

REVIEW

Spatio-temporal control of post-Golgi exocytic trafficking in plants

Liam Elliott, Ian Moore* and Charlotte Kirchhelle[‡]

ABSTRACT

A complex and dynamic endomembrane system is a hallmark of eukaryotic cells and underpins the evolution of specialised cell types in multicellular organisms. Endomembrane system function critically depends on the ability of the cell to (1) define compartment and pathway identity, and (2) organise compartments and pathways dynamically in space and time. Eukaryotes possess a complex molecular machinery to control these processes, including small GTPases and their regulators, SNAREs, tethering factors, motor proteins and cytoskeletal elements. Whereas many of the core components of the eukaryotic endomembrane system are broadly conserved, there have been substantial diversifications within different lineages, possibly reflecting lineage-specific requirements of endomembrane trafficking. This Review focusses on the spatio-temporal regulation of post-Golgi exocytic transport in plants. It highlights recent advances in our understanding of the elaborate network of pathways transporting different cargoes to different domains of the cell surface, and the molecular machinery underpinning them (with a focus on Rab GTPases, their interactors and the cytoskeleton). We primarily focus on transport in the context of growth, but also highlight how these pathways are co-opted during plant immunity responses and at the plant–pathogen interface.

KEY WORDS: Endomembrane trafficking, Plant cytoskeleton, Rab GTPases

Introduction

Like all eukaryotes, plants rely on their complex endomembrane system to maintain cellular function. Different endomembrane compartments and trafficking pathways are highly diverse in their spatial organisation within plant cells; whereas some endomembrane compartments are relatively uniformly distributed, other compartments and trafficking pathways are highly polarised (Brandizzi and Wasteneys, 2013; Furt et al., 2012; Kirchhelle et al., 2016; Seguí-Simarro and Staehelin, 2006). For instance, a quantitative survey of organelle distribution in the moss *Physcomitrella patens* revealed distinct localisation patterns for different organelles (Furt et al., 2012). Strikingly, the tips of apical caulonema, but not chloronema cells exclude chloroplasts and peroxisomes, but are significantly enriched in Golgi bodies, indicating that distinct subcellular localisation patterns are associated with specialised cell types (Furt et al., 2012). Subcellular localisation can also be associated with physiological responses; for example, in stomatal guard cells, the endoplasmic reticulum (ER) is predominantly located in the connecting region where two guard cells meet when stomata are closed, but relocalises to the dorsal cell side

when stomata open – a pattern not observed for other endomembrane organelles (Higaki et al., 2012). Polarised trafficking is particularly prevalent during growth, where different regions of cells grow at vastly different rates and cells display elaborate biochemical polarisation (reviewed by Elliott and Kirchhelle, 2019; Nakamura and Grebe, 2018). Polarised trafficking is also a prominent feature during pathogen infection and subsequent immune responses, which involve trafficking to and from discretely localised infection sites (reviewed by Rivero et al., 2019).

Organised intracellular transport relies on the motility of endomembrane compartments, which utilise the cytoskeleton of the cell as a dynamic scaffold to mediate general long-distance transport as well as more-precise targeting of different endomembrane compartments (Geitmann and Nebenführ, 2015). In contrast to what is seen in metazoans, long-distance transport in plant cells occurs mainly along actin bundles and is mediated by myosin XI motor proteins (Avisar et al., 2008; Peremyslov et al., 2008), whose maximum speeds exceed those of microtubule-associated kinesin motors (Nebenführ and Dixit, 2018). Rapid actin-myosin transport is the driving force of cytoplasmic streaming, which promotes the even distribution of endomembrane compartments and cytosolic components throughout the cell; thus, among other factors, this allows plant cells to sustain their large sizes (Tominaga et al., 2013; Verchot-Lubicz and Goldstein, 2010). Although initially believed to be less relevant in plants, microtubule-based transport is emerging as an important mechanism for targeted trafficking in plant cells (Brandizzi and Wasteneys, 2013).

To reach their appropriate domain within the cell, endomembrane compartments with different functions associate selectively with the appropriate cytoskeletal machinery. Among the many proteins that mediate interactions of endomembrane compartments with other intracellular machineries, Rab GTPases are considered key regulators (Olikkonen and Stenmark, 1997). Rab GTPases are found in all eukaryotes, and Rab-like proteins may also exist in some prokaryotes (Surkont and Pereira-Leal, 2016). Rab GTPases cycle between a GTP-bound membrane-associated active state and a GDP-bound cytosolic inactive state, regulated by interacting factors, including guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Membrane-bound Rab GTPases can recruit a myriad of other proteins involved in compartment motility (e.g. motor proteins), docking (e.g. tethering factors) and fusion [e.g. soluble N-ethylmaleimide attachment protein receptors (SNAREs)], and are thus key regulators of endomembrane compartment identity and pathway specificity (Grosshans et al., 2006). The 57 Rab GTPases of *Arabidopsis thaliana* fall into eight clades named Rab-A to Rab-H (Rutherford and Moore, 2002; see Table 1). Members of the Rab-A, Rab-E and Rab-H clades have been connected to the regulation of post-Golgi trafficking to and from the cell surface (Woollard and Moore, 2008) in the context of growth and immunity. In recent years, evidence has emerged that these Rab GTPases mediate functionally diverse trafficking routes, transporting either specific cargoes and/or delivering cargo to specific spatial domains. We are still at the beginning of

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.

*Deceased

[‡]Author for correspondence (charlotte.kirchhelle@plants.ox.ac.uk)

© L.E., 0000-0001-9925-039X; I.M., 0000-0002-1301-1329; C.K., 0000-0001-8448-6906

Table 1. Overview of the *Arabidopsis* Rab GTPases

| Rab GTPase clade | Subclade | Localisation | Suggested trafficking function | References |
|--------------------------|--------------------|--|--|---|
| Rab-A (related to Rab11) | Rab-A1 (9 members) | TGN/EE and PM | <i>De novo</i> secretion from TGN/EE to PM? Recycling from TGN/EE to PM (non-basal and basal?) Cell plate biogenesis | Asaoka et al., 2013; Berson et al., 2014; Choi et al., 2013; Feraru et al., 2012; Qi et al., 2011, Qi and Zheng, 2013 |
| | Rab-A2 (4 members) | TGN/EE and PM | <i>De novo</i> secretion from TGN/EE to PM? Non-basal recycling from TGN/EE to PM Cell plate biogenesis | Chow et al., 2008; Li et al., 2017 |
| | Rab-A3 | TGN/EE | | Chow et al., 2008 |
| | Rab-A4 (5 members) | TGN/EE | <i>De novo</i> secretion from TGN/EE to PM FLS2 endocytosis from the PM | Choi et al., 2013; Kang et al., 2011; Preuss et al., 2004, 2006; Szumlanski and Nielsen, 2009 |
| | Rab-A5 (5 members) | Cell edge compartments (and TGN/EE) | <i>De novo</i> secretion from TGN/EE to PM? Cell plate biogenesis | Kirchhelle et al., 2016, 2019 |
| | Rab-A6 (2 members) | TGN/EE? | FLS2 endocytosis from the PM | Choi et al., 2013 |
| Rab-B (related to Rab2) | Rab-B1 (3 members) | Golgi? | ER-to-Golgi trafficking | Cheung et al., 2002 |
| Rab-C (related to Rab18) | Rab-C1 | Uncertain - Golgi/post-Golgi compartments? | Unknown | Geldner et al., 2009 |
| | Rab-C2 (2 members) | | | |
| Rab-D (related to Rab1) | Rab-D1 | Golgi and TGN/EE | ER-to-Golgi trafficking | Batoko et al., 2000; Pinheiro et al., 2009; Saint-Jore et al., 2002; Zheng et al., 2005 |
| | Rab-D2 (3 members) | | | |
| Rab-E (related to Rab8) | Rab-E1 (5 members) | Golgi, TGN/EE and PM | Golgi-to-PM trafficking | Camacho et al., 2009; Speth et al., 2009; Zheng et al., 2005 |
| Rab-F (related to Rab5) | Rab-F1 | TGN/EE subdomain and LEs | TGN/EE-to-LE trafficking LE-to-PM trafficking | Ebine et al., 2011; Ito et al., 2010; Kotzer et al., 2004; Lee et al., 2004; Sohn et al., 2003; Singh et al., 2014; Ueda et al., 2004 |
| | Rab-F2 (2 members) | | | |
| Rab-G (related to Rab7) | Rab-G1 | LEs and Tonoplast | LE-to-vacuole trafficking and possibly autophagous trafficking | Bottanelli et al., 2011, 2012; Cui et al., 2014; Geldner et al., 2009; Kwon et al., 2010, 2013; Nielsen et al., 2008 |
| | Rab-G2 | | | |
| | Rab-G3 (6 members) | | | |
| Rab-H (related to Rab6) | Rab-H1 (5 members) | Golgi (and TGN/EE) | Golgi-to-PM? Required for proper exocytosis of CSCs | Chow et al., 2008; He et al., 2018; Johansen et al., 2009 |

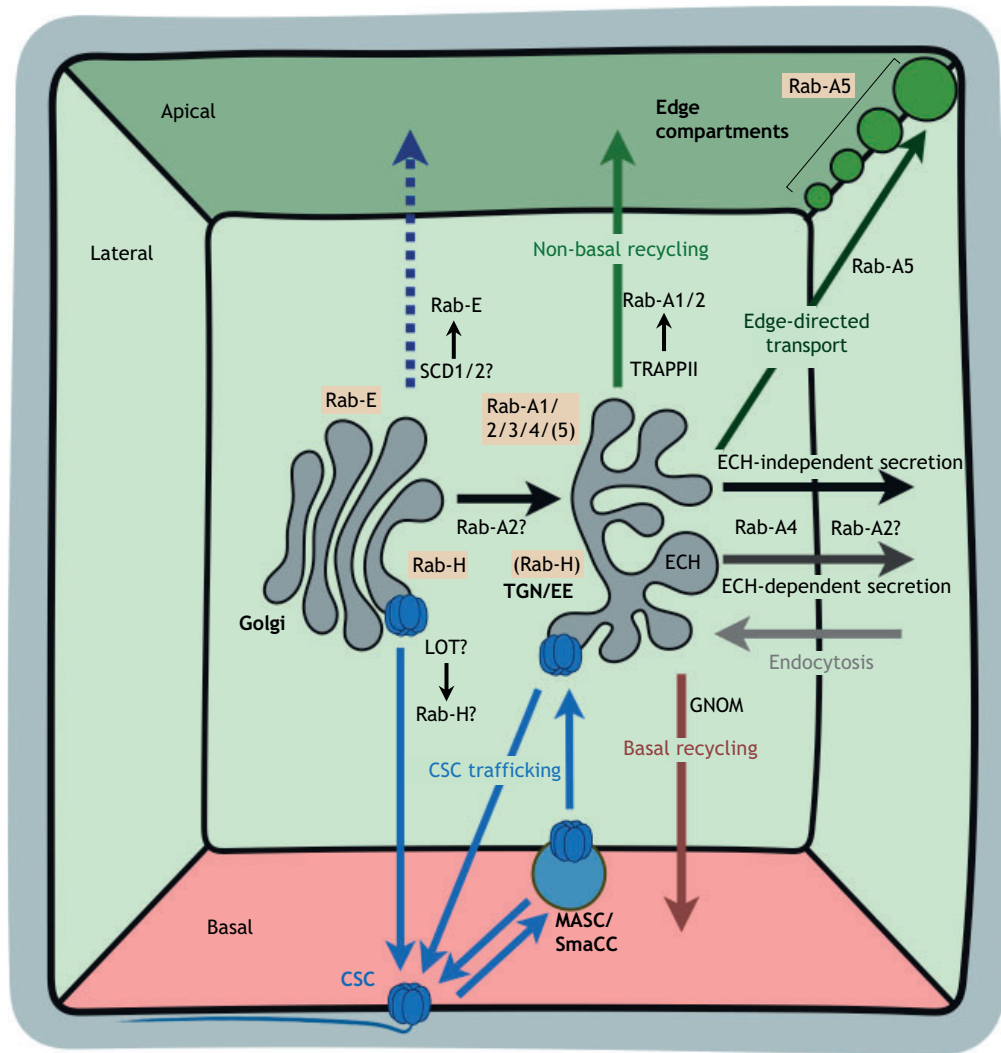
Close mammalian orthologues for all clades are listed, although it should be noted that some mammalian clades, in particular the Rab-E and Rab-F clade orthologous, have undergone substantial diversification in the mammalian lineage. For a comprehensive phylogeny, refer to Rutherford and Moore (2002).

understanding how the diverse Rab GTPases map onto these different pathways, and how they orchestrate other molecular components to control the spatio-temporal organisation of membrane pathways. In this Review, we provide an overview of recent insights into the diverse post-Golgi trafficking routes to the plasma membrane (PM) in plants, and discuss how the diverse clade of Rab GTPases contributes to it. We further highlight how cytoskeletal components and their interactors can orchestrate the spatial organisation of these pathways in developing plant cells. We conclude by reviewing how surface-bound transport routes and their associated Rab GTPases are co-opted during plant biotic interactions and plant immunity.

Many routes to the cell surface

In most growing plant cells, different regions within the same cell grow at vastly different rates and have biochemically distinct

identities (Mansfield et al., 2018; reviewed by Elliott and Kirchhelle, 2019; Nakamura and Grebe, 2018), necessitating precise spatio-temporal control of different transport routes to and from the cell surface (Fig. 1). A key organelle in this context is the *trans*-Golgi network/early endosome (TGN/EE), a tubulo-vesicular post-Golgi compartment at which secretory and endocytic pathways intersect (Chow et al., 2008; Dettmer et al., 2006; Gendre et al., 2015; Kang et al., 2011; Viotti et al., 2010). The TGN/EE arises through maturation of the *trans*-most Golgi cisterna (Gendre et al., 2015; Kang et al., 2011; Staehelin and Kang, 2008) and comprises several functional subdomains, which are specified by distinct, but partially overlapping sets of proteins (Chow et al., 2008; Ravikumar et al., 2018; Viotti et al., 2010; Woollard and Moore, 2008). Whereas certain cargo might be able to bypass the TGN/EE (Crowell et al., 2009), most secreted cargo molecules, including membrane-bound and secreted proteins and cell wall matrix polysaccharides, are



AQ3

Fig. 1. Golgi-PM trafficking pathways in *Arabidopsis*. Schematic representation of trafficking routes and associated Rab GTPases and their regulators. Steady-state Rab GTPase localisations based on immunolocalisation or fluorescent fusions are indicated with beige shading. Brackets indicate sites at which wild-type Rab GTPases accumulate to lesser extents. Trafficking pathways with experimental support are shown by solid arrows, more speculative pathways by dotted lines. Rab GTPases and their associated regulators linked to specific trafficking routes are shown next to each route, question marks indicate speculative associations. Golgi-localised RAB-H1b has been implicated in CSC secretion from the Golgi (blue arrow), and RAB-E1d has been speculated to mediate a Golgi-to-PM route (blue dotted arrow). Several secretory routes from the TGN/EE to the cell surface exist: pectins, xyloglucans, and the model cargo proteins PIN2 and PIN3, LAX and BRI1 are secreted through a route dependent on the TGN/EE-resident protein ECH (black arrow), whereas AUX1 secretion is ECH-independent (dark grey arrow). The Rab-A4 clade has been linked to secretion of pectins and xyloglucans from the TGN/EE, but it is not clear whether it, or another Rab clade, is involved in ECH-independent secretion. Another secretory route from the TGN/EE to cell edges (dark green arrow) has been identified through RAB-A5c, which specifically localises to cell edge compartments. The TGN/EE also receives endocytic cargo from the cell surface (light grey arrow). At least two recycling pathways are specified back from the TGN/EE to different domains of the PM: a GNOM-mediated, basal route (red arrow), and a non-basal route (green arrow) mediated by Rab-A1 and Rab-A2 GTPases. Rab-A2 has also been speculated to mediate a Golgi-to-TGN/EE-to-PM route. CSCs can be rapidly endocytosed into MASCs/SmaCCs and recycled back to the cell surface (blue arrows). Rab proteins involved in CSC transport to and from MASCs/SmaCCs are not known.

thought to pass through the TGN/EE (Gendre et al., 2015). For example, xyloglucan and pectin epitopes have been detected at the TGN/EE through immunolocalisation (Stierhof and El Kasmi, 2010), and mutations in the TGN/EE-resident protein ECHIDNA (ECH) and its redundantly acting interactors YPT/RAB GTPase-INTERACTING PROTEIN 4a and 4b (YIP4a and YIP4b) are impaired in pectin and xyloglucan secretion, resulting in dramatic growth defects at the cell and organ level (Gendre et al., 2013, 2011; McFarlane et al., 2013). The secretion of several polar localised PM proteins [including PIN-FORMED2 and 3 (PIN2 and PIN3), LIKE AUXIN RESISTANT1 (LAX) and BRASSINOSTEROID INSENSITIVE1 (BRI1)] from the TGN/EE is not dependent on ECH, although it is required for AUXIN RESISTANT1 (AUX1)

secretion (Boutte et al., 2013; Gendre et al., 2015, 2011), indicating that independent secretory routes exist from the TGN/EE to the cell surface (Fig. 1).

In addition to secretory trafficking, the TGN/EE is also involved in orchestrating endocytosis and recycling. Spatially confined endocytosis and polarised recycling are key mechanisms for the establishment and maintenance of polarised localisation of proteins, like PINs, at the PM of different cell faces (Dhonukshe et al., 2007; Kitakura et al., 2011; Kleine-Vehn et al., 2009, 2011). At least two recycling routes that target different spatial domains have been identified in *Arabidopsis* roots: one route involving the large ARF-GEF GNOM mediates transport to the basal cell face (Geldner et al., 2003), and an additional route was recently identified through use of

the pharmacological agent Endosidin16 (ES16; Li et al., 2017). In ES16-treated *Arabidopsis* roots, recycling to the basal face is unperturbed. However, ES16 disrupted recycling of the non-polarised PM proteins PLASMA MEMBRANE INTRINSIC PROTEIN 2a (PIP2a) and PENETRATION1 (PEN1), the laterally polarised REQUIRES HIGH BORON1 (BOR1) and NOD26-LIKE INTRINSIC PROTEIN5;1 (NIP5;1), and apically polarised PIN proteins in different cell layers, indicating that in addition to the basal route, root cells specify a 'non-basal' recycling route controlling apical, lateral and non-polarised recycling.

There is evidence that plant cells can also specify recycling routes that bypass the TGN/EE, which has been revealed through the study of cellulose synthase (CESA) complex (CSC) trafficking. The abundance and distribution of CSCs in the PM (where cellulose fibres are synthesised) is critical for appropriate cell wall assembly, as both enhanced and reduced levels of CSCs and their altered distribution at the PM are associated with cell wall morphology and growth defects (Polko et al., 2018; Polko and Kieber, 2019; Sampathkumar et al., 2013). CESA can be stable *in vivo* over days (Hill et al., 2018), vastly exceeding the estimated CSC residence time at the PM of 7–15 mins (Paredes et al., 2006; Sampathkumar et al., 2013), indicating that regulated transport and recycling of CSCs, rather than overall protein turnover are key determinants of their abundance at the PM. Intracellular reservoirs of CSCs are found in the Golgi and TGN/EE (Haigler and Brown, 1986) as well as so-called microtubule-associated cellulose synthase compartments/small CESA compartments (MASC/SmaCCs) (Crowell et al., 2009; Gutierrez et al., 2009), which are believed to play an important role in CSC recycling (Fig. 1). In response to osmotic stress or pharmacological treatment, CSCs from the PM are endocytosed to form MASCS/SmaCCs, and CSCs from this intracellular reservoir can then be rapidly recycled back to the PM (Lei et al., 2015).

Taken together, these findings demonstrate that plant cells specify independent trafficking routes to the cell surface, which may either target distinct subcellular domains, or transport specific cargo molecules. In the next section, the regulatory machinery involved in specifying these different transport routes will be discussed.

Rabs en route

Members of the Rab-A clade localise predominantly to the TGN/EE (Fig. 1; Table 1), and it has been speculated that the dramatic, lineage-specific diversification of this clade in plants allowed the functional diversification of trafficking pathways to and from the plant TGN/EE (Woollard and Moore, 2008). All members of the Rab-A2 clade and the single RAB-A3 GTPase localise to the same subdomain of the TGN/EE, which can function as an early endosome (Chow et al., 2008). In meristematic root cells, RAB-A1a, -A1b and -A1c localise to the same compartment sensitive to brefeldin A (BFA, a fungal toxin that causes aggregation of endosomal membranes in plant cells), and partially overlap with the TGN/EE markers SYNTAXIN OF PLANTS43 (SYP43) and VACUOLAR PROTON ATPASE A1 (VHA-a1), as well as VESICLE-ASSOCIATED MEMBRANE PROTEIN721 and 722 (VAMP721 and VAMP722), two largely redundant R-SNARES involved in secretory trafficking (Asaoka et al., 2013; Feraru et al., 2012). Furthermore, both RAB-A1b and RAB-A1c co-localise almost completely with RAB-A2a, indicating that Rab-A1, Rab-A2 and Rab-A3 act at the same TGN/EE domain (Feraru et al., 2012; Qi et al., 2011).

The GTPase-deficient constitutively active RAB-A2a[Q71L] and RAB-A1b[Q71L] variants reside predominantly at the PM, indicating RAB-A2a and -A1b are involved in an exocytic

trafficking route to the PM (Asaoka et al., 2013; Chow et al., 2008). The subcellular localisation of a preferentially GDP-bound inactive RAB-A2a[S26N] variant was shifted towards the Golgi, which may indicate involvement in a secretory pathway from the Golgi to the PM (Chow et al., 2008). However, RAB-A2a has also been implicated in regulating non-basal recycling based on phenotypic and biochemical interactions with ES16 (Li et al., 2017). ES16 partially protected RAB-A2a from degradation in a drug affinity-responsive target stability (DARTS) assay, indicating direct interaction, and ES16 treatment of roots caused RAB-A2a to become mislocalised into large aggregates. Furthermore, expression of the constitutively active RAB-A2a[Q71L] variant partially suppressed root inhibition caused by ES16 treatment, whereas expression of dominant-negative RAB-A2a[S26N] enhanced root inhibition (Li et al., 2017). Finally, in BFA-washout experiments in the presence of RAB-A2a[S26N], recycling of apically localised PIN2, but not of basally localised PIN1, was perturbed, phenocopying the effect of ES16.

RAB-A1b has also been implicated in non-basal recycling of PIN2 and PIP2a through the dominant-negative RAB-A1b[S156F] protein variant identified in a screen for mutants defective in exocytic trafficking in the presence of BFA (Feraru et al., 2012). However, the same protein variant intriguingly also caused defects in basal PIN1 recycling after prolonged BFA treatment (Feraru et al., 2012), suggesting either that, in contrast to RAB-A2a, RAB-A1b contributes to both basal and non-basal recycling pathways, or to unspecific perturbation of recycling under extended BFA treatment. The S156F mutation is situated in the GTP-binding domain of RAB-A1b, but is distinct from those generally used to generate Rab GTPase mutants with deficiencies in nucleotide binding or hydrolysis (Feraru et al., 2012; Rutherford and Moore, 2002). However, a preferentially GDP-bound RAB-A1b[S27N] variant displayed similar defects in PIN1 and PIN2 trafficking in the presence of BFA (Feraru et al., 2012). Further evidence that Rab-A1 and Rab-A2 members mediate non-basal recycling has emerged from studies of the transport protein particle (TRAPP), a multi-subunit complex which has been proposed to act as GEF for members of the Rab-A2 and Rab-A1 subclades at the TGN/EE (Kalde et al., 2019; Qi et al., 2011). In root cells of two TRAPPII-specific subunit mutants, in *trs120* and *trs130*, non-basal PIN2, but not basal PIN1, polarity was disrupted, a defect that could be partially rescued by overexpression of constitutively active RAB-A1c (Qi et al., 2011). Similarly, overexpression of constitutively active RAB-A2a could partially suppress the severe morphological phenotype of a *trs130* mutant (Kalde et al., 2019), indicating that a TRAPP complex acts upstream of Rab-A1 and Rab-A2 GTPases to regulate non-basal recycling from the TGN/EE to the PM during growth (Fig. 1). However, RNAi-mediated suppression of *BET5*, a core TRAPP subunit predicted to be present in all TRAPP complexes, causes defective polarisation of both PIN1 and PIN2 (Zhang et al., 2018), suggesting that distinct TRAPP complexes can function in both non-basal and basal recycling routes.

Members of the Rab-A4 subclade are also implicated in *de novo* secretory trafficking from the TGN (Fig. 1). RAB-A4b preferentially localises to budding sites of secretory vesicles at the TGN/EE in electron tomographic images and co-fractionates with membrane fractions containing xyloglucan and pectin epitopes (Kang et al., 2011). *In vitro*, RAB-A4b specifically interacts with the phosphatidylinositol 4-OH kinase PI-4Kβ1 and both colocalise at the tips of growing root hairs (Preuss et al., 2006, 2004). Loss of function of PI-4Kβ1 and the closely related PI-4Kβ2 cause aberrations in secretory vesicles budding off the TGN/EE, with

secretory vesicles being both fewer and larger in size (Kang et al., 2011; Preuss et al., 2006). Although secretory trafficking of cell wall polysaccharides appears not to be completely abolished, cell geometry in *pi-4k β 1 pi-4k β 2* double-mutants is aberrant, plants are overall stunted, and polarised root hair growth is impaired (Kang et al., 2011; Preuss et al., 2006). Rab-A4 GTPases have also been functionally linked to pollen tube tip growth. RAB-A4d is pollen specific in *Arabidopsis* and localises to the tip of growing pollen tubes (Szumlanski and Nielsen, 2009). A *rab-a4d* loss-of-function mutation causes shortened and thickened pollen tubes, a phenotype that could be partially complemented by ectopic expression of RAB-A4b in pollen tubes. Like RAB-A4b, RAB-A4d also interacts with PI-4K β 1 *in vitro*, and pollen tube growth in the *pi-4k β 1 pi-4k β 2* double-mutant is aberrant, suggesting a similar role in both tip growing systems (Szumlanski and Nielsen, 2009).

Another member of the Rab-A clade, RAB-A5c, has been implicated in a highly polarised secretory trafficking pathway to a confined spatial domain, the cell geometric edges (where two cell faces meet), in epidermal cells of lateral organ primordia (Kirchhelle et al., 2016) (Figs 1 and 2B). In addition to weakly labelling the TGN/EE, RAB-A5c localises to a set of endomembrane compartments at the geometric edges of cells, which exclude the endocytic tracer FM4-64 even after extended treatment. The subcellular localisation of a RAB-A5c[N125I] variant with reduced nucleotide affinity is shifted towards the TGN/EE, whereas a GTPase-deficient constitutively active RAB-A5c[Q71L] variant localises predominantly to the PM, indicating that RAB-A5c specifies a secretory pathway from the TGN/EE to the PM. Conditional overexpression of the dominant-negative RAB-A5c[N125I] variant severely impairs directional growth in lateral roots through a mechanism independent of cellulose arrangement at cell faces (Kirchhelle et al., 2019), indicating a previously unknown functional requirement for edge-directed trafficking in directional growth control. Inhibition of RAB-A5c function did not perturb secretion of secreted GFP (secGFP), PIN2 or the PM marker YFP–NOVEL PLANT SNARE12 (NPSN12), indicating that RAB-A5c defines a selective, highly polarised secretory route to the PM (Kirchhelle et al., 2016).

Secretory trafficking to the PM also involves other classes of Rab GTPases. Members of the Rab-E clade, which has five members in *Arabidopsis* (Pereira-Leal and Seabra, 2001), have been implicated in a secretory trafficking route from the Golgi to the PM (Speth et al., 2009; Zheng et al., 2005). RAB-E1d and other Rab-E members localise to the Golgi and (to varying degrees) to the PM in *Arabidopsis* leaf epidermal cells (Speth et al., 2009). Expression of a dominant-negative RAB-E1d[N128I] variant with reduced nucleotide affinity inhibits secretion of secGFP from the Golgi when expressed transiently in tobacco cells (Zheng et al., 2005), whereas a constitutively active RAB-E1d[Q74L] variant localises to the PM (Speth et al., 2009), indicating that RAB-E1d acts on a secretory route from the Golgi to the PM. Recently, the DIFFERENTIALLY EXPRESSED IN NEOPLASTIC VERSUS NORMAL CELLS (DENN) domain proteins STOMATAL CYTOKINESIS DEFECTIVE1 and 2 (SCD1 and SCD2) were identified as interactors and putative GEFs for Rab-E clade members; this is based on the observation that SCD1 and SCD2 interact with a preferentially GDP-bound RAB-E1c[S29N] variant, but not the constitutively active RAB-E1c[Q74L] form, and overexpression of wild-type or constitutively active RAB-E1c[Q74L] could partially rescue the severe growth defect of *scd1* mutants (Mayers et al., 2017). SCD1/2 also interacted with members of the conserved multi-subunit tethering complex exocyst which is involved in tethering secretory vesicles to the PM, suggesting Rab-E, SCD1/2 and the exocyst may act together in a secretory trafficking route to the cell surface (Mayers et al., 2017) (Fig. 1).

Recently, a member of the little-studied Rab-H GTPases in *Arabidopsis* was implicated in secretory trafficking of CSCs. RAB-H1b is predominantly expressed in the hypocotyl, where it localises to Golgi bodies and, to a lesser extent, the TGN/EE (He et al., 2018; Johansen et al., 2009; Renna et al., 2018). In *rab-h1b* knockout mutants, reduced cell wall thickness and cellulose content coincided with cell morphological defects (He et al., 2018). Exocytosis of the CSC component CELLULOSE SYNTHASE A CATALYTIC SUBUNIT6 (CESA6) is severely impaired in *rab-h1b* mutants, which might indicate that RAB-H1b orchestrates secretory trafficking from the Golgi to the PM,

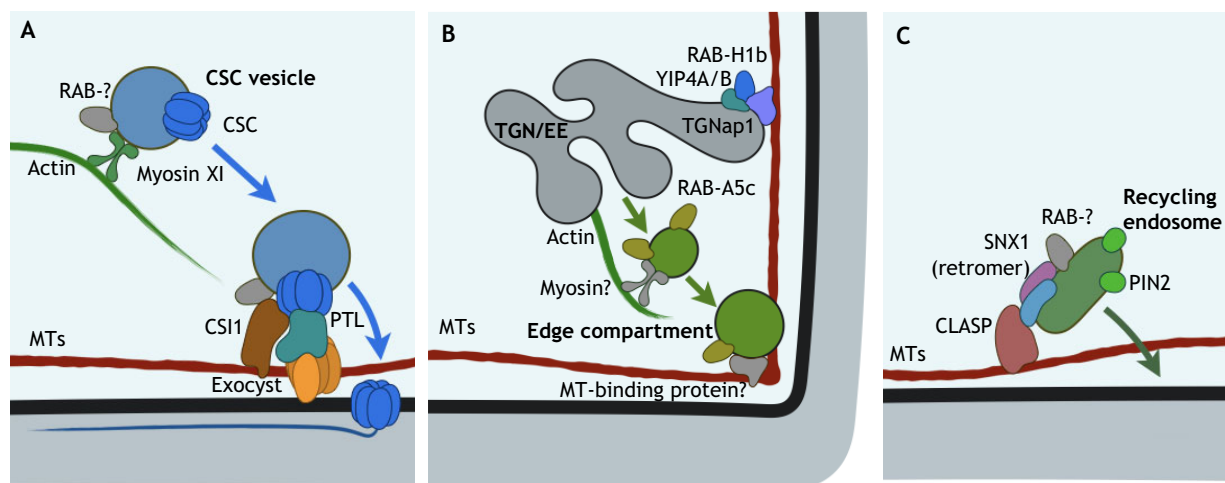


Fig. 2. Molecular mechanisms underpinning spatial control of trafficking. Schematic representation of molecular interactions underlying spatio-temporal control of various trafficking events. Speculative interactors and/or factors are depicted in grey and with a question mark. (A) Exocytosis of CSCs (blue arrows) involves myosin XI-driven transport along actin filaments, which determines delivery rate, and a module comprising microtubules, CS11, PTL and the exocyst complex to coordinate local insertion site. (B) TGNap1 mediates the interaction of the TGN/EE with microtubules through association with YIP4A/B and RAB-H1b. RAB-A5c-mediated transport from the TGN/EE to the PM through edge compartments involves both actin-based transport, and microtubules at cell edges as landmarks for compartment localisation. (C) PIN2-containing recycling endosomes associate with microtubules through a direct interaction of SNX1 with CLASP. Tethering to microtubules putatively enhances recycling rate.

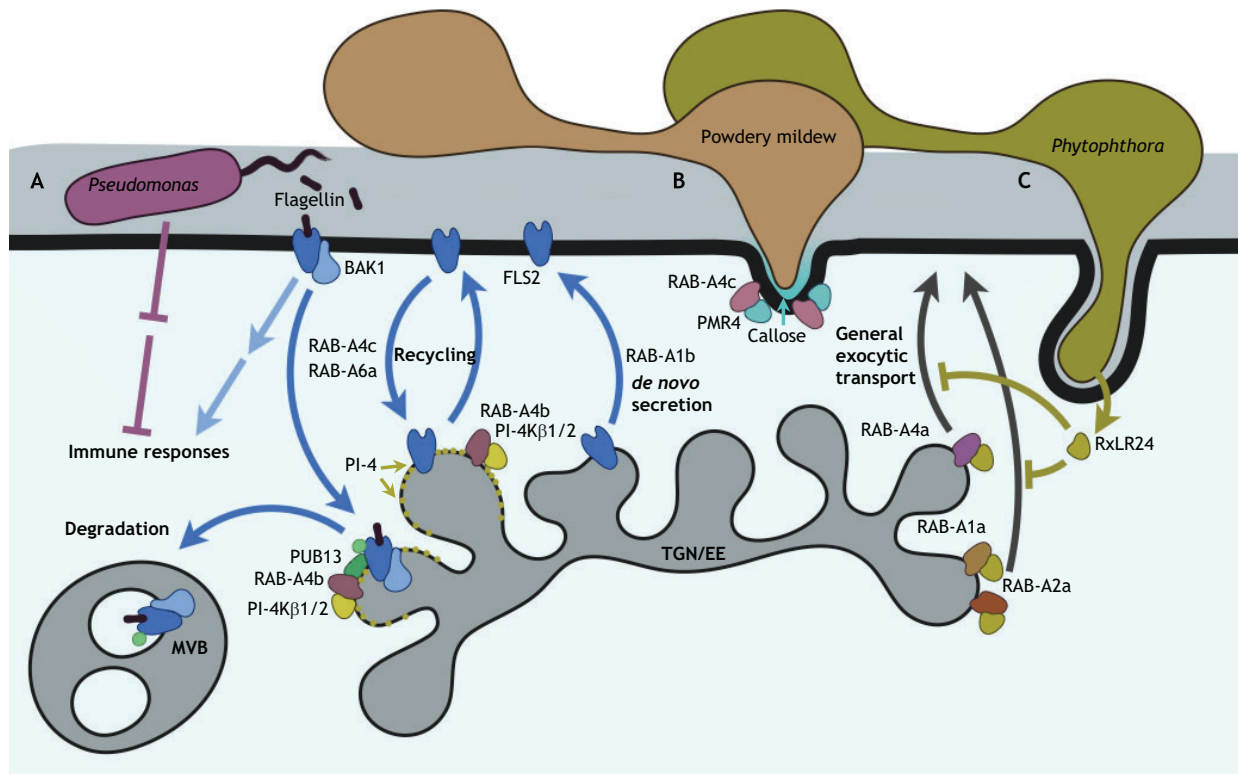


Fig. 3. Rab-A GTPases in plant–pathogen interactions. Schematic representation of molecular interactions underlying dynamic regulation of endomembrane trafficking events in biotic interactions. (A) Members of the Rab-A1, -A4 and -A6 clades have been implicated in regulated transport (blue arrows) of the flagellin receptor FLS2. (B) RAB-A4c is involved in cell wall modification during powdery mildew infection through recruitment of the callose synthase PMR4 to infection sites. (C) The *Phytophthora* effector RxLR24 blocks membrane trafficking through interaction with members of the Rab-A1, -A2 and -A4 families (green arrows). MVB, multivesicular body.

analogous to the function of its mammalian homologue Rab6 (Grigoriev et al., 2007). However, Golgi morphology and velocity, as well as the abundance of some non-cellulosic cell wall components, were also perturbed in the *rab-h1b* mutant. Whereas the latter effects are smaller than the reduction in cellulose, this could be indicative of a more-general Golgi-trafficking defect, and it has yet to be determined whether RAB-H1b influences CESA-trafficking specifically. Interestingly, loss of the Golgi-localised protein LOSS OF TGN (LOT), which was recently identified as a putative GEF for Rab-H in pollen tubes, is associated with perturbed Golgi and TGN/EE morphology, and defects in secretion of pectins and other secretory cargo (Jia et al., 2018). This might be indicative of general defects in secretory trafficking in the absence of active Rab-H (Fig. 1), although it is also possible that LOT acts upstream of other Rab GTPases.

Taken together, research advances in recent years have functionally linked distinct Rab GTPases to different transport routes to the cell surface, providing a molecular basis defining the distinct biochemical identities of these pathways. In particular, experimental data linking different members of the diverse Rab-A clade to distinct exocytic routes from the TGN/EE to the cell surface support the long-standing hypothesis that the diversification in the Rab-A clade is linked to a diversification in different trafficking pathways. Intriguingly, both *de novo* secretory routes and recycling routes to the cell surface can target specific spatial domains of the PM, indicating precise spatial control of different trafficking pathways. In the next section, we will discuss recent insights into the role of the cytoskeleton in spatially organising surface-bound transport.

Organising traffic in space

Spatially organising different trafficking pathways to and from the cell surface is a non-trivial problem as cells have to ensure uniform trafficking of some cargoes across their entire cell surface and simultaneously fine-tune exocytosis and endocytosis at the local scale. For example, at the cellular scale, consistent cell wall thickness depends on even distribution of CSCs across diffusely growing regions like cell faces (Sampathkumar et al., 2013). The requisite uniform PM delivery of CSCs across the entire growing region depends on uniform Golgi distribution throughout the cell, which in turn relies on actin-based cytoplasmic streaming (Crowell et al., 2009; Gutierrez et al., 2009; Sampathkumar et al., 2013). Fine-tuning CSC exocytosis at the local scale, however, appears to depend on both microtubules and actin (Fig. 2A). CSC trajectories in the PM are guided by cortical microtubules, an association mediated by CELLULOSE SYNTHASE INTERACTING1 [CSII; also known as POM-POM2 (POM2)] (Bringmann et al., 2012; Li et al., 2012). CSC insertion sites at the PM often coincide with microtubules, and this coincidence is proposed to optimise subsequent cellulose alignment. This hypothesis is supported by the recent finding that CSII directly interacts with the Munc13-like plant-specific protein PATROL1 (PTL1) and members of the multi-subunit tethering complex exocyst, and might thus act as a landmark to recruit CSC-containing secretory vesicles to discrete sites on microtubules (Zhu et al., 2018). Loss of CSII does not, however, affect the rate of CSC delivery to the cell wall (Bringmann et al., 2012). By contrast, recent findings indicate a role for myosin XI in determining CSC delivery rate, but not insertion site positioning (Zhang et al., 2019): in a triple *myosin xik xi1 xi2* mutant, the density of PM-localised CSCs as well

as cellulose content in the cell wall is reduced, and mutants accumulate putatively exocytic small CSC-containing compartments. Taken together, these findings suggest that the combined activity of an actin-myosin-based mechanism controlling CSC delivery rate and the microtubule–CSII–PTL1–exocyst-based mechanism controlling insertion site cooperatively orchestrate CSC delivery at the local scale (Fig. 2A).

A similar mechanism may also fine-tune non-uniform CSC delivery during secondary cell wall development (Oda et al., 2015; Vukašinović et al., 2017). During *Arabidopsis* xylem development, subunits of the exocyst complex are recruited to small microtubule-associated compartments in areas of secondary wall deposition through a mechanism that involves the coiled-coiled proteins VESICLE TETHERING1 and 2 (VETH1 and VETH2) and the conserved oligomeric Golgi (COG) protein complex (Oda et al., 2015). Two exocyst mutants, *exo70A1-1* and *exo84b-1*, partially mislocalise CSCs during secondary wall development, resulting in defects in secondary wall thickening (Oda et al., 2015; Vukašinović et al., 2017). Furthermore, exocyst subunits as well as CSII are enriched in the microtubule-associated proteome during xylem development (Derbyshire et al., 2015), and CSII is essential for patterning during early stages of xylem development (Schneider et al., 2017), consistent with a microtubule–CSII–exocyst module spatially controlling CSC insertion sites during vasculature wall development.

Microtubules have also recently been implicated in organising a secretory TGN/EE domain through the TGN-localised protein TGNap1 (Renna et al., 2018). In yeast two-hybrid and Förster resonance energy transfer (FRET) assays, TGNap1 interacted with YIP4a and YIP4b, which act in concert with ECH in the secretion of non-cellulosic cell wall components (Gendre et al., 2013) and, similar to *ech* mutants, *tnap1* mutants have reduced seed coat mucilage, indicative of defective pectin secretion (Gendre et al., 2011; Renna et al., 2018). TGNap1-associated TGN/EE can colocalise with microtubules *in vivo*, and TGNap1 can directly associate with microtubules *in vitro*, putatively through its N-terminal LisH domains. In *tnap1* mutants, movement of the TGN/EE is reduced, suggesting that the TGNap1-mediated microtubule-association might be relevant for TGN/EE motility and function (Renna et al., 2018). TGNap1 was also found to interact with RAB-H1b, the Rab GTPase recently implicated in CSC secretion (He et al., 2018; Renna et al., 2018; Figs 1 and 2B). Surprisingly, TGNap1 preferentially interacts with the GDP-bound, putatively inactive form of RAB-H1b, so the functional significance of this interaction is unclear. It might be that TGNap1 recruits inactive RAB-H1b to the TGN/EE to ensure its localised activation by a GEF. This hypothesis suggests RAB-H1b might be involved in secretion of non-cellulosic cell wall components from the TGNap1 domain, which may explain the observed differences in some non-cellulosic cell wall components in the *rab-h1b* mutant (He et al., 2018). Another possibility is that TGNap1 sequesters inactive RAB-H1b to the TGN/EE to reduce its activity at the Golgi during CSC delivery, possibly acting to moderate relative delivery rates of CSCs versus non-cellulosic cell wall components.

Furthermore, microtubules have also been linked to the secretion of non-cellulosic cell wall components at the PM through the activity of KINESIN-4A [also known as FRAGILE FIBRE1 (FRA1)]. *Arabidopsis* mutants in *fra1* are impaired in cell elongation in various organs and in the mechanical strength of their inflorescence stems (Zhong et al., 2002). FRA1–GFP labels punctate structures that do not co-localise with and move independently of both PM-localised CSCs and CSC-labelled

MASCs/SmaCCs (Kong et al., 2015; Zhu et al., 2015). Surprisingly, whereas secretion of pectin was found to be severely reduced in *fra1* mutants based on the incorporation of Fuc-alkyne into the pectic polymer rhamnogalacturonan-I, overall cell wall composition was minimally altered (Zhu et al., 2015). This indicates that although non-cellulosic cell wall components and CSCs are likely secreted independently from each other, there is significant crosstalk between both delivery systems.

Contributions of both actin and microtubule cytoskeletons have also been described in the context of organising the edge-directed secretory route mediated by RAB-A5c (Figs 1 and 2B). Pharmacological depolymerisation of both actin and microtubules leads to a loss of edge-localised RAB-A5c compartments in favour of cytosolic puncta (Kirchhelle et al., 2016). RAB-A5c compartments colocalise with cortical microtubules at cell edges, where they are stable over minutes (Kirchhelle et al., 2016, 2019), suggesting that cortical microtubules may serve as anchor points to keep these compartments within the spatially confined domain of the cell edge. Whereas only a subset of microtubules at edges colocalises with RAB-A5c compartments, the relative distribution of RAB-A5c compartments at longitudinal versus transverse edges in meristematic cells of lateral roots is significantly altered in mutants with perturbed microtubule organisation (Kirchhelle et al., 2019). This indicates that, although microtubules are necessary for localisation of RAB-A5c compartments at a given edge, they are not sufficient to explain the distribution of RAB-A5c compartments across different edges. This implies that additional ‘landmark’ factors may be involved in recruiting RAB-A5c compartments at the local scale, similar to the proposed role of CSII in CSC secretion (Zhu et al., 2018).

Microtubules might also be involved in controlling trafficking rates to different subcellular domains in some cases, as was revealed in a study examining the role of microtubules in PIN2 recycling (Fig. 2C; Ambrose et al., 2013). The microtubule-associated protein CLIP-ASSOCIATED PROTEIN (CLASP) was found to interact with the retromer complex component SORTING NEXIN1 (SNX1), a well-conserved regulator of protein recycling and lytic trafficking across eukaryotes, which has been implicated in PIN recycling (Heucken and Ivanov, 2018). SNX1-labelled endosomes associate with microtubules in a CLASP-dependent manner, and loss of CLASP reduces overall SNX1 protein levels and shifts SNX1–GFP localisation from endosomes towards the cytosol, indicating that SNX1 endosomes are stabilised through the CLASP-mediated microtubule association (Ambrose et al., 2013). Intriguingly, loss of CLASP is associated with an overall reduction of PIN2 at the PM but not a loss of polarity; this is compatible with the hypothesis that cortical microtubules could enhance recycling by locally increasing the abundance of PIN2-containing SNX1-positive endosomes (Fig. 2C). Similarly, the actin cytoskeleton has been linked to PIN trafficking, but not polarity establishment per se, based on the observation that pharmacological depolymerisation of actin led to accumulation of PIN1 and PIN2 on intracellular membranes and defects in PIN recycling from BFA bodies to the PM after BFA washout, but did not disrupt polarised localisation of PINs at the PM (Geldner et al., 2001; Glanc et al., 2019). This indicates cytoskeleton-independent mechanisms are involved in determining PIN polarity, which may include lipid composition of the PM and interactions with the cell wall (Feraru et al., 2011; Men et al., 2008). Based on computational and experimental data, however, differential PIN endocytosis at different cell faces has been proposed to be the major mechanism that drives PIN polarity (Glanc et al., 2018; Kitakura et al., 2011; Kleine-Vehn et al., 2011). The molecular mechanisms regulating

differential endocytosis at different faces are still poorly explored, but may involve differential phosphorylation of PIN proteins through activity of the closely related kinases PINOID (PID) and WAG1 and WAG2 (Glanc et al., 2018).

In summary, in recent years it has become apparent that both actin and microtubule-based mechanisms are involved in organising endomembrane trafficking, and can collaborate to fine-tune transport in space and time. Furthermore, circumstantial and direct evidence suggests that cytoskeleton-based trafficking integrates other spatial cues affecting polarised transport, including landmark proteins that may themselves interact with the cytoskeleton or cytoskeleton-independent factors, for instance, through spatially confined post-translational modification of cargo proteins. While the mechanisms described above have focussed largely on steady-state interphase cells, plant cells also have to dynamically adapt their intracellular trafficking pathways in response to developmental as well as extrinsic cues. For example, dividing plant cells construct a centrifugally growing cell plate between the two daughter cells, which involves transient re-routing of surface-bound endomembrane trafficking and its associated machinery (including Rab-A, -E and -H GTPases) to the growing cell plate (Chow et al., 2008; Kirchhelle et al., 2016; Speth et al., 2009; reviewed in detail by Livanos and Müller, 2019; Müller and Jürgens, 2016). Vesicles forming the cell plate are transported along the phragmoplast, a specialised, bi-polar cytoskeletal array containing both microtubules and actin filaments (Livanos and Müller, 2019; Müller and Jürgens, 2016).

Dynamic regulation of post-Golgi transport also plays an essential role in biotic interactions ranging from microbial perception to the establishment of plant immune responses (Ben Khaled et al., 2015; Rivero et al., 2019). Plant trafficking pathways can also be hijacked by pathogens during infection. Below, we will highlight how surface-bound trafficking routes mediated by Rab-A GTPases can be co-opted at different stages of plant biotic interactions.

Rab GTPases and the cytoskeleton in plant immunity

Pathogen perception in plants involves pattern recognition receptors (PRRs) at the PM, which can bind to microbe-associated molecular patterns (MAMPs) and trigger plant immune responses ranging from cytoskeletal remodelling to transcriptional responses (Ben Khaled et al., 2015; Li and Staiger, 2018). The *Arabidopsis* flagellin receptor FLAGELLIN-SENSING2 (FLS2), in particular, has been used to dissect the trafficking pathways involved in trafficking PRRs to and from the cell surface (Beck et al., 2012; Ben Khaled et al., 2015; Choi et al., 2013), revealing that different Rab-A proteins mediate different stages of FLS2 trafficking (Fig. 3A). Expression of a dominant-inhibitory mutant variant of RAB-A1b disrupts *de novo* secretion of FLS2, whereas dominant-negative variants of RAB-A4c and RAB-A6a disrupt distinct stages of early endocytic trafficking of FLS2 (Choi et al., 2013). The related RAB-A4b is also involved in FLS2 endocytic transport, and recruits a member of the plant U-box family of E3 ubiquitin ligases, PLANT U-BOX13 (PUB13) (Antignani et al., 2015), which ubiquitylates FLS2 upon ligand binding (Lu et al., 2011). Mutants of both *pub13* and the RAB-A4b effectors *pi-4kβ1* *pi-4kβ2* are severely impaired in growth, but more resistant against the bacterial pathogen *Pseudomonas syringae*, indicating that Rab-A4 members and their effectors act as negative regulators of the plant immune response to flagellin in addition to their roles in growth (Antignani et al., 2015).

Inhibition of other Rab-A clade members (including RAB-A2c, RAB-A3 and the edge-localised RAB-A5c) by contrast has no

effect on FLS2 trafficking, demonstrating the functional diversification within the clade (Choi et al., 2013). However, Rab-A2 members are associated with the establishment and maintenance of beneficial microbial interactions in plants (reviewed by Rivero et al., 2019). For instance, a *Phaseus vulgaris* Rab-A2 protein mediates polarised trafficking in the context of cell wall remodelling during colonisation by the symbiotic nitrogen-fixer *Rhizobium etli* (Blanco et al., 2009; Dalla Via et al., 2017). Expression of a dominant-negative Rab-A2 variant results in defects during infection thread formation, indicating that Rab-A2 is also required at later stages of infection (Dalla Via et al., 2017). In addition to its role in FLS2 endocytosis, RAB-A4c is also implicated in cell wall remodelling during infection with the powdery mildew *Golovinomyces cichoracearum* (Fig. 3B; Ellinger et al., 2014). RAB-A4c localises to the infection site where it directly interacts with the callose synthase POWDERY MILDEW RESISTANT4 (PMR4), and overexpression of RAB-A4c results in complete penetration resistance against powdery mildew, which is associated with callose accumulation at the infection site (Ellinger et al., 2014).

The machinery regulating post-Golgi trafficking includes known targets of pathogen effectors, which act to subvert plant immune responses during pathogenesis. For instance, the conserved *Phytophthora infestans* effector RxLR24 targets and presumably inhibits Rab GTPases to reduce general secretion of antimicrobial proteins (Fig. 3C, Tomczynska et al., 2018). Identified targets of RxLR24 include Rab-A1, -A2, -A4 and -G3 GTPases (Tomczynska et al., 2018), and expression of RxLR24 in *Nicotiana benthamiana* resulted in retention of secGFP in the ER, indicating a loss of bulk secretion. In addition to Rab GTPases, pathogen effectors can also target the cytoskeleton and its associated proteins to disrupt endomembrane trafficking. For instance, expression of the *Pseudomonas syringae* effector HopW1 disrupts the actin cytoskeleton and results in reduced endocytosis and enhanced virulence, possibly owing to impaired FLS2 signalling (Kang et al., 2014). By contrast, the *Pseudomonas syringae* effector HopZ1a disrupts the microtubule cytoskeleton and severely impairs secretion of secGFP into the extracellular space in *Nicotiana benthamiana* (Lee et al., 2012). The *Pseudomonas syringae* effector HopE1 similarly perturbs microtubule organisation through sequestering the microtubule-interacting protein MICROTUBULE-ASSOCIATED PROTEIN65 (MAP65), resulting in reduced secretion of secGFP and PATHOGENESIS RELATED1 (PR1) (Guo et al., 2016). Increased virulence associated with HopZ1a and HopE1 expression may thus be associated with reduced secretion of immunity-related proteins.

Taken together, transport routes to and from the cell surface constitute an essential component of symbiotic and pathogenic plant-microbe interactions. Intriguingly, the same families of regulatory Rab proteins that are involved in beneficial interactions and plant immune responses can also be targeted by pathogens, illustrating the complex dynamics of transport pathways in different developmental and physiological contexts.

Concluding remarks and perspectives

Spatio-temporal control of surface-bound transport is essential for the establishment of polarity and directional growth in plants, as well as for supporting physiologically dynamic processes such as plant immune responses. Identification and characterisation of key molecular players in post-Golgi trafficking associated with the endomembrane and cytoskeletal systems has revealed that plants rely on a highly diverse set of Rab GTPases (Figs 1 and 3; Table 1)

Box 1. Regulating the regulators – determinants of Rab GTPase localisation and function

Mechanisms driving recruitment and targeting of plant Rab GTPases at the subcellular scale are poorly understood; however, studies from metazoans can provide some mechanistic insight into their spatial patterning. GEFs mediate membrane recruitment and activation of Rab GTPases, and can directly provide targeting specificity (Blümer et al., 2013). GEFs may provide further spatial specificity through direct association with the cytoskeleton: for example, the human RAB12 GEF DENND3 binds actin through a PH domain, an association that is required for RAB12 function in autophagy (Xu et al., 2018). However, Rab membrane recruitment and targeting specificity can also occur independently of GEFs – for instance, a variant of the yeast Rab GTPase Ypt7 with a putatively enhanced intrinsic nucleotide exchange rate can localise to the target membrane even in the absence of its GEF (Cabrera and Ungermann, 2013).

Specificity in Rab–Rab-regulator interactions can be achieved either through specific interaction domains in Rabs or specific post-translational modifications (PTMs). The hypervariable C-terminal domains (HVDs) of Rab GTPases are long-standing candidates for specific interaction domains (Chavrier et al., 1991), and HVDs of the related Rho of Plants (ROP) GTPases can indeed contribute to their localisation in plants (Li et al., 2013). However, domain-swap experiments on different animal Rab proteins suggest that HVDs do not act as universal targeting signals, and might only be involved in target specificity for a small subset of Rabs (Ali et al., 2004; Li et al., 2014). Localisation of Rab GTPases can be dynamically regulated by PTMs. For instance, N-terminal phosphorylation of RAB5a is required for RAB5a localisation to the leading edge during T-lymphocyte movement (Ong et al., 2014). Similarly, phosphorylation of the yeast Rab GTPase Sec4 blocks its interaction with the tethering complex exocyst in a cell-cycle-dependent manner, co-ordinating membrane trafficking during cytokinesis (Lepore et al., 2016). PTMs of Rab-interacting factors can also indirectly regulate Rab GTPase activity, for example, the animal RAB8 GEF Rabin8 is phosphorylated by extracellular signal-regulated kinase 1 and 2 (ERK1/2; also known as MAPK3 and MAPK1, respectively), which inhibits its interaction with RAB11 and allows it to function as a GEF for RAB8 (Wang et al., 2015). Although such mechanisms have not been described yet for plant Rab GTPase-interacting factors, ROP GTPase-interacting factors can be regulated similarly through PTMs (Feiguelman et al., 2018; Klahre and Kost, 2006; Zhang and McCormick, 2007).

to specify multiple routes to the cell surface, some of which are spatially highly confined. Plants utilise both their actin and microtubule cytoskeletons to fine-tune delivery rate and spatial targeting of surface-bound trafficking routes during cell growth and biotic interactions (Fig. 2). Despite these considerable advances in recent years, there are still many open questions regarding the regulation of transport pathways to and from the cell surface, which will require detailed mapping and mechanistic characterisation of Rab GTPases and their interactors, regulators and effectors (see Box 1). In addition to greater basic molecular characterisation of components that are involved, the dynamic nature and crosstalk between different trafficking pathways will also necessitate innovative approaches addressing regulatory dynamics and emergent features of the plant endomembrane system and its interactors.

Acknowledgements

The early stages of this work were overshadowed by the untimely death of Ian Moore. We dedicate this paper to his memory.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was funded through a Biotechnology and Biological Sciences Research Council (BBSRC) studentship 1810136 to L.E., BBSRC grant BB/P01979X/1 to I.M. and C.K., and Leverhulme Trust Early Career Fellowship ECF-2017-483 to C.K.

References

- Ali, B. R., Wasmeier, C., Lamoreux, L., Strom, M. and Seabra, M. C. (2004). Multiple regions contribute to membrane targeting of Rab GTPases. *J. Cell Sci.* **117**, 6401–6412. doi:10.1242/jcs.01542
- Ambrose, C., Ruan, Y., Gardiner, J., Tamblin, L. M., Catching, A., Kirik, V., Marc, J., Overall, R. and Wasteney, G. O. (2013). CLASP interacts with sorting Nexin 1 to link microtubules and auxin transport via PIN2 recycling in Arabidopsis thaliana. *Dev. Cell* **24**, 649–659. doi:10.1016/j.devcel.2013.02.007
- Antignani, V., Klocko, A. L., Bak, G., Chandrasekaran, S. D., Dunivin, T. and Nielsen, E. (2015). Recruitment of PLANT U-BOX13 and the PI4Kbeta1/beta2 phosphatidylinositol-4 kinases by the small GTPase RabA4B plays important roles during salicylic acid-mediated plant defense signaling in Arabidopsis. *Plant Cell* **27**, 243–261. doi:10.1105/tpc.114.134262
- Asaoka, R., Uemura, T., Ito, J., Fujimoto, M., Ito, E., Ueda, T. and Nakano, A. (2013). Arabidopsis RABA1 GTPases are involved in transport between the trans-Golgi network and the plasma membrane, and are required for salinity stress tolerance. *Plant J.* **73**, 240–249. doi:10.1111/tpj.12023
- Avisar, D., Prokhnevsky, A. I., Makarova, K. S., Koonin, E. V. and Dolja, V. V. (2008). Myosin XI-K is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. *Plant Physiol.* **146**, 1098–1108. doi:10.1104/pp.107.113647
- Batoko, H., Zheng, H.-Q., Hawes, C. and Moore, I. (2000). A Rab1 GTPase is required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement in plants. *Plant Cell* **12**, 2201–2221. doi:10.1105/tpc.12.11.2201
- Beck, M., Zhou, J., Faulkner, C., MacLean, D. and Robatzek, S. (2012). Spatio-temporal cellular dynamics of the arabidopsis flagellin receptor reveal activation status-dependent endosomal sorting. *Plant Cell* **24**, 4205–4219. doi:10.1105/tpc.112.100263
- Ben Khaled, S., Postma, J. and Robatzek, S. (2015). A moving view: subcellular trafficking processes in pattern recognition receptor-triggered plant immunity. *Annu. Rev. Phytopathol.* **53**, 379–402. doi:10.1146/annurev-phyto-080614-120347
- Berson, T., von Wangenheim, D., Takac, T., Samajova, O., Rosero, A., Ovecka, M., Komis, G., Stelzer, E.H., and Samaj, J. (2014). Trans-Golgi network localized small GTPase RabA1d is involved in cell plate formation and oscillatory root hair growth. *BMC Plant Biol* **14**, 252. doi:10.1186/s12870-014-0252-0
- Blanco, F. A., Peltzer Meschini, E., Zanetti, M. E. and Aguilar, O. M. (2009). A small GTPase of the Rab family is required for root hair formation and preinfection stages of the common bean-rhizobium symbiotic association. *Plant Cell* **21**, 2797–2810. doi:10.1105/tpc.108.063420
- Blümer, J., Rey, J., Dehmelt, L., Mazel, T., Wu, Y.-W., Bastiaens, P., Goody, R. S. and Itzen, A. (2013). RabGEFs are a major determinant for specific Rab membrane targeting. *J. Cell Biol.* **200**, 287–300. doi:10.1083/jcb.201209113
- Bottanelli, F., Foresti, O., Hanton, S. and Denecke, J. (2011). Vacuolar transport in tobacco leaf epidermis cells involves a single route for soluble cargo and multiple routes for membrane cargo. *Plant Cell* **23**, 3007–3025. doi:10.1105/tpc.111.085480
- Bottanelli, F., Gershlick, D. C. and Denecke, J. (2012). Evidence for sequential action of Rab5 and Rab7 GTPases in prevacuolar organelle partitioning. *Traffic* **13**, 338–354. doi:10.1111/j.1600-0854.2011.01303.x
- Boutte, Y., Jonsson, K., McFarlane, H. E., Johnson, E., Gendre, D., Swarup, R., Friml, J., Samuels, L., Robert, S. and Bhalerao, R. P. (2013). ECHIDNA-mediated post-Golgi trafficking of auxin carriers for differential cell elongation. *Proc. Natl. Acad. Sci. USA* **110**, 16259–16264. doi:10.1073/pnas.1309057110
- Brandizzi, F. and Wasteney, G. O. (2013). Cytoskeleton-dependent endomembrane organization in plant cells: an emerging role for microtubules. *Plant J.* **75**, 339–349. doi:10.1111/tpj.12227
- Bringmann, M., Li, E., Sampathkumar, A., Kocabek, T., Hauser, M.-T. and Persson, S. (2012). POM-POM2/cellulose synthase interacting1 is essential for the functional association of cellulose synthase and microtubules in Arabidopsis. *Plant Cell* **24**, 163–177. doi:10.1105/tpc.111.093575
- Cabrera, M. and Ungermann, C. (2013). Guanine nucleotide exchange factors (GEFs) have a critical but not exclusive role in organelle localization of Rab GTPases. *J. Biol. Chem.* **288**, 28704–28712. doi:10.1074/jbc.M113.488213
- Camacho, L., Smertenko, A. P., Pérez-Gómez, J., Hussey, P. J. and Moore, I. (2009). Arabidopsis Rab-E GTPases exhibit a novel interaction with a plasma-membrane phosphatidylinositol-4-phosphate 5-kinase. *J. Cell Sci.* **122**, 4383–4392. doi:10.1242/jcs.053488
- Chavrier, P., Gorvel, J.-P., Stelzer, E., Simons, K., Gruenberg, J. and Zerial, M. (1991). Hypervariable C-terminal domain of rab proteins acts as a targeting signal. *Nature* **353**, 769. doi:10.1038/353769a0
- Cheung, A. Y., Chen, C. Y.-H., Glaven, R. H., de Graaf, B. H. J., Vidali, L., Hepler, P. K. and Wu, H.-M. (2002). Rab2 GTPase regulates vesicle trafficking between

- the endoplasmic reticulum and the Golgi bodies and is important to pollen tube growth. *Plant Cell* **14**, 945–962. doi:10.1105/tpc.000836
- Choi, S.-W., Tamaki, T., Ebine, K., Uemura, T., Ueda, T. and Nakano, A. (2013). RABA members act in distinct steps of subcellular trafficking of the FLAGELLIN SENSING2 receptor. *Plant Cell* **25**, 1174–1187. doi:10.1105/tpc.112.108803
- Chow, C.-M., Neto, H., Foucart, C. and Moore, I. (2008). Rab-A2 and Rab-A3 GTPases define a trans-golgi endosomal membrane domain in Arabidopsis that contributes substantially to the cell plate. *Plant Cell* **20**, 101–123. doi:10.1105/tpc.107.052001
- Crowell, E. F., Bischoff, V., Desprez, T., Rolland, A., Stierhof, Y.-D., Schumacher, K., Gonneau, M., Höfte, H. and Vernhettes, S. (2009). Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in arabidopsis. *Plant Cell* **21**, 1141–1154. doi:10.1105/tpc.108.065334
- Cui, Y., Zhao, Q., Gao, C., Ding, Y., Zeng, Y., Ueda, T., Nakano, A. and Jiang, L. (2014). Activation of the Rab7 GTPase by the MON1-CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in arabidopsis. *Plant Cell* **26**, 2080–2097. doi:10.1105/tpc.114.123141
- Dalla Via, V., Traubenik, S., Rivero, C., Aguilar, O. M., Zanetti, M. E. and Blanco, C. A. (2017). The monomeric GTPase RabA2 is required for progression and maintenance of membrane integrity of infection threads during root nodule symbiosis. *Plant Mol. Biol.* **93**, 549–562. doi:10.1007/s11103-016-0581-5
- Derbyshire, P., Ménard, D., Green, P., Saalbach, G., Buschmann, H., Lloyd, C. W. and Pesquet, E. (2015). Proteomic analysis of microtubule interacting proteins over the course of xylem tracheary element formation in arabidopsis. *Plant Cell* **27**, 2709. doi:10.1105/tpc.15.00314
- Dettmer, J., Hong-Hermesdorf, A., Stierhof, Y.-D. and Schumacher, K. (2006). Vacuolar H⁺-ATPase activity is required for Endocytic and secretory trafficking in Arabidopsis. *Plant Cell* **18**, 715–730. doi:10.1105/tpc.105.037978
- Dhonukshe, P., Añiento, F., Hwang, I., Robinson, D. G., Mravec, J., Stierhof, Y.-D. and Friml, J. (2007). Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in Arabidopsis. *Curr. Biol.* **17**, 520–527. doi:10.1016/j.cub.2007.01.052
- Ebine, K., Fujimoto, M., Okatani, Y., Nishiyama, T., Goh, T., Ito, E., Dainobu, T., Nishitani, A., Uemura, T., Sato, M. H. et al. (2011). A membrane trafficking pathway regulated by the plant-specific RAB GTPase ARA6. *Nat. Cell Biol.* **13**, 853–859. doi:10.1038/ncb2270
- Ellinger, D., Glöckner, A., Koch, J., Naumann, M., Stürtz, V., Schütt, K., Manisseri, C., Somerville, S. C. and Voigt, C. A. (2014). Interaction of the Arabidopsis GTPase RabA4c with its effector PMR4 results in complete penetration resistance to powdery mildew. *Plant Cell* **26**, 3185–3200. doi:10.1105/tpc.114.127779
- Elliott, L. and Kirchhelle, C. (2019). The importance of being edgy: cell geometric edges as an emerging polar domain in plant cells. *J. Microsc.* doi:10.1111/jmi.12847
- Feiguelman, G., Fu, Y. and Yalovsky, S. (2018). ROP GTPases structure-function and signaling pathways. *Plant Physiol.* **176**, 57–79. doi:10.1104/pp.17.01415
- Feraru, E., Feraru, M. I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S., Vernhettes, S., Runions, J. and Friml, J. (2011). PIN polarity maintenance by the cell wall in arabidopsis. *Curr. Biol.* **21**, 338–343. doi:10.1016/j.cub.2011.01.036
- Feraru, E., Feraru, M. I., Asaoka, R., Paciorek, T., De Rycke, R., Tanaka, H., Nakano, A. and Friml, J. (2012). BEX5/RabA1b regulates trans-golgi network-to-plasma membrane protein trafficking in arabidopsis. *Plant Cell* **24**, 3074–3086. doi:10.1105/tpc.112.098152
- Furt, F., Lemoi, K., Tüzel, E. and Vidali, L. (2012). Quantitative analysis of organelle distribution and dynamics in Physcomitrella patens protonemal cells. *BMC Plant Biol.* **12**, 70. doi:10.1186/1471-2229-12-70
- Geitmann, A. and Nebenführ, A. (2015). Navigating the plant cell: intracellular transport logistics in the green kingdom. *Mol. Biol. Cell* **26**, 3373–3378. doi:10.1091/mbc.E14-10-1482
- Geldner, N., Friml, J., Stierhof, Y.-D., Jürgens, G. and Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**, 425–428. doi:10.1038/35096571
- Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Müller, P., Delbarre, A., Ueda, T., Nakano, A. and Jürgens, G. (2003). The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* **112**, 219–230. doi:10.1016/S0092-8674(03)00003-5
- Geldner, N., Dénervaud-Tendon, F., Hyman, D. L., Mayer, U., Stierhof, Y.-D. and Chory, J. (2009). Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set. *Plant J.* **59**, 169–178. doi:10.1111/j.1365-3113.2009.03851.x
- Gendreau, D., Oh, J., Boutte, Y., Best, J. G., Samuels, L., Nilsson, R., Uemura, T., Marchant, A., Bennett, M. J., Grebe, M. et al. (2011). Conserved Arabidopsis ECHIDNA protein mediates trans-Golgi-network trafficking and cell elongation. *Proc. Natl. Acad. Sci. USA* **108**, 8048–8053. doi:10.1073/pnas.1018371108
- Gendreau, D., McFarlane, H. E., Johnson, E., Mouille, G., Sjodin, A., Oh, J., Levesque-Tremblay, G., Watanabe, Y., Samuels, L. and Bhalerao, R. P. (2013). Trans-golgi network localized ECHIDNA/Ypt interacting protein complex is required for the secretion of cell wall polysaccharides in arabidopsis. *Plant Cell* **25**, 2633–2646. doi:10.1105/tpc.113.112482
- Gendreau, D., Jonsson, K., Boutte, Y. and Bhalerao, R. P. (2015). Journey to the cell surface—the central role of the trans-Golgi network in plants. *Protoplasma* **252**, 385–398. doi:10.1007/s00709-014-0693-1
- Glanc, M., Fendrych, M. and Friml, J. (2018). Mechanistic framework for cell-intrinsic re-establishment of PIN2 polarity after cell division. *Nature plants* **4**, 1082. doi:10.1038/s41477-018-0318-3
- Glanc, M., Fendrych, M. and Friml, J. (2019). PIN2 polarity establishment in arabidopsis in the absence of an intact cytoskeleton. *Biomolecules* **9**, 222. doi:10.3390/biom9060222
- Grigoriev, I., Splinter, D., Keijzer, N., Wulf, P. S., Demmers, J., Ohtsuka, T., Modesti, M., Maly, I. V., Grosveld, F., Hoogenraad, C. C. et al. (2007). Rab6 regulates transport and targeting of exocytic carriers. *Dev. Cell* **13**, 305–314. doi:10.1016/j.devcel.2007.06.010
- Grosshans, B. L., Ortiz, D. and Novick, P. (2006). Rabs and their effectors: achieving specificity in membrane traffic. *Proc. Natl. Acad. Sci. USA* **103**, 11821. doi:10.1073/pnas.0601617103
- Guo, M., Kim, P., Li, G. Y., Elowsky, C. G. and Alfano, J. R. (2016). A bacterial effector co-opts calmodulin to target the plant microtubule network. *Cell Host Microbe* **19**, 67–78. doi:10.1016/j.chom.2015.12.007
- Gutierrez, R., Lindeboom, J. J., Paredez, A. R., Emons, A. M. C. and Ehrhardt, D. W. (2009). Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nat. Cell Biol.* **11**, 797–806. doi:10.1038/ncb1886
- Haigler, C. H. and Brown, R. M. (1986). Transport of rosettes from the golgi apparatus to the plasma membrane in isolated mesophyll cells of Zinnia elegans during differentiation to tracheary elements in suspension culture. *Protoplasma* **134**, 111–120. doi:10.1007/BF01275709
- He, M., Lan, M., Zhang, B., Zhou, Y., Wang, Y., Zhu, L., Yuan, M. and Fu, Y. (2018). Rab-H1b is essential for trafficking of cellulose synthase and for hypocotyl growth in Arabidopsis thaliana. *J. Integr. Plant Biol.* **60**, 1051–1069. doi:10.1111/jipb.12694
- Heucken, N. and Ivanov, R. (2018). The retromer, sorting nexins and the plant endomembrane protein trafficking. *J. Cell Sci.* **131**, jcs203695. doi:10.1242/jcs.203695
- Higaki, T., Kutsuna, N., Hosokawa, Y., Akita, K., Ebine, K., Ueda, T., Kondo, N. and Hasegawa, S. (2012). Statistical organelle dissection of Arabidopsis guard cells using image database LIPS. *Sci. Rep.* **2**, 405. doi:10.1038/srep00405
- Hill, J. L., Jr, Josephs, C., Barnes, W. J., Anderson, C. T. and Tien, M. (2018). Longevity in vivo of primary cell wall cellulose synthases. *Plant Mol. Biol.* **96**, 279–289. doi:10.1007/s11103-017-0695-4
- Ito, E., Uemura, T., Ueda, T. and Nakano, A. (2010). Distribution of RAB5-positive multivesicular endosomes and the trans-Golgi network in root meristematic cells of Arabidopsis thaliana. *Plant Biotechnol.* **33**, 281–286. doi:10.5511/plantbiotechnology.16.0218a
- Jia, P.-F., Xue, Y., Li, H.-J. and Yang, W.-C. (2018). Golgi-localized LOT regulates trans-Golgi network biogenesis and pollen tube growth. *Proc. Natl. Acad. Sci. USA* **115**, 12307–12312. doi:10.1073/pnas.1809206115
- Johansen, J. N., Chow, C.-M., Moore, I. and Hawes, C. (2009). AtRAB-H1(b) and AtRAB-H1(c) GTPases, homologues of the yeast Ypt6, target reporter proteins to the Golgi when expressed in Nicotiana tabacum and Arabidopsis thaliana. *J. Exp. Bot.* **60**, 3179–3193. doi:10.1093/jxb/erp153
- Kalde, M., Elliott, L., Ravikumar, R., Rybak, K., Altmann, M., Klaeger, S., Wiese, C., Abele, M., Al, B., Kalbfuß, N. et al. (2019). Interactions between Transport Protein Particle (TRAPP) complexes and Rab GTPases in Arabidopsis. *Plant J.* **100**, 279–297. doi:10.1111/tpj.14442
- Kang, B.-H., Nielsen, E., Preuss, M. L., Mastroratte, D. and Staehelin, L. A. (2011). Electron tomography of RabA4b- and PI-4Kβ1-labeled trans Golgi network compartments in Arabidopsis. *Traffic* **12**, 313–329. doi:10.1111/j.1600-0854.2010.01146.x
- Kang, Y., Jelenska, J., Cecchini, N. M., Li, Y., Lee, M. W., Kovar, D. R. and Greenberg, J. T. (2014). HopW1 from Pseudomonas syringae disrupts the actin cytoskeleton to promote virulence in Arabidopsis. *PLoS Pathog.* **10**, e1004232. doi:10.1371/journal.ppat.1004232
- Kirchhelle, C., Chow, C.-M., Foucart, C., Neto, H., Stierhof, Y.-D., Kalde, M., Walton, C., Fricker, M., Smith, R. S., Jérusalem, A. et al. (2016). The specification of geometric edges by a plant Rab GTPase is an essential cell-patterning principle during organogenesis in Arabidopsis. *Dev. Cell* **36**, 386–400. doi:10.1016/j.devcel.2016.01.020
- Kirchhelle, C., Garcia-Gonzalez, D., Irani, N. G., Jérusalem, A. and Moore, I. (2019). Two mechanisms regulate directional cell growth in Arabidopsis lateral roots. *eLife* **8**, e47988. doi:10.7554/eLife.47988
- Kitakura, S., Vanneste, S., Robert, S., Löffke, C., Teichmann, T., Tanaka, H. and Friml, J. (2011). Clathrin mediates endocytosis and polar distribution of PIN Auxin transporters in arabidopsis. *Plant Cell* **23**, 1920–1931. doi:10.1105/tpc.111.083030
- Klahre, U. and Kost, B. (2006). Tobacco RhoGTPase ACTIVATING PROTEIN1 spatially restricts signaling of RAC/Rop to the apex of pollen tubes. *Plant Cell* **18**, 3033–3046. doi:10.1105/tpc.106.045336
- Kleine-Vehn, J., Huang, F., Naramoto, S., Zhang, J., Michniewicz, M., Offringa, R. and Friml, J. (2009). PIN Auxin efflux carrier polarity is regulated by PINOID

- kinase-mediated recruitment into GNOM-independent trafficking in arabidopsis. *Plant Cell* **21**, 3839–3849. doi:10.1105/tpc.109.071639
- Kleine-Vehn, J., Wabnick, K., Martinière, A., Langowski, Ł., Willig, K., Naramoto, S., Leitner, J., Tanaka, H., Jakobs, S., Robert, S. et al. (2011). Recycling, clustering, and endocytosis jointly maintain PIN auxin carrier polarity at the plasma membrane. *Mol. Syst. Biol.* **7**, 540. doi:10.1038/msb.2011.72
- Kong, Z., Ioki, M., Braybrook, S., Li, S., Ye, Z.-H., Julie Lee, Y.-R., Hotta, T., Chang, A., Tian, J., Wang, G. et al. (2015). Kinesin-4 functions in vesicular transport on cortical microtubules and regulates cell wall mechanics during cell elongation in plants. *Mol. Plant* **8**, 1011–1023. doi:10.1016/j.molp.2015.01.004
- Kotzer, A. M., Brandizzi, F., Neumann, U., Paris, N., Moore, I. and Hawes, C. (2004). AtRabF2b (Ara7) acts on the vacuolar trafficking pathway in tobacco leaf epidermal cells. *J. Cell Sci.* **117**, 6377–6389. doi:10.1242/jcs.01564
- Kwon, S. I., Cho, H. J., Jung, J. H., Yoshimoto, K., Shirasu, K. and Park, O. K. (2010). The Rab GTPase RabG3b functions in autophagy and contributes to tracheary element differentiation in Arabidopsis. *Plant J.* **64**, 151–164. doi:10.1111/j.1365-3113X.2010.04315.x
- Kwon, S. I., Cho, H. J., Kim, S. R. and Park, O. K. (2013). The Rab GTPase RabG3b positively regulates autophagy and immunity-associated hypersensitive cell death in arabidopsis. *Plant Physiol.* **161**, 1722–1736. doi:10.1104/pp.112.208108
- Lee, G.-J., Sohn, E. J., Lee, M. H. and Hwang, I. (2004). The arabidopsis Rab5 homologs Rha1 and Ara7 localize to the prevacuolar compartment. *Plant Cell Physiol.* **45**, 1211–1220. doi:10.1093/pcp/pch142
- Lee, A. H.-Y., Hurley, B., Felsensteiner, C., Yea, C., Kukurshumova, W., Bartetzko, V., Wang, P. W., Quach, V., Lewis, J. D., Liu, Y. C. et al. (2012). A bacterial acetyltransferase destroys plant microtubule networks and blocks secretion. *PLoS Pathog.* **8**, e1002523. doi:10.1371/journal.ppat.1002523
- Lei, L., Singh, A., Bashline, L., Li, S. D., Yingling, Y. G. and Gu, Y. (2015). Cellulose synthase interactive1 is required for fast recycling of cellulose synthase complexes to the plasma membrane in Arabidopsis. *Plant Cell* **27**, 2926–2940. doi:10.1105/tpc.15.00442
- Lepore, D., Spassibojko, O., Pinto, G. and Collins, R. N. (2016). Cell cycle-dependent phosphorylation of Sec4p controls membrane deposition during cytokinesis. *J. Cell Biol.* **214**, 691–703. doi:10.1083/jcb.201602038
- Li, J. and Staiger, C. J. (2018). Understanding cytoskeletal dynamics during the plant immune response. *Annu. Rev. Phytopathol.* **56**, 513–533. doi:10.1146/annurev-phyto-080516-035632
- Li, S., Lei, L., Somerville, C. R. and Gu, Y. (2012). Cellulose synthase interactive protein 1 (CS11) links microtubules and cellulose synthase complexes. *Proc. Natl. Acad. Sci. USA* **109**, 185–190. doi:10.1073/pnas.1118560109
- Li, S., Zhou, L.-Z., Feng, Q.-N., McCormick, S. and Zhang, Y. (2013). The C-terminal hypervariable domain targets Arabidopsis ROP9 to the invaginated pollen tube plasma membrane. *Mol. Plant* **6**, 1362–1364. doi:10.1093/mp/sss098
- Li, F., Yi, L., Zhao, L., Itzen, A., Goody, R. S. and Wu, Y.-W. (2014). The role of the hypervariable C-terminal domain in Rab GTPases membrane targeting. *Proc. Natl. Acad. Sci. USA* **111**, 2572–2577. doi:10.1073/pnas.1313655111
- Li, R., Rodriguez-Furlan, C., Wang, J., van de Ven, D., Gao, T., Raikhel, N. V. and Hicks, G. R. (2017). Different endomembrane trafficking pathways establish apical and basal polarities. *Plant Cell* **29**, 90–108. doi:10.1105/tpc.16.00524
- Livanos, P. and Müller, S. (2019). Division plane establishment and cytokinesis. *Annu. Rev. Plant Biol.* **70**, 239–267. doi:10.1146/annurev-arplant-050718-100444
- Lu, D., Lin, W., Gao, X., Wu, S., Cheng, C., Avila, J., Heese, A., Devarenne, T. P., He, P. and Shan, L. (2011). Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. *Science* **332**, 1439. doi:10.1126/science.1204903
- Mansfield, C., Newman, J. L., Olsson, T. S. G., Hartley, M., Chan, J. and Coen, E. (2018). Ectopic BASL reveals tissue cell polarity throughout leaf development in Arabidopsis thaliana. *Curr. Biol.* **28**, 2638–2646.e4. doi:10.1016/j.cub.2018.06.019
- Mayers, J. R., Hu, T., Wang, C., Cárdenas, J. J., Tan, Y., Pan, J. and Bednarek, S. Y. (2017). SCD1 and SCD2 Form a complex that functions with the exocyst and RabE1 in exocytosis and cytokinesis. *Plant Cell* **29**, 2610. doi:10.1105/tpc.17.00409
- McFarlane, H. E., Watanabe, Y., Gendre, D., Carruthers, K., Levesque-Tremblay, G., Haughn, G. W., Bhalerao, R. P. and Samuels, L. (2013). Cell wall polysaccharides are mislocalized to the vacuole in *echidna* mutants. *Plant Cell Physiol.* **54**, 1867–1880. doi:10.1093/pcp/pct129
- Men, S. Z., Boutté, Y., Ikeda, Y., Li, X. G., Palme, K., Stierhof, Y.-D., Hartmann, M.-A., Moritz, T. and Grebe, M. (2008). Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. *Nat. Cell Biol.* **10**, 237–244. doi:10.1038/ncb1686
- Müller, S. and Jürgens, G. (2016). Plant cytokinesis—no ring, no constriction but centrifugal construction of the partitioning membrane. *Semin. Cell Dev. Biol.* **53**, 10–18. doi:10.1016/j.semdb.2015.10.037
- Nakamura, M. and Grebe, M. (2018). Outer, inner and planar polarity in the Arabidopsis root. *Curr. Opin. Plant Biol.* **41**, 46–53. doi:10.1016/j.pbi.2017.08.002
- Nebenführ, A. and Dixit, R. (2018). Kinesins and myosins: molecular motors that coordinate cellular functions in plants. *Annu. Rev. Plant Biol.* **69**, 329–361. doi:10.1146/annurev-arplant-042817-040024
- Nielsen, E., Cheung, A. Y. and Ueda, T. (2008). The regulatory RAB and ARF GTPases for vesicular trafficking. *Plant Physiol.* **147**, 1516–1526. doi:10.1104/pp.108.121798
- Oda, Y., Iida, Y., Nagashima, Y., Sugiyama, Y. and Fukuda, H. (2015). Novel coiled-coil proteins regulate exocyst association with cortical microtubules in xylem cells via the conserved oligomeric golgi-complex 2 protein. *Plant Cell Physiol.* **56**, 277–286. doi:10.1093/pcp/pcu197
- Oikkonen, V. M. and Stenmark, H. (1997). Role of Rab GTPases in membrane traffic. *International Review of Cytology - A Survey of Cell Biology*, vol. 176 (ed. K. W. Jeon), pp. 1–85, Academic Press.
- Ong, S. T., Freeley, M., Skubis-Zegadło, J., Fazil, M. H. U. T., Kelleher, D., Fresser, F., Baier, G., Verma, N. K. and Long, A. (2014). Phosphorylation of Rab5a protein by protein kinase Cε is crucial for T-cell migration. *J. Biol. Chem.* **289**, 19420–19434. doi:10.1074/jbc.M113.545863
- Paredes, A. R., Somerville, C. R. and Ehrhardt, D. W. (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**, 1491–1495. doi:10.1126/science.1126551
- Pereira-Leal, J. B. and Seabra, M. C. (2001). Evolution of the Rab family of small GTP-binding proteins. *J. Mol. Biol.* **313**, 889–901. doi:10.1006/jmbi.2001.5072
- Peremyslov, V. V., Prokhnovsky, A. I., Avisar, D. and Dolja, V. V. (2008). Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis. *Plant Physiol.* **146**, 1109–1116. doi:10.1104/pp.107.113654
- Pinheiro, H., Samalova, M., Geldner, N., Chory, J., Martinez, A. and Moore, I. (2009). Genetic evidence that the higher plant Rab-D1 and Rab-D2 GTPases exhibit distinct but overlapping interactions in the early secretory pathway. *J. Cell Sci.* **122**, 3749–3758. doi:10.1242/jcs.050625
- Polko, J. K. and Kieber, J. J. (2019). The regulation of cellulose biosynthesis in plants. *Plant Cell* **31**, 282–296. doi:10.1105/tpc.18.00760
- Polko, J. K., Barnes, W. J., Voiniciuc, C., Doctor, S., Steinwand, B., Hill, J. L., Jr, Tien, M., Pauly, M., Anderson, C. T. and Kieber, J. J. (2018). SHOU4 proteins regulate trafficking of cellulose synthase complexes to the plasma membrane. *Curr. Biol.* **28**, 3174–3182.e6. doi:10.1016/j.cub.2018.07.076
- Preuss, M. L., Serna, J., Falbel, T. G., Bednarek, S. Y. and Nielsen, E. (2004). The Arabidopsis Rab GTPase RabA4b localizes to the tips of growing root hair cells. *Plant Cell* **16**, 1589–1603. doi:10.1105/tpc.021634
- Preuss, M. L., Schmitz, A. J., Thole, J. M., Bonner, H. K. S., Otegui, M. S. and Nielsen, E. (2006). A role for the RabA4b effector protein PI4Kβ1 in polarized expansion of root hair cells in Arabidopsis thaliana. *J. Cell Biol.* **172**, 991–998. doi:10.1083/jcb.200508116
- Qi, X. and Zheng, H. (2013). Rab-A1c GTPase defines a population of the trans-golgi network that is sensitive to endosidin1 during cytokinesis in arabidopsis. *Mol. Plant* **6**, 847–859. doi:10.1093/mp/sss116
- Qi, X. Y., Kaneda, M., Chen, J., Geitmann, A. and Zheng, H. Q. (2011). A specific role for Arabidopsis TRAPP1 in post-Golgi trafficking that is crucial for cytokinesis and cell polarity. *Plant J.* **68**, 234–248. doi:10.1111/j.1365-3113X.2011.04681.x
- Ravikumar, R., Kalbfuss, N., Gendre, D., Steiner, A., Altmann, M., Altmann, S., Rybak, K., Edelmann, H., Stephan, F., Lampe, M. et al. (2018). Independent yet overlapping pathways ensure the robustness and responsiveness of trans-Golgi network functions in Arabidopsis. *Development* **145**, dev169201. doi:10.1242/dev.169201
- Renna, L., Stefano, G., Slabaugh, E., Wormsbaeche, C., Sulpizio, A., Zienkiewicz, K. and Brandizzi, F. (2018). TGNap1 is required for microtubule-dependent homeostasis of a subpopulation of the plant trans-Golgi network. *Nat. Commun.* **9**, 5313. doi:10.1038/s41467-018-07662-4
- Rivero, C., Traubenik, S., Zanetti, M. E. and Bianco, F. A. (2019). Small GTPases in plant biotic interactions. *Small GTPases* **10**, 350–360. doi:10.1080/21541248.2017.1333557
- Rutherford, S. and Moore, I. (2002). The Arabidopsis Rab GTPase family: another enigma variation. *Curr. Opin. Plant Biol.* **5**, 518–528. doi:10.1016/S1369-5266(02)00307-2
- Saint-Jore, C. M., Evins, J., Batoko, H., Brandizzi, F., Moore, I. and Hawes, C. (2002). Redistribution of membrane proteins between the Golgi apparatus and endoplasmic reticulum in plants is reversible and not dependent on cytoskeletal networks. *Plant J.* **29**, 661–678. doi:10.1046/j.0960-7412.2002.01252.x
- Sampathkumar, A., Gutierrez, R., McFarlane, H. E., Bringmann, M., Lindeboom, J., Emons, A.-M., Samuels, L., Ketelaar, T., Ehrhardt, D. W. and Persson, S. (2013). Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis interphase cells. *Plant Physiol.* **162**, 675–688. doi:10.1104/pp.113.215277
- Schneider, R., Tang, L., Lampugnani, E. R., Barkwill, S., Lathe, R., Zhang, Y., McFarlane, H. E., Pesquet, E., Niittyla, T., Mansfield, S. D. et al. (2017). Two complementary mechanisms underpin cell wall patterning during xylem vessel development. *Plant Cell* **29**, 2433. doi:10.1105/tpc.17.00309
- Seguí-Simarro, J. M. and Staehelin, L. A. (2006). Cell cycle-dependent changes in Golgi stacks, vacuoles, clathrin-coated vesicles and multivesicular bodies in meristematic cells of Arabidopsis thaliana: A quantitative and spatial analysis. *Planta* **223**, 223–236. doi:10.1007/s00425-005-0082-2
- Singh, M. K., Krüger, F., Beckmann, H., Brumm, S., Vermeer, J. E. M., Munnik, T., Mayer, U., Stierhof, Y.-D., Grefen, C., Schumacher, K. et al. (2014). Protein

- delivery to vacuole requires SAND protein-dependent Rab GTPase conversion for MVB-vacuole fusion. *Curr. Biol.* **24**, 1383-1389. doi:10.1016/j.cub.2014.05.005
- Sohn, E. J., Kim, E. S., Zhao, M., Kim, S. J., Kim, H., Kim, Y.-W., Lee, Y. J., Hillmer, S., Sohn, U., Jiang, L. et al. (2003). Rha1, an Arabidopsis Rab5 homolog, plays a critical role in the vacuolar trafficking of soluble cargo proteins. *Plant Cell* **15**, 1057-1070. doi:10.1105/tpc.009779
- Speth, E. B., Imboden, L., Hauck, P. and He, S. Y. (2009). Subcellular localization and functional analysis of the arabidopsis GTPase RabE. *Plant Physiol.* **149**, 1824. doi:10.1104/pp.108.132092
- Staehelein, L. A. and Kang, B.-H. (2008). Nanoscale architecture of endoplasmic reticulum export sites and of Golgi membranes as determined by electron tomography. *Plant Physiol.* **147**, 1454-1468. doi:10.1104/pp.108.120618
- Stierhof, Y.-D. and El Kasm, F. (2010). Strategies to improve the antigenicity, ultrastructure preservation and visibility of trafficking compartments in Arabidopsis tissue. *Eur. J. Cell Biol.* **89**, 285-297. doi:10.1016/j.ejcb.2009.12.003
- Surkont, J. and Pereira-Leal, J. B. (2016). Are there Rab GTPases in archaea? *Mol. Biol. Evol.* **33**, 1833-1842. doi:10.1093/molbev/msw061
- Szumliński, A. L., and Nielsen, E. (2009). The Rab GTPase RabA4d regulates pollen tube tip growth in *Arabidopsis thaliana*. *Plant Cell* **21**, 526-544. doi:10.1105/tpc.108.060277
- Tomczynska, I., Stumpe, M. and Mauch, F. (2018). A conserved RxLR effector interacts with host RABA-type GTPases to inhibit vesicle-mediated secretion of antimicrobial proteins. *Plant J.* **95**, 187-203. doi:10.1111/tpj.13928
- Tominaga, M., Kimura, A., Yokota, E., Haraguchi, T., Shimmen, T., Yamamoto, K., Nakano, A. and Ito, K. (2013). Cytoplasmic streaming velocity as a plant size determinant. *Dev. Cell* **27**, 345-352. doi:10.1016/j.devcel.2013.10.005
- Ueda, T., Uemura, T., Sato, M. H. and Nakano, A. (2004). Functional differentiation of endosomes in Arabidopsis cells. *Plant J.* **40**, 783-789. doi:10.1111/j.1365-3113X.2004.02249.x
- Verchoot-Lubicz, J. and Goldstein, R. E. (2010). Cytoplasmic streaming enables the distribution of molecules and vesicles in large plant cells. *Protoplasma* **240**, 99-107. doi:10.1007/s00709-009-0088-x
- Viotti, C., Bubeck, J., Stierhof, Y.-D., Krebs, M., Langhans, M., van den Berg, W., van Dongen, W., Richter, S., Geldner, N., Takano, J. et al. (2010). Endocytic and secretory traffic in arabidopsis merge in the trans-golgi network/early endosome, an independent and highly dynamic organelle. *Plant Cell* **22**, 1344-1357. doi:10.1105/tpc.109.072637
- Vukašinović, N., Oda, Y., Pejchar, P., Synek, L., Pečenková, T., Rawat, A., Sekereš, J., Potocký, M. and Žárský, V. (2017). Microtubule-dependent targeting of the exocyst complex is necessary for xylem development in Arabidopsis. *New Phytol.* **213**, 1052-1067. doi:10.1111/nph.14267
- Wang, J., Ren, J., Wu, B., Feng, S., Cai, G., Tuluc, F., Peränen, J. and Guo, W. (2015). Activation of Rab8 guanine nucleotide exchange factor Rabin8 by ERK1/2 in response to EGF signaling. *Proc. Natl. Acad. Sci. USA* **112**, 148-153. doi:10.1073/pnas.1412089112
- Woollard, A. A. D. and Moore, I. (2008). The functions of Rab GTPases in plant membrane traffic. *Curr. Opin. Plant Biol.* **11**, 610-619. doi:10.1016/j.pbi.2008.09.010
- Xu, J., Kozlov, G., McPherson, P. S. and Gehring, K. (2018). A PH-like domain of the Rab12 guanine nucleotide exchange factor DENND3 binds actin and is required for autophagy. *J. Biol. Chem.* **293**, 4566-4574. doi:10.1074/jbc.RA117.001446
- Zhang, Y. and McCormick, S. (2007). A distinct mechanism regulating a pollen-specific guanine nucleotide exchange factor for the small GTPase Rop in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **104**, 18830-18835. doi:10.1073/pnas.0705874104
- Zhang, J., Chen, J., Wang, L., Zhao, S., Li, J., Liu, B., Li, H., Qi, X., Zheng, H. and Lu, M. (2018). AtBET5 is essential for exine pattern formation and apical meristem organization in Arabidopsis. *Plant Sci.* **274**, 231-241. doi:10.1016/j.plantsci.2018.05.033
- Zhang, W., Cai, C. and Staiger, C. J. (2019). Myosins XI are involved in exocytosis of cellulose synthase complexes. *Plant Physiol.* **179**, 1537-1555. doi:10.1104/pp.19.00018
- Zheng, H., Camacho, L., Wee, E., Batoko, H., Legen, J., Leaver, C. J., Malhó, R., Hussey, P. J. and Moore, I. (2005). A Rab-E GTPase mutant acts downstream of the Rab-D subclass in biosynthetic membrane traffic to the plasma membrane in tobacco leaf epidermis. *Plant Cell* **17**, 2020-2036. doi:10.1105/tpc.105.031112
- Zhong, R., Burk, D. H., Morrison, W. H. and Ye, Z.-H. (2002). A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. *Plant Cell* **14**, 3101-3117. doi:10.1105/tpc.005801
- Zhu, C., Ganguly, A., Baskin, T. I., McClosky, D. D., Anderson, C. T., Foster, C., Meunier, K. A., Okamoto, R., Berg, H. and Dixit, R. (2015). The fragile Fiber1 kinesin contributes to cortical microtubule-mediated trafficking of cell wall components. *Plant Physiol.* **167**, 780-792. doi:10.1104/pp.114.251462
- Zhu, X., Li, S., Pan, S., Xin, X. and Gu, Y. (2018). CSI1, PATROL1, and exocyst complex cooperate in delivery of cellulose synthase complexes to the plasma membrane. *Proc. Natl. Acad. Sci. USA* **115**, E3578-E3587. doi:10.1073/pnas.1800182115

Summary: A review of the elaborate plant trafficking pathways transporting different cargoes to different domains of the cell surface and the molecular machinery underpinning them, with a focus on Rab GTPases.

Funding details

| S.No. | Funder name | Funder ID | Grant ID |
|-------|--|---|-------------------------|
| 1 | Biotechnology and Biological Sciences Research Council | http://dx.doi.org/10.13039/501100000268 | 1810136 BB/P01979X/1 |
| 2 | Leverhulme Trust | http://dx.doi.org/10.13039/501100000275 | |