Cytokinesis in Animal Cells

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Abstract
Cytokinesis, the final step in cell division, partitions the contents of a single cell into two. In animal cells, cytokinesis occurs through cortical remodeling orchestrated by the anaphase spindle. Cytokinesis relies on a tight interplay between signaling and cellular mechanics and has attracted the attention of both biologists and physicists for more than a century. In this review, we provide an overview of four topics in animal cell cytokinesis: (a) signaling between the anaphase spindle and cortex, (b) the mechanics of cortical remodeling, (c) abscission, and (d) regulation of cytokinesis by the cell cycle machinery. We report on recent progress in these areas and highlight some of the outstanding questions that these findings bring into focus.
INTRODUCTION

Cytokinesis completes mitosis by partitioning the contents of a single cell into two. In animal cells, cytokinesis begins with the assembly and constriction, orchestrated by signaling from the anaphase spindle, of an equatorial contractile ring. As constriction progresses, the central region of the anaphase spindle is remodeled to form a midbody, which organizes the narrow intracellular bridge between the nascent daughter cells. The midbody directs abscission, a distinct membrane scission event that requires the assembly of filaments composed of ESCRT-III proteins (see overview in Figure 1). In this review, we cover four major topics: (a) signaling between the anaphase spindle and cortex, (b) the mechanics of cortical remodeling, (c) abscission, and (d) the regulation of cytokinesis by the cell cycle. Owing to our focus on animal cell cytokinesis, we touch on only some of the literature on cytokinesis in Schizosaccharomyces pombe, a popular model system in which many important advances have been made. See Bathe & Chang (2010) and Pollard & Wu (2010) for more detailed reviews of S. pombe cytokinesis. To make the review broadly accessible, we refer to components by the most common name for the mammalian protein. A table listing protein names in different animal cell systems is included for reference (Supplemental Table 1; follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org).
Figure 1
Summary of events during cytokinesis. (a) Cytokinesis begins with signaling between the anaphase spindle and cortex to generate an equatorial zone of active RhoA (RhoA-GTP). The central spindle, a narrow zone of bundled overlapping microtubules (MTs), forms in the midzone between the separating chromosomes. The central spindle recruits the Rho-GEF, Ect2, and loads it onto the plasma membrane, where it activates RhoA. (b) Active RhoA directs assembly of the contractile ring, which constricts to change cell shape. (c) The contractile ring is a filamentous network composed of formin-nucleated actin filaments, bipolar filaments assembled from the motor, myosin II, membrane-associated septin filaments, and the filament cross-linker, anillin. (d) As the contractile ring constricts, the spindle midzone matures to form the midbody, which organizes the intercellular bridge. (e) During abscission, a distinct ESCRT-III filament system catalyzes membrane scission on either side of the midbody to generate the two daughter cells. Abbreviations: CPC, chromosomal passenger complex.
**SIGNALING BETWEEN THE ANAPHASE SPINDLE AND CORTEX**

**Specification of an Equatorial Zone of Active RhoA**

In animal cells, the site of contractile ring assembly is positioned through signaling between the anaphase spindle and cortex. Classic micromanipulation experiments in echinoderm eggs demonstrated the dynamic nature of this communication by showing that moving the spindle during anaphase causes furrow regression and formation of a new furrow above the spindle midplane (Rappaport 1985). The spindle directs formation of a narrow equatorial zone of active RhoA (Bement et al. 2006, Piekny et al. 2005). Active RhoA promotes the recruitment of effector contractile ring proteins including the cytokinesis formin, which triggers the assembly of unbranched actin filaments, and Rho kinase as well as citron kinase, which promote myosin-II activation (Bement et al. 2006, Goode & Eck 2007, Matsumura et al. 2011, Piekny et al. 2005). Consistent with a central role for RhoA in communication between the spindle and cortex, a rendition of Rappaport’s micromanipulation experiment performed in sea urchin embryos expressing an active RhoA probe showed that repositioning of the spindle leads to translocation of the RhoA activity zone followed by the appearance of the new furrow (Bement et al. 2005).

Rho family GTPases are activated by GTPase exchange factors (GEFs), which promote exchange of GTP for GDP and target the GTPase to the membrane (Rossman et al. 2005) and are inactivated by GTPase activating proteins (GAPs) that enhance their intrinsically low hydrolysis rate (Tcherkezian & Lamarche-Vane 2007). The mechanisms that allow the spindle to rapidly specify a narrow zone of active RhoA are not well understood. However, they are thought to include a bipartite mechanism in which RhoA is activated at the cell equator by signals from the spindle midzone and suppressed at the cell poles by dynamic microtubules nucleated by the centrosomal asters (D’Avino et al. 2005, von Dassow 2009). In addition to spindle-based signals, confining active RhoA to a narrow zone is proposed to require rapid GAP-mediated RhoA inactivation. In the RhoA flux model, localized GEF-mediated RhoA activation is countered by global GAP-mediated RhoA inactivation. The RhoA flux model predicts that as the rate of GAP-mediated RhoA turnover decreases, active RhoA generated by the equatorial stimulus will diffuse through the membrane, broadening the RhoA zone (Bement et al. 2006). Ect2 has been identified as the critical RhoA GEF during cytokinesis, and spindle-based mechanisms that would activate Ect2 preferentially at the cell equator (reviewed below) have been proposed; however, the identity of the GAP(s) that would oppose RhoA activation is less clear (see section discussing the centralspindlin GAP domain, below). Understanding how GAP-mediated RhoA flux contributes to the dimensions and dynamics of the RhoA zone will require clarification of the identity of the relevant opposing GAP(s).

**Assembly of the Central Spindle**

At anaphase, a bipolar microtubule array called the spindle midzone forms between the separating chromosomes. Midzone microtubules are organized with their minus ends facing the poles and their plus ends interdigitated with the plus ends of oppositely oriented microtubules near the center of the array (Euteneuer & McIntosh 1980). Reconstructions of PtK1 cell spindles from serial electron micrographs showed that antiparallel bundles containing 10–30 microtubules begin to form in early anaphase. The average length of the overlap zone in the bundles was ~5 µm in early and middle anaphase and narrowed to ~1 µm in late anaphase (Mastronarde et al. 1993). Microtubule minus ends were primarily in the region between the chromosomes and the spindle poles in early anaphase, but by late anaphase they were found between the spindle midplane and chromosomes. At that time, the regions
of antiparallel overlap were aligned so that the majority of microtubule plus ends were within 2 µm of the spindle midplane (Mastronarde et al. 1993). This narrow region of antiparallel overlap, which recruits cytokinesis signaling molecules, has been termed the central spindle (Glotzer 2009).

Central spindle formation requires the antiparallel microtubule cross-linker PRC1 and two kinesins—KIF4 (kinesin-4) and the MKLP1 (kinesin-6) subunit of the centralspindlin complex—and is regulated by the kinase activities of the chromosomal passenger complex (CPC) and Plk1 (Glotzer 2009). PRC1 is a member of the MAP65 protein family, whose members selectively cross-link microtubules in an antiparallel orientation (Bieling et al. 2010, Gaillard et al. 2008, Janson et al. 2007, Loiodice et al. 2005). In vitro imaging of dynamic microtubules in the presence of PRC1 and KIF4 demonstrated that this two-protein system can reconstitute formation of microtubule bundles with a fixed extent of antiparallel overlap (Bieling et al. 2010). In the in vitro analysis, antiparallel microtubules were bundled cooperatively by PRC1, and overlap zones increased in length as the microtubules grew. In the presence of KIF4, microtubule plus-end elongation slowed as overlap zones expanded, and it stopped when a fixed length of overlap was achieved. The extent of overlap that stopped growth was inversely correlated with the KIF4 concentration (Bieling et al. 2010).

In addition to PRC1 and KIF4, two protein complexes, centralspindlin and the CPC, are also essential for central spindle assembly (Carmena et al. 2009, Glotzer 2009, Ruchaud et al. 2007). Centralspindlin is a hetero- trameric complex (Pavicic-Kaltenbrunner et al. 2007) consisting of two molecules of the kinesin-6, MKLP1, and two molecules of CYK-4, whose distinguishing feature is a GAP domain predicted to target a Rho family GTPase. The CPC is composed of the kinase Aurora B and three additional subunits that localize and activate the kinase (Carmena et al. 2009, Ruchaud et al. 2007). The CPC phosphorylates MKLP1 and promotes recruitment of centralspindlin to the midzone (Douglas et al. 2010, Giet & Glover 2001, Guse et al. 2005, Hauf et al. 2003, Hu et al. 2008, Kaitna et al. 2000, Severson et al. 2000, Verbrugghe & White 2004, Zhu et al. 2005). Stable localization of centralspindlin to the midzone is thought to require its multimerization into clusters that are competent to travel along microtubules to their plus ends (Hutterer et al. 2009). Centralspindlin is constitutively phosphorylated on a conserved site that causes it to bind 14-3-3, and 14-3-3 binding sequesters centralspindlin, preventing it from targeting to the midzone. Phosphorylation of centralspindlin on a second, adjacent conserved site by the CPC releases it from 14-3-3 and allows centralspindlin to multimerize and accumulate at the midzone (Douglas et al. 2010).

Plk1 is not required to form the central spindle (Brennan et al. 2007, Burkard et al. 2007, Petronczki et al. 2007). However, when Plk1 is inhibited, the spindle midzone fails to elongate during anaphase (Brennan et al. 2007); KIF4 depletion restores midzone elongation in Plk1 inhibited cells. Because KIF4 does not contain Plk1 consensus sites, Hu et al. (2011) proposed...
Central spindle formation and signaling

(a) In early anaphase, PRC1, centralspindlin, and the chromosomal passenger complex (CPC) are recruited to the spindle midzone, where they promote bundling of overlapping microtubules (MTs) of opposite polarity that emanate from the two spindle poles.

(b) KIF4 is recruited to the overlap zone in midanaphase by PRC1. KIF4 translocates to MT plus ends and stops their growth, limiting the length of the overlap zone. Ect2 binds centralspindlin and is loaded onto the equatorial membrane.

(c) Ect2 converts RhoA-GDP to RhoA-GTP to promote contractile ring assembly.

Recruitment of Signaling Complexes that Promote Contractile Ring Assembly to the Spindle Midzone

Several of the protein complexes that localize to the spindle midzone play a key role in promoting contractile ring assembly (Barr & Gruneberg 2007, Carmena et al. 2009, Petronczki et al. 2008). In addition to the bundled microtubules in the central spindle, stabilized astral microtubules, whose plus ends point toward the furrow, are also proposed to promote contractile ring assembly (Figure 3a; Canman et al. 2003, Foe & von Dassow 2008, Inoue et al. 2004). Consistent with this idea,
Centralspindlin has often been observed on the tips of microtubules near the furrow (see, for example, Nishimura & Yonemura 2006). Not much is known about how signaling molecules on the central spindle or stabilized astral microtubules reach the cortex. Arguments have been made both for and against transmission of spindle-based signals by diffusion (Cao & Wang 1996, Salmon & Wolniak 1990, von Dassow et al. 2009). The CPC has been observed on actin cables extending between microtubule ends and the cortex in cells undergoing monopolar cytokinesis, which suggests that actin-based transport may play a role (Hu et al. 2008). On the basis of work in Drosophila, centralspindlin has been proposed to interact directly with anillin, which could also play a role in linking signaling microtubules to the cortex (D’Avino et al. 2008, Gregory et al. 2008).

Although PRC1, centralspindlin, and the CPC all are required to form the central spindle, their inhibition has very different effects on furrow ingression. Inhibition of PRC1 in Caenorhabditis elegans or in human cells does not prevent contractile ring assembly or constriction (Mollinari et al. 2005, Verbrugghe & White 2004), whereas inhibition of centralspindlin (Eggert et al. 2006, Glotzer 2005) or the CPC (Carmena et al. 2009, Ruchaud et al. 2007) leads to pronounced constriction defects. In PRC1-depleted C. elegans embryos, constriction kinetics are similar to those in controls, and the first cytokinesis completes successfully (Lewellyn et al. 2010, Verbrugghe & White 2004), whereas inhibition of centralspindlin or the CPC leads to a two- to threefold reduction in constriction rate and cytokinesis failure (Canman et al. 2008, Jantsch-Plunger et al. 2000, Kaitna et al. 2000, Lewellyn et al. 2011, Powers et al. 1998, Raich et al. 1998, Schumacher et al. 1998, Severson et al. 2000, Speliotes et al. 2000). The less-severe effect of PRC1 depletion could result from the fact that the CPC, and to a lesser extent centralspindlin, still targets to microtubules in the midzone in the absence of PRC1-mediated bundling (Kurasawa et al. 2004, Lewellyn et al. 2010, Mollinari et al. 2005, Verbrugghe & White 2004). Alternatively, midzone targeting may not be essential for some of the contributions of centralspindlin and/or the CPC to contractile ring constriction (Cesario et al. 2006, Mollinari et al. 2005, Nishimura & Yonemura 2006).

![Figure 3](Annu. Rev. Cell Dev. Biol. 2012.28:29-58. Downloaded from www.annualreviews.org by Universita degli Studi di Roma La Sapienza on 05/17/13. For personal use only.)

**Figure 3**

Dynamic astral microtubules confine active RhoA to the cell equator. (a) Microtubule (MT) populations that signal to the cortex to specify the dimensions of the zone of active RhoA (light green) are highlighted: midzone MTs (dark green), dynamic astral MTs (red), and stable astral MTs (purple), which terminate near the equatorial cortex. (b–d) Panels illustrate the results of experiments performed by von Dassow et al. (2009) in sea urchin embryos, which demonstrate the role that the centrosomal asters play in shaping the zone of active RhoA. Inhibition of astral MTs by treatment with low levels of nocodazole (b), or ablation of both centrosomes (c), broadens the equatorial RhoA zone, indicating that dynamic astral MTs normally confine RhoA to a narrow equatorial zone. Ablation of a single centrosome (d) shifts the RhoA zone toward the ablated aster.
Verbrugghe & White 2007). In *C. elegans* embryos, centralspindlin and the CPC are not required for the initial enrichment of contractile ring proteins in a band around the cell equator (Lewellyn et al. 2010, 2011; see Figure 4c). However, when the cortex folds in to form a furrow composed of two back-to-back plasma membranes, the mature contractile ring at the furrow tip contains substantially less myosin II when either the CPC or centralspindlin is inhibited (Lewellyn et al. 2011, Loria et al. 2012). Simultaneous inhibition of both complexes results in an additive defect in which the cortex fails to fold into a furrow, suggesting that centralspindlin and the CPC have independent roles in contractile ring assembly (Lewellyn et al. 2011). Two proposed cytokinesis CPC targets are centralspindlin and the myosin II regulatory light chain (Douglas et al. 2010, Guse et al. 2005, Murata-Hori et al. 2000, Touré et al. 2008). The CPC phosphorylates centralspindlin to promote formation of the central spindle, as discussed above. However, the additive constriction defect observed when the CPC is inhibited in a centralspindlin-null background (Lewellyn et al. 2011) suggests that the CPC also contributes to contractile ring assembly through targets other than centralspindlin. Inhibition of the phosphatase targeting myosin regulatory light chain fails to alleviate the CPC inhibition–associated furrowing defect (Lewellyn et al. 2011), which suggests that the regulatory light chain is not a critical CPC target during contractile ring assembly in vivo. Thus, important CPC targets that promote contractile ring assembly likely remain to be identified.

**Mechanism of Ect2 Rho GTPase Exchange Factor Targeting to the Central Spindle**

Two roles have been identified for centralspindlin in promoting contractile ring assembly. The first centers on the N terminus of centralspindlin’s CYK-4 subunit, which scaffolds the recruitment of the Ect2 RhoGEF to the central spindle. The second, discussed in the next section, centers on the CYK-4 GAP domain. In human cells, Ect2 is recruited to the central spindle through an interaction with the N terminus of HsCYK-4 (Chalamalasetty et al. 2006, Kamijo et al. 2006, Nishimura & Yonemura 2006, Yuce et al. 2005, Zhao & Fang 2005b). Ect2 is then loaded onto the adjacent equatorial plasma membrane through a reaction that requires the pleckstrin homology domain and adjacent polybasic region in the Ect2 C terminus (Figure 2; Su et al. 2011). Ect2 recruitment to the central spindle is regulated by the polo kinase Plk1 (Brennan et al. 2007, Burkard et al. 2007, Petronczki et al. 2007). Plk1 promotes its own recruitment to the central spindle by phosphorylating central spindle–localized targets to generate phospho-binding sites for its polo-box domain. The main polo-box domain–binding sites implicated in recruiting Plk1 to the central spindle are in PRC1 and MKLP2 (Nee et al. 2003, 2007). Once at the midzone, Plk1 phosphorylates the HsCYK-4 N terminus to generate a binding site for the BRCT domains of Ect2 (Burkard et al. 2009, Wolfe et al. 2009). In addition to phosphorylating HsCYK-4, analysis of a phosphomimetic HsCYK-4 mutant suggests that Plk1 also has a second essential function in targeting Ect2 to the central spindle (Wolfe et al. 2009), which may involve Plk1 binding to Ect2 and relieving its autoinhibition (Niiya et al. 2006). Notably, the Ect2 homologs in *Drosophila* and *C. elegans* localize to the cortex but have not been detected at the central spindle (Motegi & Sugimoto 2006, Prokopenko et al. 1999), although the N terminus of *Drosophila* CYK-4 has been reported to interact with the *Drosophila* Ect2 homolog (Somers & Saint 2003), which suggests that the mechanism concentrating the RhoGEF on the central spindle may be specific to vertebrates.

**Function of the Centralspindlin GTPase Activating Protein Domain**

The second role of centralspindlin in promoting contractile ring assembly centers on the
The CYK-4 GAP domain, which is essential for cytokinesis in every system and cell type in which it has been tested (Canman et al. 2008, Loria et al. 2012, Miller & Bement 2009, Yamada et al. 2006, Zavortink et al. 2005). Although the CYK-4 GAP domain is essential, the role of the CYK-4 GAP activity and the identity of the target GTPase are less clear. CYK-4 homologs with mutations predicted to inactivate GAP activity were shown to support cytokinesis in chicken DT40 B lymphocytes (Yamada et al. 2006) and Drosophila neuroblasts (Goldstein et al. 2005) but led to cytokinesis failure in Xenopus (Miller & Bement 2009) and Drosophila embryos (Zavortink et al. 2005). The observed differences could be due to the extent to which the different mutations compromise GAP activity or affect GAP domain structure; however, an alternative possibility is that, although the GAP domain plays an essential role in cytokinesis across systems, GAP activity is essential for cytokinesis in some cellular contexts but not in others. The target of the CYK-4 GAP domain also is not clear. Because RhoA is the Rho family GTPase implicated in cytokinesis, the CYK-4 GAP domain has been proposed to inactivate RhoA (Jantsch-Plunger et al. 2000), and a broader RhoA zone was observed in Xenopus embryos expressing GAP-dead CYK-4 (Miller & Bement 2009), consistent with the Rho-flux hypothesis (discussed above). However, work in C. elegans has suggested that the centralspindlin GAP domain promotes, rather than opposes, RhoA activation (Canman et al. 2008, Loria et al. 2012). One suggestion, based on CYK-4 GAP specificity in vitro (Jantsch-Plunger et al. 2000, Kawashima et al. 2000, Touré et al. 1998) and genetic evidence that inhibition of the Rho family GTPase Rac can suppress phenotypes resulting from CYK-4 inhibition (Canman et al. 2008, D’Avino et al. 2004), is that the CYK-4 GAP domain locally inactivates Rac, thereby promoting RhoA activation. Alternatively, the CYK-4 GAP domain could have a more direct, as yet uncharacterized, role in RhoA activation (Loria et al. 2012).

**Role of Dynamic Astral Microtubules in Confining Active RhoA to the Cell Equator**

The now-established idea that the midzone promotes contractility was suggested initially by classic experiments in grasshopper spermatocytes, echinoderm eggs, and vertebrate kidney epithelial cells (Oegema & Mitchison 1997). At the same time, other classic work, including the seminal Rappaport experiment examining cleavage in toroidal cells with two spindles, demonstrated that two adjacent asters are sufficient to induce furrow formation (Rappaport, 1996). The midzone versus aster debate was reconciled by an elegant spindle-severing experiment in the C. elegans embryo, which showed that the midzone and asters provide two normally superimposed, but experimentally separable, signals that position the furrow (Bringmann & Hyman 2005). In contrast to the positive signal generated by the midzone, the asters function by preventing the accumulation of contractile ring proteins in their vicinity, which leads to their relative enrichment at the cell equator. Support for this idea came from the C. elegans embryo, in which Werner et al. (2007) showed that formation of a small spindle in the embryo posterior restricted the accumulation of myosin to the opposite anterior cortex. Similarly, in grasshopper spermatocytes, positioning asters or a mechanically deconstructed spindle on one side of the cell led to flow of cortical actin toward the opposite side of the cell (Chen et al. 2008). Zhou & Wang (2008) also documented aster-based inhibition in vertebrate cells where, in contrast to the progressive recruitment of myosin observed on the equatorial cortex, phases of myosin recruitment were balanced by phases of loss on the cortex above the asters, which prevented net myosin accumulation.

Local inhibition of contractility by the asters results in a gradient of contractility from the poles toward the equator and can induce furrowing even in the absence of a midzone-based equatorial signal, provided that the asters are sufficiently far apart (Dechant & Glotzer
Asters positioned closer together than the minimal distance not only fail to promote furrow formation but also can prevent furrow induction by an intervening midzone (Lewellyn et al. 2010). Consistent with the idea that the division plane is specified through spatial refinement by aster-based inhibition of a positive signal from the midzone, eliminating dynamic astral microtubules in sea urchin embryos through laser ablation or other means led to a broader zone of active RhoA and contractile ring protein accumulation (Figure 3; Bement et al. 2005, Foe & von Dassow 2008, von Dassow et al. 2009). The total amount of active RhoA in the zone was not altered when asters were ablated, despite the change in RhoA zone dimensions. This result has led to the proposal that, rather than inhibiting RhoA activation, the asters corral a fixed amount of active RhoA to prevent it from spreading from the cell equator (von Dassow et al. 2009). Understanding how the asters shape the RhoA zone will require a molecular understanding of aster-based regulation, which at present is lacking.

**THE MECHANICS OF CORTICAL REMODELING**

**Contractile Ring Structure and Dynamics**

Across systems, contractile rings are composed of a thin (0.1–0.2 µm) layer of cross-linked protein filaments that forms around the cell equator beneath the plasma membrane (Schroeder 1990). In contrast to its conserved thickness, the width of the contractile ring along the plasma membrane is variable between species (ranging from <1 µm to ~20 µm) (Kamasaki et al. 2007, Schroeder 1990). Myosin decoration indicates that the actin filaments within the contractile ring are of mixed polarity. However, organization of actin filaments into sarcomere-like arrays has not been reproducibly observed (Kamasaki et al. 2007, Mabuchi et al. 1988, Maupin & Pollard 1986, Sanger & Sanger 1980). The structural components of the ring include actin filaments, bipolar filaments of the motor myosin II, and septin filaments (Eggert et al. 2006). The septins are membrane-associated filaments that bind and are recruited to the ring by anillin, a filament cross-linker that binds to all three filament types (D’Avino 2009, Piekny & Maddox 2010). Contractile rings also contain proteins that regulate actin nucleation, capping, polymerization, disassembly, and cross-linking (Eggert et al. 2006, Wu & Pollard 2005).

Work in several systems has shown that contractile rings constrict at a roughly constant rate despite their progressively decreasing perimeter (Biron et al. 2005, Calvert et al. 2011, Carvalho et al. 2009, Ma et al. 2012, Pelham & Chang 2002, Zumdieck et al. 2007). Experiments in the *C. elegans* embryo showed that the constant constriction rate is proportional to the initial perimeter of the ring. This property enables cells of different sizes to divide in the same amount of time, which may help coordinate cell separation with cell cycle progression (Carvalho et al. 2009). To account for the dependence of constriction rate on initial circumference, Carvalho et al. (2009) proposed that contractile rings are composed of units of fixed initial size arrayed around the ring in series (Figure 4a) that shorten during constriction without being lost. Consistent with Schroeder’s (1972) work measuring the actin network volume in electron micrographs, analysis in *C. elegans* revealed that anillin, the septins, and myosin II are lost in proportion to the decrease in perimeter during constriction (Carvalho et al. 2009), which indicates that the ring is disassembled as it constricts. Photobleaching data in *C. elegans* embryos and *Drosophila* spermatocytes suggest that myosin II, anillin, and the septins progressively disassemble, but exchange only slowly, if at all, with cytoplasmic and cortical pools as the ring constricts (Carvalho et al. 2009, Goldbach et al. 2010). By contrast, rapid exchange of myosin II was observed in the furrow region of HeLa cells (Kondo et al. 2011). In *Drosophila* S2 cells, some rapid turnover of myosin II was observed in the cleavage furrow, but both the rate and extent of exchange decreased dramatically as cytokinesis...
Figure 4

Contractile ring assembly in *Schizosaccharomyces pombe* versus in metazoans. (a) Contractile unit model (Carvalho et al. 2009). Contractile rings are assembled from units of fixed initial size that shorten at a constant rate during constriction. Larger rings are assembled from more units and constrict at a proportionally higher overall rate. (b) Summary of the node model for contractile ring assembly in *S. pombe*, as outlined in Pollard & Wu (2010). The anillin-like protein Mid1 is recruited to a set of ~65 nodes overlying the nucleus during interphase. The nodes mature during mitosis, recruiting the cytokinesis formin and myosin II. In early/midanaphase, the nodes nucleate actin filaments and coalesce to form a contractile ring through a search, capture, pull, and release (SCPR) mechanism in which myosin localized to the nodes captures and pulls actin filaments nucleated by other nodes. Contractile ring constriction, which is coupled with septum formation, initiates in response to mitotic exit in late anaphase. (c) Contractile ring assembly in the *Caenorhabditis elegans* embryo as outlined in Lewellyn et al. (2010). Contractile ring assembly and constriction occur simultaneously following anaphase onset. Initially, contractile ring proteins, including the cytokinesis formin, myosin II, and anillin, are recruited to a broad equatorial band via a process that requires RhoA activation. The equatorial band subsequently folds inward, placing the plasma membranes of the nascent blastomeres in a back-to-back configuration. Components accumulate at the furrow tip and disappear from the region behind the furrow tip to form the mature contractile ring.

Progressed (Uchita et al. 2010). Rapid turnover has been reported for labeled actin in the cleavage furrow of cultured vertebrate cells (Guha et al. 2005, Murthy & Wadsworth 2005). However, labeled actin subunits are not incorporated into formin-nucleated filaments in yeast or *C. elegans* (Pollard 2010). Although it is not clear if this is also the case for mammalian formins, actin turnover rates might reflect only the dynamics of Arp2/3 rather than formin-nucleated actin in the furrow region. The dynamics of the formin-nucleated actin filaments within the contractile ring during constriction, and the effects of the proportions of formin and Arp2/3-nucleated actin filaments in the furrow on ring dynamics in different systems, are key issues that need to be addressed.

**Contractile Ring Assembly in *Schizosaccharomyces pombe***

The fission yeast *S. pombe* has been a powerful model for studies of contractile ring assembly. We highlight briefly models based on work in this system for comparison with animal...
cells. For a summary of the primary literature, we refer the reader to recent reviews (Bathe & Chang 2010, Pollard & Wu 2010). In contrast to animal cells, the contractile ring in fission yeast assembles prior to mitotic exit in response to the position of the nucleus. A set of approximately 65 membrane-bound nodes containing the anillin-like protein Mid1 are distributed initially in a broad equatorial band, ~2 µm in width, that forms during interphase (Figure 4b). During mitosis the nodes recruit additional proteins, including myosin II. In the node model for ring assembly, the formin Cdc12 is recruited to the nodes and nucleates actin filaments coincident with the progressive condensation of the nodes into a narrow equatorial ring (Figure 4b; Bathe & Chang 2010, Pollard & Wu 2010). A mathematical formulation of this model proposes that the nodes coalesce into a tight ring through a search, capture, pull, and release mechanism (Vavylonis et al. 2008) in which actin filaments nucleated at the nodes are captured by myosin II in adjacent nodes, which pulls them together. Monte Carlo simulations quantitatively recapitulate experimental observations, including behaviors such as the formation of irregular or patchy rings in specific mutants (Ojkic & Vavylonis 2010, Vavylonis et al. 2008). For example, the model predicts that dynamic remodeling of the connections between nodes ("release") is essential to avoid the generation of clumps of nodes rather than rings, a prediction consistent with recent experiments showing that perturbing the actin-severing activity of cofilin results in node clumping (Chen & Pollard 2011). An alternative model, which has not yet been formulated mathematically, proposes that myosin is recruited to the nodes, but the Cdc12 formin concentrates in one or a small number of spots. Actin filaments emanate from the formin spot(s) and interact with the nodes, which coalesce into a narrow ring (Bathe & Chang 2010).

**Contractile Ring Assembly in Metazoans**

Substantially less is known about contractile ring assembly in metazoans, which is coupled to anaphase onset and requires cortical reorganization on a much larger scale than in *S. pombe*. Although myosin often displays a patchy distribution in the animal cell cortex (DeBiasio et al. 1996, Vale et al. 2009, Zhou & Wang 2008), it is unclear if contractile proteins are organized in structures analogous to the cortical nodes in *S. pombe* and to what extent cortex reorganization at anaphase onset can be compared with node aggregation in yeast. Work in the *C. elegans* embryo has suggested that the contractile ring assembles in two genetically separable steps (Lewellyn et al. 2011). In the first step, which requires RhoA activation and properly spaced asters (Lewellyn et al. 2010), contractile ring proteins accumulate on the cortex in a broad equatorial band that encircles the cell equator (Figure 4c). The second step transforms the sheetlike equatorial band into a ribbonlike contractile ring that sits at the furrow tip. During this second step, the equatorial band folds in half to form a furrow that extends into the embryo interior, and contractile ring proteins become enriched along the furrow tip (Lewellyn et al. 2011). Conversion of the equatorial band into the mature contractile ring in *C. elegans* requires centralspindlin and the CPC as well as myosin II and the cytokinesis formin (Lewellyn et al. 2011, Severson et al. 2002, Shelton et al. 1999). A similar broad band–to–tight ring conversion has been documented in sea urchin embryos (Mabuchi 1994) and in *Drosophila* neuroblasts, where it was shown to require the CPC (Szafer-Glusman et al. 2011). Although less pronounced in cultured vertebrate cells, threefold narrowing of the contractile ring has also been reported in HeLa cells (Hu et al. 2011), where it was found to require a narrow central spindle.

Although further work is needed to understand the transition from equatorial band to contractile ring, it is tempting to speculate that it may involve a myosin-II- and formin-dependent filament alignment process similar to that which occurs during contractile ring assembly in *S. pombe* (Figure 4b). Filament alignment along the cell circumference has been observed in vertebrate cells using electron
microscopy (Mabuchi et al. 1988, Maupin & Pollard 1986) and fluorescence-detected linear dichroism microscopy (Fishkind & Wang 1993). Filament alignment could occur through a mechanism analogous to the search, capture, pull, and release mechanism proposed for node alignment during contractile ring assembly in fission yeast. Alternatively, physical models of contractile ring formation in which the cortex is modeled as an active gel suggest that filament alignment could result from an intrinsic physical instability of the cortex (Zumdieck et al. 2005) and/or from cortical flows driven by gradients of myosin activity (Salbreux et al. 2009). Further work is needed to explore the degree and physical basis of actin-filament alignment during contractile ring assembly in different systems.

**Mechanics of Contractile Ring Constriction**

Understanding the dynamics of ring constriction requires addressing two fundamental questions: (a) How is contractile stress generated in the ring, and (b) what are the major forces that resist ring constriction? Several physical models have been developed that aim to define the minimal mechanical requirements and to recapitulate ring constriction dynamics. Most assume that force generation results from myosin-dependent filament sliding, as occurs in muscle (Biron et al. 2005, Carlsson 2006). However, active stresses resulting from actin-filament disassembly in the presence of end-tracking cross-linkers have also been proposed as a force generator independent of myosin activity (Dickinson et al. 2004, Zumdieck et al. 2007). Importantly, although nonmuscle myosin II is essential for cytokinesis in most animal cell types (Matsumura et al. 2011), its contributions to cytokinesis remain unclear. In a recent study in COS-7 cells, Ma et al. (2012) showed that mutant myosin IIs that are able to sustain tension, but which are not capable of translocating along actin filaments, were able to rescue cytokinesis in myosin II–depleted cells in culture and in myosin-mutant cells in mice. Although it remains unclear how force to drive constriction is generated, this study suggests that the essential function of myosin II could be to cross-link actin filaments, rather than to slide them with respect to each other. Myosin II may also promote ring constriction by accelerating actin disassembly. Actin turnover has been shown to decrease upon inhibition of myosin II activity in two cultured mammalian cells (Guha et al. 2005, Murthy & Wadsworth 2005). This is consistent with studies in migrating cells, in which myosin II has been implicated in actin disassembly at the rear of the lamellipodium (Wilson et al. 2010), and with in vitro experiments, in which actin depolymerization by myosin, at very high myosin concentrations, has been observed directly (Haviv et al. 2008). These findings highlight the importance of new efforts to understand the functions of myosin II in cytokinesis.

A physical model for ring constriction in which the contraction of a ring of parallel filaments is resisted by substrate adhesions, cell surface elasticity, and cytoplasmic viscosity highlighted the potential importance of actin treadmilling. In this model, actin-filament polymerization was important to promote ring remodeling and prevent the formation of clumped actin bundles unable to promote long-range contraction (Biron et al. 2005). A model describing the ring as a homogeneous, contractile active gel predicted that tight cross-linking of individual actin filaments into larger bundled superfilaments, as well as filament turnover, would be essential for efficient contraction. In this model, the rate of ring constriction was limited by the treadmilling rate because slow filament turnover positioned myosin close to filament plus ends, where it was unable to promote efficient contraction (Carlsson 2006). Another active gel model predicts that filament turnover will be essential for ring contraction to occur at constant velocity (Zumdieck et al. 2007), as observed experimentally. So far, most physical models have focused on force generation and the role of actin turnover. Puzzling experimental questions, such as the role of actin cross-linkers,
which can hinder motor-driven contraction (Janson et al. 1992), but whose turnover can be accelerated by myosin (Mukhina et al. 2007), remain to be explored theoretically.

**Mechanics of the Polar Cortex During Cytokinesis**

In contrast to fission yeast, in which most cortical actin is found in the contractile ring during cytokinesis, animal and amoeboid cells typically have actin and myosin II throughout the cortex, including at the poles, with relative enrichment at the cell equator. Analysis in *Dictyostelium* and mammalian cells revealed that myosin-II levels were only approximately twofold higher at the equator than at the poles (Mukhina et al. 2007, Robinson et al. 2002, Sedzinski et al. 2011). The cortex at the poles is mechanically distinct from the equatorial cortex. Atomic-force microscopy on mammalian cells showed that cortex stiffness is substantially lower for the polar cortex (Matzke et al. 2001). Additionally, fluorescence-detected linear dichroism microscopy revealed that, although actin filaments align at the equator during cytokinesis, they remain isotropically organized on the polar cortex (Fishkind & Wang 1993).

Mechanically, the globally distributed actin cortex generates drag forces that resist contractile ring constriction. An early model considering cell poles with a uniform surface tension estimated the minimal equatorial force required for furrow contraction (Yoneda & Dan 1972). This model has been developed and tested experimentally in *Dictyostelium* (Surcel et al. 2010, Zhang & Robinson 2005). In *Dictyostelium*, globally distributed actin cross-linkers, including dynacortin and fimbrin, control polar resistive forces, presumably by increasing cortical viscosity, and decreasing their levels accelerates ring constriction (Girard et al. 2004, Reichl et al. 2008). Other cross-linkers, such as cortexillin, concentrate on the equatorial cortex (Surcel et al. 2010). In this system, cross-linker distribution and the mechanical properties of the cortex appear to be regulated by microtubules via the RacE/14-3-3 pathway (Zhou et al. 2010). Dynacortin and cortexillin do not have direct orthologs in animal cells, in which much less is known about the specific molecular composition of the polar cortex and about how relative regulation of actin cross-linking controls cortical properties during cytokinesis.

In addition to generating forces that resist ring constriction, the polar cortex can also display active contractions. Polar contractions can be triggered locally by a mechanosensing response upon perturbation of cell shape (Effler et al. 2006, Surcel et al. 2010). More generally, global polar contractility can make the shape of the symmetrically dividing cell intrinsically unstable (Sedzinski et al. 2011). If polar contractile forces are not balanced precisely, shape instabilities can arise in which one pole contracts at the expense of the other (Figure 5a). Such instabilities, which often result in shape oscillations and division failure, have been observed upon depletion of a number of actin-binding proteins, including the scaffolding protein anillin (Piekny & Glotzer 2008, Straight et al. 2005, Zhao & Fang 2005a), septin 11 (Estey et al. 2010), the membrane-protein supervillin (Smith et al. 2010), and the actin regulators profilin and diaphanous (Dean et al. 2005). Disruption of the cortex at one of the cell poles can also induce shape oscillations (Sedzinski et al. 2011). A physical model based on quantitative analysis of oscillating cells proposes that cortex oscillations are an intrinsic mechanical behavior of the cellular actin cortex (Sedzinski et al. 2011); depending on the relative values of cellular mechanical parameters, the dividing cell will display various shapes that can be summarized in a stability diagram (Figure 5b). How dividing cells tune their mechanical properties to avoid shape instabilities resulting from polar contractions remains to be investigated. One possibility is that polar blebs, which are observed commonly at the poles of dividing cells (Charras & Paluch 2008), act as pressure valves, constantly releasing cortical tension and effectively maintaining the stability of cell shape (Sedzinski et al. 2011).
Polar contractility also may provide a mechanism for the generation of different-sized daughter cells. During the division of *C. elegans* QR.a neuroblasts, myosin accumulates at one of the poles, and the size difference between daughter cells appears to result from an unbalanced contraction of the myosin-rich pole (Ou et al. 2010). A similar mechanism may also drive size asymmetry during *Drosophila* neuroblast division (Cabernard et al. 2010). It remains unclear how such an imbalanced shape is stabilized and what stops the myosin-enriched pole from contracting further. Mechanical studies going beyond the investigation of equatorial forces and directly probing the mechanics of the cell poles will be necessary to understand fully the physical basis of symmetric and asymmetric cell division.

**ABSCISSION**

The cell is split topologically into the two distinct daughter cells during the final stage of cytokinesis known as abscission. As the ring closes, the spindle midzone is remodeled to form the densely packed midbody, which organizes the intracellular bridge. Because the contractile ring is beneath the plasma membrane, it cannot cut through it; the presence of the densely packed midbody microtubules further prohibits the ring from constricting to completion. Instead, recent work suggests that there is a handoff from the actomyosin contractile ring to a distinct membrane-associated ESCRT-III filament system, which brings about abscission by narrowing the connection between the cells, first on one side of the cell-cell bridge and then on the other, concurrent with disassembly of the midbody microtubules (Caballe & Martin-Serrano 2011, Guizetti & Gerlich 2012).

**Maturation of the Spindle Midzone to Form the Midbody**

In one of the first ultrastructural characterizations of the intracellular bridge in human cells, Mullins & Biesele (1977) showed that the midbody is organized by a central disc of electron-dense material with embedded microtubules that emanate out in a packed parallel arrangement on either side. Later-stage intracellular bridges had narrowed across their length (to ~0.2 μm) except at the central, electron-dense region, or stem body, which retained its initial diameter (~1.5 μm). Narrowing therefore creates two secondary ingestion sites on either side of the stem body (Figure 6). This early work also documented a series of wavelike

Figure 6
Mechanisms of the polar cortex during cytokinesis. (a) Cortical forces during cytokinesis: Strong contractile forces are exerted at the equatorial ring (green arrows); contractile forces are also exerted at the poles of the cell (red arrows) by a globally distributed actomyosin cortex. Polar tension slows down furrow ingression; when polar forces are not balanced precisely, shape instabilities can arise. (b) A mechanical model of the dividing cell (Sedzinski et al. 2011) predicts a phase diagram for cell shape. The shape adopted by the cell depends on four cellular mechanical parameters: polar cortical tension, cell elasticity, the timescale of cortical turnover, and friction related to the flow of material between poles. When polar tension is high compared with cellular elasticity, the dumbbell shape of the dividing cell becomes unstable, which leads to the contraction of one pole at the expense of the other; this can result in shape oscillations (for slow cortical turnover).
ripples with regular spacing of approximately 34–43 nm along the plasma membrane in the secondary ingestion regions (Mullins & Biesele 1977), which likely correspond to locations at which the helical ESCRT-III filament system interacts with the plasma membrane (Guizetti et al. 2011).

The midbody is derived from the spindle midzone. Maturation of the spindle midzone to form the midbody is blocked in cells treated with blebbistatin, which indicates that midbody assembly requires furrow ingestion (Hu et al. 2012a). As the contractile ring constricts, components that colocalized to the central spindle are partitioned into three groups that are directed to different regions of the maturing midbody (Figure 6; Elia et al. 2011, Hu et al. 2012a). One group, which contains KIF4 and PRC1, remains associated with the central zone of microtubule overlap (Hu et al. 2012a) (midbody core, Figure 6a). A second group of central spindle proteins, which includes centralspindlin and Ect2, is released from microtubules and concentrates in the midbody ring (Elia et al. 2011, Hu et al. 2012a), where it colocalizes with anillin, RhoA, ARF6, and Cep55. Reports differ on whether some centralspindlin and Cep55 also remain in the midbody core (Elia et al. 2011, Guizetti et al. 2011, Hu et al. 2012a). The midbody ring and the central region of the midbody core are characterized by the presence of an electron-dense material. The composition of this material, which excludes antibody staining in the central part of the midbody core, remains unclear (Hu et al. 2012a). A third group of central spindle proteins, which includes CENP-E, MKLP2, and Aurora B, colocalizes with tightly packed, parallel midbody microtubules in the regions that flank the midbody core (Hu et al. 2012a) (midbody flank, Figure 6a). The midbody flank terminates near the eventual abscission site. Plk1 is required for the partitioning of central spindle components into distinct zones during midbody assembly. In the presence of a Plk1 inhibitor, central spindle components failed to relocalize, which resulted in failure to form a stem body and failure of abscission (Hu et al. 2012a).

Maturation of the Contractile Ring to Form the Midbody Ring

As the midzone matures to form the midbody, the contractile ring is converted into the midbody ring (Kechad et al. 2012). Several contractile ring components, including anillin, the septins, citron kinase, and RhoA, are retained in the midbody ring (Gai et al. 2011, Hu et al. 2012a, Kechad et al. 2012, Madaule et al. 1998). Work in Drosophila S2 cells, in which it is possible to bypass anillin’s earlier role in helping to scaffold the contractile ring, has shown that anillin is required to assemble the midbody ring and to anchor the midbody ring to the plasma membrane. Anillin-depleted S2 cells form abnormally broad intracellular bridges, which are stable for tens of minutes but eventually open back up, and cytokinesis fails (Echard et al. 2004, Kechad et al. 2012, Somma et al. 2002). The ability of anillin to cross-link the actin and
membrane-associated septin filament systems through the actin- and septin-binding domains in its N and C termini, respectively (D’Avino 2009, Pickny & Maddox 2010), seems to be critical for its role in the midbody ring. An anillin truncation that lacks the C-terminal septin-interacting domain can support midbody ring assembly, but the ring detaches from the plasma membrane, and cytokinesis fails. Septin inhibition phenocopies the midbody ring detachment phenotype (Kechad et al. 2012), which is consistent with an essential role for the anillin-septin connection in attaching the midbody ring to the plasma membrane. Citron kinase also localizes to the midbody ring (Madaule et al. 1998) and has been implicated functionally in abscission in Drosophila S2 and vertebrate cells (Echard et al. 2004, Gai et al. 2011). Citron kinase can interact with the anillin N terminus and is required to pattern the localization of anillin and RhoA in the intracellular bridge (Gai et al. 2011). In citron kinase–depleted cells, anillin and RhoA display an extended, disorganized localization along the intracellular bridge during the early stages of abscission, and they fail to be retained in the midbody ring as the bridge matures (Gai et al. 2011). Inhibition of RhoA during abscission showed that RhoA is required for localization of anillin, but not citron kinase, to the midbody ring (Gai et al. 2011). In summary, anillin plays a critical role in assembling the midbody ring and anchoring it to the plasma membrane through its association with the septins (Figure 7); retention of anillin and RhoA in the midbody ring requires citron kinase.

**Role of the ESCRT Filament System in Abscission**

One of the most exciting recent advances in cytokinesis has been the discovery that the
ESCRT complexes, which are known for their role in membrane scission during viral budding and budding of vesicles into late endosomes to form multivesicular bodies, also play a key role in abscission (Caballe & Martin-Serrano 2011, Henne et al. 2011, Hurley & Hanson 2010, Morita 2012). Conservation of ESCRT function in cytokinesis in a subset of archaea (Lindas et al. 2008, Samson et al. 2008), which lack an endomembrane system, suggests that membrane scission during cytokinesis may be the ancestral function of the ESCRT system.

ESCRT-III proteins catalyze the scission of membrane necks by polymerizing to form a spiral membrane–associated polymer. Multiple ESCRT-III proteins, which have similar structures, copolymerize in the membrane neck in an as-yet-undefined ratio to accomplish scission (Henne et al. 2011, Hurley & Hanson 2010). ESCRT-III polymers are subsequently disassembled in an energy-consuming process by a dodecameric ring–shaped AAA+ ATPase called VPS4 (Henne et al. 2011, Hurley & Hanson 2010). Humans have seven core ESCRT-III proteins, which are called CHMPs (charged multivesicular body proteins): five ESCRT-III-related regulatory proteins; and two VPS4 homologs (Henne et al. 2011, Hurley & Hanson 2010, Morita 2012). Systematic RNA interference of these components suggested that most, if not all, of these proteins have nonredundant functions in cytokinesis (Morita 2012).

The ESCRT-III complex is recruited to mediate different membrane scission events through different adaptor complexes (Henne et al. 2011, Hurley & Hanson 2010, Morita 2012). All of these adaptors utilize the heterotrimeric ESCRT-I complex. Inhibition of the ESCRT-I subunit TSG101 (tumor susceptibility gene 101), which has only one paralog in humans, blocks all ESCRT-dependent scission reactions (Carlton & Martin-Serrano 2007, Garrus et al. 2001, Lu et al. 2003, Morita et al. 2007). During HIV budding, a viral structural protein recruits ESCRT-I and a second protein called ALIX (ALG-2-interacting protein X) through direct protein-protein interactions, and ESCRT-I and ALIX, in turn, recruit ESCRT-III (Caballe & Martin-Serrano 2011, Henne et al. 2011, Hurley & Hanson 2010, Morita 2012). Cytokinesis occurs in an analogous fashion; Cep55 is the key protein directing ESCRT recruitment, and ESCRT-I and ALIX dock onto CEP55, competing for binding to an unusual coiled-coil region in the center of the protein (Carlton & Martin-Serrano 2007, Lee et al. 2008, Morita et al. 2007).

High-resolution live and fixed imaging in HeLa (Guizetti et al. 2011) and MDCK (Elia et al. 2011) cells showed that ESCRT-I and ESCRT-III are recruited sequentially to the intercellular bridge to form a pair of partially overlapping, membrane-juxtaposed rings on either side of the midbody ring (Figure 6b; Elia et al. 2011). Immediately prior to abscission, ESCRT-III was observed extending toward/accumulating at the abscission site (Elia et al. 2011, Guizetti et al. 2011). The ESCRT-III disassembly factor VPS4 followed ESCRT-III to the abscission site (Elia et al. 2011). The peak accumulation of ESCRT-III at the abscission site coincided with an abrupt loss of the midbody microtubules distal to the abscission site as well as cell separation. A similar scission event to release a midbody remnant occurred on the other side of the midbody ∼20 min later (Elia et al. 2011, Guizetti et al. 2011). Electron tomography of cells undergoing abscission revealed helical filaments 17 nm in diameter, spaced at 35-nm intervals, spanning the region between the stem body and the abscission site (Guizetti et al. 2011). These filaments, which the authors speculate are ESCRT-III filaments, likely generate the regularly spaced electron-dense ripples in the plasma membrane observed originally by Mullins & Biese (1977). The coupling between removal of midbody microtubules and abscission is thought to be achieved through recruitment of the microtubule-depolymerizing enzyme spastin by ESCRT-III (Connell et al. 2009, Reid et al. 2005, Yang et al. 2008). Spastin inhibition delays the completion of cytokinesis (Connell et al. 2009, Guizetti et al. 2011), but this can be rescued by treatment with a microtubule-depolymerizing drug.
(Guizetti et al. 2011), which suggests that microtubule disassembly is a rate-limiting step in abscission.

Abscission timing is dictated by the inactivation of the mitotic kinases Plk1 and Aurora B. Plk1 phosphorylates the ESCRT docking protein Cep55, preventing it from associating with centralspindlin and targeting to the central spindle or midbody (Bastos & Barr 2010, Fabbro et al. 2005, Zhao et al. 2006). When Plk1 is degraded during mitotic exit, Cep55 is recruited to the midbody (Bastos & Barr 2010), where it scaffolds the recruitment of ESCRT complexes to promote abscission (Carlton & Martin-Serrano 2007, Lee et al. 2008, Morita et al. 2007). Inactivation of Aurora B kinase, which localizes adjacent to the abscission sites, also regulates abscission timing in both vertebrate cells and budding yeast (Norden et al. 2006, Steigemann et al. 2009). In human cells, Aurora B prevents premature abscission by phosphorylating a regulatory region in the C terminus of the ESCRT-III component CHMP4C (Capalbo et al. 2012, Carlton et al. 2012). Degradation of Aurora B during mitosis presumably reverses this inhibition, allowing abscission to occur.

Prior to identification of the ESCRT complex as a key player, most models proposed that abscission occurred via the accumulation and fusion of Golgi complex–or endocytic-derived vesicles transported along microtubules to the division site (Barr & Gruneberg 2007, Prekeris & Gould 2008). Consistent with this idea, numerous membrane-trafficking components, including dynamin, soluble N-ethylmaleimidesensitive factor attachment protein receptor proteins (SNAREs), exocyst components, and Rab proteins, have been localized to the intracellular bridge, and disruption of membrane-trafficking processes prevents cells from completing the late stages of cytokinesis (Barr & Gruneberg 2007, Neto et al. 2011, Prekeris & Gould 2008, Schiel & Prekeris 2010). However, during characterization of the role of the ESCRT complex, vesicles were not observed in EM images of the intracellular bridge during the final stages of abscission. Instead, light microscopy revealed that Golgi complex-derived and endosomal vesicles fuse with the plasma membrane prior to abscission, coincident with the appearance of the secondary ingression sites in the intracellular bridge (Goss & Toomre 2008, Guizetti et al. 2011, Schiel & Prekeris 2011). Abscission assays have additionally revealed that Golgi complex–derived secretion has no effect on the final step (Guizetti et al. 2011). These findings have led to a rethinking of the role of membrane trafficking in abscission and to the suggestion that trafficking pathways may have alternative roles in delivering proteins to promote furrow assembly or in directing membrane remodeling in the intracellular bridge prior to abscission. We refer the reader to recent reviews on this topic (Neto et al. 2011, Schiel & Prekeris 2010).

REGULATION OF CYTOKINESIS BY THE CELL CYCLE MACHINERY

Thresholds of Cdk1 Activity and Cytokinesis

After anaphase onset, vertebrate cells prevented from completing cytokinesis by treatment with blebbistatin or the actin polymerization inhibitor cytochalasin maintain cytokinesis competence for approximately 1 h (Martineau et al. 1995, Straight et al. 2003), a window of time that has been termed C-phase (Canman et al. 2000). Proteasome inhibition increased C-phase duration twofold, which indicates that ubiquitin-mediated proteolysis helps to terminate C-phase (Straight et al. 2003). Proteasome inhibition could lengthen C-phase by slowing cyclin-B degradation. Genetic data from the Drosophila embryo have shown that cytokinesis occurs slowly but eventually completes in the presence of stable cyclin B3; however, cytokinesis fails when slowed to a similar degree by inhibition of Drosophila Ect2. This result led to the suggestion that, although cytokinesis onset requires a reduction in Cdk1 activity over metaphase levels, a lowered level of Cdk1...
activity is required to maintain the cell in a state conducive to furrowing (Echard & O’Farrell 2003). This model suggests that C-phase is the interval between two threshold levels of Cdk1 activity.

Assembly of the central spindle and contractile ring is triggered by a reduction in Cdk1 activity. Treatment of vertebrate cells with small-molecule Cdk1 inhibitors triggers translocation of Aurora B and centralspindlin to the central spindle, formation of the central spindle, and assembly and constriction of the contractile ring (Niiya et al. 2005, Potapova et al. 2006). The same result is obtained when Cdk1 is inhibited in cells arrested at metaphase with the proteasome inhibitor MG132 (Potapova et al. 2006), which demonstrates that Cdk1 inactivation is sufficient to trigger cytokinesis onset in the absence of proteasome-mediated protein degradation.

Experiments introducing nondegradable cyclins suggest that cytokinesis initiation does not require complete inhibition of Cdk1 activity. Depending on the system and protein level, introduction of stable cyclin B arrests mitotic progression at points between metaphase and telophase (Chang et al. 2003, Gallant & Nigg 1992, Hagting et al. 2002, Wheatley et al. 1997). In one study in human cells, expression of nondegradable cyclin B1 at endogenous levels blocked cells in a metaphase-like state with separated chromosomes that remained organized in a metaphase plate; expression of nondegradable cyclin B1 at lower levels led to a telophase-like arrest with separated condensed chromosomes. In this case, cytokinesis initiated and furrow ingression completed, but abscission failed, which suggests that abscission requires a greater extent of Cdk1 inactivation than does cytokinesis initiation (Wolf et al. 2006). This work has led to the hypothesis that successive events during cytokinesis require progressively lower thresholds of Cdk1 activity (Wolf et al. 2007). The conclusion that progressive Cdk1 inhibition controls the timing of events during cytokinesis is also supported by genetic experiments in the Drosophila embryo in which reduction of cyclin B or cyclin B3 function accelerated cytokinesis relative to chromosome segregation. In the converse experiments, induction of stable cyclin B prevented cytokinesis onset, whereas induction of stable cyclin B3 delayed cytokinesis onset and slowed furrow ingression while still allowing its successful completion (Echard & O’Farrell 2003).

Inhibition of Key Cytokinesis Regulators by Cdk1 Activity

Central spindle formation and contractile ring assembly require declining Cdk1 activity, because Cdk1 phosphorylation inhibits the activity of key cytokinesis regulators (Supplemental Table 2). Cdk1 phosphorylation controls the major upstream regulator of cortical contractility, Ect2. Phosphorylation of Ect2 on two different sites prevents it from docking onto centralspindlin (Yuce et al. 2005) and from loading onto the plasma membrane (Su et al. 2011). Cdk1 is proposed to directly inhibit central spindle assembly by phosphorylating the microtubule-bundling protein PRC1, the centralspindlin component kinesin-6MKLP1, and the INCENP subunit of the CPC. Phosphorylation of MKLP1 inhibits its motor activity and is thought to prevent it from localizing to the spindle (Mishima et al. 2004), and phosphorylation of INCENP prevents its interaction with MKLP2 and targeting to the central spindle (Hummer & Mayer 2009). Phosphorylation of PRC1 by Cdk1 has been proposed to prevent its oligomerization and targeting to microtubules as well as to inhibit its ability to recruit Plk1 (Jiang et al. 1998, Mollinari et al. 2002, Neef et al. 2007, Zhu et al. 2006). However, recent work has suggested that the regulation of PRC1 by Cdk1 may not be as simple as this model would suggest. Hu et al. (2012b) showed that phosphorylation of PRC1 on T602 by Plk1 was essential to prevent premature PRC1-mediated microtubule bundling and the formation of “metaphase midzones” that also recruited centralspindlin. By contrast, they found that mutant PRC1 with all three
of its known Cdk1 sites changed to alanine did not prematurely bundle microtubules in vivo, which suggests that these sites may not be critical for PRC1 regulation during metaphase. Additional work will be needed to understand how the potent regulators of central spindle assembly and contractile ring formation are inhibited in metaphase and activated as Cdk1 activity levels decline following anaphase onset.

**Roles of the Anaphase-Promoting Complex/Cyclosome in Cytokinesis**

Another candidate for orchestrating events during cytokinesis is the anaphase-promoting complex/cyclosome (APC/C). The APC/C is a cullin-RING finger E3 ubiquitin ligase that controls Cdk1 activity by ubiquitylating cyclin B and targeting it for destruction by the 26S proteasome (Pines 2011). During anaphase, the APC/C also promotes sister chromatid separation by targeting securin and thereby activating separase, which cleaves the cohesin complexes linking sister chromatids. The APC/C plays a critical role in cytokinesis by controlling the kinetics of the reduction of cyclin B–Cdk1 activity (Pines 2011). In theory, the APC/C could also have additional functions in: (a) promoting the degradation of mitotic proteins that obstruct the progress of cytokinesis, (b) targeting cytokinesis proteins to control the timing of progression through C-phase and abscission, or (c) cleaning house after cytokinesis is complete to prevent cytokinesis proteins from interfering during subsequent cell cycle stages. Work to date has provided evidence for all three of these ideas (Supplemental Table 2). The APC/C is a multisubunit complex that depends on the presence of one of a pair of WD-40 repeat–containing coactivators, Cdc20 or Cdh1, for its activity. A switch from Cdc20 to Cdh1 during anaphase contributes to a change in APC/C substrate specificity (Pines 2011). Aurora A kinase is degraded by APC/C^Cdh1^ (Castro et al. 2002, Littlepage & Ruderman 2002, Taguchi et al. 2002). Cdh1 depletion results in a weak spindle midzone and accelerated cytokinesis timing, which can be mimicked by expression of nondegradable Aurora A (Floyd et al. 2008). This result suggests that the APC/C promotes proper cytokinesis progression by ensuring timely Aurora A degradation. Human Plk1 is degraded by APC/C^Cdc20^ (Floyd et al. 2008, Lindon & Pines 2004). Expression of stable Plk1 delays mitotic exit, anaphase, and cytokinesis (Lindon & Pines 2004). As discussed in the abscission section, loss of Plk1 activity is thought to dictate abscission timing by triggering the recruitment of the abscission regulator CEP55 (Bastos & Barr 2010). The Ect2 RhoGEF (Liot et al. 2011), the CPC component Aurora B (Floyd et al. 2008, Nguyen et al. 2005, Stewart & Fang 2005), and anillin (Zhao & Fang 2005a) are all targeted for degradation by APC/C^Cdh1^. Inhibiting the degradation of Ect2 or Aurora B has been shown to result in cell transformation (Liot et al. 2011, Nguyen et al. 2005), which highlights the importance of APC/C^Cdh1^-mediated degradation of key regulators after cytokinesis is complete.

**FUTURE PERSPECTIVES**

It is an exciting time for cytokinesis research. Understanding cell division is a cross-disciplinary effort because it requires an integrated understanding of cellular mechanics, the cytoskeleton, cell signaling, membrane dynamics, and the cell cycle. In addition to an increased understanding of the molecular processes underlying cytokinesis, cytokinesis research will benefit from systems-level approaches that provide insight into how molecular regulation is coupled to the mechanical processes underlying cell shape changes during cytokinesis. As most work to date has been performed in cultured cells, additional work is needed to determine how findings from cultured cells relate to the division of cells within tissues in vivo, and how the extracellular environment and neighboring cells influence cytokinetic events to coordinate cell division with tissue structure.
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