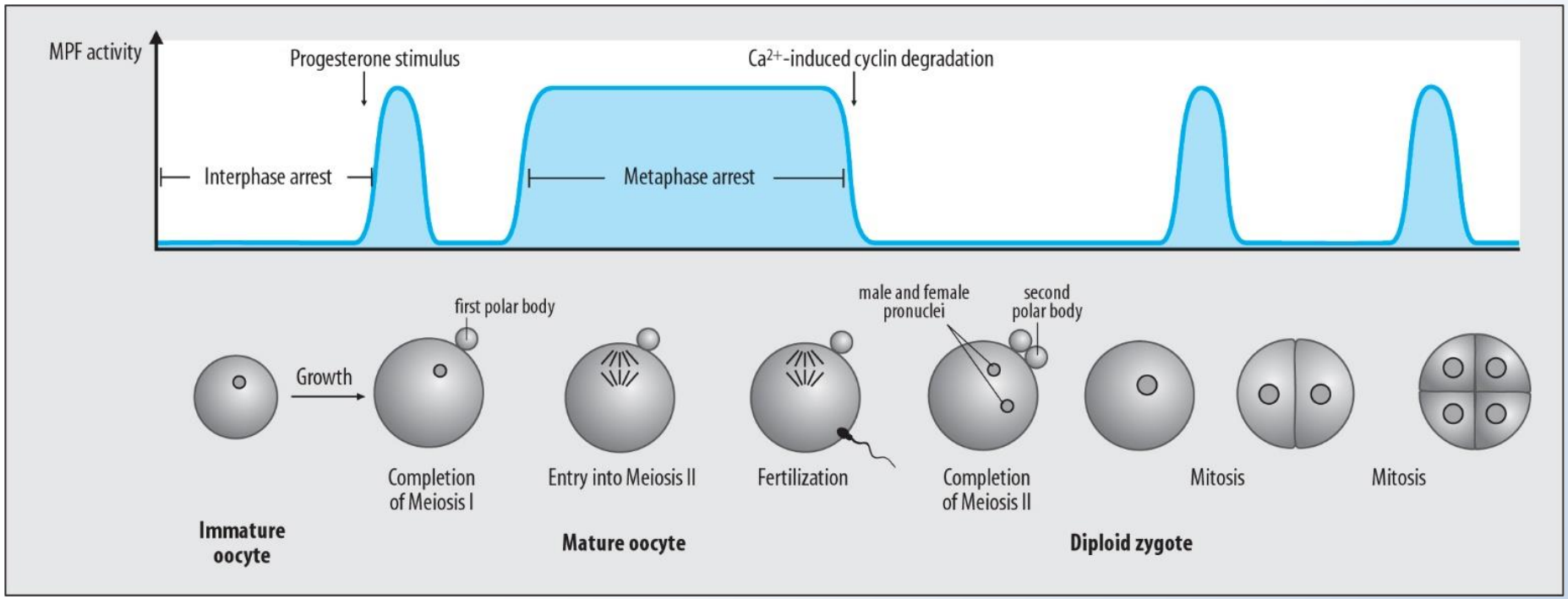
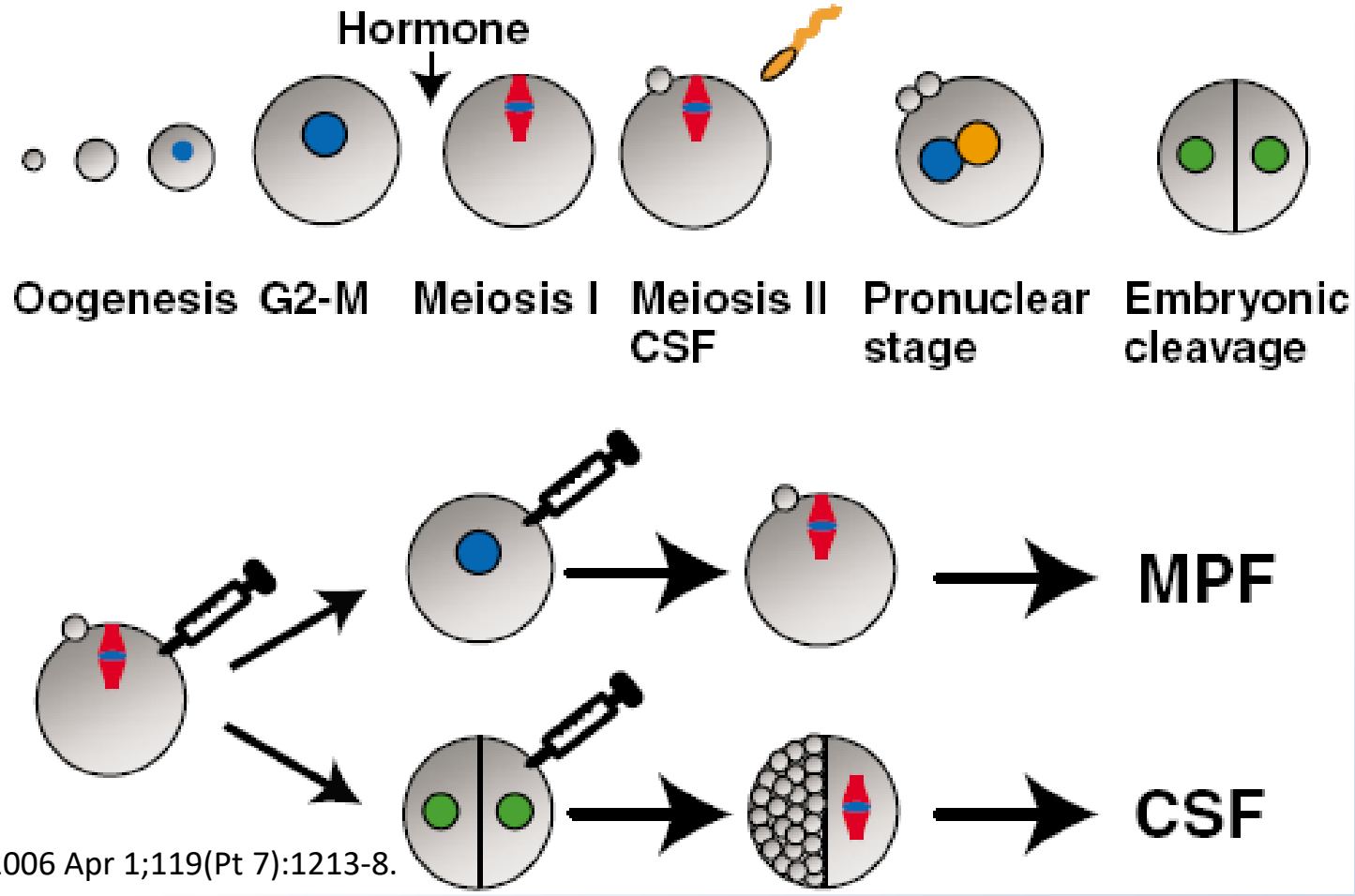
CDK1 ACTIVITY DURING MEIOTIC MATURATION



https://socratic.org/questions/where-in-the-body-do-oocytes-mature

- Mouse oocytes proceed through meiosis I and arrest at **second meiotic metaphase with high CDK1-cyclin B1 activity.**

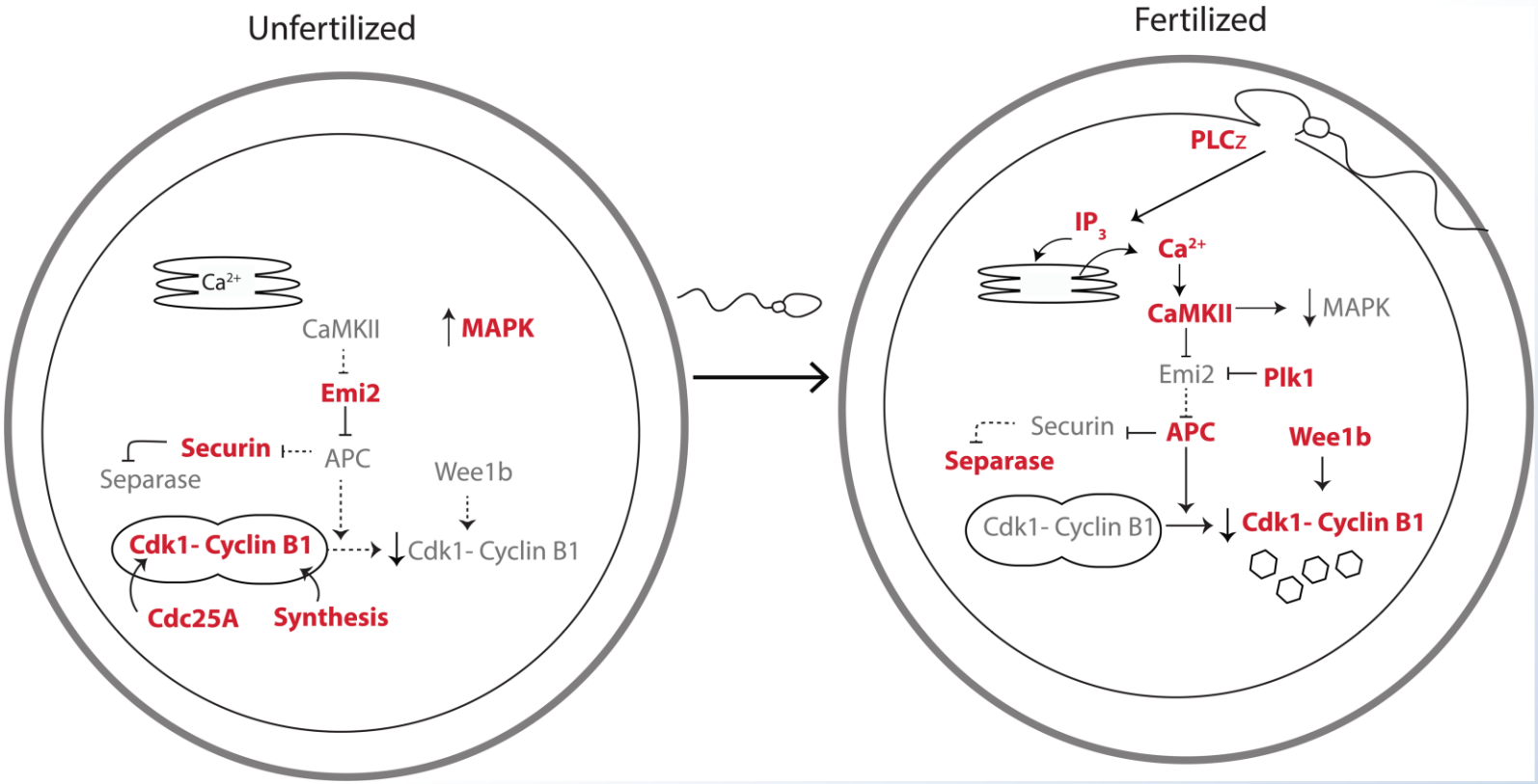
MPF AND APC ACTIVITY DURING METAPHASE II ARREST



J Cell Sci. 2006 Apr 1;119(Pt 7):1213-8.

- **Cytostatic factor (CSF)** is responsible for metaphase II arrest

METAPHASE II ARREST AND EGG ACTIVATION TRIGGERED BY FERTILIZATION

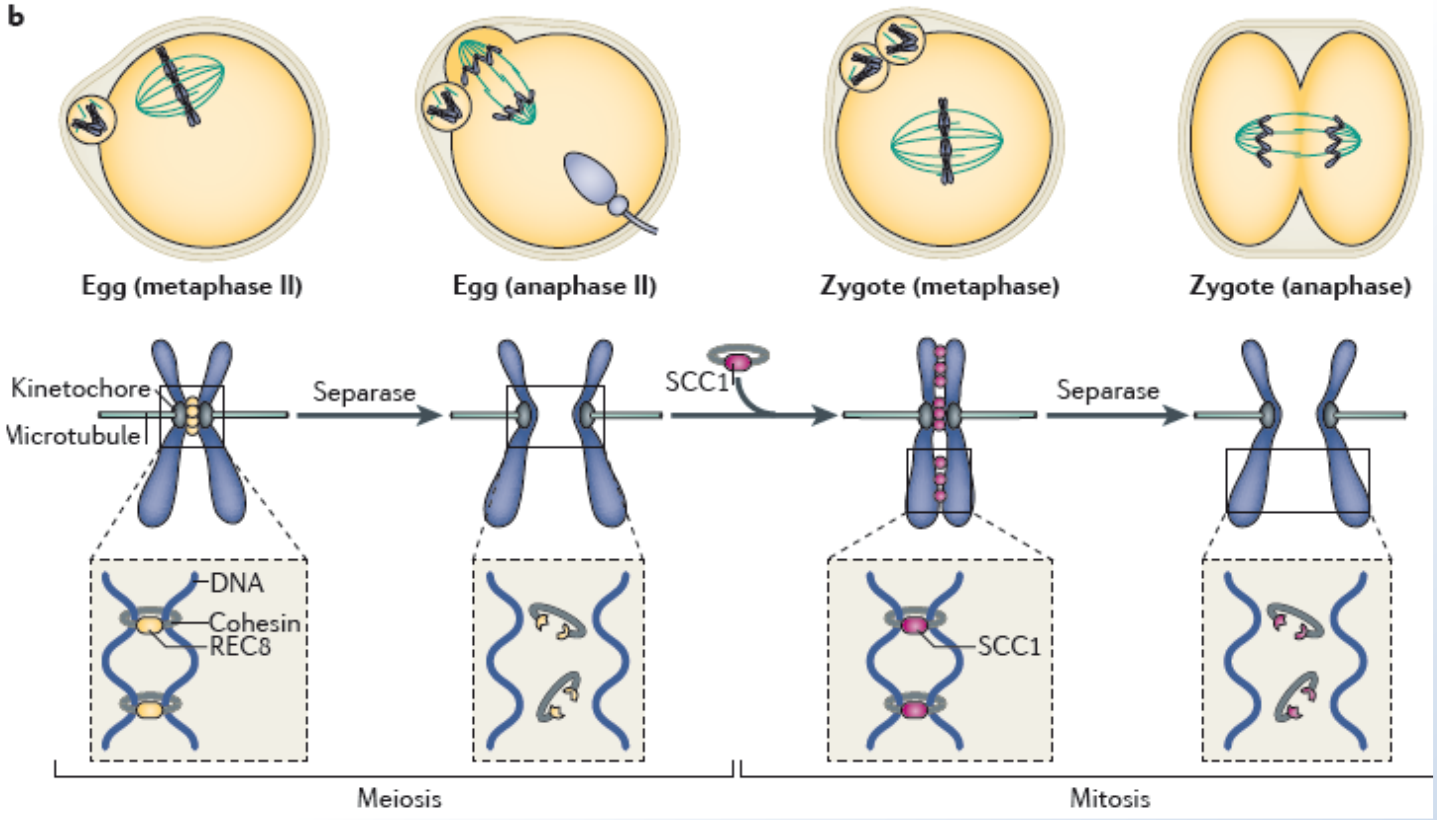


Sanders and Jones, 2018, *Biochemical Society Transactions*

PLCz - Phospholipase C zeta, CAMKII - Calcium/calmodulin-dependent protein kinase II

- **Emi2** is responsible for **metaphase II arrest**

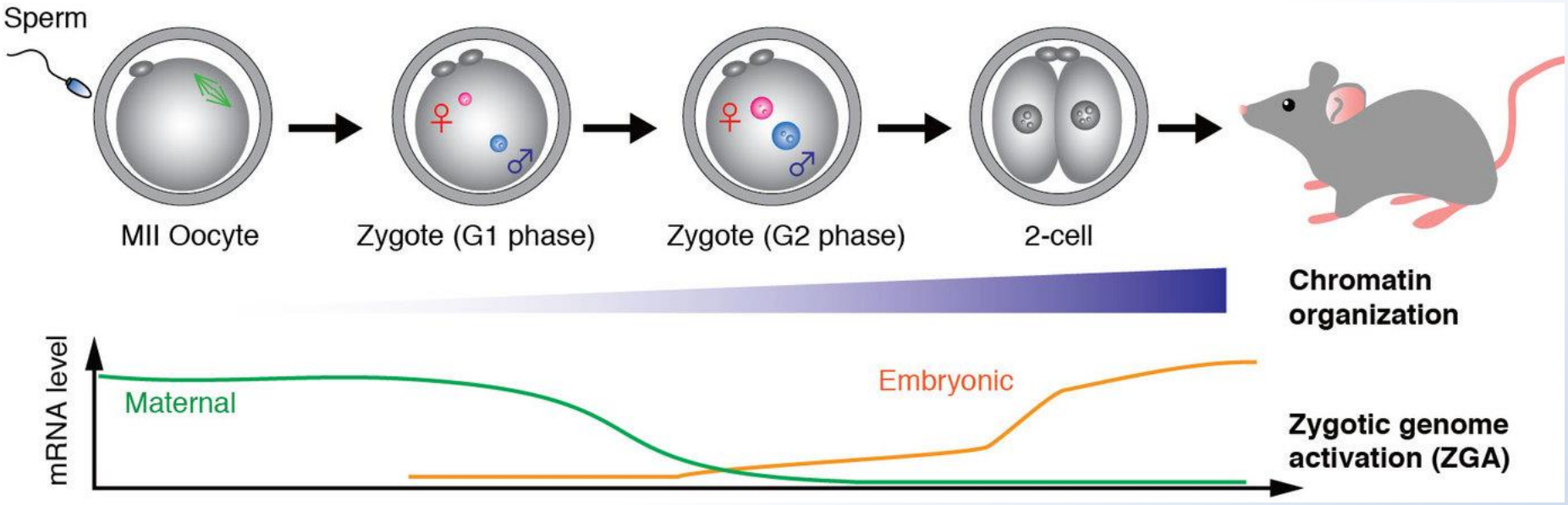
MEIOTIC-TO-MITOTIC TRANSITION



Clift, Schuh, 2013, Nat Rev Mol Cell Biol., PMID: 23942453

- **SCC1-containing cohesin complexes** are loaded onto chromosomes immediately in the zygote

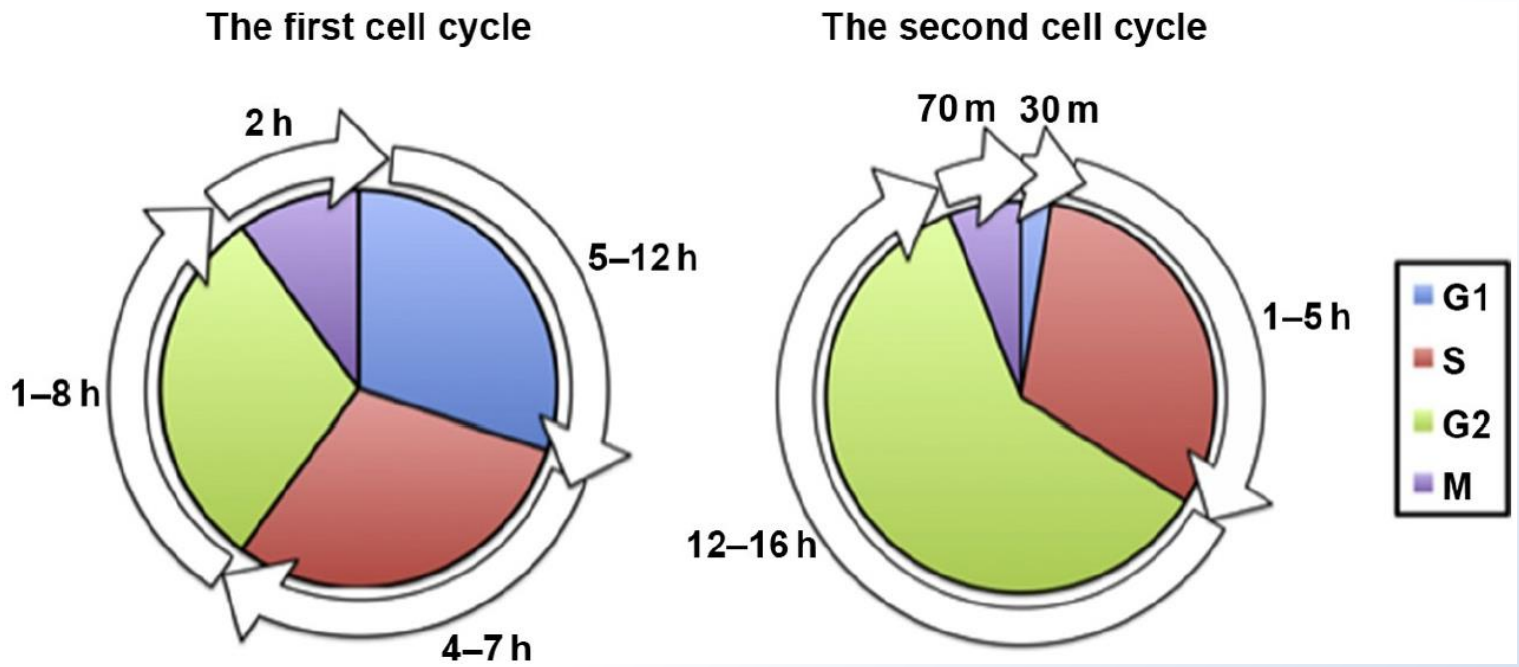
FROM A TRANSCRIPTIONALLY SILENT ZYGOTE TO THE ZYGOTIC GENOME ACTIVATION



Highlights of the mouse oocyte-to-embryo transition
https://www.biochem.mpg.de/tachibana/research

- **Maternal transcripts are degraded** and embryonic transcription in the major **zygotic genome activation** occurs **in the 2-cell mouse embryo**

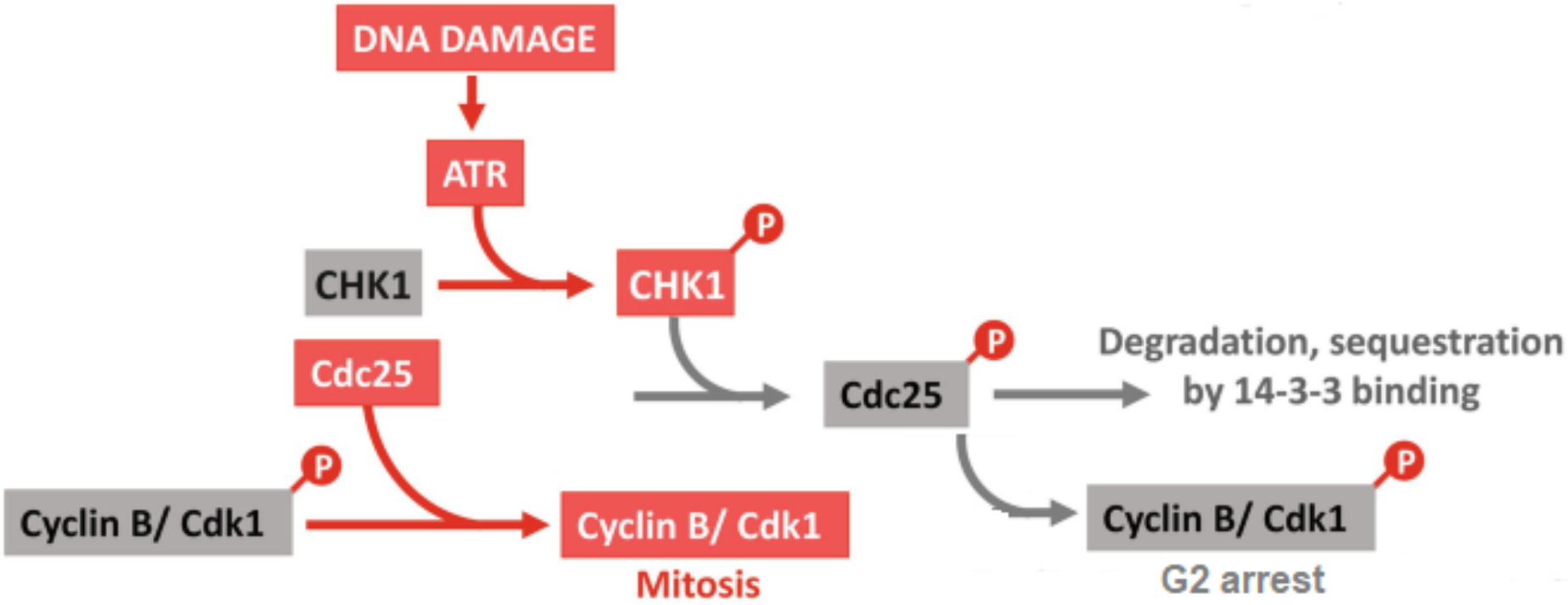
INITIAL EMBRYONIC CELL DIVISIONS ARE ACCOMPANIED BY CELL CYCLE ADAPTATIONS



The estimated duration of cell cycle stages during murine embryonic cell cycles
Palmer, Kaldis, 2016, *Curr Top Dev Biol.*

- Regulation of the transition from a **long G1 and short G2 in zygotes** to a **short G1 and long G2 in two-cell embryos** and the **mechanisms by which the cell cycle regulates genomic integrity** remain largely unknown.

CHK1 KINASE IS ESSENTIAL FOR THE GENOME INTEGRITY PROTECTION IN SOMATIC CELLS

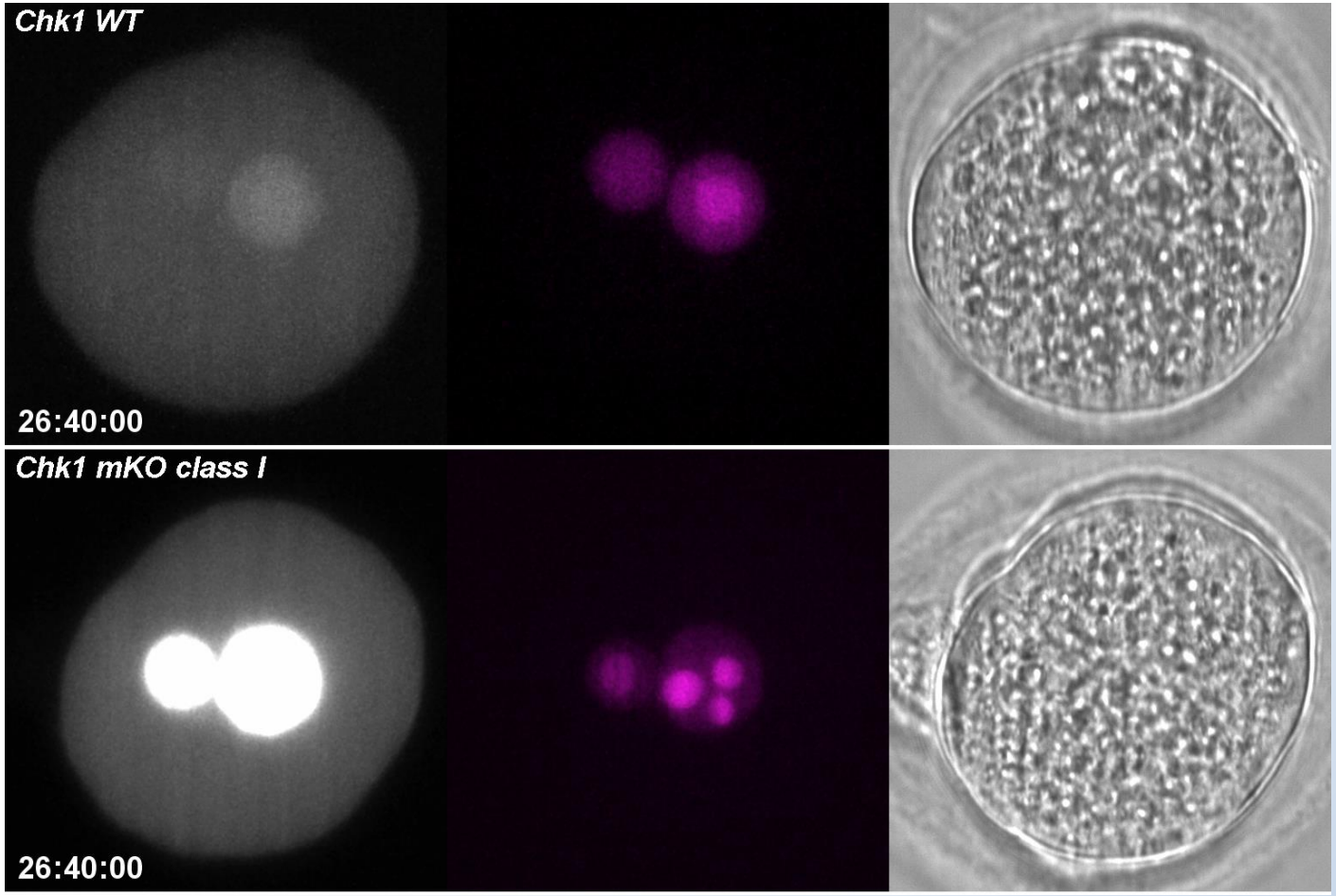


The cell cycle checkpoint pathway activated by DNA damage

Gillespie, 2018, modified

- **CHK1** activity **inhibits CDC25 phosphatases** and thus **holds the cells in the G2 phase** until ready to enter the mitotic phase after DNA repair

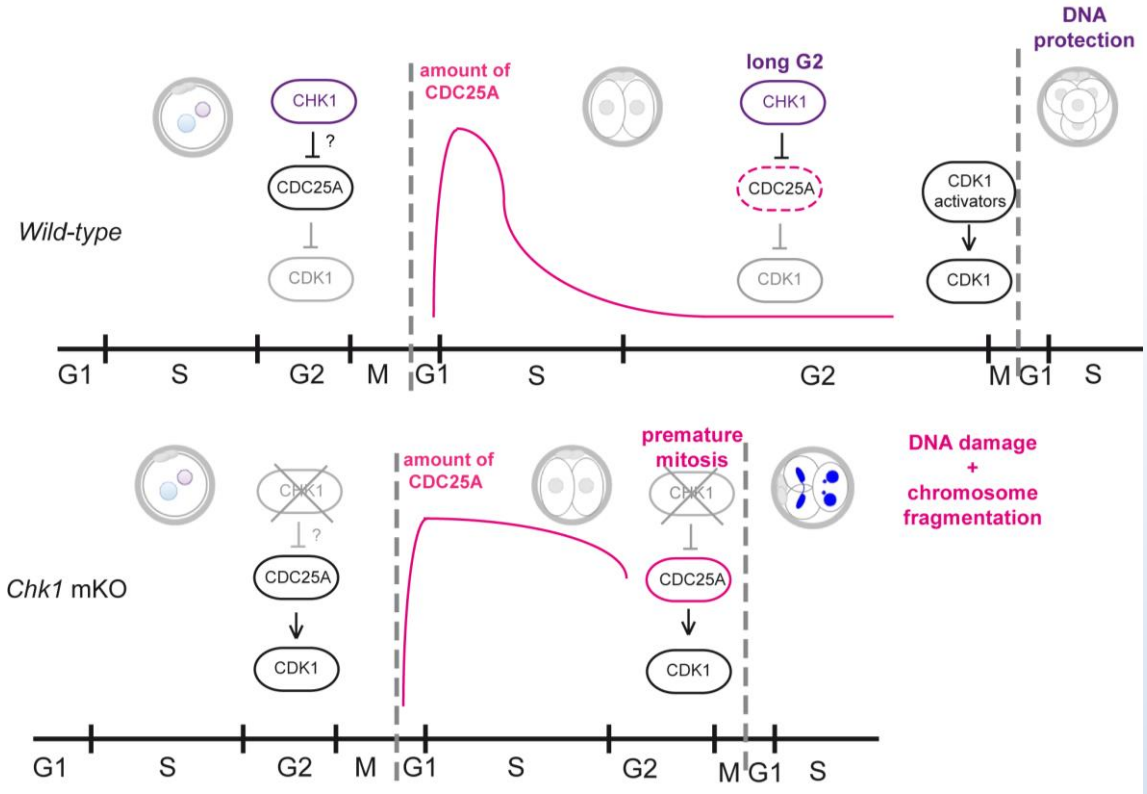
CHK1 KINASE IS ESSENTIAL FOR MAINTAINING THE LONG G2 PHASE IN TWO-CELL EMBRYOS



***Shorter G2** and **genome fragmentation** in 2-cell Chk1 mKO class I embryos*

***Chromatin**, mCDT1-EYFP, time post hCG administration (h)*

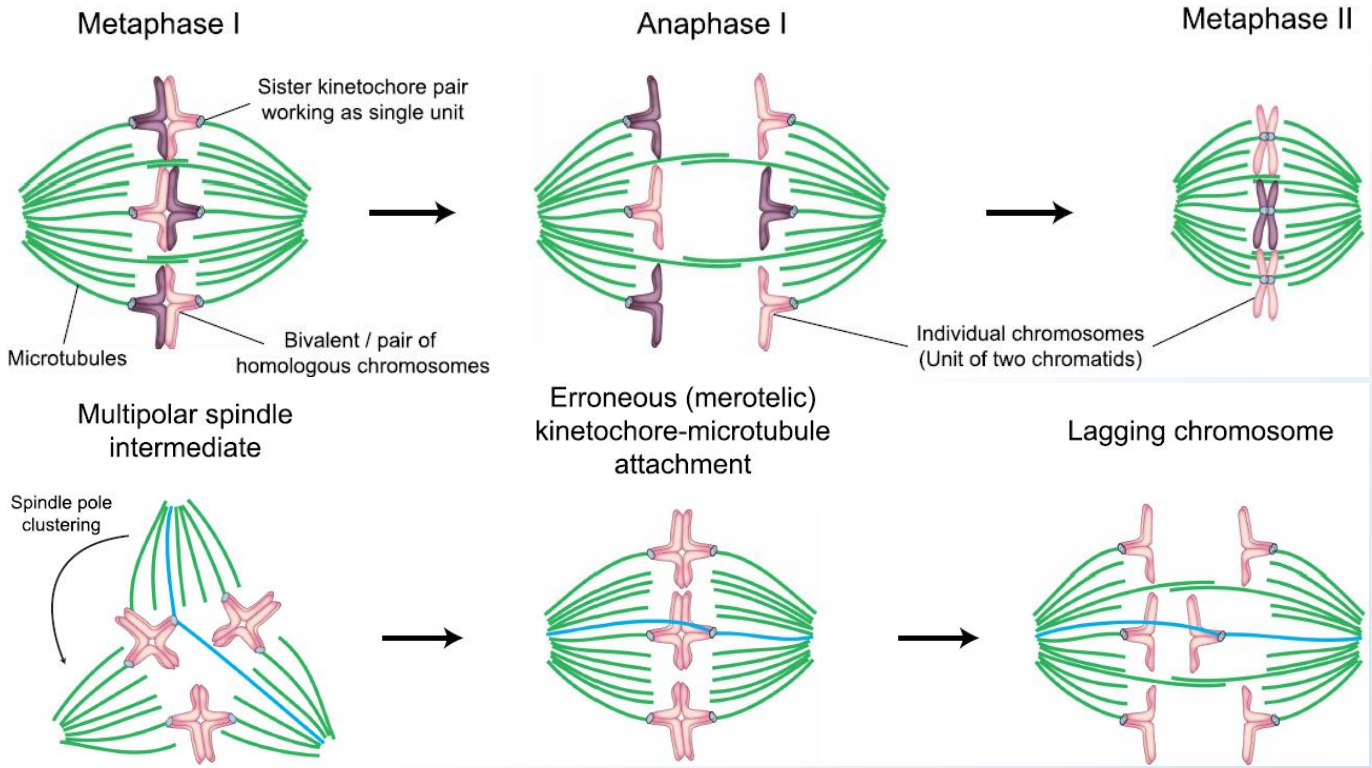
CHK1 KINASE IS ESSENTIAL FOR MAINTAINING THE LONG G2 PHASE IN TWO-CELL EMBRYOS



Knoblochova et al, 2022, *EMBO Rep.*

- **CHK1-CDC25A-CDK1 maintains a long G2 phase in 2-cell mouse embryos that protects early embryos from chromosome segregation errors** that result in aneuploidy and infertility.

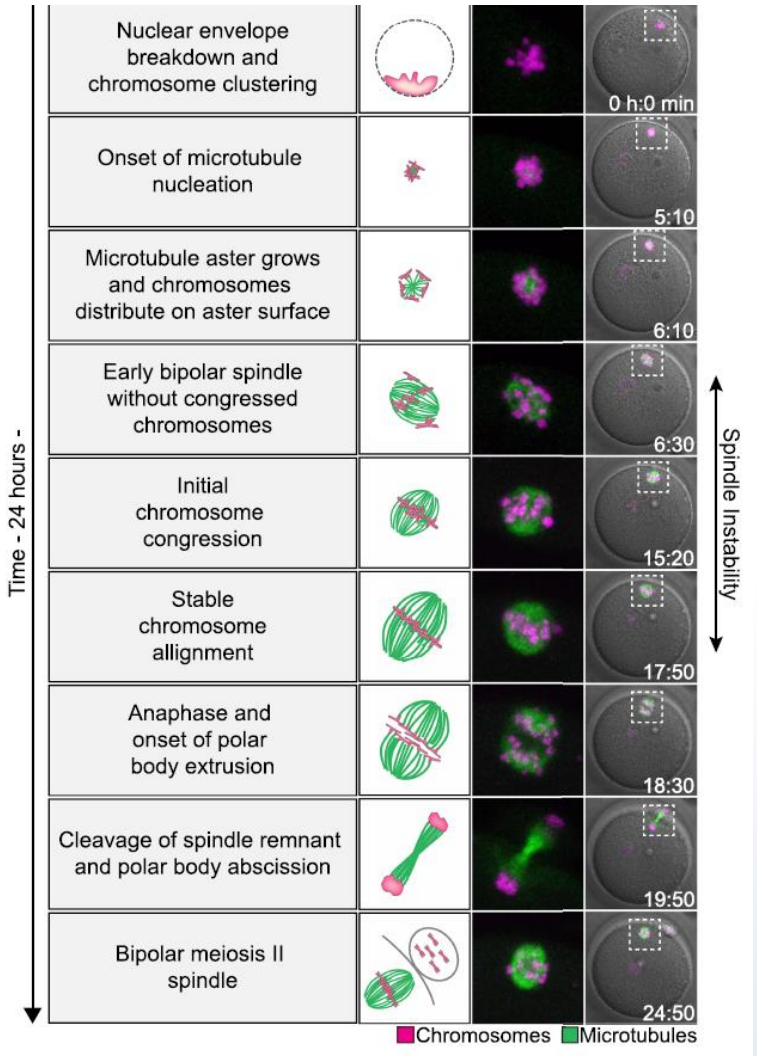
CHROMOSOME SEGREGATION AND CONFIGURATION IN OOCYTES



Thomas et al, 2021, *Biochemical Society Transaction*

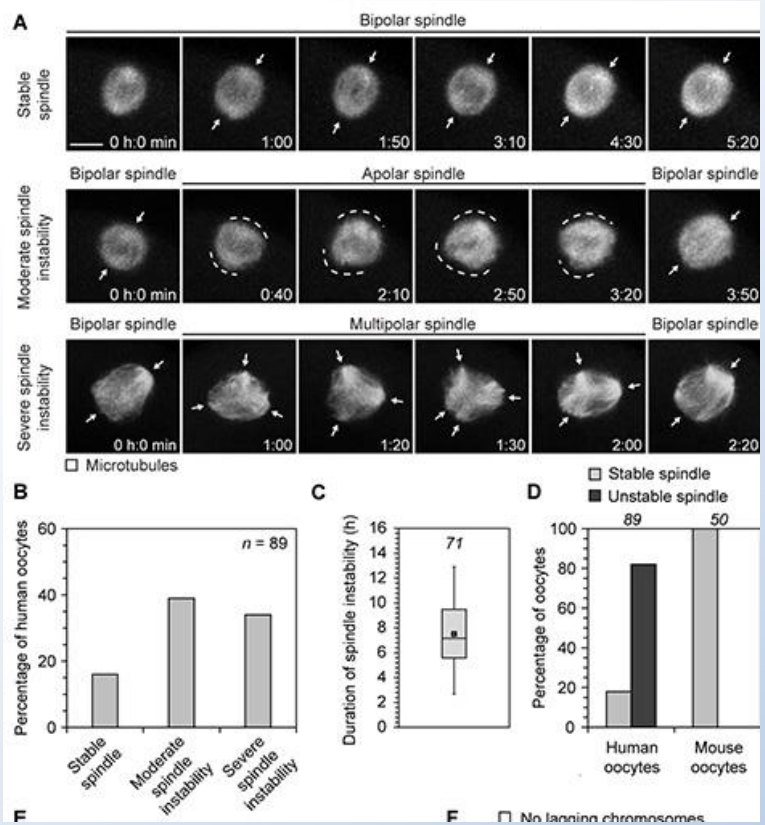
- **Merotelic attachments during the multipolar stages** are a common cause of **lagging chromosomes** in anaphase

SPINDLE ASSEMBLY IN OOCYTES – IMPLICATIONS FOR HUMAN INFERTILITY



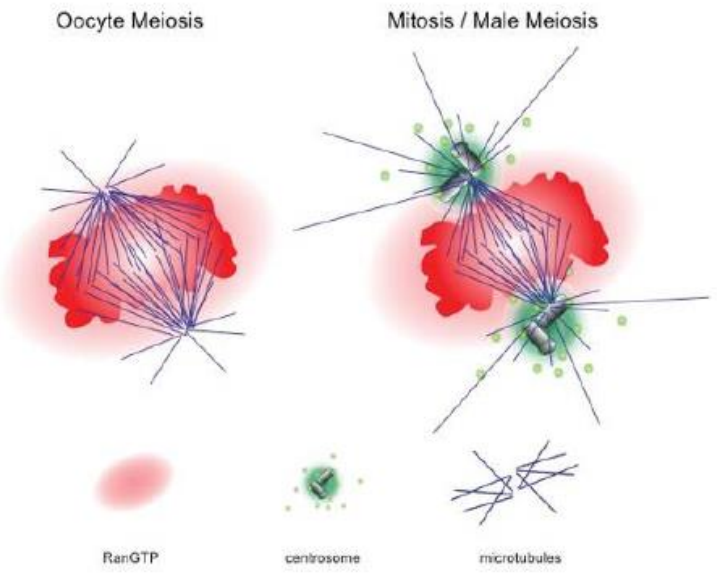
Thomas et al, 2021, *Biochemical Society Transaction*

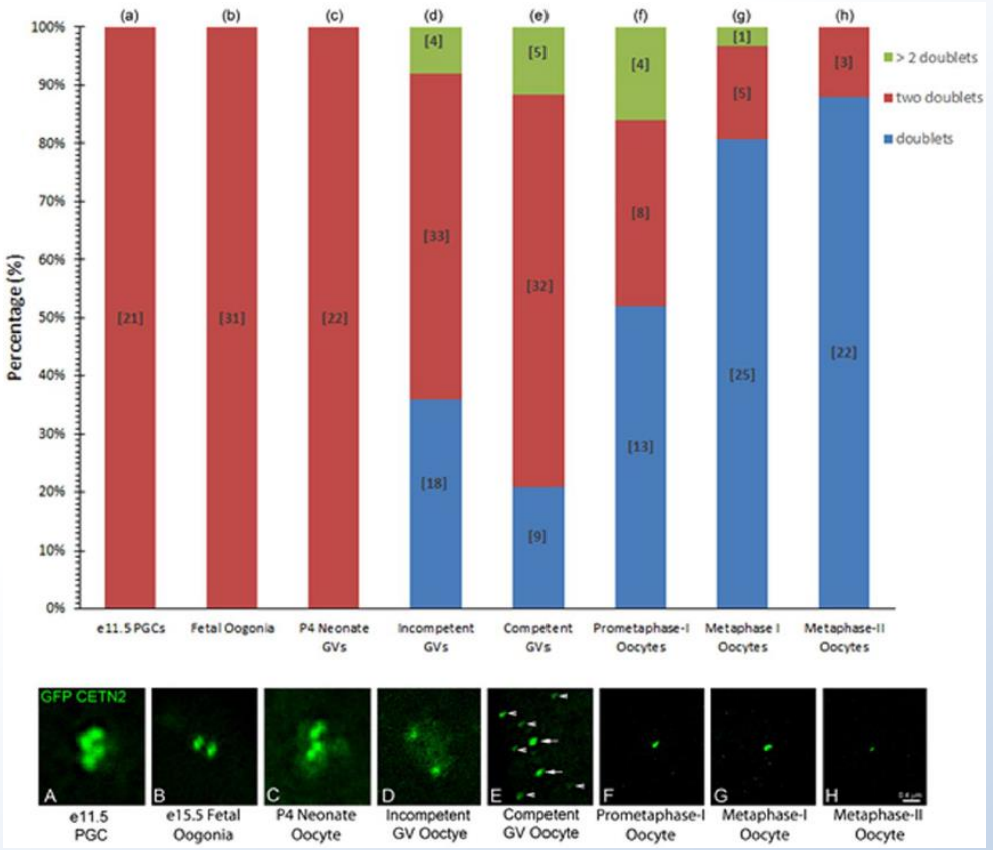
- **spindle instability** during meiosis I of human oocytes leads to **lagging chromosomes** in anaphase I



Holubcova et al, 2015, *Science*

CENTRIOLE LOSS IN MAMMALIAN OOCYTES



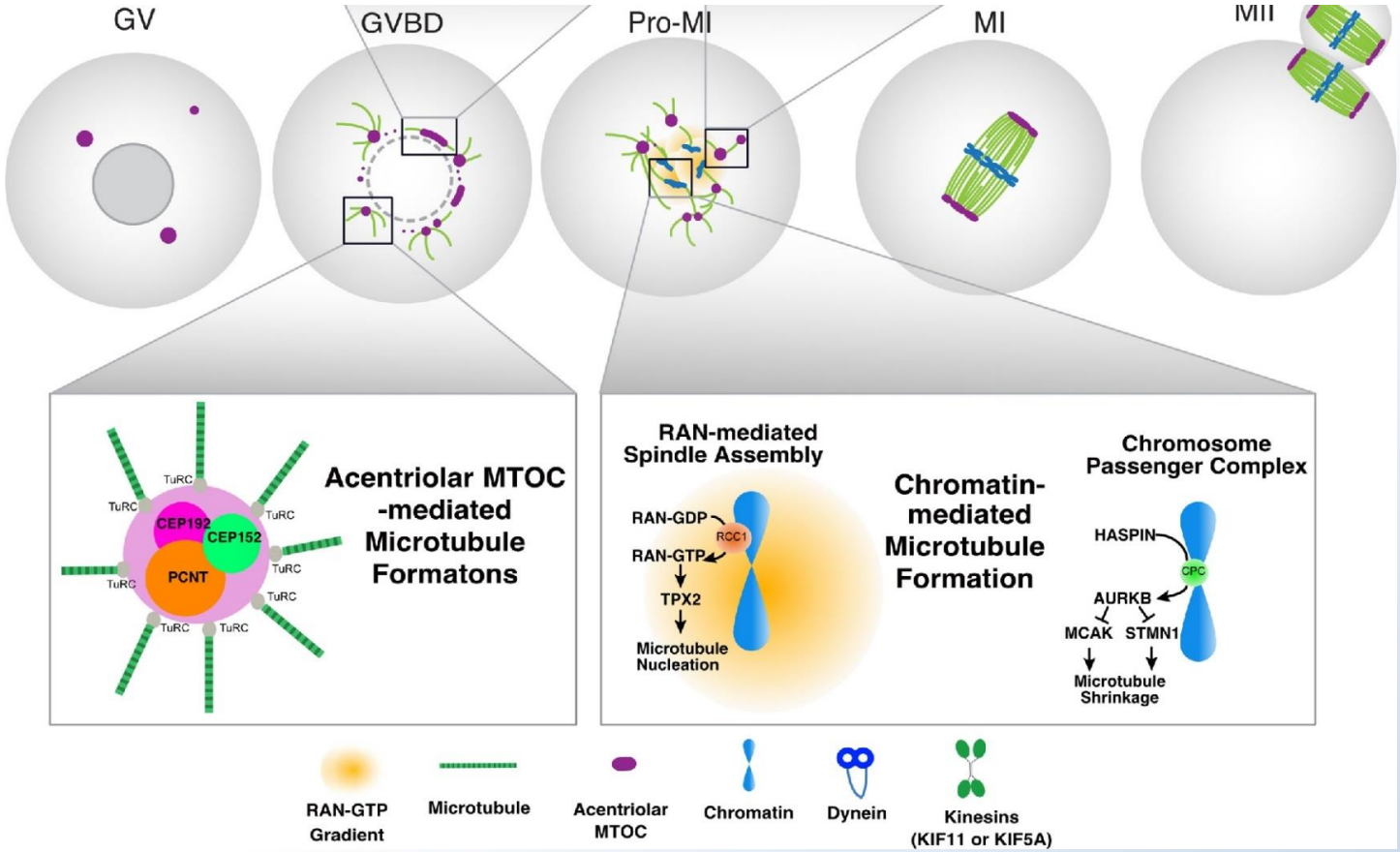


- GFP-centrin-2 transgenic mice
- PGC - primordial germ cells

Simerly et al, 2018, *Sci Rep.,* PMID: 30143724

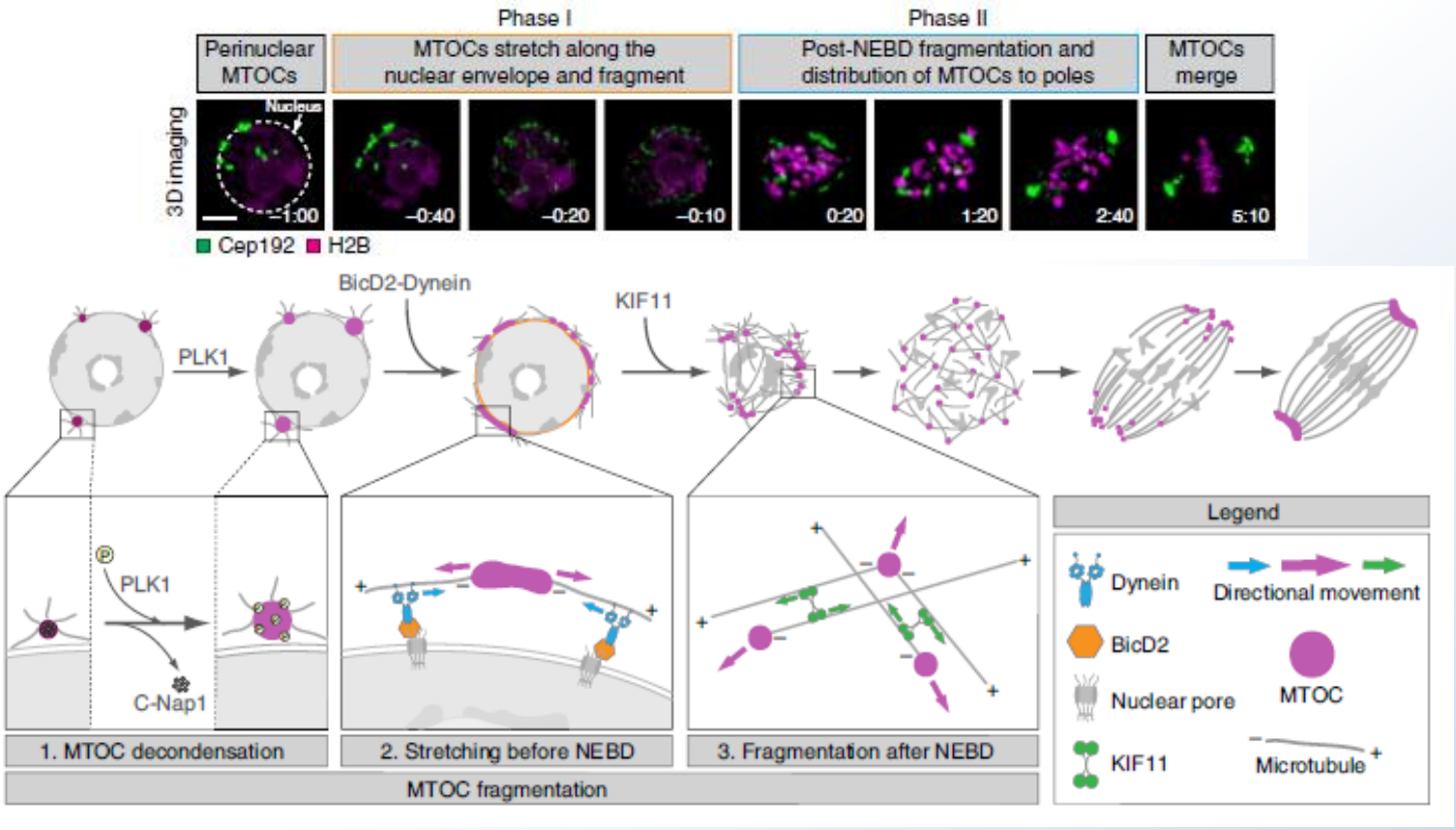
- **Separation** and **gradual loss of centrioles** **from primordial germ cells to mature oocytes** in the mouse

ACENTRIOLAR SPINDLE FORMATION IN MAMMALIAN OOCYTES



- **Acentriolar MTOCs** (microtubule organizing centers)**-dependent** and **chromatin-dependent** pathways contribute to **acentrosomal spindle assembly**
- alternative pathways

MTOCS-DEPENDENT SPINDLE ASSEMBLY IN MOUSE OOCYTES

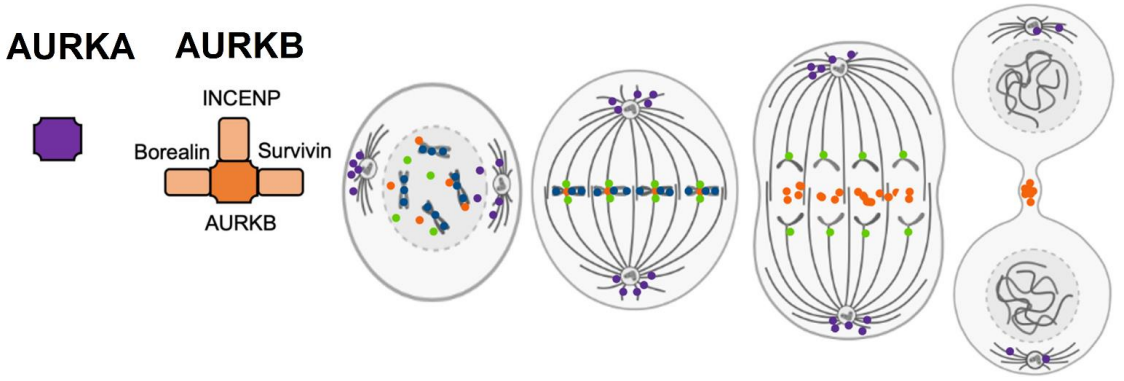


Three-step mechanism of MTOC fragmentation in mouse oocytes

Clift a Schuh, 2014, *Nature Communications*

- **Each step of MTOCs behaviour** and spindle formation **is critical** for correct chromosome segregation

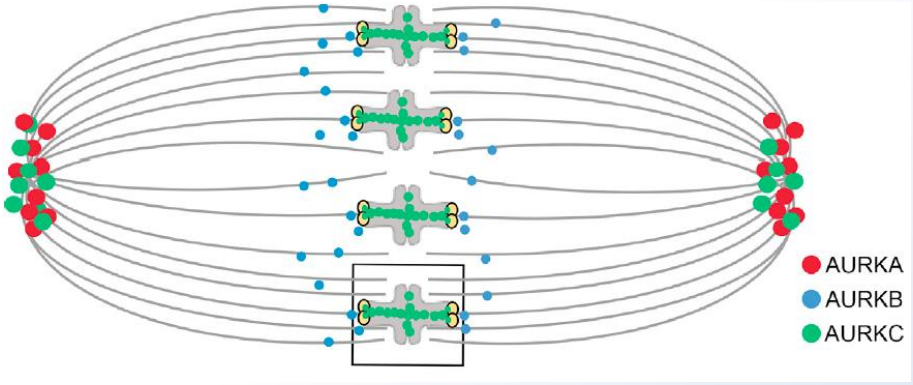
ROLE OF AURORA KINASES (AURKS) IN SPINDLE ASSEMBLY



Pajpach et al, 2021, *Genes*

Somatic cells

- **AURKA** – centrosomes
- **AURKB** – CPC (chromosomal passenger complex)



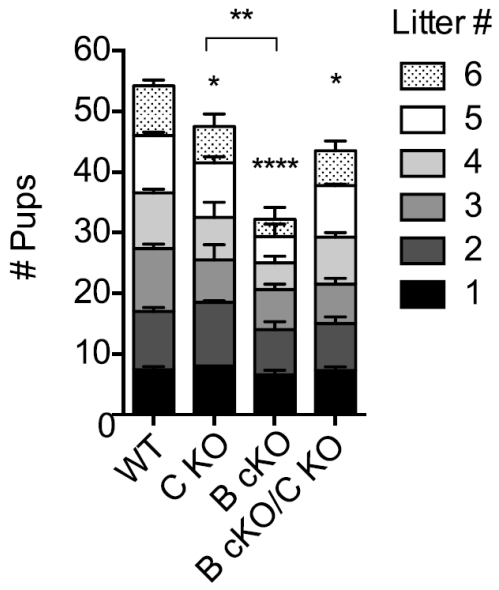
Nguyen a kol., 2018, *Current Biology*

Germ cells

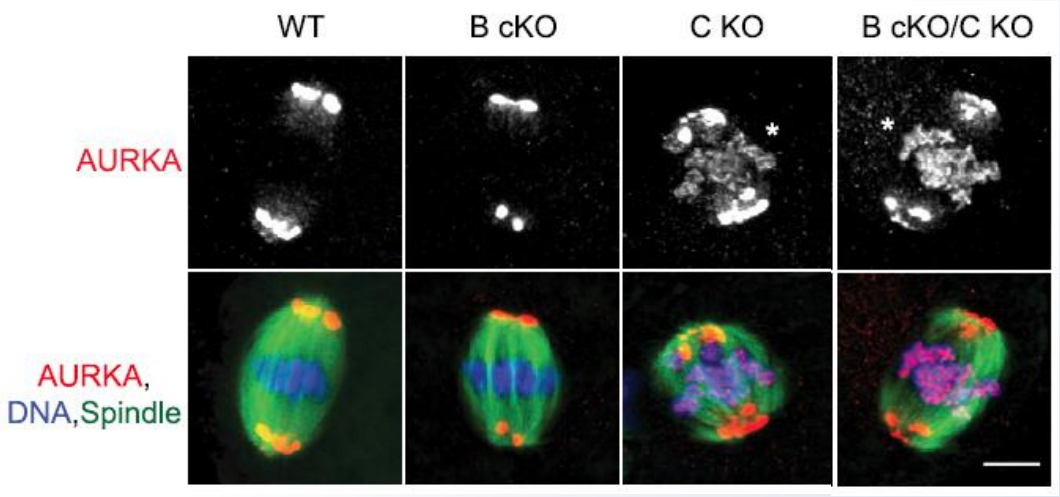
- **AURKA** – MTOCs
- **AURKB** – ?
- **AURKC** – CPC, MTOCs

- **Why germ cells express three Aurora kinases instead of two?**

A MODEL OF OOCYTE-SPECIFIC *AURKB/AURKC* DOUBLE-KNOCKOUT MICE



***Aurkb/Aurkc* double KO (B cKO/C KO) mice are subfertile**

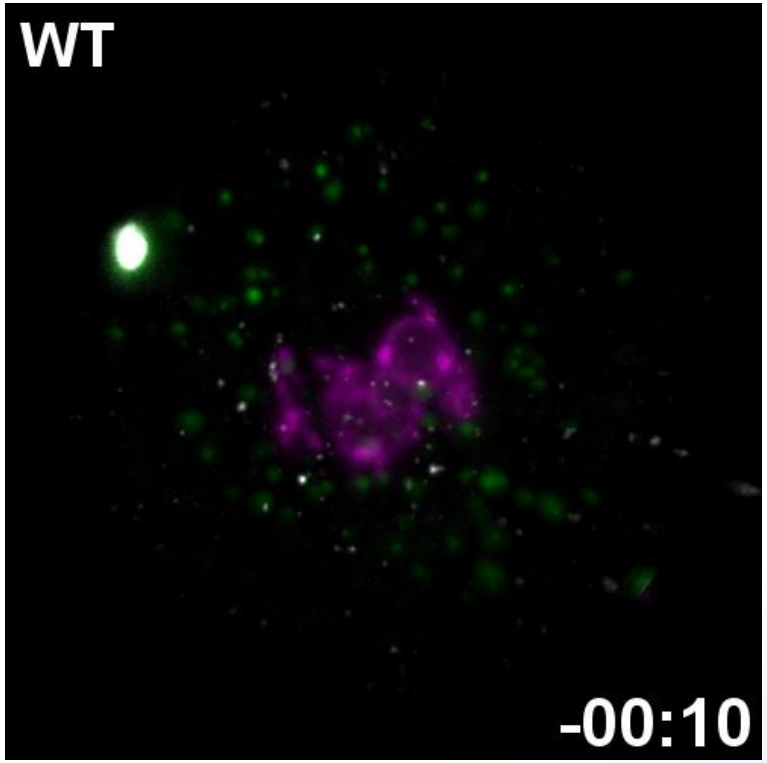


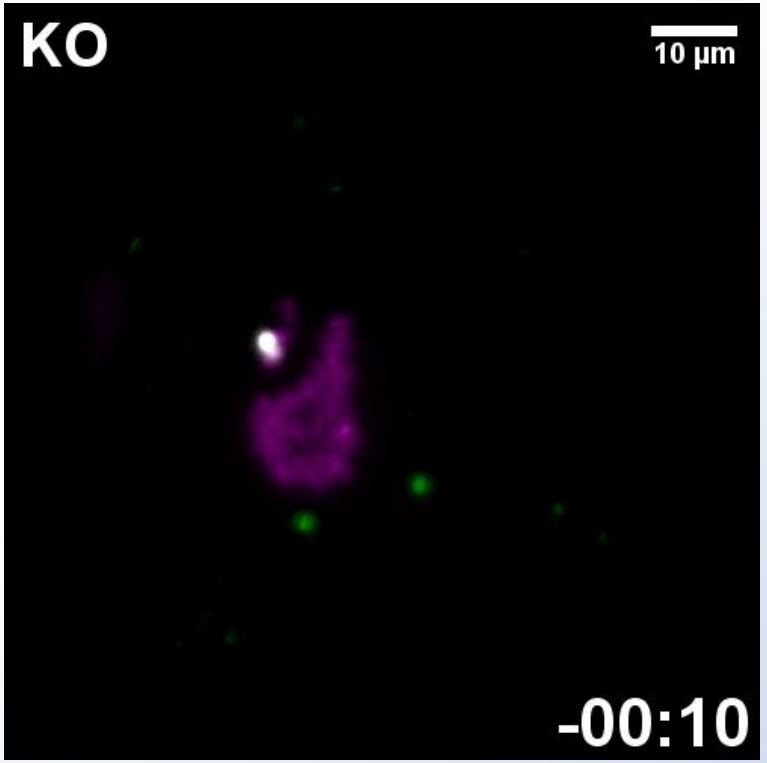
AURKA localized to chromosomes in oocytes upon deletion of *Aurkc*

Nguyen a kol., 2018, *Current Biology*

- **AURKA specifically in mouse oocytes compensates for loss of AURKB/C**

A MODEL OF OOCYTE-SPECIFIC *AURKA* KNOCKOUT MICE





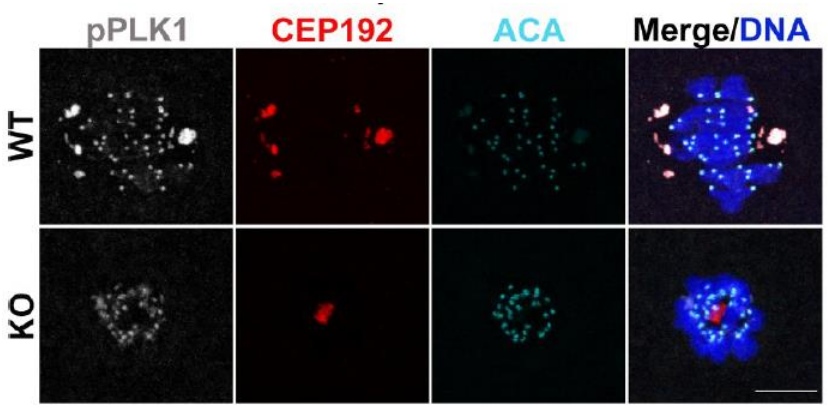
***Aurka* knockout oocytes (KO) are arrested in Metaphase I with defective spindle**

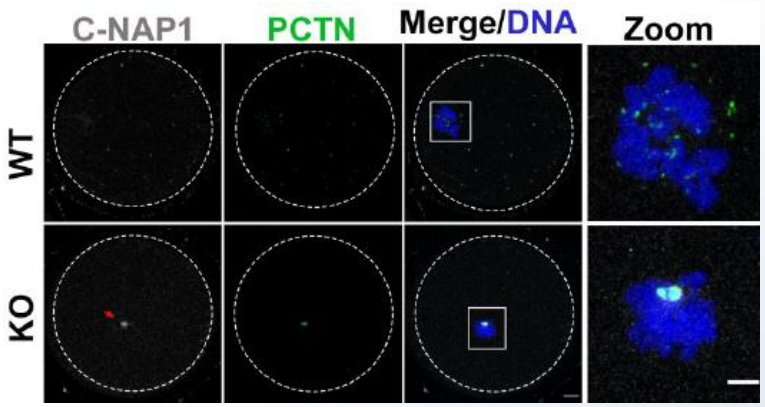
Blengini et al, 2021, *PLoS Genetics*

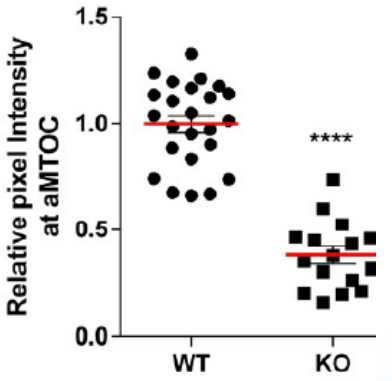
Chromosomes, spindle, MTOCs, time (hh:mm) after meiotic resumption

- **AURKA is required for spindle assembly and meiosis I-meiosis II transition in mouse oocytes**

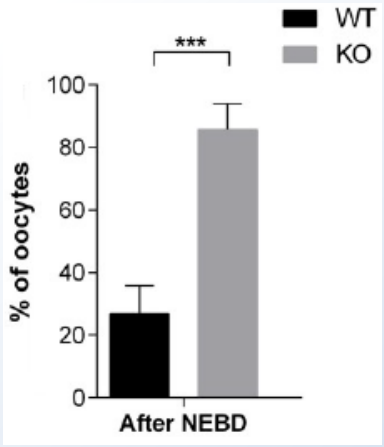
ROLE OF AURKA IN MTOCS FRAGMENTATION







Decreased pPLK1 at MTOCs in Aurka KO oocytes

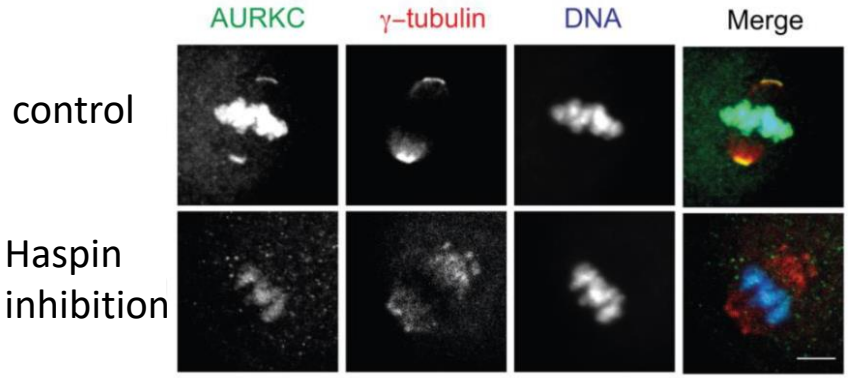


C-NAP1 persisted at MTOCs after meiotic resumption in Aurka KO oocytes

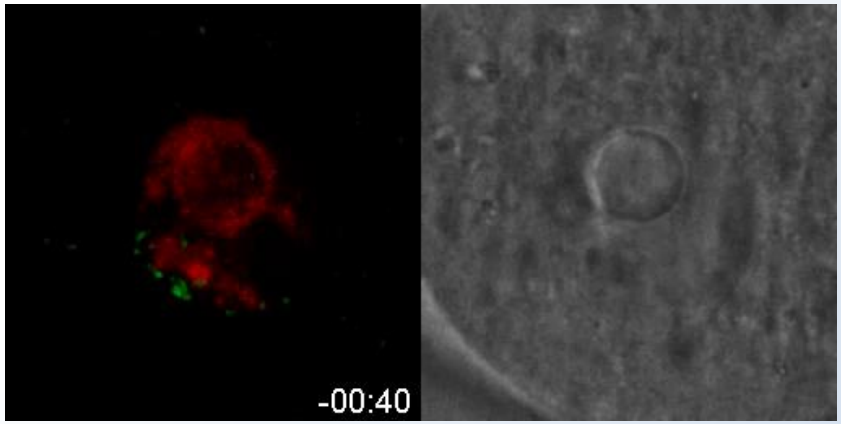
Blengini et al, 2021, *PLoS Genetics*

- **AURKA is required for full PLK1 activation to initiate MTOC fragmentation through inducing C-NAP1 release from aMTOCs**

MTOC SORTING IS REGULATED BY AURKC AND HASPIN KINASE



Haspin regulates AURKC localization on chromosomes and MTOCs



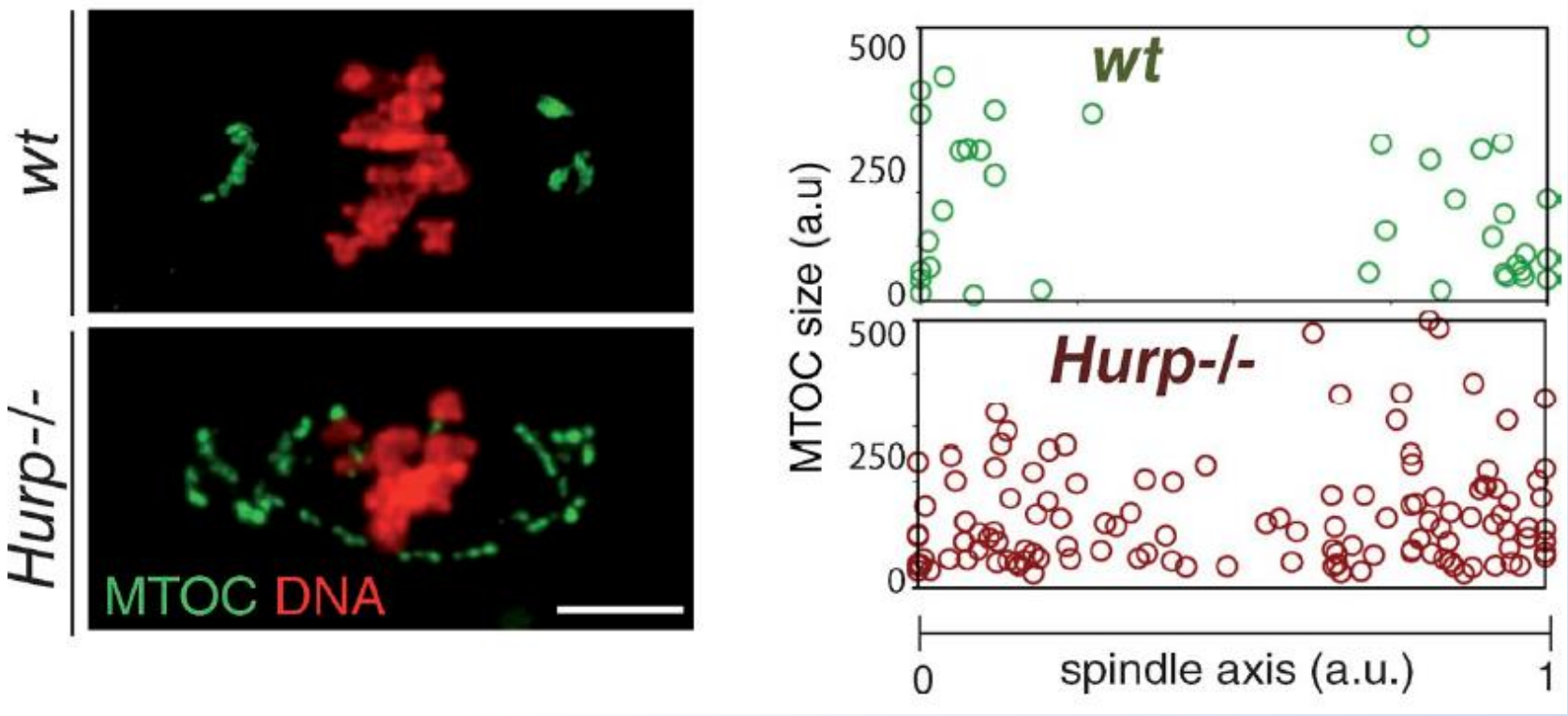
Live-cell imaging of haspin-inhibited oocytes

Balboula et al, 2016, *Journal of Cell Science*

Chromosomes, spindle, MTOCs, time (hh:mm) after meiotic resumption

- **Haspin regulates AURKC localized-function at MTOCs in mouse oocytes**
- **MTOCs clustering defects are associated with segregation errors**

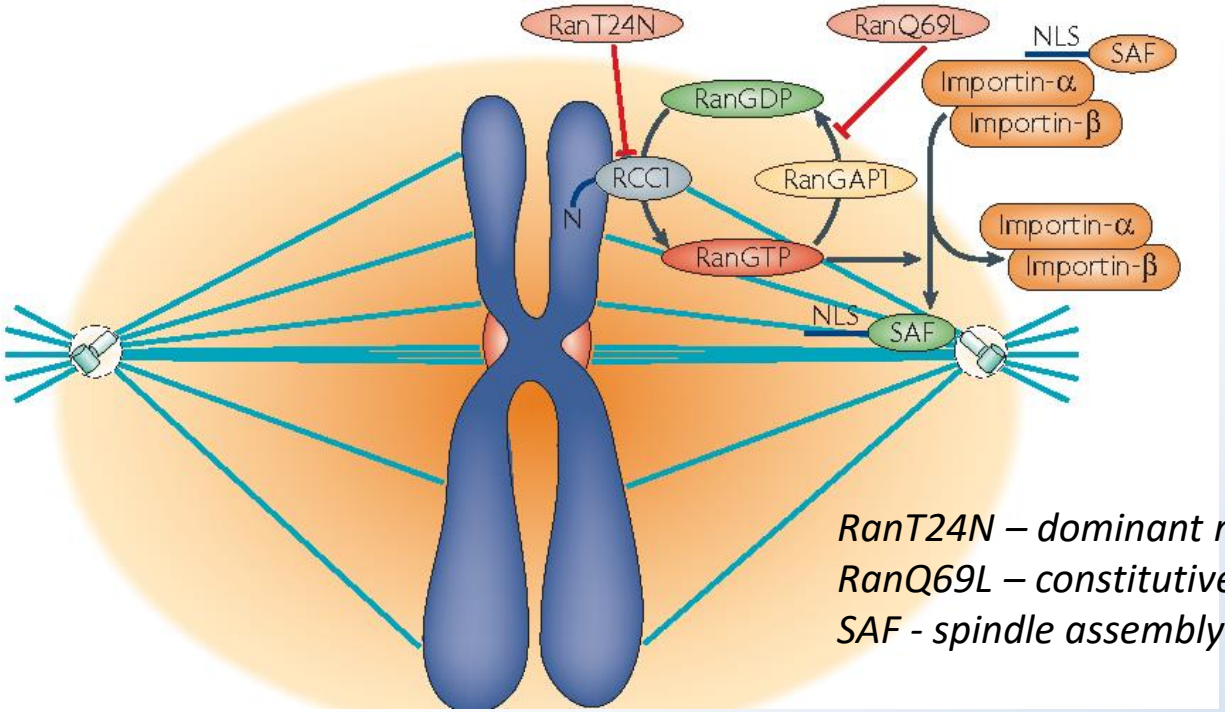
MTOC SORTING IS REGULATED BY HURP



Breuer et al, 2010

- **By promoting stability in the spindle central domain, HURP allows MTOC sorting, providing bipolarity establishment and maintenance**
- **HURP has a critical role in the clustering of extra centrosomes during mitosis in human cancer cells**

THE ROLE OF RANGTP-IMPORTIN BETA PATHWAY



RanT24N – dominant negative Ran
RanQ69L – constitutively active Ran
SAF - spindle assembly factor

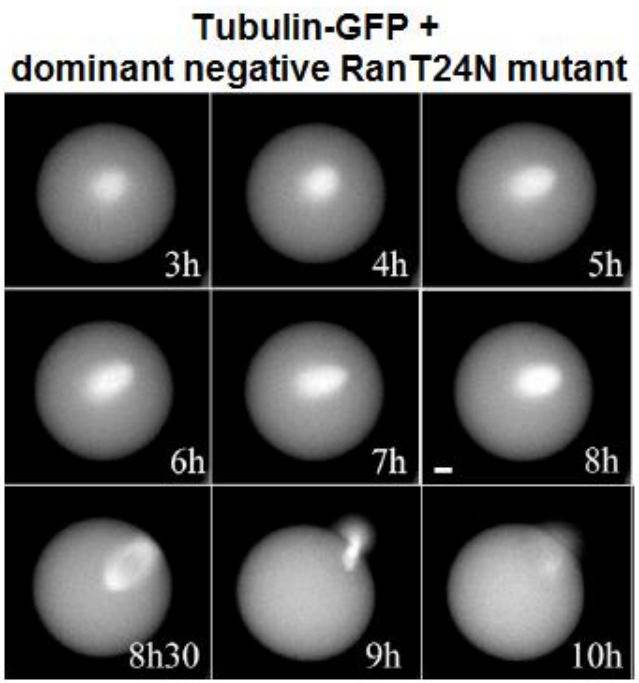
Involvement of Ran in spindle assembly

Clarke, Zhang, 2008, *Nature reviews. Molecular cell biology*

- **During cell division, high concentrations of RanGTP around the spindle assembly regions attract the importins and release NLS-containing SAFs from inhibitory importins**

INHIBITION OF RANGTP PATHWAY USING DOMINANT NEGATIVE RAN MUTANT

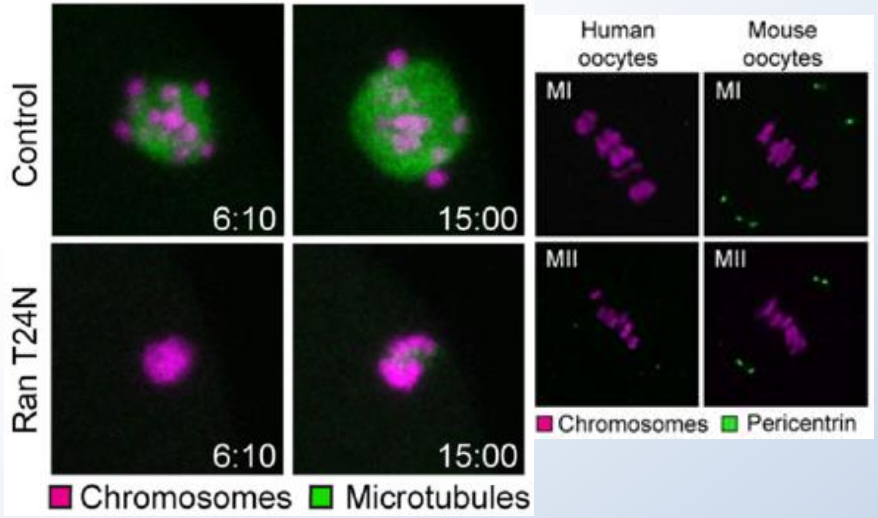
Mouse oocytes



Dumont et al, 2007, *Journal of Cell Biology*

RanGTP inhibition did not affect assembly of functional spindles

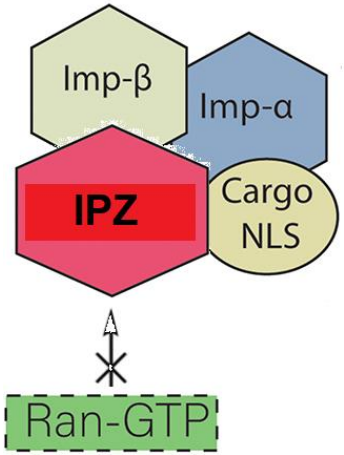
Human oocytes



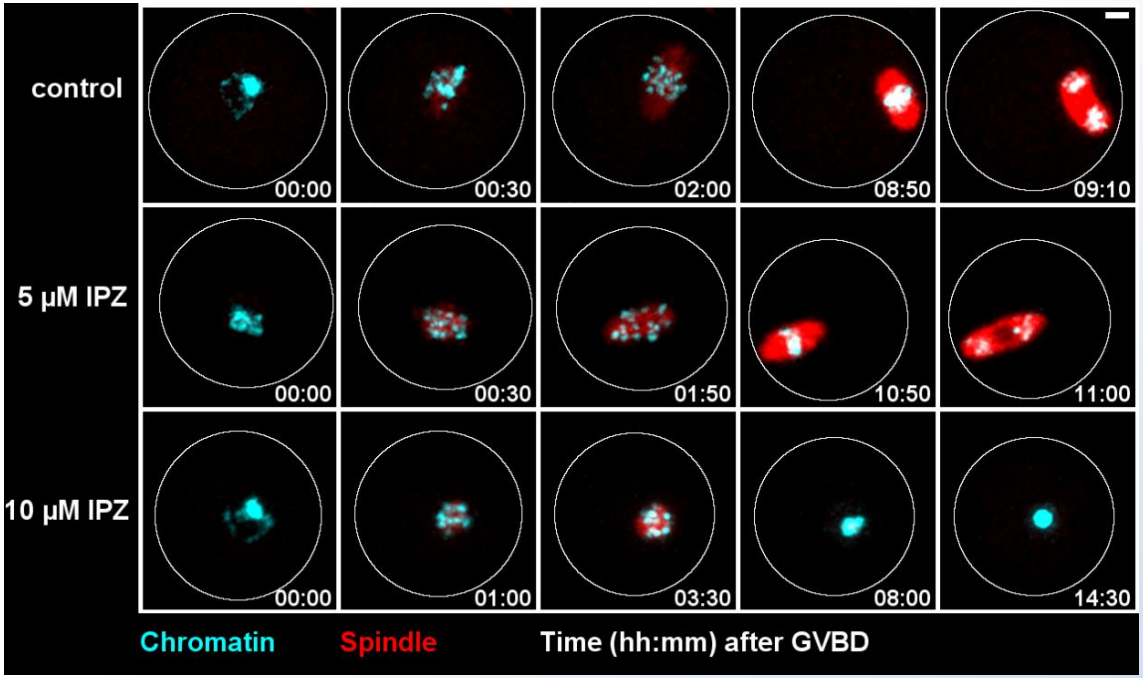
Holubcova et al, 2015, *Science*

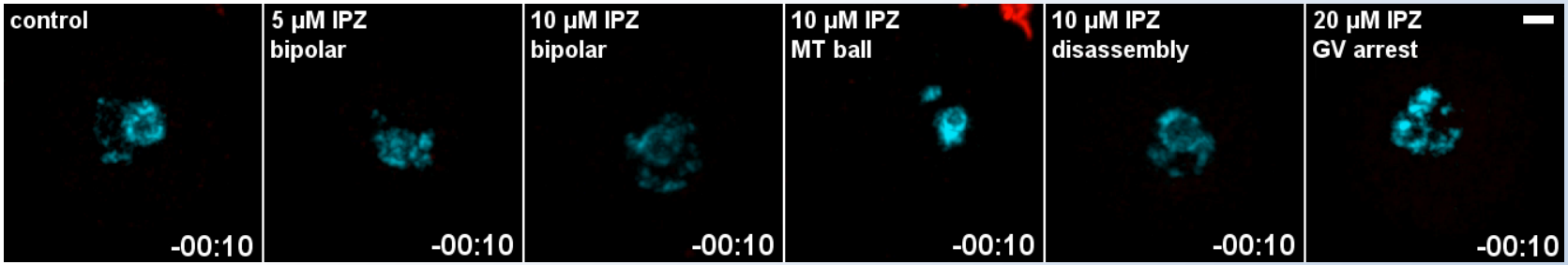
Spindle assembly is dependent on RanGTP, but independent on MTOCs

EFFECT OF IMPORTAZOLE (IPZ) ON SPINDLE ASSEMBLY IN MOUSE OOCYTES



Importin β (Imp-β) inhibitor Importazole (IPZ) blocks Ran binding

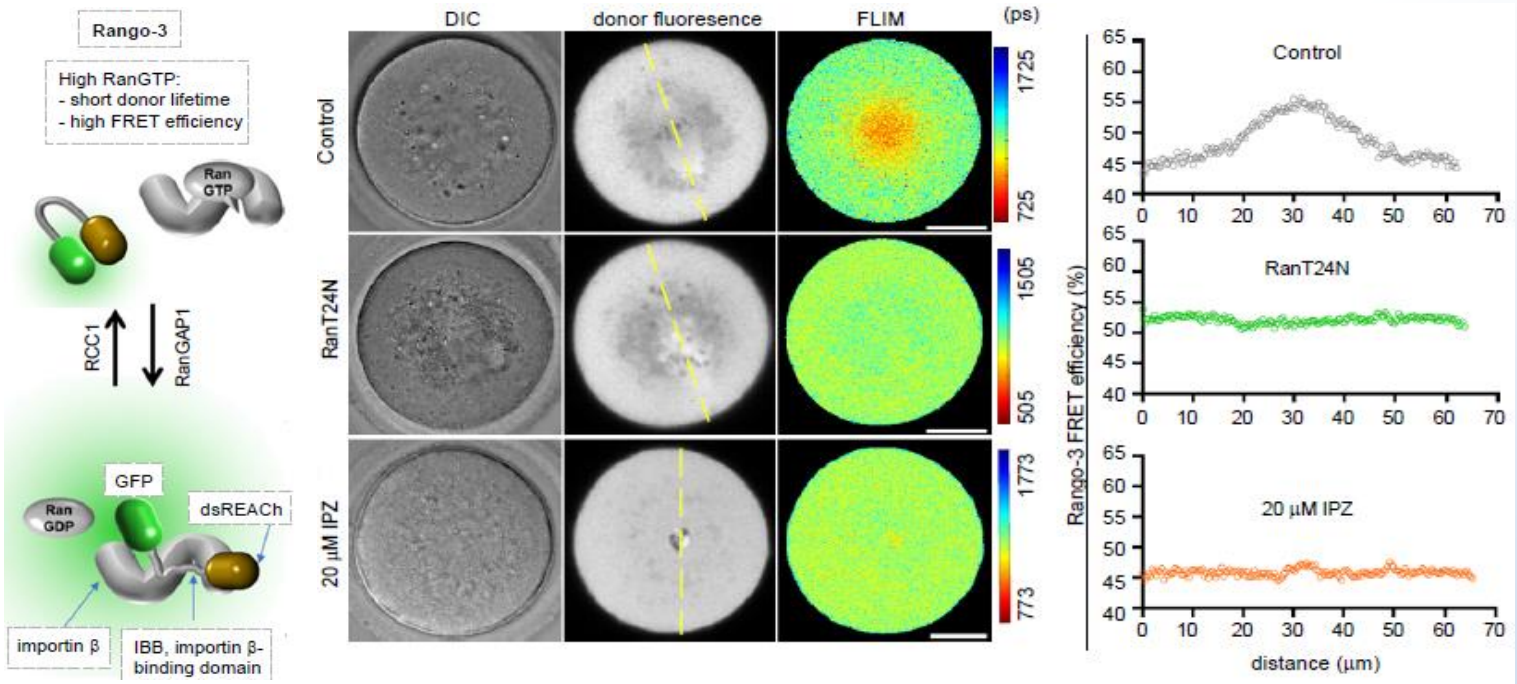




Live-cell imaging of spindle assembly in Importazole (IPZ)-treated mouse oocytes

- **RanGTP is required for the proper formation of the meiotic spindle**

EFFECT OF IMPORTAZOLE AND RANT24N ON RANGTP GRADIENT FORMATION



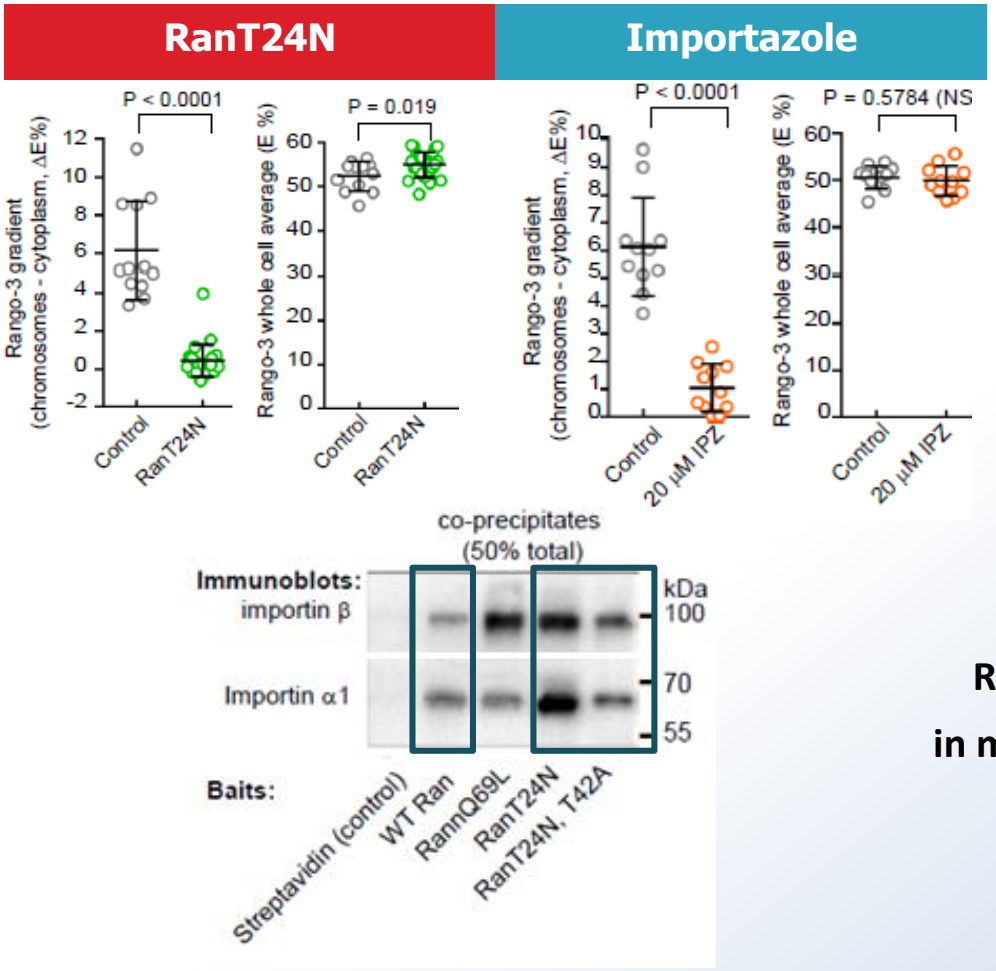
Quantitative FLIM/FRET imaging of RanGTP gradient

in RanT24N-microinjected or Importazole (IPZ)-treated oocytes

Drutovic et al, 2020, *EMBO Journal*

- **Dominant negative RanT24N, as well as Importazole, reduced the RanGTP gradient in mouse oocytes**

EFFECT OF IMPORTAZOLE AND RANT24N ON RANGTP GRADIENT FORMATION

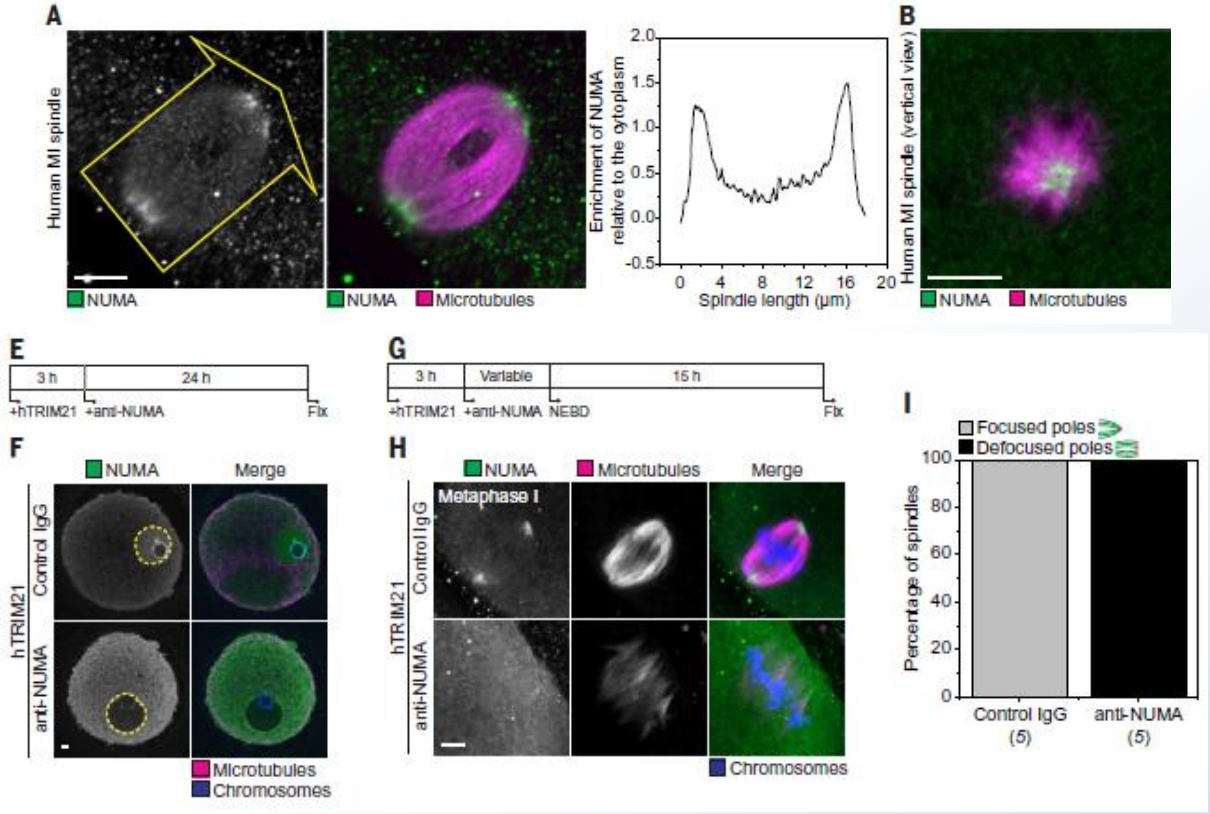


In contrast to Importazole, RanT24N increased the overall RanGTP activity

RanT24N binds to importin β in mouse oocyte through T42 site

- **RanT24N did not act as a dominant negative mutant of Ran**

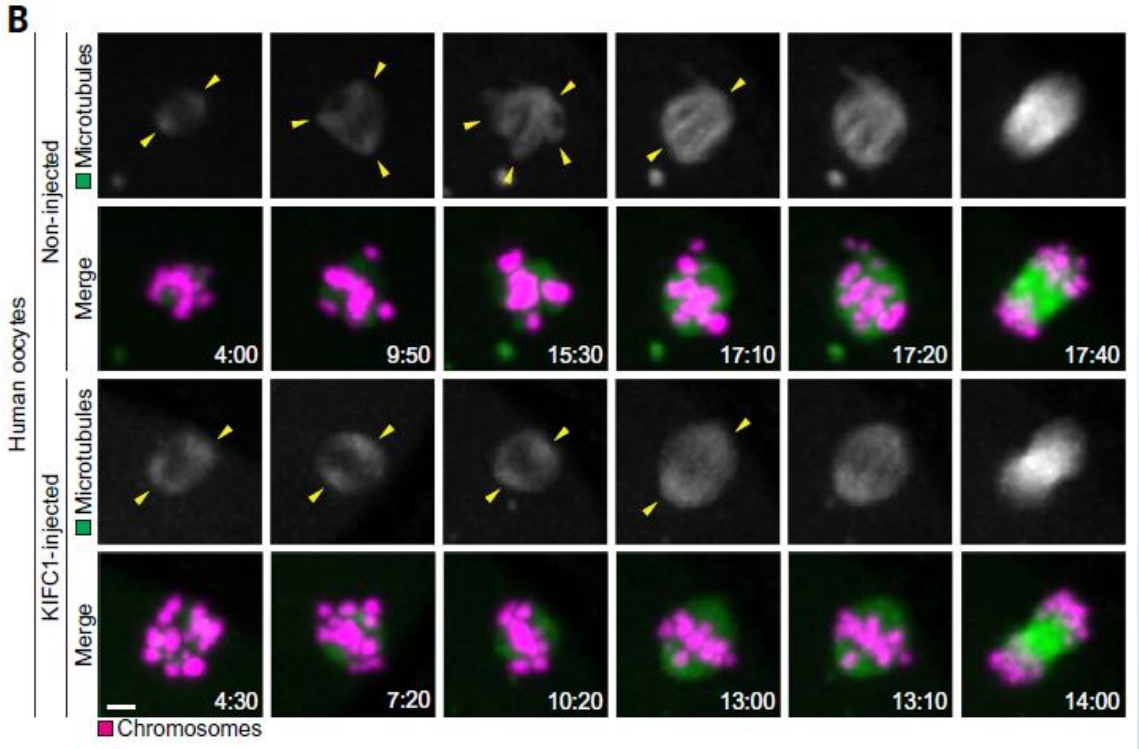
SPINDLE ASSEMBLY IN HUMAN OOCYTES



So et al, 2022, Science, PMID: 35143306

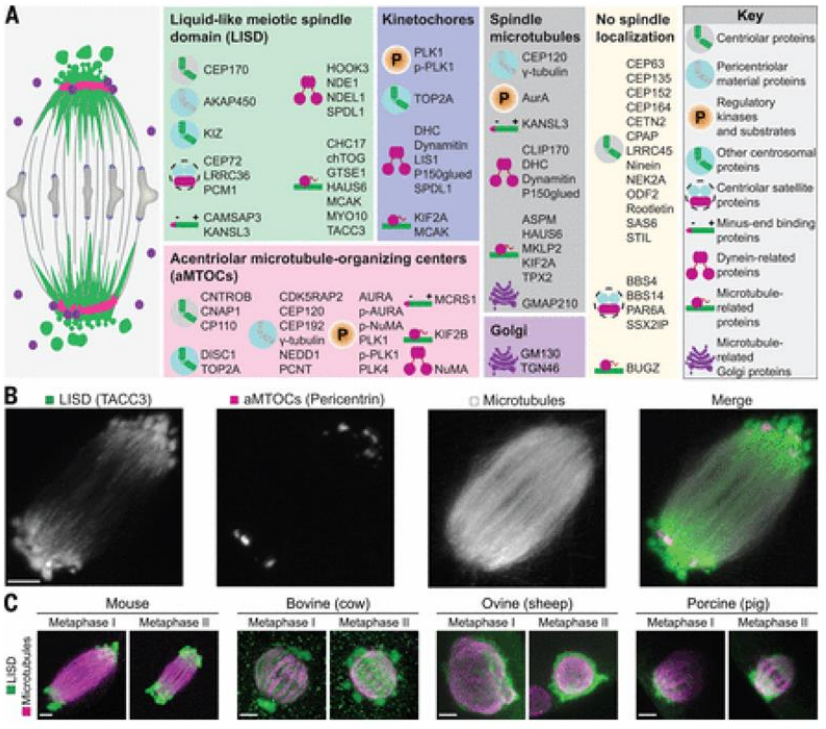
- **microtubule cross-linking protein NUMA localized to microtubule minus ends (spindle poles) in human oocytes**
- **NUMA-depleted human oocytes formed spindles with defocused poles**

SPINDLE ASSEMBLY IN OOCYTES – IMPLICATIONS FOR HUMAN INFERTILITY

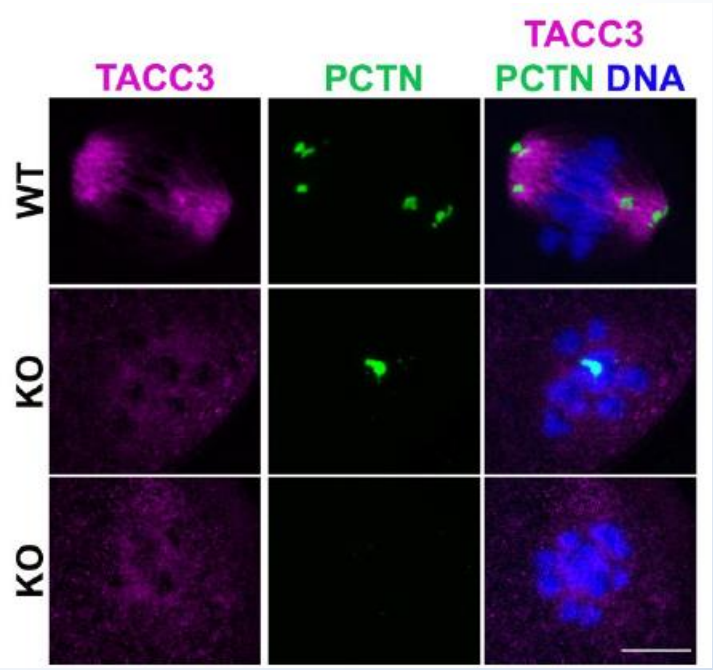


- KIFC1 stabilizes the spindle poles and prevents their fragmentation
- KIFC1 is present in other mammalian oocytes but deficient in human oocytes
- Microinjection of KIFC1 rescued stabile spindle poles formation in human oocytes

LISD AS A ALTERNATIVE STRATEGY FOR SPINDLE FORMATION IN MAMMALIAN OOCYTES



So et al, 2019, Science

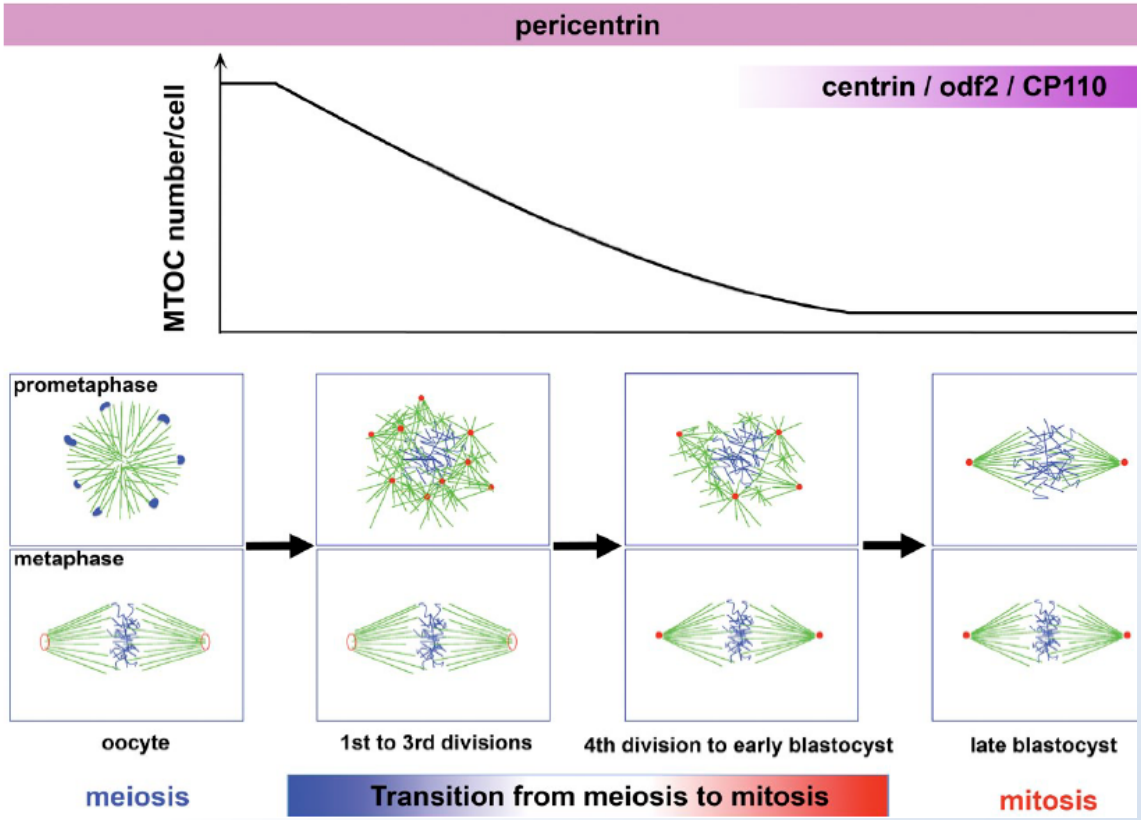


TACC3 does not localize properly in *Aurka* knockout oocytes

Blengini et al, 2021, *PLoS Genetics*

- **The LISD selectively concentrates multiple microtubule regulatory factors and allows them to diffuse rapidly within the spindle volume.**
- **LISD formation is regulated by AURKA and PLK1**

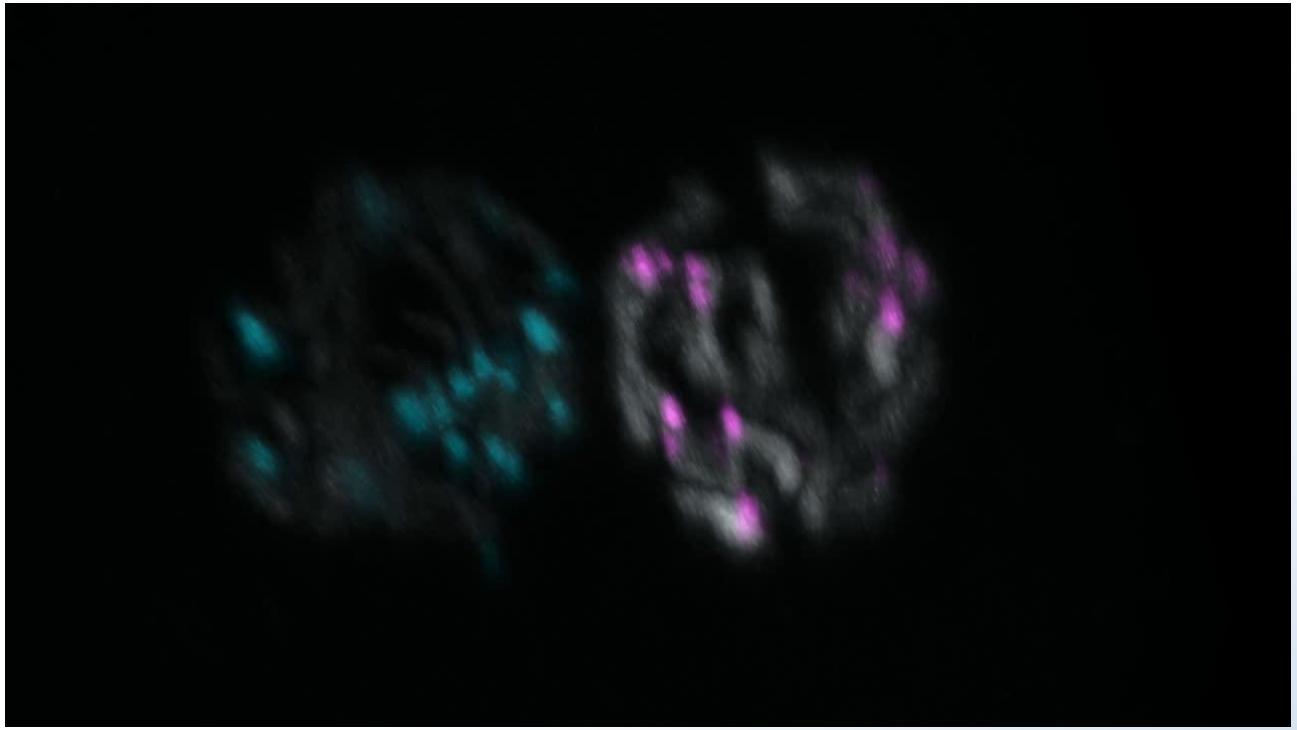
MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



Courtois et al, 2017, *J Cell Biol., PMID: 22851319*

- the number of cellular **MTOCs** progressively **decreased**, the spindle **pole** gradually became **more focused**, and **spindle length** progressively **scaled down** with cell size

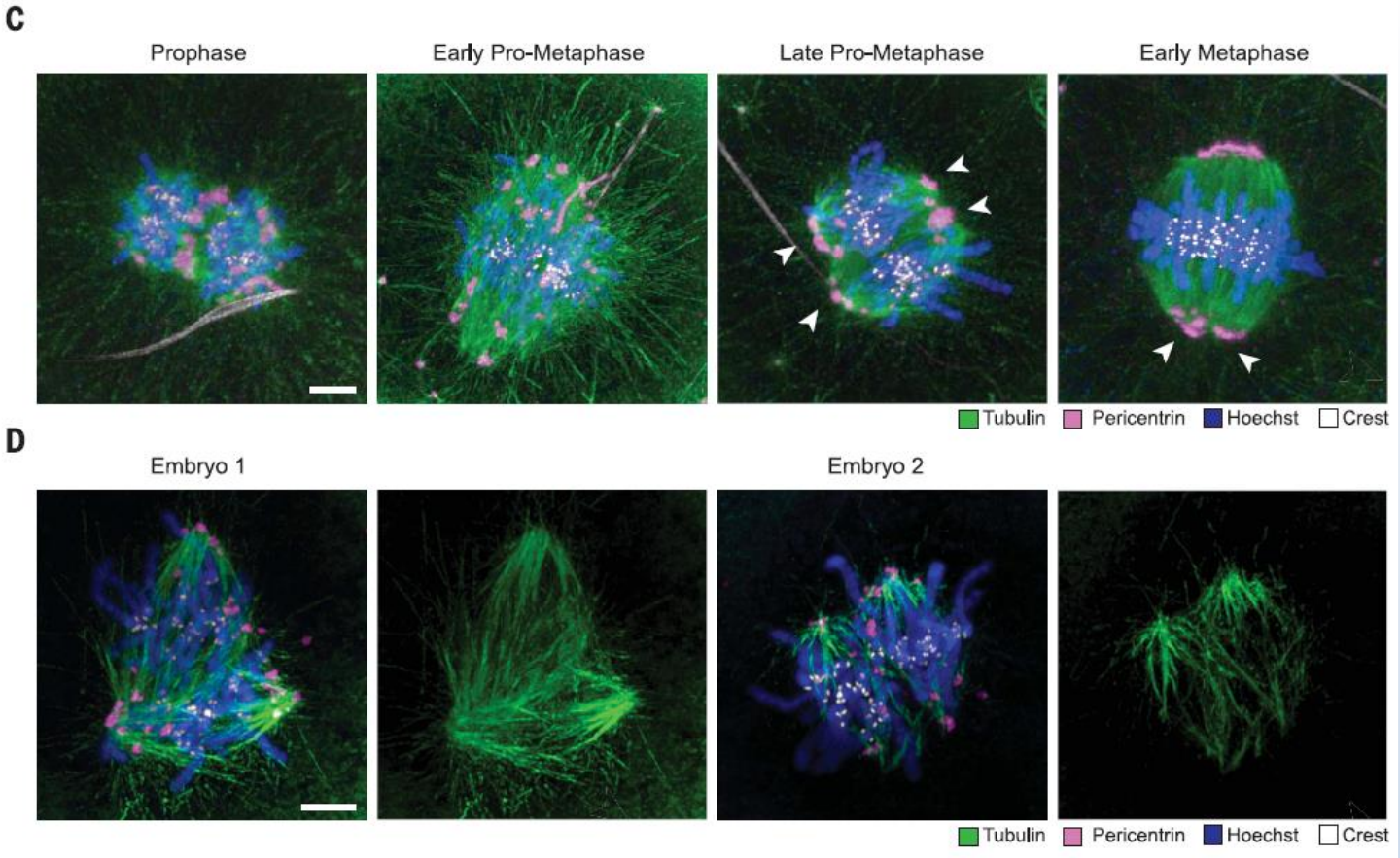
MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



Live-cell imaging of mouse zygote for differential labelling of maternal (magenta) and paternal (cyan) centromeres. Chromosome arms are labelled with H2B-mCherry (grey). Time resolution is 7.5 min, Reichmann et al, 2018, *Science, PMID:30002254*

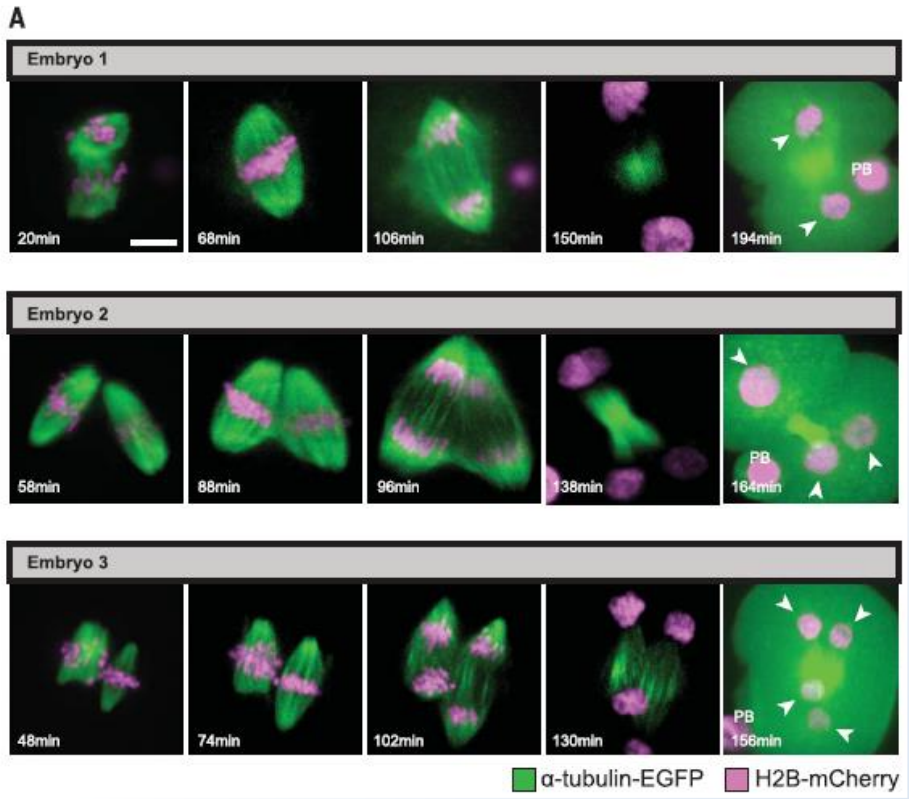
- **The paternal and maternal genome remain spatially separate throughout the first mitosis of mouse zygotes**

MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



- **Individual bipolar spindle formation around each pronucleus in mouse zygotes**

MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



Reichmann et al, 2018, *Science, PMID:30002254*

- **Failure to align the two zygotic spindles gives rise to multinucleated two-cell stage embryos**

TAKE-HOME MESSAGES

- differences between **mitosis** and **meiosis**
- **cell cycle arrest** at prophase I and metaphase II
- **meiotic recombination**
  - highly regulated process promote the formation of at least one crossover per bivalent – **prerequisity for proper chromosome segregation in meiotic divisions**
- **meiotic resumption** from the prophase I regulated by **CDK1**
- **maintaining of prophase I arrest**
- **meiotic maturation**
- alternative pathways for **spindle assembly**
- SAC lacks stringency
- **meiotic-to-mitotic transition**
- **cell cycle adaptations** in early embryos