Myosin activity drives actomyosin bundle formation and organization in contractile cells of the Caenorhabditis elegans spermatheca

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ABSTRACT Stress fibers—contractile actomyosin bundles—are important for cellular force production and adaptation to physical stress and have been well studied within the context of cell migration. However, less is known about actomyosin bundle formation and organization in vivo and in specialized contractile cells, such as smooth muscle and myoepithelial cells. The Caenorhabditis elegans spermatheca is a bag-like organ of 24 myoepithelial cells that houses the sperm and is the site of fertilization. During ovulation, spermathecal cells are stretched by oocyte entry and then coordinately contract to expel the fertilized embryo into the uterus. Here we use four-dimensional confocal microscopy of live animals to observe changes to spermathecal actomyosin network organization during cell stretch and contraction. Oocyte entry is required to trigger cell contraction and concomitant production of parallel actomyosin bundles. Actomyosin bundle size, connectivity, spacing, and orientation are regulated by myosin activity. We conclude that myosin drives actomyosin bundle production and that myosin activity is tightly regulated during ovulation to produce an optimally organized actomyosin network in C. elegans spermathecae.

INTRODUCTION Actin is one of the most highly conserved proteins across eukaryotes and plays a central role in cellular adaptation to and generation of force (Gunning et al., 2015). This is required for numerous biological processes, including cell-cycle progression (Provenzano and Keely, 2011), cell migration (Li et al., 2005), tissue morphogenesis (Wozniak and Chen, 2009; Nelson and Gleghorn, 2012), wound closure (Brugues et al., 2014), and cell/tissue adaptation to physical stress (Davies et al., 2001; Burridge and Wittchen, 2013). The actin cytoskeleton is able to produce the different structures required to meet diverse cellular needs through complex and tightly regulated protein networks (dos Remedios et al., 2003; Dominguez, 2004; Zaidel-Bar et al., 2015). Stress fibers—contractile actomyosin bundles—are common among cells exposed to physical stress (Tojkander et al., 2012) and have been well studied within the context of cell migration (Pollard et al., 2000; Pellegrin and Mellor, 2007; Naumanen et al., 2008; Burridge and Wittchen, 2013). However, contractile stress fiber–like structures are found in many specialized contractile cells, such as contractile epithelial, contractile endothelial, smooth muscle, and myoepithelial cells, and much less is known about the regulation of actomyosin contractility in these contexts (Pellegrin and Mellor, 2007; Pollard and Cooper, 2009). Alterations in actomyosin contractility underlie the pathophysiology of numerous conditions, including cardiac disease, hypertension, vasospasm, asthma, erectile dysfunction, and preterm labor (Uehata et al., 1997; Wettscoureck and Offermanns, 2002; Somlyo and Somlyo, 2003; Seguchi et al., 2007; Lavoie et al., 2009). There are few in vivo systems that allow for visualization of the actomyosin cytoskeleton in cells under dynamic mechanical conditions. Most of the in vivo work comes from elegant studies of cell movements and cell contractility during Drosophila elongation, gastrulation, egg chamber rotation, and tracheal tube development (Mason et al., 2013; Cetera et al., 2014; Kasza et al., 2014; Hannezo et al., 2015; Hosono et al., 2015). Although cell shape changes and large perturbations to the localization of the actomyosin contractile apparatus can be observed in these cell types, their small size makes imaging of...
dynamic reorganization of the actin cytoskeleton a challenge. The somatic gonad of the transparent, hermaphroditic nematode Caenorhabditis elegans is composed of a single layer of contractile myoepithelial cells (McCartner et al., 1999; Michaux et al., 2001). Because the cells of the gonad are large and clearly visible in the intact animal and the gonad naturally stretches and contracts during the ovulation process, this system is ideal for studying how cells in an intact tissue modulate their actomyosin cytoskeletons in response to stretch and contraction.

The C. elegans somatic gonad contains two symmetrical, U-shaped gonad arms connected to a common uterus. Sheath cells surround the developing oocytes, and the spermatheca—a contractile, bag-like organ of 24 myoepithelial cells—houses the sperm and is the site of fertilization (Hirsh et al., 1976; Hubbard and Greenstein, 2000). During ovulation, sheath cell contractions increase and the distal spermathecal neck opens to allow entry of the proximal oocyte to the spermatheca, where it is immediately fertilized (Hirsh et al., 1976; Ward and Carrel, 1979; Hubbard and Greenstein, 2000). After a regulated period of time, spermathecal cells coordinately contract, the spermathecal-uterine (SP-UT) valve opens, and the embryo is expelled into the uterus. This process occurs ~150 times per gonad arm, requiring robust regulation of somatic tissue contractility to successfully propel the oocyte from the sheath through the spermatheca and into the uterus (McCartner et al., 1999; Yamamoto et al., 2006).

Contractions of both sheath and spermathecal cells are regulated by the conserved Ca$^{2+}$ and RhoA signaling pathways (Clandinin et al., 1998; Wissmann et al., 1999; Bui and Sternberg, 2002; Kariya et al., 2004; Yin et al., 2004; McMullan and Nurrish, 2011; Kovacevic et al., 2013; Meighan et al., 2015; Ono and Ono, 2016), which cooperate to increase phosphorylation of the myosin regulatory light chain (p-MRLC) and actomyosin contractility in smooth muscle and nonmuscle cells (Somlyo and Somlyo, 2000). In the C. elegans gonad, two phospholipase C isoforms, Cy (PLC-3) and Cc (PLC-1), cleave phosphatidyl inositol to produce inositol 1,4,5-triphosphate (IP$_3$), which triggers Ca$^{2+}$ release from the endoplasmic reticulum (Clandinin et al., 1998; Bui and Sternberg, 2002; Kariya et al., 2004; Yin et al., 2004; Kovacevic et al., 2013). This likely increases myosin activity by activating a Ca$^{2+}$-calmodulin–dependent myosin light chain kinase (Adelstein, 1982; Somlyo and Somlyo, 2000). In both tissues, RHo-1/RhoA signaling activates Rho-associated kinase, LET-502/ROCK, which phosphorylates and inactivates the myosin-associated phosphatase regulatory subunit MEL-11 (referred to here as myosin phosphatase), leading to increased p-MRLC (Wissmann et al., 1999; McMullan and Nurrish, 2011; Meighan et al., 2015; Ono and Ono, 2016). However, unlike sheath cells, spermathecal cells lack the troponin-tropomyosin complex (Ono and Ono, 2004; Ono et al., 2007) responsible for Ca$^{2+}$ regulation of actomyosin contraction in muscle cells (Ebashi, 1984). Sheath and spermathecal cells also have distinct cytoskeletal organizations (Strome, 1986). Sheath cells exhibit long, longitudinal actin bundles that contain both nonmuscle and muscle isoforms of myosin II heavy chain (Ono and Ono, 2016), whereas the spermatheca has stress fiber–like, parallel actin bundles oriented along the long axis of each cell (Figure 1; Strome, 1986) and lacks muscle isoforms of myosin II (Ardizzi and Epstein, 1987; Ono et al., 2007). This difference in actomyosin organization may reflect differences in the contractile properties of each cell type. The sheath cells, wrapped around the maturing oocytes, experience a relatively constant degree of stretch and produce multiple contractions that increase in intensity and frequency during ovulation, whereas spermathecal cells are dramatically stretched by the incoming oocyte and produce a single smooth contraction as the oocyte is expelled into the uterus (Ward and Carrel, 1979; Hubbard and Greenstein, 2000).

We previously observed that spermathecal cells before the first ovulation have tortuous, randomly oriented actin bundles and that the mature actin cytoskeleton is not present until after the first ovulation (Kovacevic and Cram, 2010). Here we use four-dimensional (4D) confocal microscopy to observe changes to spermathecal actomyosin network organization during cell stretch and contraction. We show that 1) maturation of the actin cytoskeleton requires and is proportional to the degree of spermathecal cell stretch during the first ovulation; 2) actin network maturation coincides with the onset of cell contraction and requires Ca$^{2+}$ signaling to trigger myosin II activity; and 3) both reduced and increased myosin II activity cause changes to actomyosin network connectivity, tortuosity, spacing, and orientation. We conclude that tight spatiotemporal regulation of myosin II activity during cell stretch and contraction is required for optimal actomyosin network organization and tissue contractility in C. elegans spermathecae.

**RESULTS**

Parallel actomyosin bundle formation requires oocyte entry to the spermatheca

The most prominent features of the mature actin cytoskeleton in the C. elegans spermatheca are basal stress fiber–like actin

![FIGURE 1: Anatomy of the C. elegans spermatheca. (A) Schematic diagram showing actin bundle orientation in spermathecal cells. (B–G) Confocal images of two fixed and stained spermathecae, one that is unoccupied, sperm only (B–D), and one that is occupied, sperm and oocyte present (E–G). (B, C, E, F) Confocal maximum intensity projections of spermathecae expressing INX-12::mApple to label lateral junctions (red) stained with phalloidin to label F-actin (green). Note the difference in cell stretch in an unoccupied (C) and an occupied (F) spermatheca. (D, G) A central sagittal z-slice showing a cross section of the spermatheca with basal actin bundles (green), lateral junctions (red), and bright-field image (grayscale). Sperm and oocyte are false colored in blue and pink, respectively. Scale bar, 20 μm. In all images, the spermatheca is oriented distal to proximal.](image-url)
bundles oriented along the long axis of each cell (Figures 1 and 2; Strome, 1986). We previously reported that these parallel actin bundles are absent in spermathecae of young animals before the first ovulation (Kovacevic and Cram, 2010). To investigate what drives production of parallel actin bundles in mature adults and determine whether these actin structures are actomyosin bundles, we used phallolidin staining to visualize filamentous-actin (F-actin) and functional green fluorescent protein–labeled nonmuscle myosin II (GFP::NMY-1) to visualize myosin (Supplemental Figure S1). In late-L4 animals, parallel basal bundles are absent, and the majority of F-actin is located at lateral junctions and the apical cell surface. GFP-labeled myosin II appears diffusely throughout the cytosol and, similarly to F-actin, accumulates at lateral junctions and the apical cell surface, forming small punctae. In some cases, a few thin actomyosin bundles are visible at the basal surface (Figure 2A). By young adulthood, F-actin and myosin II colocalize into basal actomyosin bundles that differ in their organization from those seen in mature adults. Before the first ovulation, the basal actomyosin bundles are tortuous, branching, and randomly oriented (Figure 2B). After the first ovulation, branching and tortuosity decrease, and prominent, parallel actomyosin bundles aligned along the long axis of each cell are apparent (Figure 2C). These actomyosin bundles appear to result primarily from reorganization of existing F-actin. However, using DNase I to specifically bind globular actin (G-actin; Cramer et al., 2002), we did observe a slight but significant increase in the ratio of F-actin to G-actin in spermathecal cells from animals after the first ovulation, suggesting that de novo actin polymerization may also be involved (Supplemental Figure S2). Once parallel actin bundles develop, they are maintained throughout successive rounds of ovulation and are consistently seen in both occupied (oocyte present) and empty (only sperm present) spermathecae. These results demonstrate that the actin structures observed in adults (Strome, 1986) are actomyosin bundles and suggested to us that oocyte entry might be required for development of the mature actomyosin network during the first ovulation.

To determine whether animal age rather than oocyte entry per se initiates actin bundle alignment, we used RNA interference (RNAi) to block entry of the oocyte to the spermatheca and observed the cytoskeleton in larval stage 1 (L1) arrest–synchronized populations after 45 h. At this time point, wild-type (WT) animals have undergone approximately five ovulations. First, we abolished oocyte production by knocking down prp-17, a germline-expressed gene involved in regulating the sperm-to-oocyte switch (Kerins et al., 2010). Most prp-17(RNAi) animals produce only sperm (Kerins et al., 2010; Cecchetelli et al., 2016). In these animals, the immature actomyosin network is maintained, and parallel bundles do not develop. In some cases, prp-17(RNAi) animals produce and ovulate a few abnormal oocytes (Kerins et al., 2010; Cecchetelli et al., 2016). When these oocytes enter the spermatheca, the actomyosin network forms aligned bundles. Quantification of network anisotropy—a measurement of alignment—in individual cells using FibriTool (Boudaoud et al., 2014) reveals a significant increase in anisotropy in prp-17(RNAi) animals, which produced abnormal oocytes, compared with control animals after the first ovulation. Because the prp-17(RNAi) oocytes do not form an eggshell and remain deformable, this apparent increase in anisotropy over WT is probably the result of increased flattening of the spermatheca during imaging. These results suggest that knockdown of prp-17 influences spermathecal actin organization through its effect on oocyte production.

We next explored the role of oocyte entry in actomyosin maturation by inhibiting genes required for sheath cell contraction. RNAi knockdown of the phospholipase Cβ, plc-3 (Yin et al., 2004), or the ER calcium sensor STIM1, stim-1 (Yan et al., 2006), disrupts Ca2+ signaling and sheath contractions, and knockdown of scribble homologue, let-413 (Piliuk et al., 2009), or the atypical protein kinase C (PKC), pck-3 (Aono et al., 2004), disrupts dilation of the distal spermathecal neck. Knockdown of each gene prevents entry of the oocyte to the spermatheca, trapping oocytes in the oviduct, where they undergo endomitotic (Emo) DNA replication and produce abnormally large nuclei (Iwasaki et al., 1996; Mccarter et al., 1999). Although the mechanism of ovulation disruption differs for each gene, the effect on spermathecal actomyosin bundle organization is similar. In all cases, animals with an Emo phenotype have basal, poorly aligned actomyosin bundles that resemble those in control animals before the first ovulation (Figure 3). Quantification of actomyosin organization in individual cells with FibriTool shows that bundle anisotropy in animals treated with plc-3 or let-413 RNAi does not differ significantly from that in control animals before the first ovulation. RNAi knockdown of stim-1 results in a modest decrease in anisotropy compared with the preovulation control, whereas RNAi knockdown of pck-3 results in a more significant reduction in anisotropy (Figure 3). Because pck-3 is involved in establishing apicobasal polarity (Aono et al., 2004), PKC-3 may be required for wild-type morphology of preovulation actomyosin.
Kaunas, 2016) have been shown to influence actomyosin network organization. To determine whether cell stretch, contraction, or both are required for parallel actomyosin bundle production, we used confocal microscopy to capture 4D ovulation movies of live whole animals. Spermathecal actin was visualized using three different fluorescent actin reporters: GFP-labeled actin, GFP::ACT-1; GFP fused to the actin-binding calponin homology domain from utrophin, GFP::UtrCH (Burkel et al., 2007); and the moesin actin-binding domain fused to mCherry, moeABD::mCherry (Edwards et al., 1997). All spermathecal actin reporter lines recapitulate the F-actin organization observed using phalloidin staining and are capable of ovulating normally, suggesting that the actin markers accurately label endogenous actin with minimal disruption of the actin cytoskeleton (Supplemental Figure S3). Fortuitously, we found that during image acquisition, the SP-UT valve in the GFP::ACT-1 animals remains partially closed, prolonging oocyte dwell time in the spermatheca. This allows us to monitor changes in actin bundle organization during cell stretch caused by oocyte entry and subsequent cell contraction. When the embryo exits, the spermatheca returns to a compressed conformation, making it difficult to image cells during contraction (Supplemental Figure S3). For this reason, we present here data obtained using the GFP::ACT-1 line. However, similar results were obtained with the GFP::UtrCH and moeABD::mCherry lines (Supplemental Figure S3).

As previously discussed, oocyte entry stretches the cells of the spermatheca (Figure 1), and this cell stretch precedes and is required for parallel actin bundle production (Figure 2). To determine whether the degree of stretch correlates with the degree of actomyosin bundle alignment, we observed actin dynamics in animals in which the distal spermathecal neck had closed prematurely, permitting only a fraction of the oocyte to enter the spermatheca (Supplemental Movie S1). In contrast to the actomyosin bundle production and alignment evident in normally stretched cells, entry of small oocyte fragments results in minimal change to bundle organization (Figure 4 and Supplemental Movie S1). This suggests that stretch is required for bundle alignment. To determine whether actomyosin bundle alignment coincides temporally with cell stretch upon oocyte entry or instead with onset of contraction, we simultaneously tracked changes in cell length—an indirect measurement of cell stretch and contraction—and actomyosin bundle anisotropy. Immediately after oocyte entry, stretched spermathecal cells exhibit the

Parallel actin bundle formation coincides with cell contraction triggered by and proportional to cell stretch during ovulation

Cell stretch (Thoumine et al., 1995; Sears and Kaunas, 2016) and actomyosin-driven contraction (Peterson et al., 2004; Sears and

**FIGURE 3:** Oocyte entry during ovulation is required for alignment of spermathecal actin bundles. (A) Quantification of actin bundle anisotropy (degree of alignment) using FibrilTool. Each point represents a single cell, and no more than three cells were measured from the same animal. For each condition, from left to right, 44, 63, 152, 42, 27, 35, 54, and 49 cells. (B) Representative phalloidin staining results for each condition in A. Yellow line indicates a single cell selected for measurement with FibrilTool, and numbers indicate anisotropy measured for each cell. Error bars represent SEM. Unpaired t test: ns, \( p > 0.05; * p \leq 0.01; ** p \leq 0.001; **** p \leq 0.0001. \) Scale bar, 5 \( \mu \text{m}. \)

**FIGURE 4:** Actin bundle alignment is proportional to cell stretch. (A, B) Confocal maximum intensity projections showing spermathecal actin labeled with GFP (GFP::ACT-1) in a slightly stretched spermatheca containing an oocyte fragment (A) and normally stretched spermatheca containing the entire oocyte (B) at \(-20 \text{ min after oocyte entry}. \) (A’, B’) A central, sagittal z-slice from A and B. The yellow dashed line indicates measurement of spermathecal cross-sectional area. (C) Comparison of spermathecal cross-sectional area and actin network anisotropy (degree of alignment) achieved \(-20 \text{ min after oocyte entry}. \) depicting a positive relationship between spermathecal cell stretch and actin network anisotropy. Two cells were measured from nine animals (18 cells). Black line: best-fit linear regression. Scale bar, 20 \( \mu \text{m}. \)
Actomyosin in Caenorhabditis elegans

Myosin activity drives parallel actomyosin bundle formation

Because formation of the parallel actomyosin bundles coincides with contraction, we next investigated whether phospholipase-induced cell contraction is required. Phospholipase-stimulated Ca\(^{2+}\) release is predicted to lead to actomyosin contraction through the activation of myosin light chain kinase (MLCK) and the phosphorylation of MRLC. Although the MLCK active in the spermatheca has not been identified, the connection between phospholipases, IP\(_3\) production, Ca\(^{2+}\) release, and contraction in C. elegans is well established (Clandinin et al., 1998; Bui and Stemberg, 2002; Kariya et al., 2013). We showed previously that intracellular Ca\(^{2+}\) levels peak during spermathecal contraction and that loss of the phospholipase C, PLC-1, abolishes Ca\(^{2+}\) signaling and tissue contraction (Kovacevic et al., 2013). If contraction is required for actin network maturation, plc-1-null animals should retain the immature, webby actomyosin network after spermathecal cell stretch. To investigate the effect of plc-1 knockout on actomyosin organization during ovulation, we generated animals homozygous for the putative null allele, plc-1(rx1), expressing GFP-labeled actin in the spermatheca. In these animals, the oocyte enters the spermatheca normally, is fertilized, and then becomes trapped as spermatheca cells fail to contract. This phenotype allowed us to use embryonic development to roughly determine the duration of the embryo in the spermatheca and spermathecal cell stretch. We find that actomyosin network maturation is severely delayed in plc-1(rx1) animals. In wild-type animals, actin organization rapidly increases after ~500 s of cell stretch (Figure 5 and Supplemental Movie S2). In contrast, plc-1(rx1) animals require at least 2 h to form quantifiable parallel bundles (Figure 6). This suggests that cell contraction, lost in plc-1(rx1) animals, is required to drive timely actomyosin organization.

To determine whether delayed actomyosin network maturation seen in plc-1(rx1) animals can be rescued by increasing myosin activity, we used 4D confocal microscopy to capture ovulation movies of WT, plc-1(rx1), and plc-1(rx1) animals treated with RNAi against the myosin phosphatase, mel-11. If the noncontractile plc-1(rx1) phenotype is primarily the result of decreased p-MRLC levels and reduced myosin activity, then loss of the phosphatase mel-11 should rescue timely actomyosin bundle production and alignment. In WT animals, actomyosin network maturation begins during cell contraction, ~500 s after the start of cell stretch, and mature, parallel bundles are well developed after 1000–1500 s (Figures 5 and 7 and Supplemental Movie S2). During this time, plc-1(rx1) spermathecal cells do not contract and show no significant change in actomyosin bundle organization (Figure 7 and Supplemental Movie S3). Treatment of plc-1(rx1) animals with RNAi against mel-11 partially rescues timely actomyosin network maturation, and prominent bundles, similar to those in wild-type cells, are apparent after ~1500 s of cell stretch (Figure 7 and Supplemental Movie S4). Although mel-11 RNAi treatment rescues the production of prominent actomyosin bundles in plc-1(rx1) animals, these bundles are frequently misaligned with respect to the long axis of the cell, and populations of differentially oriented bundles are seen within the same cell (Figure 7). This phenotype is distinct from WT cells, which consistently develop parallel actomyosin bundles oriented along the long axis of each cell, and likely contributes to the reduced anisotropy measured in plc-1(rx1);mel-11(RNAi) animals. Overall these results suggest that the delayed actomyosin network maturation in plc-1(rx1) animals is the result of insufficient activation of myosin and suggest that precise regulation of myosin activity during cell contraction is required to achieve wild-type actin network morphology.

Both increased and decreased myosin activity alter actin network organization

To probe more directly the role of myosin activity in actin bundle formation, we used RNAi of nmy-1, the major nonmuscle myosin II heavy chain expressed in the spermatheca (Kovacevic et al., 2013), to reduce the level of myosin, and RNAi of myosin phosphatase,
mel-11, to elevate myosin activity. Spermathecal actin was visualized in whole live animals using the actin reporter lines described earlier and in N2 animals using phalloidin staining. Similar results were obtained using all techniques (Supplemental Figures S5–S7). As expected, RNAi depletion of nmy-1 reduces spermathecal cell contraction, producing the flaccid phenotype described previously (Kovacevic et al., 2013) characterized by a distended distal neck and SP-UT valve (Supplemental Figure S5). Surprisingly, we find that nmy-1(RNAi) spermathecae have basal actin bundles indistinguishable from WT animals before the first ovulation (Supplemental Figure S3 and Supplemental Movie S5). Imaging fixed whole animals mid ovulation using slow acquisition settings (see Materials and Methods) more clearly shows that GFP-myo-1 is functional and likely reports localization of endogenous myosin.

During ovulation in wild-type animals, myosin is highly dynamic. Immediately after oocyte entry, GFP-myo-1 becomes diffused throughout the cytosol. This is followed by rapid recruitment of GFP-myo-1 into contractile actomyosin bundles. However, unlike nmy-1(RNAi) spermathecae, nmy-1(RNAi) spermathecae never develop the prominent, parallel actin bundles observed in mature, postovulation, WT animals, regardless of the duration of cell stretch. Loss of mel-11 produces the opposite phenotype. After oocyte entry, the spermatheca hyperconstricts, the SP-UT valve remains tightly closed, blocking oocyte exit, and contraction continues until mounting force culminates in rupture of cell–cell contacts. Before tissue rupture, mel-11(RNAi) spermathecae develop very prominent actin bundles. These bundles are frequently thicker and spaced farther apart than bundles in WT cells and appear to result from neighboring bundles being pulled together (Figure 9 and Supplemental Figures S6 and S7). In addition, mel-11(RNAi) cells frequently contain populations of actin bundles oriented in different directions (Figure 9 and Supplemental Figure S5). Overall these results show that myosin is required for actin network maturation and suggest that myosin activity is regulated during contraction to produce WT parallel actin bundles.

**Increased myosin activity leads to clustering of myosin within highly contractile actomyosin bundles**

To better understand how altered myosin activity influences actin network organization, we used 4D confocal microscopy to visualize both actin and myosin during cell stretch and contraction. Myosin and actin were coimaged in a line expressing moeABD::mCherry and GFP::NMY-1 to label actin and myosin, respectively. To confirm that GFP does not interfere with myosin activity, we expressed GFP::NMY-1 in the nmy-1(sb115) null background. GFP::NMY-1 rescues the brood size defect of nmy-1(sb115), producing 142 ± 8.737 (mean ± SEM, n = 3) live offspring per animal, compared with only 21 ± 4.509 (mean ± SEM, n = 3) produced by nmy-1(sb115) (Supplemental Figure S1). This indicates that the GFP-myo-1 is functional and likely reports localization of endogenous myosin.
In mel-11(RNAi) spermathecae, myosin was not distributed throughout actomyosin bundles and instead accumulated in large, laterally associated clusters, creating transverse bands across several actin bundles (Figure 9). In mel-11(RNAi) spermathecae, immediately after oocyte entry, actomyosin organization appears similar to WT. However, 3–5 min later, myosin is recruited to actin bundles, producing small clusters of myosin along actin bundles. These foci rapidly increase in intensity and appear to grow by fusion with neighboring clusters along a single actin bundle until reaching a steady state, ~10 min after initiation of cell stretch, when they increase in intensity but maintain a distance of 5–10 μm along the actin bundles (Figure 9 and Supplementary Figure S9 and Supplemental Movie S6). We also observe changes in actomyosin bundle behavior, including rupture of bundles and maintenance of misaligned bundles present in the immature network (Figure 9B). Eventually, sufficient force builds up to rupture cell contacts. Rupture occurs 27 ± 2.1 min (mean ± SEM, n = 3) after oocyte entry and always at horizontal cell junctions, whereas vertical junctions remain intact. Furthermore, loss of cell-cell adhesion begins at basal cell-cell contacts. Tissue integrity is presumably maintained by apical adherens junctions until cells completely pull apart (Figure 9B and Supplemental Movie S6). Tissue rupture indicates that these actomyosin bundles are highly contractile. Together these results show elevated myosin activity influences myosin organization within actomyosin bundles and generates sufficient force to alter bundle behavior and rupture bundles and cell-cell contacts.

**DISCUSSION**

We previously observed that spermathecal basal actomyosin bundles mature from a webby, isotropic network to one with prominent, evenly spaced, and aligned bundles. Here we show that this maturation requires and is proportional to spermathecal cell stretch, suggesting that spermathecal cells are mechanoreceptive. One recently identified mechanotransducer in the spermatheca is the RhoA GAP SPV-1, which contains an F-BAR domain capable of perceiving membrane curvature (Tan and Zaidel-Bar, 2015). During cell stretch, SPV-1 is displaced from the membrane, releasing inhibition of RHO-1/RhoA (Tan and Zaidel-Bar, 2015) and triggering actomyosin contraction by LET-502/ROCK inhibition of the myosin phosphatase, MEL-11 (Wissmann et al., 1999). This is likely part of the mechanism by which cells that experience greater stretch have higher p-MRLC and active myosin than minimally stretched cells. We also show here that cell stretch alone is not sufficient to align actin bundles. Immediately after oocyte entry, spermathecal cells are fully stretched, yet retain the webby, immature, actomyosin network. Actomyosin network maturation instead coincides with cell contraction ~500 s after the initiation of cell stretch. This is contrary to some cell culture work, in which actin bundle alignment is proportional to the degree of uniaxial stretch (Sears and Kaunas, 2016) but still occurs in stretched cells when actomyosin contractility is disrupted by inhibition of ROCK (Kaunas et al., 2005; Lee et al., 2010) or MLCK (Lee et al., 2010). However, complete loss of myosin activity by simultaneous inhibition of ROCK and MLCK does prevent actin bundle formation in cultured cells (Lee et al., 2010). Similarly, we find myosin activity is required for spermathecal actomyosin bundle production and alignment. We propose that increase in active myosin during cell contraction, triggered by stretch, drives reorganization of the actomyosin network into parallel bundles. This may occur by the network contraction behavior of myosin (Verkhovsky and Borisy, 1993; Fenix et al., 2016) or poorly understood attractive forces between distant myosin bipolar filaments capable of merging actin bundles (Hu et al., 2017).

In smooth muscle and nonmuscle cells, an increase in cytosolic Ca$^{2+}$ causes cell contraction primarily through activation of the Ca$^{2+}$-calmodulin–dependent myosin light chain kinase (Adelstein, 1982). Unlike muscle myosin, nonmuscle myosin Mg$^{2+}$-ATPase activity is not activated by actin alone but requires phosphorylation of the MRLC (Sellars et al., 1981). We showed previously that intracellular Ca$^{2+}$ levels peak during spermathecal contraction and that loss of phospholipase Cε activity influences myosin organization within actomyosin bundles and generates sufficient force to alter bundle behavior and rupture bundles and cell-cell contacts.
Myosin activity influences actin bundle development and organization. (A–D) A single confocal z-slice of a spermathecal cell stained with phalloidin–Texas Red to label F-actin. (A’–D’) AnalyzeSkeleton analysis of bundles. Blue, endpoint (less than two neighbors); purple, junction (more than two neighbors); orange, slab (exactly two neighbors). Analysis indicates high actin bundle branching and/or intersection in a preovulation, WT cell (A’) and a nmy-1(RNAi) cell (C’); note increased purple pixels in A’ and C’ compared with B’ and D’. (A’–D’) OrientationJ was used to false-color actin bundles according to their orientation. Note that a postovulation, WT cell has parallel actin bundles oriented along the long cell axis. In a preovulation, WT cell (A”) and a nmy-1(RNAi) cell (C”), actin bundles are randomly oriented, as illustrated by many different colors. In a mel-11(RNAi) cell, some bundles are oriented correctly parallel to the long cell axis, but subpopulations are misaligned. (E) Quantification of actin bundle connectivity based on AnalyzeSkeleton results. The number of junctions (purple pixels) was divided by the number of endpoints (blue pixels). Each point represents analysis of a single cell with no more than two cells from the same animal and six, five, seven, and five cells for WT preovulation, WT postovulation, nmy-1(RNAi), and mel-11(RNAi), respectively. A value <1 indicates actin bundles more frequently intersected with or branched from neighboring bundles. A value <1 indicates actin bundles more frequently extended the length of the cell without contacting neighboring bundles. Note the increased actin bundle intersection and/or branching in preovulation, WT and cell without contacting neighboring bundles. Note the increased actin bundle tortuosity in preovulation, WT cells and nmy-1(RNAi) cells. Each point represents an individual bundle measurement from five to seven different cells and 241, 145, 256, and 110 bundles for WT preovulation, WT postovulation, nmy-1(RNAi), and mel-11(RNAi), respectively. Error bars represent SEM. Unpaired t-test (E) or Mann-Whitney test (F): ns, p > 0.05; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001. Scale bar, 5 μm.
explain the observation that mel-11 RNAi in a plc-1–null background frequently produces misaligned actin bundles but not the large bundles seen in mel-11 RNAi in a WT background.

Conclusion

Although actin nucleators (Hegsted et al., 2016) and cross-linkers (Kovacevic and Cram, 2010) clearly play an important role in spermathecal actin organization, we find that myosin activity is the dominant factor driving actomyosin network maturation and alignment during cell contraction. This is consistent with work in Drosophila tracheal tubes, where properties of the circumferential actomyosin bundles, including bundle production (Hannezo et al., 2015) and orientation (Hosono et al., 2015), are regulated by myosin activity. In vitro–reconstituted systems provide mechanistic insight into how modulation of myosin activity alone can alter actomyosin network properties. In vitro, actin and myosin are able to self-organize into contractile bundles (Thoresen and Gardel, 2011; Thoresen et al., 2013), and myosin is capable of cross-linking actin filaments (Haviv et al., 2008; Thoresen and Gardel, 2011), fusing actin bundles (Miyazaki et al., 2015), and producing forces sufficient to buckle (Molloy et al., 1995; Lenz et al., 2012) and depolymerize actin filaments (Haviv et al., 2008). Furthermore, in vitro phosphorylation of the MRLC is cooperative, allowing a small increase in kinase activity to cause a large increase in active myosin (Persechini and Hartshorne, 1981). This suggests that tight regulation of p-MRLC and actin–myosin interactions is required for establishment and maintenance of functional actomyosin networks in vivo.

Now that we have characterized the role of myosin activity in spermathecal actomyosin network development, we see this as an ideal system for investigating how myosin activity is regulated to maintain optimal tissue contractility in a mechanically stressed tissue. The spermatheca must undergo tissue contractility in a mechanically stressed environment through an elastic deformable substrate (Friedrich et al., 2011). Our observations suggest that myosin clusters on adjacent actomyosin bundles physically interact either directly or through an unknown binding partner. This idea is supported by the observation that myosin clusters appear to pull neighboring bundles together and hold on to ruptured bundles, preventing further retraction. We also observe precocious stabilization and reinforcement of misaligned bundles present in the immature actomyosin network. This inappropriate stabilization of nascent actomyosin bundles may long-range interactions (Hu et al., 2017) such as mechanical communication through an elastic deformable substrate (Friedrich et al., 2011). Thus, tight regulation of p-MRLC and actin–myosin interactions is required for establishment and maintenance of functional actomyosin networks in vivo.

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BD Bacto-Agar [Fisher Scientific], 0.5% nystatin [Sigma-Aldrich], 0.1 mM CaCl₂, 0.1 mM MgSO₄, 0.5% [wt/vol] cholesterol, and 2.5 mM KPO₄ agar plates seeded with OP50 Escherichia coli at 23°C (Hope, 1999). Generation of nematode strains for this study was done by standard microinjection (Mello et al., 1991). All constructs were injected at a concentration between 5 and 10 ng/μl with injection markers rol-6 and tx3p::RFP injected at 40 ng/μl. Carrier DNA, sheared, denatured salmon sperm DNA (Clontech, Mountain View, CA) was added to bring the final DNA concentration to 100 ng/μl for each injection mix. Extrachromosomal arrays were integrated by ultraviolet irradiation essentially as described in Mariol et al. (2013). Nematode observations and manipulations were performed at 23°C unless otherwise noted. The C. elegans nonmuscle myosin II allele HR1184 nmy-1(sb115) and phospholipase Cα allele PS4112 plc-1(nr1) were obtained from the Caenorhabditis Genetics Center and outcrossed at least four times with N2 (wild-type strain from Bristol). Transgene cys198[nft-1p::moeABD::mCherry, unc-119(+)] was obtained from strain NK1069, a kind gift from the Sherwood lab (Duke University, Durham, NC). For a list of strains used in this study, see Supplemental Table S1.

RNA interference

The RNAi feeding protocol was performed essentially as described in Timmons et al. (2001). To prepare seeded NGM-isopropylthio-β-galactoside (IPTG) plates, HT115(DE3) bacteria transformed with the double-strand RNA (dsRNA) construct of interest were grown overnight at 37°C in Luria broth (LB) supplemented with 40 μg/ml ampicillin. The next day, 150 μl of the culture was seeded on NGM-IPTG agar (NGM supplemented with 25 μg/ml carbenicillin and 1 mM IPTG) and incubated at room temperature for 24–72 h to induce dsRNA expression. Age-synchronized animals, prepared as described next, were then transferred to these plates.

Partially synchronized populations were obtained by alkaline lysis procedure (“egg prep”), and tightly synchronized populations were obtained by L1 arrest. For egg prep, starved dauer nematodes were allowed to recover for 48 h on NGM plates newly seeded with OP50. This produces young gravid adults for egg collection. Eggs were released using an alkaline hypochlorite solution as described in Hope (1999) and washed three times with filter-sterilized M9 buffer (22 mM KH₂PO₄, 42 mM NaHPO₄, 86 mM NaCl, and 1 mM MgSO₄; Hope, 1999). Clean eggs were then transferred to seeded NGM-IPTG plates. The L1 arrest protocol was adapted from Hope (1999). For L1 arrest, the same alkaline lysis procedure was performed, except that clean eggs were transferred onto NGM without bacteria. Eggs were allowed to hatch and develop until L1 at 16°C overnight before being transferred to seeded NGM-IPTG plates. Populations produced by egg prep are ~10 h behind L1 arrest populations developmentally. All RNAi experiments were performed at 23°C. Strains used in each RNAi experiment are indicated.

RNAi constructs were obtained from the ORFeome-RNAi v1.1 library or were constructed by PCR amplification of WT cDNA and cloned into pPD129.36 (Fire Vector Kit). Empty pPD129.36 vector was used as a negative control in RNAi experiments. All primer sequences and cloning details are available upon request.

Histochemistry

For observations of N2 and UN1534 (GFP::NMY-1), partially synchronized populations were obtained by egg prep and grown at 23°C for 50–55 h. For RNAi experiments, tightly synchronized populations were obtained by L1 arrest and grown at 23°C for 45 h. At this time point, N2 control animals have undergone approximately five ovulations, and the Emo phenotype is obvious in RNAi treatments that disrupt oocyte entry. F-actin staining was adapted from Ono et al. (2007). Briefly, animals were dissected using a 25-gauge hypodermic needle in phosphate-buffered saline (PBS), and dissected gonads were fixed in 1.85% formaldehyde in PBS for 25 min at room temperature. After fixation, gonads were washed twice with PBS, permeabilized for 15 min in PBST (PBS + 0.1% Triton X-100), and then incubated with 0.4 U/ml Texas Red-X-phalloidin in PBS (Invitrogen, Carlsbad, CA) overnight at 4°C or 4 h at room temperature. For identification of the Emo phenotype, the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO) was added at 100 ng/ml and incubated for at least 20 min at room temperature. Labeled samples were washed twice with PBS and mounted on 2% agarose pads for observation.

Confocal microscopy

For ovulation movies and observations of live or fixed whole animals, partially synchronized populations were obtained by egg prep, and animals were grown at 23°C for ~50 h, around the time of the first ovulation. Live animals were immobilized with 0.01% tetramisole and 0.1% tricaine in M9 buffer (Kirby et al., 1990; Mccarter et al., 1997) and mounted on 2% agarose pads or with 0.05-μm Polybead Microspheres (Polysciences, Warrington, PA) diluted 1:2 in water and mounted on 5% agarose pads (adapted from Wang and Audhya, 2014). In this imaging preparation, WT transits occur in ~500 s. Although Mccarter et al. (1999) show more rapid oocyte transit times (~340 s), our results agreed with earlier work from our laboratory and others (Ward and Carrel, 1979; Kovacevic et al., 2013). To prevent alteration of GFP::NMY-1 localization as was occasionally observed in excised gonads, live whole animals were fixed by immersion in 1.25% formaldehyde in PBS for 25 min at room temperature. Fixed animals were washed three times with PBS and mounted on 2% agarose pads. Confocal microscopy was performed on an LSM 710 confocal microscope (Zeiss) equipped with Zen software (Zeiss) using the Plan-Apochromat 63x/1.40 Oil DIC M27 objective. The 405-nm laser was used to excite DAPI, the 488-nm laser was used for GFP, and the 561-nm laser was used for mCherry and Texas Red. For movies, 40 z-slices were acquired at 14-s intervals for imaging a single channel or 30 z-slices at 20-s intervals for imaging two channels. Illumination of the spermathecae from animals expressing actin labeled with GFP (GFP::ACT-1) with the 488-nm laser for ~5 min before oocyte entry frequently caused the valve to remain partially closed during ovulation, increasing oocyte dwell time. For movies of all lines, live animals were imaged for ~30 min total. For still images of live and fixed animals and tissue, z-slices were acquired at 0.38-μm intervals with each slice representing the average of two or four scans for slow acquisition settings.

Image analysis

ImageJ software was used for all image analysis. For consistency, all analysis was performed on cells of the main spermathecal bag. Cells of the distal neck and cells most proximal to the valve were not used. FibriTool (Boudaoud et al., 2014) was used for quantification of actin anisotropy in selected cells. For images of fixed and stained tissue, FibriTool was used on a single z-slice, capturing only the basal surface of the cell. For movie analysis, maximum intensity projections were generated, and FibriTool was used on individual cells at 28-s intervals (every other frame). In Figure 5, anisotropy measurements were normalized by taking the average anisotropy of the first 10 frames measured and subtracting this number from each measurement. The anisotropy curve in Figure 5 was smoothed using GraphPad Prism software set to average each point according to the five nearest points on each side of it.
To quantify actin network tortuosity (length of the bundle/linear distance between bundle ends) and connectivity in images of fixed tissue, ImageJ was used to enhance image contrast of the entire image, and NeuroJ (Meijering et al., 2004) was used for computer-guided tracing of individual bundles to allow for tortuosity measurements and to generate a skeletonized image. Network connectivity of skeletonized images was measured using AnalyzeSkeleton (Arganda-Carreras et al., 2010) to determine the number of junction (more than two neighbors), endpoint (less than two neighbors), and slab (exactly two neighbors) pixels per image. Connectivity was calculated by dividing the number of junction pixels by the number of endpoint pixels for each skeletonized image of a single cell. OrientationJ (Rezkallahina et al., 2012), configured using a Gaussian fit with a pixel size of 2, was used to measure the orientation distribution of actin bundles in individual cells and generate color-coded images, where color indicates orientation, hue indicates coherency, and brightness is the brightness of the original image. Unless otherwise indicated, line-scan measurements of fluorescence intensity were conducted using line pixel width of 5. All measurements were conducted on raw images except when NeuroJ was used. Contrast is enhanced in images to clarify structures of interest. All statistical analysis was performed with GraphPad Prism software. Unpaired t test was used to determine whether the difference between the means of two data sets was significant when data had a normal distribution, and Welch’s correction was included if the different treatments were expected to have different SDs. The Mann–Whitney test was used when data did not have a normal distribution. In all cases, the statistical test used and resulting p values are noted in the figure captions. ns, p > 0.05; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

ACKNOWLEDGMENTS
We thank Ronen Zaidel-Bar for many helpful discussions. C. elegans strain N1069 was generously provided by the Sherwood lab. Some C. elegans strains used in this study were provided by the Caenorhabditis Genetics Center, which is funded by the National Center for Research Resources, National Institutes of Health. This work was supported by a grant from the National Institutes of Health/National Institute of General Medical Sciences (GM110268) to E.J.C.

REFERENCES
Adelstein RS (1982). Calsodulin and the regulation of the actin/myosin interaction in smooth muscle and nonmuscle cells. Cell 30, 349–350.
Amano M, Ito M, Fukuta Y, Chihara K, Nakano T, Matsura Y, Kaibuchi K (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). J Biol Chem 271, 20246–20249.
Aono S, Legouis R, Huse WA, Kephues KJ (2004). PAR-3 is required for Caenorhabditis elegans somatic gonad for proper cytoe development. Dev Biol 264, 55–71.
Cetera M, Ramirez-San Juan GR, Oakes PW, Llewellyn L, Fairchild MJ, Tanentzapf G, Gardel ML, Horne-Badovinac S (2014). Epithelial rotation promotes the global alignment of contractile actin bundles during Drosophila egg chamber elongation. Nat Commun 5, 5511.
Clandinin TR, DeModena JA, Sternberg PW (1998). Inositol trisphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in C. elegans. Cell 92, 523–533.
Clapham DE (2007). Calcium signalling. Cell 131, 1047–1058.
Cramer LP, Briggs LJ, Dawe HR (2002). Use of fluorescently labelled deoxyribonucleic acid to spatially measure G-actin levels in migrating and non-migrating cells. Cell Motil Cytoskeleton 38, 27–38.
Davis MJ, Wu XIN, Berry JA, Nosworthy NJ (2003). Integrins and mechanotransduction of the vascular myogenic response. Am J Physiol Heart Circ Physiol 280, 1427–1433.
Dingwall J, Burridge K, Wittchen ES (2013). The tension mounts: stress fibers as force-generating mechanotransducers. J Cell Biol 200, 9–19.
Ebashi S (1994). Ca2+ and the contractile response. J Mol Cell Cardiol 16, 2799–2903.
Ebihara S, Tanaka J, Taira C, Hasegawa Y, Nakanishi T, Efstrand J, Arenz M, Ulrich R, Kishi H, Nakamura Y, Ohno S (1996). The evolution of compositionally and functionally distinct actin filaments. Development 136, 3109–3119.
Elbashir S, Xie XS, Bartel DP, Lendeckel W, Tuschl T (2001). RNA interference is mediated by 21 and 22 nucleotide RNAs. Cell 107, 185–191.
Faham S, Peleg S, Oren M, Pines J, Lapidot M, Givol D, Giladi N, Biton A, Gazit E (2004). Breaking and promoting androgen receptor SPARC by actin depolymerization. Cancer Res 64, 5365–5371.
Fukata Y, Kimura K, Oshiro N, Saya H, Matsuura Y, Kaibuchi K (1998). Association of the myosin-β subunit of myosin phosphatase and moesin: dual regulation of moesin phosphorylation by Rho-associated kinase and myosin phosphatase. J Biol Chem 271, 1145–1147.
Fukuta Y, Kimura K, Oshiro N, Matsura Y, Kaibuchi K (1998). Association of the myosin-β subunit of myosin phosphatase and moesin: dual regulation of moesin phosphorylation by Rho-associated kinase and myosin phosphatase. J Biol Chem 271, 1145–1147.
Friedrich BM, Buxboim A, Discher DE, Safran SA (2011). Striated actomyosin fibers can reorganize and register in response to elastic interactions with the matrix. Biophys J 100, 2706–2715.
Fujiwara N, Saka Y, Naka T, Matsui K, Ogawa N, Itoh K, Yamada KM (2002). The importance of actin filaments for determining the mechanical response of cells to a soft substrate. J Cell Sci 115, 17–28.
Gally C, Wissler F, Zarehddine H, Quintin S, Landmann F, Labouesse M (2009). Myosin II regulation during C. elegans embryonic elongation: LET-502/ROCK, MRRK-1 and PAK-1, three kinases with different roles. Development 136, 3109–3119.
Gunning PW, Ghoshdastider U, Whitaker S, Popp D, Robinson RC (2015). The evolution of compositionally and functionally distinct actin filaments. J Cell Sci 128, 2009–2019.
Hanneman E, Dong B, Reho P, Joanny JF, Hayashi S (2015). Cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. Proc Natl Acad Sci USA 112, 201504762.
Haynes J, Gillingham AJ, Backouche F, Bernheim-Groswasser A, Kaibuchi K (1998). Multifunctional myosin: myosin II acts as an actin depolymerization agent. J Mol Biol 375, 325–330.
Haynes J, Srivastava J, Madson N, Wittmann T, Barber DL (2011). Dynamic actin remodeling during epithelial-mesenchymal transition depends on increased moesin expression. Mol Biol Cell 22, 4750–4764.
Hegele U, Wright FA, Votra S, Pynne D (2016). INF2 and FODH-Related formins promote ovulation in the somatic gonad of C. elegans. Cell 162, 712–728.
Hirsh D, Oppenheim D, Klass M (1976). Development of the reproductive system in Caenorhabditis elegans. Dev Biol 49, 200–219.
Hope IA (1999). C. elegans—the Practical Approach, Oxford, UK: Oxford University Press.
Hosono C, Matsuda R, Adryan B, Samakovlis C (2015). Transient junction anisotropies orient annular cell polarization in the Drosophila airway tubes. Nat Cell Biol 17, 1569–1576.
Miyazaki M, Chiba M, Eguchi H, Ohki T, Ishiwata S (2015). Cell-sized...

Michaux G, Legouis R, Labouesse M (2001). Epithelial biology: lessons from...

Mello CC, Kramer JM, Stinchcomb D, Ambros V (1991). Efficient gene...

Meighan CM, Kelly VE, Krahe EC, Gaeta AJ (2015).

McMullan R, Nurrish SJ (2011). The RHO-1 RhoGTPase modulates fertility...

Meighan CM, Kelly VE, Krahe EC, Gaeta AJ (2015). Apical domain polarization local...

Lenz M, Gardel ML, Dinner AR (2012). Requirements for contractility in...

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress...

Lavoie TL, Dowell ML, Lakser OJ, Gerthoffer WT, Fredberg JJ, Seow CY, ...

Kovacevic I, Lefebvre C, Wiesenfahrt T, Legouis R, Bossinger O (2009). Increased IP3/Ca2+ signaling compensates depletion of LET-413/DLG-1 in C. elegans epidermal junction assembly. Dev Biol 327, 34–47.

Kariya KI, Kim Bui Y, Gao X, Sternberg PW, Kataoka T (2004). Phospholipase C γ regulates ovulation in Caenorhabditis elegans. Dev Biol 274, 201–210.

Kaunas R, Nguyen P, Usami S, Chien S (2005). Cooperative effects of Rho and mechanical stretch on stress fiber organization. Proc Natl Acad Sci USA 102, 15895–15900.

Kerins JA, Hanazawa M, Dorsett M, Schedl T (2010). PRP-17 and the premRNA splicing pathway are preferentially required for the proliferation versus meiotic development decision and germline sex determination in Caenorhabditis elegans. Dev Dyn 239, 1555–1572.

Kirby C, Kusch M, Kemphues K (1990). Mutations in the par genes of Cae...

Kovacevic I, Crom EJ (2010). FLN-1/Filamin is required for maintenance of actin and exit of fertilized oocytes from the spermatheca in C. elegans. Dev Biol 347, 247–257.

Kovacevic I, Orozco JM, Crom EJ (2013). Filamin and phospholipase C-ε are required for calcium signaling in the Caenorhabditis elegans spermatheca. PLoS Genet 9, e1003510.

Lamb NJC, Fernandez A, Conti MA, Adelstein R, Glass DB, Welch WJ, Wirshing AC, Cram EJ (2010). FLN-1/Filamin is required for maintenance of actin and exit of fertilized oocytes from the spermatheca in C. elegans. Dev Biol 347, 247–257.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

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Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

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Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.

Lee C, Haase C, Deguchi S, Kaunas R (2010). Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. J Cell Biol 201, 213–220.
associated alterations of their cytoskeletal structure. Exp Cell Res 219, 427–441.
Timmons L, Court DL, Fire A (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene 263, 103–112.
Tojkander S, Gateva G, Lappalainen P (2012). Actin stress fibers—assembly, dynamics and biological roles. J Cell Sci 125, 1855–1864.
Totsukawa G, Yamakita Y, Yamashiro S, Hartshorne DJ, Sasaki Y, Matsumura F (2000). Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. J Cell Biol 150, 797–806.
Tsukita S, Yonemura S (1999). Cortical actin organization: lessons from ERM (ezrin/radixin/moesin) proteins. J Biol Chem 274, 34507–34510.
Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, et al. (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389, 990–994.
Verkhovsky AB, Borisy GG (1993). Non-sarcomeric mode of myosin II organization in the fibroblast lamellum. J Cell Biol 123, 637–652.
Verkhovsky AB, Svitkina TM, Borisy GG (1995). Myosin II filament assemblies in the active lamella of fibroblasts: their morphogenesis and role in the formation of actin filament bundles. J Cell Biol 131, 989–1002.
Wang L, Audhya A (2014). In vivo imaging of C. elegans endocytosis. Methods 68, 518–528.
Ward S, Carrel JS (1979). Fertilization and sperm competition in the nematode Caenorhabditis elegans. Dev Biol 73, 304–321.
Wettschureck N, Offermanns S (2002). Rho/Rho-kinase mediated signaling in physiology and pathophysiology. J Mol Med 80, 629–638.
Wissmann A, Ingles J, Mains PE (1999). The Caenorhabditis elegans mel-11 myosin phosphatase regulatory subunit affects tissue contraction in the somatic gonad and the embryonic epidermis and genetically interacts with the Rac signaling pathway. Dev Biol 209, 111–127.
Wozniak MA, Chen CS (2009). Mechanotransduction in development: a growing role for contractility. Nat Rev Mol Cell Biol 10, 34–43.
Yamamoto I, Kosinski ME, Greenstein D (2006). Start me up: cell signaling and the journey from oocyte to embryo in C. elegans. Dev Dyn 235, 571–585.
Yan X, Xing J, Lorin-Nebel C, Estevez AY, Nehrke K, Lamitina T, Strange K (2006). Function of a STIM1 homologue in C. elegans: evidence that store-operated Ca2+ entry is not essential for oscillatory Ca2+ signaling and ER Ca2+ homeostasis. J Gen Physiol 128, 443–459.
Yin X, Gower NJD, Baylis HA, Strange K (2004). Inositol 1,4,5-trisphosphate signaling regulates rhythmic contractile activity of myoepithelial sheath cells in Caenorhabditis elegans. Mol Biol Cell 15, 3938–3949.
Zaidel-Bar R, Zhenhuan G, Luxenburg C (2015). The contractome—a systems view of actomyosin contractility in non-muscle cells. J Cell Sci 128, 2209–2217.