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Breaking up is hard to do – membrane traffic in cytokinesis

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Summary

Throughout normal development, and in aberrant conditions such as cancer, cells divide by a process called cytokinesis. Most textbooks suggest that animal cells execute cytokinesis using an actomyosin-containing contractile ring, whereas plant cells generate a new cell wall by the assembly of a novel membrane compartment using vesicle-trafficking machinery in an apparently distinct manner. Recent studies have shown that cytokinesis in animal and plant cells may not be as distinct as these models imply – both have an absolute requirement for vesicle traffic. Moreover, some of the key molecular components of cytokinesis have been identified, many of which are proteins that function to control membrane traffic. Here, we review recent advances in this area.

Keywords

Cytokinesis; ER; Endosome; Secretory pathway; Membrane traffic

Introduction

Cytokinesis is the final stage in the cell cycle that results in the physical separation of daughter cells. After replication of the genetic material and formation of two nuclei, the mother cell divides by the formation of a furrow that constricts the cytoplasm, leaving two daughter cells connected by a thin intercellular bridge. Resolution of this bridge (abscission) results in the physical separation of the two daughter cells. Classically, cytokinesis in mammalian cells was thought to be driven by an actomyosin-containing contractile ring that assembles around the equator of the cell and serves to bisect the daughter cells upon contraction. In this model, membrane traffic was not considered to be a significant feature. By contrast, cytokinesis in plants has long been known to involve post-Golgi vesicular traffic to assemble a unique organelle (the phragmoplast), which, upon expansion by continued vesicle traffic, ultimately divides the cell (Jurgens, 2005). This model, originally proposed on the basis of elegant electron-microscopy studies, has been extensively supported by genetic studies in *Arabidopsis thaliana* that have identified members of the SNARE family (among others) and their regulatory proteins as being key players in this event (Assaad et al., 2001; Jurgens, 2005; Muller et al., 2003).

Recent years have seen an accumulation of evidence that supports a role for membrane traffic in mammalian-cell cytokinesis (Albertson et al., 2005; Finger and White, 2002; Glotzer, 2005). In large embryonic cells, such as amphibian eggs, the addition of new membrane to the plasma membrane (PM) during cytokinesis is well established (Bluemink and de Laat, 1973). Similarly, during *Drosophila melanogaster* cellularisation (the formation of the primary epithelium), the addition of new membrane through vesicle traffic to the ingressing furrow canals has long been appreciated (Albertson et al., 2005). More recent studies that use the power of model-organism genetics and proteomics have revealed a clear role for membrane traffic in mammalian-cell cytokinesis. For example, genetic studies in *Caenorhabditis elegans* and *Drosophila* have identified the small GTP-binding protein Rab11 as having a role in both cellularisation and cleavage (Pelissier et al., 2003; Riggs et al., 2003), and roles for SNARE proteins (which are involved in membrane-fusion events) (Low et al., 2003) and the large GTPase dynamin (Thompson et al., 2002) have also been established. It is also striking that proteomic analysis identified a significant number of proteins that have known roles in membrane traffic as being components of purified mammalian-cell midbodies (Skop et al., 2004) (for reviews, see Albertson et al., 2005; Barr and Greneberg, 2007; Doxsey et al., 2005; Otegui et al., 2005; Robinson and Spudich, 2000; Strickland and Burgess, 2004).

The identification of an important role for Rab11 in cytokinesis (Wilson et al., 2005), together with previous studies that implicate secretory traffic from the Golgi complex to the furrow (Hill et al., 2000), suggests that membrane traffic into the furrow and midbody during cytokinesis is of fundamental importance. These studies raise several important questions: how is vesicle traffic to the furrow or midbody controlled and is there a role for the centrosome in this process? Although the role for secretory vesicle traffic into the furrow is established, the function of traffic from the endosomal system is unclear. What happens to the vesicles upon arrival at either the furrow or midbody – do they become anchored and, if so, how is the anchoring machinery assembled? What role does the endosomal sorting complex required for transport (ESCRT) machinery have? Is there a role for other membrane stores, such as the endoplasmic reticulum (ER)? What is the role of membrane lipid in this event? Here, we will review recent studies that are relevant to such questions and attempt to integrate them into a model for membrane traffic during cytokinesis.

Centrosomes, centriolin and a potential link to secretory membrane traffic

Traffic from the trans-Golgi network (TGN) and the secretory pathway to the furrow and/or midbody of dividing cells is thought to be crucial for cytokinesis. An understanding of how secretory traffic is re-directed to specific domains of the PM, such as the furrow or midbody, during cytokinesis has remained elusive. A crucial landmark in this area was the identification of a novel protein of the maternal centriole called centriolin, which – as well as being localised to the centriole – was also found to decorate a novel structure in the midbody called the midbody ring. This structure of around 1.5 to 2 μm in diameter is enriched in γ-tubulin and colocalises with the phase-dense Flemming body within the midbody, which suggests a role in the events of late telophase (Gromley et al., 2003); it should be noted that this structure is quite distinct from the actomyosin contractile ring and is observed only in late telophase. Depletion of centriolin was found to result in an unusual

defect in cytokinesis – specifically, it was observed that depletion of centriolin resulted in cells that remain attached by long intercellular bridges that fail to cleave (Gromley et al., 2003). Such observations, together with data that implicate membrane traffic in abscission, offered a tantalising link between events at the centrosome and membrane traffic to the furrow and midbody. A physical interaction between centriolin and the exocyst machinery has offered new insight into abscission.

The exocyst was first described in yeast by Peter Novick's laboratory as a multiprotein complex (comprising Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that targets secretory vesicles to defined sites on the PM of yeast (TerBush et al., 1996). In budding yeast, the exocyst localises to the neck of the mother bud (the site of cytokinesis) (TerBush and Novick, 1995), and has been implicated in cytokinesis in yeast, flies and mammalian cells (Hsu et al., 1999). Such observations suggest that the exocyst has a role in the targeting and/or tethering of secretory vesicles in the midbody during cytokinesis.

Centriolin was found to associate with components of the exocyst complex and with SNAPIN, a SNARE-associated protein, offering a direct link to membrane trafficking pathways that operate in cytokinesis (Gromley et al., 2005). Strikingly, depletion of centriolin resulted in mis-localisation of the exocyst complex, abrogated the correct localisation of SNAPIN and SNARE proteins into the midbody and resulted in the failure of cytokinesis. This lead to the hypothesis that vesicles that are derived from the secretory pathway accumulate in the midbody through interactions with exocyst components (Gromley et al., 2005). Consistent with this, depletion of the exocyst resulted in the accumulation of intracellular vesicles in the midbody that, remarkably, appear to be derived from only one of the daughter cells. Such visually striking results led Gromley et al. (Gromley et al., 2005) to propose a model in which the anchoring of secretory vesicles by the exocyst resulted in the accumulation of vesicles within the midbody. In Fig. 1, we summarise and extend this model to include other facets of membrane traffic in cytokinesis. Briefly, upon the establishment of the midbody after furrowing (Fig. 1A), the centralspindlin complex (containing MKLP1 and MgcRacGAP, a Rho family GTPase-activating protein) arrives at the midbody ring. Centriolin becomes localised to this structure (Fig. 1B) and acts as a scaffold, recruiting SNAPIN and exocyst components (Fig. 1C). Secretory vesicles – and also endosomes (see below) – traffic through the centrosome (Fig. 1D) and accumulate in the midbody (Fig. 1E). It is thought that fusion of these vesicles, perhaps with each other (compound exocytosis) as well as with the PM, then results in abscission (Fig. 1F). The SNAREs that are involved in mammalian cytokinesis are also known to be involved in compound exocytosis in other cell types (Guo et al., 1998; Low et al., 2003), but the trigger for this final fusion event remains unclear at present.

Compound exocytosis has been demonstrated in many cell types and can be regarded as a specialised form of exocytosis in which vesicles fuse with each other as well as with the adjacent PM. Compound fusion is involved in exocytic events in pancreatic acinar cells, mast cells and neutrophils; there, it is thought that the fusion of deep-lying intracellular vesicles with the other vesicles already fused with the PM might increase the efficiency of exocytosis (Pickett and Edwardson, 2006). In certain specialised cases, compound fusion

can involve intracellular vesicles fusing with each other before their interaction with the PM (Pickett and Edwardson, 2006).

The role of compound fusion in cytokinesis remains unresolved. Although Gromley and colleagues have shown an accumulation of secretory vesicles in the midbody of dividing cells (Fig. 1E), and have suggested that a compound-fusion event is involved in abscission (Gromley et al., 2005), it is clear that this fusion event actually precedes the final abscission step. This raises the intriguing questions of what the role of this 'fusion before abscission' actually is, how it is triggered and how it is controlled (Fig. 1E). It is possible that compound fusion acts to engineer an endosomal 'platform' for the assembly of the fusion machinery, or in some way 'primes' intracellular membranes for subsequent fusion by facilitating actin remodelling. Regardless, these elegant and provocative studies offer unique insight into the integration of vesicle trafficking from the secretory pathway – and indeed from recycling endosomes (see below) – into the midbody, and suggest a link between the centrosome and midbody in this crucial event. Moreover, as discussed elsewhere (Baluska et al., 2006), such trafficking events offer striking parallels with the types of membrane traffic that underpin plant cytokinesis.

The role of recycling endosomes during cytokinesis

In addition to TGN, recycling endosomes have recently emerged as key players in mediating cleavage-furrow ingression and abscission during cytokinesis (Baluska et al., 2006; Fielding et al., 2005; Wilson et al., 2005). A number of reports have demonstrated the pronounced changes that occur in endocytic recycling during mitosis (Boucrot and Kirchhausen, 2007; Pypaert et al., 1991; Raucher and Sheetz, 1999; Sager et al., 1984) and which appear to be mediated by the effects of mitotic kinases (Rosse et al., 2003; Woodman et al., 1993). It has been reported that clathrin-dependent endocytosis and fluid-phase uptake also decrease dramatically during metaphase and anaphase (e.g. Schweitzer et al., 2005). This is also accompanied by a decrease in protein- and membranerecycling back to the PM, resulting in the accumulation of endocytic organelles as well as a decrease in PM surface area (Boucrot and Kirchhausen, 2007). By contrast, initiation of the furrow-formation and -ingression during telophase stimulates a rapid increase in membrane and protein recycling back to the PM, in particular, in the area of the cleavage furrow (Boucrot and Kirchhausen, 2007). Interestingly, these dynamic changes in the rates of endocytic recycling and the surface area of the PM seem to be required for the successful completion of cytokinesis (Boucrot and Kirchhausen, 2007). Similarly, inhibition of dynamin, a protein that is involved in clathrin-coat-dependent endocytosis, has been shown to inhibit ingression of the cleavage furrow (Konopka et al., 2006; Thompson et al., 2002). Furthermore, α-adaptin, a protein involved in receptor-mediated endocytosis, is localised near the cleavage furrow during cellularisation of the *Drosophila* embryo (Lecuit, 2004). Finally, VAMP8, a known endocytic SNARE, was also shown to be present at the cleavage furrow and is required for mitotic cell division (Low et al., 2003). So, how might endocytic traffic be regulated during cytokinesis?

Rab proteins regulate endocytic membrane trafficking in cytokinesis

Rab proteins are small monomeric GTPases that have a key role in regulating endocytic membrane traffic. At least two endocytic Rabs, Rab35 and Rab11, have been implicated in

mediating cytokinesis (Fig. 1D). Rab35 has been suggested to regulate the targeting of septins as well as phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] to the furrow (Kouranti et al., 2006). However, the mechanism of Rab35 function remains unclear, and effectors of this protein have yet to be identified. Rab11, by contrast, has a well-established role in regulating protein and membrane transport through recycling endosomes (Chen et al., 1998; Ullrich et al., 1996). Several reports have implicated the Rab11 GTPase in both furrow ingression and abscission steps of cytokinesis. In *C. elegans* embryos, depletion of Rab11 by small interfering RNA (siRNA) causes furrow regression (Skop et al., 2001). Similarly, it has been demonstrated that Rab11 has an important role in meiotic cytokinesis in *Drosophila* (Giansanti et al., 2007), and that Rab11-containing vesicles are required for furrow ingression during *Drosophila* cellularisation (Pelissier et al., 2003; Riggs et al., 2003). Finally, in mammalian cells, Rab11 has been shown to mediate targeting of the recycling endosome to the furrow during late telophase, thus regulating the abscission step of cytokinesis (Fielding et al., 2005; Wilson et al., 2005) (Fig. 1D–F). These studies have provided a clear link between membrane traffic, and both furrowing and abscission, and suggest that there is an inextricable connection between membrane traffic, and the formation of the actomyosin contractile ring and furrowing (Giansanti et al., 2007).

The cycling between GDP- and GTP-bound forms of Rab proteins regulates the recruitment of various effector proteins to cellular membranes (Zerial and Stenmark, 1993). These effector proteins regulate the targeting and fusion of endocytic organelles. In recent years, several Rab11 effector proteins have been identified and include the Rab11-family-interacting proteins (Rab11-FIPs; also known as FIPs). FIPs are divided into two main classes (I and II) on the basis of sequence homology, but all share a highly conserved 20 amino acid motif at the C-terminal region of the protein, known as the Rab11-binding domain (RBD) (Prekeris et al., 2001). Although class I FIPs have been implicated in regulating the recycling of PM receptors, class II FIPs (FIP3 and FIP4) appear to have more specialised functions. FIP3 and FIP4 were shown to mediate Rab11-dependent targeting of recycling endosomes to the cleavage furrow during cytokinesis (Fielding et al., 2005; Wilson et al., 2005). Furthermore, depletion of FIP3 using siRNA inhibits abscission (Fielding et al., 2005; Wilson et al., 2005). Similarly, nuclear fallout protein (Nuf), a *Drosophila* homologue of class II FIPs, has been shown to regulate furrow ingression during cellularisation (Hickson et al., 2003; Riggs et al., 2003). Thus, it was proposed that the FIP3-Rab11 protein complex has a key role in the delivery and targeting of recycling endosomes to the forming cleavage furrow, the process that is required for the successful completion of cytokinesis (Fig. 1) (Fielding et al., 2005; Wilson et al., 2005).

Although the requirement for the FIP3-Rab11 protein complex in targeting recycling endosomes to the cleavage furrow is established, the mechanisms that mediate this targeting remain to be fully understood. Interestingly, in addition to Rab11, FIP3 can also interact with the Arf6 GTPase (Fielding et al., 2005; Hickson et al., 2003). Rab11 and Arf6 bind to different FIP3 motifs, because FIP3, Rab11 and Arf6 can be a part of the same protein complex (Fielding et al., 2005). As Arf6 also accumulates at the furrow during cytokinesis, it has been proposed that Arf6 acts as a tethering molecule that mediates the recruitment of FIP3-Rab11 endosomes to the midbody (Fielding et al., 2005). However, recent data

demonstrate that Arf6 binds to FIP3-Rab11 endosomes before they arrive at the midbody and, thus, is unlikely to function as an endosome-tethering factor (Schonteich et al., 2007). This is consistent with recent work on *Drosophila* spermatocyte cytokinesis (Dyer et al., 2007). This study showed that cytokinesis requires Arf6 in the male germ line. Interestingly, Arf6 was observed to localise to recycling endosomes associated with the central spindle, but was not required for targeting vesicles to this location. Rather, Arf6 appeared to function in the rapid membrane addition that is needed during furrowing, thereby acting to coordinate membrane recycling with ingression of the cleavage furrow (Dyer et al., 2007). Consistent with this, Arf6 depletion using siRNA has a moderate effect on the progression of cytokinesis, but appears to modulate the stability of the furrow in HeLa cells (Yu et al., 2006).

The other potential endosome-tethering factor is the exocyst complex (see above and Fig. 1). Interestingly, the exocyst has been shown to bind to both Arf6 (through Sec10) and Rab11 (through Sec15) (Prigent et al., 2003; Wu et al., 2005). Thus, the exocyst might mediate the targeting of the FIP3-Rab11 endosomes to the cleavage furrow via two distinct pathways: one that involves Rab11 and another that involves Arf6 (Fig. 1D). This is supported by data that show that the knockdown of both Rab11 and Arf6 synergistically inhibits cytokinesis (Yu et al., 2006). In addition, recent data suggest that FIP3 also interacts with MgcRacGAP/Cyk4 (Cyk4 is the *C. elegans* homologue), a subunit of the centralspindlin complex that is localised to the midbody during cytokinesis (G. C. Simon, E. Schonteich, C. C. Wu, A. Piekny, D. Ekiert, X. Yu, G.W.G., M. Glotzer and R.P., unpublished data).

Although the contributions of all of these interactions in targeting the recycling endosomes to the cleavage furrow remain to be fully understood, they probably work as a ‘belt and braces’ to ensure the specificity of membrane targeting (Fig. 1D,E). Thus, the simultaneous interactions of the FIP3-Rab11 complex with Arf6, the exocyst and the centralspindlin complexes might ensure the spatial and temporal coordination of endosome recruitment to the midbody during cytokinesis in the face of fluctuating cellular concentrations of these proteins. However, once at the midbody, what is the function of the endosomes during cytokinesis?

Endosome function in cytokinesis

Initially, endosomes were thought to be involved in mediating the delivery of new PM to accommodate the ingressing cleavage furrow (Albertson et al., 2005). Recent work in sea urchins supports this idea by suggesting that membrane in the cleavage furrow is not derived from pre-existing PM, but rather from internal stores (Shuster and Burgess, 2002). Alternatively, endocytic membrane transport might have a role in delivering various lipids and/or proteins to the cleavage furrow, rather than to new PM. The PM in the furrow has a distinct lipid and protein composition (Ng et al., 2005), which might underscore its ability to be deformed during ingression, as well as provide the necessary signals for regulating the progression of cytokinesis. Such observations provide a compelling link between the formation of the actomyosin contractile ring and the constriction with endocytic membrane traffic (Giansanti et al., 2007). Finally, endocytic transport has also been implicated in mediating abscission, the last step of cytokinesis (Fielding et al., 2005; Baluska et al., 2006).

The exact machinery of the abscission step remains unclear, but it has been suggested that the accumulation and simultaneous fusion of multiple organelles on one side of the midbody is a key step in the separation of the two daughter cells (Gromley et al., 2005). This compound fusion is probably also accompanied by the localised disassembly of the actomyosin contractile ring, but could also be regulated by endosomal cargo. Indeed, the *Drosophila* FIP3 homologue Nuf has been shown to regulate actin polymerisation in the cleavage furrow (Rothwell et al., 1998). Arf6 and the exocyst complex are also known to regulate actin dynamics through the regulation of Rac and Rho GTPases (Prigent et al., 2003; Roumanie et al., 2005; Zhang et al., 2001). Based upon such observations, it is tempting to suggest that membrane traffic into the furrow and midbody serve to deliver key membrane-remodelling factors or abscission machinery to the correct spatial location during mitosis (Fig. 1). The identification of such factors awaits further research, but recent studies have suggested that ESCRT proteins are one such example.

ESCRT proteins and cytokinesis

Recent data have opened up a new avenue of research that is aimed at resolving the molecular basis of abscission. The ESCRT pathway is required for the terminal membrane-fission events that are involved in a range of biological processes, not least the formation of endosomal intra-luminal vesicles – which results in the formation of multivesicluar bodies (MVBs) – and HIV budding (Slagsvold et al., 2006). Current models suggest that there are four ESCRT complexes that act in a sequential manner in MVB sorting, but whether all the complexes are involved in all ESCRT-dependent functions is unclear (Slagsvold et al., 2006). In the case of virus budding, for example, retroviral late-budding domains (L-domains) drive virus release from the infected cell by facilitating a membrane-fission event that separates the virion from the PM (Bieniasz, 2006). Certain L-domains mediate this process by recruiting Tsg101 (a subunit of the ESCRT-I-protein complex), whereas others recruit an ESCRT-interacting protein called apoptosis-linked gene-2-interacting protein X (Alix) (Garrus et al., 2001; von Schwedler et al., 2003). It is thought that ESCRT I and/or Alix then recruit the core ESCRT III machinery to facilitate membrane fission.

A striking link between these topologically similar events and cytokinesis was provided by studies that showed that Tsg101 and Alix were recruited to the midbody by direct interaction with the centrosomal protein Cep55 (Carlton and Martin-Serrano, 2007). Interestingly, Cep55 has been shown to be essential for abscission (Fabbro et al., 2005), and subsequent studies have shown that both Tsg101 and Alix are also required for this process. Careful analysis has shown that direct interaction of Cep55 and either Tsg101 or Alix is required for cytokinesis, and that the localisation of Tsg101 and Alix to the Flemming body is dependent on Cep55 (Carlton and Martin-Serrano, 2007). Morita et al. suggested the possibility that Tsg101 and Alix assemble on centrosomes and are then recruited to the midbodies of dividing cells through direct interactions with Cep55 ‘hinge’ region (Morita et al., 2007). Their study also revealed that Alix and Tsg101 interact with an array of proteins that are known to be involved in cytokinesis, including ROCK1 and IQGAP, and, moreover provided evidence for a role for the ESCRT complexes by observing that mutants of Vps4, an AAA-type ATPase that is required for the recycling of the ESCRT complexes, inhibited abscission (Morita et al., 2007). Studies in *Arabidopsis* have also implicated ESCRT

components in plant-cell cytokinesis (Spitzer et al., 2006). The relative importance of Tsg101 and Alix is difficult to ascertain because different cell types appear to exhibit differential sensitivity to the depletion of these proteins (Carlton and Martin-Serrano, 2007; Morita et al., 2007). This could reflect the possibility that multiple pathways for abscission exist in cells.

So, what role might the ESCRT complex have in cytokinesis? Current models suggest that the ESCRT machinery is recruited to defined sites for different biological events (MVB formation, viral release and, now, cytokinesis) by different adaptor proteins (Slagsvold et al., 2006). Cep55 clearly fulfils this role during cytokinesis, which again hints at a potential link between centrosomal events and abscission. Cep55 is concentrated at centrosomes until prophase, following which it undergoes considerable spatial rearrangement during cytokinesis, ending up in the spindle midzone and, ultimately, in the Flemming body, (Morita et al., 2007). This seems to be controlled through a series of phosphorylation-dependant steps. Dimeric Cep55 appears to form a ring within the Flemming body that then acts to nucleate the recruitment of ESCRT complexes. Interestingly, Cep55 also influences the localisation to the midbody of a range of other late-acting cytokinesis proteins, including Aurora B, Plk1 and ECT2 (Morita et al., 2007). Thus, it is tempting to suggest that the ESCRT complex, recruited by Cep55, has a crucial role in controlling membrane-remodelling events in the midbody.

What might this role be? It is important to emphasise that the three systems in which the ESCRT machinery has been implicated all require the resolution of a thin membrane bridge by a membrane-fission event. This has led Morita and colleagues to propose that the ESCRT pathway is a ‘modular membrane-fission machine’, which is recruited to distinct intracellular compartments by a range of different adaptor proteins (Morita et al., 2007). This idea certainly has intellectual merit. However, it is important to note that whether the ESCRT complex is directly involved in abscission or has an indirect role (perhaps by controlling vesicle traffic into the furrow or protein degradation by selective retrieval of cargo from the midbody vesicles) remains unresolved. Recent studies in *C. elegans* have identified a requirement for Alix in RME-1-mediated endosomal traffic (Shi et al., 2007), which is consistent with an indirect role for the ESCRT complex in abscission. RME-1 is an EH-domain protein that was identified in a genetic screen for genes that regulate endocytosis in *C. elegans*. Interestingly, RME-1 is involved in the exit of membrane proteins from recycling endosomes, perhaps hinting at an alternative role in cytokinesis in which Alix and the ESCRT machinery function to remove inhibitory signals or cargo from endosomes in the furrow. Such issues, together with the identification of the potential role for ubiquitinylation in abscission, offer exciting areas of investigation for the years ahead.

The role of the ER in cytokinesis

So far, most studies on membrane traffic in cytokinesis have focused on secretory and endocytic pathways. However, the ER represents a potentially large membrane depot within cells that could contribute to cytokinesis. This is supported by the identification of both ERresident proteins (such as Sec13, BiP, endoplasmin and calnexin) and proteins that are involved in ER-Golgi traffic (e.g. Sec 23, Sec31 and COPI) in purified midbody proteomic

screens (Skop et al., 2004). Recent studies have also hinted at a role for the ER in cytokinesis. Studies that used GFP-tagged ER-resident proteins in plant cells have revealed that the ER undergoes pronounced reorganisation during mitosis and cytokinesis (Gupton et al., 2006). Tubules of ER were observed to accumulate around the chromosomes after nuclear-envelope breakdown, with the tubules aligned parallel to the microtubules of the mitotic spindle. In cytokinesis, the phragmoplast becomes enriched in ER, and this redistribution appears to be microtubule dependent (Gupton et al., 2006). Similarly, in mammalian cells, the ER is aligned around the contours of the spindle body and oriented along microtubules (McCullough and Lucocq, 2005) (Fig. 1E). So why should this reorganisation take place? One possibility, supported by evidence from cellularisation in *Drosophila*, is that the ER acts as a Ca^{2+} store. Cell-cycle Ca^{2+} signals are provided by the release of Ca^{2+} from the ER. Studies in flies have shown that mitotic Ca^{2+} signals are confined to so-called mitotic microdomains, which are, in turn, enclosed by the ER (Parry et al., 2005). The ER tubules that are arranged around the spindle might therefore contribute a signalling capacity to the cytokinesis machinery.

However, there might be additional roles for the ER in cytokinsesis. During cytokinesis in budding yeast, chitin synthase S (Chs2p) localises to the mother-daughter neck in telophase, where it functions in localisation of the septum. In an elegant study, Zhang and colleagues have shown that Chs2p is retained in the ER during metaphase (Zhang et al., 2006). Upon mitotic exit, there is COPII-dependent transport of Chs2p out of the ER, which is triggered by a decrease in mitotic-kinase activity. Hence, by extension, the arrangement of the ER along microtubules in the spindle might serve to localise proteins that act at defined stages of cytokinesis in an inactive ‘reservoir’ that can be selectively mobilised in response to the appropriate signals. Such a novel role for the ER awaits further experimental support, but this and other data, such as studies that implicate the unfolded protein response in regulating cytokinesis (Bicknell et al., 2007), suggest that the ER is far from a passive contributor to this complex process.

The role of phospholipids in regulating cytokinesis

Phosphatidylethanolamine in cytokinesis

In addition to proteins, phospholipids have also recently emerged as key players in regulating various cellular processes. In most biological membranes phospholipids are distributed asymmetrically, thus forming distinct PM sub-domains that are enriched in specific sub-species of phospholipids and are known to mediate multiple signalling pathways. For instance, in eukaryotic cells phosphatidylserine (PS) and phospatidylethanolamine (PE) are present predominately in the inner leaflet of the PM, whereas phosphatidylcholine and sphingomyelin reside in the outer leaflet (Zachowski, 1993). Interestingly, during the final stage of cytokinesis, PE accumulates at the outer leaflet of the PM in the cleavage furrow (Emoto et al., 2005; Emoto et al., 1996) (Fig. 1B), and CHO cell lines deficient in PE biosynthesis display cytokinesis defects (Emoto et al., 1990). The modulation of PE at the cell surface of the cleavage furrow is required for cytokinesis, because inhibition of the retro-translocation of PE to the inner leaflet by a PE-binding peptide blocks abscission (Emoto et al., 2005; Emoto et al., 1996).

The trans-bilayer distribution of phospholipids is not a static event and represents the equilibrium between the inward and outward transport of phospholipids. However, the movement of the lipids between the outer and inner leaflets is a very slow process, so additional factors are expected to mediate the transport of PE to the cell surface during the transient changes of PE distribution in dividing cells. A family of type 4 P-type ATPases, also known as flippases, has been implicated in mediating the transport of phospholipids between the inner and outer leaflets of the PM (Devaux, 1992). Thus, the localised activity of flippases might mediate the transient enrichment of PE at the cell surface in the cleavage furrow. Indeed, the yeast flippases Dnf1p and Dnf2p were shown to be required for budding in *Saccharomyces cerevisiae* (Saito et al., 2007).

Gangliosides and phosphoinositides in cytokinesis

In addition to aminophospholipids, it has been suggested that gangliosides and phosphoinositides (PIs) also have important roles in cytokinesis (Logan and Mandato, 2006; Ng et al., 2005) (Fig. 1B). For instance, the ganglioside GM1 and cholesterol were shown to accumulate at the cleavage furrow of dividing *Xenopus laevis* embryos, where they might regulate Src and PLCγ-dependent signalling (Ng et al., 2005). Recent evidence has also shown that PtdIns(4,5)P_2 accumulates at the cleavage furrow in mammalian and *Schizosaccharomyces pombe* cells (Desautels et al., 2001; Emoto et al., 2005; Field et al., 2005), whereas phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] is enriched at the poles of the dividing cells (Janetpopoulos et al., 2005) (Fig. 1B). Such a role of PtdIns(4,5)P_2 in cytokinesis has also been proposed for *Dictyostelium discoideum* (Stock et al., 1999; Weber et al., 1999).

This asymmetry of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 distribution resembles the polarised PI distribution in moving mammalian cells, as PtdIns(3,4,5)P_3 is present at the leading edge, whereas PtdIns(4,5)P_2 is enriched at the trailing end of the cell. Thus, it has been proposed that the same mechanism generates the asymmetry in PI distribution during cell movement and cell division (Janetpopoulos et al., 2005; Logan and Mandato, 2006). Consistent with this, the two enzymes that control the distribution of PtdIns(3,4,5)P_3 during cell movement, phosphatase and tensin homologue on chromosome 10 [PTEN; a phosphatase which dephosphorylates PtdIns(3,4,5)P_3 and is mutated in multiple advanced cancers], and phosphoinositide 3-kinase (PI3K), were shown to be enriched in the furrow and the poles, respectively, of the dividing cells (Janetpopoulos et al., 2005). Cells that lack PTEN and PI3K failed to complete cytokinesis and exhibited a multinuclear phenotype (Janetpopoulos et al., 2005). In addition, *Dictyostelium* cells that were treated with a PI3K inhibitor also displayed defects in cytokinesis. Similarly, phosphatidylinositol (4)-phosphate 5-kinase type-1 beta (PI5Kβ), the enzyme required for PtdIns(4,5)P_2 synthesis, accumulates at the furrow (Emoto et al., 2005; Field et al., 2005), and the inhibition of PI5Kβ by the overexpression of a kinase-inactive PI5Kβ mutant was shown to inhibit cytokinesis, a phenotype similar to the overexpression of the PtdIns(4,5)P_2 phosphatase synaptojanin (Wong et al., 2005). It is noteworthy that PI5Kβ is activated by Arf6, the GTPase that binds FIP3 and is delivered to the cleavage furrow via Rab11-containing endosomes (Schonteich et al., 2007). Thus, it is possible that one function of endosomes during cytokinesis is to regulate the generation of PtdIns(4,5)P_2 in the furrow (Kouranti et al., 2006) (Fig. 1D,E).

Phospholipid function during cytokinesis

The inhibition of PE redistribution was shown to inhibit the disassembly of the actomyosin contractile ring, a step that is required for the completion of cytokinesis (Emoto et al., 1996). Thus, it has been suggested that PE induces the disassembly of actin filaments (Emoto and Umeda, 2000). Although the exact mechanisms that mediate the crosstalk between PE and actin remain unclear, recent data suggest that PE and PS regulate the actin cytoskeleton in budding yeast by activating Rga1p and Rga2p, the proteins known to activate Cdc42p-GTPase (Saito et al., 2007). PtdIns(4,5)P_2 binds and regulates a variety of proteins that regulate actin polymerisation, namely WASP, profilin and cofilin (Logan and Mandato, 2006), so PtdIns(4,5)P_2 might also have a role in regulating the stability of the actomyosin contractile ring as well as its attachment to the PM.

Recent evidence suggests that, in addition to regulating the actin cytoskeleton, PtdIns(4,5)P_2 also serves as a source of inositol (1,4,5)-triphosphate [Ins(1,4,5)P_3] during cytokinesis (Wong et al., 2005). Although the role of Ca^{2+} during cell division remains to be fully understood, work in *Xenopus* and zebrafish embryos suggest that Ca^{2+} regulates the initiation and progression of the cleavage furrow. Consistent with this, the cell-permeable Ca^{2+} chelator BAPTA-AM causes failure of cytokinesis in *Drosophila* and mammalian cells (Wong et al., 2005) (R.P., unpublished data). Ca^{2+} is probably released from the ER, as the Ins(1,4,5)P_3-receptor-agonist 2-APB as well as the PLC inhibitor U73122 cause the regression of the cleavage furrow and formation of multinucleated cells (Wong et al., 2005). On the basis of all this evidence, the generation of Ins(1,4,5)P_3 from PtdIns(4,5)P_2 and the subsequent release of Ca^{2+} from the ER probably has an important role in cell division.

Conclusion and perspectives

It is clear that the past few years have seen a dramatic increase in our understanding of cytokinesis, and in the role of membrane traffic in this complex and beautifully orchestrated event – and we have attempted to provide a working model of some of these studies here (Fig. 1). The challenge ahead will be to extend this model to pinpoint the control mechanisms that integrate signals from specific cell-cycle events into membrane traffic both in the furrow and deep within the midbody in space and time. It should also be noted that, at present, it is not always possible to define whether some of the events alluded to the above (e.g. localisation and function of the trafficking machinery and lipid remodelling) occur using the same spatial and temporal coordinates. The advent of new tools, such as specific mitotic kinase inhibitors and the ability to knock down selective genes coupled to reverse genetics, will no doubt soon reveal more of Mother Nature’s secrets to an equitable divorce.

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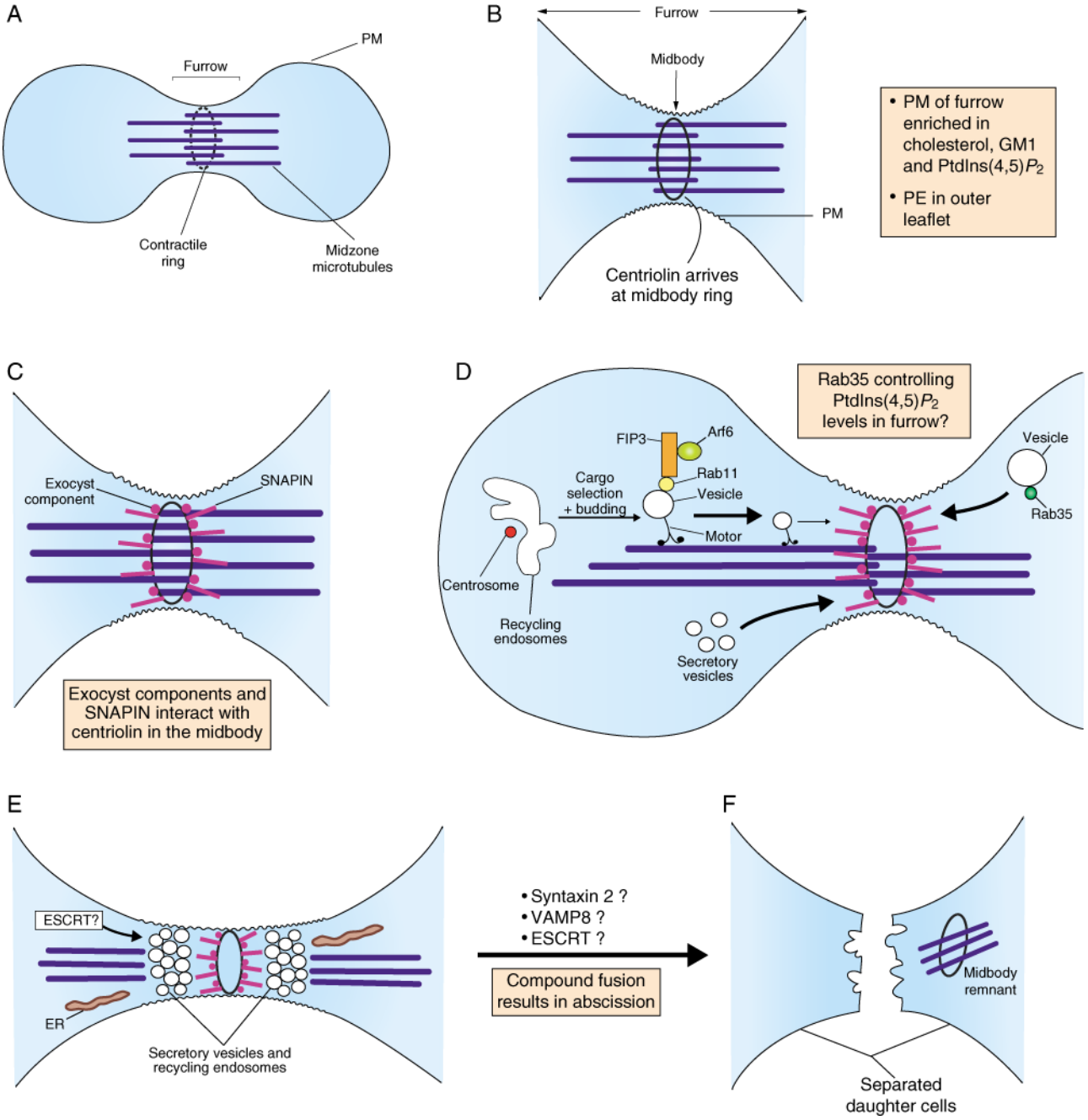

Fig. 1.

Model for membrane traffic during cytokinesis (A) MKLP1 and MgcRacGAP act to recruit the Rho guanine nucleotide-exchange factor (RhoGEF) ECT2 and activate RhoA (not shown), thereby driving contractile-ring formation and contraction (Piekny et al., 2005). The furrow then ingresses, leaving a thin intercellular connection between the cells, and a midbody-ring structure is assembled. (B) During furrowing, the composition of the plasma membrane (PM) of the furrow changes – levels of cholesterol, GM1 and PtdIns(4,5)P_2 increase (Desautels et al., 2001; Emoto et al., 2005; Field et al., 2005), PE redistributes into the outer leaflet (Emoto and Umeda, 2000). Increased levels of PtdIns(4,5)P_2 might be

controlled by Rab35 (see text). The arrival of centriolin at the midbody ring is controlled by MKLP1 (Gromley et al., 2005). (C) Centriolin then recruits SNAPIN and exocyst components to the midbody ring (Gromley et al., 2005). (D) Recycling endosomes at the centrosome bud vesicles that contain Rab11-GTP (and which interact with FIP3 and Arf6) and traffic into the midbody (Wilson et al., 2005), presumably along microtubules using an unidentified motor protein. Secretory vesicles (probably derived from the TGN) also traffic into the midbody. These vesicles might contain VAMP8 and other cargo that is required for abscission. Rab35-positive vesicles also traffic into the furrow, perhaps to establish differential lipid domains that are enriched in PtdIns(4,5)P_2 (Kouranti et al., 2006). Bold arrows indicate traffic into the furrow. (E) Endosomal (Rab35-positive and/or Rab11-positive) and secretory vesicles accumulate in the midbody through the interaction with multiple proteins (e.g. FIP3 with Arf6, Rab11 or Cyk4, or Rab11 with exocyst components) (Fielding et al., 2005). The ESCRT complex might also function here to retrieve cargo from vesicles (Carlton and Martin-Serrano, 2007; Morita et al., 2007). Note that ER tubules are often observed within midbodies and might function to control signalling molecules such as Ca^{2+}. (F) A signal for compound fusion might result in these vesicles fusing with the PM and themselves, resulting in abscission. This might include a direct role for the ESCRT machinery in resolving the thin cytoplasmic bridge. This compound fusion is possibly mediated by syntaxin 2 and VAMP8 (Low et al., 2003).