Membrane Traffic and Cytokinesis: The Final Cut

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Abstract

Cells don't simply separate at cytokinesis. While furrow contraction critically relies on myosin-II and F-actin, post-furrowing steps are less understood but involve the constriction of ESCRT-III polymer-dependent helices on the side of the midbody, which likely drive the final abscission. The first evidence that animal cell cytokinesis requires membrane traffic, as in plant cells, was provided about 15 years ago. Since then, it became increasingly clear that fusion of vesicles to the cytokinetic furrow is essential in large embryonic cells, and that membrane traffic within the intercellular bridge is crucial for its stability and successful abscission in all animal cells. Here, we review our current knowledge of the secretory and endocytic recycling pathways involved in cytokinesis, and how vesicles defined by specific Rab and Arf GTPases are targeted and fused to the membrane of the intercellular bridge thanks to different molecular motors, tethering complexes and SNARE machineries. At the functional level, we will describe how membrane traffic can remodel both phosphoinositide lipids and promote F-actin clearance necessary for ESCRT-III-dependent abscission, and identify key unanswered questions in the field. We will finally review recent evidence showing a tight coupling between membrane traffic and cytokinesis in complex processes, such as during the establishment of *de novo* apico-basal polarity.

The division of one cell into two daughter cells is fundamental for all living organisms. Cytokinesis, the final step of cell division whereby the two daughter cells physically separate, remains a challenging frontier in cell biology as extensive research aims to understand the precise mechanism of daughter cell split [1-3]. Since the 1970s and the discovery that animal cells use actin filaments (F-actin) and myosin-II to constrict a furrow required for the cleavage of the two daughter cells, more than one hundred genes have been identified to directly or indirectly contribute to cytokinesis [4-6]. While many questions remain unanswered, it was demonstrated that cytoskeletal elements, such as actin, microtubules and septins, cooperate and play a crucial role during cytokinesis [1-3, 7]. During the early steps of cytokinesis, the ingression of the cleavage furrow is driven by an actomyosin contractile ring interacting with the plasma membrane and results in the formation of a narrow cytoplasmic bridge that connects the two daughter cells. This intercellular bridge, filled with anti-parallel microtubule bundles, is eventually severed in a process termed abscission, representing the last and still the least understood step of cytokinesis [8]. At the center of this bridge, an electron-dense structure named the midbody serves as a platform for the recruitment of proteins essential for abscission. The discovery that assembly of endosomal sorting complex required for transport (ESCRT)-III filament helices on the side of the midbody, downstream of Cep55 and ALIX/TSG101, likely drives the final bridge constriction and leads to cytokinetic abscission has been a major discovery in the last ten years [8-10].

It becomes increasingly clear that cytokinesis relies on a close cooperation between cytoskeletal elements and lipid-based membranes, and that membrane traffic plays a critical role in cytokinesis in animal cells. This idea is not new since it was initially observed in the 1930's that vesicles from a cytoplasmic reservoir insert at the furrow and it was proposed that they could even drive cytokinetic ingression during the cleavage phase of large marine eggs [11]. The role of membrane traffic as a driving force for cytokinesis was abandoned after the discovery of the actin ring but resurrected later (see below). In higher plant cells, which have no myosin II and no contractile cortex, the importance of membrane traffic was recognized a century ago (BOX 1). Unexpectedly, genome-wide screens in the early 2000's using *Drosophila* cells, human cells and *C. elegans* combined with elegant genetic studies in *Drosophila* male spermatogenesis and *Drosophila* cellularization (a process akin of cytokinesis in many respects) revealed an unexpected importance of membrane trafficking genes in the late steps of cytokinesis, both for intercellular bridge stabilization and abscission [4-6]. Once believed to be totally shut down throughout cell division, it was found that

clathrin-mediated endocytosis is not interrupted and both endocytic recycling and secretion resume during mitotic exit [12-14, 15 , 16-18].

Excellent previous reviews have described the role of membrane traffic in cytokinesis [19-23] but a wealth of important information has been obtained since. In this review, we will summarize key historical findings and emphasize the recent discoveries about the role of membrane traffic and its functional significance in animal cells. We will first review the existence as well as the mechanisms of vesicle targeting, docking and fusion to the furrow and the bridge. We will then explain how this intense trafficking can remodel both phosphoinositide lipids and promote F-actin clearance necessary for ESCRT-III-dependent abscission. We will finally review how membrane traffic is tightly coupled to cytokinesis in order to establish apico-basal polarity during *de novo* lumen formation in 3D organotypic models.

Active trafficking and fusion of vesicles during early and late steps of cytokinesis

Membrane insertion and endocytosis at the cytokinetic furrow in large dividing cells

Pioneer studies in amphibian eggs [11] and later in *Xenopus*, zebrafish, *Drosophila* and sea urchin revealed that the ingressing furrow is an active site of insertion of microtubule-dependent vesicles of internal origin [14, 24-28]. Interestingly`, membrane insertion at the furrow and actomyosin ring contraction occur simultaneously [14, 24], and accommodate surface increase associated with the division of one cell into two. Reinforcing the link between membrane addition and actomyosin ring contraction, vesicles of endosomal origin were reported to deliver F-actin to the furrow in *Drosophila* embryos [28]. Complete or stable furrow ingression is sensitive to Brefeldin A treatment (which impairs secretory vesicle production) in some but not all organisms, such as in *C. elegans* embryos [29]. More specifically, it requires Golgi-dependent secretion in *Drosophila* spermatocytes, with essential function of Cog5, Cog7, Rab1, Zw10, TRAPPII and COP-I [30-35]. This likely reflects an absence of a sufficient reservoir of plasma membrane microvilli, consistent with a requirement of exocyst-dependent secretion for cell surface increase during furrow contraction [36]. Intriguingly, clathrin-coated pits and caveolae are found at the cleavage furrow in zebrafish embryos, and perturbing endocytosis results in furrow regression [37]; however the reason for furrow-specific endocytosis and in particular caveolae is an open

question. Vesicles containing endocytosed material are also found apposed to the furrow in smaller, human cells [13], in which cholesterol-dependent endocytic tubules originating from the intercellular bridge after furrow contraction are also observed [38]. Of note, the endocytic protein dynamin-1 was recently proposed to act after ingression to remove membrane from the intercellular bridge through endocytosis in *C. elegans* embryos [39]. Altogether, both secretion and endocytosis appear to be required for furrow ingression or for maintaining contracted furrows in eggs or embryonic systems (Figure 1).

Traffic of vesicles within the intercellular bridge and membrane remodeling in late cytokinetic steps

In contrast, perturbing membrane trafficking usually impairs either intercellular bridge stability after furrow contraction and abscission in other systems, including mammalian cells [20-23]. Consistently, the existence of membrane vesicles within the intercellular bridge was reported using electron microscopy (EM) in original studies, but their exact nature and function could not be addressed at that time [40]. Time-lapse microscopy using GFP-tagged secretory markers later revealed a dynamic accumulation [15] and fusion [17] of vesicles within intercellular bridges, originating equally from both connected daughter cells [17]. Contrary to initial models [15], the fusion of these vesicles, which occurs before abscission, is required for successful abscission but does not drive it directly. This is consistent with high pressure freezing EM tomography and live-cell fluorescent studies indicating that few secretory vesicles are present in late bridges, where microtubule-associated FIP3- and VAMP8-positive endosomes [39, 41, 42], as well as Rab35-positive endosomes [43, 44], accumulate close to or at the future abscission site. The fusion of FIP3 endosomes is required for the formation of a 100-200 nm diameter bridge constriction (also named secondary ingression) on the side of the midbody and ESCRT-III recruitment at this location [41, 45]. According to the current models, the final scission event occurs at the secondary ingression and is directly driven by ESCRT-III-dependent helices that assemble into spirals that pinch the plasma membrane at the abscission site [9, 42, 46-51]. Thus, fusion of FIP3 and Rab35 endosomes to the bridge appears as an important upstream event preparing abscission in human cells (Figure 1).

Unexpectedly, tomography did not reveal any helices at the abscission site during the first division of *C. elegans* embryos, contrary to human cells [39]. Consistently, ESCRT do not seem to be essential for abscission, but rather are required for outward budding and shedding

of membranes from the midbody [39], a phenomena already observed in *Drosophila* and mammalian cells [50, 52]. Thus, recent *in vivo* evidence argues for a role of ESCRTs in removing excessive membrane from the midbody, at least in *C. elegans*, suggesting that ESCRT-independent pathways exist to promote abscission.

Mechanisms of vesicle targeting and fusion to the furrow and to the intercellular bridge during cytokinesis

Several proteins are now described as being essential for vesicle transport and delivery to the furrow and the bridge (Figure 2). Fusion of vesicles with a target membrane is a sequential process that requires association of vesicles with molecular motors (i.e. kinesins, myosins or dynein), docking of vesicles by tethering complexes (e.g. exocyst) and fusion itself by interactions between cognate vesicular SNAREs (v-SNAREs or VAMPs) and target SNAREs (t-SNAREs or syntaxins) on the acceptor membrane. These steps are regulated by conserved GTPases of the Rab and Arf families, which cycle between soluble, GDP-bound state and membrane-associated, GTP-bound state that directly recruit effector proteins [53, 54]. Among the > 60 Rabs encoded in the human genome, several have been found localized at the furrow or the intercellular bridge (Rab1, Rab8, Rab10, Rab11 Rab14, Rab21, Rab24 and Rab35) [22, 35, 55-58]. Most functional studies have focused on the role of Rab11 and Rab35, as well as their effector proteins in cytokinesis. In *C. elegans*, Rab11 [29] and its GEF REI-1 are required for normal furrow ingression [59]. Rab11 and its effector FIP3 [60-64] regulate endosomal recycling and their role in cytokinesis has also been reported in *Drosophila* [65, 66]. Furthermore, Rab35 regulates a separate recycling pathway and has been identified in a systematic screen for Rab GTPases required for cytokinesis [43, 67]. While the general role of endocytosis and endocytic recycling in cell division was known previously [20, 22, 68], these two GTPases define specific pathways involved in cytokinesis.

Targeting of vesicles into the intercellular bridge by molecular motors

Bi-directional movement of Rab11/FIP3 and Rab35 endosomes in and out of early bridges have been reported in human cells, whereas they are largely stationary in late bridges [41, 44, 69-72]. FIP3 directly interacts through distinct sites with the Rab11 GTPase and with the Arf6 GTPase, and both FIP3 and Arf6 promote abscission [41, 45, 62, 70]. Interestingly, the bidirectional movement of FIP3 endosomes within the intercellular bridge is dependent on a

switch between plus-end and minus-end microtubule-based motors (conventional kinesin KIF5B and dynein, respectively), which is controlled by Arf6 and the KIF5B/dynein scaffold protein JIP4 [70]. In dog renal epithelial MDCK cells in matrigel, endosomes marked by another Rab11 effector (FIP5) are transported along microtubules toward the center of the bridge thanks to the plus-end-directed motor KIF3A/B [73], which also transports the Golgi associated spectrin syne-1 to the same location [74]. Regarding the transport of Rab8 vesicles, less is known. The actin-based motor myosin VI is required for cytokinesis and for transporting transferrin-positive vesicles in and out of the bridge [75]. Myosin VI might help transport Rab8 endosomes within the bridge since it interacts with the Rab8 partner Optineurin, but this has not been tested experimentally. In any case, DCDC5 binds to both Rab8 and dynein, and is required for Rab8 accumulation at the bridge center and for normal timing of abscission, perhaps by promoting trafficking toward the minus ends of a small number of microtubules that traverse the bridge [76]. However, it is not yet known which motors actively transport Rab35, nor secretory vesicles within the bridge in human cells or towards the furrow in large embryonic cells (see above).

Docking vesicles within the intercellular bridge by multiple mechanisms

More is known about the docking of many secretory and endosomal vesicles at the midbody, which relies on the exocyst, an octameric tethering complex (Figure 2). All subunits of the exocyst are concentrated in a ring like structure at the midbody of human cells and are required for normal abscission [15, 63]. Strikingly, Rab11/FIP3 endosomes can be docked in the bridge through several interactions: 1) Sec15 of the exocyst is a Rab11 effector; 2) FIP3 links Rab11 with Arf6 (see above), while Arf6 itself interacts with the exocyst component Sec10; 3) FIP3 binds to Cyk4, a component of the central spindling complex present in a ring-like structure at the bridge center [62, 63, 69]. In addition, Arf6 forms a complex with the MKLP1 kinesin of the centralspindlin, thus concentrates at the midbody where it is required for bridge stability and abscission [70, 71, 77-80]. Of note, Arf1 and Arf3 are also concentrated at the midbody [81]. While both Arf6 and Rab11 might act synergistically, it should be pointed out that Arf6 plays a predominant role since Rab11 is dispensable for FIP3 endosome targeting in bridges [62, 63, 70]. In addition, the RalA and RalB GTPases collaborate and are required for completion of abscission by controlling exocyst localization at the furrow and the midbody, respectively [82, 83]. Interestingly, the t-SNARE syntaxin 16 (likely together with its cognate SNARE syntaxin 6) is also crucial for the accumulation of exocyst components and Rab11/FIP3 endosomes (but not secretory vesicles) in the

intercellular bridge [84]. Remarkably, Syntaxin 16 is also required for accumulation of Cep55 and the ESCRT protein ALIX in the midbody, demonstrating a pivotal role of Syntaxin 16 in connecting the exocyst, Rab11/FIP3 endosomes and the ESCRT machinery during cytokinesis [84]. Yet another layer of crosstalk between the exocyst and endosomes exists, since the multifunctional protein BRUCE interacts with the GTPases Rab8 and Rab11, as well as the exocyst subunits Sec6 and Sec8 and is also required for abscission [85].

In addition to the exocyst, the Arf6-binding scaffolding protein MICAL-Like1 (MICAL-L1) localizes to bridges, participates to Rab11/FIP3 endosome targeting at this location and contributes to cytokinesis [86]. Of note, the MICAL-L1 interactor EHD1 interacts with the SNARE protein Snapin, which has a well-described role in cytokinesis [15]. Since MICAL-L1 interacts directly with both Rab35 and Rab8 [87, 88], future studies should determine whether it may also link Arf6 with Rab35 or Rab8 endosomes at the midbody.

Finally, another member of the MICAL family [89], MICAL3, is involved in the tethering of Rab8 vesicles at the midbody before fusion with the plasma membrane. Rab8 endosomes within bridges are distinct and arrive before FIP3 endosomes [45]. During cytokinesis, MICAL3 is localized to the intercellular bridge through a direct interaction with the centralspindlin component MKLP1, where MICAL3 also directly interacts and thus docks Rab8-positive endosomes close to the plasma membrane [90]. Although the exact cargoes important for cytokinesis that are delivered by Rab8 endosomes are unknown, Rab8 vesicles concentrate in the bridge in a MICAL3-dependent manner, and promote intercellular bridge stability prior to abscission [90, 91] (Figure 2).

Fusion of vesicles with the plasma membrane during cytokinesis

Several t-SNARE/syntaxins involved in vesicular fusion at the plasma membrane have been identified, and were among the first trafficking proteins characterized in *C. elegans* [92] and sea urchin cytokinesis [93]. The syntaxin 1 homologue Syn-4 in *C. elegans* is localized at the furrow and is required for furrow ingression in embryos [92]. In mammalian cells, Syntaxin 2, the v-SNARE VAMP8 and the SNARE complex-promoting protein Snapin are required for abscission [94], likely for the fusion of secretory vesicles to the bridge [15]. Fusion of recycling endosomes is also required for cytokinesis, and interfering with the endosomal v-SNAREs VAMP3 and VAMP7 leads to cytokinetic defects and defects in Ca^{++}-dependent surface increase in telophase [16]. Of note, perturbing endocytosis by various means leads to

cytokinetic defects, presumably due to a reduced amount of recycling endosomes [13, 16, 95]. As described above, the t-SNARE Syntaxin 16 is also required for the accumulation of Rab11- and FIP3-positive endosome accumulation within intercellular bridges, likely through its key role in exocyst delivery to the midbody. However, it remains to be investigated why vesicles do not accumulate as after exocyst inactivation [15] and instead disappear from the bridge in Syntaxin 16-depleted cells. In parallel to Syntaxin 16, it has been shown that SCAMP2/3, originally characterized as proteins regulating exocytic membrane fusion, colocalize with FIP3 in bridges and may thus promote Rab11/FIP3-endosome fusion into the intercellular bridge, a step required for the formation of the secondary ingression and ESCRT-III accumulation on the side of the midbody (see above and [41, 45]). Interestingly, SCAMP3, like FIP3 and FIP4, interacts with the ESCRT-protein TSG101, and is required for abscission, possibly connecting these endosomes with ESCRT-III localization at the abscission site [45, 96].

Altogether, a number of components of the machinery required for targeting, docking and fusion of secretory vesicles, Rab11/FIP3 endosomes and to a lesser extent Rab8 endosomes at the ingressing furrow and/or at the intercellular bridge have been identified (Figure 2). A key question is to characterize the cargoes transported by these distinct pathways and their roles in cytokinesis. In the past few years, the functions of several cargoes have been identified and found to impact local lipid and F-actin remodeling, as detailed in the next two sections.

Membrane traffic and phosphoinositide remodeling during cytokinesis

Lipid composition dramatically changes during cytokinesis, in particular in the furrow and bridge regions [97, 98]. Although phosphoinositides (PIs) represent less than 1% of total cellular lipids [99], they play critical roles in several steps of cytokinesis, as reviewed in detail recently [100]. In this section, we will focus on the direct connections between PIs and membrane traffic for maintaining bridge stability and abscission.

Phosphoinositides and intercellular bridge stability

Seven distinct PIs can be generated through selective phosphorylation of the 3, 4, 5 hydroxyl positions of the myo-inositol headgroup of phosphatidylinositol [99]. Among them, PtdIns(4,5)P_2 is enriched at the furrow and directly binds to several proteins, notably anillin,

Septin2 and MgcRacGAP, which are essential for furrow stability once contracted [100] (Figure 3A). Membrane traffic and PIs are interdependent in promoting complete furrow contraction and stability of the bridge for four reasons:

First, the subunit Exo70 of the exocyst (see above) binds to PtdIns(4,5)P_2 *in vitro,* and this interaction is likely important for the correct association of the tethering complex with the plasma membrane [101]. Second, vesicles originating from the Golgi may be important for delivering PtdIns4*P* to late ingressing furrows, where it is converted into PtdIns(4,5)P_2 [102]. Accordingly, the type III PtdIns 4-kinase (Fwd or four wheel drive in *Drosophila*) does not localize at the furrow but at the Golgi, where it produces PtdIns4*P* from phosphatidylinositol. Consistent with Fwd being important for PtdIns4*P*, thus PtdIns(4,5)P_2 enrichment in late furrows, mutation in *fwd* leads to furrow regression after contraction in spermatocytes [102]. Third, the PtdIns 4-kinase also promotes bridge stability through a kinase-independent mechanism, by directly recruiting Rab11 to the Golgi complex and on post-Golgi vesicles [102-104]. As a consequence, the inactivation of Fwd prevents the accumulation of PtdIns4*P* vesicles colocalizing with Rab11 close to the furrow. Forth, the PtdIns4*P* effector GOLPH3 (Golgi Phosphoprotein 3) forms a complex with myosin II, septins and Rab11, and may thus coordinate phosphoinositides with vesicle trafficking and furrow contraction to completion during cytokinesis [105] (Figure 3A). Interestingly, GOLPH3 is an effector of the *Drosophila* Rab1 (Omt), which controls its localization at the furrow and membrane trafficking [35]. Future studies should reveal whether the multiple roles of PtdIns 4-kinase in complete furrow contraction and bridge stability are conserved beyond *Drosophila* spermatocytes.

Phosphoinositides and abscission

There is growing evidence indicating that remodeling the PI composition within the bridge is essential for successful abscission, which requires PtdIns3*P* production and PtdIns(4,5)P_2 hydrolysis (Figure 3B). The class III PI-3-kinase VPS34 and its non-catalytic subunits Beclin-1, UVRAG and Bif-1 are found in the intercellular bridge together with PtdIns3*P*-positive endosomes [106, 107]. A key function of PtdIns3*P* endosomes during cytokinesis is to transport the PtdIns3*P*-intercting protein FYVE-CENT into the bridge both in human cells and in mouse oocytes [106, 108], which is proposed to act as a scaffold for targeting TCC19 [106]. This transport is dependent on the kinesin KIF13A. Interestingly, TCC19 interacts with the ESCRT-III subunit CHMP4B, and may thus regulate the function of ESCRT-III in

abscission [106, 107]. In addition, the G-protein Gαi and its GEF Ric-8A regulate the VPS34-dependent production of PtdIns3*P* endosomes and thus abscission in human cells [109].

While PtdIns(4,5)P_2 is crucial for bridge stability (see above), this PI must be hydrolyzed in late bridges for successful abscission. The PI 5-phosphatase OCRL is responsible for PtdIns(4,5)P_2 hydrolysis prior to abscission, and depletion of OCRL leads to delayed abscission [72]. Interestingly, OCRL is an effector of Rab35 and both proteins colocalize on endosomes moving in and out of the bridge. Thus OCRL may be targeted to the abscission site through fusion of these endosomes to the plasma membrane of the bridge. Accordingly, OCRL localization in bridges depends on Rab35 and Rab35 depletion also impairs PtdIns(4,5)P_2 hydrolysis and thus abscission [72]. Of note, Rab35/OCRL similarly control PtdIns(4,5)P_2 hydrolysis on endosomes, and complete depletion/inactivation of OCRL in *Drosophila* and human cells leads to delocalization of Septins on PtdIns(4,5)P_2-rich intracellular endosomes and thus bridge instability [67, 110-112].

Endosomal traffic promotes actin clearance in late bridges and abscission

Rab11 and Rab35 endosomes promote abscission by ensuring low levels of F-actin required for abscission (Figure 3B). Several parallel mechanisms are at stake, which not only restrict excessive F-actin polymerization but also actively induce F-actin depolymerization, as detailed below.

Restricting F-actin accumulation in the intercellular bridge by Rab11 and Rab35 endosomes

After furrow ingression, F-actin is progressively removed from the intercellular bridge until reaching extremely low levels before abscission [44, 45, 113]. Consistently, it has been proposed that F-actin accumulation could act as a physical barrier, impair vesicles trafficking and fusion to the intercellular bridge, and inhibit the assembly of ESCRT-III filaments at the abscission site.

It was first established that excess F-actin in late bridges is responsible for abscission delay by studying the consequences of Rab35 and OCRL depletion in human cells [72]. As mentioned above, Rab35 and its effector OCRL favors PtdIns(4,5)P_2 hydrolysis at the time of abscission. High levels of PtdIns(4,5)P_2 is known to stimulate actin polymerization and to inhibit the

activity of the actin-severing enzyme Cofilin [114], which could thus explain F-actin accumulation after either OCRL or Rab35 depletion [72]. Importantly, low levels of the F-actin depolymerizing drug Latrunculin-A restores normal actin levels in bridges and rescues the abscission delay associated with Rab35 or OCRL depletion, demonstrating that excessive F-actin levels indeed prevents normal abscission [72].

In parallel to the Rab35 pathway, Rab11/FIP3 endosomes transport key cargoes to the intercellular bridge, which also prevent F-actin polymerization in late bridges. As mentioned above, SCAMP2/3-dependent fusion of FIP3 endosomes to the bridge is required for the formation of the secondary ingression and ESCRT-III recruitment at the abscission site [45]. Interestingly, FIP3 depletion leads to abscission delay associated with excessive F-actin levels in bridges and defective ESCRT-III localization. Proteomic analysis of isolated FIP3-endosomes and functional studies revealed that these endosomes transport many proteins, including p50RhoGAP (ARHGAP1), a GTPase-activating protein (GAP) for RhoA, a well-established positive regulator of F-actin polymerization [1-3]. Accordingly, p50RhoGAP depletion induces high levels of F-actin in bridges and delays abscission. Thus, it is proposed that FIP3 endosomes normally prevent F-actin accumulation by inactivating RhoA through at least the delivery of p50RhoGAP to late bridges in human cells.

Active depolymerization of F-actin in the intercellular bridge by Rab35- and MICAL1-dependent oxidoreduction

The fact that F-actin levels increase after OCRL or p50RhoGAP depletion suggests that there is a balance between actin polymerization and depolymerization in late bridges. However, the mechanisms that actively depolymerize F-actin were not known until the recent discovery of a key role for the oxidoreductase MICAL1 as an effector of Rab35 in this process [44]. Like MICAL3 and MICAL-L1 (see above), MICAL1 belongs to an evolutionarily conserved family of oxidoreductases, recently reviewed in [89]. MICAL1 is known to oxidize specific methionine residues of F-actin, which leads to complete actin filament disassembly *in vitro* [44, 115]. Accordingly, depletion of MICAL1 leads to dramatic F-actin accumulation in late bridges, delays abscission in an actin-dependent manner and impairs ESCRT-III recruitment at the abscission site (but not earlier at the midbody) [44]. The function of MICAL1 in promoting actin depolymerization and abscission is conserved in human and *Drosophila* cells. Interestingly, MICAL1 localizes at the midbody and at the abscission site [44, 116, 117]. Furthermore, Rab35 binding to MICAL1 not only controls the localization of the latter at the

abscission site but also activates its oxidoreductase activity [44]. Intriguingly, the function of MICAL3 in cytokinesis does not dependent on F-actin depolymerization or on its redox activity [90] and MICAL-L1 lacks the redox domain thereby acting as a scaffolding protein [86]. This indicates that the three members of the MICAL family discussed above play a role in cytokinesis through different mechanisms. Altogether, MICAL1-dependent oxidation constitutes the first example of a role of oxidoreduction in cell division, controls F-actin depolymerization, and thus ESCRT-III recruitment and abscission. Future studies should reveal whether Rab11/FIP3/p50RhoGAP, Rab35/OCRL and Rab35/MICAL1 act in a synergistic and perhaps sequential manner to clear F-actin in late cytokinetic bridges prior to abscission.

405

Coupling membrane traffic, cytokinesis and apico-basal polarity establishment

The last decade has revealed an essential role of membrane traffic in initiation and maintenance of apico-basal polarity in 2D and 3D epithelia. This has been extensively reviewed recently [118]. It is now well established that intracellular trafficking promotes the segregation of apical determinants (such as Par6, aPKC, Par3 and Crumbs), basolateral determinants (such as Lgl, Scrib and Dlg) and specific transporters into distinct plasma membrane domains in polarized epithelial cells [118]. Remarkably, proteins involved in cytokinesis (such as the exocyst complex, Rab8, Rab11, Rab35, FIP3, FIP5, VAMP7 and ESCRT, see above) also control apical polarity in a variety of *in vivo* and tissue culture models [118]. Here, we will focus on how membrane traffic is coupled to cytokinesis during establishment of the apical domain and *de novo* lumen formation, essentially in organotypic cyst models.

420

Trafficking of key apical determinants toward the cytokinetic bridge during de novo *apical lumen formation*

When a single human Caco-2, mouse ES or canine MDCK cell is embedded inside extracellular matrix (such as Matrigel), it undergoes proliferation and morphogenesis leading to the formation of a cyst, which is a polarized epithelial monolayer surrounding a single, centrally-located apical lumen. During this *de novo* lumen formation, endocytosis and recycling pathways are crucial for delivering apical polarity determinants at the first cell-cell interface, classically referred as to the AMIS (apical membrane initiation site). In particular,

the apical determinant Crumbs3 and the lumen promoting factor PODXL/GP135 are two key transmembrane proteins internalized and trafficked into Rab11 endosomes, which are targeted to the AMIS during initial steps of cyst formation [119, 120]. A complete molecular network has been identified allowing for the movement, targeting and fusion of Rab11 vesicles containing Crumbs, PODXL and Cdc42 with the AMIS, thereby determining where the apical domain initiates and where the lumen forms [118, 121-123] (Figure 4). Briefly, Rab11 recruits its effector Rabin8 (a Rab8 GEF) to activate Rab8A, which in turn leads to Myosin-5b recruitment and Cdc42 activation on recycling endosomes. In parallel Rab11 recruits Sec15 of the exocyst to these vesicles, while other exocyst subunits are localized at the AMIS [118, 121]. Furthermore, Rab3b and Rab27a/b act together with the SNARE protein Syntaxin-3 (STX3) and the synaptotagmin-like proteins, Slp2-a and Slp4-a, to target the vesicles to the AMIS and to initiate lumen formation [122, 123]. Remarkably, it was noticed that Rab11 vesicles deliver Crumbs (and other cargoes) around the cytokinetic bridge during the first cell division, suggesting a link between cytokinesis and the position of the future apical lumen [119]. In other words, the AMIS is determined by the position of the intercellular bridge before cytokinetic abscission of the first daughter cells has occurred [73, 124, 125].

Molecular mechanisms coupling vesicle targeting and fusion to the cytokinetic bridge during polarity establishment

How are Rab11-positive endosomes transporting key apical determinants and lumen-promoting factors targeted to the first cytokinetic bridge during polarity establishment? First, Rab11 regulates the delivery of apical vesicles to the AMIS through its effector FIP5 that interacts with the sortin nexin 18 (SNX18) and the kinesin KIF3A [73, 126]. Interaction of Rab11/FIP5 with the plus-end-directed kinesin KIF3A helps to explain how Rab11 vesicles are targeted toward the cytokinetic bridge during AMIS formation (Figure 4). Besides this spatial targeting, there is a temporal regulation of FIP5/KIF3A interaction, which can only occur during cytokinesis. Indeed, this interaction is inhibited through a GSK3-dependent phosphorylation of FIP5 during metaphase and anaphase, but not during telophase and cytokinesis [124]. In addition, another FIP5 binding protein, Cingulin (CGN), has also been identified as a tethering factor that ensures the fidelity of apical endosome targeting to the AMIS [127]. CGN forms a complex with the tight junction protein ZO-1 and with the Rac1/WAVE complex, which first mediates CGN recruitment at the AMIS [127].

In parallel to the Rab11 pathway, Rab35 also acts as a docking factor for the apical vesicles containing PODXL [125]. Mechanistically, Rab35 directly interacts with the cytoplasmic tail of PODXL and acts as a tethering factor for PODXL-positive vesicles at the AMIS. As Rab35 is enriched at the ingressing furrow during cytokinesis, it likely acts upstream in the pathway of vesicle recruitment to the AMIS. Accordingly, depletion of Rab35 (as well as impairing the interaction between Rab35 and the PODXL tail) leads to a complete inversion of apico-basal polarity. Indeed, the apical vesicles that cannot fuse at the first cytokinetic bridge fuse back to the matrigel-facing membrane and initiate apical-like polarity at this abnormal location [125]. Interestingly, the vesicles tethered at the plasma membrane of the bridge not only contain the lumen-promoting factor PODXL, but also other key apical determinants such as Crumbs3, aPKC and Cdc42. Among all Rab GTPases, Rab35 was confirmed to be one whose depletion inverts polarity upon depletion in 3D cysts [123]. Intriguingly, a mutant of Rab35 that can not interact with its effector ACAP2 (an Arf6 GAP) is unable to rescue the depletion phenotype, suggesting that inversion of polarity might be due to Arf6 activation [123]. Future studies should aim to determine whether this mutant also looses the ability to bind to the PODXL tail.

Altogether, these studies demonstrate that Rab11 and Rab35 play a pivotal role in coupling cytokinesis with apical lumen establishment. Like during cytokinetic abscission, these two pathways act at different steps and both promote *de novo* lumen formation at the center of the cyst, around the first cytokinetic bridge. *In vivo,* a recent study also revealed that CCM-3 plays a role both in promoting furrow contraction to completion and in cell polarity [128]. In *C. elegans* embryos, CCM-3 regulates Rab11-dependent recycling, is required for proper localization of Anillin and non-muscle myosin II at the furrow, and controls polarity establishment in early embryogenesis likely by regulating proper Myosin-II activity.

Open questions and Perspectives

Recent years have yielded key discoveries regarding the functional link between membrane trafficking and cytokinesis (Figure 5). Remarkably, the same membrane trafficking pathways and effector proteins can be used in cytokinesis and in other important cellular functions (BOX 2). During cytokinesis, the kinesin MKLP1 plays an essential role in the spatial control of abscission, by localizing the exocyst and other docking machineries at the center of the

bridge. It is also established that Rab11/FIP3 and Rab35/MICAL1/OCRL modules control the clearing of F-actin from late bridges, an important step for ESCRT-III recruitment at the abscission site. However, a number of important questions still remains.

First, the precise timing of arrival/fusion of secretory, Rab8, Rab11 and Rab35 vesicles with respect to each other and with ESCRT-III has not been tested directly (Figure 1 represents a summary of data from several papers). The systematic use of genome-edited cell lines allowing for endogenous expression levels, as recently shown for Rab35/MICAL1, will be very helpful to address this question and to avoid artifacts of overexpression [43, 44].

Second, we still do not know the relevant cargoes transported by Rab8-positive endosomes and by secretory vesicles, which promote bridge stability and abscission, respectively. Furthermore, many cargoes are transported by Rab11/FIP3 endosomes, and functional analysis suggests that additional cargoes beyond p50RhoGAP are important for abscission [45]. It also remains to be clarified how the site of secondary ingression is defined, and whether endosomal cargoes or Rab effectors act locally at this site. Perhaps, microtubule buckling at the abscission site [41, 129] plays a role in biasing the delivery and fusion of the vesicles at this location.

Third, while Rab11 and Rab35 endosomes caught much of the attention in recent years, additional Rabs localize to the furrow or the bridge (Rab1, Rab10, Rab14, Rab21 and Rab24) and little is known about their function in cytokinesis or the cargos that they deliver [22, 35, 55-58]. It will also be important to confirm the function of the aforementioned Rab GTPases in cytokinesis *in vivo*, since most of our knowledge comes from tissue culture cells. Intriguingly, proteins involved in membrane traffic are essential for cytokinesis in *Drosophila* spermatocytes but not in somatic cells for a reason that is not fully understood [30-35].

Finally, particular lipids are largely enriched during cytokinesis by largely unknown mechanisms [98]. These lipids could potentially be delivered by membrane vesicles to promote cytokinesis. It indeed appears that ESCRT-III might be dispensable in *C. elegans* abscission [39], suggesting that alternative, perhaps lipid-based pathways exist. The lipidome of the vesicles trafficking within the bridge might shed light on this hypothesis in the future [98].

BOX 1: A key but different role of membrane traffic in cytokinesis in Plant cells

While all eukaryotes evolved from a common ancestor, plants and animal cells use different strategies for partitioning dividing cells, as recently reviewed in [130]. Plant cell cytokinesis is mediated by a microtubule and actin-based specialized platform, the phragmoplast, which delivers membrane vesicles to the plane of division. These membrane vesicles fuse with one another to form a cell plate, which grows from the center to the cell periphery until it physically separates the two daughter cells. These vesicles originate both from the secretory pathway, which plays an essential role [131] and from the endocytic pathway [132]. However, in contrast to animal cytokinesis, cell-plate formation does not involve recycling pathways. Given the role of the exocyst, Rab GTPases, t-SNARE and similar microtubule crosslinkers, it has been proposed that the phragmoplast in plant cells and the bridge/spindle midzone in animal cells might be analogous structures [133].

BOX 2: Re-use of Rab/effector modules outside cytokinesis

Identical Rab/effector modules used to achieve cytokinesis are also crucial for diverse other functions in cells, and some examples are given below. For instance, Rab6, Rab8 and MICAL3 cooperate to control the docking and fusion of exocytic carriers to the plasma membrane [134]. As during cytokinesis, the GTPase Rab35 and its lipid phosphatase effector OCRL control PtdIns(4,5)P_2 homeostasis and F-actin remodeling in endosomal trafficking and around internalized bacteria [112, 135, 136]. Rab35 together with its effector ACAP2 regulate Arf6 activity and are required for FcγR-mediated phagocytosis [137] and neurite outgrowth [138]. Rac1/Rab11/FIP3 regulate vesicle trafficking and actin remodeling during T cell activation [139]. Another important module is the ESCRT machinery that drives membrane budding and fission away from the cytosol, in wide-ranging cellular contexts including the formation of multivesicular endosomes (MVBs), cytokinetic abscission, plasma membrane repair, neuron pruning, exovesicle shedding, nuclear pore complex quality control, nuclear envelope reassembly and repair, unconventional protein secretion and virus budding (reviewed in [10]). Interestingly, while the core ESCRT-I-II-III machinery is always used, the upstream targeting events are specific for a particular function (Cep55 for cytokinesis, GAG for viral budding, HRS and STAM1/2 for MVBs, ALG-2 for plasma membrane repair).

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References

1. Green, R.A., Paluch, E., and Oegema, K. (2012). Cytokinesis in Animal Cells. Annual review of cell and developmental biology.
2. Fededa, J.P., and Gerlich, D.W. (2012). Molecular control of animal cell cytokinesis. Nature cell biology *14*, 440-447.
3. D'Avino, P.P., Giansanti, M.G., and Petronczki, M. (2015). Cytokinesis in animal cells. Cold Spring Harbor perspectives in biology *7*, a015834.
4. Skop, A.R., Liu, H., Yates, J., 3rd, Meyer, B.J., and Heald, R. (2004). Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. Science *305*, 61-66.
5. Eggert, U.S., Kiger, A.A., Richter, C., Perlman, Z.E., Perrimon, N., Mitchison, T.J., and Field, C.M. (2004). Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. PLoS Biol *2*, e379.
6. Echard, A., Hickson, G.R., Foley, E., and O'Farrell, P.H. (2004). Terminal Cytokinesis Events Uncovered after an RNAi Screen. Current biology : CB *14*, 1685-1693.
7. Pollard, T.D. (2017). Nine unanswered questions about cytokinesis. The Journal of cell biology *216*, 3007-3016.
8. Mierzwa, B., and Gerlich, D.W. (2014). Cytokinetic Abscission: Molecular Mechanisms and Temporal Control. Developmental cell *31*, 525-538.
9. Stoten, C.L., and Carlton, J.G. (2017). ESCRT-dependent control of membrane remodelling during cell division. Seminars in cell & developmental biology.
10. Christ, L., Raiborg, C., Wenzel, E.M., Campsteijn, C., and Stenmark, H. (2017). Cellular Functions and Molecular Mechanisms of the ESCRT Membrane-Scission Machinery. Trends in biochemical sciences *42*, 42-56.
11. Schechtman, A.M. (1937). Localized Cortical Growth as the Immediate Cause of Cell Division. Science *85*, 222-223.
12. Monzo, P., Gauthier, N.C., Keslair, F., Loubat, A., Field, C.M., Le Marchand-Brustel, Y., and Cormont, M. (2005). Clues to CD2-associated protein involvement in cytokinesis. Molecular biology of the cell *16*, 2891-2902.

13. Schweitzer, J.K., Burke, E.E., Goodson, H.V., and D'Souza-Schorey, C. (2005). Endocytosis resumes during late mitosis and is required for cytokinesis. The Journal of biological chemistry *280*, 41628-41635.
14. Shuster, C.B., and Burgess, D.R. (2002). Targeted new membrane addition in the cleavage furrow is a late, separate event in cytokinesis. Proceedings of the National Academy of Sciences of the United States of America *99*, 3633-3638.
15. Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C.T., Mirabelle, S., Guha, M., Sillibourne, J., and Doxsey, S.J. (2005). Centriolin Anchoring of Exocyst and SNARE Complexes at the Midbody Is Required for Secretory-Vesicle-Mediated Abscission. Cell *123*, 75-87.
16. Boucrot, E., and Kirchhausen, T. (2007). Endosomal recycling controls plasma membrane area during mitosis. Proceedings of the National Academy of Sciences of the United States of America *104*, 7939-7944.
17. Goss, J.W., and Toomre, D.K. (2008). Both daughter cells traffic and exocytose membrane at the cleavage furrow during mammalian cytokinesis. The Journal of cell biology *181*, 1047-1054.
18. Tacheva-Grigorova, S.K., Santos, A.J., Boucrot, E., and Kirchhausen, T. (2013). Clathrin-mediated endocytosis persists during unperturbed mitosis. Cell reports *4*, 659-668.
19. Albertson, R., Riggs, B., and Sullivan, W. (2005). Membrane traffic: a driving force in cytokinesis. Trends in cell biology *15*, 92-101.
20. Montagnac, G., Echard, A., and Chavrier, P. (2008). Endocytic traffic in animal cell cytokinesis. Curr Opin Cell Biol *20*, 454-461.
21. Neto, H., Collins, L.L., and Gould, G.W. (2011). Vesicle trafficking and membrane remodelling in cytokinesis. Biochem J *437*, 13-24.
22. Schiel, J.A., and Prekeris, R. (2012). Membrane dynamics during cytokinesis. Curr Opin Cell Biol.
23. Schiel, J.A., Childs, C., and Prekeris, R. (2013). Endocytic transport and cytokinesis: from regulation of the cytoskeleton to midbody inheritance. Trends in cell biology *23*, 319-327.
24. Bluemink, J.G., and de Laat, S.W. (1973). New membrane formation during cytokinesis in normal and cytochalasin B- treated eggs of Xenopus laevis. I. Electron microscope observations. The Journal of cell biology *59*, 89-108.
25. Jesuthasan, S. (1998). Furrow-associated microtubule arrays are required for the cohesion of zebrafish blastomeres following cytokinesis. J Cell Sci *111 (Pt 24)*, 3695-3703.
26. Danilchik, M.V., Bedrick, S.D., Brown, E.E., and Ray, K. (2003). Furrow microtubules and localized exocytosis in cleaving Xenopus laevis embryos. J Cell Sci *116*, 273-283.
27. Li, W.M., Webb, S.E., Lee, K.W., and Miller, A.L. (2006). Recruitment and SNARE-mediated fusion of vesicles in furrow membrane remodeling during cytokinesis in zebrafish embryos. Experimental cell research *312*, 3260-3275.
28. Albertson, R., Cao, J., Hsieh, T.S., and Sullivan, W. (2008). Vesicles and actin are targeted to the cleavage furrow via furrow microtubules and the central spindle. The Journal of cell biology *181*, 777-790.
29. Skop, A.R., Bergmann, D., Mohler, W.A., and White, J.G. (2001). Completion of cytokinesis in C. elegans requires a brefeldin A- sensitive membrane accumulation at the cleavage furrow apex. Current biology : CB *11*, 735-746.
30. Farkas, R.M., Giansanti, M.G., Gatti, M., and Fuller, M.T. (2003). The Drosophila Cog5 homologue is required for cytokinesis, cell elongation, and assembly of

specialized Golgi architecture during spermatogenesis. Molecular biology of the cell *14*, 190-200.

31. Robinett, C.C., Giansanti, M.G., Gatti, M., and Fuller, M.T. (2009). TRAPPII is required for cleavage furrow ingression and localization of Rab11 in dividing male meiotic cells of Drosophila. J Cell Sci *122*, 4526-4534.
32. Wainman, A., Giansanti, M.G., Goldberg, M.L., and Gatti, M. (2012). The Drosophila RZZ complex - roles in membrane trafficking and cytokinesis. J Cell Sci *125*, 4014-4025.
33. Belloni, G., Sechi, S., Riparbelli, M.G., Fuller, M.T., Callaini, G., and Giansanti, M.G. (2012). Mutations in Cog7 affect Golgi structure, meiotic cytokinesis and sperm development during Drosophila spermatogenesis. J Cell Sci *125*, 5441-5452.
34. Kitazawa, D., Yamaguchi, M., Mori, H., and Inoue, Y.H. (2012). COPI-mediated membrane trafficking is required for cytokinesis in Drosophila male meiotic divisions. J Cell Sci *125*, 3649-3660.
35. Sechi, S., Frappaolo, A., Fraschini, R., Capalbo, L., Gottardo, M., Belloni, G., Glover, D.M., Wainman, A., and Giansanti, M.G. (2017). Rab1 interacts with GOLPH3 and controls Golgi structure and contractile ring constriction during cytokinesis in Drosophila melanogaster. Open biology *7*.
36. Giansanti, M.G., Vanderleest, T.E., Jewett, C.E., Sechi, S., Frappaolo, A., Fabian, L., Robinett, C.C., Brill, J.A., Loerke, D., Fuller, M.T., et al. (2015). Exocyst-Dependent Membrane Addition Is Required for Anaphase Cell Elongation and Cytokinesis in Drosophila. PLoS genetics *11*, e1005632.
37. Feng, B., Schwarz, H., and Jesuthasan, S. (2002). Furrow-specific endocytosis during cytokinesis of zebrafish blastomeres. Experimental cell research *279*, 14-20.
38. Kettle, E., Page, S.L., Morgan, G.P., Malladi, C.S., Wong, C.L., Boadle, R.A., Marsh, B.J., Robinson, P.J., and Chircop, M. (2015). A Cholesterol-Dependent Endocytic Mechanism Generates Midbody Tubules During Cytokinesis. Traffic *16*, 1174-1192.
39. Konig, J., Frankel, E.B., Audhya, A., and Muller-Reichert, T. (2017). Membrane remodeling during embryonic abscission in Caenorhabditis elegans. The Journal of cell biology *216*, 1277-1286.
40. Mullins, J.M., and Biesele, J.J. (1977). Terminal phase of cytokinesis in D-98s cells. The Journal of cell biology *73*, 672-684.
41. Schiel, J.A., Park, K., Morphew, M.K., Reid, E., Hoenger, A., and Prekeris, R. (2011). Endocytic membrane fusion and buckling-induced microtubule severing mediate cell abscission. J Cell Sci *124*, 1411-1424.
42. Guizetti, J., Schermelleh, L., Mantler, J., Maar, S., Poser, I., Leonhardt, H., Muller-Reichert, T., and Gerlich, D.W. (2011). Cortical Constriction During Abscission Involves Helices of ESCRT-III-Dependent Filaments. Science *331*, 1616-1620.
43. Klinkert, K., and Echard, A. (2016). Rab35 GTPase: a central regulator of phosphoinositides and F-actin in endocytic recycling and beyond. Traffic.
44. Fremont, S., Hammich, H., Bai, J., Wioland, H., Klinkert, K., Rocancourt, M., Kikuti, C., Stroebel, D., Romet-Lemonne, G., Pylypenko, O., et al. (2017). Oxidation of F-actin controls the terminal steps of cytokinesis. Nat Commun *8*, 14528.
45. Schiel, J.A., Simon, G.C., Zaharris, C., Weisz, J., Castle, D., Wu, C.C., and Prekeris, R. (2012). FIP3-endosome-dependent formation of the secondary ingression mediates ESCRT-III recruitment during cytokinesis. Nature cell biology *14*, 1068-1078.
46. Carlton, J.G., and Martin-Serrano, J. (2007). Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. Science *316*, 1908-1912.

47. Morita, E., Sandrin, V., Chung, H.Y., Morham, S.G., Gygi, S.P., Rodesch, C.K., and Sundquist, W.I. (2007). Human ESCRT and ALIX proteins interact with proteins of the midbody and function in cytokinesis. The EMBO journal *26*, 4215-4227.
48. Lee, H.H., Elia, N., Ghirlando, R., Lippincott-Schwartz, J., and Hurley, J.H. (2008). Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55. Science *322*, 576-580.
49. Elia, N., Sougrat, R., Spurlin, T.A., Hurley, J.H., and Lippincott-Schwartz, J. (2011). Dynamics of endosomal sorting complex required for transport (ESCRT) machinery during cytokinesis and its role in abscission. Proceedings of the National Academy of Sciences of the United States of America *108*, 4846-4851.
50. Sherman, S., Kirchenbuechler, D., Nachmias, D., Tamir, A., Werner, S., Elbaum, M., and Elia, N. (2016). Resolving new ultrastructural features of cytokinetic abscission with soft-X-ray cryo-tomography. Scientific reports *6*, 27629.
51. Mierzwa, B.E., Chiaruttini, N., Redondo-Morata, L., von Filseck, J.M., Konig, J., Larios, J., Poser, I., Muller-Reichert, T., Scheuring, S., Roux, A., et al. (2017). Dynamic subunit turnover in ESCRT-III assemblies is regulated by Vps4 to mediate membrane remodelling during cytokinesis. Nature cell biology *19*, 787-798.
52. El Amine, N., Kechad, A., Jananji, S., and Hickson, G.R. (2013). Opposing actions of septins and Sticky on Anillin promote the transition from contractile to midbody ring. The Journal of cell biology *203*, 487-504.
53. Stenmark, H. (2009). Rab GTPases as coordinators of vesicle traffic. Nature reviews. Molecular cell biology *10*, 513-525.
54. Pylypenko, O., Hammich, H., Yu, I.M., and Houdusse, A. (2017). Rab GTPases and their interacting protein partners: Structural insights into Rab functional diversity. Small GTPases, 1-27.
55. Pellinen, T., Tuomi, S., Arjonen, A., Wolf, M., Edgren, H., Meyer, H., Grosse, R., Kitzing, T., Rantala, J.K., Kallioniemi, O., et al. (2008). Integrin trafficking regulated by Rab21 is necessary for cytokinesis. Developmental cell *15*, 371-385.
56. Lalioti, V.S., Ilari, A., O'Connell, D.J., Poser, E., Sandoval, I.V., and Colotti, G. (2014). Sorcin links calcium signaling to vesicle trafficking, regulates Polo-like kinase 1 and is necessary for mitosis. PloS one *9*, e85438.
57. Kelly, E.E., Horgan, C.P., Adams, C., Patzer, T.M., Ni Shuilleabhain, D.M., Norman, J.C., and McCaffrey, M.W. (2009). Class I Rab11-Family Interacting Proteins are binding targets for the Rab14 GTPase. Biol Cell.
58. Militello, R.D., Munafo, D.B., Beron, W., Lopez, L.A., Monier, S., Goud, B., and Colombo, M.I. (2013). Rab24 is required for normal cell division. Traffic *14*, 502-518.
59. Sakaguchi, A., Sato, M., Sato, K., Gengyo-Ando, K., Yorimitsu, T., Nakai, J., Hara, T., Sato, K., and Sato, K. (2015). REI-1 Is a Guanine Nucleotide Exchange Factor Regulating RAB-11 Localization and Function in C. elegans Embryos. Developmental cell *35*, 211-221.
60. Hickson, G.R., Matheson, J., Riggs, B., Maier, V.H., Fielding, A.B., Prekeris, R., Sullivan, W., Barr, F.A., and Gould, G.W. (2003). Arfophilins are dual Arf/Rab 11 binding proteins that regulate recycling endosome distribution and are related to Drosophila nuclear fallout. Molecular biology of the cell *14*, 2908-2920.
61. Horgan, C.P., Walsh, M., Zurawski, T.H., and McCaffrey, M.W. (2004). Rab11-FIP3 localises to a Rab11-positive pericentrosomal compartment during interphase and to the cleavage furrow during cytokinesis. Biochem Biophys Res Commun *319*, 83-94.
62. Fielding, A.B., Schonteich, E., Matheson, J., Wilson, G., Yu, X., Hickson, G.R., Srivastava, S., Baldwin, S.A., Prekeris, R., and Gould, G.W. (2005). Rab11-FIP3 and

FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. The EMBO journal *24*, 3389-3399.

63. Wilson, G.M., Fielding, A.B., Simon, G.C., Yu, X., Andrews, P.D., Hames, R.S., Frey, A.M., Peden, A.A., Gould, G.W., and Prekeris, R. (2005). The FIP3-Rab11 protein complex regulates recycling endosome targeting to the cleavage furrow during late cytokinesis. Molecular biology of the cell *16*, 849-860.
64. Kasahara, K., Nakayama, Y., Nakazato, Y., Ikeda, K., Kuga, T., and Yamaguchi, N. (2007). Src signaling regulates completion of abscission in cytokinesis through ERK/MAPK activation at the midbody. The Journal of biological chemistry *282*, 5327-5339.
65. Riggs, B., Rothwell, W., Mische, S., Hickson, G.R., Matheson, J., Hays, T.S., Gould, G.W., and Sullivan, W. (2003). Actin cytoskeleton remodeling during early Drosophila furrow formation requires recycling endosomal components Nuclear-fallout and Rab11. The Journal of cell biology *163*, 143-154.
66. Giansanti, M.G., Belloni, G., and Gatti, M. (2007). Rab11 is required for membrane trafficking and actomyosin ring constriction in meiotic cytokinesis of Drosophila males. Molecular biology of the cell *18*, 5034-5047.
67. Kouranti, I., Sachse, M., Arouche, N., Goud, B., and Echard, A. (2006). Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. Current biology : CB *16*, 1719-1725.
68. Prekeris, R., and Gould, G.W. (2008). Breaking up is hard to do - membrane traffic in cytokinesis. J Cell Sci *121*, 1569-1576.
69. Simon, G.C., Schonteich, E., Wu, C.C., Piekny, A., Ekiert, D., Yu, X., Gould, G.W., Glotzer, M., and Prekeris, R. (2008). Sequential Cyk-4 binding to ECT2 and FIP3 regulates cleavage furrow ingression and abscission during cytokinesis. The EMBO journal *27*, 1791-1803.
70. Montagnac, G., Sibarita, J.B., Loubery, S., Daviet, L., Romao, M., Raposo, G., and Chavrier, P. (2009). ARF6 Interacts with JIP4 to control a motor switch mechanism regulating endosome traffic in cytokinesis. Current biology : CB *19*, 184-195.
71. Takahashi, S., Takei, T., Koga, H., Takatsu, H., Shin, H.W., and Nakayama, K. (2011). Distinct roles of Rab11 and Arf6 in the regulation of Rab11-FIP3/arfophilin-1 localization in mitotic cells. Genes Cells *16*, 938-950.
72. Dambournet, D., Machicoane, M., Chesneau, L., Sachse, M., Rocancourt, M., El Marjou, A., Formstecher, E., Salomon, R., Goud, B., and Echard, A. (2011). Rab35 GTPase and OCRL phosphatase remodel lipids and F-actin for successful cytokinesis. Nature cell biology *13*, 981-988.
73. Li, D., Kuehn, E.W., and Prekeris, R. (2014). Kinesin-2 mediates apical endosome transport during epithelial lumen formation. Cell Logist *4*, e28928.
74. Fan, J., and Beck, K.A. (2004). A role for the spectrin superfamily member Syne-1 and kinesin II in cytokinesis. J Cell Sci *117*, 619-629.
75. Arden, S.D., Puri, C., Au, J.S., Kendrick-Jones, J., and Buss, F. (2007). Myosin VI Is Required for Targeted Membrane Transport during Cytokinesis. Molecular biology of the cell *18*, 4750-4761.
76. Elad, N., Abramovitch, S., Sabanay, H., and Medalia, O. (2011). Microtubule organization in the final stages of cytokinesis as revealed by cryo-electron tomography. J Cell Sci *124*, 207-215.
77. Schweitzer, J.K., and D'Souza-Schorey, C. (2002). Localization and activation of the ARF6 GTPase during cleavage furrow ingression and cytokinesis. The Journal of biological chemistry *277*, 27210-27216.

78. Dyer, N., Rebollo, E., Dominguez, P., Elkhatib, N., Chavrier, P., Daviet, L., Gonzalez, C., and Gonzalez-Gaitan, M. (2007). Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. Development *134*, 4437-4447.
79. Joseph, N., Hutterer, A., Poser, I., and Mishima, M. (2012). ARF6 GTPase protects the post-mitotic midbody from 14-3-3-mediated disintegration. The EMBO journal *31*, 2604-2614.
80. Makyio, H., Ohgi, M., Takei, T., Takahashi, S., Takatsu, H., Katoh, Y., Hanai, A., Ueda, T., Kanaho, Y., Xie, Y., et al. (2012). Structural basis for Arf6-MKLP1 complex formation on the Flemming body responsible for cytokinesis. The EMBO journal *31*, 2590-2603.
81. Hanai, A., Ohgi, M., Yagi, C., Ueda, T., Shin, H.W., and Nakayama, K. (2016). Class I Arfs (Arf1 and Arf3) and Arf6 are localized to the Flemming body and play important roles in cytokinesis. Journal of biochemistry *159*, 201-208.
82. Chen, X.W., Inoue, M., Hsu, S.C., and Saltiel, A.R. (2006). RalA-exocyst-dependent recycling endosome trafficking is required for the completion of cytokinesis. The Journal of biological chemistry *281*, 38609-38616.
83. Cascone, I., Selimoglu, R., Ozdemir, C., Del Nery, E., Yeaman, C., White, M., and Camonis, J. (2008). Distinct roles of RalA and RalB in the progression of cytokinesis are supported by distinct RalGEFs. The EMBO journal *27*, 2375-2387.
84. Neto, H., Kaupisch, A., Collins, L.L., and Gould, G.W. (2013). Syntaxin 16 is a master recruitment factor for cytokinesis. Molecular biology of the cell *24*, 3663-3674.
85. Pohl, C., and Jentsch, S. (2008). Final stages of cytokinesis and midbody ring formation are controlled by BRUCE. Cell *132*, 832-845.
86. Reinecke, J.B., Katafiasz, D., Naslavsky, N., and Caplan, S. (2015). Novel functions for the endocytic regulatory proteins MICAL-L1 and EHD1 in mitosis. Traffic *16*, 48-67.
87. Rahajeng, J., Giridharan, S.S., Cai, B., Naslavsky, N., and Caplan, S. (2012). MICAL-L1 is a tubular endosomal membrane hub that connects Rab35 and Arf6 with Rab8a. Traffic *13*, 82-93.
88. Kobayashi, H., Etoh, K., Ohbayashi, N., and Fukuda, M. (2014). Rab35 promotes the recruitment of Rab8, Rab13 and Rab36 to recycling endosomes through MICAL-L1 during neurite outgrowth. Biol Open *3*, 803-814.
89. Fremont, S., Romet-Lemonne, G., Houdusse, A., and Echard, A. (2017). Emerging roles of MICAL family proteins - from actin oxidation to membrane trafficking during cytokinesis. J Cell Sci *130*, 1509-1517.
90. Liu, Q., Liu, F., Yu, K.L., Tas, R., Grigoriev, I., Remmelzwaal, S., Serra-Marques, A., Kapitein, L.C., Heck, A.J.R., and Akhmanova, A. (2016). MICAL3 Flavoprotein Monooxygenase Forms a Complex with Centralspindlin and Regulates Cytokinesis. Journal of Biological Chemistry *291*, 20617-20629.
91. Kaplan, A., and Reiner, O. (2011). Linking cytoplasmic dynein and transport of Rab8 vesicles to the midbody during cytokinesis by the doublecortin domain-containing 5 protein. J Cell Sci *124*, 3989-4000.
92. Jantsch-Plunger, V., and Glotzer, M. (1999). Depletion of syntaxins in the early Caenorhabditis elegans embryo reveals a role for membrane fusion events in cytokinesis. Current biology : CB *9*, 738-745.
93. Conner, S.D., and Wessel, G.M. (1999). Syntaxin is required for cell division. Molecular biology of the cell *10*, 2735-2743.

94. Low, S.H., Li, X., Miura, M., Kudo, N., Quinones, B., and Weimbs, T. (2003). Syntaxin 2 and endobrevin are required for the terminal step of cytokinesis in mammalian cells. Developmental cell *4*, 753-759.
95. Smith, C.M., and Chircop, M. (2012). Clathrin-mediated endocytic proteins are involved in regulating mitotic progression and completion. Traffic *13*, 1628-1641.
96. Horgan, C.P., Hanscom, S.R., Kelly, E.E., and McCaffrey, M.W. (2012). Tumor susceptibility gene 101 (TSG101) is a novel binding-partner for the class II Rab11-FIPs. PloS one *7*, e32030.
97. Ng, M.M., Chang, F., and Burgess, D.R. (2005). Movement of membrane domains and requirement of membrane signaling molecules for cytokinesis. Developmental cell *9*, 781-790.
98. Atilla-Gokcumen, G.E., Muro, E., Relat-Goberna, J., Sasse, S., Bedigian, A., Coughlin, M.L., Garcia-Manyes, S., and Eggert, U.S. (2014). Dividing cells regulate their lipid composition and localization. Cell *156*, 428-439.
99. Schink, K.O., Tan, K.W., and Stenmark, H. (2016). Phosphoinositides in Control of Membrane Dynamics. Annual review of cell and developmental biology *32*, 143-171.
100. Cauvin, C., and Echard, A. (2015). Phosphoinositides: Lipids with informative heads and mastermind functions in cell division. Biochimica et biophysica acta *1851*, 832-843.
101. He, B., Xi, F., Zhang, X., Zhang, J., and Guo, W. (2007). Exo70 interacts with phospholipids and mediates the targeting of the exocyst to the plasma membrane. The EMBO journal *26*, 4053-4065.
102. Polevoy, G., Wei, H.C., Wong, R., Szentpetery, Z., Kim, Y.J., Goldbach, P., Steinbach, S.K., Balla, T., and Brill, J.A. (2009). Dual roles for the Drosophila PI 4-kinase four wheel drive in localizing Rab11 during cytokinesis. The Journal of cell biology *187*, 847-858.
103. de Graaf, P., Zwart, W.T., van Dijken, R.A., Deneka, M., Schulz, T.K., Geijsen, N., Coffer, P.J., Gadella, B.M., Verkleij, A.J., van der Sluijs, P., et al. (2004). Phosphatidylinositol 4-kinasebeta is critical for functional association of rab11 with the Golgi complex. Molecular biology of the cell *15*, 2038-2047.
104. Burke, J.E., Inglis, A.J., Perisic, O., Masson, G.R., McLaughlin, S.H., Rutaganira, F., Shokat, K.M., and Williams, R.L. (2014). Structures of PI4KIIIbeta complexes show simultaneous recruitment of Rab11 and its effectors. Science *344*, 1035-1038.
105. Sechi, S., Colotti, G., Belloni, G., Mattei, V., Frappaolo, A., Raffa, G.D., Fuller, M.T., and Giansanti, M.G. (2014). GOLPH3 Is Essential for Contractile Ring Formation and Rab11 Localization to the Cleavage Site during Cytokinesis in Drosophila melanogaster. PLoS genetics *10*, e1004305.
106. Sagona, A.P., Nezis, I.P., Pedersen, N.M., Liestol, K., Poulton, J., Rusten, T.E., Skotheim, R.I., Raiborg, C., and Stenmark, H. (2010). PtdIns(3)P controls cytokinesis through KIF13A-mediated recruitment of FYVE-CENT to the midbody. Nature cell biology *12*, 362-371.
107. Thoresen, S.B., Pedersen, N.M., Liestol, K., and Stenmark, H. (2010). A phosphatidylinositol 3-kinase class III sub-complex containing VPS15, VPS34, Beclin 1, UVRAG and BIF-1 regulates cytokinesis and degradative endocytic traffic. Experimental cell research *316*, 3368-3378.
108. You, S.Y., Park, Y.S., Jeon, H.J., Cho, D.H., Jeon, H.B., Kim, S.H., Chang, J.W., Kim, J.S., and Oh, J.S. (2016). Beclin-1 knockdown shows abscission failure but not autophagy defect during oocyte meiotic maturation. Cell cycle *15*, 1611-1619.

109. Boularan, C., Kamenyeva, O., Cho, H., and Kehrl, J.H. (2014). Resistance to inhibitors of cholinesterase (Ric)-8A and Galphai contribute to cytokinesis abscission by controlling vacuolar protein-sorting (Vps)34 activity. PloS one *9*, e86680.
110. Ben El Kadhi, K., Roubinet, C., Solinet, S., Emery, G., and Carreno, S. (2011). The inositol 5-phosphatase dOCRL controls PI(4,5)P2 homeostasis and is necessary for cytokinesis. Current biology : CB *21*, 1074-1079.
111. Chesneau, L., Dambournet, D., Machicoane, M., Kouranti, I., Fukuda, M., Goud, B., and Echard, A. (2012). An ARF6/Rab35 GTPase cascade for endocytic recycling and successful cytokinesis. Current biology : CB *22*, 147-153.
112. Cauvin, C., Rosendale, M., Gupta-Rossi, N., Rocancourt, M., Larraufie, P., Salomon, R., Perrais, D., and Echard, A. (2016). Rab35 GTPase Triggers Switch-like Recruitment of the Lowe Syndrome Lipid Phosphatase OCRL on Newborn Endosomes. Current biology : CB *26*, 120-128.
113. Fremont, S., and Echard, A. (2017). Studying cytokinesis and midbody remnants using correlative light/scanning EM. Methods in cell biology *137*, 239-251.
114. Echard, A. (2012). Phosphoinositides and cytokinesis: The "PIP" of the iceberg. Cytoskeleton *69*, 893-912.
115. Hung, R.J., Pak, C.W., and Terman, J.R. (2011). Direct redox regulation of F-actin assembly and disassembly by Mical. Science *334*, 1710-1713.
116. Fukuda, M., Kanno, E., Ishibashi, K., and Itoh, T. (2008). Large scale screening for novel rab effectors reveals unexpected broad Rab binding specificity. Mol Cell Proteomics *7*, 1031-1042.
117. Rai, A., Oprisko, A., Campos, J., Fu, Y., Friese, T., Itzen, A., Goody, R.S., Gazdag, E.M., and Müller, M.P. (2016). bMERB domains are bivalent Rab8 family effectors evolved by gene duplication. eLife *5*.
118. Roman-Fernandez, A., and Bryant, D.M. (2016). Complex Polarity: Building Multicellular Tissues Through Apical Membrane Traffic. Traffic *17*, 1244-1261.
119. Schluter, M.A., Pfarr, C.S., Pieczynski, J., Whiteman, E.L., Hurd, T.W., Fan, S., Liu, C.J., and Margolis, B. (2009). Trafficking of Crumbs3 during cytokinesis is crucial for lumen formation. Molecular biology of the cell *20*, 4652-4663.
120. Bryant, D.M., Roignot, J., Datta, A., Overeem, A.W., Kim, M., Yu, W., Peng, X., Eastburn, D.J., Ewald, A.J., Werb, Z., et al. (2014). A molecular switch for the orientation of epithelial cell polarization. Developmental cell *31*, 171-187.
121. Bryant, D.M., Datta, A., Rodriguez-Fraticelli, A.E., Peranen, J., Martin-Belmonte, F., and Mostov, K.E. (2010). A molecular network for de novo generation of the apical surface and lumen. Nature cell biology *12*, 1035-1045.
122. Galvez-Santisteban, M., Rodriguez-Fraticelli, A.E., Bryant, D.M., Vergarajauregui, S., Yasuda, T., Banon-Rodriguez, I., Bernascone, I., Datta, A., Spivak, N., Young, K., et al. (2012). Synaptotagmin-like proteins control the formation of a single apical membrane domain in epithelial cells. Nature cell biology *14*, 838-849.
123. Mrozowska, P.S., and Fukuda, M. (2016). Regulation of podocalyxin trafficking by Rab small GTPases in 2D and 3D epithelial cell cultures. The Journal of cell biology *213*, 355-369.
124. Li, D., Mangan, A., Cicchini, L., Margolis, B., and Prekeris, R. (2014). FIP5 phosphorylation during mitosis regulates apical trafficking and lumenogenesis. EMBO Rep *15*, 428-437.
125. Klinkert, K., Rocancourt, M., Houdusse, A., and Echard, A. (2016). Rab35 GTPase couples cell division with initiation of epithelial apico-basal polarity and lumen opening. Nat Commun *7*, 11166.

126. Willenborg, C., Jing, J., Wu, C., Matern, H., Schaack, J., Burden, J., and Prekeris, R. (2011). Interaction between FIP5 and SNX18 regulates epithelial lumen formation. The Journal of cell biology *195*, 71-86.
127. Mangan, A.J., Sietsema, D.V., Li, D., Moore, J.K., Citi, S., and Prekeris, R. (2016). Cingulin and actin mediate midbody-dependent apical lumen formation during polarization of epithelial cells. Nat Commun *7*, 12426.
128. Pal, S., Lant, B., Yu, B., Tian, R., Tong, J., Krieger, J.R., Moran, M.F., Gingras, A.C., and Derry, W.B. (2017). CCM-3 Promotes C. elegans Germline Development by Regulating Vesicle Trafficking Cytokinesis and Polarity. Current biology : CB *27*, 868-876.
129. Lafaurie-Janvore, J., Maiuri, P., Wang, I., Pinot, M., Manneville, J.B., Betz, T., Balland, M., and Piel, M. (2013). ESCRT-III assembly and cytokinetic abscission are induced by tension release in the intercellular bridge. Science *339*, 1625-1629.
130. Muller, S., and Jurgens, G. (2016). Plant cytokinesis-No ring, no constriction but centrifugal construction of the partitioning membrane. Seminars in cell & developmental biology *53*, 10-18.
131. Reichardt, I., Stierhof, Y.D., Mayer, U., Richter, S., Schwarz, H., Schumacher, K., and Jurgens, G. (2007). Plant cytokinesis requires de novo secretory trafficking but not endocytosis. Current biology : CB *17*, 2047-2053.
132. Dhonukshe, P., Baluska, F., Schlicht, M., Hlavacka, A., Samaj, J., Friml, J., and Gadella, T.W., Jr. (2006). Endocytosis of cell surface material mediates cell plate formation during plant cytokinesis. Developmental cell *10*, 137-150.
133. Otegui, M.S., Verbrugghe, K.J., and Skop, A.R. (2005). Midbodies and phragmoplasts: analogous structures involved in cytokinesis. Trends in cell biology *15*, 404-413.
134. Grigoriev, I., Yu, K.L., Martinez-Sanchez, E., Serra-Marques, A., Smal, I., Meijering, E., Demmers, J., Peranen, J., Pasterkamp, R.J., van der Sluijs, P., et al. (2011). Rab6, Rab8, and MICAL3 cooperate in controlling docking and fusion of exocytotic carriers. Current biology : CB *21*, 967-974.
135. Deschamps, C., Echard, A., and Niedergang, F. (2013). Phagocytosis and cytokinesis: do cells use common tools to cut and to eat? Highlights on common themes and differences. Traffic *14*, 355-364.
136. Kuhbacher, A., Dambournet, D., Echard, A., Cossart, P., and Pizarro-Cerda, J. (2012). Phosphatidylinositol 5-phosphatase oculocerebrorenal syndrome of Lowe protein (OCRL) controls actin dynamics during early steps of Listeria monocytogenes infection. The Journal of biological chemistry *287*, 13128-13136.
137. Egami, Y., Fukuda, M., and Araki, N. (2011). Rab35 regulates phagosome formation through recruitment of ACAP2 in macrophages during FcgammaR-mediated phagocytosis. J Cell Sci *124*, 3557-3567.
138. Kobayashi, H., and Fukuda, M. (2012). Rab35 regulates Arf6 activity through centaurin-beta2 (ACAP2) during neurite outgrowth. J Cell Sci *125*, 2235-2243.
139. Bouchet, J., Del Rio-Iniguez, I., Lasserre, R., Aguera-Gonzalez, S., Cuche, C., Danckaert, A., McCaffrey, M.W., Di Bartolo, V., and Alcover, A. (2016). Rac1-Rab11-FIP3 regulatory hub coordinates vesicle traffic with actin remodeling and T-cell activation. The EMBO journal *35*, 1160-1174.

Fig.1: Localization of vesicles and their respective origin at the different steps of cytokinesis. Secretory vesicles, endocytic vesicles, and distinct recycling endosomes (Rab11/FIP3, Rab8, Rab35) are indicated in different colors. *: applicable in large dividing cells. Microtubules are illustrated with straight grey lines.

Fig.2: Molecules involved in vesicle targeting, tethering and fusion to the intercellular bridge. The molecular motors (MKLP1 at the midbody ring, KIF5B, dynein) are shown in black, the Rab GTPases Rab8, Rab11, Rab35 in purple, green and blue, respectively, with their effectors in red. Proteins involved in fusion are shown in green-yellow. Tethering/docking involves the exocyst, Arf6 and MICAL3.

Fig.3: Regulation of phosphoinositides, actin cytoskeleton and ESCRT-III assembly by membrane traffic during early (a) and late (b) cytokinetic events. In (a), proteins required for furrow stability and that directly or indirectly bind to PtdIns(4,5)P_2 (red) are shown in grey. In (b), note the complementary roles of Rab11/p50RhoGAP, Rab35/OCRL and Rab35/MICAL1 for clearance of F-actin and thus ESCRT-III assembly at the abscission site.

Fig.4: Coupling of membrane trafficking and cytokinesis during *de novo* lumen formation and apico-basal polarity establishment in 3D models. (a) Mechanisms of apical vesicles targeted towards the AMIS (Apical Membrane Initiation Site) by Rab11/FIP5/KIF3A along microtubules of the cytokinetic bridge. (b) Mechanisms of vesicle tethering and fusion around the intercellular bridge leading to delivery of apical determinants and formation of the AMIS.

Fig.5: Known mechanisms connecting membrane traffic and cytokinesis. This diagram represents a tentative model for summarizing known direct interactions (double arrows) and direct or indirect hierarchical relationships (single arrows, A → B meaning A is required for the localization/function of B). Question marks represent unknown cargos transported by vesicles toward the intercellular bridge that control either bridge stability after furrow ingression or only abscission.

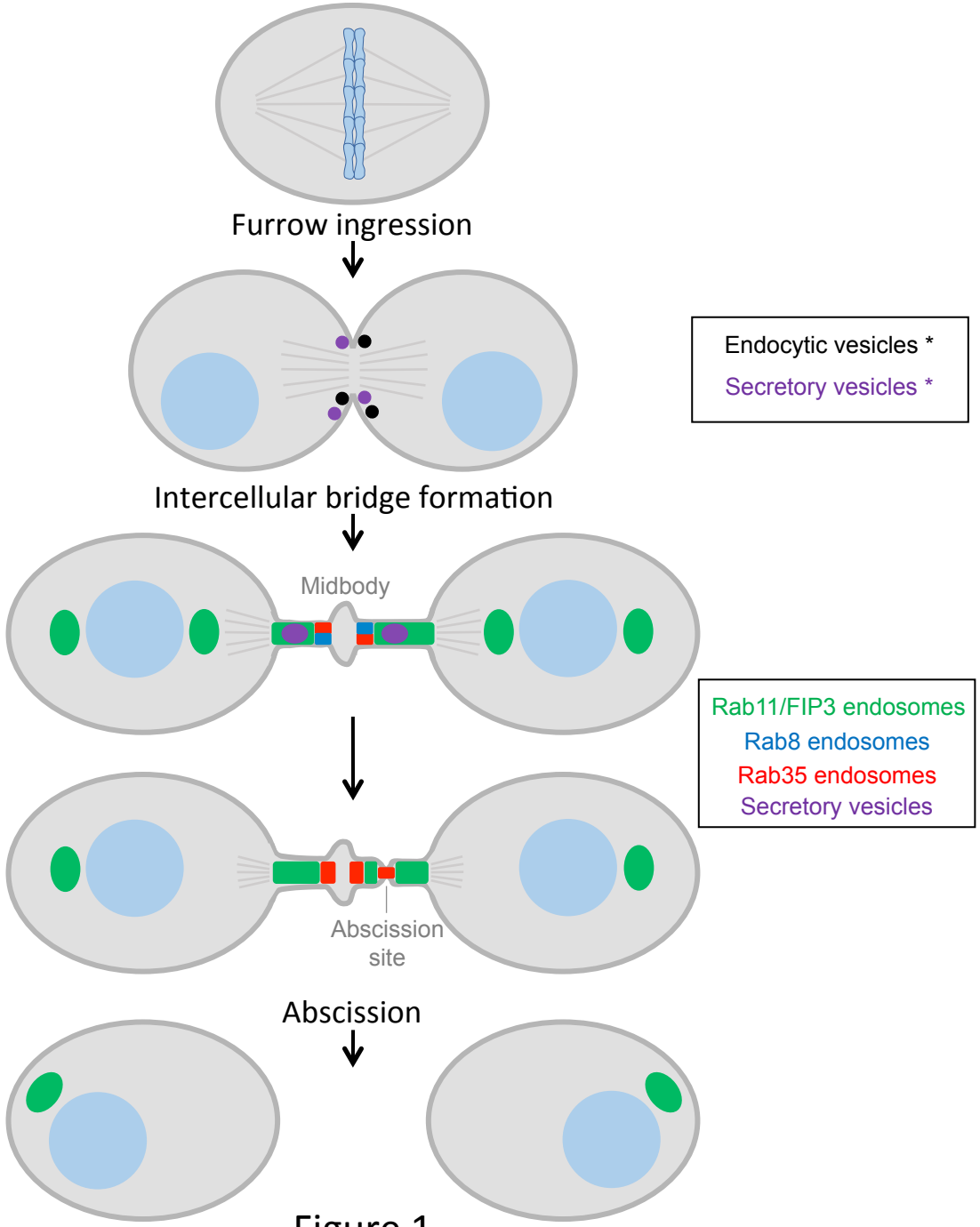

Furrow ingression
Endocytic vesicles *
Secretory vesicles *
Intercellular bridge formation
Midbody
Rab11/FIP3 endosomes
Rab8 endosomes
Rab35 endosomes
Secretory vesicles
Abscission site
Abscission

Figure 1

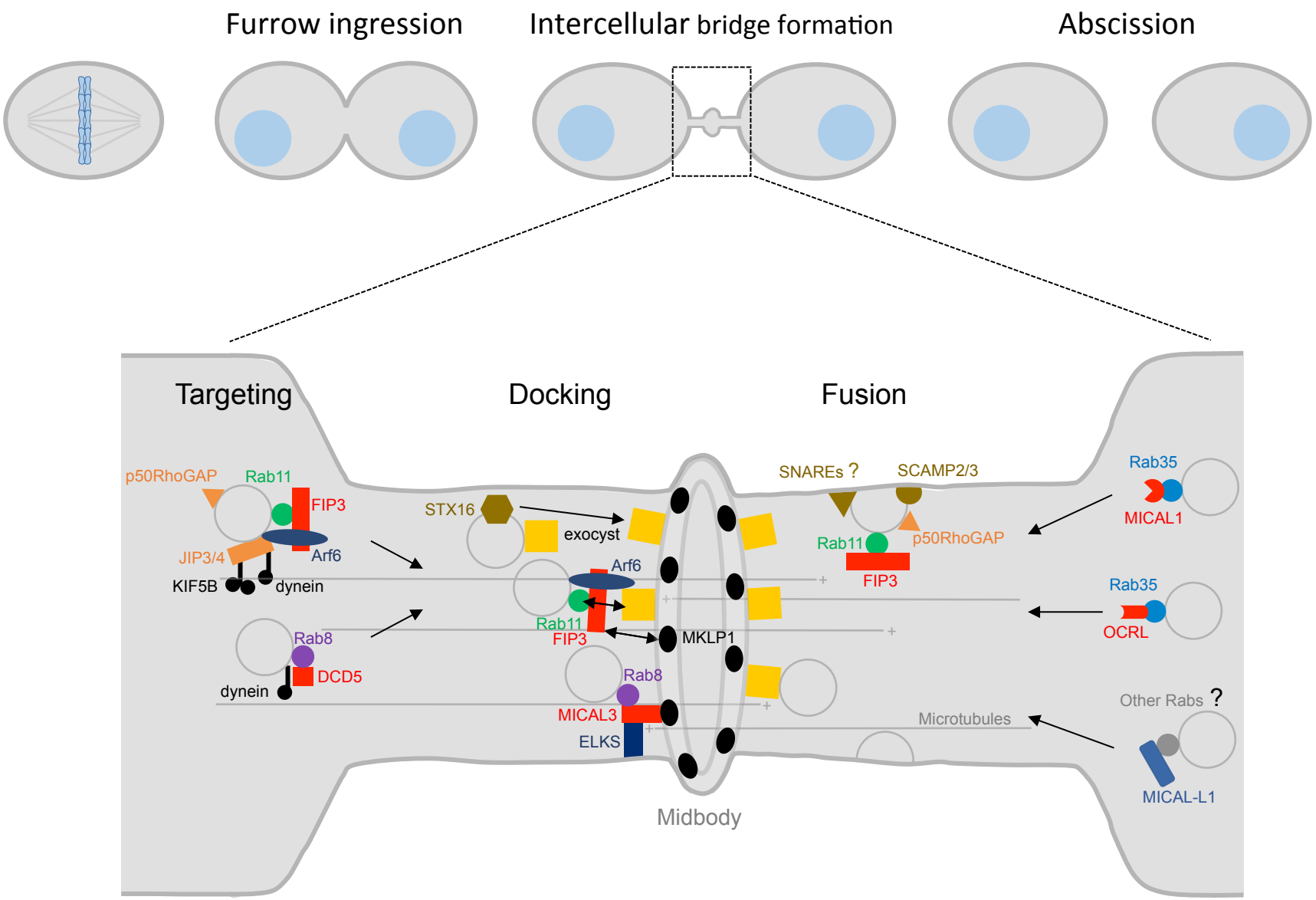

Furrow ingression
Intercellular bridge formation
Abscission
Targeting
Docking
Fusion
p50RhoGAP
Rab11
FIP3
Arf6
JIP3/4
KIF5B
dynein
Rab8
DCD5
dynein
STX16
exocyst
Arf6
Rab11
FIP3
MKLP1
Rab8
MICAL3
ELKS
Midbody
SNAREs ?
SCAMP2/3
Rab11
p50RhoGAP
FIP3
Rab35
MICAL1
Rab35
OCRL
Other Rabs ?
Microtubules
MICAL-L1

Figure 2

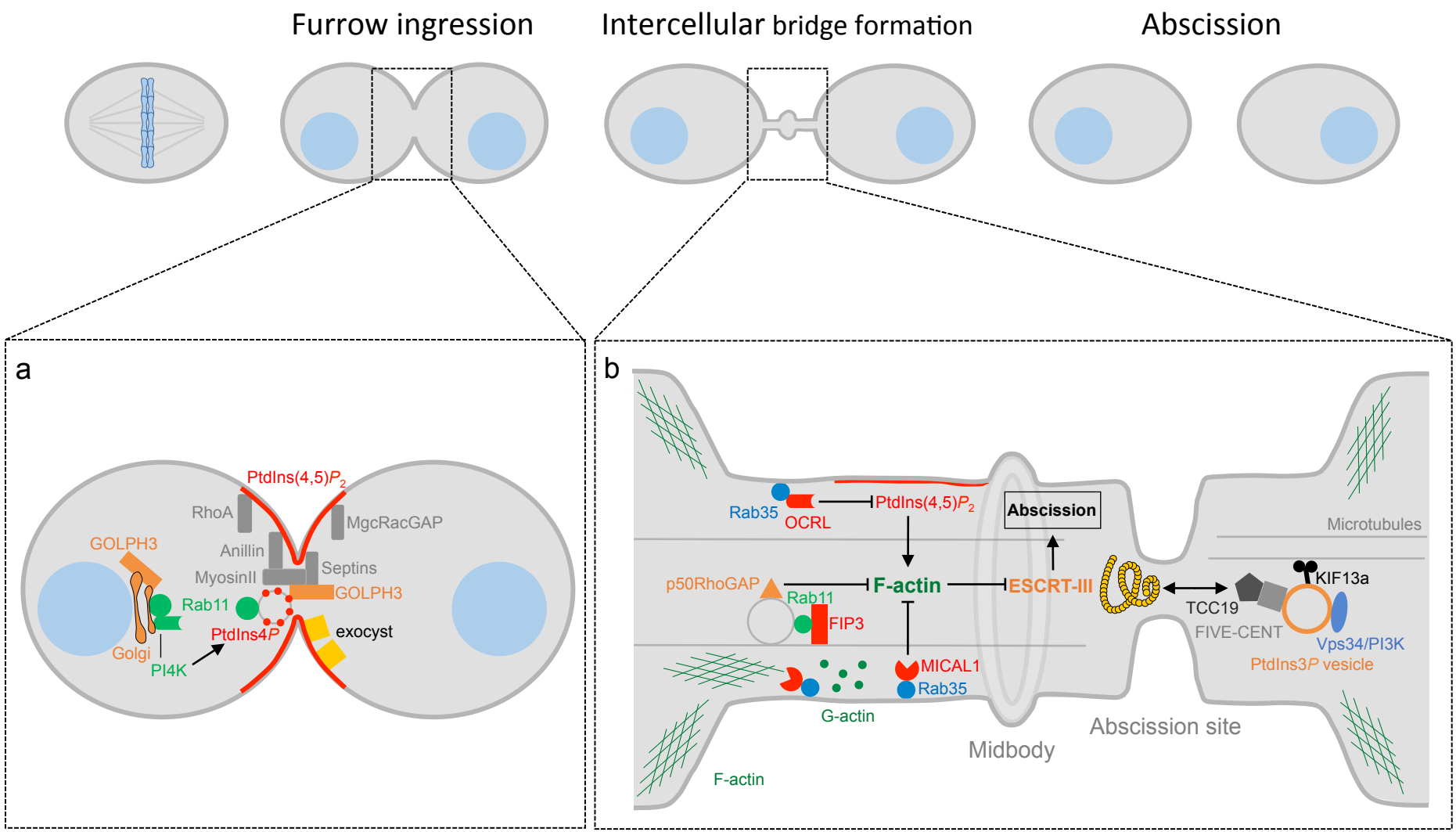

Furrow ingression
Intercellular bridge formation
Abscission
a
PtdIns(4,5)P2
RhoA
MgcRacGAP
GOLPH3
Anillin
MyosinII
Septins
GOLPH3
Rab11
PtdIns4P
exocyst
Golgi
PI4K
b
Rab35
OCRL
PtdIns(4,5)P2
Abscission
Microtubules
p50RhoGAP
F-actin
ESCRT-III
KIF13a
Rab11
FIP3
TCC19
FIVE-CENT
Vps34/PI3K
PtdIns3P vesicle
MICAL1
Rab35
G-actin
Abscission site
Midbody
F-actin

Figure 3

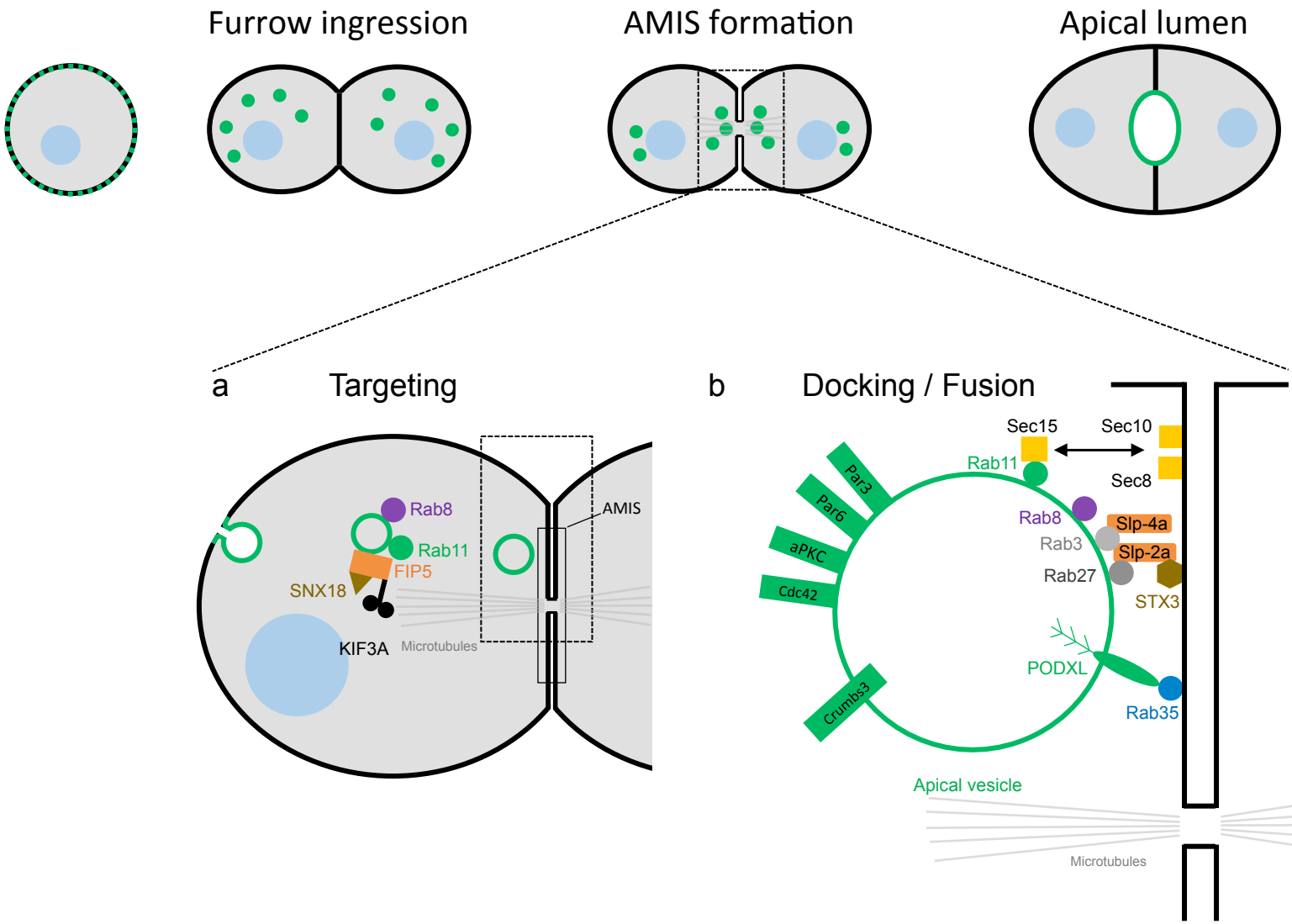

Furrow ingression
AMIS formation
Apical lumen
a
Targeting
Rab8
Rab11
FIP5
SNX18
KIF3A
Microtubules
AMIS
b
Docking / Fusion
Sec15
Sec10
Sec8
Rab11
Par3
Par6
aPKC
Cdc42
Crumbs3
Rab8
Rab3
Rab27
Slp-4a
Slp-2a
STX3
PODXL
Rab35
Apical vesicle
Microtubules

Figure 4

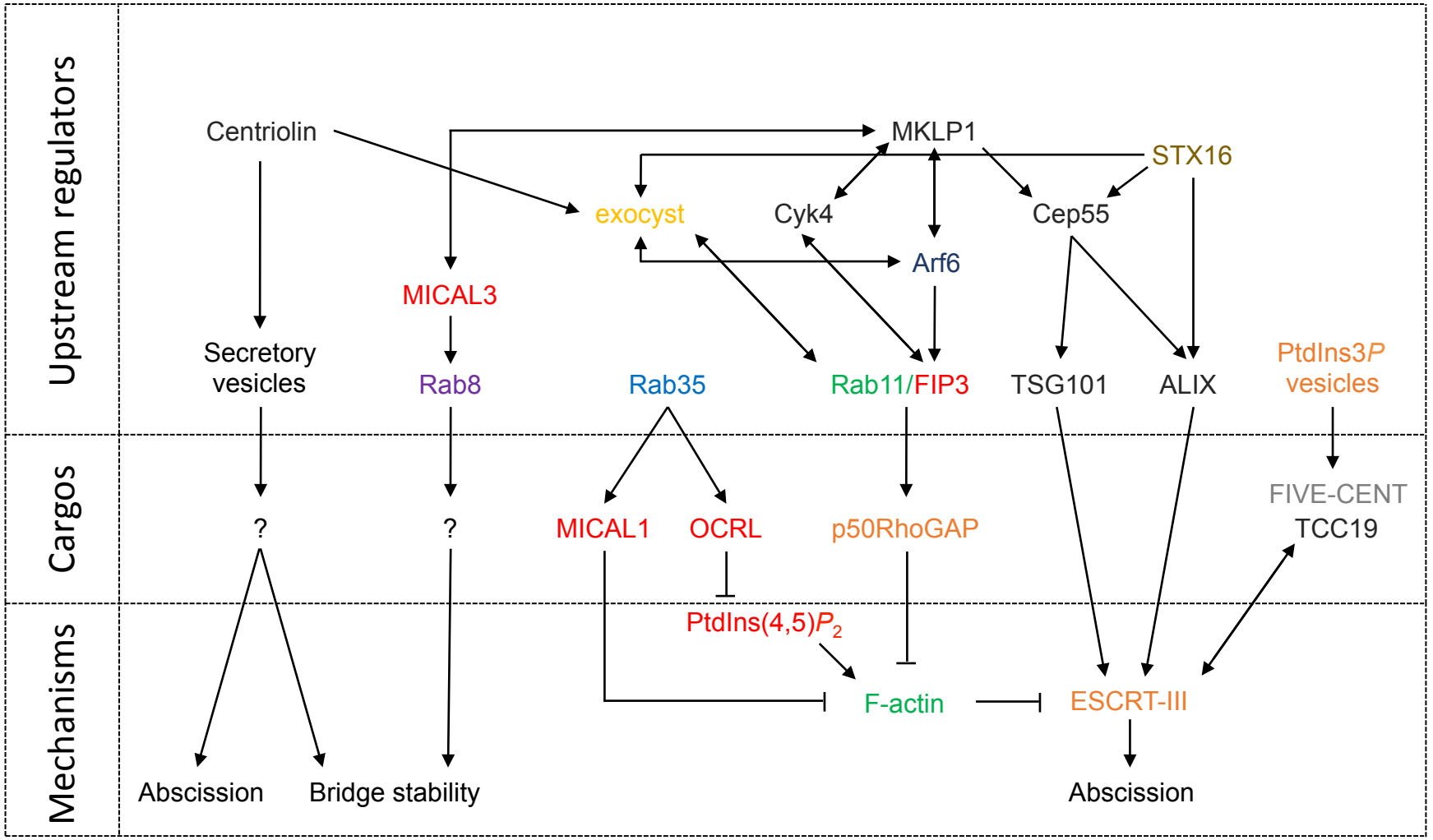

Upstream regulators
Cargos
Mechanisms
Centriolin
MKLP1
STX16
exocyst
Cyk4
Cep55
Arf6
MICAL3
Secretory vesicles
Rab8
Rab35
Rab11/FIP3
TSG101
ALIX
PtdIns3P vesicles
?
?
MICAL1
OCRL
p50RhoGAP
FIVE-CENT
TCC19
PtdIns(4,5)P2
F-actin
ESCRT-III
Abscission
Bridge stability
Abscission

Figure 5