REVIEW

Molecular control of animal cell cytokinesis

Juan Pablo Fededa and Daniel W. Gerlich

Cytokinesis is the process by which mitotic cells physically split in two following chromosome segregation. Dividing animal cells first ingress a cytokinetic furrow and then separate the plasma membrane by abscission. The general cytological events and several conserved molecular factors involved in cytokinesis have been known for many years. However, recent progress in microscopy, chemical genetics, biochemical reconstitution and biophysical methodology has tremendously increased our understanding of the underlying molecular mechanisms. We discuss how recent insights have led to refined models of the distinct steps of animal cell cytokinesis, including anaphase spindle reorganization, division plane specification, actomyosin ring assembly and contraction, and abscission. We highlight how molecular signalling pathways coordinate the individual events to ensure faithful partitioning of the genome to emerging daughter cells.

Animal cell cytokinesis is initiated during anaphase, when the mitotic spindle reorganizes to form a dense array of antiparallel microtubules midway between the two centrosomal asters — the central spindle (or spindle midzone; Fig. 1). Together with microtubules from the spindle asters, the central spindle defines the position of the division plane between the segregated chromosomes. This spatial signal is transmitted through a pathway involving the small GTPase RhoA, leading to the assembly of an actomyosin ring at the equatorial cell cortex. Contraction of the actomyosin ring results in ingression of the attached plasma membrane to form a cytokinetic furrow, which partitions the cytoplasm into two domains. At this stage, sister cells remain connected by a narrow intercellular bridge containing dense antiparallel bundles of microtubules that overlap at a central region termed the midbody (or Flemming body). Physical separation of the emerging sister cells is finally accomplished by plasma membrane fission at the intercellular bridge.

Faithful inheritance of the genome requires tight temporal coordination of cytokinesis with chromosome segregation. This is achieved by a common molecular cue, the activation of the E3 ubiquitin ligase anaphase promoting complex (APC), which initiates both chromosome segregation and cytokinetic furrow ingression[1]. The APC triggers chromosome segregation by targeting securin, an inhibitor of the protease separase that destroys the cohesive link between sister chromatids, for proteasome-mediated destruction. Simultaneous targeting of the cyclin-dependent kinase 1 (CDK1) coactivator cyclin B for degradation leads to CDK1 inactivation, resulting in dephosphorylation of many CDK1 substrates by the counteracting phosphatases[2], which promotes cytokinetic furrow ingression and mitotic exit. Finally, abscission is temporally coordinated with completion of chromosome segregation by a signalling pathway involving the Aurora B mitotic kinase[3–5].

The timing of individual events, as well as the cellular mechanism of cytokinesis, is distinct in different model organisms. For example, the division plane in budding yeast is determined by the bud neck long before mitosis, and the mitotic spindle subsequently aligns perpendicularly. Actomyosin ring contraction occurs in budding yeast, but is not essential for cytokinesis, presumably because assembly of a septum can substitute its function to ensure efficient partitioning of the cells[6]. In fission yeast, the division plane is specified by the position of the cell nucleus during early mitosis[7]. Plant cells do not contain actomyosin rings but instead assemble a separating membrane and cell wall through Golgi-derived secretion at a specialized structure termed the phragmoplast[8]. The diversity of cytokinesis mechanisms in different model organisms has been covered in several excellent reviews[9–11].

In this Review, we discuss the cellular mechanisms and signalling pathways of animal cell cytokinesis, focusing on recent advances and discussing how they have led to a refined model of this process.

Central spindle assembly

Animal cell cytokinesis is initiated during anaphase, when the decline of CDK1 activity leads to a stabilization of microtubules and reorganization of the mitotic spindle. The assembly of the central spindle is an early key event, as it contributes to division plane specification and provides the template for the midbody, a targeting platform for abscission factors (Fig. 2).

The central spindle is built from antiparallel bundles of microtubules that overlap at a central region, where microtubule-associated proteins, motor proteins and protein kinases accumulate[12]. Microtubules of the central spindle partly derive from interpolar microtubules of the metaphase spindle, but also assemble *de novo* during anaphase through non-centrosomal microtubule nucleation mediated by the augmin complex[13,14] (Fig. 2a).

The microtubules of the central spindle are spatially organized by bundling factors that bind to overlapping microtubule plus ends.

Juan Pablo Fededa and Daniel W. Gerlich: Institute of Biochemistry, Swiss Federal Institute of Technology Zurich, CH-8093 Zurich, Switzerland.
Present address: Institute of Molecular Biotechnology of the Austrian Academy of Sciences, 1030 Vienna, Austria.
e-mail: daniel.gerlich@imba.oeaw.ac.at

	Metaphase	Anaphase	Telophase	
CDK1	High	Low	Inactive	
Aurora B	Centromeres	Central spindle	Midbody	Midbody derivative; inactive
PLK1	Kinetochores centrosomes	Central spindle	Degraded	

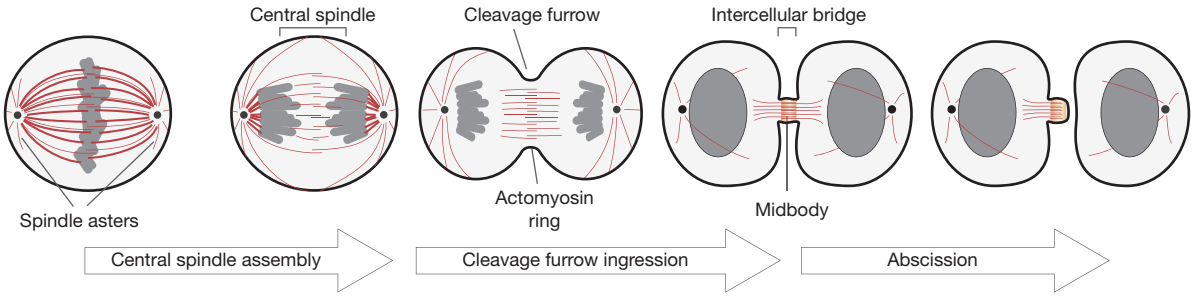

Figure 1 Overview of animal cell cytokinesis. Top: the activity and localization of key kinases. Bottom: a schematic representation showing the reorganization of an animal cell progressing through the different stages of cytokinesis. Red, microtubules; grey, chromosomes.

One key component is the microtubule bundling protein required for cytokinesis 1 (PRC1)[15], which as a homodimer selectively binds to the interface between antiparallel microtubules[16,17]. PRC1 is inhibited until anaphase onset by CDK1-mediated phosphorylation, which prevents its dimerization[18].

Another essential component of the central spindle is the tetrameric centralspindlin complex, which consists of two copies of the kinesin motor protein MKLP1 and the Rho-family GTPase activating protein (GAP) CYK-4 (also termed MgcRacGAP)[19,20]. Centralspindlin binds to the central spindle as higher-order clusters that travel along microtubules towards the plus ends to accumulate at the central region[21]. Several mechanisms control centralspindlin targeting; MKLP1 affinity to microtubules is negatively regulated by CDK1 phosphorylation of its motor domain[22], and phosphorylation of MKLP1 by Aurora B during anaphase[23] promotes centralspindlin cluster formation by triggering release from the inhibitory 14-3-3 protein[24].

The chromosomal passenger complex (CPC), a multi-subunit protein complex that contains Aurora B, is a third essential factor in central spindle assembly. During metaphase, the CPC localizes to centromeres, where it regulates attachment of chromosomes to the mitotic spindle. At anaphase onset, the CPC relocates to the central spindle, which requires the kinesin motors MKLP1 and MKLP2 in mammalian cells[25,26], but not in *Caenorhabditis elegans*[27]. This translocation depends on the removal of a CDK1 phosphorylation from the INCENP subunit of the CPC, enabling INCENP binding to MKLP2 (ref. 28). The translocation of Aurora B to the central spindle also depends on its efficient removal from anaphase chromatin, which is promoted by the E3 ubiquitin ligase Cul3 (ref. 29) and the ATPase p97 (ref. 30). An important function of the CPC is the phosphoregulation of other central spindle components such as PRC1 (ref. 31) or MKLP1 (ref. 23), but it may also mediate microtubule bundling as a structural component of the central spindle[12]. Besides its function in central spindle assembly, the CPC also contributes directly to actomyosin ring assembly[32].

A mechanism of central spindle self-assembly has been recently suggested on the basis of biochemical *in vitro* reconstitution experiments with a minimal set of components (Fig. 2b). In this system, the specific recognition of antiparallel microtubule overlaps by PRC1, which binds in a manner that supports sliding of microtubules, enables assembly of dynamic central-spindle-like structures[16,17]. The kinesin KIF4A (Xklp1 in *Xenopus laevis*) is targeted to microtubule overlap regions by PRC1 and adjusts the length of the overlap zone by inhibiting microtubule polymerization and dynamic instability[16,33].

In cells, the central spindle assembly mechanism must be more complex, because the localization of central spindle core components (PRC1, centralspindlin and the CPC) is mutually interdependent, and all these components are essential for central spindle assembly[12]. Multiple microtubule bundling factors may be required in cells to stabilize the central spindle when declining CDK1 activity simultaneously promotes disassembly of astral spindle microtubules. Experiments with chemically induced monopolar spindles showed that antiparallel microtubules are not strictly required for microtubule binding of central spindle factors and their delivery towards the cytokinetic furrow[34,35]. The finding that monopolar spindles are able to induce cell polarization and cytokinesis may be explained by interactions of microtubules with chromosomes[34] or the actin cytoskeleton[35].

Although the mitotic kinase PLK1 is not required for central spindle assembly, its localization at the central spindle is essential for division plane specification (see below). PLK1 is targeted to the central spindle through binding to PRC1 after dephosphorylation of an inhibitory CDK1 site on PRC1 (ref. 36), and by binding to PLK1-phosphorylated MKLP2 (ref. 37).

In summary, a decline of CDK1 activity at the metaphase–anaphase transition leads to dephosphorylation of inhibitory sites on multiple central spindle components. This initiates a stepwise self-assembly process that involves preferential binding of microtubule bundling factors to antiparallel microtubule overlap regions.

Division plane specification

Precise positioning of the division plane between the two masses of segregated chromosomes is essential to prevent chromosome loss. In animal cells, the division plane is specified by transmission of a spindle position signal to the cell cortex during early anaphase (Fig. 3a). The position of the spindle is controlled during tissue morphogenesis through mechanical, geometrical and biochemical signals[38–41].

How the mitotic spindle defines the division plane position has been a matter of intense debate, owing to controversial results obtained in

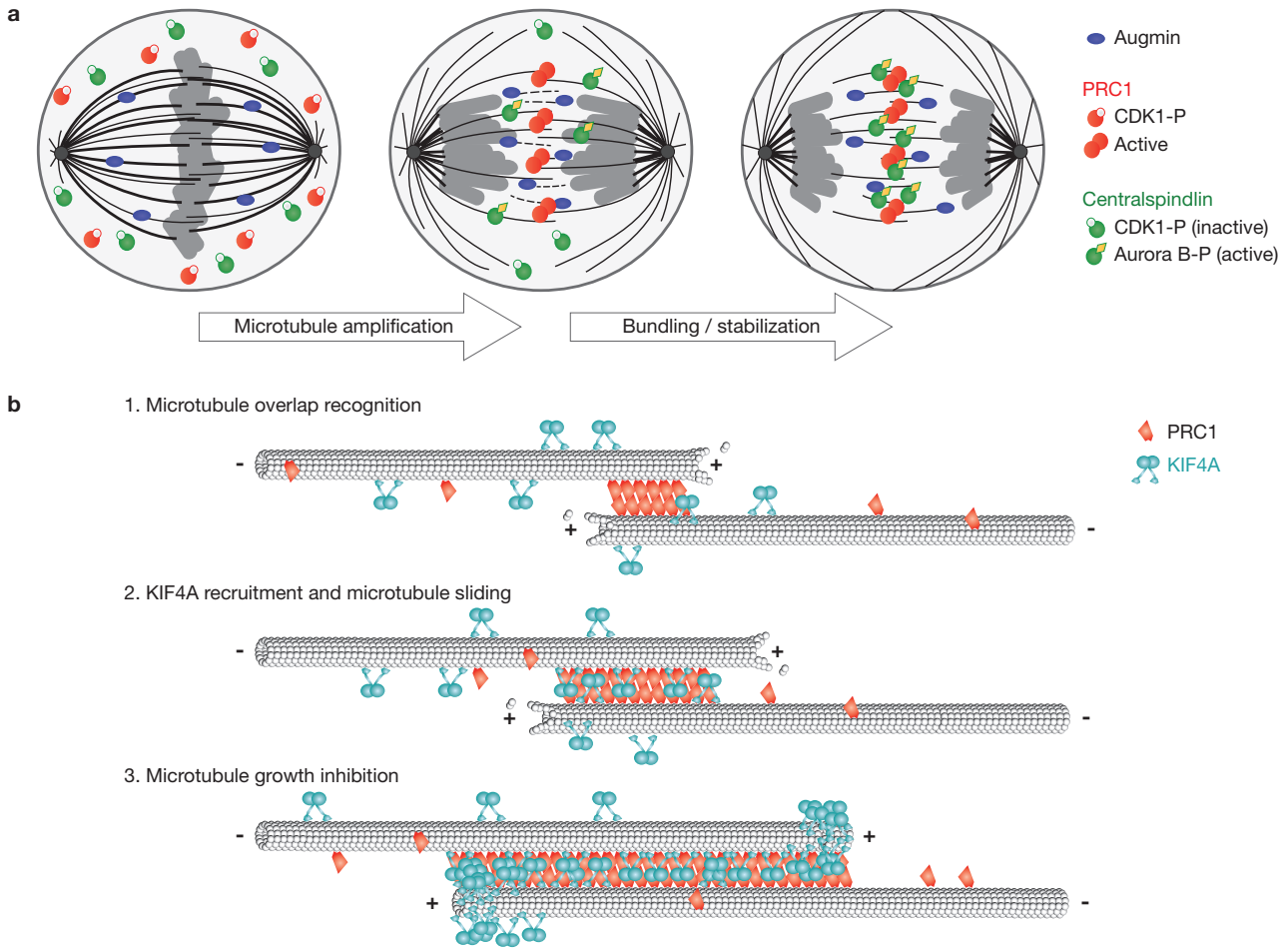

Figure 2 Central spindle assembly. (**a**) Localization and activity of central spindle assembly factors. During anaphase, the augmin protein complex promotes *de novo* assembly of microtubules (dashed black lines), which together with interpolar microtubules (solid black lines) form an array of antiparallel microtubules at the central spindle. The overlap is stabilized by PRC1 after removal of an inhibitory CDK1 phosphorylation. Dephosphorylation of a CDK1 site and phosphorylation of an Aurora B site on MKLP1 (a component of the centralspindlin complex, green) promotes its binding and bundling of central spindle microtubules. (**b**) Model for central spindle self-assembly. Dimers of PRC1 specifically recognize antiparallel microtubule overlap regions, but still allow microtubule sliding. KIF4A then binds to microtubules and moves towards the plus end to stabilize the overlap zone by inhibiting dynamic instability of microtubules. Adapted with permission from ref. 16.

different model organisms[42]. Genetic and laser-micromanipulation studies in *C. elegans* embryos eventually clarified that the spindle sends two redundant signals to the cell cortex, one originating from the central spindle, and a second signal deriving from the spindle asters[43–45]. The predominance of either signal varies between cell types and organisms[42].

Spindle microtubules can provide positional cues by direct contact with the cell cortex[34,42,45–49] (Fig. 3a). Stable microtubules have been proposed to provide signals that promote contractility at the equatorial cortex, in contrast to microtubules with higher dynamic instability that inhibit cortical contractility at the poles[49,50]. Another model proposes that microtubules generally inhibit cortical contractility, thus leading to cytokinetic furrow formation at equatorial regions with minimal microtubule density[44]. Further studies are needed to clarify the regulatory role of astral microtubules in division plane specification.

The central spindle also contributes to the specification of the division plane by promoting concentration and activation of the small GTPase RhoA at the equatorial cortex[46,51,52] (Fig. 3b). Like most other small GTPases, RhoA is regulated by guanine-nucleotide exchange factors (GEFs) and a GAP. Activation of equatorial RhoA critically depends on Rho-GEF ECT2 (Pebble in *Drosophila melanogaster*, and LET-21 or ECT-2 in nematodes), which is localized to the central spindle by binding to the CYK-4 subunit of centralspindlin following CYK-4 phosphorylation by PLK1 (refs 51–55). ECT-2 translocates to the equatorial cell cortex after CDK1 inactivation, thereby temporally coordinating cytokinesis with chromosome segregation[56]. As well as ECT2, GEF-H1 may further activate RhoA at the cell cortex[57]. However, defining the exact role of GEF-H1 in cytokinesis requires further experimental investigation, owing to the pleiomorphic loss-of-function phenotypes.

RhoA activation at the equatorial cortex also depends on the GAP activity of CYK-4 (refs 58,59). CYK-4 GAP has been suggested to promote a constant cycling of RhoA through GDP- and GTP-bound states, which may be required for RhoA activity[58]. Alternatively, CYK-4 GAP may activate RhoA by positive regulation of ECT-2 (ref. 59). It is controversial whether the GAP domain of CYK-4 also regulates another small GTPase, Rac (refs 59,60). Genetic disruption of Rac renders furrow ingression partially insensitive to mutation of CYK-4 GAP activity, which has led to the proposal that suppression of Rac by CYK-4 is needed to prevent

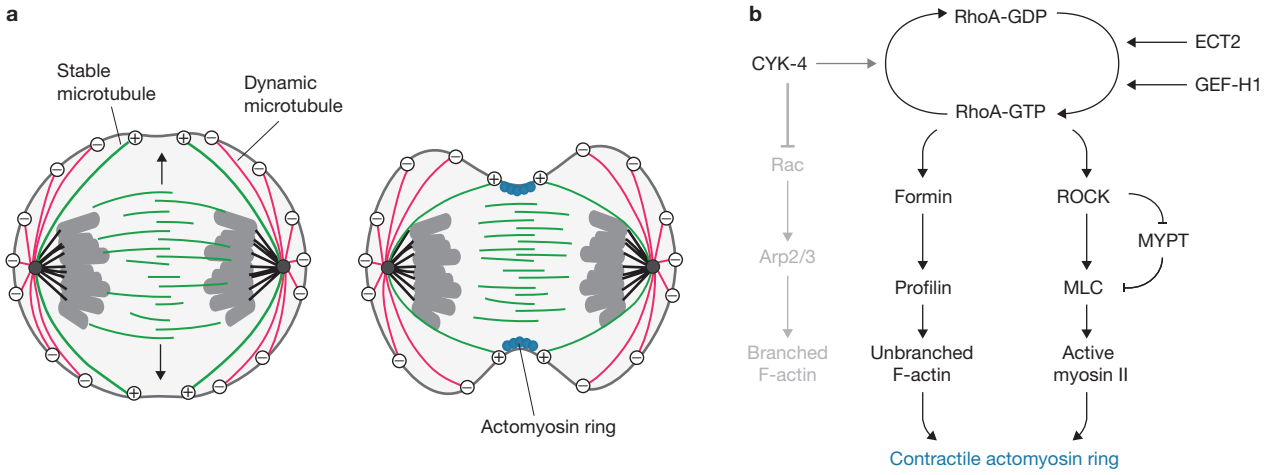

Figure 3 Division plane specification and the RhoA pathway. (**a**) The central spindle stimulates equatorial contractility (arrows). Stable microtubules (green) from the spindle asters can also stimulate cortical contractility, whereas dynamic microtubules (red) inhibit cortical contractility. The actomyosin ring assembles at the specified division site of the equatorial cortex. (**b**) ECT2, GEF-H1, and CYK-4 regulate the Rho-GTP cycle. Active RhoA-GTP promotes polymerization of unbranched actin filaments and activates myosin II to assemble and contract the actomyosin ring. CYK-4 may also inhibit Rac, which could be important to suppress branched actin filament network formation at the cytokinetic furrow.

branched actin filament nucleation by the Rac effector Arp2/3 complex at the equatorial cell cortex[60]. Genetic inactivation of Rac, however, could also facilitate cytokinetic furrow ingression independently of CYK-4; for example, by reducing cortical tension[59].

So how do spindle-localized division plane regulators reach their targets at the cell cortex? One possibility is transport along microtubules that contact the equatorial cell cortex; for example, through the motor activity of MKLP1 (ref. 61). Communication between the central spindle and cortex may also proceed along actin cables, as observed in chemically induced monopolar mitosis[35]. A fluorescence resonance energy transfer biosensor for Aurora B phosphorylation revealed a phosphorylation gradient around the central spindle[62], consistent with a diffusible signal transmission between cortex and central spindle[63]. Future studies will be needed to clarify which of these spindle–cortex communication mechanisms is most relevant for division plane specification.

Overall, these studies indicate that a combination of stimulatory and inhibitory signals from the mitotic apparatus lead to increased contractility at the equatorial cortex and relaxation at the polar cortex. The multitude and partial redundancy of signals may be required to make the system robust and to increase spatial precision.

Contractile ring assembly

The RhoA pathway promotes assembly of the actomyosin ring by two main effectors (Fig. 3b). First, RhoA stimulates nucleation of unbranched actin filaments by activation of Diaphanous-related formins[64–66]. Second, RhoA promotes myosin II activation by the kinase ROCK, which activates myosin II directly by phosphorylation of the myosin light chain and also inhibits myosin phosphatase by phosphorylation of the phosphatase-targeting subunit MYPT (ref. 67).

Actin and myosin II bind to the cell cortex independently, concentrate at the cell equator by several distinct mechanisms, and preferentially accumulate directly at the site where the contractile ring forms[45,61,68–70]. Additional actin filaments[68,71], and in some organisms also myosin II patches[45,69,70], move towards the cell equator by cortical flow. During a specialized form of cytokinesis, cellularization of *Drosophila* embryos, spindle microtubules also contribute to actin delivery to the cleavage site[72].

Besides actin and myosin II, the contractile ring contains the scaffolding protein anillin[73]. Anillin binds to actin, myosin, RhoA and CYK-4, and thereby links the equatorial cortex with the signals from the central spindle[74–76]. This is particularly important for late stages of furrow ingression[53,74,77]. Anillin has also been proposed to contribute to the linkage of the actomyosin ring to the plasma membrane[73]. The organization and function of the contractile ring further involves actin crosslinking proteins[78], septin filaments[79–82] and specific lipids[83].

Cytokinetic furrow ingression

Following its assembly, contraction of the actomyosin ring leads to ingression of the attached plasma membrane, which partitions the cytoplasm into two domains of emerging sister cells. Despite its central importance in cell division, the force-generating mechanism of actomyosin ring contraction is not understood. Several different models have been proposed on the basis of ultrastructural analysis and biophysical considerations.

A classical model proposes that bipolar myosin filaments move along actin filaments similarly to the force-generating mechanism in muscle sarcomeres[84]. Supporting this model, filamentous myosin II has been visualized at the cytokinetic furrow by total internal reflection fluorescence microscopy[61,68,69], and mutant myosin II that cannot polymerize is unable to promote cytokinesis[85]. For a 'purse-string' mechanism to generate force, alignment of filaments with the division plane is needed, and has been observed by electron microscopy in a number of organisms[84,86–88].

However, many actin filaments of the contractile ring are oriented in directions other than along the division plane[78,89]. It is not clear whether non-aligned actin filaments contribute to ring contraction, although randomly oriented actin filaments can contract *in vitro* by gelation[90]. Contraction of an interconnected actin network could be driven by motor activity, or by depolymerization when filaments are linked by end-tracking crosslinkers[91].

Photobleaching experiments revealed a dynamic turnover of actin[71] and myosin[69,70] at the contractile ring, indicating that the filament network of the actomyosin ring is constantly remodelled. However, stable pools of actin[71] and myosin[92] have also been observed at the actomyosin ring with variable abundance in different species. To what extent the stable and dynamic pools of actin and myosin filaments contribute to contractile force generation is not known, but theoretical modelling provides an interesting framework for further experimental investigation[93,94].

The concentration of actin and myosin per unit length remains constant during the early stages of furrow ingression in urchins[84] and *C. elegans*[92], indicating that contractile ring components disassemble with the same rate as they contract. The *C. elegans* furrow ingresses with a constant rate throughout the initial phases of ring contraction and the ingression speed correlates with the initial perimeter[92,95]. This has led to a model proposing that the contractile ring is built from a series of contractile units that shorten simultaneously, and where the number of units is defined by the original perimeter of the ring[92].

Efficient furrow ingression also requires reduction of contractility at polar cortex regions[48]. Misregulated polar cortex contractility (for example, by astral microtubule stabilization) leads to unstable and oscillating furrows[96]. Stabilization of the cytokinetic furrow position involves dampening of cytoplasmic and cortex fluctuations by plasma membrane blebbing[97].

The ingressing cytokinetic furrow needs a supply of additional membrane as the total cell surface increases. In embryos of echinoderms, *Xenopus, Drosophila* and *C. elegans*, this involves targeted secretion of vesicles that travel along microtubules towards the furrow[72,98–102]. Targeted secretion also delivers specific lipids to the equatorial cortex, thus contributing to the assembly and function of the contractile ring[83,103,104].

Our current knowledge thus provides a consistent picture of RhoA-stimulated actin and myosin II filament formation at the equatorial cortex. The exact spatial arrangement of the filaments and the force-generating mechanism of the contractile ring, however, remain key open questions.

Abscission

The cytokinetic furrow ingresses until the actomyosin ring has reached a diameter of about 1–2 μm. Most animal cell types then remain connected by an intercellular bridge for up to several hours until they are split by an actin-independent process termed abscission[105,106]. Abscission proceeds by removal of cytoskeletal structures from the intercellular bridge, constriction of the cell cortex, and plasma membrane fission (Fig. 4).

The intercellular bridge is filled with dense bundles of antiparallel microtubules that derive from the central spindle. These microtubules overlap at the midbody, which also contains an electron-dense matrix of unknown composition. More than 100 different proteins localize at the intercellular bridge[107], but the specific function of many components remains unclear. Generally, the midbody is thought to provide a targeting platform for the abscission machinery.

Briefly after complete cytokinetic furrow ingression, Golgi- and endosome-derived vesicles accumulate at regions adjacent to the midbody[108–110]. Vesicles in the intercellular bridge fuse with the plasma membrane before abscission[108–110], and several vesicle-targeting and tethering factors, including centriolin and the exocyst complex[108], Rab35 (ref. 111), Rab11 (ref. 112), v- and t-SNARES (refs 108,113) and BRUCE (ref. 114), are required for efficient abscission. These observations are consistent with a compound vesicle fusion model of abscission, which assumes that a separating membrane assembles inside the intercellular bridge, analogous to cytokinesis in plant cells[108,115].

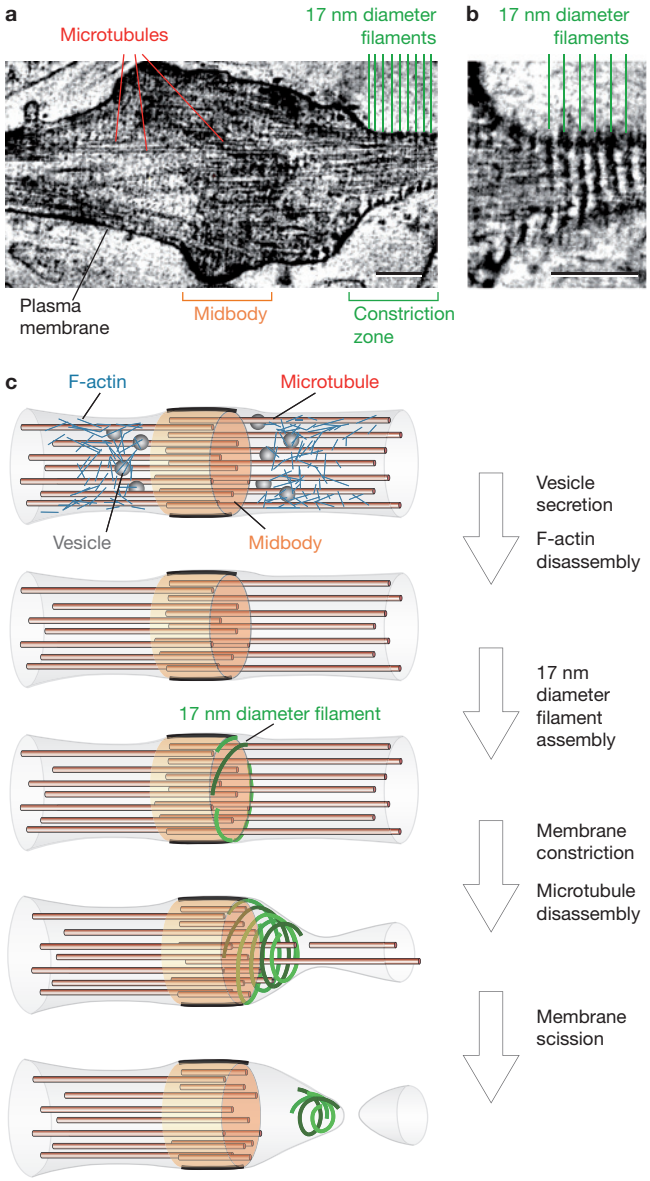

Figure 4 The intercellular bridge and abscission. (**a**) Electron tomogram of a late-stage intercellular bridge. 17 nm diameter filaments assemble at a cortical constriction zone adjacent to the midbody. (**b**) Enlarged grazing section of **a** reveals a regular array of 17 nm diameter filaments. Scale bars, 200 nm. **a** and **b** reprinted with permission from ref. 116. (**c**) Speculative model for abscission mechanism.

Kinetic studies of mammalian tissue culture cells, however, showed that vesicles disappear from the intercellular bridge before abscission[105,109,116]. Larger internal membrane structures resembling cell plate formation in plant cytokinesis have not been observed in animal cell intercellular bridges[110,116]. Electron microscopy of late-stage intercellular bridges instead revealed cortical constriction zones adjacent to the midbody that contain membrane-associated 17 nm diameter filaments forming large intertwined helices surrounding the intercellular bridge[116] (Fig. 4a,b). The secondary constriction of the cell cortex indicates that abscission may proceed by direct contact and fission of opposing plasma membranes[105,110,116] (Fig. 4c).

The endosomal sorting complex required for transport (ESCRT)-III is a candidate component of the 17 nm diameter filaments because it co-localizes with constriction zones[97,99] and is required for the formation of these filaments[116]. ESCRT-III is an essential abscission factor[116–119] that mediates membrane deformation and scission from the cytosolic face in a variety of biological processes, including virus budding, intraluminal vesicle budding and autophagy[120]. Recombinant ESCRT-III subunits can form polymers in the shape of filaments, rings, sheets or tubes *in vitro*, providing an interesting framework to speculate about mechanisms by which 17 nm filaments may generate constriction force during abscission[121]. For example, ESCRT-III may constrict the intercellular bridge by inward-directed curvature of filaments during polymerization, or by filament remodelling after assembly of 17 nm filament helices. Alternatively, ESCRT-III may narrow membrane necks by the capture and stabilization of spontaneous lipid-driven membrane bulging.

ESCRT-III accumulates at cortical regions adjacent to the midbody during late telophase[116,122]. This is regulated by the centrosomal protein Cep55, which binds to the midbody component MKLP1 after removal of an inhibitory phosphate on PLK1, once PLK1 is degraded by the APC (ref. 123). Cep55 then recruits the ESCRT-III targeting factor ALIX to the midbody[117–119]. Further factors contribute to ESCRT-III targeting to the abscission site, including Tsg101 (ref. 117) and FYVE-CENT (ref. 124).

Complete cortical constriction at the abscission site requires removal of the underlying cytoskeletal structures. Actin filament disassembly during late cytokinesis depends on the PKCε–14-3-3 complex, which inactivates RhoA after furrow ingression[125]. Actin disassembly is further controlled by the GTPase Rab35 and its effector, the phosphatidylinositol-4,5-bisphosphate 5-phosphatase OCRL (refs 111,126).

Disassembly of microtubule bundles inside the intercellular bridge depends on the microtubule severing protein spastin[116,127], which binds to midbody-localized ESCRT-III-associated protein CHMP1B (refs 116,127,128). Spastin may be targeted to the abscission site by high levels of tubulin polyglutamylation within the intercellular bridge[129]. Complementary to spastin-mediated severing, a high local curvature at the constriction site may also facilitate microtubule disassembly[110].

Despite the progress in understanding regulation of individual abscission factors, the overall temporal control of abscission is still poorly understood. Abscission occurs only after removal of all chromatin from the division site, as the abscission machinery may otherwise damage unsegregated chromosomes, or fail due to mechanical hindrance. A tight temporal coordination between chromosome segregation and cytokinesis is ensured by the Aurora B kinase, which is kept active by unsegregated chromatin at the division plane to inhibit abscission until the division plane is cleared of chromatin[3–5]. A recent study further indicates that abscission is temporally coordinated with postmitotic nuclear envelope reassembly[130].

Cytokinesis failure results in tetraploid cells with extra centrosomes that are genetically instable owing to perturbed chromosome segregation in subsequent cell divisions[131,132]. Tetraploid cells derived from experimentally perturbed cytokinesis induce cancer in a mouse model[133], indicating that understanding the molecular control of cytokinesis may help to elucidate cellular defects underlying carcinogenesis.

Following abscission, the residual midbody structure, known as the midbody derivative, can have different fates depending on the cell type. The midbody derivative is either released to the extracellular medium[116,134], degraded by autophagy[135,136] or persists in the cytoplasm[108,134,135]. Asymmetric accumulation of midbody derivatives has been proposed to contribute to the maintenance of an undifferentiated phenotype in stem cells and cancer cells[134,135]. The asymmetric accumulation of midbody derivatives is regulated by their selective removal from differentiating daughter cells either by autophagy[135] or shedding to the extracellular medium[134]. The mechanism by which midbody derivatives contribute to cell fate specification, however, is not known.

In summary, abscission proceeds by a secondary ingression of the cell cortex, involving helices of 17 nm diameter filaments spanning the intercellular bridge. Understanding the mechanism by which the plasma membrane ultimately splits and how vesicles contribute to abscission will require further investigation.

Concluding remarks and future perspectives

The molecular pathways regulating animal cell cytokinesis are now relatively well defined, owing to recent advances in imaging technology, biochemical reconstitution systems, chemical genetics and physical perturbation tools. However, the mechanisms of the force-generating structures are still poorly understood. How exactly are actin and myosin II filaments arranged in the contractile ring and generate contractile force? How do 17 nm diameter filaments assemble and how do they generate constriction force during abscission? Does the final fission of the plasma membrane proceed by a rupture-resealing mechanism, or by membrane hemifusion-fission? Answering these questions will need improved analytical tools to study these processes in cells. New super-resolution microscopy techniques[137] and new labelling strategies in electron microscopy[138] are promising developments. Dissecting the underlying molecular mechanisms will further require sophisticated biochemistry, and recent progress in reconstitution of complex structures like central spindle microtubule arrays[16,17], or ESCRT-III-mediated membrane fission[139], indicates that we face exciting times uncovering the remaining mysteries in cytokinesis.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

1. Pines, J. Mitosis: a matter of getting rid of the right protein at the right time. *Trends Cell Biol.* **16,** 55–63 (2006).
2. Wurzenberger, C. & Gerlich, D. W. Phosphatases: providing safe passage through mitotic exit. *Nat. Rev. Mol. Cell Biol.* **12,** 469–482 (2011).
3. Steigemann, P. *et al.* Aurora B-mediated abscission checkpoint protects against tetraploidization. *Cell* **136,** 473–484 (2009).
4. Norden, C. *et al.* The NoCut pathway links completion of cytokinesis to spindle midzone function to prevent chromosome breakage. *Cell* **125,** 85–98 (2006).
5. Mendoza, M. *et al.* A mechanism for chromosome segregation sensing by the NoCut checkpoint. *Nat. Cell Biol.* **11,** 477–483 (2009).
6. Bi, E. *et al.* Involvement of an actomyosin contractile ring in Saccharomyces cerevisiae cytokinesis. *J. Cell Biol.* **142,** 1301–1312 (1998).
7. Daga, R. R. & Chang, F. Dynamic positioning of the fission yeast cell division plane. *Proc. Natl Acad. Sci. USA* **102,** 8228–8232 (2005).
8. Jurgens, G. Plant cytokinesis: fission by fusion. *Trends Cell Biol.* **15,** 277–283 (2005).
9. Barr, F. A. & Gruneberg, U. Cytokinesis: placing and making the final cut. *Cell* **131,** 847–860 (2007).
10. Balasubramanian, M. K., Bi, E. & Glotzer, M. Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. *Curr. Biol.* **14,** R806–R818 (2004).
11. Otegui, M. S., Verbrugghe, K. J. & Skop, A. R. Midbodies and phragmoplasts: analogous structures involved in cytokinesis. *Trends Cell Biol.* **15,** 404–413 (2005).
12. Glotzer, M. The 3Ms of central spindle assembly: microtubules, motors and MAPs. *Nat. Rev. Mol. Cell Biol.* **10,** 9–20 (2009).

13. Uehara, R. & Goshima, G. Functional central spindle assembly requires *de novo* microtubule generation in the interchromosomal region during anaphase. *J. Cell Biol.* **191,** 259–267 (2010).
14. Uehara, R. *et al.* The augmin complex plays a critical role in spindle microtubule generation for mitotic progression and cytokinesis in human cells. *Proc. Natl Acad. Sci. USA* **106,** 6998–7003 (2009).
15. Jiang, W. *et al.* PRC1: a human mitotic spindle-associated CDK substrate protein required for cytokinesis. *Mol. Cell* **2,** 877–885 (1998).
16. Bieling, P., Telley, I. A. & Surrey, T. A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps. *Cell* **142,** 420–432 (2010).
17. Subramanian, R. *et al.* Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein. *Cell* **142,** 433–443 (2010).
18. Zhu, C., Lau, E., Schwarzenbacher, R., Bossy-Wetzel, E. & Jiang, W. Spatiotemporal control of spindle midzone formation by PRC1 in human cells. *Proc. Natl Acad. Sci. USA* **103,** 6196–6201 (2006).
19. Mishima, M., Kaitna, S. & Glotzer, M. Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. *Dev. Cell* **2,** 41–54 (2002).
20. Pavicic-Kaltenbrunner, V., Mishima, M. & Glotzer, M. Cooperative assembly of CYK-4/MgcRacGAP and ZEN-4/MKLP1 to form the centralspindlin complex. *Mol. Biol. Cell* **18,** 4992–5003 (2007).
21. Hutterer, A., Glotzer, M. & Mishima, M. Clustering of centralspindlin is essential for its accumulation to the central spindle and the midbody. *Curr. Biol.* **19,** 2043–2049 (2009).
22. Mishima, M., Pavicic, V., Gruneberg, U., Nigg, E. A. & Glotzer, M. Cell cycle regulation of central spindle assembly. *Nature* **430,** 908–913 (2004).
23. Guse, A., Mishima, M. & Glotzer, M. Phosphorylation of ZEN-4/MKLP1 by aurora B regulates completion of cytokinesis. *Curr. Biol.* **15,** 778–786 (2005).
24. Douglas, M. E., Davies, T., Joseph, N. & Mishima, M. Aurora B and 14-3-3 coordinately regulate clustering of centralspindlin during cytokinesis. *Curr. Biol.* **20,** 927–933 (2010).
25. Neef, R., Klein, U. R., Kopajtich, R. & Barr, F. A. Cooperation between mitotic kinesins controls the late stages of cytokinesis. *Curr. Biol.* **16,** 301–307 (2006).
26. Vazquez-Novelle, M. D. & Petronczki, M. Relocation of the chromosomal passenger complex prevents mitotic checkpoint engagement at anaphase. *Curr. Biol.* **20,** 1402–1407 (2010).
27. Powers, J., Bossinger, O., Rose, D., Strome, S. & Saxton, W. A nematode kinesin required for cleavage furrow advancement. *Curr. Biol.* **8,** 1133–1136 (1998).
28. Hummer, S. & Mayer, T. U. Cdk1 negatively regulates midzone localization of the mitotic kinesin Mklp2 and the chromosomal passenger complex. *Curr. Biol.* **19,** 607–612 (2009).
29. Sumara, I. *et al.* A Cul3-based E3 ligase removes Aurora B from mitotic chromosomes, regulating mitotic progression and completion of cytokinesis in human cells. *Dev. Cell* **12,** 887–900 (2007).
30. Ramadan, K. *et al.* Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. *Nature* **450,** 1258–1262 (2007).
31. Ban, R., Irino, Y., Fukami, K. & Tanaka, H. Human mitotic spindle-associated protein PRC1 inhibits MgcRacGAP activity toward Cdc42 during the metaphase. *J. Biol. Chem.* **279,** 16394–16402 (2004).
32. Lewellyn, L., Carvalho, A., Desai, A., Maddox, A. S. & Oegema, K. The chromosomal passenger complex and centralspindlin independently contribute to contractile ring assembly. *J. Cell Biol.* **193,** 155–169 (2011).
33. Hu, C. K., Coughlin, M., Field, C. M. & Mitchison, T. J. KIF4 regulates midzone length during cytokinesis. *Curr. Biol.* **21,** 815–824 (2011).
34. Canman, J. C. *et al.* Determining the position of the cell division plane. *Nature* **424,** 1074–1078 (2003).
35. Hu, C. K., Coughlin, M., Field, C. M. & Mitchison, T. J. Cell polarization during monopolar cytokinesis. *J. Cell Biol.* **181,** 195–202 (2008).
36. Neef, R. *et al.* Choice of Plk1 docking partners during mitosis and cytokinesis is controlled by the activation state of Cdk1. *Nat. Cell Biol.* **9,** 436–444 (2007).
37. Neef, R. *et al.* Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis. *J. Cell Biol.* **162,** 863–875 (2003).
38. Thery, M., Jimenez-Dalmaroni, A., Racine, V., Bornens, M. & Julicher, F. Experimental and theoretical study of mitotic spindle orientation. *Nature* **447,** 493–496 (2007).
39. Fink, J. *et al.* External forces control mitotic spindle positioning. *Nat. Cell Biol.* **13,** 771–778 (2011).
40. Gibson, W. T. *et al.* Control of the mitotic cleavage plane by local epithelial topology. *Cell* **144,** 427–438 (2011).
41. Minc, N., Burgess, D. & Chang, F. Influence of cell geometry on division-plane positioning. *Cell* **144,** 414–426 (2011).
42. Von Dassow, G. Concurrent cues for cytokinetic furrow induction in animal cells. *Trends Cell Biol.* **19,** 165–173 (2009).
43. Bringmann, H. & Hyman, A. A. A cytokinesis furrow is positioned by two consecutive signals. *Nature* **436,** 731–734 (2005).
44. Dechant, R. & Glotzer, M. Centrosome separation and central spindle assembly act in redundant pathways that regulate microtubule density and trigger cleavage furrow formation. *Dev. Cell* **4,** 333–344 (2003).
45. Werner, M., Munro, E. & Glotzer, M. Astral signals spatially bias cortical myosin recruitment to break symmetry and promote cytokinesis. *Curr. Biol.* **17,** 1286–1297 (2007).
46. Bement, W. M., Benink, H. A. & von Dassow, G. A microtubule-dependent zone of active RhoA during cleavage plane specification. *J. Cell Biol.* **170,** 91–101 (2005).
47. Motegi, F., Velarde, N. V., Piano, F. & Sugimoto, A. Two phases of astral microtubule activity during cytokinesis in *C. elegans* embryos. *Dev. Cell* **10,** 509–520 (2006).
48. Murthy, K. & Wadsworth, P. Dual role for microtubules in regulating cortical contractility during cytokinesis. *J. Cell Sci.* **121,** 2350–2359 (2008).
49. Foe, V. E. & von Dassow, G. Stable and dynamic microtubules coordinately shape the myosin activation zone during cytokinetic furrow formation. *J. Cell Biol.* **183,** 457–470 (2008).
50. Odell, G. M. & Foe, V. E. An agent-based model contrasts opposite effects of dynamic and stable microtubules on cleavage furrow positioning. *J. Cell Biol.* **183,** 471–483 (2008).
51. Yuce, O., Piekny, A. & Glotzer, M. An ECT2-centralspindlin complex regulates the localization and function of RhoA. *J. Cell Biol.* **170,** 571–582 (2005).
52. Nishimura, Y. & Yonemura, S. Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. *J. Cell Sci.* **119,** 104–114 (2006).
53. Zhao, W. M. & Fang, G. Anillin is a substrate of anaphase-promoting complex/cyclosome (APC/C) that controls spatial contractility of myosin during late cytokinesis. *J. Biol. Chem.* **280,** 33516–33524 (2005).
54. Petronczki, M., Glotzer, M., Kraut, N. & Peters, J. M. Polo-like kinase 1 triggers the initiation of cytokinesis in human cells by promoting recruitment of the RhoGEF Ect2 to the central spindle. *Dev. Cell* **12,** 713–725 (2007).
55. Wolfe, B. A., Takaki, T., Petronczki, M. & Glotzer, M. Polo-like kinase 1 directs assembly of the HsCyk-4 RhoGAP/Ect2 RhoGEF complex to initiate cleavage furrow formation. *PLoS Biol.* **7,** e1000110 (2009).
56. Su, K. C., Takaki, T. & Petronczki, M. Targeting of the RhoGEF Ect2 to the equatorial membrane controls cleavage furrow formation during cytokinesis. *Dev. Cell* **21,** 1104–1115 (2011).
57. Birkenfeld, J. *et al.* GEF-H1 modulates localized RhoA activation during cytokinesis under the control of mitotic kinases. *Dev. Cell* **12,** 699–712 (2007).
58. Miller, A. L. & Bement, W. M. Regulation of cytokinesis by Rho GTPase flux. *Nat. Cell Biol.* **11,** 71–77 (2009).
59. Loria, A., Longhini, K. M. & Glotzer, M. The RhoGAP domain of CYK-4 has an essential role in RhoA activation. *Curr. Biol.* **22,** 213–219 (2012).
60. Canman, J. C. *et al.* Inhibition of Rac by the GAP activity of centralspindlin is essential for cytokinesis. *Science* **322,** 1543–1546 (2008).
61. Vale, R. D., Spudich, J. A. & Griffis, E. R. Dynamics of myosin, microtubules, and Kinesin-6 at the cortex during cytokinesis in *Drosophila* S2 cells. *J. Cell Biol.* **186,** 727–738 (2009).
62. Fuller, B. G. *et al.* Midzone activation of aurora B in anaphase produces an intracellular phosphorylation gradient. *Nature* **453,** 1132–1136 (2008).
63. Von Dassow, G., Verbrugghe, K. J., Miller, A. L., Sider, J. R. & Bement, W. M. Action at a distance during cytokinesis. *J. Cell Biol.* **187,** 831–845 (2009).
64. Severson, A. F., Baillie, D. L. & Bowerman, B. A Formin Homology protein and a profilin are required for cytokinesis and Arp2/3-independent assembly of cortical microfilaments in *C. elegans*. *Curr. Biol.* **12,** 2066–2075 (2002).
65. Watanabe, S. *et al.* mDia2 induces the actin scaffold for the contractile ring and stabilizes its position during cytokinesis in NIH 3T3 cells. *Mol. Biol. Cell* **19,** 2328–2338 (2008).
66. Castrillon, D. H. & Wasserman, S. A. Diaphanous is required for cytokinesis in Drosophila and shares domains of similarity with the products of the limb deformity gene. *Development* **120,** 3367–3377 (1994).
67. Matsumura, F. Regulation of myosin II during cytokinesis in higher eukaryotes. *Trends Cell Biol.* **15,** 371–377 (2005).
68. Zhou, M. & Wang, Y. L. Distinct pathways for the early recruitment of myosin II and actin to the cytokinetic furrow. *Mol. Biol. Cell* **19,** 318–326 (2008).
69. Yumura, S., Ueda, M., Sako, Y., Kitanishi-Yumura, T. & Yanagida, T. Multiple mechanisms for accumulation of myosin II filaments at the equator during cytokinesis. *Traffic* **9,** 2089–2099 (2008).
70. Uehara, R. *et al.* Determinants of myosin II cortical localization during cytokinesis. *Curr. Biol.* **20,** 1080–1085 (2010).
71. Murthy, K. & Wadsworth, P. Myosin-II-dependent localization and dynamics of F-actin during cytokinesis. *Curr. Biol.* **15,** 724–731 (2005).
72. Albertson, R., Cao, J., Hsieh, T. S. & Sullivan, W. Vesicles and actin are targeted to the cleavage furrow via furrow microtubules and the central spindle. *J. Cell Biol.* **181,** 777–790 (2008).
73. Piekny, A. J. & Maddox, A. S. The myriad roles of Anillin during cytokinesis. *Semin. Cell. Dev. Biol.* **21,** 881–891 (2010).
74. Piekny, A. J. & Glotzer, M. Anillin is a scaffold protein that links RhoA, actin, and myosin during cytokinesis. *Curr. Biol.* **18,** 30–36 (2008).
75. Gregory, S. L. *et al.* Cell division requires a direct link between microtubule-bound RacGAP and Anillin in the contractile ring. *Curr. Biol.* **18,** 25–29 (2008).
76. D'Avino, P. P. *et al.* Interaction between Anillin and RacGAP50C connects the actomyosin contractile ring with spindle microtubules at the cell division site. *J. Cell Sci.* **121,** 1151–1158 (2008).
77. Echard, A., Hickson, G. R., Foley, E. & O'Farrell, P. H. Terminal cytokinesis events uncovered after an RNAi screen. *Curr. Biol.* **14,** 1685–1693 (2004).
78. Reichl, E. M. *et al.* Interactions between myosin and actin crosslinkers control cytokinesis contractility dynamics and mechanics. *Curr. Biol.* **18,** 471–480 (2008).
79. Maddox, A. S., Lewellyn, L., Desai, A. & Oegema, K. Anillin and the septins promote asymmetric ingression of the cytokinetic furrow. *Dev. Cell* **12,** 827–835 (2007).
80. Estey, M. P., Di Ciano-Oliveira, C., Froese, C. D., Bejide, M. T. & Trimble, W. S. Distinct roles of septins in cytokinesis: SEPT9 mediates midbody abscission. *J. Cell Biol.* **191,** 741–749 (2010).
81. Dobbelaere, J. & Barral, Y. Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. *Science* **305,** 393–396 (2004).
82. Joo, E., Surka, M. C. & Trimble, W. S. Mammalian SEPT2 is required for scaffolding nonmuscle myosin II and its kinases. *Dev. Cell* **13,** 677–690 (2007).

83. Brill, J. A., Wong, R. & Wilde, A. Phosphoinositide function in cytokinesis. *Curr. Biol.* **21,** R930–R934 (2011).
84. Schroeder, T. E. The contractile ring II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol.* **53,** 419–434 (1972).
85. Egelhoff, T. T., Lee, R. J. & Spudich, J. A. Dictyostelium myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization *in vivo*. *Cell* **75,** 363–371 (1993).
86. Tucker, J. B. Microtubules and a contractile ring of microfilaments associated with a cleavage furrow. *J. Cell Sci.* **8,** 557–571 (1971).
87. Maupin, P. & Pollard, T. D. Arrangement of actin filaments and myosin-like filaments in the contractile ring and of actin-like filaments in the mitotic spindle of dividing HeLa cells. *J. Ultrastruct. Mol. Struct. Res.* **94,** 92–103 (1986).
88. Kamasaki, T., Osumi, M. & Mabuchi, I. Three-dimensional arrangement of F-actin in the contractile ring of fission yeast. *J. Cell Biol.* **178,** 765–771 (2007).
89. Fishkind, D. J. & Wang, Y. L. Orientation and three-dimensional organization of actin filaments in dividing cultured cells. *J. Cell Biol.* **123,** 837–848 (1993).
90. Pollard, T. D. The role of actin in the temperature-dependent gelation and contraction of extracts of Acanthamoeba. *J. Cell Biol.* **68,** 579–601 (1976).
91. Kruse, K. & Julicher, F. Self-organization and mechanical properties of active filament bundles. *Phys. Rev. E Stat. Nonlin. Soft. Matter Phys.* **67,** 051913 (2003).
92. Carvalho, A., Desai, A. & Oegema, K. Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. *Cell* **137,** 926–937 (2009).
93. Biron, D., Alvarez-Lacalle, E., Tlusty, T. & Moses, E. Molecular model of the contractile ring. *Phys. Rev. Lett.* **95,** 098102 (2005).
94. Zumdieck, A., Kruse, K., Bringmann, H., Hyman, A. A. & Julicher, F. Stress generation and filament turnover during actin ring constriction. *PLoS One* **2,** e696 (2007).
95. Calvert, M. E. *et al.* Myosin concentration underlies cell size-dependent scalability of actomyosin ring constriction. *J. Cell Biol.* **195,** 799–813 (2011).
96. Rankin, K. E. & Wordeman, L. Long astral microtubules uncouple mitotic spindles from the cytokinetic furrow. *J. Cell Biol.* **190,** 35–43 (2010).
97. Sedzinski, J. *et al.* Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. *Nature* **476,** 462–466 (2011).
98. Bluemink, J. G. & de Laat, S. W. 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 (1973).
99. Shuster, C. B. & Burgess, D. R. Targeted new membrane addition in the cleavage furrow is a late, separate event in cytokinesis. *Proc. Natl Acad. Sci. USA* **99,** 3633–3638 (2002).
100. Danilchik, M. V., Bedrick, S. D., Brown, E. E. & Ray, K. Furrow microtubules and localized exocytosis in cleaving *Xenopus laevis* embryos. *J. Cell Sci.* **116,** 273–283 (2003).
101. Dyer, N. *et al.* Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. *Development* **134,** 4437–4447 (2007).
102. Skop, A. R., Bergmann, D., Mohler, W. A. & White, J. G. Completion of cytokinesis in *C. elegans* requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. *Curr. Biol.* **11,** 735–746 (2001).
103. Emoto, K., Inadome, H., Kanaho, Y., Narumiya, S. & Umeda, M. Local change in phospholipid composition at the cleavage furrow is essential for completion of cytokinesis. *J. Biol. Chem.* **280,** 37901–37907 (2005).
104. Ng, M. M., Chang, F. & Burgess, D. R. Movement of membrane domains and requirement of membrane signaling molecules for cytokinesis. *Dev. Cell* **9,** 781–790 (2005).
105. Guizetti, J. & Gerlich, D. W. Cytokinetic abscission in animal cells. *Semin. Cell. Dev. Biol.* **21,** 909–916 (2010).
106. Steigemann, P. & Gerlich, D. W. Cytokinetic abscission: cellular dynamics at the midbody. *Trends Cell Biol.* **19,** 606–616 (2009).
107. Skop, A. R., Liu, H., Yates, J. III, Meyer, B. J. & Heald, R. Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* **305,** 61–66 (2004).
108. Gromley, A. *et al.* Centriolin anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. *Cell* **123,** 75–87 (2005).
109. Goss, J. W. & Toomre, D. K. Both daughter cells traffic and exocytose membrane at the cleavage furrow during mammalian cytokinesis. *J. Cell Biol.* **181,** 1047–1054 (2008).
110. Schiel, J. A. *et al.* Endocytic membrane fusion and buckling-induced microtubule severing mediate cell abscission. *J. Cell Sci.* **124,** 1411–1424 (2011).
111. Kouranti, I., Sachse, M., Arouche, N., Goud, B. & Echard, A. Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. *Curr. Biol.* **16,** 1719–1725 (2006).
112. Fielding, A. B. *et al.* Rab11-FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. *EMBO J.* **24,** 3389–3399 (2005).
113. Low, S. H. *et al.* Syntaxin 2 and endobrevin are required for the terminal step of cytokinesis in mammalian cells. *Dev. Cell* **4,** 753–759 (2003).
114. Pohl, C. & Jentsch, S. Final stages of cytokinesis and midbody ring formation are controlled by BRUCE. *Cell* **132,** 832–845 (2008).
115. Baluska, F., Menzel, D. & Barlow, P. W. Cytokinesis in plant and animal cells: endosomes 'shut the door'. *Dev. Biol.* **294,** 1–10 (2006).
116. Guizetti, J. *et al.* Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments. *Science* **331,** 1616–1620 (2011).
117. Carlton, J. G. & Martin-Serrano, J. Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. *Science* **316,** 1908–1912 (2007).
118. Carlton, J. G., Agromayor, M. & Martin-Serrano, J. Differential requirements for Alix and ESCRT-III in cytokinesis and HIV-1 release. *Proc. Natl. Acad. Sci. USA* **105,** 10541–10546 (2008).
119. Morita, E. *et al.* Human ESCRT and ALIX proteins interact with proteins of the midbody and function in cytokinesis. *EMBO J.* **26,** 4215–4227 (2007).
120. Hurley, J. H. & Hanson, P. I. Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat. Rev. Mol. Cell Biol.* **11,** 556–566 (2010).
121. Guizetti, J. & Gerlich, D. W. ESCRT-III polymers in membrane neck constriction. *Trends Cell Biol.* **22,** 133–140 (2012).
122. Elia, N., Sougrat, R., Spurlin, T. A., Hurley, J. H. & Lippincott-Schwartz, J. Dynamics of endosomal sorting complex required for transport (ESCRT) machinery during cytokinesis and its role in abscission. *Proc. Natl Acad. Sci. USA* **108,** 4846–4851 (2011).
123. Bastos, R. N. & Barr, F. A. Plk1 negatively regulates Cep55 recruitment to the midbody to ensure orderly abscission. *J. Cell Biol.* **191,** 751–760 (2010).
124. Sagona, A. P. *et al.* PtdIns(3)P controls cytokinesis through KIF13A-mediated recruitment of FYVE-CENT to the midbody. *Nat. Cell Biol.* **12,** 362–371 (2010).
125. Saurin, A. T. *et al.* The regulated assembly of a PKCε complex controls the completion of cytokinesis. *Nat. Cell Biol.* **10,** 891–901 (2008).
126. Dambournet, D. *et al.* Rab35 GTPase and OCRL phosphatase remodel lipids and F-actin for successful cytokinesis. *Nat. Cell Biol.* **13,** 981–988 (2011).
127. Connell, J. W., Lindon, C., Luzio, J. P. & Reid, E. Spastin couples microtubule severing to membrane traffic in completion of cytokinesis and secretion. *Traffic* **10,** 42–56 (2009).
128. Yang, D. *et al.* Structural basis for midbody targeting of spastin by the ESCRT-III protein CHMP1B. *Nat. Struct. Mol. Biol.* **15,** 1278–1286 (2008).
129. Lacroix, B. *et al.* Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. *J. Cell Biol.* **189,** 945–954 (2010).
130. Mackay, D. R., Makise, M. & Ullman, K. S. Defects in nuclear pore assembly lead to activation of an Aurora B-mediated abscission checkpoint. *J. Cell Biol.* **191,** 923–931 (2010).
131. Ganem, N. J., Godinho, S. A. & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460,** 278–282 (2009).
132. Ganem, N. J., Storchova, Z. & Pellman, D. Tetraploidy, aneuploidy and cancer. *Curr. Opin. Genet. Dev.* **17,** 157–162 (2007).
133. Fujiwara, T. *et al.* Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. *Nature* **437,** 1043–1047 (2005).
134. Ettinger, A. W. *et al.* Proliferating versus differentiating stem and cancer cells exhibit distinct midbody-release behaviour. *Nat. Commun.* **2,** 503 (2011).
135. Kuo, T. C. *et al.* Midbody accumulation through evasion of autophagy contributes to cellular reprogramming and tumorigenicity. *Nat. Cell Biol.* **13,** 1214–1223 (2011).
136. Pohl, C. & Jentsch, S. Midbody ring disposal by autophagy is a post-abscission event of cytokinesis. *Nat. Cell Biol.* **11,** 65–70 (2009).
137. Toomre, D. & Bewersdorf, J. A new wave of cellular imaging. *Annu. Rev. Cell. Dev. Biol.* **26,** 285–314 (2010).
138. Shu, X. *et al.* A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.* **9,** e1001041 (2011).
139. Wollert, T., Wunder, C., Lippincott-Schwartz, J. & Hurley, J. H. Membrane scission by the ESCRT-III complex. *Nature* **458,** 172–177 (2009).