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REVIEW

Molecular form and function of the cytokinetic ring

MariaSanta C. Mangione and Kathleen L. Gould*

ABSTRACT

Animal cells, amoebas and yeast divide using a force-generating, actin- and myosin-based contractile ring or 'cytokinetic ring' (CR). Despite intensive research, questions remain about the spatial organization of CR components, the mechanism by which the CR generates force, and how other cellular processes are coordinated with the CR for successful membrane ingression and ultimate cell separation. This Review highlights new findings about the spatial relationship of the CR to the plasma membrane and the arrangement of molecules within the CR from studies using advanced microscopy techniques, as well as mechanistic information obtained from *in vitro* approaches. We also consider advances in understanding coordinated cellular processes that impact the architecture and function of the CR.

KEY WORDS: Cell division, Cytokinesis, Cytokinetic ring, Contractile ring, Actomyosin ring, Metazoa, Fungi, Animal cells, Yeast, Amoeba

Introduction

Cytokinesis is the final event in the cell cycle when a cell physically divides. Successful execution of cytokinesis is essential to ensure proper segregation of the genome and cytoplasmic contents, as division failure results in either cell death or multi-nucleate or aneuploid cells that can be oncogenic precursors (Storchova and Pellman, 2004; Li, 2007; Normand and King, 2010).

Organisms from two of the five eukaryotic supergroups (Burki, 2014), Amoebozoa and Opisthokonta (which includes fungi and animals), assemble an actin cytoskeleton- and myosin motor protein-based contractile ring, or 'cytokinetic ring' (CR), to accomplish cytokinesis (see Box 1) (Gu and Oliferenko, 2015; Willet et al., 2015; Balasubramanian, 2016; Srivastava et al., 2016; Bhavsar-Jog and Bi, 2017; Glotzer, 2017; Hardin et al., 2017; Jahan and Yumura, 2017), whereas the other supergroups rely on alternative mechanisms, such as other cytoskeletal proteins, motility-based mechanisms, or vesicle trafficking of membrane and cell wall to the division site (Farr and Gull, 2012; Hardin et al., 2017; Smertenko, 2018; Müller, 2019). This Review focuses on advances in understanding CR-mediated cytokinesis. The elemental steps of CR-mediated cytokinesis are shared (Fig. 1A,B); in response to cell cycle-regulated signaling, F-actin that is assembled through the action of the formins accumulates at the division plane. At the same time, the non-muscle myosin-II motor (hereafter called myosin-II) localizes to the division plane in F-actin-dependent and -independent manners (Wu et al., 2003; Motegi et al., 2004; Dean et al., 2005; Takaine et al., 2014). Myriad additional components also assemble at the division site, including membrane scaffolds (e.g. anillin, septins and F-BAR domain-containing proteins) and F-actin regulators (e.g. severing proteins and bundlers) (Eggert et al., 2006). Ultimately, signaling cues trigger myosin-II-mediated contraction of the CR, which simultaneously disassembles as it contracts.

Substantial progress has been made towards identifying the molecular participants of cytokinesis and unraveling signaling events that impact the process (for reviews, see Glotzer, 2017; Pollard and O'Shaughnessy, 2019). However, how structural components are integrated into the CR and how the CR provides constricting force are long-standing, unsettled questions. In this Review, we highlight recent studies that have employed cutting-edge imaging or *in vitro* techniques to reveal CR architecture and provide mechanistic information about CR-mediated cell division. In the final section, this new knowledge of CR-intrinsic features is placed into a cellular context.

Nanoscale architecture of the CR

Classic electron microscopy (EM) studies in animal cells, amoebas and yeast have revealed that, at the division site, F-actin forms a parallel arrangement of bi-directional filaments (Schroeder, 1972; Sanger and Sanger, 1980; Gawlitta and Stockem, 1981; Maupin and Pollard, 1986; Mabuchi et al., 1988; Mabuchi, 1994; Noguchi and Mabuchi, 2001; Kamasaki et al., 2007) (Fig. 1A,B). Some EM studies also described CR-adjacent structures that could be myosin-II filaments (Schroeder, 1972; Maupin and Pollard, 1986; Mabuchi, 1994; Kamasaki et al., 2007). However, the arrangement of myosin-II and other CR components relative to F-actin and the plasma membrane has been obscured by the resolution limit of conventional light microscopy (~200 nm) and the difficulty of identifying proteinaceous structures in EM samples. Subsequently, super-resolution fluorescence microscopy (Betzig et al., 2006; Hess et al., 2006; Rust et al., 2006; Gustafsson et al., 2008; Sydor et al., 2015) has been used to examine CR architecture in animal cells and *Schizosaccharomyces pombe*. These studies and others using improved EM and fluorescence polarization microscopy techniques have provided high-resolution information about F-actin, myosin-II and numerous other CR components, which we will discuss in the following sections.

F-actin

Observations made in classic EM studies have now been confirmed: F-actin aligned preferentially along the division plane has been detected by electron cryotomography (ECT) of contracting CRs in cryopreserved *S. pombe* (Swulius et al., 2018) and by rotary shadow platinum replica transmission EM (TEM) of CRs from the isolated cortices of sea urchin embryos (Henson et al., 2017). This arrangement is also visible in furrows of HeLa cells stained with phalloidin and imaged using structured illumination microscopy (SIM) (Fenix et al., 2016). Interestingly, even though actin filaments are ultimately aligned roughly parallel to the division plane, studies in *S. pombe* and animal cells indicate that the CR initiates as a network of randomly oriented actin filaments (Fishkind and Wang, 1993; Mabuchi, 1994; Wu et al., 2003; Wu et al., 2006). Fluorescence polarization microscopy has determined that, in

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37240, USA.

*Author for correspondence (kathy.gould@vanderbilt.edu)

M.S.C.M., 0000-0003-3894-3533; K.L.G., 0000-0002-2981-269X

Journal of Cell Science (2019) 132, jcs226928. doi:10.1242/jcs.226928

Box 1. Model organisms used to study CR-based cytokinesis

Yeast

Most yeast cytokinesis studies employ the fission yeast *S. pombe*, the budding yeast *S. cerevisiae* and, more recently, the fission yeast *S. japonicus*. Both budding and fission yeasts are single-cell walled organisms that can grow in suspension and offer many experimental advantages, including a fast cell cycle (2–4 h), morphology that reflects the cell cycle stage and genetic tractability. Budding yeast divide asymmetrically, whereas the fission yeast division plane is medially placed. Unlike animal cells and amoebas, fungi have a cell wall. Plasma membrane furrowing must be coordinated with deposition of the septum, a cell wall structure that physically separates daughter cells. Septum deposition is essential for division and a mechanism for force generation (see section 'Extracellular matrix').

Animal cells

Animal cells do not have a cell wall and instead cell shape is controlled by the cortex, which is an actin- and myosin-based network beneath the plasma membrane. Cortical dynamics are implicated in CR formation and function (see section 'Cell cortex'). In most instances, animal cells are attached to a substrate or to other cells when they divide. Attachment offers a potential alternative for force generation by traction, although how much this mechanism contributes in a wild-type setting is unknown (see section 'Extracellular matrix').

Amoebas

The most commonly encountered amoeba used in cytokinesis studies is *Dictyostelium discoideum*. Interestingly, *D. discoideum* can divide in myosin-II-dependent and -independent manners. In myosin-II-null cells, successful cytokinesis depends on adherence to a substratum, which allows daughter cells to exert traction force by migrating away from each other (see section 'Extracellular matrix').

human epithelial cells, F-actin is isotropic at the onset of anaphase and throughout early furrow ingression before re-organizing into bi-directional filaments (∼150 s after anaphase onset) (Spira et al., 2017) (Fig. 1A). This re-organization is dependent on myosin-II. It remains to be seen whether actin reorganization is required for CR contraction or is a consequence of force generation (Spira et al., 2017).

CR-generated force is transmitted to the plasma membrane, which remains closely associated with the CR throughout cytokinesis (Schroeder, 1990). How the CR–plasma-membrane association is maintained during contraction is still largely unknown (Schroeder, 1990; Pollard, 2017). ECT images revealed a gap of ∼60 nm between the plasma membrane and the actin bundles of contracting CRs in *S. pombe* (Swulius et al., 2018). A gap of ∼100 nm between the plasma membrane and F-actin was also observed in a fluorescence photoactivation localization microscopy (fPALM) study of fully formed *S. pombe* CRs pre-contraction (McDonald et al., 2017). The modest difference in measured gap size in these two studies could be explained by different sample preparation methods or, more interestingly, by a structural reorganization of the CR during contraction that brings F-actin closer to the membrane. Distinguishing between these two possibilities will require additional studies to obtain the localization and dynamics of other CR components. Importantly, however, two distinct imaging modalities reveal that CR F-actin is not immediately adjacent to the plasma membrane and is linked to the plasma membrane by largely unknown mechanisms.

Myosin-II

In multiple organisms, myosin-II first localizes to the division plane in foci that then reorganize into a ring (Maupin and Pollard, 1986; Noguchi and Mabuchi, 2001; Wu et al., 2003; Maddox et al., 2005; Vavylonis et al., 2008; Zhou and Wang, 2008; Mavrakis et al., 2014) (Fig. 1B). Recent SIM and TEM images of sea urchin embryos at sequential stages of furrowing have captured fine details of myosin-II rearrangement from a wide band of clusters at the future division site into a linear structure (Henson et al., 2017). As in other organisms, this reorganization is dependent on actin (Henson et al., 2017).

Myosin-II is a double-headed motor (Fig. 2A) and *in vitro* studies of myosin-II from animal cells have demonstrated that it can also oligomerize through its C-terminus into bipolar mini-filaments (Verkhovsky and Borisy, 1993; Ricketson et al., 2010; Billington et al., 2013) (Fig. 2B). The linearly arranged myosin-II in the CR of sea urchin embryos is presumed to represent myosin-II mini-filaments (Henson et al., 2017). Platinum-replica EM of *Saccharomyces cerevisiae* protoplasts with a CR also identified myosin-II-dependent thick filaments solely at the late stages of cytokinesis (Ong et al., 2014). Bipolar myosin-II mini-filaments in the CRs of mammalian cells have been observed by SIM, which revealed that the myosin-II mini-filaments are arranged head-to-head, parallel to the plane of division (Beach et al., 2014; Fenix et al., 2016) (Fig. 2D). Furthermore, inhibition of myosin-II motor activity impairs the assembly of myosin-II mini-filaments into larger arrays (i.e. stack formation) (Fenix et al., 2016), supporting the idea that myosin-II undergoes a molecular rearrangement during cytokinesis (Henson et al., 2017).

Unlike animal and amoeboid myosin-II, the two myosin-II heavy chains in *S. pombe*, Myo2 and Myp2, do not form filaments. The non-essential Myp2 is single-headed (Bezanilla and Pollard, 2000) whereas biochemical studies of the essential Myo2 indicate that it functions as a double-headed motor rather than a bipolar mini-filament (Pollard et al., 2017; Friend et al., 2018) (Fig. 2A). In mature CRs, the tail of Myp2 localizes ∼125 nm away from the plasma membrane, whereas the Myo2 C-terminal tail is anchored closer to the plasma membrane (McDonald et al., 2017). Both of the N-terminal motor domains extend into the cytoplasm to colocalize with F-actin (Fig. 2C). This Myo2 organization is also present in foci that exist during early CR formation that are termed cytokinesis nodes, as determined by single-molecule high-resolution colocalization microscopy (Laporte et al., 2011) (Fig. 1B). Within cytokinesis nodes, which contain approximately ten molecules of Myo2, the C-terminal tails of myosin-II have a tighter radial organization compared to the N-terminal motor domains, as determined by fPALM (Laplante et al., 2016). This organization led to a proposal that a myosin-II 'bouquet', in which N-terminal motor domains are radially arrayed to interact with F-actin, could function as a motor unit that is comparable to myosin-II mini-filaments (Laplante et al., 2016) (Fig. 2C).

Taken together, these studies reveal various myosin-II arrangements during CR-mediated cytokinesis: myosin-II mini-filaments have been definitively demonstrated in animal cell CRs in contrast to the proposed radial arrangement of fission yeast Myo2 and the entirely distinct localization of single-headed Myp2. Furthermore, SIM and EM images indicate that myosin-II in animal cells and budding yeast rearrange during cytokinesis, although more time-lapse super-resolution studies are needed to resolve these myosin-II dynamics.

Other CR proteins

Genetic studies indicate that building and contracting CRs requires many other proteins (Eggert et al., 2006), some of which are structural components that must be performing key jobs such as

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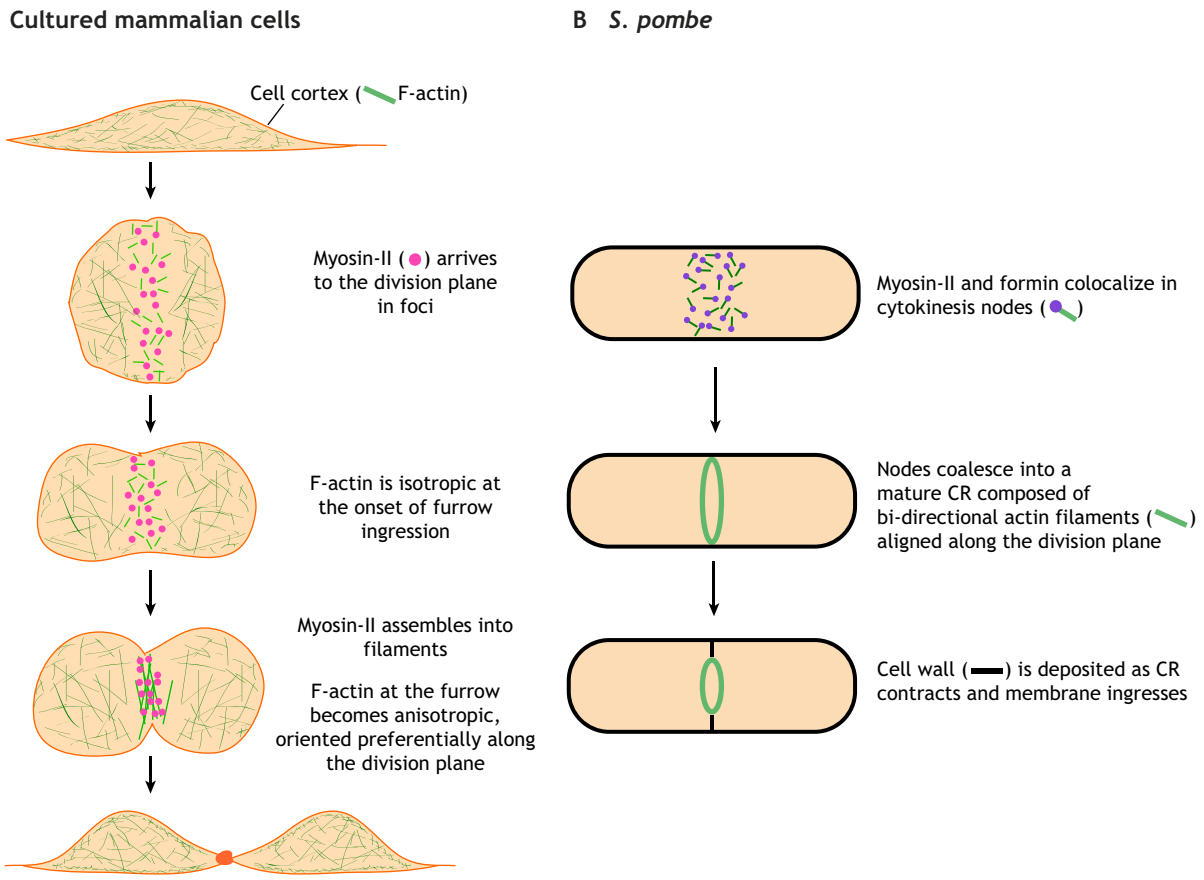

Fig. 1. Elemental steps of CR formation and contraction. CR formation and contraction is illustrated for (A) cultured mammalian cells and (B) *S. pombe*. Although not depicted, F-actin and myosin-II exhibit similar dynamics in animal embryos (e.g. *Caenorhabditis elegans* and sea urchin) and amoeboid cells.

linking the CR to the plasma membrane or possibly organizing myosin-II into motor units. However, how these are organized into the CR as a whole is unclear. Other than septins, which form filaments on the plasma membrane and bind components of the CR (Marquardt et al., 2018), most other CR components have not been detected by EM. Super-resolution microscopy can probe their organization at the nanoscale level and two such studies have been performed in *S. pombe* (Laplante et al., 2016; McDonald et al., 2017). Our research group used fPALM to measure the distance between 30 CR proteins and the plasma membrane and found that the mature CR appears to be stratified (McDonald et al., 2017) (Fig. 3). Closest to the membrane (on average, 0–80 nm from the membrane) are membrane-bound scaffolding proteins, such as anillin, septins and F-BAR-containing proteins, and also formin. Just above these proteins – 80 to 160 nm away from the plasma membrane – are most signaling proteins (e.g. kinases, phosphatases and GTPases) and a variety of accessory components scaffolded through SH3-protein domains, which are critical for ring integrity. Most distal from the membrane is F-actin, the center of which is ∼200 nm away from the plasma membrane, as well as most direct actin-binding proteins, including the N-terminal motor domain of myosin-II (Fig. 3).

The arrangement of proteins within the CR is still unclear; however, we have gained some insight into this by using fPALM to observe proteins within the plane of the CR in *S. pombe* cells. CR components in the membrane-proximal layer of pre-contracted CRs cluster, whereas proteins localizing to the middle and distal layers have a more homogenous distribution (McDonald et al., 2017). This is consistent with findings of Laplante et al. who used fPALM in live cells to discover that five cytokinesis node proteins are clustered in contracting CRs (Laplante et al., 2016). These clusters are probably distinct from the cytokinesis nodes present during CR formation given that the essential organizer for cytokinesis nodes, the anillin-like protein Mid1, leaves the CR during contraction (Wu et al., 2003). Additionally, the four node proteins examined within the plane of the CR remain clustered even in cells with deletion of the *mid1* gene and they have different patterns of clustering in the mature CR (McDonald et al., 2017). Finally, although the estimated size of cytokinesis nodes is within the range of TEM, Swulius et al. did not observe any molecular complexes in contracting CRs (Swulius et al., 2018).

Future studies to image additional CR proteins during contraction are needed to determine whether molecular complexes exist during contraction, and, if yes, their precise composition and the functional implications for contraction. Additionally, whether any of these newly revealed features of the *S. pombe* CR are shared with other model organisms is another area of future investigation.

New structural information for models of CR-mediated furrowing

The organization of proteins within the CR indicates potential modes of CR contraction and net inward force generation during cytokinesis. The identification of myosin-II stacks in the CR of animal cells (Beach et al., 2014; Fenix et al., 2016) is consistent with the classical ‘purse-string’ theory of contraction, in which myosin-II slides bi-directionally oriented F-actin filaments over one another to reduce CR diameter. Models of contraction in *S. pombe*, which

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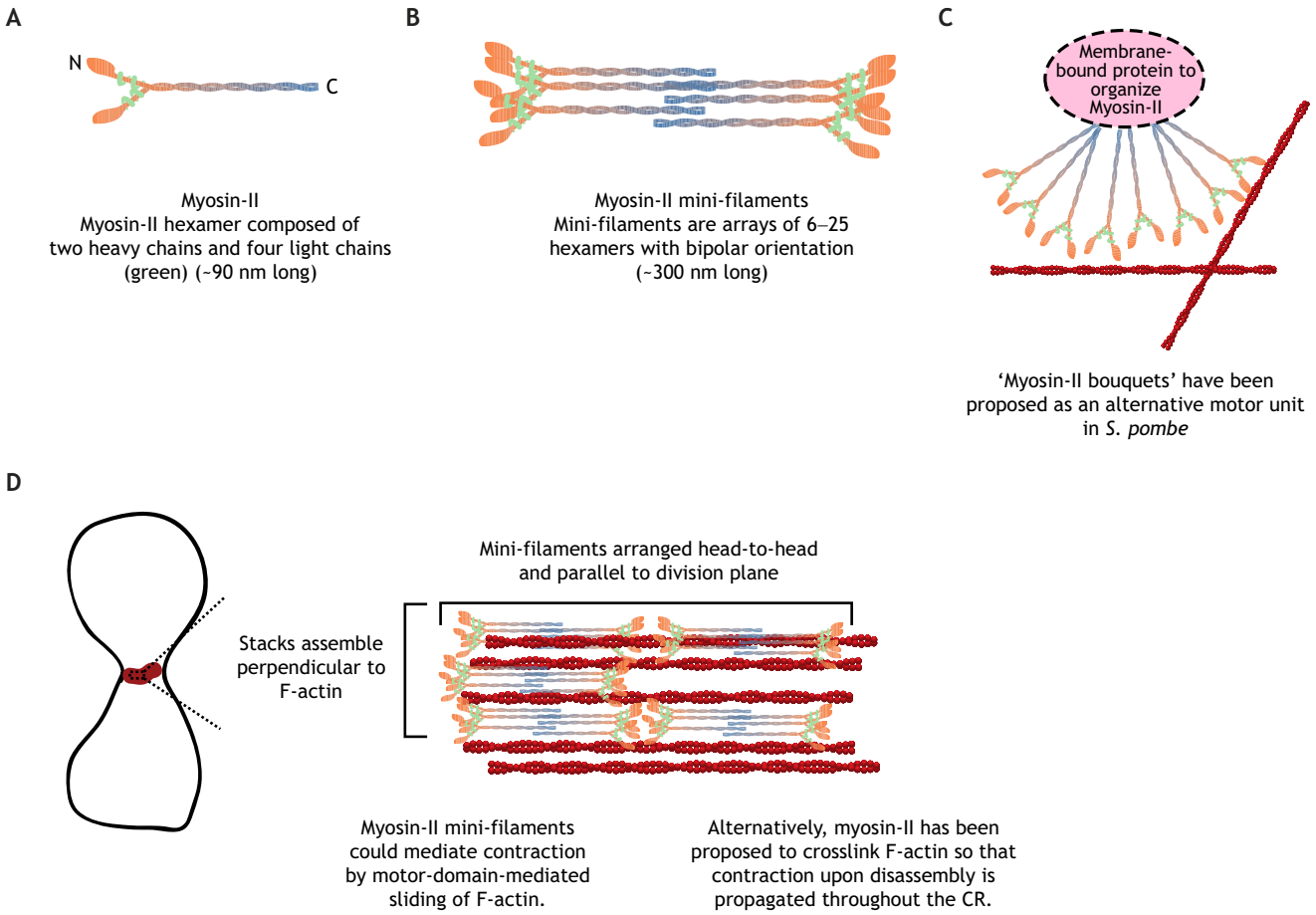

Fig. 2. Molecular structure of myosin-II. (A) Double-headed myosin-II is a hexamer. (B) Animal and amoeboid myosin-II can oligomerize via C-terminal tails into bipolar filaments. (C) Radial arrangement of myosin-II predicted to act as a motor unit in *S. pombe*, which does not form mini-filaments. Actin is red. (D) Cartoon of myosin-II arrangement in animal cell CRs. Actin is red.

lack myosin-II bipolar filaments, also converge on sliding of bi-directional actin-filaments. These models assume various myosin-II organizations, such as plasma membrane-anchored individual myosin-II motors (Nguyen et al., 2018) or myosin-II bouquets (Laplante et al., 2016; Thiyagarajan et al., 2017) (Fig. 2C). Myosin-II motors arranged at an angle to each other and bound to different actin filaments would slide the intact filaments past one another.

The idea of myosin-II bouquets functioning as a motor unit is attractive because of the observed myosin-II clustering (Schroeder, 1990; Stachowiak et al., 2014; Takaine et al., 2015; Wollrab et al., 2016; McDonald et al., 2017) and the proposal that contractile units exist in the CRs of multiple organisms (Carvalho et al., 2009; Silva et al., 2016; Thiyagarajan et al., 2017). However, the finding that furrow ingression initiates while F-actin is still randomly oriented at the division plane (Spira et al., 2017) suggests that an alternative mode of contraction could be at work, at least early in cytokinesis. One proposal is that rather than sliding almost-parallel actin filaments, myosin-II first contracts a network of isotropic F-actin (Ennomani et al., 2016; Linsmeier et al., 2016; Spira et al., 2017). Over time, F-actin in the furrow becomes preferentially aligned with the plane of division. There are also other models of CR contraction that do not rely on filament sliding. Myosin-II might pull on anchored F-actin to the point of breaking such that filament disassembly could drive CR contraction (Harasimov and Schuh, 2018) (Fig. 2D). The dispensability of the myosin-II motor domain for cytokinesis in *S. cerevisiae* (Lord et al., 2005; Wloka et al., 2013) and myosin-II motor activity for late stages of *Drosophila melanogaster* cellularization (Xue and Sokac, 2016) led to a related hypothesis wherein it is the disassembly of cross-linked F-actin filaments that drives CR contraction (Sun et al., 2010; Mendes Pinto et al., 2012, 2013).

In summary, the insights gained from the latest microscopy techniques, such as the temporal dynamics of actin and myosin-II or the spatial arrangement of molecules at nanoscale resolution, enhances theoretical models of cytokinesis, the details of which are comprehensively discussed elsewhere (Cortes et al., 2018). In the next section, we discuss *in vitro* studies, which can be used to test theoretical models and learn mechanistic information.

Cytokinesis *in vitro*

An ambition of cytokinesis researchers is to reconstitute CR formation, contraction and furrowing on a lipid bilayer (Fig. 4A). With such a system, individual proteins could be manipulated (e.g. modified to be hyper- or hypo-active, removed or added in excess) to assess impacts on the CR at different stages. Although this goal has yet to be achieved, there has been exciting progress in establishing *in vitro* systems to manipulate components, particularly using fission yeast.

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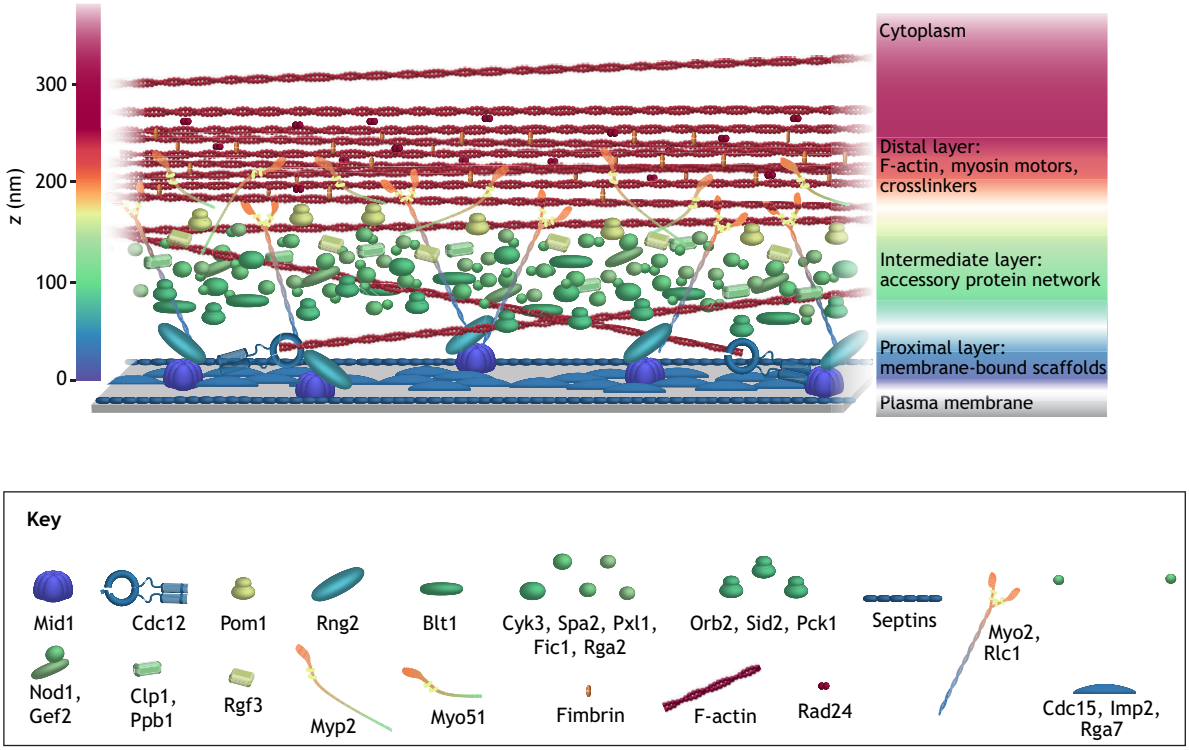

Fig. 3. A model for CR arrangement in *S. pombe*. Scale model of *S. pombe* CR architecture based on experimentally determined distances of CR proteins from the plasma membrane. The model does not incorporate stoichiometry. Panels are modified from McDonald et al., 2017, where they were published under a CC-BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

Reconstituting *S. pombe* CR formation

CR formation in *S. pombe* is well characterized, including protein abundances and the precise timing and order of molecular events (Pollard and Wu, 2010), making it a prime candidate to both model theoretically and reconstitute *in vitro*. Cytokinesis nodes, containing Myo2, the formin Cdc12 and other proteins, assemble in a broad band at the onset of cytokinesis (Wu et al., 2003, 2006; Ye et al., 2012; Guzman-Vendrell et al., 2013; Jourdain et al., 2013; Zhu et al., 2013; Akamatsu et al., 2014). Over time, nodes coalesce into a coherent ring (Wu et al., 2006). A 'search-capture-pull-release' (SCPR) model has been proposed in which formins that are anchored on the plasma membrane nucleate and elongate F-actin in random orientations ('searching'). Actin filaments are then 'captured' by myosin-II from neighboring nodes. Next, myosin-II 'pulls' nodes closer together until their attachment is 'released' by F-actin severing (Vavylonis et al., 2008; Vavylonis and Horan, 2017). Zimmermann et al. reconstituted a portion of the proposed SCPR mechanism using beads (1 µm diameter) that were conjugated to either myosin-II or a formin fragment containing formin homology (FH) 2 and FH1 domains with nucleation and elongation activity, respectively (Fig. 4B). In this system, myosin-II beads bound F-actin emanating from formin beads and pulled the beads together, recapitulating node coalescence *in vivo* and supporting the current model for how CRs assemble (Zimmermann et al., 2017).

These *in vitro* experiments also revealed that formin Cdc12 is mechanoregulated by its FH1 domain, whereby excessive tension on the FH1 domain (e.g. by myosin-II pulling) inhibits F-actin assembly (Zimmermann et al., 2017). This negative feedback could regulate the amount and/or length of actin produced at the CR to prevent abnormal clustering of nodes or to ensure the actin filament length is ideal for encircling the division plane of *S. pombe* (Lim et al., 2018). Indeed, replacing the FH1 domain of Cdc12 with that of a non-mechanosensitive formin results in cytokinesis defects *in vivo*, specifically node clumping, a longer time to CR formation, and increased F-actin in the ring (Zimmermann et al., 2017). The next challenge for this system is to more closely mimic the conditions in cells, for example by reconstituting actin assembly on a lipid bilayer (Fig. 4A), by reducing the distance between beads from 2–25 µm to the 0.6 µm that is observed *in vivo* (Vavylonis et al., 2008; Zimmermann et al., 2017) or by introducing additional proteins.

Using semi-*in vitro* systems to study CR contraction

A semi-*in vitro* method that has been optimized in yeast is the use of 'cell ghosts', which are prepared by digesting the cell wall and then permeabilizing the membrane to remove cytoplasmic material (Young et al., 2010; Mishra et al., 2013; Huang et al., 2016b; Mabuchi et al., 2017) (Fig. 4C). Mature CRs at the time of permeabilization are stable and remain associated with the plasma membrane. Adding ATP to the cell ghosts induces CR contraction, albeit at a rate 20 times faster than that observed in cells (Mishra et al., 2013). Contraction also occurs without membrane ingression; instead, the CR slides along one side of the membrane, becoming smaller in diameter concurrent with progressive disassembly of F-actin (Mishra et al., 2013). The efficiency of CR contraction varies between species, with 63% and 17% of CRs contracting in cell ghosts that are derived from *S. pombe* and *Schizosaccharomyces japonicus*, respectively (Chew et al., 2017), although the mechanism underlying this difference is unknown. Cell ghosts that are generated from cells with gene deletions or temperature-sensitive mutations have been cleverly deployed to address how

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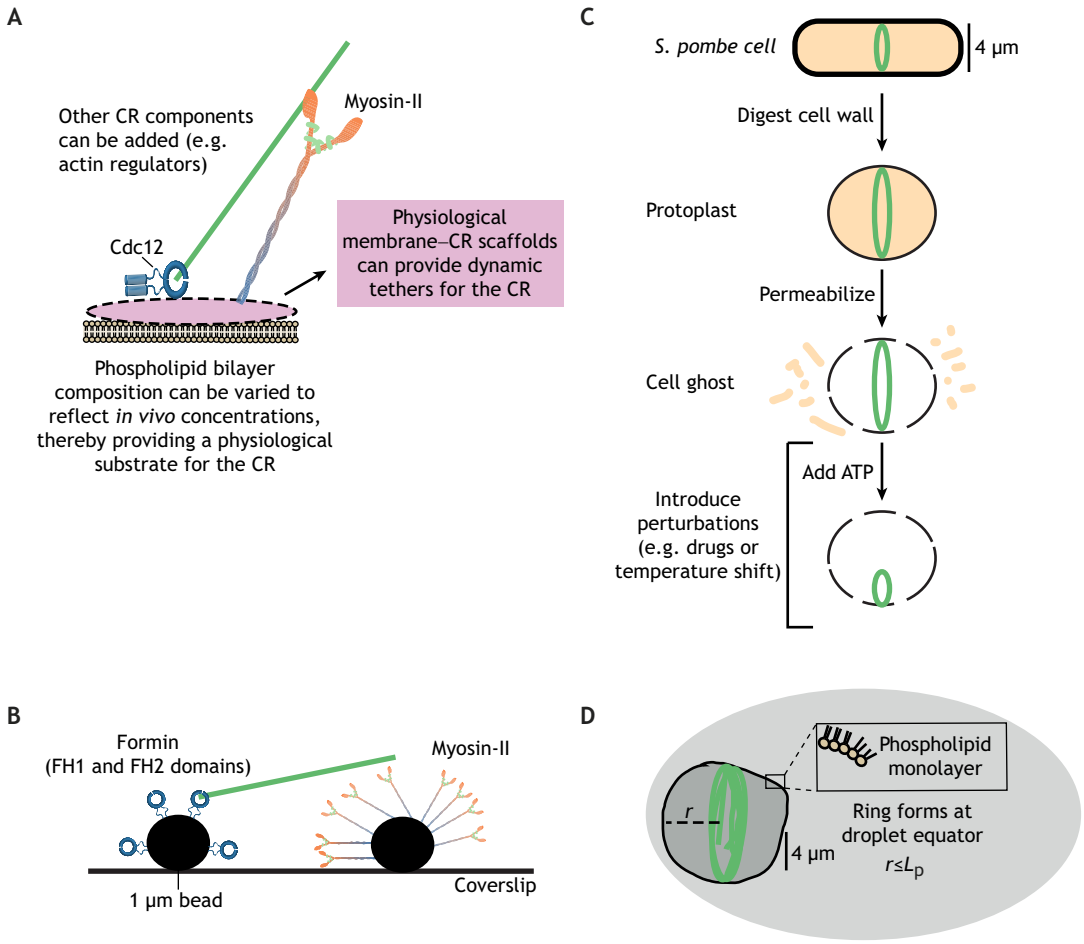

Fig. 4. *In vitro* methods to reconstitute the CR. (A) Schematic showing the method of reconstituting CRs on a phospholipid bilayer. Myosin-II and the formin Cdc12 from *S. pombe* are included as sample proteins. (B) Schematic of *in vitro* reconstitution of SCPR model of CR formation using 1 µm beads coated with FH1-FH2 fragments or myosin-II. Myosin-II is ∼90 nm (Friend et al., 2018) and is presented at ∼100 times its size relative to the bead. FH1–FH2 fragments are presented at ∼200× their size relative to the bead. (C) Method for preparing cell ghosts from *S. pombe* cells. Green represents a fluorescent CR protein. (D) Just after the initiation of actin filament formation, phospholipids are added to a solution containing G-actin and other actin-binding proteins so that F-actin rings (green) form inside of a phospholipid monolayer. The maximum radius (r) of these rings is limited by the persistence length (L_p) of the actin filament.

individual CR components, particularly those required for CR formation, contribute to contraction (Mishra et al., 2013; Huang et al., 2016a; Chew et al., 2017; Palani et al., 2017). These studies defined the minimal requirements for CR contraction in this system as the presence of F-actin, myosin-II ATPase activity and a balance of actin-crosslinking proteins (Mishra et al., 2013); surprisingly, the actin severer cofilin is not essential. Previously, cofilin was proposed to be essential for F-actin filament severing during disassembly (Mendes Pinto et al., 2012), which is well-established to occur simultaneously upon contraction (Schroeder, 1972; Carvalho et al., 2009; Mavrakis et al., 2014; Huang et al., 2016a). The dispensability of cofilin for contraction in fission yeast cell ghosts indicates the existence of alternative mechanisms for F-actin disassembly, as have been previously proposed, such as myosin-II-mediated actin filament breaking or a physical mechanism owing to compression by increased curvature as the CR diameter decreases (Huang et al., 2016a). It also suggests cofilin might have a different essential function, such as regulating F-actin length for proper CR formation. However, it remains possible that cofilin is essential for contraction in the context of continuous actin polymerization.

The absence of actin polymerization in cell ghosts likely explains why, upon ATP treatment, other CR components (e.g. myosin-II, formin and F-BAR-containing proteins) accumulate in fluorescent spots or clusters (Chew et al., 2017). Clustering is prevented by stabilizing F-actin (e.g. through jasplakinolide treatment) (Chew et al., 2017). This led to the proposal that continuous polymerization of F-actin is required to maintain circumferential CR–membrane association for even distribution of force, which is consistent with previous studies that showed actin polymerization is essential for contraction in animal cells (Schroeder, 1972; Mabuchi et al., 1988; Murthy and Wadsworth, 2005).

However, CR contraction in the presence of an actin stabilizer is inconsistent with some studies in animal cells (Schroeder, 1972) and budding yeast (Mendes Pinto et al., 2012). Therefore, although this system has defined the minimal intrinsic features for CR contraction, other factors are required for ingression and division *in vivo*, such as additional proteins, a CR rearrangement that is triggered by cytoplasmic signaling proteins or contributions from other cell processes, as are described in the final section of this Review.

Actin rings *in vitro*

An early step towards building a CR from scratch was the formation of actin rings in oil droplets. Under molecular crowding conditions,

actin polymerizes into a ring when enclosed in a water-in-oil droplet system (Miyazaki et al., 2015) (Fig. 4D). Regardless of droplet size, rings form at the equator (Miyazaki et al., 2015), which is the path of least curvature. Formation along the path of least curvature has been observed for actin rings formed in *S. pombe* protoplasts that lack all division site positional cues (Lim et al., 2018). Thus, this intrinsic feature of F-actin assembly might need to be carefully regulated during CR formation. The previously discussed mechano-regulation of formin activity and thus F-actin length could be one way this is achieved (Zimmermann et al., 2017). There are also geometric constraints on this ring; the maximum radius of the ring is equal to the persistence length (L_p) of the actin filament. Adding in heavy meromyosin (HMM; a dimer that functions as a bipolar motor) increases the maximum radius of the ring and the likelihood of ring formation, possibly due to its ability to dynamically crosslink and pull actin filaments together. In contrast, adding a motor-defective HMM mutant decreases the likelihood of ring formation, as does adding a passive crosslinker, such as α-actinin, although this can be rescued by the addition of HMM (Miyazaki et al., 2015). This finding is consistent with other observations showing that the levels of crosslinkers in the CR must be carefully balanced (Mukhina et al., 2007; Reichl et al., 2008; Mishra et al., 2013; Mavrakis et al., 2014; Takaine et al., 2015; Li et al., 2016; Descovich et al., 2018).

At high concentrations of HMM, actin rings formed in the above system can even contract. Contraction was observed to occur in a slow and fast phase, with the speed of the fast phase proportional to the initial radius of the ring, suggesting that even this self-assembled ring might have an effective contraction unit (e.g. the length of actin filaments) despite not having periodic sarcomere-like structures or even other proteins to organize the units (Miyazaki et al., 2015). Adding a small portion of full-length myosin-II that can assemble into mini-filaments enhanced ring contraction at the expense of ring assembly, suggesting that myosin-II mini-filaments might be prohibitive for ring assembly. In light of the myosin-II rearrangements that occur during cytokinesis (Fenix et al., 2016; Henson et al., 2017), this result supports the hypothesis that temporal regulation of myosin-II molecular assembly modulates its activity in order to meet the functional requirements of different stages of cytokinesis (Uehara et al., 2010; Wloka et al., 2013; Miyazaki et al., 2015).

As additional proteins are added to these minimal systems, further molecular insights are likely to be uncovered, as was the mechano-sensitivity of Cdc12 mentioned above (Zimmermann et al., 2017). The functions or localization of some CR proteins such as membrane scaffolds may also be modulated by lipid composition. Indeed, although we have discussed exclusively intrinsic properties of the CR and how CRs might be reconstituted, it is important to also consider how other factors influence cytokinesis.

The cytokinetic ring in context

Plasma membrane deposition and cortical architecture and dynamics must be coordinated with CR formation and contraction. Furthermore, although the CR generates force, other mechanisms of force generation likely exist given that yeast, amoebas and animal cells are able to divide in the absence of a robust CR under certain circumstances (Zang et al., 1997; Kanada et al., 2005; Carvalho et al., 2009; Ma et al., 2012; Mendes Pinto et al., 2012; Silva et al., 2016; Davies et al., 2018; Dix et al., 2018).

Plasma membrane

The plasma membrane serves as a direct substrate for CR assembly and furrowing and therefore it is important to consider the influence its composition and dynamics has on cytokinesis. The lipidome changes from interphase to mitosis (Atilla-Gokcumen et al., 2014), and multiple plasma membrane lipids are implicated in cytokinesis, chief among them phosphatidylinositol 4,5-bisphophate [$PI(4,5)P_2$] (Echard, 2012; Cauvin and Echard, 2015; Storck et al., 2018).

Indeed, in animal cells, $PI(4,5)P_2$ enriches in the plasma membrane at the cleavage furrow (Field et al., 2005), where it is important for maintaining the CR (Field et al., 2005; Wong et al., 2005). Consistent with these results, in *S. pombe* cells with reduced levels of plasma membrane $PI(4,5)P_2$ the CR forms equatorially – at the typical division plane – but its position is unstable and it moves away from the cell middle (Snider et al., 2017; Snider et al., 2018). Overexpressing a $PI(4,5)P_2$-binding domain also results in CR sliding, presumably by competing with CR proteins for binding to $PI(4,5)P_2$ (Snider et al., 2017). Indeed, many candidate CR-membrane linkers contain $PI(4,5)P_2$-binding domains, including anillin (Sun et al., 2015), septins (Bertin et al., 2010) and F-BAR proteins (McDonald et al., 2015; McDonald et al., 2016). However, surprisingly, the depletion of $PI(4,5)P_2$ has an additive effect with deletion of the anillin Mid1 and F-BAR protein Cdc15 (Snider et al., 2017), causing even more CR sliding and indicating that other proteins participate in CR–plasma-membrane linking in *S. pombe*, and possibly in other organisms. In addition to mediating CR scaffolding, phosphoinositides might directly influence actin-binding proteins such as profilin, cofilin or α-actinin to modulate actin cytoskeleton architecture (reviewed in Saarikangas et al., 2010) or mediate signaling cascades (Wong et al., 2005).

Not only does the composition of the plasma membrane affect cytokinesis, plasma membrane deposition is important for successful furrow formation (reviewed in Neto et al., 2011; Frémont and Echard, 2018; Gerien and Wu, 2018). Mutations that affect trafficking of membrane to the division site result in cytokinesis failure, most commonly due to failure of CR contraction and retracting furrows. It will be interesting to more deeply explore the CR not only as a force-generating molecular machine, but also as a landmark for vesicle delivery.

Cell cortex

Bound to the intracellular leaflet of the plasma membrane of animal cells and amoebas is the cellular cortex, a dynamic meshwork of F-actin, myosin and actin-binding proteins that provides structural integrity and generates contractile tension through myosin activity (Srivastava et al., 2016; Chugh and Paluch, 2018). During cytokinesis, the CR emerges as a specialized region of the cortex. In addition to the CR, the cortex contributes to cytokinesis in at least two ways. First, the phenomenon of cortical flow contributes to CR formation and has been recently implicated in contraction. Cortical flow is the mechanical compression of actin filaments towards the cell middle (Reymann et al., 2016). CR formation likely involves both localized *de novo* formin-mediated assembly of F-actin and directional transport of existing cortical F-actin (Zhou and Wang, 2008). F-actin filaments transported by cortical flow could be aligned by either compression by flow and/or myosin-II-mediated 'search and capture', similar to what is proposed for *S. pombe* CR formation. More recently, Khaliullin and colleagues proposed that cortical flow also promotes CR contraction through a positive-feedback mechanism wherein compression at the furrow drives increased cortical flow, which delivers existing cortical F-actin and myosin-II to the furrow and thus promotes further compression and flow. They posit that this maintains contraction rate even as the CR reduces in size (Khaliullin et al., 2018).

Second, the outward force that the CR has to overcome is altered by changes in cortical tension through modulation of myosin-II

activity and/or the architecture of the cortical actin network (Ennomani et al., 2016; Ding et al., 2017; Descovich et al., 2018; Koenderink and Paluch, 2018). Indeed, actin cortex thickness and tension are inversely correlated during cell cycle progression (Chugh et al., 2017). Furthermore, misregulation of cortical contractility at the cell poles can negatively affect cells, possibly resulting in aneuploidy or division failure (Sedzinski et al., 2011).

Extracellular matrix

Finally, the extracellular matrix of the cell is also a critical factor in cell division. Animal cells and amoebas with a perturbed CR can still divide by 'traction-mediated cytokinesis', which relies on force generation by pulling on a substrate as daughter cells separate (Zang et al., 1997; Kanada et al., 2005; Jahan and Yumura, 2017; Taira and Yumura, 2017; Dix et al., 2018). Cell rounding is a classic characteristic of mitotic cells, but some substrate attachments typically persist (Mitchison, 1992; Taneja et al., 2016; Dix et al., 2018; Lock et al., 2018; Taneja et al., 2019) and eliminating them in non-transformed cells causes cytokinesis failure (Dix et al., 2018). These observations raise the possibility that some proportion of force for cytokinesis during 'classical division' is dependent on proper substrate adhesion.

In yeast, the cell wall can be equated with the extracellular matrix. Current models of division propose that the major force for separation comes from cell wall deposition rather than CR contraction. *S. cerevisiae* lacking myosin-II can divide if mutation(s) activating the septum-synthesizing machinery are acquired (Tolliday et al., 2003). In *S. pombe*, biophysical measurements in protoplasts determined that the force generated by the CR is insufficient to overcome turgor pressure (Stachowiak et al., 2014). Thus, the CR may primarily serve as a landmark or mechanical signal guiding septum deposition (Proctor et al., 2012; Stachowiak et al., 2014; Thiyagarajan et al., 2015; Zhou et al., 2015) rather than a force-generating molecular machine, and this function could also exist in animal cells and amoebas.

Taken together, although essential in many eukaryotic cell types, the CR is not universally sufficient or necessary for cytokinesis, which requires coordination with the plasma membrane, membrane trafficking and other cytoskeletal structures, such as the cortex or focal adhesions, used during cell migration.

Conclusions and perspectives

How cells separate remains a fascinating and complex biophysical question. Here, we have synthesized recent findings from studies that employed multiple model organisms and experimental approaches to study CR formation and contraction. These studies offer substantial advances in our understanding of CR molecular architecture, in particular by studying F-actin dynamics with high temporal resolution, determining the molecular arrangement of myosin-II and examining other CR components at the nanoscale for the first time. We described three different *in vitro* approaches, highlighting their utility for testing existing theoretical models and for discovering mechanistic insights. We ended with a discussion of the cellular context of CR to acknowledge that, despite the advantages offered by *in vitro* techniques, the results must be interpreted with the knowledge that other cellular components such as the plasma membrane, the cortex or extracellular matrix also influence the success of cell division. Despite the significant advances in understanding CR-mediated cytokinesis, much work remains to determine how the CR contracts, how it generates force and transmits it to the plasma membrane, whether the primary purpose of the CR is to generate an inward force or serve as a landmark for membrane trafficking, whether CR-generated force is sufficient for division or if other forces are required in a wild-type context, and the degree to which these answers differ between organisms or even tissues within the same organism. Future super-resolution imaging studies on additional CR components in animal cells, progress in CR reconstitution and the framework provided by theoretical computational models will help elucidate the answers to these questions.

Acknowledgements

Many thanks to Dr Sierra Cullati, Dr Nathan McDonald, Dr Alaina Willet, Rodrigo Guillen and Chloe Snider for critical comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area was supported by Public Health Service awards from the National Institute of General Medical Studies of the National Institutes of Health [R01GM101035 (to K.L.G.), F31GM119252 (to M.Z.M.) and T32GM007347 (to the Vanderbilt Medical-Scientist Training Program)]. Deposited in PMC for release after 12 months.

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