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Abstract

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Interdependence of Filamentous Actin and Microtubules for Asymmetric Cell Division

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Asymmetric cell divisions are crucial to the generation of cell fate diversity. They contribute to unequal distribution of cellular factors to the daughter cells. Asymmetric divisions are characterized by a 90° rotation of the mitotic spindle. There is increasing evidence that a tight cooperation between cortical, filamentous actin and astral microtubules is indispensable for successful spindle rotation. Over the past years, the dynactin complex has emerged as a key candidate to mediate actin/microtubule interaction at the cortex. This review discusses our current understanding of how spindle rotation is accomplished by the interplay of filamentous actin and microtubules in a variety of experimental systems.

Key words: Ascidian embryos / Asymmetric cell division / Dynactin / Pelvetia / Saccharomyces cerevisiae / Xenopus.

Introduction

All multicellular organisms begin as a single cell. During development, the progeny of this parental cell differentiates into a wide variety of cell types. Symmetric divisions serve to amplify the existing cell pool of one specific lineage and asymmetric divisions serve to increase the variety of cell types. Asymmetric cell divisions can be generated when a polarized mother cell is capable of segregating determinants into one of the two daughter cells to initiate a distinct developmental pathway in this cell, but not in its sister. Therefore, during asymmetric cell divisions, the mother cell responds to an internal or external cue or polarity signal by activating a signal transduction cascade, which in turn sets up an internal polarity. The mitotic spindle senses this polarity and translates it into correct orientation (for a review see Goenzi and Hyman, 1996). This general principle of polarity establishment has been conserved from yeast to human.

In multicellular organisms, asymmetric cell divisions are characterized by the production of daughter cells of most-
Immunoelectron microscopy indicated that at least some of the actin patches represent coils of actin filaments surrounding membrane invaginations (Mulholland et al., 1994). These structures might anchor the cytoskeleton to the plasma membrane. Fusion of the green fluorescent protein (GFP) to the actin-capping protein Cap2p enabled the visualization of actin patches in living cells (Waddle et al., 1996). Actin patches are highly dynamic and move in the cortical region with a rate approaching 1 µm per second. Upon entry into the G1 phase of the cell cycle, actin patches are evenly distributed in the unbudded yeast cell (Figure 1). Appearance, disappearance, and movement of actin patches leads to a change in their distribution during the cell cycle. Prior to bud emergence, the actin patches form a discrete ring at one pole. Following bud emergence, the patches accumulate in the daughter cell until the bud reaches a certain size. At this time, the patches distribute fairly evenly over the mother and daughter cortex. Following this short period of isomorphic distribution, the patches redistribute to the bud neck prior to cytokinesis where they form single or double rings. The distribution of the actin patches to regions of active cell growth is consistent with a role in localized secretion (see below).

Actin cables have been proposed to represent bundles of F-actin (Adams and Pringle, 1984). Since the cables stain less intensely with phallotoxins than the patches, it is not entirely clear whether they undergo changes in distribution through the cell cycle similar to the patches. It is clear, however, that cables are oriented towards the actin ring that is formed prior to bud emergence and then are oriented into the newly formed bud (Kilmartin and Adams, 1984). Prior to cytokinesis, the cables seem to radiate from the actin patch rings (Ford and Pringle, 1991). A systematic analysis of the cytoskeletal components indicated that cables associate with patches with a frequency considerably higher than expected for a random organization (Karpova et al., 1998), suggesting that actin cables are anchored in actin patches. This conclusion is further supported by the notion that no yeast mutant exhibiting cables without patches was found whereas mutants lacking cables but displaying patches have been identified (Karpova et al., 1998).

Analysis of a collection of temperature sensitive mutants in the ACT1 gene has indicated that the actin cytoskeleton is involved in establishment and maintenance of cell polarity (e.g. bud site selection, membrane growth, and polarized secretion), in changes of cell shape (e.g. during mating), in nuclear positioning and cytokinesis, in endocytosis and the intracellular positioning of organelles, and in resistance to osmotic forces (Wertman et al., 1992; Kübler and Riezman, 1993; Holtzman et al., 1994).

The yeast microtubule system consists of microtubules, microtubule-associated proteins (MAPs), and the microtubule organizing center (MTOC) known as the spindle pole body (SPB). Microtubules are polymers of tubulin, which is a 1:1 heterodimer of α- and β-tubulin. All microtubules have the minus end at the SPB, which is embedded as a trilaminar structure in the nuclear envelope (Peterson and Ris, 1976). The SPB is thought to nucleate microtubules by binding the yeast β-tubulin complex. This complex comprises Tub4p, the yeast γ-tubulin, Spc98p, and Spc97p (Geissler et al., 1996; Knop and Schiebel, 1997; Knop et al., 1997; Nguyen et al., 1998). The components of the γ-tubulin complex have been shown to associate with the discs of the SPB facing both the cytoplasm and nucleoplasm (Spang et al., 1996). Electron microscopy, immunofluorescence using antibodies against α- or β-tubulin, and fusion of the tubulin genes to GFP (Byers and Goetsch, 1975; Adams and Pringle, 1984; Kilmartin and Adams, 1984; Stearns, 1995) has revealed the existence of two distinct sets of microtubules. The cytoplasmic (astral) microtubules start at the cytosolic face of the SPB and are directed towards the cytoplasm. The

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**Fig. 1**  The Cytoskeleton in the Yeast Saccharomyces cerevisiae. (A) Organization of the yeast actin cytoskeleton through the cell cycle. Adapted from Botstein et al. (1997). (B) Organization of the yeast microtubule cytoskeleton through the cell cycle. Adapted from Botstein et al. (1997).
nuclear microtubules are organized at the nucleoplasmic face of the SPB and constitute the spindle during mitosis.

The microtubule cytoskeleton undergoes dramatic rearrangements during the cell cycle (Figure 1). In unbudded yeast cells, microtubules grow from the single SPB and radiate into both the nucleus and cytoplasm. The number of astral microtubules that are visible is greater than at later stages of the cell cycle. Near the time of bud emergence, the SPB duplicates. Shortly thereafter, the new SPB nucleates microtubules. As soon as the bud has emerged, astral microtubules from the unseparated SPBs extend into the newly formed bud. SPB separation occurs at about the time of DNA replication and leads to the formation of a short spindle across the nucleus. At this stage, the astral microtubules from one SPB are retracted from the bud and are extended into the mother cytoplasm. Prior to mitosis, the nucleus migrates to a position adjacent to the bud neck with the spindle oriented at an angle of ~45° to the mother-bud axis. Astral microtubules from one SPB still extend to the cortex of the daughter cell. This stage is closest to metaphase in mammalian systems. During anaphase, the spindle is aligned along the mother-bud axis and is elongated through the bud neck to partition the chromosomes between the mother and daughter cell. Late anaphase spindles reach a length of ~10 μm, which corresponds to the axial length of a large-budded yeast cell (Byers and Goestch, 1977; Byers et al., 1978).

The analysis of cold sensitive mutants of the TUB2 gene, which encodes β-tubulin, has indicated that the astral microtubules are needed for nuclear positioning, migration of the nucleus into the bud during mitosis, and elongation of the spindle during anaphase (Huffaker et al., 1988; Jacobs et al., 1988), but they are not absolutely required for anaphase B in budding yeast (Sullivan and Huffaker, 1992). Nuclear microtubules are required for SPB separation and chromosome segregation during mitosis (Jacobs et al., 1988).

**Spindle Pole Body Duplication in Yeast**

SPB duplication in yeast begins in late G1 phase and occurs in several distinct steps (Winney and Byers, 1993). Ultrastructural analysis of conditional mutants defective in SPB duplication indicated that the duplication event begins with the formation of the so-called satellite. This structure is formed on one side of the SPB only and faces the cytosol. Mutants defective in the genes KAR1 or the homolog of mammalian centrin, CDC31 (Biggins and Rose, 1994), arrest with a monopolar spindle that terminates on a single SPB lacking any evidence of satellite formation (Rose and Fink, 1987). Both Kar1p (Biggins and Rose, 1994; Spang et al., 1995) and Cdc31p (Spang et al., 1993) have been localized to the SPB substructure involved in satellite formation. The essential function of Kar1p seems to be the localization of Cdc31p to the SPB (Biggins and Rose, 1994; Spang et al., 1995). Following satellite formation, the sequence of events are terminated with the assembly of the outer and central plaques of the nascent SPB mediated by the kinase Mps1p (Winney et al., 1991; Lauzé et al., 1995) and insertion of the new SPB into the nuclear envelope through the action of Mps2p, Ndc1p, and Bbp1p (Winney et al., 1991; Schramm et al., 2000).

A recent study reveals that Cdc31p interacts with Kic1p, a kinase of the PAK kinase family, to the SPB (Sullivan et al., 1998). The authors demonstrated that interaction of Kic1p with Cdc31p is essential for its kinase activity. Both kic1 and certain cdc31 mutants display defects in the polarity of the actin cytoskeleton (Sullivan et al., 1998). This opens the interesting possibility that Cdc31p regulates both duplication of the SPB and polarization of the actin cytoskeleton in G1 via the kinase Kic1p, although kic1 mutants have no evident defects in spindle pole bodies. Such a dual regulatory function for Cdc31p could coordinate two events that occur roughly at the same time of the cell cycle.

**Mechanisms of Spindle Positioning in Yeast**

Experiments using different β-tubulin mutants revealed that astral microtubules play a key role in the positioning of the nucleus at the bud neck (Huffaker et al., 1988). Mutants that specifically lack astral microtubules, but have nuclear microtubules, were found to be defective for migration of the nucleus, whereas mutants lacking nuclear microtubules but having astral ones did undergo nuclear migration. This finding led to the hypothesis that astral microtubules are anchored to actin-containing structures in the cell cortex. It was proposed that anchoring of microtubules to actin structures may provide a force-generating system for nuclear positioning. However, a careful analysis of a yeast mutant lacking detectable actin cables indicated that nuclear positioning may depend on the presence of actin cables rather than actin patches (Theesfeld et al., 1999). Recent findings question a strict actin-dependence of nuclear positioning. Theesfeld and colleagues (Theesfeld et al., 1999) determined that nuclear positioning and orientation can occur in cells with a depolymerized actin cytoskeleton if the event is delayed artificially until late in the cell cycle. This suggests that it is the time in the cell cycle, and not the event, that determines actin-dependence.

Later, two possible linker candidates between astral microtubules and F-actin in the cortex were identified. Mutations in both the dynactin complex (Li et al., 1993; McMullan and Tatchell, 1994; Muhua et al., 1994; Clark and Meyer, 1994) and in two proteins, Num1p (Farkasovsky and Küntzel, 1995) and Kar9p (Miller and Rose, 1998), were found to result in defects in spindle positioning and orientation. Dynactin (dynein activator) was originally identified as a complex that stimulates dynein-based organelle motility along microtubules (Gill et al., 1991). Knockout of components of the dynactin complex in yeast, however, suggested an involvement of the complex in transducing cell po-
larity into the corresponding spindle positioning as well as orientation. Strains lacking yeast dynein, Dyn1p, show severe defects in positioning the nucleus at the bud neck and in orienting the mitotic spindle along the mother-bud axis (Li et al., 1993) causing mitosis to take place in the mother cell. It is interesting to note that the mutant does have astral microtubules that extend into the bud (Li et al., 1993). The dynein mutant obviously senses polarity but cannot translate it into correct spindle orientation. Elimination of other proteins, which are now known to be part of the dynactin complex, resulted in identical phenotypes. Deletion of either the actin-related protein Arp1 (Muhua et al., 1994; Clark and Meyer, 1994) or J nm1p (McMillan and Tatchell, 1994) led to a failure to properly position and orient the mitotic spindle. As for dyn1Δ mutants, astral microtubules are abundant and extend into the bud curving along the cortex (McMillan and Tatchell, 1994). The phenotypes described for the mutants are consistent with dynactin mediating an interaction between astral microtubules and the cell cortex. Localization of dynein (Yeh et al., 1995) and J nm1p (McMillan and Tatchell, 1994) to punctuate structures in the bud cortex, to the SPB facing the bud, and to astral microtubules further emphasizes the importance of this complex in spindle positioning and orientation (Figure 2). Time-lapse microscopy observing pre-anaphase spindle migration provided additional support. Astral microtubules in dyn1Δ mutants exhibited less sweeping of the cortex and failed to undergo end-on interactions with the cortex as observed in wild-type cells (Carminati and Stearns, 1997). Except for the fact that an actin-related protein and traces of actin are part of the dynactin complex, evidence is lacking that proves an interaction of the complex with actin-dependent structures in the cell cortex.

Num1p and Kar9p represent another set of proteins that mediate interactions of the astral microtubules with the cell cortex (Figure 2). Cells deficient in Num1p often fail to align the spindle along the mother-bud axis and undergo nuclear division in the mother cell. As for mutants in the dynactin complex, the astral microtubules are abundant and extend into the bud (Farkasovsky and Küntzel, 1995). It is striking that Num1p localizes to the mother cell cortex during S, G2, and M phase (Figure 2). Deletion of KAR9 results in a failure to position the nucleus at the bud neck and to orient the spindle properly (Miller and Rose, 1998) (Figure 2). Deletion of KAR9 resulted in a failure to position the nucleus at the bud neck and to orient the spindle properly (Miller and Rose, 1998). In contrast to the other mutants described, the astral microtubules did not extend into the bud. This was expected for deletion of a protein that anchors astral microtubules to the bud cortex. Kar9p is apparently linked to microtubules through Bim1p, a conserved microtubule binding protein found at microtubule distal ends (Korinek et al., 2000; Lee et al., 2000). Bim1p is required for movement of the nucleus into the mother-bud neck region (Adames and Cooper, 2000).

Kar9p is the only linker protein for which an actin-dependency has been demonstrated. It was found that actin, but not microtubules, are essential for localizing Kar9p to the bud tip (Miller et al., 1999). In addition, the polarity proteins Bud6p and Bni1p, a yeast homolog of the mammalian formins, are required for proper localization of Kar9p (Lee et al., 1999; Miller et al., 1999). Mislocalization of Kar9p in bud6Δ and bni1Δ mutants results in defects in spindle positioning and orientation as described for kar9Δ. Since bud6Δ, bni1Δ and kar9Δ mutants exhibit astral microtubules that do not extend into the bud and mutants in the dynactin complex have exaggerated astral microtubules extending into the bud, the hypothesis arose that the two linker systems described might fulfill overlapping, but distinct functions. Genetic analyses showed that kar9Δ is viable in combination with bud6Δ and bni1Δ, but not in combination with dyn1Δ or arp1Δ, components of the dynactin complex (Miller and Rose, 1998; Miller et al., 1999). It is therefore currently believed that an actin-dependent complex of Bud6p, Bni1p, and Kar9p captures astral microtubules at the bud cortex whereas the dynactin complex provides the force-generating system acting on the captured microtubules, pulling them toward the cortical site defined by the Kar9p complex.

Direct involvement of formins of other species in spindle positioning and orientation has not been demonstrated (for a review, see Wasserman, 1998). However, the mouse formin limb deformity is involved in establishing embryonal pattern formation (Maas et al., 1990). The Drosophila melanogaster formin cappuccino is involved in oocyte

**Fig. 2** Mechanisms of Nuclear Positioning in Saccharomyces cerevisiae.

The dynactin complex, Kar9p, and Num1p function in moving the nucleus towards the bud neck and in elongation of the mitotic spindle through the bud neck during mitosis. Yeast cells during late G1/S phase and late anaphase are shown.
polarity (Emmons et al., 1995) and the Schizosaccharomyces pombe formin Fus3 in polarizing the actin cytoskeleton during conjugation (Petersen et al., 1998). Formins of other species therefore participate, as in S. cerevisiae, in the establishment of cell polarity. It remains to be elucidated whether they participate directly in spindle orientation in response to polarity signals as in the budding yeast.

### Involvement of the Dynactin Complex in Asymmetric Cell Division

In the next section, the involvement of the dynactin complex in spindle positioning during asymmetric cell division in higher eukaryotes will be discussed. The dynactin complex was isolated originally from chicken embryo brain due to its stimulation of dynein-based organelle motility along microtubules (Schafer et al., 1991). It was characterized biochemically and found to be composed of at least eight subunits (Gill et al., 1991). The most abundant protein of the complex is the actin-related protein Arp1. Trace amounts of F-actin are also found in the complex (Lees-Miller et al., 1991). The homolog of Drosophila Glued, p150GLUED (Gill et al., 1991), and the α- and β-subunits of the actin capping protein have been identified as additional components (Schafer et al., 1994). The complex can be visualized as a short filament of Arp1 plus a thinner, laterally oriented filament composed of p150GLUED that terminates in two globular heads (Schafer et al., 1994). The Arp1 filament is capped by the capping protein (Schafer et al., 1994). The p150GLUED filament connects the complex to microtubules (Waterman-Storer et al., 1995) and to the intermediate chain of dynein (Vaughan and Vale, 1995). The complex bound to dynein therefore has the potential to transport cargo along microtubules. Alternatively, assuming that the dynactin complex bound to dynein is anchored firmly at the cortex, it can exert a pulling force on microtubules.

In Drosophila, a large cytoplasmic structure called the fusome connects the individual cytotyes in the 2 to 16-cell cyst during oogenesis (Figure 3A). The fusome associates with one pole of each of the mitotic spindles during the cytocyte divisions. Spectrin, adducin, and ankyrin are components of the fusome. It was observed that deletion of adducin, which abolishes fusome structures, results in a random orientation of the mitotic spindles to each other during the divisions (Deng and Lin, 1997). Anchoring of the spindles to the fusome may therefore ensure proper alignment of the spindles to each other and define the asymmetry of the division. Another study showed that dynein localizes to the fusome during mitosis (McGrail and Hays, 1997) (Figure 3A). Deletion of the dynein heavy chain resulted in a similar misalignment of the mitotic spindles during oogenesis as observed for deletion of adducin (McGrail and Hays, 1997). It is striking that the fusome was still detectable at positions comparable to wild-type cysts. The spindle misalignment therefore suggested that lack of dynein disturbs anchoring of the mitotic spindles to the fusome and consequently asymmetric division. Interestingly, a component of the dynactin complex, Glued, is mislocalized in a dynein heavy chain mutant (McGrail et al., 1995). It is likely that dynein associated with the dynactin complex is anchored to the fusome via its short filament of Arp1 in a manner analogous to F-actin anchoring to erythrocyte membranes (for a review, see Bretscher, 1991). Dynein bound to the dynactin-fusome complex could capture the microtubules from one aster of the mitotic spindles. Due to its minus-end directed motor activity, dynein would pull on the spindles and align them with respect to the fusome.

Madine-Darby canine kidney (MDCK) cells are polarized epithelial cells. The apical surface, rich in microvilli, faces the outside whereas the basolateral membrane is in contact with other cells of the epithelial layer. During prometaphase, the short spindle is aligned parallel to the apical-basolateral axis. During metaphase, the spindle...
orient perpendicular to this axis, which is achieved by rotating the short, early mitotic spindle (Reinsch and Karsenti, 1994) (Figure 3B). It has recently been demonstrated that during prometaphase both dynein and dynactin localize to discrete spots on the lateral membrane just below the apical microvilli (Busson et al., 1998; Figure 3B). Some astral microtubules were seen to dock at these spots. During metaphase, the number and intensity of the dynactin/dynein spots on the lateral membrane increase as do the number of astral microtubules terminating on them. Strikingly, the dynactin caps appear prior to the beginning of spindle rotation. In addition, treatment of MDCK cells with the actin depolymerizing drug cytochalasin D inhibited assembly of the dynactin caps and interaction of the astral microtubules with the lateral cortex and prevented spindle rotation (Busson et al., 1998). In MDCK cells therefore, dynein/dynactin localizes in an F-actin dependent manner to the lateral cortex. Astral microtubules are captured by the dynactin complex on both lateral membranes (Figure 3B). Dynein probably exerts a pulling force on the astral microtubules due to its motor activity, which results in alignment of the spindle perpendicular to the polarity axis.

In the nematode Caenorhabditis elegans, the two-cell embryo undergoes defined division patterns. In the anterior or AB cell, spindle alignment occurs transverse to the anterior-posterior axis resulting in a division orthogonal to the previous one. This default pattern is observed for divisions that produce daughter cells of equal size and developmental fate. The posterior P1 cell of the two-cell embryo aligns the centrosome axis parallel to the anterior-posterior axis (Hyman and White, 1987) (Figure 3C). This results in division off the default pattern and cleavage along the axis of the previous division. This second pattern of division results in daughter cells of unequal size and developmental potential. Analysis of centrosome movement in the two-cell embryo indicated that the centrosome axis is established first orthogonal to the anterior-posterior axis in both the AB and P1 cell. This centrosomal position is maintained in the AB cell. In the P1 cell, however, the whole nucleus/centrosome complex rotates 90° resulting in alignment of the centrosome axis along the anterior-posterior axis of the embryo (Hyman and White, 1987) (Figure 3C). Time-lapse experiments indicated that, prior to rotation, the nucleus as a whole migrates towards the anterior cortex of the P1 cell (Hyman and White, 1987). This suggests that equal forces act on both centrosomes. It is only after migration that the microtubules of one centrosome prevail and induce rotation (Hyman and White, 1987). Rotation of the nucleus in the P1 cell was abolished when either the microtubule cytoskeleton was depolymerized with nocodazole or the actin cytoskeleton with cytochalasin D (Hyman and White, 1987). Laser irradiation of the cytoplasm between the centrosome rotating towards the anterior cortex and the cortex prevented rotation as well (Hyman, 1989). This provided the first evidence that an interaction of the astral microtubules with an actin-containing structure in the anterior cortex of the P1 cell may literally pull the centrosome axis into the proper orientation. The finding that both actin and capping protein localize to the anterior cortex of P1, but not AB cells (Waddle et al., 1984) supports this hypothesis. The discovery that both actin and capping protein are part of the dynactin complex (see above) presented a possible solution as to the mechanism. Shortly thereafter, it was demonstrated that p150GLUED, a well-defined component of the dynactin complex, accumulated at the anterior cortex of P1 cells in a pattern similar to actin and capping protein (Skop and White, 1998). Depletion of p150GLUED led to the disappearance of the dynactin cap and misalignment of the mitotic spindles in P1 cells (Skop and White, 1998). Depletion of dynein heavy chain resulted in an identical phenotype (Goenczy et al., 1999). Therefore, it seems that in the P1 cell the astral microtubules from one centrosome contact the actin-dynactin cap in the anterior cortex. The microtubules are captured and pulled towards this site by the action of dynein. This results in rotation of the nucleus (Figure 3C). A similar mechanism seems to operate in the one-cell embryo during the first asymmetric division when the spindle is rotated by 90° to orient along the anterior-posterior axis (Albertson, 1984). Depletion of either dynein heavy chain or the dynactin component p150GLUED by RNA-mediated interference resulted in a failure to rotate the mitotic spindle. Rotation of the spindle in both studies was only observed late in anaphase probably because of the physical constraints of the egg shell (Skop and White, 1998; Goenczy et al., 1999). Additional protein factors involved in spindle rotation during the first and second asymmetric divisions are beginning to emerge (Lorson et al., 2000). However, it remains to be elucidated whether they interact functionally with the dynactin complex.

In summary, there is good evidence that the dynein/dynactin complex anchored to specific sites of the cortex in an F-actin-dependent manner is a key player in the rotation of mitotic spindles during asymmetric cell divisions. The mechanism of dynein exerting a pulling force on captured astral microtubules seems to be conserved from yeast to human.

**Involvement of Cortical, Actin-Dependent Structures in Asymmetric Cell Division**

Below we will discuss examples of asymmetric cell divisions for which there is evidence for an interplay of cortical actin and astral microtubules. In some cases, a dynein-independent mechanism is possible, for example neuroblast division in the central nervous system of Drosophila and unequal cleavage at the posterior pole of ascidian embryos. For other examples, such as Drosophila syncytial divisions, spindle rotation during meiosis in Xenopus oogenesis, and spindle rotation in the Pelvetia embryo, there is no mechanistic insight.

During development of the central nervous system (CNS) in Drosophila, a neuroblast divides unequally to produce a large apical daughter cell and a smaller basal gan-
Involvement of Cortical, Actin-Dependent Structures during Drosophila Development

(A) During neuroblast divisions in the central nervous system, the protein inscuteable localizes to an apical crescent in the neuroblast cell (black crescent). The apical crescent disappears following incomplete cytokinesis.

(B) Nuclear spacing in the syncytium depends on a tight interplay between astral microtubules and cortical actin. An example of a syncytium is shown. The lower part, focusing on two nuclei of the syncytium, represents the induction of actin caps (grey crescents) by astral microtubules at the cell cortex.

(C) During mitosis in the syncytium, the astral microtubules induce the formation of a pseudocleavage furrow (dark grey bars) that acts as a barrier between neighbor nuclei and orients the mitotic spindle parallel to the cell surface. Following mitosis, the actin caps (grey crescents) are reformed.
formed where centrosomes from neighboring nuclei are not juxtaposed. Microtubules could therefore signal the rearrangement of actin into pseudocleavage furrows. To obtain the even spacing of the interphase nuclei in the syncytium, microtubules and cortical actin could cooperate to separate neighboring nuclei during mitosis. However, no protein has yet been identified that could link the two cytoskeletal components.

During meiosis I in Xenopus oocyte maturation, the spindle at the animal pole is first aligned orthogonally to the animal-vegetal axis (Figure 5A). A rotation of 90° then ensures its correct alignment along the axis (Gard, 1992; Figure 5A). Experimental displacement of the meiotic spindle revealed that, even though it can form at any position within the oocyte, it can only be anchored for rotation at a specific cortical site at the animal pole (Gard, 1993). F-actin was found to be concentrated at the site of attachment of the meiotic spindle (Gard et al., 1995) (Figure 5A). Additionally, treatment of the oocytes with the actin depolymerizing drug cytochalasin B during maturation prevented anchoring and rotation of the meiotic spindle (Gard et al., 1995). These experiments suggest that spindle rotation is mediated by interactions between astral microtubules and cortical F-actin.

Sea urchin embryos undergo three rounds of unequal cleavage at the posterior-vegetal pole (Conklin, 1905; Satoh, 1979). This results in the production of small posterior-most blastomeres with a distinct developmental potential. A unique structure, called the centrosome-attracting body (CAB), was found at the posterior cortex of the posterior-most blastomere pair (Hibino et al., 1998; Figure 5B). It was observed that a thick bundle of microtubules appeared between the posterior centrosome and the CAB (Hibino et al., 1998), which shortened as the nucleus was drawn towards the CAB (Figure 5B). Depolymerization of the microtubules with nocodazole prevented formation of the thick microtubule bundle and posterior migration of the nucleus (Nishikata et al., 1999). Depolymerization of the actin cytoskeleton with cytochalasin B prevented maintenance of the CAB and abolished nuclear migration towards the posterior cortex (Nishikata et al., 1999). Interestingly, kinesin – or a kinesin-like protein – was found to localize to the CAB in an actin-dependent, but microtubule-independent, manner (Nishikata et al., 1999). In analogy to the dynactin complex pulling on astral microtubules due to its motor activity (see above), kinesin anchored to the actin-dependent CAB may exert a pulling force on captured astral microtubules by inducing depolymerization from the plus end.

The fertilized egg of the brown alga Pelvetia undergoes an unequal first division producing the big thallus cell and the smaller, ellipsoid-shaped rhizoid (for a review see Kropf, 1994) (Figure 5C). Following fertilization, the centrosomes align first transverse to the thallus-rhizoid axis (Figure 5C). Subsequently, the nucleus rotates by 90° resulting in alignment of the centrosomes along the polarity axis (Figure 5C). Prior to the rotation, microtubule asters from both centrosomes are seen to contact the actin cap at the rhizoid pole (Kropf, 1992). Treatment with cytochalasin D prior to and during rotation prevents nuclear rotation and centrosome alignment along the thallus-rhizoid axis (Allen and Kropf, 1992). Depolymerization of the microtubules also abolishes spindle rotation (Allen and Kropf, 1992). The data favor a model in which the interaction of the astral microtubules from one centrosome with the rhizoid actin cap accomplishes nuclear rotation.

**Conclusions and Perspectives**

It has been known for a long time that both cortical actin and astral microtubules participate in the process of nuclear rotation and spindle alignment in response to internal...
or external polarity signals. Over the past years, studies with genetically tractable systems such as the yeast Saccharomyces cerevisiae, the fly Drosophila melanogaster, the nematode Caenorhabditis elegans, or an easily manipulable system, the frog Xenopus laevis, have increased our understanding of how cortical actin and microtubules cooperate to achieve the astonishingly precise process of asymmetric spindle alignment and positioning. The microtubule-dependent motor protein dynein and the dynein activator complex dynactin seem to play a crucial role in tethering astral microtubules to the cell cortex. It is currently not understood how the dynactin/dynein complex is anchored to the cortex, but this process is likely to involve filamentous actin that associates with the complex and that has been shown to be essential for cortical localization of the dynactin complex in MDCK cells. The general view is that the dynein/dynactin complex captures astral microtubules. The inherent minus-end directed motor activity of dynein results in migration of the complex towards the microtubule minus-end at the MTOC. The resulting shortening of astral microtubules produces a force that pulls the nucleus towards the cortical site of dynein attachment. Interestingly, kinesin has been identified as a component of the cortical attachment site in ascidian embryos. Kinesin is a plus-end directed microtubule motor. However, it is likely that it acts to depolymerize the plus ends of captured microtubules rather than functioning as a motor protein. A similar situation is thought to occur at the kinetochores during anaphase A.

Studies in Saccharomyces cerevisiae have revealed the existence of another, functionally redundant system besides the dynactin complex. It will therefore be interesting to search for homologues of the second yeast system in other organisms and to analyze a potential involvement in nuclear positioning and orientation. Given the surprising conservation of the mechanisms used to achieve nuclear positioning from yeast to mammals, it seems likely that additional, redundant mechanisms will be identified in higher eukaryotes as well.

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