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COMMENTARY

Actin-based spindle positioning: new insights from female gametes

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ABSTRACT

Asymmetric divisions are essential in metazoan development, where they promote the emergence of cell lineages. The mitotic spindle has astral microtubules that contact the cortex, which act as a sensor of cell geometry and as an integrator to orient cell division. Recent advances in live imaging revealed novel pools and roles of F-actin in somatic cells and in oocytes. In somatic cells, cytoplasmic F-actin is involved in spindle architecture and positioning. In starfish and mouse oocytes, newly discovered meshes of F-actin control chromosome gathering and spindle positioning. Because oocytes lack centrosomes and astral microtubules, F-actin networks are key players in the positioning of spindles by transmitting forces over long distances. Oocytes also achieve highly asymmetric divisions, and thus are excellent models to study the roles of these newly discovered F-actin networks in spindle positioning. Moreover, recent studies in mammalian oocytes provide a further understanding of the organisation of F-actin networks and their biophysical properties. In this Commentary, we present examples of the role of F-actin in spindle positioning and asymmetric divisions, with an emphasis on the most up-to-date studies from mammalian oocytes. We also address specific technical issues in the field, namely live imaging of F-actin networks and stress the need for interdisciplinary approaches.

KEY WORDS: Asymmetric division, F-actin, Cortex, Meiosis, Oocyte, Spindle

INTRODUCTION

Asymmetric divisions play a crucial role during metazoan development. An early step, oocyte maturation, involves one of the most extreme cases of asymmetric division (Brunet and Verlhac, 2011). Indeed, all oocytes, with the exception of *Drosophila*, divide twice in a very unequal way, expelling small polar bodies with a minimal cytoplasmic content, thus preserving their maternal stores. Maintaining energy resources in eggs is vital for species such as starfish, jellyfish or *Xenopus* that develop by spawning and whose early embryos divide outside the genital tracts of their mothers. These embryos will need considerable egg supplies until they turn into larvae that can eat and grow further. During early embryonic development, asymmetric divisions are of central importance to generate cell lineages, and later to organise and shape tissues and generate cellular diversity (Gillies and Cabernard, 2011).

There has been a plethora of excellent reviews on the control of spindle positioning for asymmetric divisions during metazoan development (e.g. Gönczy, 2002; Gönczy, 2008; Knoblich, 2010; Morin and Bellaïche, 2011). The aim of this Commentary is to give an overview of the strategies used by cells to orient the spindle, with a specific emphasis on the emerging roles of the actin cytoskeleton. We present representative work in different model systems and at different stages of development. In particular, we emphasise the most recent work and compare the role of microfilaments in female germ cells, oocytes and somatic cells.

Asymmetric division: a consequence of spindle positioning

The question of the orientation of cell division was raised in the 19th century, with the 'long-axis rule' established by Hertwig (Hertwig, 1884). According to this rule, the cleavage plane orients perpendicular to the longest cell axis, suggesting that cell shape influences the orientation of the spindle and the division plane. Many cell types orient their spindles according to pre-existing polarity cues. This is the case in budding yeast, *Caenorhabditis elegans* embryos and *Drosophila* neuroblasts (Siller and Doe, 2009). In neuroblasts, the spindle orients in relation to an axis of external polarity through the planar cell polarity (PCP) pathway, as well as relative to an axis of internal polarity through a ternary complex called the LGN–NuMa–Gαi complex in vertebrates or the Pins–Mud–Gαi complex in *Drosophila*. This complex is composed of a GoLoco-domain-containing protein (LGN), a microtubule-associated protein (NuMa) and a GTP-binding protein (Gαi) anchored in the plasma membrane (Gillies and Cabernard, 2011).

Addressing the influence of cell shape on cortical cues in these model organisms is challenging because the cells have a pre-established shape. An elegant study circumvented this problem by forcing the shape of non-polarised cultured mammalian cells by using micropatterns and provided evidence that cell geometry can influence the localisation of cortical determinants, such as ezrin or cortactin at adhesion sites, and thus constrains the orientation of the mitotic spindle (Théry et al., 2005).

Spindle positioning is a crucial event for the success of asymmetric division. Spindle position relays spatial information that is dictated by the cell itself and/or its environment in order to determine the position of the future division plane. During metaphase, the spindle can translate or rotate according to the cell or tissue polarity, the cell adhesion pattern or cell–cell signalling (Gillies and Cabernard, 2011; Siller and Doe, 2009). Then, during early anaphase, the spindle position defines the position of the division plane (Rappaport and Rappaport, 1974) by signalling of the central spindle to the facing cortex, leading to the formation of a recruitment platform for the cytokinetic machinery (Green et al., 2012). This tight coupling between spatial cues and the position of the division plane is a major issue for the dividing cell: it ensures that two cells of the right size are obtained, with the right cell fate determinants and with the same amount of genetic material.

The way the spindle is oriented in relation to cortical determinants in mitotic cells is well documented; astral microtubules emanating from centrosomes interact with cortical anchors such as the dynein family of minus-end-directed molecular motors (Fig. 1A). How dyneins produce the forces that power spindle movement at the spindle or cortex interface is well known from studies in budding yeast (Moore and Cooper, 2010). In the *C. elegans* zygote, spindle positioning in anaphase, as well as centration and rotation of the nucleus–centrosome complex, is controlled by dynein, which interacts with the NuMa orthologue Lin-5 (Couwenbergs et al., 2007; Nguyen-Ngoc et al., 2007). In *Drosophila* neuroblasts, the Pins–Mud–Gαi complex interacts with dynein to regulate spindle orientation (Bowman et al., 2006; Izumi et al., 2006; Siller and Doe, 2008; Siller et al., 2006; Wang et al., 2011). Compared with the role of dyneins in spindle tethering and orienting, much less is known about the role of actin and myosin – other key factors in this process – which we discuss below.

Actin and asymmetric division in somatic cells

F-actin is required for spindle anchoring and orientation in several cultured mammalian cell types, in which randomisation of spindle orientation occurs upon treatment with drugs that disassemble F-actin (Théry et al., 2005; Toyoshima and Nishida, 2007). Cortical F-actin is essential because the disruption of cell adhesions prevents proper anchoring and orientation of the spindle (Théry et al., 2005). Disruption of F-actin in *Xenopus* embryos also leads to spindle mispositioning in the outer epithelial cells (Woolner et al., 2008). Unconventional myosin-X, which binds both to microtubules and actin (Hirano et al., 2011; Weber et al., 2004), is required for spindle orientation in cultured cells (Toyoshima and Nishida, 2007). Indeed, a study of epithelial cells of *Xenopus* embryos shows that myosin-X and F-actin both contribute to the anchoring of the spindle at the cortex (Woolner et al., 2008).

F-actin and myosins are not only involved in spindle anchoring, but also have other roles in spindle positioning. For instance, in cultured mammalian and *Drosophila* cells, in which centrosomes do not separate before disappearance of the nuclear envelope, myosin-II- and F-actin-driven cortical contraction can also pull the nascent spindle poles away from each other (Rosenblatt et al., 2004). In *Drosophila* wing discs, planar polarisation of the atypical myosin Dachs drives changes in cell shape through a constriction of cell–cell junctions, which then direct the orientation of the spindle according to Hertwig's long-axis rule (Mao et al., 2011).
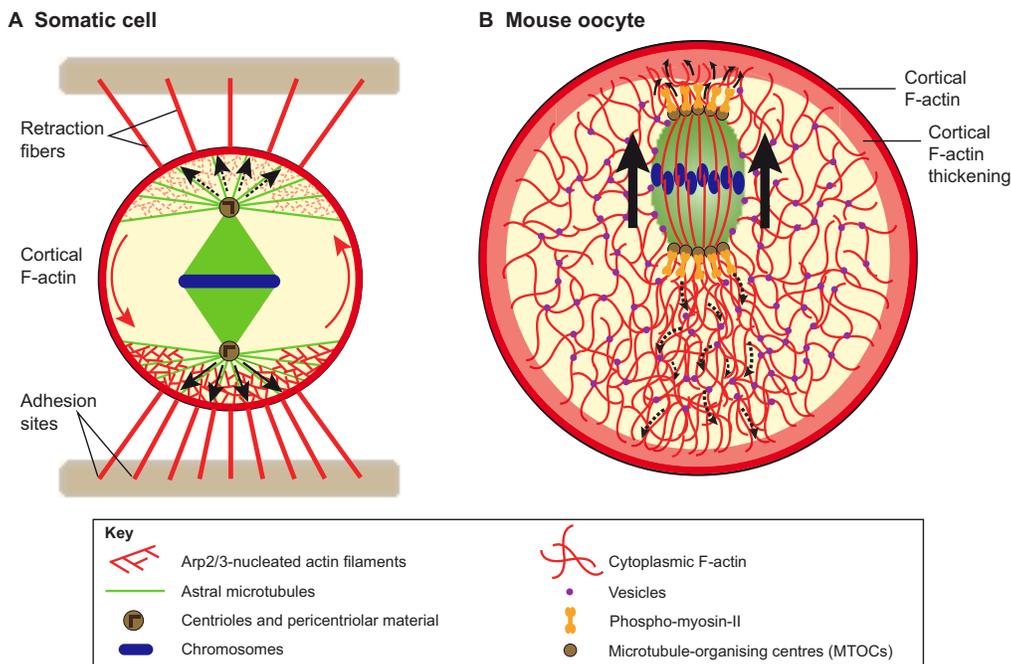

A Somatic cell

Retraction fibers

Cortical F-actin

Adhesion sites

B Mouse oocyte

Cortical F-actin

Cortical F-actin thickening

Key

Arp2/3-nucleated actin filaments	Cytoplasmic F-actin
Astral microtubules	Vesicles
Centrioles and pericentriolar material	Phospho-myosin-II
Chromosomes	Microtubule-organising centres (MTOCs)

Fig. 1. Interplay between the different F-actin pools and the spindle: implication in spindle positioning. (A) Somatic cell in metaphase of mitosis. Here, retraction fibres (in red) are anchored on adhesion sites (light brown bars). Spindle positioning is dictated by external cues, namely the geometry of cell adhesions. Cortical F-actin plays a prominent role in spindle positioning by anchoring the astral microtubules to the cortex and also by increasing cortical rigidity. A recently discovered cytoplasmic pool of F-actin that is nucleated by the Arp2/3 complex is also involved in spindle positioning. This pool appears to respond to the geometry of cell adhesions because it revolves periodically within the cell (red curved arrows; solid filaments, actual position; dotted filaments, alternative position) according to the forces transmitted by the retraction fibres. The cytoplasmic F-actin cluster is thought to position the spindle by pulling on astral microtubules (as illustrated by the black arrows), until the spindle is aligned with the cell adhesion pattern. (B) Mouse oocyte in metaphase of meiosis I. Mouse oocytes are isolated from external forces and show no sign of polarisation in prophase I. Here, migration of the centrally located spindle is triggered by a symmetry breaking event that comes from within the cell and different F-actin pools are involved in the correct spindle migration. One pool is a cytoplasmic meshwork that is nucleated by formin-2 and Spire 1 and Spire 2 from cytoplasmic vesicles and around the spindle. Another actin pool is the thickening of cortical F-actin that is nucleated by Arp2/3; it appears around 3 hours after meiosis resumes (light red inner cortical layer). An attractive model for symmetry breaking and spindle migration is provided by the interplay between these pools. Following nucleation of the cytoplasmic meshwork, the spindle becomes embedded in an actin 'cage' that is connected to the cortex. Phospho-myosin-II is present at both poles of the spindle and pulls on cytoplasmic F-actin (curved black arrows). The 'leading' pole of the spindle, which is slightly closer to the cortex, is pulled with greater strength than the opposite pole (dotted curved black arrows), bringing it even closer to the 'leading' pole and increasing the initial slight asymmetry. Concomitantly, the appearance of the deformable, thickening of cortical F-actin increases the imbalance of forces and contributes to cortical softening. Because a soft cortex is more responsive to the pulling forces exerted by myosin-II, spindle migration is enhanced.

Interestingly, the rigidity that cortical F-actin and myosins give to the cortex has a strong impact on spindle assembly and function in some *Drosophila* cell types (Kunda et al., 2008). Together with phospho-myosin-II (Maddox and Burridge, 2003), phospho-moesin, a plasma protein that crosslinks F-actin to the membrane, increases cortical stiffness at metaphase and promotes cell rounding (Fig. 2A). Interfering with this process by silencing of moesin perturbs normal spindle assembly and positioning. In turn, restriction of phospho-myosin-II and phospho-moesin at the cell equator at anaphase promotes the relaxation of the polar cortex, thus allowing spindle elongation (Carreno et al., 2008; Kunda et al., 2008).

Until recently, the study of the role of actin in spindle positioning was focused mostly on the contribution of cortical F-actin, probably because the most common method used to visualise F-actin with phalloidin strongly stains the cortex and other structures, such as retraction fibres in mitotic cells. But during the past few years, the development of new actin probes that bind to F-actin rather than G-actin, such as Lifeact or GFP–UtrCh (Burkel et al., 2007; Riedl et al., 2008), revealed other pools of F-actin in the cytoplasm or around the spindle in mitotic cells. F-actin cables were indeed detected around the spindle and between the spindle and the cortex in *Xenopus* embryo epithelial cells and were proposed to have a role in spindle positioning and regulation of spindle length (Woolner et al., 2008). In cultured cells, a cluster of cytoplasmic actin consisting of Arp2/3-nucleated branched filaments has been detected (Fig. 1A; Mitsushima et al., 2010). Moreover, this novel pool of actin is thought to have a role in the positioning of the spindle of cells that adhere on micropatterns. This actin cluster is extremely dynamic and is nucleated in the form of patches that revolve in the cell in accordance with the geometry of adhesion sites. By an unknown mechanism, forces that are exerted by retraction fibres induce the nucleation of these patches, which, in turn, align the spindle with the adhesion pattern by pulling on astral microtubules (Fig. 1A) (Fink et al., 2011).

Asymmetric division and spindle positioning in oocytes

In contrast to mitotic divisions that generate two daughter cells with the same diploid content, meiotic divisions produce gametes with haploid content. In the female gamete – the oocyte – homologous chromosomes are segregated in a tiny cell called the polar body during anaphase of the first meiotic division (meiosis I). Then, the second meiotic division (meiosis II) leads to the segregation of sister chromatids in a second polar body. These divisions are extremely asymmetric in order to preserve the very large size of the oocyte that is reached during its growth and to provide the reserves necessary for the first steps of embryonic development.

Another feature of oocytes is the absence of centrosomes and thus of associated astral microtubules (Fig. 1B) from early oogenesis onwards in many species, such as flies (Theurkauf and Hawley, 1992), worms (Albertson and Thomson, 1993), mice (Szollosi et al., 1972) and human (Sathananthan, 1997). Lack of centrioles and astral microtubules renders asymmetric divisions very challenging in oocytes, in particular with regard to spindle positioning and furrowing. During anaphase in mitotic animal cells, astral microtubules that interact with the cortex act as sensors of cell geometry and accordingly define a site for cleavage furrow ingression. This site is then validated (or not) by a second signal emanating from the central spindle, which couples the position of the cleavage plane with that of the chromosomes (Almonacid and Paoletti, 2010; von Dassow, 2009). The range of this second signal depends on the distance of the central spindle to the cortex, so that in big cells, such as sea urchin or sand dollar zygotes, astral microtubules become important players for furrowing (von Dassow, 2009).

To compensate for the lack of centrosomes and astral microtubules, oocytes use a panoply of alternative strategies, depending on the species. Transport of the genetic material to the cortex is a key step that is shared by many organisms and this can be performed before, during or after spindle assembly. We illustrate this point below with three representative examples: the oocytes of *Xenopus*, starfish and mice.

Chromosomal transport in *Xenopus* and starfish oocytes

Mature *Xenopus* oocytes are gigantic cells of 1 mm in diameter that are polarised along an A (animal)–V (vegetal) axis. The
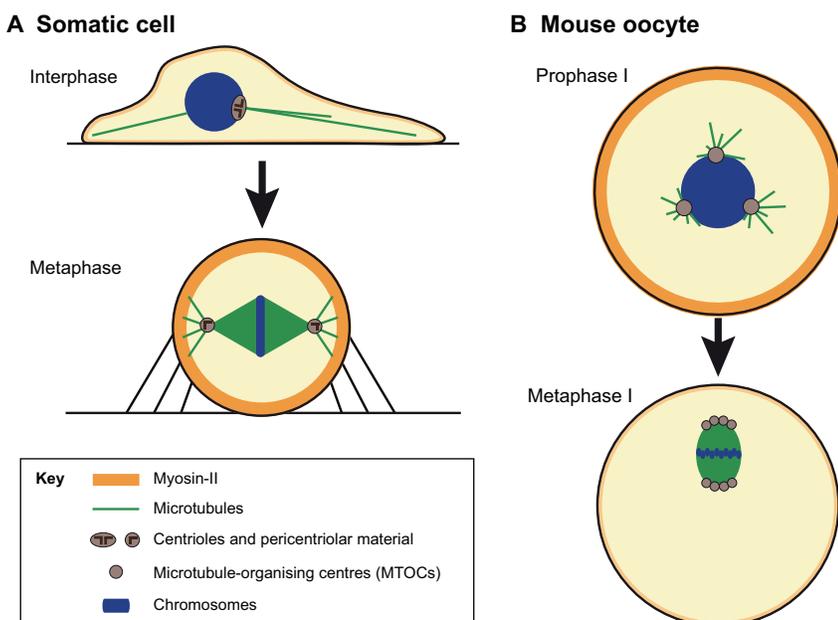

A Somatic cell

Interphase

Metaphase

B Mouse oocyte

Prophase I

Metaphase I

Key
- Myosin-II
- Microtubules
- Centrioles and pericentriolar material
- Microtubule-organising centres (MTOCs)
- Chromosomes

Fig. 2. Differences in the generation of cortical tension between somatic cells and mouse oocytes. (A) Rounding of a somatic cell at mitosis onset. At metaphase, myosin-II, together with phospho-moesin (a plasma membrane F-actin crosslinking protein that remodels cortical actin filament organisation) contribute to increase cortical stiffness and induce mitotic cell rounding. (B) Cortical softening of a mouse oocyte during meiosis I. In prophase I, before meiosis resumes, the cortex is stiffened by myosin-II. But during meiosis I, the appearance of cortical F-actin thickening, together with the drop in cortical tension that results from the departure of myosin-II from the cortex (as indicated by the thinner layer of myosin-II), both soften the cortex. Interestingly, the oocyte remains round despite this drop in cortical tension, in contrast to somatic cells with a soft cortex.

animal pole contains most organelles, including the nucleus, whereas the vegetal pole has a high yolk content (Dumont, 1972). Nucleus localisation at the animal pole is microtubule dependent because microtubule depolymerisation results in its displacement towards a more equatorial or vegetal position (Gard, 1993). In these cells, the nucleus has a diameter of 400 µm and the initial pole-to-pole length of the meiosis I spindle is 17 µm (Gard, 1992). Collection of the chromosomes from an extremely large volume to fit onto a tiny spindle requires specific adaptation mechanisms. This is thought to be achieved by a structure called the transient microtubule array (TMA), which appears after breakdown of the nuclear envelope; it collects the chromosomes from the large nuclear volume and transports them closer to the cortex where the spindle assembles in a manner that is comparable to a basket (Gard, 1992; Huchon et al., 1981). Then, the *Xenopus* meiosis I spindle rotates from an orientation that is parallel to the cortex to a perpendicular one in metaphase (Gard, 1992). The site of spindle anchoring at the cortex is overlaid by a cap of high F-actin density (Gard et al., 1995).

Microtubules and actin filaments interact during the entire process of spindle assembly, rotation and cortical anchoring as shown by drug-induced disruption of the F-actin cytoskeleton, which perturbs the normal organisation of the TMA and rotation of the meiosis I spindle perpendicular to the cortex (Gard, 1992; Huchon et al., 1981). The integrating factor of these two cytoskeletal networks again is unconventional myosin-X, which binds both F-actin and microtubules and localises to both the meiotic spindle and the cortical region overlying the spindle. Indeed, disruption of myosin-X produces phenotypes that recapitulate those of disassembly of microtubules or F-actin (Weber et al., 2004).

Starfish oocytes are 150–200 µm in diameter and are among the rare oocytes that exhibit centrosomes and astral microtubules (Miyazaki et al., 2000); they are thus somewhat outside of the scope of this Commentary. Nevertheless, they display a very interesting example of intracellular transport by an F-actin mesh. In this model system, the chromosomes are scattered inside an enormous nuclear volume (the nucleus is 80 µm in diameter), whereas the microtubule asters extend only 30 µm away from the animal pole cortex. Consequently, the chromosomes are clearly out of reach of microtubules, which makes their capture difficult as in *Xenopus* oocytes. Imaging with the GFP–UtrCh probe reveals the formation of an F-actin meshwork inside the nucleus during the breakdown of the nuclear envelope (Fig. 3) (Lénárt

et al., 2005; Mori et al., 2011). This meshwork is believed to drive chromosome gathering by sieving (chromosomes are larger than the mesh size and therefore are captured by the mesh) and by its isotropic contractile behaviour (Lénárt et al., 2005; Mori et al., 2011). Although this example relates to transport in the range of the nuclear size, it is nevertheless relevant. Because the starfish oocyte nucleus is as big as mammalian oocytes, this similarity in scale might reflect a similarity in transport mechanisms in mammalian oocytes, in particular with regard to spindle transport.

Spindle migration in mouse oocytes

Mouse oocytes are also large cells, with a diameter of 80 µm. They are isolated from external forces and like most mammalian oocytes, they show no sign of polarisation in prophase I of meiosis. In these cells, the spindle assembles in the cell center, where the nucleus was formerly located. Spindle migration and asymmetric division are thus dependent on a symmetry-breaking event that comes from within the cell (Azoury et al., 2011; Verlhac et al., 2000). The direction of spindle migration, along its long axis, is randomly defined by the one spindle pole that is closest to the cortex, which becomes the 'leading pole' and takes the shortest path to the cortex, with a velocity of 0.12 µm/minute over a period of 2–3 hours (Verlhac et al., 2000). Unlike in *Xenopus* oocytes, the first meiotic spindle reaches the cortex with its leading pole facing forward and therefore requires no further rotation (Verlhac et al., 2000). Spindle migration in meiosis I is actin dependent (Longo and Chen, 1985; Verlhac et al., 2000), similar to chromosome gathering in starfish oocytes. Recently, the same F-actin probe GFP–UtrCh that contributed to the observation of the actin cluster in mitotic cultured cells (Mitsushima et al., 2010) allowed the detection of an F-actin meshwork in the cytoplasm of mouse oocytes. Importantly, structures detected with UtrCh were also detected using fluorescent-tagged phalloidin on fixed samples (Schuh and Ellenberg, 2008; Yi et al., 2011). This meshwork, which is very dynamic, consists of numerous thin filaments and crossing points and an 'actin cage' around the spindle (Fig. 1B; Azoury et al., 2008; Schuh and Ellenberg, 2008). Formation of this meshwork is dependent on formin-2, a nucleator of straight actin filaments, in cooperation with the spire protein homologues Spire 1 and Spire 2, because the absence or inhibition of these nucleators impairs the formation of the meshwork (Azoury et al., 2008; Pfender et al., 2011; Schuh and Ellenberg, 2008). Their absence or inhibition also impairs spindle migration (Dumont et al., 2007;
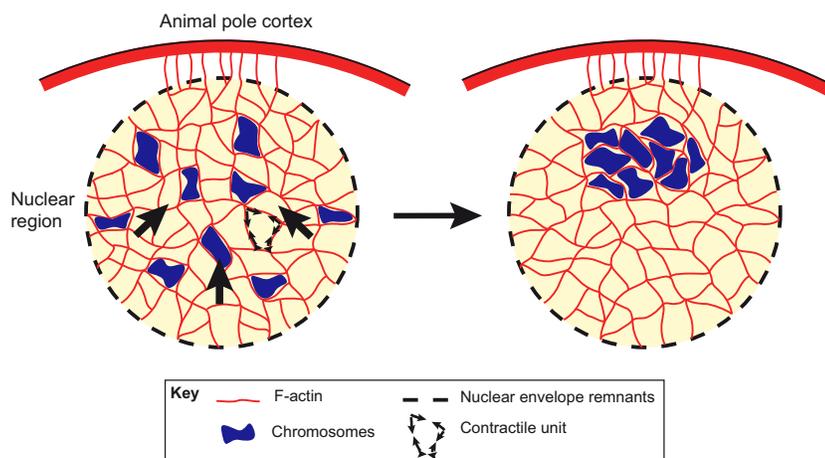

Fig. 3. Chromosome gathering in starfish oocytes. In starfish oocytes, chromosomes are scattered inside a big nuclear volume (left) and need to be brought close to the animal pole cortex for a correct asymmetric division (shown on the right where chromosomes are near the cortex). This process involves an entangled F-actin meshwork that forms inside the nucleus during the breakdown of the nuclear envelope. Chromosomal transport close to the cortex relies on three features of this meshwork. First, its mesh size is smaller than chromosomes, so they become trapped within the meshwork. Second, the trapped chromosomes passively follow the filament movements within the meshwork, which is subject to homogeneous contractions (as indicated by the small black arrows). Eventually, the direction of transport towards the cortex, despite the isotropic contraction of the meshwork, is provided by anchoring of the meshwork at the cortex.

Key
F-actin
Chromosomes
Nuclear envelope remnants
Contractile unit

Pfender et al., 2011), which confirms the involvement of the actin meshwork in this process. Although the nucleation and the architecture of this actin cytoplasmic meshwork have been described, the exact mechanisms underlying symmetry breaking and spindle migration are still under active investigation. Nevertheless, these studies have provided possible explanations for how the meshwork could drive the spindle towards the cortex.

Symmetry breaking has been proposed to require a specific destabilisation of the meshwork concomitant with a drop in the levels of formin-2 upon resumption of meiosis. Indeed, overexpression of formin-2 prevents spindle migration by trapping the assembling spindle in the cell centre in a dense mesh of actin filaments (Azoury et al., 2011).

Furthermore, the connection of the actin cage that surrounds the spindle with the actin-rich cortex (Azoury et al., 2008; Schuh and Ellenberg, 2008) together with the accumulation of active myosin-II at both spindle poles (Fig. 1B; Schuh and Ellenberg, 2008) suggest an active role for myosin-II in anchoring the spindle at the cortex and pulling on it. Impairment of spindle migration upon treatment with the myosin-II inhibitor ML7 supports this hypothesis (Chaigne et al., 2013; Schuh and Ellenberg, 2008). According to this model, the leading pole of the spindle that is initially closer to the cortex is pulled with more strength than the other pole, bringing it even closer to the cortex and therefore reinforcing the initial slight asymmetry in spindle positioning (Fig. 1B).

Another model has been put forward by concurrent work that used Lifeact instead of GFP–UtrCh to detect intracellular F-actin. This probe does not allow the detection of the cytoplasmic actin meshwork, but permits the visualisation of a cloud of actin surrounding the chromosomes. This cloud of actin is enriched in the region opposite the direction of migration of the chromosomes (Li et al., 2008). On the basis of this result, the authors proposed a model that involves DNA-induced actin polymerisation, reminiscent of the *Listeria* actin 'comet tails', that would generate pushing forces behind the chromosomes (Li et al., 2008). A number of recent reviews have already discussed the discrepancy between the use of Lifeact in oocytes compared with GFP–UtrCh (Fabritius et al., 2011; Field and Lénárt, 2011; Clift and Schuh, 2013). Future work is required to elucidate the importance of the chromosome-induced actin cloud in positioning of the meiotic spindle.

Novel aspects of actin-based spindle positioning: contribution from the mouse oocyte model

Recently, two studies from different labs revealed new levels of complexity in the organisation of the actin-based structures that drive spindle positioning in mouse oocytes. The first one, from the Schuh laboratory, proposes a vesicle-driven mechanism for the regulation of the F-actin cytoplasmic meshwork (Holubcová et al., 2013). Previous work in this laboratory revealed the existence of a collective directional movement of vesicles towards the cortex (Schuh, 2011). These vesicles, which are coated with the small GTPase Rab11a, myosin-Vb, formin-2, Spire 1 and Spire 2, are seeds for filament nucleation and were shown to indeed correspond to branching points that are observed within the mesh (Fig. 1B) (Azoury et al., 2008; Schuh and Ellenberg, 2008). The presence of the meshwork nucleators formin-2, Spire 1 and Spire 2 and the plus-end-directed motor myosin-Vb on the same vesicles allows for a very efficient outward-directed movement, in which the vesicles themselves generate the tracks for their own transport towards the cortex. Such a long-range actin-mediated vesicle

transport had not previously been reported and actin filaments have only been implicated in local vesicle transport, for instance in the actin cortex and in dendritic spines (Wagner et al., 2011; Wagner et al., 2011; Wu et al., 1998). This outward-directed movement of vesicles and actin filaments also drives asymmetric spindle positioning, because depletion of Rab11a-positive vesicles and stabilisation of the actin meshwork by overexpression of the GFP–UtrCh probe impair spindle migration (Holubcová et al., 2013).

In addition to the role of such a vesicle-based transport in meiotic spindle positioning, this study also proposes an interesting mechanism for the organisation of dynamic actin networks. It reveals an interdependent and reciprocal relationship between meshwork density and vesicle number and size; the higher the meshwork density, the smaller and the more abundant the vesicles, and the lower the meshwork density, the larger and the less abundant the vesicles. This suggests a self-regulation of the meshwork density by the number and size of Rab11-positive vesicles. In this situation, the presence of the meshwork nucleators on the vesicles themselves renders these vesicle-based actin meshworks highly robust and adaptable.

This study also sheds some light on how such an entangled actin meshwork can be generated from linear actin filaments. One would expect filament nucleation by the Arp2/3 complex to generate branched filaments, as it is the case for the actin cluster in mitotic cells (Mitsushima et al., 2010). But it has been suggested that myosin-II has a lower affinity for filaments that are generated by Arp2/3, so that these meshworks become less prone to contract (Levayer and Lecuit, 2012). Conversely, meshworks made out of linear filaments are highly contractile, which is very important for myosin-II-mediated pulling at the spindle poles in mouse oocytes.

The second study comes from our laboratory and is based on the initial observation of a thickening of cortical F-actin that appears during meiosis I (Fig. 1B) (Azoury et al., 2008), whose biological significance we aimed to address more recently (Chaigne et al., 2013). Using a biophysical approach based on micropipette aspiration, we were able to link the thickening of cortical F-actin to an increase in cortical plasticity, concomitant with a drop in cortical tension during meiosis I (Fig. 2B). The drop in cortical tension in meiosis I has been previously observed but neither its molecular basis nor its potential function had been addressed (Larson et al., 2010). We deciphered the molecular pathways that lead to the thickening of the cortical F-actin and the drop in cortical tension, both events that unexpectedly soften the cortex (Chaigne et al., 2013). First, we found the thickening of the cortical F-actin to be Arp2/3 dependent, and second, we correlated the drop in cortical tension with an exclusion of myosin-II from the cortex. Third, we found the Mos–MAPK pathway to be upstream of this cascade; its activation on one hand promotes the thickening of the cortical F-actin, at least in part, by regulating the nucleation-promoting factor Wave 2 and thus regulating the activity of the Arp2/3 complex. On the other hand, the Mos–MAPK pathway triggers the exclusion of myosin-II from the cortex.

Interestingly, cortical softening appears to have a major role in spindle migration during meiosis I. Indeed, spindle migration is impaired in $Mos^{-/-}$ oocytes that do not turn on the MAPK pathway (Verlhac et al., 2000) and in oocytes in which Arp2/3 function has been inhibited (Sun et al., 2011; Chaigne et al., 2013). Furthermore, oocytes whose cortex is artificially stiffened by treatment with concanavalin A, a tetravalent lectin that

extensively crosslinks sugar residues of glycoprotein from the plasma membrane and is often used to stiffen the cortex (Kunda et al., 2008; Larson et al., 2010; Pasternak and Elson, 1985; Pasternak et al., 1989), have spindle migration defects, thus directly demonstrating that a soft cortex is essential for spindle migration (Chaigne et al., 2013).

This result is rather puzzling because in mitosis the situation is exactly the opposite: spindle assembly and positioning are positively regulated by cell rounding, which is consequence of an increase in cortical stiffness (Carreno et al., 2008; Kunda et al., 2008). Moreover, mouse oocytes remain round during meiosis I, making these findings even more confusing. Fortunately, mathematical modelling has helped to resolve these apparent contradictions (Chaigne et al., 2013). The modelling is based on the forces that are applied on the two spindle poles by a contractile actin-myosin mesh (Schuh and Ellenberg, 2008) and on the action-reaction effect of these forces on the cortex. By introducing parameters that depend on cortex stiffness, the modelling predicted that a softer cortex actually amplifies the initial imbalance of forces that act at the poles and thus accelerates spindle movement to the cortex that is closer (Chaigne et al., 2013). The modelling also predicted that a stiff cortex would slow down spindle migration and thus the spindle would not have time to reach the cortex during the frame timing of meiosis I.

Our work actually fits well with the model for myosin-II-dependent pulling from the spindle poles (Schuh and Ellenberg, 2008). Indeed, the thickening of the cortical F-actin allows the reduction of the distance between the actin cage surrounding the spindle, favouring a pulling of myosin-II from the spindle poles on the cortex (Fig. 1B). Pulling on a soft, deformable cortex makes the transmission of forces easier and the cortex can respond to the exerted force by dragging the spindle towards the periphery. By contrast, pulling on a stiff, undeformable cortex such as that in $Mos^{-/-}$ oocytes would require considerably more force than can actually be exerted by myosin-II to drag the spindle from the centre of the cell, explaining why the spindle cannot reach the cortex in this scenario.

These recent studies, in addition to proposing a new mechanism for asymmetric spindle positioning in mouse oocytes, also introduce a time dimension through the regulation by the Mos–MAPK pathway (Chaigne et al., 2013). Moreover, they highlight the contribution that biophysical approaches can bring to cell biology, particularly in the case of large cells such as mouse oocytes, in which physical parameters including size and shape are well controlled.

CONCLUSION

The examples discussed in this Commentary clearly indicate that spindle positioning is largely dependent on actin in meiosis, either through interactions between actin and microtubules, as is the case in *Xenopus* oocytes, or through 'bulk' actin as in mouse oocytes. In somatic cells, microtubules are favoured for long range transport and their physical properties, in particular their rigidity, provide a partial explanation for this. The persistence length of microtubules, namely the distance over which the polymer can transmit force, is around 5200 µm compared with only 17.7 µm for actin (Gittes et al., 1993), so they are very well suited for intracellular trafficking, especially radial arrays such as asters. Owing to their low rigidity, actin microfilaments have to be crosslinked into bundles or organised into actin meshes in order to be able to transmit forces, and under these conditions, they can generate movement of large organelles within the cell. The forces produced can result from actin polymerisation itself; *in vitro*, linear actin filaments can induce forces in the piconewton range (Kovar and Pollard, 2004) and *in vivo*, actin polymerisation can

generate sustained forces to push against the membrane at the leading edge of a migrating cell (Pollard and Borisy, 2003). Organisation of actin filaments into antiparallel structures allows for the emergence of an important property of actin networks – contractility – which is an extensive source of force generation (Levayer and Lecuit, 2012). This contractile behaviour allows chromosome gathering in starfish oocytes and partly, spindle migration in mouse oocytes, although here the role of actin polymerisation per se remains more difficult to address. Dissection of these actin-dependent transport mechanisms in oocytes might be highly informative, in particular in view of the recent discovery of new pools of F-actin in somatic cells and their possible role in spindle positioning, such as the actin cables around the spindle and between the spindle and the cortex (Woolner et al., 2008), and cytoplasmic actin pools (Fink et al., 2011; Mitsushima et al., 2010).

Throughout this Commentary, we have introduced current actin-based mechanisms of spindle positioning that act from somatic to germinal cells, as well as in symmetric and asymmetric divisions. To further understand these mechanisms, it will be important to precisely elucidate the architecture of these actin networks. This would help to formulate the more general principles of actin network organisation that might be valid for mechanisms other than spindle positioning and intracellular transport, and for other model systems. Closely related are improvements in the imaging techniques used to visualise actin filaments and the extensive use of universal probes such as GFP–UtrCh, which are anticipated to result in new and exciting discoveries.

Dealing with actin networks can be challenging for biologists who are more familiar with molecular pathways than with collective behaviour of actin polymers. Coupling powerful imaging techniques with physical approaches and mathematical modelling will be of great help to clarify the apparently non-intuitive behaviours of actin networks and can contribute to further understanding at the level of intracellular mechanisms. Expectations for this field are high because these actin-based mechanisms are involved in pathologies such as cancer in the case of somatic cells, and infertility in the case of oocytes, but the adoption of interdisciplinary approaches has great promise in helping to gain a better understanding of these processes.

References

Albertson, D. G. and Thomson, J. N. (1993). Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. *Chromosome Res.* **1**, 15-26.

Almonacid, M. and Paoletti, A. (2010). Mechanisms controlling division-plane positioning. *Semin. Cell Dev. Biol.* **21**, 874-880.

Azoury, J., Lee, K. W., Georget, V., Rassinier, P., Leader, B. and Verlhac, M.-H. (2008). Spindle positioning in mouse oocytes relies on a dynamic meshwork of actin filaments. *Curr. Biol.* **18**, 1514-1519.

Azoury, J., Lee, K. W., Georget, V., Hikal, P. and Verlhac, M.-H. (2011). Symmetry breaking in mouse oocytes requires transient F-actin meshwork destabilization. *Development* **138**, 2903-2908.

Bowman, S. K., Neumüller, R. A., Novatchkova, M., Du, Q. and Knoblich, J. A. (2006). The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. *Dev. Cell* **10**, 731-742.

Brunet, S. and Verlhac, M. H. (2011). Positioning to get out of meiosis: the asymmetry of division. *Hum. Reprod. Update* **17**, 68-75.

Burkel, B. M., von Dassow, G. and Bement, W. M. (2007). Versatile fluorescent probes for actin filaments based on the actin-binding domain of utrophin. *Cell Motil. Cytoskeleton* **64**, 822-832.

Carreno, S., Kouranti, I., Glusman, E. S., Fuller, M. T., Echard, A. and Payre, F. (2008). Moesin and its activating kinase Slik are required for cortical stability and microtubule organization in mitotic cells. *J. Cell Biol.* **180**, 739-746.

Chaigne, A., Campillo, C., Gov, N. S., Voituriez, R., Azoury, J., Umaña-Diaz, C., Almonacid, M., Queguiner, I., Nassoy, P., Sykes, C. et al. (2013). A soft cortex is essential for asymmetric spindle positioning in mouse oocytes. *Nat. Cell Biol.* **15**, 958-966.

Clift, D. and Schuh, M. (2013). Restarting life: fertilization and the transition from meiosis to mitosis. *Nat. Rev. Mol. Cell Biol.* **14**, 549-562.

Couwenbergs, C., Labbé, J.-C., Goulding, M., Marty, T., Bowerman, B. and Gotta, M. (2007). Heterotrimeric G protein signaling functions with dynein to promote spindle positioning in C. elegans. *J. Cell Biol.* **179**, 15-22.

Dumont, J. N. (1972). Oogenesis in Xenopus laevis (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**, 153-179.

Dumont, J., Million, K., Sunderland, K., Rassinier, P., Lim, H., Leader, B. and Verlhac, M.-H. (2007). Formin-2 is required for spindle migration and for the late steps of cytokinesis in mouse oocytes. *Dev. Biol.* **301**, 254-265.

Fabritius, A. S., Ellefson, M. L. and McNally, F. J. (2011). Nuclear and spindle positioning during oocyte meiosis. *Curr. Opin. Cell Biol.* **23**, 78-84.

Field, C. M. and Lénárt, P. (2011). Bulk cytoplasmic actin and its functions in meiosis and mitosis. *Curr. Biol.* **21**, R825-R830.

Fink, J., Carpi, N., Betz, T., Bétard, A., Chebah, M., Azioune, A., Bornens, M., Sykes, C., Fetler, L., Cuvelier, D. et al. (2011). External forces control mitotic spindle positioning. *Nat. Cell Biol.* **13**, 771-778.

Gard, D. L. (1992). Microtubule organization during maturation of Xenopus oocytes: assembly and rotation of the meiotic spindles. *Dev. Biol.* **151**, 516-530.

Gard, D. L. (1993). Ectopic spindle assembly during maturation of Xenopus oocytes: evidence for functional polarization of the oocyte cortex. *Dev. Biol.* **159**, 298-310.

Gard, D. L., Cha, B. J. and Roeder, A. D. (1995). F-actin is required for spindle anchoring and rotation in Xenopus oocytes: a re-examination of the effects of cytochalasin B on oocyte maturation. *Zygote* **3**, 17-26.

Gillies, T. E. and Cabernard, C. (2011). Cell division orientation in animals. *Curr. Biol.* **21**, R599-R609.

Gittes, F., Mickey, B., Nettleton, J. and Howard, J. (1993). Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J. Cell Biol.* **120**, 923-934.

Gönczy, P. (2002). Mechanisms of spindle positioning: focus on flies and worms. *Trends Cell Biol.* **12**, 332-339.

Gönczy, P. (2008). Mechanisms of asymmetric cell division: flies and worms pave the way. *Nat. Rev. Mol. Cell Biol.* **9**, 355-366.

Green, R. A., Paluch, E. and Oegema, K. (2012). Cytokinesis in animal cells. *Annu. Rev. Cell Dev. Biol.* **28**, 29-58.

Hertwig, O. (1884). Das problem der befruchtung und der isotropie des eies. eine theorie der vererbung. *Jenaische Zeitschrift für Naturwissenschaft* **18**, 276-318.

Hirano, Y., Hatano, T., Takahashi, A., Toriyama, M., Inagaki, N. and Hakoshima, T. (2011). Structural basis of cargo recognition by the myosin-X MyTH4-FERM domain. *EMBO J.* **30**, 2734-2747.

Holubcová, Z., Howard, G. and Schuh, M. (2013). Vesicles modulate an actin network for asymmetric spindle positioning. *Nat. Cell Biol.* **15**, 937-947.

Huchon, D., Crozet, N., Cantenot, N. and Ozon, R. (1981). Germinal vesicle breakdown in the Xenopus laevis oocyte: description of a transient microtubular structure. *Reprod. Nutr. Dev.* **21**, 135-148.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T. and Matsuzaki, F. (2006). Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. *Nat. Cell Biol.* **8**, 586-593.

Knoblich, J. A. (2010). Asymmetric cell division: recent developments and their implications for tumour biology. *Nat. Rev. Mol. Cell Biol.* **11**, 849-860.

Kovar, D. R. and Pollard, T. D. (2004). Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces. *Proc. Natl. Acad. Sci. USA* **101**, 14725-14730.

Kunda, P., Pelling, A. E., Liu, T. and Baum, B. (2008). Moesin controls cortical rigidity, cell rounding, and spindle morphogenesis during mitosis. *Curr. Biol.* **18**, 91-101.

Larson, S. M., Lee, H. J., Hung, P. H., Matthews, L. M., Robinson, D. N. and Evans, J. P. (2010). Cortical mechanics and meiosis II completion in mammalian oocytes are mediated by myosin-II and Ezrin-Radixin-Moesin (ERM) proteins. *Mol. Biol. Cell* **21**, 3182-3192.

Lénárt, P., Bacher, C. P., Daigle, N., Hand, A. R., Eils, R., Terasaki, M. and Ellenberg, J. (2005). A contractile nuclear actin network drives chromosome congression in oocytes. *Nature* **436**, 812-818.

Levayer, R. and Lecuit, T. (2012). Biomechanical regulation of contractility: spatial control and dynamics. *Trends Cell Biol.* **22**, 61-81.

Li, H., Guo, F., Rubinstein, B. and Li, R. (2008). Actin-driven chromosomal motility leads to symmetry breaking in mammalian meiotic oocytes. *Nat. Cell Biol.* **10**, 1301-1308.

Longo, F. J. and Chen, D. Y. (1985). Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus. *Dev. Biol.* **107**, 382-394.

Maddox, A. S. and Burridge, K. (2003). RhoA is required for cortical retraction and rigidity during mitotic cell rounding. *J. Cell Biol.* **160**, 255-265.

Mao, Y., Tournier, A. L., Bates, P. A., Gale, J. E., Tapon, N. and Thompson, B. J. (2011). Planar polarization of the atypical myosin Dachs orients cell divisions in Drosophila. *Genes Dev.* **25**, 131-136.

Mitsushima, M., Aoki, K., Ebisuya, M., Matsumura, S., Yamamoto, T., Matsuda, M., Toyoshima, F. and Nishida, E. (2010). Revolving movement of a dynamic cluster of actin filaments during mitosis. *J. Cell Biol.* **191**, 453-462.

Miyazaki, A., Kamitsubo, E. and Nemoto, S. I. (2000). Premeiotic aster as a device to anchor the germinal vesicle to the cell surface of the presumptive animal pole in starfish oocytes. *Dev. Biol.* **218**, 161-171.

Moore, J. K. and Cooper, J. A. (2010). Coordinating mitosis with cell polarity: Molecular motors at the cell cortex. *Semin. Cell Dev. Biol.* **21**, 283-289.

Mori, M., Monnier, N., Daigle, N., Bathe, M., Ellenberg, J. and Lénárt, P. (2011). Intracellular transport by an anchored homogeneously contracting F-actin meshwork. *Curr. Biol.* **21**, 606-611.

Morin, X. and Bellaïche, Y. (2011). Mitotic spindle orientation in asymmetric and symmetric cell divisions during animal development. *Dev. Cell* **21**, 102-119.

Nguyen-Ngoc, T., Afshar, K. and Gönczy, P. (2007). Coupling of cortical dynein and G alpha proteins mediates spindle positioning in Caenorhabditis elegans. *Nat. Cell Biol.* **9**, 1294-1302.

Pasternak, C. and Elson, E. L. (1985). Lymphocyte mechanical response triggered by cross-linking surface receptors. *J. Cell Biol.* **100**, 860-872.

Pasternak, C., Spudich, J. A. and Elson, E. L. (1989). Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. *Nature* **341**, 549-551.

Pfender, S., Kuznetsov, V., Pleiser, S., Kerkhoff, E. and Schuh, M. (2011). Spire-type actin nucleators cooperate with Formin-2 to drive asymmetric oocyte division. *Curr. Biol.* **21**, 955-960.

Pollard, T. D. and Borisy, G. G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453-465.

Rappaport, R. and Rappaport, B. N. (1974). Establishment of cleavage furrows by the mitotic spindle. *J. Exp. Zool.* **189**, 189-196.

Riedl, J., Crevenna, A. H., Kessenbrock, K., Yu, J. H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T. A., Werb, Z. et al. (2008). Lifeact: a versatile marker to visualize F-actin. *Nat. Methods* **5**, 605-607.

Rosenblatt, J., Cramer, L. P., Baum, B. and McGee, K. M. (2004). Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. *Cell* **117**, 361-372.

Sathananthan, A. H. (1997). Ultrastructure of the human egg. *Hum. Cell* **10**, 21-38.

Schuh, M. (2011). An actin-dependent mechanism for long-range vesicle transport. *Nat. Cell Biol.* **13**, 1431-1436.

Schuh, M. and Ellenberg, J. (2008). A new model for asymmetric spindle positioning in mouse oocytes. *Curr. Biol.* **18**, 1986-1992.

Siller, K. H. and Doe, C. Q. (2008). Lis1/dynactin regulates metaphase spindle orientation in Drosophila neuroblasts. *Dev. Biol.* **319**, 1-9.

Siller, K. H. and Doe, C. Q. (2009). Spindle orientation during asymmetric cell division. *Nat. Cell Biol.* **11**, 365-374.

Siller, K. H., Cabernard, C. and Doe, C. Q. (2006). The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. *Nat. Cell Biol.* **8**, 594-600.

Sun, S. C., Wang, Z. B., Xu, Y. N., Lee, S. E., Cui, X. S. and Kim, N. H. (2011). Arp2/3 complex regulates asymmetric division and cytokinesis in mouse oocytes. *PLoS ONE* **6**, e18392.

Szollosi, D., Calarco, P. and Donahue, R. P. (1972). Absence of centrioles in the first and second meiotic spindles of mouse oocytes. *J. Cell Sci.* **11**, 521-541.

Théry, M., Racine, V., Pépin, A., Piel, M., Chen, Y., Sibarita, J.-B. and Bornens, M. (2005). The extracellular matrix guides the orientation of the cell division axis. *Nat. Cell Biol.* **7**, 947-953.

Theurkauf, W. E. and Hawley, R. S. (1992). Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* **116**, 1167-1180.

Toyoshima, F. and Nishida, E. (2007). Integrin-mediated adhesion orients the spindle parallel to the substratum in an EB1– and myosin X-dependent manner. *EMBO J.* **26**, 1487-1498.

Verlhac, M. H., Lefebvre, C., Guillaud, P., Rassinier, P. and Maro, B. (2000). Asymmetric division in mouse oocytes: with or without Mos. *Curr. Biol.* **10**, 1303-1306.

von Dassow, G. (2009). Concurrent cues for cytokinetic furrow induction in animal cells. *Trends Cell Biol.* **19**, 165-173.

Wagner, W., Brenowitz, S. D. and Hammer, J. A., III (2011). Myosin-Va transports the endoplasmic reticulum into the dendritic spines of Purkinje neurons. *Nat. Cell Biol.* **13**, 40-48.

Wang, C., Li, S., Januschke, J., Rossi, F., Izumi, Y., Garcia-Alvarez, G., Gwee, S. S. L., Soon, S. B., Sidhu, H. K., Yu, F. et al. (2011). An ana2/ctp/mud complex regulates spindle orientation in Drosophila neuroblasts. *Dev. Cell* **21**, 520-533.

Weber, K. L., Sokac, A. M., Berg, J. S., Cheney, R. E. and Bement, W. M. (2004). A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* **431**, 325-329.

Woolner, S., O'Brien, L. L., Wiese, C. and Bement, W. M. (2008). Myosin-10 and actin filaments are essential for mitotic spindle function. *J. Cell Biol.* **182**, 77-88.

Wu, X., Bowers, B., Rao, K., Wei, Q. and Hammer J. A., III (1998). Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function In vivo. *J. Cell Biol.* **143**, 1899-1918.

Yi, K., Unruh, J. R., Deng, M., Slaughter, B. D., Rubinstein, B. and Li, R. (2011). Dynamic maintenance of asymmetric meiotic spindle position through Arp2/3-complex-driven cytoplasmic streaming in mouse oocytes. *Nat. Cell Biol.* **13**, 1252-1258.