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Multisubunit tethering complexes in higher plants

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Tethering complexes mediate the initial, specific contact between donor and acceptor membranes. This review focuses on the modularity and function of multisubunit tethering complexes (MTCs) in higher plants. One emphasis is on molecular interactions of plant MTCs. Here, a number of insights have been gained concerning interactions between different tethering complexes, and between tethers and microtubule-associated proteins. The roles of tethering complexes in abiotic stress responses appear indirect, but in the context of biotic stress responses it has been suggested that some tethers are direct targets of pathogen effectors or virulence factors. In light of the central roles tethering complexes play in plant development, an emerging concept is that tethers may be co-opted for plant adaptive responses.

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Tethering refers to the initial contact between donor and recipient membranes and represents a highly selective trafficking step that precedes and facilitates vesicle docking and fusion (Figure 1). There are two classes of tethering factors, long coiled coil proteins and multisubunit tethering complexes (MTCs). This review focuses on MTCs. Ten distinct MTCs are known in eukaryotes [1,2]. In yeast (Figure 2a), the transport protein particle (TRAPPI) complex mediates endoplasmic reticulum (ER) to Golgi traffic, conserved oligomeric Golgi complex (COG) intra-Golgi traffic, and TRAPPII exit from the Golgi; from the trans-Golgi, secretion to the plasma membrane is mediated by the TRAPPII and EXOCYST complexes [3,4]. Endosomal trafficking between the trans-Golgi and multivesicular body (MVB) is mediated by Golgi-associated retrograde protein (GARP) or class C core vacuole/endosome tethering (CORVET), and vesicles or autophagosomes destined for the vacuole are tethered by homotypic fusion and protein sorting (HOPS) or TRAPPIII/IV complexes [5,6••]. Retrograde traffic from the Golgi to the ER is mediated by the dependence on Sly1 (DSL1) complex [3]. All known MTCs are found to be encoded by plant genomes [7,8] but it is only for some of these (Figure 2b, Table 1) that experimental data provides evidence for functions similar to those described in yeast. Much of what has been published in plants is, in fact, conjecture based on the yeast and animal literature. This review attempts to tease apart conserved versus plant-specific aspects of MTC function, with an emphasis on molecular interactions and novel insights across kingdoms. As recent reviews have covered EXOCYST function in plants [8–10], this review focuses predominantly on other MTCs, particularly TRAPPII.

Defining a tethering factor

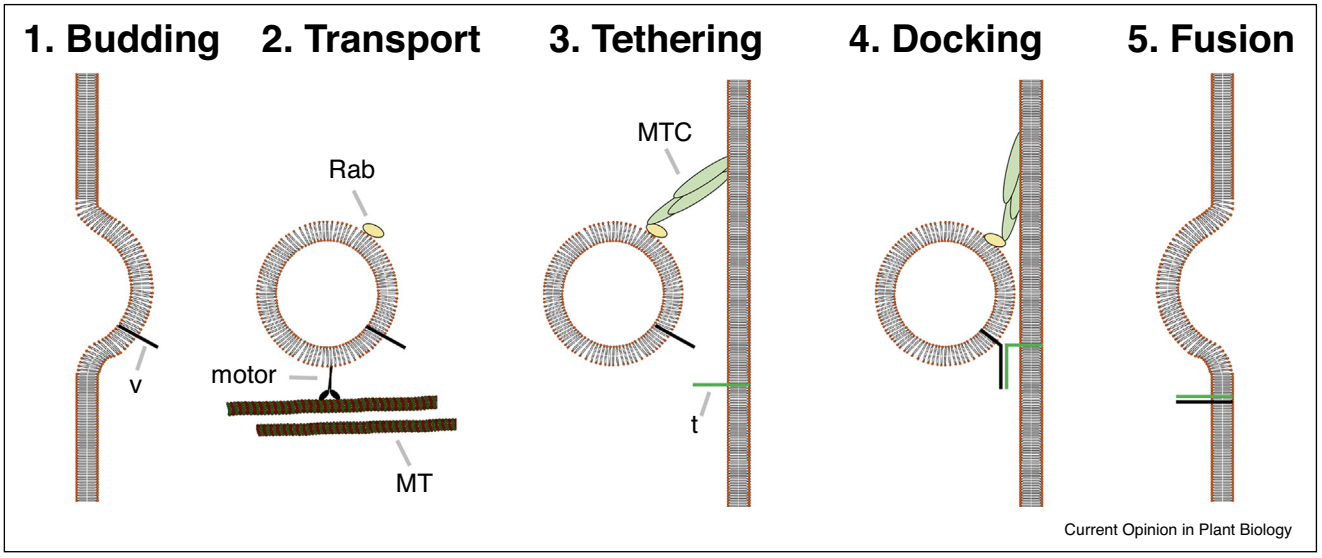
Tethers are defined as bringing donor and acceptor membranes into a proximity of approximately 300–2000 Å [11]. In contrast, SNAREs, which mediate membrane fusion, bring membranes to roughly 140 Å proximity [4,12]. Some MTCs such as HOPS and CORVET have been shown via reconstituted proteoliposome assays to be *bona fide* tethering complexes [13]. For some MTCs, especially the TRAPP complexes, the ability to act as tethers remains unclear. Depletion of TRAPP I in a cell free system resulted in the accumulation of ER vesicles, which is compatible with an at least indirect role in tethering [14].

Discovery of plant multisubunit tethering complexes

Tethering factors in Arabidopsis are often encoded by essential genes [7] or by expanded gene families [8], and this has rendered their identification laborious. The Arabidopsis HOPS complex, for example, is required for vacuole biogenesis: null mutants are embryo lethal and do not have lytic vacuoles, but appear to accumulate autophagosomes instead [15,16]. Similarly, the Arabidopsis COG complex was identified based on aberrant embryo phenotypes [17]. Events such as tip growth and cytokinesis require polarized or specialized secretion, and, as a consequence, numerous plant MTCs have been characterized on the basis of pollen tube or cytokinesis defects [7,18–23,24•,25•]. The failure of vacuolar biogenesis in HOPS mutants [15,16] and of cell plate biogenesis in TRAPPII mutants (Figure 3c) [7,22,26••] provides indirect corroborating evidence that the Arabidopsis HOPS and TRAPPII complexes are MTCs.

In contrast to the above-mentioned lethal phenotypes, viable alleles of the DSL1 complex were initially isolated in studies on the transport of seed storage protein

Figure 1



Five major steps in membrane traffic. *Budding:* a vesicle buds off a donor membrane. *Transport:* the vesicle, depicted as moving along microtubules (MT) with the help of a motor protein (motor), is delivered to the target membrane. *Tethering:* the vesicle is tethered to the target membrane by virtue of an interaction between a Rab GTPase (Rab) on the vesicle and a tethering molecule or complex (MTC) on the acceptor membrane. *Docking:* the tethered vesicle becomes tightly docked when the v- (v) and t (t) -SNAREs form a SNARE pin, or a trans-SNARE complex. *Fusion:* The vesicle and target membranes fuse as a cis-SNARE complex zippers and pulls them together, thereby delivering vesicle cargo to the target compartment.

precursors between the ER and the Golgi [27]. As regards expanded families of tethering factors, the most striking example is provided by EXO70. This EXOCYST subunit is encoded by 23 different paralogs in Arabidopsis, and these appear to have tissue-specific roles or to be required for plant adaptive responses. Examples of this includes the requirement for EX070H4 in trichome development [28], or the roles of EXO70B1, EXO70B2 and EXO70H1 in plant–pathogen interactions [29,30,31•]. In addition, EXO70B1 has been implicated in the fusion of autophagsomes with the vacuole [32], though EXO70E2-positive organelles have since been shown to be distinct from autophagosomes [33].

Modularity of MTCs

MTCs can exist in a variety of modular forms as a result of subunit exchange [34]. HOPS and CORVET, for example, both contain four common subunits that form a core complex to which two additional subunits are added in a modular fashion: HOPS-specific subunits mediate interactions with vacuolar or lysosomal Rab GTPases, whereas CORVET-specific subunits mediate interactions with Rab GTPases on early endosomes [35]. Similarly, TRAPP complexes exist in four modular forms in yeast (Figure 2a). Yeast TRAPPI or core-TRAPP consists of four core subunits. TRAPPII consists of core-TRAPP, three additional subunits (including Trs33), as well as two large TRAPPII-specific subunits, Trs120 and Trs130 [4]. TRAPPIII consists of core-TRAPP together with an adaptor and a specific subunit, Trs85 [4,6••]. Finally, addition of Trs33 to core-TRAPP generates the TRAPPIV complex [6••]. The observation that Arabidopsis *trs33*, *trs120* and *trs130/club* mutants share the same cytokinesis defective phenotypes [7] and that the three gene products are all found to copurify upon immunoprecipitation [26••] suggests that TRS33 can be found in an Arabidopsis TRAPPII complex; whether it exists in a TRAPPIV complex as in yeast, and whether TRAPPI and TRAPPIII exist in plants remains to be determined.

A third example of modularity is comprised by the mammalian GARP and endosome-associated recycling protein (EARP) complexes; these share three common subunits (Vps51, Vps52, and Vps53) but the fourth subunit differs, with the GARP-specific subunit Vps54 being replaced by syndetin in the EARP complex. Whereas GARP resides on the Trans-Golgi-network (TGN), where it is required for retrograde traffic from endosomes to the Golgi, EARP resides on recycling endosomes and is required for bringing recycling proteins/cargo to the cell surface [36••]. The *unhinged* allele of Arabidopsis VPS51 is characterized by expanded PIN1 expression domains and aberrant leaf venation patterns, and these phenotypes have been attributed to an impairment in vacuolar targeting [37]. However, the double mutant phenotype of *unhinged* in combination with SNAREs required for PIN1 vacuolar targeting was additive [37], suggestive of independent trafficking steps. Furthermore, VPS51 is a core subunit common to both the GARP and EARP complexes, and the *unhinged* phenotype is more readily explained by an