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# Role of MAP kinase and myosin light chain kinase in chromosome-induced development of mouse egg polarity

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**Abstract**

During maturation, the mouse oocyte is transformed into a highly polarized egg, characterized by an actin cap and cortical granule-free domain (CGFD) overlying the meiotic spindle that is in close proximity to the cortex. The presence of spindle/chromosomes or microinjected sperm chromatin in the cortical region initiates this cortical reorganization, but the pathway is unknown. We report that cortical reorganization induced by microinjected sperm chromatin is blocked by inhibitors of microfilament assembly or disassembly. Active mitogen-activated protein kinase (MAPK), which becomes enriched in the region of sperm chromatin, is required for cortical reorganization, because microinjected sperm chromatin fails to induce cortical reorganization in *Mos*$^{-/-}$ eggs, which lack MAPK activity. Last, myosin light chain kinase (MLCK), which can be directly phosphorylated and activated by MAPK, appears involved, because the MLCK inhibitors ML-7 and Peptide 18 prevent sperm chromatin-induced cortical reorganization. These results provide new insights into how cortical reorganization occurs independently of extracellular signals to generate egg polarity.


*Keywords:* Mitogen-activated protein kinases; Myosin light chain kinase; Actins; Cell polarity; Ovum

## Introduction

Mouse oocytes, which are arrested in the first meiotic prophase, are radially symmetrical with respect to cortical components, for example, microvilli, cortical actin, and cortical granules (CG) (Fig. 1A). During maturation to metaphase II (MII), the centrally positioned meiotic spindle translocates to the cortex in a microfilament- (Wassarman et al., 1976) and formin-2-dependent (Leader et al., 2002) manner. Spindle translocation results in CG redistribution and consequent formation of a CG-free domain (CGFD), loss of microvilli in the region overlying the meiotic spindle, and cortical actin thickening to form an actin cap (Deng et al., 2003; Longo and Chen, 1985; Maro et al., 1986; van Blerkom and Bell, 1986). When spindle translocation does not occur, for example, in formin-2 or *Mos* null oocytes, cortical reorganization does not occur (Leader et al., 2002; Verlhac et al., 2000). Furthermore, myosin II colocalizes with the actin cap (Simerly et al., 1998), positioning components of the contractile apparatus over the metaphase II (MII) spindle. These changes, which are subsequently referred to as "cortical reorganization", result in the formation of a highly polarized egg (Fig. 1B). Of note, recent work suggests that the position of the actin cap, in conjunction with the site of sperm entry, defines the first cleavage plane in the mouse embryo (Piotrowska and Zernicka-Goetz, 2001).

What is intriguing about the development of mouse egg polarity and the underlying cortical reorganization is that it occurs in the absence of any obvious external ligand. This contrasts with the more common ligand-mediated induction of polarity, for example, chemoattractant-induced cell motility (Kutsuna et al., 2004), and suggests that the signal is intrinsic to the maturing oocyte. Because these cortical changes occur in the vicinity of the meiotic spindle, the

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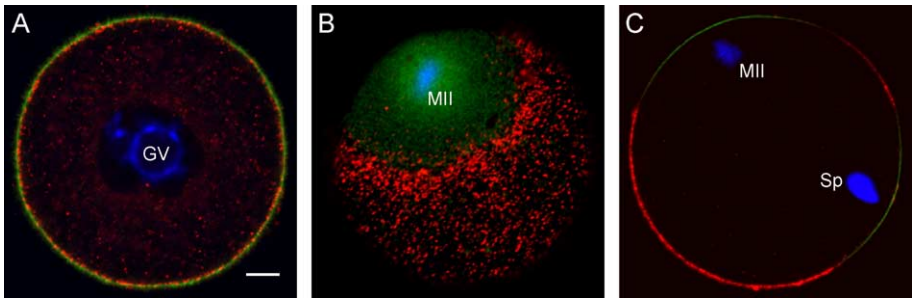


Fig. 1. Confocal images highlighting the development of egg polarity. GV-intact oocytes, MII eggs, or sperm chromatin-injected MII eggs were stained for DNA (blue), actin (green), or CGs (red). (A) In the GV-intact oocyte, actin and CGs are uniformly distributed in the cortex. (B) In the MII egg, an actin cap and CGFD are seen overlying the MII chromosomes (and spindle, not shown). Also note the higher density of CGs at the periphery of the CGFD adjacent to the actin cap. (C) In the sperm chromatin-injected MII egg, an actin cap and CGFD are also found in the region of the injected sperm chromatin. In this and subsequent figures, MII and Sp indicate the egg chromosomes and sperm chromatin, respectively. Unless otherwise noted, DNA is blue, actin is green, and CGs red; scale bar corresponds to 10 μm.

spindle is an obvious candidate to induce the response. Disrupting the MII spindle with nocodazole, however, can result in chromosome scattering with the formation of a CGFD and accumulation of cortical actin in the region of cortically localized chromosomes (Connors et al., 1998). A similar cortical reorganization is observed when either individual metaphase chromosomes are mechanically placed in the egg cortex (van Blerkom and Bell, 1986) or sperm chromatin incapable of inducing calcium oscillations is injected into an MII egg (Deng et al., 2003) (Fig. 1C). These findings suggest that chromosomes alone or in association with other cellular components induce cortical reorganization.

Although this phenomenon was noted some 20 years ago, there is little information regarding the pathway mediating cortical reorganization in mammalian eggs. We report that MAP kinase (i.e., ERK1 and ERK2; mitogen-activated protein kinase (MAPK)) is required for cortical reorganization, because sperm chromatin-induced cortical reorganization is not observed in *Mos*$^{-/-}$ eggs, which lack any MAPK activity. MAPK may promote the changes in cortical actin dynamics by phosphorylating myosin light chain kinase (MLCK), a known substrate for MAPK, because the MLCK inhibitors ML-7 and Peptide 18 inhibit cortical reorganization. These findings suggest that chromosomes can serve as a novel trigger that modulates the actin cytoskeleton.

## Materials and methods

### *Chemicals*

All chemicals were from Sigma unless stated otherwise.

### *Oocyte and egg collection, and culture*

Oocytes and MII-arrested eggs were harvested from female CF-1 mice (Harlan Sprague–Dawley, Indianapolis, IN) as previously described (Mehlmann and Kline, 1994). The collection and culture media were as previously described (Deng et al., 2003). *Mos*$^{-/-}$ female mice were the generous gift of John Eppig (Jackson Laboratory, Bar Harbor, ME). To prepare *Mos*$^{-/-}$ MII eggs, germinal vesicle (GV)-intact oocytes were collected from *Mos*$^{-/-}$ mice and matured in CZB for 15 h (Chatot et al., 1989). Oocytes that extruded the first polar body were used for sperm chromatin injection. GV-intact oocytes collected from C57BL/6J (Jackson Laboratory) and matured under the same conditions as *Mos*$^{-/-}$ oocytes served as controls. Unless otherwise indicated, oocytes and eggs were cultured at 37°C in a humidified atmosphere of 5% $CO_2$ in air.

### *Drug treatments*

MII-arrested eggs were injected with sperm chromatin and within 10 min transferred to medium containing 500 μM latrunculin A (Lat A) in 4-well culture dishes (Nunc, Denmark) with no oil overlay. Lat A treatment at this concentration for as short as 5 min resulted in complete loss of cortical actin over the MII spindle. This experimental protocol was used because of the poor survival rate of eggs microinjected in the presence of Lat A. Following injection, eggs were cultured for 90 min in Lat A-containing medium and then processed for CG and actin staining. For ML-7 treatment, eggs were similarly microinjected with sperm chromatin and then incubated in medium containing 5 μM ML-7 for 90 min.

Staurosporine and jasplakinolide were used to inhibit protein kinases and actin dynamics, respectively. MII-arrested eggs were incubated in medium containing either 10 μM staurosporine or 100 nM jasplakinolide for 30 min prior to sperm chromatin injection in the continued presence of the drug. Following injection, the eggs were cultured for 90 min in drug-containing medium and then processed for CG and actin staining.

### *Microinjection of eggs*

Sperm chromatin was injected into the egg cortex as previously described (Deng et al., 2003) as far as possible

from the MII spindle to maximize visualization of the sperm chromatin-induced cortical reorganization. The sperm head was injected into the cortex, because cortical chromatin positioning is required to observe cortical reorganization (Deng et al., 2003). It should be noted that in all control eggs in which the injected sperm head was in close proximity to the plasma membrane there was evidence of cortical reorganization, whose extent appeared positively correlated with proximity. Eggs in which the injected sperm head did not remain in the cortex were excluded from the analysis; this was typically about 10% of the injected eggs. Sperm chromatin-injected eggs were cultured for 1.5–2 h to allow induction of the cortical response (Deng et al., 2003). To stabilize F-actin, 5–10 pl of 6.3 mM FITC-phalloidin dissolved in methanol was injected into MII-arrested eggs. The phalloidin-injected eggs were allowed to recover for 40 min prior to sperm chromatin injection.

To assess the cortical response to an injected sperm head when Cdk1 activity was low, eggs were activated by incubating them for 1 h in $Ca^{2+}$-free Whitten's medium (Whitten, 1971) containing 10 mM $SrCl_2$. The eggs were allowed to recover for 0.5 h in $Ca^{2+}$-containing Whitten's medium and then only eggs that had emitted the second polar body were injected with a sperm head. This protocol ensured that only eggs that contained basal levels of Cdk1 activity were injected (Ducibella et al., 2002; Moos et al., 1995). The eggs were incubated in Whitten's medium for an additional 1.5 h before being processed for immunocytochemistry as described below.

MII eggs were also injected with the myosin light chain kinase (MLCK) inhibitory Peptide 18 (Calbiochem, Cat#475981) that was diluted in NIM (123 mM KCl, 2.6 mM NaCl, 7.8 mM $NaH_2PO_4$, 1.4 mM $KH_2PO_4$, 3 mM EDTA; Kuretake et al., 1996) and coinjected with sperm chromatin such that the final intracellular peptide concentration was ~10 μM. Control MII eggs were coinjected with a protein tyrosine kinase Peptide inhibitor (Calbiochem, Cat#657015) to the same final concentration as the MLCK inhibitory peptide. The eggs were then cultured for 1.5 h as described above and then fixed and processed for actin and CGs as described below.

### *Immunocytochemistry and confocal microscopy*

Eggs were fixed for 20–30 min in 3.8% paraformaldehyde in PBS and then blocked with PBS containing 3 mg/ml BSA, 0.01% Tween 20, and 0.1 M glycine for at least 1 h. The fixed eggs were permeabilized in PBS containing 3 mg/ml BSA and 0.1% Triton X-100 for 10 min. Lens culinaris agglutinin staining of CGs was conducted as previously described (Deng et al., 2003), except that streptavidin-conjugated Alexa Fluor 546 (Molecular Probes) was used at a 1:200 dilution in PBS containing 3 mg/ml BSA and 0.01% Tween 20.

To visualize F-actin, eggs were stained with 6.6 μM Alexa Fluor 488-conjugated phalloidin (Molecular Probes) for 15 min. To detect the active, phosphorylated form of MAPK (pMAPK), eggs were fixed and permeabilized as described above and then incubated with a rabbit anti-pMAPK antibody (Santa Cruz Biotechnology, sc-7976-R) at a final concentration of 40 μg/ml for 60 min, followed by a 40-min incubation with an anti-rabbit Alexa Fluor 488-conjugated secondary antibody (1:100). Specificity was established by including excess blocking peptide (5-fold by weight) (Santa Cruz Biotechnology, sc-7976-P). Eggs were mounted in Vectashield (Vector Laboratories) containing 1.5 μg/ml DAPI for chromatin visualization and observed with a laser-scanning confocal microscope (Leica DMRE). Images were taken with a 40× Plan APO objective N.A. 0.75. Images were acquired with Leica confocal software and processed using Adobe Photoshop.

## Results

### *Rationale for the model system*

As described in the Introduction, chromosomes have the capacity to induce cortical reorganization. We chose to inject exogenous chromosomes rather than monitor the activity of the endogenous chromosomes because many of the treatments or conditions described below inhibit the movement of the spindle to the cortex and therefore cortical reorganization would not be observed. Likewise, chromosome scattering that occurs following disruption of the MII spindle does not consistently position chromosomes in the cortex. In contrast, microinjection affords a high degree of control over the positioning of chromatin and timing of events of cortical reorganization not readily achievable during oocyte maturation. Furthermore, although the MOS pathway has been proposed to have a role in cortical reorganization, the failure of spindle translocation in $Mos^{-/-}$ oocytes confounds this interpretation (Verlhac et al., 2000). Our approach of microinjecting sperm heads in close proximity to the plasma membrane of $Mos^{-/-}$ oocytes bypasses this problem and permits direct analysis of the MOS pathway in cortical reorganization.

We chose sperm heads as a source of chromatin because the procedure to isolate sperm chromatin results in a preparation that lacks membranes, and contains condensed chromatin that is easily manipulated during microinjection. Moreover, procedures used to isolate somatic cell nuclei are more laborious, and detergent-extracted nuclei are extremely difficult to microinject. Last, by injecting MII-arrested eggs, analysis of the actin cap and CGFD in the region of the MII spindle provides an internal control for actin and CG organization and the efficacy of drug treatments in modulating microfilament assembly/disassembly. Note that hereafter we will refer to the sperm head as sperm chromatin, because the preparation is largely composed of chromatin, although there are some nonchromatin proteins that remain associated (Kimura et al., 1998).

*Cortical reorganization requires MAP kinase activity*

Because protein phosphorylation is commonly used in signaling pathways, we first globally inhibited protein kinases with staurosporine to examine their involvement in cortical reorganization. Eggs were incubated in medium containing 10 μM staurosporine, a concentration that induces apoptosis in preimplantation mouse embryos (Weil et al., 1996), presumably by globally inhibiting protein kinases. This treatment inhibited the ability of the injected sperm chromatin to induce an actin cap and a CGFD, but did not disrupt the actin cap overlying the MII spindle (data not shown). This inhibition could not be attributed to compromised egg viability, because staurosporine treatment for 4 h induced parthenogenetic activation in control eggs (Sun et al., 1998) (data not shown).

The observation that sperm chromatin microinjected into a GV-intact oocyte does not induce cortical reorganization (data not shown) suggests that the responsible protein kinase(s) exhibits a maturation-associated increase in activity. Cyclin-dependent protein kinase 1 (Cdk1, also known as maturation-promoting factor) and MAPK are two such protein kinases whose activity is very low in GV-intact oocyte and peaks in MII-arrested eggs (Gebauer and Richter, 1997). Furthermore, the signal that induces cortical reorganization is likely associated with the spindle, as is true for Cdk1 and MAPK.

To test whether Cdk1 activity was required for cortical reorganization, we ascertained the ability of injected sperm chromatin to induce cortical reorganization under conditions in which Cdk1 activity is essentially absent but MAPK activity is high. To do this, we capitalized on the observation that Cdk1 activity is reduced to basal levels within 1 h following egg activation, whereas MAPK activity only starts to decline after 4–5 h (Ducibella et al., 2002; Moos et al., 1995). We found that sperm chromatin induced the cortical response when injected into $SrCl_2$-activated eggs that had emitted the second polar body; these eggs were analyzed 3 h following initiation of $SrCl_2$ treatment (Fig. 2). Thus, the eggs were injected with sperm chromatin and cultured when Cdk1 activity is absent. These results indicate that Cdk1 activity is not required for cortical response.

To determine if MAPK activity is required for sperm chromatin-induced cortical reorganization, we obtained $Mos^{-/-}$ eggs by culturing GV-intact $Mos^{-/-}$ oocytes to MII; these eggs do not contain detectable MAPK activity (Verlhac et al., 1996). Control oocytes of the same strain were similarly matured in vitro. Sperm chromatin was injected into these eggs that were then examined for cortical reorganization. We found that the injected sperm chromatin did not induce this response in $Mos^{-/-}$ eggs (Fig. 3A) but did in the controls (Fig. 3B). In toto, these results provide strong evidence that MAPK (and not Cdk1) is a component of the sperm chromatin-initiated pathway leading to cortical reorganization.

*Active MAP kinase accumulates in the region of the microinjected sperm chromatin*

The spatial relationships of the actin cap and CGFD to the metaphase II spindle suggest that the active phosphorylated form of MAPK (pMAPK) (Nishida and Gotoh, 1993; Pelech and Sanghera, 1992) would localize in this region. The findings that chromosomes or microinjected sperm chromatin can induce cortical reorganization suggest that active MAPK may localize to areas containing chromatin. When MII-arrested eggs were stained with an antibody that recognizes pMAPK, similar to previous reports (Hatch and Capco, 2001), we observed staining of the spindle microtubules that was more intense than that in the cytoplasm, but no apparent staining of the chromosomes (Figs. 4A and B). As anticipated, pMAPK was also enriched in the region of the injected sperm chromatin (Figs. 4C–F); staining was specific because the signal was abolished when the antibody was incubated with the blocking peptide (Figs. 4G and H).

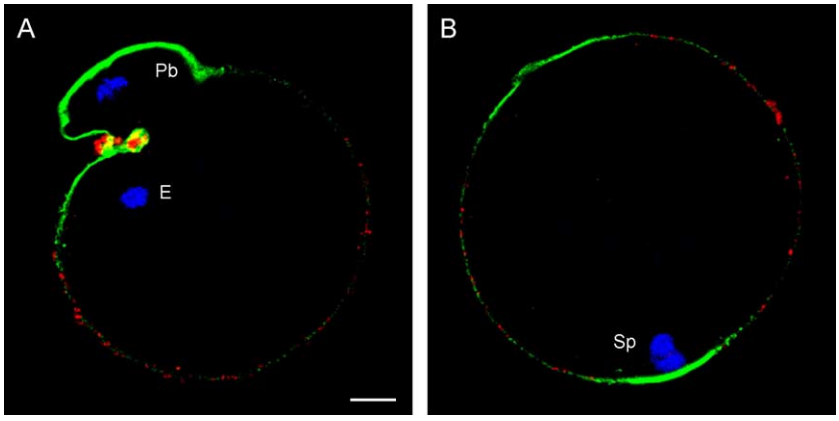


Fig. 2. Cortical reorganization in the absence of Cdk1 activity. The eggs were stained for actin, cortical granules, and DNA. (A) Confocal plane through egg chromatin. (B) Confocal plane through sperm chromatin. Note the reduced number of CGs in these activated eggs. Pb, polar body; E, egg chromatin; Sp, sperm chromatin. A total of 17 eggs were injected, of which 15 were suitable for analysis and showed the response; shown is a representative example.

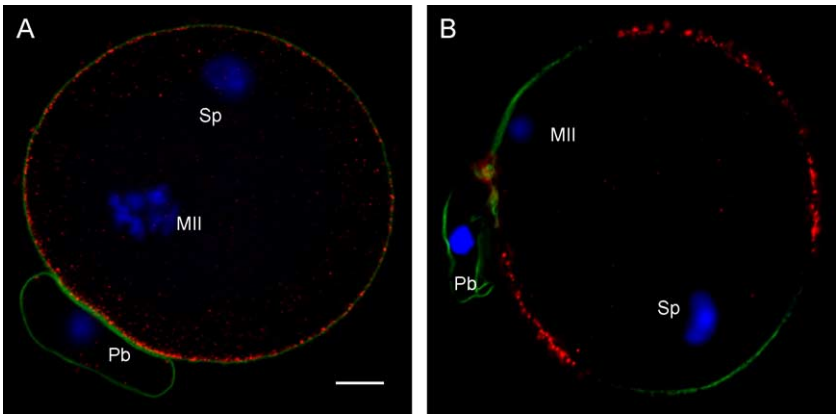


Fig. 3. Requirement for MAPK activity in cortical reorganization. (A) *Mos*$^{-/-}$ MII egg. Note the large polar body (Pb), which has been previously observed (Choi et al., 1996; Verlhac et al., 1996). (B) Control MII egg. The experiment was repeated three times; a total of 33 eggs were analyzed and none showed the response.

### *Sperm chromatin-induced cortical reorganization requires microfilament assembly/disassembly*

As described in the Introduction, loss of microvilli occurs during cortical reorganization. One formal possibility was that the presence of chromatin in the cortex resulted in incorporation of intact microfilament bundles into the cortical actin cap rather than requiring microfilament assembly/disassembly, which would suggest different targets for MAPK action. We used Lat A (Spector et al., 1989) to disrupt microfilament assembly; Lat A binds to G-actin adjacent to the nucleotide-binding cleft, thereby sequestering all available G-actin monomers and preventing their incorporation into F-actin (Coue et al., 1987).

MII-arrested eggs treated with Lat A were injected with sperm chromatin and then assessed for cortical actin and CGs. Lat A treatment resulted in complete loss of cortical actin over the MII spindle (Figs. 5A and B). Although a CGFD remained in the region of the MII spindle in treated eggs, sperm chromatin did not induce either an actin cap or a CGFD. This effect was reversible, because a cortical actin cap and CGFD formed when these Lat A-treated and sperm chromatin-injected eggs were cultured in Lat A-free medium for 2 h (data not shown). These findings also suggest that CG redistribution requires actin assembly.

To determine if microfilament disassembly was required for sperm chromatin-induced cortical reorganization, MII-arrested eggs were either microinjected with FITC-phalloidin or incubated in medium containing 100 nM jasplakinolide, each of which stabilizes microfilaments by binding to F-actin (Bubb et al., 1994; Cooper, 1987). In both cases, the injected sperm chromatin failed to induce formation of the actin cap as well as a CGFD (Figs. 5C and D). These results, in conjunction with those obtained using Lat A, suggest that microfilament assembly/disassembly—and not incorporation of intact microfilaments derived from microvilli—is required for cortical reorganization. Microfilament assembly/disassembly, however, is not required for maintenance of the CGFD once it has formed.

### *Cortical reorganization requires myosin light chain kinase activity*

The previous results suggested that proteins involved in actin dynamics were candidate downstream targets for MAPK. Members of the Rho family of small G proteins are frequently implicated in actin dynamics (Burridge and Wennerberg, 2004; Hall, 1998). These proteins can activate MAPK (Burridge and Wennerberg, 2004), but we found no evidence from the literature that MAPK can activate Rho family members. Because phosphorylation and activation of MLCK by MAPK can induce changes in actin dynamics (Klemke et al., 1997), we first tried to determine if pMLCK could be detected in the region of the microinjected sperm head, and if so, whether inhibiting activation of MAPK by either U0126 or using *Mos* null oocytes prevented this phosphorylation. Regrettably, the antibodies available were not suitable for immunocytochemistry in our system. Accordingly, we examined the effect of MLCK inhibitors on the ability of the injected sperm chromatin to induce cortical reorganization. The inhibitors used were ML-7, which is a competitive inhibitor with respect to ATP (Saitoh et al., 1987), and a peptide inhibitor Peptide 18, identified by functional genomics to identify short auto-inhibitory sequences in MLCK that do not interact with calmodulin (Lukas et al., 1999).

Results of these experiments indicated that ML-7-treated eggs did not undergo cortical reorganization in the region of the injected sperm chromatin (Figs. 6A and B). The failure of the CGFD to form suggests that CG redistribution requires myosin II function, that is, formation of the CGFD is a consequence of local alterations in actin dynamics. Moreover, the actin cap overlying the metaphase II spindle appeared somewhat disrupted. The actin cap was lost when the eggs were incubated for an additional 1.5 h in ML-7 (data not shown), suggesting that MLCK activity is required to maintain the actin cap. The CGFD was still present at this time, consistent with the CGFD remaining after complete disruption of the actin cap overlying the MII spindle with Lat A (see above). These results suggest that once formed,

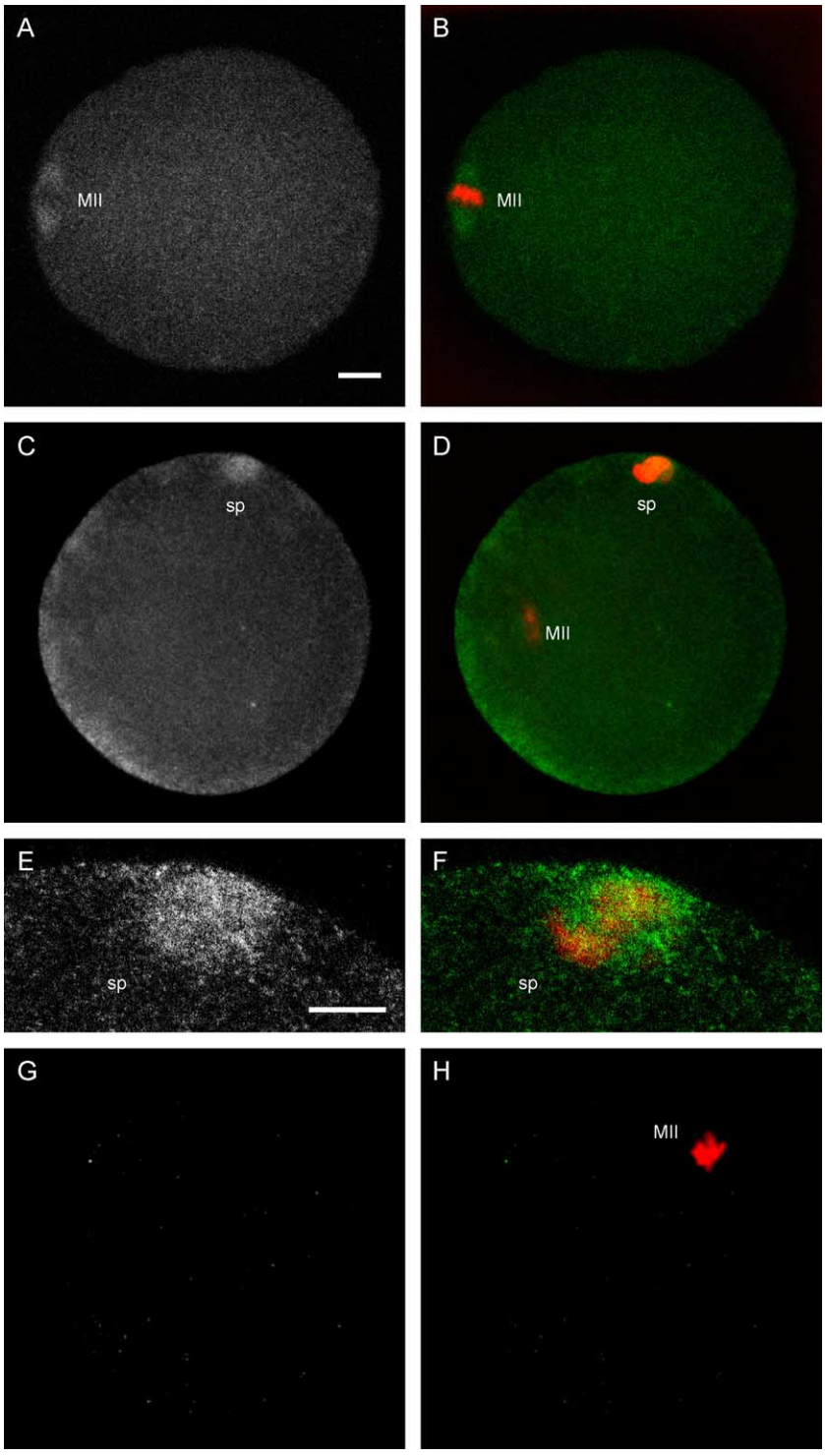


Fig. 4. Association of pMAPK with MII spindle and injected sperm chromatin. The eggs were stained for pMAPK (grey/green) and DNA (red). (A, B, G, and H) Control MII eggs. (C and D) Sperm chromatin-injected egg. (E and F) Same egg as in C and D, but at higher magnification; scale bar corresponds to 5 μm. Specificity of staining was confirmed by the absence of staining when blocking peptide was used (G and H). Left panels show just pMAPK staining, and right panels show merged image of pMAPK and DNA. The experiment was performed three times and a total of 21 sperm chromatin-injected eggs were analyzed and all showed the association.

the CGFD is stable and does not require the continued presence of microfilaments.

A similar phenotype was observed when eggs were injected with Peptide 18, but not the same concentration of a peptide inhibitor to a protein tyrosine kinase (Figs. 6C and D). The similar phenotype induced by two inhibitors whose mechanisms of action differ strongly suggests that MAPK is responsible for MLCK phosphor-

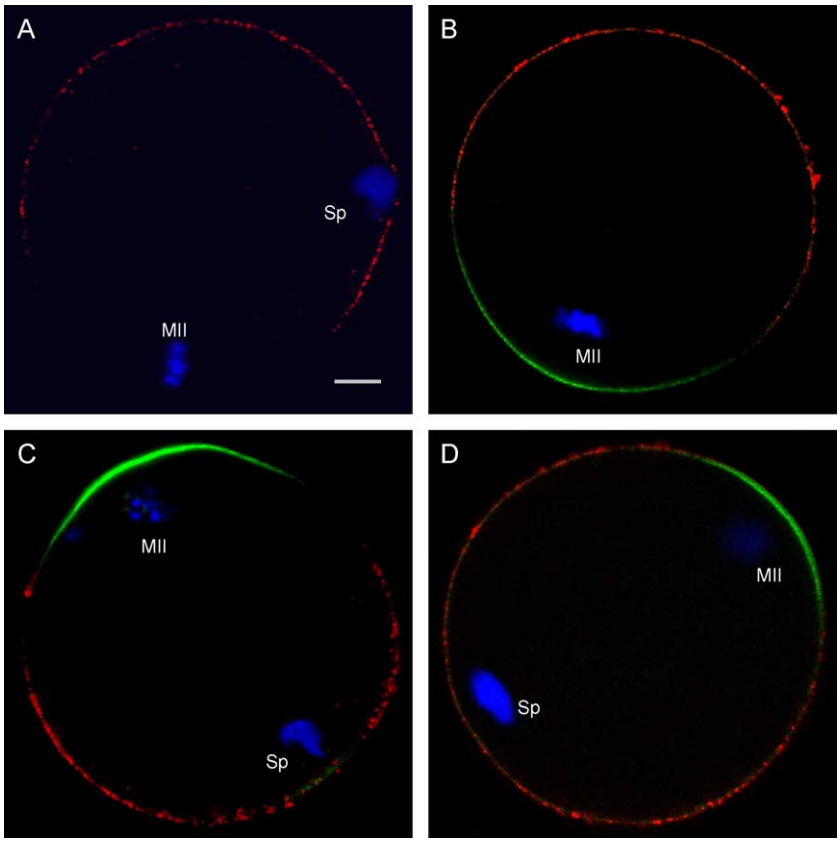


Fig. 5. Effect of modulators of actin dynamics on sperm chromatin-induced cortical reorganization. (A) Lat A-treated MII egg. Note the absence of an actin cap in the region overlaying/over the MII spindle and sperm chromatin. Also note the continued presence of a CGFD of the MII spindle. (B) Control, MII egg. (C) Phalloidin-injected MII egg. (D) Jasplakinolide-treated MII egg. In panels C and D, note that the actin cap overlying the MII spindle is thicker than that in panel B. The Lat A experiments were repeated three times; a total of 32 Lat A-treated eggs were analyzed. The phalloidin experiments were repeated two times and a total of 11 phalloidin-injected eggs were analyzed. The jasplakinolide experiments were repeated four times and a total of 41 jasplakinolide-treated eggs were analyzed. In each treatment group, no cortical reorganization was observed.

ylation and activation that is in turn linked to cortical reorganization.

## Discussion

The results reported here provide new insights into how chromatin initiates the dramatic reorganization of the egg cortex that occurs during maturation. The model that emerges from these studies is that active MAPK becomes spatially restricted to spindle microtubules or microtubules assembling on the injected sperm chromatin (unpublished observations) and directly phosphorylates MLCK that in turn results in localized cortical actin polymerization. Microvillar actin is likely to be one source of the actin incorporated into the actin cap, because the region overlying the MII spindle/chromosomes is amicrovillar. It is also likely that cytoplasmic actin is recruited because the cortical actin in the actin cap is significantly thicker than cortical actin in other regions of the egg that still contain microvilli.

The model accounts for the following observations. Compared to the oocyte, MAPK activity is markedly elevated in the egg. Nevertheless, there is no apparent difference in the thickness of cortical actin in either oocytes or MII eggs, except in the region overlying the spindle. This could be explained by a requirement for a threshold of MAPK activity that is provided by its association with the spindle. Consistent with this idea is that cortical reorganization is not observed unless the spindle or microinjected sperm chromatin is located close to the cortex. For example, in most oocytes deficient in formin-2, neither spindle migration nor cortical reorganization occurs (Leader et al., 2002). In the few instances, however, in which the spindle does migrate to the cortex, cortical reorganization occurs; this finding also demonstrates that formin-2 is unlikely to be critical for cortical reorganization. The model also accounts for the smaller size of the actin cap overlying chromosomes not associated with a spindle, for example, nocodozole-treated or sperm chromatin-injected eggs, when compared to that overlying a spindle (Deng et al., unpublished observations).

Enrichment of active MAPK in the region of chromosomes/spindle may also regulate actin dynamics in other cellular processes. Sperm–egg plasma membrane fusion places sperm chromatin in the egg cortex. The presence of active MAPK in the region of sperm DNA could account for formation of the fertilization cone that is highly enriched in

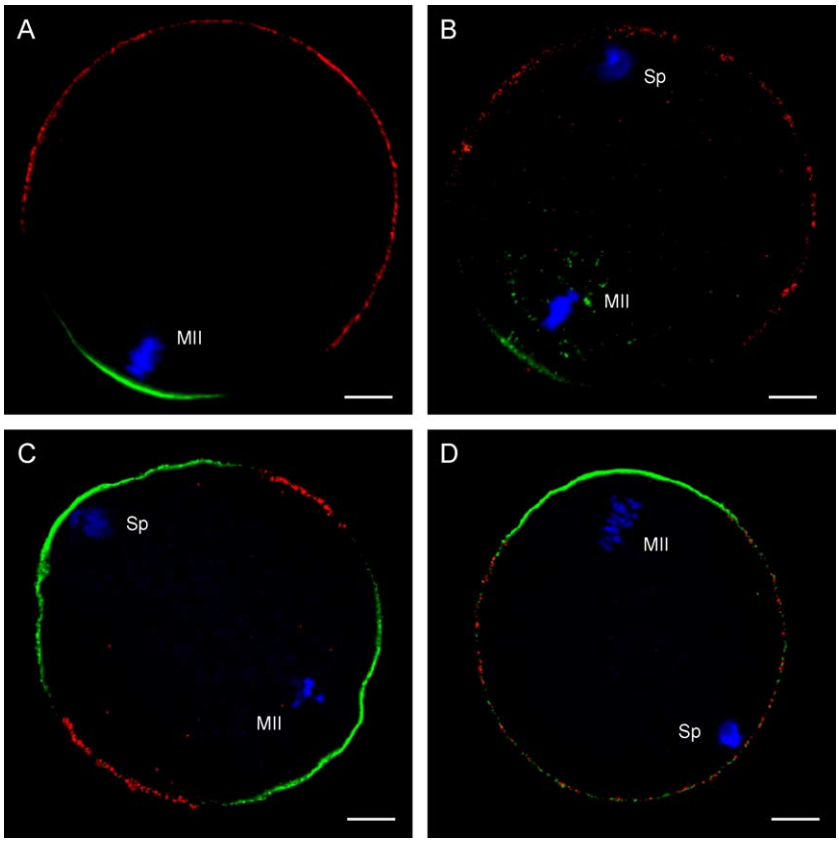


Fig. 6. Effect of inhibiting MLCK on cortical reorganization and stability. (A) Control MII egg cultured for 1.5 h. (B) Sperm chromatin-injected MII egg cultured in the presence of ML-7 for 1.5 h. (C) Control MII egg injected with protein tyrosine kinase inhibitor. (D) MII egg injected with MLCK inhibitory Peptide 18. For A and B, the experiment was performed twice and a total of 21 ML-7-treated eggs were analyzed, none of which showed the response. For C and D, the experiment was repeated four times. A total of 24 Peptide 18-injected eggs were analyzed and none showed the response.

filamentous actin (Simerly et al., 1998). Association of active MAPK at the spindle and chromosomes may define the position of the contractile ring by promoting local assembly of contractile ring components, for example, actin. In fact, such a function is observed in plants in which active MAPK localizes to the plane of cell division (Bogre et al., 1999). The association of MAPK with kinetochores during mitosis in animal cells may also contribute to defining the position of the cleavage plane (Shapiro et al., 1998; Zecevic et al., 1998).

Our results strongly implicate MAPK as an upstream activator of MLCK. There are, however, other pathways that lead to myosin light chain (MLC) phosphorylation and activation. One of these involves direct activation of MLC by $Ca^{2+}$/calmodulin-dependent protein kinase CaMKII, which is associated with the meiotic spindle (Hatch and Capco, 2001). Although CaMKII could participate in cortical reorganization, the observation that cortical reorganization does not occur in *Mos*$^{-/-}$ eggs (that presumably have normal levels of CaMKII activity) makes it unlikely that CaMKII activity is sufficient. Rho kinase can phosphorylate MLC and inhibit MLC phosphatase, thereby maximally activating myosin activity (Burridge and Wennerberg, 2004). Rho family proteins are implicated in sperm incorporation into mouse eggs (Kumakiri et al., 2003), suggesting a function for these proteins in cortical reorganization required for fertilization cone assembly. A role for Rho family members in cortical reorganization is under current study.

The lack of a large microtubule astral array may allow cortical positioning of the meiotic spindle. This contrasts with mitotic cells in which astral arrays are likely involved in positioning the spindle at a more central location. The ability of the meiotic spindle to reach the cortex ensures local recruitment of contractile machinery components required for cytokinesis and therefore dictates that a highly asymmetric cell cleavage occurs during polar body emission. A consequence of this asymmetric cell division is the conservation in the egg of macromolecules and organelles accumulated during oocyte growth and required for embryo development.

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