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Endocytická mašinerie v cytokinezi

Endocytic transport in cytokinesis

Bakalářská práce

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Poděkování

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Kristina Koudelová

Abstract

Cytokinesis represents a very complex and highly orchestrated process. For many years, the mechanism of animal cell cytokinesis was described as a result of actomyosin ring constriction. By contrast, in plant cells the division was seen as an outcome of vesicle fusion at the cell plate region between two daughter cells. Recent studies, however, uncover the involvement of vesicular trafficking in animal cell cytokinesis. This thesis aims to highlight the importance of endocytic transport and the necessity of its proper regulation. At first, the origin of vesicles is debated. Afterwards, three main types of endocytic vesicles are examined – Rab11/FIP3 endosomes, Rab35-endosomes and PI(3)P-enriched endosomes, along with their function and interacting partners. Finally, the attention is given to the mechanism of abscission and midbody inheritance. Ongoing processes are accompanied by changes in membrane composition, cytoskeleton reorganization and targeted delivery of distinct cargo molecules. Failure in cytokinesis has been implicated in the etiology of many diseases, such as cancer. Therefore, better understanding of associated endocytic trafficking may provide us with new therapeutic strategies.

Key words:

Cytokinesis, endosomes, ESCRT-III complex, FIP proteins, midbody, microfilaments, microtubules, motor proteins, Rab GTPases

Abstrakt

Cytokineze představuje velice složitý a pečlivě organizovaný proces. Po mnoho let byla cytokineze živočišných buněk popisována jako výsledek zaškrcení pomocí aktinomyosinového prstence. Naproti tomu na cytokinezi rostlinných buněk bylo pohlíženo jako na výsledek splývání váčků v oblasti buněčné přepážky mezi dvěma dceřinými buňkami. Nedávné studie však odhalily účast váčkového transportu i v cytokinezi živočišných buněk. Snahou této práce je zdůraznit význam endocytického transportu v dělení živočišných buněk a potřebu jeho řádné regulace. Nejprve je probírán původ váčků. Následně jsou rozebírány tři hlavní typy endocytických váčků – Rab11/FIP3 endozómy, Rab35 endozómy a endozómy bohaté na PI(3)P, spolu s jejich funkcí a interakčními partnery. Nakonec je věnována pozornost mechanismu rozdělení buňky a dědičnosti midbody. Probíhající procesy jsou provázeny změnami ve složení membrán, reorganizací cytoskeletu a cíleným doručováním jednotlivých transportovaných molekul. Poruchy v cytokinezi jsou zřejmě příčinou řady chorob, včetně některých typů rakoviny. Lepší porozumění úloze endocytického transportu v cytokinezi by tedy mohlo poskytnout nové možnosti terapie.

Klíčová slova:

Cytokineze, ESCRT-III komplex, endozómy, FIP proteiny, midbody, mikrofilamenta, mikrotubuly, motorové proteiny, Rab GTPázy

Abbrevations

AAA – ATPases associated with diverse cellular activities
ALIX – Apoptosis-linked gene 2-interacting protein X
AMIS – Apical membrane initiation site
Arf6 – ADP ribosylation factor 6
ATP – Adenosine triphosphate
ATPase – Adenosine triphosphatase
CHMP4B – Charged multivesicular body protein 4B
CEP55 – Centrosomal protein of 55 kDa
ECT2 – Epithelial cell transforming 2
ESCRT-I – Endosomal sorting complexes required for transport I
ESCRT-III – Endosomal sorting complexes required for transport III
Exo70p – Exocyst 70 protein
FIP – Family of interacting proteins
FIP3 – Family of interacting proteins 3
FIP4 – Family of interacting proteins 4
FIP5 – Family of interacting proteins 5
FRAP – Fluorescence recovery after photobleaching
FRET – Fluorescence resonance energy transfer
FYVE-CENT – FYVE domain-containing centrosomal protein
FYVE – Fab1, YOTB, Vac1, EEA1
GAP – GTPase activating protein
GEF – Guanosine exchange factor
GFP – Green fluorescence protein
GDI – Guanosine dissociaition inhibitor
GDP – Guanosine diphosphate
GTP – Guanosine triphosphate
GTPase – Guanosine triphosphatase
JIP3 – JNK-interacting protein 3
JIP4 – JNK-interacting protein 4
JNK – c-Jun N-terminal kinase
KIF13A – Kinesin family member 13A
NSF – *N*-ethylmaleimide-sensitive factor
Nuf – Nuclear fallout
OCRL-1 – Lowe oculocerebrorenal syndrome protein 1
p50RhoGAP – Rho GTPase activating protein 1
PI(3)K II – Phosphatidylinositol 3-kinase II
PI(3)K III – Phosphatidylinositol 3-kinase III
PI(3)P – Phosphatidylinositol 3-phosphate
$PI(4,5)P_2$ – Phosphatidylinositol 4,5-bisphosphate
Rab – Ras-related in brain

Rab4 – Ras-related in brain 4
Rab5 – Ras-related in brain 5
Rab7 – Ras-related in brain 7
Rab8 – Ras-related in brain 8
Rab11 – Ras-related in brain 11
Rab14 – Ras-related in brain 14
Rab21 – Ras-related in brain 21
Rab35 – Ras-related in brain 35
Ras – Rat sarcoma
Rho – Ras homology
RhoA – Ras homology gene family member A
RNAi – RNA interference
SCAMP2/3 – Secretory carrier protein 2/3
Sec3 – Secretory 3
Sec8 – Secretory 8
Sec10 – Secretory 10
Sec15 – Secretory 15
SNARE – Soluble NSF attachement protein receptor
TSG101 – Tumor susceptibility gene 101
TTC19 – Tetratricopeptide repeat domain 19
VAMP8 – Vesicle-associated membrane protein 8
VPS4 – Vacuolar protein sorting 4
VPS34 – Vacuolar protein sorting 34
UVRAG – UV radiation resistant associated

Contents

1 Introduction

Cytokinesis is a vital and highly complex process leading to the creation of two daughter cells by the division of one mother cell. In plants, the site of division is predicted by the assembly of a preprophase band and the cytokinetic process itself is mediated by the phragmoplast, a structure containing a concentrated cylinder of antiparallel microtubules between two daughter cell nuclei (reviewed in Wasteneys, 2002). It gradually centrifugally expands towards the cell wall and its microtubules serve as tracks for the transport of Golgi-derived secretory vesicles with newly synthetized building material, as well as cell wall- and plasma membrane-derived endocytic vesicles containing preexisting recycled material. The content of these vesicles is essential for cell plate initiation and expansion (Dhonukshe et al., 2006). Once the cell plate reaches the plasma membrane, the phragmoplast disappears (Gu and Verma, 1997) and cell plate transforms into new cell wall separating two daughter cell (Samuels et al., 1995).

Though the importance of vesicular trafficking in plant cell cytokinesis is well established, when it comes to animal cells, the necessity of targeted vesicular transport in the late stages of cytokinesis is still poorly understood. For many years, it was assumed that the abscission is executed by the assembly of an actomyosin contractile ring at the cell equator, which subsequently constricts, resulting in the formation of two daughter cells. As an unexpected twist, new studies have shown that many features formerly considered as plant-specific are also valid for animal cells going through cytokinesis (reviewed in Baluska et al., 2006). Moreover, recent evidence suggests that endocytic transport may play an important role not only in cytokinesis, but also in the post-mitotic cellular events such as midbody inheritance and degradation (reviewed in Schiel et al., 2013). Several distinct endocytic pathways has been taken into consideration as possible significant contributors to successful abscission.

The aim of this Bachelor's thesis is to sum up the current knowledge regarding endocytosis and endosomal trafficking during the process of cytokinesis and point out the complexity of its regulation. The events comprising early stages of cytokinesis have been the subject of many excellent reviews and therefore will be not covered in depth in this thesis.

1.1 Cytokinesis in animal cell

Cytokinesis represents a final step of the cell cycle and leads to the physical separation of two dividing daughter cells. Successful abscission is vital for cell proliferation and vitality. The cytokinetic process is tightly temporally and spatially controlled and consists of a series of events (**Figure 1**), including changes in membrane composition and cell shape, cytoskeleton reorganization and targeted vesicular transport.

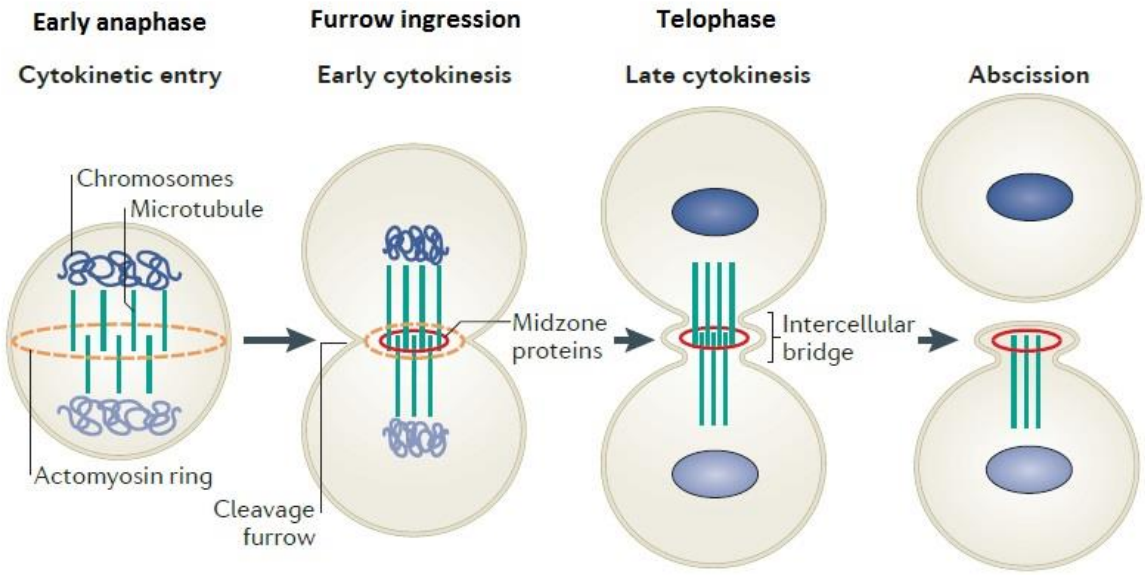

Figure 1: The major stages from cytokinesis to abscission. Reprinted and modified from Chen et al., 2012 (review).

At the onset of mitosis, the mother cell rounds up. Cytokinesis starts after the equal segregation of replicated sister chromatids during anaphase. In this stage of early cytokinesis actomyosin contractile ring starts to assemble at the cell equator beneath the plasma membrane. Its gradual constriction leads to the formation of a cleavage furrow in the midzone area. The ingression, also called the primary ingression, is caused by the activity of motor protein myosin II, which uses energy from ATP (adenosine triphosphate) hydrolysis to move along the actin filaments. This movement leads to the gradual ingression. The assembly of the actomyosin contractile ring is regulated by RhoA (Ras homology gene family member A) GTPase (guaninosine triphosphatase), which controls the actin polymerization and myosin II activation (reviewed in Jordan and Canman, 2012).

GTPases are enzymes that can bind and hydrolyze GTP (guanosine triphosphate) to GDP (guanosine diphosphate). By switching between GTP- and GDP-bound forms, proteins cycle between their active and inactive state. The exchange is mediated by various

GAP (GTPase activating protein) and GEF (guanosine exchange protein) proteins. GAP activates or accelerates the GTP hydrolysis leading to the protein inactivation, whereas GEF ensures the release of GDP from the protein and therefore returns GTPase to its empty state, which enables potential GTP binding. Moreover, GEF can also mediate the dissociation of GDI (guanosine dissociation inhibitor), which can block the protein in its inactive state (reviewed in Jaffe and Hall, 2005).

Activation and localization of RhoA to the cleavage furrow, and subsequent formation of actomyosin ring, are mediated by RhoA GEF protein ECT2 (epithelial cell transforming 2), which is targeted to the cleavage area by the centralspindlin complex at the central spindle microtubules in the midzone area (Nishimura and Yonemura, 2006). Later dissociation of ECT2 enables the interaction of recycling endosomes with centralspindlin complex and plasma membrane. Proteins delivered by these vesicles cause cortical actin depolymerization (Schiel et al., 2012). Cortical actin network hinders the vesicle fusion on the plasma membrane (Vitale et al., 1995), thus this step is essential for further vesicle fusion in the area.

Midzone is a region of antiparallel overlapping arrays of microtubules assembled in between the separated chromosomes. It is also called the central spindle region. As cell proceeds towards late cytokinesis, which usually correlates with telophase, the ingression continues and microtubules gradually compact, creating a narrow bridge in between the two prospective daughter cells, called the intercellular bridge. In the middle of the intercellular bridge is the midbody, also referred as the Flemming body, an electron-dense structure consisting of overlapping plus ends of central spindle microtubules. The midbody is easily recognizable from its bulky appearance at the center of the intracellular bridge. The small dense disk in the middle of midbody is called the stembody (reviewed in Eggert et al., 2006).

When the primary furrow ingression, mediated by actomyosin ring, is completed, the diameter of the intracellular bridge is approximately 1.5-2 μm (Schiel et al., 2011). Prior to the final step of cytokinesis, the abscission, the thickness is reduced to roughly 100-200 nm (Schiel et al., 2011). This so-called secondary ingression occurs along the entire length of the intercellular bridge or only at one side of the midbody and is preceded by depolymerization of the cortical actin (Schiel et al., 2012). The reduction of the diameter is essential for the localization of the ESCRT-III (endosomal sorting complexes required for transport III) complex to the site of abscission (see chapter 4.2).

Interestingly, while microtubules first serve as tracks for vesicle delivery and also play a stabilization role for the long and narrow intercellular bridge, as the cytokinesis proceeds towards its final stage, the microtubules need to be severed to allow vesicle fusion and successful abscission (Schiel et al., 2011). However, the precise mechanism of microtubule severing and depolymerization is still poorly understood.

During late cytokinesis, vesicles are delivered along microtubules to the intercellular bridge or may arise by plasma membrane endocytosis in sites adjacent to the midbody area. At first, the tethering of the vesicles is probably mediated by Rab (Ras-related in brain) GTPases and the exocyst complex. After the microtubules are severed, the vesicle fusion occurs by the interaction of SNARE (soluble NSF attachement protein receptor) proteins and exocyst complex as well (reviewed in Barr and Gruneberg, 2007).

Finally, the abscission occurs, presumably by the action of ESCRT-III machinery (reviewed in Barr and Gruneberg, 2007). It is noteworthy that the abscission usually takes place at only one side of the midbody and therefore the midbody inheritance is often asymmetric (Gromley et al., 2005).

1.2 Intracellular trafficking pathways

Two major pathways can be distinguished in intracellular vesicle trafficking. One of them is the secretory pathway, the other one is the endocytic pathway. Although both of them are hypothesized to contribute, to a certain extent, to the process of abscission, this thesis is going to focus mainly on the role of the endocytic pathway.

Generally speaking, the secretory pathway traffics vesicles with newly synthetized macromolecules from the endoplasmic reticulum, through the Golgi apparatus until they finally fuse with the plasma membrane. Alternatively, the vesicles are sent to lysosomes (reviewed in Pelham, 1996). The endocytic pathway, on the other hand, transports vesicles the opposite way. The vesicles bud from the plasma membrane and are subsequently delivered to other membrane compartments, such as the early and late endosomes, lysosomes and recycling endosomes (reviewed in Grant and Donaldson, 2009).

In endocytic pathway at least two endocytic recycling pathways have been described. First, a direct or a fast recycling pathway acts from peripheral endocytic compartments. Second, named a slow recycling pathway transports cargo molecules back to the plasma membrane from the compartments, which lie deeper in the endocytic pathway (reviewed in Grant and Donaldson, 2009). Endocytic pathways are highly coordinated and under control of distinct GTPases (reviewed in Somsel Rodman and Wandinger-Ness, 2000).

2 Endocytic trafficking throughout mitosis

More than three decades ago, it was thought that endocytosis and endosomal trafficking does not occur during mitosis (Sager et al., 1984) and is only recovered during the early G_1 phase of the cell cycle (Berlin and Oliver, 1980). At that time, it was believed that endocytic processes were depressed during mitosis (Sager et al., 1984). Later research has shown that, although indeed the recycling part of the membrane endocytosis back to the cell surface ceases during early stages of mitosis, it resumes not after the cell division is completed, but already during the late mitosis (Schweitzer et al., 2005). Furthermore, following studies confirmed an ongoing endocytosis throughout all stages of cell division as well (Boucrot and Kirchhausen, 2007).

2.1 Plasma membrane endocytosis and endocytic compartments

During the mitotic phase of the cell cycle, the plasma membrane undergoes visible changes in its total area. However, not only the amount of plasma membrane changes (Boucrot and Kirchhausen, 2007), but also its composition alters, especially in the area of the intercellular bridge (Field et al., 2005).

At the onset of mitosis the mother cell ceases to recycle membrane from endosomal compartments back to the plasma membrane, consequently leading to the decrease of total cell surface, accumulation of endocytic organelles and rounding up. As mitosis proceeds to anaphase, endosomal recycling is resumed and stored vesicles provide the excess membrane required for the two daughter cells. In other words, endocytosis continues normally throughout all phases of mitosis, only the recycling part of the endocytosis to the cell surface slows considerably and is accelerated again at anaphase. This action seems to be independent of the secretory pathway (Boucrot and Kirchhausen, 2007).

Moreover, recycling endosomes are also essential for proper spindle formation and subsequent cell division. This is because recycling endosomes carry microtubule nucleating, anchoring and regulatory proteins to the spindle poles. The failure of proper spindle formation caused by recycling endosomes disruption results in insufficient tension on the chromosomes (Hehnly and Doxsey, 2014). Indeed, interference with these processes leads to the incorrect cell division (Boucrot and Kirchhausen, 2007; Hehnly and Doxsey, 2014).

As the mitosis proceeds, several other events take place. The distribution of the compartments of the endocytic pathway changes based on the position of the mitotic

spindle. Endosomal compartments display visible clustering around separated centrosomes of future daughter cells. The localization is densest at the minus ends of the forming mitotic spindle (Dunster et al., 2002). Prominent pools of endosomes appear around the poles of mitotic spindle as well as at both extremities of the central spindle later in cytokinesis (**Figure 2**) (Schweitzer et al., 2005; Takatsu et al., 2013). Formation of the clusters seems to be dependent on microtubule motor protein dynein (Takatsu et al., 2013). These clusters may later serve as a reservoir for following cytokinetic events, based upon the fact that numerous studies reported that the endosomes transported to the cleavage furrow originate from the area around the centrosomes (Takahashi et al., 2011).

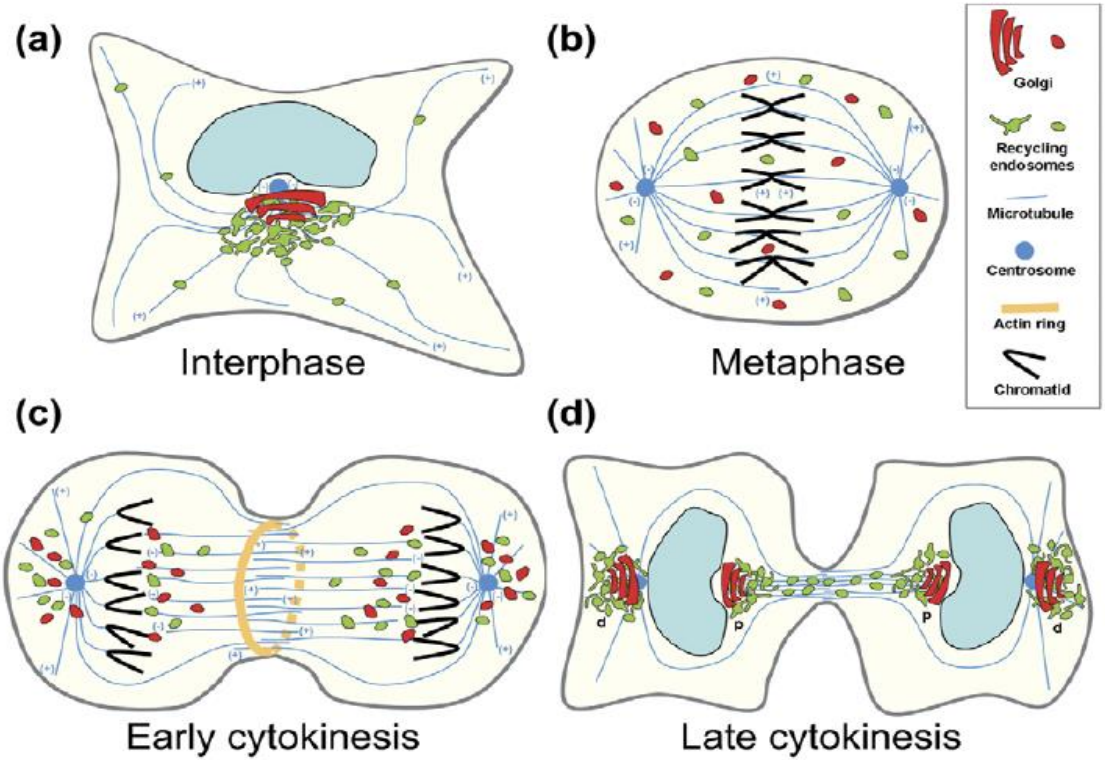

Figure 2: Distribution of organelles during cell cycle. Reprinted from Montagnac et al., 2008 (review).

2.2 Receptor-mediated endocytosis in mitosis

Moreover, vesicles delivered to the cleavage furrow may not originate exclusively from endosomal cluster deposits, given the fact that they could arise by endocytosis adjacent to the midbody area (Boucrot and Kirchhausen, 2007).

Schweitzer et al. conducted an experiment using labeled transferrin, where they proved that even though there was no detectable internalization of this ligand in prophase

and metaphase, as the cell proceeded to anaphase and telophase, the internalization was not only observed, but also significantly increased, especially in telophase (Schweitzer et al., 2005). Later studies confirmed this observation (Takatsu et al., 2013).

The endocytosis resumes first at the polar region near the mitotic spindle poles during cleavage furrow ingression. Endocytic vesicles are subsequently transported to the midbody area during late telophase and cytokinesis. Moreover, in addition to the initial uptake at the polar region of dividing cells during early cytokinesis, at the later stage of cell division, endocytosis takes place at the midbody area as well. Endocytic vesicles may deliver components needed for the cytokinetic events (Schweitzer et al., 2005).

Schweitzer et al. also expressed an opinion that endocytosis at the midbody may help to physically seal off daughter cell membranes and thus facilitate the final step of cytokinesis (Schweitzer et al., 2005). However, according to a later study, the membrane amount may not be sufficient to promote the cell division (Schiel et al., 2011) as will be discussed in chapter 4.

2.3 Origin of the membrane

The fact that the new plasma membrane is deposited to the cleavage furrow has been known for many years now (Bluemink and de Laat, 1973). Nevertheless, to this date, the source of the membrane that is transported to the midbody remains to be fully understood.

The necessity of the secretory pathway trafficking is still under debate and needs further investigation. Results from treatment with brefeldin A, an inhibitor of secretory pathway, are not uniform across experimental models. For example, it does not appear to block cytokinesis in sea urchin zygotes (Shuster and Burgess, 2002). On the contrary, it can do so in *Caenorhabditis elegans* embryos (Skop et al., 2001). Finally, in some studies, there has been no effect on deposition of new membrane at the cleavage furrow in HeLa cells (Boucrot and Kirchhausen, 2007), whereas another team has observed late stage cytokinesis defects (Gromley et al., 2005). Taken together, these inconsistent results may suggest that different membrane trafficking pathways are used in various animal cells.

In contrast, the importance of endocytic transport during the late stages of cytokinesis is comparatively well established. Endocytic trafficking is known to be involved in processes such as secondary ingression formation (Schiel et al., 2011) or reorganization of actin network at the abscission site (Dambournet et al., 2011; Schiel et al., 2012).

Taken together, it is probable that majority of the vesicles transported to the midbody prior to the abscission are of endocytic pathway origin and thus the secretory pathway is probably not essential for the cell division and may play only a minor role in cytokinesis. However, it is also possible that the mechanism is not identical for all organisms and secretory pathway may play an important role in distinct species. Without a doubt, further research is needed to answer these questions.

3 Targeting to the midbody

Why cells need endosomal transport towards intercellular bridge and what is the purpose of endosomal fusion there during cytokinesis? Two likely explanations were proposed. First, endosomes may supply new membrane (Boucrot and Kirchhausen, 2007) and specific lipids and proteins necessary for the forming intracellular bridge (Sagona et al., 2010). Second, endosomes may facilitate rapid localized delivery of regulatory proteins needed for the reorganization of the cytoskeleton (Riggs et al., 2003) and formation of the secondary ingression (Schiel et al., 2012).

Prominent regulators of the vesicle targeting and fusion are Rab proteins, monomeric G proteins, members of the Ras superfamily of small GTPases. Their function is coupled with the cyclic activation and inactivation and subsequent cytosolic and membrane localization. Like other GTPases, Rab GTPases also switch between an inactive, GDP-bound state in cytoplasm, and an active, GTP-bound state, in which they interact with their effector proteins. Interacting effector proteins subsequently regulate targeting and fusion of the transported vesicles (revieved in Jaffe and Hall, 2005). Based on the subcellular localization of different Rab proteins, endosomes can be distinguished from one another. For example, Rab4 and Rab5 serve as a marker of early endosomes (Bucci et al., 1992; Van Der Sluijs et al., 1991), Rab7 represents a hallmark of late endosomes (Feng et al., 1995) and Rab11 is typical for a subset of recycling endosomes (Ullrich et al., 1996).

In the following chapter three main types of endosomes will be discussed along with their role in cytokinesis.

3.1 Rab11/FIP3 endosomes

Rab11, is a small GTPase involved in the regulation of slow recycling pathway. It was reported that this protein associates with pericentrosomal endocytic recycling compartment (Ullrich et al., 1996). As was mentioned in chapter 2, endosomal clusters appear at the pericentrosomal region near the spindle poles prior to cytokinesis and are subsequently targeted to the midbody (Dunster et al., 2002). These clusters are confirmed to be derived from endocytic recycling compartment (Takatsu et al., 2013). The involvement of Rab11 in the delivery of new membrane to the cleavage furrow was originally demonstrated in *Caenorhabditis elegans* embryos (Skop et al., 2001). Later studies have shown that Rab11 also regulates cytokinesis in mammalian cells (Wilson

et al., 2005), where it regulates trafficking to the midbody region. Moreover, Rab11 recycling endosomes seems to be also essential for successful cellularization in *Drosophila melanogaster* embryos (Pelissier et al., 2003; Riggs et al., 2003).

In cytokinesis, FIP proteins (family of interacting proteins) represent a common interacting partner for these endosomes (Fielding et al., 2005; Hickson et al., 2003; Kelly et al., 2010). Especially, FIP3 is well-established functional partner of Rab11 recycling endosomes in the intercellular bridge, therefore are these vesicles often referred as FIP3-endosomes (Simon et al., 2008). In accordance with this custom, in this thesis, the term FIP3-endosomes is used to make reference to FIP3-containing Rab11 recycling endosomes.

FIP3, also known as Eferin or Arfophilin (Horgan et al., 2004), is together with FIP4 a member of class II FIP (reviewed in Prekeris, 2003). It was demonstrated that FIP3 regulates the temporal and spatial dynamics of the recycling endosomes during cytokinesis and mediates the abscission (Fielding et al., 2005; Simon et al., 2008; Wilson et al., 2005). The recruitment of FIP3 to the membrane of the recycling vesicles relies on the interaction with Rab11 (Wilson et al., 2005; Collins et al., 2012).

The precise function of these vesicles will be discussed in greater detail in chapter 4.

3.1.1 Recruitment of recycling endosomes by Rab11 and FIP3

In early anaphase, FIP3-endosomes rapidly accumulate around centrosomes with no detection in the midzone region. As cytokinesis proceeds, FIP3-endosomes gradually translocate to the cleavage furrow (Fielding et al., 2005; Wilson et al., 2005). During the late stage of cytokinesis, FIP3-endosomes are localized near the intercellular bridge and relocalize to the midbody shortly before the abscission (Takahashi et al., 2011).

It is noteworthy that furrowing and midbody formation is Rab11 and FIP3 independent (Wilson et al., 2005), whereas the formation of the secondary ingression seems to depend on FIP3-endosomes (Schiel et al., 2011). Interestingly, the translocation of FIP3-endosomes coincides with the formation of the midbody (Wilson et al., 2005) and marks the progression to late telophase (Schiel et al., 2012).

In spite of the fact that Rab11, activated by GTP loading, recruits FIP3 to the endosomes, it was demonstrated that Rab11 is not involved in FIP3 and FIP4 localization to the midbody. The role of Rab11 is likely to recruit the recycling endosomes from endosome clusters for traffic along microtubules mediated by kinesin-1 (Montagnac et al., 2009) motor protein into the cleavage furrow area, where an interaction with active Arf6

(ADP ribosylation factor 6) is likely to facilitate docking via cooperation with other tethering factors prior to the fusion with plasma membrane (Fielding et al., 2005). In other words, whereas FIP3 association with recycling endosomes is dependent on Rab11, its midbody localization is not (Fielding et al., 2005; Wilson et al., 2005). Conversely, another study concluded that targeting of FIP3 and FIP4 to the cleavage furrow and midbody is Rab11 dependent (Horgan et al., 2004). The mechanism of interaction among Rab11, FIP3 or FIP4 and Arf6 is still not fully understood and will be discussed again later.

Time-lapse and FRAP (fluorescence recovery after photobleaching) analysis has shown that while FIP3-endosomes are continuously moving in and out of the midbody region during early and mid-telophase, the movement is significantly reduced at the onset of late telophase. FIP3-endosomes accumulation in the midbody area is mediated by an interaction with centralspindlin complex components (Simon et al., 2008). Centralspindlin is well known for its regulation of the cleavage furrow through the recruitment of the RhoA GEF ECT2 (Nishimura and Yonemura, 2006), but it has been shown that it has an additional role in the translocation of recycling endosomes to the midbody region during cytokinesis as well (Simon et al., 2008). However, it is important to note that centralspindlin does not transport the recycling endosomes, but rather works as a tethering factor. Furthermore, motility of FIP3-endosomes appears to be dependent on microtubules, since treatment with nocodazole, a depolymerizing agent of microtubules, inhibits movement of FIP3-endosomes. Motor protein, responsible for the delivery along the central spindle microtubules is probably kinesin-1 (Montagnac et al., 2009). Interestingly, experiments monitoring the fluorescence of FIP3-GFP also indicate that endosomes do not exchange between prospective daughter cells and stay at their side of the furrow (Simon et al., 2008).

FIP3 on Rab11 recycling endosomes binds to the centralspindlin complex in late telophase (**Figure 3**). By contrast, during metaphase and early anaphase, when is the binding region occupied by ECT2, FIP3 cannot interact with centralspindlin. At the onset of late telophase, ECT2 dissociates from the centraspindlin complex allowing FIP3 to bind instead. This fact points to the conclusion that ECT2 and FIP3 form mutually exclusive complexes with centraspindlin and consequently ECT2 interaction regulates FIP3-endosomes recruitment to the midbody (Simon et al., 2008). Dissociation of ECT2 therefore precedes FIP3 and recycling endosomes recruitment. However, FIP3 does not

appear to direct the removal of ECT2. Indeed, knock-down of ECT2 does not cause premature association of FIP3 with centralspindlin (Simon et al., 2008).

Moreover, in addition to Rab11, FIP3 also binds to Arf6 (Fielding et al., 2005; Wilson et al., 2005) and may therefore serve as a scaffolding protein for Arf6 interaction (Schonteich et al., 2007).

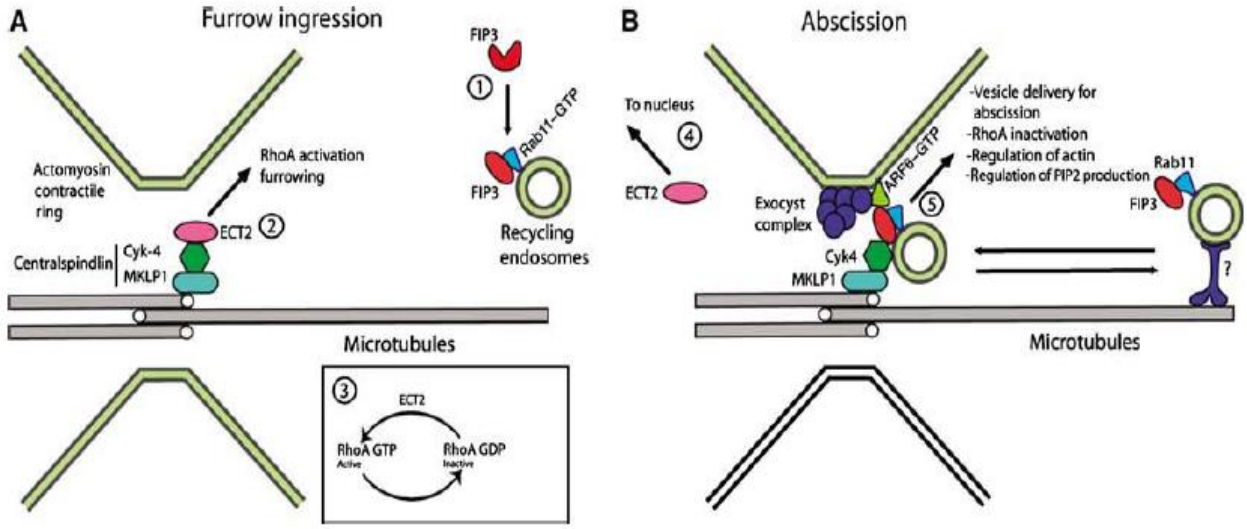

Figure 3: One of the possible targeting models of FIP3-endosomes to the cleavage furrow. Reprinted from Simon et al., 2008.

3.1.2 Localization to the midbody region

Arf6 is another key regulator of cytokinesis in animal cells and has been reported to interact with both FIP3 and FIP4. Both of these FIP proteins are capable of binding Rab11 and Arf6 simultaneously, creating a ternary complex (Fielding et al., 2005). The interaction of FIP3 and Arf6 was originally identified in yeast two-hybrid screen (Hickson et al., 2003).

During interphase, Arf6 is localized to the plasma membrane and intracellular vesicles (Fielding et al., 2005). However, as cell proceeds to late telophase and cytokinesis, the localization of Afr6 shifts to the furrow and subsequently to the midbody, where it is in its activated GTP-bound form (Fielding et al., 2005; Schweitzer and D'Souza-Schorey, 2002; Takahashi et al., 2011). However, the exact mechanism of Arf6 translocation to the cleavage furrow region and its interaction with FIP proteins and Rab11 remains a subject of controversy.

Up to date, there are two main possible ways of recruiting recycling endosomes to the midbody region. First, Arf6 is already present at the plasma membrane in the cleavage

furrow and serves as a tethering factor binding FIP3 located on the Rab11-containing endosome (Fielding et al., 2005). Second, based on FRET (fluorescence resonance energy transfer) analysis, Arf6 first interacts with FIP3 and Rab11, forming Af6-FIP3-Rab11 complex on recycling endosome, which is subsequently transported to the cleavage furrow (Schonteich et al., 2007), where an interaction with exocyst or SNARE proteins mediates the fusion. Indeed, it was reported that Arf6 interacts with Sec10 (Prigent et al., 2003) and Exo70p (Fielding et al., 2005) subunits of exocyst; Rab11 binds to Sec15 subunit (Zhang et al., 2004) and probably also Sec3, Sec8 and Exo70p subunits (Neto et al., 2013).

To sum it up, the exact mechanism of targeting recycling endosomes to the cleavage furrow and midbody is controversial. One of the newest studies suggested that a possible reason for this discrepancy may be caused by the fact that previous studies determined the localization of overexpressed FIP3, FIP4, Rab11 and Arf6, rather than endogenous proteins (Takahashi et al., 2011). Takahashi et al. used optimized immunological detection of endogenous FIP3, Rab11 and Arf6, in combination with RNAi (RNA interference) and time-lapse imagining of GFP-tagged proteins. According to this study, transport of FIP3-endosomes relies on Rab11 and both, FIP3 and Rab11, can be detected in the area surrounding the intercellular bridge during late telophase, but not in the midbody. However, immediately prior to the abscission, only FIP3 moves to the midbody and this translocation is dependent upon Arf6. Arf6 is present in the cleavage furrow in telophase, when FIP3 is still accumulated near the spindle poles, concluding that targeting of Arf6 to the midbody is FIP3 independent. After cytokinesis, both FIP3 and Arf6 remain associated with the midbody remnant in one of the daughter cells (Takahashi et al., 2011). Without a doubt, additional research is needed to confirm the exact mechanism of recycling endosome targeting to the midbody.

3.2 Rab35-endosomes

Rab35-endosomes regulated pathway is another essential endocytic pathway involved in the coordination of cytokinesis and abscission. Rab35 controls fast recycling pathway and localize to clathrin-coated vesicles (Kouranti et al., 2006). It is noteworthy that Rab35 is phylogenetically conserved among metazoans (Pereira-Leal and Seabra, 2001).

During early cytokinesis this GTPase has been found in the cleavage furrow region and in the intercellular bridge at later stages of cytokinesis (Kouranti et al., 2006). In both cases, Rab35 has co-localized with $PI(4,5)P_2$ (phosphatidylinositol 4,5-bisphosphate)

enriched domains (Kouranti et al., 2006) that has been previously shown to be abundant in the intercellular bridge during cytokinesis (Emoto et al., 2005; Field et al., 2005). While these PI(4,5)P_2 domains are probably responsible for initial stability of the cleavage furrow, they hinder terminal steps of cytokinesis as will be discussed further (Field et al., 2005).

Molecular mechanism regulating the activation of Rab35 and consequently its interaction with OCRL (Lowe oculocerebrorenal syndrome protein 1) phosphatase, which is responsible for PI(4,5)P_2 dephosphorylation, is depicted in **Figure 4**. Rab35 and Arf6 act antagonistically in the same endocytic recycling pathway, significant for cytokinesis, where GTP-Arf6 promotes conversion of active Rab35 GTP-bound to an inactive GDP-bound form. Unexpectedly, this interaction has been reported to occur in clathrin-coated pits (Chesneau et al., 2012), invaginated membrane regions coated with protein clathrin, which subsequently bud off as coated vesicles involved in receptor-mediated endocytic pathway (reviewed in Mousavi et al., 2004). The clathrin-dependent endocytic pathway, however, can only be entered by GTP-Rab35 (Chesneau et al., 2012).

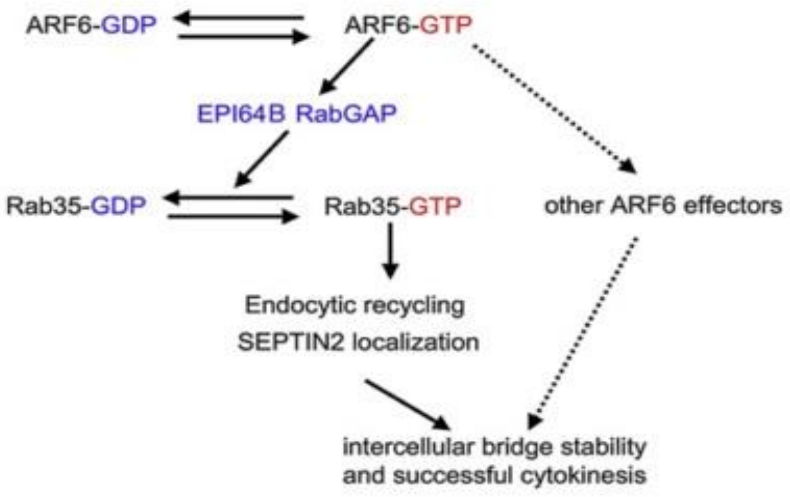

Figure 4: Molecular model of the Arf6/Rab35 interaction cascade regulating Rab35 activation in endocytosis and cytokinesis. Reprinted from Chesneau et al., 2012.

Taken together, the activation of Arf6 must be precisely regulated, for Arf6 over-activation inhibits loading of Rab35 in the endocytic pathway and thus leads to the intercellular bridge instability (Chesneau et al., 2012).

Rab35 is involved in the initial stabilization of the intercellular bridge, after the furrow ingression, by controlling localization of cytoskeletal proteins, such as Septin2

(Kouranti et al., 2006). At later stages of cytokinesis, Rab35 controls abscission through the function of the PI(4,5)P_2 5-phosphatase OCRL-1 (Lowe oculocerebrorenal syndrome protein 1) (**Figure 5**). OCRL-1 directly interacts with GTP-Rab35 and is transported to the cleavage furrow area through the association with Rab35-containing endosomes (Dambournet et al., 2011). PI(4,5)P_2 5-phosphatase OCRL-1, which is mutated in Lowe syndrome patients cells (reviewed in Lowe, 2005), is an effector of the Rab35 GTPase and promotes PI(4,5)P_2 lipid hydrolysis required for F-actin removal from the intercellular bridge in late stages of cytokinesis (Dambournet et al., 2011). This dephosphorylation step is essential, since PI(4,5)P_2 mediates adhesion of the contractile ring components to the plasma membrane (Field et al., 2005) and thus promotes actin polymerization at the furrow. In other words, it is a limiting factor for subsequent abscission step, since cortical F-actin hinders the vesicle fusion with plasma membrane and ESCRT-III complex assembly (reviewed in Echard, 2012) (for further details see chapter 4.1.1.).

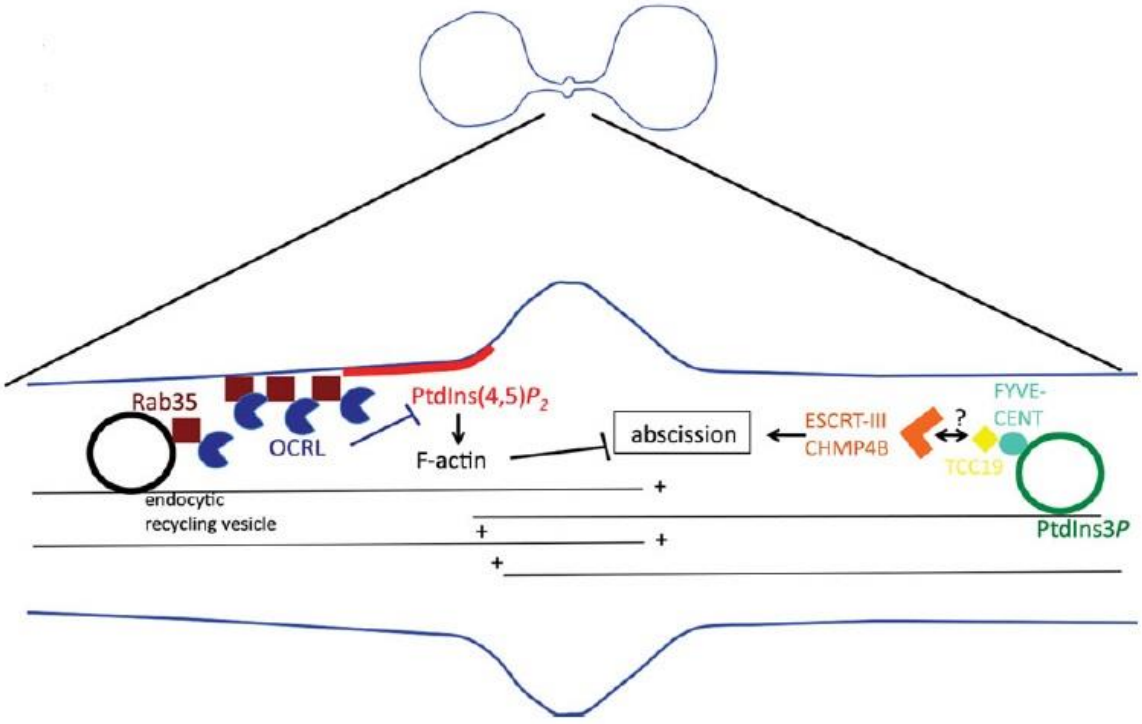

Figure 5: General model of Rab35-endosomes and PI(3)P-enriched endosomes interactions inside the intercellular bridge during late cytokinesis. Reprinted and modified from Echard, 2012 (review).

Depletion of Rab35 or OCRL-1 in cells, for instance such as in Lowe syndrome patient cells, results in over-stabilized intercellular bridges that contain abnormally high levels of PI(4,5)P_2 and cortical F-actin, leading to an abscission failure. Interestingly, after

treatment with very low doses of latrunculin A, an F-actin depolymerizing drug, the cytokinesis defects were rescued (Dambournet et al., 2011). Moreover, defective Rab35 results in decreased levels of endocytic recycling and accumulation of intracellular vacuoles rich in endocytic markers (Kouranti et al., 2006).

3.3 Other Rab GTPases

Aside from Rab11 and Rab35, other Rab GTPases such as Rab4, Rab8, Rab14 and Rab21 are also present within the midzone or the intercellular bridge. However, none of them seems to be absolutely essential for the successful completion of cytokinesis (reviewed in Schiel and Prekeris, 2013).

Targeted trafficking of integrins was shown to be required for successful cytokinesis in adherent cells. Transport of integrin-containing vesicles is regulated by activation of small GTPase, Rab21. Loss of Rab21 function leads to impaired integrin localization to the cleavage furrow and defective cytokinesis (Pellinen et al., 2008).

Rab14 has been demonstrated to interact with class I FIPs and to co-localize with transferrin receptor-containing recycling endosomes. Moreover, Rab14 localizes to the cleavage furrow and midbody region during cytokinesis (Kelly et al., 2010).

Rab8-positive tubular endosomes represents another Rab compartment involved in the material and membrane supply for the midbody region (Pohl and Jentsch, 2008).

Finally, Rab4 interacts with Arf6 on recycling endosomes at the central spindle and ensures delivery of material to the cleavage furrow during cytokinesis (Dyer et al., 2007).

3.4 PI(3)P-enriched endosomes

Apart from Rab11/FIP3 and Rab35-endosomes, which have been recognized as significant contributors to the successful abscission, there are also so-called PI(3)P-enriched (phosphatidylinositol 3-phosphate) endosomes. PI(3)P is an endosomal lipid typical for early endosomes (Gillooly et al., 2000), but is also present on the cytosolic leaflet of the plasma membrane and certain intracellular membranes. Phosphatidylinositols are well known regulators of diverse cellular processes such as signaling, vesicular trafficking and cytoskeletal dynamics. Moreover, they also serve as a platform for recruiting specific effector proteins (reviewed in Echard, 2012).

PI(3)P localization was studied using a GFP-tagged tandem FYVE-domain, a probe for PI(3)P distribution, in HeLa cells (Sagona et al., 2010). FYVE-domain specifically binds to PI(3)P (Gillooly et al., 2000). During late cytokinesis, PI(3)P-enriched vesicles

were detected inside the intercellular bridge and in the vicinity of the midbody. Moreover, they have been shown to co-localize with the recycling endosomal marker transferrin. PI(3)P endosomes in the midbody probably serve as an interacting platform for ESCRT-III complex (pivotal role in abscission, see chapter 4.2) components (Sagona et al., 2010).

The synthesis of PI(3)P is done by phosphorylation mediated by class II and III phosphatidylinositol 3-kinases (PI(3)K II and III). It is noteworthy that dysfunction of either VPS34 (vacuolar protein sorting 34), the catalytic subunit of PI(3)K III, or tumor suppressor Beclin-1, its accessory subunit, leads to an increased number of binucleated and multinucleated cells. PI(3)K III, together with PI(3)P, has been observed in the midbody region during late cytokinesis (Sagona et al., 2010).

The interacting machinery (**Figure 5 and 6**) acts as follows – in interphase, protein FYVE-CENT (FYVE domain-containing centrosomal protein) is located on centrosomes and remains associated with spindle poles during mitosis. However, FYVE-CENT and its binding partner, protein TTC19 (tetratricopeptid repeat domain 19), translocate from centrosomes to the midbody during cytokinesis. Their trafficking is mediated by mictorubule-dependent motor protein KIF13A (kinesin family member 13A) in telophase. At the midbody, protein FYVE-CENT docks to PI(3)P-enriched membrane, presumably on PI(3)P-enriched endosomes, which seem to serve as an interacting platform. Protein TTC19 binds to CHMP4B (charged multivesicular body protein 4B), a subunit of ESCRT-III. Finally, ESCRT-III complex performs the abscission (Sagona et al., 2010).

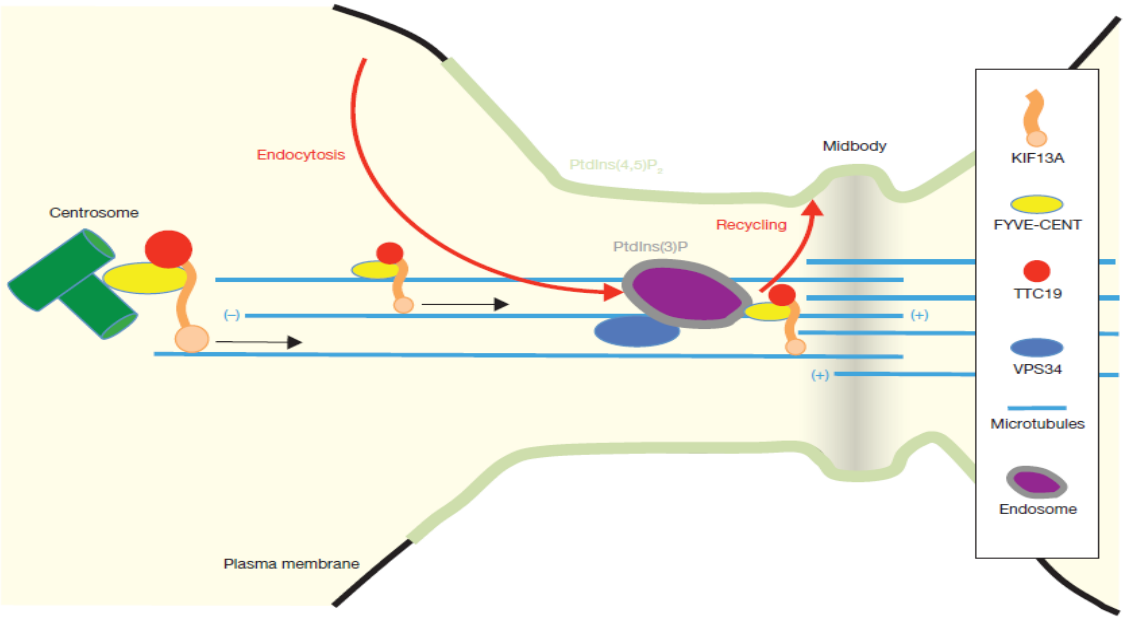

Figure 6: Model of PI(3)P-enriched endosomes interacting machinery. Reprinted from Montagnac and Chavrier, 2010 (review).

This machinery not only leads to dynamic remodeling of the lipid and protein composition of the midbody plasma membrane, but also ensures the final step by providing interacting partners for ESCRT-III complex. PI(3)P is required for the recruitment of PI(3)P-binding protein FYVE-CENT and its interacting partners TTC19 and KIF13A. Depletion of FYVE-CENT, VPS34 or KIF13A causes arrest in early cytokinesis (Sagona et al., 2010). Moreover, defective FYVE-CENT protein seems to be associated with hereditary spastic paraplegia (Hanein et al., 2008).

3.5 Motor proteins in endocytic trafficking

Endocytic trafficking is tightly coupled with motor proteins. Several motor proteins were identified as possible candidates for targeted endosome transport during cytokinesis. It is likely that various types of motor proteins are required for distinct pathways and in different cell types.

Trafficking of FIP3-containing Rab11 recycling endosomes in and out of the intercellular bridge is mediated mainly by kinesin-1 and dynactin/dynein motors and regulated by Arf6 (**Figure 7**). Kinesin-1 and dynactin complex interact with second leucine zipper domain of JIP3 (JNK-interacting protein 3) and JIP4 (JNK-interacting protein 4).

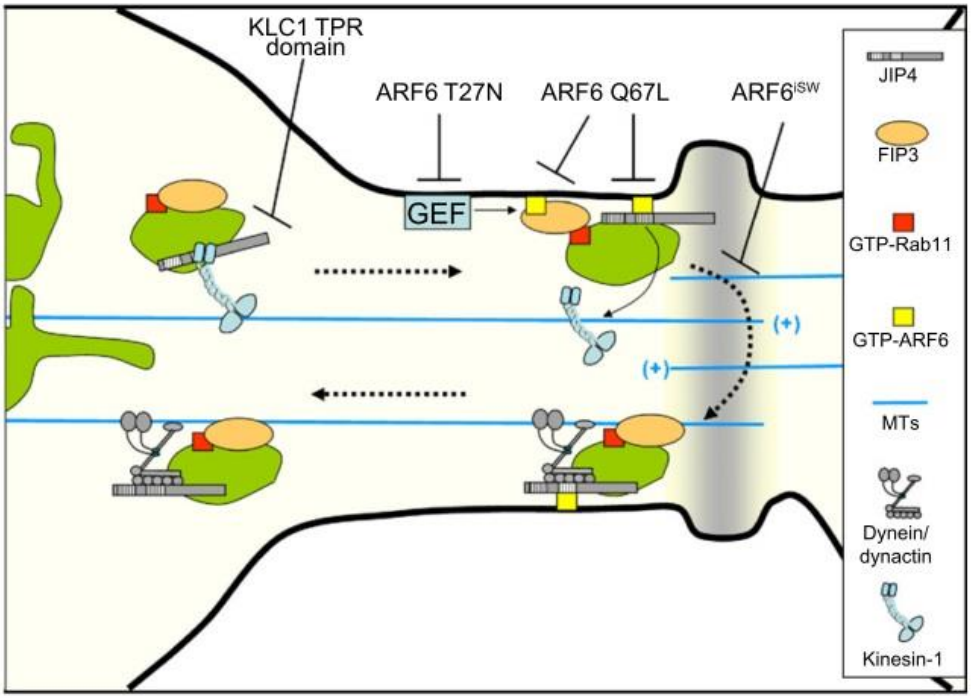

Figure 7: Model of Arf6/JIP4 regulation of kinesin-1/dynactin-dependent trafficking in and out of the intercellular bridge. Reprinted from Montagnac et al., 2009.

However, these interactions are mutually exclusive. In other words, either kinesin-1 binds to one of the JIPs or the dynactin complex. Furthermore, the binding site on second leucine zipper domain is also mutually exclusive for kinesin-1 and GTP-Arf6. Without the presence of GTP-Arf6, JIP3 and JIP4 interact with kinesin-1 and move the cargo along microtubules toward plus ends at the midbody. Interaction with GTP-Arf6, which is located at the midbody, leads to the replacement of kinesin-1 by dynactin complex on JIP3 or JIP4. In effect, this whole dynactin complex moves toward microtubule minus ends (Montagnac et al., 2009).

Montagnac et al.'s finding is in agreement with Simon et al.'s study, which showed that during early and mid-telophase FIP3-endosomes are moving in and out of the intercellular bridge region (Montagnac et al., 2009; Simon et al., 2008). However, in late telophase the movement is decreased significantly (Simon et al., 2008). This movement is probably mediated by kinesin-1 and dynactin complex motors and ensures trafficking of FIP3-endosomes from endosomal clusters to the intercellular bridge and midbody, where they interact with GTP-Arf6 and other tethering factors, such as centralspindlin and exocyst complex and SNARE proteins.

Besides kinesin-1 and dynactin complex, another motor protein has been identified as a possible regulator of endocytic transport in cytokinesis – myosin VI. Myosin VI was previously demonstrated to participate in clathrin-mediated endocytosis (Buss et al., 2001). During early cytokinesis myosin VI can be detected co-localized with the actomyosin ring. As cytokinesis proceeds, myosin VI is recruited and concentrated on the walls of the midbody region. Furthermore, this motor protein was also confirmed to be present on vesicles moving in and out of the midbody region. At late cytokinesis, when intercellular bridge is increasing in length, myosin VI-vesicles are gradually moving away from the midbody into the cytoplasm. This leads to a conclusion that at late stages of cytokinesis, myosin VI is possibly responsible for the endocytic transport out of the midbody region to the cell (Arden et al., 2007). This finding is in an agreement with Schweitzer et al.'s study stating that endocytic transport resumes in late cytokinesis in the midbody area (Schweitzer et al., 2005). The movement of myosin VI-interacting vesicles from the centrosomal area into cleavage furrow and midbody region seems to be coupled with the interaction with specific binding partners. Furthermore, targeting to the cleavage furrow may also involve its C-terminal domain that shows high affinity for $PI(4,5)P_2$, which is enriched in the cleavage area (Arden et al., 2007).

Apart from kinesin-1, kinesin-2 also emerged as a motor protein involved in cytokinesis. Recent studies of apical lumen formation in epithelial morphogenesis show interesting connection between cytokinesis and targeted delivery of FIP5-containing Rab11 recycling endosomes (Li et al., 2014a), which are transported to the midbody region by motor protein kinesin-2 (Li et al., 2014b). Kinesin-2 carries these endosomes from apical endosomes, which are accumulated around centrosomes during early telophase, along microtubules to the midbody in late telophase (Li et al., 2014b) (**Figure 8**). Vesicles fuse with apical membrane initiation site (AMIS), which forms around the midbody. Their cargo consists of apical proteins essential for *de novo* lumen formation (Li et al., 2014a).

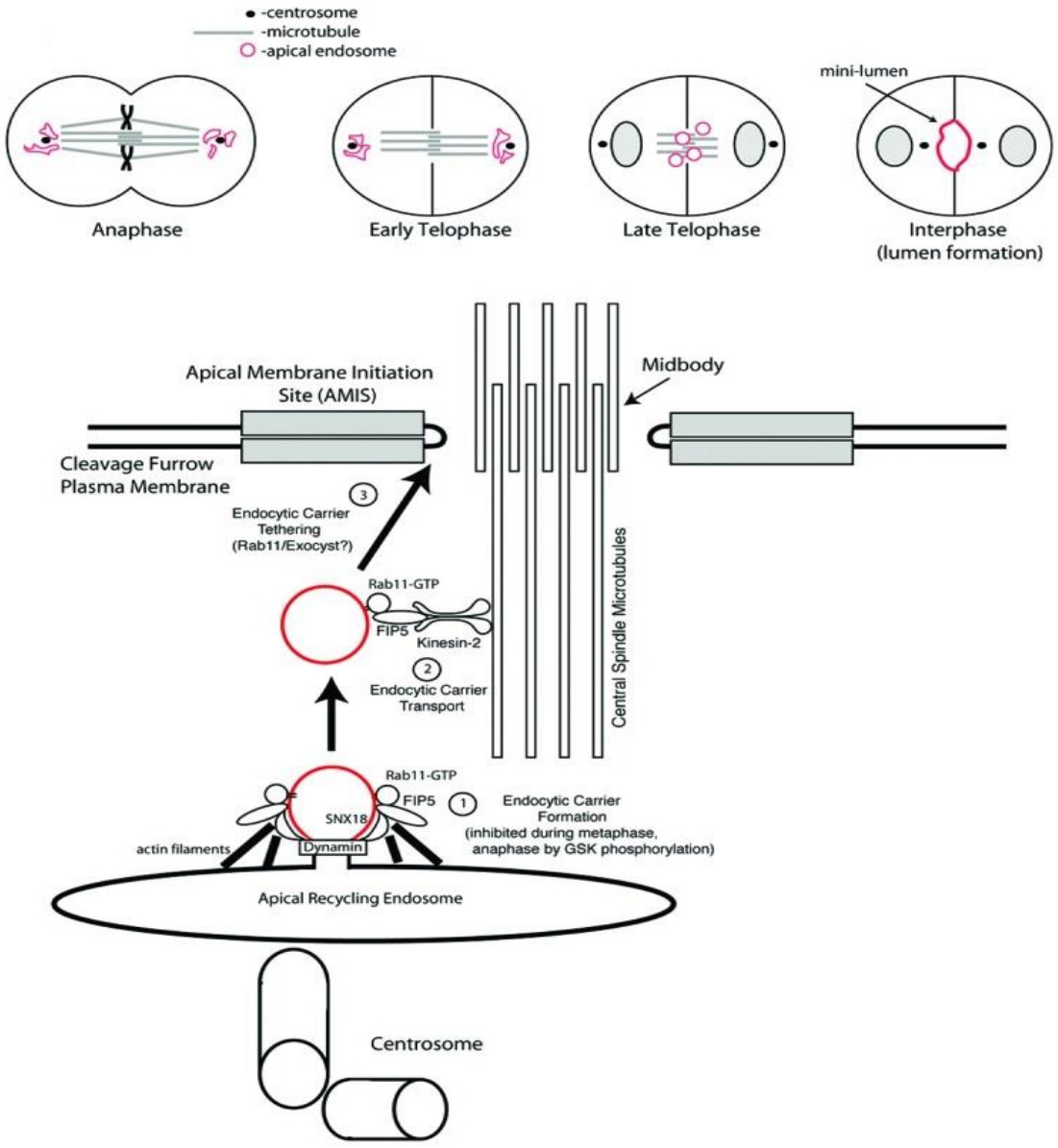

Figure 8: Models of kinesin-2-directed transport during apical lumen formation in cytokinesis. Reprinted and modified from Li et al., 2014b.

Last but not least, the kinesin-3 family plus end microtubule motor protein, KIF13A, is responsible for FYVE-CENT delivery to the midbody in late telophase, where FYVE-CENT interacts with PI(3)P-enriched endosomes (Sagona et al., 2010). Interestingly, KIF13A was also identified as a motor protein involved in the morphogenesis of Rab11 recycling endosome tubules (Delevoye et al., 2014). It is tempting to speculate that this motor protein may be also involved in the targeting of Rab11 recycling endosomes to the midbody. One way or another, KIF13A depletion is associated with cytokinetic defects (Sagona et al., 2010).

There are still many questions to be answered about the mechanism, possible interacting partners and motor proteins that may contribute to the delivery of endocytic vesicles to the midbody.

4 Abscission

Abscission is the final and irreversible step in cytokinesis, leading to the physical separation of two daughter cells.

As a matter of fact, two possible scenarios of the abscission step are discussed (reviewed in Baluska et al., 2006). First, the plugging mechanism, where the vesicles fuse with each other and with the plasma membrane at the abscission site (Schweitzer et al., 2005; Gromley et al., 2005). Second, the furrowing mechanism, where the vesicles fuse with the furrowed plasma membrane of the mother cell (Low et al., 2003; Schiel et al., 2011). Although it is likely that these two mechanisms are not mutually exclusive and both of them play their role in the cytokinesis, according to the newer studies the amount of membrane provided by these vesicles would not be sufficient for the plugging leading to the separation of the daughter cells (Schiel et al., 2011). Therefore, the furrow mechanism seems to be more accurate.

At late stage of cytokinesis, prior the abscission, secondary ingression occurs (see 4.1.) and as a result, two daughter cells are connected by a narrow intercellular bridge with specific membrane composition and cytoskeleton organization.

4.1 Secondary ingression prior the abscission

After the primary ingression, which is mediated by actomyosin ring, the diameter of the intercellular bridge is still too large for ESCRT-III complex assembly and subsequent scission (Schiel et al., 2012). Therefore, the secondary ingression, a further reduction of the intercellular bridge diameter mediated by vesicle fusion, is required. Secondary ingression is an important two-step process, which takes place during late telophase and is essential for the following abscission. At first, the thickness of the intracellular bridge is decreased from 1.5–2 μm to approximately 100-200 nm. Afterwards, this secondary ingression expands and forms a thin bridge, on either one or both sides of the midbody, and then undergoes the scission. Formation of the secondary ingression is necessary, given that even if all vesicles fused at one plane of the intercellular bridge, it would provide only roughly 25 % of the membrane required to fully bridge the intercellular bridge (Schiel et al., 2011).

Endocytic traffic is crucial for the formation of the secondary ingression. Especially, FIP3-endosomes have been implicated as important, since they are responsible for the delivery of numerous regulation proteins, such as SCAMP2/3 (secretory carrier protein 2/3) or p50RhoGap (Rho GTPase activating protein 1) (Schiel et al., 2012) and

their fusion with plasma membrane is coupled with the formation and expansion of the secondary ingression (Schiel et al., 2011).

SCAMP proteins were described as regulators of exocytotic membrane fusion in pore formation in PC12 cells, where SCAMP2 interacts with Arf6 (Liu et al., 2005). However, as it turned out, SCAMP proteins are also essential for proper cytokinesis. SCAMP3 interacts with TSG101 (tumor susceptibility gene 101), an ESCRT-I (endosomal sorting complexes required for transport I) complex component (Elia et al., 2011). Furthermore, SCAMP 2/3 additionally helps with the recruitment of CHMP4B, ESCRT-III protein, to the abscission site (Schiel et al., 2012). Therefore it may be responsible for the recruitment of ESCRT-III complex to the midbody prior the abscission (Schiel et al., 2012).

During late telophase, FIP3-endosomes fuse inside the intercellular bridge with plasma membrane, which is followed by an dramatic increase in the dynamics of plasma membrane in the area and creation of membranous waves (Schiel et al., 2011). The fusion of FIP3-endosomes is, apart from previously mentioned factors in chapter 3, also mediated by SCAMP2/3 (Schiel et al., 2012) and SNARE proteins, such as VAMP8 (vesicle-associated membrane protein 8), which has been also observed to localize with FIP3-endosomes (Schiel et al., 2011).

It should be also mentioned that actin and microtubule network remodelling, especially depolymerization, is required for the secondary ingression to take place. It was observed that microtubule buckling and severing, which leads to a local microtubule depolymerization, coincides with the site of secondary ingression iniciation, presumably by allowing an increased FIP3-endosomes fusion with the plasma membrane (Schiel et al., 2011). Schiel et al. hypothetized that microtubules may limit lateral movement of the vesicles and thus hinder the fusion with the plasma membrane (Schiel et al., 2011). Moreover, spastin-containing endosomes were also detected among vesicles transported to the midbody area. Spastin is a microtubule-severing enzyme, which associates with microtubules in the midbody forming a ring structure (Connell et al., 2009). However, according to the newer studies, spastin is involved in the efficient organization of microtubules to bundles (Schiel et al., 2011) rather than in their severing (Connell et al., 2009). Spastin seemingly clears off the tracks for FIP3-endosomes delivery to their fusion site (Schiel et al., 2012). Consistent with this theory, depletion of spastin does not interfere with the completition of cytokinesis, but it does delay the process (Schiel et al., 2011).

4.1.1 Reorganization of actin network and plasma membrane

Proper abscission of two daughter cells requires dissociation of cortical actin network. Whereas F-actin is necessary during primary ingression to stabilize the cleavage furrow, it must be disassembled prior to the secondary ingression. Several reasons have been proposed as the possible explanation why F-actin hinders the terminal steps of cytokinesis. Firstly, it blocks the vesicle fusion in the intercellular bridge region. Secondly, it interferes with ESCRT-III complex machinery. Thirdly, it causes a mechanical obstacle for the abscission (reviewed in Echard, 2012).

It seems that there are two major players, which control local actin network, FIP3-endosomes with their cargo protein p50RhoGAP (Schiel et al., 2012) and Rab35-endosomes, which deliver OCRL-1 phosphatase (Dambournet et al., 2011).

Whereas p50RhoGap inactivates Rac/Rho GTPases in the cleavage furrow and thus induces F-actin depolymerization (Schiel et al., 2012), OCRL-1 mediates the hydrolysis of $PI(4,5)P_2$, which is greatly enriched at plasma membrane in the furrow area during cytokinesis (Dambournet et al., 2011). While initial function of $PI(4,5)P_2$ is to stabilize the forming furrow after ingression, likely by serving as an interaction and adhesion platform for actomyosin ring components, its hydrolysis is a limiting step for subsequent abscission, as it leads to the dissociation of F-actin (Field et al., 2005).

Taken together, FIP3-endosomes and Rab35-endosomes fusion within the intercellular bridge leads to the decrease of the intercellular bridge diameter, creation of a region with highly curved membrane, local dissociation of actin network and delivery of interacting partners for abscission complex (Dambournet et al., 2011; Schiel et al., 2011; Schiel et al., 2012). Moreover, PI(3)P-enriched endosomes transported to the area may serve as an interaction platform for a number of regulatory proteins, including subunits of ESCRT-III machinery (Sagona et al., 2010). Namely, TTC19, which interacts with ESCRT-III interacting protein CHMP4B that is delivered to the midbody probably through the interaction with SCAMP2/3 on FIP3-endosomes (Schiel et al., 2012).

In addition, according to the Liao et al.'s study, SCAMP2 is able to interact with $PI(4,5)P_2$ in electrostatic manner (Liao et al., 2007), and may thus control the distribution of this phosphatidylinositol phosphate and subsequent actin polymerization and ESCRT-III recruitment (Sagona et al., 2010). Protein SCAMP3 has been reported to bind another ESCRT-III associated protein, TSG101 (Schiel et al., 2012). All in all, SCAMP2/3 proteins

may be involved in the regulation and positioning of the ESCRT-III complex at the midbody.

To sum it up, regulated delivery and fusion of various endosomal vesicles significantly contributes to the creation of the abscission site and recruitment of the scission complex.

However, it is noteworthy that in case of *Drosophila melanogaster* embryos, the role of Rab11 recycling endosomes containing Nuf (nuclear fallout), fly ortholog of FIP3, is to promote furrow integrity by initiation F-actin assembly during cellularization (Cao et al., 2008; Riggs et al., 2003). Moreover, in *Drosophila* embryos, there have been reported endosome-derived actin-positive vesicles that move along the microtubules and supply actin to the cleavage furrow area during later stages of cytokinesis. Interestingly, these vesicles do not seem to be crucial for actomyosin ring assembly, but rather act as actin and membrane supply in mid-to-late cytokinesis (Albertson et al., 2008).

4.2 ESCRT-III complex making the final cut

The final step of cytokinesis, the abscission, is mediated by ESCRT-III complex (**Figure 9 and 10**). The scission usually occurs at only one side of the midbody. As was mentioned earlier, targeted endosome delivery plays an important role in determining, where the abscission takes place. It was observed that ESCRT-III preferentially associates with highly curved membranes (Fyfe et al., 2011). Therefore, the secondary ingression, which ensures the reduction of the intercellular bridge diameter, is essential for the abscission to occur (Schiel et al., 2012; Schiel et al., 2011).

In early telophase, first protein which arrives at the midbody is CEP55 (centrosomal protein of 55 kDa). CEP55 binds to the centralspindlin complex and is essential for the targeting of the ESCRT complex to the intercellular bridge, where it creates a solid disc (Elia et al., 2011). Subsequently, CEP55 recruits its binding partners, TSG101 and ALIX (apoptosis-linked gene 2-interacting protein X), which are components of ESCRT-I complex. At that time, FIP3-endosomes are still accumulated in pericentrosomal area (Schiel et al., 2012). During G2/M phase, CEP55, as well as its interacting proteins ALIX and TSG101, are concentrated on centrosomes (Morita et al., 2007). TSG101 subunit, which directly interacts with CEP55, localizes to the intercellular bridge as two distinct rings adjacent to the midbody center and associate with cortical surface of the intercellular bridge. Arrival of TST101 is followed by the accumulation of ESCRT-III component, CHMP4B (Guizetti et al., 2011; Elia et al., 2011).

In contrast to previously mentioned proteins, CHMP4B is recruited to the midbody region during late telophase, when spindle microtubules are still present within the intercellular bridge. Protein CHMP4B is also distributed as two cortical rings adjacent to the midbody center. Each of the two rings is positioned next to the one of TSG101 rings. The interacting complex thus consists of CHMP4B, which binds to CEP55, ALIX and TGS101. Furthermore, CHMP4B also binds to TTC19. Motor protein KIF13A transports FYVE-CENT with bound TTC19 to the intercellular bridge, where FYVE-CENT can dock to PI(3)P and TTC19 can interact with CHMP4B (Sagona et al., 2010). After the formation of the secondary ingression and microtubule disassembly, CHMP4B and other ESCRT-III core components extend away from the midbody towards the abscission site, creating a constriction and marking the abscission site (Guizetti et al., 2011; Schiel et al., 2012). Secondary CHMP4B pool, derived from one of the rings, forms spiral-shaped 17nm-diameter filaments around the scission site (**Figure 9**) (Elia et al., 2011).

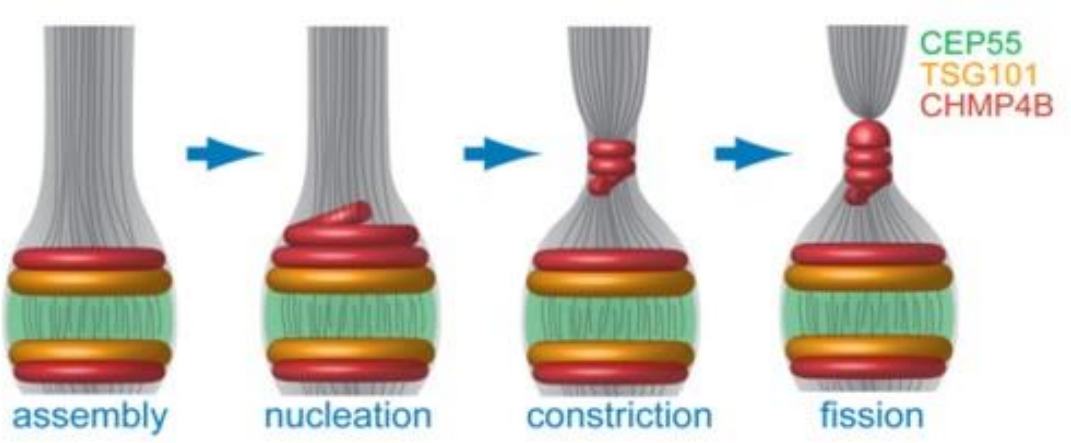

Figure 9: Model of ESCRT-mediated abscission. Reprinted from Elia et al., 2011.

The abscission occurs closely after the interaction of AAA-ATPase (ATPases associated with diverse cellular activities) protein VPS4 (vacuolar protein sorting 4), with CHMP4B (Elia et al., 2011). The mechanism of scission is as follows, VPS4 removes one ESCRT-III subunit after another, which leads to the shrinking of the filament and the final scission (Saksena et al., 2009).

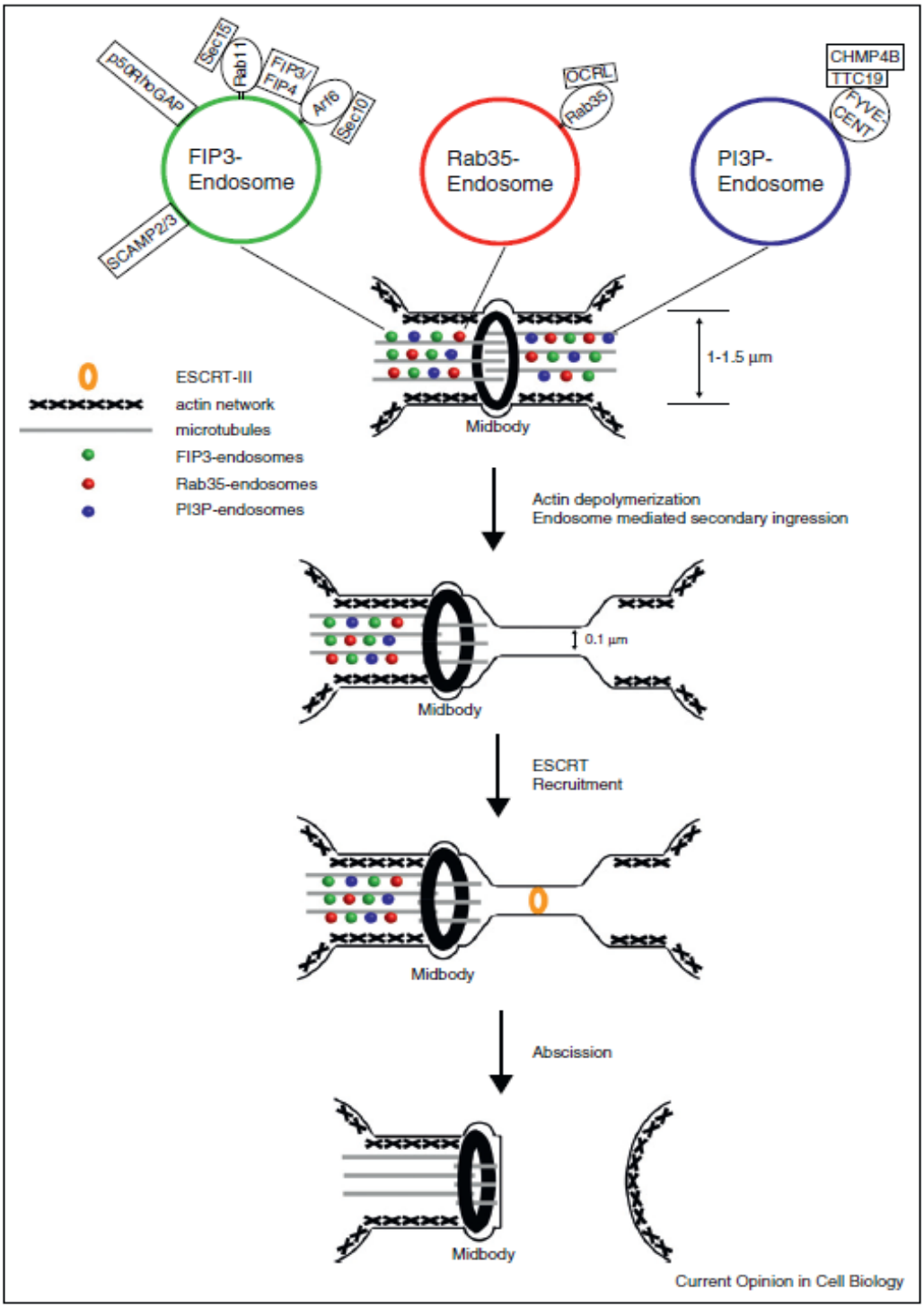

Figure 10: Model describing mechanisms proposed to regulate the abscission. Three main types of endosomes with their interacting and cargo proteins localize to the actin stabilized intercellular bridge. Endosomes mediated actin depolymerization and secondary ingression formation. ESCRT-III complex is recruited and mediates the final scission. Reprinted from Schiel and Prekeris, 2013 (review).

4.2.1 Midbody inheritance

In the last step of abscission, the midbody is severed from the rest of the daughter cell on either one side (asymmetric abscission), or on both sides (symmetric abscission).

Interestingly, it was reported that the midbody is preferentially inherited by the cell with the older centrosome (Pohl and Jentsch, 2009), which leads to the conclusion that the determination of the abscission site is not stochastic. One of the theories has suggested that autophagy and lysosomal degradation may play an important role in the severing of the midbody (Pohl and Jentsch, 2009). Indeed, PI(3)P-enriched endosomes have been observed to interact with UVRAG (UV radiation resistant associated) protein complex containing Beclin-1 (Thoresen et al., 2010), which has been confirmed to regulate autophagosome formation (Liang et al., 2006). Furthermore, FIP3-endosomes are known to preferentially accumulate and fuse on one side of the intercellular bridge, which leads to the secondary ingression and subsequent scission by ESCRT-III complex (Schiel et al., 2012). Finally, secretory vesicles are delivered from only one of prospective daughter cells and thus possibly determine the side of vesicular fusion and subsequent scission. The midbody is inherited by the cell which lies opposite to the site of vesicle delivery (see **Figure 10**) (Gromley et al., 2005) and is subsequently degraded by autophagy (Pohl and Jentsch, 2009). Alternatively, in the case of symmetric abscission, midbody can be cut off on both sides and released into extracellular space (Ettinger et al., 2011). Recently, a third possible pathway was proposed. Despite the fact that the midbody remnant is completely cleaved on both side of the midbody and freed, it tightly associates with the cell surface, move over the cell for several hours and finally is engulfed by phagocytosis and subsequently degraded in lysosomes (Crowell et al., 2014). The fate of the midbody seems to be coupled with the fate of the cell and likely determines whether the cell will proliferate or differentiate (Crowell et al., 2014; Ettinger et al., 2011).

In conclusion, the midbody inheritance can be possibly regulated by more than only one mechanism. Further research is needed to reveal components and interactions involved in this process.

5 Conclusions

Endocytic membrane transport is a key process required for the successful completion of cytokinesis. Endosomal machinery encompasses a wide range of interactions that needs to be delicately orchestrated. Endocytic transport ensures delivery of new membrane and specific factors needed for remodeling of the plasma membrane and change of local lipid composition. Moreover, it alters cytoskeleton organization and prepares a platform for subsequent scission.

In this thesis, three main types of vesicles, involved in the process of cytokinesis, were presented – Rab11/FIP3 endosomes, Rab35-endosomes and PI(3)P-enriched endosomes. FIP3-endosomes are essential for the creation of the secondary ingression and delivery of interacting partners for ESCRT-III complex. Together with Rab35-endosomes, FIP3-endosomes are responsible for the local dissociation of actin network and changes in plasma membrane curvature. PI(3)P-enriched endosomes alternate plasma membrane properties and serve as an interaction platform for subunits of ESCRT-III machinery. After the abscission site is prepared, ESCRT-III machinery facilitates the final scission and daughter cell separation.

The central role of proper cell division in development and health of any organism is undeniable. Failure in cytokinesis may lead to severe developmental defects, diseases, such as hereditary spastic paraplegia (Hanein et al., 2008) or Lowe syndrome (Dambournet et al., 2011), cancer, sterility (Dyer et al., 2007). Deeper understanding of the cytokinetic process may also provide new means of therapeutic treatment, a potential target for drugs. Moreover, better understanding of processes underlying endosomal trafficking may provide us with finding a way how excessive cell proliferation, as occurs in cancer, can be prevented. Inability to complete cytokinesis has been shown to promote aneuploidy and this has been associated with tumorigenesis (Fujiwara et al., 2005).

There is still a long way to go to fully understand the endocytic machinery leading to the abscission and creation of two daughter cells. Many questions are left unanswered and various riddles remain to be deciphered.

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