Prospects

Cytokinetic abscission: Phosphoinositides and ESCRTs direct the final cut†

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†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcb.26066]

Received 13 April 2017; Accepted 14 April 2017
Journal of Cellular Biochemistry

DOI 10.1002/jcb.26066

Abstract

Cytokinetic abscission involves the fine and regulated recruitment of membrane remodeling proteins that participate in the abscission of the intracellular bridge that connects the two dividing cells. This essential process is mediated by the concomitant activity of the endosomal sorting complex required for transport (ESCRT) and the vesicular trafficking directed to the midbody. Phosphoinositides (PtdIns), produced at plasma membrane and endosomes, act as molecular intermediates by recruiting effector proteins involved in multiple cellular processes, such as intracellular signalling, endo- and exo-cytosis and membrane remodelling events. Emerging evidences suggest that PtdIns have an active role in recruiting key elements that control the stability and the remodelling of the cytoskeleton from the furrow ingression to the abscission, at the end of cytokinesis. Accordingly, a possible concomitant and coordinated activity between PtdIns production and ESCRT machinery assembly could also exist and recent findings are pointing the attention on poorly understood ESCRT subunits potentially able to associate with PtdIns rich membranes. Although further studies are required to link PtdIns to ESCRT machinery during abscission, this might represent a promising field of study.

Introduction

Cytokinesis is the last step of cell division, leading to the physical separation of the two daughter cells. In animal cells, it involves an elaborate control of cell shape and cytoskeleton rearrangements that progressively constrict the plasma membrane until its cleavage (Agromayor and Martin-Serrano, 2013; Barr and Gruneberg, 2007; Fededa and Gerlich, 2012; Nahse et al., 2017). After the anaphase onset, chromosomes segregate in opposite directions and a plasma membrane cleavage furrow ingresses at the cell equatorial plane. This constriction requires the assembly of an actomyosin cortical network, mainly composed by RhoA and myosin II, that drives cleavage furrow contraction during the early steps of cytokinesis (Yoshida et al., 2006). The two dividing cells are then connected by a long intracellular bridge for most of the cytokinesis and the stability of this structure depends on actin cytoskeleton and Septins {Fung, 2014 #127}. The polarisation of the cytoskeleton requires signalling from the plasma membrane, and this communication involves the localised production of phosphoinositides (PtdIns), particularly phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P3) (Suetsugu et al., 2014). Among other lipids, PtdIns are essential phospholipids involved in membrane trafficking and cytoskeletal remodelling both during furrow ingression and intercellular bridge abscission. (Cauvin and Echard, 2015) Through cytokinesis, membrane trafficking plays a crucial role, by increasing the cell surface, delivering specific proteins to the midbody/intracellular bridge and by remodelling the local lipid composition of the plasma membrane (Echard, 2008). Finally abscission occurs by the sequential assembly of Endosomal Sorting Complex Required for Transport (ESCRT) components that progressively constrict plasma membrane until its cleavage (Nahse et al., 2017). Direct connections between phosphoinositide production, recruitment of cytoskeleton remodelling proteins and ESCRT machinery assembly are progressively improving our understanding of this fundamental process during cells division.

How to detect PtdIns in live cell and organisms.

PtdIns are involved in many aspects of membrane homeostasis and signalling as well as in human physiology and disease. The identification of probes specifically recognising intracellular PtdIns and able to follow their dynamic changes in levels and localization, might represent an important tool to study the role of PtdIns in several processes during the cell cycle. The first approach was the identification of purified recombinant proteins to probe PtdIns in fixed cells by both immunofluorescence and electron microscopy. These probes were able to recognize and provide quantitative data regarding PtdIns(4,5)P2 (Fujita et al., 2009; Hammond et al., 2009; James

et al., 2008; Watt et al., 2002), PtdIns(3,4)P2 (Watt et al., 2004), PtdIns(3,4,5)P3 (Lindsay et al., 2006) and PtdIns3P (Gillooly et al., 2000) levels in cells. However fixation processes and cell permeabilization can partially alter PtdIns accessibility and localization, sometimes resulting in mislabelling and unspecific staining. More importantly, this technique precludes the analysis of the dynamic distribution of the lipids in living cells (Schnell et al., 2012). To avoid these problems, alternative pioneering approaches firstly proposed to use PtsIns binding domain as biosensors by their fusion to fluorescent proteins (Stauffer et al., 1998; Varnai and Balla, 1998). These probes can be ectopically expressed in living cells or even whole organisms (Hardie et al., 2015), providing specific enrichment in membrane compartments. A fundamental requirement in this approach is the high affinity of the binding probe for a specific PtdIns species and only for that species (Balla et al., 2000). The sensor would thus report the dynamic changes of the PtdIns concentration visualizing its enrichment in specific subcellular regions. This approach can be significantly relevant to study the lipid composition during the membrane remodelling events that occur during abscission. The most commonly used fluorescent probes to detect PtdIns are summarized in Fig 2. They mainly derive from lipid binding domains taken form proteins that exhibited a high binding affinity for a certain PtdIns. One of the firstly characterized and widely used in several studies is the FYVE-domain of the Early Endosomal Antigen 1 protein (EEA1) (Burd and Emr, 1998; Gaullier et al., 1998). This probe, with a very low K_d of 45nM, specifically recognizes PtdIns3*P* and became mainly localized on early endosomal compartments. PtdIns(4,5)P2, the most abundant PtdIns at plasma membrane, can also be visualized in live cell imaging by the PH-PLCD1 probe (Garcia et al., 1995; Lemmon et al., 1995). However this probe, also partially binds to the cytosolic PtdIns(1,4,5)P3 (Hirose et al., 1999). A more specific and recently developed probe used to mark PtdIns(4,5)P2 is the ENTH/ANTH domain fused to an organic fluorophore that exhibits a blue shift upon membrane binding. This approach, by distinguishing between the green cytosolic vs blue membrane-bound fluorescence of the probe permits a quantitative imaging of PtdIns(4,5)P2 (Yoon et al., 2011). Even the less abundant PtdIns(3,4,5) and PtdIns(3,4)P2 can be revealed by PtdIns binding probes. Particularly, the PH-Akt or the PH-Btk and TAPP1-PH-CT can allow the visualization of PtdIns(3,4,5) and PtdIns(3,4)P2 respectively (Manna et al., 2007). The P4M domain from Legionella SidM was recently developed to permit the study of PtdIns4P production, both in dissociated cells (Hammond et al., 2014), and in a whole organism such as the eyes of intact living flies. Although the importance of this techniques to study PtdIns production and function in live cells and organisms, some limitations of this application exist. Particularly, these probes can partially interfere and compete to the endogenous proteins for the binding to PtdIns, resulting in of target effects and eventually cell death (Schnell et al., 2012). To avoid this problem is crucial to

monitor the expression of the PtdIns binding probes, maintaining it at sufficiently low levels during live studies. For example, as shown in *Field et al*, ten-fold overexpression of GFP-PLC-PH resulted in separation of actin from the plasma membrane and binucleation, due to competition for PtdIns(4,5)P2 binding to the plasma membrane at the cleavage furrow {Field, 2005 #69}. On the contrary, maintaining low levels of the same probe allows to visualize the PtdIns(4,5)P2 levels and localization during cell division without interfering to the abscission process {Field, 2005 #69}.

PtdIns production and function during cytokinesis

PtdIns(4,5)P2 and PtdIns4P. PtdIns(4,5)P2 is the major phosphoinositide at the plasma membrane (Figure 1) (Di Paolo and De Camilli, 2006) that is uniformly localized during early steps of mitosis (from prophase to anaphase) and then accumulates at the cleavage furrow and midbody during cytokinesis (Figure 2) in a variety of organisms ranging from mammalian to fission yeast (Emoto et al., 2005; Kouranti et al., 2006; Zhang et al., 2000). The main source of PtdIns(4,5)P2 at the furrow results from the activity of type I PI 5-kinase that phosphorylates PtdIns4P. Consistent with their role in abscission, PtdIns4P 5-kinases are enriched at the cytokinesis furrow and in late cytokinesis to midbody in yeast (Its3 kinase) (Zhang et al., 2000), in Drosophila (Skitles kinase) (Roubinet et al., 2011) and in mammalian (PtdIns4P5-kinase β) (Emoto et al., 2005) cells. In addition, PtdIns(4,5)P2 could partially result from the dephosphorylation of PtdIns(3,4,5)P3 driven by the phosphatase activity of PTEN (Janetopoulos et al., 2005). Several evidences point out to the importance of the synthesis of PtdIns(4,5)P2 during abscission. For example, the overexpression of protein modules that selectively bind PtdIns(4,5)P2 increases the number of binucleated cells by competing to endogenous proteins for the lipid binding (Field et al., 2005). Similarly, overexpression of phosphatases that hydrolyse PtdIns(4,5)P2 (Synaptojanin) or overexpression of kinase inactive form of PtdIns4P 5-kinases α and β strongly perturb cytokinesis in both mammals (Emoto et al., 2005; Field et al., 2005) and *Drosophila* (Wong et al., 2005). Of note, the cytokinetic furrowing and contraction still occur after perturbing either PtdIns(4,5)P2-producing enzymes (Emoto et al., 2005; Roubinet et al., 2011; Wong et al., 2005), or after acute removal of PtdIns(4,5)P2 by targeting 5-phosphatase domain (Roubinet et al., 2011). Indeed, PtdIns(4,5)P2 does not appear to be required for furrow ingression but mainly involved in the maintenance of post-furrowing bridge stability by providing a docking region between the plasma membrane and multiple cytoskeleton elements such as RhoA, Anillin, septins and MgcRacGAP (Brill et al., 2011). This accumulation of PtdIns(4,5)P2 at the intracellular bridge appears to be dynamic because, at

least in mammals, its levels rapidly decrease in late stages of cytokinesis and this reduction is important for terminal abscission events (Brill et al., 2011; Dambournet et al., 2011).

The hydrolysis of PtdIns(4,5)P2 to PtdIns4P can be achieved by several 5-phosphatases (Figure 1) including Oculo-cerebro-renal syndrome of Lowe (OCRL), Synaptojanin, PIPP, INPP5E, SKIP and SHIP (Pirruccello and De Camilli, 2012; Vicinanza et al., 2008). Recent findings demonstrate the requirement of at least the OCRL phosphatase during abscission (Ben El Kadhi et al., 2012; Dambournet et al., 2011; Prekeris, 2011). The impairment of the phosphatase activity of OCRL by inactivating mutation, is responsible for a rare genetic disease, the Oculo-cerebro-renal syndrome of Lowe, characterized by congenital cataracts, glaucoma, intellectual disability, dysfunction in proximal renal tubules and altered glomerular function (Lowe, 2005; Pirruccello and De Camilli, 2012). In renal cell lines from Lowe patient and in HeLa cells, depletion of OCRL results in normal furrow ingression but strongly delays or inhibits abscission as a consequence of PtdIns(4,5)P2 and actin accumulation (Dambournet et al., 2011). A phosphatases-dead version of OCRL is unable to restore the normal cytokinesis in OCRL depleted cells. PtdIns(4,5)P2 is known to stimulate actin polymerization and to inhibit the actin-severing enzyme cofilin, which can explain the increased actin levels in OCRL-depleted cells. In late cytokinesis, F-actin accumulation is likely to provide a physical barrier, thus impairing vesicle fusion at the intracellular bridge and potentially inhibiting the assembly of the ESCRT machinery and its association at the abscission site (Dambournet et al., 2011; Fremont et al., 2017; Prekeris, 2011).

The phosphatase activity of OCRL and other 5-phosphatases during abscission, results in the production of PtdIns4P at the intracellular bridge (Figure 2). Similarly, PtdIns4P can be generated by the activity of PtdIns 4-kinases (PI4Ks) (Figure 1) in mammals and *four wheel drive* (*fwd*) in *Drosophila*, a type III PI4K that uses PtdIns as a substrate (Brill et al., 2000; Polevoy et al., 2009). Although the precise function of PI4Ks during abscission is not well understood, Drosophila *fwd* was shown to be required for synthesis of PtdIns4P on Golgi membranes and for the formation of PtdIns4P-containing secretory organelles that localize to the midzone during cytokinesis (Figure 2). *Fwd* binds to and colocalizes with Rab11 on Golgi membranes, and is required for the localization of Rab11 in dividing cells. One possible function of the vesicles containing PtdIns4P might be the delivery of this PtdIns during late cytokinetic furrowing, where it could serve as a precursor for the PtdIns(4,5)P2 synthesis and thus promoting furrow stability (Polevoy et al., 2009). Intriguingly, genetic analysis indicates that Fwd contributes to cytokinesis both in a kinase-dependent and in a kinase-independent manner (Polevoy et al., 2009). This is possibly due to the fact that Drosophila Fwd and mammalian PI4Kβ have non enzymatic function and directly recruit Rab11 on Golgi membranes. Given that Rab11 can bind simultaneously to effectors and to PI4K (Burke et al., 2014)

and function downstream to Fwd in Drosophila (Polevoy et al., 2009), one key function of PI4K in abscission might be to regulate Rab11 association with membranes directed to the furrow during cytokinesis. Rab11 has been shown to regulate F-actin organization and remodelling at the intracellular bridge during cytokinesis (Cao et al., 2008; Hickson et al., 2003; Schiel et al., 2012). It would thus be important to determine whether PI4Ks and PtdIns4P are also involved in F-actin dynamics and delivery of Rab11 vesicles during cytokinesis beyond Drosophila.

PtdIns(3,4,5)P3 and PtdIns(3,4)P2. PtdIns(3,4,5)P3 is mainly produced by the kinase activity of class I PI3K and converted in PtdIns(4,5)P2 by the phosphatase activity of PTEN (Figure 1) (Martini et al., 2014). So far there is no evidence for a direct role of class I PI3K or PTEN during either cytokinesis or abscission in mammalian cells. Nonetheless, studies in *Dictostelium* indicate that during cell division, PtdIns(3,4,5)P3 accumulates in membrane ruffles at the polar cortex but not at the cleavage furrow (Figure 2) (Janetopoulos et al., 2005). The idea beyond this study is that PtdIns(3,4,5)P3 and PtdIns(4,5)P2 define the "front" and the "back" of polarized cells, meaning that the asymmetry of PtdIns(3,4,5)P3 and PtdIns(4,5)P2 might be important for a successful cytokinesis, possibly by promoting ruffling at the poles and by suppressing actin protrusion at the furrow (Hehnly and Doxsey, 2012). In line with this view, increased cytokinetic defects were observed after deletion of PTEN, PI3K1 or PI3K2 and penetrant cytokinesis failure is observed, apparently after furrow ingression, in cells lacking the three enzymes (Janetopoulos et al., 2005). Further studies are required to better understand if this process might be also relevant for cytokinesis in mammals, alternatively this may reflect different requirements for PtdIns(3,4,5)P3 in different organisms.

Class I PI3K not only convert PtdIns(4,5)P2 into PtdIns(3,4,5)P3 but also PtdIns4*P* into PtdIns(3,4)P2 (Figure 1). Similarly, Class II PI3Ks have been recently described to produce PtdIns(3,4)P2 at plasma membrane during Clathrin-mediated endocytosis During this process PtdIns(3,4)P2 has a fission activity on Clathrin coated vesicles, functioning by recruiting Sortin Nexin 9 (SNX9) and dynamin to the vesicle neck and promoting vesicle detachment (Figure 2) (Posor et al., 2013). Proteins involved in Clathrin mediated endocitosys such as, α-adaptin, CALM, epsin, eps15, endophilin II, syndapin II and the dynamin II have been associated with completion of the abscission stage of cytokinesis, by functioning in an endocytic-dependent manner. In support of this concept, overexpression of epsin(S357D), which blocks endocytosis, induces multinucleation (Smith and Chircop, 2012). An interesting point to address would be to understand if PtdIns(3,4)P2 might have a role in controlling clathrin mediated endocytosis during abscission or if it could be produced from the PtdIns4P that accumulates at the intracellular bridge, thus resulting in a new PtdIns at the abscission site.

PtdIns3P and myotubularin. Class II and Class III PI3Ks catalyse the production of PtdIns3P on early and recycling endosomal membranes as well as on lysosomes during autophagy (Figure 1) (Marat and Haucke, 2016; Nascimbeni et al., 2016). Recent works also reveal a role for PtdIns3P in abscission (Sagona et al., 2010). Using the specific probe GFP-2xFYVE, PtdIns3P-positive endosomes were observed along the intracellular bridge and finally accumulating at the midbody during late cytokinesis (Figure 2). This source of PtdIns3P derived from the catalytic activity of class III PI3K (VPS34) was observed to be enriched at the intracellular bridge but excluded from the midbody. In agreement, depletion of VPS34 resulted in formation of binucleated cells and a strong abscission delay (Sagona et al., 2010). Accessory subunits of the class III PI3K such as Beclin1, UVRAG and BIF-1 also localize at the intracellular bridge. Depletion of VPS15, UVRAG and BIF-1 modestly delays abscission, while Beclin removal leads to a more severe cytokinetic defects (Sagona et al., 2011; Thoresen et al., 2010).

In addition to PtdIns3P production, the hydrolysis of PtdIns3P is likely required for cytokinesis completion. In *Drosophila* S2 cells, depletion of the myotubularin *mtm*, the analogous of MTM1 in mammals, that hydrolyses PtdIns3P into PtdIns (Figure 1), leads to giant and binucleated cells (Ben El Kadhi et al., 2011). A recent study also reveals that, in mammalian cells, both depletion or overexpression of either myotubularin-related protein 3 (MTMR3) or myotubularin-related protein 4 (MTMR4) results in abnormal midbody morphology and cytokinesis failure. Strikingly, MTMR3 and MTMR4 do not exert their effects through lipid regulation at the midbody, but regulate abscission during early mitosis, by interacting with the mitotic kinase polo-like kinase 1, and with centrosomal protein of 55 kDa (CEP55), an important regulator of abscission (St-Denis et al., 2015).

After abscission that occurs at one side of the midbody, a "midbody remnant" (MBR) is asymmetrically distributed to one of the two daughter cells (Chen et al., 2013; Gromley et al., 2005). Several studies proposed the MBR can significantly influence cell proliferative capacities and modulate the balance between differentiation and proliferation program (Kuo et al., 2011). After abscission, MBRs are sequestered in LC3-positive membranes and degraded by lysosomal-dependent autophagy (Isakson et al., 2013; Kuo et al., 2011; Pohl and Jentsch, 2009). Accordingly, prolonged pharmacological inhibition of class III PI3K, leads to a dramatic increase of the number of MBRs in the cell population. Depletion of the class III PI3K activating subunit Beclin 1 also increases the number of MBRs (Pohl and Jentsch, 2009). This suggests that class III PI3K and PtdIns3P production have a significant role in the processes of controlling the clearance of the midbody, possibly by controlling autophagy and lysosomal-mediated MBRs degradation.

ESCRT and PtdIns3P: potential connections and future directions in cytokinetic abscission

The ESCRT complex directs membrane constriction and fission from the cytosolic surface of membranes in several cellular processes, including budding of endosomal vesicles and viruses (Christ et al., 2017). Among the four multi-subunit protein complexes (ESCRT 0-I-II-III), extensive studies reported the fundamental role of ESCRT-III in abscission (Capalbo et al., 2016; Carlton et al., 2012; Carlton and Martin-Serrano, 2007; Eikenes et al., 2015; Lafaurie-Janvore et al., 2013; Morita et al., 2007). ESCRT-III becomes enriched at the midbody, an organelle at the intercellular bridge, which acts as a platform for the recruitment and organisation of various proteins involved in both the control and execution of the abscission process. ESCRT-III localization at the intracellular bridge results from sequential recruitment of its targeting factors. This is initiated during late cytokinesis when CEP55 binds to MKPL1, a component of the motor complex centralspindlin localized to the midbody (White and Glotzer, 2012). CEP55 then mediates the accumulation of ALIX and TSG101, a component of the ESCRT-I complex, which targets ESCRT-III subunits to cortical rings at both sides of the midbody. During constriction, the CHMP4B component of the ESCRT-III complex starts to extend from one side of the midbody, growing spiral filaments that progressively reduce intracellular bridge diameter until the formation of a 17 nm structure, named secondary ingression, where abscission finally occurs (Figure 3) (Christ et al., 2017; Mierzwa and Gerlich, 2014). This model of abscission involves an element of interaction between ESCRT-III components and the membrane. ESCRT-III proteins exhibit a preference for acidic membranes in *in vitro* binding studies (Muziol et al., 2006) and have been associated with PtdIns3P or cholesterol rich membranes during multivesicular body formation and viral budding, in which analogous ESCRT-III-dependent membrane scission events occur (Lin et al., 2005; Whitley et al., 2003; Yorikawa et al., 2005).

Recently, it was observed that endosomes play a fundamental role in determining the site of abscission through the initiation of the secondary ingression and possibly by recruiting ESCRT-III (Schiel et al., 2012). Phosphatidilinositol 3-phosphate (PtdIns3P) was directly involved in CHMP4B recruitment to the midbody. This phosphoinositide mainly derived by the kinase activity of class III phosphoinositide 3-kinase (PI3KC3) on early and recycling endosomes. During abscission PtdIns3P containing vesicles transport the FYVE-CENT-TTC9-CHMP4B complex to the intracellular bridge (Figure 3) and removal of PI3KC3 correlates with cytokinesis failure and multinucleation of the dividing cells (Sagona et al., 2010). This was the first finding that establishes a functional connection between PtdIns3P production and ESCRT-III during abscission, although the existence of a direct binding between PtdIns3P and CHMP4B was never observed. The

requirement of PtdIns3P and PI3KC3 is evolutionary conserved, since TbVps34 is also required for cytokinesis completion in *Trypanosoma* (Hall et al., 2006). This is fully consistent with an essential role of endosomes during late steps of abscission that needs to be further clarified (Montagnac et al., 2008; Neto et al., 2011; Schiel et al., 2013).

Among ESCRT complex, not only ESCRT-I and ESCRT-III are involved in abscission. Recently, two independent studies reported that ESCRT-II subunits can also participate in cell division (Figure 3) (Christ et al., 2016; Goliand et al., 2014). ESCRT-II, together with ESCRT-0, is the only component of the ESCRT machinery that can directly interact with membrane phosphoinosites (Wollert et al., 2009). Particularly, the Vps27 subunit of ESCRT-0 contains a FYVE-domain able to interact with PtdIns3P but a direct role of ESCRT-0 in abscission has so far never been shown (Williams and Urbe, 2007). Conversely, the ESCRT-II subunit, VPS36, has a GLUE-domain with a structural similarity to the pleckstrin homology (PH) domain, that can associate to PtdIns (Slagsvold et al., 2005; Teo et al., 2006; Teo et al., 2004). Recent findings firstly described the localization of VPS36 to the midbody during cytokinesis (Goliand et al., 2014). Although the experiments have been conducted in protein overexpression using GFP-tagged proteins, VPS36 was involved in mediating the recruitment of the ESCRT-III subunit CHMP6 to the midbody. Previous reports (Teo et al., 2006) demonstrated that the full length VPS36 binds most efficiently to PtdIns3P-containing lipid vesicles but also significantly to PtdIns(3,4)P2 and moderately to PtdIns(4)P and PtdIns(3,5)P2. These results, together with the finding of VPS36 localization to the midbody, suggest that ESCRT-II could have a role in targeting ESCRT complex to membranes during cell division by the binding to locally produced PtdIns. Similarly to the previous study, a more recent work also reports the role of another ESCRT-II subunit, Vps22, in abscission, suggesting the existence of an alternative way to the ALIX mediated recruitment of CHMP4B to the midbody (Figure 3) (Christ et al., 2016). Although a direct link between Vps22 and PtdIns has not been described yet, it was reported that Vps22 from various species is particularly rich in basic residues (~30% in human Vps22) (Teo et al., 2004) and could be involved in membrane targeting of ESCR-III during abscission. This hypothesis that a specific PtdIns produced at the abscission zone in late cytokinesis can drive the functional assembly of the ESCRT machinery could be imagined by functioning either via the chemical nature of the PtdIns, physically interacting with an ESCRT subunit, or by changing the physical nature of the membrane, perhaps by the induction of curvature and promoting a progressive constriction {Viaud, 2015 #125}.

Conclusions and perspectives

PtdIns are involved in many cellular processes and new functions in intracellular signalling as well as in cell growth, proliferation and differentiation are rapidly emerging. This is reasonably due to the multiple roles that PtdIns play in membrane trafficking, in the local recruitment of membrane-binding proteins and in the establishment of cytoskeleton-membrane interactions. An interesting feature of PtdIns is their dynamicity. PtdIns can be rapidly synthetized by the activity of lipid kinases or hydrolysed by phosphatases. Thus, particularly relevant appears, during abscission, the control of the conversion of PtdIns(4,5)P2 into PtdIns4P or PtdIns(3,4,5)P3 and vice versa. This directly influences the stability of the intracellular bridge and the integrity of the midbody, that largely relies on the recruitment or the release of cytoskeleton-remodelling proteins from the cleavage furrow as a consequence of rapid PtdIns interconversion. New insight in the comprehension of the cytokinetic process might then derive from the identification of novel PtdIns involved in abscission and by the characterization of new PtdIns-binding proteins and their roles in cell division. Particularly, the recruitment and maintenance of the ESCRT subunits at the cleavage furrow and intracellular bridge during abscission might be strictly related to the production of PtdIns that became specifically enriched to the cellular midzone during cytokinesis. However, not only the ESCRTs can be downstream effector of PtdIns during cells division. Accordingly, in a recent quantitative mass spectrometry analysis have been identified more than 400 PtdIns-binding proteins, most of them with potentially new domains involved in PtdIns interaction (Jungmichel et al., 2014). Similarly to ALIX, that functions by bridging ESCRT-I and ESCRT-III at the midbody, further in depth analysis might define specific roles for certain of the identified proteins providing a more robust link between PtdIns and ESCRT during cytokinesis. This might largely increases the understanding of the complexity of the molecular framework beyond the abscission process.

Acknowledgements

This work was supported by grants from AIRC, Italy (to E.H.) and FIRC, Italy (to F.G).

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Figure legends

Figure 1. Schematic representation of the six PtdIns involved in cytokinesis. Red and blue arrows indicate kinases and phosphatases respectively that interconverts PtdIns into each other.

Figure 2. Schematic representation of PtdIns(4,5)P2, PtdIns4P, PtdIns(3,4,5)P3, PtdIns(3,4)P2 and PtdIns3P localization during early and late cytokinesis and list of main PtdIns-binding probes able to detect PtdIns in fixed and live cell imaging.

Figure 3. Schematic representation of the assembly of the ESCRT machinery during late cytokinesis. Green and blue background represents the ALIX and the ESCRT-II -mediated recruitment of CHMP4B to the abscission site respectively

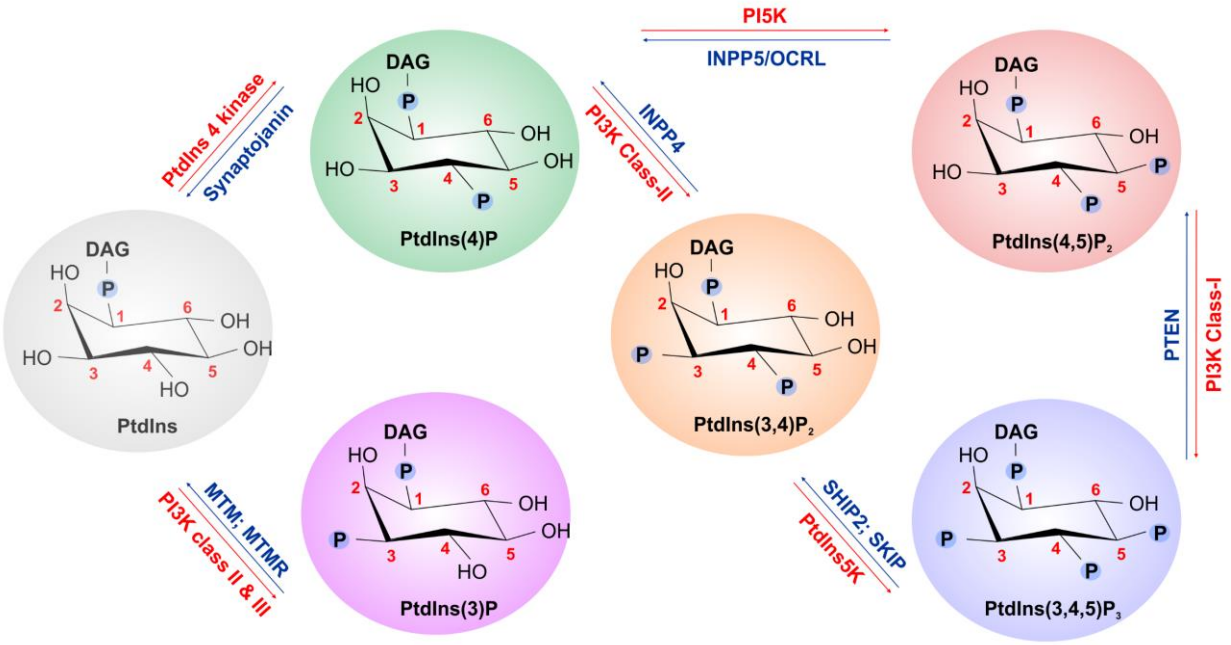


Figure 1

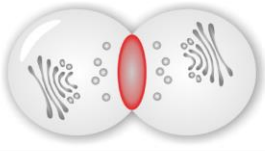
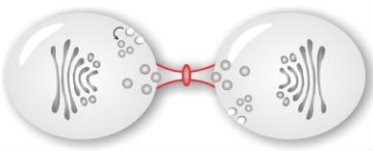
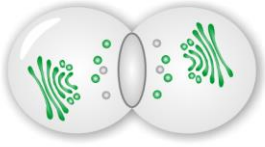
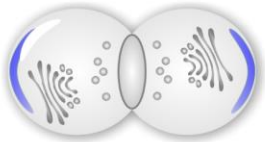
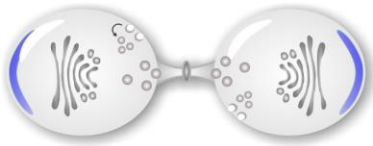
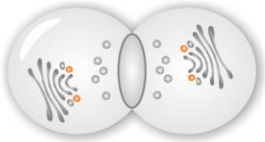
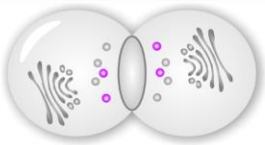
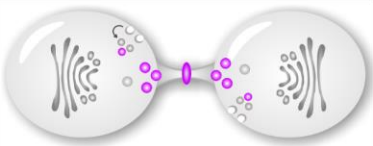
	early cytokinesis	late cytokinesis	PtdIns binding probes
PtdIns(4,5)P2			*PH-PLCD1* *PH-PLCD4* *ENTH/ANTH*
PtdIns4P			*P4M-SidM* *PH-OSBP* *PH-FAPP1*
PtdIns(3,4,5)P3	 *Dictostelium* *Mammals?*	 *Mammals?*	*PH-Akt* *PH-Btk*
PtdIns(3,4)P2			*TAPP1-PH*
PtdIns3P			*FYVE-EEA1* *FYVE-Hrsx2* *PX-p40phox*

Figure 2

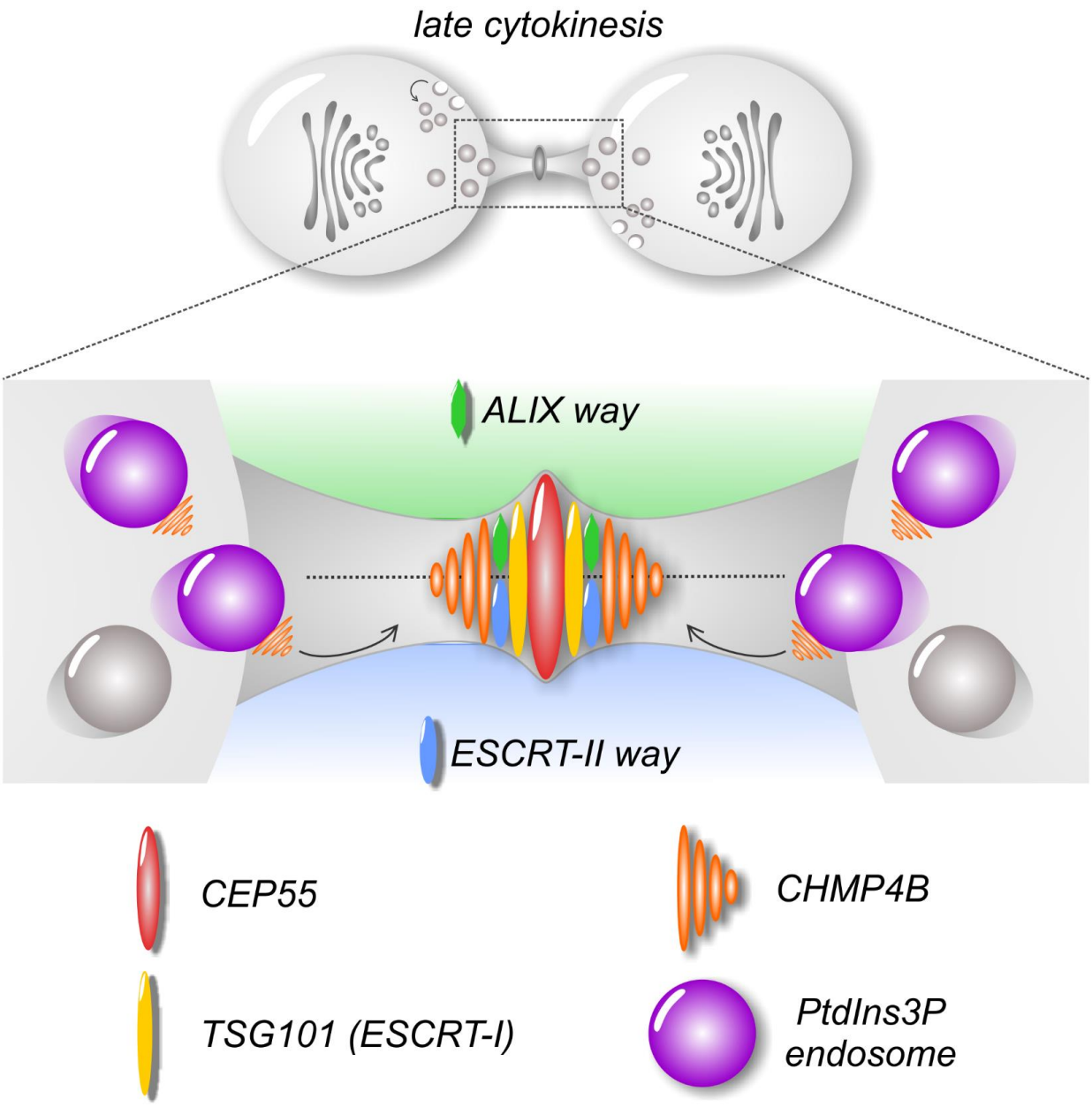


Figure 3