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Cytokinesis series

Membrane traffic: a driving force in cytokinesis

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Dividing animal and plant cells maintain a constant chromosome content through temporally separated rounds of replication and segregation. Until recently, the mechanisms by which animal and plant cells maintain a constant surface area have been considered to be distinct. The prevailing view was that surface area was maintained in dividing animal cells through temporally separated rounds of membrane expansion and membrane invagination. The latter event, known as cytokinesis, produces two physically distinct daughter cells and has been thought to be primarily driven by actomyosin-based constriction. By contrast, membrane addition seems to be the primary mechanism that drives cytokinesis in plants and, thus, the two events are linked mechanistically and temporally. In this article (which is part of the *Cytokinesis* series), we discuss recent studies of a variety of organisms that have made a convincing case for membrane trafficking at the cleavage furrow being a key component of both animal and plant cytokinesis.

Introduction

Basic light-microscopic observations highlight the differences between plant and animal cytokinesis. During plant cell division, a specialized structure known as the phragmoplast forms at the cell center. The phragmoplast consists of membrane, microtubules and microfilaments, and promotes the concentration and fusion of secretory vesicles [1]. Thus, plant cytokinesis proceeds from the cell center towards the cortex, relying primarily on vesicle fusion. This process involves delivery of cell wall and membrane components, and requires actomyosin for expansion and fusion of the newly formed membrane with the plasma membrane. By contrast, cytokinesis in animal cells relies on a pronounced actomyosin contractile ring that drives plasma membrane invagination from the cortex. An important feature of this 'purse-string' model is that, with the exception of the terminal sealing event, it does not require cleavage furrow ingression and membrane addition to be linked temporally and spatially. According to this basic model, additional membrane could be incorporated at any time and anywhere along the plasma membrane before the terminal events of cytokinesis. Reinforcing the distinction between plant and animal cytokinesis is the fact that secretion remains active throughout the cell cycle in plants but is dramatically downregulated during mitosis in animal cells [2,3].

Recent advances in microscopy, a new generation of fluorescent probes and sophisticated functional approaches have begun to highlight the similarities between plant and animal cytokinesis. Specifically, studies of a variety of animal systems have revealed that, like plants, membrane trafficking has an important role during animal cytokinesis (Figures 1,2 and Tables 1,2). These studies demonstrate that targeted membrane addition during cleavage furrow formation is a fundamental and widely conserved mechanism of animal cytokinesis. This insight has opened new avenues of investigation that are now rapidly being addressed: what are the sources of membrane; when and where is membrane added and removed during cytokinesis; how do membrane-trafficking pathways regulate membrane delivery to and from the cleavage furrow; what role does the cytoskeleton have in this process; and why are the distinct protein and lipid compositions at the furrow important for cytokinesis? In this article, we highlight recent progress in these areas (for further reviews about specific aspects of plant and animal cytokinesis, see Refs [3–9]).

Cytokinesis in animal cells often requires on-time delivery of Golgi-derived membrane

The additional membrane required for cytokinesis has several possible origins such as excess membrane stored within the plasma membrane, and internal membrane derived from secretory, endocytic or recycling pathways (Figure 2). In amphibian embryos, microvilli observed during the initiation of cytokinesis might provide the required membrane [10]. In cellularizing *Drosophila* embryos, excess membrane stored as microprojections is a possible source [11]. Studies have also emphasized the role of vesicle-mediated delivery of membrane from internal pools during cytokinesis [12–16] (Table 1). Perhaps the most striking evidence comes from scanning electron microscopy (SEM) and live confocal microscopy studies of *Xenopus* embryos that demonstrate large clusters of exocytic fusion pores near the base of the invaginating furrow [17] (Figure 3a). These are the sites of recent exocytic events that required microtubules. Estimates based on fusion pore distribution and density suggest that localized exocytosis is sufficient to provide

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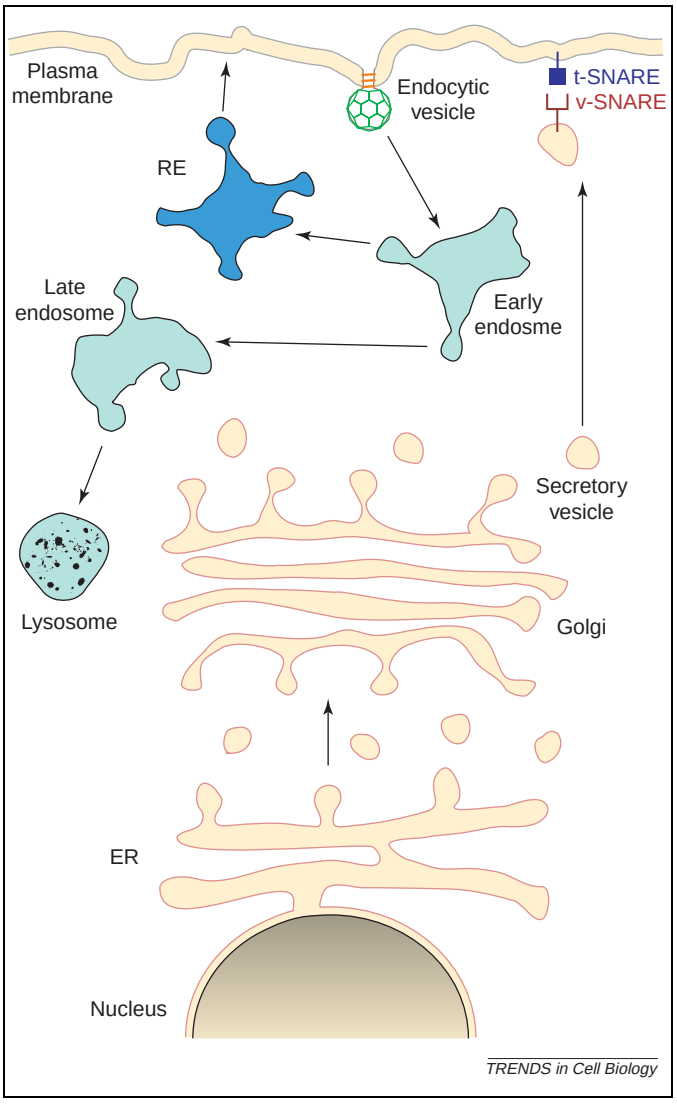

Figure 1. Intracellular membrane-trafficking pathways. Components of secretory, endocytic, lysosomal and recycling pathways are shown. Transport steps are indicated by arrows. Secretion involves vesicle transport from the ER to the Golgi apparatus and then to the plasma membrane. Vesicle fusion involves membrane-specific t-SNARE and v-SNARE (blue and red, respectively) protein interactions that are promoted through several adaptor proteins (not shown). Endocytic vesicles budding from the plasma membrane are coated with clathrin (green); clathrin coats include a membrane-specific complex of adaptor proteins (not shown). The GTPase dynamin assembles into rings (orange) at the neck of clathrin-coated endocytic vesicles; dynamin ring constriction facilitates the 'pinching off' of vesicles from the plasma membrane. One destination for endocytic vesicles is the early endosome, which directs vesicles back to the plasma membrane (through the RE) or to lysosomes for degradation.

most of the new membrane required to complete cytokinesis. These and other studies clearly demonstrate that major vesicle addition is associated with cleavage furrow formation [18].

Golgi-derived vesicles are the primary source of membrane in plant cytokinesis [5]. Evidence of a similar role in animal cytokinesis came initially from functional studies using brefeldin A (BFA), an Arf GTPase inhibitor that blocks anterograde transport from the endoplasmic reticulum (ER) and, ultimately, results in Golgi disassembly [19]. During the first zygotic divisions in *Caenorhabditis elegans* embryos, initiation and progression of furrows proceed normally in the presence of BFA. However, BFA prevents the terminal sealing event and furrow regression occurs [20]. Because BFA treatment also results in ectopic furrowing, it is unlikely that defects during the terminal stages of cytokinesis are due to a general lack of membrane; it is more likely that they are caused by a specific requirement for Golgi-derived vesicles at this stage.

Whereas Golgi-based vesicle delivery is crucial during the terminal stages of embryonic *C. elegans* cytokinesis, it is required for the initial stages of furrow progression in *Drosophila* spermatocytes. Mutants for *four way stop (fws)*, a Cog5 (conserved oligomeric Golgi) homolog, establish cleavage furrows, although ingression fails in these mutants [21] (Figure 1f). Mammalian Cog5 is a member of an eight-protein Golgi-associated complex believed to have a role in Golgi vesicle targeting [22]. Cog5 is nonessential but increases the efficiency of Golgi-based trafficking. Similarly, Fws is not generally required for cytokinesis and cell division throughout *Drosophila* development, raising the possibility that the large (20 μm) and rapidly cleaving (20 min) spermatocytes place exceptional demands on Golgi-based vesicle trafficking during cytokinesis. *fws* mutations also disrupt spermatid elongation – a process that requires extensive membrane addition. Further supporting the role of Golgi-based vesicle trafficking in spermatocyte cytokinesis is the finding that hypomorphic mutations in the *Drosophila* syntaxin5 homolog, a Golgi-localized soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) required for ER-to-Golgi traffic, result in male sterility due to failed cytokinesis [23].

In cellularizing *Drosophila* embryos, there is accumulating evidence that indicates that Golgi-mediated membrane delivery is required throughout the process of furrow invagination. Cellularization is a specialized form of cytokinesis in which furrows that are compositionally and structurally equivalent to cleavage furrows encompass the ~6000 interphase nuclei aligned on the cortex. Electron microscopy (EM) studies indicate that some of the membrane required for cellularization is derived from extensive microprojections. However, quantifications of cell surface area indicate that excess plasma membrane is insufficient to complete cell division; thus, it is likely that additional internal stores are also required [11]. Live analysis of Golgi movement during cellularization shows that Golgi move towards and closely associate with the advancing furrow, suggesting that they might be a source of membrane [15]. BFA injections severely disrupt cellularization, providing functional support for this interpretation [15]. Functional studies using antibodies directed against a *Drosophila* Golgi-associated protein, Lava Lamp, result in severe disruptions of Golgi morphology and distribution, in addition to failed furrow formation [15]. A separate study using fluorescent surface and newly synthesized transmembrane markers also concluded that mobilization of internal membrane stores through the ER and Golgi is a primary source of membrane during cellularization [16].

Proteomic analysis of midbodies purified from mammalian cells provides additional support for the role of Golgi-derived membrane in cytokinesis. Approximately a quarter of the proteins associated with the midbody are Golgi

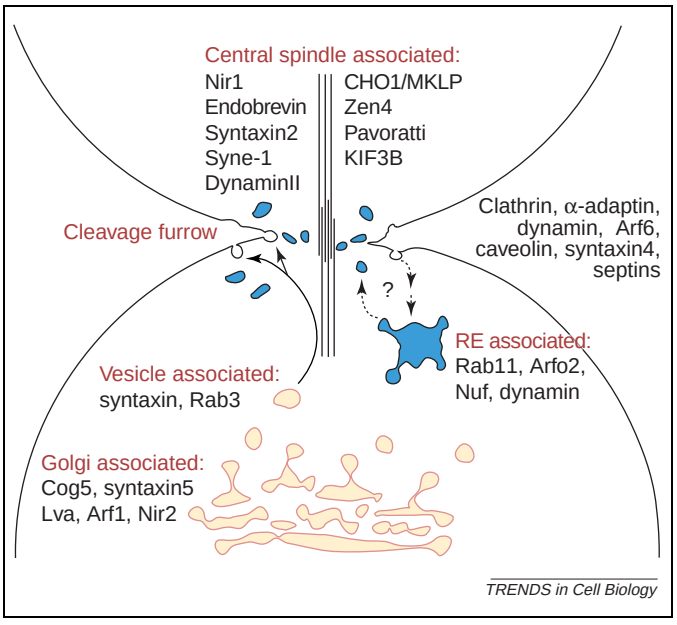

Figure 2. Membrane-trafficking components involved in animal cytokinesis. Evidence from a variety of organisms has identified proteins within exocytic, endocytic and recycling pathways that have essential roles in cytokinesis. Polarized targeting of vesicles to invaginating cleavage furrows provides new membrane, and perhaps regulatory proteins, essential for furrow elongation. Vesicle delivery might be guided by the central spindle and associated proteins. SNARE proteins such as syntaxins are likely to facilitate furrow-specific vesicle targeting and fusion. Currently, it is unclear precisely where vesicle fusion occurs along the furrow. Vesicles might be derived directly from the Golgi or from intermediate trafficking organelles such as endosomes. Also, cell-cycle-mediated Golgi disassembly might trigger the release of Golgi-associated cytokinetic regulatory proteins. It is likely that cleavage furrow membrane is recycled through clathrin-coated vesicles and clathrin-associated proteins such as dynamin and α-adaptin. Endocytic vesicles can be re-routed back to the furrow by the RE.

derived [24]. RNA interference (RNAi)-based functional analysis in *C. elegans* embryos revealed that many of these Golgi-associated proteins are essential in the early and late stages of cytokinesis [24]. These observations suggest that vesicle transport near the cleavage furrow involves Golgi that are closely associated with the midbody.

Golgi disassembly is required for cytokinesis

Golgi have at least two distinct roles in promoting cytokinesis: supplying membrane through Golgi-mediated vesicle addition and supplying cytokinetic regulatory proteins through cell-cycle-mediated release of Golgi-associated proteins [14] (Figure 2). In many cell types, Golgi undergo an ordered stepwise disassembly as the cells enter mitosis [25]. A recent study has identified the small GTPase Arf1 as being a regulator of Golgi mitotic disassembly [26]. As cells enter mitosis, Arf1 becomes inactive, the Golgi disassembles and Golgi-associated proteins disperse into the cytoplasm. Arf1 inhibitors and constitutively active Arf1 prevent Golgi disassembly and the release of Golgi-associated components. Importantly, the cells enter mitosis but exhibit chromosome segregation defects and failure of cleavage furrow ingression. Proteins released from the Golgi to the cytoplasm during mitosis include COPI, ankrin, spectrin, cullins, myosin IIa and Cdc42 [26], some of which are known to promote cytokinesis [27]. Together, these findings suggest that Arf1-mediated mitotic Golgi disassembly is necessary for the release and proper function of key proteins required for cytokinesis. Further support for this model comes from a study of Nir2, a Golgi-associated protein that transits to the cleavage furrow and is essential for cytokinesis [28]. Cdk1 phosphorylation of Nir2 promotes its dissociation from the Golgi and its accumulation at the cleavage furrow and midbody. Nir2 phosphorylation is required for binding of PLK1 and proper cleavage furrow ingression [29]. Thus, in addition to directly providing membrane for cleavage furrow invagination, it is likely that Golgi mediate the coordinated release of proteins required for cytokinesis and other cytoplasmic events as the cell progresses through mitosis.

Endocytosis and cytokinesis

Considering that cytokinesis requires the incorporation of additional membrane, it is surprising that endocytosis-based membrane trafficking is also required for cytokinesis (Figure 2, Table 1). In *Dictyostelium discoideum*, the completion of cytokinesis is dependent upon clathrin and dynamin, which are membrane-associated proteins that promote vesicle budding from the plasma membrane [30]. Other studies support a role for endocytosis-associated proteins in promoting animal cytokinesis. In *Drosophila*, the clathrin-associated protein α-adaptin is targeted to the leading edge of furrows, and the dynamin homolog shibire is required for cellularization [31,32]. Similarly, dynamin in *C. elegans* localizes to cleavage furrows and is necessary for cytokinesis completion [33]. A striking illustration of the role of endocytosis in cytokinesis comes from studies using zebrafish embryos. Using fluorescent markers for fluid-phase and plasma membrane uptake, it was shown that furrow-specific endocytosis occurs from early to late stages of cytokinesis [18] (Figure 3d). In addition, clathrin-coated pits, caveolin and dynamin II localize specifically to the cleavage furrow, and inhibitors of endocytosis prevent completion of cytokinesis [18]. It is unclear how endocytic events at the cleavage furrow contribute to cytokinesis. One possibility is that endocytosis is required for the recycling of components previously delivered to cleavage furrows by exocytosis [17].

Membrane traffic within the endocytic pathway proceeds through a series of organelles, including early, late and recycling endosomes (REs) (Figure 1). Recent studies indicate that the RE is crucial for proper membrane addition during cytokinesis [34]. This organelle has several features that make it well suited to this role. Although the RE can transport vesicles to Golgi and other endosomal components, the primary destination of RE-derived vesicles is the plasma membrane. In addition to membrane, the RE sorts specific proteins to this location. Furthermore, the RE associates with microtubules and localizes to the microtubule-organizing center (MTOC), a key element in contractile ring positioning (Box 1).

Functional evidence implicating the RE in cytokinesis came initially from RNAi-induced inhibition of Rab11 in early *C. elegans* embryos [20]. Rabs are small GTPases that provide specificity to endosome-based vesicle trafficking. Rab11 localizes preferentially to the RE and is required for proper RE organization and the recycling of vesicles to the plasma membrane [35,36]. Inhibition of

Table 1. Summary of Golgi-associated and endocytic-trafficking proteins in animal cytokinesis

Component	Organism	Inhibitor or mutation	Cytokinesis defect	Localization	Refs
Golgi-associated proteins					
Cog5	*Drosophila* spermatocyte	Null mutation: *four way stop* (*fws*)	Furrow ingression fails	Golgi associated	[21]
Syntaxin5	*Drosophila* spermatocyte	Hypomorphic mutation	Cytokinesis fails	Golgi associated	[23]
Lava-lamp (Lva)	Drosophila cellularization	Antibodies	Inhibition of furrow progression	Golgi associated	[15]
Arf1	Cell culture	Mutation	Cleavage furrows regress	Golgi associated	[26]
Nir2	Cell culture	Dominant–negative	Furrow ingression fails	Golgi to midbody; cleavage furrow	[28,29]
Golgi Function	*Caenorhabditis elegans* embryo	BFA	Furrow regression; ectopic furrows	N/A	[20]
	Drosophila cellularization	BFA	Furrow ingression fails late	N/A	[15]
Endocytosis and recycling					
Clathrin	*Dictyostelium discoideum*	Null mutation	Furrow ingression fails, abnormal furrow morphology	N/A	[30]
	Zebrafish embryo	Drug: chlorpromazine	Furrow regression, cytokinesis completion fails	Cleavage furrow	[18]
Caveolin	Zebrafish embryo	Drug: methyl-B-cyclodextrin	Furrow regression, cytokinesis completion fails	Cleavage furrow	[18]
Dynamin II	Zebrafish embryo	Drug: chlorpromazine	Furrow regression, cytokinesis completion fails	Cleavage furrow	[18]
Rab11	*C. elegans* embryo	RNAi	Furrow regression	RE	[20]
	Drosophila syncytial divisions and cellularization	Hypomorphic mutation	Failed actin and membrane recruitment	RE	[34,37]
Arfophilin-2 (Rab11 effector)	*Drosophila* syncytial divisions and cellularization	Null mutation: *nuclear-fallout* (*nuf*)	Failed actin and membrane recruitment	RE	[34,40]
Arf6	Cell culture	Dominant–negative	Late cytokinesis defects	Cleavage furrow	[83]
Dynamin	*Drosophila* cellularization	Temperature-sensitive mutation: *shibire* (*shi*)	Early cellularization defect	RE	[32]
	C. elegans embryo	Temperature-sensitive mutation; RNAi	Late cytokinesis defects	Cleavage furrow	[33]
Dynamin II	Cell culture	N/A	N/A	Spindle midbody	[33]

Rab11 does not interfere with the initial events of cytokinesis, although furrow regression occurs during later stages [20]. Functional studies in syncytial and cellularizing *Drosophila* embryos also demonstrate that the RE is required for proper cytokinesis; injection of a dominant–negative form of Rab11 causes cleavage furrowing defects specifically during the initial slow phase of invagination [37]. In addition, these studies also provide evidence that dynamin controls vesicle trafficking through a Rab11-positive endosome (presumably the RE).

In addition to these studies supporting a role for the RE during furrow ingression, analysis of Rab11-deficient *Drosophila* embryos indicates that the RE is also required for cellular furrow formation [34]. In these embryos, the recruitment of both actin and membrane fails during the initial stages of furrow formation, suggesting that this

Box 1. The RE, centrosomes and Rappaport furrows

Among endocytic organelles, the close association of the RE with the centrosome is unique. A consequence of this association is that centrosomes influence the position of the RE within the cell. Thus, either developmentally programmed or experimentally induced positioning of centrosomes to a specific region of the cell cortex not only increases the number of microtubules interacting with the cortex but might also target RE-mediated vesicle delivery to specific cortical regions. Rappaport's classic experiments in marine invertebrates, in addition to more-recent work, highlight the role of the MTOC and midzone microtubules in establishing furrow invagination [8,72]. The furrow-inducing activity of the MTOC is thought to be mediated primarily through stimulatory and inhibitory interactions between the plus ends of microtubules and the cortex [6] (see Figure 2 in main text).

The positive effect microtubules have on furrow formation is also likely to be the result of activities directly associated with the MTOC. The MTOC has an important role in organizing and positioning the RE. Positioning the MTOC to a specific region of the cell also positions the RE and might direct RE-mediated vesicle trafficking to the site of furrow invagination (see Figure 2d in main text). Additional support for this idea comes from studies demonstrating that the RE is crucial for establishing epithelial membrane polarity. In Madin–Darby canine kidney (MDCK) cells, the RE is required for the final step in transcytosis: delivering specific components to the apical plasma membrane [73]. Importantly, the RE is apically positioned and depends on microtubules for its integrity. Thus, in both cytokinesis and cell polarity, the role of microtubules probably includes organizing and positioning the RE.

Table 2. Summary of kinesin-related proteins, SNAREs and membrane lipids in animal cytokinesis

Component	Organism	Inhibitor or mutation	Cytokinesis defect	Localization	Refs
Kinesin-related proteins					
CHO1/MKLP	Cell culture	RNAi	Furrow scission fails	Spindle midzone	[74]
Zen4	*Caenorhabditis elegans*	RNAi	Furrows regress	Spindle midzone	[79,80]
Pavarotti	*Drosophila*	Mutation	Failed furrow initiation and contractile ring assembly	Spindle midzone	[81]
MPP1	Cell culture	RNAi	Furrow scission fails	Spindle midzone	[78]
Rab6-KIFL	Cell culture	Antibodies	Cleavage furrow regression	Cleavage furrow, midbody	[75]
KLP3A	*Drosophila* spermatocytes	Mutations	Failed furrow initiation and contractile ring assembly	Spindle midzone	[76,77]
KIF3B	Mammalian NRK cells	Dominant–negative	Loss of syntaxin at midbody; cytokinesis fails	Spindle midzone	[82]
MKLP-2	Cell culture	Antibodies	Furrow ingression fails	Spindle midzone	[85]
Syne-1 (spectrin)	Cell culture	Dominant–negative	Failed cytokinesis	Central spindle	[82]
Secretory pathway and SNAREs					
Syntaxins					
Syntaxin1	*Drosophila* cellularization	Hypomorphic germline clones	Patches of failed actin recruitment	Newly formed lateral surface of invaginating furrows	[51]
Syntaxin4	*C. elegans* embryo	RNAi	Furrow ingression fails	Ingressing cleavage furrow	[53]
Syntaxin	Sea urchin	Botulinum neurotoxin C1; α-syntaxin antibodies	Cytokinesis fails	Vesicles at cortex	[54]
Syntaxin2	Cell culture	Dominant–negative	Furrow scission fails	Midbody	[55]
Endobrevin	Cell culture	Dominant–negative	Furrow scission fails	Midbody	[55]
Lipids and plasma membrane					
PtdEtn	Major eukaryote plasma membrane phospholipid	PtdEtn-binding peptide	Contractile ring disassembly; cytokinesis failure	Membrane leaflets of cleavage furrows	[64,65]
PtdIns	Crane fly spermatocytes	Inhibitors of PtdIns recycling, phosphorylation and hydrolysis	Slowed furrow progression and regression; actin depletion at furrow	Minor eukaryote plasma membrane phospholipids	[70]
PtdIns 4-kinase	*Drosophila* spermatocytes	Null mutation: *four-wheel drive* (fwd)	Unstable cleavage furrow	N/A	[67]
PtdIns4*P*5-kinase[a]	*Schizosaccharomyces pombe*	Temperature sensitive	Late cytokinesis defects	Cleavage furrow	[69]
Sterol	*S. pombe*	Filipin	Abnormal actomyosin ring positioning	Cleavage furrow; growing polar tips	[63]

[a]Abbreviation: phosphatidylinositol 4-phosphate 5-kinase.

recruitment is coupled through RE-mediated vesicle delivery. Additional support for RE function during early cytokinesis comes from studies of the mammalian Rab11-binding protein Arfophilin (Arfo)-2. Arfo-2 binds to Rab11 in a GTP-dependent manner and colocalizes with it at the centrosome. Functional studies demonstrate that Arfo-2 is required for normal organization of the RE [38]. Nuclear-fallout (Nuf), the *Drosophila* homolog of Arfo-2 [39], and Rab11 interact physically and are interdependent for their localization at the RE [34]. Embryos lacking Nuf or Rab11 cannot recruit membrane and actin during furrow initiation; consequently, syncytial and cellularization furrow formation fails [34,40]. A plausible model is that Rab11 and Nuf are localized to distinct domains that create specific vesicle-fusion sites within the RE [41].

These studies establish a link between RE-based membrane delivery and recruitment of actin to the cleavage furrow, although the molecular basis of this linkage is unclear. Immunofluorescent analysis reveals that actin puncta are often associated with vesicles and that these seem to be delivered as a unit to the cellularization furrow [40]. Although this has not been observed in cytokinesis in other organisms, actin filaments often associate with endocytic vesicles [42]. Alternatively, vesicles destined for the cleavage furrow might include actin-remodeling factors [43]. Live analysis in *Drosophila* reveals that myosin puncta are delivered to the invaginating furrow, suggesting that myosin recruitment is also mediated by vesicles [44].

Cytokinesis involves homotypic and heterotypic membrane fusion

EM analysis of cleaving *Xenopus* embryos provides dramatic visual evidence that vesicle fusion has a role in cytokinesis [17] (Figure 3a). The region immediately behind the leading edge of the cleavage furrow is heavily pockmarked with fusion pores. Vesicle fusion is central to many membrane-based cellular processes, including synaptic transmission, organelle inheritance and epithelial polarity, and much is known about the underlying

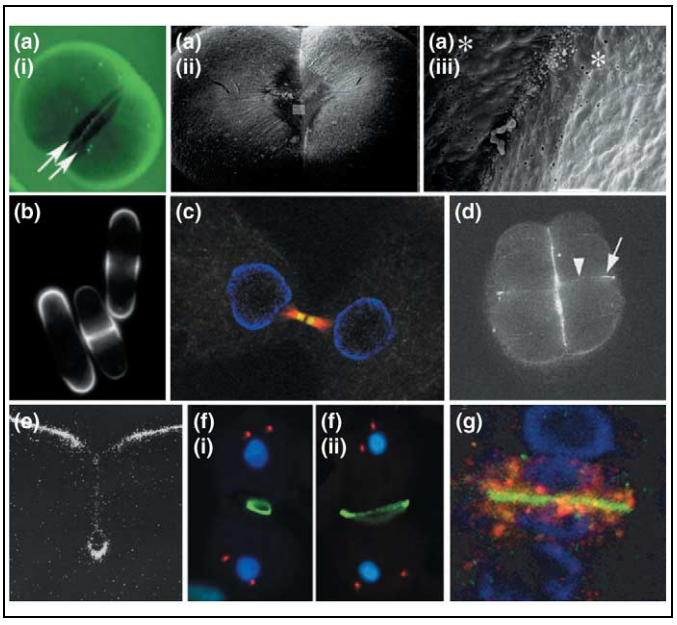

Figure 3. Membrane dynamics in animal cytokinesis. **(a)(i–iii)** Dividing *Xenopus* embryos. (i) Embryo incubated with fluorescein isothiocyanate (FITC)–soybean agglutinin and observed using epifluorescence. (ii) Low-magnification SEM showing new membrane in the furrow region. (iii) High magnification of area represented by the box in (ii). Many pits with raised edges appear in clusters (asterisks). Reproduced, with permission, from Ref. [17]. **(b)** Sterol localization visualized by filipin staining in *Schizosaccharomyces pombe* cells. Sterol localizes to sites of active growth and cytokinesis. Reproduced, with permission, from Ref. [63]. **(c)** Mammalian NRK cells at cytokinesis. Syntaxin2 is shown in green; α-tubulin is shown in red; and 4′,6-diamidino-2-phenylindole (DAPI) is shown in blue. Reproduced, with permission, from Ref. [55]. **(d)** Zebrafish embryos incubated in fluorescent markers 1–43 to mark sites of endocytosis. Arrow indicates peripheral furrow region; arrowhead indicates central furrow region. Reproduced, with permission, from Ref. [18]. **(e)** Light-microscope autoradiography of *Xenopus* embryo at the first cleavage. Reproduced, with permission, from Ref. [12]. **(f)(i–ii)** *Drosophila* spermatocytes at cytokinesis. *four way stop* mutants (ii) have furrow ingression failure compared with wild type (i). Phalloidin is shown in red; β-tubulin is shown in green; and DAPI is shown in blue. Reproduced, with permission, from Ref. [21]. **(g)** Localization of syntaxins in *Arabidopsis thaliana* seedling root cytokinetic cells. Knolle is shown in green; Myc–Pep12 is shown in red; and DAPI is shown in blue. Reproduced, with permission, from Ref. [71].

molecular mechanisms. Membrane fusions are classified as being either heterotypic or homotypic. The former involves fusion of membrane from different sources, with one providing the target (t)-SNARE and the other providing the vesicle (v)-SNARE. The latter involves fusion of membrane from the same source, between equivalent vesicles for example, and involves symmetrical v-SNARE and t-SNARE interactions (Figures 4,5).

Although SNAREs are known to promote heterotypic fusion events, studies in plants suggest that syntaxin might also mediate homotypic vesicle fusion. In *Arabidopsis thaliana*, three SNARE-associated proteins – knolle (a syntaxin homolog [45]), keule (a Sec1 homolog [46]) and AtSNAP33 (a SNAP25 homolog [47]) – are required for membrane addition during cytokinesis. In plant cytokinesis, rather than incorporating individual vesicles into the cleavage furrow, vesicles fuse with one another before associating with the invaginating membrane. The microtubule-based phragmoplast guides recruitment of Golgi-derived vesicles to the cell equator (Box 2). The presence of hourglass vesicle intermediate structures suggests that homotypic fusion of individual Golgi-derived vesicles is an early event in plant cytokinesis. These vesicles then fuse with a 20-nm fusion tube and, ultimately, create a contiguous tubular network across the equator [48]. Several lines of evidence indicate that knolle is required specifically for cytokinesis. Knolle is membrane associated, it accumulates preferentially at the site of phragmoplast formation, and the expression of other syntaxins in a knolle-mutant background cannot rescue cytokinesis defects [49] (Figure 3g). Furthermore, EM analysis of *knolle* mutations reveals abnormal aggregates of vesicles at the cell plate, which indicates that vesicles are delivered but fail to fuse [45]. A reasonable interpretation of these results is that knolle-mediated homotypic fusion of Golgi-derived vesicles is a key step in plant cytokinesis.

The role of syntaxins in animal cytokinesis has been more difficult to study because no cytokinesis-specific syntaxins have been identified. Thus, specialized hypomorphic or temperature-sensitive alleles, rather than null alleles, must be used. For example, *Drosophila* syntaxin1 has an essential role in *Drosophila* synaptic transmission [50] and oogenesis [51], and null alleles of *syntaxin1* are cell lethal [51]. Yet, by creating germline clones of hypomorphic *syntaxin1* alleles, it was shown that syntaxin is required for elongation of the cellularization furrow [51]. Syntaxin1 localizes preferentially along the plasma membrane during cellularization, and mutations cause large acellular patches, indicating that syntaxin is required during early stages of cellularization. These studies are consistent with models proposing that cellularization proceeds through the direct fusion of vesicles with invaginating plasma membrane [52]. Syntaxin1 at the plasma membrane might be functioning as a t-SNARE, mediating heterotypic fusion events with specific vesicle populations.

RNAi, rather than specialized alleles, was used to analyze the role of syntaxins during cytokinesis in the initial *C. elegans* embryonic divisions [53]. Of the eight syntaxin gene products examined, syntaxin-4 produced defects in cytokinesis and nuclear-envelope assembly. RNAi-induced knockdown of syntaxin-4 produced both complete and partial failure of furrow ingression. Syntaxin4 localizes primarily to the region of ingressing cleavage furrows, suggesting that it mediates heterotypic vesicle–membrane interaction during furrow progression.

Syntaxins are also required for cytokinesis in sea urchin embryos. Reducing syntaxin function with Botulinum neurotoxin C1 or antibodies against the single sea-urchin syntaxin blocks cytokinesis and karyokinesis [54]. In contrast to the studies of *Drosophila* and *C. elegans*, sea urchin syntaxin localizes to a vesicle population concentrated at the cortex rather than the plasma membrane. Although these results can be interpreted in terms of heterotypic vesicle–membrane fusion, they also raise the possibility that syntaxins mediate homotypic vesicle-fusion events required for cytokinesis.

Overexpression and dominant–negative studies in mammalian cells demonstrate that syntaxins are required during the terminal stages of cytokinesis [55]. The t-SNARE syntaxin2 and the v-SNARE Endobrevin localize specifically at the midbody of dividing cells [55] (Figure 3c). Inhibition of these proteins has no effect on the initial stages of cytokinesis or furrow ingression but it

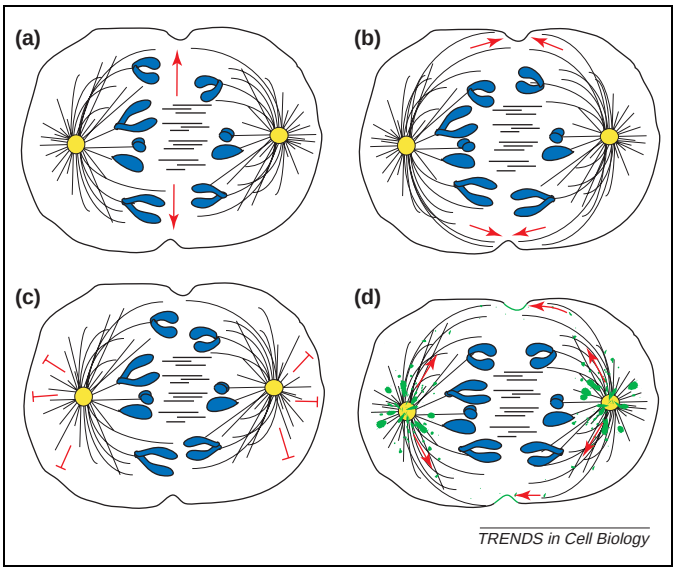

Figure 4. Models of the role of microtubules in cleavage furrow induction. **(a)** Midzone antiparallel overlapping microtubules stimulate cleavage furrow formation (red arrows). **(b)** Furrow induction through interaction of astral microtubule plus ends with the equatorial cortex [equatorial stimulation (red arrows)]. **(c)** Inhibitory interactions (red) of astral microtubule plus ends at the polar cortices promote furrow formation at the equator (polar relaxation). **(d)** Microtubule-dependent events at the MTOC are required for organizing the RE (green). Furrow induction is promoted through delivery (red arrow) of RE-derived vesicles to the invaginating furrow (green part of cell outline).

does prevent scission. Because these proteins are also expressed in non-dividing cells, they are likely to provide additional functions beyond the completion of cytokinesis. Unlike in *Drosophila* and *C. elegans* cytokinesis, syntaxin2 is not concentrated at the plasma membrane or cleavage furrow in mammalian cells. One interpretation of these results is that, similar to what occurs in plants, these proteins are mediating the homotypic vesicle fusion required to complete mammalian cytokinesis.

Cytokinesis is linked to establishment and maintenance of distinct cortical domains

Cortical domains, which contain a distinct protein and lipid composition, represent functionally specialized regions of the plasma membrane that are necessary for several biological processes. Establishment and maintenance of cortical domains require: (i) the site-specific targeting of proteins and lipids; (ii) the restriction of cortical proteins and lipids within the domain; and (iii) the exclusion of protein and lipid diffusion from adjacent domains and the cytoplasm. Animal cells use several mechanisms to establish and maintain cortical domains. For example, they can be established by reorganizing the plasma membrane through site-specific endocytosis, recycling and exocytosis [56]. Cortical domains in neuronal axons and epithelial cell junctions are established and maintained, in part, through extensive protein–protein interactions mediated through PDZ (PSD-95, Discs large, ZO1) domains. This class of proteins serves as a scaffold for protein targeting and anchoring, and provides barriers against lateral diffusion. Targeted delivery of membrane and proteins to cortical domains is often promoted through polarized secretion: for example, by relocating Golgi to specific cell regions [57].

Establishment and maintenance of cortical domains within the cleavage furrow are essential for cytokinesis. The link between cell polarity and cleavage furrow ingression is particularly evident during *Drosophila* cellularization, in which the lateral furrow membrane is subdivided into multiple domains that are separated from an apical membrane domain [16,58]. The novel protein Slow-as-molasses (Slam) localizes to the lateral membrane, at which it organizes cell polarity and promotes membrane addition [59]. Reduced Slam function causes both loss of apical–basal polarity and failure of furrow ingression, highlighting the tight coordination between

Box 2. Kinesin-related proteins

During plant cell division, an antiparallel array of microtubules forms within the phragmoplast that facilitates the delivery of vesicles containing cell-wall material and membrane. Animal cells possess a similar structure – the spindle midzone – that forms during anaphase and also consists of an antiparallel array of microtubules. Several kinesin-related proteins, including CHO1/MKLP, KLP3A, Rab6-KIFL and MPP1, localize to the midzone, where they promote spindle midbody formation, stability and function [53,74–78]. Each of these proteins is required for cytokinesis, although they might function during different stages (see Table 2 in main text). The use of RNAi against the mammalian gene products CHO1/MKLP and MPP1 and against the *Caenorhabditis elegans* CHO1/MKLP homolog Zen4 does not disrupt contractile ring assembly, and dividing cells initiate cleavage furrow ingression. However, furrows then regress, which is indicative of a late role during cytokinesis [74,78–80]. By contrast, mutants of the *Drosophila* CHO1/MKLP homolog Pavarotti seem to have an early role during cytokinesis; these mutants cannot initiate furrow ingression and they mislocalize contractile ring components such as septin, anillin and actin [81]. Similarly, *Drosophila* KLP3A is required for actomyosin ring assembly and cleavage furrow formation [76,77]. These observations indicate that kinesin-related proteins probably share common roles in promoting central spindle organization and cytokinesis, although how they function specifically to promote cleavage furrow initiation and ingression is unknown. The appearance of defects at different stages of cytokinesis suggests that kinesin-related proteins employ distinct mechanisms in achieving these goals.

In addition to their role in bundling midzone microtubules, kinesin-related proteins might promote vesicle-trafficking events during cytokinesis. Recent reports suggest that kinesins mediate microtubule-based transport of proteins and vesicles to the midzone that are subsequently required for cleavage furrow ingression and cell fission. In mammalian NRK cells, the kinesin II subunit KIF3B colocalizes with syntaxin-containing vesicles to the central spindle and midbody during telophase [82]. Expression of the dominant–negative tail of KIF3B results in a marked reduction in levels of syntaxin at the midbody and subsequent cytokinesis defects. KIF3B also interacts physically with the spectrin syne-1 and targets it to the central spindle and midbody; furthermore, reduced syne-1 function leads to an identical phenotype to that caused by expression of the dominant–negative tail of KIF3B [82]. Because spectrins interact with membranes, a reasonable suggestion given is that spectrins link vesicles to kinesins that transport the vesicles along microtubules to the spindle midbody [82]. Vesicles might then fuse with each other and the advancing cleavage furrow to promote cell fission [82]. Other studies have shown that ARF6, a regulator of membrane traffic and cytoskeletal rearrangements, accumulates at the cleavage furrow, is required for cytokinesis and interacts physically with CHO1/MKLP [83,84]. These reports raise the possibility that kinesin-related proteins link cytoskeletal rearrangements and membrane-trafficking events at the midzone with ARF family GTPases.

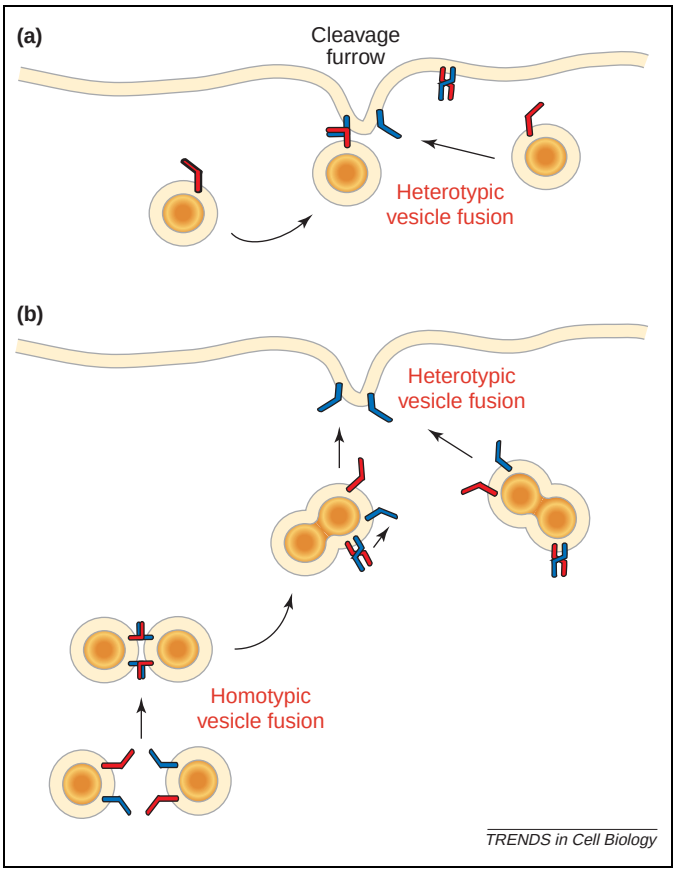

Figure 5. Homotypic membrane fusion facilitates furrow ingression by increasing the surface area that is available for fusion. **(a)** Heterotypic fusion involves interactions between vesicles containing v-SNARE–synaptobrevin (red) and membranes containing the complementary t-SNARE–syntaxin (blue). Furrow invagination during animal cytokinesis probably relies on direct vesicle–plasma-membrane heterotypic fusion events. **(b)** Membranes derived from the same source can undergo homotypic fusion events that involve symmetrical v-SNARE and t-SNARE interactions. Plant cytokinesis relies extensively on homotypic fusion because it accelerates furrow formation by generating multiple large units of membrane before incorporation into the growing furrow. There is evidence that this strategy might also be used in animal cytokinesis [11].

these processes. In conventional cytokinesis, a key upstream regulator in the establishment of cleavage furrow domains is the formin homology (FH) protein Diaphanous. The loss of Diaphanous causes mislocalization of essential contractile ring components such as actin, septins and anillin [60]; consequently, cleavage furrow ingression fails. In addition to components of the contractile ring, membrane-trafficking proteins such as SNAREs are targeted to the cleavage furrow in various organisms (Table 2). The establishment of a polarized cortex probably promotes furrow ingression by providing spatial cues for contractile ring assembly and specific target sites for vesicle delivery.

Recent work indicates that, in addition to protein composition, the lipid composition of the furrow is distinct and promotes cytokinesis. Photobleaching-based experiments in yeast and mammalian cells suggest that maintenance of the cleavage furrow domain depends on a barrier to lateral protein diffusion [61,62]. Diffusion rates across the cleavage furrow for different membrane proteins vary depending on how the protein is anchored. Fluorescently tagged membrane proteins embedded in the inner leaflet of the plasma membrane by myristyl and palmityl moieties, and transmembrane proteins have markedly slower diffusion rates across the cleavage furrow than at other regions of the plasma membrane [62]. During late cytokinesis, these proteins are excluded from the midbody. By contrast, a fluorescently tagged membrane protein embedded in the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor showed free diffusion across the cleavage furrow and was present at a constant density across the midbody. These observations suggest that a diffusion barrier in the cleavage furrow of mammalian cells exists but is effective only in preventing diffusion of proteins containing a cytosolic domain [62]. A furrow-associated diffusion barrier could be provided by septins. In yeast, septins form rings adjacent to the contractile ring and are required for proper localization of proteins needed for cytokinesis. Recent work using fluorescence recovery after photobleaching (FRAP) and temperature-sensitive septin alleles demonstrates that, in fission yeast, septin rings function as barriers to restrict diffusible cortical components, such as the exocyst, to the cleavage site [61].

Additional reports further highlight the importance of establishing a specialized lipid composition at the plasma membrane in cytokinesis (Table 2). In *Schizosaccharomyces pombe*, sterols are enriched at the growing tips and at the site of cytokinesis [63] (Figure 1b). Disruption of these domains results in improper positioning of the actomyosin ring. Phosphatidylethanolamine (PtdEtn), which is the major eukaryotic plasma membrane phospholipid, resides preferentially on the inner leaflet of the lipid bilayer. However, PtdEtn is redistributed at cytokinesis so that it is abundant on the outer, in addition to the inner, leaflet during cytokinesis [64]. Functional studies demonstrate that this reorganization of PtdEtn is essential for contractile ring disassembly and scission [65]. Phosphatidylinositol (PtdIns), which is a minor but important regulatory plasma membrane lipid, also has a key role in cytokinesis. Functional studies in several organisms indicate that PtdIns and its phosphoderivatives are required throughout cytokinesis [28,66–70]. Although it is tempting to conclude that PtdIns is exerting its effects at the cleavage furrow, a recent study suggests that some of its action, at least, is at the poles. Taking advantage of the synchronous cell cycles in crane fly spermatocytes, Saul *et al.* [70] examined the cell-cycle effects of U7, a drug that inhibits hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] specifically. This phosphorylated form of PtdIns is known to promote actin polymerization. Surprisingly, U7 treatment caused depletion of actin at the furrows, accumulation of actin at the poles and, ultimately, cleavage furrow regression. This raises the intriguing possibility that PtdIns(4,5)P_2 turnover at the poles is required for the global actin reorganization that occurs during cytokinesis.

Concluding remarks

The realization that membrane dynamics have an active role in animal cytokinesis forces us to reconsider many of the outstanding issues in the field. Foremost among these is the identification of the factors and mechanisms responsible for establishing cleavage furrow position.

Influenced by developmental biologists and their successful hunt for morphogens, the field has been anticipating that a protein will prove to be the mythical cleavage furrow determinant. It is likely that the plasma membrane will also have a direct role in furrow establishment. Studies demonstrating dramatic changes in lipid composition at the leading edge of the furrow provide tantalizing hints that link membrane composition to furrow induction. Another key issue in animal cytokinesis is the composition, structure and formation of the contractile ring. Although many of the core conserved protein components have been identified, the relationship between them and the associated plasma membrane is not well understood. Compromising the contractile ring disrupts the organization and integrity of the cleavage furrow. This indicates an intimate linkage between the two components, and elucidating the nature of this linkage will, undoubtedly, be a focus of future research.

Another outstanding issue is the mechanism of furrow progression. There is a growing consensus that, in many cell types, furrow progression involves both actomyosin-based constriction and membrane addition. Obvious issues that are now being addressed rapidly are the sources of membrane, the mechanisms of membrane addition, and when and where this occurs in the growing cleavage furrow. One issue that is currently being tackled is the understanding of the mechanisms by which these processes are coordinated. Both endocytosis and exocytosis occur during furrow progression, and much work needs to be done to characterize this relationship and the role of these reciprocal events in furrow formation. It is becoming clear that the final stage of cytokinesis – scission – is a complex process that requires sophisticated mechanisms to dismantle the contractile ring while enabling the joining of the invaginating furrows. Recent work demonstrates that Golgi-derived vesicles have a prominent role in this process and it strongly suggests that these final events are analogous to phragmoplast-based cytokinesis in plants. Although much work remains to be done to understand these final stages, the good news is that the excellent work on plant cytokinesis can be used as a guide. In addition, much is known about the mechanisms of vesicle fusion in other contexts, and many of these mechanisms are likely to be employed in animal cytokinesis.

References

1 Segui-Simarro, J.M. *et al*. (2004) Electron tomographic analysis of somatic cell plate formation in meristematic cells of *Arabidopsis* preserved by high-pressure freezing. *Plant Cell* 16, 836–856
2 Kreiner, T. and Moore, H.P.H. (1990) Membrane traffic between secretory compartments is differentially affected during mitosis. *Cell Regul.* 1, 415–424
3 Bednarek, S.Y. and Falbel, T.G. (2002) Membrane trafficking during plant cytokinesis. *Traffic* 3, 621–629
4 Schweitzer, J.K. and D'Souza-Schorey, C. (2004) Finishing the job: cytoskeletal and membrane events bring cytokinesis to an end. *Exp. Cell Res.* 295, 1–8
5 Verma, D.P.S. (2001) Cytokinesis and building of the cell plate in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 751–784
6 Glotzer, M. (2004) Cleavage furrow positioning. *J. Cell Biol.* 164, 347–351
7 Guertin, D.A. *et al*. (2002) Cytokinesis in eukaryotes. *Microbiol. Mol. Biol. Rev.* 66, 155–178
8 Maddox, A.S. and Oegema, K. (2003) Deconstructing cytokinesis. *Nat. Cell Biol.* 5, 773–776
9 Straight, A.F. and Field, C.M. (2000) Microtubules, membranes and cytokinesis. *Curr. Biol.* 10, R760–R770
10 Denis-Donini, S. *et al*. (1976) Morphological changes of the surface of the eggs of *Xenopus laevis* in the course of development. 2. Cytokinesis and early cleavage. *J. Ultrastruct. Res.* 57, 104–112
11 Fullilove, S.L. and Jacobson, A.G. (1971) Nuclear elongation and cytokinesis in *Drosophila montana*. *Dev. Biol.* 26, 560–577
12 Byers, T.J. and Armstrong, P.B. (1986) Membrane-protein redistribution during *Xenopus* first cleavage. *J. Cell Biol.* 102, 2176–2184
13 Bluemink, J.G. and de Laat, S.W. (1973) New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of *Xenopus laevis*. I. Electron microscope observations. *J. Cell Biol.* 59, 89–108
14 Leaf, D.S. *et al*. (1990) The secretory pathway is blocked between the *trans*-Golgi and the plasma membrane during meiotic maturation in *Xenopus* oocytes. *Dev. Biol.* 141, 1–12
15 Sisson, J.C. *et al*. (2000) Lava lamp, a novel peripheral Golgi protein, is required for *Drosophila melanogaster* cellularization. *J. Cell Biol.* 151, 905–917
16 Lecuit, T. and Wieschaus, E. (2000) Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. *J. Cell Biol.* 150, 849–860
17 Danilchik, M.V. *et al*. (2003) Furrow microtubules and localized exocytosis in cleaving *Xenopus laevis* embryos. *J. Cell Sci.* 116, 273–283
18 Feng, B. *et al*. (2002) Furrow-specific endocytosis during cytokinesis of zebrafish blastomeres. *Exp. Cell Res.* 279, 14–20
19 Donaldson, J.G. and Jackson, C.L. (2000) Regulators and effectors of the ARF GTPases. *Curr. Opin. Cell Biol.* 12, 475–482
20 Skop, A.R. *et al*. (2001) Completion of cytokinesis in *C. elegans* requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. *Curr. Biol.* 11, 735–746
21 Farkas, R.M. *et al*. (2003) The *Drosophila Cog5* homologue is required for cytokinesis, cell elongation, and assembly of specialized Golgi architecture during spermatogenesis. *Mol. Biol. Cell* 14, 190–200
22 Ungar, D. *et al*. (2002) Characterization of a mammalian Golgi-localized protein complex, COG, that is required for normal Golgi morphology and function. *J. Cell Biol.* 157, 405–415
23 Xu, H. *et al*. (2002) Syntaxin 5 is required for cytokinesis and spermatid differentiation in *Drosophila*. *Dev. Biol.* 251, 294–306
24 Skop, A.R. *et al*. (2004) Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* 305, 61–66
25 Colanzi, A. *et al*. (2003) Cell-cycle-specific Golgi fragmentation: how and why? *Curr. Opin. Cell Biol.* 15, 462–467
26 Altan-Bonnet, N. *et al*. (2003) A role for Arf1 in mitotic Golgi disassembly, chromosome segregation, and cytokinesis. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13314–13319
27 Drechsel, D.N. *et al*. (1997) A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos. *Curr. Biol.* 7, 12–23
28 Litvak, V. *et al*. (2002) Nir2, a human homolog of *Drosophila melanogaster* retinal degeneration B protein, is essential for cytokinesis. *Mol. Cell. Biol.* 22, 5064–5075
29 Litvak, V. *et al*. (2004) Mitotic phosphorylation of the peripheral Golgi protein Nir2 by Cdk1 provides a docking mechanism for Plk1 and affects cytokinesis completion. *Mol. Cell* 14, 319–330
30 Gerald, N.J. *et al*. (2001) Cytokinesis failure in clathrin-minus cells is caused by cleavage furrow instability. *Cell Motil. Cytoskeleton* 48, 213–223
31 Dornan, S. *et al*. (1997) α-Adaptin, a marker for endocytosis, is expressed in complex patterns during *Drosophila* development. *Mol. Biol. Cell* 8, 1391–1403
32 Swanson, M.M. and Poodry, C.A. (1981) The Shibirets mutant of *Drosophila* – a probe for the study of embryonic-development. *Dev. Biol.* 84, 465–470
33 Thompson, H.M. *et al*. (2002) The large GTPase dynamin associates with the spindle midzone and is required for cytokinesis. *Curr. Biol.* 12, 2111–2117

34 Riggs, B. *et al*. (2003) Actin cytoskeleton remodeling during early *Drosophila* furrow formation requires recycling endosomal components Nuclear-fallout and RAb11. *J. Cell Biol.* 163, 143–154

35 Dollar, G. *et al*. (2002) Rab11 polarization of the *Drosophila* oocyte: a novel link between membrane trafficking, microtubule organization, and oskar mRNA localization and translation. *Development* 129, 517–526

36 Ullrich, O. *et al*. (1996) Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* 135, 913–924

37 Pelissier, A. *et al*. (2003) Trafficking through Rab11 endosomes is required for cellularization during *Drosophila* embryogenesis. *Curr. Biol.* 13, 1848–1857

38 Hickson, G.R.X. *et al*. (2003) Arfophilins are dual Arf/Rab 11 binding proteins that regulate recycling endosome distribution and are related to *Drosophila* nuclear fallout. *Mol. Biol. Cell* 14, 2908–2920

39 Rothwell, W.F. *et al*. (1998) Nuclear-fallout, a *Drosophila* protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization. *Development* 125, 1295–1303

40 Rothwell, W.F. *et al*. (1999) The *Drosophila* centrosomal protein Nuf is required for recruiting Dah, a membrane associated protein, to furrows in the early embryo. *J. Cell Sci.* 112, 2885–2893

41 Zerial, M. and McBride, H. (2001) Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2, 107–117

42 Engqvist-Goldstein, A.E.Y. and Drubin, D.G. (2003) Actin assembly and endocytosis: from yeast to mammals. *Annu. Rev. Cell Dev. Biol.* 19, 287–332

43 Sugihara, K. *et al*. (2002) The exocyst complex binds the small GTPase RaIA to mediate filopodia formation. *Nat. Cell Biol.* 4, 73–78

44 Royou, A. *et al*. (2004) Reassessing the role and dynamics of nonmuscle myosin II during furrow formation in early *Drosophila* embryos. *Mol. Biol. Cell* 15, 838–850

45 Lauber, M.H. *et al*. (1997) The *Arabidopsis* KNOLLE protein is a cytokinesis-specific syntaxin. *J. Cell Biol.* 139, 1485–1493

46 Assaad, F.F. *et al*. (2001) The cytokinesis gene *KEULE* encodes a Sec1 protein that binds the syntaxin KNOLLE. *J. Cell Biol.* 152, 531–543

47 Heese, M. *et al*. (2001) Functional characterization of the KNOLLE-interacting t-SNARE AtSNAP33 and its role in plant cytokinesis. *J. Cell Biol.* 155, 239–249

48 Staehelin, L.A. (2003) Chloroplast structure: from chlorophyll granules to supra-molecular architecture of thylakoid membranes. *Photosynth. Res.* 76, 185–196

49 Muller, J.M.M. *et al*. (1999) An NSF function distinct from ATPase-dependent SNARE disassembly is essential for Golgi membrane fusion. *Nat. Cell Biol.* 1, 335–340

50 Schulze, K.L. *et al*. (1995) Genetic and electrophysiological studies of *Drosophila* syntaxin-1a demonstrate its role in nonneuronal secretion and neurotransmission. *Cell* 80, 311–320

51 Burgess, R.W. *et al*. (1997) The synaptic protein syntaxin1 is required for cellularization of *Drosophila* embryos. *J. Cell Biol.* 138, 861–875

52 Loncar, D. and Singer, S.J. (1995) Cell-membrane formation during the cellularization of the syncytial blastoderm of *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 2199–2203

53 Jantsch-Plunger, V. and Glotzer, M. (1999) Depletion of syntaxins in the early *Caenorhabditis elegans* embryo reveals a pole for membrane fusion events in cytokinesis. *Curr. Biol.* 9, 738–745

54 Conner, S.D. and Wessel, G.M. (1999) Syntaxin is required for cell division. *Mol. Biol. Cell* 10, 2735–2743

55 Low, S.H. *et al*. (2003) Syntaxin 2 and endobrevin are required for the terminal step of cytokinesis in mammalian cells. *Dev. Cell* 4, 753–759

56 Tuma, P.L. and Hubbard, A.L. (2003) Transcytosis: crossing cellular barriers. *Physiol. Rev.* 83, 871–932

57 Horton, A.C. and Ehlers, M.D. (2003) Neuronal polarity and trafficking. *Neuron* 40, 277–295

58 Mazumdar, A. and Mazumdar, M. (2002) How one becomes many: blastoderm cellularization in *Drosophila melanogaster*. *Bioessays* 24, 1012–1022

59 Lecuit, T. *et al*. (2002) *slam* encodes a developmental regulator of polarized membrane growth during cleavage of the *Drosophila* embryo. *Dev. Cell* 2, 425–436

60 Afshar, K. *et al*. (2000) Functional analysis of the *Drosophila* Diaphanous FH protein in early embryonic development. *Development* 127, 1887–1897

61 Dobbelaere, J. and Barral, Y. (2004) Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. *Science* 305, 393–396

62 Schmidt, K. and Nichols, B.J. (2004) A barrier to lateral diffusion in the cleavage furrow of dividing mammalian cells. *Curr. Biol.* 14, 1002–1006

63 Wachtler, V. *et al*. (2003) Sterol-rich plasma membrane domains in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* 116, 867–874

64 Emoto, K. *et al*. (1996) Redistribution of phosphatidylethanolamine at the cleavage furrow of dividing cells during cytokinesis. *Proc. Natl. Acad. Sci. U. S. A.* 93, 12867–12872

65 Emoto, K. and Umeda, M. (2000) An essential role for a membrane lipid in cytokinesis: regulation of contractile ring disassembly by redistribution of phosphatidylethanolamine. *J. Cell Biol.* 149, 1215–1224

66 Becchetti, A. and Whitaker, M. (1997) Lithium blocks cell cycle transitions in the first cell cycles of sea urchin embryos, an effect rescued by *myo*-inositol. *Development* 124, 1099–1107

67 Brill, J.A. *et al*. (2000) A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. *Development* 127, 3855–3864

68 Desautels, M. *et al*. (2001) Cdc4p, a contractile ring protein essential for cytokinesis in *Schizosaccharomyces pombe*, interacts with a phosphatidylinositol 4-kinase. *J. Biol. Chem.* 276, 5932–5942

69 Zhang, Y. *et al*. (2000) Phosphatidylinositol 4-phosphate 5-kinase Its3 and calcineurin Ppb1 coordinately regulate cytokinesis in fission yeast. *J. Biol. Chem.* 275, 35600–35606

70 Saul, D. *et al*. (2004) Continuous phosphatidylinositol metabolism is required for cleavage of crane fly spermatocytes. *J. Cell Sci.* 117, 3887–3896

71 Muller, I. *et al*. (2003) Syntaxin specificity of cytokinesis in *Arabidopsis*. *Nat. Cell Biol.* 5, 531–534

72 Rappaport, R. (1996) *Cytokinesis in Animal Cells (Developmental and Cell Biology Series)*, Cambridge University Press

73 Hoekstra, D. *et al*. (2004) The subapical compartment: a traffic center in membrane polarity development. *J. Cell Sci.* 117, 2183–2192

74 Matuliene, J. and Kuriyama, R. (2002) Kinesin-like protein CHO1 is required for the formation of midbody matrix and the completion of cytokinesis in mammalian cells. *Mol. Biol. Cell* 13, 1832–1845

75 Hill, E. *et al*. (2000) The Rab6-binding kinesin, Rab6-KIFL, is required for cytokinesis. *EMBO J.* 19, 5711–5719

76 Giansanti, M.G. *et al*. (1998) Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. *Genes Dev.* 12, 396–410

77 Williams, B.C. *et al*. (1995) The *Drosophila* kinesin-like protein Klp3a is a midbody component required for central spindle assembly and initiation of cytokinesis. *J. Cell Biol.* 129, 709–723

78 Abaza, A. *et al*. (2003) M phase phosphoprotein 1 is a human plus-end-directed kinesin-related protein required for cytokinesis. *J. Biol. Chem.* 278, 27844–27852

79 Raich, W.B. *et al*. (1998) Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. *Mol. Biol. Cell* 9, 2037–2049

80 Powers, J. *et al*. (1998) A nematode kinesin required for cleavage furrow advancement. *Curr. Biol.* 8, 1133–1136

81 Adams, R.R. *et al*. (1998) *pavarotti* encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis. *Genes Dev.* 12, 1483–1494

82 Fan, J. and Beck, K.A. (2004) A role for the spectrin superfamily member Syne-1 and kinesin II in cytokinesis. *J. Cell Sci.* 117, 619–629

83 Schweitzer, J.K. and D'Souza-Schorey, C. (2002) Localization and activation of the ARF6 GTPase during cleavage furrow ingression and cytokinesis. *J. Biol. Chem.* 277, 27210–27216

84 Boman, A.L. *et al*. (1999) Arf proteins bind to mitotic kinesin-like protein 1 (MKLP1) in a GTP-dependent fashion. *Cell Motil. Cytoskeleton* 44, 119–132

85 Neef, R. *et al*. (2003) Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis. *J. Cell Biol.* 162, 863–875