Cytokinesis in Metazoa and Fungi

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SUMMARY

Cell division—cytokinesis—involves large-scale rearrangements of the entire cell. Primarily driven by cytoskeletal proteins, cytokinesis also depends on topological rearrangements of the plasma membrane, which are coordinated with nuclear division in both space and time. Despite the fundamental nature of the process, different types of eukaryotic cells show variations in both the structural mechanisms of cytokinesis and the regulatory controls. In animal cells and fungi, a contractile actomyosin-based structure plays a central, albeit flexible, role. Here, the underlying molecular mechanisms are summarized and integrated and common themes are highlighted.

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1 INTRODUCTION

Cell multiplication requires replication and segregation of the genome and partitioning of the cell contents into two daughter cells through cytokinesis. For this to result in viable, genetically identical daughter cells, the plane of cell division needs to be coordinated with the position of the mitotic spindle. This can be accomplished by the mitotic spindle directing the position of the division plane or by positioning the mitotic spindle with respect to a previously established division plane. Once positioned, the biochemical or structural mark that defines the division plane must give rise to a physical barrier that subdivides the mother cell. The barrier can be created by vesicle fusion and/or constriction of a contractile apparatus that draws in the plasma membrane. Finally, the membrane that serves as the barrier between the cells needs to be fully separated—a distinct process termed abscission.

Although the above account constitutes a minimal description of cell division, in some cell types the process is substantially modified. For example, cytokinesis can be coupled with growth to maintain cell size, the division plane can be coordinated with the asymmetric segregation of cortical or cytoplasmic factors, the process of cytokinesis can be completely suppressed as in a syncytial division, or just the final step can be suppressed, generating daughter cells that remain connected but that function autonomously.

Cytokinesis is less well-conserved than nuclear division among eukaryotes. The core molecules that mediate cell cycle progression and nuclear division can be recognized throughout eukaryotes; however, the molecules that facilitate cytokinesis vary among the eukaryote lineages. Members of the opisthokonta clade, including metazoa and fungi (Adl et al. 2012), contain both filamentous (F-) actin and myosin II that assemble into a contractile ring that drives cell division. However, myosin II is absent from many other eukaryotes, including plants, ciliates, and algae (Richards and Cavalier-Smith 2005; Sebé-Pedrós et al. 2014). Cytokinetic mechanisms in opisthokonts, as exemplified by metazoans, and budding and fission yeast are reviewed. As each step in the process of cytokinesis is discussed, the commonalities and differences in cytokinetic mechanisms in animal cells and fungi will be highlighted.

2 CELLULAR STRUCTURES THAT ORCHESTRATE CYTOKINESIS

Cytokinesis involves a large-scale reorganization of the contents of the cell. A reorganization of this scale necessitates machinery that can induce cellular-scale rearrangements. The principle driving force for cytokinesis

in yeast and metazoa is the actomyosin-based contractile ring. Microtubules play a regulatory role in positioning of the division plane and facilitate the abscission step of cytokinesis.

2.1 Contractile Ring

Pioneering analyses in sea urchin embryos showed that the contractile ring contains actin filaments closely apposed to the plasma membrane and aligned circumferentially along the cell equator (Schroeder 1972). In early embryonic divisions, the sea urchin contractile ring is $\sim 8\text{-}\mu\text{m}$ wide and projects $\sim 0.2\text{ }\mu\text{m}$ into the cytoplasm. In these cells, the ring width and thickness is fairly constant during constriction, implying that significant disassembly of actin filaments occurs while the ring constricts. Individual filaments are relatively short, $\sim 0.25\text{ }\mu\text{m}$. Follow-up studies showed the presence of myosin, some assembled into minifilaments, located in the furrow region and associated with actin filaments of mixed polarity (Fujiwara and Pollard 1976; Mabuchi and Okuno 1977; Sanger and Sanger 1980; Yumura et al. 1984; Maupin and Pollard 1986). Many of these essential conclusions have been confirmed by live-cell imaging with fluorescently labeled actin and myosin in diverse animal species and fungi (Bi et al. 1998; Bezanilla et al. 2000; Yumura 2001; Murthy and Wadsworth 2005).

2.2 Accessory Factors of the Contractile Ring

The components of the contractile ring have been most thoroughly inventoried in the fission yeast *Schizosaccharomyces pombe*. The major components enriched in the fission yeast contractile ring are actin, myosin II and its essential and regulatory light chains, the anillin-related protein Mid1, the F-BAR protein Cdc15, the formin Cdc12, the actin-bundling protein α -actinin Ain1, the actin-stabilizing protein tropomyosin Cdc8, and the IQGAP Rng2 (Wu and Pollard 2005). Orthologs of most of these proteins also accumulate in contractile rings in metazoan cells, with the exception of the F-BAR protein Cdc15.

The recruitment mechanisms for many regulatory proteins are reasonably well understood and will be discussed below. In contrast, the mechanisms that control the accumulation of specific actin-binding proteins to the contractile ring as compared with other actin-containing structures in the cell are less well understood. Competition between various actin-binding proteins for binding to particular classes of filaments contributes to this sorting process (Skau and Kovar 2010). For example, fimbrin and tropomyosin mutually antagonize each other's binding to endocytic patches and the contractile ring, respectively.

2.3 Accessory Structures in Fission and Budding Yeast

Ultrastructural analysis of the *S. pombe* contractile ring revealed an arrangement of actin filaments similar to that of animal cells, albeit at a smaller scale (Kamasaki et al. 2007). In the mature ring, the actin filaments average ~600 nm in length and show interdigitation. Live-imaging studies with fluorescent proteins revealed that the contractile ring assembles from a series of cortical nodes that contain myosin motors and simultaneously nucleate actin assembly (Vavylonis et al. 2008). These nodes assemble into rings by means of a pathway that will be discussed in detail below.

Ultrastructural studies in budding yeast revealed a strikingly ordered set of 10-nm filaments tightly associated with the plasma membrane and restricted to the bud neck (Byers and Goetsch 1976). Subsequent analysis revealed that these filaments are formed by a class of protein called septins (Haarer and Pringle 1987). Multimeric septin complexes constitute the building blocks of these filaments (Sirajuddin et al. 2007). These heteromultimers assemble longitudinally and pair through the carboxy-terminal coiled-coil domains, ultimately forming paired, antiparallel filaments. Despite being highly ordered, the overall arrangements of septin filaments undergo dynamic rearrangement during the cell cycle, being primarily aligned with the axis of the bud neck early in the cell cycle and, later, aligning circumferentially after a single septin ring splits into two rings just before the onset of cytokinesis (Demay et al. 2011). However, septin rings are not contractile; instead, budding yeast assemble an actomyosin contractile ring between the split septin rings.

Yeast cells are surrounded by a rigid cell wall; therefore, cytokinesis must be tightly coupled with remodeling the old cell wall and assembly of a new wall or septum. In budding yeast, the actomyosin-based ring and exocytic deposition of septal material constitute parallel pathways for narrowing the bud neck, either of which can support successful cytokinesis (Vallen et al. 2000).

2.4 Anaphase Spindle

In animal cells, the anaphase spindle plays a central role in cytokinetic processes. The anaphase spindle consists primarily of astral arrays of dynamic microtubules and bundled, nonkinetochore, antiparallel spindle microtubules, forming the central spindle. In addition, antiparallel astral rays can sometimes interact, allowing the formation of midzone-like structures in the cell periphery (Su et al. 2014).

As will be explained below, the central spindle participates in most steps of cytokinesis. It facilitates division-

plane positioning, RhoA activation, assembly of the midbody, and finally abscission, in which the plasma membrane is abscised to generate two separate daughter cells (for a review, see White and Glotzer 2012).

An assortment of microtubule-associated proteins (MAPs) and kinesin motor proteins mediates bundling of the antiparallel, central spindle microtubules (reviewed by Glotzer 2009). Prominent among these microtubule regulators is the centralspindlin complex. Centralspindlin is a heterotetrameric complex consisting of a dimer of the kinesin-6 MKLP1 (or its orthologs [*Caenorhabditis elegans* ZEN-4, *Drosophila melanogaster* Pavarotti; MKLP1 hereafter]) stably bound to a dimer of Cyk4/MgcRacGAP (or its orthologs C.e. CYK-4, D.m. RacGAP50C/Tumbleweed; Cyk4 hereafter) (Mishima et al. 2002). Another important protein in the spindle midzone is the MAP Prc1, which preferentially binds to antiparallel microtubules (Jiang et al. 1998; Subramanian et al. 2010). In animal cells, Prc1 complexes with kinesin-4 and concentrates at microtubule plus ends, where it facilitates bundling of antiparallel microtubules (Subramanian et al. 2013). Prc1 is evolutionarily ancient, being found in diverse eukaryotes, including metazoa, yeast, and plants. In contrast, centralspindlin is restricted to metazoans (Frédéric et al. 2013; M Glotzer, unpubl.).

The chromosome passenger complex is another prominent component of the central spindle. This four-protein complex regulates numerous mitotic events by spatially restricting the localization of its catalytic subunit aurora B (Ruchaud et al. 2007). During mitosis, the complex localizes at kinetochores and, during anaphase, it localizes to both the spindle midzone and the equatorial cortex.

3 SPATIOTEMPORAL CONTROL—INTRODUCTION

Cytokinesis is usually coupled to cell cycle progression. However, the degree to which the two processes are coupled varies in different cell types, as do the underlying mechanisms. In metazoan cells, cytokinesis is an anaphase event; cytokinetic events are inhibited until the metaphase-to-anaphase transition, whereas, in yeasts, some aspects of division-plane positioning begin soon after the previous division.

Diverse mechanisms contribute to division-plane positioning in different organisms (Oliferenko et al. 2009). However, these processes can be conceptually unified by considering division-plane positioning to be a form of cell polarization wherein a defined domain of the cell acquires special characteristics. Similarly, during cytokinesis, the division plane, frequently the equator, acquires characteristics distinct from the remainder of the cell. This per-

spective is applicable to essentially all dividing cells, despite the existence of divergent mechanisms. In some cell types, cytokinesis is inextricably coupled to cell polarization. Additionally, the molecular mechanisms that mediate certain types of cell polarization and cytokinesis overlap, both being highly dependent on Rho-family GTPases.

There are a number of pathways by which the cell cortex becomes polarized during cytokinesis. In animal cells, this polarization is manifested by the local activation of the small GTPase RhoA, which is directly involved in assembly of the cleavage furrow. Local RhoA activation creates a membrane-associated domain that favors actin polymerization and myosin activation, ultimately building a contractile ring. In fission yeast, the initial polarization is manifested by the accumulation of a set of relatively stable protein-based nodes in the equatorial cortex (Wu et al. 2006). In budding yeast, the division plane is coincident with the site of bud formation, which is triggered by the local activation of the conserved Rho-family GTPase Cdc42. In the following sections, each of these cases will be discussed in detail.

3.1 Temporal Control in Metazoa

Most metazoan cells round up during mitosis, thereby altering cell–substrate and cell–cell associations. A number of mechanisms cooperate to induce this cell rounding, including an increase in osmotic pressure that exerts force against a uniformly contractile actomyosin cortex (Stewart et al. 2011) induced by a modest activation of RhoA (Maddox and Burridge 2003; Matthews et al. 2012). During mitosis, the cyclin-dependent kinase Cdk1–cyclin-B phosphorylates centralspindlin, inhibiting its association with the spindle (Mishima et al. 2004), and Ect2, inhibiting its association with both the plasma membrane and centralspindlin (Yüce et al. 2005; Su et al. 2011). Similarly, inhibitory phosphorylation by Cdk1 and the polo-like kinase Plk1 on PRC1 limits PRC1 localization during the cell cycle (Zhu et al. 2006; Hu et al. 2012). PRC1 shows multiple modes of microtubule binding, depending on its interaction partners in the midzone, notably Kif4 (Subramanian et al. 2013).

Cytokinetic events, per se, do not commence until the metaphase-to-anaphase transition, when the anaphase-promoting complex/cyclosome (APC/C) is activated, triggering destruction of cyclin B and inactivation of Cdk1. Removal of inhibitory mitotic phosphorylations activates cytokinetic regulators, including centralspindlin. Activation of RhoA during anaphase also requires activating phosphorylations, including phosphorylation of the centralspindlin subunit Cyk4 by Plk1. Phospho-Cyk4 binds to the Rho GDP–GTP exchange factor (GEF) Ect2 (Burkard et al. 2009; Wolfe et al. 2009), relieving its autoinhibition.

3.2 Spindle-Dependent Division-Plane Positioning in Metazoa

The anaphase spindle is the primary determinant of positioning of the cleavage furrow (Rappaport 1986). It contains bundled antiparallel microtubules forming the spindle midzone and dynamic spindle pole–anchored astral microtubules. These structures appear to function coordinately, the dominant structure depending on the cell type in question (Fig. 1A). Occasionally, spindle-independent mechanisms influence the position of the division plane.

The centralspindlin complex is required for division-plane specification in metazoan cells. This complex localizes prominently to the spindle midzone, which lies at variable distances from the cell membrane, raising the question of how it impacts events on the membrane when they are well separated. Indeed, a second, smaller pool of centralspindlin that localizes to the plasma membrane has recently been shown to play a role (Basant et al. 2015). The Cyk4 subunit of centralspindlin contains a weak membrane-binding domain (Lekomtsev et al. 2012). During anaphase, centralspindlin undergoes regulated oligomerization. Oligomerization promotes the microtubule association of centralspindlin (Hutterer et al. 2009) and membrane localization (Basant et al. 2015) through distinct domains. Centralspindlin activates the RhoGEF Ect2, which activates RhoA at the plasma membrane; thus, these interactions could direct positioning of the division plane (Fig. 1B,C) (Yüce et al. 2005; Burkard et al. 2009; Zhang and Glotzer 2015).

The equatorial localization of centralspindlin is proposed to be under control of the chromosome passenger complex that accumulates at the site of the presumptive furrow (Cooke et al. 1987; Basant et al. 2015). Aurora B kinase—the catalytic subunit of this complex—promotes centralspindlin oligomerization (Douglas et al. 2010). The mechanism of Aurora B localization is incompletely understood, although the kinesins of the Kif20 family (also called MKLP2) and Kif4 are required in vertebrate systems (Gruneberg et al. 2004; Nguyen et al. 2014). Although the membrane-bound pool of centralspindlin is important when the midzone and cortex are far apart, in cells in which they are closer, or once the furrow has begun to ingress, in larger cells, the midzone pool of centralspindlin and associated ECT-2 likely sustain furrow ingression (Yüce et al. 2005; Lekomtsev et al. 2012).

3.3 Spindle-Independent Positioning of the Division Plane in Metazoa

Although the centralspindlin complex promotes furrow formation, in a number of cell types furrow formation can occur when this complex is disrupted, aurora B is inhibited, or the spindle midzone is disrupted. For example,

Figure 1. Cytokinesis in metazoa. (A) Overview of the mechanism of division-plane positioning. Furrow position is determined by the position of the mitotic spindle. Positive signals from the spindle midzone and bundled astral microtubules cooperate with inhibitory signals from dynamic astral microtubules to position the division plane. (B) Pathway that induces accumulation of contractile ring components. Components conserved between metazoa and yeast are highlighted in blue. (C) Spatial organization of the activators of contractile ring components and schematic depicting the contractile ring assembled from short actin filaments. GAP, GTPase-activating protein; GEF, GDP–GTP exchange factor.

in *C. elegans* embryos, a second, NOP-1-dependent pathway for RhoA activation can promote furrow formation in centralspindlin-defective embryos (Raich et al. 1998; Tse et al. 2012). NOP-1 primarily promotes contractility and polarization of the fertilized zygote and secondarily induces global activation of RhoA and its effectors during cytokinesis. Cultured mammalian cells depleted of the kinesin-6 subunit of centralspindlin can also form furrows (Yüce et al. 2005). In both these cases, the polarized accumulation of RhoA appears to result from local inhibition by the astral microtubules of the anaphase spindle (Murthy and Wadsworth 2008; Tse et al. 2012). The inhibitory mechanism is not fully understood; however, one component involves local inhibition of anillin function by astral microtubules (Piekný and Glotzer 2008; Tse et al. 2011). These additional mechanisms confer robustness and could be particularly relevant in cleaving embryonic blastomeres.

Recently, the use of optogenetic tools in the context of cytokinesis revealed that spatial and temporal control of cytokinesis is largely achieved through the control of RhoA activation. Creation of a zone of active RhoA can

induce furrow formation in cells at various cell cycle stages and irrespective of the position of the spindle (Wagner and Glotzer 2016). In certain animal cells, furrow formation is independent of spindle microtubules. *Drosophila* pole cells, which ultimately become germ cells, are formed by a budding-like process that encapsulates the germ cell nucleus (Cinalli and Lehmann 2013). This contractility depends on RhoA and its effectors, as well as on a protein called germ cell-less. An analogous process can be elicited in dividing *Drosophila* neuroblasts. Depolymerization of the spindle microtubules eliminates the conventional furrow, but a secondary furrow that requires cortical polarity proteins can occur in these cells at the onset of anaphase (Cabernard et al. 2010).

3.4 Temporal Control of Cytokinesis in Fission Yeast

In *S. pombe*, contractile ring assembly begins earlier in the cell cycle than in metazoan cells. The contractile ring assembles from equatorially positioned, cortically associated, cytokinetic nodes (large, membrane-associated multimo-

lecular complexes). Cytokinetic nodes are derived from the fusion, during G₂ phase, of two classes of interphase nodes that differ in protein composition, subcellular position, and mobility (Akamatsu et al. 2014). Components of the contractile ring join one of the two classes of cortical nodes at different times throughout the cell cycle (see below). Following association with class 1 nodes shortly after division, the anillin ortholog Mid1 ultimately concentrates in the equatorially localized cytokinetic nodes in G₂ (Akamatsu et al. 2014). Myosin II is also recruited to these nodes before spindle assembly, and other factors, including a nucleator of actin filaments, are recruited during M phase (Wu et al. 2003). Ring constriction begins ~25 min after assembly of a complete contractile ring, which is ~10 min after the spindle has fully elongated.

A signaling cascade called the septation initiation network (SIN) regulates both classes of interphase nodes and maturation of the contractile ring. The SIN includes a GTPase, scaffold proteins, and kinases from both the Ste20 and Ndr kinase families (see Simanis 2015 for review). This pathway becomes activated during anaphase and localizes prominently at spindle pole bodies (SPBs) (some components accumulate specifically on one of the two SPBs); the most-downstream components—the Ndr kinase Sid2 and its associated activating subunit Mob1—localize to the contractile ring.

Mutations in SIN pathway components prevent complete ring assembly (Hachet and Simanis 2008), and the SIN regulates several contractile ring components. SIN activation promotes disassembly of type 1 nodes and maturation of type 2 nodes (Pu et al. 2014). The SIN also regulates contractile ring components, including the formin Cdc12 and the F-BAR protein Cdc15. Cdc15 regulation by the SIN is likely indirect, as Cdc15 is activated by dephosphorylation. Indeed, the SIN activates the phosphatase Clp1, which dephosphorylates Cdc15 (Roberts-Galbraith et al. 2010). Dephosphorylation of Cdc15 allows its oligomerization, its association with the membrane, and allows it to interact with its binding partner Cdc12 (Fig. 2) (Roberts-Galbraith et al. 2010).

Not only is the SIN required for maturation of the contractile ring, its hyperactivation can induce contractile ring assembly and constriction at inappropriate times. Direct activation of the SIN (Schmidt et al. 1997), or its indirect activation by means of polo kinase Plo1 overexpression (Cullen et al. 2000; Tanaka et al. 2001), induces assembly of contractile rings and septa at various stages of the cell cycle. More downstream, overexpression of a truncated Cdc12 formin is also sufficient to induce ring assembly in interphase (Yonetani and Chang 2010). Because SIN activation promotes formin activation (Bohnert et al. 2013), these perturbations might share a common mechanistic basis.

Figure 2. Cytokinesis in the fission yeast *Schizosaccharomyces pombe*. (A) Overview of the mechanism of division-plane positioning. The contractile ring assembles at a site that is determined by the position of the nucleus in G₂ phase. Interphase microtubules serve to position the nucleus near the cell center. (B) Pathway that induces accumulation of components of the contractile ring. Components conserved between yeast and metazoa are highlighted in blue. (C) Schematic depicting the assembly of an actomyosin-based contractile ring from a broad band of cytokinetic nodes that progressively align by means of the so-called search-capture-pull-release mechanism. Note also the relationship between the ingressing contractile ring and the required remodeling of the cell wall materials through septum deposition.

3.5 Contractile Ring Positioning in Fission Yeast

In fission yeast, as in metazoan cells, multiple pathways cooperate to position the division plane. These pathways for division-plane positioning converge on Mid1, an anillin-like scaffold protein. Deletion of Mid1, although not lethal, causes severe defects in ring positioning and assembly (Paoletti and Chang 2000). Mid1 has a cryptic C2 domain, a pleckstrin-homology (PH) domain, and an amphipathic helix that permits it to associate directly with the membrane (Sun et al. 2015). Its membrane localization is regulated by several pathways (Almonacid et al. 2009; Lee and Wu 2012). One pathway involves regulated export of Mid1 from the nucleus (Fig. 2A), promoting membrane association in the vicinity of the nucleus (Celton-Morizur et al. 2004; Lee and Wu 2012); an additional regulatory pathway involves the kinase Pom1, which regulates cell polarity and prevents Mid1 accumulation at cell tips (Celton-Morizur et al. 2006; Padte et al. 2006). A third pathway that controls Mid1 localization involves cortical nodes of proteins that accumulate during interphase. Two types of nodes accumulate during interphase in wild-type cells (Akamatsu et al. 2014). Type 1 nodes coordinate growth and the cell cycle (Moseley et al. 2009). These nodes are nucleated by the cell cycle–regulating kinase Cdr2 and recruit both Mid1 and myosin II. Type 2 nodes contain the adaptor protein Blt1 and a RhoGEF called RhoGEF2 (Moseley et al. 2009; Guzman-Vendrell et al. 2013). Type 1 nodes accumulate in the cell center, whereas type 2 nodes are more mobile. These two types of nodes fuse in late G₂ in the cell center, and some type 1 node components dissipate from these hybrid, cytokinetic nodes during M phase (Fig. 2C) (Guzman-Vendrell et al. 2013). Cytokinetic nodes, loaded with approximately 20 to 40 molecules of Mid1 and myosin II, recruit a small number of Cdc12 formin dimers, approximately 20 molecules of the F-BAR protein Cdc15, and other ring components (Wu and Pollard 2005; Laporte et al. 2011). These nodes then undergo a well-characterized ring assembly process that will be discussed below (Vavylonis et al. 2008). Mid1 likely induces local concentration of contractile ring components, as artificial targeting of several ring components to nodes can largely restore cytokinesis in mid1 mutant cells (Tao et al. 2014). This is likely to reflect a conserved function of the anillin family of scaffold proteins.

Despite their central role in cytokinesis, neither type of node appears essential for viability. Deletion of node components Cdr2, Blt1, and Gef2, either alone or in some pairwise combinations, delays, but does not prevent, contractile ring assembly (Almonacid et al. 2009; Moseley et al. 2009; Akamatsu et al. 2014; Goss et al. 2014). Contractile ring positioning in these cells is sensitive to perturbations that

would otherwise have mild effects. This robustness could be due to the presence of additional cytokinetic regulators in the nodes, notably the nonessential RhoGEF Gef2 (Ye et al. 2012; Guzman-Vendrell et al. 2013). These results indicate that both types of nodes play a central role in ring assembly and make the process rapid, properly timed and robust to perturbations. However, the relatively mild phenotypes of mutants in both types of nodes suggest the existence of other mechanisms for contractile ring assembly.

Consistent with this model, contractile ring assembly in the related fission yeast *Schizosaccharomyces japonicus* does not require Mid1 (Gu et al. 2015). When Mid1 is absent, myosin II does not detectably accumulate on cortical nodes during interphase—it first appears in anaphase. Therefore a robust, Mid1-independent pathway exists in this species; the aforementioned kinase Pom1 plays a key role in ring positioning (Gu et al. 2015).

3.6 Bud Site Selection in Budding Yeast

The spatial control of cytokinesis in budding yeast is directly coupled to bud site selection, as cell division occurs at the bud neck (Fig. 3A). During mitosis and anaphase, one of the two spindle poles, usually the older one, is drawn through the bud neck to coordinate mitosis with the plane of cell division. Despite the fact that the spindle moves relative to the plane of cell division rather than the converse in metazoa, conceptual commonalities exist between the process of bud site selection in yeast and division-plane positioning in metazoan cells. Both processes involve local accumulation of an activated Rho-family GTPase, which in yeast budding is Cdc42 (see Bi and Park 2012 for review). This is accomplished by the cell cycle–regulated activation and localization of a GEF, which in yeast is Cdc24. Local accumulation of Cdc42 at a cortical site in G₁ phase promotes assembly of septin rings at the bud neck, through adaptor proteins. Ultimately, septin filaments template assembly of the contractile ring by recruiting specific factors at defined cell cycle stages.

3.7 Temporal Control of Cytokinesis in Budding Yeast

Contractile ring assembly appears to initiate early in the cell cycle in budding yeast, as myosin II accumulates at the bud neck during G₁ phase. However, two sequential and independent pathways promote myosin accumulation to this site: One pathway is active during G₁, and the second is active during anaphase; only the latter is required for cytokinesis (Fang et al. 2010). Other important events in ring assembly are temporally regulated by a polo kinase ortholog, Cdc5. Cdc5 promotes accumulation of the RhoGEF

Figure 3. Cytokinesis in the budding yeast *Saccharomyces cerevisiae*. (A) Overview of the mechanism of division-plane positioning. The contractile ring assembles at the neck of the bud, which is positioned in early G₁ phase. The spindle is positioned relative to the bud neck during anaphase. (B) Pathway that induces accumulation of components of the contractile ring. Components conserved between metazoa and yeast are highlighted in blue. (C) Spatial organization of the yeast contractile ring, highlighting the relationship between the ingressing contractile ring and the required remodeling of the cell wall materials through septum deposition. GAP, GTPase-activating protein; GEF, GDP–GTP exchange factor.

Tus1 at the bud neck shortly after anaphase (Yoshida et al. 2006). Tus1, in turn, activates Rho1, inducing local recruitment of the formin Bni1, which nucleates assembly of the actin filaments in the contractile ring (Fig. 3B,C).

3.8 Spatiotemporal Regulation—Summary

Although diverse, the examples above do not encompass all the known mechanisms for division-plane positioning in eukaryotic cells. Plant cell cytokinesis diverges significantly, and other organisms, such as protozoa, divide through distinct mechanisms (Farr and Gull 2012). However, despite this diversity, a number of themes recur. First, redundant pathways ensure proper positioning in many cells. Second, lipid binding, multiprotein scaffolds recruit the structural proteins of the contractile ring. Third, polo-like kinases play a major role in promoting cytokinetic events, although their substrates vary between organisms. Fourth, there is an inverse correlation between the degree of order of the structure that templates the division plane and the degree of flexibility in the division-plane positioning mechanism. For example, the septin ring that organizes the contractile ring of budding yeast is highly ordered and stable at the bud neck, and this is the only site at which cytokinesis occurs in this organism. The nodes and nucleus of fission yeast that position the division plane are moderately mobile and allow the division plane to be positioned relative to the position of the cell nucleus. In animal cells, division-plane positioning is highly plastic and allows repositioning after its initial assembly (Rappaport 1985). It lacks large structures or stable aggregates, yet each compo-

nent of the multiprotein complex that positions the contractile ring has membrane-binding motifs, suggesting cooperative membrane association.

4 CONTRACTILE RING ASSEMBLY AND CONSTRICTION

Once the upstream pathways induce accumulation of contractile ring components at the equatorial cortex, they then assemble into a ring that constricts and deforms the overlying plasma membrane. This process involves assembly of bundles of actomyosin, coordinated constriction, and filament disassembly, and dynamic, yet force-generating, association of contractile bundles with the membrane. Many of the key proteins—cytoplasmic myosin, actin, formin, actin-bundlers, the actin-depolymerizing factor cofilin, and anillin—accumulate on contractile rings in metazoa and budding and fission yeast, suggesting that the underlying assembly process is generally conserved. However, they are not yet fully understood.

4.1 The Role of RhoA and Its Effectors in Metazoa

Activated Ect2 generates a metastable cortical zone of active RhoA, likely through positive feedback (Zhang and Glotzer 2015). Active RhoA directly stimulates the Rho kinase ROCK, which induces myosin light chain phosphorylation, filament assembly, and motor activation (Kosako et al. 2000). RhoA also directly activates formins, which nucleate assembly of unbranched actin filaments (Otomo et al. 2005; Watanabe et al. 2008, 2013). RhoA recruits anillin,

a scaffold protein that can interact with RhoA, F-actin, and myosin (Fig. 1B) (Straight et al. 2005; Piekny and Glotzer 2008; Sun et al. 2015). These direct RhoA effectors generate myosin minifilaments and actin filaments that recruit tropomyosin, α -actinin (Mukhina et al. 2007), and septin. The process by which these components assemble into a well-ordered ring is not fully understood, in part, because the underlying events involve rapidly rearranging and highly concentrated assemblages of filaments that are challenging to resolve in the light microscope.

4.2 Membrane Association of the Contractile Ring

The creation of a furrow that partitions the daughter cells requires association of the contractile ring with the plasma membrane. Note that the contractile ring need not encircle the cell for furrow ingression to occur as there are numerous examples of unilateral furrowing in which the furrow ingresses from one side before it is fully assembled, including in amphibians and other species. This indicates that membrane association of filaments in the contractile ring can support force generation. The nature of this association is not well characterized. Defects in this linkage would result in contractile ring constriction accompanied by a failure to draw in the plasma membrane.

There are some molecules that appear to contribute to this bridging function. Manipulation of the levels of phosphatidylinositol (4,5)-bisphosphate (PIP2) disrupts the association of the ring with the membrane in a minority of cells (Field et al. 2005). Lipid kinases and phosphatases responsible for controlling PIP2 levels impact cytokinesis at a variety of stages (Ben El Kadhi et al. 2011; Dambournet et al. 2011). The complete list of relevant PIP2-binding factors has not been unambiguously identified, although a class of good candidates is FERM (4.1 protein–ezrin–radixin–moesin)-domain-containing proteins (Fehon et al. 2010). In *Dictyostelium*, cells deficient in the FERM-domain-containing protein talin show compromised linkage between the ring and membrane (Tsujioka et al. 2012); ERM proteins also suppress cortical blebbing during cytokinesis, particularly at cell poles (Roubinet et al. 2011). In both *S. pombe* and *Drosophila*, F-BAR-containing proteins might serve as linkers between the membrane and cytoskeleton (Roberts-Galbraith et al. 2010; Takeda et al. 2013); in *S. pombe*, the F-BAR protein Cdc15 also promotes accumulation of a polysaccharide synthetase (Arasada and Pollard 2014), which also contributes to productive furrowing (Liu et al. 1999). Cytoplasmic myosin II associates with anionic phospholipids (Li et al. 1994). Thus, there might not be a single, specific tether for the contractile ring, but rather a constellation of diverse contributing factors. Finally, late in cytokinesis, the membrane-binding C1 domain of

the centralspindlin subunit Cyk4 is required for membrane linkage to the central spindle at the midbody stage (Lekomtsev et al. 2012), which is also stabilized by the scaffold protein anillin (D'Avino et al. 2008; Kechad et al. 2012).

4.3 Contractile Ring Assembly in Fission Yeast

The process of ring assembly has been most clearly defined in fission yeast. The aforementioned nodes that contain myosin and formin nucleate actin filaments that are captured by neighboring nodes and pulled together (Vavylonis et al. 2008). The barbed end of the growing actin filament is anchored by the nucleating formin at the donor node, and the pointed end of the filament is captured by myosin at the acceptor node (Laporte et al. 2011). This interaction results in node congression at ~ 30 nm/sec for a duration of ~ 20 sec. Internode connections are severed by cofilin (Chen and Pollard 2011), and this process repeats until the nodes and filaments are aligned. This is known as the search–capture–pull–release model (Fig. 2C) (Vavylonis et al. 2008). This process has been modeled using physiological parameters that recapitulate observations in wild-type and mutant conditions. Some F-actin in the ring is recruited from filaments that assemble at peripheral sites of nucleation (Huang et al. 2012). However, as discussed previously, other mechanisms might suffice for functional ring assembly in fission yeast.

4.4 Myosin Function during Cytokinesis

Ever since actin and nonmuscle myosin II were identified as important components of the contractile ring (Carter 1967; Schroeder 1968; Fujiwara and Pollard 1976; Mabuchi and Okuno 1977), the prevailing model for the process of constriction has involved myosin-II-mediated sliding of actin filaments. In a wide variety of cell types, including mammalian cells, *Drosophila*, *C. elegans*, and fission yeast, nonmuscle myosin II is required for successful cytokinesis. Myosin V can contribute to cytokinesis in *S. pombe* (Lapante et al. 2015). The situation in mammals is complicated by genetic redundancy because of multiple isoforms of nonmuscle myosin II that show partial overlap among isoforms in certain tissues (Takeda et al. 2003; Conti et al. 2004; Bao et al. 2005; Ma et al. 2010). Nevertheless, there is strong evidence that supports a role for myosin II motor activity in cytokinesis.

However, myosin II is not invariably required for cytokinesis. Notable exceptions include plants, which lack nonmuscle myosin II altogether, and *Dictyostelium*, in which adherent—although not detached—cells have a myosin-II-independent pathway for cytokinesis (Neujahr et al. 1997; Zang et al. 1997). In budding yeast, there is clear evidence

that myosin performs functions other than filament sliding in cytokinesis. Remarkably, the requirement for Myo1 can be provided by a headless version lacking the motor domain. This truncated myosin is therefore not performing a filament-cross-linking function—more likely it functions as a scaffold that guides deposition of septal materials (Lord et al. 2005; Fang et al. 2010; Wloka et al. 2013). Although headless myosin fulfills its essential function, loss of the head has consequences—for example, the motor domain of myosin is needed to accelerate ring constriction and the rate of actin disassembly during constriction (Mendes Pinto et al. 2012). Models for force generation have been compared and contrasted in a recent review (Mendes Pinto et al. 2013).

4.5 Constriction of the Contractile Ring

A number of factors influence furrow ingression during cytokinesis, including active force generators at the cleavage furrow, active protrusion at cell poles, overall cortical tension, the viscoelasticity of the cytoplasm, and interactions of the cell with its environment (Poirier et al. 2012). Most cells round up during mitosis, and cell cycle-regulated changes in cortical tension have been measured. However, the timing of these changes during mitosis is not consistent across species (Hiramoto 1990; Ramanathan et al. 2015).

Contractile rings in marine invertebrate blastomeres exert forces on the order of 1–10 nN (Rappaport 1967; Hiramoto 1975). Contractile rings in fission yeast, in contrast, show much smaller forces, although these contractile rings are much smaller in cross section than those of marine invertebrate blastomeres. However, the two types of rings generate similar tensions, on the order of 10 nN/ μm^2 (Stachowiak et al. 2014). As has been extensively discussed, ring ingression in these cells requires myosin motor activity, and sufficient motors are present in the fission yeast ring to generate these forces, assuming that the actin filaments are properly oriented (Wu and Pollard 2005; Stachowiak et al. 2014).

In sea urchin embryos, contractile ring width and thickness remain fairly constant during constriction, reflecting significant disassembly of actin filaments as it constricts (Schroeder 1972). Observations of fluorescently tagged myosin indicate that, although the total number of myosin motors decreases as the ring constricts, the local concentration can increase severalfold or remain relatively constant in fission yeast or *C. elegans* blastomeres, respectively (Wu and Pollard 2005; Carvalho et al. 2009).

The slime mold *Dictyostelium* provides a clear example of an alternative means of force generation in the furrow. Although myosin II is essential for furrow formation in such cells when grown in suspension (De Lozanne and

Spudich 1987), knockout cells divide with nearly normal kinetics when allowed to attach to the substrate (Neujahr et al. 1997). Nonuniform changes in cortical tension and hydrodynamic pressures can account for furrowing in such cells (Poirier et al. 2012).

4.6 Septum Deposition in Yeast

Although constriction of the contractile ring is largely driven by the actomyosin-based contractile ring in metazoan cells, in budding yeast, regulated secretion of cell wall material makes a significant contribution (Figs. 2C and 3C). Indeed, in budding yeast, deletion of the gene encoding myosin II is tolerated in some strain backgrounds. Proteins that act in a parallel pathway to actomyosin contractility would be predicted to be essential in cells that lack myosin. Hof1, which is related to the fission yeast F-BAR protein Cdc15, fits these criteria (Vallen et al. 2000). Hof1 binds to chitin synthase II, which is essential for secretion of primary septum material. Hof1 also binds to the tail of yeast myosin II, suggesting that Hof1 coordinates the contractile ring and the enzyme crucial for cell wall deposition (Oh et al. 2013). Similar interactions exist in fission yeast. Thus, in fungi, the contractile ring could function to guide deposition of septal material (Zhou et al. 2015).

5 RECONSTITUTION OF THE CONTRACTILE APPARATUS

Aspects of cytokinesis have been reconstituted in a number of in vitro systems. Contractile ring constriction has been shown in both permeabilized vertebrate cells (Hoffman-Berling 1954; Cande 1980) and isolated contractile rings (Mabuchi et al. 1988). Actin-filament-stabilizing drugs inhibit ring constriction; thus, constriction depends on actin filament disassembly. This approach has received renewed attention in budding and fission yeast (Young et al. 2010; Mishra et al. 2013). In the former case, ring constriction occurs in the presence of cytoplasmic extracts (Young et al. 2010). In *S. pombe*, constriction occurs in permeabilized cells largely devoid of cytoplasmic components (Mishra et al. 2013). During constriction of the ring, myosin II becomes progressively concentrated, whereas a significant portion of the filamentous actin disassembles. However, in this case, stabilization of actin filaments does not impair ring constriction. The genetic tractability of this system should permit dissection of this important step in cytokinesis.

The spatially regulated signaling events of cytokinesis have been reconstituted in unfractionated *Xenopus* egg extracts on supported lipid bilayers (Nguyen et al. 2014). In this system, microtubules polymerized from neighboring

synthetic asters interact, forming discrete overlap zones that recruit central spindle components in a manner dependent on aurora B kinase. These zones induce RhoA activation in the adjacent membrane.

Cytokinesis-like events have also been modeled in a fully defined system, producing striking results (Miyazaki et al. 2015). Actin polymerization in phospholipid-delimited aqueous droplets in oil results in assembly of filament bundles that encircle the droplets. Successful ring formation requires the droplet diameter to be smaller than the persistence length of single actin filaments ($\sim 5\text{--}15\ \mu\text{m}$). Actin polymerization in the presence of double-headed heavy meromyosin fragments generated constricting rings. The constriction rate is proportional to ring size, similar to that seen in *C. elegans* embryos (Carvalho et al. 2009). During constriction, the rings slide along the inner leaflet of the droplet, rather than inducing deformations, as seen in permeabilized fission yeast and spheroplasts (Mishra et al. 2013; Stachowiak et al. 2014). Importantly, ring constriction under these conditions depends on motor activity—rigor conditions impair the assembly of rings seen in the absence of myosin. Thus, some characteristic features of the contractile ring can be solely attributed to interactions between spatially constrained actin filaments and myosin motor proteins.

6 ABSCISSION

Following cleavage furrow ingression, dividing cells still need to resolve the single membrane that encapsulates the two nascent daughter cells, a process known as abscission (see Mierzwa and Gerlich 2014 for review). This process is topologically similar to viral budding and formation of multivesicular bodies, in which the neck of the constriction is exposed to extracellular space, as compared with endocytosis, in which the neck is exposed to the cytoplasm (Carlton and Martin-Serrano 2007). This requires that cytoplasmic machinery that drives membrane scission must be specialized to constrict the membrane without sterically interfering with membrane scission. These processes are mediated by a protein complex, called “endosomal sorting complex required for transport III” (ESCRT-III), that assembles into a spiral arrangement of filaments that associates with the cytoplasmic face of the plasma membrane and induces membrane deformation (Fig. 4) (see Henne et al. 2011 and Agromayor and Martin-Serrano 2013 for review).

6.1 Evolutionary Conservation of ESCRT-III

The ESCRT-III complex contains four structurally related subunits, called charged multivesicular body proteins (CHMPs), each of which can polymerize on an appropriate

membrane (Shim et al. 2007). However, in vivo and in vitro, the four subunits have distinct functions. CHMP6/Vps20 is thought to nucleate assembly of longer CHMP4/Snf7 polymers that are terminated by CHMP3/Vps24 and CHMP2/Vps2 (Teis et al. 2008; Wollert et al. 2009). The two terminal ESCRT-III subunits induce a conformational change in the CHMP4/Snf7 polymers, promoting membrane deformation. They also recruit an AAA-ATPase complex, Vps4, that disassembles and recycles ESCRT-III components (see Mierzwa and Gerlich 2014 for review). Abscission timing is closely linked to recruitment of Vps4 (Elia et al. 2011).

The ESCRT-III complex could be one of the most ancient cytokinesis factors as it is found not only in eukaryotes but also in some archaea. The archaea contains multiple phyla, including the well-characterized Crenarchaeota and Euryarchaeota. Genomic comparisons of these two phyla suggest that they use distinct molecular mechanisms for cell division. Like many eubacterial species, many Euryarchaeota species contain FtsZ, a tubulin-related protein that participates in cell division. However, FtsZ is lacking in many Crenarchaeota species. These species do, however, contain CdvB, which is related to ESCRT-III subunits (Lindås et al. 2008), and CdvC, which is related to the Vps4 AAA-ATPase that promotes disassembly of ESCRT-III filaments. *Cdv* genes are induced before cell division, and the Cdv proteins accumulate between segregated nucleoids (Lindås et al. 2008; Samson et al. 2008). Overexpression of an ATPase-defective variant of CdvC prevents cell division, providing functional evidence for its role in cell division. Thus, the involvement of ESCRT-III proteins in eukaryotic cell division likely reflects an ancient role. Intriguingly, related proteins are also found in Lokiarchaeota, an archaeal phylum that also contain proteins similar to eukaryotic actin and GTPases (Spang et al. 2015).

Despite this strong evolutionary connection and the compelling evidence for its role in mammalian cells, there is limited evidence implicating the ESCRT-III complex in cytokinesis in other model organisms. For example, in *C. elegans*, depletion of various ESCRT subunits affects endocytosis and other process—however, it does not cause defects in cytokinesis (Kim et al. 2011). Residual ESCRT-III subunits might obscure a role for this machinery in cytokinesis. In *Drosophila*, ESCRT-III subunits accumulate at the midbody, but a functional role in cytokinesis has not yet been shown (Capalbo et al. 2012). Interestingly, in budding yeast, in which the ESCRT system was first characterized, ESCRT-III subunits are not essential; however, there are weak genetic interactions with mutations in known cytokinesis genes, including genes encoding septins (McMurray et al. 2011). Septal deposition might provide an

Figure 4. Abscission in metazoa. Cell separation is mediated by endosomal sorting complex required for transport III (ESCRT-III) filaments that assemble in constriction zones that flank the centralspindlin-dense midbody. The remaining microtubules are depolymerized by spastin, and the membrane scission process itself is driven by ESCRT-III filaments that constrict the neck of the cytoplasmic bridge between the two daughter cells.

alternative means to drive final constriction of the neck of the cell.

6.2 ESCRT-III Recruitment

Recruitment of ESCRT-III to the cytoplasmic bridge in mammalian cells requires centralspindlin and an interacting adaptor protein, Cep55 (Zhao et al. 2006), which in turn recruits two additional adaptors, Alix and the ESCRT-I subunit TSG101 (Carlton and Martin-Serrano 2007; Morita et al. 2007; Lee et al. 2008). Alix and TSG101 directly recruit CHMP4 subunits of the ESCRTIII complex (McCullough et al. 2008). CHMP4 accumulates in filaments at regions of secondary constrictions that flank the central midbody (Guizetti et al. 2011). For these secondary constrictions to proceed to completion, microtubules in the midbody must be removed as they obstruct the intercellular bridge. ESCRT-III directs recruitment of the microtubule-severing complex spastin by means of CHMP1B, a peripheral subunit of the ESCRT-III complex (Yang et al. 2008).

6.3 Regulation of Abscission

The numerous steps required for abscission provide opportunities for regulation. Chromatin stuck in the midbody prevents or delays completion of cytokinesis (Mendoza et al. 2009; Steigemann et al. 2009). This delay involves activation of the chromosome passenger complex by the trapped DNA, causing midbody stabilization (Steigemann et al. 2009). Aurora B phosphorylates CHMP4C (Capalbo et al. 2012; Carlton et al. 2012), which delays abscission, perhaps by acting as an inhibitor of the CHMP4B isoform. This model is based on the finding that CHMP4C is not essential for cytokinetic abscission—in fact, it acts as an inhibitor of the process (Carlton et al. 2012).

The cellular microenvironment impacts the timing of abscission. The time interval between formation of the midbody and abscission is highly variable. Some variability might be due to the variable rate of daughter cell spreading that exerts tension on the intercellular bridge. Counterintuitively, increased tension on the bridge induces a delay in abscission (Lafaurie-Janvore et al. 2013). Experimentally

severing one side of an intercellular bridge under tension releases the tension and induces rapid severing on the other side of the bridge. Recruitment of ESCRT-III subunits is tension sensitive, providing a possible underlying molecular mechanism.

6.4 Fate of Midbody Remnants

Once the two daughter cells fully separate, a portion of the cytoplasmic bridge or midbody remains. In principle, the structure could be internalized into autophagosomes directly, released into the extracellular milieu, or the particle could be later phagocytosed by the dividing cell or its neighbors and ultimately degraded by lysosomal processes (Crowell et al. 2014). The balance between these fates varies in a cell type-dependent manner (Ettinger et al. 2011). Division remnants have been suggested to alter cellular behavior (Ettinger et al. 2011; Kuo et al. 2011), but the perturbations used to alter the fate of internalized midbodies might have indirect effects (Crowell et al. 2014).

7 CONTEXT DEPENDENCE OF CYTOKINETIC MECHANISMS

It is tempting to posit that cytokinesis is a singular event that is largely similar in animal cells and fungi. Indeed, there are common requirements that appear in many organisms and cell types. However, it is not difficult to find different mechanisms in different species. In fact, the requirements for cytokinesis vary within a given organism, depending on the cellular context. For example, during the cleavage stages of embryogenesis, when cells divide without growing, cell size changes over multiple orders of magnitude, and there are likely to be adaptations required for division of large blastomeres. Indeed, in aqueous droplets that reconstitute contractile rings, droplet flattening, or myosin addition increases the size of the droplets that assemble intact rings (Miyazaki et al. 2015). Several *Drosophila* mutants show spermatocyte-specific cytokinesis defects, providing additional evidence for context-dependent requirements (Giansanti et al. 2004). Likewise, in *Drosophila*, septins are essential for division of epithelial cells that divide in the plane of the epithelia, but not those that divide in the orthogonal plane (Founounou et al. 2013). Similarly, as contractile rings ingress against cortical tension, defects in force generation in the ring might be compensated by reductions in cortical tension. In *C. elegans*, mutations in CYK-4 that limit RhoA activation can be partially suppressed by mutations in Arp2/3 or its activator Rac1 (Canman et al. 2008; Loria et al. 2012).

Abscission regulators also show cell-type-specific requirements. For example, the RhoA-activated kinase citron

(Gai et al. 2011) contributes to abscission in cultured cells, but its requirement is tissue specific in vivo. Mice possessing a mutant citron kinase are grossly normal at birth, but they show postnatal neurological defects (Di Cunto et al. 2000). Multinucleate cells are observed at low frequency specifically in the brain; this could result from differential clearance of multinucleate cells. However, it seems more likely that the cytokinetic requirement for citron depends on growth conditions. Thus, the mechanical or biochemical properties of a cell or tissue can influence the genetic requirements for cytokinesis.

8 CONCLUSION

Despite the remarkable progress in defining the molecular mechanism of cytokinesis, much remains to be learned about this essential cellular process. The mechanism by which the contractile ring assembles, constricts, and generates force remains quite opaque in many systems. Likewise, there are open questions concerning the mechanism and regulation of membrane abscission. Finally, accumulating results show that, despite significant shared components and mechanisms, different cells and tissues of a given organism have specific requirements for cytokinesis owing to differences in cell size, cellular microenvironment, or the panoply of biochemical regulators. The number of such variations increases when multiple species are considered. Thus, it is crucial to consider the cellular context when studying cytokinesis. Our understanding of cytokinesis in intact tissues and animals is particularly limited. Last, some subtle cytokinetic defects could affect particular cell types and result in disease (Liljeholm et al. 2013).

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