Journal of Cell Science 111, 2283-2295 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JCS5007

COMMENTARY

Mechanisms of nuclear positioning

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Published on WWW 30 July 1998

SUMMARY

The mechanisms underlying two types of microtubule-dependent nuclear positioning are discussed. 'MTOC-dependent nuclear positioning' occurs when a nucleus is tightly associated with a microtubule organizing center (MTOC). 'Nuclear tracking along microtubules' is analogous to the motor-driven motility of other organelles and occurs when the nucleus lacks an associated MTOC. These two basic types of microtubule-dependent nuclear positioning may cooperate in many proliferating animal cells to achieve proper nuclear positioning. Microtubule polymerization and dynamics, motor proteins, MAPs and specialized sites such as cortical anchors function to control nuclear movements within cells.

Key words: Nucleus, Microtubule, Dynein, MTOC, Centrosome-nucleus connection

TWO TYPES OF MICROTUBULE-DEPENDENT NUCLEAR POSITIONING

When we represent a eukaryotic cell with a centrally located nucleus we do not always appreciate that this central position depends on active mechanisms which move nuclei within the cytoplasm and maintain them in the correct cellular location. Nuclear positioning can be crucial for proper cellular behavior. For instance, in *Saccharomyces cerevisiae*, nuclear migration to the bud neck is required for correct segregation of genetic material to daughter cells (reviewed by Stearns, 1997). In the *Drosophila* syncytial embryo, migration of nuclei to the cortex is a prerequisite for their inclusion in newly forming cells (Foe and Alberts, 1983). In many species, following fertilization, movements of the male and female pronuclei towards each other are essential for zygote formation (Wilson, 1928).

Nuclear positioning in many cells is a microtubule-dependent process. While some cases of nuclear positioning involve the actin cytoskeleton in addition (Lloyd et al., 1987; Swope and Kropf, 1993; Meindl et al., 1994; Grolig, 1998) or exclusively (von Dassow and Schubiger, 1994; Guild et al., 1997; Maniotis et al., 1997), the focus of this review will be on mechanisms of microtubule-dependent nuclear positioning.

There are two basic types of microtubule-dependent nuclear positioning. The predominant type occurs when the nucleus is tightly associated with a microtubule organizing center (MTOC) such as a centrosome or spindle pole body (SPB). In these instances the position of the nucleus follows that of the MTOC. We term this first type 'MTOC-dependent nuclear positioning'. A second type occurs when the nucleus is not associated with a MTOC. In this instance nuclear positioning is analogous to the motility of other cellular organelles along microtubules. We term this second type 'nuclear tracking along microtubules'.

The two basic types of nuclear positioning are illustrated by the migrations of the male and female pronuclei in the newly fertilized egg of many species (Fig. 1). The movement of the male pronucleus corresponds to the first type of nuclear positioning. The male pronucleus is tightly associated with the centrosome, which nucleates microtubules to form the sperm aster. The growth of the sperm aster drives the centrosome and associated male pronucleus from the cell cortex towards the center of the egg. The movement of the female pronucleus represents the second type of nuclear positioning. In contrast to the male pronucleus, the female pronucleus has no associated centrosome nor microtubule-nucleating activity. Nevertheless, the female pronucleus moves along microtubules from the cell cortex towards the centrosome located in the center of the sperm aster.

In this review, we consider the mechanisms underlying the two basic types of microtubule-dependent nuclear positioning in turn. We then postulate that these two types may, in fact, cooperate in many cells to achieve proper nuclear positioning. Throughout this review we focus on nuclear positioning in embryos of higher eukaryotes, while mentioning other systems when appropriate.

MTOC-DEPENDENT NUCLEAR POSITIONING

MTOCs nucleate polar arrays of microtubules with the minus-ends tethered at the MTOC and the plus-ends extending away from it (reviewed by Kellogg et al., 1994). MTOCs are associated with nuclei in many cells. In fungi, the MTOC is the SPB which is embedded in the nuclear membrane

(reviewed by Snyder, 1994). Multinucleate myotubes (Tassin et al., 1985) and plant cells (reviewed by Baluska et al., 1997) have perinuclear MTOCs; microtubules emanate from the periphery of the nuclear membrane. In proliferating animal cells, the centriole-containing centrosome is the principal MTOC (reviewed by Kellogg et al., 1994), and is generally closely associated with the nuclear membrane.

The separation of nuclei and centrosomes in some instances reveals the fundamental role of the MTOC in directing nuclear positioning; centrosomes are able to position appropriately within cells even if they lose their association with the nucleus or if the nucleus is altogether absent. In the *Drosophila* syncytial embryo, the nuclei and associated centrosomes normally undergo seven cycles of division within the interior of the egg and then migrate out to the cortex (Foe and Alberts, 1983). Treatment with the DNA polymerase inhibitor aphidicolin leads to the cessation of nuclear divisions, while centrosomes continue to duplicate. As a result, there are many free centrosomes which migrate to the cortex at the appropriate time (Raff and Glover, 1988). Similarly, Boveri (1888) described cases of fertilized sea urchin eggs in which the sperm nucleus lost its association with the centrosome; the nucleus remained near the cortex, while the aster nonetheless centered in the egg. In amphibians, injection of purified centrosomes or basal bodies into unfertilized eggs can cause parthenogenetic development (Heidemann and Kirschner, 1975; Manes and Barbieri, 1977; Klotz et al., 1990). In this case, the centrosome assembles a microtubule aster that correctly moves to the center of the egg in the absence of the sperm nucleus. The above examples demonstrate that appropriate centrosome positioning is independent of the presence of an associated nucleus and suggest that it is the position of the centrosome or other associated MTOC that normally determines that of the nucleus.

MTOCs are often positioned in the geometric center of a cell. For instance, when fibroblast or epithelial cells migrate into an experimental wound, the centrosome remains in the vicinity of the constantly shifting geometric center (Euteneuer and Schliwa, 1992). What are the mechanisms which enable centrosomes and other MTOCs to be positioned at the cell center? Many experiments have demonstrated the requirement for an intact microtubule cytoskeleton. For instance, treatment of sea urchin embryos with the microtubule-depolymerizing agents nocodazole or colchicine prevents movement of the sperm aster from the cortex to the center of the egg (Schatten and Schatten, 1981). In the two-cell stage *C. elegans* embryo, nocodazole treatment leads the normally centrally located nucleus and associated centrosomes of the anterior blastomere to assume a cortical position (Hyman and White, 1987). Such experiments demonstrate that microtubules are required for movement of the centrosome and its associated nucleus to the center of cells as well as for their maintenance at that position.

In the following sections we first discuss two types of forces which may enable MTOCs and associated nuclei to move to the cell center. We then discuss how cortical anchors participate in enabling MTOCs to depart from their central position in particular circumstances. Some aspects of MTOC-dependent nuclear positioning which are not discussed in the main text are presented in the accompanying Boxes.

FORCES RESULTING FROM MICROTUBULE POLYMERIZATION

A simple model for explaining how MTOCs can be positioned in the cell center invokes forces resulting from microtubule polymerization. There is substantial evidence that microtubule polymerization can generate pushing forces against movable objects such as chromosomes or membranes (reviewed by

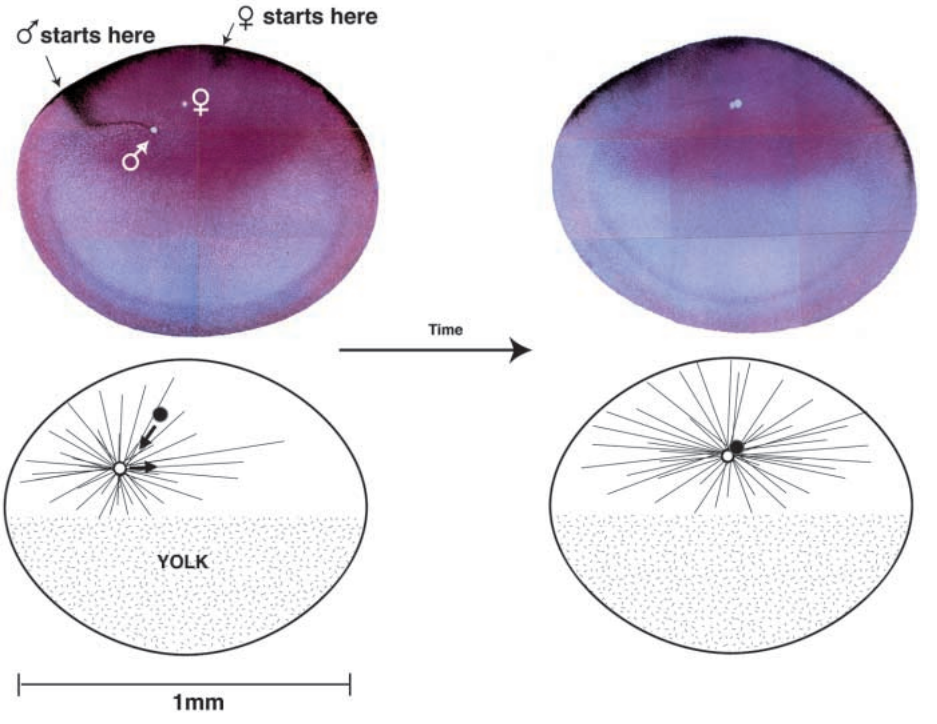

Fig. 1. Movements of male and female pronuclei in the newly fertilized *Xenopus* egg. The positions of the male and female pronuclei are depicted 30 minutes (left panels) and 40 minutes (right panels) after fertilization. Sections of *Xenopus* eggs stained with Azure B are shown on top, and corresponding schematic representations below. Thin black lines: microtubules; white and black disks: male and female pronuclei respectively. The male pronucleus is associated with the centrosome, which nucleates microtubules to form the sperm aster. The growth of the sperm aster drives the male pronucleus from the cell cortex towards the center of the egg. Organelle-like motility propels the female pronucleus along microtubules from the cell cortex towards the centrosome located in the center of the sperm aster. After pronuclear meeting, the aster and the associated male and female pronuclei continue to migrate until the center of the egg is reached. (Histological sections reprinted with permission from Hausen and Riebessell, 1991.)

MTOC-mediated nuclear positioning in multinucleate cells

In some multinucleate cells, astral microtubules from adjacent MTOCs overlap and generate anti-parallel microtubule arrays. The action of microtubule motors on these arrays has been proposed to either bring MTOCs and their associated nuclei together as during karyogamy in *S. cerevisiae* or apart as in *Drosophila* syncytial embryos. During mating in *S. cerevisiae*, the kinesin motor Kar3p is required to bring the two nuclei together prior to nuclear fusion (reviewed by Rose, 1996). In vitro, Kar3p has been shown to have minus-end directed motor activity and to depolymerize microtubules at their minus-ends (Endow et al., 1994). Kar3p has been proposed to cross-bridge anti-parallel microtubules emanating from the two SPBs and to move towards their minus-ends. After reaching the SPB, Kar3p is postulated to bring the nuclei together by shortening microtubules at their minus-ends. Therefore, microtubule-microtubule interactions may act here in concert with controlled microtubule depolymerization to generate movement of the two SPBs and associated nuclei towards each other. In *Drosophila* syncytial embryos, centrosomes and their associated nuclei migrate from the center of the embryo to the cortex at the end of division cycle 7 (Foe and Alberts, 1983). Short microtubules extend from the moving centrosomes toward the cortex, while longer microtubules extend behind them (Baker et al., 1993). These trailing microtubules appear to make anti-parallel arrays with microtubules emanating from centrosomes on the opposite side of the embryo. Here centrosomes and associated nuclei are proposed to be pushed apart by the action of a plus-end directed motor acting on these anti-parallel arrays.

Inoue and Salmon, 1995). However, when a growing microtubule abuts a 'fixed' object, addition of tubulin dimers at the plus-end will displace the microtubule away from the object. The MTOC tethered at the microtubule minus-end will likewise be displaced away from the object (Fig. 2a). In cells, the MTOC could be positioned by the combination of polymerization forces generated by all of the associated astral microtubules against 'fixed' objects at their plus ends. For instance, polymerizing microtubules of the sperm aster may push against the cortex of the egg, the 'fixed' object in this instance. This would result in the migration of the centrosome away from the cortex and towards an equilibrium position in the cell center where polymerization forces would be balanced. Indeed, microtubule polymerization forces exerted against the walls of a small chamber can center an aster composed of pure tubulin (Holy et al., 1997). Polymerization forces appear to be similarly involved in positioning the SPB in *S. cerevisiae* interphase cells. This was revealed by observing the behavior of microtubules and SPB in living cells: when growing astral microtubules abutted the cell cortex, the SPB and associated nucleus moved away from the cortex (Shaw et al., 1997).

Could polymerization forces similarly center an aster in large cells such as newly fertilized eggs? This has been proposed for sea urchin and sand dollar eggs (80-150 μm diameter) (Hamaguchi and Hiramoto, 1980; Schatten, 1982), where microtubules extend all the way to the cortex and movement of the sperm nucleus correlates with growth of the aster (Chambers, 1939; Allen, 1954; Schatten, 1982). In the still larger *Xenopus* egg (1 mm diameter), aster assembly has been proposed to drive nuclear movement (Subtelny and Bradt, 1963; Manes and Barbieri, 1977). Although microtubules of the sperm aster have not been visualized in live *Xenopus* eggs, studies on fixed specimens are compatible with microtubule polymerization driving movement of the sperm aster here as well. Below we consider the feasibility of this mechanism in *Xenopus* eggs since their large size pushes the model of polymerization driven movement to its limits. In addition, several physical parameters important for calculations of force generation have been determined in *Xenopus*.

Two conditions must be met in order for polymerization forces to account for aster centering. First, the rate of microtubule polymerization must equal or exceed that of aster movement towards the cell center and second, the microtubules which push against the cortex must generate sufficient force to work against the drag acting on the aster. In *Xenopus*, the first condition appears to be met since microtubules in interphase *Xenopus* egg extracts grow at a rate slightly exceeding the measured speed of the male pronucleus and associated centrosome (16 μm/minute) (Stewart-Savage and Grey, 1982; Tournebize et al., 1997). To evaluate whether the second condition is also met, we will first estimate the drag acting on the aster and then the number of microtubules that would need

What multinucleate fungi can tell us about human disease

Nuclei are regularly spaced in the syncytial hyphae of fungi (Oakley and Morris, 1980). Proper spacing of nuclei requires cytoplasmic dynein and components of the dynactin complex as well as several other proteins (reviewed by Xiang et al., 1995a; Holleran et al., 1998). One of these is NudF, which encodes an evolutionarily conserved protein containing β-transducin repeats (Xiang et al., 1995b) which is proposed to interact directly with both tubulin and dynein (Willins et al., 1997). Mutations in LIS1, the human homologue of NudF, result in defects in neuronal migrations during development of the cerebral cortex (Reiner et al., 1993). Such neuronal migrations are believed to occur by a process termed 'perikaryal translocation' during which the nucleus of the migrating neuron moves through an elongated neuronal process (Book et al., 1991; Liesi, 1992; Hager et al., 1995). LIS1 binds to microtubules and affects microtubule dynamics by reducing the incidence of depolymerization events (Sapir et al., 1997). This means that LIS1 would normally act to make microtubules longer and possibly more stable. While the story may be complicated in mammals by the fact that LIS1 also functions as a subunit of brain platelet-activating factor (Hattori et al., 1994), it is tempting to speculate that LIS1 may act both during nuclear spacing and neuronal migration by regulating microtubule dynamics. Such potential mechanistic links underscore the fact that studying nuclear positioning in simple genetically tractable organisms may help us unravel the causes of some human diseases.

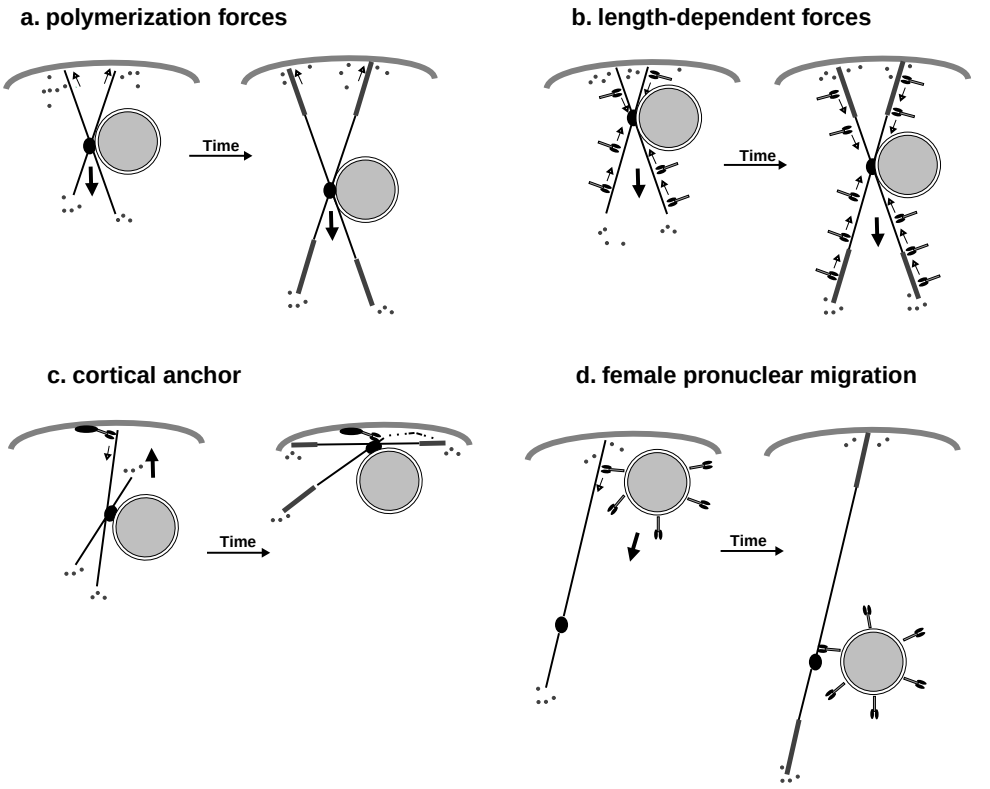

Fig. 2. Force-generation mechanisms and nuclear positioning. Four different mechanisms that may be involved in nuclear positioning are depicted. For each mechanism, two panels are presented to illustrate the consequence of force generation over time. Thick shaded line: cell cortex; small black oval: centrosome; large shaded double circle: nucleus; thin black lines: microtubules; shaded dots: tubulin dimers, shaded lines: newly polymerized segments of microtubules. Minus-end directed microtubule motors are depicted with two small rod-like tail domains and two globular head domains. Small arrows: direction of force generation; large black arrow: direction of nuclear movement. (a) Microtubule polymerization forces. The plus-ends of growing microtubules abut the cell cortex. Addition of tubulin dimers displaces the microtubules and hence the centrosome and associated nucleus away from the cortex. (b) Length dependent forces. Minus-end directed motor proteins anchored and evenly distributed in the cytoplasm generate force as a function of microtubule length since longer microtubules contact more motors than do shorter ones. An aster with the accompanying nucleus moves in the direction of the longest microtubules away from the cortex. (c) Cortical anchor. Cortical anchors (black oval) are proposed to anchor minus-end directed motors during asymmetric centrosome positioning. Anchored motors capture astral microtubules and move towards their minus-end, thereby bringing the centrosome and nucleus towards the cortical site. (d) Female pronuclear migration. Minus-end directed motors such as cytoplasmic dynein are bound to the nuclear membrane and translocate the female pronucleus towards the microtubule minus-end at the centrosome.

to be pushing on the cortex to overcome that drag. We will use Stoke's law to estimate the drag exerted on the aster, assuming that a sphere is a good first approximation of an aster. Stoke's law states $F=6\pi\eta rV$, where η is the viscosity of the medium, r the radius of the sphere and V its velocity. We set $\eta=1$ gcm^{-1} $second^{-1}$ which is intermediate between published values determined in sea urchin eggs (reviewed by Hiramoto, 1970), and V=16 μm/minute which is the observed speed for the sperm aster of a *Xenopus* egg (Stewart-Savage and Grey, 1982). The drag is calculated to be ~100 pN when the aster is 200 μm in radius, and therefore midway through the centering process.

To estimate the number of microtubules needed to overcome 100 pN of drag, we need to establish how much force each microtubule can generate. Microtubule polymerization forces are in the range of 3 pN for pure tubulin and may be as high as 35 pN in vivo (Dogterom and Yurke, 1997; reviewed by Hill and Kirschner, 1982; Desai and Mitchison, 1997). However, these values apply to forces generated by short microtubules. The actual forces exerted by long microtubules during centering are likely to be significantly lower because microtubules are flexible rods that buckle when the force applied to their ends exceeds a critical value, the buckling force (Bjerknes, 1986; Dogterom and Yurke, 1997). We will assume that the maximum force a microtubule can generate while tethered at the centrosome and pushing against the cortex is equal to its buckling force given by the formula $Fc=2\pi EI/L^2$, where EI=rigidity of the microtubule and L=length. The rigidity of microtubules in *Xenopus* egg extracts is $25\text{-}50\times10^{-24}$ Nm^2 (T. Salmon, personal communication). Using the larger value, we calculate that the buckling force for a microtubule 200 μm long is 0.008 pN. Therefore, midway through centering, 12,000 microtubules would have to push against the cortex to generate a total force sufficient to overcome the estimated 100 pN of aster drag. Because the sperm aster is unlikely to have that many microtubules, these estimates argue against a model for microtubule polymerization alone driving sperm aster movement. However, it should be noted that several factors could alter this conclusion. For instance, microtubule polymerization forces could be much greater since buckled microtubules may still be able to generate significant force (T. Holy and M. Dogterom, personal communication). And bundling of microtubules by microtubule-associated proteins (MAPs) could increase their rigidity and force generation capability (reviewed by MacRae, 1992; Mandelkow and Mandelkow, 1995), and hence also decrease the number of microtubules required.

LENGTH-DEPENDENT FORCES

An alternative model for MTOC positioning invokes microtubule-based motors anchored in the cytoplasm which generate length-dependent forces. The existence of such a possible mechanism was revealed in experiments conducted in

newly fertilized sand dollar eggs (Fig. 3) (Hamaguchi and Hiramoto, 1986), which capitalized on the fact that colcemid, a drug which affects microtubule polymerization, can be inactivated by UV light (Aronson and Inoue, 1970). Colcemid was globally applied, inhibiting microtubule polymerization throughout the egg. The drug was then locally inactivated by UV light, allowing microtubules to grow only in irradiated areas. Colcemid inactivation in the vicinity of the male pronucleus resulted in aster growth from the centrosome and centering of the sperm aster within any area where colcemid was inactivated. This happened even if the inactivated area did not contact the cortex of the egg.

Although the mechanism for centering in this experiment is still not clear, two formal possibilities exist. First, centering could be accounted for by microtubule polymerization forces, as described above, provided the border between the inactivated and activated area represented a 'fixed' object against which growing microtubules could push. However, there is no reason a priori to believe that such a border would exist in an otherwise contiguous cytoplasm. Alternatively, centering may result from length-dependent force generation by minus-end directed motor proteins anchored and evenly distributed in the cytoplasm (Fig. 2b). As a result of force generation by anchored minus-end directed motors, a microtubule moves in the direction of its plus-end (Paschal and Vallee, 1993). If the motors are evenly distributed in the cytoplasm, longer microtubules contact more motors than shorter ones. Centrosome position is the result of the balance of forces acting on all individual microtubules. Therefore, the centrosome moves in the general direction of the longest microtubules. Aster asymmetry is essential for movement in this model: once the aster has become symmetric, all forces are balanced and movement ceases.

Length-dependent forces have also been proposed to account for the movements of asters during centrosome separation in some mitotic cells (Waters et al., 1993). In these cells, if the centrosomes are widely separated upon breakdown of the nuclear envelope, the chromosomes make attachments to the microtubules of only one aster. Therefore, the two asters are not connected by chromosomes and can move independently of one another. Observation in live cells indicated that asters consistently moved in the direction of the longest microtubules. For these cultured cells, it was proposed that motors are anchored and evenly distributed on the plasma membrane and thus generate length-dependent forces to move the asters in the direction of their longest microtubules.

Individual dynein molecules can generate forces in the range of 1 pN (Gittes et al., 1993). In theory, if 100 pN were required to overcome the drag exerted on the sperm aster as estimated above, then only a relative excess of ~100 anchored dynein molecules pulling on the side of the aster with the longer microtubules would be required in this scenario.

Where could minus-end directed motors be anchored in the egg? The answer to this question is not known, but possible structures include the actin cytoskeleton and intermediate filaments. In addition, the endoplasmic reticulum (ER) may play a role. Indeed, the ER extends throughout the cortical and cytosolic areas of the egg (Singal and Sanders, 1974; Terasaki and Jaffe, 1991). Moreover, the minus-end directed motor cytoplasmic dynein is bound to ER membranes in interphase *Xenopus* egg extracts (Allan, 1995b). These observations raise the possibility that cytoplasmic dynein anchored to ER could mediate length-dependent forces that help center the sperm aster. Similarly, it has been proposed that myosin anchored on the ER generates force against actin filaments in the cortical cytoplasm to produce cytoplasmic streaming in characean algal cells (Nothnagel and Webb, 1982; Kachar and Reese, 1988).

In many cells, both microtubule polymerization and length-dependent forces may contribute to MTOC and hence nuclear positioning. While estimating whether each type of mechanism can generate sufficient force to account for centering may be a useful exercise, experiments such as that proposed in Fig. 4 are required to distinguish between the relative contribution of each type to specific cases of MTOC centering.

THE ROLE OF ASTER SIZE AND REGULATION OF MICROTUBULE DYNAMICS

For either microtubule polymerization to push or length-dependent forces to pull an MTOC to the center of a cell, microtubules must be as long as the radius of the cell. For instance, when microtubules are experimentally shortened using low doses of nocodazole in migrating cells at the edge of a wound, the centrosome is unable to follow the geometric center (Euteneuer and Schliwa, 1992). In addition to this length requirement, the number of microtubules must also be proportional to the volume of the cell in order to effectively

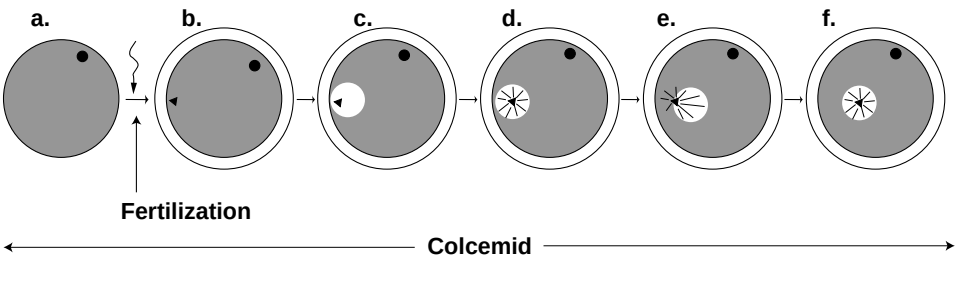

Fig. 3. Length-dependent forces in sand dollar eggs. Triangle: male pronucleus and associated centrosome; black circle: female pronucleus; thin lines: microtubules: gray shading: colcemid; white circles: UV irradiated area. (a) Unfertilized sand dollar egg globally treated with the microtubule-depolymerizing agent colcemid. (b) Upon fertilization, the sperm aster fails to grow, due to the presence of colcemid throughout the egg. (c-d) UV-irradiation results in local inactivation of colcemid. When the irradiated area contains the centrosome and male pronucleus, microtubules grow to form a sperm aster which moves to the center of the irradiated area. (e) When the UV-irradiated area is displaced, microtubules grow into the new area, while microtubules outside of the area will depolymerize, owing to the action of colcemid. (f) The sperm aster migrates to the center of the new irradiated area, even though microtubules do not contact the cortex in this case. Such observations led the authors of these experiments to postulate the existence of forces that are proportional to the length of microtubules. (Adapted, with permission, from Hamaguchi and Hiramoto, 1986.)

steer the centrosome. Asters with large numbers of microtubules will be less affected by movements caused by individual microtubule dynamics or buckling. Thus in fertilized eggs, an aster containing thousands of microtubules may be essential for efficient steering of the nucleus to the center.

Once an MTOC has reached the cell center, limitation of microtubule length might be important to maintain the MTOC in a central position. In the experiments described above with asters of pure tubulin in small chambers (Holy et al., 1997), the centrosome moved to the center driven by microtubule polymerization. If microtubules continued to polymerize once the centrosome reached the center, some microtubules would buckle, causing the force distribution to become uneven, and the centrosome to move off-center. The most favorable position of the centrosome in terms of balancing forces was often observed to be off-center with a few long buckled microtubules and many shorter straight ones. These observations raise the possibility that control of microtubule length in vivo may be important for the MTOC to maintain a central position (Holy et al., 1997). Such control could be achieved by regulating the parameters of dynamic instability of microtubules through the activity of MAPs and other factors (reviewed by Desai and Mitchison, 1997).

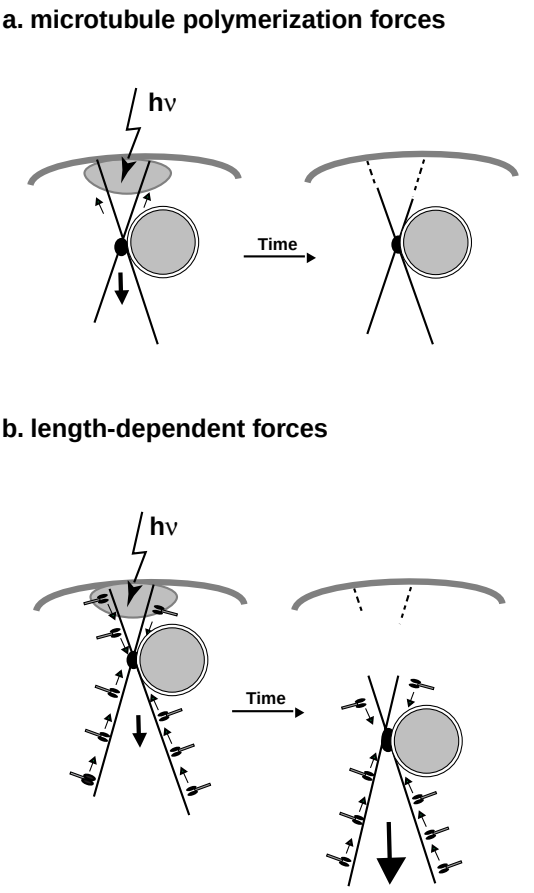

Fig. 4. Testing the contribution of microtubule polymerization forces vs length-dependent forces in vivo. Two types of forces are proposed to cause centering of the sperm aster: polymerization forces that push growing microtubules away from the cell cortex or length-dependent forces that pull them towards the cell center. In principle, it should be feasible to experimentally discriminate between the contribution of each type of force. (a) If microtubules extending from the aster to the cortex were specifically destroyed, for instance by severing with a laser, centering of the sperm aster should slow or stop if microtubule polymerization forces pushing away from the cortex were mainly involved. (b) In contrast, the same treatment should accelerate centering of the sperm aster if length-dependent forces were mainly involved. In this case, the forces normally acting along the microtubules directed towards the cortex would no longer be present to counteract those acting along centrally directed microtubules. (Symbols in this Figure are the same as in Fig. 2.)

ASYMMETRIC NUCLEAR POSITIONING AND CORTICAL ANCHORS

While MTOCs and associated nuclei are often positioned in the cell center, this is not always the case. Notably, they assume an eccentric location prior to many asymmetric cell divisions. For instance, in the 8-cell stage sea urchin embryo, the nucleus of each of the four vegetal blastomeres moves from the cell center to the cortex, led by a centrosome (Dan, 1979, 1984). As a result, the cleavage divisions are unequal, giving rise to four macromeres and four micromeres which differ in developmental potential (Hörstadius, 1928).

Eccentric centrosome positioning cannot be accounted for by simple microtubule polymerization forces or length-dependent forces. Nonetheless we speculate that spatial regulation of these two basic force-generating mechanisms may underlie some cases of asymmetric centrosome positioning. For instance, if microtubules were selectively destabilized in one region of the cell, polymerization forces would become unbalanced, resulting in asymmetric aster

Extrinsic signals and nuclear positioning

In most cases the mechanisms of microtubule-dependent nuclear positioning appear to be cell-intrinsic. However, there are cases in which cell-extrinsic factors play an essential role as well. For instance, in the 4-cell stage *C. elegans* embryo, a signal from a cell called P_2 is required for proper orientation of the centrosome pair in the neighbouring cell called EMS (Goldstein, 1995). The signal coming from P_2 is encoded by a member of the Wnt family of secreted glycoproteins (Thorpe et al., 1997). Reception of this signal in EMS must then somehow be translated into appropriate alterations in centrosome positioning. Cell-extrinsic influences are also apparent in the positioning of nuclei in vertebrate myotubes. Nuclei move freely within cultured myotubes via a microtubule-dependent mechanism (Englander and Rubin, 1987). However, if acetylcholine receptor (AChR) clustering is induced by the application of extracellular matrix from Torpedo electric tissue, nuclei stop moving when they encounter such a cluster. If clustered AChRs later disperse, these nuclei resume movement (Englander and Rubin, 1987). In muscles, nuclei are also localized at synapses beneath clusters of acetylcholine receptors (Kelly and Zacks, 1969; Bruner and Bursztajn, 1986; Fontaine et al., 1988; Jennings and Burden, 1993) suggesting that nuclei similarly respond to cell-extrinsic cues in vivo.

positioning. Similarly, if cytoplasmic dynein were not present or inactive in one region of the cell, length-dependent forces would become unbalanced, also resulting in asymmetric aster positioning.

A popular model to explain asymmetric MTOC positioning invokes minus-end directed microtubule motors anchored at specialized cortical sites. In this model, force generation by these motors moves the MTOC towards the cortical site. The existence of specialized regions of the cell cortex which anchor centrosomes or spindle poles has been recognized for almost a century. Early experiments with oocytes of *Chaetopterus* and *Crepidula* investigated the nature of the asymmetric positioning of the meiotic spindle to the cell cortex (Lillie, 1909; Conklin, 1916, 1917). Upon centrifugation, the spindle was displaced towards the center, but remained firmly attached to the cortex. As a result, the spindle became elongated and an indentation was observed at the site of cortical attachment, suggesting a mechanical link between a spindle pole and the cortical site (Conklin, 1917). While studies in *Xenopus* oocytes have shown that attachment of the meiotic spindle can occur anywhere along the cortex (Gard, 1993), the site is spatially restricted in several other cases. For instance, micromanipulation studies have demonstrated that the cortical site is restricted to a particular area of *Chaetopterus* oocytes (Lutz et al., 1988). In the vegetal blastomeres of the 8-cell stage sea urchin embryo, the centrosome invariably moves towards a region of the cortex characterized by an absence of vesicles (Dan et al., 1983; Dan, 1984).

What is the nature of such specialized cortical sites, and how do they function to asymmetrically position an MTOC? Although the answer to these questions is far from known, studies in *C. elegans* and *Drosophila* have yielded some insight. In the posterior daughter of the two-cell stage *C. elegans* embryo, one of the centrosomes moves towards a site in the anterior cortex, imparting a 90° rotation to the centrosome/nucleus complex (Hyman and White, 1987). During this movement, there is a slight indentation of the cortex, again suggesting a mechanical link between the centrosome and cortex. Movement of the centrosome is abolished by nocodazole and by localized laser irradiation either at the cortical site or between the centrosome and the site (Hyman and White, 1987; Hyman, 1989). This indicates that movement relies on astral microtubules projecting from the centrosome to the cortical site. Rotation is also sensitive to inhibitors of actin polymerization, and both actin and the barbed-end capping protein accumulate at the cortical site (Waddle et al., 1994). Since capping protein is a component of dynactin, a multi-protein complex which is required for targeting of cytoplasmic dynein (Gill et al., 1991), the authors proposed a mechanism involving dynactin and dynein (Fig. 2c) (Waddle et al., 1994). In this model, dynactin localized at the cortical site anchors cytoplasmic dynein, which captures astral microtubules and moves them and the coupled centrosome towards the cortical site by minus-end directed motility. During *Drosophila* oogenesis, an analogous mechanism is proposed to align the mitotic spindles via anchorage of one spindle pole to a specialized structure called the fusome (McGrail and Hays, 1997). Here, the model is supported both by immunolocalization of dynein to the fusome and by analysis of dynein mutants in which spindle alignment is affected.

Symmetric and asymmetric spindle positioning

In eggs of most species, the centrosome duplicates during the first cell cycle, and a bipolar spindle sets up in the middle of the egg. As a consequence, daughters of equal size are generated at cell division. In contrast, in *Tubifex* embryos, the centrosome does not duplicate during the first cell cycle, and the bipolar spindle has an aster at only one pole (Ishii and Shimizu, 1997). This aster centers within the egg, just like a single centrosome would do. As a consequence, the spindle is pushed off-center and an asymmetric cell division ensues. *Tubifex* eggs can be artificially induced to have two centrosomes, in which case spindles with two asters are generated and cleavage is symmetric (Ishii and Shimizu, 1997). Therefore, asymmetric spindle position in *Tubifex* follows from the rules that govern centrosome positioning in any cell. In a less extreme case, two asters of different size would also lead to an asymmetric spindle position. Differences in aster size have been characterized during unequal divisions in molluscs and sea urchins (Dan, 1979; Dan and Ito, 1984; Holy and Schatten, 1991), but whether such differences are a cause or a consequence of asymmetric spindle positioning remains to be determined.

NUCLEAR POSITIONING IN *S. CEREVISIAE*

Analysis of nuclear positioning in *S. cerevisiae* (reviewed by Stearns, 1997) provides additional insights into the mechanism of MTOC positioning via cortical anchors. In yeast, nuclear positioning occurs via cytoplasmic microtubules which emanate from the SPB. Since the mitotic spindle is assembled within the nuclear membrane, the nucleus must be precisely positioned at the mother-bud neck for the genetic material to be correctly segregated at cell division. Elongation of the intranuclear spindle results in one nuclear mass being distributed to the mother cell and one to the bud cell. A failure in nuclear positioning leads to elongation of the spindle within the mother cell, resulting in the generation of binucleate mother cells and anucleate bud cells.

Binucleate cells have been observed in strains lacking astral microtubules, the heavy chain of cytoplasmic dynein or dynactin components (Palmer et al., 1992; Eshel et al., 1993; Li et al., 1993; Muhua et al., 1994; Yeh et al., 1995; Shaw et al., 1997). These genetic data suggested a mechanism for nuclear positioning in *S. cerevisiae* analogous to that proposed for asymmetric centrosome positioning in *C. elegans*: dynein anchored by dynactin at the bud cortex would capture an astral microtubule and pull it and the coupled SPB towards the bud by minus-end directed motility. However, recent evidence indicates that this model requires modifications to incorporate novel cortical components and a potential role for regulation of microtubule dynamics.

The proteins Jnm1p, Kar9p and Num1p are candidates for cortical anchor components during nuclear positioning in *S. cerevisiae*. Mutations in any of these genes results in nuclear positioning defects, and all three molecules are cortically localized. Jnm1p has been proposed to be the yeast equivalent of the p50 subunit of dynactin (Echeverri et al., 1996) and additional genetic data support the proposed function of Jnm1

in the dynein pathway (Geiser et al., 1997). A Jnm1p-lacZ fusion localizes either to a single site in the bud cortex in anaphase or to the spindle pole oriented towards the bud (McMillan and Tatchell, 1994). Together, these observations lend support to the model of dynactin acting as a cortical anchor at the bud cortex, at the least during anaphase. Kar9p encodes a novel molecule and a functional GFP-Kar9 fusion protein localizes to a single cortical dot in the bud into which cytoplasmic microtubules are directed (Miller and Rose, 1998). Num1p localizes to multiple cortical dots in the mother cell (Farkasovsky and Kuntzel, 1995) and shares significant homology with apsA, a protein required for microtubule-dependent nuclear positioning in *Aspergillus* (Fischer and Timberlake, 1995). Perhaps Kar9p and Num1p function as cortical anchors for astral microtubules or motor proteins in the bud and the mother cell, respectively. The actual mechanism of Kar1p and Num1p action as well as the nature of their potential interaction with dynactin remain to be elucidated.

Observations of astral microtubule behavior in wild-type and dynein mutant cells revealed novel aspects of nuclear positioning in *S. cerevisiae* (Carminati and Stearns, 1997; Shaw et al., 1997). Directed movement of the SPB/nucleus towards the cortex was shown to occur following: (1) a sweeping motion of the microtubule end along the cortex, as if searching, and (2) a depolymerization event, with the microtubule end continuously remaining in contact with the cortex (Carminati and Stearns, 1997). In a dynein mutant (dyn1), both types of interaction are affected and directed movement of the SPB/nucleus rarely occurs. It was concluded that these two types of directed movement correspond to two distinct mechanisms of pulling the SPB/nucleus towards the cortex: (1) by anchored dynein motor activity, as in the previous model, and (2) by microtubule depolymerization while the microtubule plus-end remains attached to the cortex, perhaps through dynein. In vitro, microtubule motors coupled to beads are able to follow depolymerizing microtubules (Lombillo et al., 1995). Therefore, it is plausible that dynein localized to the cortex may remain attached to shrinking microtubules. This study also revealed that microtubule dynamics in the dyn1 mutant are altered: shrinkage rates are diminished, resulting in longer average microtubules (Carminati and Stearns, 1997). Therefore, altered microtubule dynamics may, in fact, be responsible for the dyn1 mutant phenotype; perhaps longer microtubules cannot allow normal searching of the cortex or proper depolymerization-coupled movement. Interestingly, a GFP-dynein fusion protein that can rescue the dyn1 null mutant phenotype is localized to astral microtubules and spindle pole bodies (Shaw et al., 1997). This localization is compatible with dynein driving nuclear positioning through regulation of global microtubule dynamics.

More support for the importance of regulating microtubule dynamics for proper nuclear positioning in *S. cerevisiae* comes from analysis of the kinesin family motors Kar3p, Kip3p and Kip2p (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Saunders et al., 1997). While single mutants in each of the corresponding genes as well as in dyn1 are viable despite defects in nuclear positioning, double and triple mutant combinations have different and informative phenotypes. For instance, deleting kip2 suppresses the growth defects of dyn1 kip3 double mutants and rescues the lethality of dyn1 kar3 double mutants. This led to the hypothesis that nuclear positioning may be achieved by antagonistic motor activities, with dynein, Kar3p and Kip3p exerting a force on one SPB and Kip2p exerting an opposing force on the other one (Cottingham and Hoyt, 1997). However, the distribution of astral microtubules in mutant strains suggested an alternative explanation. Compared to wild-type, astral microtubules are longer in dyn1 and kip3 single mutants, shorter in kip2 single mutants, and close to normal in dyn1 kip3 kip2 triple mutants (Cottingham and Hoyt, 1997). This led the authors to suggest that alterations of microtubule dynamics may be causing the nuclear positioning defects in dyn1, kar3, kip3 or kip2 mutant cells (Cottingham and Hoyt, 1997). In vitro studies have established that motor proteins can indeed modulate microtubule dynamics (Endow et al., 1994; Walczak et al., 1996), in addition to their classical role in force generation via translocation along microtubules. Moreover, over-expression studies with cytoplasmic dynein in *S. cerevisiae* indicate that motors may regulate microtubule dynamics in a cell-cycle specific way. Over-expression of cytoplasmic dynein causes astral microtubules in the bud to elongate more than normally during anaphase, while their dynamic parameters are comparable to wild-type during the remainder of the cell-cycle (Shaw et al., 1997).

In summary, during MTOC-dependent nuclear positioning, the nucleus is positioned by forces acting on microtubules emanating from an associated MTOC. The positioning of MTOCs and associated nuclei to the cell center can result from at least two types of forces: (1) microtubule polymerization forces that push MTOCs away from the cell cortex, and (2) length-dependent forces mediated by minus-end directed microtubule motors that pull MTOCs in the direction of their longest microtubules. In cases of asymmetric MTOC positioning, cortically anchored motor proteins such as dynein may capture astral microtubules and move the MTOC towards the cortex by minus-end directed motility. However, analysis of nuclear positioning in *S. cerevisiae* has raised the possibility that microtubule-based motors ensure proper MTOC positioning primarily by modulating microtubule dynamics. Further studies should elucidate whether similar mechanisms may contribute to asymmetric MTOC and nuclear positioning in higher eukaryotes as well.

NUCLEAR TRACKING ALONG MICROTUBULES

A second type of nuclear positioning occurs when nuclei lack an associated MTOC. Experiments have shown that in many species the centrosome disintegrates during oogenesis and is restored to the egg by the sperm at fertilization (reviewed by Schatten, 1994). Therefore, in many cases the oocyte nucleus and the female pronucleus lack an associated centrosome. That no microtubules are nucleated from the vicinity of the female pronucleus has been directly demonstrated in fertilized sand dollar eggs (Hamaguchi and Hiramoto, 1986).

Early investigators recognized the importance of the sperm aster for female pronuclear motility long before it was known that the rays emanating from the aster center corresponded to microtubules. In several echinoderm species, it was observed that the female pronucleus begins moving when it first contacts astral rays (Conklin, 1894; Chambers, 1939). In one experiment, the motility of the female pronucleus was drastically reduced in newly fertilized sea urchin eggs when

the sperm aster was suppressed by treatment with ether (Wilson, 1901). Moreover, a detailed analysis in sea urchin eggs demonstrated that the trajectories followed by the female pronucleus were always directed towards the current position of the sperm aster (Wilson, 1928; Chambers, 1939).

The advent of pharmacological agents which perturb the cytoskeleton demonstrated that female pronuclear motility is, in fact, dependent on the presence of microtubules which emanate from the sperm aster. Thus, treatment with nocodazole or other microtubule-depolymerizing agents abolishes female pronuclear motility in sea urchin, *C. elegans* and other species (Rouviere et al., 1994; reviewed by Schatten, 1982; Strome and Hill, 1988). Taken together, such studies established the dependence on the microtubules of the sperm aster for female pronuclear motility.

DYNEIN-DEPENDENT NUCLEAR MOVEMENT

The current model for the movement of the female pronucleus involves its translocation along the microtubule lattice using the minus-end directed motor dynein (Rouviere et al., 1994; Schatten, 1994; Allan, 1996; Reinsch and Karsenti, 1997), in a manner analogous to organelle motility (Allan, 1995a).

Studies in polyspermic embryos of the comb jelly *Beroe ovata* documented that the female pronucleus can switch between microtubule tracks (Carre and Sardet, 1984; Carre et al., 1991; Rouviere et al., 1994). The cytoplasm of these large marine eggs is restricted to a thin cortical shell, allowing for convenient visualization of the pronuclear movements. Polyspermy is common in *Beroe*; each sperm organizes a large microtubule aster that remains at each sperm entry site. When asters overlap, the female pronucleus may migrate for a while on one aster, and then change onto a second or third aster. Similar observations were made in a case of dispermy in sea urchin (Chambers, 1939). Such changes in directionality from one aster to another in polyspermic eggs suggest that, like other organelles, the female pronucleus can switch from one microtubule to another during migration.

In motility of other organelles, plus- or minus-end directed microtubule-dependent motors are linked to the organelle membrane, and thus translocate the organelle towards the microtubule plus- or minus-end, respectively. Due to the observed directionality (minus-end directed) and speed (0.2 to 1.5 μm/second), cytoplasmic dynein was proposed to be the motor during female pronuclear migration (Schatten, 1982; Rouviere et al., 1994; Schatten, 1994; Allan, 1996).

Confirmation of the model for dynein in this type of nuclear positioning has recently been afforded by the development of a cell-free reconstituted system (Reinsch and Karsenti, 1997). In this system nuclei are assembled in bulk in *Xenopus* egg extracts using DNA coupled to magnetic beads. These nuclei lack an associated centrosome and, therefore, bear resemblance to female pronuclei. The purified nuclei move on microtubules towards the centrosome in *Xenopus* extracts. Both the directionality (minus-end directed) and speed (0.36 to 1.6 μm/sec) are consistent with this reconstituted system mimicking female pronuclear migration (Schatten, 1981; Stewart-Savage and Grey, 1982; Rouviere et al., 1994). Importantly, motility in this cell free system was abolished in the presence of an antibody to the intermediate chain of cytoplasmic dynein or of 15 μM vanadate (Reinsch and Karsenti, 1997), a concentration indicative of dynein-mediated motility (reviewed by McIntosh and Porter, 1989). Disruption of the dynactin complex by addition of excess bacterially expressed p50/dynamitin (Echeverri et al., 1996) also blocks motility (S. Reinsch, unpublished observation). Taken together, these results demonstrate that cytoplasmic dynein drives nuclear movement along microtubules in this reconstituted system. Perhaps cytoplasmic dynein is linked to the nuclear membrane via dynactin and thus translocates the purified nucleus towards the microtubule minus-end, at the centrosome (Fig. 2d). Again using Stoke's law, an estimated force of 40 pN and hence 40 active dynein molecules, would be required to move the female pronucleus in *Xenopus* eggs at the observed velocity (r=20 μm, V=1 μm/second). In vivo, dynein is likely to be required in numerous processes in addition to female pronuclear movement. Therefore it will be challenging to design genetic or direct perturbation experiments to confirm the role of dynein in vivo as the motor for female pronuclear migration.

Could nuclear tracking along microtubules merely be a manifestation of ER motility, since the outer nuclear membrane is continuous with the ER? The ER from *Xenopus* eggs can extend tubules along microtubules in *Xenopus* egg extracts in a dynein-dependent fashion (Allan, 1995b) and ER tubules were seen to extend from the isolated nuclei in the cell free system described above. However, extension-retraction cycles of ER tubules were faster than the longer lasting episodes of nuclear motility, and showed a different sensitivity to inhibition by vanadate (Reinsch and Karsenti, 1997). Moreover, ER tubule extensions ahead of a nucleus were generally not followed by nuclear motility. Thus, although joined, the nucleus and the ER appear to have distinct modes of dynein-dependent movement along microtubules. These considerations raise the possibility that there are two kinds of domains on the outer nuclear membrane, both harboring dynein. Perhaps some domains only support ER tubule elongation from the outer nuclear membrane, while others with links to the inner nuclear membrane and nucleoplasm mediate nuclear motility (Fig. 5).

Is dynein-dependent tracking along microtubules also involved in other cases where nuclei lack an associated MTOC? In *Drosophila* oocytes, the MTOC is localized to different regions of the cortex at different stages of oogenesis (Theurkauf et al., 1992, 1993; reviewed by Theurkauf, 1994). The position of the oocyte nucleus correlates with that of the MTOC both in wild-type and in mutants in which MTOC position is altered (Theurkauf et al., 1992, 1993; Theurkauf, 1994; Roth et al., 1995; Micklem et al., 1997). Treatment with colchicine results in random nuclear position (Theurkauf et al., 1993). When colchicine is inactivated, microtubules regrow from the cortical MTOC and the nucleus is observed to move to the MTOC (W. Theurkauf, personal communication). This provides direct evidence that the positioning of the nucleus is microtubule-dependent and raises the possibility that it could be driven by minus-end directed motor activity. Dynein is required for early stages of oogenesis, precluding the assessment of its involvement in nuclear movement at this later stage (McGrail and Hays, 1997). Therefore, it remains to be determined whether the mechanism of positioning the oocyte nucleus in *Drosophila* or other nuclei lacking associated

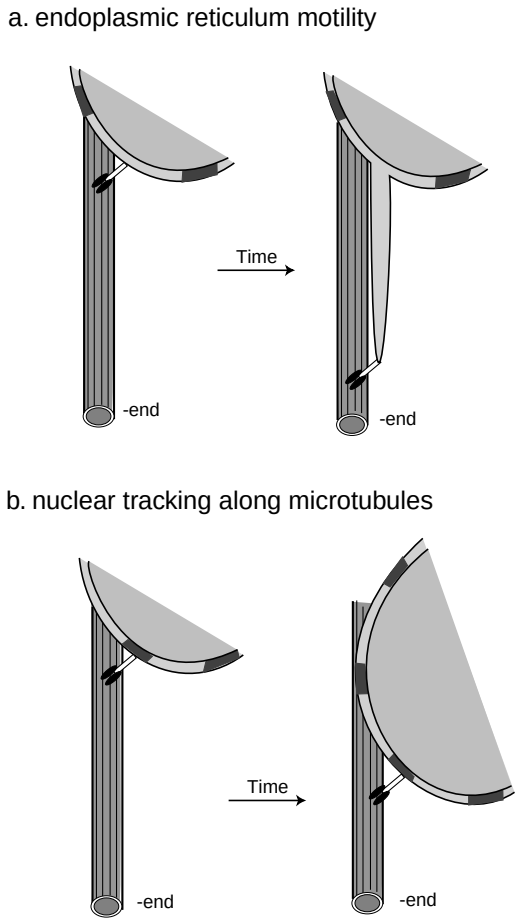

Fig. 5. Distinct domains on the nuclear membrane may mediate dynein-dependent endoplasmic reticulum and nuclear motility. A nucleus is attached to a microtubule; dynein is represented with two small rod-like tail domains and two globular head domains. Perhaps there are two kinds of domains on the outer nuclear membrane, both harboring dynein. (a) One domain (lighter areas) would only support ER tubule elongation from the outer nuclear membrane. (b) The other domain (darker areas) would have links to the inner nuclear membrane and nucleoplasm and mediate nuclear motility.

MTOC involves dynein-dependent tracking along microtubules.

In summary, during nuclear tracking along microtubules, the position of nuclei lacking an MTOC depends on the activity of motors such as cytoplasmic dynein. Motors are presumably bound to specialized sites on the outer nuclear membrane and thus transport the nucleus along the microtubule lattice.

THE NUCLEUS-CENTROSOME CONNECTION IN NUCLEAR POSITIONING

In this final section, we explore how nuclear tracking along microtubules may generally serve to maintain a tight association between the nucleus and the centrosome in proliferating animal cells, thereby enabling the MTOC-dependent nuclear positioning to determine nuclear location.

Separation of centrosomes from nuclei has been notoriously difficult to achieve after cell lysis (Bornens, 1977). However, this association seems to be in fact quite dynamic in vivo as demonstrated, for instance, in an experiment conducted in the two-cell stage sea urchin embryo (Aronson, 1971). Normally, each of the two blastomeres contains a centrally located centrosome and associated nucleus. Embryos were centrifuged in the presence of low doses of colcemid, thus globally diminishing microtubule polymerization. Conditions were chosen which resulted in the separation of centrosomes and nuclei: the centrosome stayed in the center of the blastomere, while the nucleus was displaced to the cell periphery. Colcemid was then inactivated by UV light in an area which comprised both the centrally located centrosome and the peripheral nucleus. As microtubules regrew from the centrosome, the nucleus moved along microtubules towards the center of the aster. This experiment demonstrated that such a nucleus is still capable of nuclear tracking, in a manner analogous to the migration of the female pronucleus. This raises the possibility that the association between centrosome and nucleus in the two-cell stage sea urchin embryo, and perhaps in other cells, derives from the same mechanism that drives female pronuclear migration. Thus tracking of nuclei along microtubules may play a role in maintaining the nucleus-centrosome association during interphase.

At the end of mitosis, the nuclear envelope reforms around decondensing chromosomes. In polarized epithelial cells, the centrosomes separate from the reforming nuclei and move to the apical plasma membrane some 10 μm away (Reinsch and Karsenti, 1994). Centrosomes in these cells do not interact with the nuclei again until the onset of prophase. In prophase cells, dynein is localized to the nuclear membrane and possibly mediates the interaction of the nucleus with the centrosome at this stage (Busson et al., 1998). In the vast majority of proliferating cells, however, the centrosome and nucleus renew their association just at the end of mitosis. Observations indicate that this reassociation may occur in a manner reminiscent of tracking along microtubules. In a *Xenopus* reconstituted system established for studying anaphase motility, small reforming nuclei were directly visualized moving towards the center of asters that departed from the spindle remnant (Murray et al., 1996). We speculate that such migrations towards the centrosome may generally take place after nuclear division, but be difficult to follow at the light microscopic level, since nuclei usually reform in the vicinity of centrosomes. Just like for female pronuclear migration, cytoplasmic dynein or another minus-end directed motor would be expected to be involved in associating the reforming nuclei with the centrosome.

In conclusion, we speculate that the two types of nuclear positioning cooperate in many proliferating animal cells. Nuclear tracking driven by cytoplasmic dynein may serve to maintain a tight association between the nucleus and the centrosome. In turn, this would enable microtubule polymerization forces and length-dependent forces transduced to the centrosome to position the associated nucleus. Usually, the tight association between the centrosome and nucleus masks the existence of nuclear tracking along microtubules, although this very mechanism may be key in maintaining that association. Only in some cases, like in the newly fertilized egg of most species, are the two types of nuclear positioning

spatially and temporally separated, and thus ideally suited for experimental dissection.

For helping us improve the manuscript, we thank Viki Allan, Marileen Dogterom, Suzanne Eaton, Chris Echeverri, Michael Elowitz, Anne Ephrussi, Tim Holy, Tony Hyman, Eric Karsenti, Stanislas Leibler and Ted Salmon. Pierre Gönczy was supported by fellowships from the Human Frontier Science Program (LT-202/96) and the Swiss National Science Foundation (TMR 83EU-045376).

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