

Review

Membrane Trafficking Components in Cytokinesis

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Key Words

Cytokinesis • Exocyst • Membrane trafficking • Rab

Abstract

Cytokinesis, the last major step of cell division, is a complex multistage process involving specific rearrangement of cellular cytoskeleton and a flurry of vesicular transport activities at the cell division plane. Vesicular traffic from the exocytic pathway and the endocytic/recycling pathway, operating again after being shut down since prophase, are engaged to supply the mitotic midzone with materials that would facilitate furrowing, midbody thinning and subsequent abscission of daughter cells. Cytokinesis is spatial and temporally regulated by mitotic kinases, and involves modulation by Arf and Rab small GTPases and their effectors. The latter include vesicle targeting and tethering molecules such as motor proteins, tethering complexes and SNAREs. The process of abscission requires the ultimate engagement of endosomal sorting complex required for transport (ESCRT) complexes. Although a good deal of details remains to be deciphered, cytokinesis in eukaryotes could essentially be visualized as a specialized cellular event requiring complex spatial and temporal regulated processes of membrane traffic.

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Introduction

At the end of mitosis, a dividing cell undergoes cytokinesis, a process that is tightly regulated spatially and temporally to ensure proper segregation of chromosomal and cytoplasmic content to both daughter cells. For cell wall-bearing plants, cytokinesis is mediated by a microtubule and actin-based specialized platform, the phragmoplast [1]. Membrane vesicles were transported to the cell division plane to form the cell plate [2], which begin as a tubulovesicular network generated by homotypic fusion of these vesicles, that subsequently grows into a fenestrated membrane sheet. The cell plate growth eventually reaches the cell cortex, where it fuses with the plasma membrane.

Animal cells, on the other hand, do not appear to generate a cell plate equivalent structure during cytokinesis. As mitosis progresses from anaphase to telophase, the overlapping of microtubules emanating from each poles at the centre of the mitotic spindle forms the spindle midzone. Actin filaments and the actin motor myosin II (actomyosin) accumulate in telophase to form a contractile ring at the plane of division [3]. This contractile actomyosin ring is attached to the plasma membrane, and its activity drives the formation of a cleavage furrow. Completed cleavage furrow ingression leaves the daughter cells connected by a thin cytoplasmic bridge densely packed with cytoskeletal structures [4]. Assembled from condensed, antiparallelly positioned microtubules and many other associated proteins, this intercellular bridge or midbody will gradually thin until the final abscission of the daughter cells occurs. The midbody acts as the platform whereby a membrane abscission machinery is assembled, which then executes the final cleavage of this intercellular bridge.

Cytokinesis requires extensive and tightly regulated membrane vesicle trafficking and targeting activities, both to, and from, the cleavage furrow. During the most part of mitosis in animal cells, exocytic membrane traffic, particularly from the endoplasmic reticulum (ER) to the Golgi apparatus, is shut down [5]. One way how this shutdown is known to occur in animal cells is via the phosphorylation of a trafficking component, p47, by Cdk1/Cdc2 [6]. Furthermore, key membrane compartments of the pathway, such as the Golgi apparatus, are fragmented to allow their equivalent inheritance by the daughter cells [7]. This fragmentation is also mediated by the phosphorylation of proteins that maintains the integrity of the Golgi stack, such as GM130 [8] and Golgi reassembly stacking protein of 65 kDa (GRASP65) [9] by mitotic kinases. Endocytic membrane traffic was also shown to be inhibited or at least temporarily attenuated during the early phase of mitosis [10-12], while endosomal to plasma membrane recycling is likewise greatly diminished [13].

Both the exocytic and endocytic/recycling pathway activities, which recovered during telophase, are both involved in cytokinesis. A wide range of components of the membrane trafficking machinery, including Rab/Arf small GTPases [14], tethering complexes [15], N-ethylmaleimide-sensitive fusion protein (NSF)-attachment protein receptors (SNAREs) [16] and the endosomal sorting complex required for transport (ESCRT) complexes [17], have all been implicated in the cytokinesis [18, 19]. The paragraphs below discuss the involvement of the eukaryotic membrane trafficking machinery and their known roles in this final act of cell division, with a focus on animal cells, and with analogous processes in invertebrate and plant cytokinesis also mentioned where relevant.

Spatiotemporal regulation of cleavage furrow and midbody formation

During the initiation of cytokinesis at telophase, organized positioning of microtubules in anti-parallel arrays forms an anchor for cleavage furrow formation, with subsequent furrowing mediated by the contractile actomyosin ring assembled [20]. Both the dynamics of furrowing midbody formation and its function are influenced by the mitotic kinases, directly or indirectly. In general, disruption of cyclin B and inactivation of the mitotic kinase Cdk1-cyclin B by the anaphase promoting complex/cyclosome (APC/C) [21] precedes cytokinesis [22]. Polo-like kinase 1 (Plk1) regulates multiple aspects of mitosis [23], and its functions

in cytokinesis include the regulation of cyclin B destruction by APC/C, as well as other substrates more directly involved in the various steps leading up to the final abscission [24]. The initiation of cytokinesis is regulated temporally and spatially at the midbody, where key components for the process such as the protein regulating cytokinesis 1 (PRC-1), the centralspindlin complex (which contains the Rho family GTPase activating protein Cyk-4/MgcRacGAP and the mitotic kinesin-like protein 1, MKLP1) and Plk1 are recruited. PRC1, a microtubule binding protein that could facilitate anti-parallel microtubule cross linking [25], is a Cdk substrate [26]. Transport of PRC1 to the spindle by the kinesin motor Kif4 was indeed shown to be essential for both midzone formation and cytokinesis [27].

Interestingly, while Cdk1 activity controls the docking of Plk1 onto PRC1 from the centrosomes during metaphase, Plk1 creates its own docking site on PRC1 at the central spindle at the midbody during anaphase [28]. Plk1 phosphorylates components of the centralspindlin complex and facilitates recruitment of the RhoA guanine nucleotide-exchange factor (GEF), the epithelial cell transforming sequence 2 (ECT2). The latter could itself be phosphorylated by Plk1 *in vitro* [29]. Plk1 can also phosphorylate other GEFs to regulate RhoA activity, as well as phosphorylating its downstream effector Rho dependent kinase 2 (ROCK2) [30]. Inhibition of Cdk1 by specific inhibitors could aberrantly induce cytokinesis without chromosome segregation in an ECT2 and MgcRacGAP-dependent manner [31]. The intricate interconnections between Cdk1, Plk1 and components of the midbody have yet to be fully worked out. However, what is now known attests to the notion that Cdk1 and Plk1 orchestrate the formation and functionality of the machinery required for furrowing and subsequent steps in cytokinesis.

Regulation of membrane traffic during cytokinesis – the small GTPases and their effectors

Cytokinesis requires extensive and highly regulated membrane vesicle trafficking and targeting activities, both to, and from, the cleavage furrow. Membrane trafficking is required for midzone delivery of cytoskeletal and regulatory molecules (eg. the Rho GTPases and their interacting partners) during its earlier phase [32], as well as components of the membrane abscission machinery during its later phase [33]. Exocytic traffic is clearly essential, and secretory vesicles marked by exocytic cargoes have been demonstrated to move towards the midbody in both an asymmetric [34] as well as a symmetric [35] manner from the forming daughter cells. However, endocytic and recycling traffic play equally important, non-redundant roles in this regard [36, 37] (Fig. 1A).

A role in cytokinesis has been implicated for several membrane traffic modulating members of the Rab small GTPases family. In *Arabidopsis*, Rab-A2 and Rab-A3 that are localized to a post-Golgi compartment are both important for cell plate formation [38]. A role for the recycling endosomes in cytokinesis in animal cells was first gleaned from observations that the recycling endosome-associated Rab11 and its interacting effector molecule *nuclear fallout* (*nuf*) are required for cell division in *Drosophila* embryos [39]. In mammalian cells, the *nuf* orthologue, Rab11-family interacting protein 3 (Rab11-FIP3) [40], which appears pericentrosomal in interphase cells, accumulates together with Rab11 at the cleavage furrow during cytokinesis [41] and functions to deliver recycling endosome materials the cleavage furrow [37]. Targeting of FIP3 *per se* to the midbody, however, appeared independent of Rab11 activity [37]. Interestingly, FIP3 is a phosphoprotein, and its phosphorylation status appears to be cell cycle-regulated. One of its phosphorylation sites (Ser102) is a target of Cdk1 *in vitro* [42]. Ser102 is phosphorylated in metaphase, but is dephosphorylated as cells enter telophase, an observation which correlated with Rab11-FIP3's translocation from the cytosol to membranes during telophase.

Members of the Arf and Rab small GTPases act as molecular switches, and in their GTP-bound, active state, engages effectors that modulate membrane dynamics. Both Rab11-FIP3 and another Rab11 effector, Rab11-FIP4, interact with the Arf family member of small

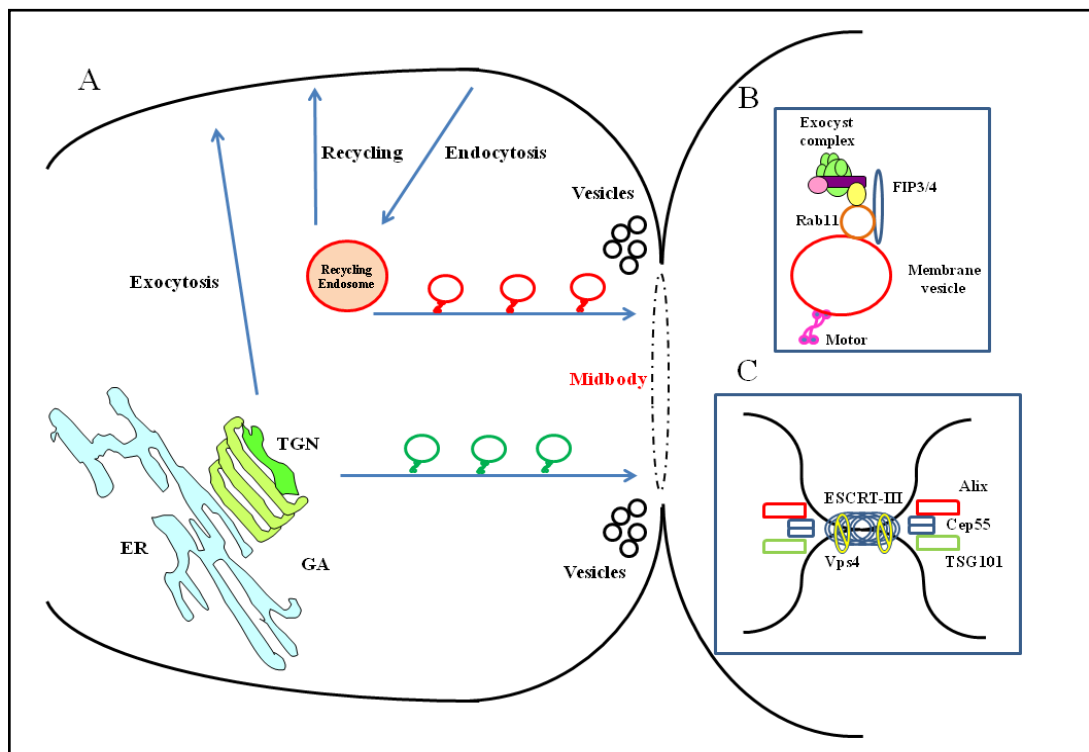


Fig. 1. A schematic diagram depicting membrane traffic processes and components required for cytokinesis in animal cells. A) Both exocytic and endocytic/recycling pathways are engaged in vesicle traffic to the midbody during cytokinesis. ER – endoplasmic reticulum, GA – Golgi apparatus, TGN- trans-Golgi network. B) A transport vesicle/carrier decorated by Rab11 and engaging effectors such as exocyst and Rab11 family interacting proteins (FIPs). C) Abscission machinery components engaged in the final steps of cytokinesis. Alix - ALG-2-interacting protein X, Cep55 – centrosome protein of 55 kDa, ESCRT - endosomal sorting complex required for transport, TSG100 - tumor susceptibility gene 101, Vps4 – vacuolar protein sorting 4.

GTPase that is involved in post-Golgi traffic, namely Arf6. The latter's roles in endocytosis and endocytic recycling are well known [43]. Arf6 could be localized to the cleavage furrow region, and a ternary complex formed between both the Rab11-FIPs and Rab11-Arf6 could be detected [44]. Another more recently uncovered role for Arf6 in cell division pertains to its ability to stabilise the midbody by interacting with the centralspindlin complex, which is essential for the formation of microtubule bundle structures of the midbody. Centralspindlin assembles in a sharp band at the centre of the midbody, and this concentrated localization could be disrupted by 14-3-3 proteins [45]. ARF6 appears to preserve midbody structural integrity by competing with 14-3-3 for binding to centralspindlin [46].

Another Rab family member known to function in cytokinesis is Rab35 [47]. Rab35 is known to regulate endocytic recycling [48], and has distinct actin-modulating activities [49, 50]. One of the key processes occurring at the cleavage furrow is modulation of actin dynamics. Rab11 and Arf6-controlled Rab11-FIP3/*nuf* apparently act in this regard by promoting actin polymerization [51, 52]. Rab35 and its effectors with known actin-modifying activity such as fascin [49] are likely to act in the same vein. Another recently described Rab35 effector in connection with cytokinesis is the protein mutated in Oculocerebrorenal Syndrome of Lowe (OCRL), a phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) 5-phosphatase named OCRL-1 [53]. Rab35 binds OCRL-1 and regulates its localization at the intercellular bridge, and silencing of either molecule inhibits abscission, with the dividing cell accumulating abnormal amounts of PtdIns(4,5)P₂ and F-actin at the midbody [54]. This is a defect that is also manifested by cell lines derived from Lowe Syndrome patients. As previous genetic

analysis in *Drosophila* have demonstrated PtdIns(4,5)P₂'s function at the cleavage furrow [55], this finding further strengthened the notion that is important for normal cytokinesis. In fact, a number of other aspects of phosphoinositide metabolism are also important for cytokinesis. It was shown in *Drosophila* spermatocytes that the phosphatidylinositol (PI) 4-kinase III β gene product *four wheel drive* (*Fwd*) is key for the formation of PI4P-containing secretory carriers and Rab11 localization to the midzone [56].

There appears to be a complex interplay of feedback regulations between the small GTPase involved in cytokinesis. As mentioned above, Rab11 and Arf6 could both regulate the same subset of effector. Recent findings have now revealed an intricate reciprocal regulation of activities between Rab35 and Arf6. Rab35 may modulate Arf6 activity, as its effector centaurin- β 2, acting as an Arf6-GTPase-activating protein (GAP), has been recognized in Rab35's particular role in modulating neurite outgrowth [57]. Conversely, Arf6 activity could in turn influence Rab35 function, as the Rab35 GAP EPI64B is also an effector of Arf6 [58].

During mitosis, animal cells' adherence to the substratum, at least in culture, appeared somewhat impaired as mitotic cells commonly adopt a rounded appearance. Cell adhesion however, is critical for cytokinesis at least in some contexts. For example, unanchored fibroblasts could not complete cytokinesis unless augmented by oncogenic signals [59]. The focal adhesion complex and its components have also been shown to be important for cytokinesis. Disruptions of talin A could detach the actomyosin contractile ring from the cell membrane, and result in concomitant regression of the cleavage furrow [60]. Integrins, a key cell adhesion molecule family, is known to be important for mitotic spindle positioning and orientation [61]. An interesting finding relating integrins to cytokinesis is that proper endocytosis and recycling of integrins is required for the process, and that this is regulated by Rab21 [62]. Chromosomal deletion and loss of Rab21 gene expression is manifested by some cancer cells, and re-expression of Rab21 reversed a "multinuclear" phenotype exhibited by these. Abnormal integrin traffic was also linked with the generation of aneuploidy and cell transformation *in vitro* and *in vivo*, while downregulation of Rab21 correlates with increased malignancy in human prostate and ovarian cancer samples [63].

Membrane tethers and motor proteins in cytokinesis

Other than the Rab11-FIPs, another key effector that is engaged by Rab11 and Arf6 in cytokinesis is the exocyst complex (Fig. 1B), which has a well known, more general role in various forms of polarized exocytosis ranging from yeast budding to neurite outgrowth [64]. The exocyst component Exo70p co-immunoprecipitates both Rab11-FIP3 and Rab11-FIP4, and silencing of Exo70p leads to impaired cytokinesis and dislocalization of Rab11 and Rab11-FIP3 from the midbody [44]. Exocyst function in cytokinesis is important for cellularization of *Drosophila* embryo [65], and cell plate maturation in plants [66]. The Ral GTPases (RalA and RalB) that have been heavily implicated in tumorigenesis [67] are also known principal regulators of exocyst activity. It is thus not surprising that this pair of small GTPases also have apparent roles in cytokinesis [68, 69]. The midbody protein centriolin interacts with both the membrane tethering exocyst complex and the SNARE complexes required for the actual vesicle fusion step (see below), and may serve to regulate membrane vesicle docking/fusion processes in cytokinesis [34].

The exocyst belongs to a functionally defined class of multiprotein complexes that act in tethering of transport vesicles or membranous carriers to target membrane prior to fusion [70, 71]. Other than the exocyst, the Transport Protein Particle (TRAPP) II complex [72, 73] has also been implicated in cytokinesis. Mutations in the *Drosophila* gene *brunelleschi* (*bru*), the orthologue of the yeast Trs120p subunit of TRAPP II, resulted in failed furrow ingression in male meiotic cells, and localization of dRab11 to the cleavage furrow requires functional *bru* [74]. The TRAPP II complex was also shown to be required for cytokinesis in *Arabidopsis* [75]. In the *AtTRS120* mutants, vesicles accumulated at the equator of dividing cells, but failed to assemble into a cell plate.

Other than actin modulators and the membrane tethering complexes, another type of effector logically expected to be engaged by the small GTPases in vesicle transport during cytokinesis would be microtubule-based motor proteins and/or their adaptors. Indeed, the c-jun N-terminal kinase (JNK)-interacting proteins 3 and 4 (JIP3 and JIP4), which are adaptors of kinesin-1 and dynactin complex, are both effectors of Arf6 [76]. Binding of activated Arf6 to the second leucine zipper domain of JIP3 and JIP4 attenuates association with kinesin-1, but enhanced JIPs' interactions with the dynactin complex. The interactions between Arf6, JIPs, kinesin-1, and the dynactin complex therefore control the trafficking of recycling endosomes to and from of the midbody structure [76].

SNAREs and vesicle fusion in cytokinesis

As mentioned above, vesicles derived from both the exocytic and endocytic pathways contributed to the formation of the cell plate in plants. Cell plate formation definitely requires vesicle fusion, which is mediated by SNAREs and their regulators [77]. The syntaxin-like molecule KNOLLE [78] has been long recognized as a target membrane (t)-SNARE protein critical for cytokinesis and cell plate formation in plants. KNOLLE interacts with the Sec1-like KEULE [79], and another t-SNARE, which is the neuronal SNAP25 homologue AtSNAP33 [80]. An association with cytokinesis has also been reported for another SNARE-like molecule, NPSN11 [81]. Although the exact SNARE complex (or complexes) involved are not yet clear, possible cognate vesicle (v)-SNARE partners to the t-SNAREs above, namely VAMP721 and VAMP722, have recently been reported [82]. Plant cytokinesis may also involve another membrane fusion factor, namely the AAA-ATPase Cdc48p/p97, engaging alternative SNAREs such as the plant homologue of animal syntaxin 5, SYP31 [83].

The exact role of vesicle fusion in animal cell cytokinesis is less clear. Membrane vesicles move to and from the midbody, but towards the later part after furrow formation, there is an accumulation of vesicles at the midbody. While furrow formation does not appear to require SNARE-mediated fusion *per se* in mammalian cells, the subsequent abscission process does appear to require the t-SNARE syntaxin 2 and the v-SNARE VAMP8/endobrevin [84]. In *Drosophila*, a hypomorphic allele of the *Drosophila* syntaxin 5 homologue, dSyx5 led to a phenotype of germ cells failing to complete cytokinesis, and defects in spermatid elongation and maturation [85]. However, in this case it is unclear if the role of dSyx5 is specific, as disruption of Golgi-localized syntaxin 5 is likely to also cause a general disruption of Golgi traffic. Another animal SNARE that has been implicated in cytokinesis is syntaxin 16, a TGN-localized SNARE that could be recruited to the midzone and midbody [86].

The exact mechanistic role for SNAREs in cytokinesis in animal cells has not yet been resolved. In general, while there clearly is fusion between vesicles targeted and the plasma membrane at the midbody, it is unclear what roles these fusions serve, and how these fusions might impact on, or may indeed be required for, the final abscission step. Vesicle targeting and accumulation at the midbody may aid abscission by facilitating the gradual thinning the midbody, or may simply serve to deliver components of the abscission apparatus.

Abscission and the role of endosomal sorting complex required for transport III (ESCRT-III)

The final resolution of the midbody and separation of daughter cells [3] requires various vesicle tethering complexes describe above [87] as well as ESCRT-III, which likely serve the role in executing the final scission [33, 88]. The ESCRT complexes 0, I, II, and III play critical roles in topologically equivalent membrane processes of multivesicular body (MVB) biogenesis, enveloped virus budding, as well as cytokinesis [89, 17]. ESCRT-I and ESCRT-II direct membrane budding in an "unconventional" manner, (away from the cytosol) while ESCRT-III cleaves these bud from their cytosolic faces [89]. Different processes involving

ESCRT utilize divergent pathways that lead to ESCRT-III assembly within the neck of a bud structure, ultimately resulting in membrane separation [90]. ESCRT-III-mediated membrane neck cleavage as a generic mechanism is now known to be crucial for many cellular processes, including receptor signalling, autophagy, establishment of cell polarity, cell migration, and even mRNA transport [91].

ESCRT-III in animal cells is composed of a number of homologous proteins grouped under charged multivesicular body proteins 1-7 (CHMP1-7), and the increased sodium tolerance-1 (IST1) protein [89, 92]. In a recruitment order established for the yeast complex, CHMP6 is first recruited by ESCRT-II to nucleate the assembly of two CHMP4. These are capped by CHMP3 and CHMP2s, which in turn recruits an ATPase, Vps4 [93], to catalyze eventual disassembly of ESCRT-III and recycling of components. A requirement for the ESCRT machinery in cytokinesis was first noted when the tumor susceptibility gene 101 (TSG101), a subunit of ESCRT-I, and ALG-2-interacting protein X (Alix), an ESCRT-associated protein, were apparently recruited to the midbody during cytokinesis by centrosome protein of 55 kDa (Cep55) [94] (Fig. 1C). CEP55 resides at the centrosome through most part of the cell cycle, but then migrates to the midbody at the start of cytokinesis, and its action there is required for abscission. Interestingly, CEP55 is a substrate for both Cdk1 and Plk1 [95]. Upon mitotic entry, centrosome dissociation of Cep55 is triggered by Erk2/Cdk1-dependent phosphorylation at Ser425 and Ser428. Ser425/428 phosphorylation is required for CEP55's interaction with Plk1, which phosphorylates Cep55 at Ser436. Phosphorylation-deficient mutant forms of Cep55 caused defective cytokinesis. The relocation of Cep55 from the centrosome to the midbody is therefore regulated by the mitotic kinases to function in the final steps of cytokinesis.

TSG101 and the Alix compete for binding to the ESCRT and Alix-binding region (EABR) of CEP55 [96]. Live-imaging analysis indicated that TSG101 and CHMP4B are sequentially recruited to the midbody, forming a series of cortical rings [97]. Both CHMP4B and the ESCRT disassembly factor Vps4 is recruited to the narrow constriction site where abscission occurs late in cytokinesis, often immediately preceding cell separation. This precise correlation in time and exact spatial location of ESCRT-III and Vps4 with abscission suggests that these complexes play a direct role in abscission. A separate report has also implicated yet another ESCRT-III subunit, CHMP4C, in abscission timing [88]. CHMP4C functions in the Aurora B kinase-dependent abscission checkpoint [98] to prevent premature abscission of intercellular chromosome bridges and accumulation of DNA damage. The ESCRT machinery may therefore protect against genetic damage by coordinating midbody abscission with the abscission checkpoint.

Exactly how ESCRT-III executes abscission is not yet completely understood. Tantalizing clues has recently came from high resolution electron tomography of the midbody undergoing abscission, which revealed helical structures made up filaments of ~17-nm in diameter [99]. These appeared to constrict the intercellular membrane bridge into to a single stalk. ESCRT-III co-localized with the constriction zones and was apparently required for the assembly of these filaments. Components of ESCRT-III are known to polymerize into various filamentous and tubular structures [100-102], and spiral-shaped ESCRT-III filaments, in the context of MVB formation or viral budding, assemble around the neck of the forming vesicle to result in abscission. It is therefore plausible that ESCRT-III-based filaments, positioned perpendicular to the microtubules parallel to the midbody, could potentially generate the contractile force necessary to drive membrane deformation and initiate midbody abscission [33]. Future work would be required to reveal the mechanistic details of the final abscission step.

Epilogue

Recent findings have begun to shed some light on the cellular and molecular events that occur during the final stages of cell division, leading up the complete physical separation of daughter cells. Of note, it is clear that vesicular traffic from both the exocytic and the endocytic

pathways is involved, and vesicular membrane transport delivers fresh membrane lipids as well as protein components required for cytokinesis. Membrane vesicle (or carrier) delivery and fusion is regulated by Arf/Rab GTPases, and mediated by their effectors (motors, tethering factors) as well as SNARE proteins, with the final abscission step involving ESCRT-III.

Amongst the issues that have remained unresolved is a description, in sufficient detail, of the succession of steps that occurs at the midbody leading to abscission. The intricate and complex reciprocal interplay between Rab11, Arf6 and Rab35 aside, the exact roles of their effectors, the order of their functions, and the link to abscission are also not yet clear. In this regard, a very recent finding of a functional interaction between Rab11-FIP4 and TSG101 could connect Rab11-controlled processes and components of the ESCRT pathway [103]. Other questions that remained include what are the nature of vesicles observed to accumulate at the midbody in late telophase, and how exactly do these fuse eventually (and with which membrane). Pertaining to abscission, how exactly does ESCRT-III mediate the final abscission, and how is this final step timed in relation to preceding events requires some resolution. A textbook-like description with some degree of molecular and structural clarity of cytokinesis may require breakthroughs in super-resolution imaging, as well as molecular tools (mutants, inhibitors) to visualize various steps arrested or morphologically frozen at particular steps at the ultrastructural level.

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