A glossary of plant cell structures: Current insights and future questions

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REVIEW ARTICLE

A glossary of plant cell structures: Current insights and future questions

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Short title: A glossary of plant cell organelles

One-sentence summary: A collection of short reviews of plant cell organelles covering our up-to-date understanding, novel findings, and future research outlooks.

ABSTRACT

In this glossary of plant cell structures, we asked experts to summarize a present-day view of plant organelles and structures, including a discussion of outstanding questions. In the following short reviews, the authors discuss the complexities of the plant cell endomembrane system, exciting connections between organelles, novel insights into peroxisome structure and function, dynamics of mitochondria, and the mysteries that need to be unlocked from the plant cell wall. These discussions are focused through a lens of new microscopy techniques. Advanced imaging has uncovered unexpected shapes, dynamics, and intricate membrane formations. With a continued focus in the next decade, these imaging modalities coupled with functional studies are sure to begin to unravel mysteries of the plant cell.

INTRODUCTION

For the Cell Biology Special Focus Issue, we wanted readers to have a modern view of plant cell structures that are sure to come up in research articles and other reviews. A common theme found throughout is how advancements in microscopy have illuminated fascinating new aspects of the plant cell, in particular the ability to generate 3-dimensional images using electron tomography whereby thicker specimens are imaged through a tilt series in the electron microscope, enabling the generation of a 3-dimensional image. In this glossary, we gathered experts in the field to share their striking images as well as their intellectual insights on how plant cell structures are understood today. This journey through the plant cell begins at the nucleus, where new insights into the protein composition of the nuclear envelope and its connections with the cytoplasm are beginning to decipher the functional diversity of the nucleus and how the nucleus is organized and linked to cytoplasmic status. The plant nucleus also hosts many phase-separated

biomolecular condensates, making it an ideal place to study this exciting new cell biological phenomenon.

Continuous with the nuclear envelope, the endoplasmic reticulum (ER) in all of its spatial and temporal complexity holds many unresolved questions. The Golgi, of central importance in polysaccharide biosynthesis for building the plant body, has several plant-specific features. The trans-Golgi network (TGN) and endosomes comprise a nexus of membrane-intricate compartments with vastly different shaping mechanisms, ultimately linking trafficking to the plasma membrane (PM) or the vacuole. The vacuole is the largest organelle in mature plant cells, playing multiple roles from cellular homeostasis, storage, growth, and development to plant responses to biotic/abiotic stresses. Long known to be a reservoir for lipids, lipid droplets (LDs) are emerging as important for plant responses to environmental stress. New insights into the molecular mechanisms driving LD formation from the ER are discussed.

Recent imaging of peroxisomes in developing seedlings has revealed strikingly complex membrane topologies. Imaging of live mitochondria demonstrates how dynamic plant mitochondria are, with many fusion and fission events occurring to generate a syncytial mitochondrial network within cells. High-resolution imaging of chloroplasts during developmental transitions underscores the structural complexity of these organelles and provides new models for populating the essential thylakoid membranes. Contact sites couple organelles to each other, creating a mechanism to communicate status across different subcellular structures. Plasmodesmata (PD) connect cells to each other, providing routes for short and long-distance communication. Finally, the cell wall not only patterns the cell but also builds the plant body and protects the plant from both abiotic and biotic stresses. The research described in this review has been performed in *Arabidopsis thaliana* unless otherwise stated.

THE PLANT NUCLEUS: A GIANT IN THE ORGANELLE GALAXY

(Written by Yu Tang and Yangnan Gu)

The nucleus can be thought of as a gigantic organelle defined by a double-layered membrane structure called the nuclear envelope. The nuclear envelope sequesters the nuclear genome and spatially separates transcription from translation, an evolutionary invention that enables remarkable functions and regulatory mechanisms that are fundamentally important to the eukaryotic cell (e.g. intricate spatial-temporal regulation of gene expression and signal transduction). Here, we briefly summarize current views in key aspects of the plant nucleus, including structure, composition, dynamics, and function, from the surface to the interior.

Nuclear envelope protein composition and function

The nuclear envelope surrounds the nucleus (Figure 1A) and is composed of the outer and inner nuclear membranes (Figure 1B), both of which harbor distinct collections of proteins that make the nuclear envelope a platform for versatile functions and communication. Plant nuclear envelope proteins have been reported to function in nuclear calcium signaling (Capoen et al., 2011; Charpentier et al., 2016), chromatin organization and dynamics (Pawar et al., 2016; Gumber et al., 2019), immune activation (Gu et al., 2016), cell cycle progression (Wang et al., 2019), mechanical shielding (Goswami et al., 2020), and so on (Figure 1D). Among these protein complexes, the LINC (linker of nucleoskeleton and cytoskeleton) complex is one of the best characterized. The LINC complex is composed of the inner nuclear membrane-localized SUN (Sad1/UNC48 homology) protein and the outer nuclear membrane-localized KASH (Klarsicht/ANC-1/Syne Homology) protein, with the former associated with the nucleoskeleton and chromatin and the latter bound with cytoskeleton and motor proteins (Figure 1D). SUN and KASH physically interact in the perinuclear region, thus establishing a molecular structure that enables the translation of cytoplasmic mechanical forces into nuclear movement and chromatin activities. The plant LINC complexes have been shown to play critical roles in stomatal development and responses to light and hormone signals (Gumber et al., 2019; Biel et al., 2020a, b), male gametophyte development (Tamura et al., 2013; Varas et al., 2015; Zhou et al., 2015; Moser et al., 2020), and plant-microbe interactions (Zhou et al., 2014; Newman-Griffis et al., 2019). Nonetheless, compared with

animals and yeast, we still lack a comprehensive understanding of nuclear envelope protein composition and function in plants. Recent applications of advanced proteomic tools in plants (e.g. proximity labeling proteomics), however, have empowered the identification of novel nuclear envelope components (Goto et al., 2019; Tang et al., 2020a) and nuclear envelope-specific biological processes (e.g. inner nuclear membrane-associated membrane protein degradation (Huang et al., 2020)). Future studies using continuously evolving proteomics and microscopy techniques will greatly expand our view of the global protein landscape of the plant nuclear envelope and unravel both eukaryote-conserved and plant-specific nuclear envelope functions.

The nuclear pore complex: more than a conduit for nucleocytoplasmic transport

The nucleus, a special membrane compartment, evolved a sophisticated communication system that allows remarkably efficient but highly selective exchange of materials across the nuclear envelope. The outer and inner nuclear membranes fuse at numerous sites to form physical openings, each ~120 nm in diameter, termed nuclear pores (Figure 1C). The surface of individual plant nuclear pores is covered by ~1,000 nucleoporin proteins of ~40 different types, which are assembled into a structurally conserved mega protein complex called the nuclear pore complex (Tamura et al., 2010; Mosalaganti et al., 2018) (Figure 1D). The central channel of the nuclear pore complex is filled with a protein meshwork made up of intrinsically disordered phenylalanine-glycine (FG)-rich nucleoporins, which are capable of interacting with nuclear transport receptors (importin and exportin) that carry out selective transport of cargo molecules. Besides playing a conserved role in mediating nucleocytoplasmic transport, individual plant nucleoporins have been reported to play specific roles in regulating flowering time, hormone signaling, and activation of abiotic and biotic stress responses, suggesting that the nuclear pore complex may function as a versatile signaling platform in addition to a conserved trafficking apparatus in plants (Meier et al., 2017; Gu, 2018; de Leone et al., 2020; Li and Gu, 2020). Efforts in identifying novel nucleoporins and dissecting their functional importance in different aspects of plant physiology are still undergoing (Tang et al., 2020b), which may help to address fundamental biological principles underlying the nuclear pore complex in both plants and animals.

The nucleoskeleton

Underneath the inner nuclear membrane lies the plant nucleoskeleton, assembled by long coiled-coil lamin-like proteins (e.g., CRWNs, named for the crowded nuclei mutants) and CRWN-associated proteins (e.g. KAKU4), which bear no sequence homology with animal lamin proteins. These proteins are required for proper nuclear morphology (Wang et al., 2013; Goto et al., 2014; McKenna et al., 2021) and potentially interact extensively with the nuclear pore complex basket (Mermet et al., 2021) and membrane-bound inner nuclear membrane proteins to form the plant nuclear lamina. CRWNs were recently shown to also interact with histone modifiers and to be necessary for tethering chromatin to the inner nuclear membrane to suppress stress-related gene expression (Hu et al., 2019; Mikulski et al., 2019; Choi and Richards, 2020; Sakamoto et al., 2020; Wang et al., 2021). These studies suggest a critical role of the plant nuclear lamina in maintaining heterochromatin organization and repression at the nuclear rim, similar to what was found in animals. Future studies will determine whether other plant nuclear lamina components, such as inner nuclear membrane proteins, also contribute to this process.

Organization of the nuclear interior

Within the nucleus, the genome is organized three-dimensionally with chromosomes occupying specific territories and active and inactive chromatin regions separated from each other. Most heterochromatic regions and chromocenters are typically positioned near the nuclear periphery. However, the distribution of telomeres and some other transcriptionally quiescent regions varies between plant species (Figure 1D). For example, most telomeres are attached to the nuclear surface in wheat and barley but are associated with the nucleolus in Arabidopsis and maize (*Zea mays*) (Pontvianne et al., 2016). Recent genome-wide high throughput chromosome conformation capture (Hi-C) analyses in both diploid and polyploid plant species revealed extensive inter- and intra-chromosomal interactions that define higher-order chromosomal packing during interphase (Bi et al., 2017; Liu et al., 2017; Dong et al., 2018; Concia et al., 2020). Both the spatial positioning (nuclear envelope tethering) and the three-dimensional organization of chromatin are tightly linked to local epigenetic states and can profoundly

influence chromatin activities, such as transcription regulation and the timing of DNA replication (Grob et al., 2014; Wear et al., 2017; Karaaslan et al., 2020; Sakamoto et al., 2020; Bishop et al., 2021).

Like chromatin, many biomolecules are also organized in a dynamic and heterogeneous manner within the nucleus. Spontaneous nucleation of biomolecules drives the formation of many membrane-less compartments observed in plant nuclei, including nucleoli, Cajal bodies, photobodies, dicing bodies, splicing speckles, DNA damage foci, and immune-activated condensates (Emenecker et al., 2020; Zavaliev et al., 2020; Huang et al., 2021b) (Figure 1D). In these nuclear bodies, multivalent proteins/nucleic acids capable of forming extensive inter- and intra-molecular interactions undergo liquid-liquid phase separation, a physical principle that compositionally demixes a homogenous solution into distinct liquid phases, to concentrate functionally relevant molecules and create a specific subnuclear environment that is integral to nuclear functions such as ribosome biogenesis, mRNA and miRNA processing, transcription activation, and signaling (Liu et al., 2012; Van Buskirk et al., 2012; Fang et al., 2019; Powers et al., 2019; Jung et al., 2020; Zavaliev et al., 2020; Huang et al., 2021a; Huang et al., 2021b). Further exploring the role of phase separation-promoted biomolecular condensates in plants and elucidating how phase separation may be regulated by internal and external signals represents an exciting new research area for plant science in the next decade.

Movement and dynamics of the nucleus

Like most other organelles, the entire nucleus is capable of directional movement triggered by environmental and developmental cues (e.g., towards pathogen-invading loci or with the rapid elongation of pollen tubes) (Griffis et al., 2014) and can establish connections with other organelles (e.g. chloroplast stromules) for signal exchange (Caplan et al., 2015; Gu and Dong, 2015). Plant nuclei also exhibit distinct morphology in different cell types and membrane dynamics during cell cycle progression. As an extreme example, the nuclear envelope undergoes a complete breakdown and subsequent reformation during mitosis. These aspects of plant nuclear dynamics have been extensively reviewed elsewhere (Meier et al., 2016; Meier et al., 2017; Groves et al., 2018;

Groves et al., 2020; Goto et al., 2021), and mechanisms that regulate plant nuclear movement, nuclear envelope dynamics, inter-organellar communication, and their functional importance are currently under active investigation.

OPEN QUESTIONS ON THE NETWORK STRUCTURE OF THE PLANT ER

(Written by Federica Brandizzi)

The ER is a large membrane-extension organelle at the core of the secretory pathway. The ER is responsible for several important processes that are essential for the life of the cell and the entire organism. For example, the ER initiates the biosynthesis of secretory proteins and essential lipids, functions as a calcium storage organelle, and houses several receptors of hormone signaling. Morphologically, the plant ER network is composed of interconnected tubules and cisternae that form a highly dynamic membrane network (Figure 2), which is anchored to the PM, similar to a spider web hanging off surfaces. ER tubules connect with other tubules and flatten themselves in enlarged areas, also known as cisternae, forming small, triangular sheets that are called three-way junctions (Shemesh et al., 2014) (Figure 2). In fully expanded plant cells, much of the cell volume is occupied by the vacuole. As a consequence, the bulk of the plant ER is distributed at the cell cortex where it is sandwiched between the PM and the tonoplast (vacuolar membrane), in continuum with the nuclear envelope and the transvacuolar strands. The transvacuolar strands form a tightly packed meshwork of ER tubules and cisternae that connect distal portions of the ER across the cell through tonoplast invaginations. The nature of the plant ER cisternae is unknown: they may be continuous membrane sheets and tightly packed tubules or perforated sheets of membranes, as described in non-plant species (Nixon-Abell et al., 2016; Schroeder et al., 2019).

The ER network undergoes continuous remodeling through processes that include homotypic fusion of ER tubules and the interconversion of ER tubules and cisternae due to the action of ER shapers, the cytoskeleton and associated motors, and ER-cytoskeleton connectors (Brandizzi, 2021). Together, these processes and ER shapers

contribute to the overall movement or streaming of the ER. This is distinct from the movement of other organelles (e.g., peroxisomes, mitochondria, endosomes), which translocate across the cytoplasm. The relative abundance of ER tubules and cisternae varies during cell growth. As cells expand, the ER shape transitions from a more predominantly cisternal form, typical of non-expanded cells, to a more tubular form that is visible in mature cells (Ridge et al., 1999; Stefano et al., 2014), through mechanisms that are yet to be established.

In vitro and in vivo experiments have demonstrated that the ER membrane-associated GTPase ROOT HAIR DEFECTIVE3 (RHD3) is responsible for the homotypic fusion of the ER membrane (Chen et al., 2011; Stefano et al., 2012; Zhang et al., 2013; Ueda et al., 2016) in a manner similar to the mammalian and yeast homologs atlastins and Sey1p, respectively (McNew et al., 2013); however, the mechanisms underlying the fast and dynamic interconversion of ER tubules and cisternae are yet to be discovered. A redistribution of membrane curvature-inducing proteins, such as the conserved reticulons (Tolley et al., 2008; Sparkes et al., 2009b), and the three-way junction-stabilizing LUNAPARK proteins (Lnps; named for the amino acid sequence LNPARK) (Kriechbaumer et al., 2018; Ueda et al., 2018; Sun et al., 2020a), is likely responsible for the dynamic interconversion of ER forms, but the underlying regulatory mechanisms remain largely unknown.

The biological function of the reshaping of the plant ER is still unclear. Confocal microscopy analyses have demonstrated that ER movement increases during cell growth concomitant with an increase in the streaming of other organelles with whom the ER is in close association, such as Golgi stacks, mitochondria, peroxisomes, and endosomes. Furthermore, defects in ER network structure due to the loss of RHD3 compromise cell expansion as well as the streaming of the ER and closely associated organelles (Stefano et al., 2014; Stefano et al., 2015). Therefore, the ER contributes to the dynamics and spatial organization of other organelles, possibly through ER-organelle contact sites, and this may be necessary for the organelles' functions. This is supported by the finding that in an *rhd3* loss-of-function mutant, the streaming of endosomes is reduced and clathrin-

mediated endocytosis is compromised (Stefano et al., 2015). These results support the hypothesis that the streaming of the ER and closely associated organelles is ultimately important for cell growth, but the underlying mechanisms are yet to be fully elucidated.

A double loss-of-function mutant of the two Arabidopsis *Lnps* shows an increased abundance of ER sheets with dense fenestration and ER conglomerates (Kriechbaumer et al., 2018; Ueda et al., 2018; Sun et al., 2020a). Combined, the finding that the localized distribution of Lnps in the ER depends on the cellular availability of their interacting protein RHD3, and that Lnps antagonize the role of RHD3 in ER shaping and induce RHD3 degradation via the proteasome pathway (Sun et al., 2020a) mechanistically support the notion that certain ER shapers are dependent on the abundance of other ER shapers for their distribution and function in the ER. Curiously, mutants with a loss of RHD3 alone are viable and show only limited phenotypic defects in plant growth (Stefano et al., 2012); however, the loss of RHD3 with either member of the RHD3-like family of proteins, RHD3-like 1 or RHD3-like 2, is either lethal or causes pollen defects, respectively (Zhang et al., 2013). Conversely, mutants with the loss of both *Lnps* are viable, with only minor defects in plant growth (Sun et al., 2020a). Therefore, certain ER shapers may have a more relevant role in the life of the cell than others, either because associated ER shaping events are essential compared to others or because the shapers carry out other functions, in addition to ER reshaping. For example, maize reticulons 1 and 2 function in shaping the ER but also as autophagy receptors and are involved in degradation of the ER through the regulated process known as ER-phagy (Zhang et al., 2020). Furthermore, RHD3 has been found to interact with ARK1, an armadillo-repeat containing kinesin, which is thought to pull an ER tubule toward another tubule (Sun et al., 2020b). While these findings support the earlier discovery that the remodeling of a subset of ER tubules depends on their sliding on pre-existing microtubules (Hamada et al., 2014), they also highlight additional functions of RHD3 besides its fusogenic activity of the plant ER membranes.

Future characterization of the broader roles of the plant ER shapers may provide opportunities to establish how physiologically and developmentally relevant processes are connected to ER network integrity. For example, the loss of RHD3 leads to an

attenuation of signaling in the unfolded protein response (Lai et al., 2014), a conserved cytoprotective pathway that is designed to attenuate proteotoxic stress in the ER (Pastor-Cantizano et al., 2020). While these findings support the idea that the homeostasis of the ER network structure is critical for cell health, a challenge for the future is to establish a mechanistic framework connecting ER shape integrity with the functions of essential signaling pathways.

Despite the functional conservation of shapers such as RHD3, reticulons, and Lnps, the plant ER structure depends on plant-unique factors. For example, a minor role for microtubules in ER reshaping is consistent with the predominant role of actin in this process (Sparkes et al., 2009a); this is markedly different from the dependence of ER network shaping on microtubules in mammalian cells (Waterman-Storer and Salmon, 1998; English et al., 2009). The existence of plant-unique ER-actin interactors (i.e., SYP73 and NETWORKED 3B) (Cao et al., 2016; Wang and Hussey, 2017), plant-specific molecular motors (i.e. Myosin XI family) (Peremyslov et al., 2010; Ueda et al., 2010), and the absence in plants of CLIMP63, the connector of the mammalian ER to microtubules and a spacer of the cisternal lumen (Klopfenstein et al., 2001; Shibata et al., 2010), further support the notion that plants have developed specific mechanisms of ER shaping across kingdoms. An obvious challenge for the future is to determine the nature or such mechanisms via the identification of additional players. For example, proteomics of cellular compartments or targeted proteomics based on pull-downs of ER shapers have yielded opportunities to identify proteins making up the plant ER (Dunkley et al., 2006; Kriechbaumer et al., 2018), but the challenge ahead is to define a functional pipeline to identify proteins specifically involved in ER structure. Forward genetics screening based on confocal microscopy analyses of Arabidopsis seedlings expressing fluorescent markers to identify mutants with defective organization of secretory organelles (Faso et al., 2009; Nakano et al., 2009; Takagi et al., 2013) offers a realistic opportunity to identify mutations that compromise the ER, although an innate limitation of these screens is their labor-intensive nature. Automation of this type of screen, along with the implementation of software capable of quantitatively analyzing the dynamics of the ER (Pain et al., 2019),

will likely offer a platform for the rapid identification of modifiers of ER shape and dynamics.

PLANT GOLGI STACKS: VERSATILE GLYCOSYLATION FACTORIES ON THE MOVE

(Written by Byung-Ho Kang)

The Golgi lies at the center of the secretory pathway, importing cargoes from the ER, adding glycosyl groups, and exporting these cargoes to post-Golgi compartments or the extracellular space (Alberts et al., 2014). The role of the Golgi as a processing trader is illustrated in its polarized stack architecture, where entry (*cis*) and exit (*trans*) sides can be discerned (Figure 3A and C) (Farquhar and Palade, 1981; Moore et al., 1991). In addition to serving as the site of protein and lipid glycosylation, the plant Golgi synthesizes non-cellulosic cell wall polysaccharides (Zhang and Staehelin, 1992; Carpita and McCann, 2000). The Golgi in plants consists of many discrete stacks (Figure 3B) whose numbers per cell vary from dozens to hundreds (Dupree and Sherrier, 1998). Each stack is thought to function independently (Nebenfuhr and Staehelin, 2001). The stacks travel in the cytosol at speeds of up to several microns per second; this movement is dependent on myosin motors (Boevink et al., 1998; Madison et al., 2015). The decentralized organization of plant Golgi contrasts with that of mammalian Golgi, whose stacks are stitched side-by-side to form a ribbon or a complex next to the nucleus (Ito et al., 2014). Therefore, ER-to-Golgi transport and post-Golgi secretion require long-distance vesicular trafficking to and from the Golgi (Gillingham and Munro, 2016). Mobile Golgi stacks in plants, by contrast, can visit ER export sites (ERES), concentrate to sites of secretion, and redistribute for cell division (Nebenfuhr et al., 2000; Kang and Staehelin, 2008; Ndinyanka Fabrice et al., 2017).

Transport through the plant Golgi

During ER-to-Golgi transport, Golgi stacks slow down at ERES and receive COPII-type vesicles (Nebenfuhr et al., 1999; Yang et al., 2005). In mammalian cells, the ER-to-Golgi

intermediate compartment (ERGIC) assembles at the ERES, and ER-resident proteins are retrieved from the ERGIC (Appenzeller-Herzog and Hauri, 2006). Plant cells lack ERGICs, as COPII vesicles are directly transferred to the cis-side of Golgi stacks in association with the ER. ERES in mammalian cells are marked by ERGICs (Weigel et al., 2021). Due to the absence of discrete ERGICs, plant ERES are spotted under an electron microscope based on their COPII buds and Golgi stacks in their vicinity. Biosynthetic activities are observed from the medial Golgi after the recycling of ER proteins is complete in the cis-Golgi (Donohoe et al., 2013), indicating that the cis cisternae take the place of the ERGIC in plant cells (Ito and Boutte, 2020).

Among the models describing intra-Golgi transport, the cisternal progression/maturation model has been supported by electron microscopy studies of the plant Golgi (Robinson, 2020). It is evident from electron micrographs of plant Golgi stacks that Golgi cisternae are peeled off from the trans-side, supporting the notion that Golgi cisternae are transient entities (Day et al., 2013). Electron tomography analysis has revealed assembly intermediates of new cisternae on the *cis*-side that exhibit highly diverse sizes and shapes (Donohoe et al., 2013). Cell wall polysaccharides were detected in the cisternal lumen but not in COPI-type vesicles at the cisternal margins, which are thought to retrieve Golgi-resident proteins against the cisternal membrane flux (Donohoe et al., 2007).

On the trans-side, TGN compartments arise from the trans-most cisternae. This transformation involves a significant reduction in the amount of membrane, suggesting that Golgi-resident proteins are retrieved from the TGN (Kang et al., 2011). Secretory vesicles carrying cell wall polysaccharides, clathrin-coated vesicles, and COPI vesicles arise from the TGN. In cotyledon cells, darkly stained vesicles, termed dense vesicles, transport globulins from the TGN to protein storage vacuoles (Robinson, 2020).

The aforementioned transport steps occur within a ribosome-excluding matrix that encloses the region from COPII vesicles to TGN cisternae (Figure 3D) (Staehelin and Kang, 2008). The matrix likely corresponds to a dense network of proteins involved in Golgi membrane assembly, maturation, TGN formation, and fastening cisternae into a

stack. Golgins are Golgi-localized long coiled-coil proteins, and some of them are tethering factors (Latijnhouwers et al., 2005) and, given their rod-like shape, they constitute scaffolds for the matrix. Mammalian Golgins are required for Golgi integrity, vesicular trafficking to the Golgi, and protein glycosylation (Wong and Munro, 2014; Liu et al., 2017; Witkos et al., 2019). Arabidopsis Golgins have been shown to play roles in COPII vesicular transport (Kang and Staehelin, 2008) and interactions of cis-Golgi with ERES (Osterrieder et al., 2017).

Biosynthesis in the plant Golgi

Production and export of cell wall matrix polysaccharides distinguish the plant Golgi from its animal counterpart. The reaction cascades for polysaccharide synthesis are arranged sequentially over the stack from the *cis*-to-*trans* direction. As the amounts of glycosyltransferases and sugar transporters per stack are small, their localization within the Golgi has been investigated using overexpressor lines or by localizing reaction products (Chevalier et al., 2010; Meents et al., 2019). The constitutive secretion of cell wall polysaccharides and several mechanisms for retaining Golgi proteins from the bulk flow have been characterized (Brandizzi, 2002; Gao et al., 2014; Schoberer et al., 2019).

TGN cisternae consist of distinct domains where secretory and vacuolar cargoes are separately packaged (Shimizu et al., 2021). Electron tomography imaging of Golgi/TGN complexes revealed that varying ratios of secretory and clathrin-coated vesicle buds in a TGN cisterna, suggesting that the biosynthetic functions of each Golgi stack are not uniform in a plant cell (Staehelin and Kang, 2008). Golgi stacks appear to be versatile factories whose activities are determined by the proteins imported from the ER. Golgi stacks enriched with enzymes for synthesizing cell wall polysaccharides would give rise to more secretory vesicles than Golgi stacks that process proteins for the vacuole.

Future research perspectives

The structures and functions of Golgi stacks change as plant cells differentiate, but the molecular mechanisms governing their remodeling remain elusive. For example, small Golgi stacks in root meristem cells undergo sequential remodeling as meristem cells

develop into gravity-sensing columella cells and eventually into mucilage-secreting border cells in the root cap (Figure 3E and F) (Wang et al., 2017). Since several cell-specific markers for the Arabidopsis root cap have been identified (Kamiya et al., 2016), it would be possible to uncover novel genes involved in cell type-specific polysaccharide synthesis and protein targeting in the Golgi after performing single-cell sequencing of root cap isolates (Shaw et al., 2021). Indeed, a proteomic analysis of fractions enriched with cis-, medial-, or trans-Golgi expanded the list of Golgi-resident proteins with their cisternal localization (Parsons et al., 2019). Expression profiling of genes encoding Golgi proteins during cell differentiation will provide insights into the regulation of Golgi functions.

Correlative light and electron microscopy refers to protocols in which macromolecules are first localized with fluorescence microscopy and the volume enclosing the macromolecules is then imaged by electron microscopy. This correlative approach will be useful for analyzing organelles composed of heterogeneous members (Wang et al., 2019). Golgi stacks labeled by specific fluorescent markers could be examined by electron microscopy to characterize their nanoscale architectures and interactions with other organelles. Examining the dynamics of Golgi subpopulations under stress conditions will shed light on how the secretory pathway reorganizes in response to threats from the outside. As export from the Golgi is mediated by the TGN, this analysis should be combined with exploring TGN dynamics (Uemura et al., 2019).

Advances in cryo-electron microscopy and sample processing technology allowed for electron tomography analysis of frozen-hydrated cells to visualize macromolecular complexes *in situ* (Otegui and Pennington, 2018). Due to the limitation in section thickness for electron microscopy, frozen cells must either be sliced or thinned by focused ion beam milling (FIB). Golgi vesicles and intraluminal filaments were delineated in Chlamydomonas cells by cryo-electron tomography (Engel et al., 2015). Although intact plant tissues are too thick for FIB, *in vitro* germinated pollen tube tips are amenable to FIB thinning (Liu et al., 2021). As Golgi stacks produce numerous secretory vesicles to sustain tip growth, it would be exciting to capture images of plant Golgi stacks by cryo-

electron tomography to uncover novel features not observed in plastic-embedded electron microscopy samples.

PLANT ENDOSOMES: PROTEIN SORTING MASTERS

(Written by Marisa Otegui)

The ability to regulate the composition of the PM and the endomembrane system is critical for cell survival. Endosomes play a central role in this process by regulating protein and lipid (cargo) trafficking in the endomembrane system through both the anterograde and retrograde pathways. As part of the anterograde pathways, that is, transport from the site of synthesis to the place of residence and function, proteins and lipids synthesized in the ER are typically transported in vesicles to the Golgi, to the TGN, and from there, either to the PM (exocytosis) or to the vacuole. Retrograde pathways mediate the transport of cargo or trafficking factors in the opposite direction from the anterograde pathway, usually back to their original donor compartments. Proteins removed from the PM in vesicles through clathrin-mediated endocytosis are delivered to early endosomes, where they can be either recycled to the PM or carried to multivesicular endosomes (MVEs, also referred to as multivesicular bodies or prevacuolar compartments) for further sorting into intralumenal vesicles and subsequent degradation in the vacuolar lumen (Valencia et al., 2016) (Figure 4A-D). In plants, the TGN functions as the early endosome since it is the first compartment that receives endocytosed cargo (Dettmer et al., 2006; Lam et al., 2007). Thus, in contrast to animal cells, plant cells do not have separate early endosomes but instead combine both endocytic and biosynthetic sorting at the TGN (Viotti et al., 2010).

TGNs and MVEs, the two types of plant endosomes, arise, mature, and are consumed as part of their membrane trafficking function. Therefore, both types of organelles are in continuous flux and can be found as subpopulations at different stages of maturation.

The TGN

As part of the endosomal pathway, the TGN receives PM cargo, which is either recycled back to the PM or retained for further sorting in MVEs and degradation in vacuoles. As part of the secretory pathway, the TGN produces both vesicles carrying cargo (proteins, membrane lipids, and cell wall polysaccharides) to the PM and vesicles containing vacuolar cargo (Rosquete et al., 2018). The TGN mediates retrograde recycling back to the Golgi and ER through COPI- (Bykov et al., 2017) and retromer-mediated trafficking (Niemes et al., 2010).

The TGN forms largely through cisternal maturation of the trans-most Golgi cisterna (Golgi-associated TGN or GA-TGN) but eventually detaches from the Golgi, becoming an independent organelle (free or Golgi-independent TGN) that fragments into vesicles (Toyooka et al., 2009; Kang et al., 2011; Uemura et al., 2014; Uemura et al., 2019) (Figure 4A and B). There are approximately 35 Golgi stacks and GA-TGNs in an Arabidopsis shoot apical meristematic cell at interphase (Segui-Simarro and Staehelin, 2006). In Arabidopsis root cells, as the *trans*-most cisterna matures into the TGN, it develops numerous vesicle buds, loses 30-35% of its total membrane surface area, and becomes enriched in the Rab GTPases RAB-A2a and RAB-A4b, the phosphatidylinositol 4-kinase PI4Kb1, the vacuolar V-ATPase subunit VHA1a (Figure 4D), and the SNAREs (Soluble N-ethylmaleimide sensitive factor Attachment protein Receptor) SYP61 (Syntaxin of Plants 61), SYP43 , VAMP721 (Vesicle-Associated Membrane Protein 721), VAMP722, and VAMP727 (Dettmer et al., 2006; Chow et al., 2008; Kang et al., 2011; Zhang et al., 2011). As the Golgi-associated TGN detaches from the Golgi stacks to become free/Golgi-independent TGNs, the budding profiles become more abundant (Figure 4B).

These Golgi-associated and Golgi-independent TGN subpopulations play distinct roles in trafficking (Renna et al., 2018; Uemura et al., 2019; Ito and Boutte, 2020). For example, GA-TGN but not free/Golgi-independent TGNs label with the endocytic tracer FM4-64 (Uemura et al., 2019), suggesting that endosomal function is carried out by the GA-TGN, whereas free Golgi-independent TGNs seem to be primarily involved in exocytosis. The different trafficking functions of the TGN are spatially separated in subdomains that differ both in their protein and membrane lipid composition (Wattelet-Boyer et al., 2016; Shimizu

et al., 2021) and their ability to recruit specific vesicle-forming coat proteins, such as clathrin. Thus, within the GA-TGN, there are at least two “zones”, the secretory (exocytic) and the vacuolar-trafficking zones. The secretory zone generates exocytic vesicles and is enriched in the SNARE VAMP721, the adaptor complex AP-1, the accessory protein EPSIN1, and clathrin. The vacuolar trafficking zone sends vesicles to MVEs for vacuolar delivery and is enriched in VAMP727, the adaptor complex AP-4, and the accessory protein MODIFIED TRANSPORT TO THE VACUOLE1 (MTV1) (Heinze et al., 2020; Shimizu et al., 2021). In addition, a plant-specific TRAPPII complex is thought to mediate the recruitment/tethering of endocytosed vesicles to subdomains of the TGN (Rosquete et al., 2019).

The TGN not only has subdomains for exocytic, endocytic, and vacuolar trafficking, but it also associates with protein complexes that control the trafficking of specific cargo proteins. Thus, for example, the TGN-localized protein ECHIDNA controls the secretion of only a subset of PM proteins, such as the auxin influx carrier AUX1 (Boutte et al., 2013). By contrast, a module formed by seven transmembrane domain-containing proteins (7TM) and components of guanine nucleotide-binding (G) protein signaling function together at the Golgi and TGN to regulate the exocytosis of cellulose synthases, but not the endocytosis or general exocytosis of soluble or PM cargoes (McFarlane et al., 2021).

MVEs

MVEs arise from membranes derived from the TGN and are characterized by a rounded shape, the presence of intralumenal vesicles (Figure 4A and B), and their association with RAB-F GTPases such as ARA6, ARA7, and RHA1 (Figure 4C) (Haas et al., 2007). There are approximately 17-20 MVEs in interphase meristematic cells, which are usually found in close proximity to the GA-TGN (Segui-Simarro and Staehelin, 2006). PM proteins targeted for degradation are usually ubiquitinated at the PM, internalized by endocytosis, and delivered first to the TGN and then to MVEs (Figure 4A).

The MVE intralumenal vesicles contain cargo proteins targeted for degradation in the vacuole. Failure to properly sort PM components into intralumenal vesicles results in the

accumulation of PM proteins in the vacuolar membrane (Figure 4A), which leads to severe developmental defects, and most frequently, to lethality.

At the surface of the MVE limiting membrane (the single membrane that surrounds the MVE), a group of cytosolic proteins called ESCRTs (Endosomal Sorting Complex Required for Transport) bind, cluster, and sort the ubiquitinated cargo into membrane domains that bend away from the cytoplasm, forming the intralumenal vesicles typical of these organelles. This membrane bending event occurs in the reverse (negative) topology of the better understood process of vesiculation, such as clathrin-mediated endocytosis. Although it has long been assumed that ESCRTs orchestrate the formation and release of a single endosomal vesicle at a time, studies performed in Arabidopsis have shown that at least in plants, these vesicles do not bud off individually but form in concatenated networks (Buono et al., 2017; Goodman et al., 2021).

In general, ESCRT proteins are well conserved across organisms, from Archaea (Makarova et al., 2010; Dobro et al., 2013; Pulschen et al., 2020) to Eukarya. In fungi and metazoans, five multimeric ESCRT complexes have been identified: ESCRT-0 to III and the triple AAA ATPase SKD1 (SUPPRESSOR OF K^+ TRANSPORT GROWTH DEFECT1) with its activator LIP5. Plants contain putative orthologs for most of the ESCRT proteins originally identified in metazoans and fungi (Spitzer et al., 2006; Haas et al., 2007; Spitzer et al., 2009; Kalinowska et al., 2015; Buono et al., 2016; Yu et al., 2016; Wang et al., 2017a), with the exception of ESCRT-0 (Winter and Hauser, 2006), which is an early acting complex that binds phosphoinositide-3-phosphate (PI3P), a lipid enriched in endosomal membranes that is critical for the recruitment of ESCRT proteins to endosomes. However, a group of proteins called TOL (TOML1-LIKE) are likely to play the role of ESCRT-0 in plants (Korbei et al., 2013; Moulinier-Anzola et al., 2020).

How do ESCRT proteins mediate intralumenal vesicle formation and sequestration of cargo proteins? ESCRT-0, -I, and -II contain ubiquitin-binding domains and contribute to the clustering of ubiquitinated cargo on the endosomal membrane and to membrane deformation (Liese et al., 2020). De-ubiquitinating enzymes remove the ubiquitin on cargo

before their final sequestration into intralumenal vesicles. Critical for the final steps in vesicle formation is the presence of membrane cargo (Chiaruttini et al., 2015) as well as ESCRT-III and ESCRT-III-associated proteins, which are able to trigger membrane deformation and neck constriction (Hanson et al., 2008; Fyfe et al., 2011; McCullough et al., 2013; Chiaruttini et al., 2015).

Plants commonly contain several isoforms for each ESCRT subunit and even have evolved plant-specific ESCRT proteins, such as PROS (Positive Regulator of SKD1, which enhances SKD1 activity) (Reyes et al., 2014), FREE1/FYVE1(Gao et al., 2014), and FYVE4 (Liu et al., 2021a). Interestingly, both proteins contain FYVE domains able to bind PI3P. FREE1 interacts with ESCRT-I subunits and is essential for endosomal sorting (Gao et al., 2014), whereas FYVE4 is required for the recruitment of ESCRT-III subunits (Liu et al., 2021a).

In Arabidopsis, the loss of critical ESCRT subunits such as CHMP1 and FREE1 results in serious protein mis-sorting defects and embryo and/or seedling lethality (Spitzer et al, 2009; Gao et al 2014), whereas the loss of the SKD1-activator LIP5 causes abnormal root gravitropic responses (Buono et al 2016), reduced tolerance to heat and drought stress (Wang et al 2015; Xia et al 2016), and compromised resistance to pathogens (Wang et al 2014).

Future perspectives

Our understanding of endosomal biogenesis and the molecular machinery mediating its multiple sorting functions has increased dramatically during the past decades. However, new regulatory and sorting components are being discovered and many more remain elusive, making it still challenging to comprehend how sorting functions are both segregated and integrated in TGNs and MVEs. The plant endosomes have many distinct features that make them different from their counterparts in other organisms. For example, whether the concatenation of MVE intralumenal vesicles in complex networks is unique to plants or is a universal mechanism in all eukaryotes is presently unknown. It is tempting to speculate that the evolution of unique ESCRT components and the drastic

diversification of some ESCRT isoforms may have contributed to the unique features of intralumenal vesicle formation in plants.

THE MULTIFUNCTIONAL VACUOLE

(Written by Kai Dünser and Jürgen Kleine-Vehn)

The plant vacuole fulfills a plethora of indispensable and sometimes seemingly contradictory functions. This multifunctional compartment ensures lytic degradation, but also stores proteins, carbohydrates, and secondary metabolites. The vacuole is a place for detoxification of harmful molecules, but also accumulates allelochemicals for plant defense against herbivory. It is central in pH as well as ion homeostasis, thereby also contributing to the control of turgor pressure (reviewed in Wink 1993; Marty, 1999; Eisenach and De Angeli 2017; de Brito Francisco and Martinoia 2018; Krüger and Schumacher 2018; Hara-Nishimura and Hatsugai, 2011) (Figure 5A and B). Besides all this, the vacuole fulfills a remarkable space-filling function, enabling enormously rapid plant cell enlargement with little de novo production of cytosolic components (reviewed in Dünser and Kleine-Vehn, 2015; Kaiser and Scheuring, 2020), but on the other hand must get out of the way to allow cell division (Figure 5A and B).

Vacuolar biogenesis

Genetic interference with vacuole biogenesis, as observed in Arabidopsis *vacuoleless1* mutants, leads to embryonic lethality, which indicates that the formation of vacuoles is essential for plant cells (Rojo et al., 2001). Depending on the cell type and developmental context, vacuoles may be formed *de novo* or inherited to daughter cells during cell division (reviewed in Cui et al., 2020). Mechanisms for vacuole biogenesis in roots include the so called provacuoles and the small vacuoles. Provacuoles are double membrane, tubular structures that bud off from the ER, constituting a major membrane source for the establishment of large vacuolar structures (Viotti et al. 2013). On the other hand, whole-cell electron tomography proposed that multivesicular bodies (also called Pre-Vacuolar

Compartments (PVC) in plants) undergo homotypic fusion to form small vacuoles prior to their fusion, resulting in the development of large central vacuoles (Cui et al., 2019).

Specialized vacuoles and their functions

Although some vacuoles fulfill multiple roles simultaneously, others specialize. Different types of vacuoles carry distinct sets of vacuolar membrane (tonoplast) marker proteins, and different types can co-exist in some plant cells (Frigerio et al., 2008). The lytic vacuole, often considered equivalent to the animal lysosome, is most common and plays central roles in virtually all tissues. By contrast, protein storage vacuoles are predominantly found in seeds and serve as nutrient reservoirs during germination (Ludevid et al., 1992; Höfte et al., 1992; Rojo et al., 2001). Vacuolar identity can be dynamic and undergo transitions, such as lytic vacuole to protein storage vacuole or *vice versa,* often marking crucial developmental fate changes (Gattolin et al., 2011; Zheng and Staehlin, 2011; Feeney et al., 2018).

Autophagy is the regulated degradation of proteins and organelles. During autophagy, the autophagic body is released into the vacuole lumen for degradation by hydrolytic enzymes. Hence, lytic vacuoles contribute to the autophagic processes that maintain basal cellular homeostasis, act in environmental stress responses, or play roles during pathogen defense, not the least of which is the vacuolar contribution to programmed cell death (reviewed in Su et al., 2020; Bassham et al., 2006; Yoshimoto and Ohsumi 2018; Merkulova et al., 2014; Phillips et al., 2008; Chung et al., 2010). Age-related developmental transitions are marked by senescence-associated vacuoles, which are implicated in the degradation of chloroplasts by autophagy (Otegui et al., 2005).

pH, ion, and water homeostasis

Vacuolar pH, ion, and water homeostasis are crucial for all of its functions. Vacuole acidification is essential for the lytic degradation of various cellular components. The vacuolar H^+-pyrophosphatase (V-PPase) AVP1 and two vacuolar H^+-ATPase (V-ATPase) proton pumps are the main actors in vacuolar pH. In addition, the P-type H^+-ATPase AHA10 contributes to vacuolar acidification in some cell types (Appelhagen et al., 2015).

V-ATPase VHA-a1 activity at the TGN likely contributes to vesicle-based delivery of protons to the vacuole, suggesting that other endomembranes can also affect the pH of the vacuole (Kriegel et al., 2015).

Cellular ion homeostasis is maintained by a myriad of transporters and channels that are energized by either the proton gradient (ΔpH) or the membrane potential difference (Δψ) (reviewed in Martinoia et al., 2012; Martinoia 2018). The vacuolar contribution to cellular ion homeostasis is, among other processes, important for the regulation of turgor pressure (Barragán et al., 2012). Reversible stomatal movements are controlled by changes in guard cell volume, accompanied by drastic changes in vacuole morphology and volume (Franks et al., 2001; Shope et al., 2003; Tanaka et al., 2007; Gao et al., 2009; Bak et al., 2013; Eisenach and De Angeli, 2017). Stomatal opening has mainly been linked to the accumulation of K^+ within the vacuole, whereas stomatal closure is facilitated by K^+ release from the vacuole (Barragán et al., 2012; Andrés et al., 2014; Wege et al., 2014; De Angeli et al., 2013; Gobert et al., 2007; Isner et al., 2018). Water channels (aquaporins) such as TIP1;1 contribute to the water permeability of the tonoplast and buffering the water content of the cytoplasm. Because the expression of TIP1;1 correlates with the onset of cell elongation, it may link intracellular water exchange with cellular enlargement (Beebo et al., 2009).

Vacuolar size and its impact on cell size control

The size of the vacuole correlates with cell size in plants, implying that vacuoles are involved in cell size determination (Owens and Poole 1979; Berger et al., 1998; Löfke et al., 2013; Dünser and Kleine-Vehn, 2015). Comparisons of whole-cell 3-D reconstructions in the meristem and elongation zone show that the cellular space occupied by the vacuole gradually increases during cellular elongation, while the cytoplasmic volume remains relatively constant. Therefore, the space-filling function of vacuoles enables rapid cell expansion in a metabolically cost-effective way by obviating the need for considerable *de novo* production of cytosolic content (Dünser and Kleine-Vehn, 2015; Dünser et al., 2019) (Figure 5B). Vacuolar size is controlled by the phytohormone auxin, which restricts the rate of cellular expansion (Löfke et al., 2015). Auxin interferes with the delivery and fusion

of vesicles to the tonoplast, as well as with actin/myosin-dependent constriction of the vacuole, contributing to the volume of the vacuole and its cellular occupation (Löfke et al., 2015; Scheuring et al., 2016; Kaiser et al., 2019). A cell wall sensing mechanism allows for the alignment of cell wall acidification/loosening with intracellular vacuole expansion, consequently ensuring cytosol homeostasis required for rapid cell expansion (Dünser et al., 2019; reviewed in Herger et al., 2019).

Multiple cargos and multiple trafficking routes towards the vacuole

The vacuolar membrane is a highly connected part of the endomembrane system that receives cargos and membrane from various trafficking routes. Anterograde vacuolar cargo sorting to the vacuole occurs early in the secretory pathway, at the level of the ER and the Golgi apparatus, and includes cargo binding to vacuolar sorting receptors. Upon reaching the TGN, cargo proteins are typically released, and the vacuolar sorting receptors are recycled back to the Golgi and the ER (Künzl et al., 2016), although vacuolar storage proteins in dense vesicles may already be sorted at the cis-cisternae of the Golgi (Hillmer et al., 2001).

Multiple trafficking routes from the TGN to the vacuole exist, including delivery via PVCs in a RAB5 and RAB7-dependent manner as well as through clathrin-coated vesicles, which are formed in an adaptor protein complex-dependent fashion (Cui et al., 2014; Ebine et al., 2014; Singh et al. 2014; Heinze et al. 2020; Feraru et al., 2010; Zwiewka et al., 2011) (Figure 5A). Notably, two of the most abundant tonoplast proteins, the vacuolar H^+-ATPase VHA-a3 and the vacuolar H^+-pyrophosphatase AVP1/VHP1, completely bypass the PVC and/or Golgi trafficking route and are delivered to the vacuole via ER-derived provacuoles (Viotti et al. 2013).

The vacuole is the endpoint of the endocytic pathway, through which ubiquitylated membrane proteins are likely directed to sub-compartments of the TGN. These sub-compartments mature into or transit towards the PVC (Scheuring et al., 2011).

693 The incorporation of PVCs, AP1-, AP4-, and AP-3/RAB5 vesicles, provacuoles, small
694 vacuoles, and autophagosomes into the central vacuole requires membrane tethering,
695 and finally membrane fusion. Recent findings confirm that class c core vacuole/endosome
696 tethering (CORVET) and homotypic fusion and protein sorting (HOPS) complexes are
697 involved in mediating tethering events for different vacuolar transport pathways in plants.
698 They ultimately activate the vacuolar soluble N-ethylmaleimide-sensitive-factor
699 attachment receptor (SNARE) complex, which selectively catalyzes the fusion of adjacent
700 membranes (Takemoto et al., 2018; Ebine et al., 2008; Uemura et al., 2010).
701
702 **Open questions about vacuolar functions**
703 Due to its multifunctional roles, the vacuole needs to process multiple and possibly
704 conflicting information. As such, it is a central integrative signaling hub for plant cells. Very
705 little is known about how its functions evolved over time. Evolutionary analysis could shed
706 further light on this and could also tackle some conceptual questions on vacuolar
707 biogenesis.
708
709 The multitude of trafficking pathways reflects the plethora of cargoes that need to traffic
710 independently to the vacuole; these processes have been subject to intense study,
711 leading to our quite detailed understanding. On the other hand, mechanisms that control
712 the dimension of the vacuole and its contribution to the plant-specific lifestyle are less well
713 understood. We don’t yet understand how plant cells monitor the size of the vacuole,
714 which seems especially challenging considering the dazzling complexity of membrane
715 flow towards the vacuole and the dynamic housekeeping processes that the vacuole
716 coordinates.
717
718 The control of vacuole size is not only crucial for its role in rapid cell expansion but also
719 cell division, if only because the vacuole can physically occupy the location specified for
720 cell plate formation. Formative, asymmetric cell divisions that initiate distinct cell fates
721 require the dedicated control of intracellular space and nuclear migration. Therefore, it is
722 not surprising that cells undergoing formative/asymmetric cell divisions contain small,
723 fragmented vacuoles (as seen in lateral root founder cells) or polarized vacuoles (as seen

in zygotes (Jansen et al., 2012; Kimata et al., 2019; Matsumoto et al., 2021). Interestingly, the steric control of vacuolar shape during cell division or nuclear migration is somewhat reminiscent of statolith sedimentation in gravitropic shoots, which also requires constant reshaping of the central vacuole (Kato et al., 2002).

It is apparent that a feedback-based, dynamic remodeling of the vacuole is required to ensure basic cellular functions, but the underlying mechanisms are largely unknown. These open questions ensure that research on vacuoles will continue to amaze us in the future.

LIPID DROPLETS: SPECIALIZED SUBCELLULAR HYDROPHOBIC COMPARTMENTS

(Written by Kent Chapman)

Like all cells, plant cells accumulate storage lipids in their cytoplasm as discrete LDs, most often consisting of a hydrophobic core of non-bilayer forming lipids such as triacylglycerols (TAGs) or sterol esters surrounded by an emulsifying monolayer of phospholipids (Pyc et al., 2017a; Huang, 2018; Ischebeck et al., 2020). Although less commonly considered, rubber particles of rubber-producing plant species with a polyisoprenoid hydrophobic core share the same overall structure (Yamashita and Takahashi, 2020). This thermodynamically stable structure was originally observed in transmission electron microscopy (TEM) micrographs and described by various terms such as lipid bodies, oil bodies, spherosomes, or oleosomes (Wanner and Theimer, 1978). However, the contemporary, unifying terminology of “lipid droplets” emphasizes the evolutionary conservation of this compartment across kingdoms of life where there are increasing reports of functions beyond the efficient storage of carbon (Lundquist et al., 2020).

754 In plants, LDs are most commonly associated with oilseeds and oleaginous fruits, where
755 they compartmentalize the well-known “vegetable oils” (Chapman et al., 2012). However,
756 LDs are present in essentially all cell types in plants, ranging from a few LDs per cell in
757 leaves to thousands of LDs per cell in seeds. Because the most abundant LD proteins in
758 seeds—oleosins—are not produced in most plant cell types, recent efforts to identify LD
759 proteins through proteomics approaches in non-seed tissues (Horn et al., 2013; Brocard
760 et al., 2017; Kretzschmar et al., 2018; Fernandez-Santos et al., 2020) have expanded the
761 inventory of LD proteins in plant cells. These proteins and their partners have begun to
762 suggest previously unrecognized participants in LD formation, stability, turnover, and
763 functions.
764
765 Among the recently recognized LD proteins are the so-called LDAPs (LIPID DROPLET-
766 ASSOCIATED PROTEINs), which share homology with small rubber particle proteins
767 from rubber producing plants. LDAPs were identified as prominent proteins in purified
768 LDs isolated from avocado (*Persea americana*) mesocarp (Horn et al., 2013) and have
769 since become appreciated for their widespread occurrence throughout the plant kingdom
770 (Gidda et al., 2016; Brocard et al., 2017; de Vries and Ischebeck, 2020) as well as for
771 their induction by drought stress (Kim et al., 2016). The LDAPs are relatively small
772 proteins without extended hydrophobic regions, and they have been shown to localize
773 specifically to the LD surface, perhaps through their extensive amphipathic helices.
774 Screens for potential protein interactors, which might serve as protein recognition sites
775 for LDAPs on the organelle surface, identified the protein LDIP (LDAP-INTERACTING
776 PROTEIN), which also is widely distributed in the plant kingdom (Pyc et al., 2017b; Coulon
777 et al., 2020) (de Vries and Ischebeck, 2020). LDAPs and LDIP are expressed in both
778 seed and non-seed tissues of plants and are suspected of playing broader roles in
779 compartmentalization of neutral lipids in cells beyond those found in seed tissues.
780
781 Another recently identified LD protein is the PLANT UBX DOMAIN-CONTAINING
782 PROTEIN10 (PUX10). PUX10 localizes to LDs through a hydrophobic polypeptide
783 sequence and recruits the AAA-type ATPase CELL DIVISION CYCLE48 (CDC48) protein
784 to the LD surface (Deruyffelaere et al., 2018; Kretzschmar et al., 2018). This interaction

785 is believed to support the selective extraction of LD surface proteins, such as oleosins
786 and LDAPs, for their ubiquitin-mediated protein degradation. This LD-associated
787 degradation pathway likely operates in all cells of plants to repurpose the surface and/or
788 contents of the LD compartment during development or in response to environmental
789 stresses.
790
791 **LD formation at the ER**
792 Like in most eukaryotes, LD formation in plant cells originates in the ER where the
793 enzymes for storage lipid assembly are present (Figure 6). Ultrastructural studies
794 frequently reveal intimate connections of LDs with the ER (Figure 6B) (Herman, 2009;
795 Brocard et al., 2017), which can also be captured by confocal fluorescence laser scanning
796 microscopy (Figure 6A). The process of LD proliferation can be capitulated at the
797 subcellular level in *Nicotiana benthamiana* cells. In these cells, LDs are normally low in
798 abundance, and the transient expression of cDNAs encoding proteins implicated in LD
799 formation can readily be studied, such as the transcription factor LEAFY COTYLEDON 2
800 (LEC2), which is preferentially expressed in developing seeds (Figure 6A). A transient
801 system for LD studies also has been developed using tobacco pollen tubes (Muller et al.,
802 2017), which has been particularly useful for protein localization studies due to the large
803 number of LDs normally present in these cells.
804
805 Existing models for LD formation suggest that newly synthesized TAGs aggregate and
806 form foci or “lipid lens” structures between the two leaflets of the ER bilayer (Figure 6C)
807 (Pyc et al., 2017a). An oligomeric protein complex comprising SEIPIN subunits in the ER
808 bilayer coordinates these TAG foci as they grow (Chapman et al., 2019). SEIPIN proteins
809 direct a bulge of newly accumulating neutral lipids to emerge into the cytoplasm covered
810 with a monolayer of ER-derived phospholipid. Unlike fungi and metazoans, plants have
811 multiple genes encoding SEIPIN isoforms (Cai et al., 2015). In Arabidopsis, *SEIPIN1* is
812 expressed mostly in seed and seedling tissues, whereas *SEIPIN2* and *SEIPIN3* are
813 expressed in essentially all issues. While loss-of-function mutants in a single *SEIPIN* gene
814 in Arabidopsis resulted in negligible phenotypes, double and especially triple *seipin*
815 mutants showed dramatic cellular disruptions in normal LD formation (Taurino et al.,

2018). Seeds and pollen of *sei1 sei2 sei3* mutants accumulated large aberrant-shaped LDs, sometimes observable in the nucleus and ER lumen in addition to the cytoplasm. These results indicate that SEIPINs play critical and partially redundant roles in the normal formation of LDs in plant cells. Structural models based on homology with known structures of Drosophila and human SEIPIN suggest that the three Arabidopsis SEIPINS can form homo-oligomeric structures with different numbers of subunits (Chapman et al., 2019), but further work is required to understand the functional interactions of the three SEIPIN proteins in plant cells, their potential for hetero-oligomeric interactions, as well as their partners in LD biogenesis.

The loss-of-function of two other Arabidopsis genes led to similar, large and aberrant LD phenotypes in seeds, reminiscent of *seipin* mutants. These two genes encode VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN 27-1 (VAP27-1) and LDIP, respectively, which were both shown to interact with SEIPINs and with LDs (Pyc et al., 2017b; Greer et al., 2020). In other work, higher order oleosin mutants also displayed aberrant formation of LDs during early seed development, which resulted from changes in the fusion dynamics of very small LDs, not necessarily during LD formation at the ER (Miquel et al., 2014). LDAPs also occur in seeds but at lower amounts than oleosins (Kretzschmar et al., 2018). Finally, while LDAPs are interactors of LDIP (Pyc et al., 2017b), their loss-of-function in *ldap* mutants did not result in dramatic alterations of LD morphology in seeds (Gidda et al., 2016), although there may have been some increase in LD size in the leaves of *ldap* knockdown mutants (Brocard et al., 2017). Future work will be required to piece together the mechanistic associations between SEIPINs, LDIP, VAP27-1, oleosins, LDAPs and other LD proteins; nevertheless, results to date support the notion that these proteins play cooperative roles in the cellular process of LD formation in plant cells (Greer et al, 2020; Pyc et al., 2021).

Engineering the LD compartment

Because of their high energy density and caloric value, LDs have become a target compartment for metabolic engineering strategies to overproduce storage lipids in the vegetative parts of plants. This process has met with remarkable success, leading to

tobacco plants with lipid yields from their leaves equivalent to oil yields from oilseed crops. This overall engineering process has been described as the “push, pull, and protect” concept for the efficient production and packaging of storage lipids in plant tissues (Vanhercke et al., 2017; Vanhercke et al., 2019). Apparently, the accumulation of lipids in leaves is at the expense of transient starch (Chu et al., 2020), illustrating a plasticity in leaves for carbon storage that may ultimately be exploited for bioenergy and/or feed energy-densification applications.

In addition to bioenergy applications, LDs offer a stable compartment for the sequestration of various hydrophobic compounds. As such, several recent reports indicate that manipulation of the LD machinery can be exploited for the subcellular storage of secondary metabolites. For example, (Sadre et al., 2019) engineered the accumulation of sesquiterpenes (patchoulol) and diterpenes (abetadiene) into cytoplasmic LDs. Elsewhere, the expression of lipogenic proteins from mouse dramatically elevated LD levels in *N. benthamiana* leaves and supported the increased accumulation of the sesquiterpene phytoalexin, capsidiol, along with TAGs (Cai et al., 2019). With the preponderance of bioactive hydrophobic secondary metabolites, these studies illustrate the utility of engineering the cytoplasmic LD compartment in plants as a repository for additional high-value isoprenoids in the future.

Future prospects for LD biology

In the last decade, increasing attention on cytoplasmic LDs has revealed a growing inventory of proteins that support the formation, stability, and turnover of this compartment in plant cells. Some proteins appear to have specific plant lineages, while others are conserved across kingdoms. The identification of this LD machinery will support a mechanistic examination of the interplay of these and other proteins in LD biogenesis, both in oilseeds and in non-seed tissues of plants. In addition, functions beyond neutral lipid storage continue to be revealed for LDs in different plant cell types, including as a reservoir for membrane lipid remodeling (Xu and Shanklin, 2016), a platform for the production of lipophilic signals (Shimada et al., 2014; Fernandez-Santos et al., 2020), and responses to environmental stress (Yang and Benning, 2018) (Lu et al., 2020). Finally,

878 an improved understanding of the cellular processes for LD formation, neutral lipid
879 deposition, and LD stability will accelerate and expand promising applications for lipid
880 engineering.
881
882
883 **THE DYNAMIC NATURE OF PEROXISOME STRUCTURES, ABUNDANCE, AND**
884 **SUBCELLULAR INTERACTIONS**
885
886 (Written by Bethany Zolman)
887
888 Peroxisomes compartmentalize diverse oxidative reactions, allowing metabolic,
889 signaling, and detoxification roles to be carried out while limiting the potential for damage
890 (Kao et al., 2018; Pan et al., 2020). Peroxisomes are a closed system, permeable only to
891 small (300-400 Da) molecules (Charton et al., 2019; Plett et al., 2020). Membrane
892 transporters import lipid substrates and ATP, NAD^+, and CoA cofactors into peroxisomes
893 (Charton et al., 2019; Plett et al., 2020), whereas enzymes are imported by cytosolic
894 receptors that recognize one of two Peroxisomal Targeting Signals (PTS1/PTS2;
895 (Reumann and Chowdhary, 2018; Pan et al., 2020). Plant peroxisomes are indispensable
896 during early development, when seedlings rely on lipid breakdown prior to photosynthetic
897 initiation (Graham, 2008). They are also crucial for photorespiration in leaf cells and
898 reactive oxygen species (ROS) and nitrogen species metabolism throughout
899 development and under changing conditions (Del Rio and Lopez-Huertas, 2016; Kao et
900 al., 2018; Corpas et al., 2020; Pan et al., 2020; Su et al., 2020). These organelles are
901 essential for life in all eukaryotes and have many evolutionarily conserved pathways and
902 proteins (Gabaldon, 2010).
903
904 Peroxisome abundance varies based on cell type, developmental stage, and
905 environmental conditions. Although peroxisome abundance has not been characterized
906 systematically, 10-100 peroxisomes per cell have been observed (for example, (Germain
907 et al., 2001; Orth et al., 2007; Lingard et al., 2008; Kim et al., 2013; Shibata et al., 2013).
908 Peroxisome numbers increase in response to stress, including salt (Mitsuya et al., 2010;

Fahy et al., 2017; Frick and Strader, 2018), light (Desai and Hu, 2008), and cadmium stress (Rodríguez-Serrano et al., 2016; Terron-Camero et al., 2020), as well as prior to cell division (Lingard et al., 2008). Peroxisome division occurs via fission or the budding of pre-peroxisomes from the ER (Agrawal and Subramani, 2016; Kao et al., 2018; Pan et al., 2020; Su et al., 2020). Peroxisomes can be degraded via pexophagy, an organelle-specific type of autophagy (Young and Bartel, 2016; Su et al., 2019), as part of a natural turnover (Kao et al., 2018; Yamauchi et al., 2019) or when excess organelles are not necessary following stress (Calero-Muñoz et al., 2019) or developmental transitions (Kim et al., 2013).

Peroxisomes are small, measuring 1-2 μm in diameter in Arabidopsis (Rinaldi et al., 2016) but with notable variability. Larger structures can be visualized 3-4 days post imbibition (Rinaldi et al., 2016), with some peroxisomes over 10 μm in diameter in 4-day-old seedlings. This expansion is temporary and is thought to occur following an influx of seed-storage lipids (Rinaldi et al., 2016). Although their morphology can differ, peroxisomes are primarily spherical.

Since their identification, peroxisomes have been considered simple organelles, with typical definitions highlighting their small size, lack of a genome, and a single membrane surrounding a defined matrix. However, recent investigations by Wright and Bartel (2020) have led to an enhanced description of peroxisomes, one in which extensive internal membranes are present. The authors combined two high-sensitivity fluorescence reporters: an mRuby3-PTS to visualize the peroxisome interior and mNeonGreen tagged with an mPTS membrane peroxisomal targeting signal to label the membrane (Figure 7A-B) (Wright and Bartel, 2020). This combination revealed the unexpected presence of internal structures, coined intralumenal vesicles (ILVs).

In 3 to 4-day-old Arabidopsis seedlings, membrane reporters localized around these structures, but also within the interior of the organelles (Wright and Bartel, 2020). Many peroxisomes contained numerous internal vesicles, which varied in size (Figure 7A-B). As discussed above, 5-day-old seedlings showed expanded organelles that rapidly

940 decreased in size. These size changes were concurrent with increasing ILV and
941 internalized membrane contents. By 8 days, the seedlings continued to show membrane
942 reporters within the peroxisome lumen, with some images showing membrane signals
943 throughout the entire structure. Following this process, dense packing likely occurred over
944 time that precluded the observation of individual vesicles, such that the membrane
945 reporter appeared uniform within the lumen at this age (Wright and Bartel, 2020). These
946 seedling experiments suggest how peroxisomes mature, beginning as larger, variable
947 structures but stabilizing at a smaller size as membranes are internalized and lipid
948 metabolism slows.
949
950 These microscopic images led to an enhanced understanding of peroxisomal structure:
951 peroxisomes have an outer membrane surrounding the lumenal space that contains
952 imported matrix proteins, as well as a dynamic number of membrane-bound vesicles clear
953 of matrix proteins (Figure 7A-B) (Wright and Bartel, 2020). Indeed, two proteins with
954 unique peroxisomal localization (SNOWY COTYLEDON3/UNKNOWN PROTEIN9;
955 (Albrecht et al., 2010; Quan et al., 2013) accumulated within a subset of ILVs that lacked
956 matrix proteins (Wright and Bartel, 2020). This apparent segregation yields at least three
957 distinct spaces within peroxisomes, potentially housing unique proteins, substrates,
958 cofactors, and/or environments.
959
960 Mutants with disrupted β-oxidation showed alterations in ILV number, size, composition,
961 and orientation, suggesting that β-oxidation activity is required for inner membrane
962 formation (Wright and Bartel, 2020). Long-chain seed storage lipids are insoluble; Wright
963 and Bartel (2020) hypothesized that membrane internalization may reduce the solubility
964 challenges associated with lipid mobilization in an aqueous matrix. Lipids could be
965 degraded from the membrane, with the subsequent release and degradation of the
966 shorter, more soluble substrates, leading to the reduced organelle size common in older
967 seedlings.
968
969 **Association with other organelles**

Beyond this structural understanding, imaging and biochemical studies have revealed the physical associations of peroxisomes with LDs, plastids, mitochondria, and the ER (Figure 7C; (Shai et al., 2016) (Oikawa et al., 2019). Peroxisomal enzymes catalyze specific reactions within metabolic pathways, which often extend to two (or more) subcellular spaces. These organelle interactions are dynamic: peroxisomes in seedlings associate with LDs, for instance, whereas peroxisomes in leaves associate with chloroplasts and mitochondria (Oikawa et al., 2019). Such interaction points enhance the transfer efficiency of pathway intermediates. These sites also may facilitate the transfer of hydrogen peroxide and other reactive species from other organelles to peroxisomes for sequestration and degradation (Shai et al., 2016; Su et al., 2019).

As detailed above, many plant species contain LDs that store TAGs for energy reserves (Esnay et al., 2020). Peroxisome-LD association facilitates the efficient transfer of stored material for metabolism via fatty acid β-oxidation and the glyoxylate cycle. Extended interactions and peroxisomal clusters in proximity to LDs occur in β-oxidation mutants (Hayashi et al., 2001; Rinaldi et al., 2016), while exogenous sucrose reduces this association (Cui et al., 2016), suggesting that such interactions are critical during development and are mediated by cellular requirements for lipid mobilization.

Peroxisomes and chloroplasts can form specific pairs that remain intact over time (Oikawa et al., 2015). Changes in peroxisome shape expand the surface area to increase chloroplast interactions. In the light, peroxisomes extend into an elliptical shape versus a spherical shape in darkness. Tethering factors connecting peroxisomes and chloroplasts may facilitate this interaction (Oikawa et al., 2015; Gao et al., 2016), potentially including the PEROXIN10 (PEX10) RING finger protein (Schumann et al., 2003; Sparkes et al., 2003; Schumann et al., 2007). A dominant-negative PEX10 line had clustered peroxisomes that did not associate with chloroplasts; this line had phenotypes similar to photorespiration mutants (Schumann et al., 2007), which is consistent with a role for organelle association in efficient metabolic transfer.

Mitochondria also appear in close proximity to both peroxisomes and chloroplasts in the light, which is consistent with their interactive metabolic roles (Oikawa et al., 2015). Peroxisomes associate with mitochondria under stress conditions as well; increasing interactions are seen in cells exposed to high ROS levels and might be important for ROS neutralization (Jaipargas et al., 2016; Mathur, 2021).

Finally, peroxisomes show a close proximity with the ER (Barton et al., 2013; Oikawa et al., 2019). Interestingly, one of the two mPTS signals used by Wright and Bartel (2020) revealed accumulation in peroxisomes and reticular membranes thought to be ER. This finding is consistent with the hypothesis that the membrane protein was trafficked through the ER or has a dual function at both sites (Wright and Bartel, 2020).

Another shape change in peroxisomes is the formation of thin organelle protrusions known as peroxules (Mathur, 2021). These structures are up to 15 μm in length, dramatically increasing the surface area (Sinclair et al., 2009; Barton et al., 2013). The formation of these organelle extensions is transient and dynamic (Mathur, 2021). The interactions between organelles described above may be mediated by peroxules, including the proposed interactions with LDs (Thazar-Poulot et al., 2015), chloroplasts (Schumann et al., 2007), mitochondria (Jaipargas et al., 2016), and the ER (Sinclair et al., 2009). Extended structures are seen following H_2O_2, UV-A, and hydroxyl radical stress, but retract when stress is minimized (Sinclair et al., 2009). In addition, elongations are common during the constriction and fission steps of peroxisome division (Sinclair et al., 2009; Barton et al., 2013). Cadmium induces ROS production and leads to peroxule formation that results in division to increase peroxisome numbers (Rodríguez-Serrano et al., 2016). These ROS-induced increases in peroxule frequency led to the hypothesis that these extensions facilitate neutralization to prevent or reduce damage (Sinclair et al., 2009; Barton et al., 2013; Rodríguez-Serrano et al., 2016). Separately, peroxule-mediated contacts might assist in protein localization. The SUGAR-DEPENDENT1 (SDP1) lipase (Eastmond, 2006) localizes to peroxisomal membranes and then the LD, a transition concurrent with peroxule development (Thazar-Poulot et al., 2015).

The refined visualization of peroxisome structures using advanced microscopy techniques and our increasing understanding of organelle interactions have led to an enhanced view of peroxisomes compared to the previously simple model. Many open questions about peroxisome biology remain. What is the mechanism for (and importance of) dynamic membrane changes for peroxisomes in adult tissues and under changing environmental conditions? How do peroxisome substructures form, and how are membrane and matrix proteins sorted to create unique environments or to provide specific functionality? Understanding such details about peroxisome structures, as well as the factors promoting and mediating peroxisome interactions with other organelles, will continue to increase our understanding of these dynamic organelles.

PLANT MITOCHONDRIA

(Written by Shin-ichi Arimura)

In plants, mitochondria provide a large portion of the ATP in the cytosol through oxidative phosphorylation. In addition, these organelles are the sites of metabolism of some amino acids, nucleic acids, lipids, and plant hormones. Plant mitochondria also control redox balance when photosynthesis is on, off, or fluctuating (Noguchi and Yoshida, 2008; Finkemeier and Schwarzlander, 2018) and play roles in cellular signaling (Huang et al., 2016; Welchen et al., 2021) and in resistance to diseases (Fuchs et al., 2020). In agriculture, cytoplasmic male sterility, which is caused by genes encoded in the mitochondrial genome, is used for the production of F1 hybrid seeds in diverse crops, including vegetables. The fine structure and dynamics of plant mitochondria are briefly reviewed here.

Mitochondria contain two lipid bilayers that form the outer and inner membrane. Some parts of the inner membrane are invaginated to form sacs, called cristae, which increase the area of oxidative phosphorylation complexes. Five diverse eukaryotic-conserved complexes are embedded in the cristae membrane. By contrast, plant-specific proteins

(alternative oxidases and extra NDH and NDPH dehydrogenases) for alternative respiration pathways mainly reside in the non-cristae parts of the inner-membrane (Schwarzlander and Fuchs, 2017). Plant ATP synthase dimers (complex V) are located in the cristae membrane, where they contribute to its curvature (Zancani et al., 2020. Complexes I to V play roles in oxidative phosphorylation. Some of these complexes form super-complexes for functional efficiency and to regulate oxidative phosphorylation (Braun, 2020). Protein-protein interactions and metabolite channeling are also observed in the TCA cycle in the matrix (Zhang, 2017). Additionally, glycolysis enzymes in the cytosol dynamically associate on the outer surfaces of mitochondria (Giege et al., 2003; Graham et al., 2007), probably to more efficiently transport metabolites.

The mitochondrial outer membrane contains the most abundant protein in plant mitochondria, the Voltage-Dependent Anion Channel (VDAC1). A single mitochondrion contains 40,000 VDACs out of a total of 1.4 million proteins (Fuchs et al., 2020). The outer membrane does not just encapsulate the inner membrane but also sometimes extends into the cytosol and other organelles (without extending the inner-membrane); occasionally, the extensions are pinched off to form small vesicle-like structures (Yamashita et al., 2016); Figure 8A). In mammals, mitochondria-derived vesicles that do not contain inner membranes are involved in the transport of specific proteins to peroxisomes, endosomes, and multivesicular bodies (Sugiura et al., 2014) and in the biogenesis of peroxisomes (Sugiura et al., 2017).

Each Arabidopsis leaf cell contains 300-450 mitochondria. Many plant mitochondria move along actin microfilaments at 0.05 - 3 µm/sec (Doniwa et al., 2007, Oikawa et al., 2021). This speed is approximately an order of magnitude faster than that of mammalian and yeast mitochondria, which mainly move along microtubules. Some plant mitochondria stop and wiggle, as if they were anchored to the cytoskeleton or other organelles, such as plastids and peroxisomes (Jaipargas et al., 2016; Oikawa et al., 2021). Moving plant mitochondria can change their speed and can also change their shapes from granular to linear to attach to other organelles in response to the presence of sucrose or light (Jaipargas et al., 2016). A single plant cell can contain mitochondria with different shapes

(Jaipargas et al., 2015), different DNA contents (Figure 8B) (Arimura et al., 2004b; Preuten et al., 2010), and transiently fluctuating membrane potentials (Schwarzlander et al., 2012). In addition, as shown in Figure 8C, differently groups of mitochondria stained in different colors in a cell achieve a unified color in two hours, indicating that mitochondria undergo frequent fusion and fission, resulting in the sharing of internal proteins. Mitochondria involved in such dynamic sharing of materials in a plant cell are referred to as a dynamic syncytium (Lonsdale et al., 1988), and the collective mitochondria in a cell are thought to exist as a discontinuous whole (Logan, 2017). Fusion of mitochondria results in the formation of elongated and/or branched mitochondria in some meristematic tissues, such as shoot apices (Segui-Simarro and Staehelin, 2006), germinating seeds (Paszkiewicz et al., 2017), and dedifferentiating protoplasts (Sheahan et al., 2005; Rose and McCurdy, 2017).

Mitochondrial fission is achieved by dynamin-related proteins that are well-conserved in eukaryotes (e.g. DRP3A and 3B in Arabidopsis (Arimura and Tsutsumi, 2002; Arimura et al., 2004a; Arimura et al., 2004b; Fujimoto et al., 2009), Figure 8D), which polymerize to form ring-like structures outside mitochondria (Ingerman et al., 2005). Plant-specific ELM1, an outer surface protein, localizes DRP3s from the cytosol to the mitochondria (Arimura et al., 2008). An outer-membrane embedded protein that is conserved in eukaryotes (Fis1) functions as a molecular adapter for DRP in budding yeast (Okamoto and Shaw, 2005). Fis1 had been thought to carry out similar functions but is now thought to play only a rather minor and indirect role in mitochondrial fission in both mammals (Otera et al., 2010; Giacomello et al., 2020) and plants (Nagaoka et al., 2017; Arimura, 2018). Other factors may be involved in plant mitochondrial fission, such as factors involved in cold-induced fission (Arimura et al., 2017) or factors that are independent of DRP and specific to Brassicaceae (Aung and Hu, 2011). On the other hand, no orthologs, factors, or molecular mechanisms are known with certainty to be involved in mitochondrial fusion in plants. However, FRIENDLY (FMT) is thought to mediate inter-mitochondrial association before mitochondrial fusion because in Arabidopsis *fmt* mutants, mitochondria gather together (Logan et al., 2003) but do not fuse (El Zawily et al., 2014).

Mitochondrial-specific autophagy (mitophagy) has been extensively studied in mammals and yeasts (Onishi et al., 2021), where it is involved in mitochondrial quality control. In these organisms, degraded mitochondria with low membrane potential could not fuse with other “healthy” mitochondria, but they were specifically recognized, captured, and engulfed by autophagosome membranes (Figure 8E). The engulfed mitochondria were transported to the vacuole to be digested to prevent accidental ROS generation and/or other negative effects. Therefore, mitophagy, fission, and fusion are thought to function as a quality control system for all the mitochondria in a cell (Twig et al., 2008). Mitochondrial-specific degradation in Arabidopsis has also been observed in several situations, including during leaf senescence (Broda et al., 2018), during the greening of cotyledons (Ma et al., 2021), after UV-irradiation (Nakamura et al., 2021), and after treating the inner membrane with ionophores (Ma et al., 2021). Orthologs of factors specific to mitophagy in mammals and yeasts have not yet been found in plant genomes, although FMT was recently reported to be involved in mitophagy in Arabidopsis (Ma et al., 2021).

Super resolution microscopy is a promising new technique that can clarify the internal structures of mitochondria, with their diverse physiology and functions, in more detail. In addition, recent trials to understand the types and numbers of molecules in an average single mitochondrion (Fuchs et al., 2020) or in a hypothetical single mitochondrion (Moller, 2016) will hopefully give rise to the next stage of analysis of the exact number of individual mitochondria. Such information would help uncover the actual quantitative dynamics of molecules among diverse mitochondria underlying the functions of each cell. Until recently, the transformation of mitochondrial genomes in multicellular plants had been impossible, but new genome editing methods (Kazama et al., 2019; Arimura et al., 2020) have opened the door to analyzing the functions of mitochondrial genes, as well as regulating their expression in order to breed crops with agriculturally important characteristics.

CHLOROPLAST: A PLANT’S POWERHOUSE WITH TUNABLE PERFORMANCE

(Written by Helmut Kirchhoff)

A unique endosymbiotic event more than 900 million years ago was the starting point for the evolution of the chloroplast from a free-living cyanobacterial precursor (Sibbald and Archibald, 2020). Every second, the thylakoid membrane system of a modern chloroplast in *Viridiplantae* can convert energy from the sun into up to 80 million ATP and NADPH + H^+ molecules. This fuels a number of anabolic reactions localized in the chloroplast stroma, including the synthesis of sugars, lipids/fatty acids, amino acids, nucleotides, pigments, alkaloids, hormones, and vitamins (Kirchhoff, 2019). Furthermore, a battery of membrane-embedded chloroplast envelope transporters makes the capacity for photosynthetic energy transformation available to the entire cell and beyond (Weber and Linka, 2011). In C3 plants, a typical leaf cell contains 20-100 chloroplasts in the palisade parenchyma and 10-50 in the spongy parenchyma (Antal et al. 2013).

Chloroplast lifecycle

During the last decade, electron tomography has provided detailed structural insights into the morphological transitions from an undifferentiated, non-photosynthetic proplastid to a mature chloroplast in the shoot apical meristem for illuminated shoots (Adam et al., 2011; Charuvi et al., 2012) or via the etioplasts, with its characteristic para-crystalline prolamellar body (Kowalewska et al., 2016). The correlation between the sequential appearance of proteins such as photosystem I and II, light-harvesting complex II, CURT1 proteins, ATPase, protochlorophyllide oxidoreductase, and plastidial ribosomes on the one hand and structural development of the plastid on the other hand provides a first glimpse of the roles of particular proteins in proplastid-chloroplast differentiation (Kowalewska et al., 2016; Liang et al., 2018a; Floris and Kuehlbrandt, 2021). Proplastid development requires the massive import of proteins from the cytoplasm into the chloroplast, mainly by the TOC/TIC translocase system (Aronsson and Jarvis, 2008; Ling et al., 2012), since ~95% of chloroplast proteins are encoded in the nucleus.

Currently, there are two major non-exclusive models describing how hydrophobic nucleus-encoded proteins (along with lipids and pigments) that are synthesized at the

plastid envelope membranes are transported through the aqueous stroma to reach their thylakoid membrane destination: (1) invaginations of the inner envelope membrane/direct contact sites with thylakoids and (2) vesicle transport (Lindquist and Aronsson, 2018; Mechela et al., 2019). Evidence exists that the invagination/direct contact site pathway is realized only in the proplastid-to-chloroplast transition, whereas vesicle transport seems to be dominant in mature chloroplasts (Vothknecht and Westhoff, 2001; Andersson and D'ormann, 2008; Lindquist and Aronsson, 2018). For the latter, the roles of typical vesicle-forming proteins such as COPI, COPII, SNARE, and VIPP1 in plastid biogenesis remain to be determined (Mechela et al., 2019). However, for cyanobacterial VIPP1, a structure-based molecular understanding was recently achieved (Gupta et al., 2021).

In contrast to proplastids, mature chloroplasts propagate by binary fission (Osteryoung and Pyke, 2014; Yoshida, 2018). The plastid division machinery is made up of four physically connected supramolecular ring structures: two outside (an outer polyglucan plastid-dividing ring and a dynamin-related ring) and two inside the chloroplast (an inner plastid-dividing ring and a tubulin-like FtsZ-ring beneath the inner envelope membrane). In a concerted mechanism, the rings generate the mechanical force required for plastid constriction and eventually division. An example of the crucial role of regulatory proteins in plastid morphogenesis, such as the FZL-fusion protein, is visualized in Figure 9. Open questions in the field are the composition of the inner plastid-dividing ring, how thylakoid membranes divide, and how chloroplast division is coordinated with the division of cells and other organelles (Osteryoung and Pyke, 2014; Yoshida, 2018).

At the end of their lifespan, chloroplasts enter highly coordinated dismantling processes with the goals of minimizing ROS production and recycling their abundant macromolecules to sink tissues of the plant (Avila-Ospina et al., 2014). Strikingly, chloroplasts hold ~80% of leaf nitrogen (Makino and Osmond, 1991), making their recycling very valuable for plant resource management. It seems that ROS-dependent retrograde signaling plays a key role in coordinating chloroplast degradation via multiple breakdown pathways including chlorophagy (Woodson, 2019; Dominguez and Cejudo, 2021). Current research focuses on elucidating how particular environmental conditions

trigger specific dismantling pathways and deciphering the corresponding signal cascades.

Interactions of chloroplasts with other organelles

Chloroplast metabolism is highly integrated into plant cell metabolism. Two prominent examples of the tight functional cooperation between chloroplasts and other organelles are photorespiration and lipid trafficking. The oxygenation of ribulose-1,5-bisphospate by Rubisco in the chloroplast stroma can lead to a loss of up to 30% of fixed carbon (Walker et al. 2016) and the production of cell-toxic 2-phosphoglycolate (2-PG). 2-PG is detoxified by the photorespiratory pathway, which converts two 2-PG molecules into one molecule of glycerate (recycled to the Calvin-Benson cycle) and CO_2. Photorespiratory metabolization of 2-PG requires the metabolic competence of three organelles: the chloroplast, peroxisomes, and mitochondria. The efficient exchange of photorespiratory metabolites between these three organelles is tuned and controlled by organellar membrane transport proteins (Kuhnert et al. 2021) and the spatial interaction of the three organelles. For example, the area of physical contact between peroxisomes and chloroplasts increases significantly under photorespiratory conditions fostered by changes in peroxisome shape from spherical to elliptical (Oikawa et al. 2015).

Another intriguing example of tight organelle cooperation is lipid trafficking (Hurlock et al. 2014). Chloroplast lipids are synthesized both entirely in the chloroplast (prokaryotic pathway) and by the cooperation between chloroplasts and the ER (eukaryotic pathway) (Hölz and Dörmann 2019). Some plants such as pea (*Pisum sativum*; also known as 18:3 fatty acid plants) have lost their ability to synthesize lipids via the prokaryotic pathway, depending entirely on the eukaryotic one (Roughan and Slack 1984, Mongrand et al. 1998). Due to their crucial roles in membrane function, integrity, and maintenance, as well as storage (triacylglycerol) and determining the composition of extracellular hydrophobic components (i.e. waxes), acclimative changes in chloroplastic fatty acid and lipid composition is key for plant survival under unfavorable conditions or during plant development (Hölz and Dörmann 2019). This plasticity of lipid composition relies heavily

on the dynamic interaction between chloroplasts, the ER, lipid bodies, Golgi, and mitochondria (Hurlock et al. 2014).

Structural membrane dynamics as a means to control energy conversion

The fact that photosynthetic energy conversion has to integrate and balance significant fluctuations in both cell metabolism (including CO_2 availability) and energy input by sunlight in an oxidizing environment calls for its strict regulation to minimize toxic ROS production. In the last decade, a central regulatory element for tuning photosynthetic performance in plants has been uncovered: the dynamic adjustment of lateral and transversal geometric (grana) thylakoid dimensions that regulate electron transport, light-harvesting, and protein repair (Kirchhoff et al. 2011, Herbstova et al. 2012, Hepworth et al. 2021). For example, changes in the vertical width of the thylakoid lumen as well as the lateral diameter of the grana disc were reported to control the mobility of the small electron carriers plastoquinone and the lumen-hosted plastocyanin and therefore linear electron transport from water to ferredoxin (Kirchhoff et al. 2011, Hepworth et al. 2021). Furthermore, lateral shrinkage of the grana diameter is beneficial for the repair of photodamaged, grana-hosted PSII complexes, since the shrinkage makes it easier for PSII to reach the protein repair machinery localized in distant (separated by a few hundred nanometers) unstacked thylakoid domains (Herbstova et al. 2012). It is an open question how reversible protein phosphorylation, physicochemical membrane properties, and protein composition dynamics work together to control architectural thylakoid features and subsequently energy conversion.

Future perspectives

Over the next five to ten years, the rapid methodical and technological development of (cryo)electron tomography (Bussi et al. 2019, Wietrzynski et al. 2020) is expected to provide detailed new insights into chloroplast structure-function relationships not only for the mature plastid but also for its biogenesis and dismantling. Furthermore, studying chloroplast diversity in non-model, less commonly studies species as well as in specialized plant tissues and organs (including transitions between different plastid types) will gain increasing attention because it will uncover the metabolic plasticity and diversity

of this organelle. Along these lines, current and future bioengineering and synthetic biology tools for chloroplasts offer the potential for improving crop plants by tuning processes such as non-photochemical quenching (Kromdijk et al. 2016) or photorespiratory pathways (South et al. 2016, Roell et al. 2021) or for using the anabolic competence of the plastid to employ these organelles as metabolic factories for valuable chemicals (Bock 2021).

PLANT MEMBRANE CONTACT SITES: QUESTIONS FROM THE MEMBRANE INTERFACE

(Written by Emmanuelle Bayer, Federica Brandizzi, Yvon Jaillais, Miguel A. Botella, Pengwei Wang, and Abel Rosado)

Membrane contact sites: Does one definition fit all?

Membrane Contact Sites (MCS) are evolutionarily conserved structures where the physical proximity between two or more membrane-bound organelles enables the direct exchange of molecules and facilitates coordinated inter-organelle adaptive responses (examples of MCS membrane proximity using TEM are shown in Figure 10A-B). Recent advances in plant cell imaging and the development of novel genetic and molecular tools have fueled an emerging field of research devoted to the investigation of their structural organization, dynamics, and physiological functions. This interest is uncovering plant-specific MCS structures and molecular mechanisms, but it is also exposing some limitations of the commonly accepted definitions and physiological functions inferred from different model organisms. As in yeast and animal cells, the plant ER is an interconnected organelle that establishes MCS with multiple cellular structures including the PM, mitochondria, endosomes, peroxisomes, Golgi ,and TGN (Barton et al., 2013; Stefano et al., 2014; Wang et al., 2014; Perez-Sancho et al., 2015; Wang et al., 2019b; Brandizzi, 2021). Unique to plants, however, are the functional interactions at ER-plastid MCS for lipid synthesis and transport (Liu and Li, 2019), the control of intercellular communication through PD MCS-regulated intercellular bridges (Tilsner et al., 2016), and the MCS

activities driven by a super-continuum that encompasses the cell wall, PM, ER, and cytoskeleton (Wang et al., 2014; Perez-Sancho et al., 2015; Zang et al., 2021). These plant-specific features are placing MCS research in plants at the forefront of discovery, broadening the definition of MCS beyond yeast and animal systems.

In plants, MCS can be defined as environmentally and developmentally regulated microdomains with an intermembrane gap as small as 3 nm in PD and an arbitrarily defined upper limit of 80-100 nm. Plant MCS are enriched with a variety of protein-protein, and/or protein-cytoskeleton tethering assemblies, such as those including the SYT1 and VAP27 tethers (Zang et al., 2021; Rosado and Bayer, 2021, Figure 10C-E). These complexes establish dynamic interactions with membrane phospholipids and/or the cell wall and carry out essential cellular functions, including (but not restricted to) the maintenance of membrane lipid homeostasis, cell-to-cell communication, organelle biogenesis, autophagy, endocytosis, receptor kinase signaling, and the regulation of Ca^{2+}-dependent stress responses (reviewed in Perez-Sancho et al., 2016; Wang and Hussey, 2017; Liu and Li, 2019; Petit et al., 2020; Rosado and Bayer, 2021).

Lipid transfer at MCS: Is that what plant tethers do?

Due to their hydrophobicity, the transport of lipids between organelles relies on either vesicle-mediated delivery mechanisms or MCS-localized lipid transport proteins (LTPs) (Scorrano et al., 2019). Most MCS-localized LTPs contain an internal hydrophobic cavity adapted to solubilize water-insoluble molecules (Wong and Levine, 2016), are anchored to the ER by either transmembrane domains or stable interactions with ER-anchored proteins (Scorrano et al., 2019), and interact with the opposing membrane, mainly through domains that bind anionic lipids (Perez-Lara and Jahn, 2015). In animal cells or yeast, direct lipid transport using MCS-localized LTPs may be one of the best characterized and documented MCS functions. The lipid species transferred using this mechanism include sterols, ceramides, phosphatidylserine (PS), phosphatidylinositol 4-phosphate (PI4P), and diacylglycerol (DAG) (Wu et al., 2018). Similarly, in plants, the emerging view is that MCS-localized LTPs participate in the delivery of lipids between the ER and organelles not linked by vesicular trafficking (e.g. mitochondria and plastids) but also in the bulk

transport of lipids between organelles connected by the secretory pathway. In a recent landmark study, Ruiz-Lopez et al. showed that stress signals regulate the activity of two members of the Synaptotagmin (SYT) family of LTPs at ER-PM MCS (SYT1 and SYT3) and demonstrated their function as LTPs that transfer DAG between the PM and the ER *in vivo* (Ruiz-Lopez et al., 2021). The authors propose a geometrical model where SYT activities counteract the stress-induced build-up of conically shaped DAG at the PM and prevent the generation of areas of negative membrane curvature that could disrupt the stability of the PM during stress episodes.

MCS in motion: What controls MCS plasticity and dynamics?

The molecular composition, geometry, and plasticity of inter-organelle junctions determine their ability to integrate and respond to cellular signals. Recent studies have provided an emerging picture in which MCS tethers do not act in isolation but instead interact with anionic lipids and cytoskeletal elements and regulate the plasticity, function, and dynamics of these cellular microdomains.

Anionic phospholipids represent only a few percent of total lipids, but they are critical biochemical and biophysical landmarks of membrane identity (Noack and Jaillais, 2020). Within the endomembrane system, anionic phospholipids, including the phosphoinositides (PIPs), phosphatidic acid (PA), and phosphatidylserine (PS), determine the electrostatic potential of each membrane, which is highest at the PM, intermediate in endosomes, and low in the ER (Platre et al., 2018; Dubois and Jaillais, 2021). *In vitro* or *in silico* data for MCS tethers such as the synaptotagmins (SYTs), Multiple C2 domains and transmembrane region (MCTPs), and Vesicle-associated membrane protein (VAMP)-associated proteins (VAPs) families support the notion that anionic lipids profoundly affect the structure and function of MCSs by enabling protein-lipid interactions that regulate the association of the ER with the PM, TGN, and early endosomes (Perez-Sancho et al., 2015; Stefano et al., 2018; Brault et al., 2019; Ruiz-Lopez et al., 2021). Interestingly, these interactions appear to be mostly non-specific, the primary determinants being the negative charge carried by the anionic lipids and, in some cases, the presence of Ca^{2+} (Schapire et al., 2008). Accordingly, electrostatic interactions

between phosphatidylinositol-4-phosphate (PI4P) and SYT1/SYT3 underpin the localization of SYT1/SYT3 to ER-PM MCS (Ruiz-Lopez et al., 2021), MCTP4 to PD-MCS, (Brault et al., 2019), and the remodeling of SYT1 ER-PM MCS in response to rare-earth elements (Lee et al., 2020). Similarly, the accumulation of phosphatidylinositol 4,5-biphosphate [$PI(4,5)P_2$] at the PM enables interactions with SYT1 and correlates with the rearrangement and expansion of ER-PM MCS in response to ionic stress (Lee et al., 2019).

MCS plasticity is also controlled by components that crosslink the actin cytoskeleton at MCS and create trapping mechanisms that influence MCS architecture and expansion. In plants, this cross-linking seems to be carried out by a plant-specific complex that includes the actin-associated NET3C protein and the microtubule-associated Kinesin light chain related (KLCRs) and IQ67-Domain (IQD) proteins (Zang et al., 2021) (Figure 10E). Remarkably, in plants, the presence of cell walls underlies the formation of a plant-specific supramolecular assembly known as the MCS super-continuum. This super-continuum encompasses the cell wall, PM, ER, and cytoskeleton and renders MCS with distinct kinetics, shapes, geometries, and functions (Rosado and Bayer, 2021; Zang et al., 2021). Recent studies proposed that the MCS super-continuum serves as a nexus that limits the mobility of MCS tethering assemblies (Wang et al., 2016; Lee et al., 2019; Zang et al., 2021) and controls their activities. Examples of regulation mediated by this continuum include the activity of receptor-like kinases in pollen and/or stomatal cells (Ho et al., 2016; Duckney et al., 2021) and the regulation of phospholipase C-mediated stress signals at the PM (Ruiz-Lopez et al., 2021). Finally, a unique type of regulation occurs at PD MCS where the transfer of molecules occurs parallel to the membranes, as opposed to orthogonal to them. In these ER-PM MCS, the intermembrane space may not be solely regulated by the tethers, lipids, and cytoskeleton elements in the super-continuum, but also by wall polymers (e.g. callose), which are locally synthesized around the PD structure (Petit et al., 2020).

Future MCS research: What's in the plant toolkit?

MCS are microdomains with an intermembrane distance below the resolution limit of conventional fluorescence microscopy and with a dynamic behavior that requires the use of live-cell compatible techniques (McFarlane et al., 2017). In recent years, advances in super-resolution microscopy (e.g. Total Internal Reflection Fluorescence, Structure illumination microscopy, (Figure 10F), and electron tomography (Figure 10G) are providing for the first time detailed high-resolution visualizations and 3D reconstructions of the MCS ultrastructure in plants (Baillie et al., 2020). In parallel, the use of optical laser tweezers to manipulate plant MCS *in vivo* is facilitating the characterization of putative MCS components such as the AtCASP tether identified at ER-Golgi MCSs (Osterrieder et al., 2017) and the mitochondria-associated GTPase AtMiro2 at ER-mitochondria contact sites (White et al., 2020). Plant MCS research is also adopting genetically encoded tools such as synthetic tethers that bridge nearby membranes (e.g. MAPPER-GFP, (Lee et al., 2019), or split-fluorescence systems (e.g. split super-folder (sp) GFP proteins, (Li et al., 2020) to visualize MCS contacts. These artificial systems, however, have limited use in functional studies, as their expression could induce non-physiological changes in the MCS structure. Additional molecular tools with broad applications, such as inducible phosphoinositide depletion systems (Doumane et al., 2021) and phosphoinositide fluorescent markers (Simon et al., 2014) are currently being adopted for MCS research and represent promising avenues to elucidate the roles of anionic phospholipids in plant MCS function and dynamics.

We predict that the combination of collaborative research, technical advances, and novel molecular tools in this quickly evolving field will provide breakthroughs that will transcend plant MCS research.

DIVERSITY IN PD FORM AND FUNCTION

(Written by Tessa M. Burch-Smith)

General PD structure

PD evolved multiple times in the plant lineage and are present in some groups of algae and in all land plants (Brunkard et al., 2015; Azim and Burch-Smith, 2020), 2020). In general, PD provide continuity of the PMs and cytoplasm across cell walls. In land plants and some algae, PD also include a central strand of the ER (Botha, 1992),(Ding et al., 1992; Franceschi et al., 1994; Cook et al., 1997). The cytoplasmic and membrane connectivity provided by the PD is the route for intercellular trafficking of numerous biomolecules, effectively rendering the plant a continuous cytoplasm (a symplast). PD are therefore essential for plant growth, development and environmental responses. Some molecules traffic through PD by passive diffusion, and their movement depends on the size of the molecules and the trafficking capacity of the pores. Other molecules are targeted to PD through the use of the endomembrane system (Spiegelman et al., 2019). A typical cell wall is pervaded by hundreds or thousands of PD that are often clustered into groups (pitfields), and as such the continuity between adjacent cells can be extensive.

PD are nanopores with outer diameters (delimited by the PMs of connected cells) ranging from 25 to 50 nm, depending on the tissue and species, and they extend for the length of cell wall thickness. Thus, much of what is known about PD structure is derived from TEM (Figure 11). The center of land plant PD is occupied by a structure called the desmotubule (DT). The DT was observed to be continuous with the cortical ER of connected cells and is now recognized as an intercellular strand of modified ER. The DT diameter is constrained to approximately 15-20 nm (Ding et al., 1992; Schulz, 1995), and so the desmotubule comprises the most tightly curved biological membranes described to date (Tilsner et al., 2011). The DT does not include a typical ER lumen. Instead, the space is largely occupied by proteins (Tilney et al., 1991), whose likely function is to enable the tight curvature of the membranes, e.g. the ER tubulating reticulon proteins (Tilsner et al., 2011; Knox et al., 2015; Kriechbaumer et al., 2015). The DT is tightly connected to the PM of the PD by structures originally described as spokes (Ding et al., 1992). The cytosol-filled space between the DT and PM is called the cytoplasmic sleeve or annulus and is likely the main route for PD trafficking, although the spoke proteins divide it into nanochannels 2-3 nm wide.

Analysis of PD isolated from Arabidopsis suspension cell culture identified 1,341 proteins as the putative PD proteome (Fernandez-Calvino et al., 2011). Of these, 21% were membrane proteins and included proteins previously identified as PD resident, e.g. PDLP1 (Thomas et al., 2008) and ATBG_papp (Levy et al., 2007). In addition, several GPI (glycosylphosphatidylinositol**)**-anchored proteins and proteins associated with the secretory pathway were identified. Further refinement of the PD proteome identified Multiple C2 domains and transmembrane region proteins (MCTPs) as PD constituents, and they have been designated as the likely spokes of PD (Brault et al., 2019). The spokes control spacing between the DT and PM, and this distance is correlated with the developmental states of PD (Nicolas et al., 2017a). Interestingly, in Arabidopsis roots, PD lacking cytoplasmic sleeves apparently had a higher trafficking capacity than PD with cytoplasmic sleeves (Nicolas et al., 2017a), raising questions about how trafficking via those PD is achieved. There are a few reports of trafficking through the DT lumen, although the DT membranes appear to provide a surface for cell-to-cell movement (Guenoune-Gelbart et al., 2008; Barton et al., 2011). The DT membranes are important conduits for the transport of at least some viruses between cells (Guenoune-Gelbart et al., 2008). The routes for PD trafficking and the contributions of the membranes and spaces to the movement of cargo molecules remain open questions in PD biology.

The lipid composition of the PM of PD is also distinct from the bulk PM. The PM of PD from Arabidopsis suspension cells is enriched in sterols and sphingolipids with saturated very long chain fatty acids (Grison et al., 2015), which is consistent with the presence of lipid microdomains akin to lipid rafts in PD (Raffaele et al., 2009; Tilsner et al., 2013) and GPI-anchored proteins in the PD proteome (Fernandez-Calvino et al., 2011). PD lipid composition is also important for PD protein composition and function, as changes in lipids affect the ultrastructure and permeability of PD (Grison et al., 2015; Yan et al., 2019; Iswanto et al., 2020; Liu et al., 2020). As described in the section on plant MCS, a modern view of PD considers both its unique lipid and protein composition to describe PD as specialized MCS (Brault et al., 2019; Petit et al., 2019; Ishikawa et al., 2020). A simple generalized PD structure is represented in Figure 11A.

PD formation and distribution

PD are intrinsic components of the cell walls found in almost all connected cell walls in a plant. Primary PD form at the end of cell division, during cytokinesis, when strands of ER become encased in the developing cell plate. The reticulon proteins RTNLB3 and 6 and MCTPs are involved in this process (Knox et al., 2015; Brault et al., 2019). The presence of substructures like the DT in newly formed PD is uncertain, as revealed by TEM (Ehlers and van Bel, 2010). Secondary PD form across existing cell walls where cell division is not occurring. The insertion of these new PD is likely necessary to establish or maintain symplastic connectivity, as in graft unions or when cells divide and grow (Ehlers and Kollmann, 2001). Studies in Arabidopsis trichomes suggest that new secondary PD form in close proximity to existing PD, as described in the multiple twinning model (Faulkner et al., 2008). It is proposed that PD divide by fission, although the mechanism for this is unclear.

PD may also be removed from existing cell walls by a still unknown process. Studies on cambial division and vascular differentiation have shown that PD numbers can increase and decrease over the lifespan of a given cell-cell interface; this would necessarily involve the removal and insertion of PD at a given interface (Ehlers and van Bel, 2010; Fuchs et al., 2010). In other instances, PD can be drastically modified or even truncated to disrupt intercellular trafficking. For example, guard cell initials contain PD, and PD trafficking is critical for guard cell development (Guseman et al., 2010). As stomata develop, however, PD are lost from the guard-cells, rendering them symplastically isolated (Wille and Lucas, 1984). It may be that PD removal is a more common occurrence in plant cell development and differentiation than previously reported. How secondary PD form and how PD are removed are other open questions that await exploration: advanced imaging approaches hold promise for generating answers to these questions.

Structure-function relationships in PD

PD are often depicted as simple linear structures traversing the cell wall (Figure 11A), but PD structure is much more diversified. PD are often branched, consisting of multiple pores

1525 that connect in the vicinity of the cell wall middle lamella (Figure 11B-D). This is captured
1526 by studies on PD structure using three-dimensional approaches such as electron
1527 tomography.
1528
1529 The formation and origins of branched PD are unclear, but they likely arise through
1530 modification of existing simple PD (Burch-Smith et al., 2011). This diversity in structure
1531 suggests diversity in function. Exemplary studies of PD in tobacco leaves undergoing the
1532 sink-source transition demonstrated that simple PD were converted to branched PD
1533 contemporaneously with decreased import of fluorescent dye (Oparka et al., 1999;
1534 Roberts et al., 2001). Another common variation of PD form is the dilation of PD pores
1535 away from the PD openings or the constriction of PD at their necks (region just below the
1536 opening, Figure 11B). This PD variation seems to correlate with PD maturation (Nicolas
1537 et al., 2017b) or with trafficking capacity (Ding et al., 1992). Mathematical modeling
1538 supports the notion that dilation increases trafficking capacity as the cell wall thickens
1539 (Deinum et al., 2019), a correlation previously observed by TEM (Nicolas et al., 2017b).
1540 Another PD form that has a specialized role in trafficking is the 'funnel PD' in sink root
1541 tissue (Ross-Elliott et al., 2017). These PD have wide openings at the phloem sieve
1542 elements that narrow considerably as they cross the cell wall and open on the phloem-
1543 pole pericycle, creating a 'funnel' shape (Figure 11B). The specialized PD shape appears
1544 to facilitate the unloading of sucrose in the root phloem. Mathematical modelling supports
1545 the need for this unusual PD form to allow phloem unloading at physiological sucrose
1546 concentrations.
1547
1548 Specialized PD forms have also been reported at sites where sugars are loaded into the
1549 phloem in source tissues. For example, PD at the phloem parenchyma-companion cell
1550 interface in Arabidopsis leaf veins have many openings to the phloem parenchyma but
1551 only one to the companion cells (Haritatos et al., 2000). These distinct PD forms correlate
1552 with specialized functions, and they raise the possibility of PD sub-functionalization
1553 between tissues and even at a given cell-interface. PD sub-functionalization is an
1554 intriguing concept that has proven difficult to investigate due to the lack of experimental
1555 approaches that allow perturbation of specific PD. The development of appropriate

genetic, imaging, and computational methods will be necessary to address this critical aspect of PD function. Undoubtedly, a comprehensive understanding of PD will enable novel approaches to engineering solutions to help overcome challenges in plant growth and development.

SO MUCH MORE THAN BRICKS AND MORTAR: PLANT CELL WALLS AS DYNAMIC EXTRACELLULAR "ORGANELLES"

(Written by Charles T. Anderson)

Much as our skin protects us from the environment but is also itself an organ, the plant cell wall can be thought of as a protective "organelle" for the plant cell; however, it is not bound by a membrane but instead encases the PM-delimited protoplast that contains the intracellular organelles. Our understanding of cell wall composition, structure, and mechanics has expanded rapidly over the past decade due to advances in high-resolution imaging (Zeng et al., 2017; Rydahl et al., 2018; Voiniciuc et al., 2018; Zhao et al., 2019), biochemical and spectroscopic analyses of wall polymers and their interactions down to single-molecule and nanoscale levels (Voxeur et al., 2019; Zhao et al., 2020; Cai et al., 2021), and new computational modeling methods that relate wall mechanics to the deformations, movements, and interactions of individual wall polymers (Zhang et al., 2021). In contrast to its previous conception as simply "dead wood" that is the inert product of polymer secretion by plant cells, the plant cell wall is starting to be appreciated as a dynamic structure that changes over time and encompasses specialized metabolic processes, including the polymerization, coalescence, binding/unbinding, cleavage, and re-ligation of wall polymers that facilitates both plant growth and the processing of plant biomass for human use (Obro et al., 2011). Cell walls serve as conduits of intercellular transport of nutrients, secreted peptides, hormones, and other metabolites (Ramakrishna and Barberon, 2019), arenas where extracellular vesicles can deliver small RNAs to silence virulence genes in plant pathogens (Cai et al., 2018), and surveillance zones where plants can sense pathogen-generated wall fragments (Vaahtera et al., 2019) to

help maintain wall integrity (Rui and Dinneny, 2020). Together, these ideas highlight how the apoplast, the extracellular compartment in which the cell wall resides, enables previously unappreciated forms of trafficking and acts as a molecular frontier in the interactions between plants and their abiotic and biotic environments.

Cell wall assembly and structure

Extending the analogy with human skin, the plant cell wall can expand along with the cell it encases, and it also helps sense and transduce important environmental and mechanical information. However, the analogy is not perfect: as a biomaterial with elements approaching the tensile strength of steel, the cell wall also acts as a flexible but strong coating that shapes its occupying cell, determining its final shape. Cellulose, the most abundant biopolymer on Earth, forms the “girders” of the cell wall as its primary load-bearing component. Cellulose is extruded directly into the apoplast by multi-subunit Cellulose Synthase Complexes (Wilson et al., 2021), which move through the PM along trajectories that are likely driven by the force of polymerization and are guided by either cortical microtubules (Figure 12) or existing wall patterning (Chan and Coen, 2020). The estimated 18 catalytic subunits in each Cellulose Synthase Complex (Nixon et al., 2016) produce strands of β-1,4-linked glucose that coalesce into cable-like microfibrils that are predicted to have 18-24 chains (Yang and Kubicki, 2020). Forming the “cross-beams” and “insulation” between the cellulose “girders” are matrix polysaccharides that include pectins and hemicelluloses. Pectins are acidic polysaccharides that are composed of homogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II domains (Anderson, 2019), whereas hemicelluloses contain mostly neutral sugars and include xyloglucans, xylans, and mannans (Scheller and Ulvskov, 2010). In growing cells, matrix polysaccharides initially interact with cellulose upon their secretion at the cell surface, following polymerization in the Golgi lumen and post-Golgi trafficking (Hoffmann et al., 2021) (Figure 12). Both pectins and hemicelluloses can associate with the surfaces of cellulose microfibrils, potentially preventing cellulose agglomeration and thus assembling a strong but deformable wall that also contains glycoproteins, enzymes, metabolites, ions, and water (Cosgrove, 2018). In the secondary walls produced by certain cell types, a polyphenolic, hydrophobic compound called lignin is also deposited (Dixon and Barros,

2019). In many cell types, the wall is deposited in layers with differing cellulose orientations, conferring multidirectional resistance to mechanical failure.

Dynamics and functions of plant cell walls

What happens to the strong but flexible wall as a plant cell grows? Atomic force microscopy (Zhang et al., 2017) and coarse-grained modeling (Zhang et al., 2021) indicate that cellulose microfibrils bend, bundle, unbundle, and slide during experimentally imposed or computationally simulated wall deformation, respectively. One open question is exactly how cellulose behaves in the growing cells of living plants, where wall deposition is often ongoing, matrix polysaccharides can also undergo reorganization (Anderson et al., 2012), and wall-modifying enzymes act to modulate cell growth (Xiao et al., 2014). Also unclear is the extent to which extracellular ATP and other energetic compounds might be used in wall metabolism, in addition to their functions as signaling molecules (Pietrowska-Borek et al., 2020). PD allow for rapid communication and transport between adjacent plant cells; however, some cell types, such as stomatal guard cells, lack these connections but must nonetheless transmit and receive information with other cells, underscoring the importance of apoplastic trafficking as a mode of intercellular communication in plants. Membrane receptors on the cell surface link events in the cell wall to intracellular signaling pathways (Vaahtera et al., 2019), allowing the plant cell to adapt to changing environmental conditions and withstand pathogen attack, although the extent to which these receptors sense biochemical, chemical, and/or mechanical cues has not been fully worked out. Cell walls are highly diverse across plant tissues and taxa (Hoffmann et al., 2021) and allow cells to adopt myriad shapes and perform specialized functions that include nutrient and water absorption (e.g., root epidermal cells), transport (e.g., xylem and phloem), and secretion (e.g., aerial epidermal and nectary cells). Autodegradation of the plant cell wall allows for developmental processes that include organ abscission and pollen dehiscence, and might allow for the recycling of some wall components to produce new wall polymers (Barnes and Anderson, 2018). Overall, the plant cell wall is a fascinating biological environment, one that we are only beginning to be able to understand well enough to be able to engineer ourselves.

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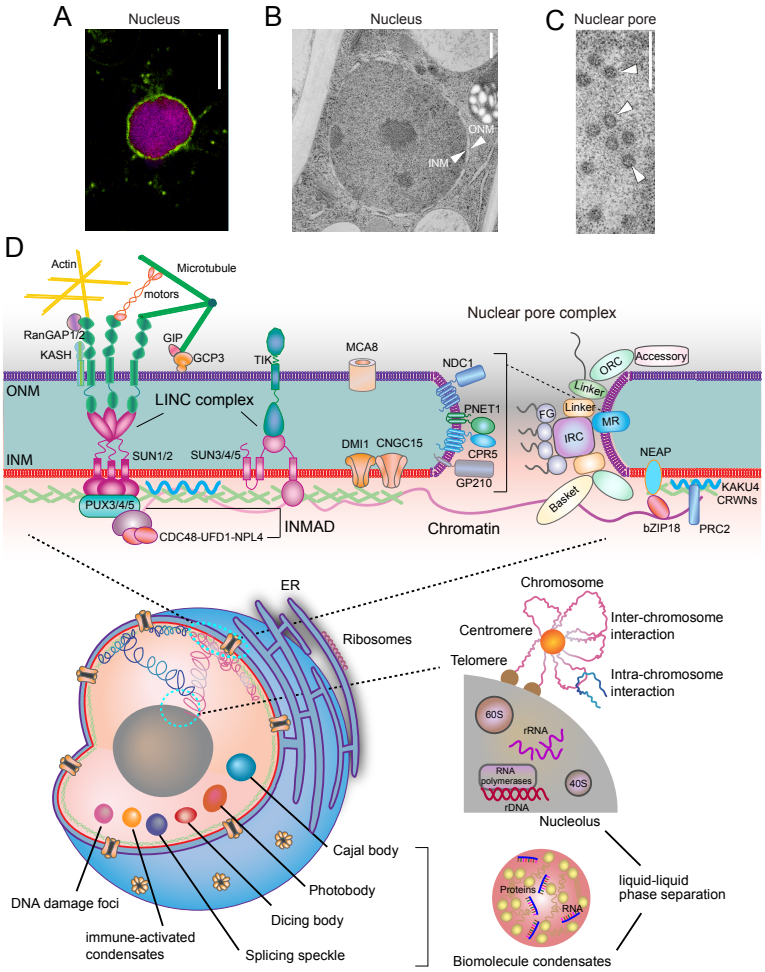

Figure 1. The nucleus and its constituents. A, A fluorescence micrograph of a nucleus in a *Nicotiana benthamiana* epidermal cell. The nuclear envelope (NE)-localized CPR5 protein (pseudo-colored in green) was coexpressed with the nucleoplasmic-localized cyclin kinase inhibitor SIM (pseudo-colored in magenta). B, An electron micrograph of a nucleus in an Arabidopsis root cell. Arrowheads indicate the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) of the NE. C, An electron micrograph showing a tangential section through the nuclear envelope in an Arabidopsis root cell. Arrowheads indicate nuclear pores distributed at the surface of the NE. Scale bars are 10 μm in A and 500 nm in B and C. D, The nucleus is defined by the double-layered NE composed of the ONM and the INM, which join at the nuclear pore membrane. The NE hosts a specific population of proteins. SUN and KASH proteins comprise the LINC complex and function in various aspects of plant cell biology and physiology, as discussed in the main text. CPR5, PNET1, GP210, and NDC1 are structural components of the plant nuclear pore complex (NPC) membrane ring (MR). CNGC15, DMI1, and MCA8 regulate nuclear calcium transport and signaling and affect symbiotic interaction with arbuscular mycorrhiza. GCP3 and GIP proteins are part of the microtubule nucleation complex and regulate nuclear stiffness. CRWN and KAKU4 proteins assemble the plant nuclear skeleton and also function as a platform to interact with INM proteins and regulate chromatin organization by binding to chromatin-associating proteins (such as the PRC2 complex). NEAP proteins bind to the transcription factor bZIP18 and may also influence chromatin organization. The CDC48-UFD1-NPL4 trimeric complex and PUX3/4/5 proteins mediate plant INM-associated protein degradation (INMAD). The nuclear interior is organized heterogeneously. Heterochromatic regions and chromocenters are typically located near the nuclear periphery and the nucleolus. Other multivalent biomolecules (e.g., proteins and RNAs) aggregate to form various types of membrane-less condensates via the liquid-liquid phase separation mechanism.

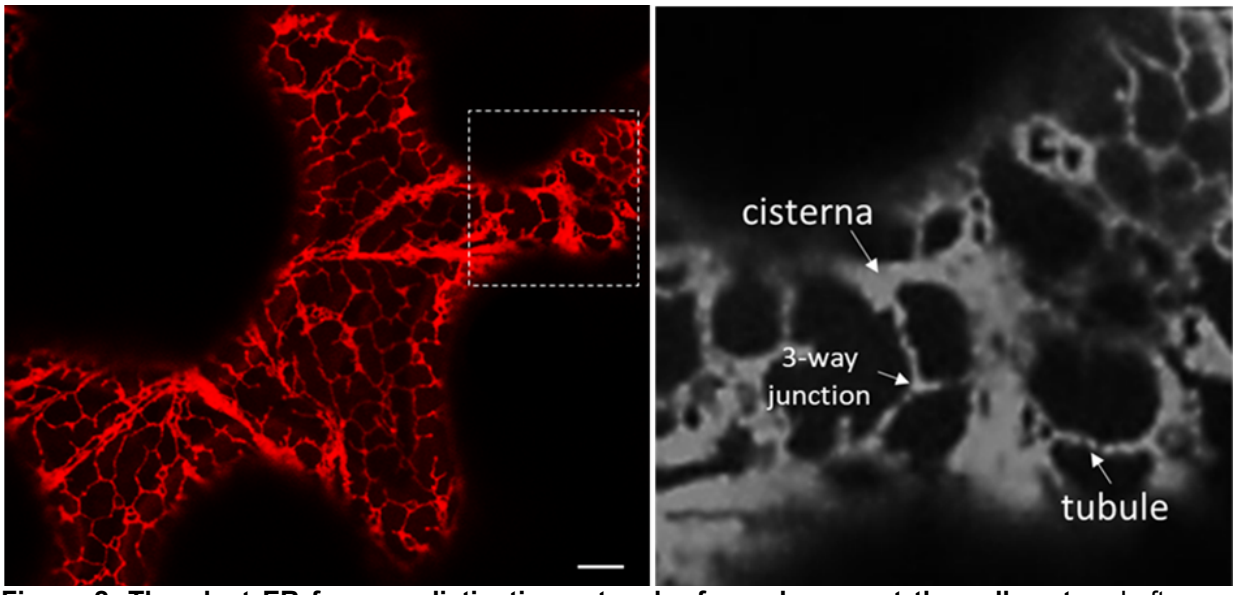

Figure 2. The plant ER forms a distinctive network of membranes at the cell cortex. Left panel: Confocal microscopy image of a *Nicotiana benthamiana* leaf epidermal cell transiently expressing the fluorescent lumenal marker ER-mCherry (Nelson et al., 2007), which labels the lumen of the bulk ER network. Scale bar = 40 mm. Right panel: magnified view of the boxed region in the left panel highlighting some of the characteristic ER structures discussed in the main text.

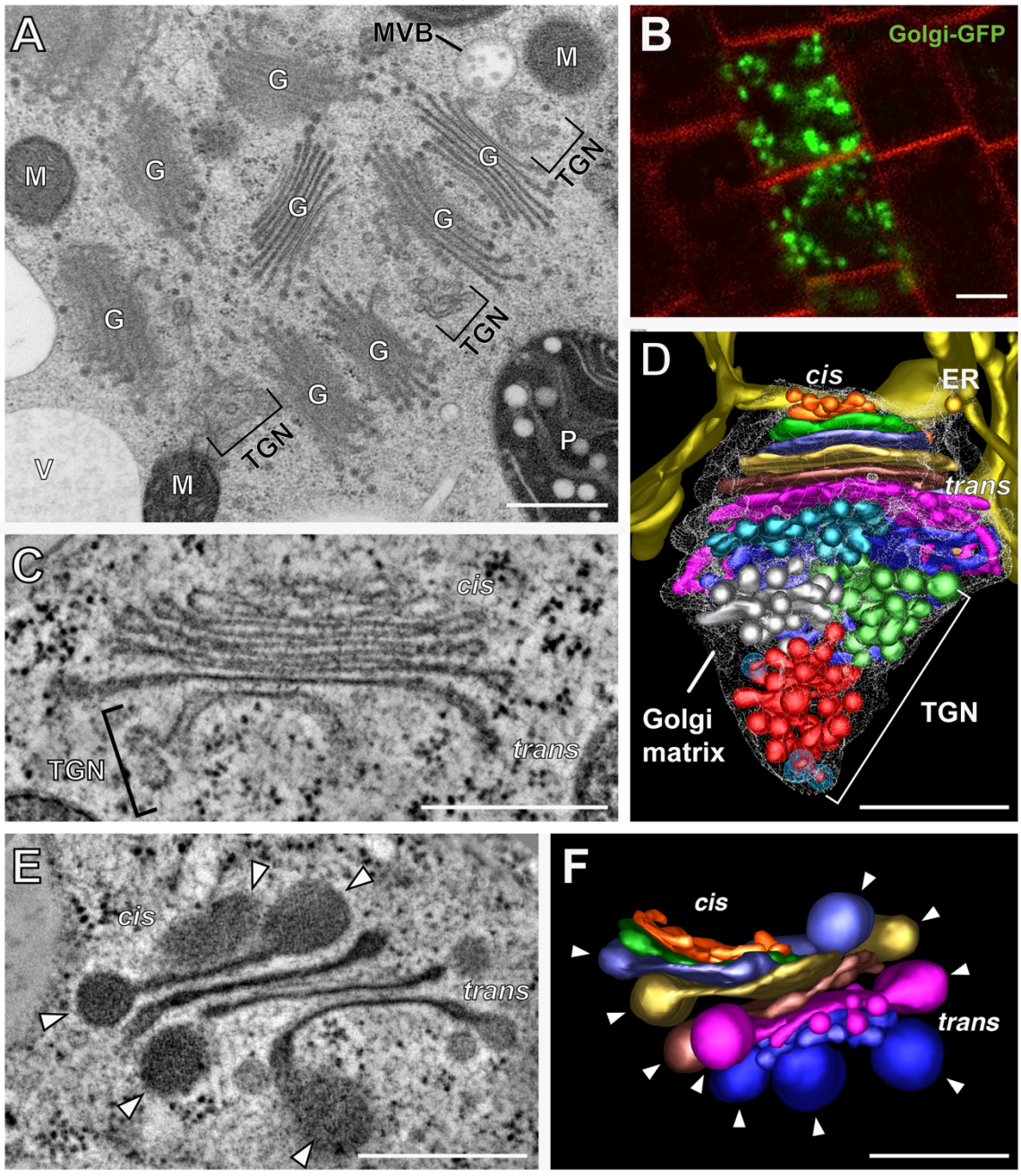

Figure 3. Plant Golgi stacks. A, Transmission electron micrograph showing a cluster of Golgi stacks in an Arabidopsis root tip cell. Plastids (P), mitochondria (M), and vacuoles (V) are marked. B**,** Confocal laser scanning micrograph of Arabidopsis root tip cells expressing a Golgi-localized green fluorescent protein. The plasma membrane was counterstained. C**,** ET slice image of a Golgi stack. The cis-side, trans-side and trans-Golgi network (TGN) are labeled. D, ET model of an Arabidopsis Golgi stack associated with the endoplasmic reticulum (ER). The entire Golgi and TGN are encompassed by a ribosome-ribosome excluding matrix (Golgi matrix). E, ET slice image of a Golgi stack in a root cap border cell. F, ET model of the Golgi in E. Swollen cisternal margins containing mucilage are marked with arrowheads in E and F. Scale bars in A, B, D, E, and F: 500 nm. Scale bar in C: 10 μm.

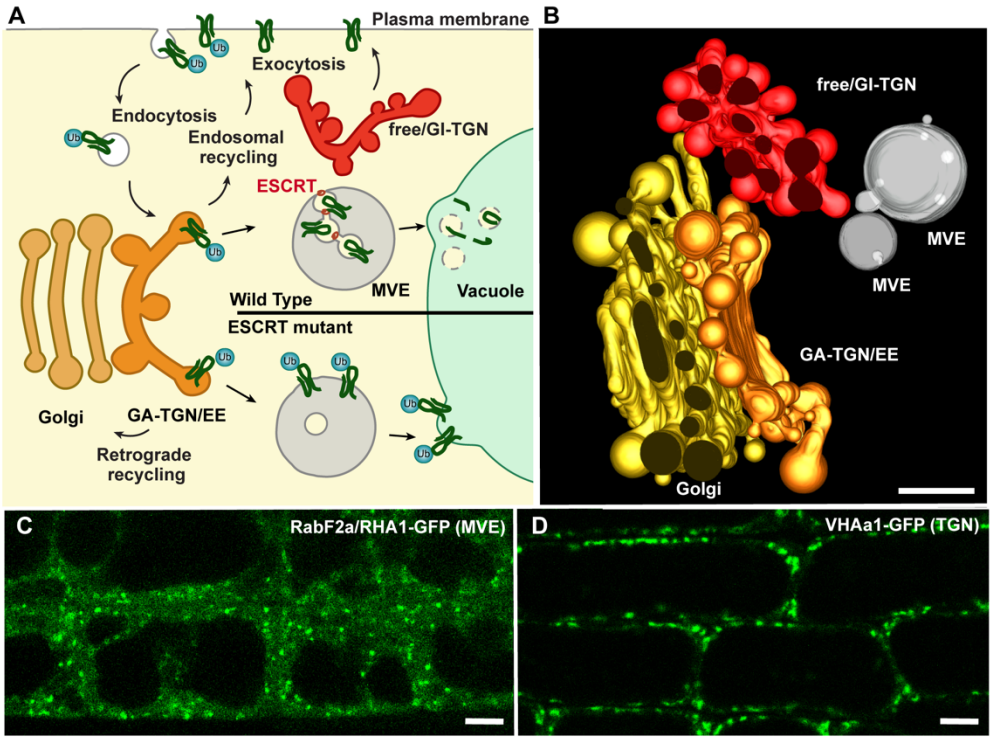

Figure 4. Plant endosomes. A, Diagram of plant endosomes and the major associated pathways, highlighting the effects on MVE mis-sorting in ESCRT mutants. B, Tomographic reconstructions of a Golgi stack, Golgi-associated TGN (GA-TGN), and free/Golgi independent (GI-TGN) in an Arabidopsis embryo cells. C-D, Confocal images of MVE-localized RabF2a/RHA1-GFP (C) and TGN-localized VHAa1-GFP (D) in Arabidopsis root cells. Scale bar = 200 nm in (B) and 5 mm in (C) and (D).

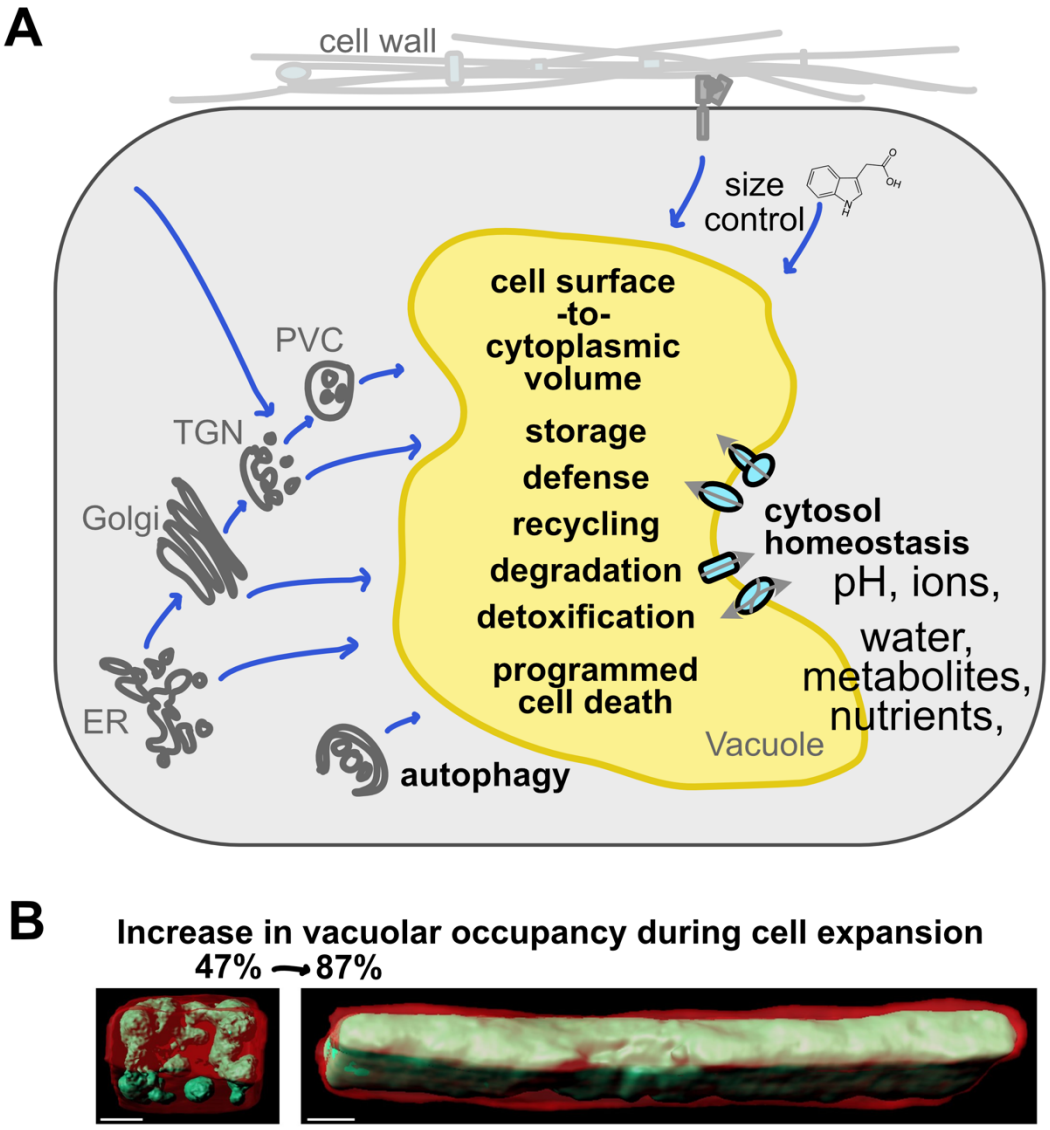

Figure 5. The multifunctional roles of the plant vacuole. A, There are various trafficking routes towards the vacuole, including pathways from the pre-vacuolar compartment (PVC), trans-Golgi network (TGN), Golgi, endoplasmic reticulum (ER), and autophagosomes. The vacuole carries out numerous indispensable functions as indicated. B, Confocal-based 3D reconstruction of the cell (in purple, based on cell wall staining with propidium iodide) and the vacuole (in green, based on BCECF-AM staining) visualizes the vacuolar occupancy of meristematic (left) and elongating cells (right). Scale bars: 6 μm.

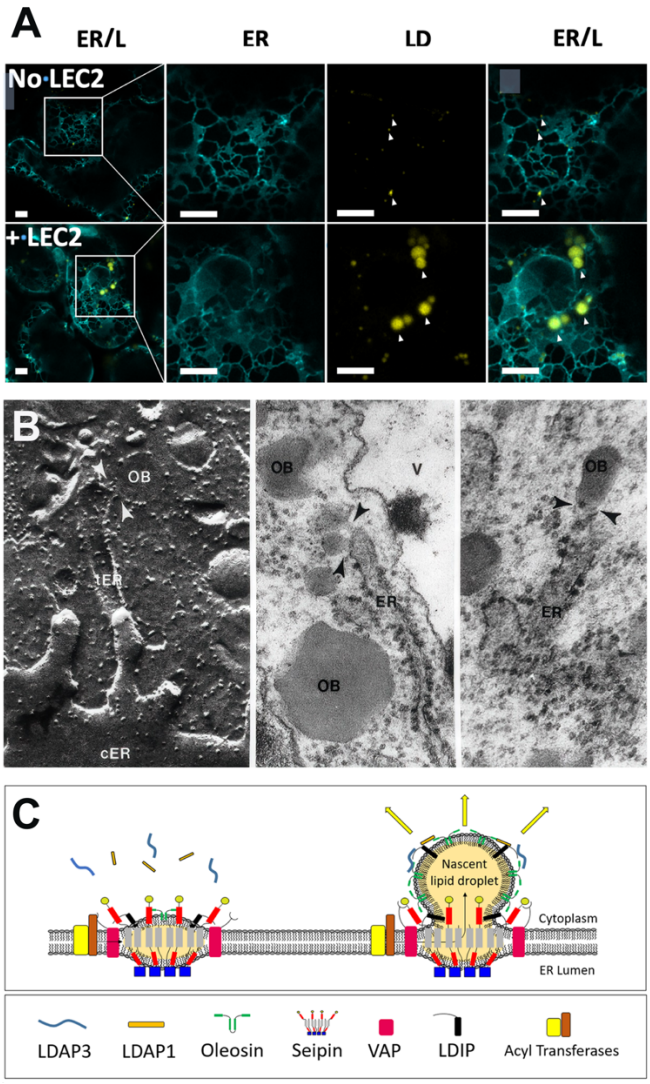

Figure 6. Spatial association of LDs with the ER and a model of LD biogenesis. A, Enhanced-resolution fluorescence imaging of the relationship of the ER to LDs in leaf mesophyll cells of *N. benthamiana* infiltrated with an ER marker and stained with the LD-specific fluorescent dye BODIPY 493/503. The ER network was marked in cyan with the ER-lumen marker protein Kar2-CFP-HDEL, and LDs are false-colored in yellow (white arrows). In leaves, small LDs are normally associated with the ER (top row). In this system, LDs were induced to proliferate by expressing lipogenic factors to study LD proteins and their roles in LD formation (second row). Here this process is illustrated by expressing LEAFY COTYLEDON2 (LEC2) in these leaves; this transcription factor is preferentially expressed in developing seeds and promotes storage lipid synthesis and LD formation. Under semi-normal conditions, the LD phenotype of tobacco shows few small LD's intimately connected to the ER. Scale bars: 5 µm. B, TEM micrographs showing LDs (labeled as OB for oil body) emerging from the ER in cells of developing soybean (*Glycine max*) cotyledons. Left to right- freeze-fracture; cryofixation; chemical fixation. Arrows mark ER-LD junctions. For scale, ribosomes on the ER membrane are approximately 20 nm in diameter. Electron micrographs are courtesy of Dr. Eliot Herman, University of Arizona. C, Diagram illustrating the current, general model for LD biogenesis. Initial LD formation begins with the coalescence of the "lipid lens" within the ER bilayer. Various LD-associated proteins such as SEIPINs, LDIP, LDAPs, VAP27-1, oleosins (in seeds), and LDIP are recruited, which together facilitate the formation and stabilization of the nascent LD as it emerges into the cytoplasm. Adapted in part from a model presented and described in Greer et al. (2020).

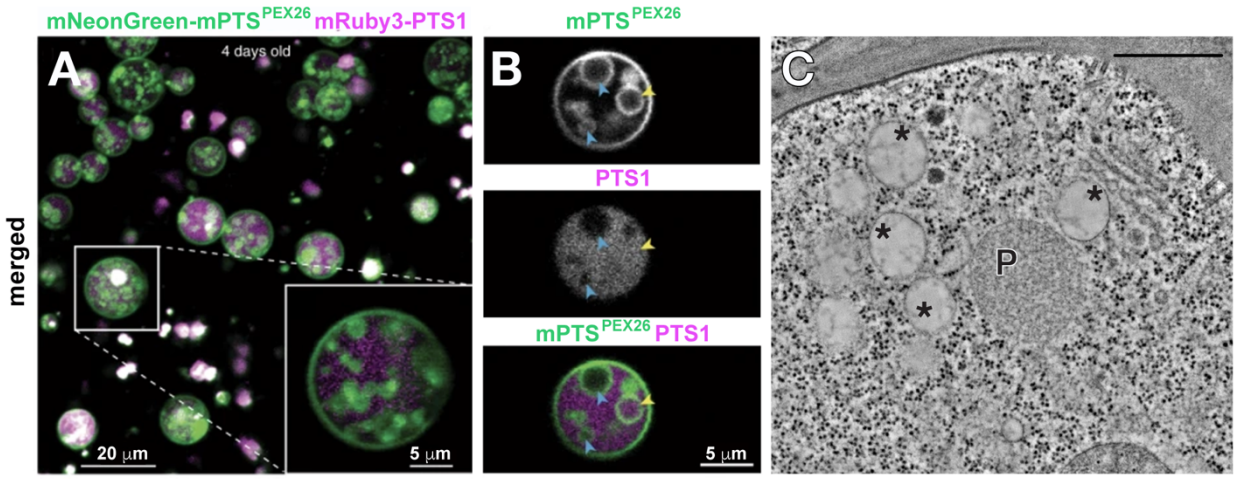

Figure 7. Microscopic images of peroxisomal structures in Arabidopsis cells. A, Microscopic image of 4-day-old Arabidopsis cotyledons expressing mNeonGreen with a membrane peroxisomal targeting signal (mNeonGree-mPTSPEX26; green) and mRuby with a matrix-bound peroxisomal targeting signal (mRuby3-PTS1; magenta) showing the presence of intralumenal vesicles (ILVs) in peroxisomes. The close-up image highlights the variable sizes of the vesicles. B, Separate images of fluorescent molecules in the membrane (green) and matrix (magenta) highlight the different substructures within the peroxisome, including ILVs with (yellow arrowheads) or without (blue arrowheads) matrix proteins and a separate area with denser membrane accumulation. Images in (A) and (B) are Figures 1G and 6A from Wright and Bartel (2020; reprinted with permission). C, ET slice image of a young root cell highlighting the interactions between a peroxisome (P), lipid droplets (*), and other organelles. Scale bar: 500 nm.

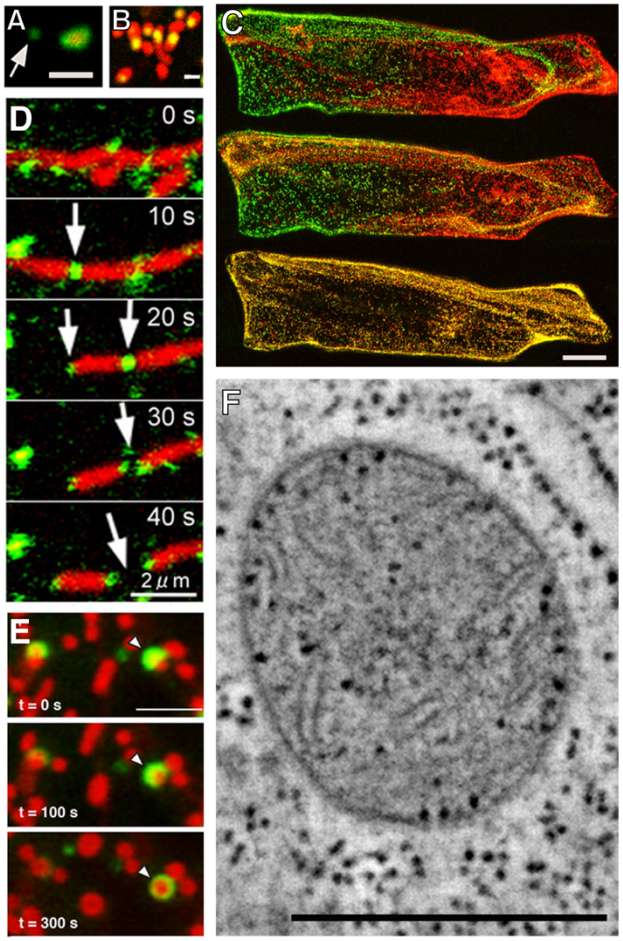

Figure 8. Microscopy imaging of plant mitochondrial dynamics. A, An apparent mitochondrial outer-membrane derived vesicle (MDV) (arrow) in an Arabidopsis cell. On the right is a mitochondrion whose outer membrane was stained with ELM1-GFP and whose matrix was stained with RFP. The MDV contains only the outer membranes and no matrix (Yamashita et al., 2016, reprinted with permission). B, Heterogeneity of DNA contents in mitochondria. The mitochondria were stained red with MitoTracker Red and DNA was stained with SYBR Green I. Green signals in red regions are shown in yellow. Therefore, red particles with yellow dots represent mitochondria containing DNA, and red mitochondria without yellow dots represent mitochondria lacking DNA (Arimura et al., 2004, reprinted with permission). C, Fusion of mitochondria in an onion bulb epidermal cell. The cell contains thousands of mitochondria. The mitochondria on the left and right sides of the cell were labeled green and red, respectively, by the (irreversibly) color-changing fluorescent protein Kaede. The photographs show the movement and mixing of the mitochondria after 10 minutes (upper), one hour (middle), and two hours (bottom). Yellow mitochondria are the result of fusion between green and red mitochondria (Arimura et al., 2004, reprinted with permission). D, Five consecutive frames showing mitochondria fission in a tobacco BY-2 cell. The mitochondria were stained with MitoTracker Red and dynamin-related protein 3A was labeled with GFP (Arimura 2018, reprinted with permission). E, Progression of mitophagy in an Arabidopsis cell, in which the mitochondria were stained with MitoTracker Red and autophagosomes were visualized by expression of YFP-ATG8e. The autophagosome on the right (arrowhead) is shown engulfing a mitochondrion over a 300 s interval (Ma et al., 2021, reprinted with permission). F, ET image of a mitochondrion in an Arabidopsis root meristematic cell. Black dots in the cytosol and mitochondrial matrix are ribosomes. Scale bars. A, D, and E, 2 μm; B, 1 μm; C, 40 μm; F, 500 nm.

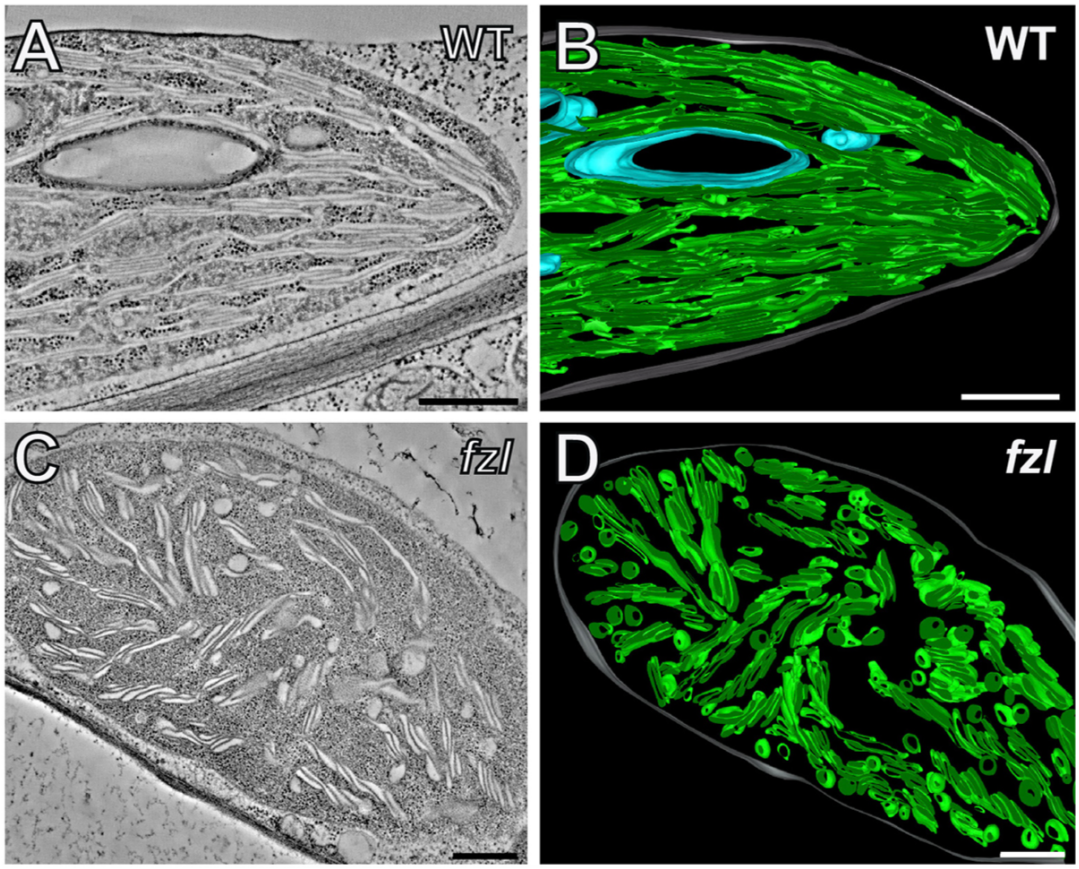

Figure 9. **Chloroplast morphogenesis is a highly regulated process.** A, ET slice image of a normal-sized wild-type (WT) chloroplast with typical thylakoid differentiation into stacked (grana) and unstacked domains. B, 3D model based on the chloroplast in (A). Green represents thylakoid membrane; blue represents starch grains. C, ET slice image of an oversized chloroplast (compare scale bars) with aberrant thylakoid membrane organization in an Arabidopsis *flz* mutant (Liang et al., 2018b). FLZ is a dynamin-like protein, and thylakoid fusion is inhibited in the mutant (Gao et al., 2006; Findinier et al., 2019). Instead of a stroma-wide network, thylakoids form discrete spirals in the mutant. D, 3D model based on the chloroplast in (C). Scale bars = 500 nm.

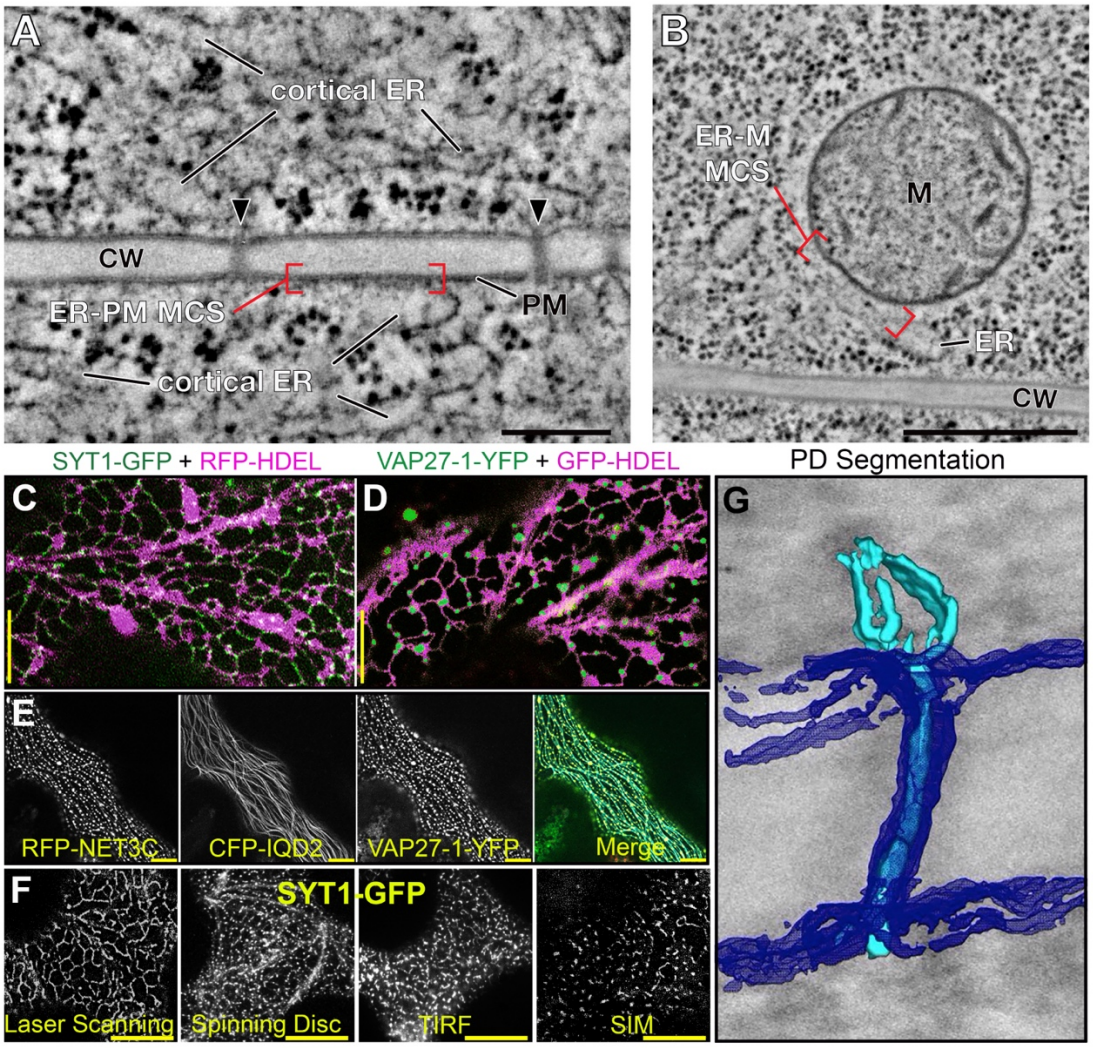

Figure 10. Examples of membrane contact sites in plants. A-B, ET slice images showing different MCS present in plant cells; an ER-PM contact site (A) and an ER-mitochondrion contact site (B) are shown. Arrowheads mark plasmodesmata. CW: cell wall, M: mitochondrion. Scale bars = 500 nm. C-D, The distribution of SYT1-GFP- and VAP27-1-YFP-labelled tethering assemblies in different regions of the cortical ER (indicated by the RFP-HDEL or GFP-HDEL markers) highlights the presence of spatially separated ER-PM MCS within the cell. E, The co-expression of the actin-associated NET3C cytoskeletal adaptor, the microtubule-associated IQ67-domain 2 (IQD2) bridging component, and the VAP27-1 tether highlights the interaction of the Arabidopsis ER-PM MCS with the cortical cytoskeleton. Scale bars in (C-E) = 10 μM. F, The appearance of putative SYT1-GFP labelled ER-PM contact sites changes depending on the microscopy technique used. The intermembrane distances at MCS are below the light diffraction limit and are not properly resolved using conventional confocal microscopy (Laser Scanning/Spinning Disc, left two panels). More accurate visualizations are obtained using super-resolution techniques (TIRF/SIM, right two panels). Scale bar in (F) = 20 μM. G, Advances in electron tomography techniques are enabling accurate 3D reconstructions of PD MCS. In the current functional models, the cytosolic space between the ER and the PM inside the PD serves as a trafficking conduit for mobile molecules, and the adjustment of its width is believed to regulate their flow rate, effectively controlling inter-cellular trafficking. Dark blue: Plasma Membrane. Light Blue: Cortical ER across the PD pore. (Panel E is from Zang et al. 2021, reprinted with permission.)

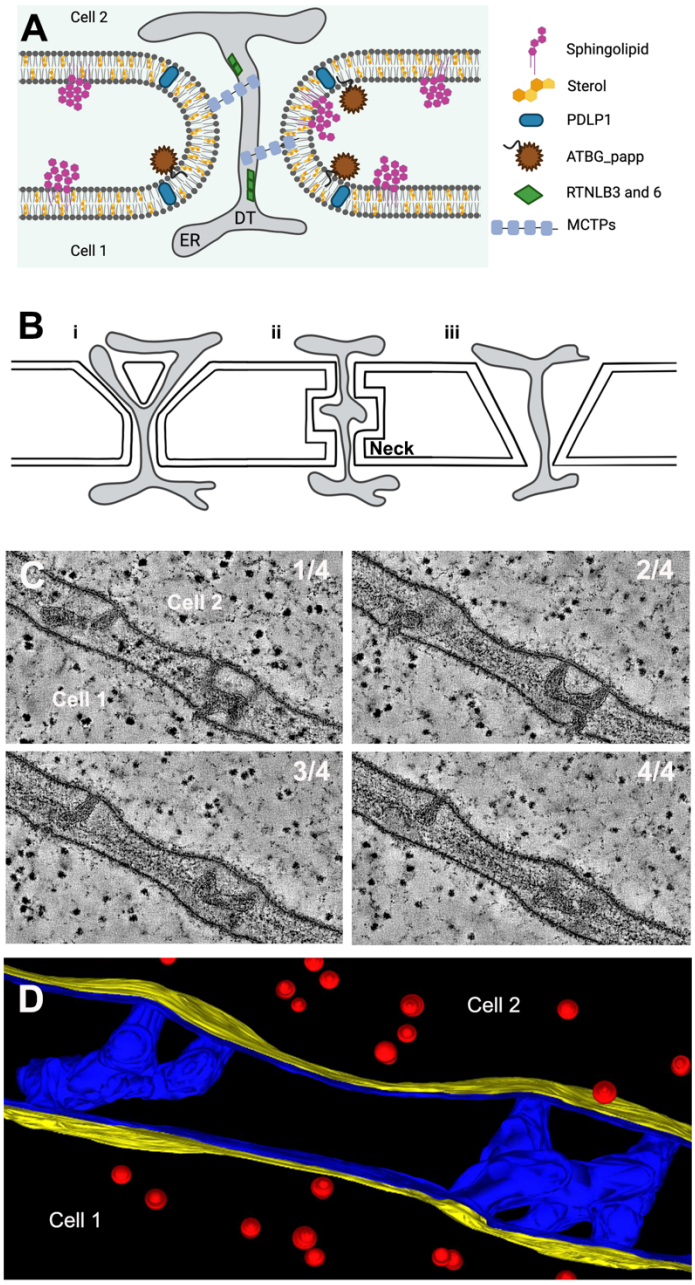

Figure 11. Structural diversity in PD and their constituents. A, A cartoon depiction of a simple plasmodesma showing details of the plasma membrane, lipid composition, and select protein constituents as described in the text. ER, endoplasmic reticulum; DT, desmotubule. B, Cartoons depicting some PD morphologies. (i) is a branched PD with a 'Y' shape, (ii) represents a simple pore with constrictions near the openings (necks) and dilation of the central region of the DT; (iii) is a funnel plasmodesma. (A) and (B) were drawn with BioRender. C-D, Structure of branched PD in Arabidopsis leaf tissue revealed by ET. (C) Four representative individual frames from a tomogram (1/4 - 4/4). While the PM is readily visible in these images, the desmotubule is difficult to discern. Central cavities are found in the vicinity of the middle lamella. (D) 3D model of PD generated by tracing the inner (yellow) and outer (blue) leaflets of the PM in the tomogram in (C). The PD on the left consists of two pores in Cell 2 and one in Cell 1. The PD on the left has two openings to Cell 2 but three to Cell 1. Ribosomes (red) are shown for scale. (C) and (D) were generated in the author's lab.

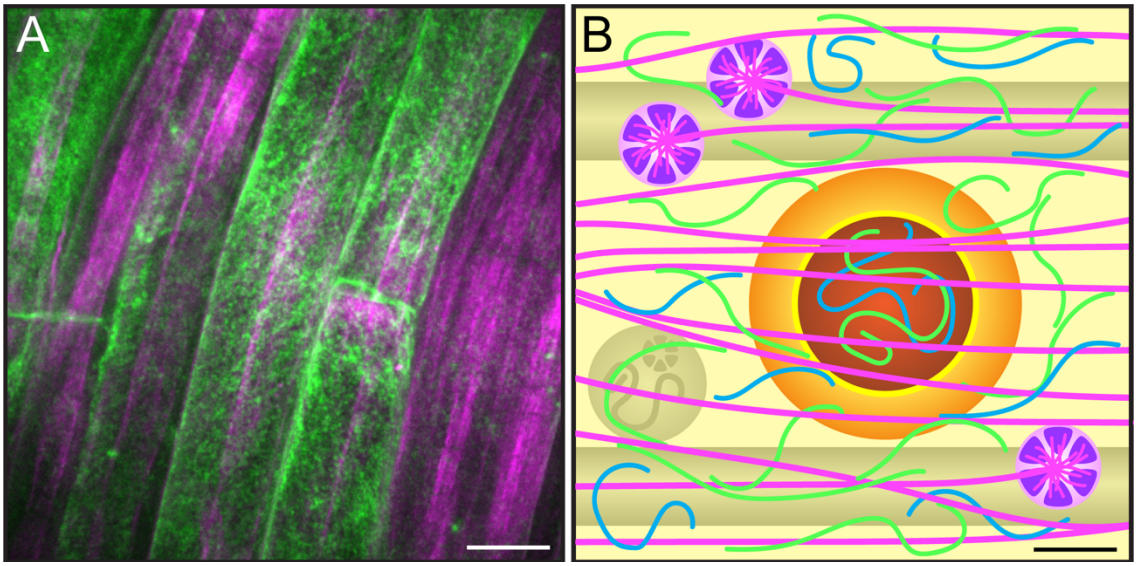

Figure 12.

Micrograph and model of the plant cell wall, showing wall patterning at the tissue and nanometer scales. A, Cellulose labelled with Pontamine Fast Scarlet 4B (S4B, magenta) and newly synthesized pectin labelled with fucose-alkyne and Alexa488-azide (green) in epidermal cells of the root differentiation zone in a 5-day-old Arabidopsis seedling. Note oblique, punctate labelling of the Alexa488 signal, predominantly longitudinal labelling of the S4B signal, and variation in intensity of the Alexa488 signal between different cells. Bar = 10 μm. B, Model of cell wall assembly viewed from outside the plasma membrane (yellow), showing Cellulose Synthase Complexes (purple) producing cellulose microfibrils (magenta) and a vesicle (orange) fusing with the plasma membrane to deliver pectin (green) and hemicellulose (cyan) to the wall. Cortical microtubules and an intracellular vesicle are shown in grey in the background. Objects are drawn approximately to scale, bar = 25 nm. Part B of this figure was inspired by a dynamic model of cell wall assembly created by Drew Berry for the Australian Research Council Center of Excellence in Plant Cell Walls and directed by Tony Bacic (University of Melbourne), Monika Doblin (University of Melbourne), and Mike Gidley (University of Queensland), which can be viewed on YouTube (https://youtu.be/zp2WW2TYcng).