Cdk1 inactivation induces post-anaphase-onset spindle migration and membrane protrusion required for extreme asymmetry in mouse oocytes

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Female meiotic divisions are extremely asymmetric, producing large oocytes and small polar bodies (PBs). In mouse oocytes, the spindle relocates to the cortex before anaphase of meiosis I (MI). It is presumed that by displacing the future midzone, pre-anaphase spindle repositioning alone ensures asymmetry. But how subsequent anaphase events might contribute to asymmetric PB extrusion (PBE) is unknown. Here, we find that inactivation of cyclin-dependent kinase 1 (Cdk1) induces anaphase and simultaneously triggers cytoplasmic formin-mediated F-actin polymerisation that propels the spindle into the cortex causing it to protrude while anaphase progresses. Significantly, if post-anaphase-onset spindle migration fails, protrusion and asymmetry are severely threatened even with intact pre-anaphase migration. Conversely, post-anaphase migration can completely compensate for failed pre-anaphase migration. These data identify a cell-cycle-triggered phase of spindle displacement occurring after anaphase-onset, which, by inducing protrusion, is necessary for extreme asymmetry in mouse oocytes and uncover a pathway for maximising unequal division.
Highly asymmetric divisions in mouse oocytes represent the culmination of a number of coordinated steps. First, bipolar spindles assemble in the absence of canonical centrosomes close to the oocyte centre and migrate to the cortex before anaphase-onset. Anaphase then occurs when separate is activated to cleave the molecular glue, cohesion, holding chromosomes together. Separation activation requires anaphase-promoting complex (APC)-mediated destruction of the separate inhibitor, securin, and inactivation of the master cell-cycle kinase, cyclin-dependent kinase 1 (CdK1), brought about through APC-mediated proteolysis of the CdK1 co-activator, cyclin B1. Finally, formation of the polar body (PB) incorporates membrane outpocketing (or protrusion), membrane furrowing and cytokinesis. The actomyosin cytoskeleton is known to be important for events on either side of protrusion, that is, pre-anaphase spindle migration and contractile ring formation that directs furrowing. formidable proteolysis of the Cdk1 co-activator, cyclin B1. Moreover, contrasting opinions exist regarding how relaxation is induced to protrude. In contrast, around the time of anaphase in meiosis I (MI), cytokinesis occurs before anaphase-onset. The actomyosin cytoskeleton is known to be important for events on either side of protrusion, that is, pre-anaphase spindle migration and contractile ring formation that directs furrowing. Furrowing is mediated proteolysis of the Cdk1 co-activator, cyclin B1. The actomyosin cytoskeleton is known to be important for events on either side of protrusion, that is, pre-anaphase spindle migration and contractile ring formation that directs furrowing. Furrowing is caused by an increase in membrane height (Fig. 1e–g) consistent with half of the spindle (~37 µm; P = 0.24; Student’s t test) and associated with narrowing of the spindle midzone (Fig. 1a, c, d). We repeated analyses using green fluorescent protein (GFP)-tagged MAP7, a microtubule marker used previously to study spindle dynamics in meiotic oocytes. We found that, during anaphase, the changes in spindle length and midzone width for MAP7-GFP-labelled spindles were indistinguishable from those of SiR-Tubulin-labelled spindles (Supplementary Fig. 2a–c and Supplementary Movie 2).

Thus, in MI mouse oocytes, chromosomes travel polewards throughout anaphase, with spindle elongation and midzone narrowing occurring during the first and second phases, respectively (Fig. 1d). As this differs from canonical anaphase-A (chromosome separation to poles) and anaphase-B (spindle elongation), we use the terms anaphase-1 and anaphase-2 (note that this is numerical 1 and 2 versus Roman numerals, I and II, which refer to first and second meiosis, respectively).

**Post-anaphase-onset migration correlates with protrusion.** During MI, two empty protrusions form in relation to anaphase chromosomes, one of which forms the second PB after becoming occupied by one of the pale polars following spindle rotation. During MI, it is unknown whether protrusion also happens first, before ingress of the bipolar spindle. We found that, concurrent with anaphase progression, the membrane protruded as reflected by an increase in membrane height (Fig. 1e–g and Supplementary Movie 3). Significantly, the first sign of protrusion always occurred during anaphase-1 (Fig. 1f) and never before (20 of 20 oocytes) (Supplementary Fig. 3 and Supplementary Movies 4 and 5). Using a fluorescent utrophin probe (UtrCH-mCherry), which binds to and labels F-actin, we confirmed that the cortex underwent changes identical to the membrane beginning after anaphase-onset (Supplementary Fig. 4 and Supplementary Movie 6).

During anaphase, and while the outpocketing enlarged, the leading pole of the anaphase spindle became increasingly enveloped by the protrusion (Fig. 1f and Supplementary Fig. 4). Indeed, by the end of anaphase-2, the maximal protrusion height (~18 µm) was half of the total length of the spindle (~37 µm; Fig. 1b, e–g and Supplementary Fig. 4) consistent with half of the anaphase spindle becoming enclosed within the protrusion. Thus, following migration to the cortex, the spindle does not enter a pre-formed cortical protrusion. Instead, protrusion of the cortex/membrane begins after anaphase-onset and enlarges as anaphase progresses.

It is presumed that protrusion is mostly accounted for by anaphase spindle extension. However, this was clearly not the case since total anaphase spindle elongation was 8.2 ± 0.4 µm (Fig. 1b) and only roughly half of this or 4–5 µm would extend towards the protrusion. We did observe an overall retraction of the oocyte’s antero-posterior diameter that accounted for a further 5.3 ± 0.6 µm of protrusion height (Fig. 1g) altogether leaving ~8–9 µm still unaccounted for.
Unexpectedly, between anaphase-onset and completion of protrusion, the lagging pole and the midzone of the spindle travelled almost exactly this distance (~8–9 µm) away from the posterior cortex (Fig. 1e–i). This was a substantial travel distance representing 12.1 ± 1.9% of the pre-anaphase oocyte diameter, over two-fold greater than the displacement reported in C. elegans embryos (4.5%)24. We also found that spindle displacement correlated strongly with protrusion size (Fig. 1j). Notably, MAP7-GFP-labelled spindles also migrated a very similar distance post-anaphase-onset (8.9 µm) in association with membrane protrusion (Supplementary Fig. 2c, d and Supplementary Movie 2). Collectively, these data support that the spindle migrates again after anaphase-onset and could be critical for protrusion.

Fig. 1 Spindle migration following anaphase-onset induces protrusion. a-c Stages of anaphase (a) and quantification of stage-specific spindle dimensions (b, c) derived from live oocytes with fluorescently labelled spindles and chromosomes. One-way ANOVA used for statistical analysis. d, e Schematics depicting stages of anaphase (d) and measurements (e). f Example of protrusion (white arrowheads) and spindle migration into the membrane occurring after anaphase-onset in a live oocyte (see Supplementary Movie 3). g–i Quantification of protrusion height, spindle displacement and oocyte dimensions (g, n = 12, error bars are mean ± SEM) (see e); total posterior pole displacement (h) and comparison with total midzone displacement (i) between anaphase-onset and anaphase-completion. Two-tailed Student’s t test used for statistical analysis. j Correlation between spindle migration and protrusion height (n = 12). Times in panels are hours:minutes relative to anaphase-onset. Scale bars, 10 µm. Box plots depict median (horizontal line), mean (crosses), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). Oocyte numbers are shown in parenthesis. P values represented as **P < 0.01, ***P < 0.001 and ****P < 0.0001, ns denoted P > 0.05.
Oocytes were untreated (mean (crosses), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). Movie 9) from 6 h post-GVBD. Quantification of time of anaphase-onset (d) and protrusion-onset (e). Two-tailed Student’s t test (d) or one-way ANOVA (e) used for statistical analysis. P values represented as ***P < 0.001 and ****P < 0.0001, ns denoted P > 0.05. Box plots depict median (horizontal line), mean (crosses), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). f–h Flavopiridol induces protrusion despite lack of proteolysis. Oocytes were treated either with inhibitor, APCIN, followed by Supplementary Movie 11) or the 26S proteasome inhibitor, MG132, followed by flavopiridol (h; see Supplementary Movie 12). Anaphase failure reflects lack of proteolysis resulting in chromosome entrapment within the furrow. Times in panels are hours: minutes post-GVBD. Scale bars, 10 µm. White arrowheads indicate protrusion-onset. Oocyte numbers are shown in parentheses from a minimum of three independent experiments.

**Cdk1 inactivation triggers post-anaphase-onset migration.** Since the second phase of spindle migration occurred strictly after anaphase-onset, we asked whether mechanisms that initiate anaphase might also induce spindle migration/protrusion. Anaphase requires APC-mediated proteolysis of securin and cyclin B1 inactivation of Cdk1 and inactivation of Cdk1. To test the importance of Cdk1 inactivation, we used the potent Cdk1 inhibitor, flavopiridol, at the same dose (5 µM) used previously in mammalian somatic cells and mouse oocytes. Under our conditions, 5 µM of flavopiridol completely abolished GVBD (n = 30), confirming highly effective inhibition of Cdk1 (Supplementary Fig. 5).

We first treated oocytes at 6 h post-GVBD when proteolysis was beginning (Supplementary Fig. 6) and most pre-anaphase spindles had migrated to the cortex (Supplementary Fig. 1). Flavopiridol treatment at 6 h post-GVBD induced markedly earlier anaphase-onset and protrusion than either untreated or dimethyl sulphoxide (DMSO)-treated controls (Fig. 2a–e and Supplementary Movies 7–9) consistent with accelerated PBE observed previously. Flavopiridol addition at 4 h post-GVBD—when a bipolar spindle had formed and spindles were nearing the cortex (Supplementary Fig. 1) but proteolysis had not yet begun (Supplementary Fig. 6) hence leaving the majority of securin intact—also induced spindle migration to the cortex and then into the membrane, and hence protrusion, in the same accelerated manner as oocytes treated at 6 h post-GVBD (Fig. 2e, f). However, owing to lack of securin destruction, chromosomes did not segregate and became trapped within the cleavage furrow (Fig. 2f and Supplementary Movie 10) showing that spindle migration/protrusion did not require anaphase.

These data suggested that Cdk1 inactivation rather than accompanying proteolysis was key for migration and protrusion. We tested this using the APC-specific inhibitor, APCIN, and the 26S proteasome inhibitor, MG132, which blocked proteolysis of securin and cyclin B1 (Supplementary Fig. 6). When Cdk1 was inactivated in APCIN- or MG132-treated oocytes, protrusion was still accelerated (Fig. 2e, g, h), but because of stable securin, chromosomes again became trapped within the cleavage furrow (Fig. 2g, h and Supplementary Movies 11 and 12). These effects were specific to Cdk1 inhibition since another Cdk1 inhibitor, RO-3306, also markedly accelerated protrusion (Fig. 2e), whereas protrusion onset was unaltered by inhibiting mitogen-activated protein kinase (MAPK) late in MI (Fig. 2e).

Thus, Cdk1 inactivation, but not proteolysis, is critical for inducing post-anaphase-onset migration required for protrusion. These data also show clearly that the post-anaphase-onset phase of migration that induces protrusion is not merely a passive continuation of pre-anaphase migration because, first, it requires Cdk1 inactivation and, second, metaphase spindles do not induce
protrusion even after long dwell times at the cortex (Supplementary Fig. 3 and Supplementary Movies 4 and 5).

Sustained protrusion requires an intact spindle. Consistent with F-actin being highly stable in the polar region during late MF, we found that cortical F-actin thickness was greatest within the protrusion (Supplementary Fig. 7). Hence, outpocketing is unlikely to result solely from cortical relaxation and might instead require a force from within the oocyte. Since protrusion is spatially restricted to the leading spindle pole and, together with spindle migration, is preceded by Cdk1 inactivation, we hypothesised that Cdk1 inactivation triggers a force that is transmitted via the spindle.

If the spindle is required for force transmission, protrusion should fail without a spindle. To test this, we used nocodazole to depolymerise the spindle after it had migrated to the cortex. We found that neither a sustained protrusion nor PB formation occurred (16 of 16 oocytes), even following forced Cdk1 inactivation (21 of 21 oocytes; Fig. 3a–e and Supplementary Movies 13–16). In contrast, if oocytes were washed out of nocodazole to allow a spindle to reassemble, protrusions and PBs occurred (22 of 22 oocytes; Supplementary Movie 17).
formed in relation to spindle poles shortly after Cdk1 was inactivated by flavopiridol (22 of 22 oocytes; Fig. 3f and Supplementary Movie 17).

To investigate the requirement for spindles further, we made use of the spindle collapse that eventually occurs after flavopiridol-mediated Cdk1 inactivation induces an interphase-like state28. We treated oocytes with flavopiridol at ~2–3 h post-GVBD, before migration to the cortex had occurred (Supplementary Fig. 1). The spindle collapsed in most oocytes prior to reaching the cortex (29 of 37 oocytes), and in these cases, a sustained protrusion was never observed (29 of 29 oocytes; Supplementary Fig. 8a and Supplementary Movie 18). In contrast, in the minority of oocytes, in which the spindle remained intact all the way to the cortex and during advancement into the membrane, a sustained protrusion followed by furrowing occurred (4 of 4 oocytes; Supplementary Fig. 8b and Supplementary Movie 19). Strikingly, we observed that in four oocytes, the spindle collapsed after protrusion began but before PBE was complete, and in all cases, the protrusion then dramatically receded (Supplementary Fig. 8c and Supplementary Movie 20). Thus Cdk1 inactivation-induced protrusion requires an intact spindle supporting our hypothesis that protrusion involves a force transmitted via the spindle. Conversely, since sustained protrusions do not occur with chromosomes only when Cdk1 is inactivated, polar relaxation secondary to chromosome-induced cortical modifications could not be the basis for protrusion.

**Actin polymerisation is necessary for spindle migration.** Next, we investigated the nature of the propulsive force that Cdk1 inactivation might initiate. In MI mouse oocytes, F-actin levels increase by ~50% around the time of anaphase3. Since actin polymerisation can generate pushing forces32, it was therefore possible that Cdk1 inactivation might trigger actin polymerisation to displace the spindle.

We quantified F-actin intensity within the cytoplasmic region adjacent to the posterior pole during exit from MI, incorporating the ~2–3 h period during which cyclin B1 degradation and hence Cdk1 inactivation occurs (see Supplementary Fig. 6). We compared F-actin profiles in oocytes undergoing anaphase (reflecting intact Cdk1 inactivation) with profiles from oocytes that were simultaneously imaged under identical conditions in the same experiment but remained arrested at metaphase (reflecting compromised Cdk1 inactivation). In oocytes that did not undergo anaphase, F-actin levels were largely unchanged until ~1 h prior to the average time of anaphase-onset when they exhibited a slow increase (Fig. 4a). In contrast, when anaphase occurred, we observed two patterns of cytoplasmic F-actin increase. The first was a slow increase of ~28.5% from 2 h pre-anaphase-onset to 30 min pre-anaphase-onset (0.3% per min) coincident with the period when cyclin B1 destruction was underway, suggesting that it could be linked with Cdk1 inactivation. Strikingly, this slow increase changed to a steep increase ~20–30 min prior to anaphase-onset (coinciding with the period when Cdk1 activity is approaching its lowest levels) resulting in an ~47.3% increase in F-actin from 30 min pre-anaphase-onset to 10 min post-anaphase-onset (1.2% per min; Fig. 4a, b and Supplementary Movie 21). Note that the increase in cytoplasmic F-actin is more apparent when the UtrCH-Cherry signal is represented in monochrome rather than as a red pseudo-colour. Significantly, the F-actin spike coincided with the period of spindle migration and protrusion (Fig. 4a, b). To more closely investigate the relationship between F-actin and cyclin B1, we simultaneously imaged in the same oocyte cyclin B1-GFP with UtrCH-mCherry and fluorescent tubulin. We found that anaphase initiated ~10 min prior to the nadir of cyclin B1 (Fig. 4c) confirming that anaphase initiation coincided with almost complete Cdk1 inactivation. Consistent with our previous findings, F-actin levels began increasing steeply during the final 20–30 min of cyclin B1 destruction, with F-actin levels peaking 10 min before cyclin B1 levels were at their lowest (Fig. 4c). Thus F-actin levels undergo a biphasic increase contemporaneously with Cdk1 inactivation, including a steep and transient spike that coincides with near-complete Cdk1 inactivation, anaphase-onset and spindle migration.

To directly test whether inactivation of Cdk1 could induce F-actin polymerisation, we inactivated Cdk1 at a time when its activity is normally high by adding flavopiridol at 4 h post-GVBD, prior to the onset of cyclin B1 destruction (see Supplementary Fig. 6). By introducing flavopiridol while live-cell imaging was underway, the small but distinct oocyte displacement caused when the drug was pipetted into the media served as a visual cue for the precise time that Cdk1 inactivation commenced (see Supplementary Movie 22, Flavopiridol addition frame). Significantly, F-actin intensity increased steeply within 5 min of drug addition and peaked by 15 min (Fig. 4d, e and Supplementary Movie 22). If increased F-actin polymerisation generates a force to push the spindle, then preventing actin polymerisation should prevent spindle migration and protrusion. To test this, we added cytochalasin D2 to disrupt the actin meshwork at 6 h post-GVBD, after spindles had migrated to the cortex. We found that the F-actin spike previously associated with anaphase-onset was completely abolished by cytochalasin (Fig. 4f and Supplementary Movie 23). Highly significantly, these anaphase spindles did not migrate and no protrusions formed (29 of 29 oocytes; Fig. 4g and Supplementary Movie 23). Thus Cdk1 inactivation induces F-actin polymerisation that is critical for spindle displacement and protrusion.

**Formins mediate anaphase-onset F-actin polymerisation.** In mouse oocytes, formins and Arp2/3 predominantly nucleate cytoplasmic and cortical actin, respectively5,23. To determine which actin nucleator mediated the F-actin spike, we employed either the formin-specific inhibitor, SMI-FH233,34, or the Arp2/3-specific inhibitor, CK66623,35, late in MI after pre-anaphase spindle migration was complete.

We found that treatment with SMI-FH2 prevented the spike in cytoplasmic F-actin (Fig. 5a, b) normally associated with anaphase-onset (see Fig. 4a), whereas CK666 did not (Fig. 5c, d). CK666 did, however, blunt cortical thickening following anaphase-onset (Fig. 5e), consistent with Arp2/3’s known role in modulating cortical F-actin. Significantly, post-anaphase-onset spindle migration and protrusion occurred in 86% of CK666-treated oocytes (30 of 35 oocytes; Fig. 5d and Supplementary Movie 24) compared with only 33% of SMI-FH2-treated oocytes (5 of 15 oocytes; Fig. 5b). Cytoplasmic actin polymerisation in CK666-treated oocytes was important for spindle migration and protrusion since both were abrogated in oocytes co-treated with CK666 and cytochalasin D (22 of 22 oocytes; Fig. 5f).

Collectively, these data show that Cdk1 inactivation triggers marked formin-mediated cytoplasmic F-actin polymerisation that is critical for spindle migration and protrusion. Since protrusion is reliant on changes initiated within the cytoplasm and is unperturbed by disrupting changes in cortical F-actin, these data further support that protrusion is not an independent cortical event.

**Protrusion maximises asymmetry.** We reasoned that the shift in midzone position of ~8 µm brought about by anaphase spindle migration (see Fig. 11) could play a hitherto unrecognised role in influencing asymmetry by displacing the midzone-directed site of
furrowing. We imaged the spindle and the cortex and found that acute furrowing occurred at the base of the protrusion, midway along the length of the spindle (Supplementary Fig. 9 and Supplementary Movie 25), confirming that the midzone at the end of anaphase spindle migration defines the site of furrowing.

Since post-anaphase-onset spindle migration determines midzone position and hence the site of furrowing, the extent of migration could be critical for determining the degree of asymmetry. To better interrogate the effects of post-anaphase-onset migration, we sought to exaggerate the distance available for this phase of migration by inhibiting pre-anaphase migration. Our previous data indicated that, to leave post-anaphase-onset migration intact, we needed to target pathways that would not disrupt cytoplasmic F-actin.

To do this, we inhibited myosin II using two small molecule inhibitors, ML-7 and Y-27632, which impaired pre-anaphase spindle migration (Supplementary Fig. 10a) consistent with previous results. Remarkably, timelapse imaging revealed that, after undergoing anaphase at the oocyte-centre following inhibitor treatment, 17 of the 27 ML-7-treated and 43 of the 64 Y-27632-treated spindles then migrated towards the cortex to varying extents before furrowing (Supplementary Fig. 10b, c). As expected, for spindles that completed anaphase at the oocyte centre without migrating, near-symmetrical cleavage occurred.
Increased cytoplasmic F-actin is required for spindle migration. **a, b** UtrCH-mCherry fluorescence changes following treatment with SMIFH2 from 6 h post-GVBD ($n=10$). Note the absence of migration and protrusion following anaphase-onset. **c, d** Cytoplasmic UtrCH-mCherry fluorescence changes in relation to anaphase-onset following CK666 treatment from 6 h post-GVBD ($n=19$; see Supplementary Movie 24). Error bars are mean ± SEM. **e** Cortical UtrCH-mCherry fluorescence intensity within the protrusion in relation to anaphase-onset for control and CK666-treated oocytes. Box plots depict median (horizontal line), mean (crosses), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). One-way ANOVA used for statistical analysis. P values represented as ***$P<0.001$, **$P<0.01$ and *$P<0.05$. **f** Lack of anaphase-onset-related increase in UtrCH-mCherry fluorescence following co-treatment with CK666 + cytochalasin D from 6 h post-GVBD. Times in panels are hours:minutes relative to anaphase-onset. Scale bars, 10 μm. Oocyte numbers are shown in parentheses from a minimum of three independent experiments.

Mos depletion impairs post-anaphase-onset spindle migration. Because the Mos/MAPK pathway is known to be important for asymmetric division3,37, we were curious as to whether it might also impact post-anaphase-onset events in addition to its known role in pre-anaphase migration. To investigate this, we depleted Mos using a previously validated morpholino37,38. Consistent with effective knockdown, we found unusually large PBs in 49% of oocytes (53 of 108) and 15% of oocytes extruded a second PB (16 of 108; Supplementary Fig. 12), very similar to previously published rates using this same morpholino sequence37. In contrast, enlarged PBs occurred in only 14% (9 of 64) of mock-depleted oocytes with none extruding a second PB (Supplementary Fig. 12).

We were struck that, in 10 of the 29 oocytes with large PBs, the spindle underwent anaphase close to the cortex, yet large PBs were produced (Fig. 7a, b and Supplementary Movie 29), indicating that asymmetry was compromised despite intact pre-anaphase spindle migration. Unexpectedly, we found that post-anaphase-onset migration was markedly impaired in Mos-depleted oocytes regardless of the position of the spindle relative to the cortex when anaphase initiated (Fig. 7c). We note that cytoplasmic F-actin is unaffected in MOS$^{−/−}$ oocytes before anaphase-onset23, but wondered whether lack of Mos might...
impact the post-anaphase-onset period. Although an F-actin spike of similar magnitude to control oocytes was observed at anaphase-onset in Mos-depleted oocytes, the duration of the spike was noticeably shorter (Fig. 7d, e). Also, and consistent with earlier data\textsuperscript{13}, we found that anaphase-spindle elongation was significantly greater following Mos depletion than in controls (Fig. 7f, g).

Thus our data support that, following Mos depletion, at least two factors might impair spindle migration after anaphase-onset and thereby compromise asymmetry: shorter-lasting F-actin spikes and larger anaphase spindles. We note as well that, by positioning the midzone deep within the oocyte, longer anaphase spindles would predispose to large PBs independently of spindle migration\textsuperscript{13}. This genetic approach therefore strongly supports our previous findings using small molecule inhibitors that post-anaphase-onset spindle migration is critical for asymmetry.

**Fig. 6** Extreme asymmetry requires anaphase-spindle migration and protrusion. a–c Live oocytes treated with either ML-7 (a, b; see Supplementary Movie 26) or Y-27632 (c, see Supplementary Movie 28) undergoing anaphase at the oocyte centre followed by either no spindle migration (a; see Supplementary Movie 26), migration to the cortex without protrusion (b; see Supplementary Movie 27) or migration and protrusion (c; see Supplementary Movie 28) as depicted in schematics. d–g Polar body (PB) dimensions in ML-7 (d, e) and Y-27632 (f, g) treated oocytes. Note that Pre-anaphase and Post-anaphase (f and g) refer to pre-anaphase and post-anaphase spindle migration, respectively. Box plots depict median (horizontal line), mean (crosses), 25th and 75th percentiles (boxes) and 5th and 95th percentiles (whiskers). One-way ANOVA used for statistical analysis. P values represented as *P < 0.05, **P < 0.01, and ****P < 0.0001, ns denoted P > 0.05. Times in panels are hours:minutes relative to anaphase-onset. Scale bars, 10 μm. Oocyte numbers are shown in parentheses from a minimum of three independent experiments.

**Migration and midzone-to-cortex distance influence furrowing.** Most oocytes initiated anaphase with their spindles oriented perpendicular to the cortex (Fig. 8a, b). In such cases, a midzone was present for at least 15–20 min without acute furrowing, which only occurred when the midzone arrived at the base of the protrusion coincident with anaphase-2 completion (Fig. 8c and Supplementary Movie 30; see also Fig. 1f and Supplementary Fig. 9). In contrast, in the case of non-perpendicular spindles, furrowing occurred around 10 min earlier during anaphase-1 (n = 11; Fig. 8d and Supplementary Movie 31); hence, very shortly after formation, the midzone is fully capable of inducing...
furrowing. How then is furrowing ordinarily delayed in the case of perpendicular spindles until the very end of anaphase-2? One obvious difference was that, for non-perpendicular spindles, the mean midzone-to-cortex distance at anaphase-onset was 7.1 µm ($n = 14$), whereas for perpendicular spindles it was over threefold greater (23.8 µm; $n = 16$; $P < 0.0001$; Student’s t test). This suggested that spindle position in relation to the cortex could influence the strength of the midzone-derived signal reaching the cortex. In line with distance effects, non-perpendicular spindles induced furrowing at the nearby cortex but not at the contralateral (more distal) side when in an off-centre location ($n = 11$; Fig. 8d), whereas two surfaces furrowed almost simultaneously when the spindle was centrally located ($n = 6$; Fig. 8e and Supplementary Movie 32). Therefore, the interval between anaphase-onset and furrowing is dependent upon midzone-to-cortex distance consistent with previous findings during MII.12.

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**Fig. 7** Mos depletion impacts anaphase-spindle migration and spindle length. a Ratio of PB:oocyte diameter for mock-depleted oocytes and Mos-depleted oocytes having cortical-proximal spindles at the time of anaphase-onset. Oocyte numbers are shown in parentheses from a minimum of three independent experiments. b Large PB produced following Mos depletion despite the spindle being adjacent to the cortex at anaphase-onset (see Supplementary Movie 29). c Quantification of spindle displacement (due to the marked anaphase spindle elongation [see d], midzone displacement rather than posterior pole displacement was quantified) between anaphase-onset and anaphase-completion for Mos- and mock-depleted oocytes ($n = 11$). For Mos-depleted oocytes, results were grouped based on whether spindles underwent anaphase close to the oocyte centre (MosMO (away from cortex), $n = 12$) or close to the cortex (MosMO (near cortex), $n = 10$). d Cytoplasmic UtrCH-mCherry fluorescence in relation to anaphase-onset for mock- and Mos-depleted oocytes ($n = 20$ and $n = 17$, respectively). e Live Mos-depleted oocyte in which spindle undergoes anaphase close to the oocyte centre associated with increased cytoplasmic F-actin. f, g Changes in spindle lengths between anaphase-onset and anaphase-completion for live mock- and Mos-depleted oocytes ($n = 11$ and $n = 10$, respectively). Times in panels are hours:minutes relative to anaphase-onset. Scale bars, 10 µm. Two-tailed Student’s t test (a, f) or one-way ANOVA (c) used for statistical analysis. $P$ values represented as **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$. 

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Notably, however, during MI, such distance relationships are strongly influenced by relatively subtle changes in the orientation of the long spindle axis relative to the cortex.

Highly significantly, for non-perpendicular spindles undergoing anaphase at the cortex, division asymmetry was influenced by whether migration with protrusion occurred. If neither pole induced protrusion and the midzone remained stationary relative to the overlying cortex, the furrow then tracked through the oocyte center resulting in near-symmetrical cleavage (Fig. 8d and Supplementary Movie 31). Notably, an almost identical pattern of cleavage was obtained if the cortex is compressed at the site of the metaphase I spindle thereby immobilising it and preventing migration and protrusion39. In striking contrast, if one pole of a non-perpendicular spindle became anchored to the cortex after anaphase-onset, furrowing was delayed in favour of spindle migration and protrusion until the end of anaphase-2, ultimately leading to asymmetric division (n = 13; Fig. 8f and Supplementary Movie 33). This strongly suggests that at least two cooperative events, the midzone-to-cortex distance and anaphase spindle migration, reduce the ability of the midzone to sufficiently modify a focal region within the overlying cortex thereby disfavouring furrowing until the midzone arrives at the base of the protrusion where the midzone-to-cortex distance is minimal (Fig. 8g).

Discussion

Here we identify a phase of spindle displacement that occurs post-anaphase-onset secondary to Cdk1 inactivation. Reduced...
Cdk1 activity also induces spindle displacement in *C. elegans* zygotes\(^4\) supporting a broader role for Cdk1 in spindle motility. In contrast to *C. elegans* zygotes, however, spindle migration in mouse oocytes occurs after anaphase-onset and, importantly, results in protrusion that is critical for maximal asymmetry (Fig. 8h). High Cdk1 activity is known to inhibit separase\(^26\), explaining the close relationship between Cdk1 inactivation, anaphase-onset and spindle migration.

Since F-actin polymerisation is well known to generate pro-pulsive force\(^12\), we hypothesise that formin-mediated F-actin polymerisation induced by Cdk1 inactivation generates a force. We propose that this force pushes the anaphase spindle (manifested as anaphase-spindle migration) against the cortex thereby inducing protrusion. In this model, the spindle constitutes a mechanical transmitter as supported by the requirement of an intact spindle for sustained protrusions (Fig. 3). We do not rule out the possibility that short-range signals emanating from the cortex-proximal anaphase spindle could also contribute to protrusion formation. However, our data do not support that such signals, if they exist, are sufficient for protrusion (see Figs. 4g and 5f in which a cortex-proximal spindle undergoes anaphase, but in the absence of the cytoplasmic F-actin spike, no spindle migration and no protrusion occur). We did not measure tension within the protrusion and therefore do not rule out the possibility that cortical weakening could also facilitate protrusion although we note that this is not supported by the F-actin thickening we observed within the protrusion (Supplementary Fig. 7). Additionally, thinning of the protrusion cortex following Arp2/3 inhibition did not appear to predispose to overt PB enlargement (Fig. 5c, d) as might be expected if reduced cortical tension was critical for forming the protrusion. The transient nature of the F-actin spike is likely important for limiting force duration and, consequently, protrusion size. Conversely, as our data from Mos-depleted oocytes suggest, too short a spike could potentially compromise migration and hence protrusion. Increased oocyte force is also triggered concurrently with anaphase I in *C. elegans* oocytes, although in this case, force is myosin II mediated\(^41\). In *C. elegans* oocytes, force generation requires separase activation\(^41\), which is also likely important in mouse oocytes since PBE is impaired in separase-knockout oocytes\(^7\) and separase is required for inactivating Cdk1\(^42\).

Employing the same cell-cycle cue (Cdk1 inactivation) for inducing both anaphase and protrusion ensures that these two exit events remain tightly coupled. Notably, in mouse oocytes, Cdk1 inactivation might also activate the Rho-GEF, Ect2, and downstream Rhoa-mediated furrowing\(^16\). Cdk1 inactivation could therefore underpin key events required for MI exit. If triggered by a common cue, how would these events be constrained to occur in the correct temporal sequence? Our data support that at least one mechanism for enforcing a delay between anaphase-onset and furrowing is spindle migration. In *C. elegans* oocytes, the leading spindle pole remains stationary and the cortex is pulled down over the spindle\(^41\). Although distinctly different from spindle migration, the net effect in both cases is relative movement between the midzone and overlying cortex, pointing to common underlying events for delaying furrowing. Proper ordering of events during MI exit could also involve event-specific Cdk1 activity thresholds, akin to the model proposed for mitotic exit\(^43\). Since anaphase begins around 10 min before cyclin B1 levels are at their lowest, it is possible that each subsequent event is triggered as lower Cdk1 activity thresholds are reached during this terminal window of Cdk1 inactivation. Late furrowing could also involve the chromosomal passenger complex (CPC) since oocytes in which the catalytic CPC components, Aurora kinases B and C, are disrupted using a dominant-negative construct undergo protrusion but furrowing fails\(^44,45\).

Exactly which Cdk1 substrate(s) undergoes dephosphorylation to directly or indirectly augment F-actin polymerisation is not currently known but several components of actin-related pathways are Cdk1 substrates\(^46\). Interestingly, the activity of the fission yeast formin, Cdc12, is modulated by Cdk1-dependent phosphorylation\(^47\). It is significant in this regard that our results support that formins mediate F-actin polymerisation post-Cdk1 inactivation. Intriguingly, Formin2 expression increases during late MI in mouse oocytes and peaks around the time of anaphase-onset\(^48\) raising the possibility that high formin levels could prime the peri-anaphase actin surge. Formin2-mediated actin polymerisation also promotes spindle displacement during early MI\(^49\). This raises the possibility that formin-dependent spindle mobilisation occurs during periods of relatively low Cdk1 activity (early MI and post-anaphase-onset). Conversely, Arp2/3 could predominate in high-Cdk1-activity environments, explaining the importance of Arp2/3-mediated cytoplasmic flow during late pre-anaphase spindle migration\(^48\) and in MI\(^50\) but not in the intervening low-Cdk1-activity period (our data).

Our findings in Mos-depleted oocytes suggest that the other major oocyte kinase and target of Mos, MAPK, is also important for post-anaphase-onset migration, in part by influencing the duration of the F-actin spike as well as indirectly by restraining spindle growth during anaphase. Given that MAPK modulates APC-mediated cyclin B1 destruction in late MI\(^51\), it is possible that Mos depletion impacts MAPK-dependent control of Cdk1 inactivation and hence F-actin polymerisation.

In closing, we note that the GV, and hence spindle, often assumes an off-centre position in follicle-enclosed mouse oocytes, suggesting that surrounding somatic cells contribute to asymmetry\(^21\). Since our experiments required denuded oocytes for detailed imaging, we cannot exclude that additional somatic inputs also contribute to protrusion formation in follicle-enclosed oocytes in vivo.

**Methods**

**Oocyte isolation, culture and microinjection** All animals were housed in a specific pathogen-free environment in filter-top cages and fed a standard diet. All work involving animals complied with all relevant ethical regulations and was approved by the Animal Ethics Committee at the University of Queensland. Oocytes were isolated from the ovaries of 3–4-week-old B6CBAF1 female mice (44–46 h following intraperitoneal injection of 7.5 international units (IU) of pregnant mare’s serum gonadotrophin (PMSG)). Dissected ovaries were immediately transferred to the laboratory in pre-warmed αMEM HEPES-buffered medium (Sigma–Aldrich) containing 50 μM 3-isobutyl-1-methylxanthine (IBMX; Sigma–Aldrich), which prevents oocytes from undergoing GVBD\(^42,43\). Ovarian wall follicles were punctured in IBMX-treated media in 35 × 10 mm dishes using a 27 G needle under direct vision on the stage of a stereomicroscope (M165C, Leica Microsystems). Only fully grown cumulus-covered oocytes were isolated and used for further experiments. For longer-term culture and for all confocal imaging, oocytes were cultured in micro-drops of M16 media (Sigma–Aldrich) under embryo-tested light mineral oil (Sigma–Aldrich) at 37 °C in an atmosphere of 5% CO₂ in air\(^52–54\).

For undertaking microinjection\(^52–54\), GV-stage oocytes in IBMX-treated medium were stabilised using suction applied through a hydraulic syringe to a pre-fabricated holding pipette (inner diameter 15 μm, outer diameter 75 μm, 35° bend; The Pipette Company). Microinjection needles were pulled from capillary tubes (0.86 mm inner diameter, 1.5 mm outer diameter; Harvard Apparatus) to a pre-determined calibre using a vertical pipette puller (P30 vertical micropipette puller, Sutter Instruments). The tip of the microinjection pipette was advanced across the zona pellucida and oolemma into the cytoplasm of the oocyte aided by a brief electrical pulse delivered by an intracellular electroporator (IE-2S1A, Warner Instruments). A precise volume roughly equal to 5% of the oocyte volume of test solution was then delivered to the oocyte using a Pneumatic PicoPump (PV-820, World Precision Instruments). The rate of oocyte death following microinjection was consistently <10%.

**cRNA constructs and morpholinos**. The mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit (Ambion) was used to produce cRNA constructs by T7-polymerase driven in vitro transcription from linearised DNA template\(^55,56\). Constructs used in this paper were histone 2B (H2B)-RFP, Securin-GFP, Cyclin B1-mCherry, UtrCH-mCherry (a gift from William Bement; Addgene plasmid #
Drug additions. Stock solutions of all small molecule inhibitors were made in DMSO at the highest possible concentration that enabled complete solubilisation thereby minimising the volume of DMSO added to media. For inhibiting Cdk1 activity, flavipridol (SelleckChem; 10 mM stock solution) was dissolved in media for a final concentration of 5 μM as used previously in mammalian somatic cells47 and mouse oocytes28,29. RO-330631 (Sigma-Aldrich; 20 mM stock solution) was also used to inhibit Cdk1 activity at 10 μM as used with the APC-Cdk2 inhibitor, APCIN (Sigma-Aldrich; 50 mM stock solution30), or the 26S proteasome inhibitor, MG132 (SelleckChem; 10 mM stock solution), to be used to inhibit proteolysis at 4 h post-GVBD before proteolysis has begun (Supplementary Fig. 6) at 150 and 5 μM, respectively. To inhibit MAPK activity late in meiosis, at 6 h post-GVBD, an Mek1/2 inhibition described earlier, U0126 (Sigma-Aldrich; 20 mM stock solution) was used at a concentration of 50 μM. Myosin II activity was inhibited prior to spindle migration at 2 h post-GVBD (Supplementary Fig. 1) by addition of either Y-2763216,36 (50 μM) (Sigma-Aldrich; stock solution 10 mM) or ML-728 (50 μM) (Sigma-Aldrich; stock solution 30 mM). Nocodazole was used to depolymerise the spindle at a concentration of 5 μM (Sigma-Aldrich; stock solution 10 μM) for 20 min. Actin polymerisation was disrupted at 6 h post-GVBD using cytochalasin D at 1 μM (Sigma-Aldrich; stock solution 2 mM), SMI-FH2 at 25 μM (Sigma-Aldrich; stock solution 50 mM) or CK666 at 200 μM (Sapphire Bioscience; stock solution 50 μM). As a control, DMSO, with or without inhibitors was added to microinjected oocytes to determine at successive time points throughout anaphase. Migration distance was then derived from the posterior pole displacement, which was the difference between the posterior pole-to-membrane distance at one time point and that at subsequent time points (y – x). During spindle migration, the leading pole was considered as the pole that migrated into the anterior oocyte membrane following anaphase-onset. The (lagging) pole was the posterior pole, which was closest to the posterior oocyte membrane (see Fig. 1e). To quantify migration, the distance between the posterior pole and the posterior oocyte membrane (posterior pole-to-membrane distance), was determined at successive time points throughout anaphase. Migration distance was then derived from the posterior pole displacement, which was the difference between the posterior pole-to-membrane distance at one time point and that at subsequent time points (y – x) (see Fig. 1e).

The term prometaphase was used to refer to the outpocketing that occurred following anaphase-onset, partially enveloping one-half of the spindle and extended above the level of the surrounding oocyte membrane. Prometaphase duration was determined as the distance from the base of the prometaphase (at the level of the surrounding oocyte membrane) to the most extreme aspect of the outpocketing (determined from either the membrane or the cortical F-actin signal) along the line of the long axis of the spindle (Fig. 1e).

Spindles were categorised as perpendicular or non-perpendicular using the criteria shown in Fig. 8a. The plane of the membrane/cortex was determined as the plane of the highest intensity of F-actin (see Fig. 1e). To analyse F-actin levels in anaphase and non-anaphase spindles, we used the Leica TCS SP8 microscope to generate z-stack images of the entire spindle using the HyD ultra-sensitive detection system, allowing us to use automated z-stack analysis to determine spindle dimensions with high precision. For each experiment, images were acquired in 3 z-stacks with an increment of 2 μm. Spindles were categorised as those with a long axis in the horizontal plane (parallel spindle) or in the horizontal plane throughout anaphase (perpendicular spindle). Spindles were classified as either parallel or perpendicular to the plane of the membrane/cortex using the angle of the long axis of the spindle, which was measured relative to the plane of the membrane/cortex (see Fig. 8c).

Quantification of spindle dimensions and cortical dynamics. For analysis of spindle size and migration, only oocytes having bipolar spindles that were oriented in the horizontal plane and that remained strictly in the same orientation through the entire period of anaphase, prometaphase, and cytokinesis were included in analyses. Indeed, spindles from single-cell embryos were excluded only from spindles formed from the bicell lineages in the horizontal plane throughout anaphase (see Fig. 1f). Spindle length was measured manually as the pole-to-pole distance along the long axis of the spindle. Spindle midzone dimensions were measured manually, as the maximal distance between the extreme limits of the spindle along a line positioned roughly midway between the posterior and anterior poles of the oocyte. The angle between the long axis of the spindle and the perpendicular to the plane of the membrane/cortex was measured using the angle between the posterior pole and the equatorial aspect of the spindle, which was determined as the distance from the base of the prometaphase to the equatorial aspect of the spindle and the perpendicular to the plane of the membrane/cortex (see Fig. 8d).

To analyse F-actin levels in anaphase and non-anaphase spindles, we used the Leica TCS SP8 microscope to generate z-stack images of the entire spindle using the HyD ultra-sensitive detection system, allowing us to use automated z-stack analysis to determine spindle dimensions with high precision. For each experiment, images were acquired in 3 z-stacks with an increment of 2 μm. Spindles were categorised as either parallel or perpendicular to the plane of the membrane/cortex using the angle of the long axis of the spindle, which was measured relative to the plane of the membrane/cortex (see Fig. 8c).

Quantification of cytokaplastic F-actin. UtrCH-mCherry fluorescence intensity was used for quantifying F-actin levels in live oocytes23,24. Cytoskeletal UtrCH-mCherry intensity was determined by measuring the mean background-corrected fluorescence intensity within a cortex-free region drawn immediately adjacent to the lagging spindle pole. To analyse F-actin levels in anaphase and non-anaphase oocytes in Fig. 4a, absolute fluorescence intensity values were plotted over time since the true scale of the difference between the two groups would be lost if normalised values were used. Because oocytes from both groups were analysed within the same experiment, they were microinjected with near-identical cRNA quantities and imaged using identical exposure conditions, allowing us to use changes in absolute fluorescence intensities as a reliable means for comparing within-group changes over time as well as for comparing between-group differences. For all other plots of UtrCH-mCherry fluorescence, intensity was normalised to the maximum value for each individual oocyte.
shown within parenthesis. All experiments were repeated a minimum of three times.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Author contributions**
H.A.H. conceived the project, designed experiments, analysed and interpreted data and wrote the paper. Z.W. and J.G. contributed equally to undertaking most experiments, analysing data and preparing figures. C.Z. undertook experiments involving nocodazole treatment.

**Additional information**
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**Competing interests:** H.A.H. is a co-founder of a company, JumpStart Fertility, whose intention is to develop novel agents for improving oocyte quality. All remaining authors declare no competing interests.

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