The role of zinc during mammalian meiosis and egg activation: the inorganic signature of life

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There are no disclosures

NORTHWESTERN UNIVERSITY

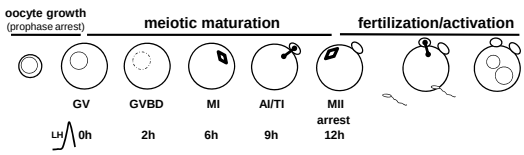
Overview

I. The events and purpose of meiosis
II. Known cell cycle regulators of meiosis
III. Zinc – the inorganic side of meiosis
   A. Levels and localization
   B. Roles in regulating
      - Prophase I arrest
      - MI-MII transition
      - Completion of meiosis and egg activation
IV. Summary

Kim *et al.* 2010

Kim *et al.* 2011

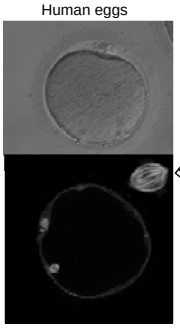
Oocyte meiosis



- Protracted (weeks to decades)
- Punctuated by two arrest points (prophase I and metaphase II)
- Meiotic resumption occurs in response to LH surge *in vivo* or spontaneously upon removal of the oocyte from the follicle
- Involves two asymmetric cell divisions to sequentially segregate homologous chromosomes and sister chromatids while minimizing cytoplasmic loss
- Occurs in the absence of any appreciable transcription

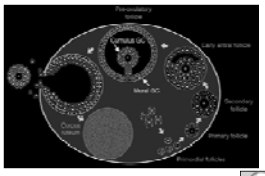
Importance of meiosis

Human eggs



Live offspring

Endocrine function

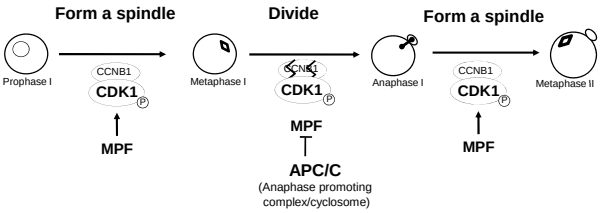


Duncan, unpublished

Cell cycle regulation of oocyte meiosis

Meiotic Progression

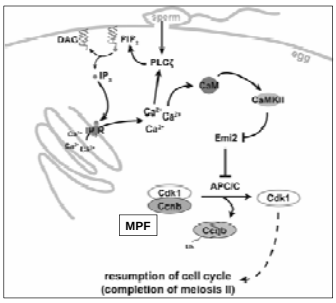
Maturation Promoting Factor (MPF)



Cell cycle regulation of oocyte meiosis

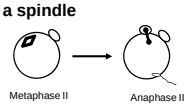
Completion of meiosis

Maturation Promoting Factor (MPF)



Maintain a spindle

Divide

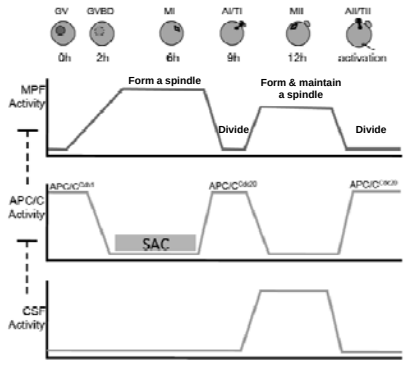


Metaphase II

Cytostatic Factor (CSF)

Anaphase II

Cell cycle regulation of oocyte meiosis



• MPF activity is dynamic because meiosis involves two cell divisions without an intervening round of DNA replication

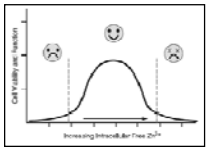
•This requires coordination of three cellular activities

Transition metal physiology

Transition metal "fingerprints" are similar across cell types and are thought to be maintained in a conserved range to prevent toxicity. Deviations from this range provide insight into a cell's specialized physiological function.

THE METAL QUOTA

	mM		
	26 Fe	29 Cu	30 Zn
E. coli[a]	0.3	0.04	0.2
S. cerevisiae[b,c]	0.2	0.01	0.4
mouse fibroblasts[c,d]	0.5	0.04	0.6
red blood cells[c]	**12.5**	0.01	0.2



Colvin, R. A. *et al. Eur. J. Pharmacol.* **479**, 171-185 (2003)

[a]Outten *et al. Science* **292**, 2488-2492 (2001)
[b]MacDiarmid *et al. EMBO J.* **19**, 2845-2855 (2000)
[c]Unpublished data courtesy of R. Marvin (O'Halloran Lab)
[d]Suhy *et al. J. Biol. Chem.* **274**, 9183-9192 (1999)

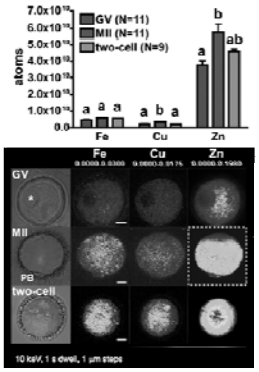
The zinc profile of the oocyte

- Mammalian female gametes are rare, precious, and difficult to obtain
- Bulk methods for elemental analysis are therefore not feasible

Synchrotron-based x-ray fluorescence microscopy was used to analyze the elemental composition of individual oocytes, eggs, and embryos

AM Kim *et al*, 2010, *Nat. Chem. Biol.*

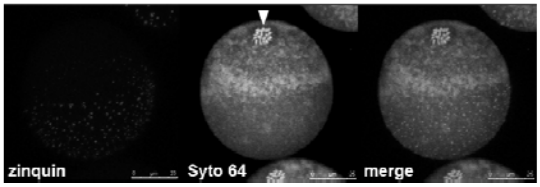
Total zinc levels and localization during meiosis



- Zinc is the most abundant transition metal in the oocyte, egg, and embryo
- A dramatic increase in zinc content occurs during meiosis accompanied by a decrease following fertilization (~$2x10^{10}$ atoms)
- Total zinc has a polarized distribution in the egg

AM Kim *et al*, 2010, *Nat. Chem. Biol.*

Labile zinc localization during meiosis

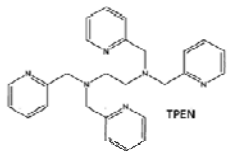


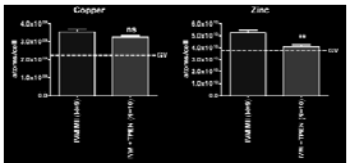
Labile zinc localizes has a polarized distribution in the vegetal pole of the egg

AM Kim *et al*, 2011, *ACS Chemical Biology*

A potent tool to study zinc function during meiosis

N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine

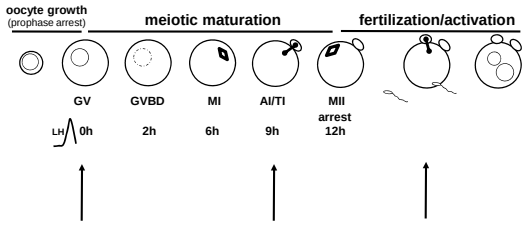




- Small-molecule chelator that sequesters kinetically accessible intracellular pools of metals
- High affinity for transition metals (zinc, copper, iron)
- Low affinity for other metals (magnesium and calcium)
- In the oocyte and egg, TPEN specifically perturbs levels of intracellular zinc and not copper

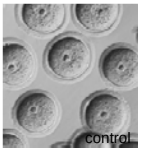
AM Kim *et al*, 2010, *Nat. Chem. Biol.*

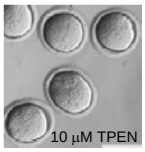
The role of zinc during oocyte meiosis



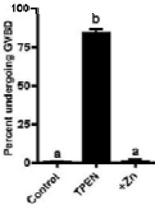
1) Prophase I arrest? 2) Meiotic progression? 3) Completion of meiosis?

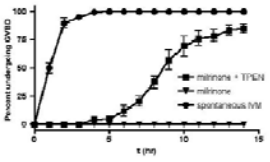
1) The role of zinc during prophase I arrest





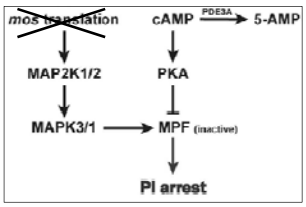
- Treatment of prophase I-arrested oocytes with TPEN results in an override of the arrest
- >80% of oocytes resume meiosis in the presence of TPEN; kinetics are slower



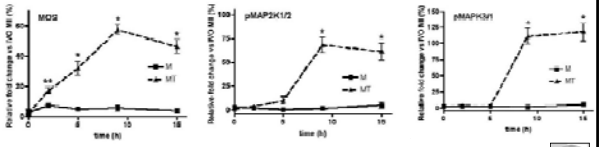


BY Kong *et al*, under review, *Biol Reprod*

1) The role of zinc during prophase I arrest



- Zinc insufficiency causes a premature activation of the MOS/MAPK pathway
- This pathway activates MPF and explains the observed release from Prophase I arrest



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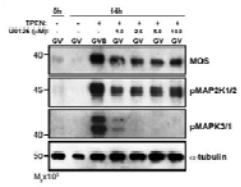

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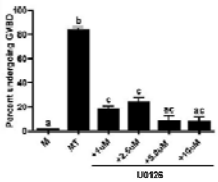
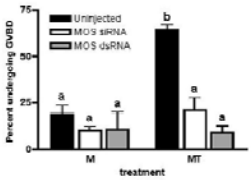


Mos knockdown

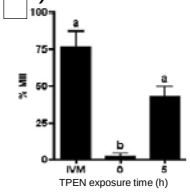


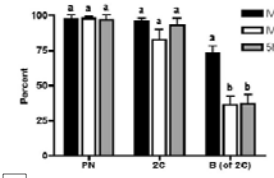
- Disruption of the MOS-MAPK pathway by several approaches prevents TPEN-induced resumption of meiosis

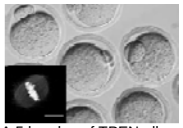
•Zinc likely functions through the MOS-MAPK pathway to regulate the Prophase I arrest

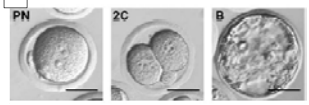
BY Kong *et al*, under review, *Biol Reprod*

1) The role of zinc during prophase I arrest





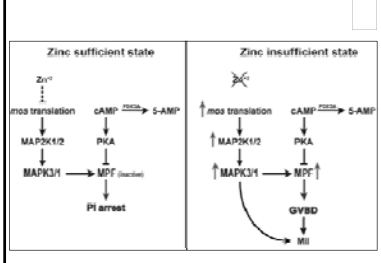


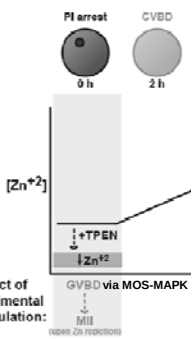


- A 5 h pulse of TPEN allows meiotic resumption followed by normal progression to MII.
- Eggs formed by this method have developmental competence equivalent to that observed during *in vitro* maturation.

BY Kong *et al*, under review, *Biol Reprod*

1) The role of zinc during prophase I arrest

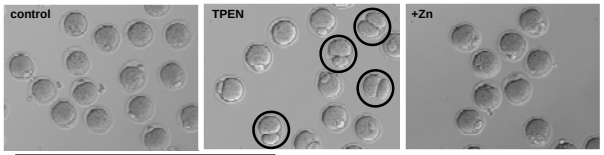


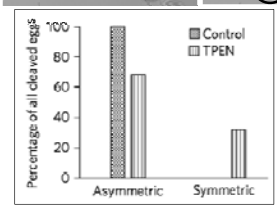


Effect of experimental manipulation: GVBD via MOS-MAPK ↓ MII (upon Zn replction)

BY Kong *et al*, under review, *Biol Reprod*

2) The role of zinc during meiotic progression



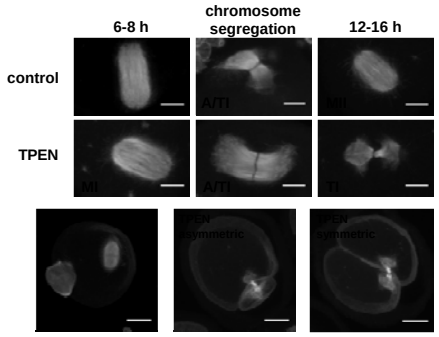


- Maturation *in vitro* in the continuous presence of TPEN results in failure of an asymmetric cell division

•Polar bodies in TPEN-treated eggs are significantly larger compared to controls

AM Kim *et al*, 2010, *Nat. Chem. Biol.*

2) The role of zinc during meiotic progression



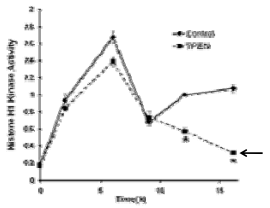
α-tubulin
DNA
F-actin

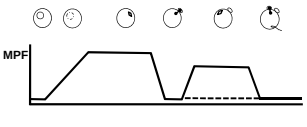
•Oocytes matured in the continuous presence of TPEN fail to reach metaphase of meiosis II

•Cells arrest at a telophase-I like state

AM Kim *et al*, 2010, *Nat. Chem. Biol.*

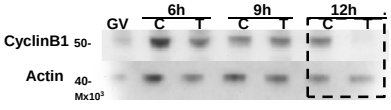
2) The role of zinc during meiotic progression





•MPF activity remains low in TPEN-treated oocytes due to a failure to reaccumulate CyclinB1

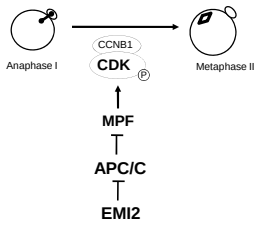
•This explains why cells can not reach metaphase of meiosis II



Bernhardt ML, *et al*, 2011, *Biol Reprod.*

2) The role of zinc during meiotic progression

EMI2 is a likely target of zinc action during the MI – MII transition



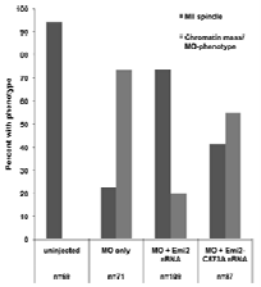
EMI2:

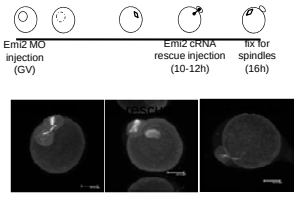
- APC/C inhibitor required for the MI-MII transition
- Is active during the MII arrest
- Contains a zinc-binding region (ZBR)
- Knockdown experiments show similar phenotype to TPEN-treatment

Hypothesis: Zinc insufficiency mediated by TPEN leads to compromised EMI2 function (can not inhibit the APC) resulting in failure to re-establish proper MPF activity

Bernhardt ML, *et al,* 2012, *Biol Reprod.*

2) The role of zinc during meiotic progression





- An intact ZBR is required for EMI2 to mediate progression to MII
- Zinc likely regulates the MI-MII transition by modulating EMI2 activity

Bernhardt ML, *et al,* 2012, *Biol Reprod.*

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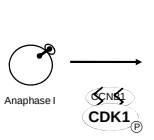
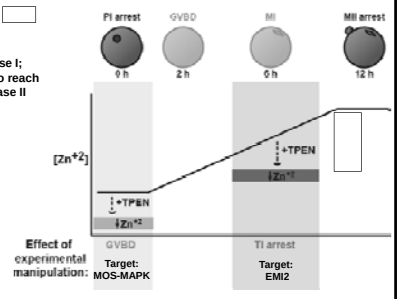


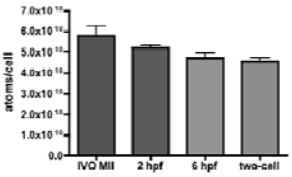
Telophase I; failure to reach metaphase II

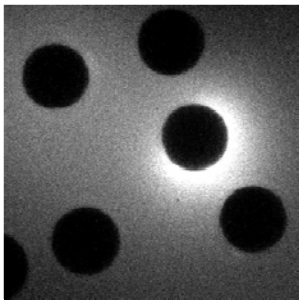


Bernhardt ML, *et al,* 2012, *Biol Reprod &* adapted from BY Kong *et al,* under review, *Biol Reprod*

3) The role of zinc in completion of meiosis

Total zinc content post-fertilization



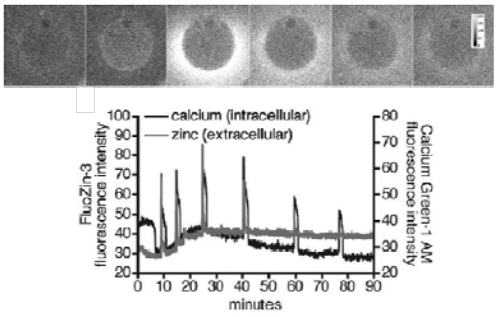


FluoZin-3
Extracellular zinc fluorophore

- Total zinc levels decrease post-fertilization
- Zinc is released in a series of repetitive secretory events termed “zinc sparks”
- Zinc sparks occur during fertilization and parthenogenetic activation
- Zinc sparks are conserved in mouse and primates

AM Kim *et al*, 2011, *ACS Chemical Biology*

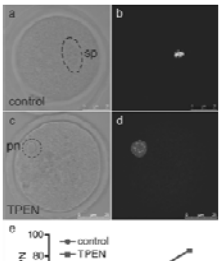
3) The role of zinc in completion of meiosis

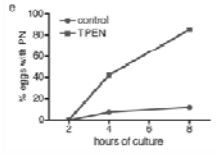


- Physiologic zinc sparks are preceded by and may depend on calcium oscillations that begin early during egg activation

AM Kim *et al*, 2011, *ACS Chemical Biology*

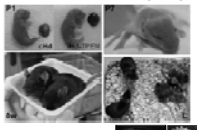
3) The role of zinc in completion of meiosis





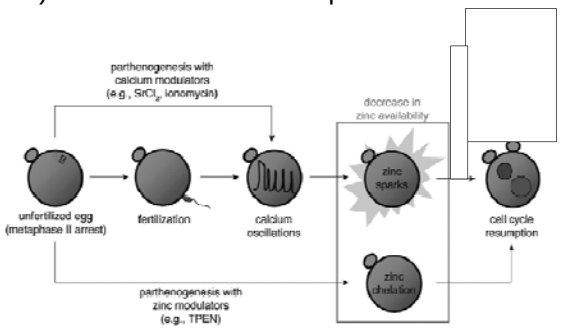
- Treatment of MII-arrested eggs with TPEN causes egg activation and pronuclear formation by mimicking physiological zinc loss via zinc sparks
- This activation is sufficient to promote full-term development in the mouse

Full-term mouse development by abolishing Zn^{2+}-dependent metaphase II arrest without Ca^{2+} release



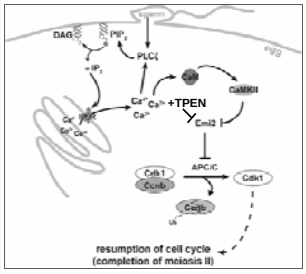
AM Kim *et al*, 2011, *ACS Chemical Biology*

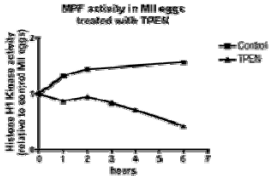
3) The role of zinc in completion of meiosis



AM Kim *et al*, 2011, *ACS Chemical Biology*

3) The role of zinc in completion of meiosis

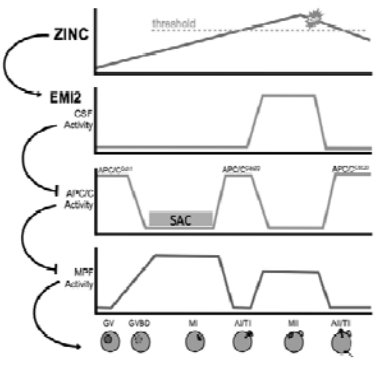




- MPF activity is decreased following zinc sequestration following TPEN treatment
- This mirrors what occurs during physiological egg activation at fertilization
- Zinc could be modulating EMI2 activity by altering the metal occupancy of its ZBR

Bernhardt ML, *et al*, 2012, *Biol Reprod.*

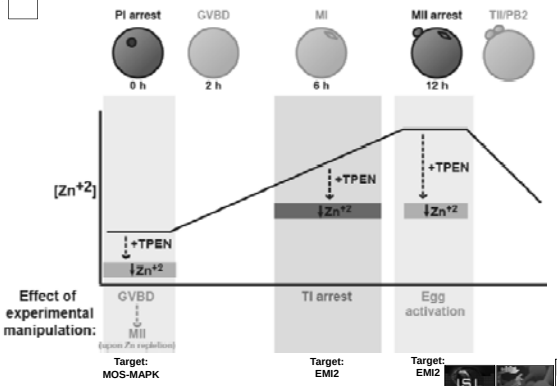
3) The role of zinc in completion of meiosis



Zinc as a component of CSF:
1) Increases over the course of meiotic maturation
2) Present and necessary for the MII arrest
3) Lost rapidly at the time of fertilization

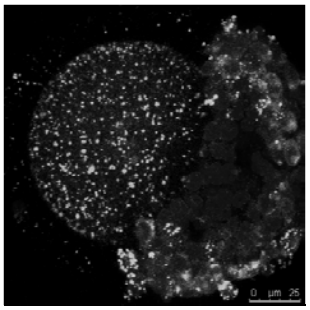
Bernhardt ML, *et al*, 2012, *Biol Reprod.*

Summary



Adapted from BY Kong *et al*, under review, *Biol Reprod*

Future directions



Fluozin3 staining of a human GV-intact oocyte

Duncan and Kong, unpublished

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