Vesicles modulate an actin network for asymmetric spindle positioning

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Actin networks drive many essential cellular processes, including cell migration, cytokinesis and tissue morphogenesis. However, how cells organize and regulate dynamic actin networks that consist of long, unbranched actin filaments is only poorly understood. This study in mouse oocytes reveals that cells can use vesicles as adaptable, motorized network nodes to regulate the dynamics and density of intracellular actin networks. In particular, Rab11a-positive vesicles drive the network dynamics in a myosin-Vb-dependent manner, and modulate the network density by sequestering and clustering the network’s actin nucleators. We also report a simple way by which networks of different densities can be generated, namely by adjusting the number and volume of vesicles in the cell. This vesicle-based mechanism of actin network modulation is essential for asymmetric positioning of the meiotic spindle in mouse oocytes, a vital step in the development of a fertilizable egg in mammals.

Dynamic actin networks have many essential functions in cells: they are required for cell migration1,2, cytoplasmic organization1, ingression of the cytokinetic furrow3,4,5, cortical flows6,7 and tissue morphogenesis8,9. Many actin networks consist of long, unbranched actin filaments, such as those that are nucleated by formins. However, little is known about how these linear filaments are reorganized into dynamic networks and how the dynamics and the density of these networks are controlled. More is known about Arp2/3-dependent actin networks. Here, network assembly depends on Arp2/3-dependent branching of actin filaments from the side of existing filaments. Arp2/3-nucleated networks are very dense and show relatively low contractility because they consist of crosslinked filaments6,10. Thus, network reorganization requires unbranching and filament severing2,11,12. We and others reported that mouse oocytes are filled with a highly dynamic actin network, which is nucleated by cooperation between two types of actin nucleator that assemble linear actin filaments: the formin Fmn2 and the recently discovered actin nucleators Spire1 and Spire213–16. We reported recently that this actin network mediates long-range transport of vesicles17. In particular, we found that vesicles that are positive for the small GTPase Rab11a, which determines the vesicles’ identity and function by recruiting vesicle-specific effector proteins18, move over distances of up to 30 µm along actin filaments towards the oocyte surface17. This was surprising given that long-range vesicle transport is generally thought to occur along microtubules in animal cells, whereas transport along actin filaments is thought to be most relevant over short distances in specialized subcellular compartments such as dendritic spines and the actin cortex9,20.

In this study, we set out to investigate the function of the Rab11a-positive vesicles that are transported by this actin-dependent mechanism in mouse oocytes. Unexpectedly, we found that these vesicles function as cytoskeletal modulators. The vesicles drive the dynamics of the actin network by recruiting myosin Vb to reorganize actin filaments, and regulate the network density by sequestering and clustering the nucleators of the network. The dynamic actin networks that are generated by this vesicle-dependent mechanism are robust, adaptable and rapidly restore a dynamic steady state after reversal of perturbation. We also show that Rab11a-positive vesicles, myosin Vb and the vesicle-dependent dynamics of the actin network are required for asymmetric spindle positioning during oocyte maturation, an essential step in the development of a fertilizable egg in mammals. On the basis of our data, we suggest a model in which the movement of the spindle to the oocyte’s surface is driven by the outward-directed dynamics of the vesicles and their associated actin filaments.

RESULTS
Asymmetric spindle positioning in oocytes requires Rab11a-positive vesicles

To investigate the function of the Rab11a-positive vesicles, we expressed a dominant-negative variant of Rab11a, Rab11a S25N. This led to the loss of Rab11a-positive vesicles from oocytes (Fig. 1a,b), which we confirmed by analysing the localization of the vesicles’ cargo transferrin (Supplementary Fig. S1). We then analysed how the loss of Rab11a-positive vesicles affected oocyte maturation, the process by which an oocyte matures into a fertilizable egg. In the first step of

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Asymmetric spindle positioning in oocytes requires Rab11a-positive vesicles. (a) mEGFP–Rab11a localization in control oocytes expressing mEGFP–Rab11a and in oocytes expressing mEGFP–Rab11a together with mCherry–Rab11a S25N (Rab11a S25N) (z-projection, 5 sections, every 0.66 μm). Outlined regions are magnified in inset. Scale bar, 10 μm. Isosurfaces of the oocyte (light grey), nucleus (dark grey) and vesicles (magenta) were reconstructed from oocytes shown on the left. (b) The number of Rab11a-positive vesicles in control and dominant-negative Rab11a-expressing oocytes. Vesicles were identified and counted with the spot detection function of Imaris in an oocyte segment of 20 × 20 × 20 μm³. The number of analysed oocytes is specified in brackets above each bar (aggregation over 3 independent experiments). Data are mean, with error bars showing s.d. P values were calculated with Student’s t-test. (c) Spindle movements in live oocytes expressing EGFP–MAP4 (magenta, microtubules, merged with differential interference contrast (DIC)). Oocytes express mCherry fused to Rab11a (Rab11a), dominant-negative Rab11a (Rab11a S25N), dominant-negative Rab5a (Rab5a S34N) or dominant-negative Rab27a (Rab27a T23N). White ovals mark initial spindle positions. Scale bar, 10 μm. (d) The spindle was tracked in 3D data sets (11 sections, every 8 μm) of oocytes as shown in c and spindle movements were plotted. The number of analysed oocytes is specified in brackets to the right of each oocyte type (aggregation over 3–4 independent experiments). Data are mean, with error bars showing s.d. (e) The spindle speeds were determined from the plots in d. The box plot shows median (line), mean (small square), and 25th, 75th (boxes), 5th and 95th percentile (whiskers) of spindle speeds. The number of analysed oocytes is specified in brackets above each box. (f) The efficiency of asymmetric spindle positioning in oocytes as shown in c. The number of analysed oocytes is specified in brackets above each plot (aggregation over 4–9 independent experiments).

maturation, the nucleus breaks down and a spindle assembles in the centre of the oocyte. The spindle is then asymmetrically positioned, which is a prerequisite for the asymmetric meiotic division that ensures that the egg contains sufficient storage material for embryo development and is crucial for fertility. At the oocyte surface, the spindle segregates the homologous chromosomes and extrudes half of them in a very small cell termed the polar body. The other half of the chromosomes remains in the large egg, which is now ready for fertilization. Depletion of Rab11a-positive vesicles affected neither the morphology (Supplementary Fig. S2a and Fig. 1c) nor the polarization of the oocytes, as indicated by the formation of an actin-rich and cortical-granule-free domain at the cortex in proximity of the spindle (Supplementary Fig. S2b,c). Likewise, the fraction of oocytes that underwent nuclear envelope breakdown, spindle assembly and anaphase, and also the timing of meiotic progression were not significantly different (Supplementary Fig. S2d–h). Instead, we found that depletion of Rab11a-positive vesicles prevented asymmetric spindle positioning (Fig. 1c,f). To quantify this defect, we recorded three-dimensional (3D) data sets of spindle movement and tracked the spindle automatically. This confirmed that the spindle was static in
Figure 2 Rab11a-positive vesicles drive the actin network dynamics. (a) Oocyte expressing EGFP–UtrCH (F-actin) together with mCherry–Rab11a (vesicles). A merge of the F-actin (magenta) and vesicle (green) localization is shown on the left, and the vesicle localization separately on the right. Representative example from 4 independent experiments (>40 oocytes total). Scale bar, 5 µm. (b) F-actin dynamics in oocytes expressing EGFP–UtrCH (F-actin) together with mCherry–Rab11a (control) or dominant-negative Rab11a (Rab11a S25N). Projections are time-coloured in RGB (green, blue and red with increasing time). Scale bar, 5 µm. (c) The percentage of oocytes with a dynamic or static actin network. An image correlation coefficient below 0.5 after 30 s was scored as dynamic and above 0.5 as static. The number of analysed oocytes is specified in brackets above each bar (aggregation over 2 independent experiments). (d) Oocytes expressing EGFP–UtrCH (F-actin; single section) together with mCherry–Rab11a (control) or dominant-negative mCherry–Rab11a (Rab11a S25N). Chromosomes (magenta; z-projection: 3 sections every 1.2 µm) were labelled with Hoechst. The outlined region is magnified below. Representative examples from 3 independent experiments (30 oocytes total for each condition). Same oocytes as in Supplementary Video S4. Scale bar, 10 µm.

dominant-negative Rab11a-expressing oocytes (Fig. 1d,e). This defect was specifically due to the depletion of Rab11a-positive vesicles, because dominant-negative variants of other Rab GTPases, such as Rab5a or Rab27a, did not affect asymmetric spindle positioning (Fig. 1c–f).

The dynamics of an actin network depend on Rab11a-positive vesicles

We next analysed the reason why asymmetric spindle positioning failed when Rab11a-positive vesicles were depleted. We and others
reported previously that asymmetric spindle positioning requires the oocyte’s cytoplasmic actin network. Thus, we investigated whether asymmetric spindle positioning might fail because the actin network was affected. We reported recently that the vesicles localize to nodes in the actin network (Fig. 2a and Supplementary Video S1), that they recruit the nucleators of the network and that they serve as sites of actin nucleation. Thus, we reasoned that the vesicles might be required to assemble the cytoplasmic actin network. However, oocytes were still filled with a network of intersecting actin filaments when Rab11a-positive vesicles were depleted (Fig. 2b and Supplementary Video S1). Only the prominent nodes of the actin network, which correspond to the position of the vesicles, were missing. This suggests that the nucleators of the actin network are still active if Rab11a-positive vesicles are absent. Unexpectedly though, the actin network, which is normally very dynamic, was static when Rab11a-positive vesicles were lost (Fig. 2b and Supplementary Video S1). To quantify this defect, we recorded high-resolution time series of the network dynamics in control and dominant-negative Rab11a-expressing oocytes (Supplementary Video S1) and performed an image correlation analysis (for details see Methods). This confirmed that the dynamics of the actin network were severely impaired when the function of Rab11a-positive vesicles was blocked (Fig. 2c and Supplementary Fig. S3a). This result was surprising because it reveals that the actin network dynamics depend on Rab11a-positive vesicles.

The dynamics of the vesicles and their associated actin filaments drive asymmetric spindle positioning

Next, we investigated why asymmetric spindle positioning failed when the vesicle-dependent network dynamics were blocked. We reported previously that the spindle poles locally contract the network’s actin filaments in a myosin light chain kinase-dependent manner and thereby pull on the network. Our recent work suggests that the Rab11a-positive vesicles and the actin filaments that are nucleated from their surface continuously move towards the plasma membrane. Thus, an attractive hypothesis is that the pulling from the spindle poles couples the spindle to the outward-directed movement of the vesicles and their associated actin filaments. In this way, the spindle could move to the plasma membrane similarly to how vesicles move to the plasma membrane. The movement of the spindle to the cell surface could also involve direct temporary associations of vesicles with the spindle, which we observed throughout the entire process of asymmetric spindle positioning (Supplementary Video S2). Weak coupling and higher drag forces could explain why the spindle moves more slowly than the vesicles. To investigate whether the outward-directed dynamics of the vesicles and their associated actin filaments drive asymmetric spindle positioning, we first compared the interaction of the spindle with the vesicle–actin network in control oocytes and in oocytes that expressed dominant-negative Rab11a. Whereas the spindle interacted dynamically with the vesicle–actin network in control oocytes, the spindle was immobile and trapped in the static actin network when Rab11a-positive vesicles were lost (Fig. 2d and Supplementary Fig. S3b, Video S3 and S4). The spindle and the actin network were so static that we could even generate a high-resolution 3D reconstruction of the trapped spindle within the actin network from live oocytes (Supplementary Video S5). To investigate directly whether the dynamics of the vesicles and their associated actin filaments were required for asymmetric spindle positioning, we analysed whether we could artificially trap the spindle by blocking the network dynamics. For this purpose, we stabilized the vesicle–actin network by overexpressing very high amounts of the actin-binding domain of utrophin (Fig. 3a). This did indeed efficiently block asymmetric spindle positioning (Fig. 3b–e). Consistent with this result, the actin-stabilizing drug jasplakinolide also blocked asymmetric spindle positioning (Supplementary Fig. S4). Together, these data are consistent with a model in which the outward-directed vesicle and actin filament dynamics drive asymmetric spindle positioning.

Myosin Vb mediates the actin network dynamics and asymmetric spindle positioning

We then investigated the mechanism that would explain the requirement of vesicles for the dynamics of the actin network. We reasoned that the vesicles must recruit a force generator that mediates the network dynamics by reorganizing filaments of the network. Myosin II has long been implicated in rearranging actin filaments, and recent in vitro data demonstrated that other myosins can also drive the reorganization of actin networks. We reported recently that Rab11a-positive vesicles recruit myosin Vb in mouse oocytes. To investigate whether the actin network dynamics were myosin-Vb-dependent, we blocked myosin Vb function by expressing a dominant-negative tail construct. Dominant-negative myosin Vb blocked the network dynamics (Fig. 4a and Supplementary Video S6), as quantitatively confirmed by image correlation analysis (Fig. 4b and Supplementary Fig. S5a).

Consistent with the model that asymmetric spindle positioning requires the outward-directed vesicle and actin filament dynamics, dominant-negative myosin Vb also blocked asymmetric spindle positioning (Fig. 4c–f). This defect was specifically due to blocking the function of myosin Vb because dominant-negative constructs of related myosins did not affect asymmetric spindle positioning (Fig. 4c–f). Consistent with this result, depletion of myosin Vb by RNA-mediated interference (RNAi) also blocked asymmetric spindle positioning (Supplementary Fig. S5b–e).

Rab11a-positive vesicles modulate the actin network density

To investigate whether the vesicles also modulated other properties of the actin network in addition to its dynamics, we quantified the actin network density in control oocytes and in oocytes expressing dominant-negative Rab11a. In support of the model that the vesicles act as network modulators, the network density was significantly increased in dominant-negative Rab11a-expressing oocytes (Fig. 5a,b). This increase was specifically due to the loss of Rab11a-positive vesicles, because dominant-negative variants of other Rab GTPases did not affect the network density (Fig. 5a,b). Next, we investigated whether a denser network is automatically less dynamic. To this end, we increased the network density to the same degree that we observed on dominant-negative Rab11a expression by moderately overexpressing the network nucleators Fmn2 and Spire2 (Supplementary Fig. S6a,b). Our quantitative network dynamics assay revealed that the network was still dynamic (Supplementary Fig. S6c,d). Also the dynamics of asymmetric spindle positioning were not affected (Supplementary Fig. S6e–h), suggesting that the block of asymmetric spindle positioning in dominant-negative Rab11a-expressing oocytes is not due to the ~2-fold increase in actin

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Figure 3 Blocking vesicle–actin network dynamics prevents asymmetric spindle positioning. (a) An oocyte expressing very high amounts of EGFP–UtrCH (F-actin). Projection is time-coloured in RGB. Scale bar, 5 µm. (b) Spindle movements (magenta, mCherry–MAP4; microtubules, merged with differential interference contrast (DIC)) in a live control oocyte (control) and an oocyte expressing very high amounts of EGFP–UtrCH to stabilize F-actin (UtrCH-stabilized F-actin). White oval marks initial spindle positions. Scale bar, 10 µm. (c) The efficiency of asymmetric spindle positioning in oocytes as shown in b is plotted. The number of analysed oocytes is specified in brackets above each bar (aggregation over 2 independent experiments). (d) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in b and spindle movements were plotted. The number of analysed oocytes is specified in brackets (aggregation over 2 independent experiments). Data are mean, with error bars showing s.d. (e) The spindle speeds were determined from the plots in d. Box plot as in Fig. 1e. The number of analysed oocytes is specified in brackets above each box.

network density, but caused by the absence of the outward-directed dynamics of the vesicles and their associated actin filaments.

Although the increased network density in dominant-negative Rab11a-expressing oocytes was not directly relevant for asymmetric spindle positioning, it still seemed important to investigate the mechanism by which the vesicles modulated the actin network density to improve our understanding of how dynamic actin networks of different densities can be generated. As reported above, we found recently that the nucleators of the actin network localize to the Rab11a-positive vesicles, from where actin filaments were nucleated. We reasoned that recruitment of the nucleators to Rab11a-positive vesicles might help to modulate the precise topology and mesh size of the network. If the nucleators are sequestered and clustered on vesicles, the network density should be decreased (Fig. S1). The Rab11a-positive vesicles do indeed seem suitable for sequestering and clustering the nucleators: their membrane surface is likely to be highly increased because Rab11a, the nucleators of the actin network, the membrane probe FM 1-43 and transferrin were found throughout the entire vesicle by light or electron microscopy (Fig. 5c–g and Supplementary Fig. S7a). This is unlikely to represent localization to the lumen, but might reflect that the membrane of the Rab11a-positive vesicles is highly folded or that the large Rab11a-positive vesicles that we observe consist of aggregates of smaller Rab11a-positive vesicles. As only the fraction of actin nucleator on the periphery of the vesicle is in direct contact with the cytoplasm and thus able to contribute to network formation, the Rab11a-positive vesicles could effectively
limit the amount of nucleator that can participate in network assembly. Expression of dominant-negative Rab11a would release the nucleators from the vesicles and could explain the increase in network density that we observed (Fig. 5l).

To investigate whether the vesicles modulate the network density by sequestering and clustering the nucleators, we first examined how the loss of Rab11a-positive vesicles affected the distribution of the actin nucleators Spire1, Spire2 and Fmn2. We found that all nucleators were released from vesicles in dominant-negative Rab11a-expressing oocytes (Fig. 5h and Supplementary Fig. S7b,c). To recruit overexpressed Fmn2 to Rab11a-positive vesicles, also Spire1 or Spire2 had to be overexpressed, which is consistent with previous studies17,22. To quantify the redistribution of the nucleators, we developed an assay that allowed us to acutely deplete Rab11a-positive vesicles from the cell. We reasoned that brefeldin A (BFA), a drug that has been reported to affect the formation of recycling vesicles in other cell types, might be suitable for this purpose23–26. Indeed, acute addition of BFA blocked the formation of Rab11a-positive vesicles and led to the stepwise loss of vesicles over a period of ∼15–20 min due to their outward-directed movement (Fig. 5i,j and Supplementary Fig. S8 and Video S7). In some oocytes, a few vesicles in the centre of the oocyte remained, which is consistent with our previous observation that vesicles in the oocyte's...

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**Figure 4** Myosin Vb mediates the actin network dynamics and asymmetric spindle positioning. (a) Oocytes expressing EGFP-UtrCH (F-actin) together with mCherry fusion proteins of dominant-negative myosin Va (Myosin Va tail) or dominant-negative myosin Vb (Myosin Vb tail; lower row). Projections are time-coloured in RGB. Scale bar, 5 µm. (b) The percentage of oocytes with a dynamic or static actin network is shown. An image correlation coefficient below 0.5 after 30 s was scored as dynamic and above 0.5 as static. The number of analysed oocytes is specified in brackets above each bar (aggregation over 2 independent experiments). (c) Spindle movements in live oocytes expressing EGFP–MAP4 (magenta, microtubules, merged with differential interference contrast (DIC)). Oocytes express mCherry fused to dominant-negative myosin Va (Myosin Va tail), dominant-negative myosin Vb (Myosin Vb tail) or dominant-negative myosin VI (Myosin VI tail). White ovals mark initial spindle positions. Scale bar, 10 µm. (d) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in c and spindle movements were plotted. The number of analysed oocytes is specified in brackets above each point (aggregation over 2–3 independent experiments). Data are mean, with error bars showing s.d. (e) The efficiency of asymmetric spindle positioning in oocytes as shown in c. The number of analysed oocytes is specified in brackets above each bar (aggregation over 2–4 independent experiments). (f) The spindle speeds were determined from the plots in d. Box plot as in Fig. 1e. The number of analysed oocytes is specified in brackets above each box.
Figure 5 Rab11a-positive vesicles modulate the actin network density. (a) Oocytes expressing mCherry–Rab11a (Control), dominant-negative mCherry–Rab11a (Rab11a S25N), dominant-negative mCherry–Rab5a (Rab5a S34N) or dominant-negative mCherry–Rab27a (Rab27a T23N) were fixed and stained for F-actin. Scale bar, 5 µm. (b) The mean intensity of F-actin in the cytoplasm (labelled by fluorescent phalloidin) was measured in oocytes as shown in a. Data are mean, with error bars showing s.d. The number of analysed oocytes is specified in brackets above each bar (aggregation over 3 independent experiments). P values were calculated with Student’s t-test. (c,d) Live oocytes expressing mEGFP–Spire1 and mCherry–Rab11a (c) or mEGFP–Spire2 and mCherry–Rab11a (d). Representative examples from 2 (c) or 6 (d) independent experiments (>15 oocytes total for each condition). Scale bar, 2 µm. (e) A live oocyte labelled with FM 1-43 and Alexa Fluor 488–transferrin. Representative example from 2 independent experiments (>10 oocytes total). Scale bar, 2 µm. (f) FM1-43-labelled membrane compartment in the same oocyte as shown in e, imaged with identical imaging conditions as in e. Representative example from 2 independent experiments (>10 oocytes total). Scale bar, 2 µm. (g) mEGFP–Spire2 localization in anti-GFP immunogold-labelled oocytes. The outlined region is magnified to the right. Representative example from 5 independent experiments (23 oocytes total). Two other examples are shown in Supplementary Fig. S7d. Scale bar, 200 nm. (h) mEGFP–Spire2 localization in oocytes expressing mCherry–Rab11a (Control) or dominant-negative mCherry–Rab11a (Rab11a S25N) (z-projection, 5 sections, every 0.66 µm). Representative examples from 6 independent experiments (>70 oocytes total for each condition). Scale bar, 10 µm. The outlined regions are magnified on the right. (i) mEGFP–Spire2 localization in live oocytes on addition of 10 µM BFA (z-projection, 5 sections, every 0.66 µm). Scale bar, 10 µm. The outlined regions are magnified below. A corresponding control experiment with methanol addition is shown in Supplementary Fig. S8a. (j) The number of Spire2-positive vesicles (j) and the intensity of mEGFP–Spire2 in the cytoplasm (k) on addition of BFA or methanol (control) was quantified in data sets as shown in i and Supplementary Fig. S8a. Vesicles were identified and counted with the spot detection function of Imaris in an oocyte segment of 20 × 20 × 20 µm³ and normalized to the initial number of vesicles. Data are mean, with error bars showing s.d. The number of analysed oocytes is specified in brackets to the right of each treatment (aggregation over 3 independent experiments). (l) Schematic illustrating the localization of actin nucleators and changes in actin network density in oocytes with different numbers of Rab11a-positive vesicles.
centre are less likely to move to the surface\textsuperscript{17}. The loss of Rab11a-positive vesicles could also explain why asymmetric spindle positioning fails in BFA-treated oocytes\textsuperscript{27}. We then analysed how vesicle depletion by BFA affected the distribution of the actin nucleators. Quantification of the cytoplasmic mEGFP–Spire2 intensity showed that the amount of nucleator in the cytoplasm increased as the number of vesicles decreased

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**Figure 6** The actin network density and the number of vesicles correlate in different stages of oocyte maturation. (a) Scheme illustrating different stages of oocyte maturation. The images and data points below b-f correspond to these stages. (b) Vesicles in different stages of maturation in oocytes expressing mEGFP–Spire2 (z-projection: 5 sections, every 0.66 µm). (c) The number of vesicles was quantified in data sets as shown in b. Vesicles were identified and counted with the spot detection function of Imaris in a volume of 20 × 20 × 20 µm\textsuperscript{3}. The number (n) of analysed oocytes is specified (aggregation over 2 independent experiments). Box plot as in Fig. 1e. (d) The vesicle volume was quantified in data sets as shown in b. The vesicle volume was measured using the region growing function in Imaris in a volume of 20 × 20 × 20 µm\textsuperscript{3}. The number (n) of analysed oocytes is specified (aggregation over 2 independent experiments). Box plot as in Fig. 1e. (e) Oocytes were fixed at the different stages of oocyte maturation shown in a and stained for F-actin using fluorescent phalloidin. Scale bar, 5 µm. (f) The mean intensity of the cytoplasmic phalloidin staining was measured in oocytes as shown in e. Error bars show s.d. P values relative to the Before NEBD stage were calculated with Student’s t-test. The number of analysed oocytes is specified on each bar (aggregation over 3 independent experiments).
Time after BFA washout

Control

18 min

6 min

8 min

Vesicles (Spire2)

(Fig. 5i–k and Supplementary Video S7). Consistent with this, also Spire1 and Formin-2 were released from vesicles in BFA-treated cells (Supplementary Fig. S8b,c). Together, these data suggest that vesicles modulate the density of the actin network by sequestering and clustering the network’s actin nucleators Spire1, Spire2 and Fmn2.

Actin networks of different densities can be generated by adjusting the number and volume of Rab11a-positive vesicles

Our model predicts that the assembly of a dense actin network should involve a high number of vesicles whereas a wide-meshed actin network should involve only a low number of vesicles (Fig. 5l). To investigate this prediction, we first quantified the number and volume of the Rab11a-positive vesicles (Fig. 6b–d) and measured the density of the actin network (Fig. 6e,f) in different stages of oocyte maturation (Fig. 6a). Before nuclear envelope breakdown, oocytes contained a very high number of small vesicles. Correspondingly, the actin network was very dense (Fig. 6b–f, Before NEBD). Around nuclear envelope breakdown, the number of vesicles dropped as small vesicles merged into larger vesicles. This was accompanied by a strong decrease in the network density (Fig. 6b–f, NEBD), which is consistent with a previous study. The number of vesicles stayed low while the spindle assembled (3 h after NEBD) and moved to the cell surface (6 h after NEBD). Accordingly, the network density stayed low in these stages as well (Fig. 6b–f, 3 h and 6 h after NEBD). These data demonstrate that the actin network density correlates with the number of vesicles.

Next, we examined whether we could artificially modulate the network density by altering the number of vesicles. We reasoned that we should be able to generate a high number of small vesicles by first depleting Rab11a-positive vesicles from oocytes using BFA and by subsequently washing out BFA to trigger the simultaneous reformation of a high number of vesicles. To investigate this possibility, we quantified the number and volume of vesicles on BFA washout. Indeed, we found that the oocytes contained a high number of small vesicles four minutes after BFA washout (Fig. 7a). Consistent with our model, the network density was significantly increased (Fig. 7a,d). Interestingly, we observed that the system quickly restored its original configuration after BFA washout. In a stepwise manner, the small vesicles merged into larger vesicles (Fig. 7a–c). The resulting decrease in the number of vesicles was accompanied by a stepwise decrease in actin network density (Fig. 7a,d). After about 18 min, the oocytes...
were not distinguishable from control oocytes anymore (Fig. 7a–d). Together, these data suggest that the actin network density can be modulated by altering the number of Rab11a-positive vesicles and that the dynamic actin networks that are generated by this vesicle-based mechanism are robust and rapidly restore a dynamic steady state after reversal of perturbation. To investigate whether vesicle abundance and network density are also coupled when the network density is altered by the amount of nucleator in the oocyte, we highly co-overexpressed the nucleators Fmn2 and Spire2, which strongly increases the network density (Fig. 7c). Consistent with coupling, the increase in network density was accompanied by a large increase in the number of Rab11a-positive vesicles and a decrease in their volume (Fig. 7f–h). Together, these data are consistent with the model in which the generation of actin networks of different densities involves an adjustment of the number of Rab11a-positive vesicles.

**DISCUSSION**

In conclusion, this study reveals an unexpected vesicle-based mechanism by which cells can generate dynamic actin networks of different densities. The vesicles drive the dynamics of the actin network in a myosin-Vb-dependent manner and modulate the network density by clustering and sequestering the nucleators of the network. In this way, the vesicles act as force generators as well as actin-nucleating centres at the same time. The dynamic actin networks that are generated by this vesicle-based mechanism are robust, adaptable and rapidly restore a dynamic steady state after reversal of perturbation.

Assembling dynamic actin networks by clustering nucleators of unbranched actin filaments and myosins on membranes in actin-organizing centres might represent a general principle of cytoskeletal organization. In fission yeast, the contractile ring is initially made up of a coarsely meshed actin network that converges at plasma-membrane-bound nodes. Like Rab11a-positive vesicles in mouse oocytes, these membrane-bound nodes are enriched in a formin, a myosin and actin. During cytokinesis, these membrane-bound nodes merge with each other to form a contractile ring. On the basis of these similarities, it is tempting to speculate that dynamic cortical actin networks can be organized from clusters of actin nucleators and myosins at the plasma membrane, whereas intracellular dynamic actin networks can be organized by clustering of actin nucleators and myosins on vesicles, as shown in this study. Indeed, morphologically similar actin networks that converge at bright actin nodes, which might correspond to membrane-associated actin-organizing centres, have been reported in a range of cell types, including *Xenopus laevis* epithelial cells, *Drosophila melanogaster* embryos and oocytes, and *Caenorhabditis elegans* embryos; and actin nucleation has been observed on vesicles in *Xenopus* egg extracts and zebrafish embryos.

In addition to revealing an elegant mechanism for organizing dynamic actin networks, this work also provides important insights into the mechanism of asymmetric spindle positioning in oocytes. Oocytes need to divide extremely asymmetrically to preserve sufficient storage material for the development of the embryo. In mammals, including humans, this requires that the spindle moves from the centre to the surface of the oocyte using an actin-dependent mechanism. Our data reveal a vital function for Rab11a-positive vesicles in this process: if the function of Rab11a or myosin-Vb is blocked the spindle is unable to move to the oocyte’s surface. Our data are consistent with a model in which myosin light chain kinase-dependent pulling from the spindle poles and direct association of Rab11a-positive vesicles with the spindle couple the spindle to the outward-moving vesicles and the actin filaments that are nucleated from their surface (Fig. 8).

Dynamic actin networks have many essential functions in cells. This study reveals a vesicle-based mechanism by which cells can generate dynamic actin networks of different densities. This function of vesicles as cytoskeletal modulators is essential for asymmetric positioning of the meiotic spindle in oocytes. As defects during asymmetric oocyte division result in pregnancy loss and infertility, this mechanism of vesicle-based actin network organization is not only of fundamental scientific interest but also vital for mammalian reproduction.

**METHODS**

Methods and any associated references are available in the online version of the paper.

*Note:* Supplementary Information is available in the online version of the paper.
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AUTHOR CONTRIBUTIONS

G.H. analysed the localization of Spire2 by electron microscopy. Z.H. and M.S. designed and carried out all other experiments. M.S. and Z.H. wrote and edited the manuscript, respectively.

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Preparation and culture of oocytes. All mice were maintained in a specific-pathogen-free environment according to UK Home Office regulations. Oocytes were isolated from ovaries of 8-week-old FVB mice, cultured and microinjected as described.

Oocytes were microinjected with mRNA encoding fluorescently labelled proteins were prepared in dbcAMP until protein was expressed. Oocytes were then released into dbcAMP-free medium. In some experiments, oocytes were treated with 10 μM BFA (brefeldin A, Sigma), 50 nM jasplakinolide (Calbiochem), 5 μM FM 1-43 (Molecular Probes), Alexa Fluor 488-labelled transferrin (Molecular Probes) or Cy5-labelled transferrin (gift from B. Nichols, MRC LMB, Cambridge, UK). The live oocytes in Fig. 2d were briefly treated with 0.5% saponin in 20 mM phosphate buffer and 150 mM sodium chloride.

Washes in phosphate buffer at pH 7.4, inactivation of reactive aldehyde groups using 0.5% saponin in 20 mM phosphate buffer and 150 mM sodium chloride.

mRNA concentrations were determined on ethidium bromide agarose gels using an RNA standard (Ambion).

Images were acquired with a Zeiss LSM710 confocal microscope equipped with a Tokai Hit Stage Top Incubator, with a ×40 C-Apochromat 1.2 NA water-immersion objective lens for live oocytes, and a ×40 ×40 40× confocal microscope. Images were acquired with identical imaging conditions and care was taken that images were not saturated during acquisition. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

Determination of the cytoplasmic actin network density. To quantify the density of the cytoplasmic actin network in oocytes, the mean intensity of the Alexa Fluor 488 phalloidin staining was measured in the cytoplasm and in a region outside the oocyte for background subtraction. Images in control and perturbed situations were acquired with identical imaging conditions and care was taken that images were not saturated during acquisition. We then correlated each image in the time series with the first time frame in ImageJ using the Image correlation plugin. In particular, images were correlated by calculating mean intensities in a local region of 5 pixels. The number of vesicles in a defined oocyte volume and the distance of the spindle to the cortex, the data sets were aligned to time points corresponding to the end of asymmetric spindle positioning in controls. The distance of the spindle to the alignment position was calculated for each time point by processing the spindle coordinates in Excel and plotted over time. To calculate average spindle speeds, the spindle velocity in each oocyte was calculated by linear regression analysis of the displacement plots. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

Automated 3D tracking of the spindle. To measure the kinetics of asymmetric spindle positioning, we injected oocytes with mRNA encoding fluorescently labelled MAP4 to label microtubules. We then recorded z-stacks of the entire oocyte volume during asymmetric spindle positioning using Zeiss’ MultiTime Series macro (11 sections; spacing: 8 μm; time interval: 10 min). We corrected for drifts during image acquisition with the correct drift function of the image analysis software Imaris (Bitplane) after segmenting the oocyte volume by applying a low threshold on the soluble pool of the fluorescent reporter. Afterwards we segmented the spindle by applying a higher threshold and tracked the spindle’s centre of mass during spindle movement using Imaris. For averaging the spindle movements in different oocytes, we temporally aligned the different data sets to the time when the spindle slowed down owing to arrival at the cortex. In oocytes, where the spindle did not reach the cortex, the data sets were aligned to time points corresponding to the end of asymmetric spindle positioning in controls. The distance of the spindle to the alignment position was calculated for each time point by processing the spindle coordinates in Excel and plotted over time. To calculate average spindle speeds, the spindle velocity in each oocyte was calculated by linear regression analysis of the displacement plots. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

Automated quantification of the number and volume of vesicles. To label vesicles, oocytes were microinjected with mRNA encoding fluorescently labelled Rab11a or Spire2 and arrested in dbcAMP until the fluorescent reporter protein was expressed. Oocytes were then released into dbcAMP-free medium. Three- dimensional data sets of vesicles were acquired using Zeiss’ MultiTime Series. The number of vesicles was determined using the spot detection function in Imaris. The vesicle volume was reconstructed by using the region growing function during spot detection in Imaris. The number of vesicles in a defined oocyte volume and the vesicle volume were then exported into Excel. In Fig. 5j, the number of vesicles was normalized to the average number of vesicles within the first three minutes of BFA or methanol addition. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

Quantification of cytoplasmic mEGFP–Spire2 intensity on BFA addition. The intensity of mEGFP–Spire2 in the cytoplasm on BFA or methanol addition in Fig. 5k and Supplementary Fig. S8a was measured in ImageJ. Vesicles were excluded from the evaluated area by thresholding. For background subtraction, the intensity in an area outside the oocyte was measured. Measurements were repeated for all five z-sections and averaged. Intensities were normalized relative to the intensity in the first frames after BFA or methanol addition. As imaging was started at slightly variable times after BFA addition, the data sets were aligned to the time when the increase in mEGFP–Spire2 intensity had reached half its maximum.

**METHODS**

**Preparation and culture of oocytes.** All mice were maintained in a specific-pathogen-free environment according to UK Home Office regulations. Oocytes were isolated from ovaries of 8-week-old FVB mice, cultured and microinjected as described. Oocytes were microinjected with mRNA encoding fluorescently labelled proteins were prepared in dbcAMP until protein was expressed. Oocytes were then released into dbcAMP-free medium. In some experiments, oocytes were treated with 10 μM BFA (brefeldin A, Sigma), 50 nM jasplakinolide (Calbiochem), 5 μM FM 1-43 (Molecular Probes), Alexa Fluor 488-labelled transferrin (Molecular Probes) or Cy5-labelled transferrin (gift from B. Nichols, MRC LMB, Cambridge, UK). The live oocytes in Fig. 2d were briefly treated with 0.5% saponin in 20 mM phosphate buffer and 150 mM sodium chloride.

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**Quantification of actin network dynamics.** To quantify the dynamics of the cytoplasmic actin network we expressed UtrCH–EGFP to label F-actin and recorded high-resolution time series of the actin network in live mouse oocytes around the time of asymmetric spindle positioning (time interval 1.5 s). Images in control and perturbed situations were acquired with identical imaging conditions and care was taken that images were not saturated during acquisition. We then correlated each image in the time series with the first time frame in ImageJ using the Image Correlation plugin. In particular, images were correlated by calculating mean intensities in a local region of five pixels. The average correlation coefficients for the entire image were then plotted against time (Supplementary Figs S3a and S5a). For Figs 2c and 4b, actin networks were considered as dynamic if the correlation coefficient after 30 s was below 0.5 and as static if the correlation coefficient after 30 s was higher than 0.5. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

**Automated 3D tracking of the spindle.** To measure the kinetics of asymmetric spindle positioning, we injected oocytes with mRNA encoding fluorescently labelled MAP4 to label microtubules. We then recorded z-stacks of the entire oocyte volume during asymmetric spindle positioning using Zeiss’ MultiTime Series macro (11 sections; spacing: 8 μm; time interval: 10 min). We corrected for drifts during image acquisition with the correct drift function of the image analysis software Imaris (Bitplane) after segmenting the oocyte volume by applying a low threshold on the soluble pool of the fluorescent reporter. Afterwards we segmented the spindle by applying a higher threshold and tracked the spindle’s centre of mass during spindle movement using Imaris. For averaging the spindle movements in different oocytes, we temporally aligned the different data sets to the time when the spindle slowed down owing to arrival at the cortex. In oocytes, where the spindle did not reach the cortex, the data sets were aligned to time points corresponding to the end of asymmetric spindle positioning in controls. The distance of the spindle to the alignment position was calculated for each time point by processing the spindle coordinates in Excel and plotted over time. To calculate average spindle speeds, the spindle velocity in each oocyte was calculated by linear regression analysis of the displacement plots. Average, standard deviation and statistical significance based on Student’s t-test were calculated in Excel.

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**Statistics.** Average (mean), s.d. and statistical significance based on Student’s *t*-test (always two-tailed) were calculated in Excel. The sample size was chosen on the basis of Student’s *t*-test. All box plots show median (line), mean (small square), 1st, 99th (crosses), 5th, 95th (whiskers) and 25th and 75th percentile (boxes).

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**Figure S1** Loss of Rab11a-positive vesicles leads to mislocalization of the vesicles’ cargo transferrin. (a) Transferrin localization in oocytes expressing mEGFP-Rab11a and oocytes expressing dominant-negative Rab11a (Rab11a S25N) (z-projection, 3 sections, every 0.66 µm). Boxed regions are magnified in inset. Scale bar, 10 µm. The number of transferrin-positive vesicles in control and dominant-negative Rab11a expressing oocytes is shown. Vesicles were identified and counted with the spot detection function of Imaris in an oocyte segment of 20x20x20 µm³. (b) The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d.. P values were calculated with Student’s t-test.
Figure S2 Rab11a-positive vesicles are dispensable for oocyte viability and progression through meiosis. (a) Live oocytes expressing H2B-mRFP (magenta, chromosomes, merged with DIC) and mEGFP-Rab11a (control) or mEGFP-Rab11a S25N (Rab11a S25N). Representative examples from 2 independent experiments (>10 oocytes total for each condition). Scale bar, 10 µm. (b) Control and mCherry-Rab11a S25N expressing oocytes from (C57BL x CBA) F1 females were fixed and stained with Hoechst (chromosomes, cyan) and fluorescent phalloidin (F-actin, pseudocoloured). The arrowheads highlight the cortical enrichment of actin in proximity of the chromosomes, which is independent of the presence of Rab11a-positive vesicles. Representative examples from 2 independent experiments (>10 oocytes total for each condition). Scale bar, 10 µm. (c) Control and mCherry-Rab11a S25N expressing oocytes from (C57BL x CBA) F1 females were fixed and stained with Hoechst (chromosomes, cyan) and fluorescent Lens culinaris agglutinin to label cortical granules (pseudocoloured). The arrowheads highlight the cortical granule free domain in proximity of the chromosomes, which is independent of the presence of Rab11a-positive vesicles. Representative examples from one experiment (>5 oocytes total for each condition). Scale bar, 10 µm. (d) The percentage of oocytes undergoing NEBD was quantified by live cell microscopy of oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 4 independent experiments). (e) The percentage of oocytes that form a bipolar spindle was quantified by live cell microscopy of oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 4 independent experiments). (f) The percentage of oocytes that progress into anaphase was quantified by live cell microscopy of oocytes expressing EGFP-MAP4 to label the spindle together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 4 independent experiments). (g) The time between NEBD and bipolar spindle assembly was measured in live oocytes expressing EGFP-MAP4 to label the spindle together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Box plot as in Figure 1e. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). (h) The time between NEBD and anaphase onset was measured in live oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Box plot as in Figure 1e. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments).
Figure S3  The spindle is trapped in a static actin network if the function of Rab11a-positive vesicles is blocked. (a) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (b) Live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Projections are time-coloured in RGB. Scale bar, 5 µm.
Figure S4 Network stabilization with jasplakinolide prevents asymmetric spindle positioning. (a) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC]) in live oocytes treated with DMSO (Control) or oocytes treated with 50 nM jasplakinolide (Jasplakinolide). White ovals mark initial spindle positions. Scale bar, 10 µm. (b) The efficiency of asymmetric spindle positioning in control and jasplakinolide treated oocytes is shown. The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). (c) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (a) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). Data are mean, with error bars displaying s.d. (d) The spindle speeds were determined from the plots in (c). The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). Box plot as in Figure 1e.
Figure S5 Myosin Vb mediates the actin network dynamics and asymmetric spindle positioning. (a) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-myosin-Va-tail or mCherry-myosin-Vb-tail. Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (b) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC]) in live oocytes injected with control siRNA (Control) or with siRNAs targeting myosin Vb (Myosin Vb RNAi). White ovals mark initial spindle positions. Scale bar, 10 µm. (c) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (b) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). Data are mean, with error bars displaying s.d. (d) The efficiency of asymmetric spindle positioning in oocytes injected with control siRNA (Control) or with siRNAs targeting myosin Vb (Myosin Vb RNAi) is shown. The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). (e) The spindle speeds were determined from the plots in (c). The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). Box plot as in Figure 1e.
Figure S6 A moderate increase of network density does not block network dynamics or asymmetric spindle positioning. (a) Control oocytes (Control) and oocytes in which the actin nucleators Fmn2 and Spire2 were moderately overexpressed (Fmn2/Spire2 moderately upregulated) were fixed and stained for F-actin. Scale bar, 5 µm. (b) The mean intensity of F-actin in the cytoplasm (labelled by fluorescent phalloidin) was measured in oocytes as shown in (a). Error bars display s.d. The number of analysed oocytes is specified in italics. *P* values were calculated with Student's t-test. (c) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH in control oocytes (Control) and in oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2 (Fmn2/Spire2 moderately upregulated). Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics. Data are mean, with error bars displaying s.d. (d) The percentage of oocytes with a dynamic or static actin network is shown. An image correlation coefficient below 0.5 after 30 s was scored as dynamic and above 0.5 as static. The number of analysed oocytes is specified in italics. (e) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC] in control live oocytes (Control) and oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2. White ovals mark initial spindle positions. Scale bar, 10 µm. (f) The efficiency of asymmetric spindle positioning in control oocytes (Control) and in oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2 (Fmn2/Spire2 moderately upregulated) is shown. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). (g) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (e) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (h) The spindle speeds were determined from the plots in (g). The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Box plot as in Figure 1e.
Figure S7 Localization of the network’s actin nucleators in the presence and absence of Rab11-a positive vesicles. (a) Two additional examples of mEGFP-Spire2 localization in anti-GFP immunogold labelled oocytes. Boxed regions are magnified next to overview. Representative examples from 5 independent experiments (23 oocytes total). Scale bar, 200 nm. (b) Z-projection (5 sections, every 0.66 μm) of live oocytes expressing mEGFP-Spire1 with either mCherry-Rab11a (top panel) or mCherry-Rab11a S25N (bottom panel). Boxed regions are magnified in inset. Representative examples from 2 independent experiments (>15 oocytes total for each condition). Scale bar, 10 μm. (c) Z-projection (5 sections, every 0.66 μm) of live oocytes expressing Fmn2-mCherry together with mEGFP-Spire1 and mEGFP-Rab11a (top panel) or mEGFP-Spire1 and mEGFP-Rab11a S25N (bottom panel). Representative examples from 3 independent experiments (>30 oocytes total for each condition). Scale bar, 10 μm.
Figure S8 BFA addition releases the network’s actin nucleators from vesicles. (a) Control for BFA addition experiment in Fig. 5i. mEGFP-Spire2 localization upon addition of methanol. (z-projection, 5 sections, every 0.66 µm). Scale bar, 10 µm. Boxed regions are magnified below. (b) mEGFP-Spire1 localization in live oocytes upon addition of 10 µM BFA. Representative example from 4 independent experiments (17 oocytes total). Scale bar, 10 µm. Boxed region is magnified below. (c) Fmn2-mCherry localization in live oocytes co-overexpressing mEGFP-Spire1 upon addition of 10 µM BFA. Only a single section is shown. Representative example from 2 independent experiments (6 oocytes total). Scale bar, 10 µm. Boxed region is magnified below.
Supplementary Video Legends

**Supplementary Video S1.** Rab11a-positive vesicles driving actin network dynamics.
Time-lapse imaging (single confocal section; time interval: 3.1 s) of the cytoplasmic actin network during asymmetric spindle positioning in oocytes expressing EGFP-UtrCH (F-actin; magenta) together with either mCherry-Rab11a (vesicles; green) or mCherry-Rab11a S25N. Please note that vesicles appear white in merge.

**Supplementary Video S2.** Rab11a-positive vesicles temporarily associate with the spindle during asymmetric spindle positioning.
Time-lapse imaging (z-projection of three sections, every 1.5 μm; time interval: 6.35 s) of an oocyte expressing mEGFP-Rab11a (vesicles; green) and mCherry-MAP4 (microtubules; magenta) during asymmetric spindle positioning.

**Supplementary Video S3.** The spindle is trapped in a static actin network if the function of Rab11a-positive vesicles is blocked.
Time-lapse imaging (single confocal section; time interval: 3.6 s) of the spindle area in oocytes expressing EGFP-UtrCH (F-actin) together with either mCherry-Rab11a or mCherry-Rab11a S25N.

**Supplementary Video S4.** Asymmetric spindle positioning requires Rab11a-positive vesicles.
Time-lapse imaging of F-actin (EGFP-UtrCH, white; single confocal section; time interval: 10 min) and chromosomes (Hoechst 33342, magenta, z-projection of three confocal sections, every 1.2 μm) in control or mCherry-Rab11a S25N expressing oocytes. Same oocyte as shown in Fig. 2d

**Supplementary Video S5.** 3D volume reconstruction of the trapped spindle shown in Supplementary Video S3.
Rotating 3D volume reconstruction of the trapped spindle in oocytes expressing EGFP-UtrCH (F-actin) together with mCherry-Rab11a S25N.

**Supplementary Video S6.** Myosin Vb drives the actin network dynamics.
Time-lapse imaging (one confocal section; time interval: 1.57 s) of the cytoplasmic actin network during asymmetric spindle positioning in oocytes expressing EGFP-UtrCH (F-actin) together with either mCherry-myosin-Va-tail or mCherry-myosin-Vb-tail.

**Supplementary Video S7.** mEGFP-Spire2 is released from vesicles and the plasma membrane into the cytoplasm upon BFA addition.
Time-lapse imaging of mEGFP-Spire2 (z-projection of five sections, every 2 μm; time interval: 13 s) in live oocyte. Time-lapse series starts directly after addition of BFA or corresponding amounts of methanol (Control).