Review

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Change your Tplate, change your fate: plant CME and beyond

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Clathrin-mediated endocytosis (CME) is the predominant and evolutionarily conserved pathway by which eukaryotes internalize cargoes (i.e., plasma membrane proteins, lipids, and extracellular material) that are engaged in a variety of processes. Initiation of CME relies on adaptor proteins, which precisely select the cargoes for internalization, recruit the clathrin cage, and start membrane curvature. The recently identified CME early adaptor complex, the TPLATE complex (TPC), is essential for CME in plants. Phylogenetic analyses suggest that the TPC evolved from an ancient protein complex involved in vesicle trafficking in early eukaryotes, which raises questions about CME evolution and adaptation within the eukaryotic Kingdoms. In this review, we focus on the early events of plant CME and explore evolutionary aspects related to CME in other eukaryotes.

Clathrin-mediated endocytosis: a selective uptake

Endocytosis is an essential process by which eukaryotic cells internalize integral membrane proteins, lipids, and extracellular material (together termed 'cargo'), to regulate plasma membrane protein turnover and signaling at the cell surface. This mechanism is vital for the cell to respond quickly to extracellular stimuli, such as stress, nutrient availability, and hormone perception, and for cell plate formation and differentiation to occur [1]. CME (see Glossary) is the predominant and also evolutionarily conserved endocytic pathway acting at the plasma membrane [1]. CME is mediated by clathrin scaffolding proteins that facilitate cage enclosure of the invaginating bilayer. Our current understanding of the complex network of proteins and membrane lipids required for endocytosis largely comes from yeast and mammalian cells. In these organisms, CME is viewed as a step-wise process that includes nucleation and cargo selection, vesicle coat assembly or maturation, scission, and vesicle uncoating [1]. The initiation of CME seems to be hallmarked by the association of a coat nucleation module constituted by adaptors (including FCHo/Syp1, AP-2, AP180/CALM, and Ede1/Eps15) and clathrin triskelia to the plasma membrane [2–4]. The adaptor proteins are those structural components of the CME machinery that function in cargo recognition, curvature formation and stabilization, and clathrin and dynamin recruitment. The priming complex recruits additional adaptors and clathrin triskelia that aid in the initiation and stabilization of the bilayer invagination that precedes vesicle formation. After sufficient membrane deformation, dynamin GTPases are recruited to the invaginated membrane structure, where they form a spiral that encloses the neck of the emerging vesicle. Activation of dynamin activity finally causes the vesicle to pinch off from the donor membrane [1] (Figure 1A). Subsequently, the nascent vesicle is uncoated, traffics through the cytosol via the cytoskeleton, and fuses with its destination compartment within the endomembrane system [1]. Notably, the clathrin scaffolding proteins are also involved in coated vesicle formation for other trafficking pathways. Accordingly,

Glossary

Adaptor protein 2 complex (AP-2): a heterotetramer that works as an adaptor in CME.
***Arabidopsis thaliana* EH domain containing protein 1/2 (AtEH1/2; AT1G20760 and AT1G21630, respectively):** both components of the TPLATE complex.
Clathrin-mediated endocytosis (CME): a predominant and evolutionarily conserved pathway by which eukaryote cells internalize cargoes.
Dynamin-related proteins (DRP): during CME, DRPs are recruited to the neck site of the forming bud and induce the scission of vesicle from the donor membrane.
Epidermal growth factor receptor substrate 15 (EPS15; AAH54006): acts as an adaptor within the coat nucleation module in CME in mammalian cells.
FCH domain-only protein 1/2 (FCHo 1/2; AAH41130 and AAI37071, respectively): endocytic hubs for CME in mammalian cells.
Longin-like protein interacting with TPLATE adaptor (LOLITA; AT1G15370): a component of the TPLATE complex.
Phosphatidylinositol 4,5-bisphosphate (PIP2): a phospholipid enriched at the plasma membrane, where it is implicated in signaling and vesicle trafficking, including CME.
TPLATE complex (TPC): an eight-core-subunit protein complex acting as early adaptor module that drives CME in plants.
TPLATE muniscin like (TML; AT5G57460): a component of the TPLATE complex.
TPLATE SET (TSET): the alternative name for TPC in *Dictyostelium*.
TPLATE-associated SH3 domain containing protein (TASH3; AT2G07360): a component of the TPLATE complex.
TPLATE-associated WD40 domain containing protein 1/2 (TWD40-1/2; AT3G50590 and AT5G24710, respectively): components of the TPLATE complex.

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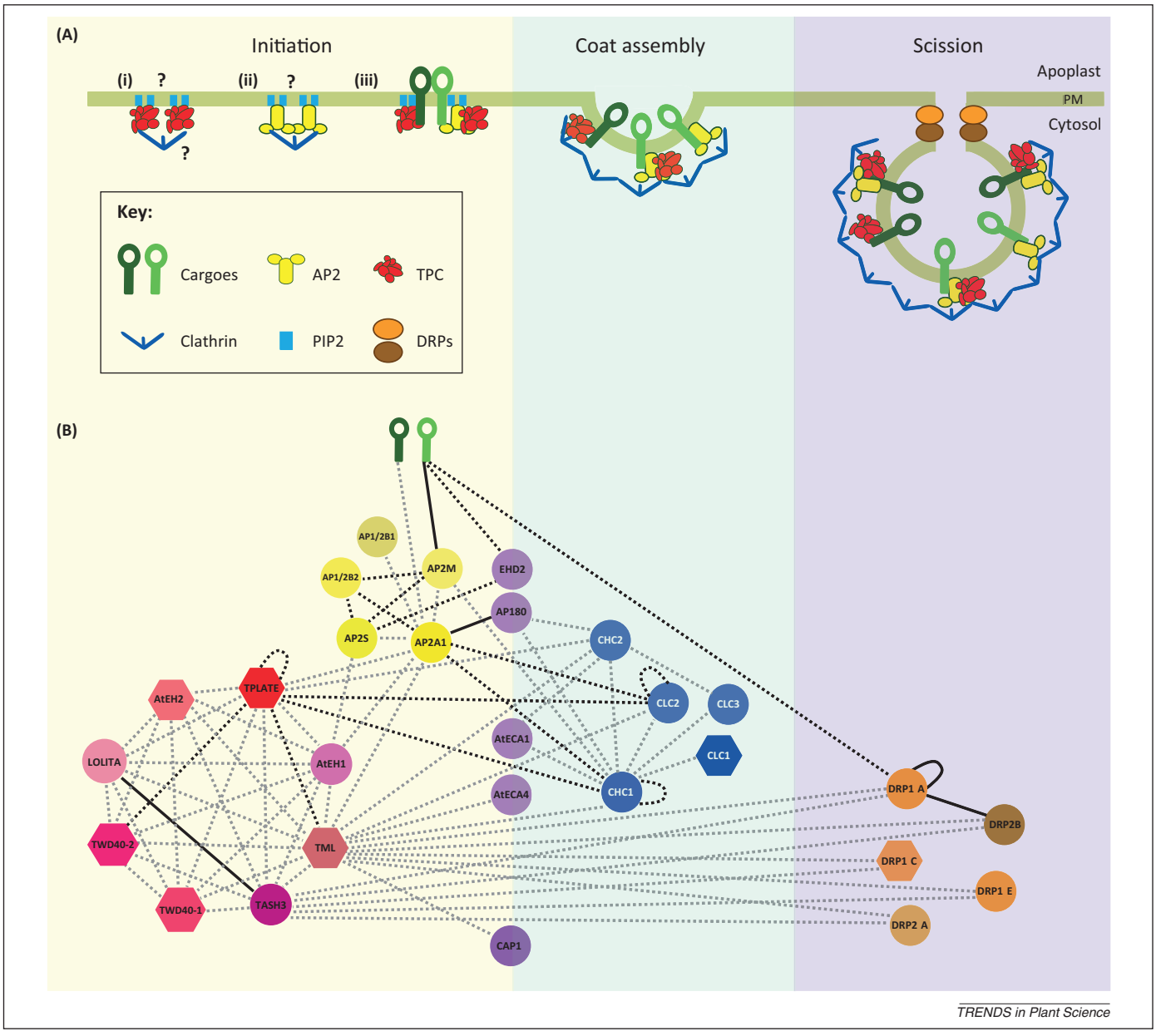

Figure 1. Clathrin-mediated endocytosis (CME) in plants. **(A)** Model for plant CME. CME might start through stochastic association of the adaptors (i) TPLATE complex (TPC) and/or (ii) adaptor protein 2 complexes (AP-2) with phosphatidylinositol 4,5-bisphosphate (PIP2) at the plasma membrane (PM); if the association with the cargo is stable enough, CME will proceed; or (iii) assembly of CME components induced by cargo sequestration. The initial adaptor proteins recruit additional clathrin triskelia, which polymerize and lead to coat assembly. After vesicle maturation, dynamin-related proteins (DRPs) are recruited to the neck site of the forming bud, where they polymerize and induce the scission of vesicle. Once detached, the vesicles are uncoated (not shown). Question marks indicate speculative events of plant CME: adaptor recruitment independently of cargo; TPC association with PIP2; and clathrin association with TPC independently of AP-2. **(B)** The interactome of plant CME proteins. The interactions among proteins involved in different CME stages are shown. The polygonal node shape of some subunits refers to their male sterile phenotype when mutated. Unbroken black lines represent interactions that were confirmed by yeast-two-hybrid (Y2H) or *in vitro* binding. Broken black lines indicate interactions observed by Bimolecular Fluorescence Complementation (BiFC) or Förster Resonance Energy Transfer (FRET). Gray broken lines represent interactions that were proved by co-immunoprecipitation (Co-IP), affinity purification/mass spectrometry (AP/MS) or tandem affinity purification (TAP). All indicated connections are based on available experimental data from [7,20,34,36–38,50,51,71–73].

other adaptor complexes (AP-1, AP-3, AP-4, and AP-5), functioning either with or without clathrin, have been identified in eukaryotes [5,6].

Similar to yeast and animal cells, plant cells rely on CME for many important processes [7–16]. Plant lines defective in CME functions typically display severe growth defects, including pollen lethal phenotypes [17–19]. In recent years, significant progress has been made towards elucidating the function of CME for plant development and the components involved [5]. However, knowledge regarding plant proteins that initiate CME remained scarce until recently. The discovery of the TPC [20] as a novel octameric early adaptor complex provided evidence that plant CME, in contrast to previous beliefs, is regulated differently from CME in animals and yeast. While some of the TPC components have predicted domains that are also found in CME effector proteins in other eukaryotes, the TPC subunits have no obvious counterparts in yeast and animal proteins. However, homologs of some TPC subunits have been identified and shown to form a complex in other organisms, including the amoeba *Dictyostelium discoideum* [21]. These findings raise important evolutionary