Published in final edited form as:
Trends Microbiol. 2010 January ; 18(1): 38. doi:10.1016/j.tim.2009.10.002.

Cytokinesis and the contractile ring in fission yeast: towards a systems-level understanding

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Abstract

Cytokinesis is the final stage of cell division in which the cytoplasm of a cell divides to form two daughter cells. In eukaryotes, it includes the placement, assembly and contraction of an actomyosin-based contractile ring. A highly conserved set of proteins and pathways involved in the process have been identified and characterized. Additionally, fluorescent protein fusion technology enables high-resolution imaging of protein dynamics in living cells. Thus, the study of cytokinesis is now ripe for quantitative, systems-level approaches. Here, we review our current understanding of the molecular mechanisms of contractile ring dynamics in the model organism *Schizosaccharomyces pombe* (fission yeast), focusing on recent examples that illustrate a synergistic integration of quantitative experimental data with computational modeling. A picture of a highly dynamic and integrated system consisting of overlapping networks is beginning to emerge, the detailed nature of which remains to be elucidated.

Modeling of cytokinesis

Cytokinesis is the process in which the cytoplasm and genetic material of a cell divide equally to create two daughter cells. In animals and fungi, cytokinesis is achieved by the action of an actin-based contractile ring that forms at the site of division. Understanding how the cell positions, forms and contracts this ring provides fundamental insight into cell division and its regulation. Studies on systems ranging from model organisms, such as yeast, to human cells have revealed that about 20 proteins in the contractile ring are conserved among otherwise divergent eukaryotes [1]. At the same time, however, it is becoming clear that not all aspects of cytokinesis are conserved, particularly the regulatory mechanisms.

The fission yeast *Schizosaccharomyces pombe*, a rod-shaped cell that divides medially, is a leading experimental model for eukaryotic cytokinesis. In *S. pombe*, a discrete contractile ring is assembled at the future division site in early mitosis. This ring persists through mitosis until the end of anaphase, when it begins to contract and appears to guide the formation of the cell

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wall septum. After ring closure, the inner part of the septum is digested away, leading to the completion of cell separation.

A number of features of *S. pombe*, including its genetic tractability, its structurally compact ring that is amenable to imaging, and the relatively large percentage of time that it spends in cytokinesis during its short cell division cycle, make it particularly attractive for studying this process. Forward genetic screens for temperature-sensitive mutants have defined a core set of proteins that are required for cytokinesis [2-4], and genome-wide analyses promise to reveal near complete sets of the involved factors. For instance, a genome-wide green fluorescent protein (GFP)-localization study has identified more than 200 proteins situated at the division plane [5]. Although null mutants of many of these proteins do not produce a clear effect on cytokinesis, it is likely that these factors do contribute to various aspects of the process, but are functionally redundant or contribute in a more subtle way [6-8].

There is now an impressive accumulation of detailed and statistically significant data at the molecular level in *S. pombe*, particularly for the core cytokinesis proteins. Rigorous genetic analyses of individual components have defined functions *in vivo*—the consequences of removing or overexpressing a given component are known. Biochemical studies have defined activities *in vitro* as well as protein–protein interactions. The time-dependent localization and behavior of individual protein components have been visualized using functional GFP fusions, which, importantly, are often expressed near endogenous levels in yeast. Localization dependencies have been investigated, and protein dynamics in the ring have been probed using time-lapse microscopy and photobleaching-based approaches [9-12]. Finally, quantitative fluorescence measurements have yielded estimates for the aggregate numbers of molecules in the ring and elsewhere in the cell [13].

This dataset now presents a relatively well-characterized, self-contained system that is becoming amenable to systems-level computational modeling. Since its inception, mathematical modeling has played an active role in cytokinesis research [14]. However, in early years, theoretical research largely out-paced the ability to test posited models experimentally. Significant technical advances have now relieved this limitation, facilitating a close connection between experiment and theory. One particularly impressive example of the synergy between systems-level modeling and experiment is in the oscillatory Min system in bacterial cytokinesis [15]. There, mechanistic modeling of reaction-diffusion dynamics of the Min proteins responsible for selecting the *Escherichia coli* division site was used together with fluorescence correlation spectroscopy to provide important insight into this basic biological process [15,16].

A principal aim of mechanistic, systems-level modeling of cytokinesis is to relate dynamic protein levels in the cell to the positioning, assembly and contraction of the discrete ring structure that is formed and regulated precisely in space and time. In addition to describing system behavior quantitatively, models ideally suggest new, testable hypotheses that help to guide experimentation and provide non-intuitive insight into underlying biological mechanisms. Models can be evaluated critically and subsequently refined in an iterative manner by using, for example, chemical or genetic perturbations. Here, we describe some examples in which the synergy of computation and quantitative measurement in fission yeast is helping to shape our systems-level understanding of this ubiquitous and fundamental cellular process.

How does the cell position the ring?

Placement of the contractile ring in fission yeast occurs at a medial location that corresponds to the position of the pre-divisional nucleus [17,18]. A key factor in ring positioning is mid1p, which has some functional and structural features in common with anilin, a cytokinesis factor

in animal cells [17,19-22]. The mid1p protein localizes to a series of more than 50 cortical dots (termed 'midsome' structures) in the middle of the cell during interphase; these midsomes then recruit other ring components to this region of the cortex in early mitosis (Figure 1). An important unanswered question is how mid1p is positioned at this medial location on the cortex. The position of the nucleus is a positive spatial cue. For instance, moving the yeast nucleus using cell centrifugation demonstrates that cortical accumulation of mid1p follows the position of the nucleus in a dynamic manner [18].

It is now clear that multiple processes insure that mid1p is localized properly on the cortex. First, mid1p is part of a complex of proteins represented by these cortical dots; these proteins function not only in cytokinesis, but also in cell cycle regulation: cdr2p (a Nim1-like protein kinase), wee1p (a cell-cycle regulatory kinase), klp8p (a kinesin-like protein), and blt1p [23-25]. Localization of mid1p is dependent on cdr2p, but not the other proteins; in *cdr2* mutants, mid1p is less abundant on the cortex but is capable of promoting a normal cytokinesis [26].

Second, nuclear shuttling of mid1p appears to be important for its cortical localization near the nucleus [26]. Mutant mid1p that cannot enter the nucleus has weak defects in cortical localization in the cytoplasm. However, when combined with a *cdr2* mutation, the mutant mid1p dots are spread in an abnormally broad distribution on the cortex and promote assembly of defective or multiple rings. Nuclear shuttling might contribute to coupling mid1p to nuclear position by increasing transiently the cytoplasmic concentration of this protein in the peri-nuclear region [20].

Third, several studies have suggested a role for inhibitory factors that prevent contractile rings from forming at cell tips [27-29]. One of these factors is pom1p (a protein kinase of the DYRK family), which forms a gradient from the cell tip in a manner that is dependent on the microtubule-associated +TIP proteins tea1p and tea4p [28,30]. The pom1p kinase is required to exclude mid1p from one cell end (specifically the non-growing cell end), which indicates that there is an additional, as-of-yet unknown, process for inhibiting mid1p from the other cell tip.

In the study of Padte and collaborators [28], computational modeling of mid1p dynamics played an integral role in the discovery of pom1p. The spatial distribution of mid1p in the cell was modeled using a one-dimensional reaction-diffusion equation that incorporates nuclear–cytoplasmic exchange of mid1p, as well as membrane association and dissociation. Using reasonable cytoplasmic and membrane diffusion coefficients, a model based solely on nuclear shuttling does not account for the physiological mid1p distribution observed *in vivo*. However, physiological mid1p distributions do result when hypothetical inhibitory factors are included that increase the local rate of mid1p dissociation from the membrane at cell tips. This result motivated an experimental screen for such cell tip inhibitory factors, which led to the discovery of pom1p as one of these putative inhibitors. This modeling work now helps to drive new experimentation targeted at testing modeling assumptions and obtaining measurements for key parameters, including local protein concentrations, diffusion coefficients, dissociation rates of key binding partners, and the identities of additional inhibitors. Modeling studies might be especially helpful to test how these multiple nonlinear, coupled processes are integrated.

In addition to regulation of mid1p, genetic studies suggest that pom1p and other cell tip factors have additional roles in ring placement; for example, mutations in the *gene pom1* have a deleterious effect even in cells lacking mid1p and give rise to cells that often form rings at the cell tips [27]. Thus, in general, work in fission yeast is revealing a complex network of positive and inhibitory mechanisms involved in positioning the division plane.

How is the ring assembled?

The contractile ring assembles in early mitosis from sites specified by the more than 50 mid1p medial cortical dots. Myosin type II, its light chains and IQGAP are recruited by mid1p to form distinct cortical 'nodes' [30-32]. Myosin heavy chains might be activated to associate with these nodes by tail dephosphorylation [31]. These cortical nodes move in an actin-dependent manner (incoherently) to form a discrete ring structure [33-35]. In this process, a broad web of disorganized, dynamic actin filaments forms in the medial region and resolves gradually into a highly organized and tightly bundled ring. These actin filaments are assembled by the formin cdc12p, an actin nucleator that functions with profilin [12,36,37]. In addition, a set of actin-binding proteins that includes tropomyosin, ADF (actin-depolymerizing factor), fimbrin and α-actinin, modulates actin dynamics and contributes to bundling of actin in the ring [8, 38,39].

Two non-exclusive models have been proposed as mechanisms for ring assembly [40]. The first model, termed 'search-and-capture, pull and release' or 'node' model (Figure 2a) demonstrates how these cortical nodes can be drawn together into a ring through the action of tensile actomyosin forces [34,35]. A computational model [35] postulates that actin filaments polymerize in random directions from a small number of cdc12p formin molecules present at each node. Growing filaments, or small bundles thereof, randomly 'search' for neighboring nodes. When they encounter nodes, the filaments are 'captured' and pulled transiently by myosin minifilaments operating near the maximum, stall-force of the motor. These transient pulling forces between nodes dynamically move nodes together on the cylindrical surface of the cell. Stochastic fluctuations in the positions of nodes enter the model by randomness associated with filament capture and rupture (or turnover) events that are incorporated using experimentally observed rates. The movement of nodes on the cylindrical surface of *S. pombe* in this manner results in their collective coalescence into a discrete ring structure, as observed experimentally. This model is consistent with the observed movements of nodes and rare sightings of actin cables that capture and pull individual connected nodes. This model also predicts the behavior of two neighboring rings, which move towards each other and sometimes merge to form a single ring if they are close enough together [18]. Several key assumptions of the model remain to be validated, including the assumption that cdc12p dimers function at each node and that tension switches off cdc12p-mediated actin polymerization.

A second model for ring formation, termed the cdc12p 'spot,' 'leading cable' or 'aster' model, derives from a series of experimental observations on the asymmetric localization of actin filaments and cdc12p [36,41] (Figure 2b). In the majority of cells analyzed, cdc12p–3GFP fusion protein expressed at endogenous levels appears in one (or a small number of) discrete particle, called the cdc12p 'spot' [42,43]. A clear demonstration of the presence of cdc12p in the more than 50 cortical nodes has not been reported, except under certain conditions (such as latrunculin treatment), although we note that the presence of a very small number of molecules, as required by the first model, cannot be ruled out [13,32,43]. The cdc12p spot moves on actin and microtubules during interphase and subsequently moves to the future site of division during entry into mitosis, spreading into a ring by an as yet undetermined, actin-dependent mechanism [42]. Examination of actin structures in the developing ring has revealed that early in ring assembly many of the actin bundles emanate from an actin 'aster' [44]. In addition, a similar aster-like actin structure nucleated by the cdc12p spot is often seen in myosin light chain mutants, which suggests that myosin activity organized by mid1p is needed to progress from the aster to the ring structure [3,42]. Partial rings of cdc12p are consistent with the asymmetric assembly around the cell circumference [36]. This view of a ring that is organized by a single cdc12p spot is consistent with recent data on the orientation of actin filaments in the ring. Single-filament resolution transmission electron microscopy (TEM) of myosin-S1 decorated actin [44] reveals that in some early rings, most actin filaments are

oriented in parallel so that they appear to emanate from a central nucleation site (Figure 3). However, because of experimental limitations and the small sample size examined, it cannot be concluded that the rings examined in this study are generally representative of all rings.

How might actin and cdc12p interact with myosin nodes to distribute around the circumference of the cell to form the ring? One possibility is that they organize using a variation of the search-and-capture theme, whereby numerous actin cables emanating from the spot travel around the cell circumference in random directions and search for another cable of opposite polarity. Myosin-containing nodes could help to transport these filaments while keeping them anchored to the cortex. Eventually, when cables or filaments of opposite polarity do span the cell circumference and contact one another, they are pulled into ring structure in an orthogonal orientation via the action of myosin, which pulls it into the shortest structure that is possible on the surface of a cylinder. Tension from contractile actomyosin forces might not only help to form the ring, bring nodes together, and orient the ring, but might also enhance its structural integrity. One possibility is that tension upregulates formin activity to produce more actin for the ring. Although the effects of tension on formin activity have not yet been demonstrated directly, similar tension-dependent activation has been proposed to act in actin stress fibers and focal adhesions [45,46], and yeast actin cables [47].

This model of ring assembly is also consistent with observation of ring formation in the absence of mid1p and functional nodes (Figure 3). In this mutant, cdc12p and myosin appear in a spot associated with one or two strands emanating from it [36,48]. Time-lapse imaging shows that one of these strands eventually forms a ring that is generally off center. Notably, these rings are often less well formed than wildtype rings, and not oriented perpendicular to the cell axis, suggesting that these rings might have less internal tension. These rings have been seen to have membrane attachment defects [49] and certainly could have defects in myosin activity. However, in a cps1 mutant strain which is defective in cell wall septum assembly, which is delayed in septation, normal orthogonally-oriented rings are ultimately formed, possibly because these rings have more time to be pulled taut [48].

It is important to emphasize that while the node- and the spot-based models of ring assembly are distinct, they are not mutually exclusive. These models differ, in part, in the initial distribution of cdc12p, which is either in nodes or in a smaller number of spots. Although several laboratories have detected cdc12p only in the spots and not in nodes, a new study (J. Q. Wu, personal communication) reports sighting of cdc12p in nodes; further investigation is needed to reconcile these differences. Notwithstanding, current data do suggest that early ring assembly can proceed using multiple distinct molecular mechanisms. Each might operate selectively under distinct environmental conditions and pressures, or possibly in parallel (Figure 2c). Indeed, there is growing evidence that at least two partially overlapping mechanisms for ring assembly are operating normally; an “early” and “late” pathway. The ring assembly mechanisms described above are proposed as “early” mechanisms. The SIN (septation initiation network) pathway, which includes a G-protein and protein kinase cascade, is responsible for driving the “late” pathway [40, 50]. The SIN is critical for triggering ring contraction and septation, and functions also to maintain rings after anaphase has completed. In the absence of the SIN pathway, rings initially form but are not maintained after anaphase. Hyperactivation of the SIN pathway causes repeated rounds of ring formation and septation, even in interphase cells [51]. Notably, the SIN pathway might drive ring formation using somewhat different components and mechanisms; ring formation by this route might be independent of nodes and mid1p, but instead function through the PCH protein cdc15p [40].

This hypothesis consisting of multiple mechanisms has certain similarities to the situation proposed in *Caenorhabditis elegans*, in which two signals, in this case one originating in the microtubule asters and one from the spindle mid-body, are thought to contribute to cytokinesis

at different stages of mitosis [52,53]. Like most critical biological processes, multiple redundant pathways probably endow cytokinesis with robustness in the face of variable cellular and environmental conditions.

How does the ring maintain itself and then constrict?

Once assembled, the mature ring remains a highly dynamic structure [12]. Mean numbers of specific molecules in the ring have been estimated at various time points from fluorescence intensity measurements of YFP-fusion proteins in living cells [13]. Estimates include 150 cdc12p dimers and 2900 myo2p molecules in the mature ring. Electron micrographs show that there are 1000–2000 actin filaments of average length of 0.6 μm in the ring, and support the estimate that approximately 50% of the nearly one million G-actin monomers in the cell (8.7 μM) reside in the ring [44,54]. Interestingly, although the basic dimensions of the mature ring remain constant for approximately 30 minutes during prometaphase, photobleaching studies indicate that most components, including cdc12p and myosin complexes, turn over on much shorter timescales of up to a minute [55-57]. Ring components appear to be turning over on somewhat different timescales [57]. In addition, although there is no functional GFP–actin fusion for use in photobleaching studies, latrunculin treatment shows that actin disassembles from the ring on a similar timescale [55]. Under normal conditions, this turnover of actin might be due both to loss of pre-existing filaments and nucleation of new filaments, as opposed solely to treadmilling of pre-existing filaments. Moreover, mutations in cdc12p that reduce its actin-polymerization efficiency slow down rates of turnover of both actin filaments and cdc12p, which indicates that the dynamics of these components are somehow coupled with one another [43]. Finally, ring dynamics are also modulated by clp1 (a cdc14-like phosphatase) that is recruited to the ring by mid1p, suggesting a possible role for cell cycle-dependent phosphorylations on regulating these dynamics in different phases of cytokinesis [57]. Taken together, these data suggest that protein components of the ring are highly dynamic during ring homeostasis. Turnover rates might reflect transient protein-protein or protein-membrane interactions, as well as possible tangential motion of actin filaments and myosin minifilaments. The collective dynamical behavior of the ring proteins associated with actin at the plasma membrane might utilize similar design principles as focal adhesions [58,59], allowing individual protein components to turnover rapidly while stably maintaining the ring's dimensions and position.

The dimensions and composition of the ring change in anaphase when the ring begins to contract. A key unanswered question is what changes in the ring to bring about this contraction. Whereas the number of myosin II molecules remains approximately constant throughout constriction, the numbers of actin filaments, formins, IQGAP and α-actinin decrease proportionately with ring volume [13]. Actin filaments become shorter on average, decreasing from a mean length of 0.6 to 0.4 μm [44]. As the ring contracts slowly over approximately 30 minutes, the dynamics of protein components appear to remain largely unchanged during this contractile phase [12]. Thus, ring contraction in *S. pombe* might represent an iterative process of gradual remodeling involving continual assembly and disassembly of macromolecular cytoskeletal assemblies. Ring closure is thought to be regulated by the SIN pathway, in which the sin2 protein kinase might regulate ring components by phosphorylation [50,60]. Recent work in *C. elegans* highlights the relationship between cell size and the rate of ring contraction [61]; the rate of constriction increases proportionately as cell size increases. The mechanism responsible for this scaling and whether it applies universally remain to be determined.

Although the ring is thought to exert significant contractile forces in large animal cells, its function in fission yeast remains unclear. One major difference is that ring contraction is accompanied by assembly of the cell wall septum in these fungal cells. While it is possible that ring contraction provides the physical force that drives cell division, to date there is no evidence

that a ring alone is capable of dividing fission yeast in the absence of a cell wall or the septum [62]. Functionally, it is equally possible that the role of the ring in cytokinesis is merely to mark the site of cell wall growth and septation, where internal ring tension serves principally to stabilize and position the ring, ensuring orderly closure of the cell wall. Systems-level molecular modeling constrained by experimental dynamical protein-protein interaction data will help to elucidate the molecular design principles governing ring homeostasis and contraction in the cell.

Concluding remarks and future directions

Cytokinesis is a complex and highly conserved cellular process that involves dynamic interactions among many protein components that assemble, maintain and contract a discrete ring structure. The continued accumulation of detailed knowledge of cytokinesis in fission yeast now calls for systems-level approaches to develop and refine our mechanistic understanding of this universal cellular process at the molecular level. Although the tenets of 'systems biology' are still evolving, we advocate a tightly integrated approach that incorporates mechanistic computational data analysis and data-driven modeling with quantitative cell biological and genome/proteome-wide experimentation (Figure 4). '-Omics'-based approaches help to discover and identify new components involved in cytokinesis and to develop systems-wide interaction and functional networks. These approaches are likely to reveal new functional links between the ring, signaling networks, and numerous other functional sub-modules in the cell. In contrast, high-resolution cell biological studies that incorporate *in vivo* genetics with *in vitro* biochemistry and quantitative microscopy are essential to develop an in-depth and quantitative understanding of molecular mechanisms and dynamics that enable cell division. In particular, advances in quantitative live-cell imaging approaches such as spatiotemporal image correlation spectroscopy [63], fluorescent speckle microscopy [64] and super-resolution, single-molecule imaging [65], are likely to reveal important dynamic properties at the molecular level that may be integrated with high resolution structural data from cryo-electron tomography and microscopy [44,66]. Computational modeling that accounts both for protein structural information and physicochemical protein-protein interactions consistent with quantitative spatiotemporal experimental data provides a venue for simulating quantitative hypotheses that can be tested, for example, using genetic and chemical perturbations *in vivo* in conjunction with parameter-variation *in silico*. The success of this integrative approach depends crucially on synergistic collaborative interactions among researchers from various disciplines.

A general theme that emerges from the studies presented in this review is that cytokinesis is an 'overbuilt' system with multiple levels of redundancy in functional networks that mediate its assembly, maintenance, and contraction. Biological systems are often faced with significant internal and external sources of variability and noise [67] and operate in unpredictable environments that have likely forced the cell to develop robust overlapping mechanisms to ensure the success of critical cellular events such as cytokinesis, thereby promoting a fitness advantage. For these reasons, perhaps it is not surprising that multiple pathways for ring assembly and placement are being elucidated. Often, these mechanisms involve functional interactions between different parts or components of the cell that operate at distinct time points in the cell cycle. For example, the highly dynamic nature of ring proteins might reflect an active feedback control mechanism that allows the cell to regulate and repair this structure actively during chromosome duplication and segregation. One as-yet unexplored factor in this system would be mechanical tension. The use of systems-level approaches to elucidate the complexities of signaling and structural networks involved in this process is likely to reveal novel and unexpected principles of nature's design.

Acknowledgments

F.C. is supported by NIH grant GM05683 and M.B. is supported by MIT faculty start-up funds and the Samuel A. Goldblith Career Development Professorship. We thank Martin Howard, David Burgess, and Douglas Lauffenburger for helpful discussions and Nilah Monnier for reading the manuscript critically. We acknowledge the summer research program at the Marine Biological Laboratory for initiating this work.

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Glossary

cdc12p	a formin responsible for the assembly of actin filaments for the ring.
Latrunculin	an actin polymerization inhibitor that acts by sequestering actin monomers.
mid1p	a key factor in positioning the site of ring assembly. Possible orthologue to metazoan anillin.
Myosin type II	an actin motor protein in the ring that bundles and slides actin filaments.
Midsome	a cortical membrane complex with function in ring assembly and regulation of the cell cycle. Its components include mid1p and cdr2p (a cell cycle regulatory kinase). It is localized on the cortex in the vicinity of the nucleus and present throughout interphase. Also known as the “interphase node.”
Node	a cortical membrane structure that organizes the assembly of the ring. Defined here as a mitotic structure that contains the midsome complex plus additional contractile ring components recruited during mitosis, such as myosin and actin.
pom1p	a protein kinase of the DYRK family that forms a gradient from cell tips. It functions in cell polarity, cell cycle, and placement of the cell division plane.
Septation initiation network (SIN)	a signaling pathway that includes a GTPase and a protein kinase cascade located primarily on the spindle pole body. It is necessary for septation and ring closure, and for driving a “late” cytokinesis pathway for ring assembly and maintenance. Orthologue to the mitosis exit network (MEN) in budding yeast.

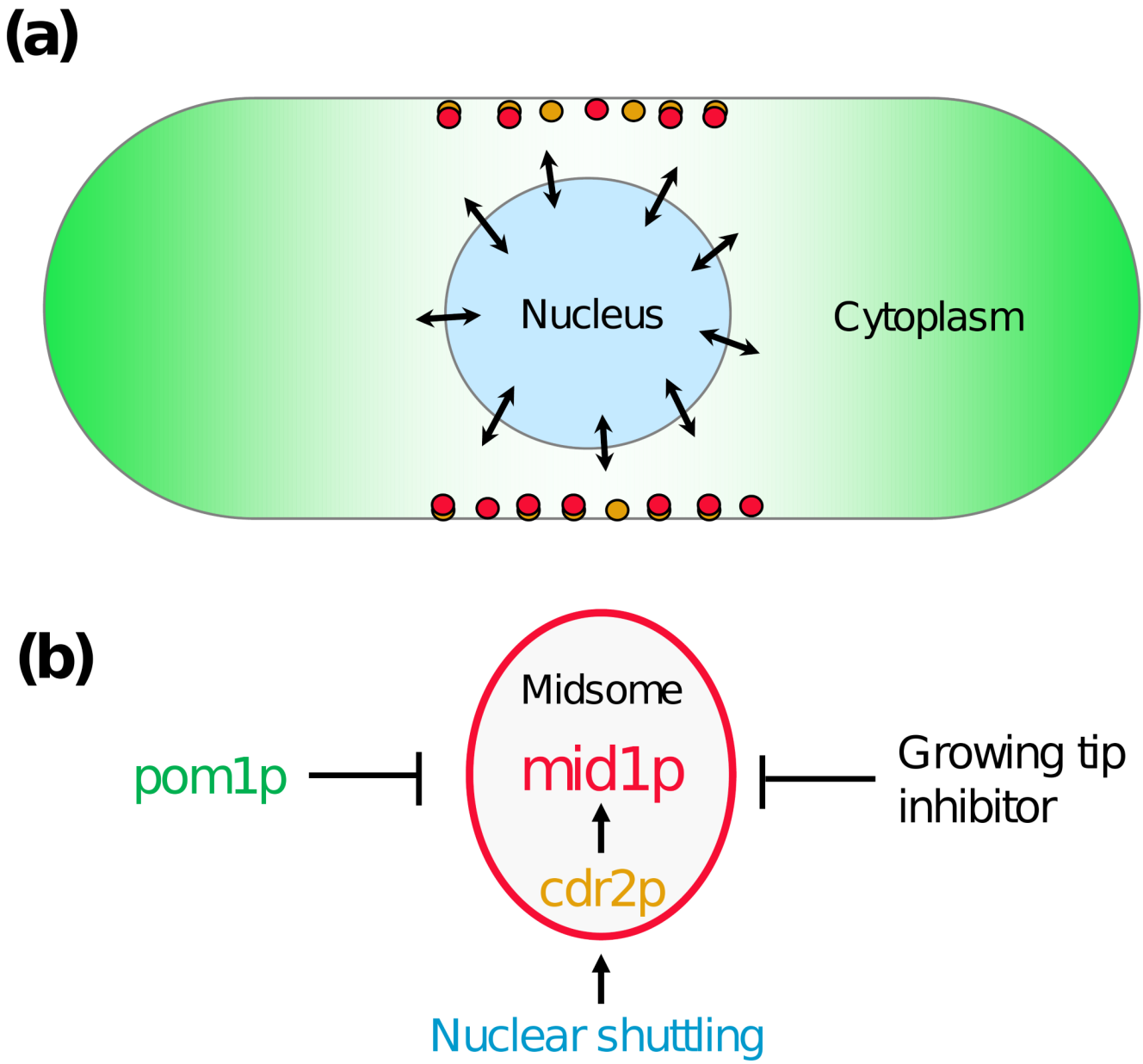

Figure 1.

Model for ring positioning. The site of ring assembly is regulated by mid1p, which forms dots on the medial cortex at a site adjacent to the nucleus **(a)**. The mid1p protein is part of a cortical protein complex termed 'midsome' **(b)** and requires another protein in the complex, cdr2p, for proper localization. The positioning of the mid1p and cdr2p are regulated by positive cues from nuclear shuttling and negative factors such as a gradient of the protein kinase pom1p from the cell tips. The pom1p protein is required for inhibition from non-growing cell tips, suggesting the existence of a as-yet unidentified inhibitor for growing cell tips.

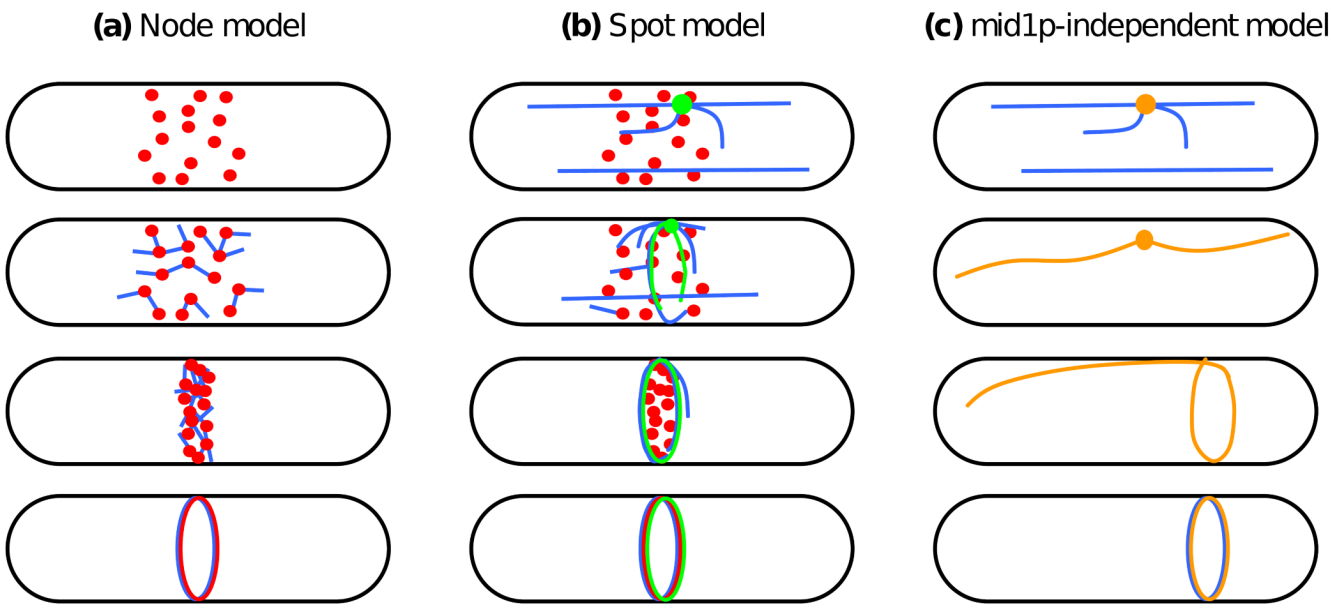

Figure 2.

Models for ring assembly. **(a)** Node model: membrane-bound mid1p recruits myosin, formins (green) and other ring factors (collectively in red) to 50+ cortical node structures. These nodes nucleate and assemble actin filaments and are brought together by a search, capture, and pull mechanism driven by contractile myosin II activity. **(b)** Spot model: similar to the node model, myosin is recruited to membrane-bound mid1p nodes in the vicinity of the nucleus. Formin (green), however, is in this model not distributed evenly at mid1p-myosin-containing nodes (red), but rather concentrates in one or a small number of spots. Actin filaments emanate from the formin spot in random directions, and are guided on the cortex by the myosin-containing nodes, eventually forming the ring structure that may be stabilized by a tension-dependent mechanism. **(c)** mid1p-independent model: cells can assemble a ring in the absence of mid1p and nodes, through the formation of strands containing myosin and formin (collectively in orange).

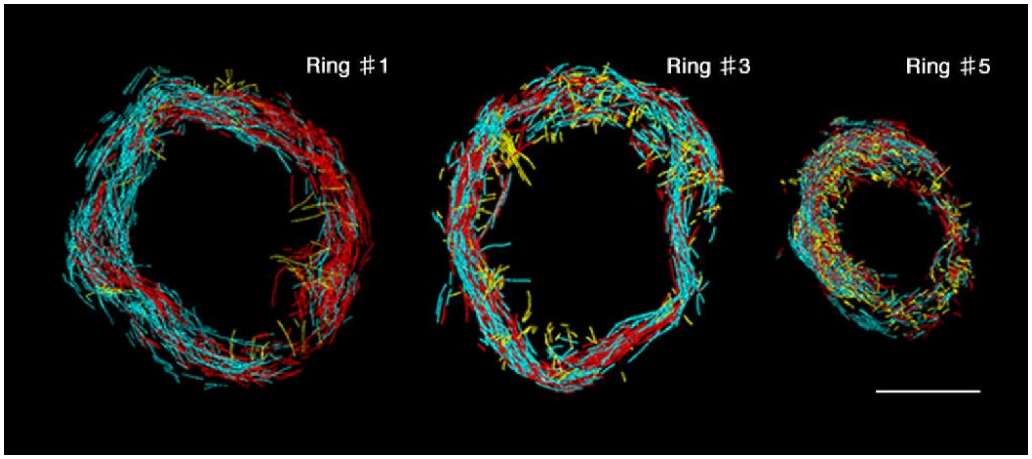

Figure 3.

Actin filament organization in the contractile ring of *S. pombe*. Electron microscopy reconstruction of fission yeast contractile rings. Red, blue and yellow denote filaments of clockwise, counterclockwise, and unknown pointed-end polarity in the ring, respectively. Ring 1 is an 'early ring' in early mitosis; ring 3 is a mature mid-mitotic ring; and ring 5 is a contracting ring. Note that many of the actin filaments in parts of ring 1 are of the same polarity. Scale bar is one micron. Reproduced with permission from Ref. [44].

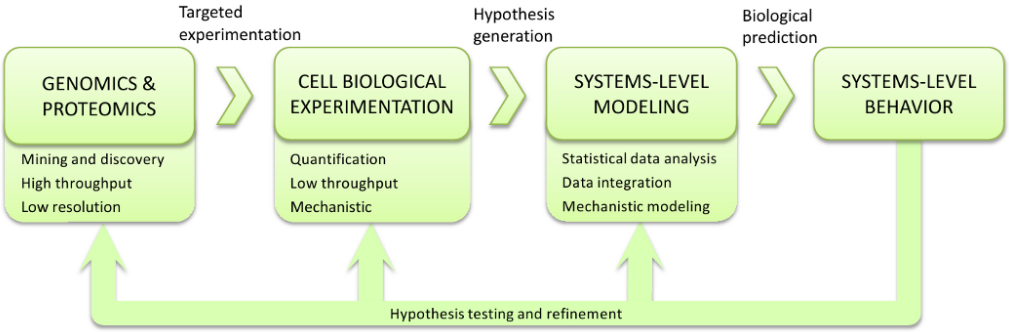

Figure 4.

Proposed approach to integrate experimentation with systems-level modeling in cell biology. Experimental methods include high-throughput "-omics-based" approaches for mining and discovery of genes, proteins and their interactions, combined with high-resolution quantitative cell biological and molecular methods. The systematic statistical analysis and integration of diverse data sets provide quantitative data for the development of mechanistic, systems-level computational models. Together, these data sets and models help to establish distinct hypotheses for systems-level function. Models are tested using targeted experimentation, including genetic and chemical perturbations. Iterative cycles of data analysis and integration, mechanistic systems-level modeling, prediction and targeted experimentation are used to refine our molecular-level and mechanistic understanding of cellular function.