Establishment of a cellular axis in fission yeast

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Recent studies in fission yeast *Schizosaccharomyces pombe* reveal how cells establish a cellular axis that specifies domains as the functional 'ends' and 'middle' of the cell. During interphase, dynamic microtubules position the nucleus at the middle of the cell and orientate microtubule 'plus' ends towards the ends of the cell. At the cell ends, the microtubule plus ends might establish a zone of polarized cell growth and actin assembly by depositing factors such as Tea1p. At the cell middle, the nucleus might specify the position of the actin contractile ring and the future cell division site by positioning cytokinesis factors such as Mid1p.

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One aim of modern cellular biology is to understand how all the cellular components are assembled into a whole, living cell. How does a single cell develop? Although countless individual proteins and protein–protein interactions are identified each day, little is known about how cellular components self-assemble and organize at a cellular level. How do cellular components sense cell size, measure distances and position cellular components at specific locations inside the cell? How does pattern formation occur in the single cell to establish distinct functional domains?

The fission yeast *Schizosaccharomyces pombe* has emerged as an excellent genetic system in which to study cellular morphogenesis. Fission yeast are simple rod-shaped cells that grow at the cell tips and divide by medial fission. Polarized cell growth is regulated during the cell cycle: after cell division, cells grow first in a unipolar manner at the 'old' end and then, at a transition point in G2 phase known as 'new-end take off' (NETO), begin to grow in a bipolar manner[1]. One feature that makes fission yeast so ideal for these studies on spatial control is that its shape, size and division habits are extremely reproducible. Therefore, it is possible to identify mutants that have aberrant cell shape, cell size or cell division patterns[2–5] (Fig. 1). Fission and budding yeasts are evolutionarily distant, and certain aspects of fission yeast cell division and cell polarity are more similar to processes in animal cells than the budding yeast processes are. For instance, as in some animal cells, localization of the sites of cell polarity and polarized growth are dependent on MICROTUBULES (see Glossary) in fission yeast, but this is not the case in budding yeast. The sequencing of the genome, coupled with excellent genetics and cytology, now makes fission yeast a potent system for addressing many important questions in cell biology.

Here, we discuss recent work in fission yeast that is beginning to reveal how a single cell establishes its 'ends' and 'middle'. The interphase nucleus and microtubules are positioned by dynamics of the microtubule cytoskeleton. These components establish a cellular axis that positions cell polarity and cytokinesis factors. The plus ends of microtubules define the cell 'ends' by delivering cell polarity factors such as TEA1p to the cell tips. The position of the nucleus determines the 'middle' through cytokinesis factors such as MID1p, which is involved in positioning the cell division plane in the vicinity of the nucleus. Thus, these recent advances reveal concepts underlying cellular organization and also begin to identify conserved molecular pathways that link global organization with processes of cell polarity and cell division.

Setting up the axis: the role of microtubule dynamics in positioning the nucleus and microtubules

During interphase, the fission yeast nucleus is positioned in the middle of the rod-shaped cell. This medial position is maintained even as the cell grows asymmetrically, suggesting that there is an active process that monitors the position of the cell ends and defines the middle position. As in many eukaryotes[6], nuclear positioning is a microtubule-dependent process, as the nucleus is no longer positioned properly in cells that have defective microtubules[7,8].

Interphase microtubules are organized in multiple (3–5) microtubule bundles that extend along the long axis of the cell forming a basket around the nucleus[9,10]. The organization of these microtubules was poorly understood, but two recent papers used green-fluorescent protein (GFP) fusions and live-cell imaging to show that each linear microtubule element is in fact a bundle of dynamic microtubules organized in an anti-parallel configuration[8,11] (Fig. 2a). Although each half of a microtubule bundle behaves independently, the halves exhibit similar behaviors with similar dynamics. Measurement of microtubule dynamics coupled with fluorescent-speckle microscopy analysis[8] or photobleaching[11] suggest that microtubule ends facing the cell tip are plus ends, with the minus ends embedded within the medial overlap region. Generally, each individual microtubule grows (from the plus end) to the cell tip, keeps growing at the cell tip for an average of 70–100 seconds, and then exhibits 'microtubule catastrophe', shrinking back to a medial bundled region. It then grows back toward the cell tip again[8,11,12], with each cycle lasting ~4–6 min. The behavior of the microtubules upon depolymerization

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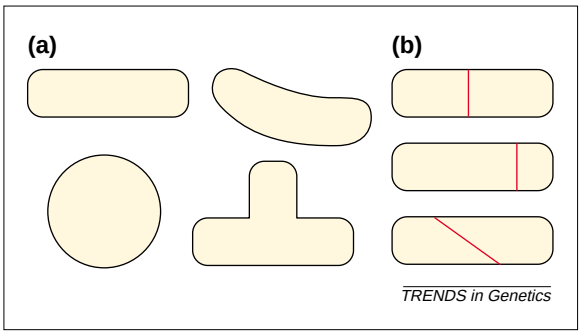

Fig. 1. Examples of *S. pombe* mutant phenotypes affected in cell polarity and cell division. (a) Cell shapes: wild-type rod shape, bent, round and branched shapes. (b) Cell-division patterns: wild-type medial division, asymmetric division and tilted septum. Genetic screens for these types of mutants have identified many factors involved in cell polarity, cytokinesis and cytoskeletal organization.

and repolymerization suggests that each bundle is organized from a medial dot that is very stable with regard to depolymerization[8].

A key parameter in the organization of the interphase microtubules is their regulated dynamics: microtubules generally shrink 100 seconds after hitting the cell tip. In addition, they usually only shrink after hitting the region of the cell tip, and do not shrink when they hit the sides of the cell[8,12]. This behavior could explain how most of the microtubules come to be orientated along the long axis of the cell, as the continued growth of microtubules would position the microtubules in this manner. The regulated dynamics of microtubules suggests that there are factors concentrated at the cell tips that promote microtubule catastrophe. In addition, there are also factors located at the ends of microtubules (Fig. 2b). Tip1p is a member of the CLIP170 family of microtubule-binding proteins, which accumulate at plus ends of microtubules in animal cells[13,14]. Tip1p is located in dots at the plus ends of growing microtubules (but not shrinking ones)[12] and in other cytoplasmic dots. In a mutant cell lacking Tip1p (*tip1*Δ), microtubules have normal rates of growth and shrinkage, but undergo catastrophe whenever they contact any plasma membrane, not just at the cell tips. Thus, one model postulates that Tip1p is a component of a cap at the microtubule plus end that stabilizes a microtubule when it hits the cell surface. At the cell tips, there could be catastrophe factors that remove the cap, thereby causing the microtubule to shrink.

Numerous other gene products are implicated in the regulation of microtubule dynamics (Fig. 2b). Mutations in these genes cause the interphase microtubules to be either too short or too long. Tea2p, a Kip2-like kinesin, is localized in discrete cytoplasmic dots along the length of the microtubule bundle and at microtubule ends[15]. Mal3p, a functional homologue of EB1, is located along microtubules when overexpressed as a GFP fusion[16]. *tea2* and *mal3* mutants are viable but have shorter microtubules[4,15,16]. Moe1p could be a destabilizing factor, as *moe1* mutants have microtubules that are hyperstable to depolymerization. Moe1p interacts with the polarity factor Scd1/Cdc24 and with Mal3p (Refs 17,18), but how these interactions regulate microtubule dynamics at the cell tip is not yet clear. Some point mutations in genes encoding the γ-tubulin complex stabilize microtubules, so that the microtubules wind around the cell tip[19,20]. How these γ-tubulin factors located presumably at the minus ends of microtubules affect microtubule dynamics (particularly at the plus end) is still a mystery.

Dynamic microtubules might position the nucleus by providing frequent, transient pushing forces from the cell tips on the nucleus. The nuclear envelope, spindle pole body and chromosomes all exhibit small, microtubule-dependent oscillations during interphase[8,21]. Direct observations of microtubules and the nuclear envelope show that at least one microtubule bundle is attached to the nuclear envelope, causing frequent small deformations of the nuclear envelope. These deformations occur during the 1–2 minutes when the microtubule polymerizes at the cell tip and pushes the microtubule lattice and nucleus away from that cell tip (Fig. 2a)[8]. This behavior is not consistent with other possible mechanisms such as microtubule pulling or tracking. It has been suggested that immediately after anaphase, the nucleus is pulled to the middle of the daughter cell[21,22]. However, careful analysis of microtubule dynamics shows that even at this stage, microtubules position the nucleus by pushing forces (P. Tran, unpublished).

These findings suggest a simple mechanism for how the cell senses the position of its cell tips and centers the nucleus between the cell tips[8]. The organization of microtubule bundles specifies that similar numbers of microtubules are pushing on the nucleus from each cell tip, producing a balance of forces. An offset nucleus would encounter a net centering force, both from more frequent pushes and from stronger pushes, because shorter microtubules buckle less readily than long ones.

Glossary

F-actin: Dynamic polymers of actin monomers. Actin has roles in cell growth, secretion, endocytosis and cytokinesis. In fission yeast, F-actin is present in actin patches and cables and is reorganized into a medial contractile ring during cell division. F-actin dynamics and nucleation are regulated by numerous actin-binding proteins and can provide contractile or propulsive forces using actin polymerization or depolymerization, or in conjunction with myosin motor proteins.

Microtubules: Dynamic polymers of tubulin dimers. Microtubules are required for nuclear positioning, mitosis, organelle distribution and cell polarity. Microtubules are used in the cell to provide pushing forces (acting like sticks), pulling forces (acting like ropes) or tracks for movement of particles and organelles. Microtubules have plus and minus ends that exhibit different properties; they are generally nucleated from the minus end at a microtubule-organizing center. The growth and shrinkage of microtubules are regulated in part by proteins that bind to their plus ends, such as Tip1p, a CLIP170-like protein, and by microtubule motor proteins.

Mid1p: A novel protein with a pleckstrin-homolgy domain. Mid1p is localized in a cortical band overlying the nucleus and is required for positioning the cytokinesis site in the vicinity of the nucleus.

Tea1p: A kelch repeat protein with weak similarity to ERM proteins. Tea1p is present on the plus ends of microtubules and plays a role in cell polarity establishment at the cell tip.

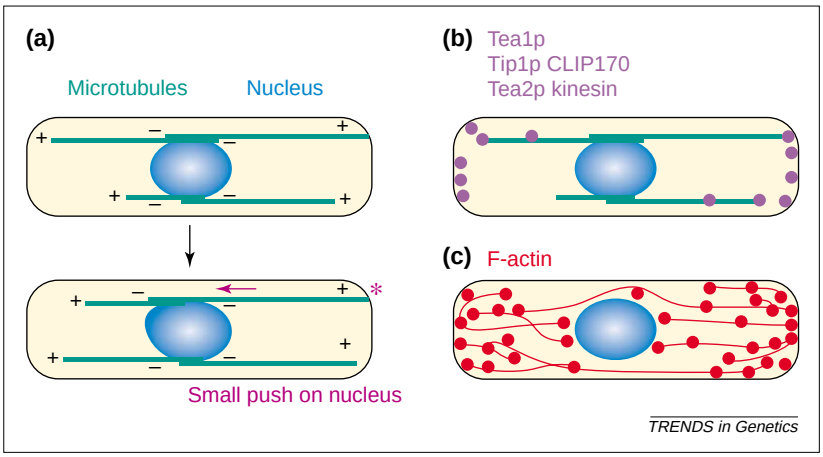

Fig. 2. Establishment of a cellular axis and establishment of cell polarity are dependent on dynamic microtubules. (a) A mechanism to position the nucleus and the microtubules. Microtubules are organized in bundles in an anti-parallel configuration, with plus ends facing the cell tips. Microtubules grow at their plus end toward the cell tip and only shrink 100 sec after they hit a region at the cell tip. Bundles of microtubules are attached to the nucleus and exert transient, small pushes on the nucleus when a microtubule end hits the cell tip and continues to grow at the tip (asterisk). A balance of these pushing forces could center the nucleus. (b) Factors at the ends of microtubules involved in cell polarity. Tea1p, Tip1p and Tea2p are localized in dots at the plus ends of microtubules as well as along the microtubules themselves. Tea2p is a kinesin that might move these factors to the end of the microtubule. These factors might be deposited at the cell tips when the microtubule contacts the cell tip, and they have various roles in the regulation of cell polarity and microtubule dynamics. (c) The actin cytoskeleton is organized in a polarized distribution of actin patches and cables.

Computer modeling suggests that this pushing mechanism is indeed sufficient to center the nucleus[8]. This mechanism illustrates how dynamic microtubules can provide a way for a cell to measure distances and define its middle.

S. pombe organizes its microtubules and positions its nucleus in a manner distinct from other cells examined to date. Nuclear positioning in interphase fission yeast is dynein independent[23]. In cells from other fungi, slime mold and animals, centrosomes and the associated nucleus or mitotic spindle are positioned using dynein-dependent mechanisms based upon microtubules pulling from the cell surface[24–29]. In fission yeast during meiosis, the nucleus exhibits a 'horse-tail movement' that is dependent on dynein[23]. Microtubule pushing-based mechanisms might be used in some round animal cells to center the centrosome during interphase, as *in vitro* studies show that a dynamic microtubule aster will center itself when placed in a round glass dish[30]. Fission yeast might have devised a pushing mechanism using microtubule bundles as the most efficient way to position the nucleus in a cylindrical cell. For example, a mutant (*rsp1*) with an aster-like microtubule configuration cannot center the nucleus properly (F. Chang, unpublished). It will be interesting to examine whether other cell types with elongated cell shapes can use similar nuclear positioning mechanisms.

How the ends of microtubules establish cell polarity at the cell tips

The establishment of cell polarity in animal cells is often dependent on the interaction between microtubules and actin[31–33]. Microtubules are thought to send signals to organize the actin cytoskeleton at sites of cell polarity, but the molecular nature of such a pathway is not well understood. Recent studies suggest that cell polarity in fission yeast is also dependent on microtubules and actin[32].

Cell polarity is reflected by the polarized organization of the F-ACTIN cytoskeleton at sites of cell growth and septation. F-actin is organized into discrete foci known as actin patches and an associated network of actin cables[34,35] (Fig. 2c). Although the precise function of the actin patches is not yet known, their polarized distribution could be important for polarized cell growth. Actin patches contain F-actin and components of the actin polymerization machinery (such as Arp2/3 complex components), and they serve as sites of actin polymerization (Ref. 35). Actin patches move using an actin polymerization-based mechanism with an average velocity of 0.3 μm/sec (Ref. 35). The patches are associated with actin cables, and some have been observed to move along cables. Actin patches do not appear to stream toward the tips, and thus their polarized distribution might be a result of preferential patch assembly at the cell tips[35].

Microtubules are not required for the maintenance of polarized growth or for the proper localization or movement of actin patches[35,36], but they might be involved in the establishment or organization of new cell-growth sites. Cells with defective microtubule organization still exhibit polarized growth but often have cell shape defects, such as bent or branched cells, or monopolar growth patterns[4,36,37] (Fig. 1). Thus, microtubules might be required to establish a defined zone of polarized cell growth that recruits the actin cytoskeleton and the formation of actin patches at the cell tips to initiate cell growth.

Tea1p represents a link between microtubules and the cell polarity machinery. *tea1* mutant cells exhibit unipolar growth, with bent and occasionally T-shaped cells[3,4,38] (Fig. 1). Tea1p has N-terminal kelch repeats[39] and a C-terminal coiled-coil region and possesses some similarity to the ezrin/moesin/radixin family of cytoskeleton-associated proteins[40]. Tea1p is located in discrete dots at the ends of microtubules (the microtubule plus ends), at the sides of microtubules and at both cell tips, including cell tips that are not growing (Fig. 2b). Tea1p localization at the cell tips is dependent on microtubules and not actin[38]. These observations suggest that Tea1p on the plus end of a microtubule is deposited at the cell tip when the microtubule touches the cell tip. Tea1p then acts to establish the cell tip as a functional 'end' capable of polarizing the actin cytoskeleton and producing polarized cell growth.

The kinesin Tea2p and the CLIP170-like protein Tip1p are required to localize Tea1p to the microtubule plus end[12,15]. In *tea2* or *tip1* mutants, Tea1p is no longer concentrated at the microtubule

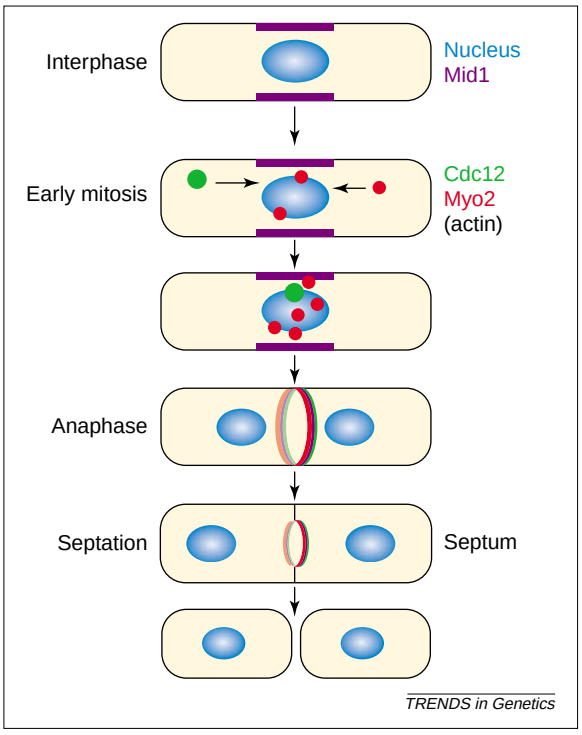

Fig. 3. A model for how the nucleus might position the cell-division plane. During interphase, a broad band of Mid1p forms at the cell surface in the region overlying the nucleus. During mitosis, Mid1p (and possibly other factors) might recruit other contractile ring factors such as F-actin, the Cdc12p spot and myosin particles to this medial zone, where they assemble a discrete contractile ring that marks the future site of cell division.

ends, but is distributed in dots along the length of the microtubule[12,15]. This abnormal distribution is also found in cells with a specific *tip1*-mutant allele in which microtubule lengths are still normal. Tea2p and Tip1p are located in discrete dots along microtubules and at the tips of microtubules (Fig. 2b). In a *tea1* mutant, Tea2p and Tip1p dots are still localized along microtubules and at microtubule ends, but are less concentrated at the microtubule ends[12,15]. These findings suggest that these three factors could be in particles that deliver Tea1p to the plus ends of microtubules (Fig. 2b). However, co-localization or physical interactions between these factors have yet to be described.

How Tea1p functions in regulating cell polarity is not well understood. Tea1p probably does not organize actin directly, but it could help to localize or activate downstream polarity factors. One possible downstream target is Pom1p, a protein kinase[41]. *pom1* mutants have a polarity phenotype similar to *tea1* and also have a defect in division-site positioning. Pom1p localization at cell tips is dependent on microtubules and Tea1p. A PAK kinase, Orb2p (also known as Pak1p or Shk1p), which might function downstream of the small GTPase Cdc42p, is required for 'end' identity[42]. Sawin *et al*. used various fusion proteins as markers for cell 'ends' and 'sides'. An *orb2*-mutant cell never initiates cell growth at the new end, and, furthermore, the new end has a 'side' marker and not an 'end' marker. Thus, not only was one of the cell tips in the *orb2* mutant deficient in cell growth, but it also has properties of the cell 'side'. Tea1p is still properly localized at both cell tips in the *orb2* mutant, suggesting that Orb2p could function downstream of Tea1p (Ref. 38). Downstream polarity factors might be tethered or regulated by F-actin, as a transient disruption of the actin cytoskeleton causes some monopolar cells to grow in a bipolar manner[43]. Thus, further study of Tea1p and its downstream targets could reveal mechanisms by which microtubules instruct the actin cytoskeleton.

Many important details in cell polarity need to be elucidated. The cell-cycle periods when Tea1p acts and polarity is established are not yet defined. A deeper question is: how are the ends initially determined? Although Tea1p and microtubules certainly are important for polarity establishment, the organization of microtubules and the deposition of Tea1p at the cell ends depend on factors already present at the cell tips. Are there microtubule-independent polarity factors that precede Tea1p, or might there be positive feedback loops based on Tea1p and other microtubule-dependent factors that gradually establish polarity at the cell tip?

How the nucleus specifies the cell division plane at the cell middle

Fission yeast divide in the middle, presumably to ensure successful partition of cellular components, such as nuclei, and to maximize their growth rate. The cellular axis described above positions the nucleus and the minus ends of microtubules at the geometric center of the cell, establishing the cell 'middle'. One important function of the cell middle is to assemble, in early mitosis, an actin–myosin-based contractile ring for cytokinesis[34,44–46]. In late mitosis, the ring marks the site of cell-wall septum formation, and it also contracts to close the plasma membrane and guide the septation process (Fig. 3). In animal cells, the plane of cell division is often dictated by the position of the mitotic spindle. By contrast, in *S. pombe*, the cell-division site appears to be determined by the position of the nucleus or by some structure associated with the nucleus, not by the mitotic spindle[5] or by markers fixed on the cell surface such as birth scars[46]. For instance, mutant cells that do not form a mitotic spindle still assemble a contractile ring in the proper location[5]. Furthermore, the positions of the nucleus and cell-division plane correlate, even in cells with an asymmetrically positioned nucleus[44]. One reason why the nucleus is used instead of the mitotic spindle could be the simple fact that the nuclear envelope does not break down during mitosis in fission yeast, as it does in animal cells.

Many factors required for both assembly of this contractile ring and its placement have now been

identified[46]. One factor required for ring placement, Mid1p, represents a link between the nucleus and the contractile ring. Mid1p is required for proper placement of the actin ring: *mid1* mutants form an actin ring at random sites and angles in the cell[5] (Fig. 1). Mid1p is a novel protein with a C-terminal pleckstrin-homology domain (PH domain), and it has some weak similarity to the cytokinesis factor anillin[47,48]. The localization of Mid1p varies during the cell cycle, and Mid1p localization and activity are regulated by the polo kinase Plo1p (Refs 47,49,50). During interphase, Mid1p is located in a broad band on the cortex overlying the nucleus (Fig. 3), defining a 'middle zone'. In addition, it shuttles in and out of the nucleus during interphase. During mitosis it exits the nucleus and colocalizes with the contractile ring. The position of this interphase broad band is linked to the position of the nucleus, suggesting that this cortical band somehow senses the position of the nucleus throughout most of the cell cycle[50]. How this cortical zone and the nucleus are linked is not yet clear. The microtubule and actin cytoskeletons are not required. One attractive possibility is that nuclear shuttling concentrates Mid1p near the peri-nuclear region. However, when most Mid1p nuclear shuttling is inhibited by a mutation of its nuclear localization signal, Mid1p still exhibits normal cytoplasmic localization and function[50]. One function of Mid1p, which arrives before the other ring proteins, could be to position other components of the contractile ring at a site near the nucleus during ring assembly (Fig. 3).

Recent analysis of other contractile ring proteins shows that some of these proteins arrive at the future ring site in the form of large particles (Fig. 3). Cdc12p is a member of the formin family of proteins (also known as the FH family), which have conserved roles in cytokinesis and cell polarity[51,52]. Cdc12p is localized during interphase to a discrete, motile cytoplasmic particle (the Cdc12p spot), which moves to the future site of cell division and, at the onset of mitosis, spreads out into the contractile ring[51,53]. This Cdc12p spot moves using microtubule and actin-based mechanisms, and is associated with both an F-actin cloud and microtubules. In a *mid1* mutant, Cdc12p is not properly localized[51]. How Cdc12p might travel to the medial future division site is not known. For instance, it might stick to the Mid1p central zone, associate with an actin web, travel down to the minus ends of microtubules, or use a combination of these mechanisms.

Myo2p, a type II myosin heavy chain required for contractile ring formation, is in discrete particles that congregate in the medial region and associate with a loose medial F-actin web, before myosin and actin coelesce into a ring[54,55]. Mutants in myosin heavy and light chains accumulate disorganized F-actin in the medial region, suggesting that one role of myosin during cytokinesis is to bundle up actin filaments into an actin ring[5,56]. Myp2/Myo3, a second type II myosin, is present in another motile particle that associates with microtubules and incorporates into the contractile ring late in mitosis, well after Myo2p does[55].

Numerous cytokinesis factors are also localized to the spindle pole body, a medial microtubule organizing center structure associated with the nuclear envelope. The IQGAP Rng2p and the polo kinase Plo1p are located on the spindle pole body and have roles in ring assembly and placement[49,57]. Many factors involved in the regulation of septation – members of the Sid/Sin pathway – are also located on the spindle pole body[49,58]. My preliminary observations show that the precise site of the spindle pole at the onset of mitosis (at spindle pole body separation) does not necessarily correlate with the site of cell division, suggesting that the spindle pole body is not the only spatial cue for the placement of the cell-division site.

These studies lead to a working model in which Mid1p establishes a central cortical zone in the region of the nucleus during interphase. This central zone, and possibly additional spatial cues such as the spindle pole body and minus ends of microtubules, might target contractile ring proteins to this region, leading to assembly of the contractile ring and ultimately cell division at the middle of the cell (Fig. 3).

Conclusions

S. pombe is emerging as a useful genetic system in which to study conserved processes such as cell polarity, cytokinesis, microtubule dynamics, actin organization and nuclear positioning. These studies in fission yeast reveal fundamental mechanisms of how cells use dynamic polymers, such as microtubules and actin, as tools to measure distances, place components in certain locations and promote cellular organization. In fission yeast, dynamic microtubules position the microtubule plus ends at the cell tips and the nucleus at the middle, thereby establishing a cellular axis for cell polarity and cell division. Spatial organization in other cell types could be established using similar cytoskeletal mechanisms. Interactions between microtubules and actin are thought to establish sites of cytokinesis and cell polarity in many animal cells[31,46]. The developmental axes of *Drosophila* are established by the nucleus and the ends of microtubules in the single cell oocyte – the position of the nucleus in the oocyte dictates the dorsal–ventral axis, whereas polarized transport of determinants to the oocyte ends using actin and microtubules are responsible for establishment of the anterior–posterior axis[59,60]. Thus, these mechanisms for the establishment of a cellular axis are important in understanding cellular function and organization as well as spatial patterning during development of multicellular organisms.

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