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Endosomal trafficking in animal cytokinesis

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1. ABSTRACT

Cytokinesis is the terminal stage of eukaryotic cell division in which the cytoplasm of a dividing cell is partitioned between two daughter cells. In animal cells, this multifaceted cellular process is spatially and temporally regulated and requires dramatic remodeling of the cytoskeleton and plasma membrane. Animal cytokinesis proceeds when the acto-myosin contractile-ring, formed at the equatorial cortex of a dividing cell, advances inward like a 'purse string' and is a major driving-force for the separation of the two daughter cells. In this review, we highlight many of the recent advances in our understanding of the function and mechanisms of action of the endocytic protein machinery that control animal cytokinesis. This includes regulation of endosome delivery and targeting by Rab and ARF GTPases, their effectors FIP3, FIP4 and JIP4, the exocyst and centralsplindlin complexes and phosphoinositides. Roles for endosomal SNAREs, BRUCE and the ESCRT pathway in the membrane remodeling processes that lead to abscission are also discussed.

2. INTRODUCTION

Cytokinesis is a meticulously orchestrated eukaryotic cellular process in which the cytoplasm and cellular organelles of a dividing cell are segregated to complete the formation of two daughter cells. In plant cells, cytokinesis occurs via the cell plate, a disc-like structure formed by homotypic fusion of Golgi-derived vesicles at the cell center which expands towards the cell periphery and eventually fuses with the plasma membrane (1). In contrast, animal cytokinesis requires multifaceted changes in cell shape which necessitate assembly and activation of a constricting acto-myosin contractile-ring between the poles of the mitotic spindle; this is a major driving-force for the physical partitioning of the cytoplasm.

Animal cytokinesis begins during anaphase with the formation of the central spindle (midzone microtubules). The central spindle is a set of anti-parallel non-kinetochore microtubules that extend from the spindle poles and overlap for a short distance at their plus-ends

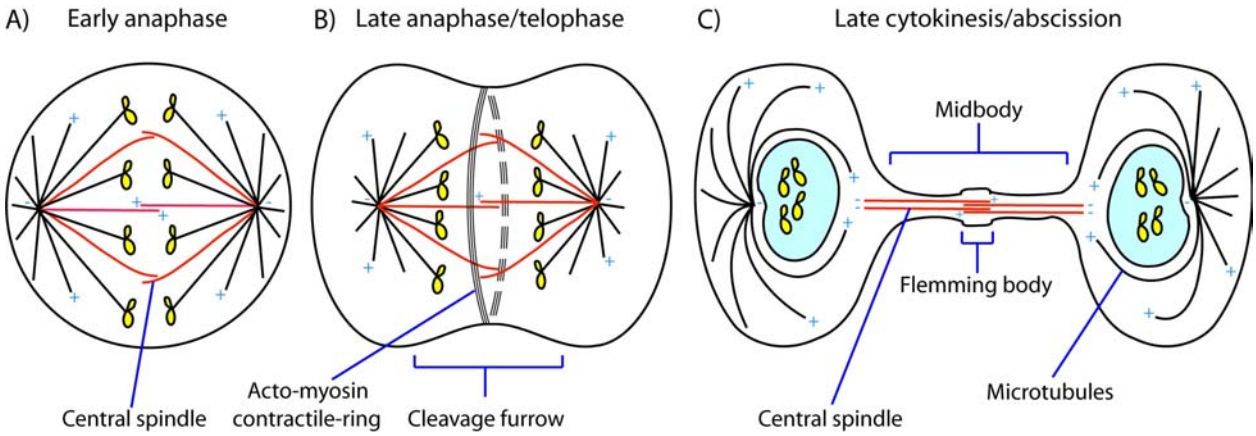

Figure 1. Cellular structures involved in animal cytokinesis. *A*) Animal cytokinesis begins during anaphase with the assembly of central spindle. *B*) The central spindle assembles and activates the acto-myosin contractile-ring which constricts creating an indentation in the plasma membrane known as the cleavage furrow. *C*) As the cleavage furrow further ingresses, the central spindle is compacted into a slender membrane-bounded intercellular canal called the midbody. The process of scission of the midbody, which occurs in the vicinity of the Flemming body, is called abscission and completes cytokinesis.

(Figure 1A) (2). They dictate the positioning of the cleavage furrow and function in its assembly and activation (2, 3). The next step in animal cytokinesis is the establishment and constriction of the acto-myosin contractile-ring which leads to the formation and ingression of the cleavage furrow, an inward-pinching groove in the plasma membrane at the cells equatorial cortex which occurs after anaphase (Figure 1B) (4). Ingression of the cleavage furrow is driven by mechano-chemical forces generated by the myosin II motor protein which translocate actin filaments causing the contractile-ring to constrict, thereby also constricting the plasma membrane and bundling the central spindle into a membrane-bounded intercellular bridge called the midbody (Figure 1C) (4). The terminal step in animal cytokinesis is known as abscission and involves scission of the midbody and sealing of the plasma membrane of the daughter cells. This occurs in the vicinity of the electron-dense centre of the midbody where the central spindle overlaps in a proteinaceous structure known as the Flemming body (alternatively known as the midbody-ring) (5) (Figure 1C).

While the acto-myosin contractile-ring is vital for ingression of the cleavage furrow, it is not the sole mechanism that drives this process as insertion of new membrane into the furrow is also required. Membrane trafficking is also crucial for abscission. In this review, we highlight many of the recent advances in our understanding of the proposed functions and mechanisms of action of the endocytic protein machinery implicated in animal cytokinesis.

3. ENDOSOMAL FUNCTION DURING ANIMAL CYTOKINESIS

Animal cytokinesis requires dramatic remodeling of the plasma membrane and significant increases in overall cell surface area as the two daughter cells are created (6). Both of these requirements necessitate the deposition of 'new' membrane into the cell surface. As the cleavage furrow has distinct membrane and protein composition, this membrane comes from internal stores which are specifically targeted to the furrow, and does not occur simply by expansion of the existing plasma membrane (7-11). Membrane trafficking is also essential for closure of the intercellular bridge during abscission (12). In animal cells, the secretory pathway contributes to the membrane required for cytokinesis (13-16); however, recent evidence indicates that the endocytic system is a central-source of the membranous-material which is deposited at the furrow and midbody during cytokinesis.

Among the first implications of the endocytic machinery in animal cytokinesis emerged from studies in *Dictyostelium discoideum* whereby mutations affecting endocytic protein function caused cytokinesis defects (17-19). Since then, an expanding collection of evidence has been forthcoming which has unequivocally implicated endosomal proteins in multiple aspects of animal cytokinesis.

3.1. Targeted delivery of the membranous cargo: from endosomal GTPases to lipid domains

In recent years, endosome-associated members of the Rab and ARF small GTPase families have emerged as key regulators of membrane trafficking events implicated in animal cytokinesis. This emergence began in 2001 when Skop *et al.* demonstrated that RNAi-mediated depletion of Rab11 in *Caenorhabditis elegans* embryos inhibited completion of cytokinesis (13). During interphase, Rab11 primarily localizes to the juxta-centriolar endosomal-recycling compartment (ERC) (alternatively know as recycling endosomes) and controls endosomal trafficking through this organelle (20-23). During cytokinesis, Rab11 accumulates near the cleavage furrow and within the midbody, and RNAi and mutant expression studies in *Drosophila melanogaster* and mammalian cells indicate that Rab11 regulates targeted membrane delivery to the furrow and midbody (24-29).

Like all GTPases, Rab11 cycles between inactive (GDP-bound) and active (GTP-bound) states which have distinct conformations (30). This conformational GTPase cycle is the primary driving-force for the on/off switch mechanism of Rab proteins and the main determinant of their ability to bind downstream effector proteins. Two Rab11 effectors, Rab11-FIP3 (FIP3) and to a lesser degree Rab11-FIP4 (FIP4), which are members of an evolutionarily-conserved Rab11-effector protein family, have been implicated in Rab11-mediated trafficking events during cytokinesis (31). Consistent with this, embryos deficient in *nuf* (*nuclear fallout*), the FIP3 and FIP4 orthologue in *D. melanogaster*, fail in the completion of cellularization (32, 33). During interphase, FIP3 is associated with the juxta-centriolar ERC, and during the early stages of cell division FIP3 is present on diffusely-distributed endosomal vesicles (24, 26). However, when cells enter anaphase, FIP3-positive vesicles associate with the centrosomes at opposite poles of the cell and then upon constriction of the acto-myosin contractile-ring, the FIP3-positive material moves to the furrow, and later, both FIP3 and FIP4 are significantly enriched within the midbody (34, 24-26). Disruption of FIP3 function by RNAi or mutant expression causes failure of cytokinesis completion, while having minimal effects on furrowing (26). These data support the hypothesis that FIP3 is necessary for abscission.

Interestingly, ARF6, another endosome-associated GTPase, appears to play a role in the recruitment of incoming FIP3 and FIP4-positive vesicles to the midbody (34-36). During cytokinesis, ARF6 localizes to the cleavage furrow and midbody, and this distribution temporally correlates with a transient spike in the activated-levels of ARF6 (37). Data from *D. melanogaster* and mammalian cells indicate that ARF6 is required for ingression of the furrow and completion of cytokinesis, respectively (37-39). In support of a role for ARF6 in the recruitment of FIP3 and FIP4 to the furrow and midbody, ARF6 and Rab11 can form ternary complexes with FIP3 and FIP4 and a Rab11-binding deficient mutant of FIP3, which retains its ability to bind ARF6 (FIP3 I738E), can be recruited to the midbody (34, 40, 26). In addition, overexpression of a constitutively active ARF6 mutant (ARF6 Q67L) enhances the recruitment of FIP3 and FIP4 to the furrow and midbody, whereas a constitutively inactive mutant (ARF6 T27N) blocks their recruitment (34).

Other protein machinery has also been implicated in the recruitment of incoming FIP3/FIP4-positive endosomes to the midbody during cytokinesis. The octa-meric exocyst complex is involved in tethering of exocytic vesicles with the plasma membrane during interphase and cytokinesis (41-44). Rab11 and ARF6 are among the small GTPases that regulate the assembly, localization and function of the exocyst complex, as Rab11 binds the Sec15 exocyst subunit and ARF6 binds Sec10 (45-50). It is possible that midbody-localized exocyst complexes serve as a tethering factor for incoming Rab11/FIP3 and Rab11/FIP4-positive endosomes and that these tethering events are regulated by Rab11 and/or midbody-localized ARF6 during abscission. Indeed, this appears to be the case as Exo70, another exocyst subunit, which localizes to the cleavage furrow and midbody and is required for completion of cytokinesis, can co-immunoprecipitate Rab11, FIP3 and FIP4, and its RNAi-mediated depletion reduces Rab11 and FIP3 localization to the furrow and midbody (34).

Centralsplindlin has also emerged as a further protein complex which regulates the recruitment of FIP3-positive endosomes to the midbody during cytokinesis. Centralsplindlin is a heterotetrameric protein complex with microtubule-bundling activity which is composed of the Rho GTPase-activating protein CYK-4/MgcRacGAP and the kinesin-like motor protein ZEN-4/MKLP-1 (51, 52). In addition to regulating formation of the central spindle, centralsplindlin regulates the formation of the acto-myosin contractile-ring by recruiting ECT2, a Rho GTPase guanine-nucleotide exchange factor, which activates RhoA and leads to contractile-ring formation and constriction (53-55). FIP3 was recently identified as a CYK-4/MgcRacGAP-binding protein that competes with ECT2 for CYK-4/MgcRacGAP-binding within the midbody (56). During early cytokinesis, FIP3 is inhibited from binding CYK-4/MgcRacGAP by ECT2, but at late telophase, ECT2 dissociates from CYK-4/MgcRacGAP and is sequestered back to the nuclei, thus allowing FIP3 to associate with CYK-4/MgcRacGAP (56). In this manner, the dissociation of ECT2 from CYK-4/MgcRacGAP may allow temporal regulation of the tethering of FIP3-positive endosomes at the midbody.

The data discussed above indicate that ARF6 and the exocyst and centralsplindlin complexes co-mediate the tethering of Rab11/FIP3-bearing endosomes at the midbody during cytokinesis, but until recently the identity of the motor proteins that mediate the movement of this endosomal-material to the midbody remained unclear. During cytokinesis, ERC membranes accumulate at the spindle poles and at both edges of the intercellular bridge (at the minus-end of the midzone microtubules) (57, 9, 58). In *C. elegans*, accumulation of Rab11-positive endosomes at the minus-end of microtubules is necessary for successful cytokinesis and is dependent on dynein and the RACK1 (Receptor for activated C kinase-1) scaffolding protein (59). Localization of endosomes at the minus-end of microtubules makes them ideally-positioned for the plus-end-directed vesicular transport along both astral and midzone microtubules which occurs during cytokinesis (26, 57, 60). Consistent with this, the plus-end-directed kinesin-1 motor protein is reported to co-immunoprecipitate with Rab11 and FIP3 and its depletion blocks the delivery of FIP3 to the cleavage furrow and midbody during cytokinesis [reported as unpublished data in (61)]. Furthermore, a study by Montagnac *et al.* found that in cells depleted of kinesin-1 subunits, transferrin-positive endosomes are retained at the minus-end of the midzone microtubules and are thus not trafficked into the midbody (62). Intriguingly, this study also demonstrated that ARF6 is a key regulatory switch for the motor proteins that traffic endosomal-material in opposing directions along microtubules within the midbody. The JNK-interacting proteins, JIP3 and JIP4, were identified as ARF6 effector proteins that also bind kinesin-1 and the dynactin/dynein complex in a mutually-exclusive manner; and JIP4 was shown to be required for kinesin-1-dependent trafficking of endosomal-material into the midbody (62). At the midbody, activated-ARF6 binds JIP4 to promote and stabilize its association with dynactin/dynein, while interfering with its

kinesin-1 association, and thus drives the trafficking of dynactin/dynein cargo out of the intercellular bridge, which was also shown to be necessary for abscission (62).

Other endosome-associated members of the Rab GTPase family have also been implicated in cytokinesis. Rab8-positive endosomes are a source of the membranous-material trafficked to the midbody during abscission [(63) – discussed in further detail below]. Rab14 also displays strong localization to the cleavage furrow and midbody during cytokinesis, although the functional significance of this cytokinetic distribution remains to be clarified (64). Rab21, an endosomal Rab that associates with multiple integrin subunits, regulates the targeting of integrins to and from the cleavage furrow, where they anchor the furrow to the surrounding matrix or trigger matrix-induced signaling events required for cytokinesis (65, 66). Pellinen et al. also demonstrated that successful cytokinesis failed when the Rab21 activity was disrupted by RNAi or mutant expression, and further mutagenesis experiments verified that the Rab21/integrin association and integrin endocytosis are both necessary for successful cytokinesis (66). A study by Kouranti et al. investigated the effects of RNAi-mediated depletion of each of the Rab genes in D. melanogaster S2-cultured cells and found that depletion of Rab35 results in post-furrowing cytokinesis defects (67). That study demonstrated that Rab35 regulates a 'fast' endosomal-recycling pathway and is responsible for midbody-localization and maintenance of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2] and the GTPase SEPT2 (septin-2), both of which are essential for successful cytokinesis (67-70). In this context, Rab35 is believed to direct the targeting of phosphoinositide kinases, such as phosphatidylinositol 4-phosphate 5-kinase (PtdIns4P 5-kinase), to the cleavage furrow and midbody whereby local lipid domains are established following regulated activation of phosphoinositide kinases at the plasma membrane (71-74). Midbody-localized PtdIns(4,5)P2 may then allow the recruitment of PtdIns(4,5)P2-binding proteins implicated in cytokinesis such as ERM (ezrin/radixin/moesin) proteins and SEPT2, and may also have additional roles such as regulating cytoskeletal remodeling or local enrichment of signaling molecules required for successful cytokinesis (75, 71, 72).

Phosphatidylinositol-3-phosphate [PtdIns(3)P], an endosomal phosphoinositide, has also recently been implicated in abscission (75). In a study by Sagona *et al.*, the authors report that PtdIns(3)P-positive endosomes localize to the midbody during cytokinesis and that disruption of the activity of VPS34 (vacuolar protein sorting 34), a class III phosphatidylinositol-3-OH kinase (PI(3)K-III) required for synthesis of PtdIns(3)P, or its accessory subunit Beclin 1, results in cytokinesis failure (75). At the midbody, PtdIns(3)P recruits additional proteins required for abscission such as the centrosomal protein FYVE-CENT (FYVE-domain-containing centrosomal protein) and its binding-partner TTC19 (tetratricopeptide repeat domain 19), which are translocated to the midbody by KIF13A (kinesin superfamily protein 13A), a plus-end-directed microtubule motor protein which binds FYVE-CENT (75).

3.2. Remodeling the midbody membrane to close the bridge: from endosomal SNAREs to ESCRTs

As outlined above, ingression of the cleavage furrow causes compaction of the central spindle into the membrane-bounded midbody. While the entire functional significance of the midbody remains to be fully elucidated, the physical obstacle of the midbody itself needs to be removed and some topological transformation of the plasma membrane must take place in order to 'plug the hole' in the plasma membrane and allow completion of cytokinesis. In this regard, a number of endosome-associated proteins appear to contribute to the membrane remodeling events within the midbody that lead to separation of the daughter cells.

SNAREs (soluble NSF attachment protein receptors) are integral membrane proteins that localize to distinct vesicle (v-SNARE) and target (t-SNARE) membranes and, through the formation of what is believed to be unique v-SNARE and t-SNARE complexes, mediate the fusion of vesicles with their target compartments (76). As a number of endosomal SNAREs are required for successful cytokinesis, it seems likely that SNARE-mediated membrane fusion plays a role in membrane remodeling events during cytokinesis (9, 77, 78). Consistent with this, mutant expression studies of two v-SNAREs, VAMP-3 and VAMP-7, demonstrate that these proteins are required for deposition of membrane into the plasma membrane during cytokinesis (9). Further mutant expression studies from different systems indicate that the VAMP-8/syntaxin-2 and VAMP-2/SNAP-25 SNARE complexes, which are enriched within the midbody, are required for abscission (77, 78). These data suggest that the VAMP-8/syntaxin-2 and VAMP-2/SNAP-25 SNARE complexes may, in a manner analogous to cell plate formation in plant cells, mediate the fusion between endosomal vesicles or endosomal vesicles and the plasma membrane within the midbody.

BRUCE (BIR repeat-containing ubiquitin-conjugating enzyme) is a further endosome-associated protein required for abscission (63). BRUCE, which is targeted to the midbody during cytokinesis, can co-immunoprecipitate the endosomal Rab8 and Rab11 GTPases as well as the exocyst components Sec6 and Sec8, and disruption of its function by RNAi-mediated depletion or mutant expression approaches blocks abscission and causes cytokinesis-associated apoptosis (63). It is believed that BRUCE may serve as a platform within the midbody for the tethering of endosomal vesicles and may coordinate the assembly of a membranous 'diffusion barrier', creating a physical blockade between the imminent daughter cells. In addition, as BRUCE possesses ubiquitin-conjugating activity and is necessary for midbody-localization of ubiquitin, it is possible that BRUCE may catalyze ubiquitylation events within the midbody that serve structural or regulatory functions required for successful cytokinesis (63). This intriguing study by Pohl *et al.* also found that in cells in which the activity of BRUCE is disrupted by RNAi-mediated depletion or mutant expression, cells accumulated large vesicles close to only one side of the midbody-ring, while intracellular trafficking

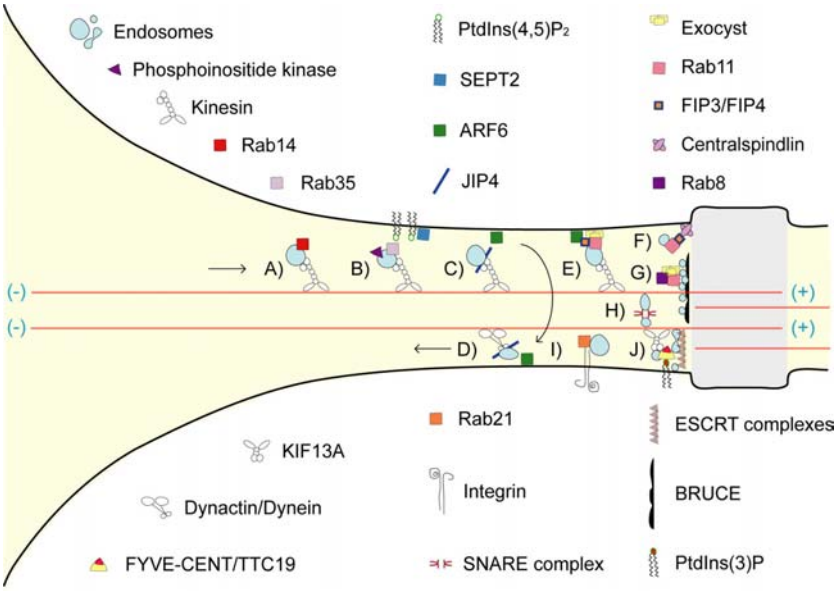

Figure 2. Integrated model of proposed endosomal functions within the midbody. Half of the midbody is portrayed and the events depicted are not necessarily independent of one another. A) Rab14 localizes to the cleavage furrow and midbody during cytokinesis. B) Rab35 directs PtdIns(4)P kinases to the midbody where local lipid domains are established which recruit proteins involved in cytokinesis. C and D) ARF6 regulates motor protein-mediated trafficking within the midbody. ARF6 binds JIP4 which inhibits JIP4 binding to kinesin-1 and promotes its association with the dynactin/dynein complex and drives minus-end-directed trafficking out of the midbody. E) Rab11/FIP3 and Rab11/FIP4-positive endosomes are tethered within the midbody via interactions with activated-ARF6 and the exocyst complex. F) Centralsplindlin mediates temporal recruitment of Rab11/FIP3 complexes to the midbody. G) BRUCE serves as a platform within that midbody for the tethering of endosomal vesicles and may coordinate the assembly of a membranous 'diffusion barrier' between the mother and daughter cell. BRUCE may also mediate ubiquitylation events that serve structural and/or regulatory functions required for abscission. H) v-SNARE and t-SNARE complexes mediate vesicle/vesicle and vesicle/plasma membrane fusion within the midbody which plays a role in membrane remodeling during cytokinesis. I) Rab21 regulates targeting of integrins to the furrow and midbody which anchor these structures to the surrounding matrix or trigger matrix-induced signaling events required for cytokinesis. J) ESCRT complexes induce membrane deformation within the midbody that contributes to the membrane remodeling events which lead to abscission. The KIF13 motor protein delivers FYVE-CENT and TTC19 to the midbody where they are recruited by midbody-localized PtdIns(3)P. TTC19 may regulate ESCRT activity within the midbody. PtdIns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; SEPT2, septin-2; JIP4, JNK-interacting protein 4; FIP3, Rab11-family-interacting protein 3 (Rab11-FIP3); FIP4, Rab11-family-interacting protein 4 (Rab11-FIP4); KIF13A, kinesin superfamily protein 13A; FYVE-CENT, FYVE-domain-containing centrosomal protein; TTC19, tetratricopeptide repeat domain 19; SNARE, soluble NSF attachment protein receptor; ESCRT, endosomal sorting complex required for transport; BRUCE, BIR repeat-containing ubiquitin-conjugating enzyme; PtdIns(3)P, phosphatidylinositol-3-phosphate.

in control cells appeared symmetrical (63). These data are in agreement with previous reports that abscission has an asymmetrical component whereby the scission event occurs at the daughter-centrosome-containing cell side of the midbody-ring, while the midbody-ring itself, along with BRUCE, are inherited by the mother-centrosome-containing cell where the midbody-ring is eventually disassembled (43, 63, 79, 80).

The ESCRT (endosomal sorting complex required for transport) pathway consists of four multi-protein complexes (ESCRT-0, -I, -II and –III) which are recruited to endosomes via protein and lipid (phosphoinositide) interactions (81). They serve to sort ubiquitinated proteins into multivesicular bodies (MVBs) and regulate MVB formation by facilitating the invagination and 'pinching-off' processes required for inward vesiculation (81). ESCRT complexes also have roles in additional cellular processes including viral budding and abscission during cytokinesis (82-84). Thus, ESCRT complexes are necessary for three cellular processes requiring topologically-analogous membrane scission events: inward vesiculation within MVBs, viral budding, and abscission (85, 86, 82, 87). Distinct adapter molecules recruit ESCRT components to the sites of membrane fission events. In this respect, the centrosomal protein CEP55, which is required for abscission, was identified as a binding-partner for TSG101 (tumor susceptibility gene 101), an ESCRT-I component, and found to recruit TSG101 and the ESCRT-associated protein ALIX (apoptosis-linked gene-2-interacting protein X) to the midbody (81, 86, 88). RNAi-mediated depletion of TSG101 and ALIX, both of which can associate with additional proteins involved in cytokinesis (CD2AP, ROCK1, and IQGAP1), results in abscission failure; and expression of a dominant-negative mutant of VPS4 (vacuolar protein sorting 4), an AAA+-type ATPase required for ESCRT-I function, results in similar effects (86, 87). Furthermore, the ESCRT-III

component CHMP3 (charged multivesicular body protein-3) also localizes to the midbody and CHMP3 mutant expression studies implicate it in cytokinesis (89). Given that recent studies indicate that ESCRT complexes can induce membrane deformation, it is possible that the ESCRT machinery contributes to the membrane remodeling events that allow closure of the intercellular bridge during abscission (90-92). These ESCRT-mediated membrane remodeling events may be regulated by the aforementioned TTC19 protein as TTC19 was recently shown to interact with the ESCRT-III component CHMP4B (charged multivesicular body protein-4B) (75).

4. PERSPECTIVES

Considerable advances in our understanding of the roles of the endocytic protein machinery have been achieved over the past decade. This information reveals a picture whereby several different mechanisms ensure appropriate targeted delivery of endosomes which serve multiple functions during the final stages of cell division (Figure 2). These data also underscore the crucial requirement for spatial and temporal regulation of numerous components of the cytokinetic protein machinery ranging from small GTPases to ESCRT complexes. So what specifically are the primary endosomal functions during cytokinesis? While endosomal function during cytokinesis is multifaceted, the preponderant evidence indicates that endosomes are crucial for the delivery of proteins needed for abscission. It seems likely that within the midbody these proteins serve diverse functions such anchoring the midbody to the surrounding matrix, tethering incoming vesicles at the correct spatial location, delivering and facilitating local enrichment of signaling molecules required to bring about completion of cytokinesis, and remodeling the membrane topology during abscission. These intriguing possibilities, as well as identification of the entire signaling events that lead to abscission, await further research and represent exciting challenges for the future.

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