## CELL SCIENCE AT A GLANCE

# Functions of actin in mouse oocytes at a glance

Julia Uraji, Kathleen Scheffler and Melina Schuh*

## ABSTRACT

Gametes undergo a specialized and reductional cell division termed meiosis. Female gametes (oocytes) undergo two rounds of meiosis; the first meiotic division produces the fertilizable egg, while the second meiotic division occurs upon fertilization. Both meiotic divisions are highly asymmetric, producing a large egg and small polar bodies. Actin takes over various essential function during oocyte meiosis, many of which commonly rely on microtubules in mitotic cells. Specifically, the actin network has been linked to long-range vesicle transport, nuclear positioning, spindle migration and anchorage, polar body extrusion and accurate chromosome segregation in mammalian oocytes. In this Cell Science at a Glance article and the accompanying poster, we summarize the many

Department of Meiosis, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany.

*Author for correspondence (melina.schuh@mpibpc.mpg.de)

J.U., 0000-0002-1441-4074; M.S., 0000-0003-0025-8952

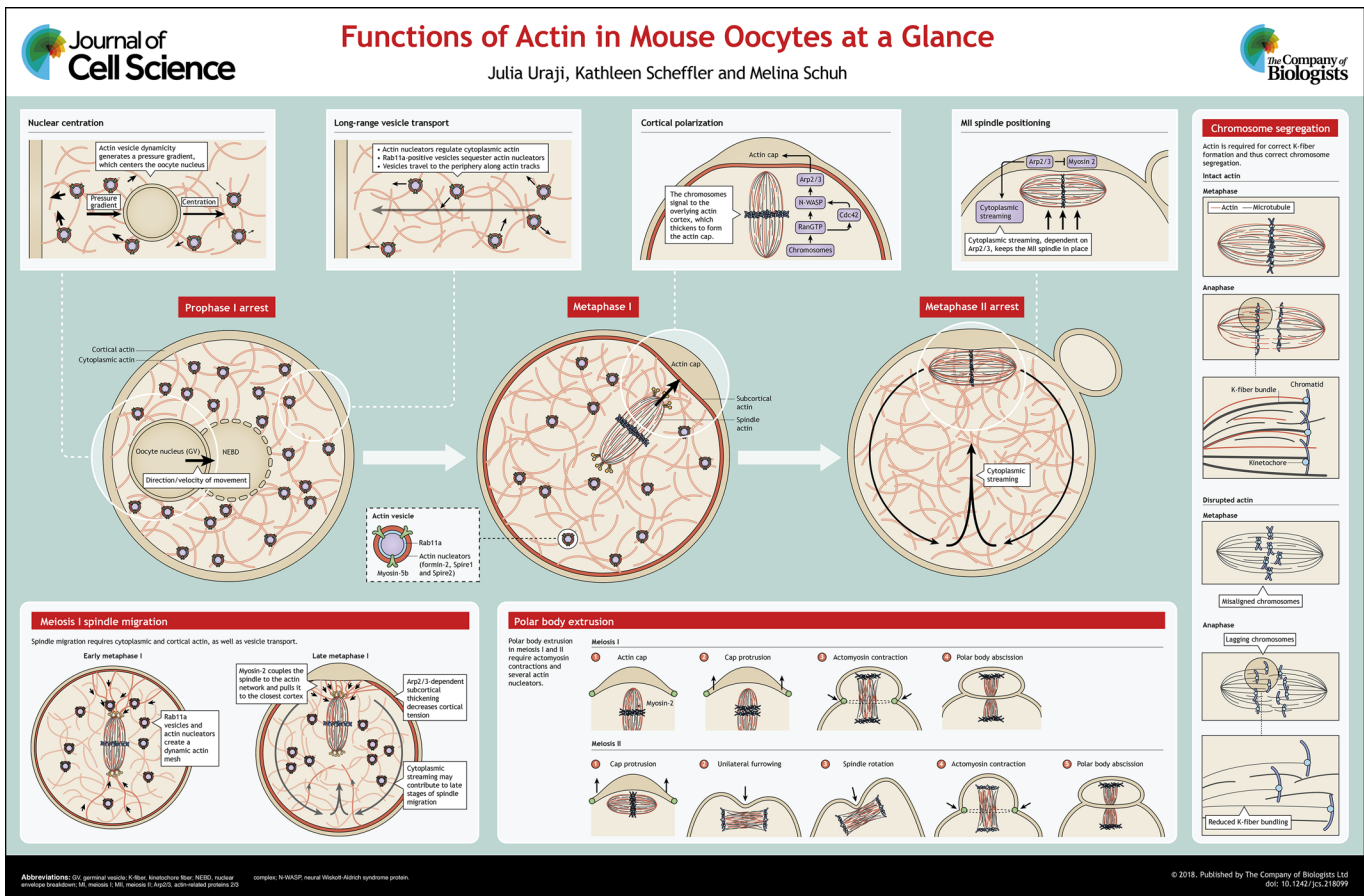functions of the actin cytoskeleton in oocytes, with a focus on findings from the mouse model system.

### Introduction

In female reproduction, the fertilizable egg is formed from progenitor cells called oocytes. In female mammals, oocytes are stored and nurtured inside follicles of the ovaries from birth onwards. Here, they remain arrested in prophase of meiosis I, with their homologous chromosomes recombined. Once every reproductive cycle, a subset of prophase I-arrested oocytes resumes meiosis. The nuclear envelope breaks down, and the meiotic spindle forms around the chromosomes. The spindle migrates towards the cortex, where the homologous chromosomes are segregated and the first polar body is extruded. The oocyte then arrests in metaphase of meiosis II. Upon fertilization, the second meiotic division is completed, and half of the remaining sister chromatids are eliminated into the second polar body (for a general overview of female meiosis in mammals, see reviews by Coticchio et al., 2015;

Marlow, 2018; Verlhac and Terret, 2016; Webster and Schuh, 2017; Clift and Schuh, 2013).

Female meiosis has several unique features. Most remarkably, its two rounds of cell division are highly asymmetric. This allows the formation of small polar bodies, which degenerate in most species (Schmerler and Wessel, 2011), and one large fertilizable egg. It is commonly thought that these asymmetric divisions are required to ensure the retention of sufficient cytoplasmic resources, including mRNA, proteins and mitochondria, to support embryonic development until implantation (reviewed by Chaigne et al., 2017).

To achieve such asymmetric divisions, the oocyte has a highly specialized cytoskeleton (reviewed by Mogessie et al., 2018). As oocytes of most species lack classical centrosomes and long astral microtubules (reviewed by Schatten and Sun, 2011), the actin cytoskeleton has taken over several roles commonly associated with microtubules in mitotic systems. The roles of actin identified so far in mouse oocytes include: (1) long-range vesicle transport towards the plasma membrane, (2) centration of the nucleus if oocyte, termed the germinal vesicle (GV), prior to nuclear envelope breakdown (NEBD), (3) spindle migration during meiosis I, (4) spindle anchorage to the cortex during metaphase-II arrest, (5) spindle rotation prior to anaphase II, (6) the support of microtubule functions in chromosome alignment and segregation, and (7) cytokinesis during polar body extrusion.

In this Cell Science at a Glance article and accompanying poster, we will give an overview of these functions of actin in oocytes, with a focus on findings from the mouse model system.

### Actin-dependent long-range vesicle transport

Oocytes have a thick actin cortex, lining the plasma membrane and stabilizing the oocyte surface (see poster). Advances in microscopy assays and actin reporters have revealed that mouse oocytes are also filled with a highly dynamic network of actin filaments that converge at nodal points. (Azoury et al., 2008; Schuh and Ellenberg, 2008).

The cytoplasmic actin meshwork is nucleated through a cooperation between formin-2 and Spire1 and Spire2 (Spire1/2). Formin-2 belongs to the Rho GTPase-independent family of formins and assembles straight actin filaments. Formin-2 requires the Spire1/2 proteins for efficient actin filament elongation (Montaville et al., 2014; Pfender et al., 2011; Quinlan et al., 2007; Rosales-Nieves et al., 2006), and loss of formin-2 function leads to loss of the cytoplasmic actin network (Azoury et al., 2008; Dumont et al., 2007a; Schuh and Ellenberg, 2008).

Formin and Spire1/2 nucleators are not randomly distributed in the oocyte, but are focused at the oocyte surface and on Rab11a-positive vesicles. These vesicles serve as sites of actin nucleation, and cluster and sequester actin nucleators from the cytoplasm to the vesicle surface (Pfender et al., 2011). Loss of Rab11a-positive vesicles, upon treatment with brefeldin A or expression of the dominant-negative variant Rab11a$^{S25N}$ leads to the release, of actin nucleators into the cytoplasm and an increase of the cytoplasmic actin density (Holubcová et al., 2013).

These vesicles also drive the dynamics of the actin network. To this end, they recruit the motor protein myosin-5b, promoting vesicle motion along actin filaments (Pfender et al., 2011). Inhibition of myosin-5b activity blocks outward-directed vesicle movement, and the actin network becomes static. While local vesicle movement is random, there is a global movement towards the plasma membrane, which is likely to be favored by an enrichment of the rapidly polymerizing actin barbed-ends at the plasma membrane (Holubcová et al., 2013; Pfender et al., 2011). Thus, a model has been proposed in which vesicles move towards each other along connecting actin filaments; the most peripheral vesicles are transported along cortical actin filaments to the cell surface, thereby dragging the entire vesicle-actin-network outwards (Schuh, 2011) (see poster).

Taken together, the special architecture and organization of this actin–vesicle network allows for transport of vesicles over long distances, a mechanism that solely relies on microtubules in mitotic cells (reviewed by Barlan and Gelfand, 2017). This actin–vesicle network is also used by other structures to migrate outward, such as Rab27a-positive cortical granules (Cheeseman et al., 2016), which move along actin and 'hitchhike' on Rab11a-positive vesicles to reach the cell surface, where they are required to prevent polyspermy.

Interestingly, vesicles appear not only to support movement to the plasma membrane but also to the oocyte center, as recently described for the oocyte nucleus (Almonacid et al., 2015).

### Nuclear positioning in prophase I-arrested oocytes

While the oocyte grows within the follicle, the oocyte nucleus is often located at the oocyte periphery. Once the oocyte is in the final stages of follicle development, before NEBD and meiotic resumption, the nucleus usually moves to the oocyte center (Brunet and Maro, 2007) (see poster).

This migration of the oocyte nucleus depends entirely on the actin network, as opposed to the microtubule network, which is often involved in nuclear positioning in somatic cells (Reinsch and Gonczy, 1998). Nuclear centration requires the assembly of a cytoplasmic actin network mediated by formin-2; in the absence of formin-2, nuclei are positioned at the oocyte periphery (Dumont et al., 2007a), and microinjection of formin-2 in $Fmn2^{-/-}$ oocytes rescues nuclear centration (Almonacid et al., 2015).

Furthermore, a vesicle-dependent pressure gradient has been proposed to promote nucleus centering in oocytes (Almonacid et al., 2015). Vesicle velocity and activity in prophase-arrested oocytes have been found to be higher at the cortex and decrease towards the center, and blocking vesicle dynamics by inhibiting myosin-5b leads to a failure in nuclear centration (see poster). A gradient of vesicle activity would thus generate a pressure that is higher close to the oocyte surface and weaker at the center, thus promoting the motion of the nucleus towards the oocyte center. Furthermore, vesicle dynamicity may make the cytoplasm more fluid and facilitate nuclear migration (Almonacid et al., 2015).

In summary, mouse oocytes employ an actin-dependent mechanism to center their nuclei during the final stages of follicle development.

### Spindle migration during meiosis I

Upon NEBD, the spindle assembles at the center of a mouse oocyte. The newly formed spindle then relocates towards the cell surface, where the chromosomes will be segregated.

In mitosis, spindle positioning is largely dependent on microtubules; long astral microtubules emanate from centrosomes at the spindle poles and interact with the cortex. Cortical dynein generates pulling forces along these astral microtubules to promote spindle motion (reviewed by Kotak and Gönczy, 2013; McNally, 2013). Pushing forces of astral microtubules against the cortex have also been recently proposed to be involved in spindle positioning in *Caenorhabditis elegans* embryos (Howard and Garzon-Coral, 2017; Pecreaux et al., 2016). However, the oocytes of most organisms eliminate centrioles during their growth phase, and thus, meiotic spindles lack canonical centrosomes and only have a few or no astral microtubules (Sathananthan et al., 2006; Schuh and Ellenberg, 2007; Szollosi et al., 1972). In mouse oocytes, it has been found that meiotic spindle migration is independent of microtubules, but is driven by an F-actin-dependent mechanism

(see poster). Indeed, perturbation of the actin cytoskeleton (via cytochalasin D or Spire1/2 siRNA treatment or upon *Fmn2* knockout,) prevents spindle migration, whereas microtubule depolymerization (with nocodazole) does not (Azoury et al., 2008; Dumont et al., 2007a; Longo and Chen, 1985; Pfender et al., 2011; Schuh and Ellenberg, 2008; Verlhac et al., 2000).

More specifically, several actin-dependent pathways have been proposed to play a role in this spindle migration by coupling the spindle pole to the cortex. First, myosin-2 has been implicated in spindle migration, as it is activated at spindle poles and pulls on the network (Holubcová et al., 2013; Schuh and Ellenberg, 2008; Simerly et al., 1998). In support of a functional role of myosin-2 in spindle migration, it has been shown that inhibition of myosin light chain kinase, which is involved in activating myosin-2, slows down spindle relocation. (Holubcová et al., 2013; Schuh and Ellenberg, 2008). Additionally, the same outward-moving actin–vesicle network that is responsible for long-range vesicle transport to the oocyte surface may aid migration of the meiotic spindle to the cortex. Indeed, spindle migration fails upon blocking of myosin-5b-dependent network dynamics (Holubcová et al., 2013). Thus, spindle-pole-associated myosin-2 may pull at surrounding actin filaments, thereby coupling the spindle to the actin–vesicle network, which shows a continuous net movement towards the plasma membrane. In a similar fashion to what occurs during nuclear migration, vesicles may also contribute to the fluidity of the cytoplasmic actin mesh, thus allowing spindle migration.

The above model of spindle migration relies on pulling forces of the spindle coupled to the dynamic cytoplasmic actin. However, a second model has been proposed, in which pushing promotes asymmetric spindle migration (Li et al., 2008; Yi et al., 2013). Here, formin-2 might nucleate F-actin, which accumulates behind the lagging pole of the migrating spindle. This actin 'cloud' might exert pushing forces on the spindle. Once the spindle begins relocating, its migration may be further supported by cytoplasmic streaming, which is mediated by Arp2/3 accumulating at the actin cortex proximal to the approaching chromosomes (Sun et al., 2011; Yi et al., 2013). This Arp2/3-dependent pathway is also responsible for maintaining the spindle in meiosis II arrest beneath the surface, which will be described in detail below.

In addition to the cytoplasmic meshwork, the actin cortex has been proposed to have a crucial role in asymmetric spindle positioning. After meiosis I resumption, the actin cortex becomes thicker and an inner, less dense, actin layer forms (Chaigne et al., 2013). This cortical thickening is independent of the formin-2 or Spire1/2 proteins, but requires the activity of the Arp2/3 complex, a nucleator of branched actin networks (Azoury et al., 2008; Chaigne et al., 2013; Pfender et al., 2011). This subcortical thickening correlates with cortical tension (Chaigne et al., 2013), the regulation of which appears to be crucial for correct spindle migration, as an either too high or too low cortical tension inhibits migration (Chaigne et al., 2013, 2015). The exclusion of myosin-2 from the cortex after NEBD decreases cortical tension, and transduces pulling forces from spindle poles to the cortex, promoting spindle migration (Chaigne et al., 2013; Larson et al., 2010) (see poster).

Thus, spindle migration from the center to the cortex in meiosis I (MI) oocytes relies on a variety of actin-dependent pathways. Recently, asymmetries of the spindle that are present prior to spindle migration were suggested to predict the direction of migration to the cortex (Wu et al., 2018); however, how these link to the described actin pathways involved in spindle migration is not yet known. Once the spindle has reached its peripheral position, an actin cap forms, which is described in the next section.

## Chromosome-induced cortical polarization

Prophase I-arrested oocytes are not polarized and are surrounded by a uniform layer of cortical actin; their surface is covered homogenously by microvilli promoting the binding of sperm to the egg and fusion (Runge et al., 2007). During spindle migration, the cortex becomes polarized. First, the actin cortex directly overlying the meiotic apparatus thickens, forming the actin cap (Longo and Chen, 1985), which is thought to be involved in anchoring the spindle during meiosis II (see poster). Furthermore, microvilli are lost in this area to prevent entry of sperm in the vicinity of the maternal chromosomes and the possibly fatal capture of sperm chromosomes by the maternal meiosis II spindle (Luo et al., 2009).

The cortical polarization above the meiotic spindle is dependent on DNA, and does not require the microtubules of the spindle (Deng et al., 2007). The small GTPase Ran, known for its role in spindle assembly around chromosomes (reviewed in Clarke and Zhang, 2008), concentrates and forms a gradient around meiotic chromosomes (Dumont et al., 2007b). Furthermore, Ran-GTP regulates the recruitment and activation of Arp2/3 through the nucleation-promoting factor N-WASP (also known as WASL) in the adjacent cortex (Yi et al., 2011). Consistent with this, DNA-coated beads proximal to the cortex are sufficient to induce actin cap formation, and cortical polarization fails after injection of dominant-negative $Ran^{T24N}$ (Deng et al., 2007). Inhibition of the Rho GTPase Cdc42 results in loss of the actin cap above meiotic spindles due to N-WASP delocalization and subsequent Arp2/3 deactivation (Dehapiot et al., 2013; Yi et al., 2011). Thus, chromosomes themselves provide the Ran-GTP-dependent spatial cue to induce actin polymerization.

## Spindle anchorage during meiosis II arrest

After completion of meiosis I and extrusion of the first polar body, the second meiotic spindle forms around the remaining chromosomes adjacent to the cortex. The oocyte arrests at metaphase II until fertilization, while the meiosis II spindle remains stably anchored below the actin cap (Liu et al., 2000; Maro et al., 1984) (see poster).

Like in meiosis I, spindle positioning in meiosis II relies on an intact actin cytoskeleton (Maro et al., 1984; Yi et al., 2011; Zhu et al., 2003). The same key players responsible for the actin cap formation are required for this spindle anchorage, namely Arp2/3, which is enriched in the actin cap, and possibly N-WASP (Yi et al., 2011). Arp2/3-dependent actin nucleation is thought to generate an actin flow, which streams away from the cortical cap, along the cell periphery, and converges at the opposite oocyte pole, circulating back towards the oocyte center (see poster). Thus, the actin flow may translate into cytoplasmic streaming, which has been proposed to push the spindle towards the cortex. Upon Arp2/3 inhibition, the direction of this cytoplasmic streaming is reversed, which pushes the spindle away in a myosin-2-dependent manner (Yi et al., 2011). This suggests that Arp2/3 maintains the meiotic spindle at the cortex by counteracting or preventing myosin-2-driven contractions from pushing the spindle towards the oocyte center. Arp2/3 and myosin-2 function at the actin cap are predominantly regulated by Ran and its downstream effectors Cdc42 and Rac, as loss of function of any of these GTPases results in detachment of the meiosis II spindle (Dehapiot et al., 2013; Halet and Carroll, 2007; Yi et al., 2011).

## Polar body extrusion and spindle rotation

Actin is crucial for cytokinesis in mitotic cells, as it forms a contractile ring that drives membrane constriction between daughter cells. Indeed, the same applies to female gametes. In mouse oocytes,

several key factors for polar body extrusion have been identified, although it remains unclear whether all these factors are equally important in meiosis I and II. We aim here to give an overview of the main findings.

Polar body extrusion of meiosis I occurs after spindle migration to the cortex, whereas that of meiosis II is only initiated following egg activation. Polar body extrusion relies on the enrichment of actin and myosin-2 in the actin cap (Simerly et al., 1998). Although actin and myosin-2 initially colocalize, myosin-2 later forms a ring surrounding the actin cap (Wang et al., 2011) (see poster). This ring formation in meiosis II relies on Ran, Cdc42 and Mos, although the latter two do not appear to be required in meiosis I (Dehapiot et al., 2013).

Next, an outward protrusion of the membrane, which contains the actin cap and the cortex-proximal set of chromatids, forms in a myosin-2 dependent manner. This membrane protrusion is required for cytokinesis and is also dependent on actin nucleation by the Arp2/3 complex (Sun et al., 2011), Spire1/2 proteins (Pfender et al., 2011) and formin-2 (Dumont et al., 2007a; Pfender et al., 2011) in meiosis I (see poster). In the case of meiosis II, a unilateral membrane furrow appears above the anaphase spindle midzone, and the spindle rotates prior to cytokinesis. This rotation requires actin (Gard et al., 1995; Zhu et al., 2003), myosin-2 (Wang et al., 2011) and RhoA (Zhong et al., 2005) (see poster).

Following this, the membrane ingresses bilaterally, engulfing half of the meiotic spindle through constriction of the actomyosin ring. Although chromosomes alone induce cortical polarization, cytokinesis is only initiated in the presence of the microtubule-based spindle (Deng and Li, 2009). However, unlike in mitosis, actomyosin ring assembly and contraction may be distinct processes that are controlled by separate pathways; RhoA and Ect2, for example, appear to be specifically required for initiation of actomyosin contraction (Elbaz et al., 2010).

The important role of actin in cytokinesis is well established in mitotic cells, and thus its function during polar body extrusion is not surprising. However, in the final section, we will describe a novel function for actin in meiotic chromosome segregation that has been only recently discovered (Mogessie and Schuh, 2017).

## Chromosome segregation and capture in meiosis

While microtubules have long been known to be the main component of the meiotic spindle in oocytes, actin filaments have been found to associate with and permeate the spindle in a variety of species (Azoury et al., 2008; Mogessie and Schuh, 2017; Schuh and Ellenberg, 2008). Importantly, our recent study found that F-actin is crucial for protecting the mouse oocyte from chromosome segregation errors and thus aneuploidy (Mogessie and Schuh, 2017). We observed that the perturbation of actin with cytochalasin D or via *Fmn2* knockout caused a significant increase in misaligned chromosomes in meiosis II metaphase, and lagging chromosomes during both meiosis I and meiosis II anaphase. Furthermore, acute addition of cytochalasin D to oocytes with chromosomes already aligned at the metaphase II plate also caused chromosome misalignment, indicating a function of actin in maintaining correct chromosome alignment. Actin did not promote chromosome segregation directly, but was instead required for the formation of kinetochore-fibers (K-fibers), the specialized microtubule bundles that are attached to the kinetochores of the chromosomes and are required to align and separate chromosomes (see poster). Consistent with this, increasing the amount of actin in the spindle also increased K-fiber bundling and induced chromosome misalignment, indicating that a dynamic actin network is required for accurate chromosome segregation (Mogessie and Schuh, 2017). Although previous work in spermatocytes of various insects has shown

the importance of actin and actomyosin for chromosome movements during anaphase (Fabian and Forer, 2007; Forer and Pickett-Heaps, 1998; Silverman-Gavrila and Forer, 2003), our recent study provides the first evidence of a function of spindle actin in oocytes.

Whether actin might fulfill other functions in chromosome segregation in oocytes still remains to be tested. Studies in starfish oocytes, which have a large nucleus with scattered chromosomes, show that a contractile F-actin mesh helps to bring chromosomes together so that they can be captured by spindle microtubules for proper alignment and segregation (Bun et al., 2018; Lénárt et al., 2005). While these findings have not been tested in mouse, they are a further example of the plethora of functions of the actin cytoskeleton in oocytes, which owing to their size and asymmetric divisions, often appear to make use of non-classical cytoskeletal mechanisms for meiosis.

## Perspectives

Our knowledge of the cytoskeleton and its functions in mitosis and meiosis has increased vastly over the past decades of research. New findings have shown that the actin cytoskeleton plays a role in a surprising number of processes in oocytes, including long-range vesicle transport, positioning of the germinal vesicle, spindle migration and anchorage, polar body extrusion and now also chromosome segregation, a process generally thought to rely solely on microtubules. While we cannot speculate whether other entirely undiscovered functions of actin in oocytes remain to be discovered, even the known functions are still not fully understood. Recent technical advances to acutely manipulate protein levels and function in oocytes may be used to explore the precise interactions between components of the actin network and other structures. As oocytes are transcriptionally silent, and target proteins may have low turnover, techniques that acutely affect protein levels, such as the TRIM-Away method (Clift et al., 2017), or inducible systems based on light (Akera et al., 2018) or auxin (Miura et al., 2018), may prove useful. Furthermore, the CRISPR/Cas system has greatly reduced the amount of time and effort required to create transgenic mouse lines for functional studies (reviewed by Hsu et al., 2014; Liu et al., 2017). Additionally, investigating the functions of actin not only in oocytes but also in zygotes and early embryos may provide further insight. For example, it has been shown that actomyosin contractions initiated at the fertilization cone in zygotes correlate with developmental competence (Ajduk et al., 2011), and that actin is required for pronuclear migration in mouse zygotes (Chaigne et al., 2016). Whether actin has a role in other processes during the oocyte-to-embryo transition, and how these roles change as mitotic mechanisms take over, will be an interesting topic for future research.

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A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.218099.supplemental

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