Published in final edited form as:

Semin Cell Dev Biol. 2010 May ; 21(3): 290–299. doi:10.1016/j.semcdb.2010.01.017.

Kinesins to the core: the role of microtubule-based motor proteins in building the mitotic spindle midzone

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Abstract

In mammalian cultured cells the initiation of cytokinesis is regulated – both temporally and spatially – by the overlapping, anti-parallel microtubules of the spindle midzone. This region recruits several key central spindle components: PRC-1, Polo-like kinase 1 (Plk-1), the centralspindlin complex, and the Chromosome Passenger Complex (CPC), which together serve to stabilize the microtubule overlap, and also to coordinate the assembly of the cortical actin/myosin cytoskeleton necessary to physically cleave the cell in two. The localization of these crucial elements to the spindle midzone requires members of the kinesin superfamily of microtubule-based motor proteins. Here we focus on reviewing the role played by a variety of kinesins in both building and operating the spindle midzone machinery during cytokinesis.

Keywords

mitosis; cytokinesis; microtubule; motor protein; kinesin

Introduction

Following the disjunction and segregation of sister chromatids during anaphase, the mitotic cell cleaves in two in a process known as cytokinesis. Cytokinesis involves the assembly of a cortical actin/myosin ring in the cleavage plane, which functions to constrict the cell, and physically divides it into a pair of daughters. The initiation of cytokinesis is a highly regulated process, which must be coordinated with the onset of anaphase. In order for proper cytokinesis to take place, the cell must position the presumptive cleavage furrow in the region where chromosome congression had occurred (reviewed in [1]). This cleavage plane may be at the cell center, but it can also be biased towards one pole or another, if that particular cell is destined to undergo an asymmetric cleavage. Finally, at the end of cytokinesis the cell must recruit membrane vesicles and associated docking complexes to the constricted furrow region to complete the process of abscission (reviewed in [2]). In order to properly orchestrate these various functions, the cell builds a transient microtubule structure known as the spindle midzone (reviewed in [3]). This midzone consists of anti-parallel

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microtubules emanating from opposite spindle poles – which overlap in the center of the mitotic spindle – coupled with several key protein complexes that are recruited to the microtubule overlap. The midzone itself can be envisioned as the region of the spindle left in the wake of the retreating chromosomes, and this has led to the idea that the chromosomes themselves influence the position of the cleavage plane through their interaction with the spindle midzone. Indeed, as the sister chromatids migrate towards their respective poles, several key factors are thought to travel from the chromosomes to the midzone microtubules [4, 5]. However, the results of several elegant micromanipulation experiments suggest that chromosomes do not play a role in the positioning of the presumptive cleavage furrow [6, 7], and this idea has been supported by molecular genetic studies suggesting that so-called chromosome passenger proteins do not have to bind to the chromosomes before they can localize to the midzone microtubules themselves (discussed in [8]). Whether or not the chromosomes are necessary as a jumping off point, the overlapping interpolar microtubules and midzone components recruited to these microtubules after anaphase are necessary for the successful completion of cytokinesis in cultured cells (reviewed in [1]). These structural/signaling components in the middle of the midzone are sometimes referred to as the central spindle. However, the terms spindle midzone and central spindle are often used interchangeably.

Importantly, the formation and function of the spindle midzone requires the activity of several members of the kinesin superfamily of motor proteins (see [9] for naming conventions of kinesins; also see [10]). Kinesin 4 (KIF4, a chromokinesin) serves to transport and organize a microtubule-associated protein known as PRC-1 (*protein required for cytokinesis*), a key player in midzone assembly [11]. Kinesin 6 (MKLP-1) is a component of the centralspindlin complex, and is required to localize the Rho activating activity of centralspindlin to the midzone [12]. Another kinesin 6 family member, MKLP-2 (also known as Kif20A), appears to be required for the localization and function of the Chromosome Passenger Complex (CPC), which redistributes from the centromere region of the chromosomes to the midzone microtubules themselves, and serves to localize Aurora B kinase to the central spindle [13]. In addition, MKLP-2 is required to localize Plk-1 (polo-like kinase) activity to the midzone [14].

While both kinesin 4 and kinesin 6 represent major players in the formation and function of the central spindle – and have been extensively studied in a variety of model organisms – there are several other kinesin family members linked to the midzone by localization studies, but their functions remain less well understood. CENP-E (kinesin 7) has been shown to localize to the spindle midzone [15]. However, in contrast to its role at the kinetochore, the role of CENP-E at the midzone remains to be determined. The same holds true for both MPP1 (M-phase phosphoprotein 1, yet another member of the kinesin 6 family; [16]) and Kif14 (a member of the kinesin 3 family; [17]). Both have also been implicated in function at the midzone, yet a mechanistic understanding of their roles during cytokinesis has not been determined. And finally, there is Eg5 (kinesin 5), which localizes to the microtubule overlap, and serves to maintain spindle polarity [18]. However, the functions of kinesin 5 are expertly reviewed elsewhere in this issue (Ferant et al.), and will not be reiterated here.

Once the cleavage plane has been determined, the cortical actin/myosin becomes activated in the cleavage furrow, and constricts the dividing cell, as well as the spindle midzone itself. The compacted midzone/central spindle is then known as the midbody, which connects the pair of daughter cells prior to abscission [19]. Here again, the organization of these microtubules and their associated components plays a key role in the completion of cell division. The midbody is central to the completion of cytokinesis, as its formation is required for final membrane fusion during abscission. Many of the molecular components of

the central spindle become localized to the midbody, though it is often not clear whether they have a direct role in abscission, or are merely compacted *in situ* during late cytokinesis.

It is important to point out at the onset of this review that the midzone is not the only region of the mitotic spindle implicated in positioning or signaling the cleavage furrow. Several studies conducted in early cleavage-stage embryos – most notably sea urchins and sand dollars – strongly support the hypothesis that astral microtubules create an overlapping zone that is key to establishing the cleavage furrow in these large cells (reviewed in [20]). These studies include the series of classical experiments carried out by Rappaport and colleagues, which really defined the early field of cytokinesis research (discussed in [1, 21, 22]). In addition, experimentally fused cultured cells that contain two independent spindles generate a cleavage furrow between the spindles, without intervening chromosomes or midzone microtubules [23], though they do assemble microtubule bundles [24]. Also, mammalian cultured cells utilize both the spindle midzone, and astral microtubules to localize Aurora B to the region of the presumptive cleavage plane [25]. And finally, mammalian cultured cells with experimentally induced monopolar spindles can induce cleavage furrows, and accumulate midzone components on microtubules that do not generate an overlap [26]. Here we have focused on reviewing mechanisms for midzone assembly and function. There are several excellent reviews that deal with the issue of midzone microtubules vs. astral microtubules in signaling for cytokinesis [1, 3, 20, 22, 27, 28]; and we will not address this issue here, except to acknowledge that the debate over the respective roles of the midzone vs. the asters continues.

Origins and organization of the midzone microtubules

As stated above, the spindle midzone is organized by anti-parallel microtubules at the mitotic cell center. These microtubules are classically referred to as interpolar microtubules, to differentiate them from the kinetochore fibers (microtubules running from the spindle poles to the kinetochores), and astral microtubules, which grow from the spindle poles, and have plus-ends that are either free and dynamic, or embedded in the cell cortex. To illustrate the formation of the spindle midzone, a series of maximal projections of two individual BSC-1 (monkey kidney) cells expressing α tubulin-GFP is shown in Figure 1 (cells described in [29]). In these images, the events of midzone microtubule formation can be observed as the cells progress from metaphase through cytokinesis. In panel A, both cell 1 and cell 2 are in metaphase, with compact spindles containing a mixture of microtubule classes – the large numbers of brightly labeled kinetochore fibers cause the spindle fluorescence to saturate the CCD camera. In panel B, cell 1 has proceeded through the metaphase-anaphase transition, and the spindle midzone can be seen (arrow). Note that the dense kinetochore fibers are still brightly fluorescent at the polar region, and that cell 2 remains in metaphase. In panel C, cell 1 is initiating furrowing in the position of the central spindle (arrow), and cell 2 is entering anaphase. The cleavage furrow in panel D, cell 1 is constricting the spindle midzone, whereas cell 2 in early anaphase has a distinct spindle midzone, and brightly fluorescent kinetochore fibers at the poles. Cytokinesis is complete in cell 1, panel E, and the midzone has become compacted into the midbody. In panel F, midbodies are readily visible in both cells. These images illustrate the transitions from a compact metaphase spindle through to an elongated central spindle in anaphase, and finally, the formation of a dense midbody during cytokinesis.

What is not readily apparent from these live-cell image stacks is the organization of the minus ends of the midzone microtubules. This is an important question, although a largely unresolved one. It has been difficult to image the minus-ends of the midzone microtubules, because the dense kinetochore fibers – along with the dynamic astral microtubules – serve to obscure the organization of the minus ends of the interpolar microtubules at the spindle pole.

What little that is known about the organization of the minus ends of the spindle midzone came from an extensive ultrastructure study of mammalian spindle microtubules [30]. This group used serial section electron microscopy and computer-based image processing to map spindle microtubules in fixed PtK₁ cells. This work revealed that the minus ends of interpolar microtubules (ipMTs, aka the midzone microtubules) are further away from the spindle poles than kinetochore microtubules, as early in mitosis as metaphase. They also found that these microtubule minus-ends moved further away from the spindle poles following anaphase onset. This suggests that there is an organizing activity associated specifically with interpolar microtubules, and this activity could account for the apparent broadening of the central spindle pole that has been observed (Figure 1, cell 1, small arrows).

Such an organizing activity – unique to the midzone microtubules – could have two possible origins. One possibility is that the ends of the interpolar microtubules, destined to become the midzone, detach from the spindle pole, and remain organized by a minus-end bundling activity. In this case, the central spindle poles would become re-organized following anaphase onset. Another contrasting view, put forth by Wright and colleagues [31] is that new microtubules of the midzone are nucleated by a special microtubule organizing activity, that is active only in telophase, and that this activity contributes to a central spindle polar organizer that is distinct from the actual spindle pole (see below).

In both of the above-mentioned scenarios, a major role is played by γ -tubulin. In interphase cells, γ -tubulin serves to nucleate the centrosomal microtubules [32]. In mitosis, there is a substantial increase in the amount of γ -tubulin localized to the centrosomes, coordinate with the increase in microtubule nucleating capacity observed at the mitotic spindle pole [33]. In addition to localizing to the spindle poles, γ -tubulin and its associated ring complexes (γ -TuRCs), have been localized to the minus-ends of the midzone microtubules [34]. Microinjection of antibodies specific to γ -tubulin into anaphase cells causes the cell to assemble an aberrant midzone, and they were prevented from forming a midbody [31]. In addition, a component of the γ -TuRC -NEDD-1- associates with the minus ends of the midzone microtubules as well [35]. NEDD-1, also called GCP WD, is thought to anchor γ -tubulin to the centrosome, and may play the same role in targeting γ -tubulin to the central spindle pole ([36]; reviewed in [37]). Therefore, γ -TuRCs at the minus ends of midzone microtubules may cap these tubes, and either prevent depolymerization, or function to nucleate new microtubules of the midzone. Whether γ -TuRCs contribute to the behavior of the microtubule plus-ends, as has been suggested by a recent report, remains to be determined [38].

One potential candidate protein that has also been proposed to organize the minus-ends specifically of the midzone microtubules is the abnormal spindle protein (Asp), identified in *Drosophila* [39, 40, 41, 42]. Asp is enriched at spindle poles during mitosis, where it maintains the integrality of the microtubule-organizing center [40, 43]. Asp also localizes to the punitive minus ends of central spindle microtubules in telophase [41]. In *asp* mutants, central spindles either fail to form, or are highly defective. This leads to failure of cytokinesis, and suggests that central spindle organization requires proper organization of the minus-ends of midzone microtubules. These studies also suggest that the minus-ends of midzone microtubules become unattached from the spindle pole following anaphase [42].

In mammals, the ortholog to Asp is called ASPM (Abnormal spindle pole like microencephaly gene, also known as MCPH-5). The ASPM is associated with the centrosome in interphase, and localizes to a broad region of the spindle pole throughout mitosis [44]. ASPM is also localized to the midbody during cytokinesis [45]. Interestingly, homozygous mutations in ASPM are associated with primary microcephaly, a

neurodevelopmental disorder [46]. Another midbody component, citron kinase, is also associated with microcephaly in rodents [47], is required for cytokinesis [48] and has been shown to interact with ASPM [45]. Current thinking in the field is that mutations in ASPM impair proper spindle orientation, leading to aberrant cytokinesis and neuronal cell death early in development [49].

Another question relates to the organization of the plus-ends of interpolar microtubules during anaphase, telophase and cytokinesis. The formation of a plus-end microtubule overlap is thought to be the targeting region for many of the central spindle components. This notion is supported by the observation that in experimentally derived cultured cells with two independent spindles, the region between two astral arrays can induce cytokinetic furrow formation [23] and these overlapping microtubules form midzone like bundles, complete with the midzone components INCENP and MKLP-1 [24]. However, it is not clear that a microtubule overlap is absolutely essential for recruitment of central spindle components. One such component – INCENP – the scaffold for the Chromosome passenger complex, becomes recruited to non-overlapping plus ends of microtubules in experimentally derived monopolar spindles that were induced to undergo anaphase [26]. Also, cells that have had their centrosomes experimentally ablated show defects in cytokinesis, and it not clear the effect of the loss of the centrosome has on midzone formation [50, 51]. Thus, it remains to be determined if these midzone microtubule factors require a plus-end overlap, or just target to midzone plus ends. If they do target to plus-ends, then it remains to be determined what differentiates the plus end of a midzone microtubule from that of an astral microtubule. It is formally possible that midzone microtubules are defined by their minus ends (released from the spindle pole) and that this can influence the behavior of the plus end.

As the chromosomes separate and are drawn to the poles during anaphase, the plus-ends of the interpolar microtubules incorporate new tubulin subunits as the spindle elongates [52, 53]. However, the extent to which this process is regulated, and by what factors remains unclear ([54, 55]; also see kinesin 5 review by Ferantz et al. in this issue). It stands to reason that the elongation of these microtubules must be constrained; otherwise they could invade across the midzone into the opposite daughter cell. It will be interesting to identify the mechanisms used for length control of the midzone microtubule plus-ends, and how midzone microtubules are able to specifically recruit these factors.

In addition to the spatial organization of the spindle midzone, there is the question of how is the formation of this key structure coupled with anaphase onset. An important observation came from live-cell imaging of cultured cells injected with fluorescent tubulin. In this study, it was shown that proper midzone formation requires a drop in Cdk1-cyclin B kinase activity [56]. To examine this, non-degradable cyclin B (cyclin B $\Delta 90$: [57]) was injected into NRK (normal rat kidney) cells during prometaphase. These cells entered into mitosis, and progressed through the metaphase/anaphase transition (via activation of the anaphase promoting complex, and the proteolytic destruction of endogenous protein targets). However, because cyclin B $\Delta 90$ could not be proteolyzed (but could function as the regulatory subunit for the Cdk-1 complex), the activity of Cdk-1 cyclin B did not drop. In these cells, the chromosomes segregated to the poles, and the poles themselves separated apart from each other. However, a prominent midzone microtubule overlap did not form. Also, these cells eventually cleave in a manner akin to cytofission (described in [1]), and when they do, no midbody was observed. From live-cell imaging experiments of cells injected with cyclin B $\Delta 90$ and fluorescent tubulin, these cells appear to have two independent half-spindles, which move apart, but never maintain the overlap between the interpolar spindles. Thus, the distribution of the components of the central spindle are regulated at the gross level, by Cdk-1 kinase activity.

In sea urchin zygotes injected with cyclin B $\Delta 90$, the two half spindles separate, then are pushed back together, forcing the disjointed chromosomes together into a central bunch [58]. No midzone overlap was observed in these cells; their centriole pairs split apart during the prolonged mitosis, and generated tetrapolar spindles, but these cells never underwent cytokinesis or cytofission. When sea urchin zygotes injected with cyclin B $\Delta 90$ were subjected to physical deformation, bringing the spindle closer to the cell cortex, cytokinesis was induced (see [59]; discussed in [60]). Together, these observations suggest that the drop in Cdk-1 cyclin B kinase activity is necessary for midzone microtubule formation and cytokinesis, and indeed, many of the components of the midzone are regulated by Cdk1-cyclin activity [61, 62]. However, in large cells, such as sea urchin embryos, the drop in Cdk-1 kinase activity is not sufficient to induce cytokinesis; factors delivered by the post-anaphase astral microtubules (mimicked experimentally by physically moving the spindle closer to the cortex) are also required [59].

Finally, there is the question of spindle bipolarity. Multipolar cell division – whether experimentally induced, or as a consequence of centrosome amplification associated with cellular transformation – appears to be highly rare [63, 64, 65, 66, 67, 68, 69]. It appears that multipolar mitotic spindle (or bipolar spindles with multiple bundled centrosomes) pre-dispose the cell to generate merotelic kinetochore attachments, which may partially explain the increase in chromosome instability observed in cells with multiple centrosomes [67, 68, 69]. During such multipolar divisions, when multiple furrows do form, they often retract, leaving one major furrow. This situation is one of many ways in which a cell with multiple centrosomes (and therefore, the potential to assemble multiple spindle poles) undergoes a bipolar division. Cells with multiple centrosomes also bundle or cluster these into two poles, passing on the multiple centrosomes without generating rapid and lethal aneuploidy (discussed in [65]). Figure 2 shows the consequences of multipolar division. Here, a BSC-1 cell expressing GFP- α tubulin has assembled a tetrapolar spindle. As the cell enters anaphase (panel C) five distinct cleavage furrows form between the separated asters (arrows). The furrows at the bottom right (asterisks) are the furthest away from the spindle. As the cell progresses through anaphase and cytokinesis, three furrows ingress, while the forth and fifth (asterisks) regress. The cell eventually divides into three daughters, with two cells connected by a major midbody, and the third connected by a slender process (Figure 2, panel F, arrow). Understanding the geometry and organization of aberrant midzone formation during multipolar mitosis will undoubtedly be important in identifying the mechanisms used to assemble bipolar midbodies.

CHO-1 and Rho activation at the midzone – The centralspindlin complex

The first indication that kinesin played a role in the spindle midzone came from the discovery of CHO-1, an antigen identified by monoclonal antibodies raised against isolated mitotic spindles from CHO (Chinese Hamster Ovary) cells [70]. CHO-1 recognized two polypeptides of 95 and 105 kDa. The CHO-1 epitope turned out to be an isoform of a plus-end directed kinesin of the MKLP-1 family (kinesin 6), also known as ZEN-4 in worms [71, 72] and *Pavarotti* in flies [73]. The proposed function of MKLP-1 was to cross-link and bundle the midzone microtubules and this is consistent with the observations that kinesin 6 is required for cytokinesis (reviewed in [8]). The CHO-1 variant of MKLP-1 turns out to have unique activity associated with late cytokinesis (discussed below), but represented an important conceptual move forward, as the obvious implications for a motor cross-linking the interpolar microtubules on anaphase spindle elongation [74].

In addition to its role in interacting with the spindle midzone microtubules, MKLP-1 has been shown to play a major role in positioning the presumptive cleavage furrow – another important conceptual leap in understanding the regulation of cytokinesis. It does this by

localizing factors that lead to the activation of Rho GTPase, and Rho-kinase, which regulate myosin contractile ring assembly and cleavage furrow ingression [75, 76]. The midzone microtubule bundling and Rho-kinase activation are coordinated through MKLP-1/ZEN-4 forming a complex with two regulators of Rho GTPase activity. These are CYK-4/MgcRacGAP (the guanine activating protein responsible for inactivating Rho), and Pebble/ECT2 (the guanine exchange factor or GEF that activates Rho). The complex formed between MKLP-1/ZEN-4 and CYK-4 turns out to be essential for the function of both – neither the motor nor the GAP can localize to or bundle midzone microtubules without first forming the complex. This motor/Rho-GAP complex has been termed centralspindlin, and is required for midzone assembly and cytokinesis [12]. Centralspindlin in turn is also required for the localization of Pebble/ECT2, the Rho GEF that is required for cytokinesis [77, 78, 79].

Another kinesin 6 family member, MKLP-2, appears to play critical roles in the localization of both Aurora B kinase [13], and Plk-1 (polo-like kinase 1; [14]). Aurora B functions as the catalytic kinase activity of the Chromosome passenger complex (CPC). The CPC consists of a scaffold protein, called INCENP, along with survivin [80], borealin, with Aurora B kinase as the catalytic subunit (reviewed in [5]). These passenger proteins are found at the inner centromere region during metaphase, and also at the central spindle during anaphase [4]. Though this dual localization has implied that the CPC “jumps ship” from the chromosomes to the midzone at anaphase, there is evidence that the CPC does not have to first bind to the chromosome before it can localize to the midzone (discussed in [8]). However, these experiments do not rule out the cycling of the CPC. The regulation of the CPC and its localization to the midzone have important consequences for the organization of midzone microtubules and the completion of cytokinesis. INCENP itself appears to have microtubule bundling activity, and thus, may coordinate the bundling of microtubules with the localization of Aurora B kinase activity to the midzone [80].

Another key regulator of central spindle function is Plk-1, which directly interacts with MKLP2, and together, becomes targeted to the central spindle. This interaction is itself mediated by MKLP2 phosphorylation by Plk-1. Loss of MKLP2 by siRNA inhibits localization of Plk-1 and coordinately, causes failure of cytokinesis [14]. It has been suggested that the mechanism behind the failure of cytokinesis in MKLP2 deficient cells is the loss of Plk-1 activity at the midzone, which may be required for proper positioning of the cleavage furrow. One potential Plk-1 target is Cyk-4 (Rho GAP), which functions to recruit ECT2 (Rho GEF) to the midzone, thereby activating Rho [81, 82]. Whether MKLP2 activity is absolutely required for Plk-1 function is not clear, because flies and worms lack this second kinesin 6 family member [8].

While there have been several mechanistic studies showing MKLP-1/2 are crucial for midzone microtubule assembly and function, less is known about a kinesin 7 family member that becomes re-distributed from the chromosomes to the midzone: CENP-E [15]. CENP-E plays a critical role in microtubule attachment to kinetochores [83], and gliding of monooriented kinetochores to the metaphase plate [84, 85] and re-distributes to the spindle midzone (Liao et al., 1994). CENP-E is a target for Cdk-1 kinase activity, and phospho-CENP-E cannot bundle microtubules in vitro [15]. This suggests that upon anaphase onset, CENP-E is released from the kinetochores, and can cross-link microtubules of the midzone. However, siRNA knock-down experiments in *Drosophila* S2 cells revealed that the fly CENP-E ortholog does not play a functional role during anaphase or cytokinesis [10]. Also, CENP-E does not localize to overlapping astral microtubules in cell experimentally manipulated to contain two separate spindles [24]. Thus, it remains to be determined in CENP-E function during anaphase can be compensated for by another activity, and whether or not it is required to crosslink the microtubules of the midzone overlap.

Finally, we return to CHO-1, which is a variant of MKLP-1. CHO-1 has some unique properties, which are distinct from the MKLP-1 that comprises the centralspindlin complex. First, CHO-1 has an exon, not found in other MKLP-1 variants, that mediates interactions with F-actin [86]. Second, CHO-1 appears to be required specifically to form the midbody matrix during cytokinesis [87, 88]. One possible explanation is that CHO-1 serves to connect the midzone microtubules to the cell cortex, through the actin-binding region of its tail domain [88]. The details of this interaction remain to be determined, but it is clear that MKLPs of the kinesin 6 family have multiple roles in the successful coordination of cytokinesis.

PRC-1 and KIF4 – the microtubule connection

Another kinesin that plays a central role in midzone microtubule assembly and the regulation of cytokinesis is KIF4, a member of the kinesin 4 family. KIF4 is a member of the broad class of chromokinesins [89]. The Kid/nod group, thought to mediate the oscillation and congression of sister chromatids [90], and the xklp1/Klp3A/KIF4 involved in chromosome positioning, bipolar spindle stabilization, and central spindle formation [91, 92, 93]. Recent work has shown that the function of KIF4 in midzone assembly is mediated through the evolutionarily conserved microtubule associated protein PRC-1 (*protein required for cytokinesis*: [94]). Figure 3 shows images of CHO cells labeled with antibodies against PRC-1. PRC-1 is distributed along spindle fibers prior to the metaphase/anaphase transition (see Figure 3 A), and then becomes concentrated at the central spindle region during anaphase; PRC-1 ultimately becomes concentrated at the midbody during cytokinesis (Figure 3 B-C). PRC-1 is a target of Cdk1-cyclin B kinase activity, which is thought to regulate its interaction with the spindle midzone [95]. The targeting of PRC-1 to the midzone is mediated by the motor activity of KIF4 [11]. In KIF4-depleted cells, PRC-1 cannot become concentrated at the spindle midzone, which in turn does not properly form. Instead, PRC-1 remains associated with a broad region of the central spindle, further supporting the idea that KIF4 serves to move PRC-1 to the central spindle. However, loss of KIF4 does not disrupt the interaction of PRC-1 with microtubules, even after the metaphase-anaphase transition [11]. In the absence of KIF4 – and coordinate mis-localization of PRC-1 – other components of the central spindle, such as MKLP-1 and the Aurora B/Chromosome passenger complex, also become mis-localized. Interestingly, depletion of KIF4 does not cause the half spindles to separate, as is the case for cells depleted of PRC-1 [94]. This observation suggests that in the absence of KIF4, PRC-1 can still interact with microtubules, and interconnect the two half-spindles at the midzone. However, when PRC-1 does not have its associated kinesin 4, its localization becomes broadened out from the central spindle region, and the proper microtubule overlap cannot assemble [11].

PRC-1 has also been implicated in the inhibition of MgcRacGAP – the GAP for Rho GTPase [96]. MgcRacGAP binds PRC-1 in a yeast two-hybrid assay. Also, PRC-1 inhibited MgcRacGAP activity towards Cdc42 (another small GTPase) prior to the metaphase/anaphase transition, and this inhibition was required for proper spindle assembly.

Another study suggests that PRC-1 forms a direct interaction with multiple kinesins: KIF4, MKLP-1, MKLP-2, and KIF14 [17]. In this study, KIF14's interaction with PRC-1 serves to localize citron kinase to the midzone. Citron kinase is another Rho effector kinase, that phosphorylates myosin light chain and regulates cleavage furrow formation [76]. Loss of citron kinase at the central spindle correlates with cytokinesis failure and the accumulation of binucleate cells [17]. Interestingly, depletion of KIF-14 does not disrupt the localization of other midzone components, or the midzone structure, suggesting that citron kinase may function late in cytokinesis [17]. Citron kinase has also been implicated in cytokinesis failure during early neuronal development in mice [47].

PRC-1 also appears to play an important role in the recruitment of Plk-1 to the midzone [62]. The interaction between PRC-1 and Plk-1 is mediated by Cdk-1 kinase activity. Up to the metaphase/anaphase transition, Plk-1 is bound to Cdc25 phosphatase due to phosphorylation by Cdk-1 cyclin B [62]. When cyclin B is destroyed at the metaphase/anaphase transition, Cdk-1 kinase activity drops, and Plk-1 preferentially binds its anaphase target – PRC-1 – on the midzone microtubules.

Another microtubule-associated protein that plays a role in both PRC-1 localization to the central spindle and in the organization of midzone microtubules is tektin 2 [97]. Tektins were purified from axonemal doublet microtubules in cilia and flagella [98, 99]. Tektins represent a family of elongated proteins that assemble into extended filaments, similar in structure to intermediate filaments (IFs), but tektins are distinct from IF proteins [100, 101]. Previous work revealed that one member of the sea urchin tektin family, tektin B (i.e. tektin 2, the mammalian ortholog of the sea urchin protein *tektin B*.), associated with the centrosomes, and the spindle remnant following Ca^{2+} -induced microtubule depolymerization [102, 103]. Antibodies against mouse tektin 2 decorate the centrosomes, spindle poles and midbody in mammalian cultured cells [97]. When tektin 2 was knocked down by shRNA, the cells failed cytokinesis, resulting in binucleate daughters. Examination of the midzone organization in tektin knock-down cells revealed that the microtubules themselves were disorganized. MKLP-1, and Aurora B localized to the disorganized midzone microtubules in these knock-down cells, but these central spindle components could not be concentrated to the central spindle region. When the distribution of PRC-1 was examined, its mis-localization was even more dramatic. Figure 4 shows PRC-1 localization patterns in late anaphase tektin 2 knock-down cells. What is striking about the localization of PRC-1 is that in the absence of tektin 2, it is associated with microtubules throughout the central region of the cell. Also, the microtubule organization in these cells is highly disrupted, compared wild-type. There appears to be a lack of microtubule bundling in the midzone, and there also appears to be excess microtubule polymerization (also see [97]). It remains to be determined whether or not the mislocalization of PRC-1 is due to a disruption of microtubule regulation, or a specific interaction between tektin 2 and PRC-1.

Abscission

The final stage of cytokinesis is the process of abscission [104], where the furrow seals off the connection between the two daughter cells by depositing new membrane [105, 106]. Abscission requires a functional midzone/midbody, and the recruitment of several key proteins, which are re-localized from the centrosome to the midbody (reviewed in [107, 108, 109]). These proteins include Centriolin [110, 111] and Cep 55 [112, 113], both of which require MKLP-1 for their function. Cep55 directly interacts with MKLP-1, and is required to build the midbody and to recruit SNAREs necessary for final membrane deposition during abscission [112, 113]. Centriolin builds a ring structure at the midbody that is required for targeting vesicle-targeting and vesicle-fusion complexes [111]. Depletion of MKLP-1 by siRNA abolished centriolin's localization to the midbody ring, though the mechanism for this is not clear [111]. Thus, MKLP-1 appear to play key roles both in the recruitment of midzone components necessary to position and establish the contractile ring, and in the formation of the midbody ring required for membrane deposition and the completion of cytokinesis.

Future directions

In examining the organization of the spindle midzone microtubules and their associated central spindle components it is clear that there are several key questions that remain unanswered. Most notably, the organization of the ends of the midzone microtubules. Taken

together, observational evidence suggests that the midzone microtubules detach from the spindle poles during the metaphase/anaphase transition, and the minus-ends shorten while the plus-ends elongate as the cell proceeds through anaphase. As the midzone is compacted during cytokinesis to form the midbody, these midzone microtubules then shrink to form the stable overlapping microtubules found in the midbody. These observations suggest the possibility that the midzone is under strict length control, ensuring that both the plus and minus ends grow and shrink in concert. The regulation of midzone minus ends appears particularly important in light of the severe phenotypes associated with mutations in the ASPM gene, thought to play a role in binding the midzone minus ends [44, 45]. Whether or not precise length control at the midzone microtubule plus end contributes to the formation of the central spindle also remains to be determined. It is interesting to speculate that microtubule-associated proteins, such as tektins, may play a role in midzone microtubule length control and/or altering the organization of these microtubules to allow binding of specific central spindle components, as they have been hypothesized to do in flagellar microtubules [114, 115, 116].

Acknowledgments

EHH wishes to thank Ron Vale for teaching him how to purify “real” kinesin (and calamari) during the 1992 MBL Physiology course in Woods Hole, MA. JEH is supported by a postdoctoral training grant from the National Institutes of Health (T32 CA080621). Work in the Hinchcliffe lab is supported by a research grant from the National Institutes of Health (R01 GM072754).

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Figure 1.

Assembly of the spindle midzone microtubules. Time-lapse 3-D image stacks of two mammalian cells (monkey kidney) progressing from metaphase through cytokinesis. The microtubules are labeled with GFP- α tubulin. A. Both cells are in metaphase. B. Cell 1 has undergone anaphase, and as the spindle elongates, the midzone microtubules can be visualized (arrow). C. Cell one is undergoing cytokinesis, with the cleavage furrows ingressing towards the central spindle. Cell 2 is undergoing anaphase – its midzone is just apparent. D. The midzone in cell 1 is becoming compacted by the cleavage furrow (white arrow). The apparent position of the midzone minus ends can also be seen (small black arrows). The spindle in cell 2 has elongated, revealing the midzone. E. The midzone microtubules in cell 1 have become compacted by the cleavage furrow into a characteristic midbody with Flemming body bulge (arrow). The cleavage furrow in cell 2 is beginning ingression. F. Completion of cytokinesis in both cells – the midzone microtubule arrays have each become compacted into a midbody. Spinning disk confocal fluorescence microscopy – images are maximal projections of 3-D image stacks collected over time. Time in minutes in upper right corner. Bar = 10 μ m.

Figure 2.

Anaphase and cytokinesis in a cell with a multipolar spindle. A mammalian cell (monkey kidney) with a spontaneous (non-experimentally induced) tetrapolar spindle. A. Metaphase with four distinct poles. B. Early anaphase, the bright kinetochore microtubules are retracting towards the poles, and a mass of midzone microtubules are visible. Note that the organization of the midzones connecting opposite poles is not readily apparent. C. Early cytokinesis. Three distinct cleavage furrows have formed (plain arrows), each between a pair of asters, where midzone arrays are presumed to have formed. Two other cleavage furrows have formed near the bottom right pair of asters (arrows w/asterisks). These furrows are not well defined, nor do they appear robust. D. The three major furrowed have compacted the midzone microtubules into a presumptive midbody, with a smaller furrow to the top left. The weaker furrows are beginning to regress (arrows w/asterisks). E. A single large midbody has formed (white arrow) with a small microtubule process extending to the daughter cell in the upper left (black arrow). The weak furrows at the bottom right continue to regress (arrows w/asterisks). F. The cell has completed cleavage, dividing into three unequal daughters, separated by a prominent midbody (white arrow) and midbody strand that lacks a central Flemming body (black arrow). The two cleavage furrows at the bottom right have retracted (asterisks). Spinning disk confocal fluorescence microscopy – images are maximal projections of 3-D image stacks collected over time. Time in minutes in upper right corner. Bar = 10 μ m.

Figure 3.

Localization of PRC-1 to the mitotic spindle of mammalian cells. Immunofluorescence images of CHO cells in metaphase (A-A''), anaphase (B-B''), and cytokinesis (C-C''). Cells are immunolabelled with anti- α -tubulin antibodies (A-C), anti-PRC-1 antibodies (A'-C'), and shown merged with DAPI DNA stain (A''-C''). Note in metaphase, the PRC-1 is distributed along the length of the spindle fibers. In anaphase, PRC-1 labeling is restricted to the central spindle region. During cytokinesis, PRC-1 is localized to the presumptive midbody region. Fluorescence optics, images are presented as a maximal projection of a 3-D image stack. Bar = 10 μ m.

Figure 4.

Mislocalization of PRC-1 in response to shRNA knock-down of tektin 2 in mammalian cells. Immunofluorescence images of three separate CHO cells in late anaphase – tektin 2 has been knocked down by expression of tek 2 specific shRNAs. Cells are labeled with anti- α -tubulin (A-C), anti-PRC-1 (A'-C') and merged with DAPI DNA stain (A''-C''). Note that in contrast to the wild-type cells in Figure 3, PRC-1 localization during anaphase is not restricted to the central spindle in these knock-down cells. Also, the cells lack a defined spindle midzone, and there is an extensive microtubule network throughout the cell (see panel B, arrow). For experimental details, see Durcan et al., (2008). Fluorescence optics, images are presented as a maximal projection of a 3-D image stack. Bar = 10 μ m.