Membrane traffic and polarization of lipid domains during cytokinesis

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Abstract
Growing evidence indicates that membrane traffic plays a crucial role during the late post-furrowing steps of cytokinesis in animal cells. Indeed, both endocytosis and exocytosis contribute to stabilizing the intercellular bridge that connects the daughter cells and to the final abscission in diverse organisms. The need for several intracellular transport routes probably reflects the complex events that occur during the late cytokinesis steps such as local remodelling of the plasma membrane composition, removal of components required for earlier steps of cytokinesis and membrane sealing that leads to daughter cell separation. In this mini-review, I will focus on recent evidence showing that endocytic pathways, such as the Rab35-regulated recycling pathway, contribute to the establishment of a PtdIns(4,5)P2 lipid domain at the intercellular bridge which is involved in the localization of cytoskeletal elements essential for the late steps of cytokinesis. Possible cross-talk between Rab35 and other endocytic pathways involved in cytokinesis are also discussed.

Lipid and protein polarization during cytokinesis
Cytokinesis leads to the physical separation of the daughter cells and is characterized in animal cells by a complex series of cell shape changes and membrane remodelling. At mitosis entry, RhoA activation drives the increase in cortical tension and cell rounding [1]. The spherical geometry adopted in metaphase is converted in anaphase into an elongated ovoid shape due to the redistribution of activatable contractile elements at the equatorial cortex and polar relaxation [2–5]. The changes of RhoA/myosin II localization, from being uniform in metaphase to polarized at the cell equator, is likely to drive cleavage furrow contraction during early stages of cytokinesis. Daughter cells are then connected for most of cytokinesis by an intercellular bridge before the final cut or abscission. At that time, RhoA/myosin II proteins that were enriched at the cleavage furrow revert to a much more uniform localization along the entire cell cortex (Figure 1). During those late, post-furrowing steps of cytokinesis that remain largely to be understood, the stability of the intercellular bridge that was initially dependent on the actin cytoskeleton subsequently relies on other mechanisms [6]. Several non-redundant but interconnected pathways contribute to those cell shape changes and to the bridge stability, and implicate the septin cytoskeleton, anillin and ERM (ezrin/radixin/moesin) proteins [6–11]. Interestingly, anillin, septin complexes and phosphorylated (activated) ERM proteins are uniformly localized at the cell cortex in metaphase/early anaphase and concentrate to the cleavage furrow and intercellular bridge at late anaphase/telophase, as described for RhoA and myosin II [11–14] (Figure 1).

Similarly, lipid composition at the plasma membrane is also profoundly remodelled during cell division (Figure 1). For instance, an enrichment of cholesterol has previously been shown in the furrow region relative to the polar regions in dividing sea urchin embryos [15] or in the fission yeast [16]. Again, polarized accumulation of cholesterol-rich domains at the furrow is reverted to a uniform distribution in late cytokinesis [15]. These cholesterol-rich domains may be required for the activation of signalling molecules, such as the Src family tyrosine kinases, whose functions are important for the late steps of cytokinesis [15,17]. The phospholipid PE (phosphatidylethanolamine) also exhibits striking localization changes during cytokinesis: PE normally resides asymmetrically in the inner leaflet of the plasma membrane, but is exposed to the outer leaflet of the cleavage furrow/bridge during cytokinesis [18,19]. Either reduction of endogenous PE or immobilization of PE at the outer leaflet inhibits cytokinesis abscission, probably because the disassembly of myosin II, actin and ERM proteins from the intercellular bridge is prevented. Finally, local changes in localization of phosphoinositides at the cleavage furrow/intercellular bridge appears to be essential for post-furrowing steps of cytokinesis (see [20] and [21] for reviews). PtdIns(4,5)P2 is a major phosphoinositide at the plasma membrane [22] that is uniformly localized at metaphase/anaphase and accumulates at the furrow/intercellular bridge during cytokinesis in mammalian cells or in fission yeast [19,23–25] (Figure 1). Production and turnover of PtdIns(4,5)P2 are required for cytokinesis in a variety of organisms [15,19,23–26], and importantly, it has been shown that PtdIns(4,5)P2 localization at the bridge is essential for bridge stability and cytokinesis completion in mammalian cells [19,25]. Conversely, PtdIns(3,4,5)P3, which is only present at low levels and is not polarized in metaphase,
Several molecules essential for late cytokinesis events are uniformly distributed at the cell cortex or plasma membrane at metaphase/early anaphase and become concentrated in the cleavage furrow/intercellular bridge at late anaphase/telophase. The polarization of lipid domains during cytokinesis can be explained by different and non-mutually exclusive mechanisms: (i) remodelling of existing lipid domains by membrane traffic (local endocytosis and delivery towards other regions or membrane addition by directed secretion); (ii) cortical flow to the equatorial region and local immobilization; (iii) local production and consumption by lipid-modifying enzymes.

Endocytic recycling may have a pivotal role in remodelling the plasma membrane composition during cytokinesis. It has been shown that perturbing recycling from endosomes to the plasma membrane leads to post-furrowing cytokinesis defects [25,30–33]. Endosomal recycling resumes at anaphase/telophase when the surface area of the cell increases [33], and could possibly reorganize lipid domains. Indeed, there is evidence that the endocytic marker transferrin is internalized at the poles and recycled to the equatorial region [34] and that clathrin-dependent internalization is locally modulated at the cleavage furrow in a complex spatiotemporal manner [35]. In the case of PtdIns(4,5)P_2 polarization during cytokinesis, however, it is unlikely that directed transport of this lipid from polar to equatorial regions by endocytic internalization and recycling could account for its enrichment at the cleavage furrow/intercellular bridge, as the internal endosomal pool of PtdIns(4,5)P_2 is believed to be negligible [22]. Rather, it is accepted that local production of PtdIns(4,5)P_2 from PtdInsP_4P_5-kinases is key, besides a potential involvement of a cortical flow-dependent mechanism. Indeed, it has been shown that such phosphoinositides kinases are enriched at the furrow/bridge [19] and that PtdInsP_5-kinase activity is essential for cytokinesis in fission yeast or mammalian cells [19,23]. Recent data, which is further discussed below, supports a more complex picture indicating that membrane traffic is involved in the establishment of polarized PtdIns(4,5)P_2 domains during cytokinesis, probably by locally directing lipid kinases [25].

Figure 1 | Polarization of lipid domains and establishment of a cortical polarity during cytokinesis

Intracellular transport is essential for animal cytokinesis, as highlighted by genome-wide RNAi (RNA interference)-based screens [6,36,37]. In particular, membrane traffic appears to have a key contribution after furrow ingression during the terminal steps of cytokinesis for stabilizing the intercellular bridge and for its final abscission (for reviews, see [38–41] and references cited therein). Interestingly, both the secretory and the endocytic pathways have been implicated in these late phases of cytokinesis in different organisms, but the exact cargoes that are transported and the specific roles of these pathways are still unclear. Among the key regulators of membrane trafficking in eukaryotes, Rab GTPases represent a large family of proteins (> 60 in mammals) that define particular routes within the secretory and endocytic pathways [42,43]. Rab GTPases are localized at the cytoplasmic face of discrete intracellular compartments and control several transport steps such as vesicle formation, motility, docking and fusion. We recently undertook a systematic RNAi-based approach in order to identify which Rab proteins and thus transport routes are essential for cytokinesis [25]. Inactivation of each Rab gene was initially preformed in Drosophila S2 cells, which display few RabS minimizing the risk of redundant isoforms and in which RNAi experiments are easy to perform [no transfection that usually perturbs cell cycle progression and use of a single long double-stranded RNA that simultaneously generates multiple siRNAs]. Previous genome-wide screens in Drosophila cells already revealed that many novel cytokinesis genes encode proteins involved in membrane traffic, but were not exhaustive because of the long persistence of some proteins after RNAi [6,36]. Using a time course of RNAi-based inactivation and the appearance of binucleated cells as an indicator of cytokinesis failure, we identified the

Rab35 regulates an endocytic pathway involved in PtdIns(4,5)P_2 polarization during cytokinesis

In Drosophila and mammals, Rab35 has been shown to regulate endocytosis and recycling of the endocytic marker transferrin [42,43]. Here, we report that Rab35 is enriched in the intercellular bridge and for its final abscission (for reviews, see [38–41] and references cited therein). Interestingly, both the secretory and the endocytic pathways have been implicated in these late phases of cytokinesis in different organisms, but the exact cargoes that are transported and the specific roles of these pathways are still unclear. Among the key regulators of membrane trafficking in eukaryotes, Rab GTPases represent a large family of proteins (>60 in mammals) that define particular routes within the secretory and endocytic pathways [42,43]. Rab GTPases are localized at the cytoplasmic face of discrete intracellular compartments and control several transport steps such as vesicle formation, motility, docking and fusion. We recently undertook a systematic RNAi-based approach in order to identify which Rab proteins and thus transport routes are essential for cytokinesis [25]. Inactivation of each Rab gene was initially preformed in Drosophila S2 cells, which display few RabS minimizing the risk of redundant isoforms and in which RNAi experiments are easy to perform [no transfection that usually perturbs cell cycle progression and use of a single long double-stranded RNA that simultaneously generates multiple siRNAs]. Previous genome-wide screens in Drosophila cells already revealed that many novel cytokinesis genes encode proteins involved in membrane traffic, but were not exhaustive because of the long persistence of some proteins after RNAi [6,36]. Using a time course of RNAi-based inactivation and the appearance of binucleated cells as an indicator of cytokinesis failure, we identified the
Rab35 GTPase as a novel protein contributing to cytokinesis (Figure 2).

Functional inactivation of Rab35 (by RNAi or dominant-negative GDP-bound mutant overexpression) indicates that it is involved in post-furrowing steps of cytokinesis in both *Drosophila* and human cells. Further characterization in human cells showed that endogenous Rab35 is mainly found at the plasma membrane during interphase and cytokinesis, where it partially colocalizes with PtdIns(4,5)P2. Rab35 is also found on endosomal compartments and regulates a fast-recycling pathway from endosomes to the plasma membrane. Inactivation of Rab35 function leads to the accumulation of the transferrin receptors on internal vacuoles that probably result from the fusion of endosomal vesicles whose recycling is inhibited. Strikingly, PtdIns(4,5)P2 accumulation at the intercellular bridge is lost when Rab35 is functionally inactivated, which is consistent with the observed late cytokinesis defects. Instead, PtdIns(4,5)P2 abnormally accumulates at the surface of the intracellular vacuoles that trap the transferrin receptor. Thus the Rab35-regulated recycling pathway is essential for proper PtdIns(4,5)P2 polarization at the intercellular bridge during cytokinesis. Our current model is that the Rab35 endocytic pathway contributes to the targeted delivery of PtdIns4P 5-kinases to the intercellular bridge in order to establish this lipid domain locally, which implies a regulated activation of the kinase activity at the plasma membrane.

**Functions of PtdIns(4,5)P2 lipid domains in cytokinesis**

Polarized membrane domains may have multiple functions in regulating signalling pathways, targeted membrane fusion, actin remodelling and protein recruitment to specific locations. In particular, high levels of PtdIns(4,5)P2 are associated with actin cytoskeleton polymerization through the regulation of actin nucleation, severing, bundling and contractility [20]. However, when PtdIns(4,5)P2...
accumulation is perturbed, the cleavage furrow contracts and F-actin is not significantly impaired. Cleavage furrows or intercellular bridges are nevertheless unstable (possibly due to the dissociation of the actin ring from the plasma membrane) and eventually regress, causing cytokinesis failure [19,23–26]. Interestingly, we recently found that the inactivation of the unique Drosophila ERM protein that connects the plasma membrane to F-actin upon activation by PtdIns(4,5)P₂ also leads to post-furrowing cytokinesis defects [11]. Thus parallel and non-redundant pathways help stabilize intercellular bridges during cytokinesis.

Importantly, PtdIns(4,5)P₂ turnover and clearance at the intercellular bridge appears as important as its accumulation [26], and immobilization of PE at the outer leaflet of the plasma membrane leads to PtdIns(4,5)P₂ over-accumulation in the intercellular bridge, prevents actin disassembly and inhibits abscission [19]. Abscission defects are also observed after Rab35 inactivation [25] and could thus result from an additional role for the Rab35-regulated pathway in PtdIns(4,5)P₂ remodelling in the late intercellular bridge.

PtdIns(4,5)P₂ is able to bind, and possibly recruit, many proteins through interactions with PH domains and basic domains [20]. As mentioned previously, septins are cytoskeletal elements that polarize during cytokinesis to the cleavage furrow/intercellular bridge during cytokinesis and are essential for cytokinesis [13]. Interestingly, the septin SEPT2 shares a domain with SEPT4 that has been shown to bind directly to PtdIns(4,5)P₂ in a GTP-dependent manner [44], and accumulation of SEPT2 at the intercellular bridge was lost after Rab35 inactivation [25]. This is consistent with the Rab35 phenotype, as previous work showed that antibody injection against SEPT2 or RNAi inactivation also leads to bridge instability and abscission defects [9,45]. We thus propose that the Rab35 recycling pathway contributes to the late steps of cytokinesis by controlling the localization of septins at the cleavage furrow/intercellular bridge during cytokinesis, and the Rab35-regulated pathway is essential for the late steps of cytokinesis by controlling the localization of septins at the bridge.

In addition, Arf6 is one of the many identified activators of PtdIns4P kinases [52]. Thus an interesting possibility currently being investigated is that the Rab35 and the Arf6/Rab11-regulated recycling pathways have convergent and non-redundant inputs into PtdIns(4,5)P₂ lipid domain polarization essential for the late steps of cytokinesis.

**Note added in proof (received 4 April 2008)**

Work from Kunda et al. [53] found, as described in [15], that ERM proteins play an essential role in mitotic cell shape.

I thank Dr Ilektra Kouranti and Dr Gilles Hickson for critical reading of this manuscript, and Ilektra Kouranti for Figure 3. This work is supported by an ANR (Agence Nationale pour la Recherche) grant JC07-188506.

**Perspectives: crosstalk between several recycling pathways during cytokinesis**

Beside Rab35, Arf6 and Rab11 are two other small GTPases regulating endocytic recycling and are involved in late cytokinesis [30–32,46–48]. Cross-talk and co-operation between Arf6 and Rab11 GTPases during cytokinesis could occur at multiple levels, as they share common effectors such as FIP3 and components of the exocyst complex. Interestingly, FIP3 and the exocyst are also implicated in intercellular bridge stability and the abscission step of cytokinesis [35,36,49]. It has been proposed that late cytokinesis events depend on the Rab11-mediated recruitment of FIP3 on endosomes that are subsequently targeted to the intercellular bridge through interactions with Arf6 and exocyst components [31,32].

Why several recycling pathways contribute to cytokinesis is not yet known. Interestingly, it has been shown that during cytokinesis septin rings act as diffusion barriers that restrict exocyst components to the site of cleavage in budding yeast [50] (see also Figure 3). Septins could thus reinforce and define the limits of specific lipid domains. FRAP (fluorescence recovery after photobleaching) experiments in mammalian cells show that proteins localized to the inner leaflet of the plasma membrane are prevented from freely diffusing through the cleavage furrow/intercellular bridge during cytokinesis, although it is not yet known whether this compartmentalization also depends on septins [51]. Thus, the Rab35 pathway could indirectly contribute to the exocyst localization during cytokinesis by controlling the localization of septins at the bridge. In addition, Arf6 is one of the many identified activators of PtdIns4P kinases [52]. Thus an interesting possibility currently being investigated is that the Rab35 and the Arf6/Rab11-regulated recycling pathways have convergent and non-redundant inputs into PtdIns(4,5)P₂ lipid domain polarization essential for the late steps of cytokinesis.

**References**

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