Cortical Mechanics and Meiosis II Completion in Mammalian Oocytes Are Mediated by Myosin-II and Ezrin-Radixin-Moesin (ERM) Proteins

Stephanie M. Larson,*† Hyo J. Lee,*† Pei-hsuan Hung,* Lauren M. Matthews,* Douglas N. Robinson,‡§ and Janice P. Evans*

*Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, and ‡Department of Cell Biology, and §Department of Pharmacology and Molecular Sciences, School of Medicine, Johns Hopkins University, Baltimore, MD 21205

Submitted January 28, 2010; Revised July 1, 2010; Accepted July 12, 2010
Monitoring Editor: Yu-Li Wang

Cell division is inherently mechanical, with cell mechanics being a critical determinant governing the cell shape changes that accompany progression through the cell cycle. The mechanical properties of symmetrically dividing mitotic cells have been well characterized, whereas the contribution of cellular mechanics to the strikingly asymmetric divisions of female meiosis is very poorly understood. Progression of the mammalian oocyte through meiosis involves remodeling of the cortex and proper orientation of the meiotic spindle, and thus we hypothesized that cortical tension and stiffness would change through meiotic maturation and fertilization to facilitate and/or direct cellular remodeling. This work shows that tension in mouse oocytes drops about sixfold during meiotic maturation from prophase I to metaphase II and then increases ~1.6-fold upon fertilization. The metaphase II egg is polarized, with tension differing ~2.5-fold between the cortex over the meiotic spindle and the opposite cortex, suggesting that meiotic maturation is accompanied by assembly of a cortical domain with stiffer mechanics as part of the process to achieve asymmetric cytokinesis. We further demonstrate that actin, myosin-II, and the ERM (Ezrin/Radixin/Moesin) family of proteins are enriched in complementary cortical domains and mediate cellular mechanics in mammalian eggs. Manipulation of actin, myosin-II, and ERM function alters tension levels and also is associated with dramatic spindle abnormalities with completion of meiosis II after fertilization. Thus, myosin-II and ERM proteins modulate mechanical properties in oocytes, contributing to cell polarity and to completion of meiosis.

INTRODUCTION

The meiotic divisions of the oocyte have significant impact on reproductive and developmental success, even though the first of these divisions occurs before a mammalian embryo is even created and the second occurs shortly after sperm has penetrated. In these two meiotic cell divisions, chromosomes must be segregated evenly between the daughter cells, as most aneuploidies are lethal or cause congenital birth defects (Hassold and Hunt, 2001). The other arrest at metaphase II (MII) in most mammals (which can last for days and up to years, depending on the species), then another arrest at metaphase II (MII) in most mammals (which can last for hours), and finally creation of the haploid maternal genome component occurring only after fertilization occurs.

Progression through meiosis is accompanied by changes in cortical architecture. These changes in the egg cortex are important for several reasons. The egg cortex has long been appreciated as having a key role in embryogenesis in localizing maternal determinants and in axis determination (reviewed in Sardet et al., 2002). Recent work also shows that the egg cortex functions in earlier stages of embryo development, involved in responses to sperm and the egg-to-embryo transition (Esposito et al., 2007; FitzHarris et al., 2007; Maruyama et al., 2007; Stitzel et al., 2007; Li et al., 2008b; Cheng et al., 2009; Parry et al., 2009). Additionally, remodeling of the cortex accompanies progression of the prophase I, germinal vesicle-intact (GVI) oocyte to the MII stage, and this cortical remodeling is part of the creation of cellular asymmetry (Brunet and Maro, 2005). The MII-arrested mouse egg has two distinct regions of the egg plasma membrane and cortex: the amicrovillar domain to which sperm bind and fuse, and small polar bodies. Female meiosis also has unique temporal challenges, with meiosis occurring in a staggered manner, characterized by an arrest at prophase I (which can last for months after publication it is available to the public under an Attribution–Noncommercial–Share Alike 3.0 Unported Creative Commons License (http://creativecommons.org/licenses/by-nc-sa/3.0).
Ran have been implicated through a variety of studies (gene knockout studies, RNAi, pharmacological perturbation, antibody injection) in the movement of the spindle to the cortex during metaphase I (MI) and/or the associated remodeling of cortical actin (Simerly et al., 1998; Leader et al., 2002; Na and Zernicka-Goetz, 2006; Deng et al., 2007; Halet and Carroll, 2007; Li et al., 2008a; Schuh and Ellenberg, 2008). However, the effects of cortical dynamics on meiosis, particularly the second meiotic division, are still not fully understood.

Given the morphological changes that occur during oocyte maturation and fertilization, we hypothesized that the mechanical characteristics of the oocyte would vary with these developmental transitions to facilitate and/or direct cellular remodeling. The mechanical properties of a cell are a critical determinant governing cell shape changes, such as those occurring during mitotic cytokinesis or cellular morphogenesis, and affecting cellular characteristics such as deformability (Zhang and Robinson, 2005; Kunda et al., 2008; Reichl et al., 2008). Here, we use micropipette aspiration (MPA) to measure the mechanical properties of mouse oocytes. Because cells are neither purely elastic solids nor fluids with constant surface tensions, the response to applied pressure may reflect multiple mechanical properties. Therefore, we measure an effective tension ($T_{eff}$), which is an energy cost for adding a unit of surface area and reflects both the tension and the stretch or expansivity modulus (Derganc et al., 2000; Reichl et al., 2008). This quantitative metric reflects the biochemical and structural features of the cortex, which are mediated by force generation from actin assembly and myosin-II motor activity, along with the structural organization of actin polymers and linkages between the polymers and the membrane. Actin-cross-linking proteins and filament-membrane–tethering proteins can also contribute to this structural organization and to mechanical properties of the cell by mediating the associations between actin polymers.

Cellular mechanical properties have been studied in echinoderm and amphibian eggs and early embryos, providing insights into how these mechanical properties were affected by temperature, by culture conditions, and most importantly, by fertilization and progression through embryonic mitosis (Cole, 1932; Cole and Michaelis, 1932; Harvey and Fankhauser, 1933; Mitchison and Swann, 1954, 1955; Mitchison and Swann, 1954, 1955; Selman and Waddington, 1955; Woltfer, 1966; Rappoport, 1967; Sawai and Yoneda, 1974; Hiramoto, 1976), although there is only limited information about mechanical transitions in different cellular regions of eggs or during meiosis (Sawai and Yoneda, 1974; Hiramoto, 1976; Nakamura and Hiramoto, 1978). This article presents about mechanical transitions in different cellular regions of eggs or during meiosis (Sawai and Yoneda, 1974; Hiramoto, 1976; Nakamura and Hiramoto, 1978). This article presents the first characterization of quantifiable mechanics in mammalian eggs, showing that there are dramatic dynamics in cellular mechanics in mouse oocytes with progression through meiosis as well as a mechanical polarity established at MII arrest. We also demonstrate the contributions of actin, nonmuscle myosin-II, and Ezrin/Radixin/Moesin (ERM) proteins to cellular mechanics in oocytes. The members of the ERM family mediate cytoskeletal-membrane interactions (Bretscher et al., 2002; Fehon et al., 2010). Flies deficient in Moesin (the only Drosophila ERM protein) have abnormalities in oocyte polarity, actin organization, and localization of certain maternal determinants such as Oskar and Staufen (Jankovics et al., 2002; Polese et al., 2002). RNAi studies of cultured Drosophila cells and of Dicyostelium cells show that ERM proteins contribute to cortical mechanics (moesin in Drosophila; Carreno et al., 2008; Kunda et al., 2008; and enlazin, the closest ERM relative in Dicyostelium; Octtaviani et al., 2006). Radixin is among the most abundant mRNAs detected in mouse oocyte transcriptomes (e.g., Eviskov et al., 2006, and Unigene library IDs 18552, 10029, and 14142), and mouse eggs have been reported to express ezrin protein (Louvet et al., 1996). Here, we find that mouse eggs also express radixin and moesin proteins, and activated forms of these proteins enrich in the microvillar domain, complementary to actin and moesin II, which are enriched in the amivillar domain. The disruption of ERM function by expression of a dominant-negative form of radixin, perturbation of actin, or inhibition of moesin-II reduced effective tension in MII eggs, as well as produced dramatic spindle abnormalities during exit from MII after fertilization.

**MATERIALS AND METHODS**

**GVI Prophase I Oocytes, Metaphase II Eggs, Sperm, and Zygotes**

Collection of oocytes from CF1 mice (Harlan, Indianapolis, IN) and zona pellucida (ZP) removal were performed as described (Evans et al., 2000). Whitten's medium (Whitten, 1971) was supplemented with 2.5 μM milrinone (Sigma-Aldrich, St. Louis, MO) or 100 mM dbcAMP (Sigma-Aldrich) to maintain prophase I arrest (Cho et al., 1974; Tsafri et al., 1996). Oocytes were maintained in vitro in medium with added 1 μg/ml 8-bromo-AMP (Cayman Chemicals, Ann Arbor, MI). Oocytes were nucleated at 6–8 h of culture after meiotic breakdown (GVBD) stage and MI samples were prepared at 4 h and 9 h after milrinone/dbcAMP removal, respectively. For some experiments, MII eggs were collected from superfused females at ~13 h after human chorionic gonadotropin injection as previously described (Gardner et al., 2007). Zygotes were generated by in vitro fertilization as described (Gardner et al., 2007). T$_{eff}$ measurements of zygotes were taken at 90 min after insemination (ZP-free eggs were inseminated with 150,000 sperm/ml for 20 min, washed, and then cultured for 70 min).

Eggs were treated with 100 μg/ml concanavalin A (ConA; Sigma-Aldrich; diluted in Whitten's medium compatible buffer) for 30 min before and during T$_{eff}$ measurement by MPA (see below). Treatment with the actin filament disruptor cytochalasin D (5 μg/ml; Calbiochem, Gibbstown, NJ) or the myosin light-chain kinase inhibitor ML-7 (15 μM; Calbiochem, La jolla, CA; or Sigma-Aldrich) was done as previously described (McAvey et al., 2002; Matson et al., 2006) for 60 min before and during T$_{eff}$ measurement by MPA. Controls were treated with a matching concentration of the solvent, DMSO. We also attempted to disrupt myosin-II activity with blebbistatin treatment, but found that this did not have consistent effects on T$_{eff}$. This may be linked with photoactivation of blebbistatin (Kolega, 2004) and is consistent with a report that blebbistatin treatment did not have effects on first polar body emission (Schuh and Ellenberg, 2008).

**$T_{eff}$ Measurements by MPA**

ZIP-free oocytes, eggs, and zygotes were subjected to MPA using a motorized system (Effler et al., 2006). Borosilicate capillaries (o.d., 1.0 mm, i.d., 0.75 mm; Sutter Instruments, Novato, CA) were pulled to an inner diameter of ~5 μm (PMP102 micropipette puller; Sutter Instruments) and broken on a microforge to smaller diameters; the average diameter of pipettes used here was 15 μm (range, 12–22 μm). Temperature of the culture medium for the T$_{eff}$ measurements was maintained at 32–37°C with a miniature temperature controller (MTC; Bioscience Tools, San Diego, CA). Cells were measured in Whitten's medium containing 15 mM HEPES, 0.05% PVA, and, when needed, 5 μg/ml DAPI. The critical aspiration pressure ($P_A$) that aspirated a tether length ($L_T$) that equaled the pipette radius ($R_p$) was measured (Figure 2B; Octtaviani et al., 2008). The $P_A$ was calculated using the Law of Laplace ($\Delta P = 2T_{eff}(R_p - 1/R_e)$, where $R_e$ is the radius of the cell outside the micropipette. All T$_{eff}$ measurements were taken on microvillar regions of unfertilized and fertilized eggs unless otherwise noted, observing morphology and/or DAPI so that cortical regions over chorionatin could be avoided. Phase contrast images of cells during MPA were captured using a 10× objective and a 1.6× objective on an IXP8 microscope equipped with Meta-Morph software ( Molecular Devices, Sunnyvale, CA). In a subset of the T$_{eff}$ analyses of dominant-negative radixin (DN-RDX) or RNA-injected eggs, injected cells with reduced T$_{eff}$ measurements were recovered after MPA and subjected to immunofluorescence analysis with anti-cmyc and anti-phospho-MAP (pERM) antibodies (see below).

**Dominant-Negative Disruption of ERM Function in Eggs**

The region of encoding the N-terminal ERM association domain (NERMAD) of mouse radixin (amino acids 1-317) tagged with the c-myc epitope (EQKLISEEDLN) was cloned into pIVT plasmid (gift of Dr. Carmen Williams, NIEHS), and verified by DNA sequencing. This construct gives rise to a dominant-negative radixin with a cmyc epitope, which will be referred to as...
DN-RDX. (A cmyc tag was used as we have been thus far unsuccessful in expressing a GFP-tagged version of the radixin N-terminal ERM association domain.) cRNA was synthesized from linearized pIVT-NERMAD using the mMessage Machine kit (Ambion, Austin, TX). cRNA (1 µg/µl) was injected using an Eppendorf FemtoJet injector on a Nikon TE2000-S inverted microscope (Melville, NY), with the oocytes in Whitten’s medium containing 15 mM HEPES, 0.05% PVA, and 2.5 µM milrinone. After injection, oocytes were cultured in Whitten’s medium containing 0.05% PVA and 2.5 µM milrinone for 3–7 h and then matured in vitro as described above. The term “DN-RDX-expressing eggs” refers to the subset of cells that expressed detectable DN-RDX-cmyc and had reduced pERM.

Immunocytochemistry and Immunoblots

Oocytes and eggs were stained with the following primary antibodies: anti-phospho-myosin-II regulatory light chain (pMRLC; Cell Signaling Technology, Danvers, MA), anti-radixin (Sigma-Aldrich), anti-pERM (Cell Signaling Technology, Beverly, MA), or anti-α-tubulin (Invitrogen), or anti-α-tubulin (AA4.3; Developmental Studies Hybridoma Bank, University of Iowa, Department of Biology, Iowa City, IA), followed by secondary antibodies from Jackson ImmunoResearch (West Grove, PA). Sodium orthovanadate (100 µM; Sigma) was included for staining with the anti-pMRLC antibody. Fluorescently labeled phalloidin (Sigma-Aldrich) was used to stain actin. Immunoblots of protein lysates were probed with anti-ERM or anti-pERM (Cell Signaling Technology) or anti-radixin (Sigma), followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch). ImageJ (http://rsb.info.nih.gov/ij/) was used to quantify band intensity.

Assessment of Soluble and Polymeric Actin in Oocytes and Eggs

Lysates of oocytes or eggs (200–250 per sample) were prepared in 25 µl of lysis buffer (50 mM NaCl, 1 mM MgCl2, 1 mM EGTA, 10 mM HEPES, 1% Triton X-100) and immunoblotted using anti-α-tubulin (Developmental Studies Hybridoma Bank), anti-β-tubulin (BUD1; Developmental Studies Hybridoma Bank), or anti-α-tubulin (AA4.3; Developmental Studies Hybridoma Bank, University of Iowa, Department of Biology, Iowa City, IA), followed by secondary antibodies from Jackson ImmunoResearch (West Grove, PA). Sodium orthovanadate (100 µM; Sigma) was included for staining with the anti-pMRLC antibody. Fluorescently labeled phalloidin (Sigma-Aldrich) was used to stain actin. Immunoblots of protein lysates were probed with anti-ERM or anti-pERM (Cell Signaling Technology) or anti-radixin (Sigma), followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch). ImageJ (http://rsb.info.nih.gov/ij/) was used to quantify band intensity.
Figure 2. Effects of actin manipulation on oocyte effective tension. (A) Phalloidin staining of a GVI oocyte (left) and a metaphase II (MII) egg (right). Polarity develops during meiotic maturation, such that the metaphase II egg has an actin-rich cap over the meiotic spindle. Red, actin; blue, DNA. Scale bar, (left) 20 μm; (right) 22 μm. (B) Effects on effective tension (Teff) of actin manipulation by cytochalasin D treatment of GVI oocytes (*p < 0.0001). Numbers of cells analyzed: cytochalasin D-treated GVI oocytes, 75; DMSO control GVI oocytes, 43. (C) Cellular concentration (nM) of total actin, and actin in the phalloidin-stabilized, polymeric fraction (polymer) and in the soluble fraction in GVI oocytes (□) and MII eggs (■). Values for GVI oocytes show the mean ± range from two experiments and for MII eggs, mean ± range from three experiments.

RESULTS

The properties and functions of the membrane and cortex differ significantly in prophase I oocytes and MII eggs (Figure 1A). (Note: Throughout this article, the term “oocyte” will be used to refer to the female gamete generically and also for GVI, prophase I oocytes; the term “egg” connotes MII arrest.) Oocytes progress through meiotic maturation, characterized by progression through GVBD and MI, and then arrest at MII. Meiotic maturation in vivo occurs with ovulation and transit of the ovulated egg(s) to the oviduct; meiotic maturation can also occur in vitro, with the culture of prophase I oocytes in medium that supports a decrease in CDK1 activity (Mehlmann, 2005). The MII egg is characterized by distinct domains of membrane and underlying cortex: the microvillar (MV) domain to which sperm bind and fuse, and the amicrovillar (AMV) domain, overlaying the meiotic spindle and characterized by an actin-rich cap, contrasting the uniform distribution of actin and microvilli at prophase I stage (Figure 1A). Exit from MII is triggered by fertilization.

We hypothesized that the mechanical properties of oocytes would vary through these developmental transitions, given the changes that occur during meiosis and fertilization. Teff measurements using MPA (Figure 1B) showed that as oocytes matured from prophase I (GVI) to MII, the effective tension varied about sixfold. GVI oocytes had a mean ± SEM Teff value of 5.9 ± 0.2 nN/μm (Figure 1C, Figure S1), making oocytes among the most rigid cell types studied (Hochmuth, 2000). Teff decreased through GVBD and metaphase I, reached a nadir at MII, and then increased after fertilization (Figures 1C, Figure S1). Interestingly, MII eggs were found to have mechanical polarity, with a nearly threefold difference in Teff detected between the microvillar and amicrovillar domains (Figure 1D, Figure S1).

This work includes studies using injections of prophase I oocytes followed by in vitro maturation to MII, so we compared the Teff levels in MII eggs that had been matured in vivo, with gonadotropin-induced ovulation, to the Teff levels in eggs that had been matured in vitro. Teff was not different between the microvillar domains of oovulated and in vitro matured eggs (ovulated, 0.84 ± 0.023 nN/μm [n = 200]; in vitro matured, 0.80 ± 0.018 nN/μm [n = 187]; Figure 1E, Figure S1). On the other hand, Teff differed between the amicrovillar domains. Amicrovillar domains of oovulated eggs measured 2.3 ± 0.085 nN/μm (n = 91), and amicrovillar domains of in vitro matured eggs measured 2.0 ± 0.069 nN/μm (n = 84); this difference was statistically significant (p = 0.002) (Figure 1E, Figure S1). Because the MII spindle is sequestered in the amicrovillar domain, we used anti-tubulin staining to investigate if these differences in effective tension correlated with differences in the morphology of the MII spindle between ovulated eggs and in vitro matured eggs. Examples of these are shown in Figure 1F; spindles in ovulated eggs tended to be shorter and often with narrower poles, whereas spindles in in vitro matured eggs were longer with broader poles, consistent with other studies using similar in vitro maturation methods (Sanfins et al., 2003, 2004; Barrett and Albertini, 2007); these references provide extensive characterization of spindle morphology in ovulated and in vitro–matured MII eggs.

The actin cytoskeleton plays a major role in cortical dynamics and tension in other cell types, and we show here that this is true for oocytes as well. Cytochalasin D treatment, which disrupts actin polymers, reduced Teff in GVI oocytes by 95% (Figure 2B, Figure S2). Cytochalasin D–treated MII eggs were aspirated into the pipette once aspiration pressure was applied, making it impossible to obtain precise Teff values but suggesting that these eggs had extremely low Teff. Because of the sixfold difference in Teff between oocytes and eggs, we hypothesized that actin levels would be higher in GVI oocytes as compared with MII eggs.
However, quantitative immunoblot analysis revealed that oocytes and eggs had comparable concentrations of total, polymeric, and soluble actin (Figure 2C), prompting us to consider additional tension-regulating factors to account for this Teff difference between GVI oocytes and MII eggs.

Myosin-II contractility works with actin to mediate cortical tension in other cell types, and is regulated by phosphorylation of the myosin-II regulatory light chain. We find that the active, phosphorylated form of the myosin-II regulatory light chain (hereafter referred to as pMRLC) is detected in the oocyte/egg and is enriched in the cortex of oocytes and in the amicrovillar domain of eggs (Maro et al., 1984; Simerly et al., 1998). The role of myosin-II in cortical tension and cell shape in mitotic cells has been characterized through manipulation of myosin-II–mediated contractility (Pasternak and Elson, 1985; Pasternak et al., 1989; Lucero et al., 2006; Kunda et al., 2008); we used these methods to assess myosin-based mechanics in oocytes and eggs. Treatment of cells with concanavalin A (ConA) typically induces a myosin-II–dependent increase in cortical tension (Pasternak and Elson, 1985; Pasternak et al., 1989; Kunda et al., 2008). ConA-treated eggs showed an increase in T eff in the microvillar domain compared with untreated controls (50%; p < 0.001, Mann-Whitney U-test) and a modest decrease in T eff of the amicrovillar domain relative to controls (18%; p = 0.14, Mann-Whitney U-test; Figures 3L, Figure S3). These observations suggest that ConA treatment alters the normal mechanical polarity in MII eggs. The localizations of pMRLC and phosphorylated ERM (addressed below) were not dramatically different between controls and ConA-treated eggs (Figure S3).

Inhibition of myosin light-chain kinase (MLCK) activity with ML-7 affects cortical contractility in sea urchin zygotes (Lucero et al., 2006) and alters certain events of mouse oocyte maturation and egg activation (Matson et al., 2006; Li et al., 2008a; Schuh and Ellenberg, 2008). We show that ML-7–treated oocytes and eggs had a ~50% decrease in T eff; this decrease in T eff in MII eggs was observed in both the microvillar and amicrovillar domains (Figures 3M, Figure S2).
These effects of ML-7 treatment suggest a connectedness of the cortical cytoskeleton as well as the fact that low concentrations of myosin II and/or low levels of myosin II activation (i.e., in the microvillar vs. amicrovillar domains, respectively) are sufficient to impact egg mechanics (Zhang and Robinson, 2005; Kunda et al., 2008; Reichl et al., 2008).

We next examined the ERM protein family. Moesin in Drosophila and enlazin, the closest ERM relative in Dictyostelium, contribute to cortical mechanics (Octtaviani et al., 2006; Kunda et al., 2008). Interestingly, radixin is very abundantly represented in the mouse oocyte transcriptome (e.g., Evsikov et al., 2006; Unigene, www.BioGPS.org), but nothing is known of its function. We show here that mouse eggs express radixin and moesin (Figure 4, A–C), complementing reports of ezrin expression (Louvet et al., 1996). Radixin is detectable in lysates of five eggs (125 ng protein) with a band of comparable intensity to that detected in 1 μg of mouse liver lysate. (D) Representative blots of oocyte and egg lysates (20 cells per lane) probed with anti-pERM (i and iii) or anti-ERM (ii and iv) antibodies. Oocyte meiotic maturation (GVII to MI) and fertilization (iii and iv) were analyzed in separate blots with different exposure times, to optimize capturing the range of signals (e.g., the blot in iii was exposed to show the increase in signal from unfertilized eggs to fertilized eggs). (E and F) Quantification of band intensities of anti-pERM (B) and ERM (C) levels during oocyte maturation (E) or the egg-to-embryo transition (F; zygote sample prepared at 90 min after insemination); sample blots are shown above in D. Values were normalized to metaphase II (MII) eggs.

Figure 4. ERM family in mouse eggs. (A and B) Immunofluorescence analysis of radixin (A) and pERM (B) in GVI oocytes (Ai–iv; Bi–iii) and MII eggs (Av–xiiv and Biv–ix), showing anti-radixin (Aii, vi, and x), anti-pERM (Biii, v, and viii), DNA (Aiii, vii, and xi and Biii, vi, and ix), or anti-β-tubulin (Aiv, vii, and xii). Scale bar, 15 μm. (C) Immunoblot analysis of ERM (i) or radixin (ii) expression in eggs. In C, the solid arrowhead indicates the electrophoretic mobility of ezrin and radixin, the open arrowhead indicates moesin. Radixin is detectable in lysates of five eggs (125 ng protein) with a band of comparable intensity to that detected in 1 μg of mouse liver lysate. (D) Representative blots of oocyte and egg lysates (20 cells per lane) probed with anti-pERM (i and iii) or anti-ERM (ii and iv) antibodies. Oocyte meiotic maturation (GVII to MI) and fertilization (iii and iv) were analyzed in separate blots with different exposure times, to optimize capturing the range of signals (e.g., the blot in iii was exposed to show the increase in signal from unfertilized eggs to fertilized eggs). (E and F) Quantification of band intensities of anti-pERM (B) and ERM (C) levels during oocyte maturation (E) or the egg-to-embryo transition (F; zygote sample prepared at 90 min after insemination); sample blots are shown above in D. Values were normalized to metaphase II (MII) eggs.
had a mean $T_{\text{eff}}$ of $0.75 \pm 0.048 \, \text{nN/\mu m}$ (Figure S2; $p = 0.16$; [Mann Whitney U-test] when these eggs were compared with the entire population of in vitro matured MII eggs $[0.80 \pm 0.018 \, \text{nN/\mu m}]$, but this distribution in $T_{\text{eff}}$ values in DN-RDX–injected eggs is likely due to the fact that only about a third of these eggs express detectable DN-RDX protein. We were able to recover some eggs after the $T_{\text{eff}}$ measurements and examine these for DN-RDX expression and reduction of pERM. The cRNA-injected eggs that had detectable DN-RDX protein, and reduced pERM levels had a mean $T_{\text{eff}}$ of $0.28 \pm 0.085 \, \text{nN/\mu m}$, which was significantly different from control eggs (Figure 5C, Figure S2). We also observed that a small number of DN-RDX–injected eggs deformed as soon as aspiration pressure was applied, similar to the cytochalasin D–treated eggs, suggesting that these eggs had extremely low $T_{\text{eff}}$.

We next sought to examine the biological effects associated with disrupted effective tension in eggs. Meiotic maturation and emission of the first polar body in DN-RDX cRNA-injected oocytes to MII appeared to occur normally. To determine if $T_{\text{eff}}$ disruption affected function of MII egg function and the second meiotic division, we performed in vitro fertilization with control and DN-RDX–injected eggs as well as with cytochalasin D– and ML-7–treated eggs. These eggs could be fertilized but developed significant spindle abnormalities during exit from MII. In normal completion of meiosis II in control eggs (solvent [DMSO]–treated, uninjected, water–injected; Figure 6, A–C, J–M, and Q–T), the meiotic spindle elongates in anaphase II and then undergoes spindle rotation from its orientation parallel to the membrane. This rotation produces the second polar body, with the polar body developing from one of the chromatin-containing protrusions that forms before spindle rotation; the other protrusion resolves (Figure 6). Defects in second polar body emission and spindle rotation were observed in cytochalasin D– and ML-7–treated eggs, in agreement with previous studies (Maro et al., 1984; Matson et al., 2006); interestingly, spindle rotation failed in DN-RDX–expressing eggs but the spindle abnormalities differed from those in cytochalasin D– and ML-7–treated eggs. (Note: Polar body emission and spindle rotation appeared to be normal in ConA–treated eggs; see Figure S3 for details. This lack of an effect on polar body emission could be due to having to introduce ConA treatment after zygote creation, and/or due to the ConA-induced change in tension being insufficient to induce significant abnormalities during polar body emission.) In cytochalasin D–treated eggs, ML-7–treated eggs, and DN-RDX–expressing eggs, spindle elongation occurred but spindle rotation failed. The cytochalasin D–treated and ML-7–treated eggs arrested at this anaphase II–like stage and no polar body was formed, with only very modest protrusions over the chromatin (Figure 6, D–I; Maro et al., 1984; Matson et al., 2006). Fertilized DN-RDX–expressing eggs developed spindles that were distorted and curved; a pair of extended polar body–like structures was associated with the chromatin at the ends of these distorted spindles (Figure 6, N–P). These PB–like structures did not resolve, still persisting at 4 h after inseminations (Figure 6, U–BB).

DISCUSSION

Cellular mechanics are a critical determinant governing the cell shape changes that occur as the cell progresses through the cell cycle (Reichl et al., 2005; Pollard, 2009). Cell morphogenesis during mitosis and many other processes is the result of an intricate interplay between three elements—biochemistry, mechanics, morphology—each of which is

Figure 5. Perturbation of ERM action in eggs. (A) Analysis of DN-RDX–cmyc and pERM by immunofluorescence in uninjected (i–iii), water–injected (iv–vi), and DN-RDX–cRNA–injected eggs (vii–ix). DN–RDX–cmyc protein was detected in $\sim33\%$ of eggs injected with DN-RDX–cmyc cRNA. (viii and ix) Two eggs, one with detectable cmyc staining (viii) and reduced pERM staining (ix), and a second egg (*) that does not have detectable cmyc staining (viii) and has cortically localized pERM (ix). (B) Assessment of pERM and actin levels in control (uninjected and water–injected) and DN-RDX–expressing egg through quantification of $I_{\text{cortical}}/I_{\text{whole}}$ signals (described in Materials and Methods). This analysis shows that cortical pERM (■) is reduced in DN-RDX–expressing eggs ($p < 0.0001$), whereas the ratio of cortical/whole actin polymers is unaffected. Numbers of cells analyzed: uninjected, pERM, 5; uninjected actin, 8; water–injected, pERM, 4; water–injected, 4; cRNA–injected, pERM, 22; and cRNA–injected, actin, 14. (A sample of phalloidin staining of a metaphase II egg is shown in Figure 2.) (C) Effects of DN-RDX expression and reduction of pERM on effective tension ($T_{\text{eff}}$) levels in eggs. Eggs were subjected to $T_{\text{eff}}$ measurements, then recovered, and analyzed for c-myc expression (indicative of DN-RDX expression) and reduction of pERM. Number of cells analyzed: uninjected, 10; water–injected, 7; and DN-RDX–expressing, 8. The difference between uninjected and water–injected eggs is not statistically significant, whereas the differences between the cRNA–injected eggs and the other two groups is significant ($p < 0.001$). Furthermore, a small number of cRNA–injected eggs deformed as soon as aspiration pressure was applied, making it impossible to obtain a $T_{\text{eff}}$ measurement but suggesting that these eggs had extremely low $T_{\text{eff}}$.  

S. M. Larson et al.
inextricably linked with the other. Biochemistry clearly influences morphology and mechanics, whereas less intuitively but just as significantly, morphology and mechanics also can influence biochemistry (Zhang and Robinson, 2005; Ren et al., 2009). These three elements work to dictate dramatic cell shape changes such as during mitosis (Reichl et al., 1993).
Asymmetric cell divisions present unique challenges (Gönczy, 2008), and the mouse egg is an especially interesting case. The mouse egg undergoes a highly asymmetric cell division after an extended MII arrest, and yet maintains a relatively simple, largely spherical, cell morphology. Thus, in the case of the mouse egg, cell morphology does not change significantly, but the other two elements, biochemistry and mechanics, do change. There are detectable redistributions of actin, myosin-II, and ERM proteins (changes in local biochemistry) and a major modulation of effective tension. An approximately sixfold decrease in effective tension occurs as oocytes progress from prophase I to MII, and then a ~1.5-fold increase occurs with MII exit and the egg-to-embryo transition. Known parameters that affect cellular mechanics include actin concentration and polymer length, cross-linking proteins, and myosin mechanoschemistry. We find that actin is central to the mechanics of mouse eggs and that myosin-II and ERM proteins contribute about twice of the effective tension. This is consistent with reported contributions of actin-associated proteins to cortical mechanics (e.g., myosin-II and Rac have up to about threefold effects on mechanics in *Dictyostelium* cells; Gerald et al., 1998; Girard et al., 2004; Zhang and Robinson, 2005; Octtaviani et al., 2006; Kunda et al., 2008; Reichl et al., 2008).

We have also discovered a new aspect of cell polarity in the MII-arrested egg, as we find there is a ~2.5-fold mechanical tension differential between amicrvillar and microvillar domains. These two domains have well-characterized differences in morphology, molecular composition and functionality (Nicosia et al., 1977; Brunet and Maro, 2005; Azoury et al., 2008), with sperm-egg fusion occurring on the microvillar domain and the meiotic spindle being sequestered in the amicrovillar domain. The lower tension in the microvillar domain of the mouse egg does not seem to be a contributing factor to sperm fusion occurring preferentially in this region, nor does the increased tension in the zygote appear to be the foundation of the membrane block to polyspermy (Gardner and Evans, 2006), as even higher tension is present in GV oocytes and these cells can be fertilized by sperm. The tension in the egg, instead, appears to be critical for meiotic spindle function. These mechanical properties of the egg are regulated by myosin-II, enriched in the amicrovillar domain, and pERM, enriched in the microvillar domain, and disruption of actin, myosin-II, or pERM function is associated with spindle defects with exit from MII upon fertilization. This work reveals an entirely new function for the egg cytoskeleton, in addition to its appreciated roles in spindle positioning (Maro et al., 1984; Longo, 1987; Simerly et al., 1998; Matson et al., 2006; Azoury et al., 2008; Li et al., 2008a; Schuh and Ellenberg, 2008), establishment of the cortical endoplasmatic reticulum during meiotic maturation (FitzHarris et al., 2007), and organization of a subcortical complex that appears to be critical for progression to early cleavage stages of development (Li et al., 2008b; Yurttas et al., 2008).

The discovery of this ~2.5-fold mechanical differential between the microvillar and amicrovillar domains in mouse eggs sheds important light on asymmetric cell division. The mechanics that produce symmetrically sized daughter cells are coming into view, with evidence for fluid-like (i.e., Laplace-like) pressures contributing alongside myosin-II–generated contractility to drive furrow ingression (Zhang and Robinson, 2005; Reichl et al., 2008). The results presented here suggest that the egg prepares for second polar body emission during MII arrest by establishing at MII a rigid “cytoskeletal plaque” enriched in actin and myosin-II (i.e., the amicrovillar domain; Figure 7). The amicrovillar domain then serves to isolate the MII spindle and then the developing polar body from the rest of the egg; this in turn allows myosin-II–mediated contractility to deform the cortex in this specific region, facilitating asymmetric cell division (Figure 7). Interestingly, we observe a difference in Teff in the amicrovillar domains of ovulated eggs and in vitro matured eggs, which also have differences in spindle morphology (Sanfins et al., 2003; Barrett and Albertini, 2007). This difference in amicrovillar Teff may be a mechanistic factor associated with these spindle morphology differences, combined with γ-tubulin distribution, which has also been associated with the spindle size differences observed between ovulated and in vitro-matured eggs (Barrett and Albertini, 2007). Additional studies are required, of course, to flesh out the implications of this for cytokinesis, but these observations provide an important starting point. This work is the first report of the mechanical properties in mammalian oocytes, how they impact processes such as spindle rotation, and what the molecular underpinnings of these mechanics are. The coordinated action of cytoskeletal proteins is at work during cytokinesis in *Dictyostelium*, with actin cross-linkers, including enlazin, mediating resistive stresses that control myosin-II–dependent stress generation in the furrow region (Zhang and Robinson, 2005; Reichl et al., 2008). It is intriguing to speculate that similar forces, such as pERM providing a resistive function to balance myosin-II contractility in the amicrovillar domain, direct morphological changes in the mouse egg. In addition to expanding our understanding of the functions of the egg cortex, these results provide significant insights into how mechanical factors are at work for a very asymmetric, meiotic cell division.
ACKNOWLEDGMENTS

We thank Carmen Williams (NIHES; Research Triangle Park, NC) for the pIVT plasmid and Amelia Mackenzie for one of the images of the anti-tubulin-stained oocytes. This work was supported by the National Institutes of Health Grants GM066817 to D.N.R. and HD037696 and HD045671 to J.P.E. and the March of Dimes Grant 6-FY-04-59 to J.P.E.

REFERENCES


