How the oocyte becomes a zygote:

Cytoskeletal and nuclear dynamics in the oocyte-to-egg and egg-to-embryo transitions

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Going from one cell to trillions
(The challenge of growth)

- Everybody started as just one cell (a fertilized egg), with one set of 23 chromosomes from mom and one set of 23 from dad.
- The human adult body has trillions of cells.
- ***Every one of these cells needs just the right amount of DNA.***
  - Deviations from this "just right amount" can lead to death or defects

Genome integrity for an oocyte

- Fertilization by one and only one sperm (prevention of polyspermy)

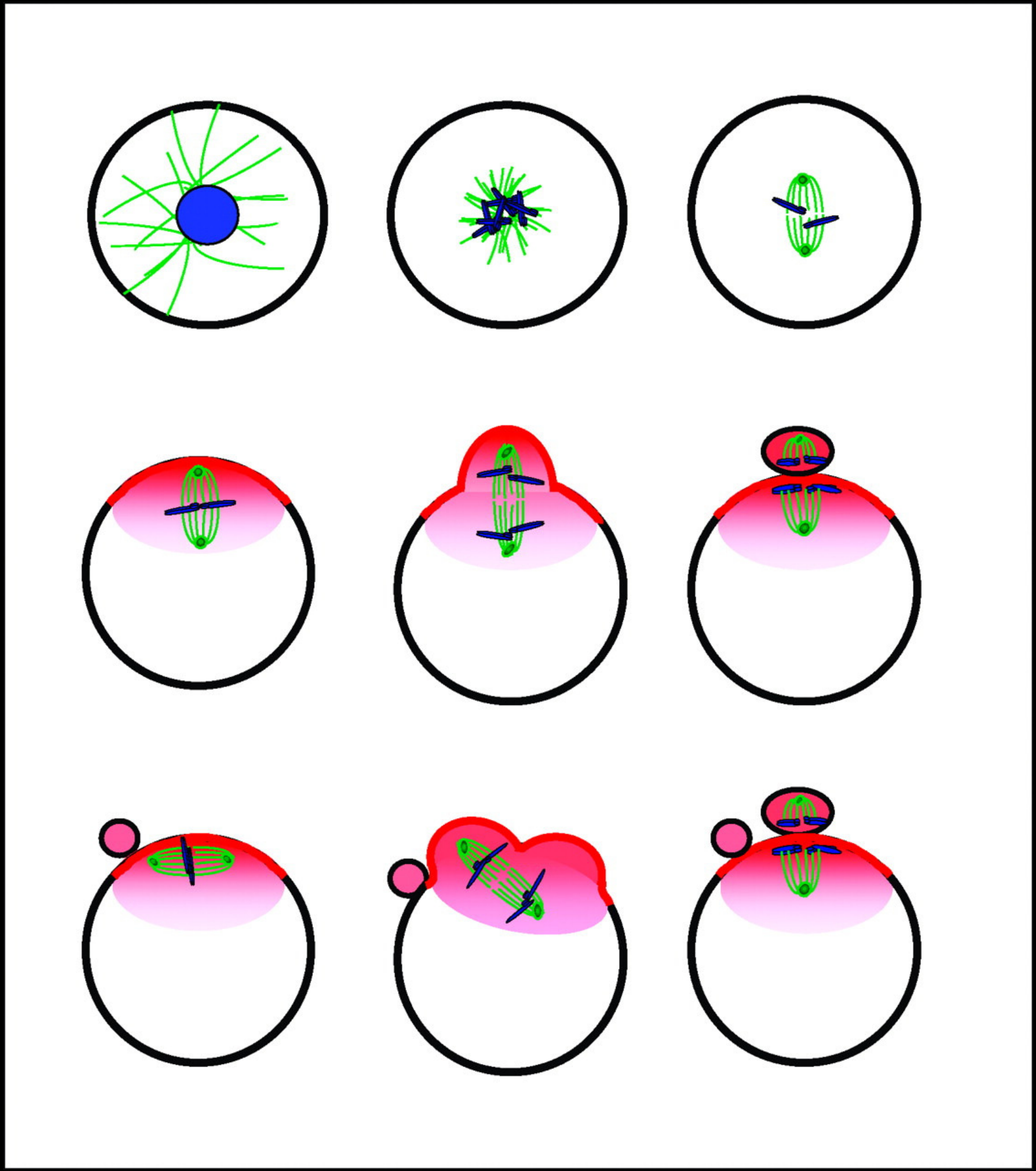
- Appropriate meiotic divisions
  - Oocyte meiotic maturation
    - prophase I to metaphase II
  - Fertilization / egg activation
    - exit from metaphase II arrest and progression into the embryonic cell cycle

Spatial and temporal challenges of female meiosis

- **Timing of meiotic divisions**
  - Prophase I arrest can last for days up to years, depending on the species.
  - Metaphase II arrest can last for hours.
  - Creation of the haploid maternal genome component occurs only *after* fertilization occurs.
- **Spatial control and localization of meiotic divisions**
  - Chromosomes must be segregated evenly between the daughter cells.
  - The other cellular contents must be distributed very asymmetrically so that the egg cytoplasm retains the materials that were stockpiled during oogenesis to support early embryo development.

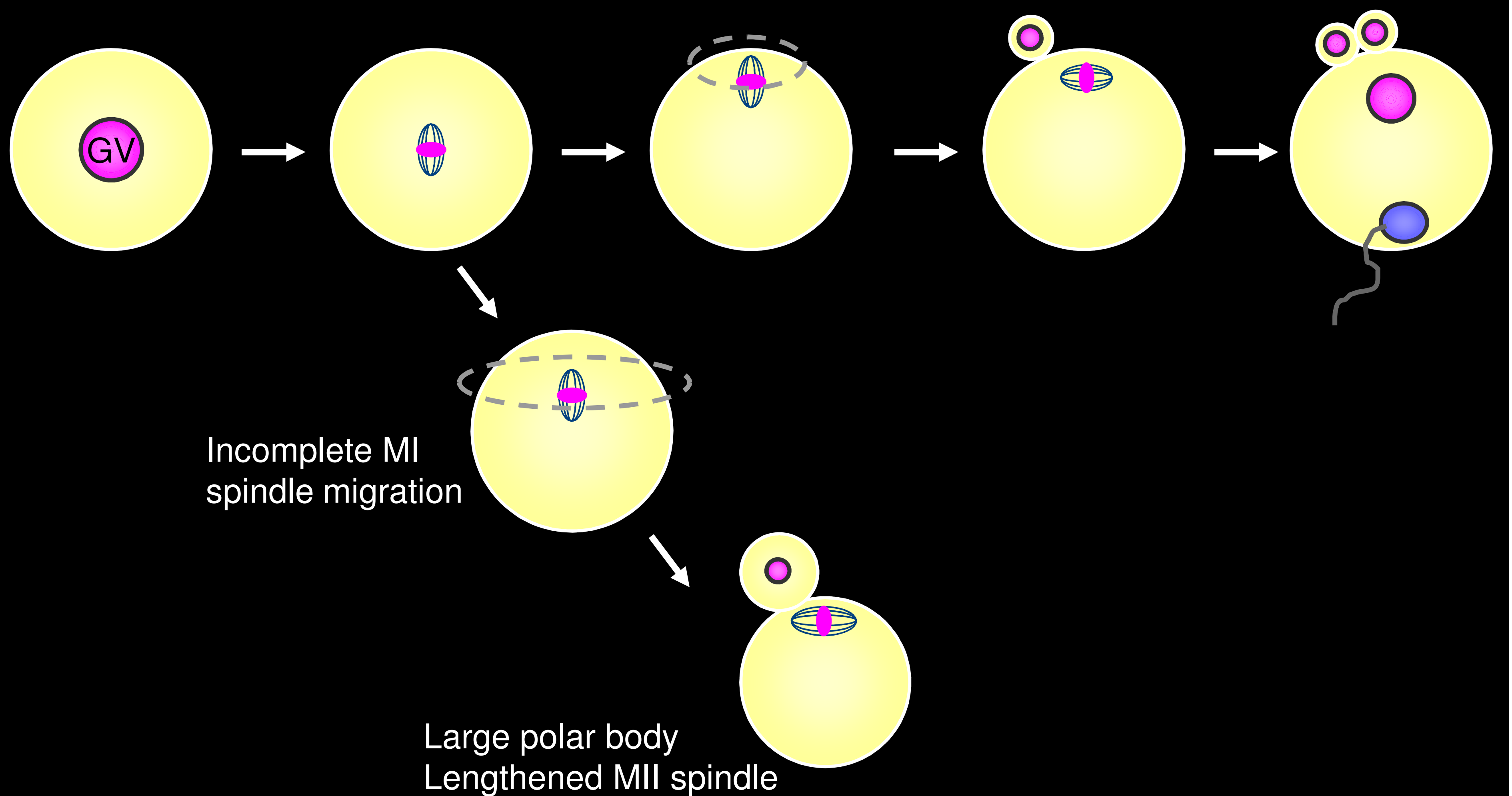
The cellular events of meiotic maturation in mouse oocytes

Green - microtubules
Blue - DNA
Red - cortex (actin)



Brunet and Maro (2005)
Reproduction. 130: 801-811

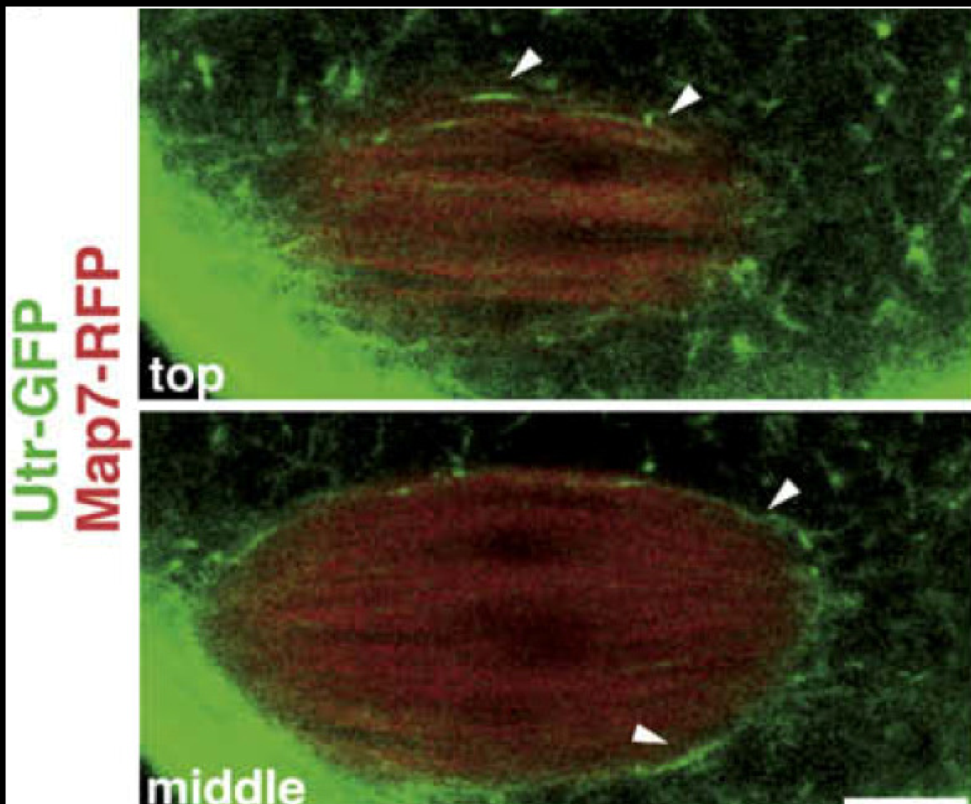
Spatial challenges of female meiosis



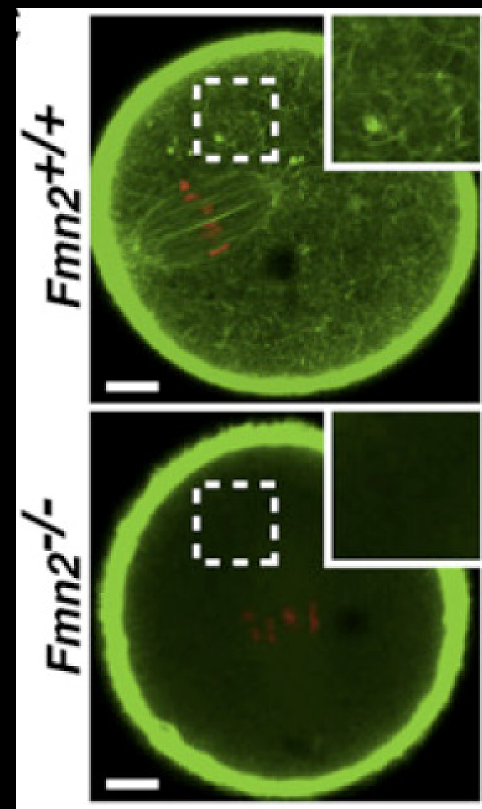
Summary:
Broad picture

- The egg's actin cytoskeleton and actin-associated proteins play important roles in multiple events:
  - Migration of the metaphase I spindle
  - Positioning of the metaphase I spindle → first polar body emission
  - Domain to sequester the metaphase II spindle
  - Positioning of the metaphase II spindle → second polar body emission
  - Pronuclear migration

Cytoplasmic actin driving metaphase I spindle relocation

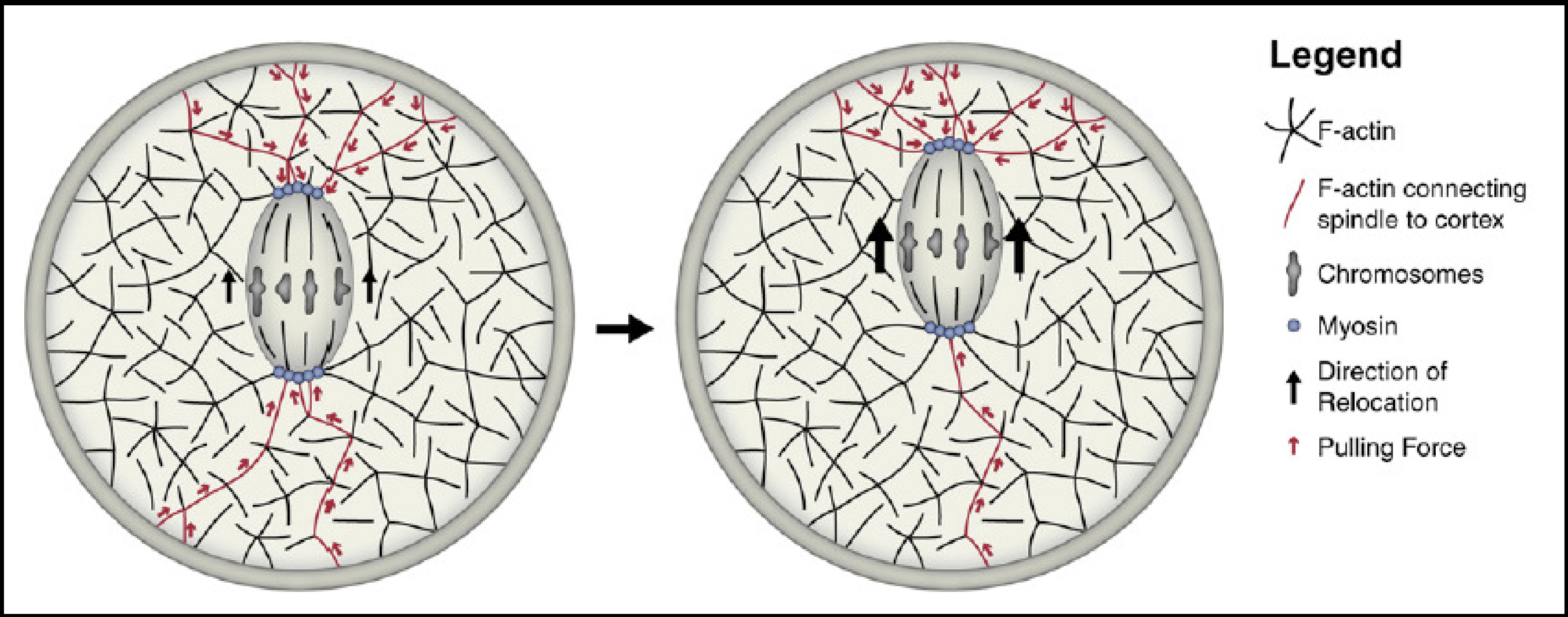


DNA
Actin



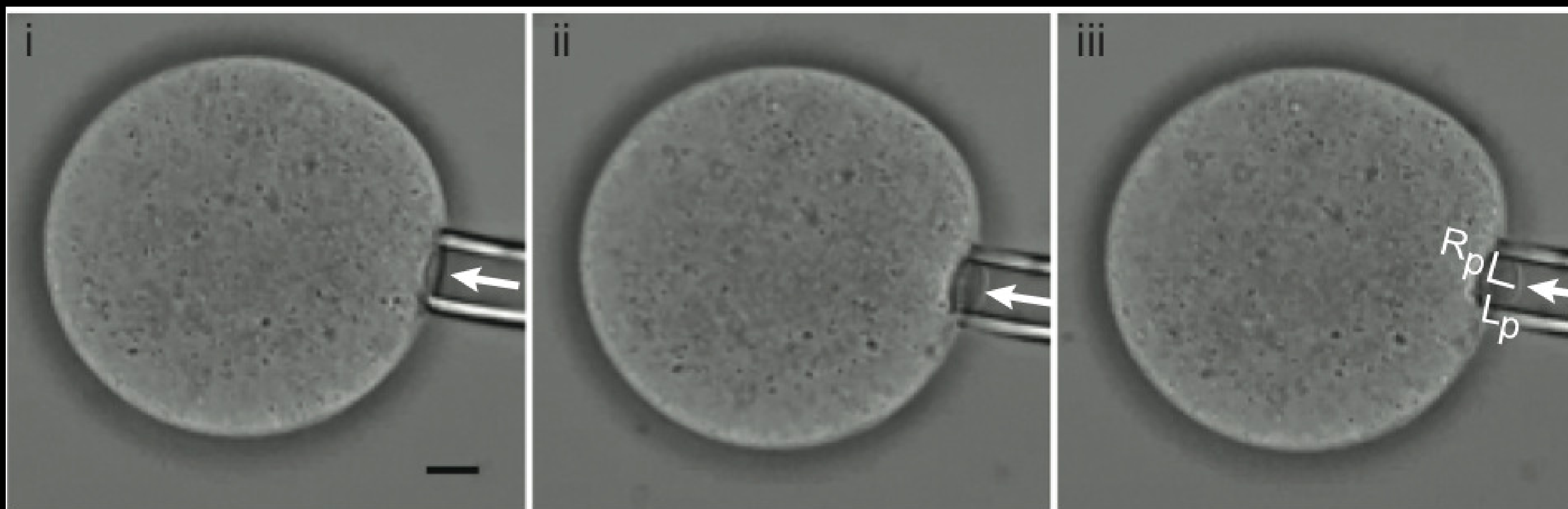
- Schuh and Ellenberg (2008) *Current Biology*, 18:1986-1992
- Azoury et al. (2008) *Current Biology*, 18:1514-1519

Cytoplasmic actin driving metaphase I spindle relocation



- Schuh and Ellenberg (2008) *Current Biology*, 18:1986-1992
- Complementary / similar studies:
  - Azoury et al. (2008) *Current Biology*, 18:1514-1519
  - Li et al. (2008) *Nature Cell Biol*, 10:1301-1308

Cortical tension studies: Micropipet aspiration of mouse eggs



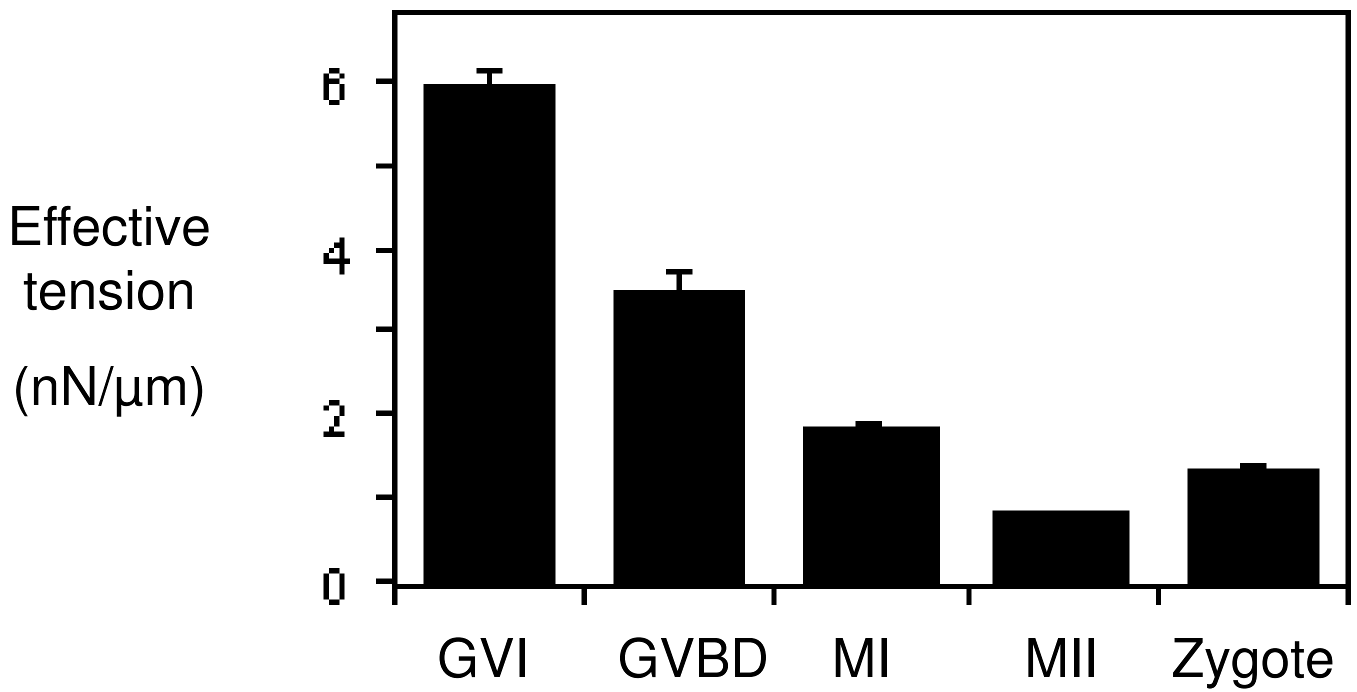
T_{eff} = Aspiration pressure / (2 x (1/pipet radius - 1/cell radius))

- R_p = pipette radius
- R_c = cell radius
- ΔP = aspiration pressure when $L_p = R_p$

T_c = cortical tension [nN/µm]

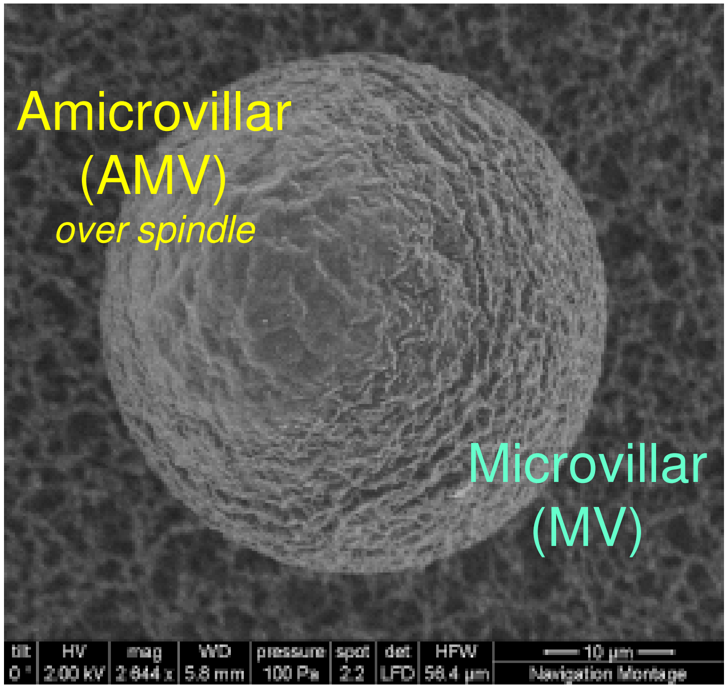
Larson, Lee, Hung, Matthews, Robinson, and Evans. *Mol Biol Cell.* 21: 3182-3192..

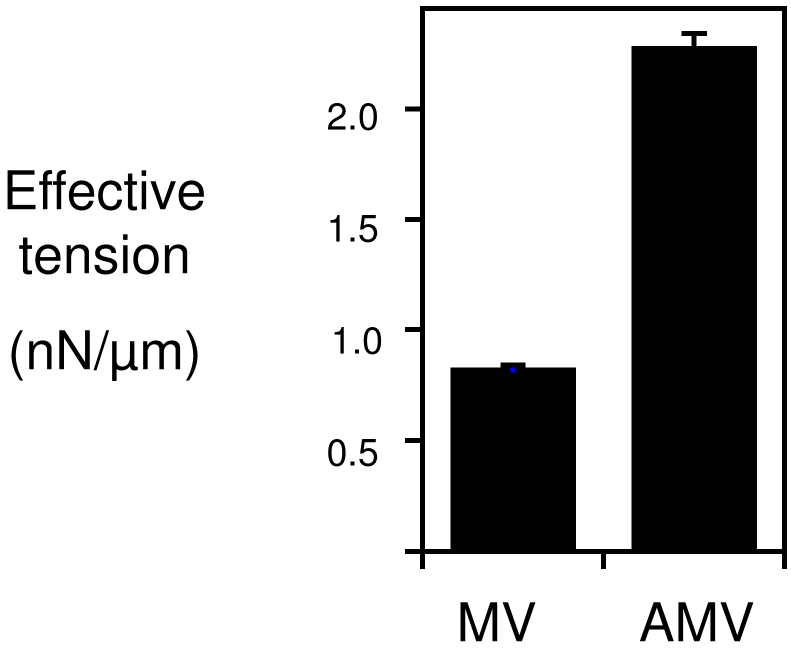
Tension changes through meiotic maturation and fertilization



Larson, Lee, Hung, Matthews, Robinson, and Evans. *Mol Biol Cell*. 21:3182-3192.

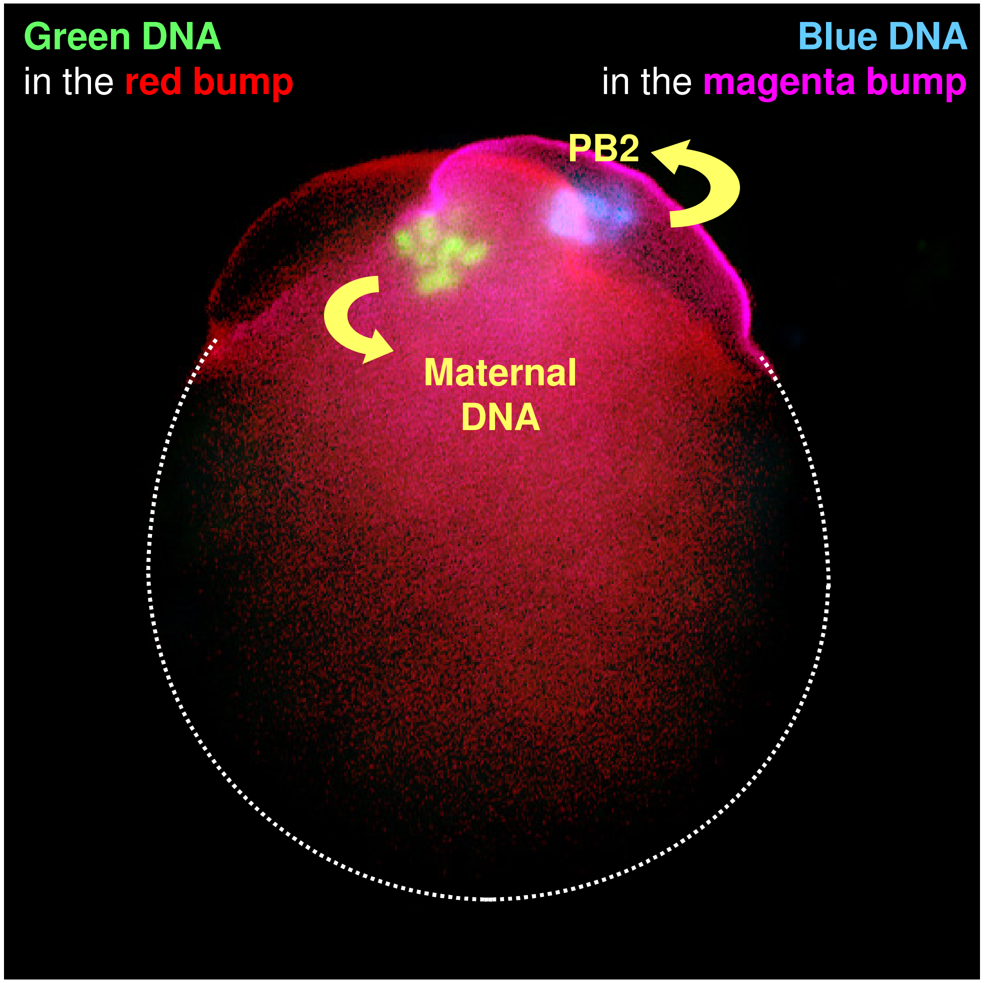
Polarity in cortical tension in metaphase II eggs





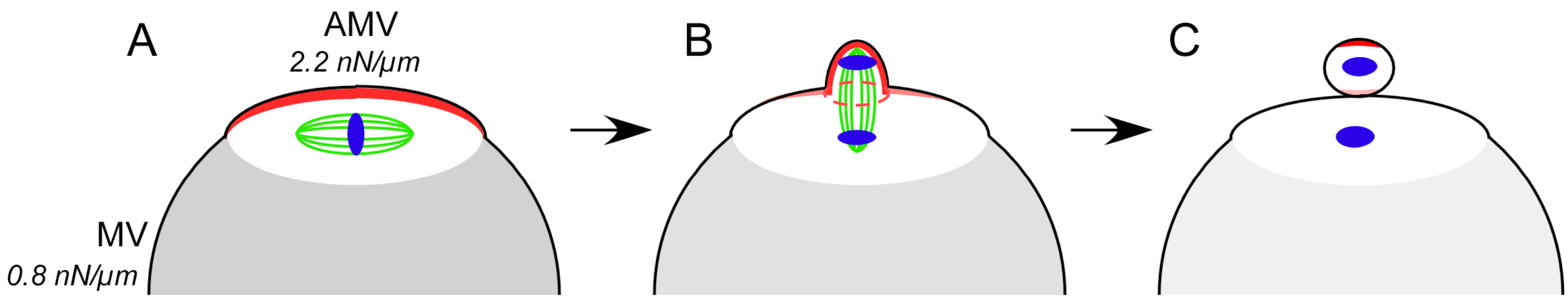
Larson, Lee, Hung, Matthews, Robinson, and Evans. *Mol Biol Cell,* 21: 3182-3192.

Exit from metaphase II arrest: spindle rotation, leading to polar body emission

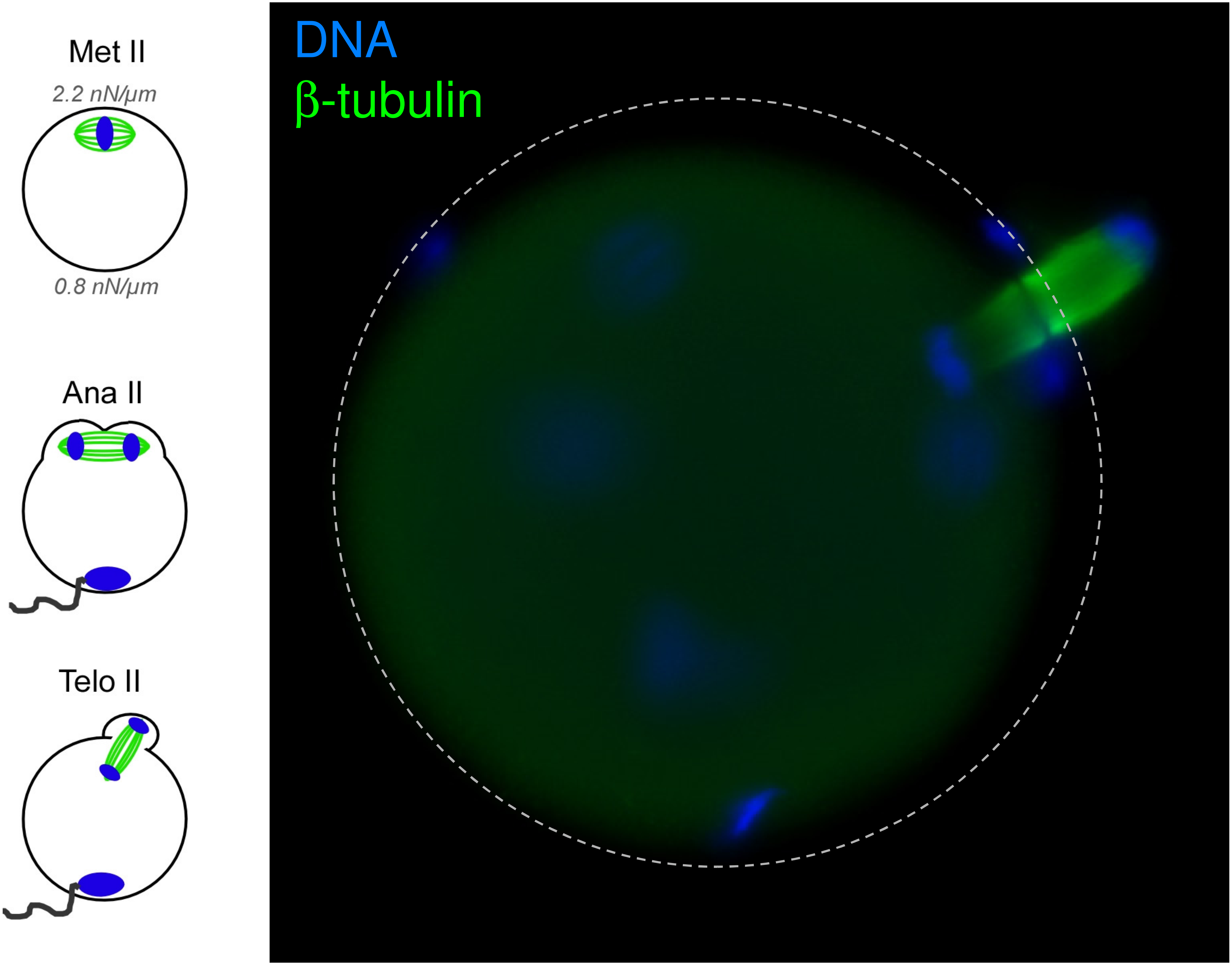


DAPI: green and blue
Phalloidin: red and magenta

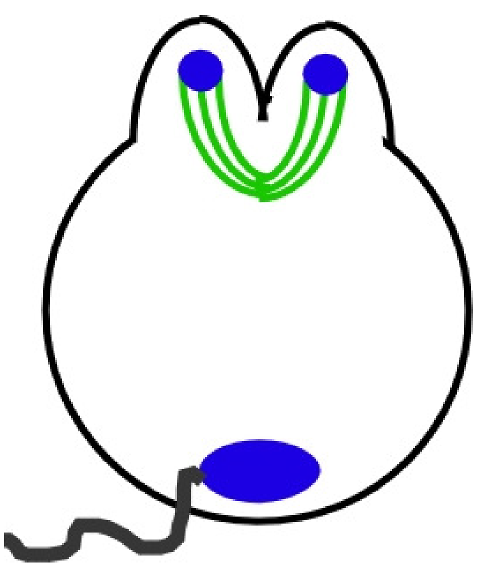
A microdomain for asymmetric cytokinesis in metaphase II eggs

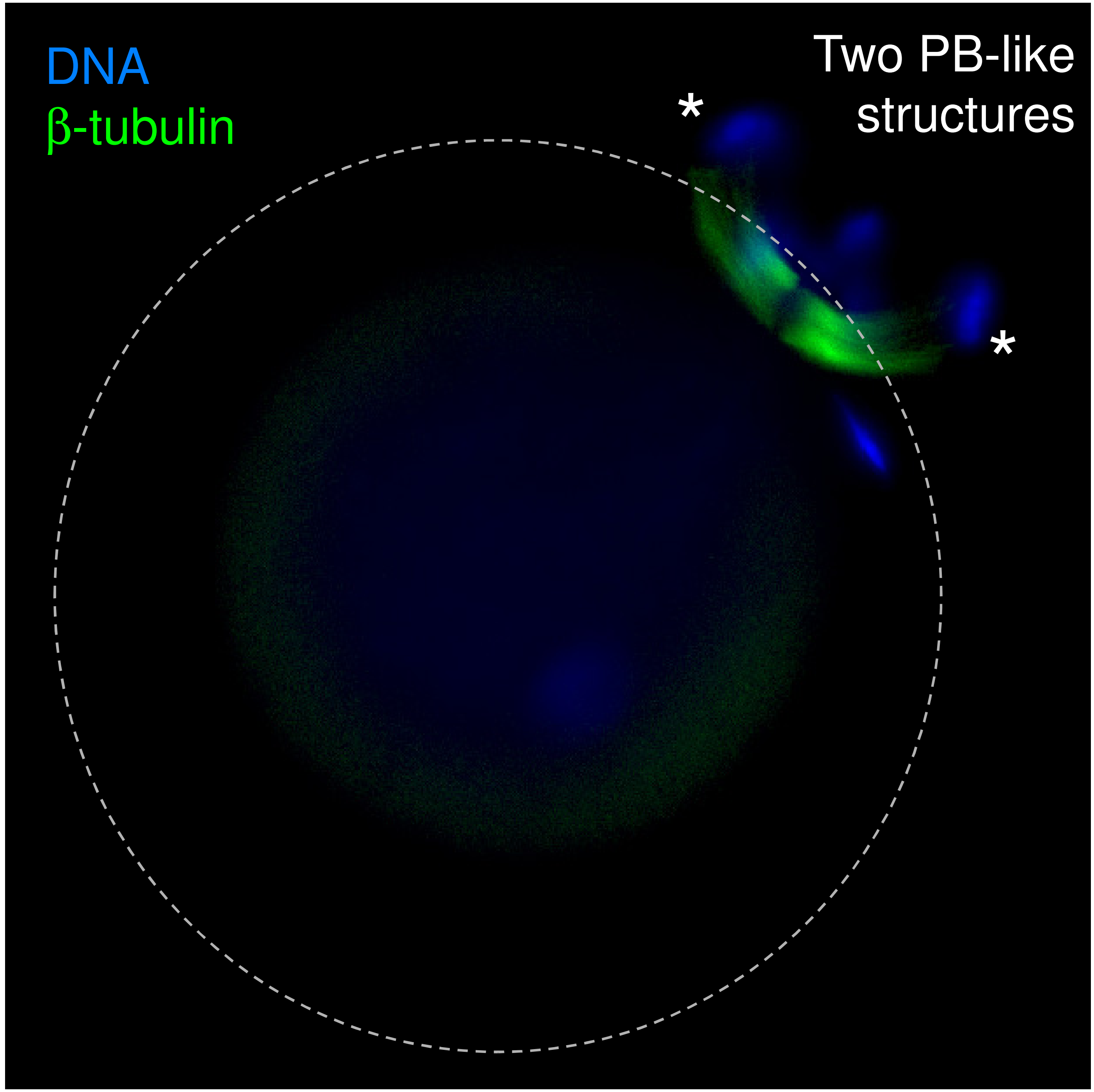


Larson, Lee, Hung, Matthews, Robinson, and Evans. *Mol Biol Cell,* 21: 3182-3192.

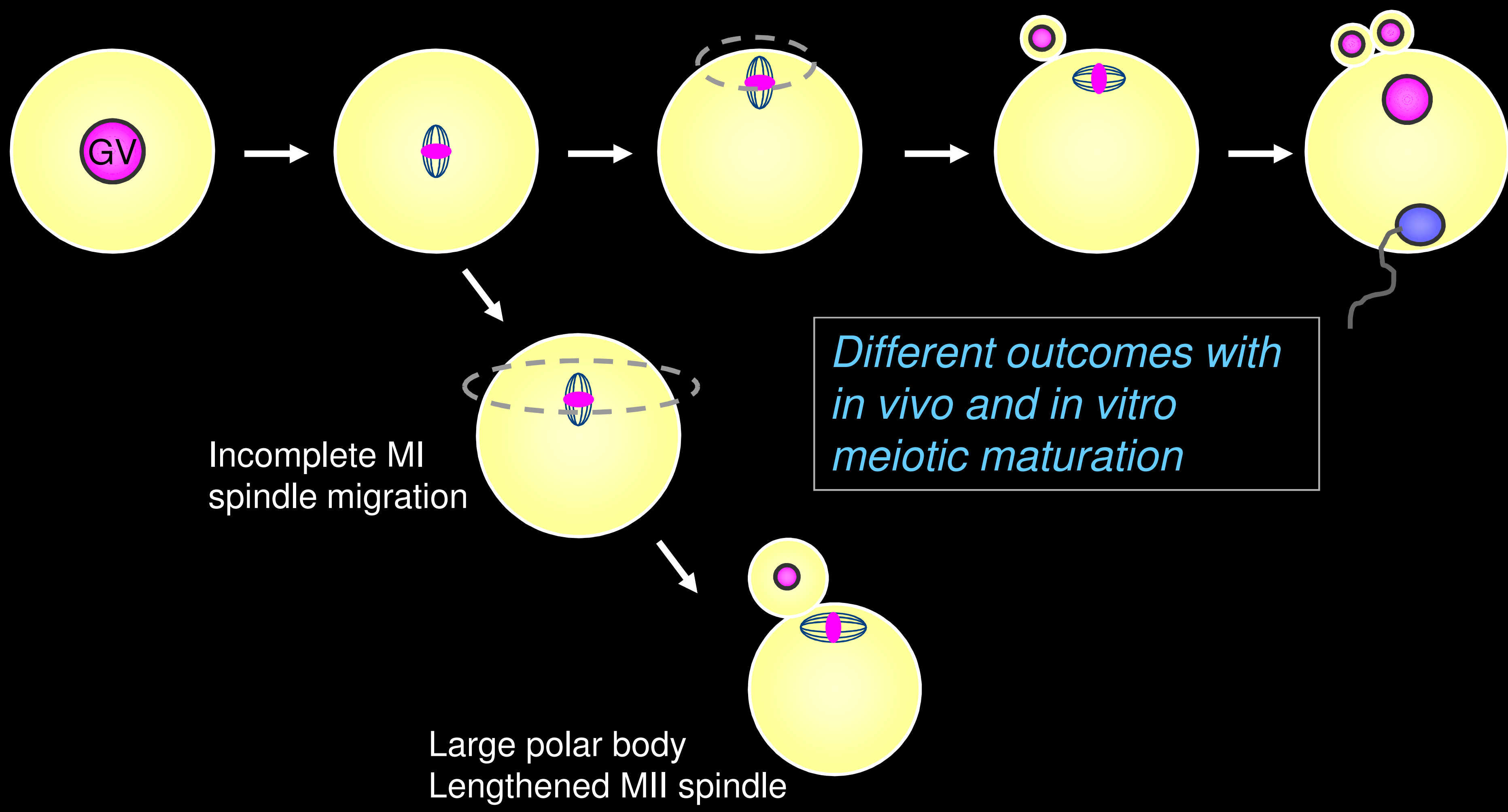

Met II
2.2 nN/μm
0.8 nN/μm
Ana II
Telo II
DNA
β-tubulin

With impaired tension - *failure of 2nd PB emission*





Spatial challenges of female meiosis



Meiotic maturation *in vivo* and *in vitro*: Oocyte and first polar body sizes

	In vitro matured	Ovulated
Oocyte	$1.2 \times 10^5 \pm 8700\ \mu m^3$	$1.4 \times 10^5 \pm 3600\ \mu m^3$
Polar body	$4900 \pm 600\ \mu m^3$	$3600 \pm 600\ \mu m^3$

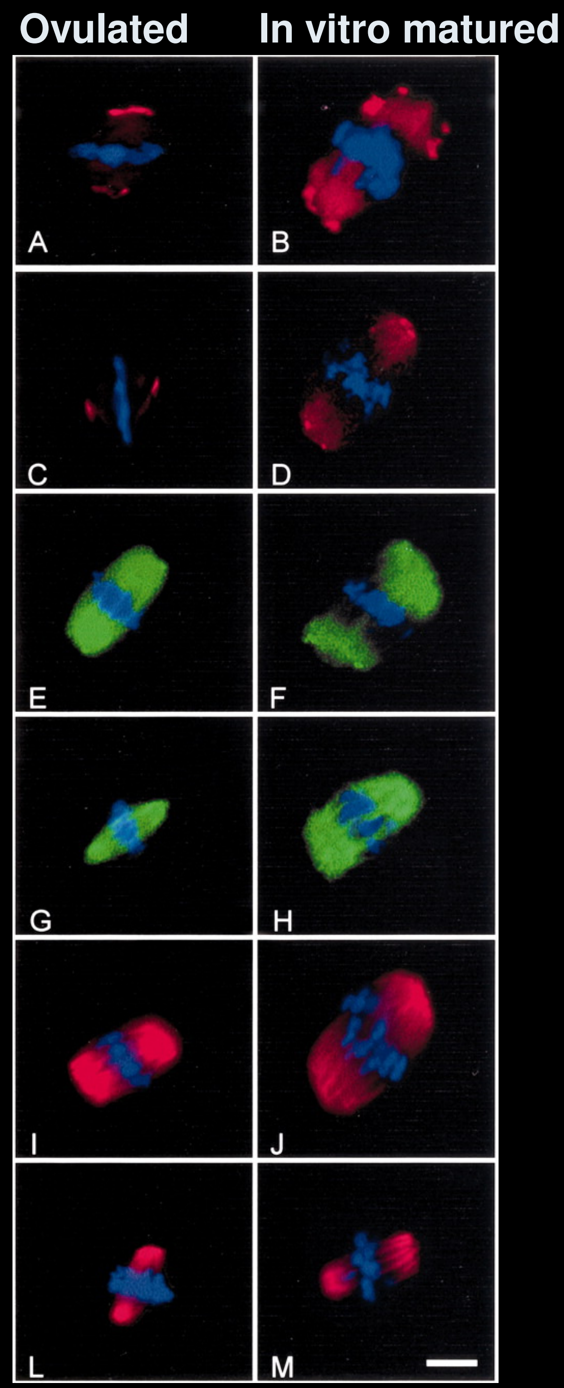
Larger PB (and smaller oocyte) with in vitro meiotic maturation

Barrett and Albertini (2007) Biol Reprod 76:949-957

Meiotic maturation *in vivo* and *in vitro*: Spindle morphology

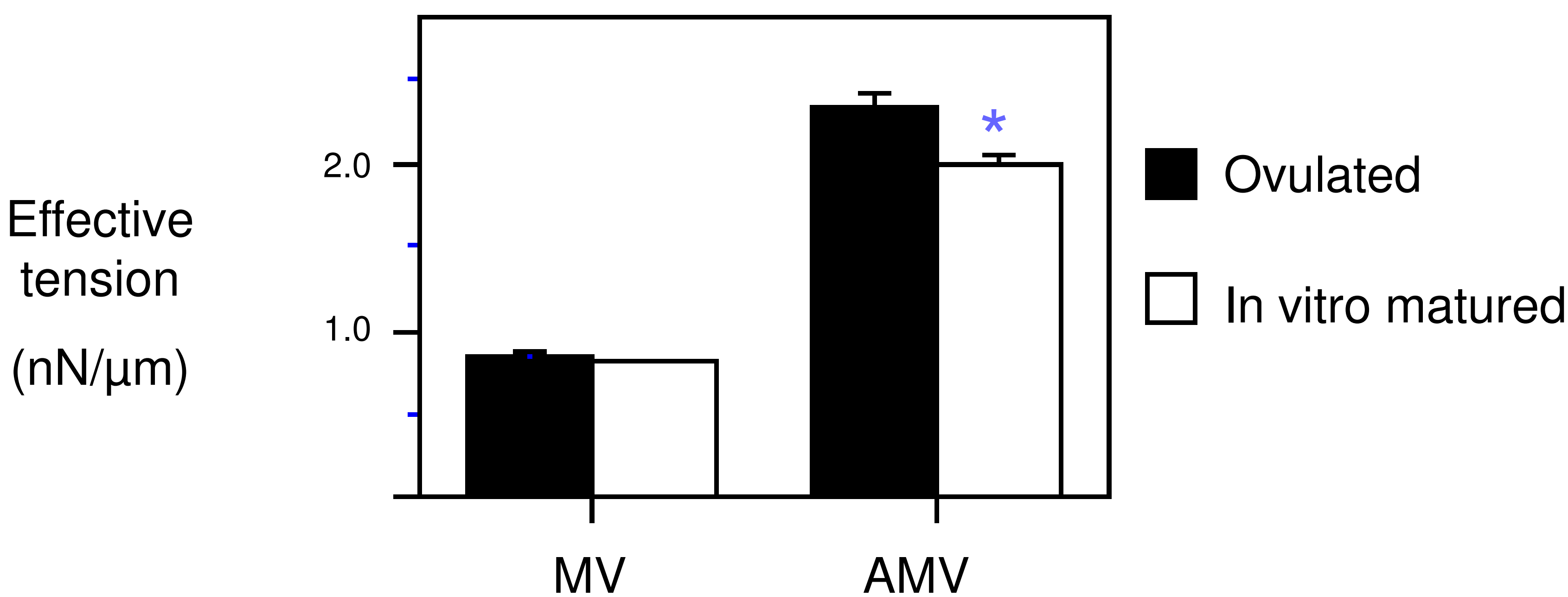
Representative spindle images of IVO (left) and IVM (right) oocytes at M-I (A, B, E, F, I, and J) or M-II (C, D, G, H, L, and M) labeled with γ-tubulin (A–D), MPM-2 (E–H), and acetylated-tubulin.

Different spindle morphology with in vitro meiotic maturation



Sanfins A et al. (2003) *Biol. Reprod.* 69:2059-2067

Tension in metaphase II eggs matured *in vivo* vs. *in vitro*

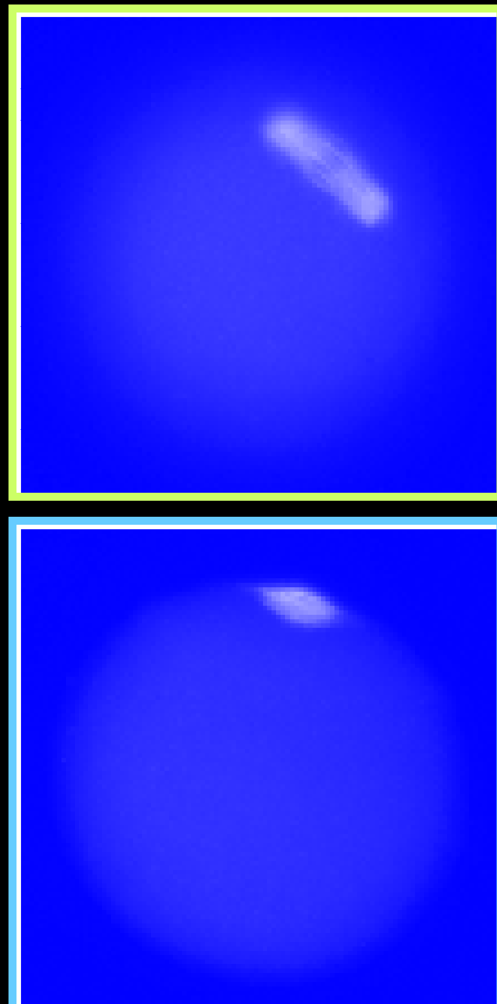


Larson, Lee, Hung, Matthews, Robinson, and Evans. *Mol Biol Cell,* 21: 3182-3192.

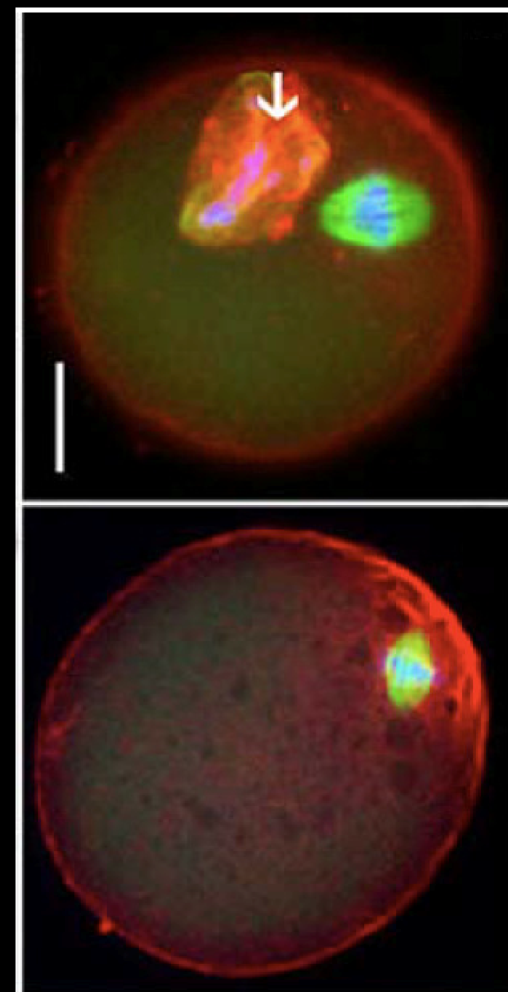
Spindle morphology in *in vivo* vs. *in vitro* matured oocytes

In vitro matured

Ovulated
(in vivo matured)

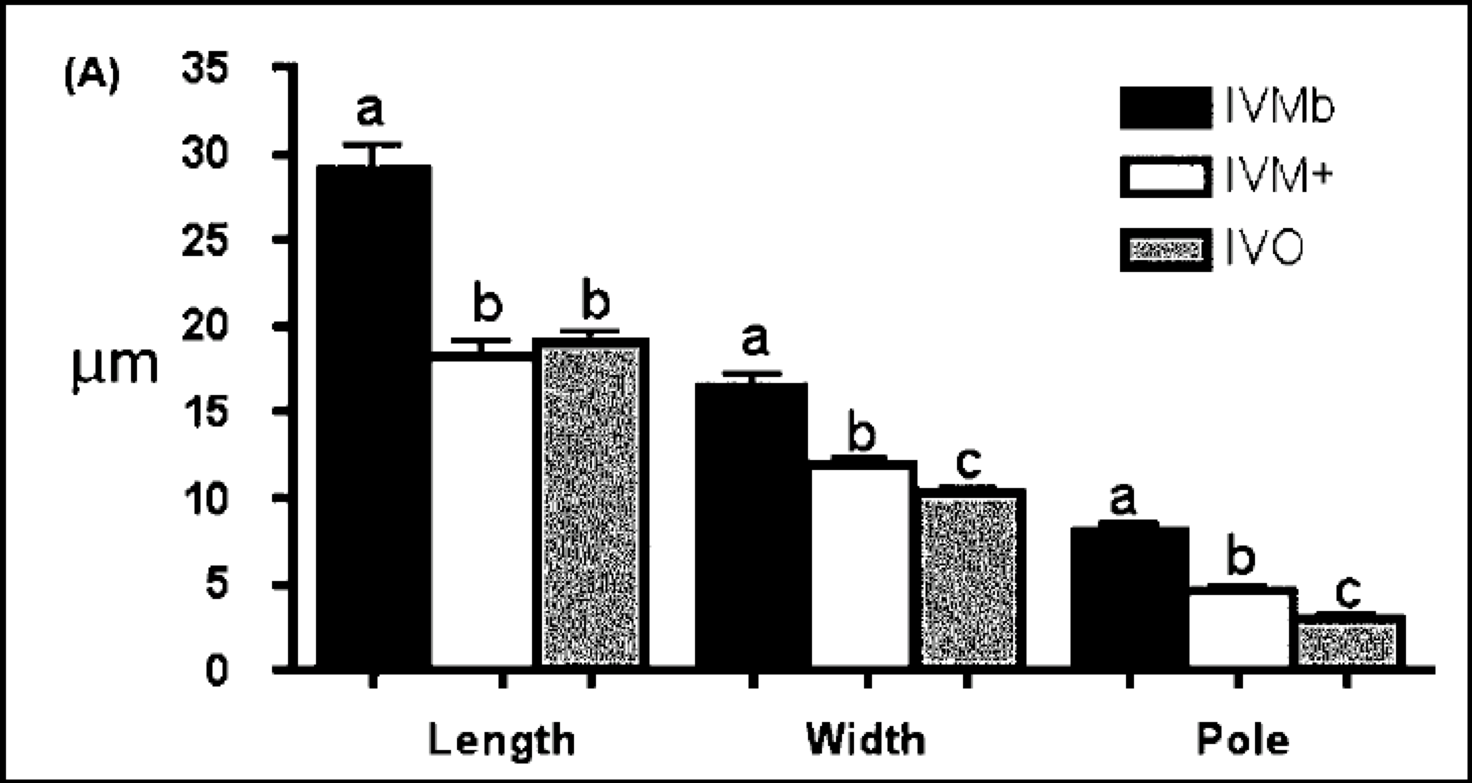


Larson, Lee, et al.
Mol Biol Cell. 21:3182-3192.



Barrett and Albertini (2007)
Biol Reprod. 76:949-957

Spindle dimensions in *in vivo* vs. *in vitro* matured oocytes



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Summary:
Details of studies addressed here

- A dynamic actin network mediates migration of the metaphase I spindle to the periphery of the oocyte.
  - This actin network is dependent on the actin nucleating protein formin-2.

- Cortical tension changes dramatically during mammalian female meiosis, dropping ~6-fold during meiotic maturation from prophase I to metaphase II, then increases ~1.6-fold upon fertilization.

Summary:
Details of studies addressed here

- The metaphase II egg is polarized, with cortical tension differing ~2.5-fold between the cortex over the meiotic spindle and the opposite cortex.
  - This suggests that meiotic maturation is accompanied by assembly of a cortical domain with stiffer mechanics as part of the process to achieve asymmetric cytokinesis.
- Oocytes resulting from meiotic maturation *in vivo* vs. *in vitro* have differences in polar body size and spindle morphology.
- Recent work shows that tension levels differ in the cortical domain over the metaphase II spindle in oocytes matured in vivo and in vitro, suggesting that cellular mechanics could be a contributing factor to asymmetric cell divisions in oocytes.

For further reading

- Brunet S, Maro B. (2005) Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reproduction. 130: 801-811.
- Leader B, Lim H., Carabatsos MJ, Harrington A, Ecsedy J, Pellman D, Maas R, Leder P. (2002) Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. *Nat Cell Biol*. 4: 921-8.
- Dumont J, Million K, Sunderland K, Rassinier P, Lim H, Leader B, and Verlhac MH. 2007. Formin-2 is required for spindle migration and for the late steps of cytokinesis in mouse oocytes. *Dev Biol*. 301: 254-65.
- Larson SM, Lee HJ, Hung P, Matthews LM, Robinson DN, Evans JP. (2010) Cortical mechanics and meiosis II completion in mammalian oocytes are mediated by myosin-II and Ezrin-Radixin-Moesin (ERM) proteins. *Mol Biol Cell*. 21:3182-3192.

For further reading

- Azoury J, Lee KW, Georget V, Rassinier P, Leader B, Verlhac MH. (2008) Spindle positioning in mouse oocytes relies on a dynamic meshwork of actin filaments. *Curr Biol*. 18:1514-1519.
- Schuh, M, Ellenberg J. (2008) A new model for asymmetric spindle positioning in mouse oocytes. *Curr Biol*. 18:1986-1992.
- Li, H, Guo F, Rubinstein B, Li R. (2008) Actin-driven chromosomal motility leads to symmetry breaking in mammalian meiotic oocytes. *Nat Cell Biol*. 10:1301-1308.

For further reading

- Sanfins A, Lee GY, Plancha CE, Overstrom EW, Albertini DF (2003) Distinctions in meiotic spindle structure and assembly during in vitro and in vivo maturation of mouse oocytes. *Biol Reprod.* 69: 2059-2067.
- Sanfins A, Plancha CE, Overstrom EW, Albertini DF. (2004) Meiotic spindle morphogenesis in in vivo and in vitro matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. *Hum Reprod*. 19: 2889-99.
- Barrett SL, Albertini DF (2007) Allocation of gamma-tubulin between oocyte cortex and meiotic spindle influences asymmetric cytokinesis in the mouse oocyte. *Biol Reprod* 76: 949-957.

Periodic surface contractions of the egg cortex

Colchicine-treated *Xenopus* egg (no vitelline envelope) parthenogenetically activated

Time in minutes

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Hara, Tydeman, and Kirschner. (1980) A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. PNAS USA. 77:462-466.