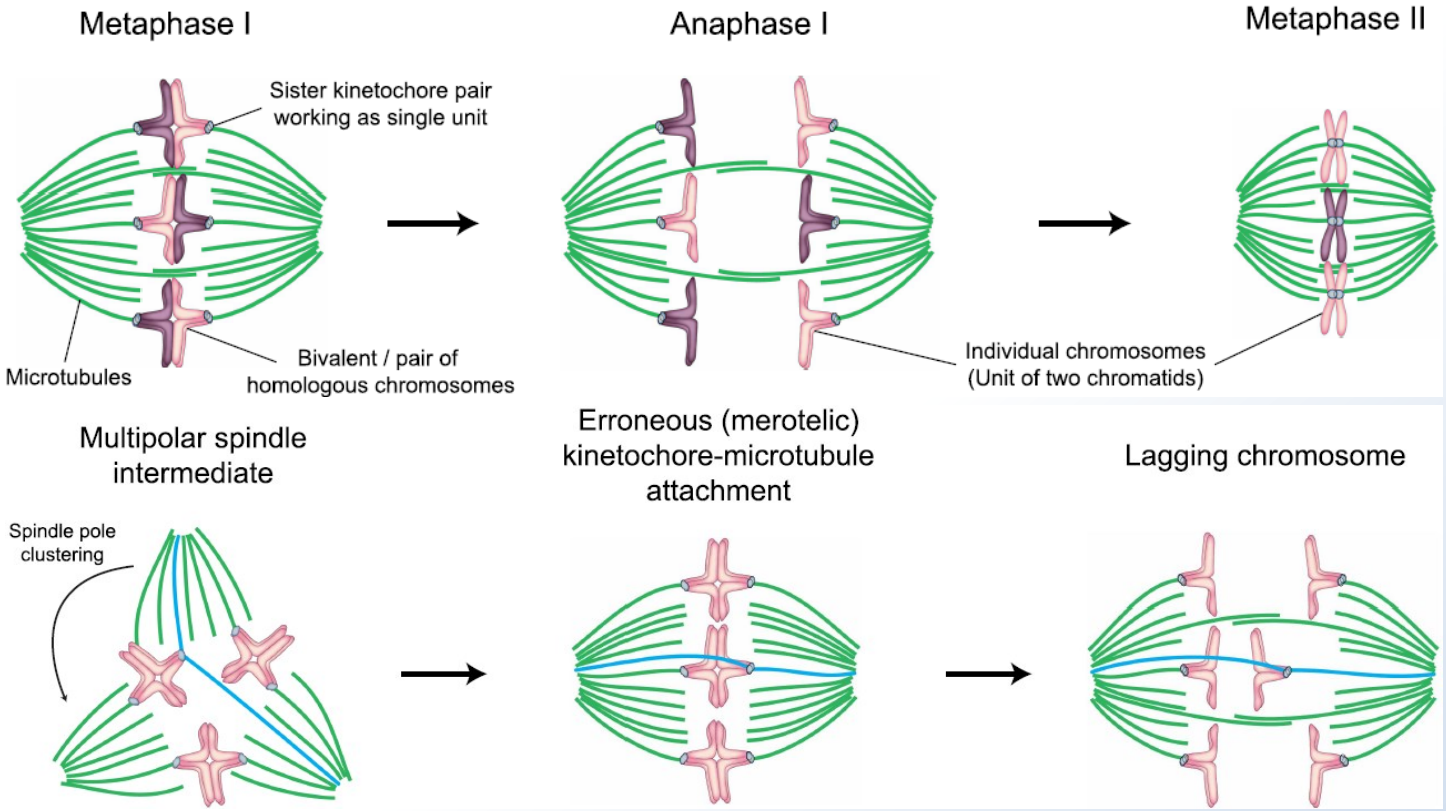
CHROMOSOME SEGREGATION AND CONFIGURATION IN MAMMALIAN OOCYTES

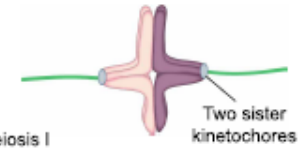


Thomas et al, 2021, *Biochemical Society Transaction*

- **Aneuploidy in human eggs: contributions of the meiotic spindle**

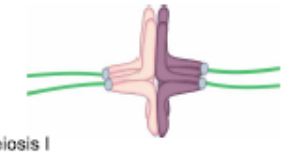
CHROMOSOME MISSEGREGATION IN MAMMALIAN OOCYTES

i. Correct alignment in meiosis I



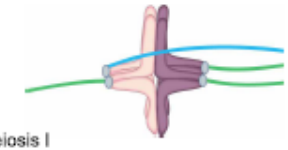
- In meiosis I, sister kinetochores of a single chromosome should act as one functional unit.
- In this way, sister kinetochores face the same spindle pole and whole chromosomes can segregate in anaphase I.

ii. Sister kinetochore separation



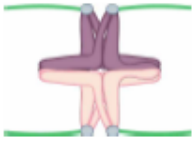
- Sister kinetochores separate as women become older.
- Separated sister kinetochores interact with microtubules independently.

iii. Merotelic attachment



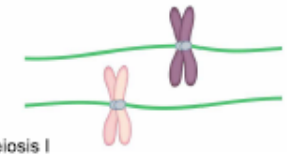
- Separation of kinetochores increases the probability of merotelic attachments.
- Merotelic attachments increase with maternal age.

iv. Bivalent rotation



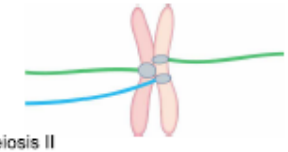
- Separation of sister kinetochores allows bivalents to rotate on the spindle.
- Here, sister kinetochores orient like in mitosis.
- This could result in the reverse segregation pattern of chromosome segregation.

v. Bivalent splitting



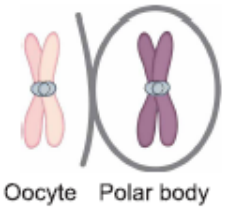
- Bivalents may prematurely separate into univalents prior to anaphase I.
- These univalents can align on the spindle and could give rise to PSSC and reverse segregation.

vi. Kinetochore fragmentation



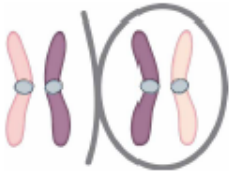
- Kinetochores fragment into multiple lobes in oocytes.
- Fragmented kinetochores frequently form incorrect attachments with spindle microtubules in meiosis II.

B i. Normal segregation

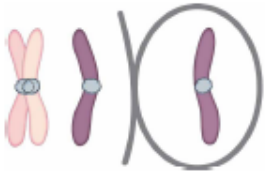


ii. Nondisjunction

iii. Reverse segregation



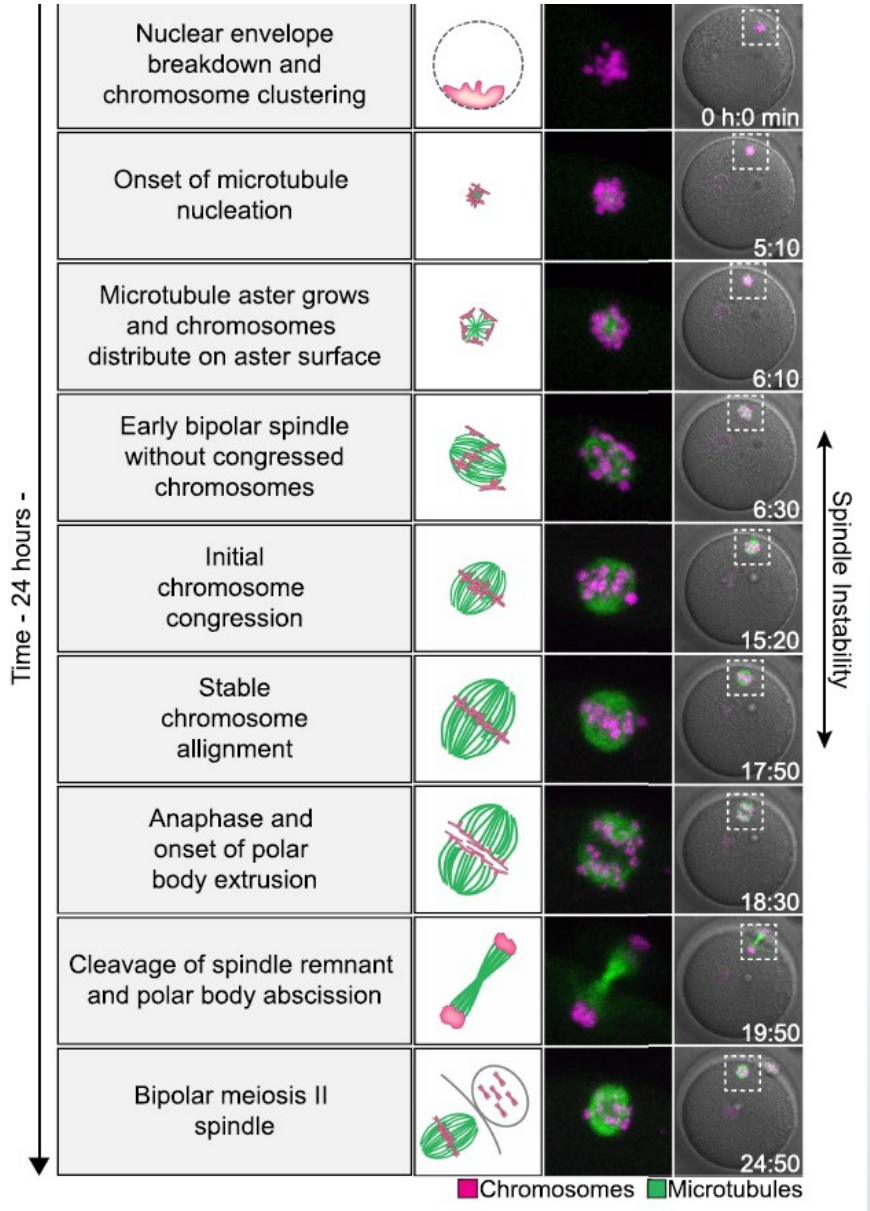
iv. Premature separation of sister chromatids (PSSC)



Thomas et al,, 2021,

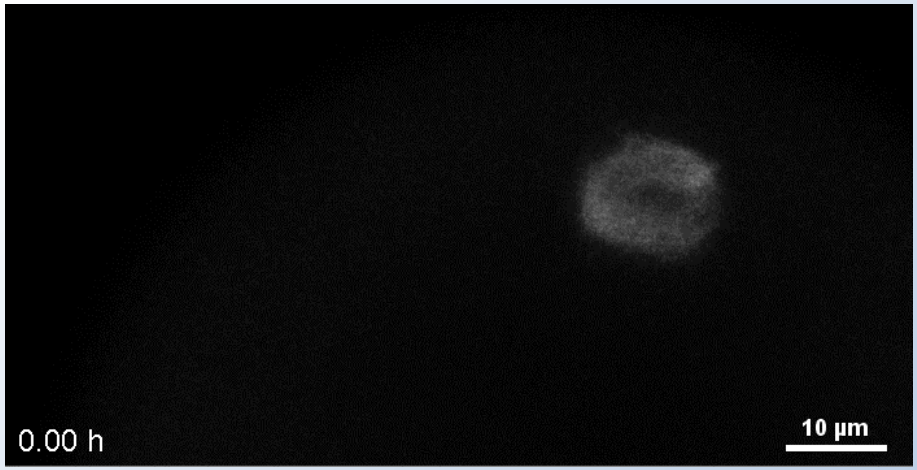
Biochemical Society Transactions

SPINDLE ASSEMBLY IN OOCYTES – IMPLICATIONS FOR HUMAN INFERTILITY



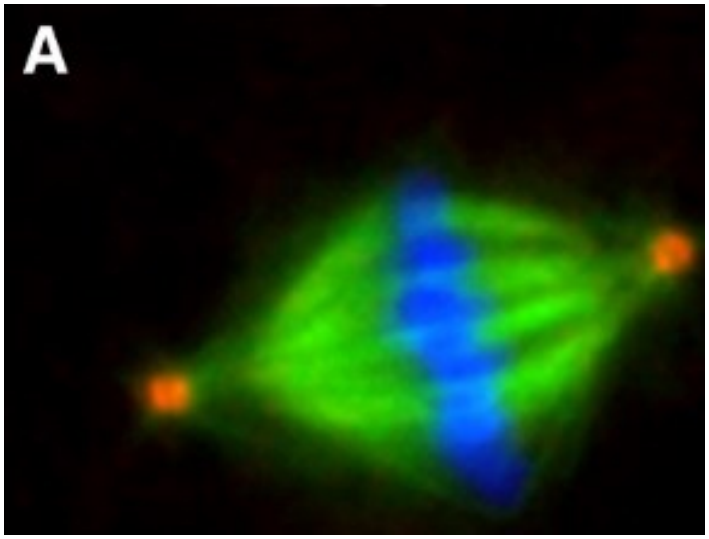
Thomas et al, 2021, *Biochemical Society Transaction*

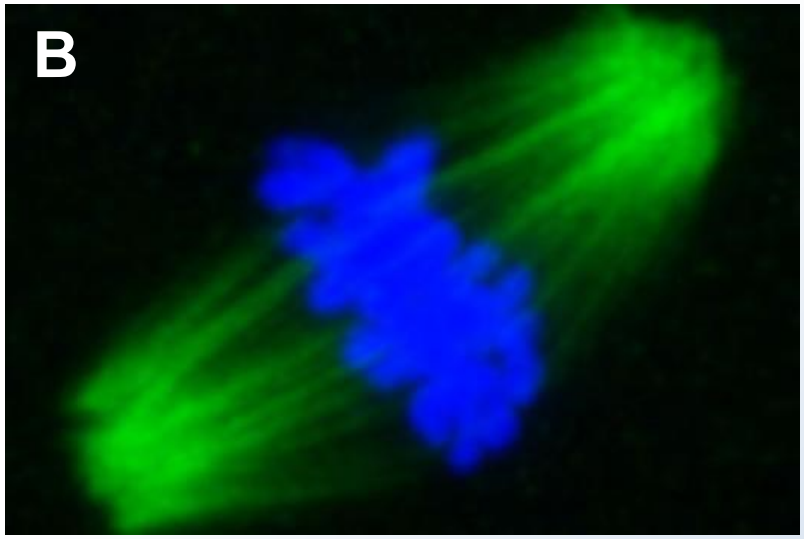
- **spindle instability** during meiosis I in human oocytes leads to lagging chromosomes in anaphase I



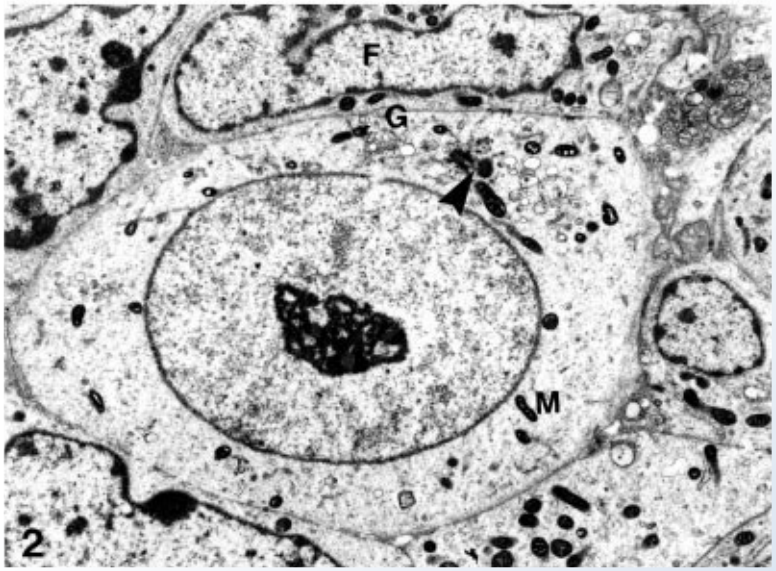
Holubcova et al, 2015, *Science*

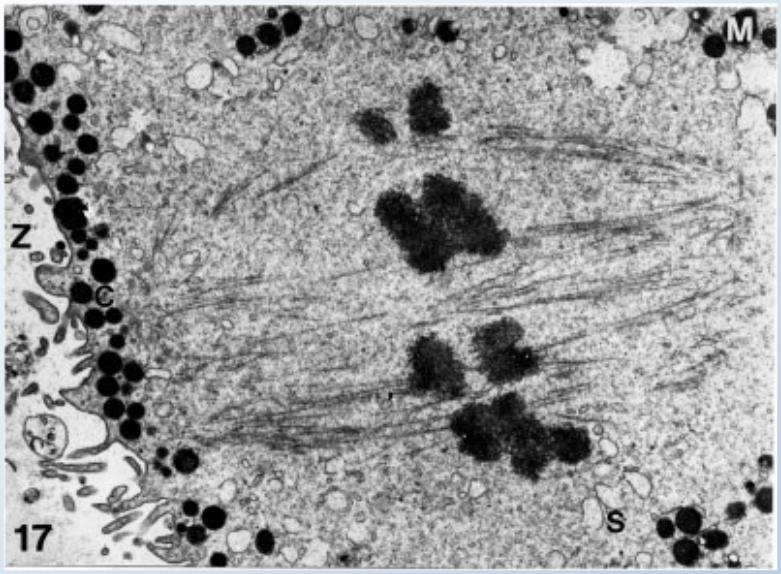
MITOTIC VS MEIOTIC SPINDLE



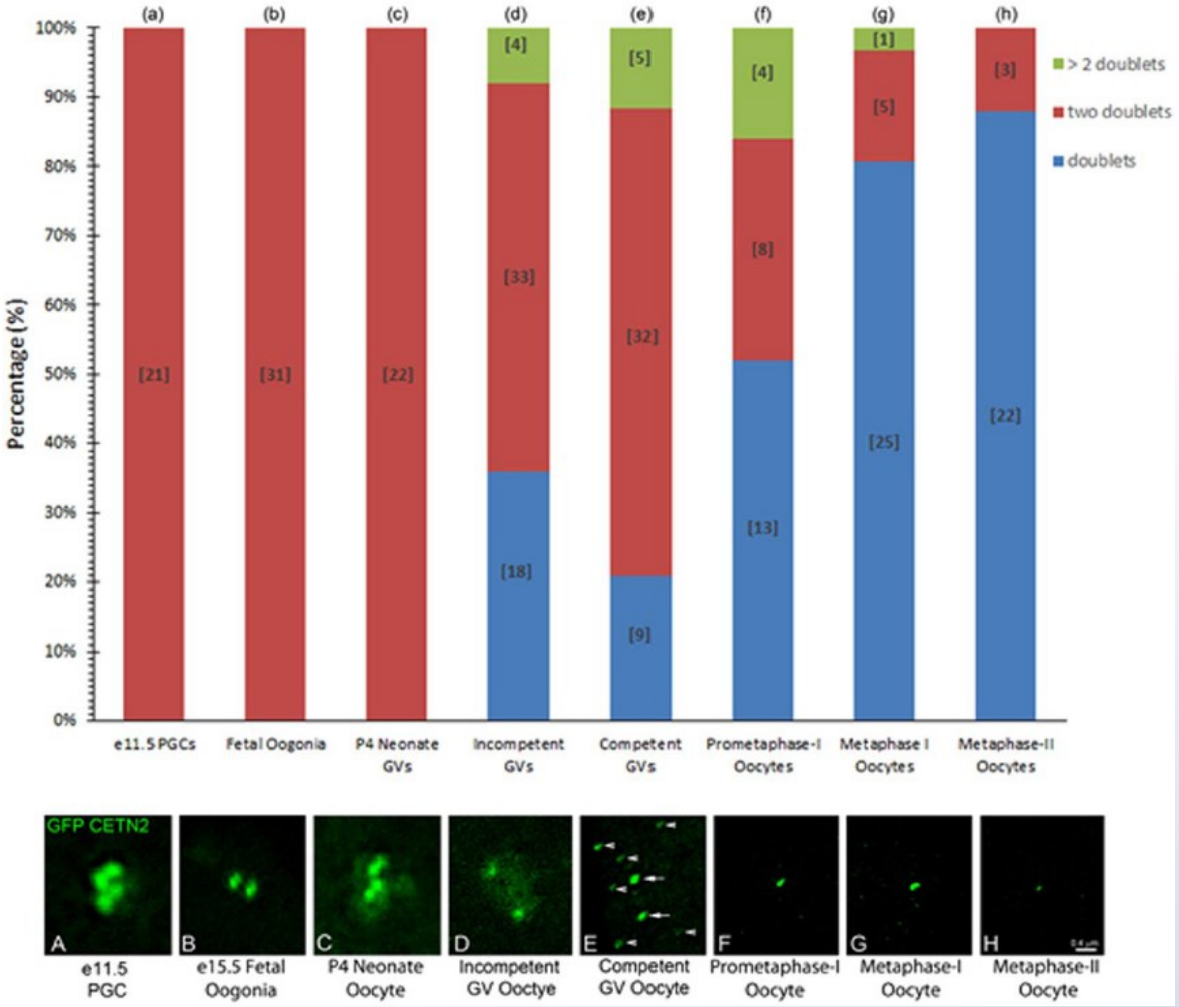


- **centriole loss** in matured eggs was detected by **electron microscopy**





CENTRIOLE ARE NOT LOST UNTIL THE COMPLETION OF MOUSE OOCYTE MATURATION

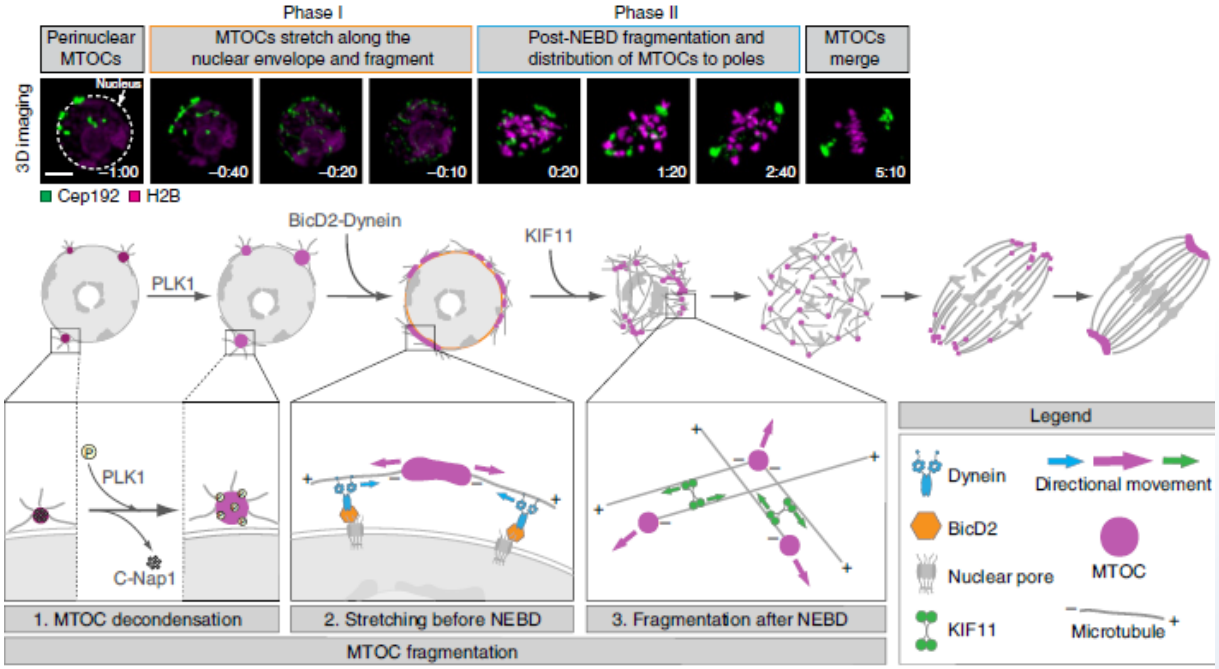


- GFP-centrin-2 transgenic mice
- PGC - primordial germ cells

Tracking GFP CETN2 centriole loss and separation from PGCs through maturation to metaphase-II arrest

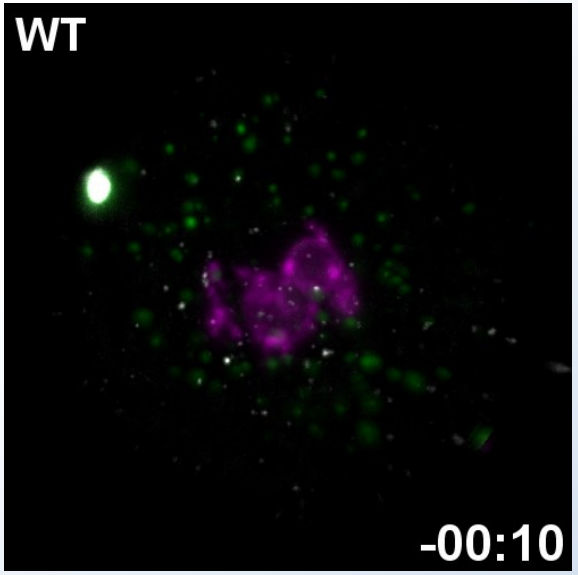
Simerly et al, 2018, *Sci Rep.,* PMID: 30143724

ALTERNATIVE STRATEGIES FOR SPINDLE BUILDING - MICROTUBULE ORGANIZING CENTRES (MTOCS) IN MOUSE OOCYTES



Three-step mechanism of MTOC fragmentation in mouse oocytes

Clift a Schuh, 2014, *Nature Communications*

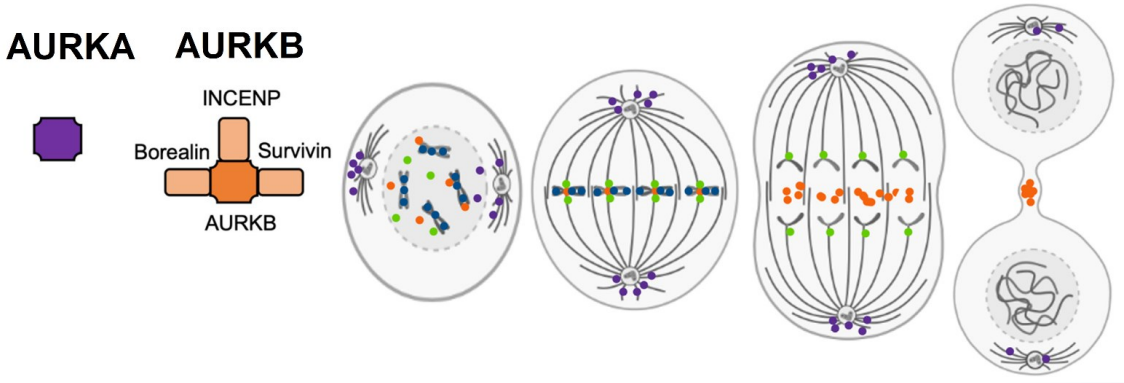


Blengini et al, 2021, *PLoS Genetics*

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

- **Failure to fragment or cluster MTOCs** leads to defects in spindle assembly, **which increase risk of aneuploidy.**

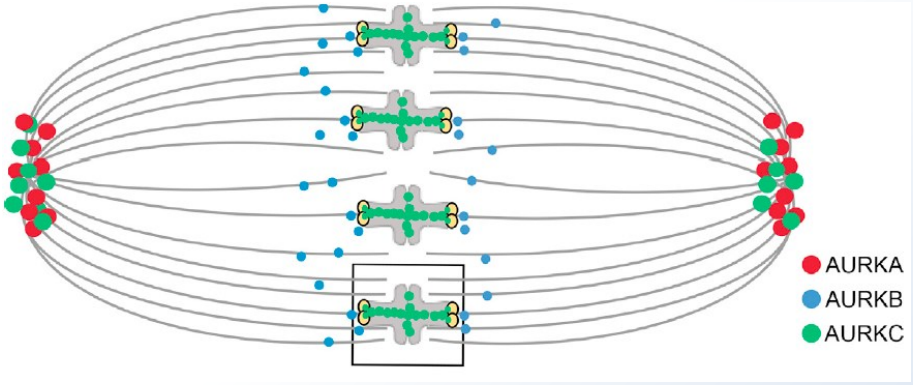
ROLE OF AURORA KINASES (AURKS) IN SPINDLE ASSEMBLY AND MTOCS BEHAVIOUR IN MOUSE OOCYTES



Pajpach et al, 2021, *Genes*

Somatic cells

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



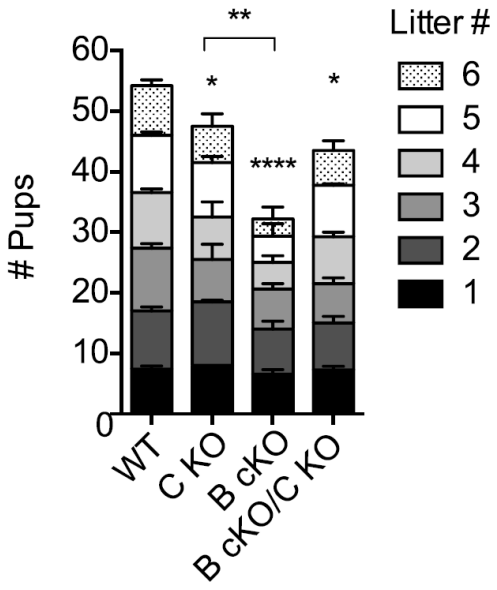
Nguyen a kol., 2018, *Current Biology*

Germ cells

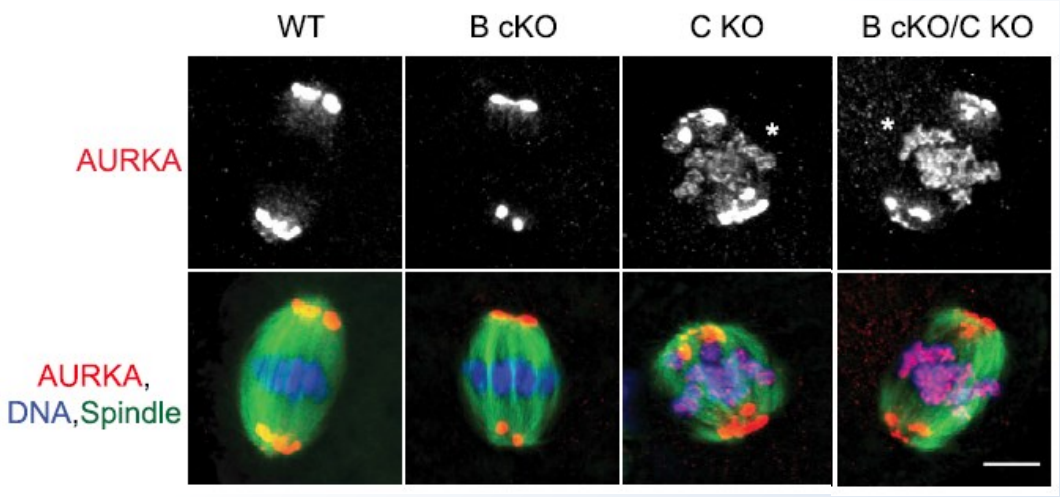
- **AURKA** – MTOCs
- **AURKB** – ?
- **AURKC** – CPC, MTOCs

- 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**

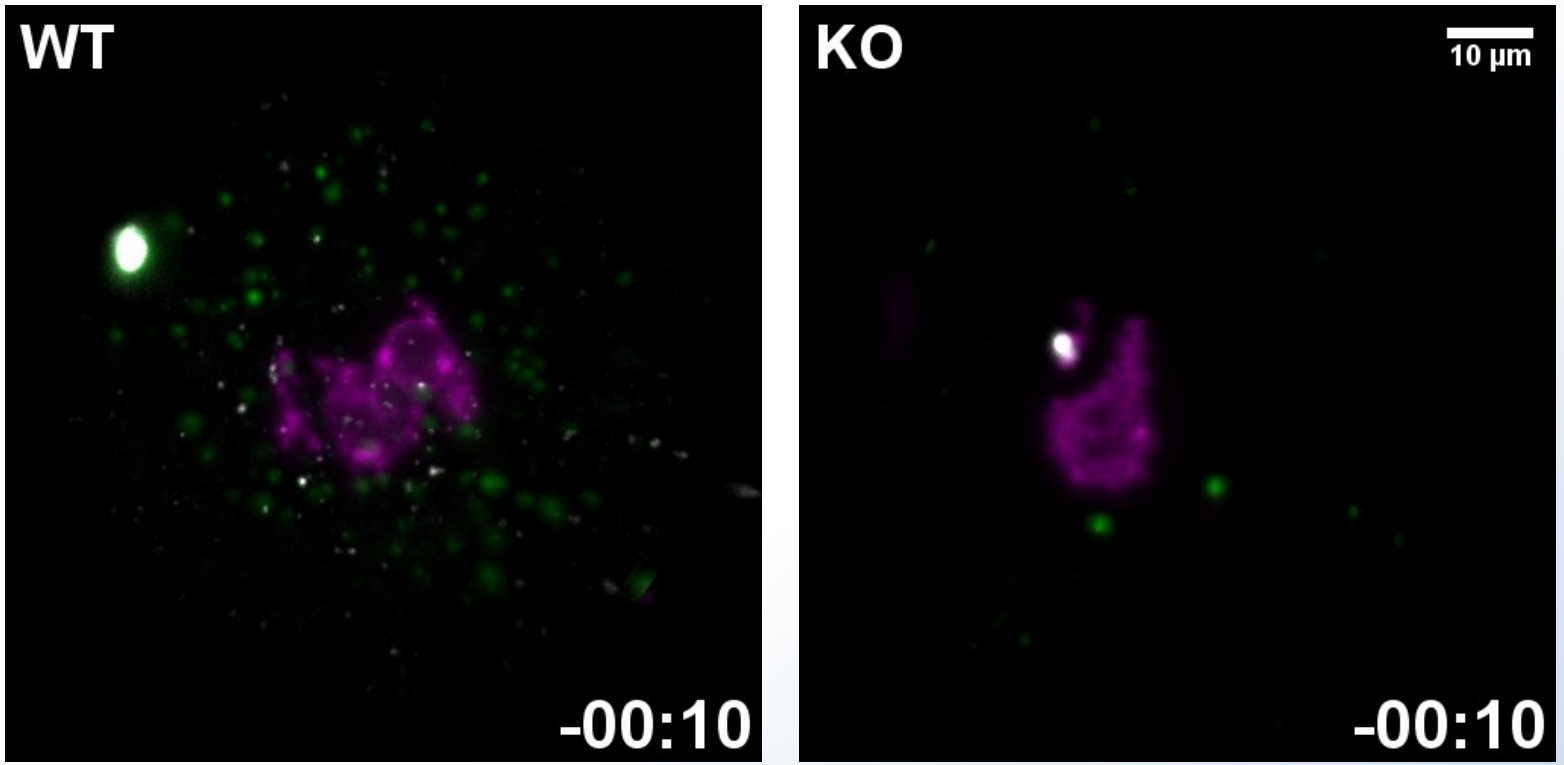


AURKA localized to chromosomes in oocytes upon deletion of *Aurkc*

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



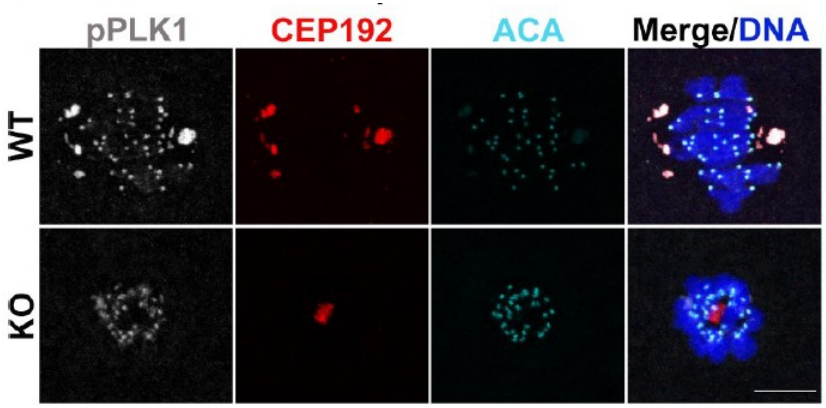
***Aurka* knockout oocytes (KO) are arrested in Metaphase I with defective spindle**

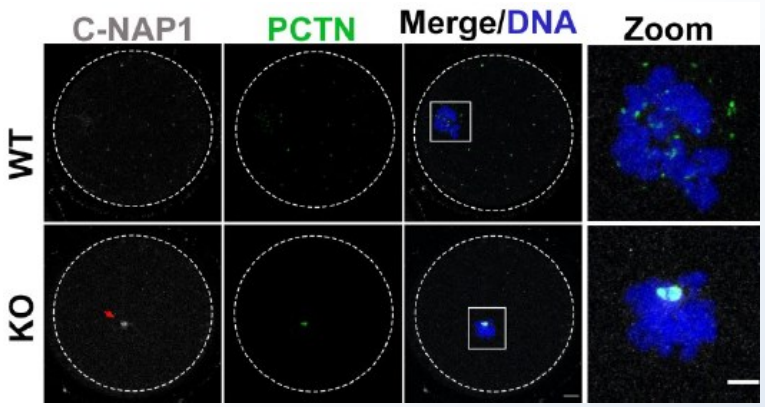
Blengini et al, 2021, *PLoS Genetics*

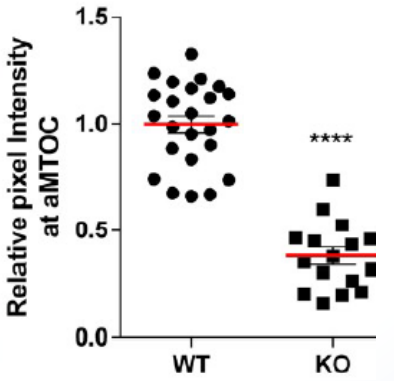
Chromosomes, spindle, MTOCs, time (hh:mm) after meiotic resumption

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

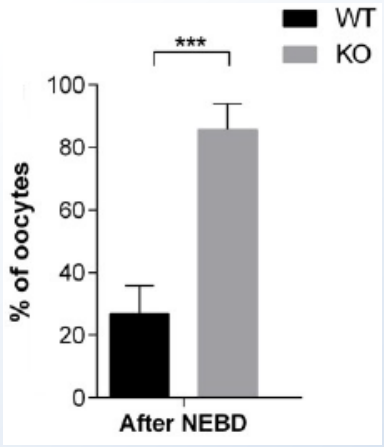
ROLE OF AURKA IN MTOCS FRAGMENTATION







Decreased pPLK1 at MTOCs in Aurka KO oocytes

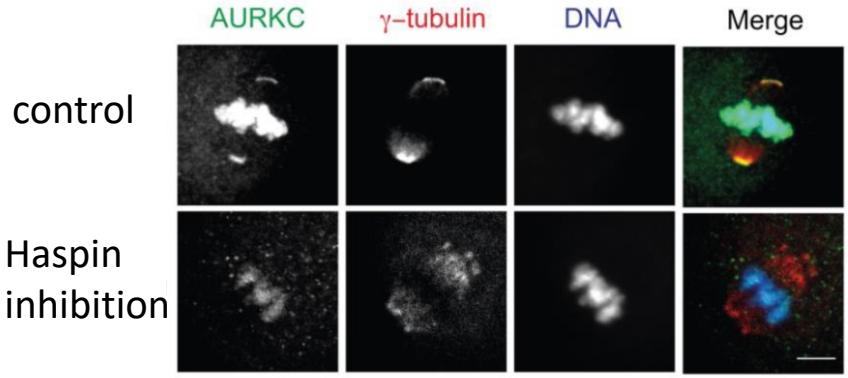


C-NAP1 persisted at MTOCs after meiotic resumption in Aurka KO oocytes

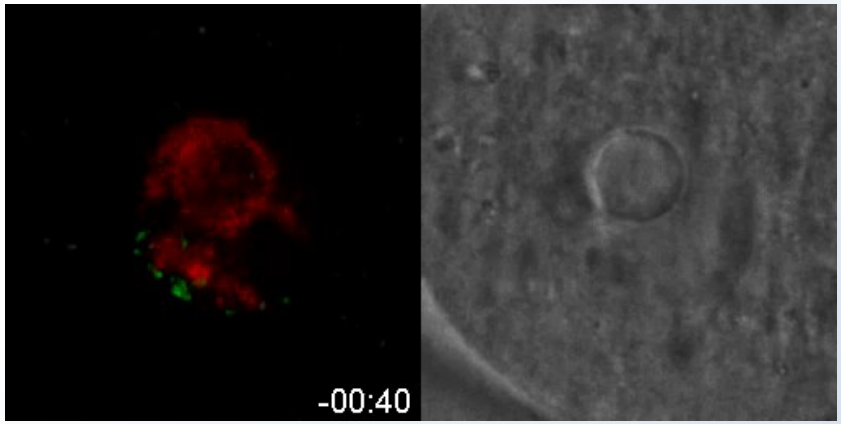
Blengini et al, 2021, *PLoS Genetics*

- **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



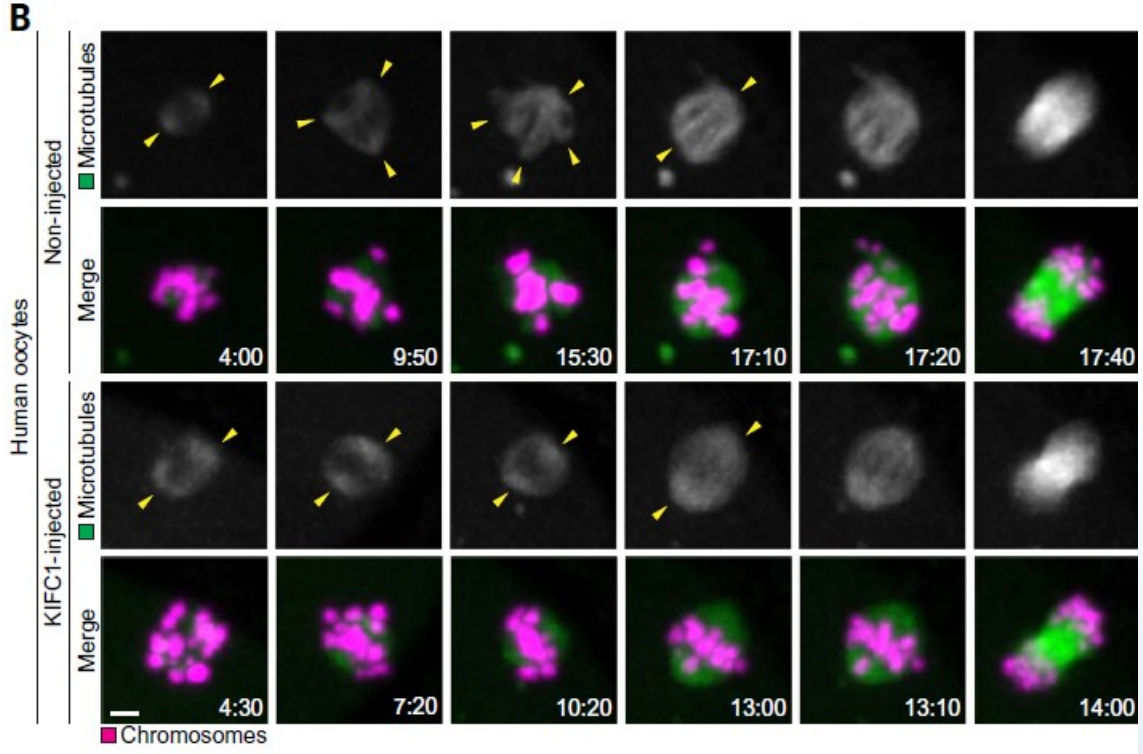
Live-cell imaging of haspin-inhibited oocytes

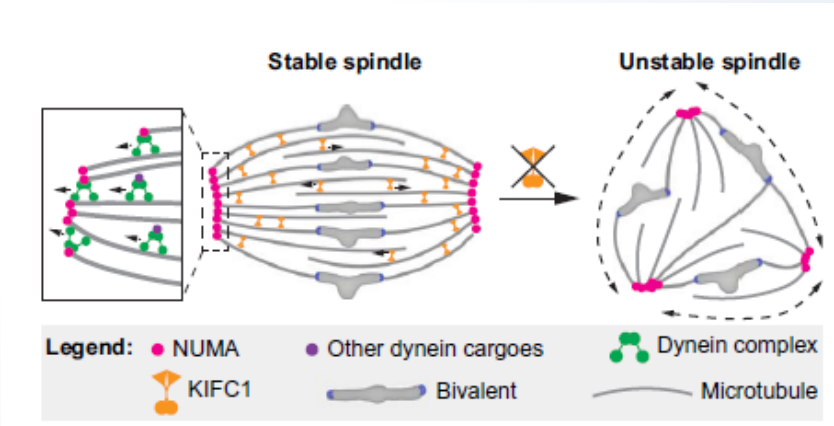
Balboula et al, 2016, *Journal of Cell Science*

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**

SPINDLE INSTABILITY IN HUMAN OOCYTES



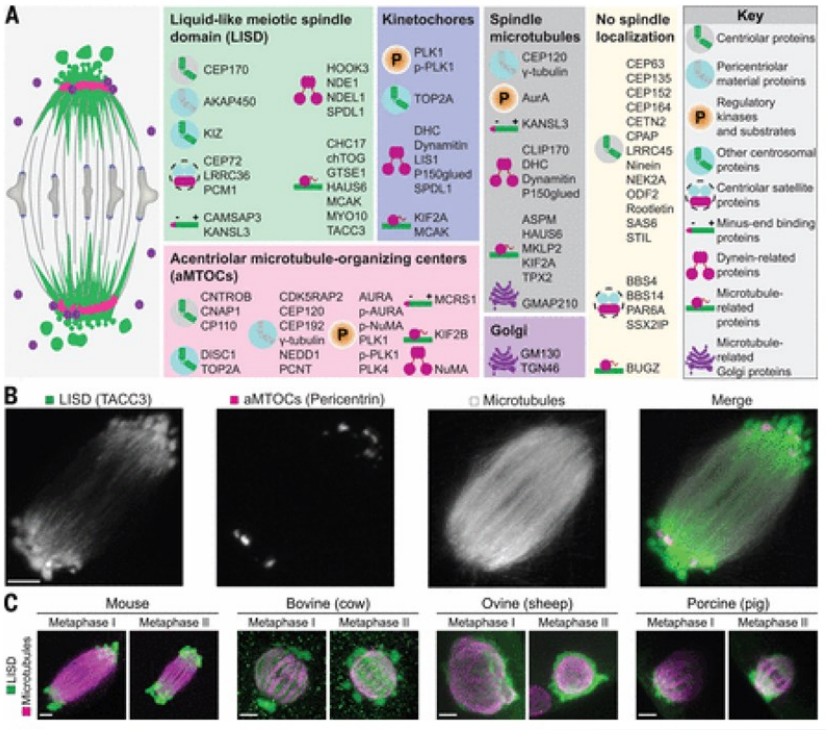


- **KIFC1** as a **spindle-stabilizing factor** that is **deficient in human oocytes** but expressed in other mammalian oocytes species

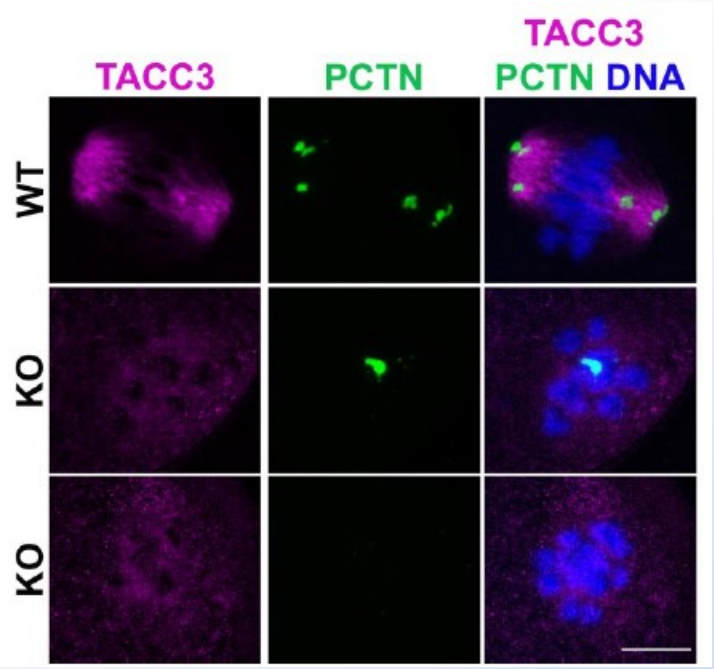
- **Introduction of exogenous KIFC1 rescues spindle instability and reduces the risk of aneuploidy in human oocytes**

So et al, 2022, Science, PMID: 35143306

LISD AS A ALTERNATIVE STRATEGY FOR SPINDLE FORMATION IN MAMMALIAN OOCYTES



So et al, 2019, Science

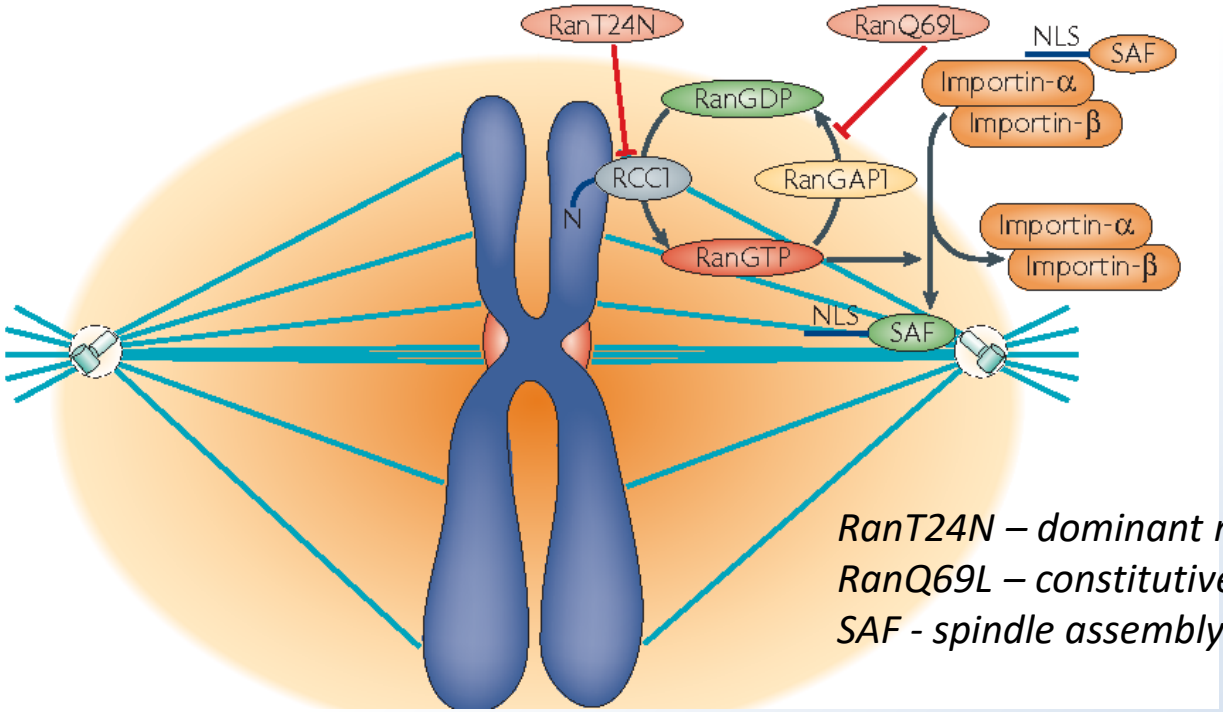


TACC3 does not localize properly in *Aurka* knockout oocytes

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**

THE ROLE OF RANGTP-IMPORTIN BETA PATHWAY IN SPINDLE ASSEMBLY



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

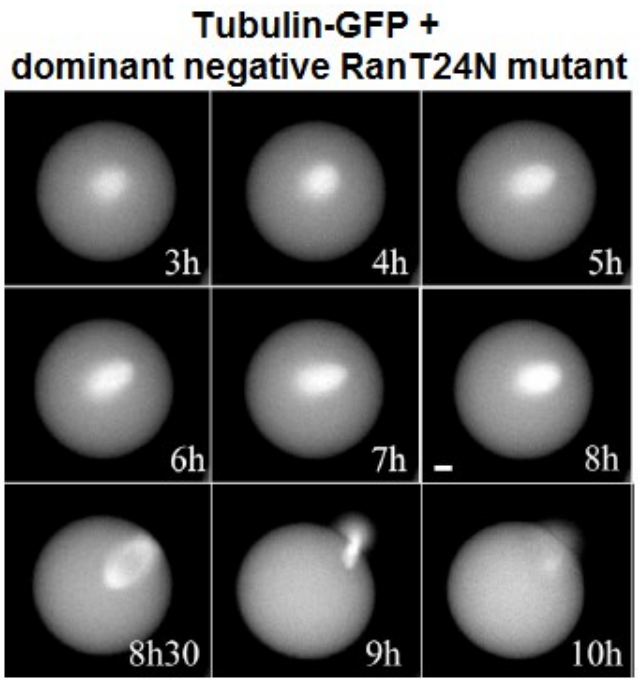
Involvement of Ran in spindle assembly

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

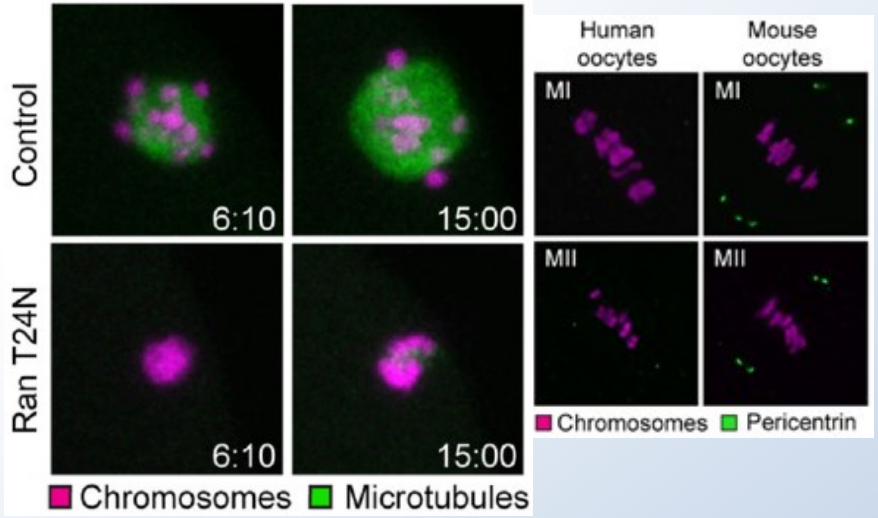
Mouse oocytes



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

RanGTP inhibition did not affect assembly of functional spindles

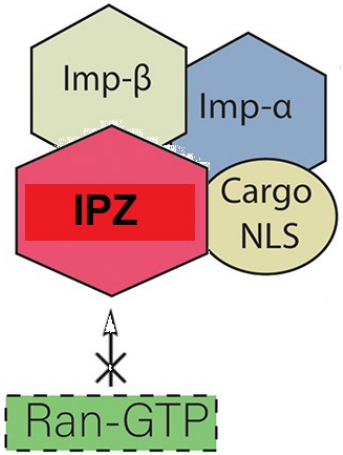
Human oocytes



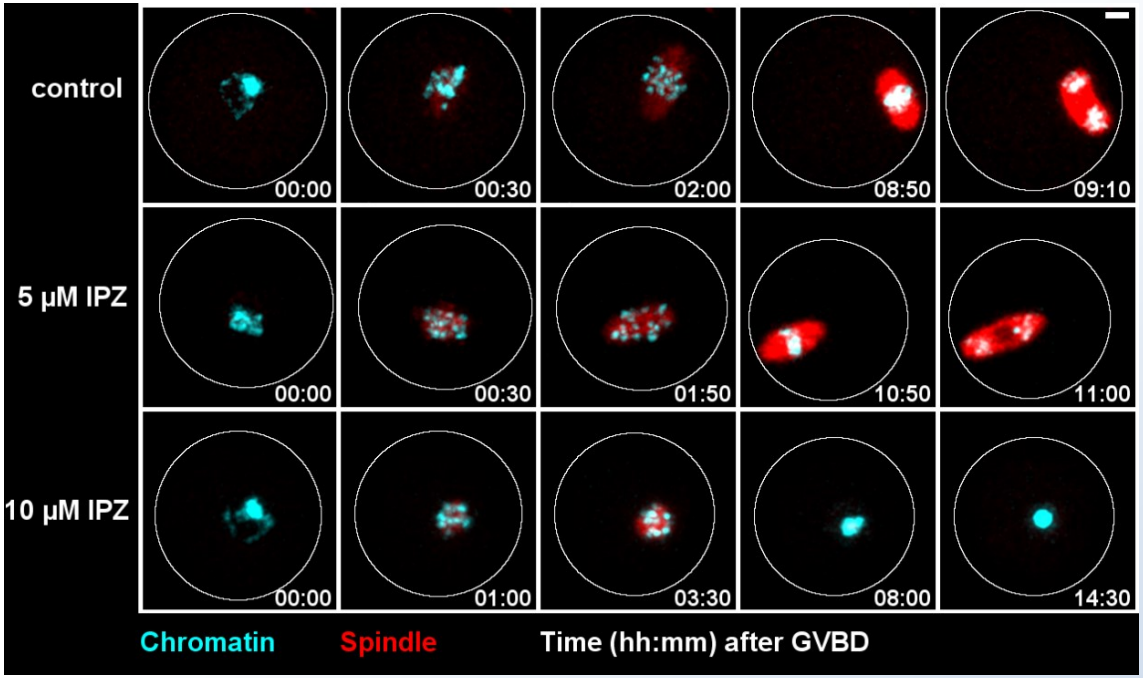
Holubcova et al, 2015, *Science*

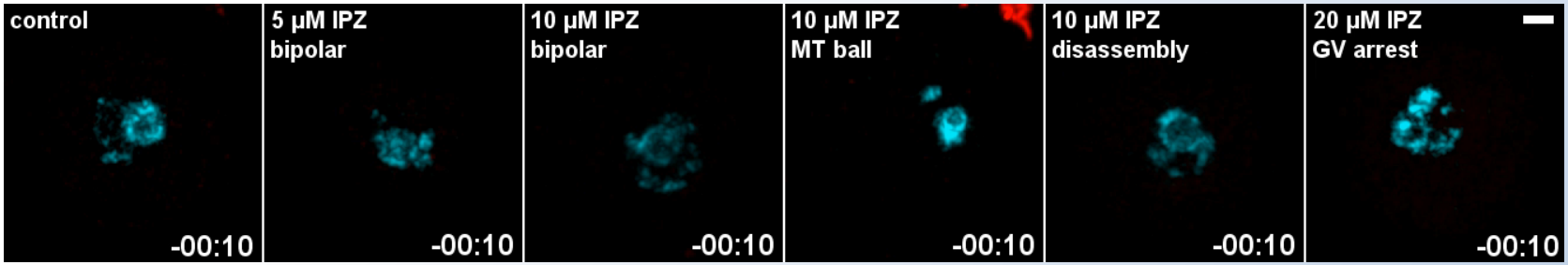
Spindle assembly is dependent on RanGTP, but independent on MTOCs

EFFECT OF RAN-GTP INHIBITOR IMPORTAZOLE (IPZ) ON SPINDLE ASSEMBLY IN MOUSE OOCYTES



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

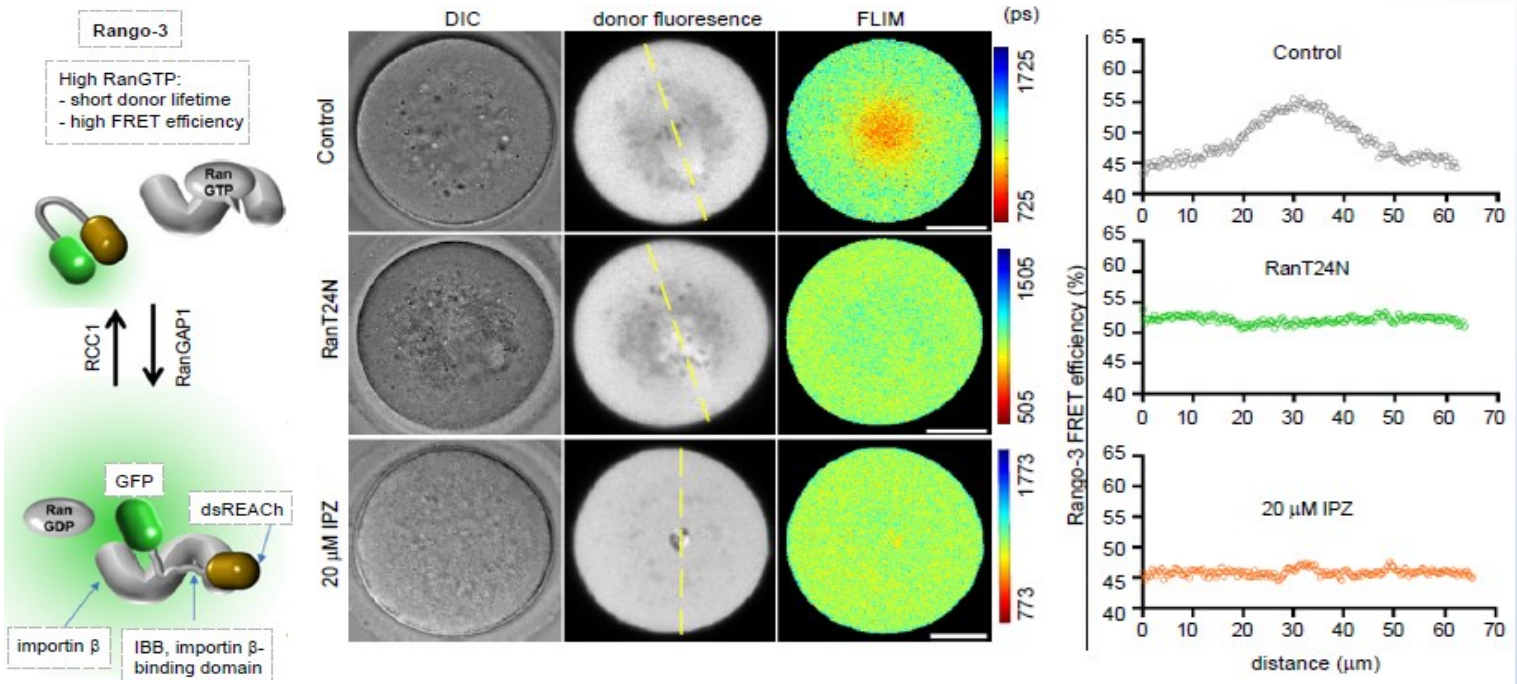




Live-cell imaging of spindle assembly in Importazole (IPZ)-treated mouse oocytes

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

EFFECT OF IMPORTAZOLE AND RANT24N ON RANGTP GRADIENT FORMATION



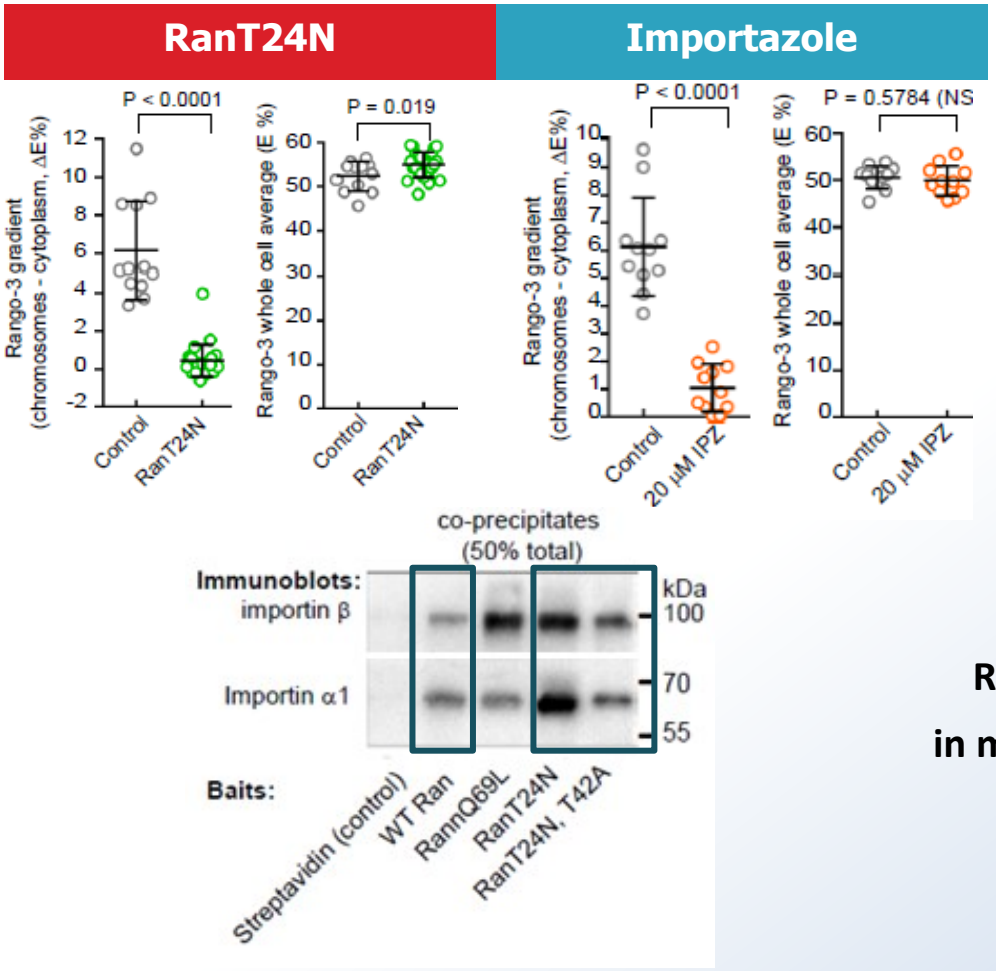
Quantitative FLIM/FRET imaging of RanGTP gradient

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

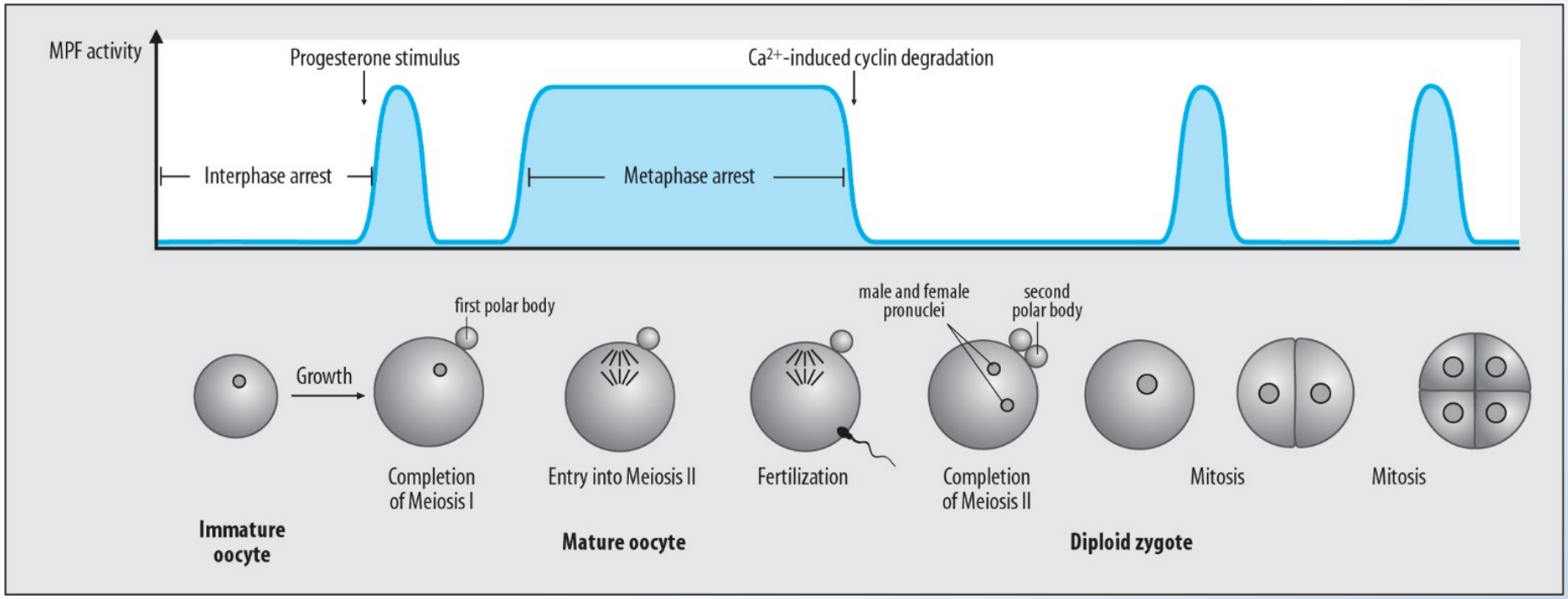


In contrast to Importazole, RanT24N increased the overall RanGTP activity

RanT24N binds to importin β in mouse oocyte through T42 site

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

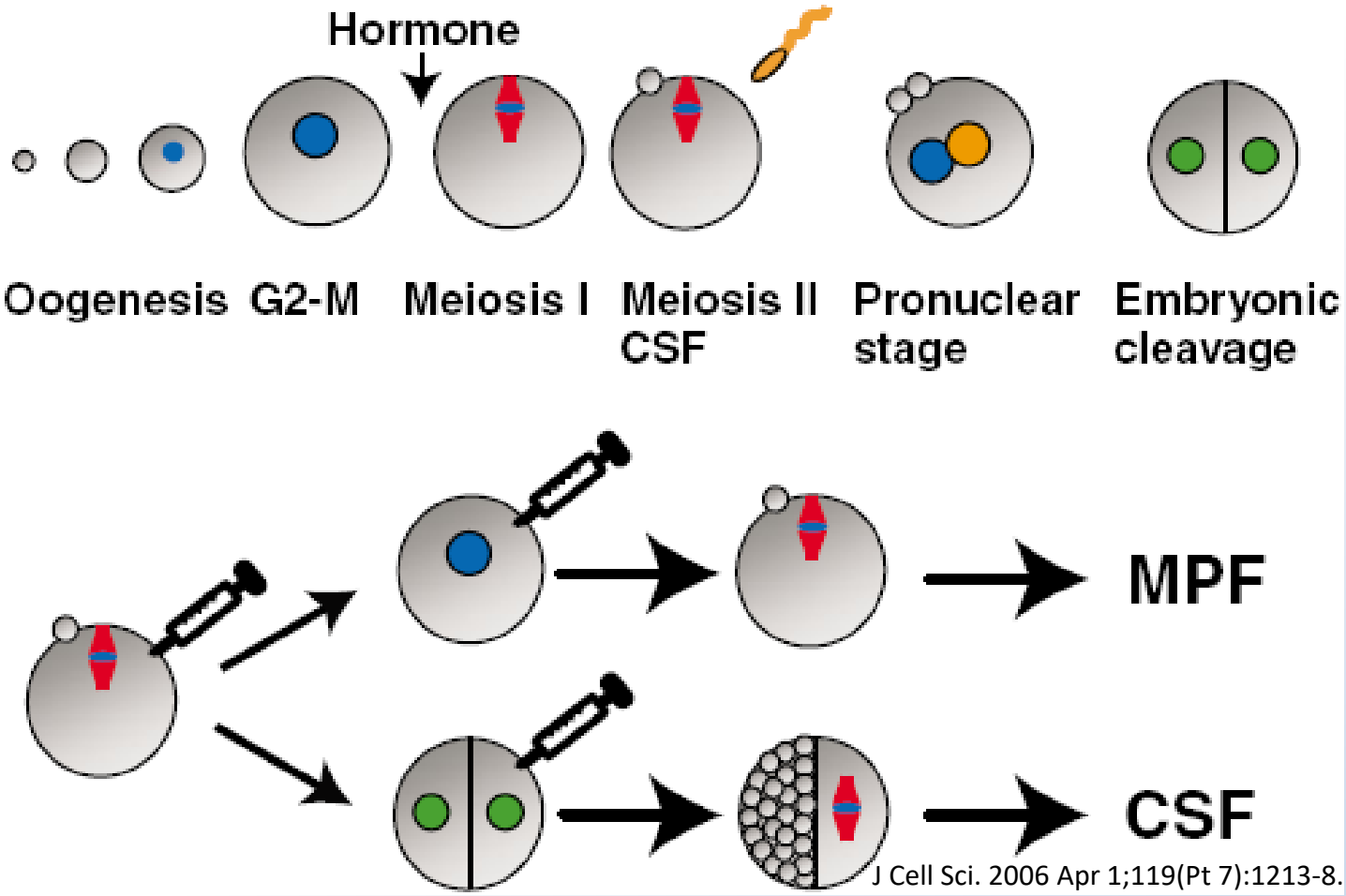
CDK1 ACTIVITY DURING MEIOTIC MATURATION



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

- mammalian oocytes proceed through meiosis I and arrest at **second meiotic metaphase with high CDK1-cyclin B1 activity.**
- **meiosis II** - absence of DNA replication

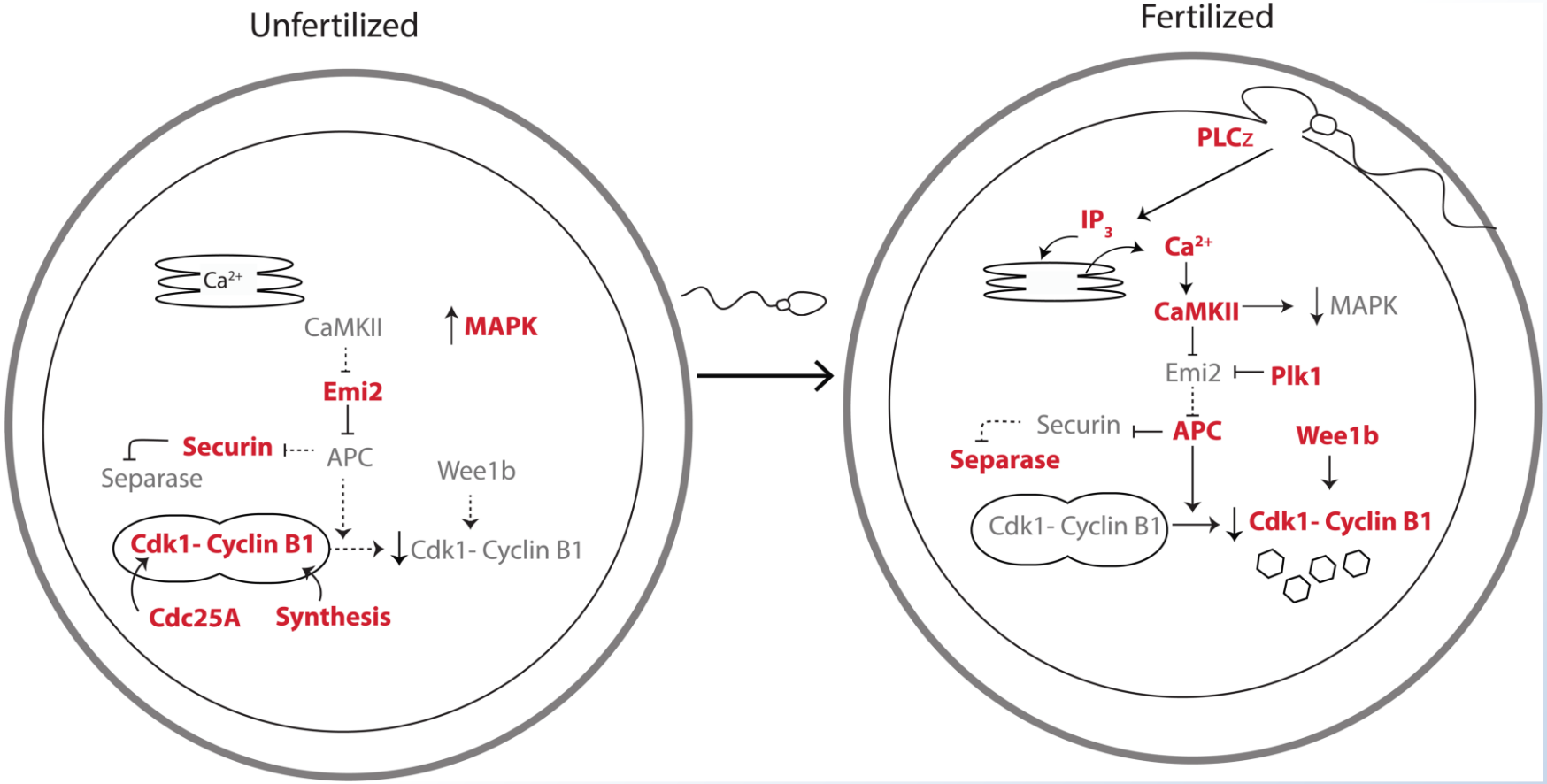
MPF AND APC ACTIVITY DURING METAPHASE II ARREST



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

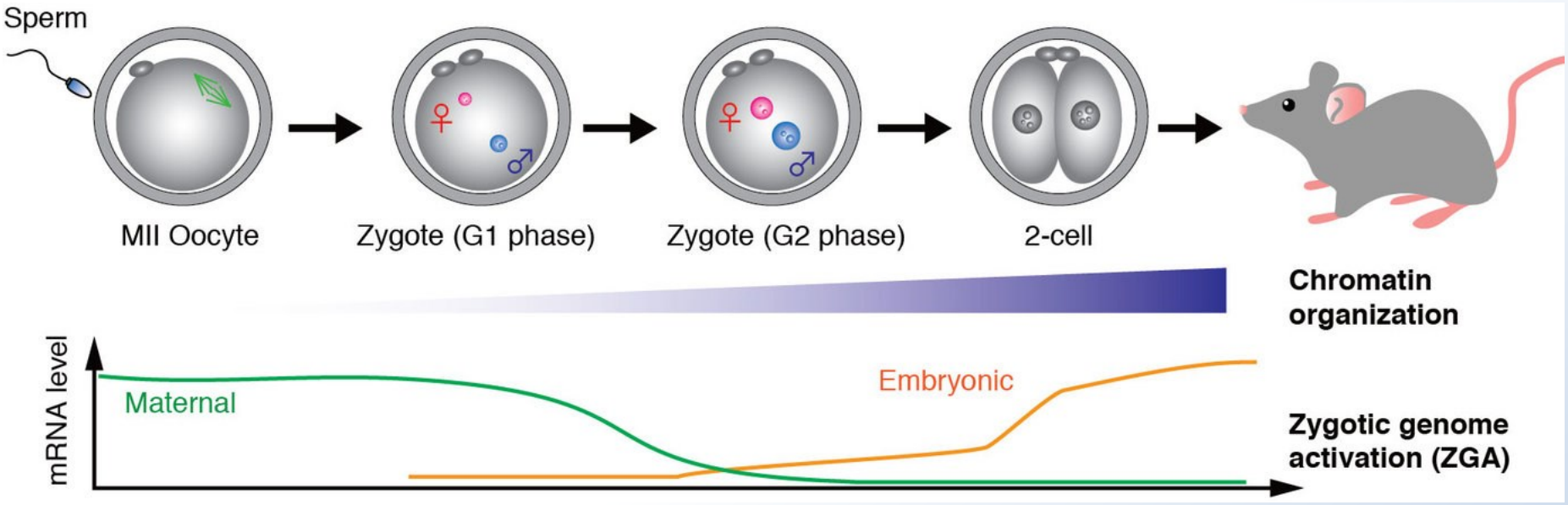


Sanders and Jones, 2018, *Biochemical Society Transactions*

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

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

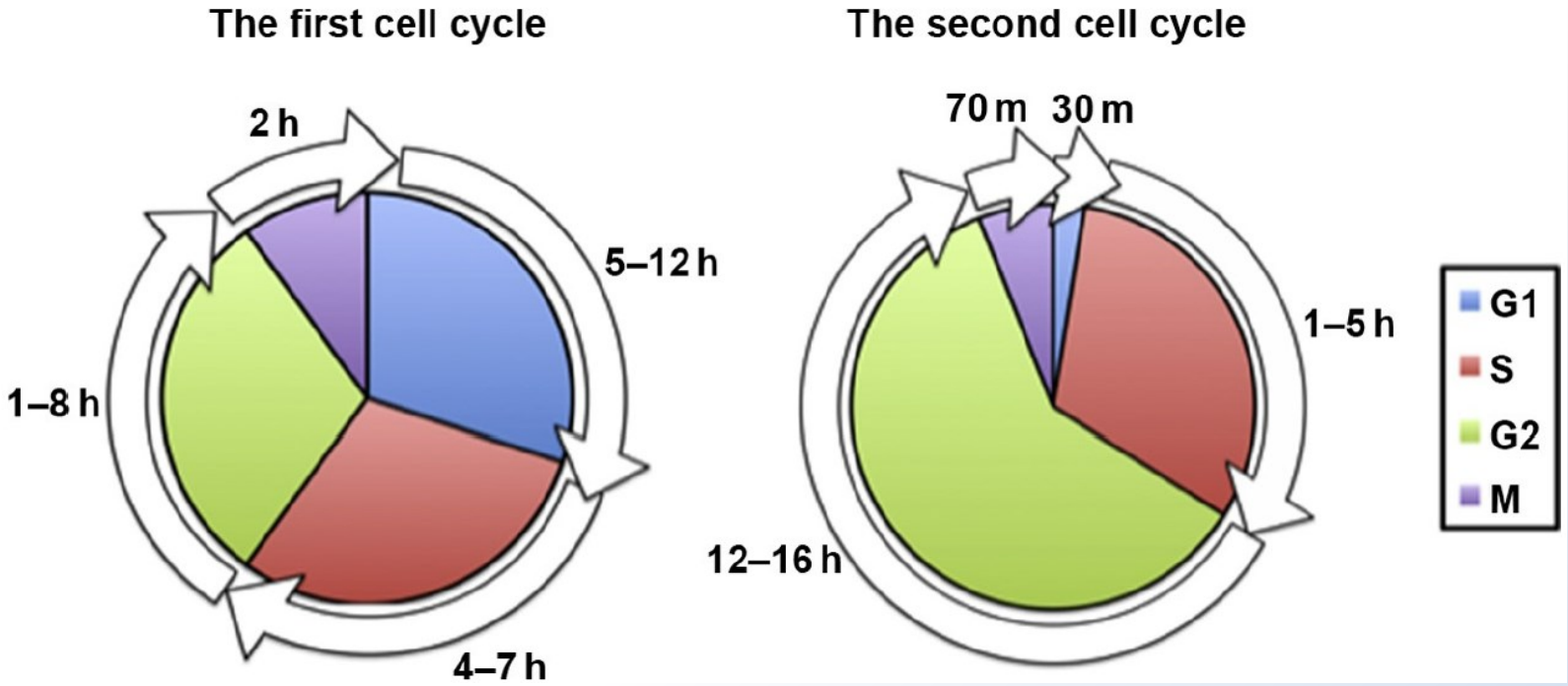
FROM A TRANSCRIPTIONALLY SILENT ZYGOTE TO THE ZYGOTIC GENOME ACTIVATION



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**

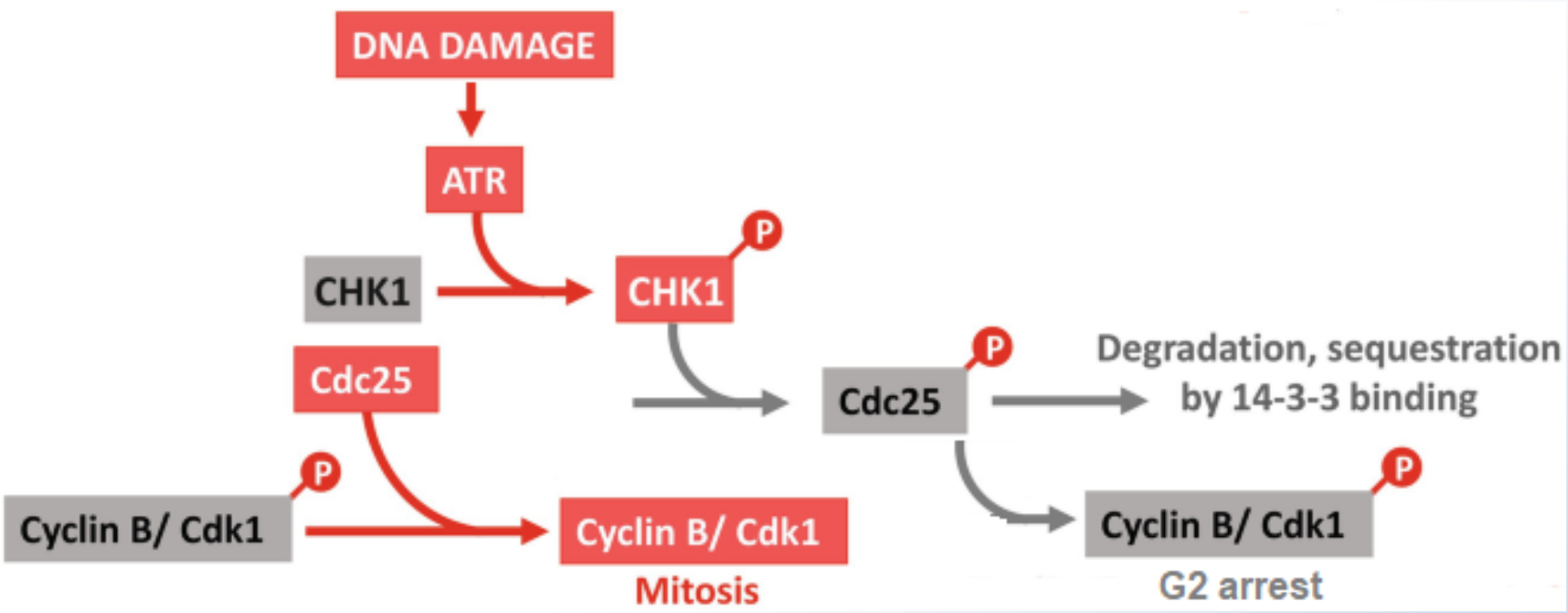
INITIAL EMBRYONIC CELL DIVISIONS ARE ACCOMPANIED BY CELL CYCLE ADAPTATIONS



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

- **Checkpoints** mainly act as gatekeepers of **cell division integrity**, their ability to regulate cell cycle progression has also been employed for **developmental purposes**

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

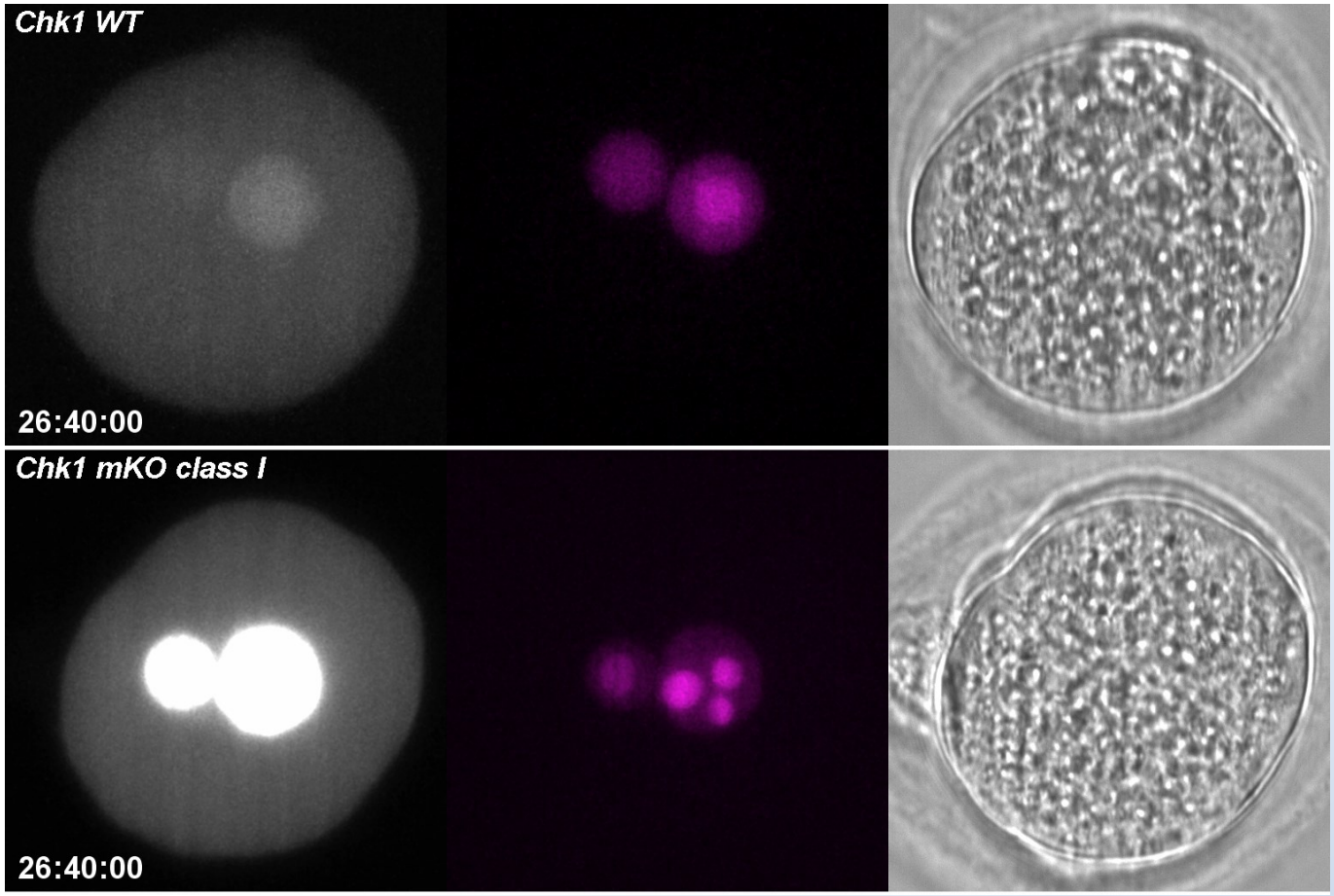


The cell cycle checkpoint pathway activated by DNA damage

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

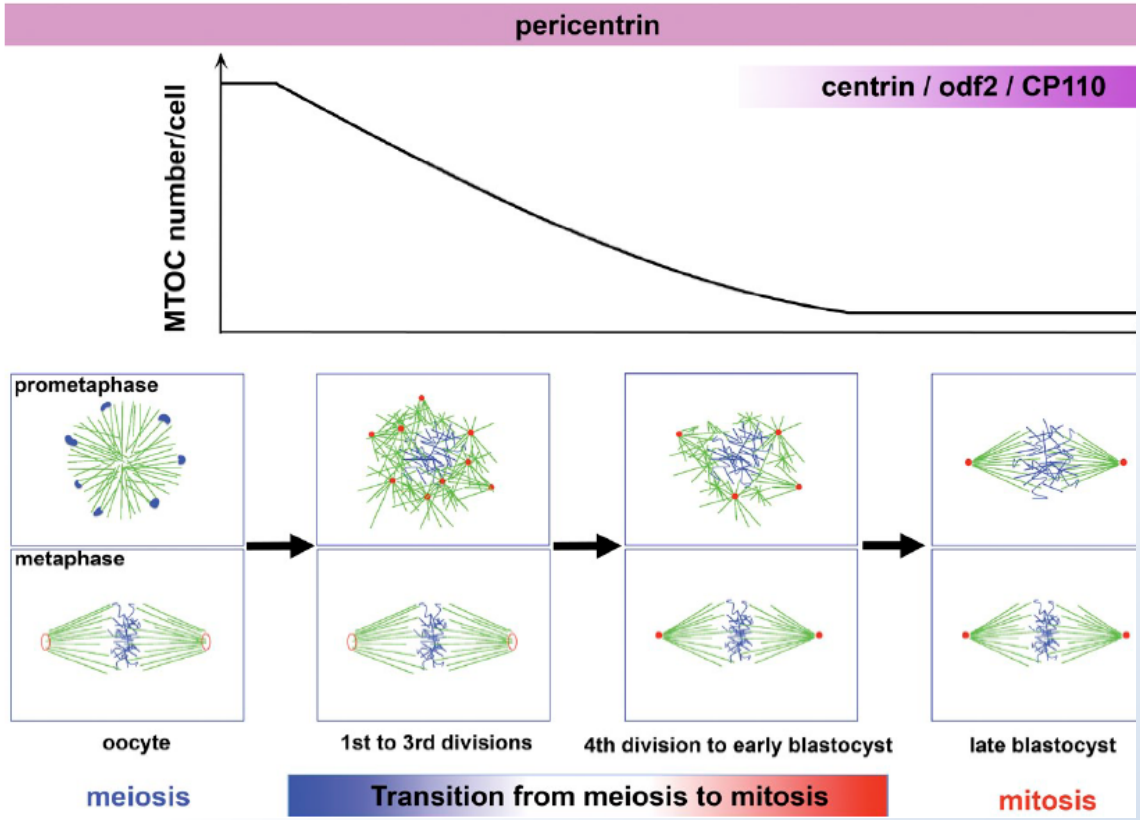
CHK1 MAINTAINS A LONG G2 PHASE IN 2-CELL MOUSE EMBRYOS THAT PROTECTS THEM FROM CHROMOSOME MISSEGREGATION



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

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

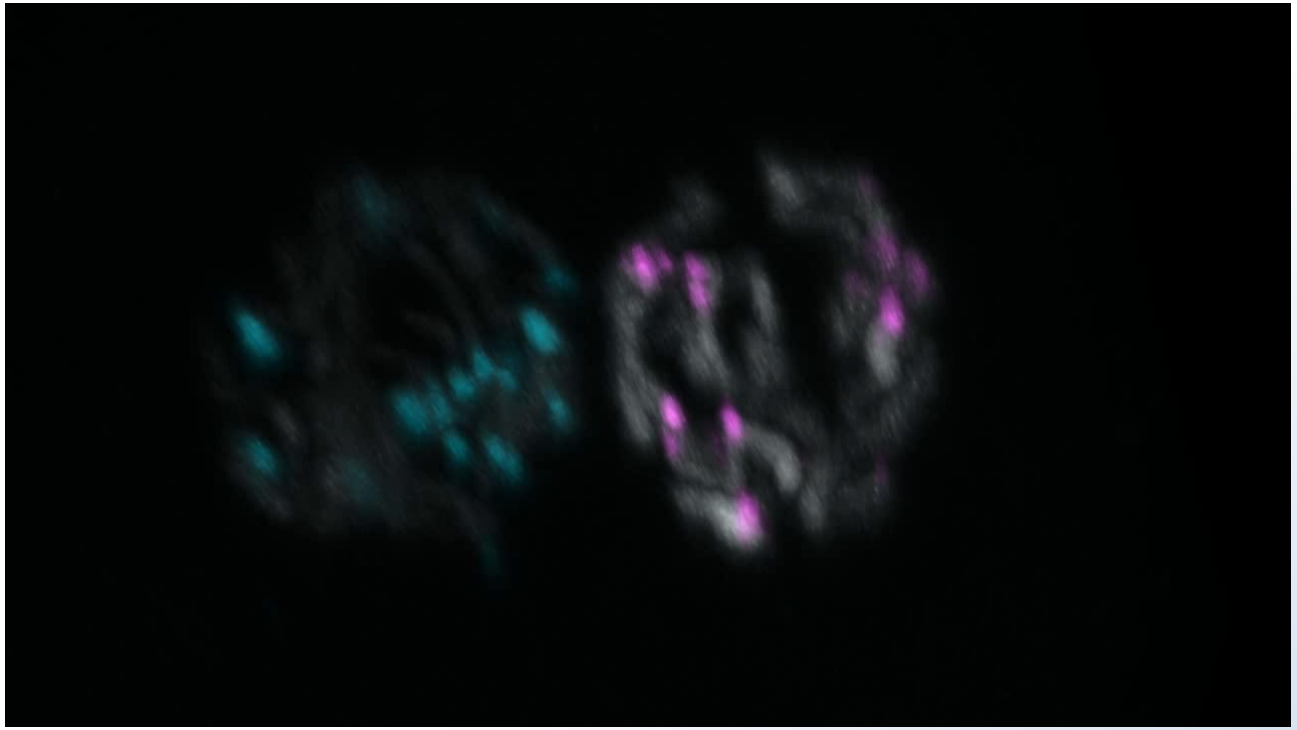
MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



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

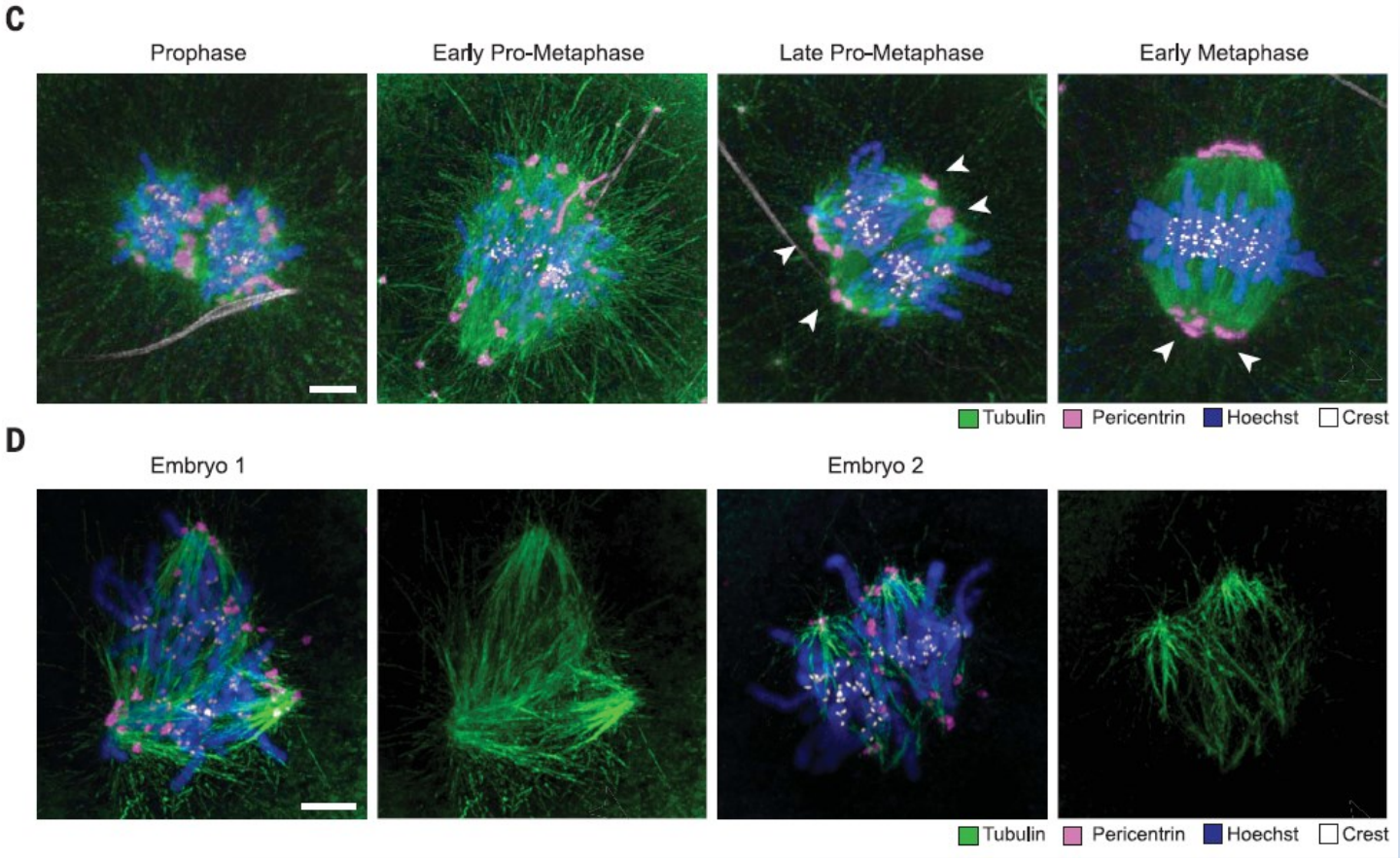
MEIOTIC-TO-MITOTIC SPINDLE TRANSITION IN MOUSE



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*

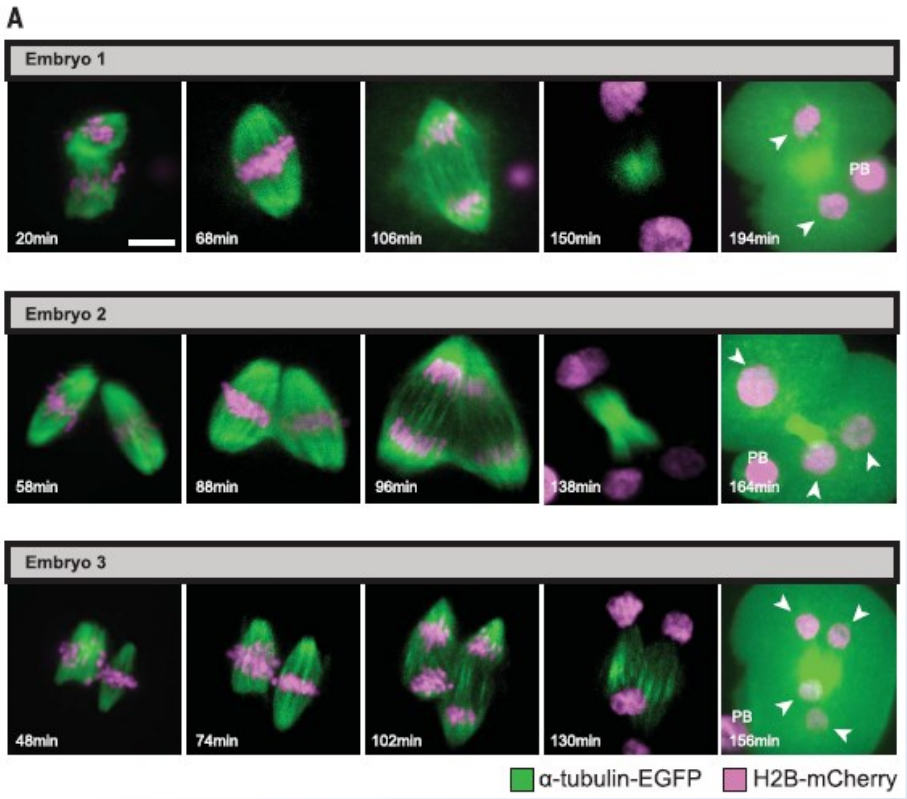
- **The paternal and maternal genome remain spatially separate throughout the first mitosis of mouse zygotes**

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