes of the large EXO70 family in *Arabidopsis* offer an opportunity to determine whether the plant exocyst plays a role in exocytosis in different types of processes.

Different plant exocyst subunits have been knocked out and the consequences on plant development have been studied. Lines with T-DNA insertions that disrupt expression of *Arabidopsis* exocyst subunits encoded by single genes such as SEC6 and SEC8 (see **Table 1**) invariably fail to produce progeny homozygous for these T-DNA insertion (Cole et al. 2005; Hála et al. 2008). Also insertion in some subunits that are encoded by two genes fail to produce progeny that is homozygous for the T-DNA insertion (double mutants with both disrupted SEC5A and SEC5B; SEC15A). The failure to produce homozygous knockouts for these insertions can be traced to a male transmission defect, caused by defects in pollen tube germination and/or pollen tube growth (Cole et al. 2005; Hála et al. 2008). Due to the failure to produce homozygous plants with the above mentioned exocyst genes it is only feasible to study the role of the exocyst in pollen using T-DNA insertion lines. However, the prominent defects in pollen tube growth strongly suggest that the exocyst in *Arabidopsis* plays an essential role in polar exocytosis, a requirement for tip growth of pollen tubes.

Besides these pollen lethal mutants, a T-DNA insertion in AtEXO70A1, causes the disruption of root hair growth and the formation of the stigmatic papillae. Plants with homozygous T-DNA insertions in AtEXO70A1 also have shortened hypocotyls in etiolated seedlings, smaller organs, loss of apical dominance and reduced fertility (Synek et al. 2006). The shortened etiolated hypocotyl phenotype of the AtEXO70A1mutant was enhanced in double mutants with sec8 or sec5 alleles that normally do not display vegetative phenotypic defects (Hála et al. 2008).

Recently, it was showed that AtEXO70A1 plays critical role in self-imcompatibility in crucifer species (Samuel et al. 2009). In *Brassica napus*, the self-incompatibility is mediated through the interaction of S-locus Cys-rich/S-locus protein 11 (SCR/SP11) carried by pollen coat and the stigma-specific S Receptor Kinase (SRK; Schopfer et al. 1999; Takasaki et al. 2000; Silva et al. 2001). Following the attachment of self pollen to the stigma, SCR/SP11 binds to the membrane-localized SRK, activates SRK, which then interacts with and phosphorylates the ARM-repeat containing 1 (ARC1), an E3 ubiquitin ligase, to trigger rejection of self pollen (Goring and Walker 2004; Murase et al. 2004; Kakita et al. 2007). ARC1 interacts directly with Axo70A1 in vitro, and thus could trigger inhibition of EXO70A1 via degradation in a self-incompatible response (Samuel et al. 2009). Consistent with this idea, additional experiments showed that EXO70A1 can be considered to be a compatibility factor in the stigma. Both *Brassica* and *Arabidopsis* lines with reduced EXO70A1 in the stigma (by RNAi or mutation) are incapable of accepting compatible pollen. By contrast, overexpression of EXO70A1 in *Brassica* is sufficient to partially overcome the self pollen rejection response in self-incompatible *Brassica* plants. These experiments for

the first time bring the exocyst to the context of protein degradation-based self-imcompatibility response in the stigma (Samuel et al. 2009). Of course whether EXO70A1 participates in pollen-stigma interactions through a role in exocytosis, and which cellular materials could be exported during this process remain to be studied.

Known phenotypes of T-DNA insertions in *Arabidopsis* genes that encode exocyst subunits and a list of the other *Arabidopsis* exocyst genes are given in Table 2. Interestingly, disruption of the Roothairless1 (RTH1) gene in maize, which encodes a homologue of SEC3, resulted in defects in root hair elongation (Wen et al. 2005). Since there are at least SEC3 genes present in the genome of maize, this mild phenotype in comparison to that of sec3b knockout lines in *Arabidopsis* possibly is likely to reflect redundancy with the other SEC3 isoforms. Even so, the tip growth of root hairs is a clear type of polarized

Table 2. List of *Arabidopsis* exocyst genes

Gene	Protein	Developmental defects in knockout lines	Reference
At1g47550	SEC3A	unknown	
At1g47560	SEC3B	unknown	
At1g76850	SEC5A	Defects in pollen germination and pollen tube growth in sec5a and sec5b double mutant	Hála et al. 2008
At1g21170	SEC5B	Defects in pollen germination and pollen tube growth in in sec5a and sec5b double mutant	Hála et al. 2008
At1g71820	SEC6	Defects in pollen germination and pollen tube growth	Hála et al. 2008
At3g10380	SEC8	Defects in pollen germination and pollen tube growth	Cole et al. 2005
*At5g12370**	SEC10	unknown	
At3g56640	SEC15A	Defects in pollen germination	Hála et al. 2008
At4g02350	SEC15B	unknown	
At1g10385	EXO84A	unknown	
At5g49830	EXO84B	unknown	
At1g10180	EXO84C	unknown	
At5g03540	EXO70A1	Defects in polarized cell growth and plant development (see text)	Synek et al. 2006
At5g52340	EXO70A2	unknown	
At5g52350	EXO70A3	unknown	
At 5g58430	EXO70B1	unknown	
At1g07000	EXO70B2	unknown	
At 5g13150	EXO70C1	unknown	
At5g13990	EXO70C2	unknown	
At1g72470	EXO70D1	unknown	
At1g54090	EXO70D2	unknown	
At3g14090	EXO70D3	unknown	
At3g29400	EXO70E1	unknown	
At5g61010	EXO70E2	unknown	
At5g50380	EXO70F1	unknown	
At4g31540	EXO70G1	unknown	
At1g51640	EXO70G2	unknown	
At3g55150	EXO70H1	unknown	
At2g39380	EXO70H2	unknown	
At3g09530	EXO70H3	unknown	
At3g09520	EXO70H4	unknown	
At2g28640	EXO70H5	unknown	
At1g07725	EXO70H6	unknown	
At5g59730	EXO70H7	unknown	
At2g28650	EXO70H8	unknown	

*The *At5g12370* locus contains two tandemly repeated *SEC10* genes (Hála et al. 2008).

The table gives AGI numbers, exocyst subunit names, developmental defects in knockout lines and references to this work.

exocytosis (Emons AMC 2009), in which the exocyst is involved in *Arabidopsis* (the AtEXO70A1 mutant has a root hair growth phenotype (Synek et al. 2006)) and also in maize.

Hála et al. (2008) performed yeast two hybrid assays to assess interactions between different subunits within the plant exocyst, and showed that SEC6 interacts with SEC8, SEC10 interacts with SEC15B and SEC3A interacts with EXO70A1. The interactions between SEC6 and SEC8 and the interaction between SEC10 and SEC15 are conserved, but the interaction between SEC3 and an EXO70 subunit appears to be unique for plants. Besides interactions conserved in yeast and mammals, unique interactions have also been found in these organisms (**Figure 1**). These non-conserved interactions may reflect differences in for example exocyst aggregation in different organisms (see below). When compared to animal cells and yeast, the number of interactions between different exocyst subunits in *Arabidopsis* found by Hála et al. (2008) seems low (**Figure 1**). It is likely that this reflects the limited amount of research into the plant exocyst rather than major differences in exocyst functioning between plants and other species.

The function of the plant exocyst in polarized exocytosis in tip growing pollen tubes suggests its presence in the tips of growing pollen tubes. Hála et al. (2008) used antibodies against SEC6, SEC8 and EXO70 A1 for an immunolocalisation study in Tobacco pollen tubes. Although all these exocyst subunits examined localized to the tip-region of growing pollen tubes, it appears that none of them are really localized locally to the expanding membrane. However, the imaging/sample preparation techniques that were used in these experiments do not adequately assess this protein localization; the samples were fixed in paraformaldehyde (and not by rapid freeze fixation), and examined only at the light microscope level (Zonia and Munnik 2009). Nevertheless, SEC6 and SEC8 do preferentially associate with the membrane fraction in Tobacco pollen (Hála et al. 2008). Even so, it is still not clear which protein(s) define the target of the exocytosis for pollen tube tip growth. Even so, the apical localization of exocyst subunits in pollen tubes fits with its putative role in polarized secretion in plants and is very similar to the accumulation of the exocyst in the bud region in budding yeast. Chong et al. (Chong et al. 2009) studied the localization of *Arabidopsis* exocyst subunits in Tobacco BY-2 suspension cultured cells. The exocyst subunits of AtSEC5a, AtSEC15a, AtSEC15b and AtEXO84b localize to large globular-like structures in the perinuclear region of the cell. These structures included TGN/early endosomes membranes as well as late endosome membranes. AtSEC6, AtSEC8 and AtEXO70A1 showed a diffuse cytosolic distribution. Chong et al. (2009) suggest that the equal cytoplasmic fluorescence may be caused by the undifferentiated and unpolarized state of BY-2 cells. However, since these cells elongate, they are polarized cells. AtEXO70E2 and AtEXO70G1 localized to TGN/early endosome markers and late endosome/prevacuolar compartment markers labeled punctuate structures throughout the cytoplasm. Co-expression with other fluorescently tagged exocyst subunits (AtSEC5a, AtSEC8, AtSEC15a, AtSEC15b and AtEXO84b) recruited these proteins to the same locations. This suggests that AtEXO70E2 and AtEXO70G1 may be triggering the formation of high-order complexes at these locations. However, one has to realize that all the above localization studies have been carried out by overexpression of exocyst subunits, which may cause localization artifacts. Structural studies on the plant exocyst have not been performed so far. However, electron tomographs of high-pressure-frozen cells at the post-meiotic cytokinesis during pollen development and cell plate formation of the somatic cells in *Arabidopsis* revealed 24 nm-long structures tethering the membrane vesicles (Otegui and Staehelin 2004; Segui-Simarro et al. 2004), which resemble the mammalian exocyst complex visualized by transmission electron microscopy (Hsu et al. 1998). If these structures observed in plants represent the plant exocyst, the exocyst in plant cells has a similar rod-shaped structure with helical bundles as the yeast exocyst. Furthermore, showing that these structures are indeed the plant exocyst would also suggest that the conserved rod-shape is required for exocyst complex function.

Co-purification experiments provide additional evidence that the different plant exocyst subunits function as a complex. Hála et al. (2008) found that most of the *Arabidopsis* exocyst subunits are present in high molecular mass fractions. It is worth noting that Hála et al. (2008) estimated the molecular mass of the exocyst at 900 kD, larger than the predicted mass of 760 kD when each subunit is represented as a single protein. It is possible that non-exocyst proteins co-purify with the exocyst, a phenomenon which is also observed in mammals (Yeaman et al. 2004). Alternatively, due to the shape of the complexes, their mobility may be decreased. Hála et al. (2008) found that seven plant exocyst subunits (SEC3, SEC5, SEC6, SEC8, SEC10, SEC15 and EXO70A1) co-fractionated during a purification procedure that is commonly used for non-plant exocyst purification. A reduction in the amount of SEC5 and SEC15 in relation to the other exocyst subunits was found after the purification procedure, which suggests that these subunits are loosely associated with the exocyst, whereas other subunits serve as a more tightly associated core of the exocyst. Hála et al. (2008) were not able to detect EXO84 in their experiments, but suggest that it will be in the same fractions. Once the structure and interaction patterns within the plant exocyst have been determined, a more comprehensive comparison between the exocyst complexes of yeast, mammals and plants can be made.

Although in *Arabidopsis*, it has been shown that Rab GTPase RabA4b localizes to the tips of growing root hair cells (Preuss et al. 2004) and RabA4d to the tips of growing pollen tubes,

where it has a clear role in pollen tube growth (Szumlanski and Nielsen 2009), direct interactions between exocyst subunits and small GTPases have not been reported yet. However, Lavy et al. (2007) showed that ICR1, a novel effector of an activated ROP (Rho of Plants) GTPase interacts with SEC3A. Ectopic expression of ICR1 causes similar growth defects as expression of constitutively active ROPs, such as changes in the cellular morphology of leaf-epidermis-pavement and root-hair cells. This phenotype may well be caused by ectopic activation of the exocyst. The interaction of Sec3A with the ROP effector ICR1 and unique plant specific interactions between the plant exocyst and the exocyst in other organisms shows that, although conserved in structure and very likely in its general function (exocytotic vesicle tethering to the plasma membrane), the plant exocyst may have unique features. Unique features, both in terms of exocytosis for a variety of processes in which it is involved, and in terms of assembly and activation and localization. It is likely that over the coming years, the exciting, novel features of the plant exocyst will be the focal point of numerous studies and more and more detailed information about the plant exocyst and plant polarized exocytosis in general will become available. Especially live cell imaging of the localization of different plant exocyst subunits will give valuable information about exocyst functioning in secretion for different purposes.

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