Embryologie I
OOGENESIS

autumn 2024

Oocyte meiosis

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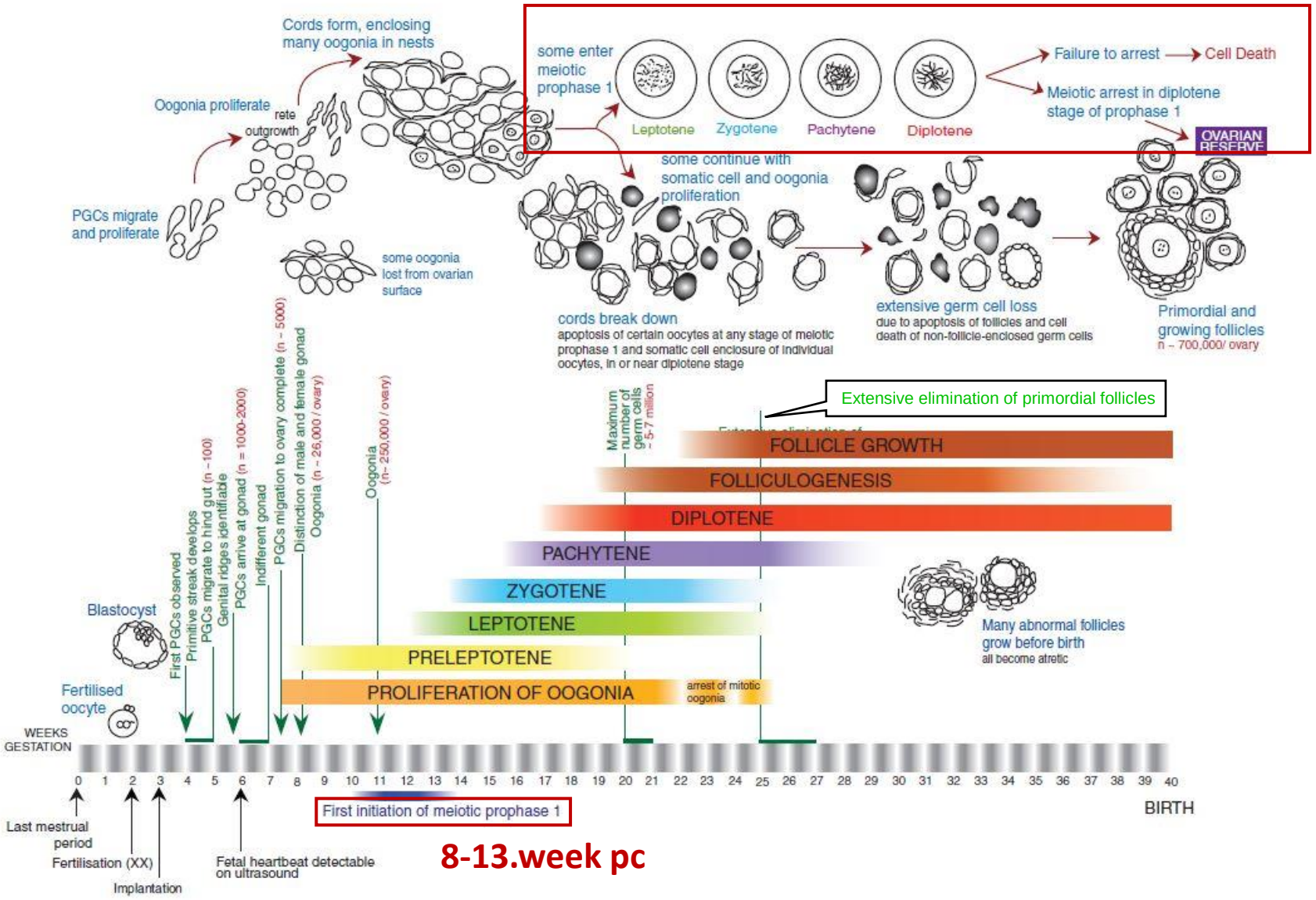
Prenatal stage of oogenesis



8-13.week pc

Entry to meiosis

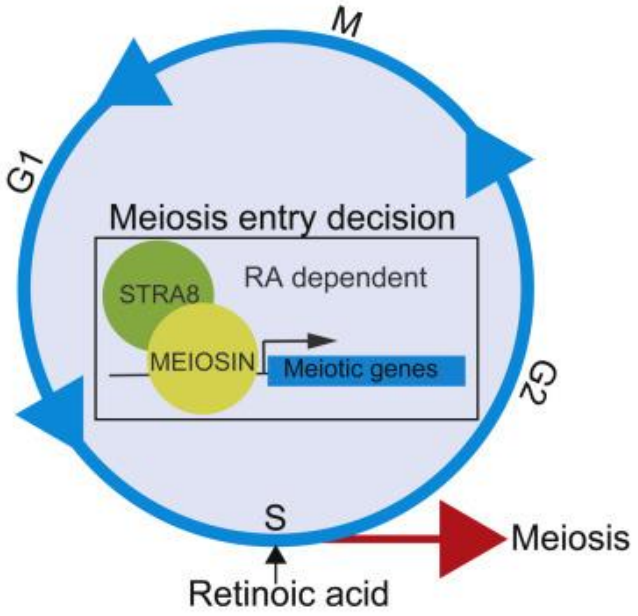
„MITOTIC-TO-MEIOTIC TRANSITION“

Retinoic acid (RA)

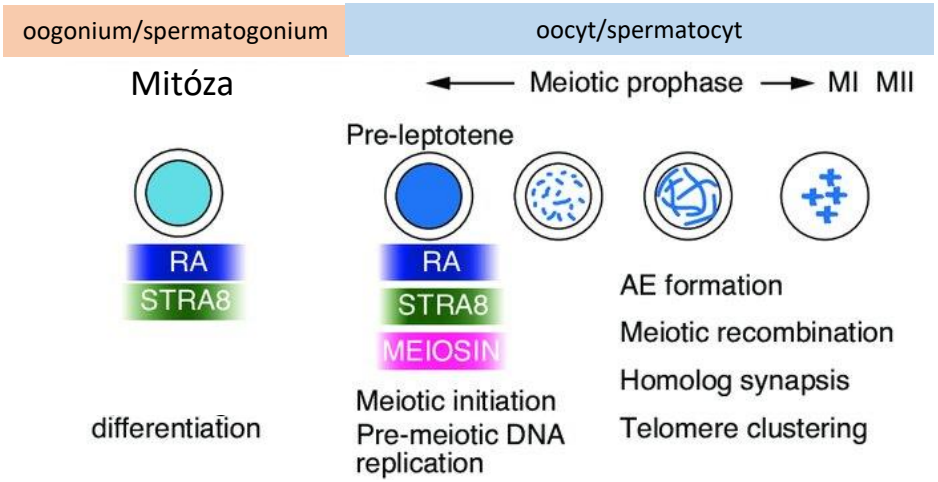
Stra8

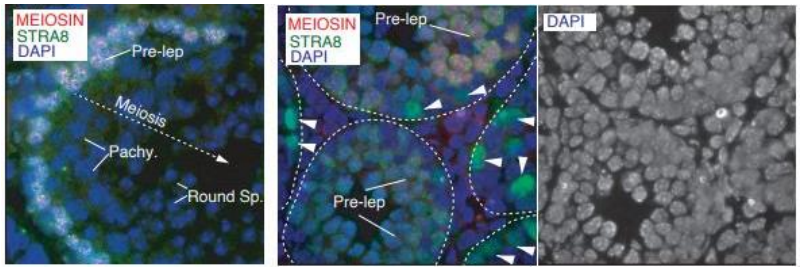
meiosin

= meiosis initiator



XX - prenatally
XY – postnatally





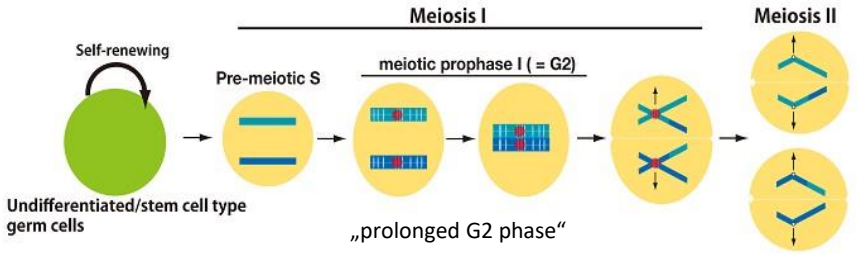
Ishiguro et al 2020

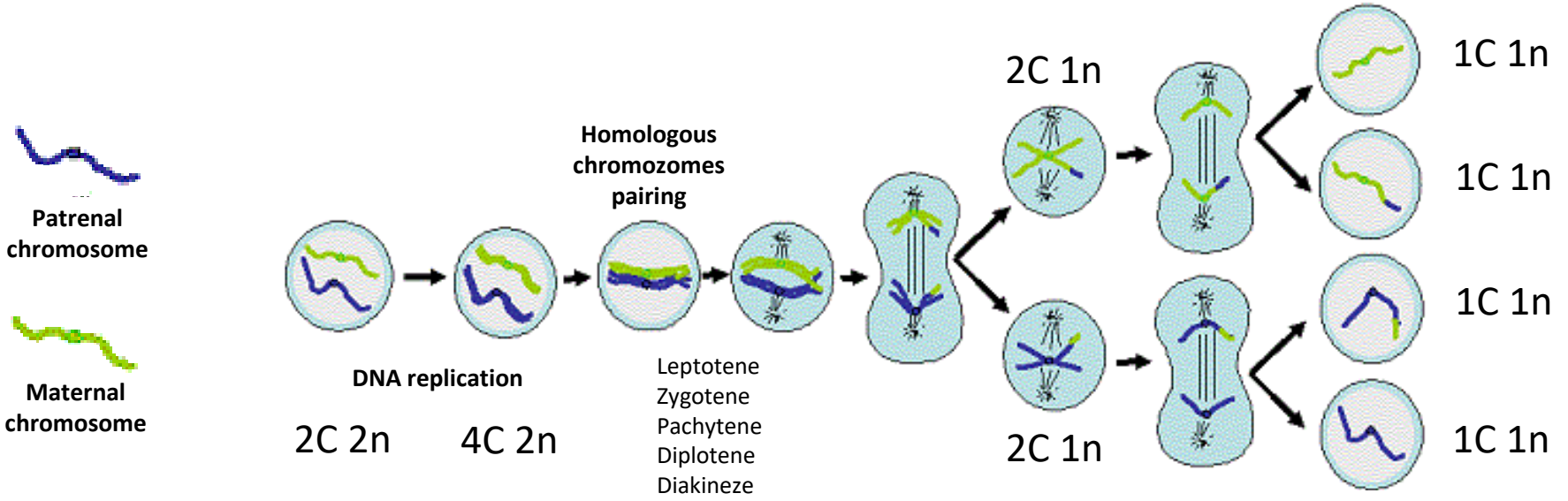
Meiosis

(1) Premeiotic S phase
- DNA replication

(2) Meiosis I
- separation of homologous chromosomes

(3) Meiosis II
- separation of sister chromatids

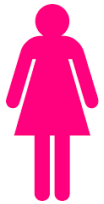




Meióza

SPERMATOGENEZE

OOGENEZE



Primordiální zárodečná buňka
(2C 2n)

Mitóza

Spermatogonie
(2C 2n)

Meióza I

Primární
spermatocyt
(4C 2n)

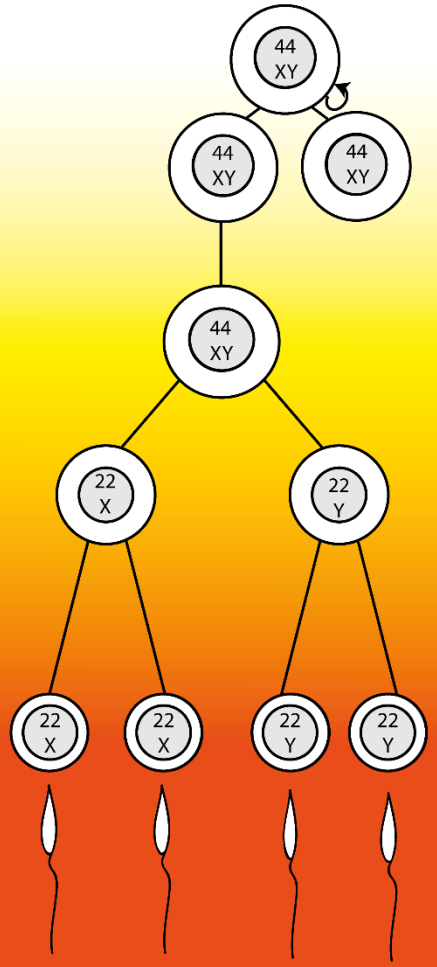
Sekundární
spermatocyt
(2C 1n)

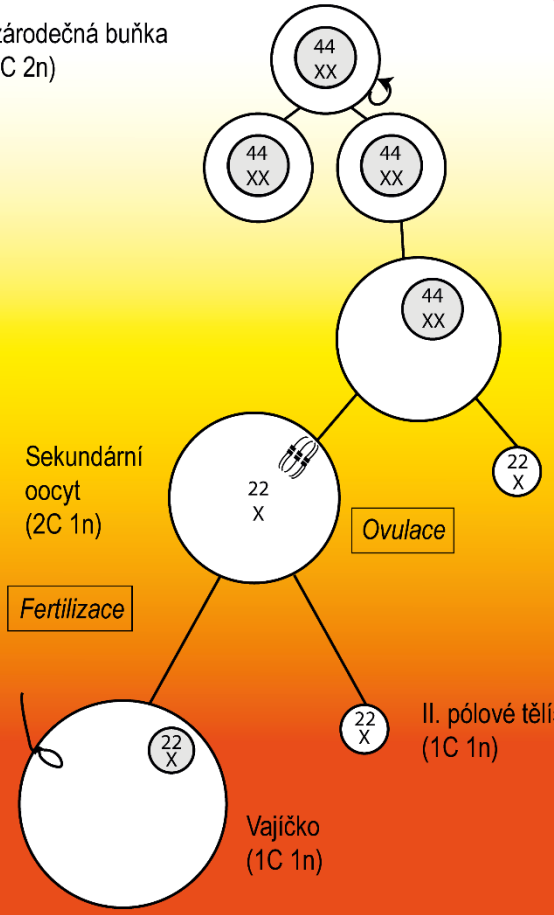
Meióza II

Spermatidy
(1C 1n)

Spermiogeneze

Spermatozoa (1C 1n)





Mitóza

Oogónie
(2C 2n)

Meióza I

Primární
oocyt
(4C 2n)

Sekundární
oocyt
(2C 1n)

I. pólové
tělísko
(2C 1n)

Meióza II

II. pólové tělísko
(1C 1n)

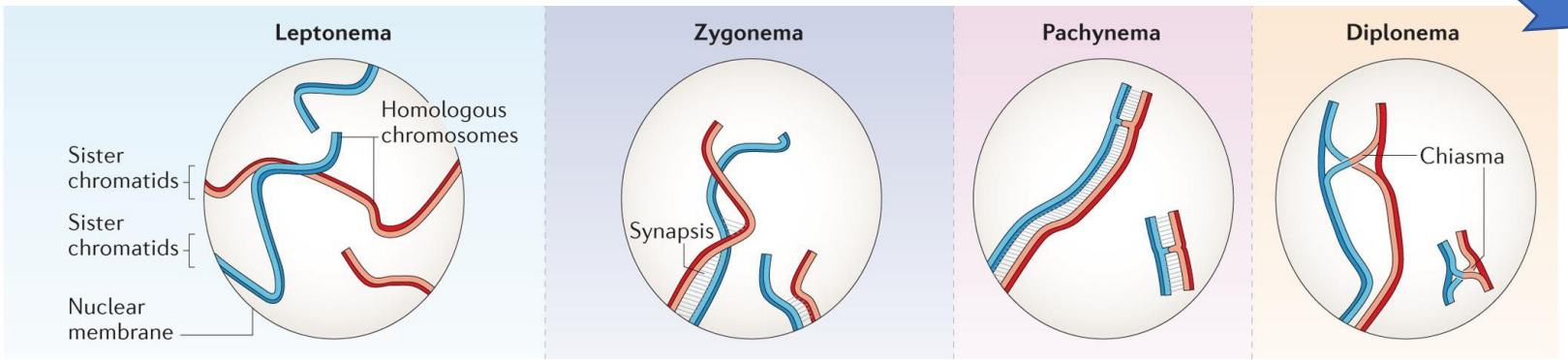
Vajíčko
(1C 1n)

n...počet sad chromozomů
C...počet kopií každého genu

Meiosis

➢ Prophase I

(1) leptotene (2) zygotene (3) pachytene (4) diplotene



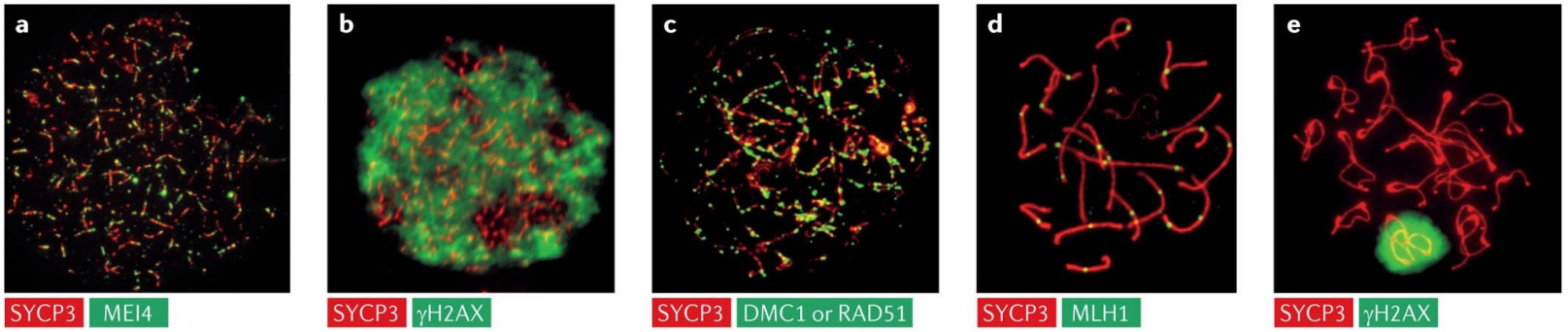
Prolonged arrest
= dictyatene

Condensation of replicated DNA

Synapsis of homologous chromosomes

Recombination of homologous chromosomes (**crossing-over**)

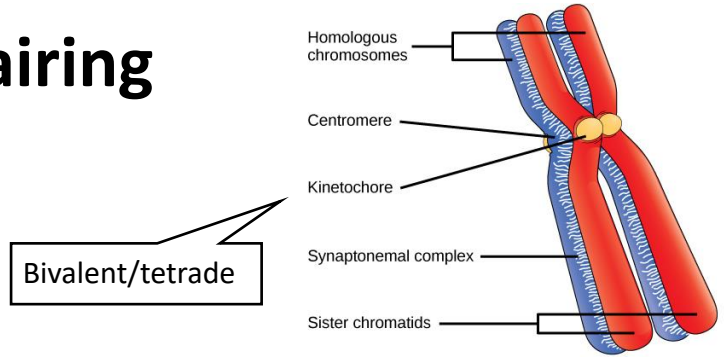
Synapsis disolution → bivalents with chiasmata



Meiosis

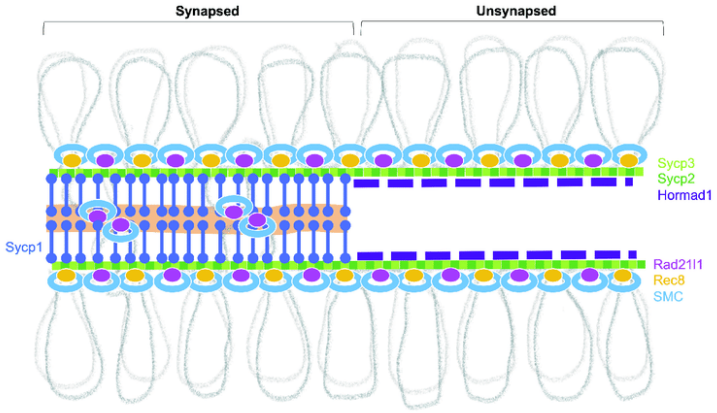
➢ Homologous chromosomes pairing

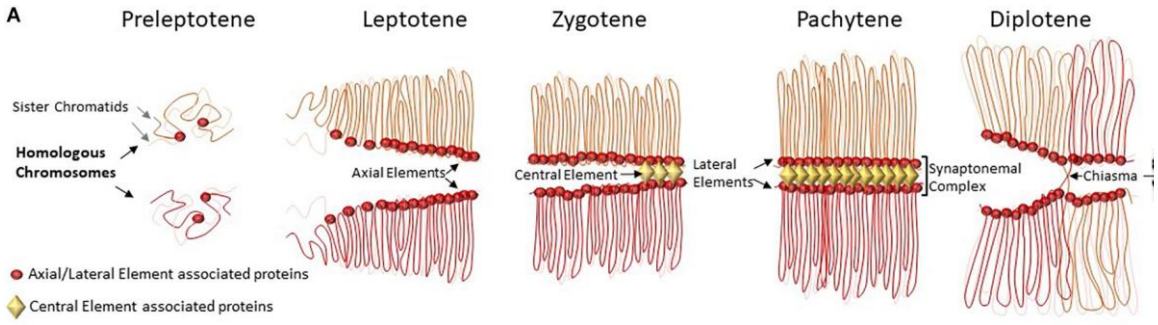
- **synapsis** = physical association of homologous chromosomes
- homolous sequence pairing

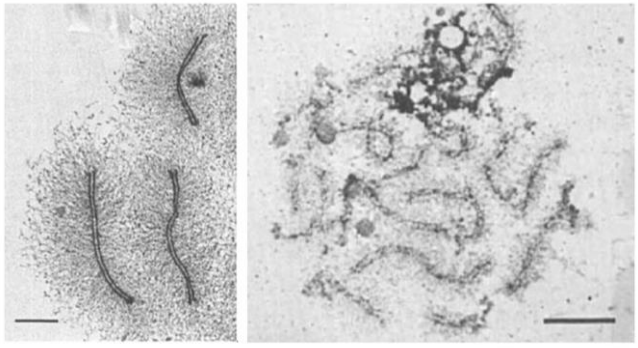


- **Synaptonemal complex**
  - axial elements - SYCP3+SYCP2
  - central element - dimer SYCP1
  - lateral elements – cohesins (Rad21, Rec8)

- gradual formation of SYCP3 a SYCP2 foci
- axial element built by coalescence of SCP3 and SCP2 foci with cohesin complex proteins
- axial element stabilized by cross-linking with SYCP1 dimer, which forms central element



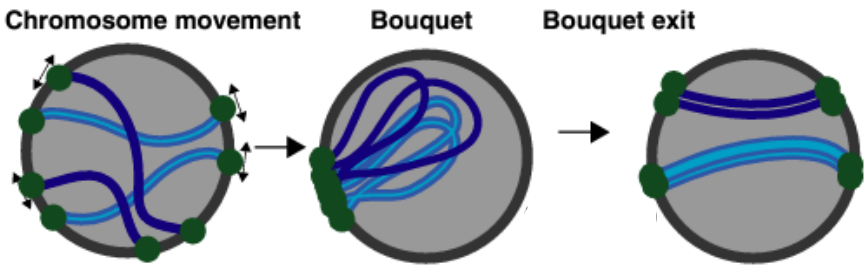




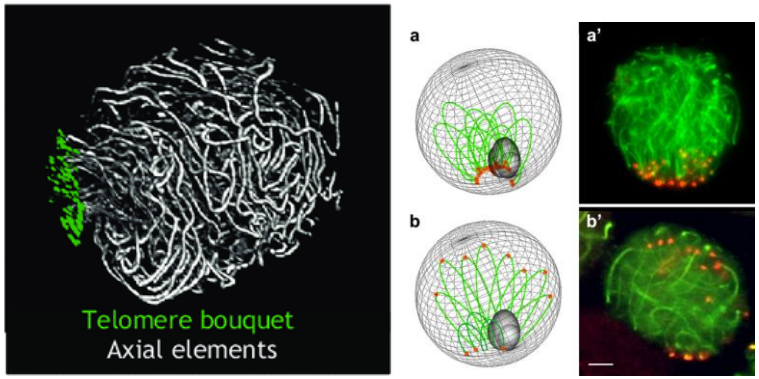
from Moens and Pearlman 1988

➢ Homologous chromosomes pairing

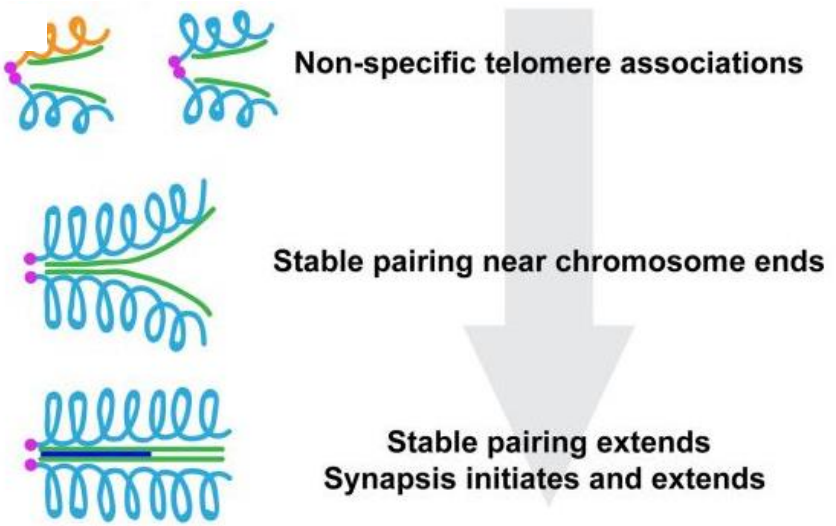
- pairing of distant homologous chromosomes accomplished by congregation of telomeres attached to nuclear membrane
  → tzv. „telomere bouqouet“

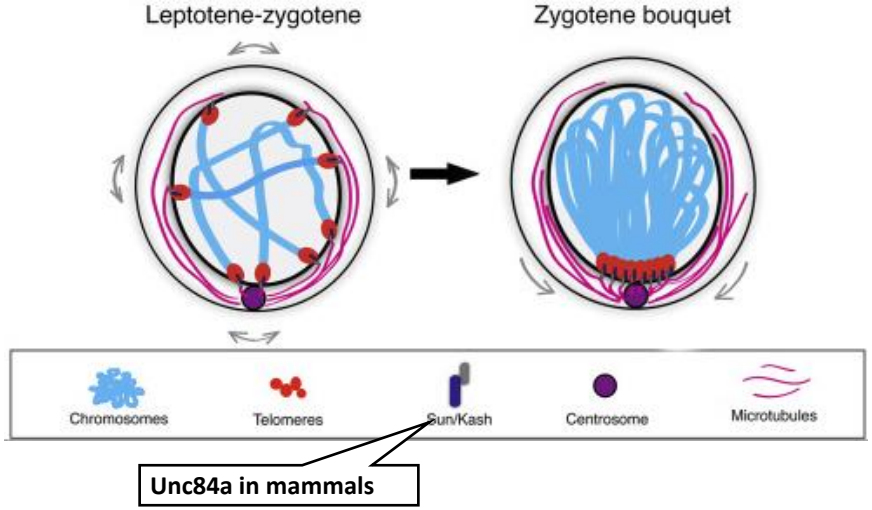


Ishiguro 2018



Berrios et al 2013



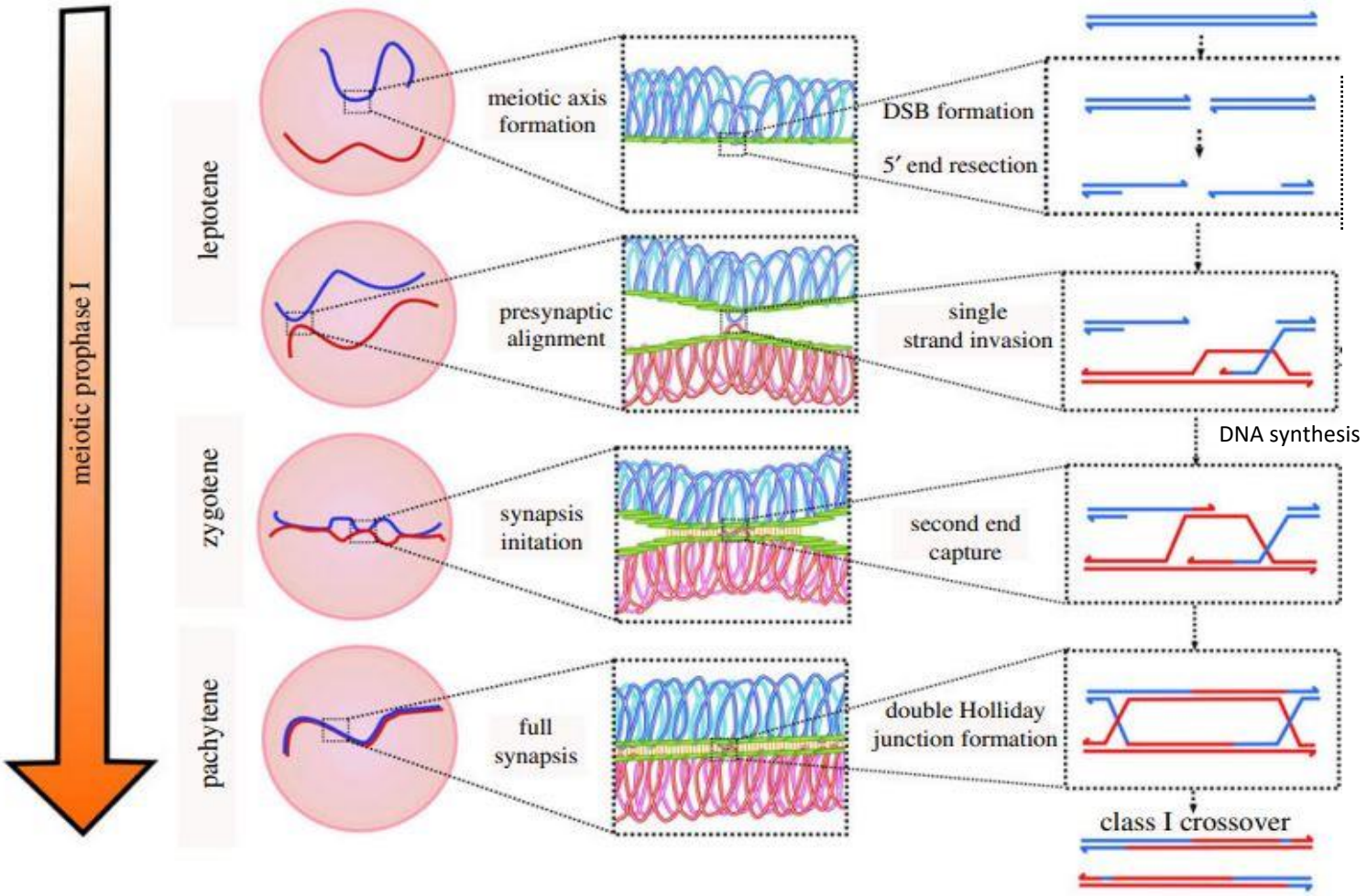


Zhou et al 2012

Elkouby and Mullins 2017

Meiosis

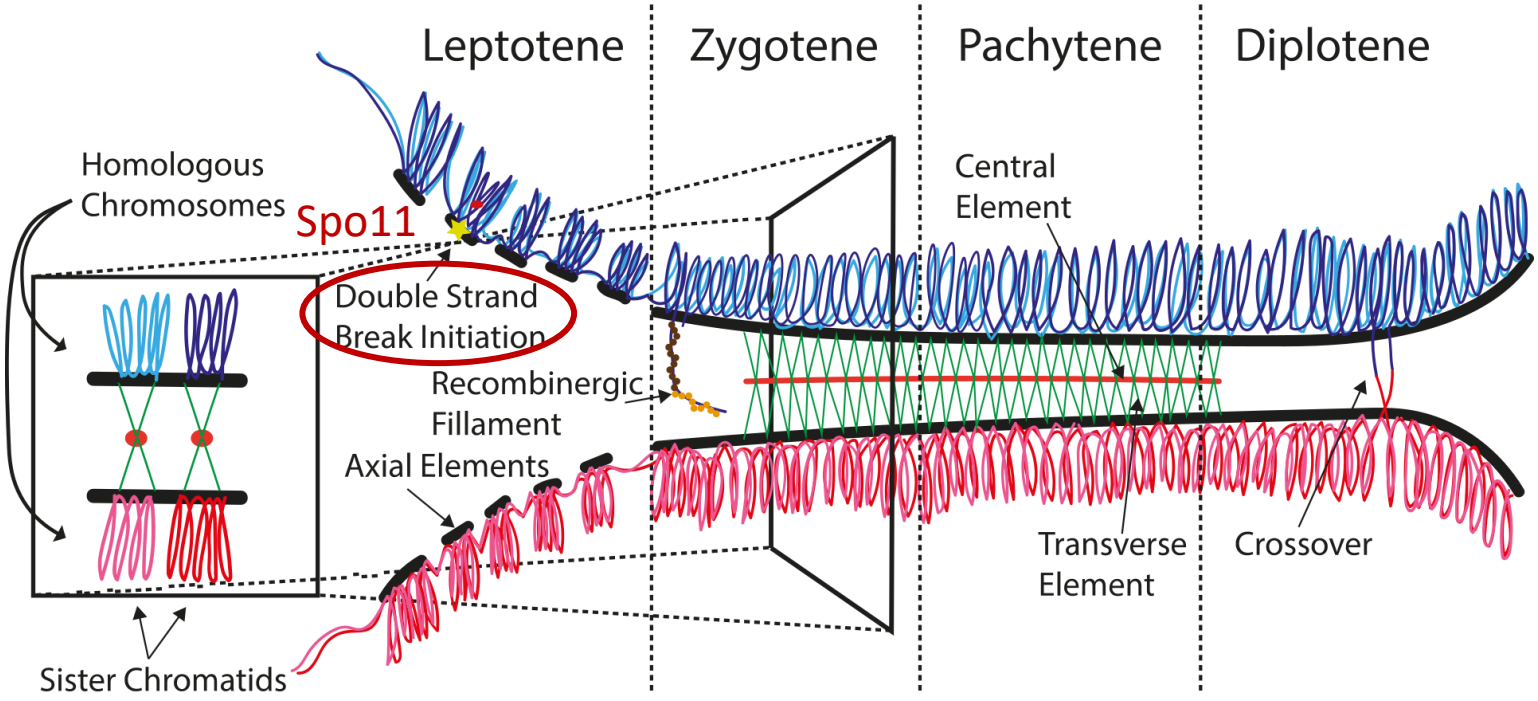
➢ Homologous recombination



Meiosis

➢ Homologous recombination

- DNA double strand breaks (DSB) precede synapsis formation

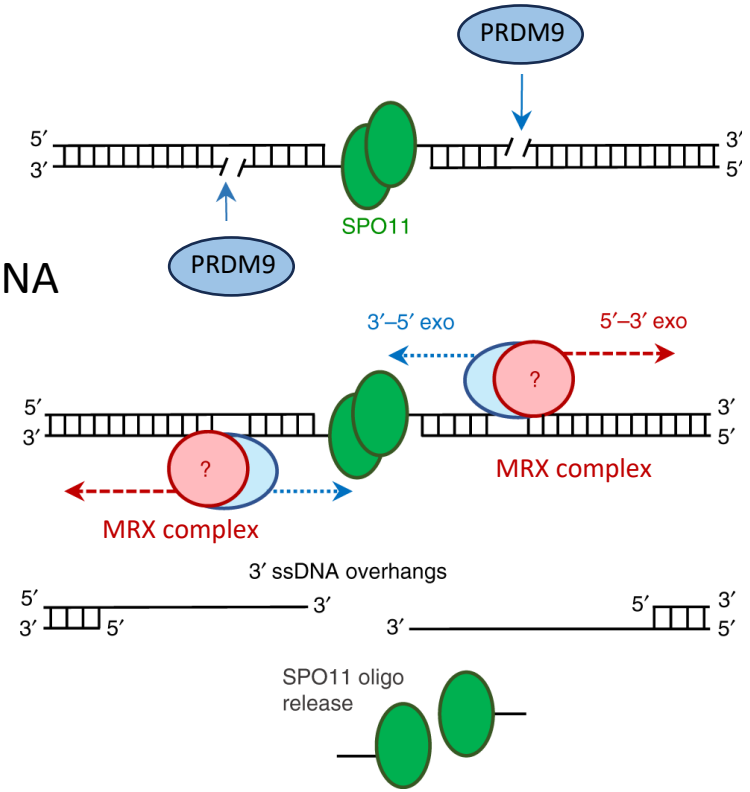


Meiosis

➢ Homologous recombination

- PRDM9
  - histon metyltransferase
  - recognition and epigenetic modification of specific DNA sequences (recombination hotspots)

- Spo11
  - DNA endonuclease
  - its dimer forms DSB
  - each monomer then binds 5´ end of ssDNA
  - Spo11+oligonucleotide cleaved away by exonuclase → free 3´end



-/-

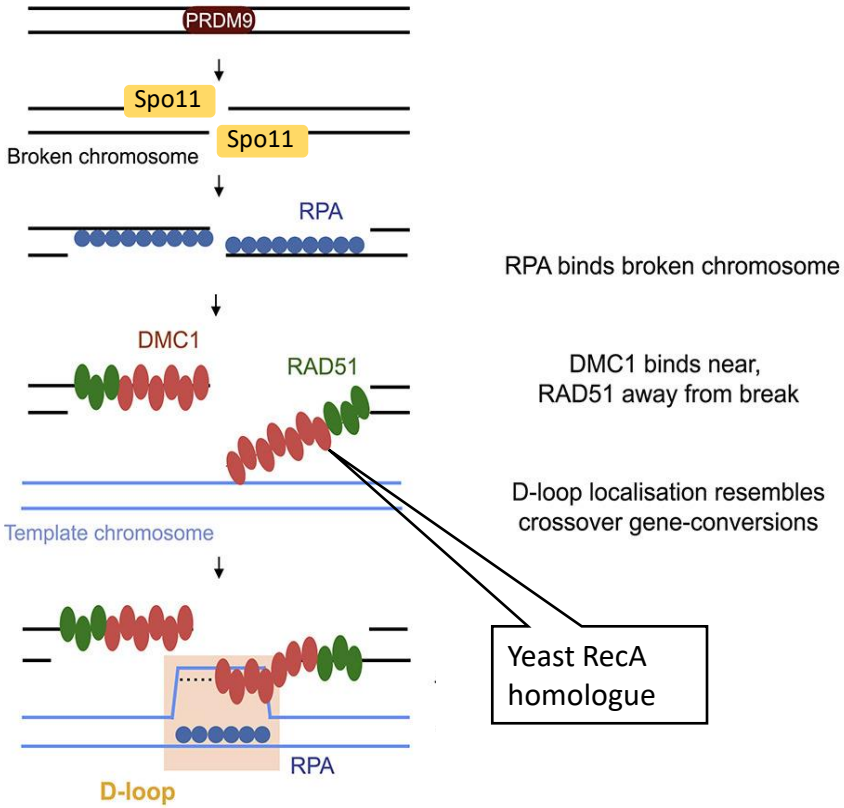
- mouse Spo11-/- males sterile
- Spo11 polymorfism in male infertility

Meiosis

➢ Homologous recombination

- RPA (replication protein A)
  - binds free 3´end
  - recruits DMC1 and Rad51
  - during invasion binds DNA template strand and stabilizes D-loop

- DMC1 + Rad51
  - meiotic recombinases
  - bind and navigate free 3´end of ssDNA to invade dsDNA of homologous chromosome



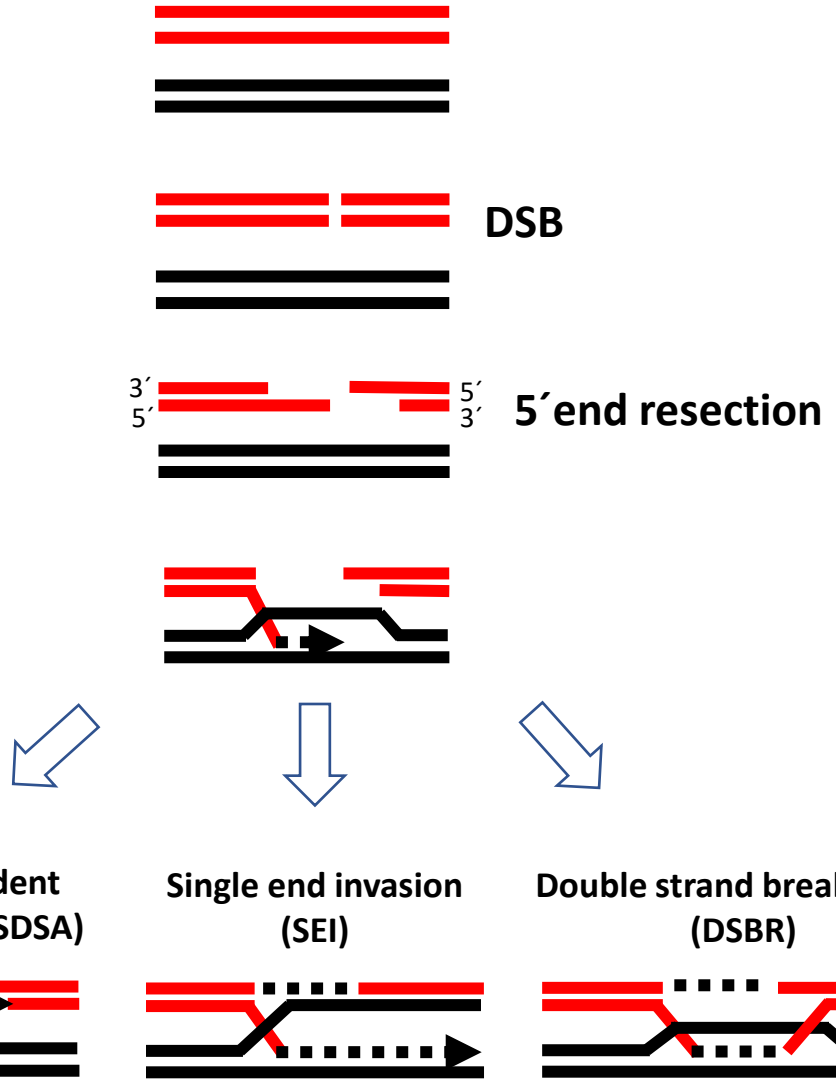
Hinch et al 2020

Meiosis

➢ Homologous recombination

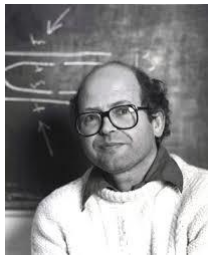
- DNA syntesis of DNA 3´end using non-sister chromatid as a template

+ strand ligation



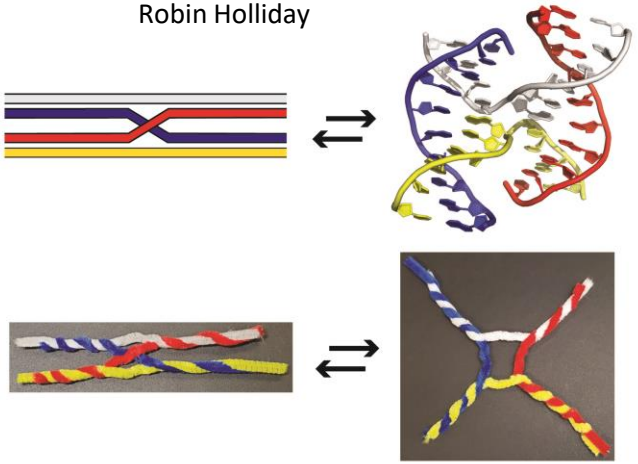
Meiosis

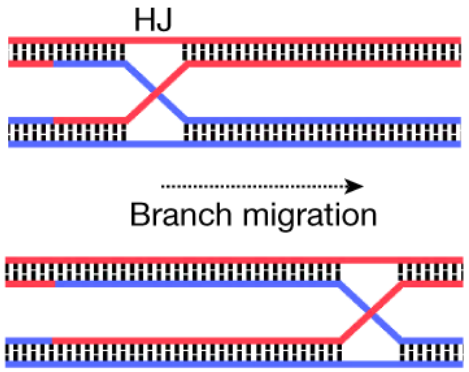
➢ Homologous recombination

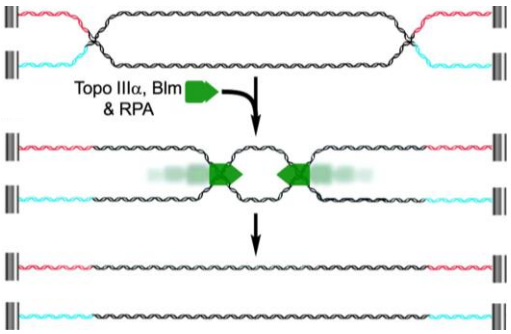


Robin Holliday

- **Holliday junction (HJ)**
  - named after Robin Holliday, who proposed its existence in 1964
  - DNA duplex – physical linkage of two DNA doublehelixes
  - intermediate of homologous recombination and DSB repair mechanism
  - visible in electron microscope
  - HJ can move, double HJ can be resolved







Meiosis

➢ Homologous recombination

DSB

non-crossover ~ 90

- no gene conversion

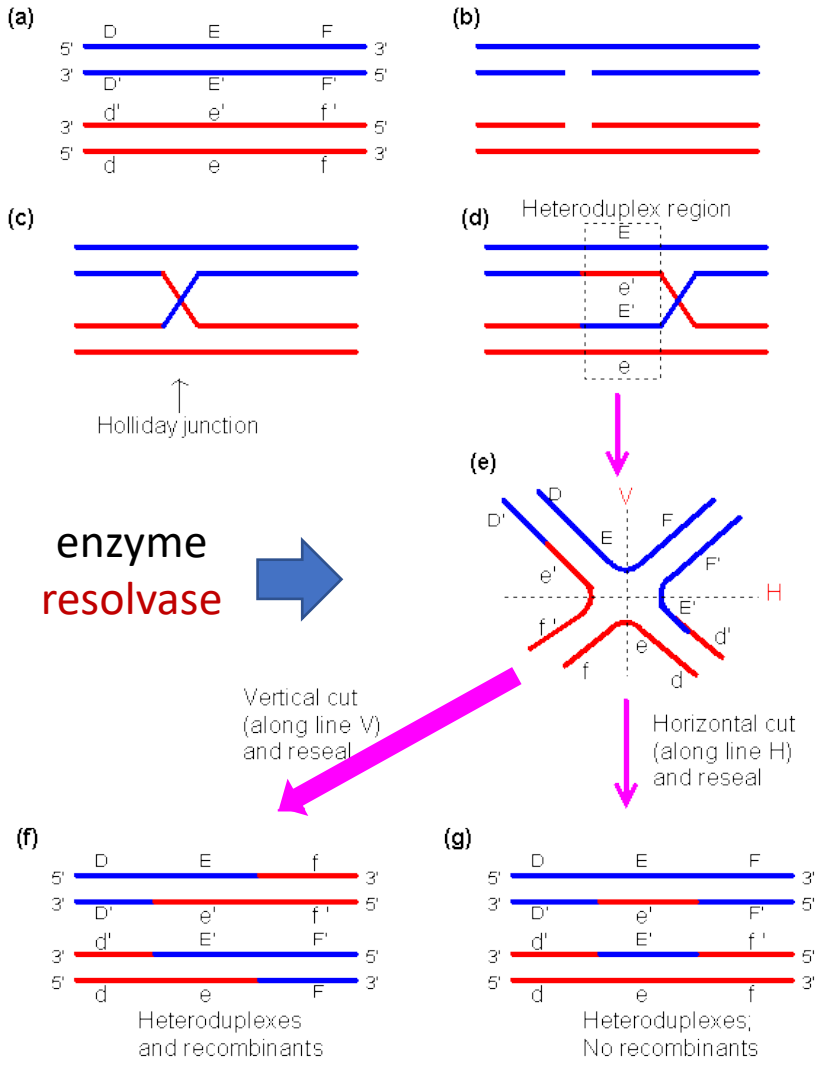
(a) D-loop resolution
→ DNA synthesis according to complementary strand

(b) convergent branch migration resulting in resolution of HJs

(c) strand exchange and HJ resolution without gene conversion

crossover ~10%

- gene conversion occurs at both chromatids
- HJ resultion produces new combination of genes



https://www.youtube.com/watch?v=3qgBKrAZCLg

Meiosis

➢ Homologous recombination

- **Chiasmata (chi-structure)**

- physical contact sites of homologous chromosomes marking crossing-over regions

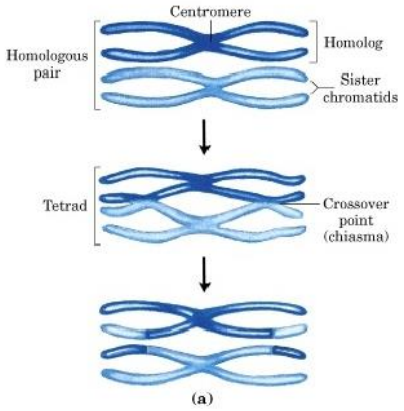
- visible after synaptonemal complex disolution during diplotene

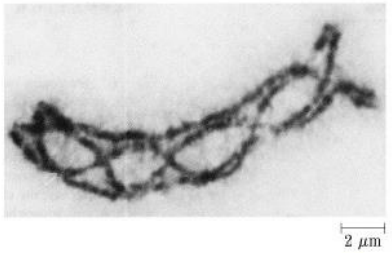
- links homologous chromosomes together in the form of bivalents (tetrades)

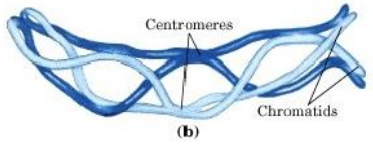
- disapprear as homologous chromosomes separate during anaphase I

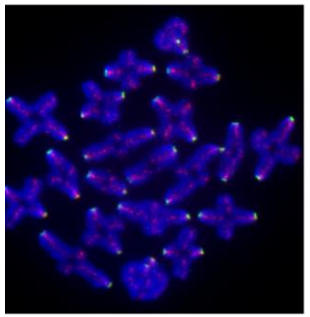
- sex differences in location and number of chiasmata (more distal in males)

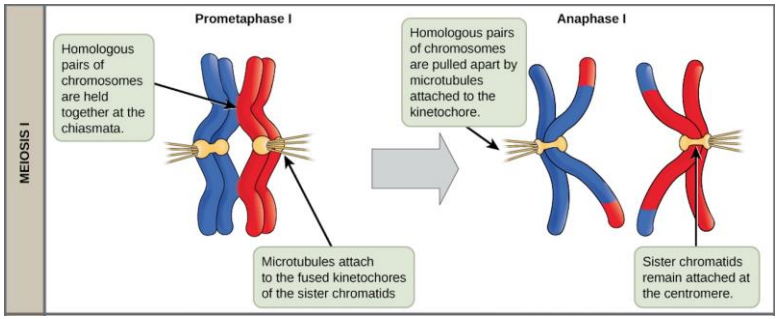
- altered number of chiasmata and location of chiasmata associated with aneuploidy



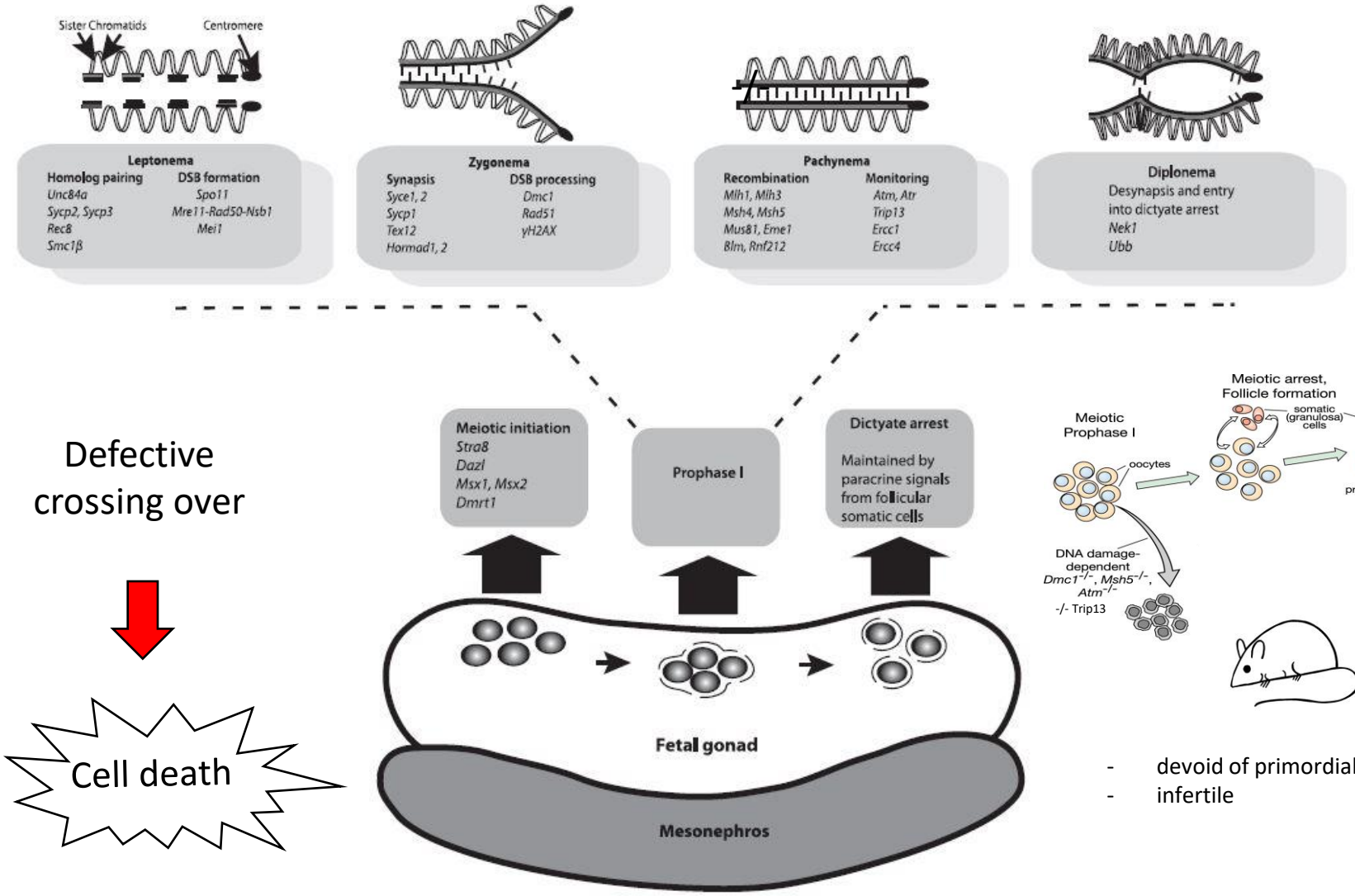








Regulation of meiotic prophase overview



Defective crossing over

Cell death

- devoid of primordial follicles
- infertile

Chromatin configuration during diplotene arrest

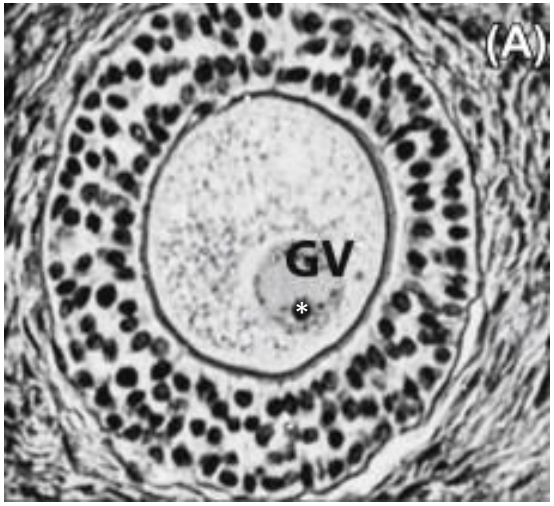
- **dictyate** stage = prolonged diplotene arrest

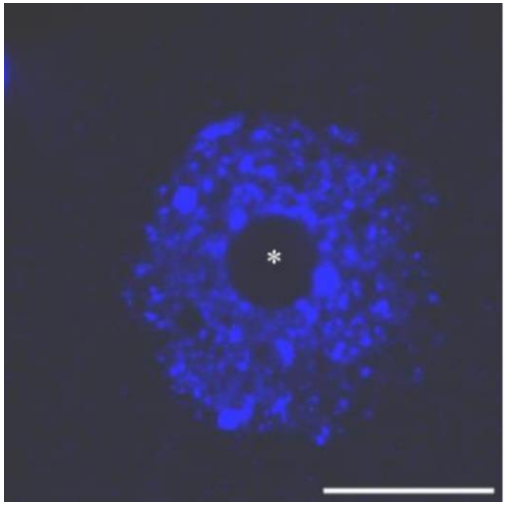
- chromosomes become dispersed, less distinct and form faint network

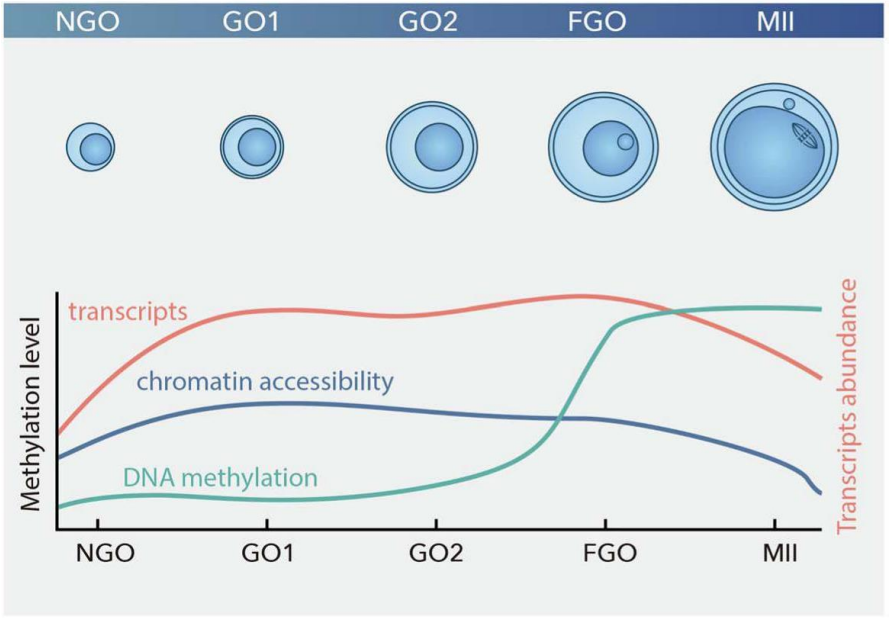
- germinal vesicle (GV)
  = prophase nucleus
  - ~30-40 µm

- nucleoli*
  = „nucleolar-like body (NLB)“, „nuclear remnant“
  - structure containing electron dense fibrilar/granular material

- during oocyte growth phase, chromosomes decondense and chromatin becomes transcriptionally active allowing for accumulation of cellular mass

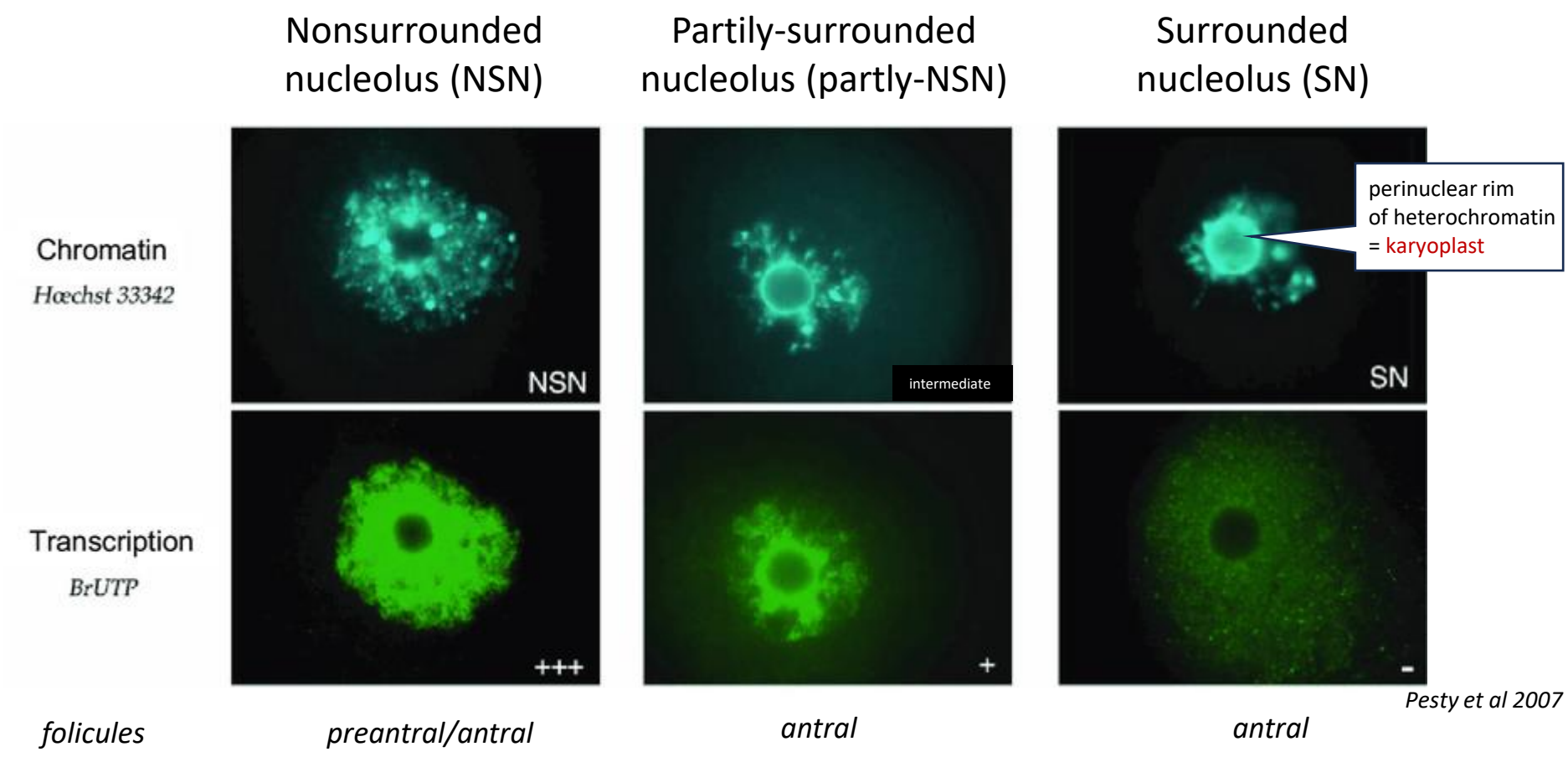






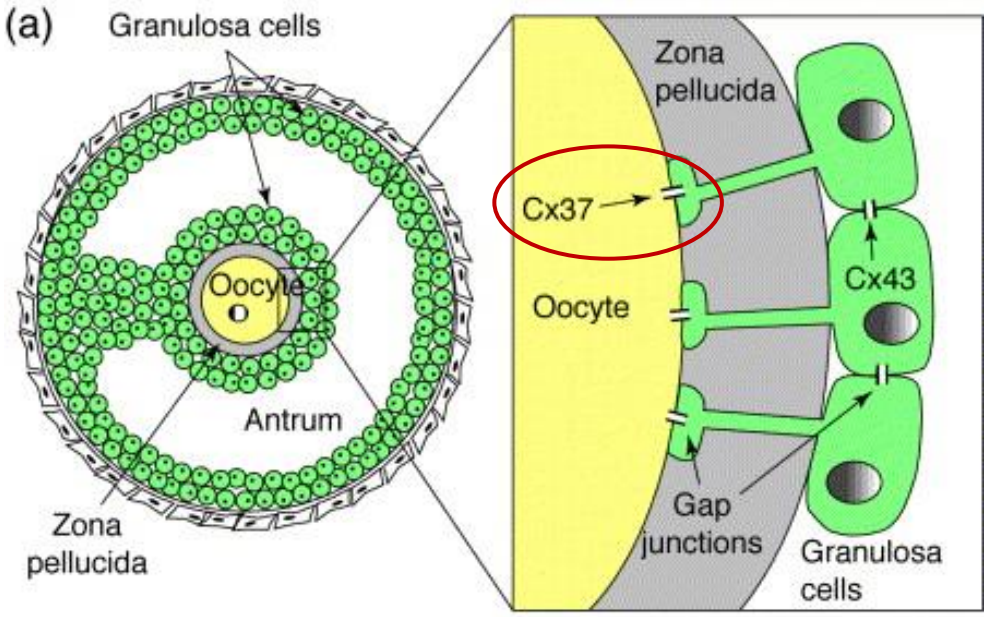
Qian and Guo 2022

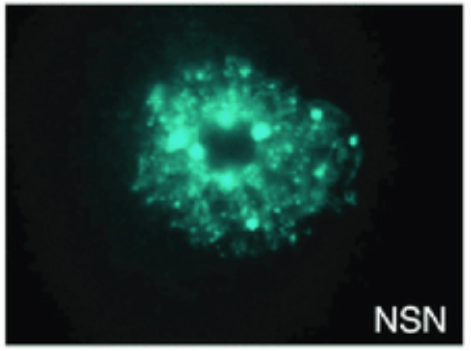
Large scale chromatin remodelling



Pesty et al 2007

Large scale chromatin remodelling

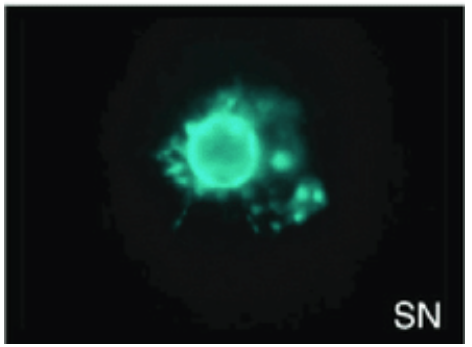




FSH

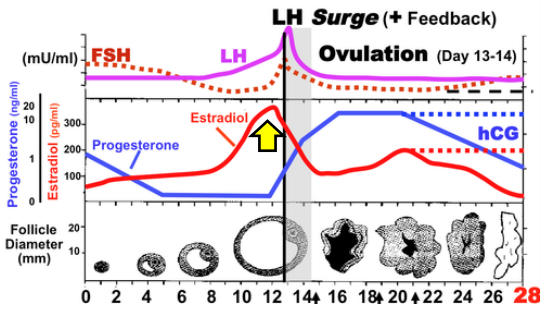
(+COC in IVM)

-/- Connexin37



- bidirectional GCs-oocyte comunication is necessary for **timely** coordination of chromatin decondensation and onset of transcriptional silencing

Resumption of meiosis

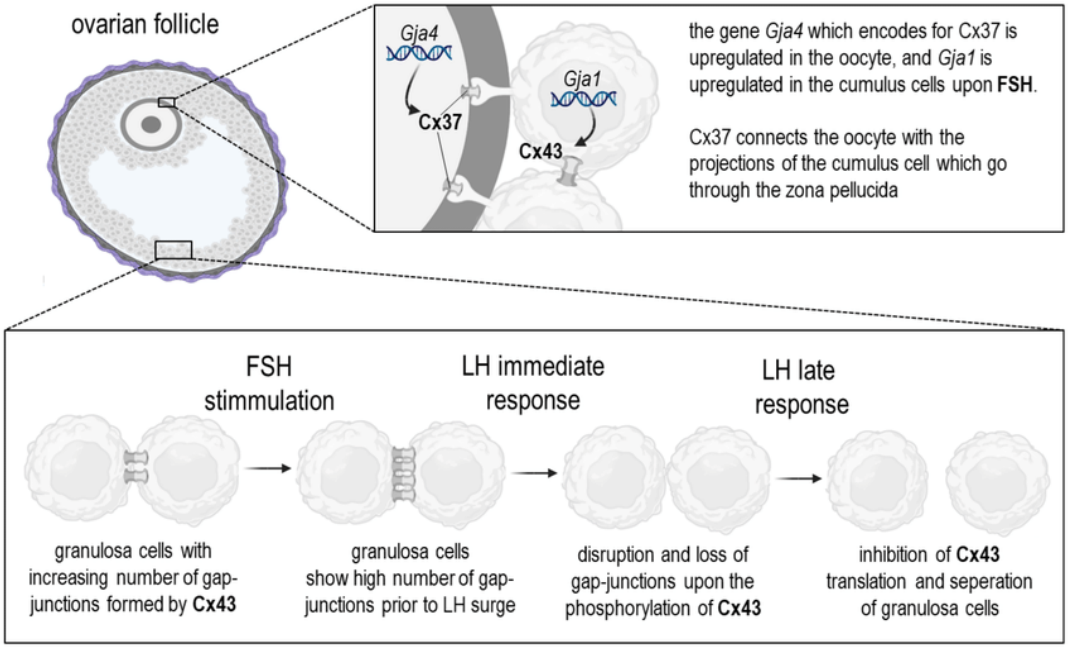


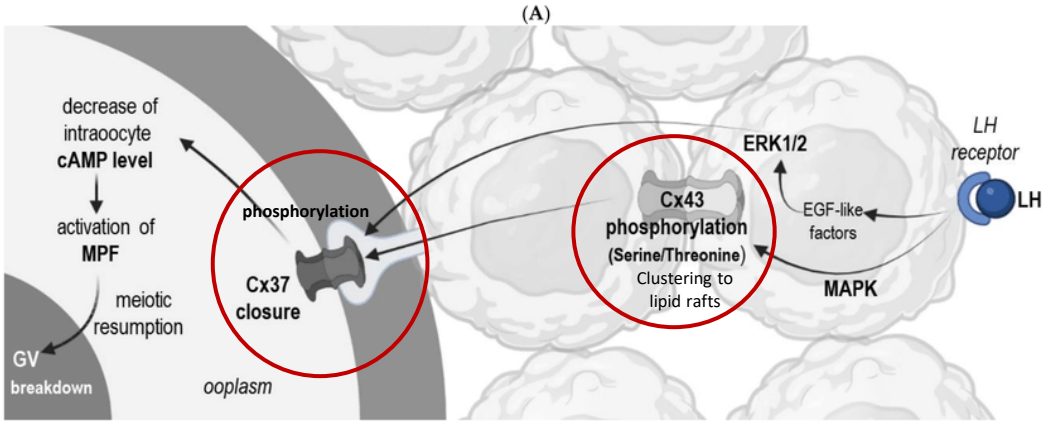
↑ estrogen

LH surge

oocyte-cGCs uncoupling

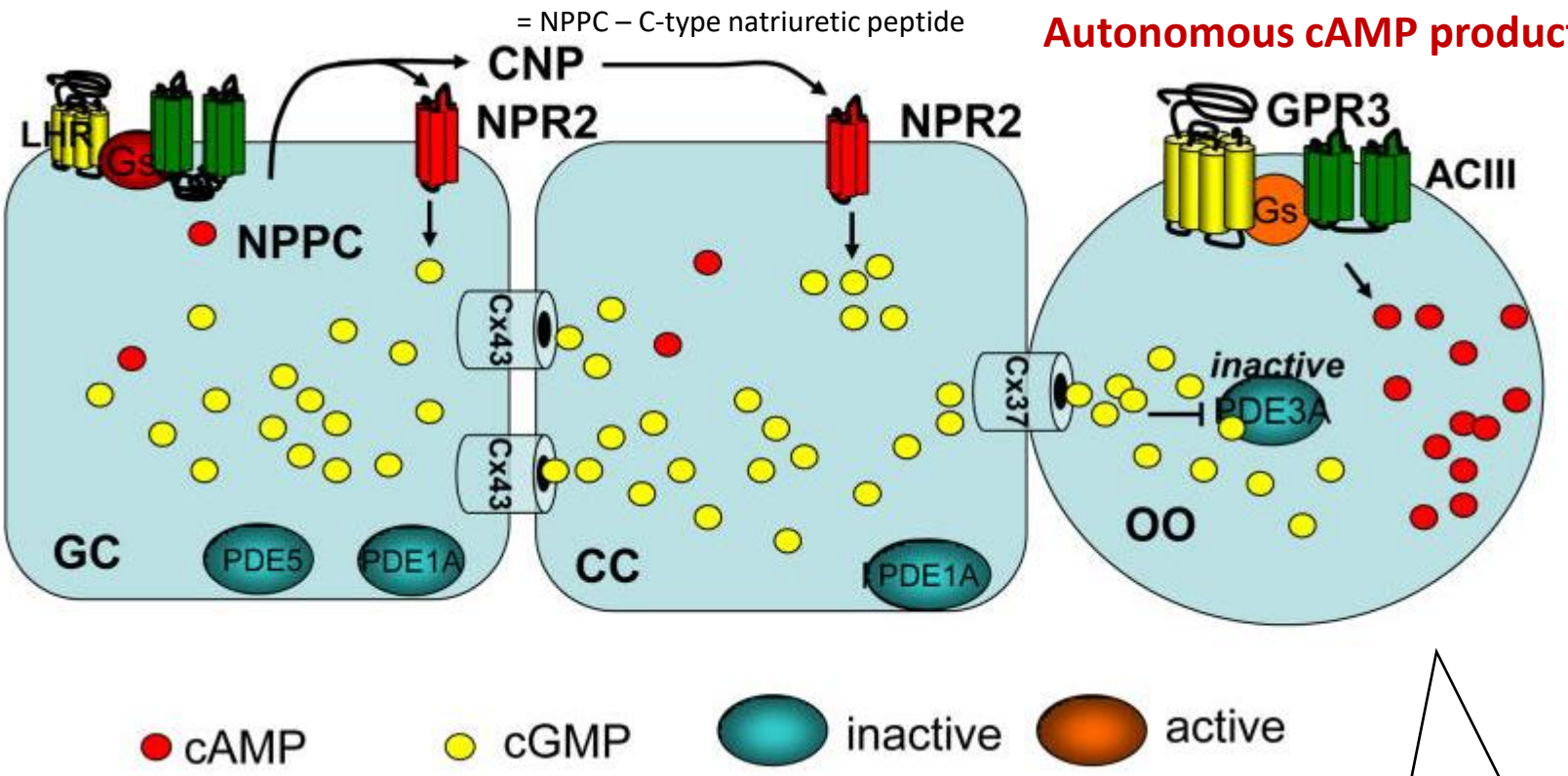
release from GV arrest





Kordowitzki et al 2021

Control of prophase arrest



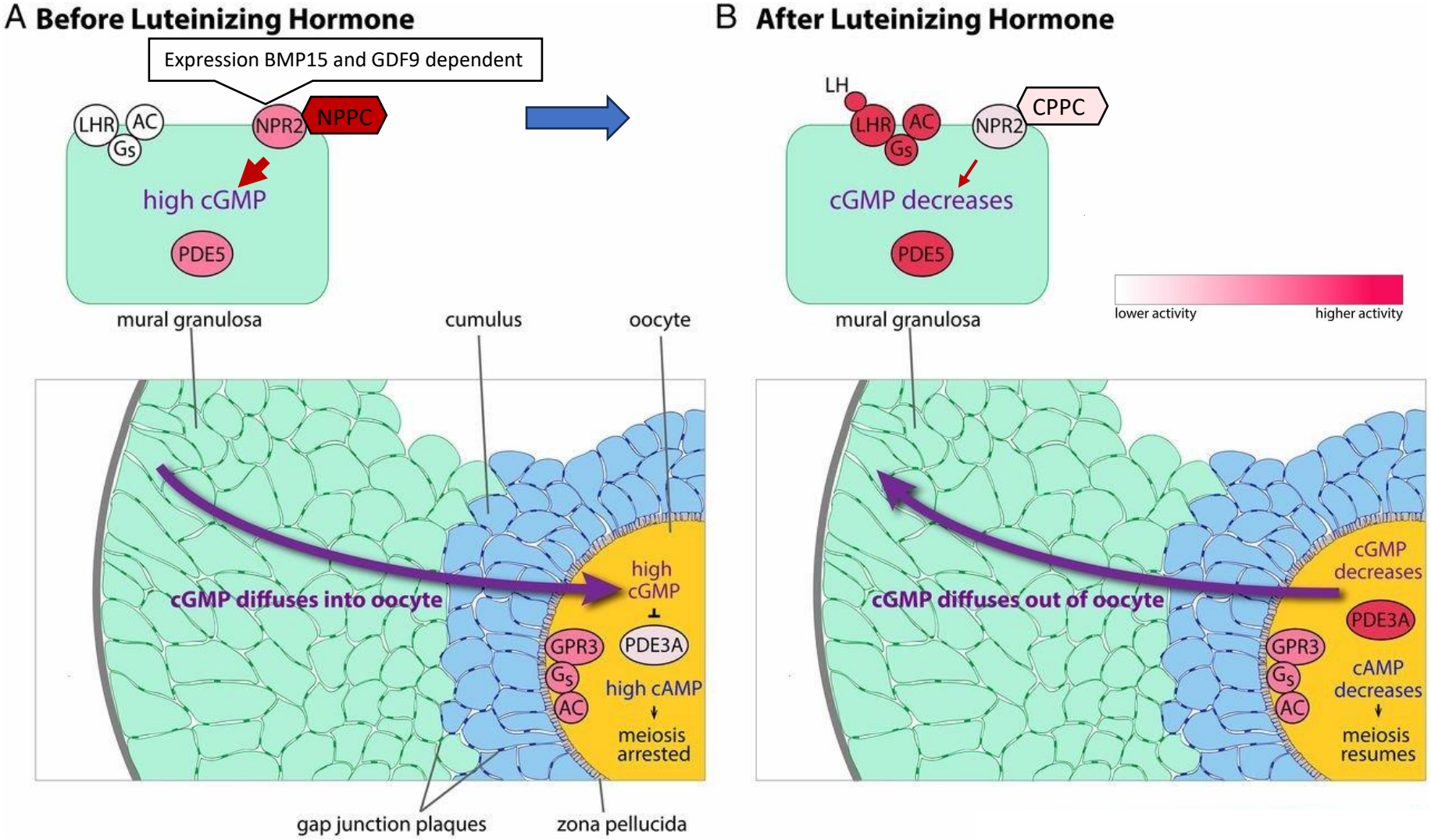
Conti et al 2011

Supplementation of cGMP by cGCs balances cAMP degradation by PDE

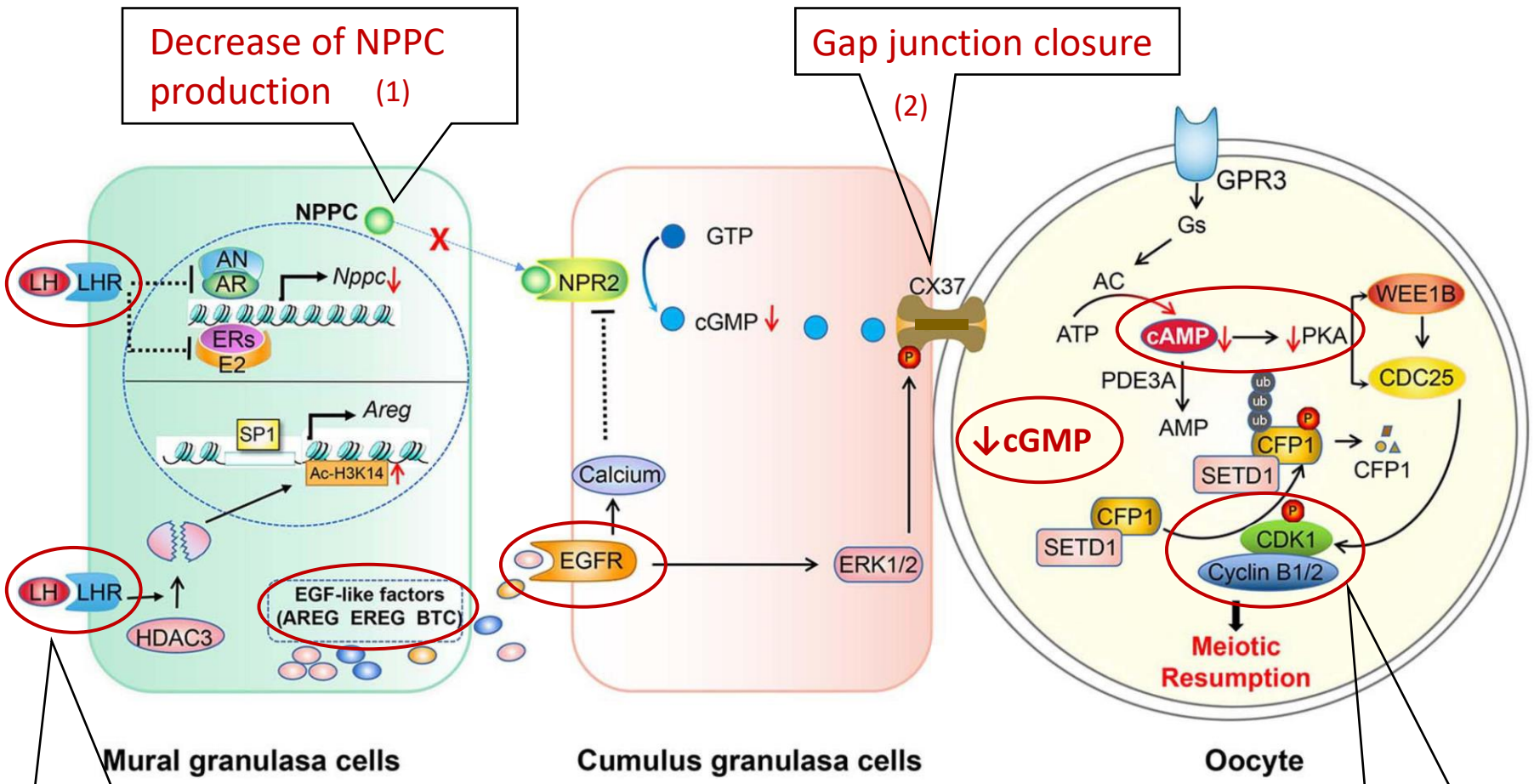
Prophase arrest is dependent on high levels of cAMP in ooplasm

* Oocyte incubation in cAMP analogs or PDE inhibitors prevents spontaneus maturation in vitro

Resumption of meiosis



Resumption of meiosis



Oocyte and cumulus GCs in preovulatory follicles lack LHR!

LH surge (/hCG trigger)

1) ↓ cGMP synthesis in cGC
2) ↓ diffusion of cGMP from cGC to the oocyte

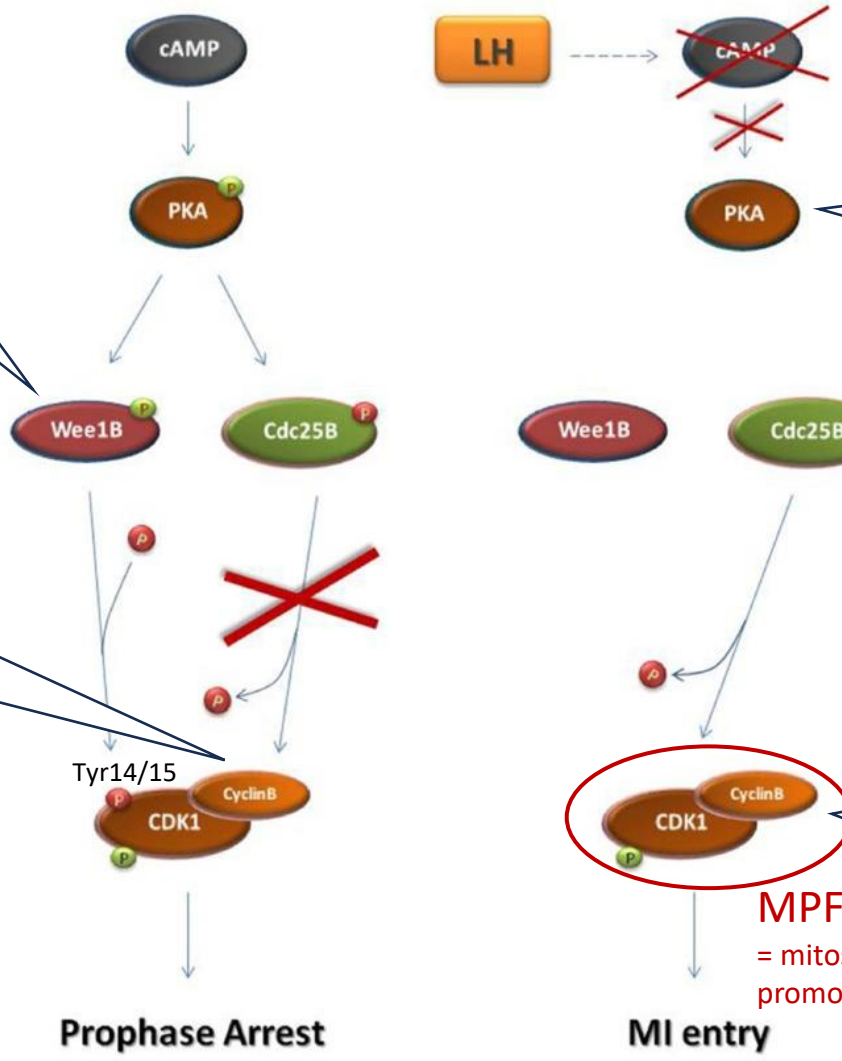
Decrease of cAMP and PKA activity leads to activation of meiosis promoting complex (**MPF**) and resumption of meiosis

He et al 2021

Resumption of meiosis

Active Wee2 and inactive Cdc25B maintain prophase I arrest

- cycB accumulated during oocyte growth
- mouse oocytes with 80% of full size (60-65µm) have sufficient stockpile to resume meiosis



LH-induced drop of cAMP level leads to PKA inactivation

Active cdc25B removes inhibitory phosphate from CDK1

MPF
= mitosis/meiosis promoting factor

- in 3-4 hours in mouse oocytes
- In 12 hours in human oocytes

Resumption of meiosis



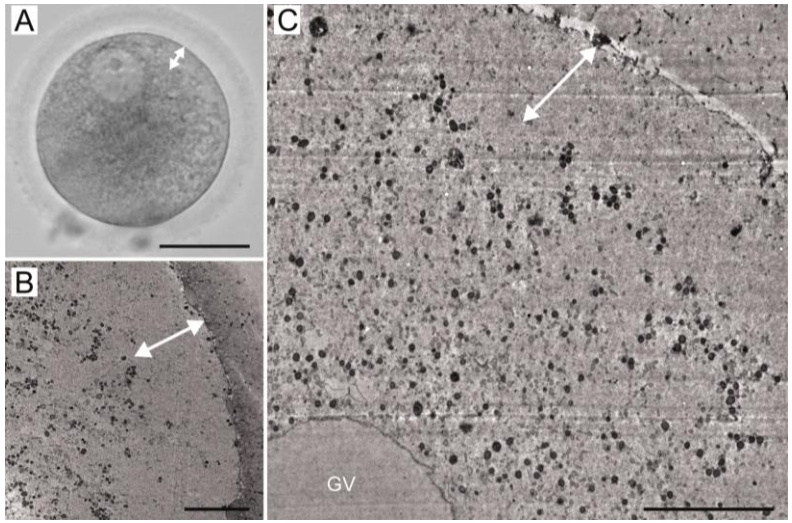

Visible sign of meiotic reactivation is

nuclear envelope breakdown (NEBD)
also known as **GV breakdown (GVBD)**
= meiotic **diakinesis**

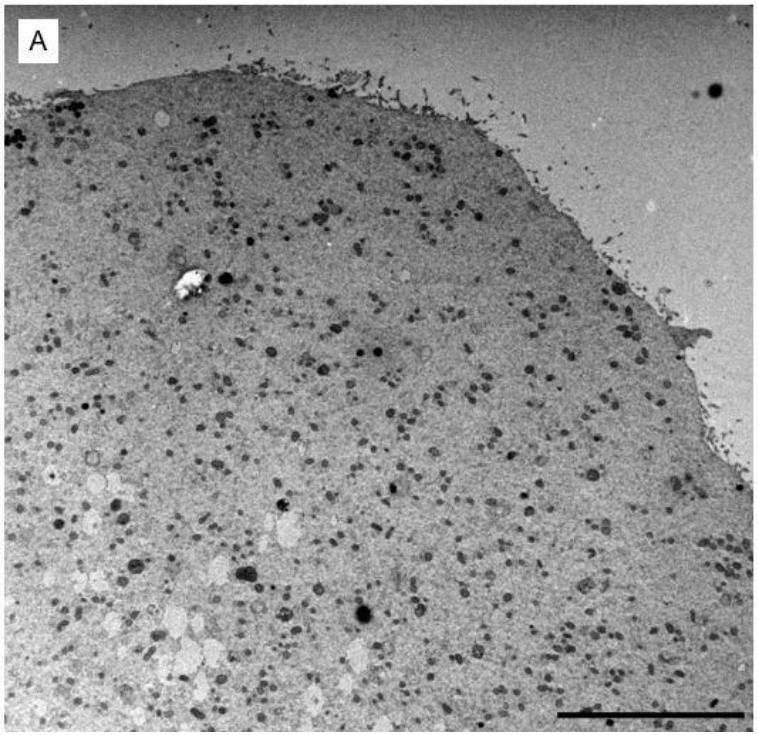
Preceded by:
- chromatin congression
- relocation of nucleus towards oolema
- GV belt disapperance

Resumption of meiosis

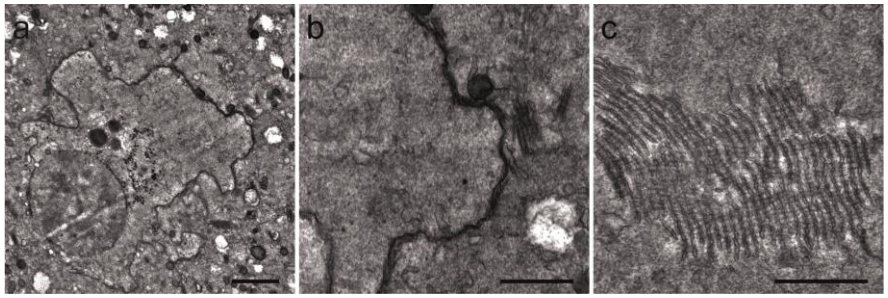
GV



GV belt – subcortical region depleted from cellular organelles



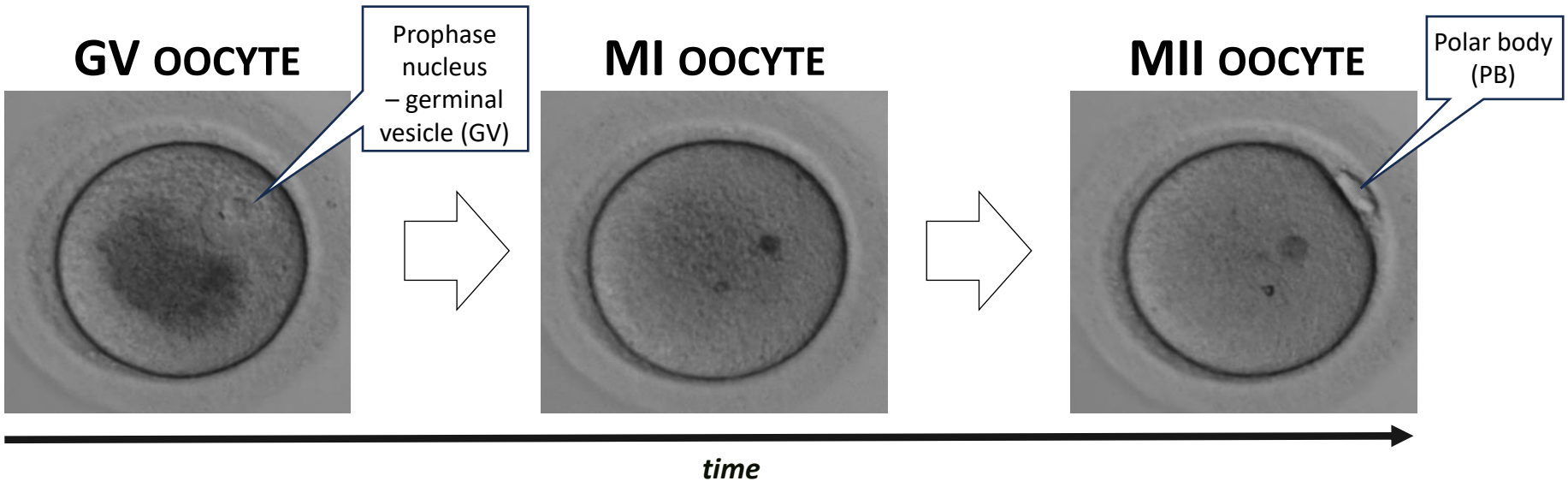
GVBD



- GV collaps
- arrays of membrane fragments *(annulate lamelae)*
- organelles populate subcortical region

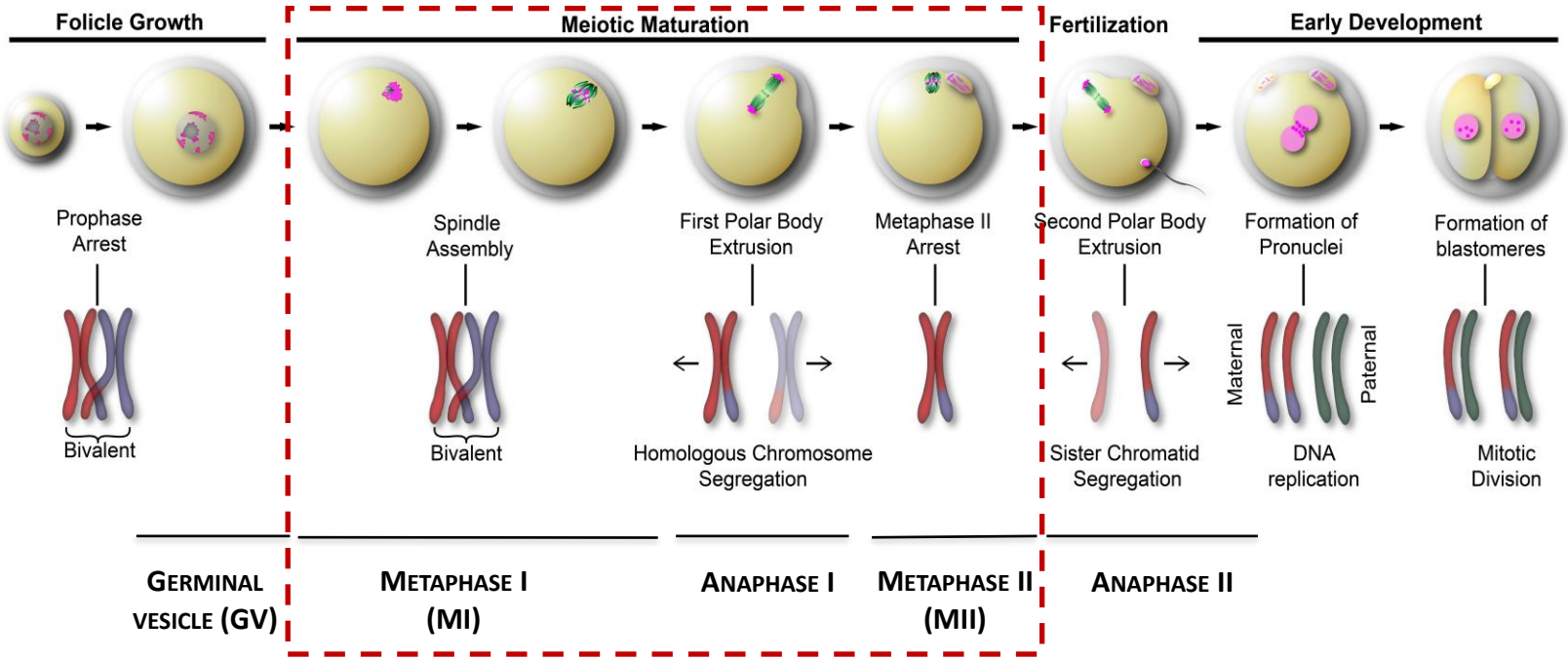
Trebichalska et al 2021

Oocyte maturation



- hormonally-primed oocytes spontaneously mature in vitro when denuded from cumulus cells

Oocyte maturation



Nuclear maturation

- chromosomal segregation
- polar body (PB) extrusion
- MII arrest
- 2n 4C oocyte → 1n 2C egg

Cytoplasmic maturation

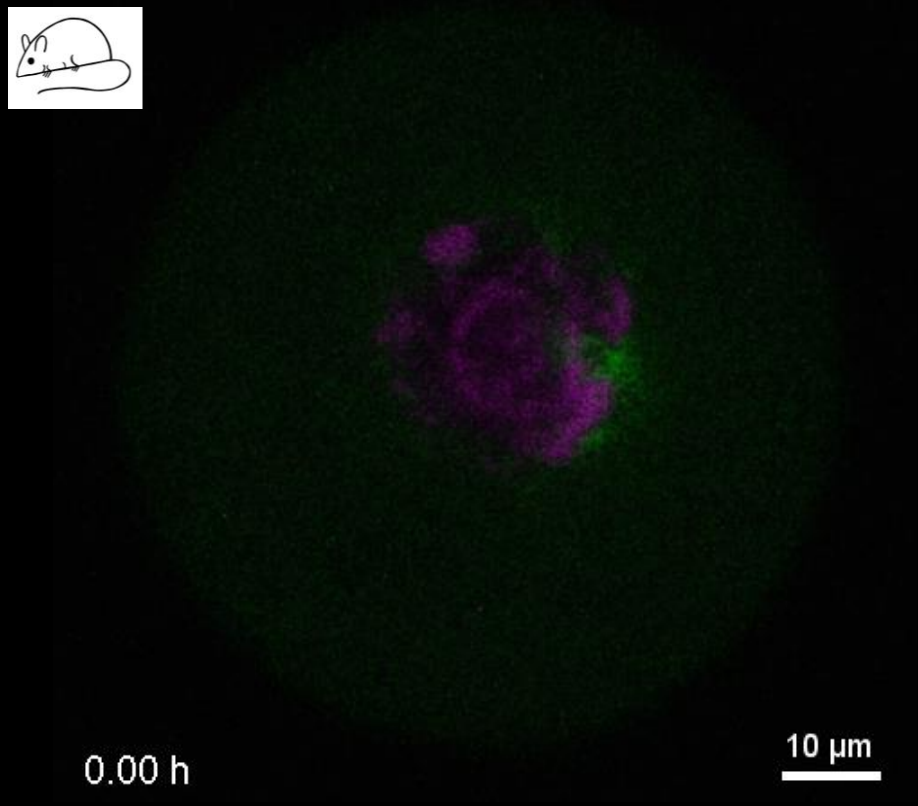
- structural and functional modification of organelles
- global changes in organelle arrangement
- mRNA translation and posttranslational modifications
- synthesis/degradation of maternal factors

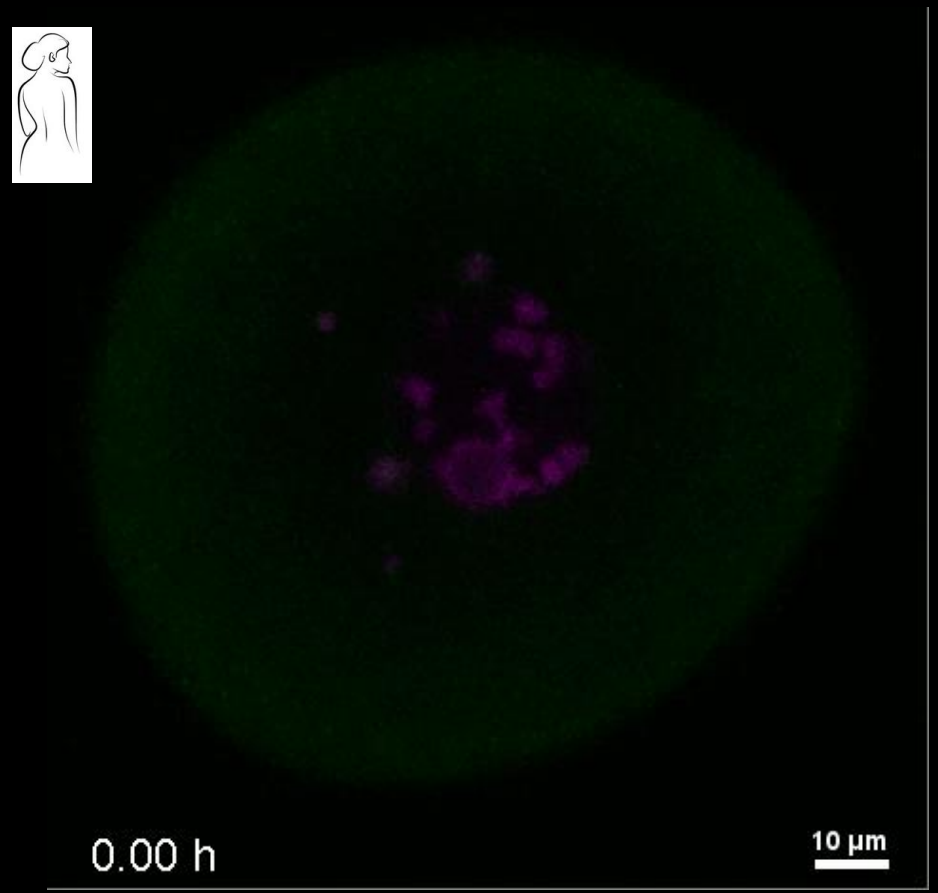
Acquisition of fertilization and developmental competence

Nuclear maturation in mammalian oocytes

(1) Nuclear envelope break-down
(2) Chromatin condensation and chromosome individualisation
(3) Chromosome alignment at spindle
(4) Homologous chromosome segregation (asymmetic cytokinesis)
(5) MII spindle formation – MII arrest

MAP4 – microtubules
H2B - DNA





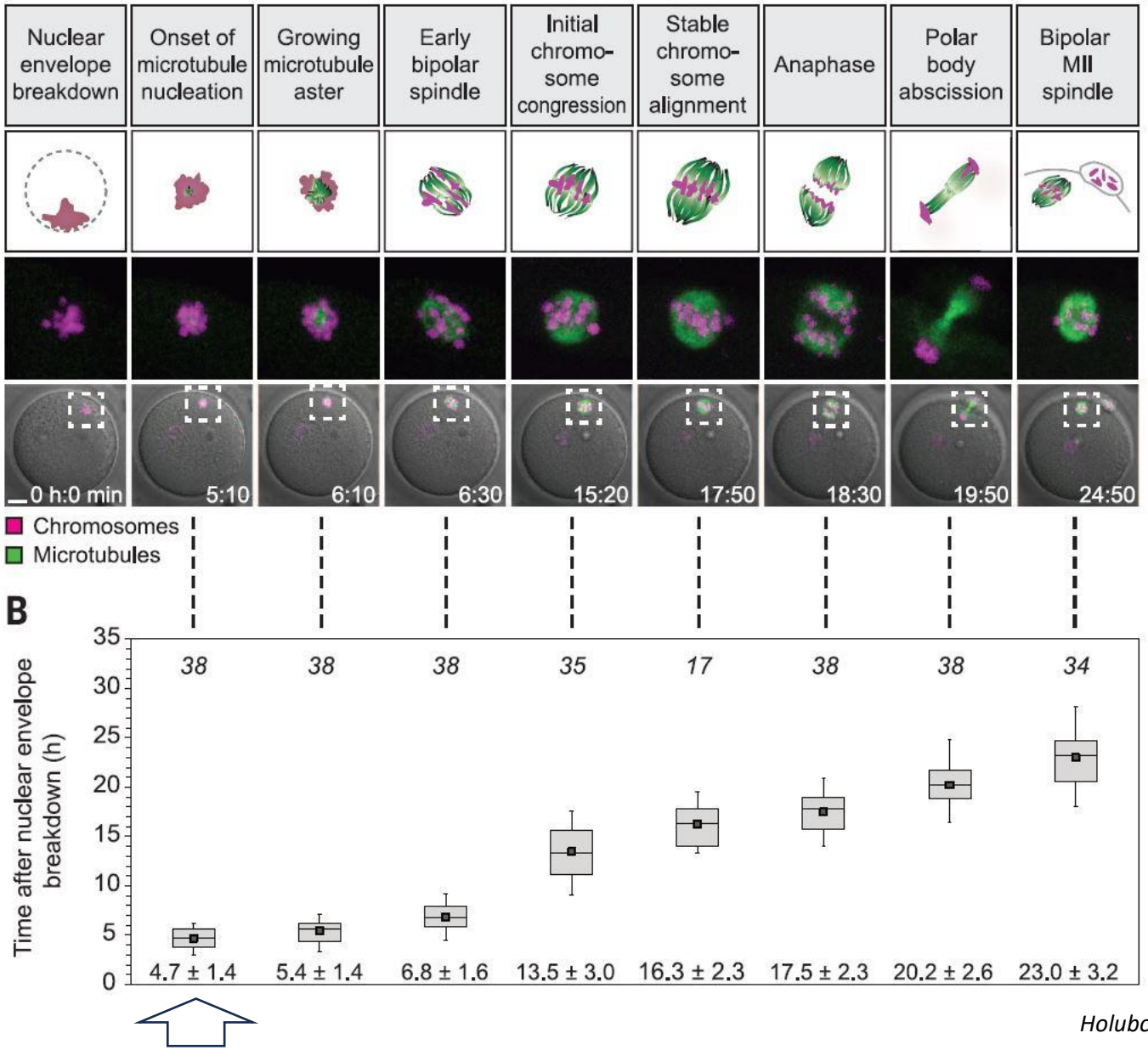
Schuh and Ellenberg, Cell 2007

~ 12 hours

~ 24 hours

Holubcova et al Science 2015

Nuclear maturation in human oocyte

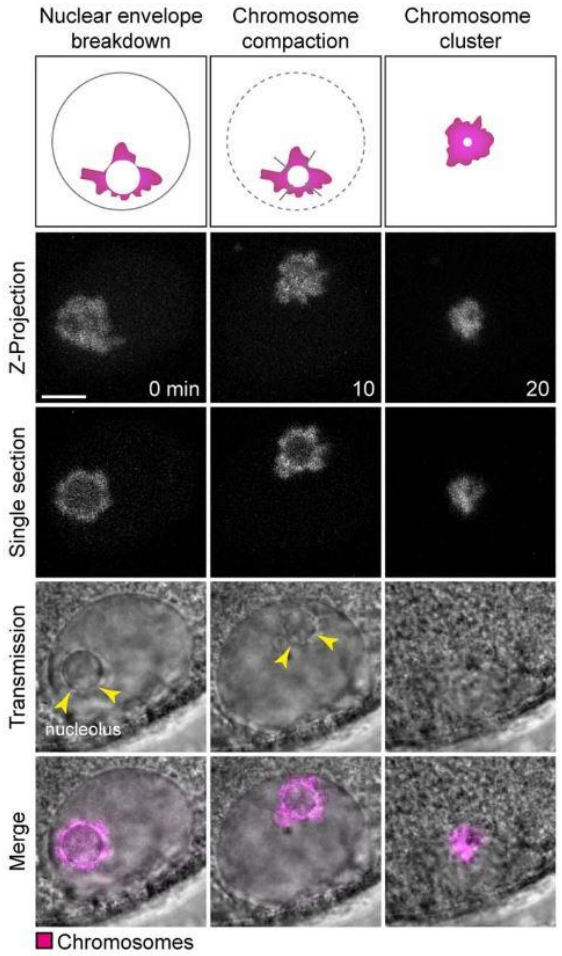


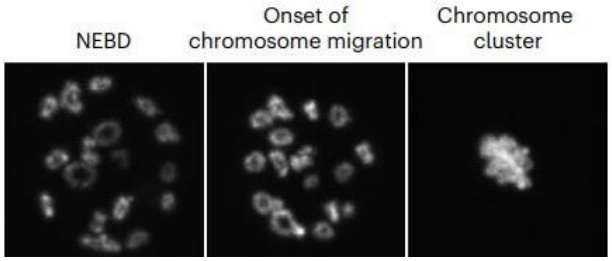
Holubcova et al Science 2015

Nuclear maturation in human oocyte

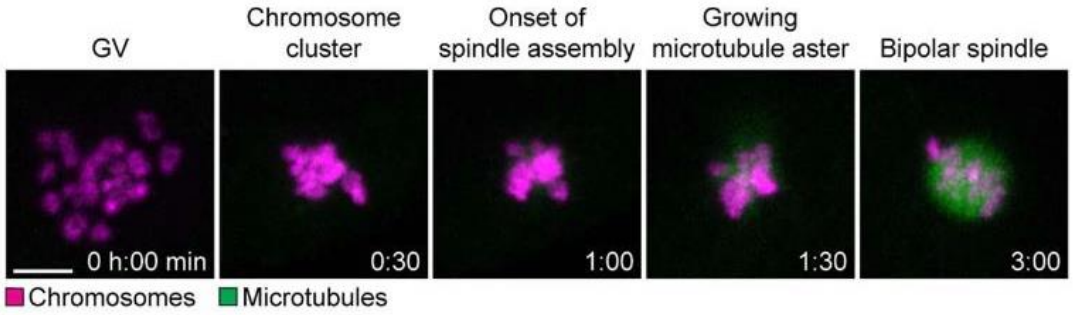
❖ Chromosome clustering

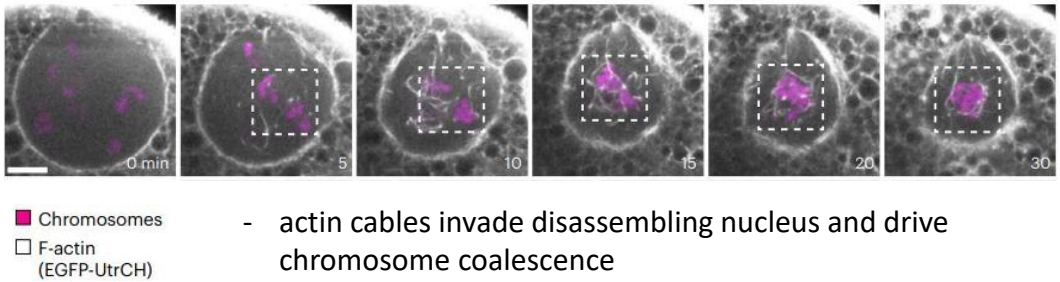
- transient stage of chromatin aggregation
- after NEBD and before onset of spindle assembly





Melina Schuh





- actin cables invade disassembling nucleus and drive chromosome coalescence

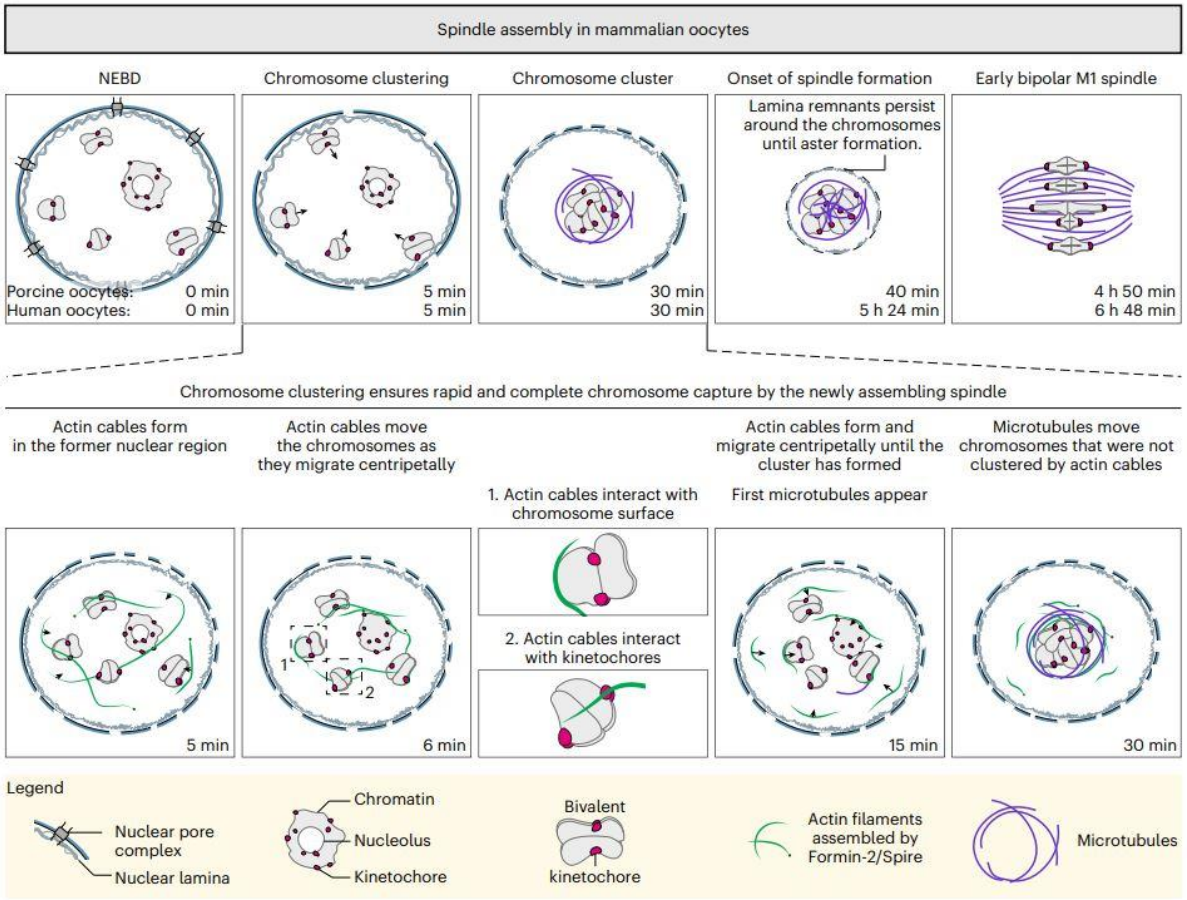
Harasimov et al, Nature Cell Biology 2023

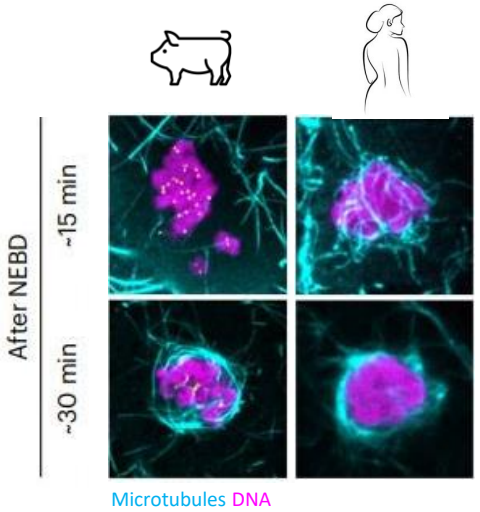
Nuclear maturation in human oocyte

❖ Chromosome clustering

- promotes rapid capture of chromosomes by acentrosomal spindle and prevents chromosome losses in the long gap phase between nuclear envelope breakdown and the onset of spindle assembly

Melina Schuh

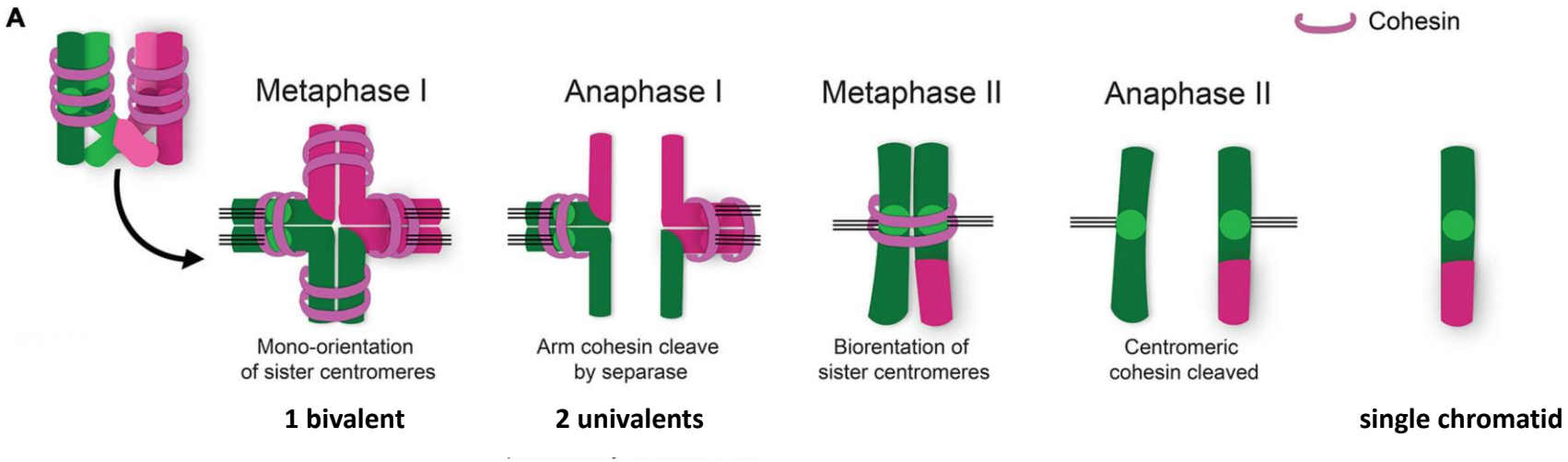


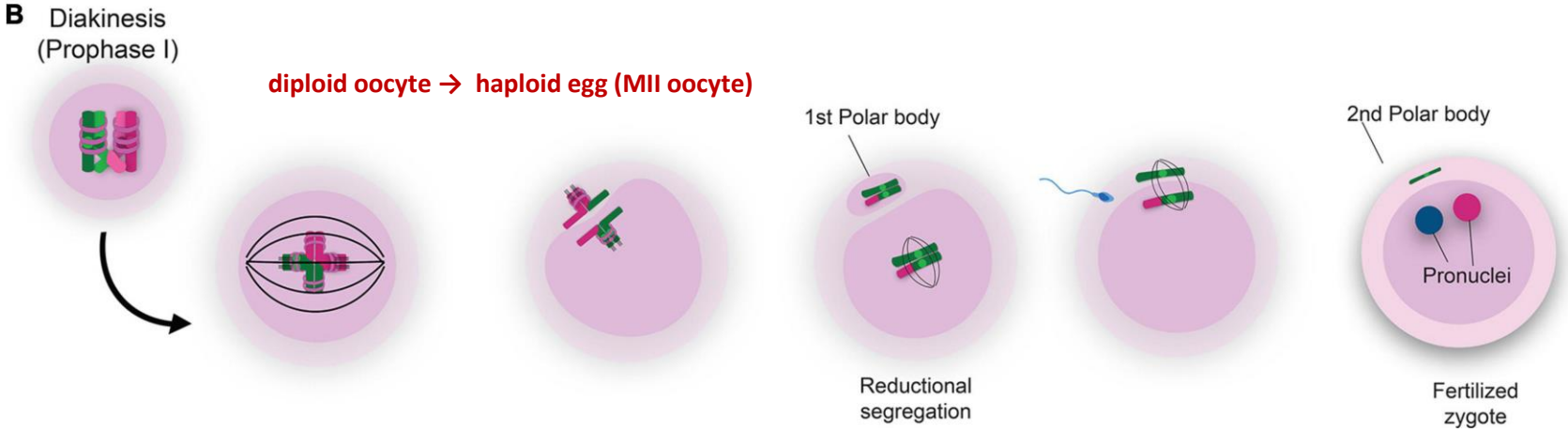


Microtubule „cage“

Harasimov et al, et al. 2023

Chromosome segregation



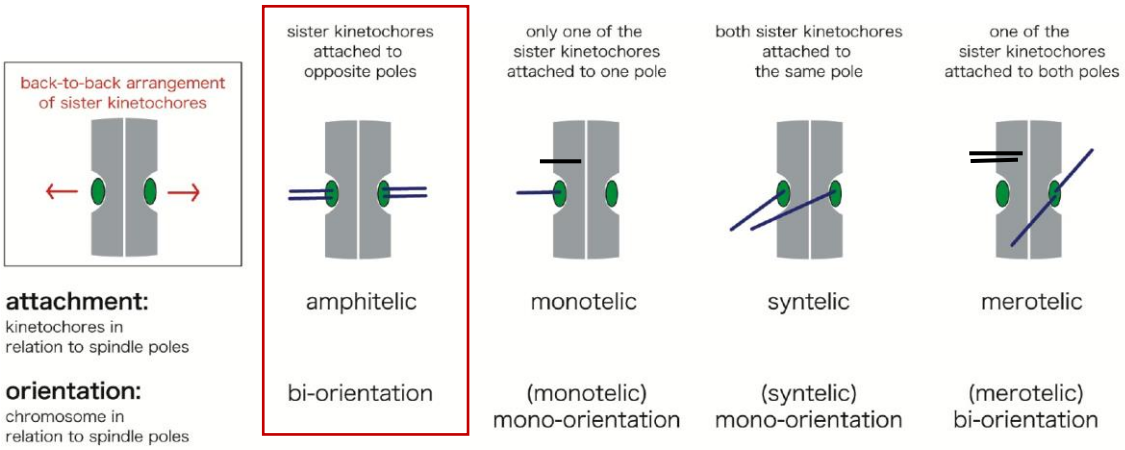


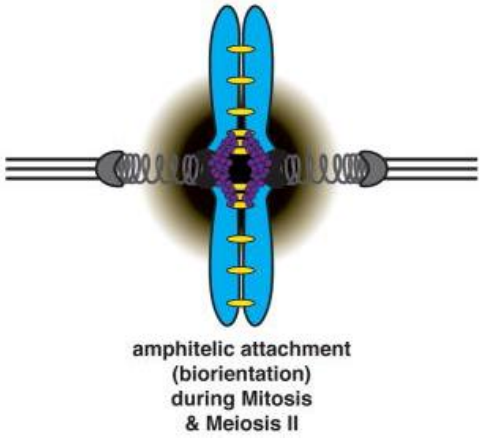
- **Meiosis I**
  - separation of homologous chromosomes
  - haploid set of chromosomes eliminates to 1st PB
  - 2n 4C → 1n 2C

- **Meiosis II**
  - separation of sister chromatids
  - one set of chromatids eliminated to 2nd PB
  - 1n 2C → 1n 1C (+ 1n 1C from sperm)

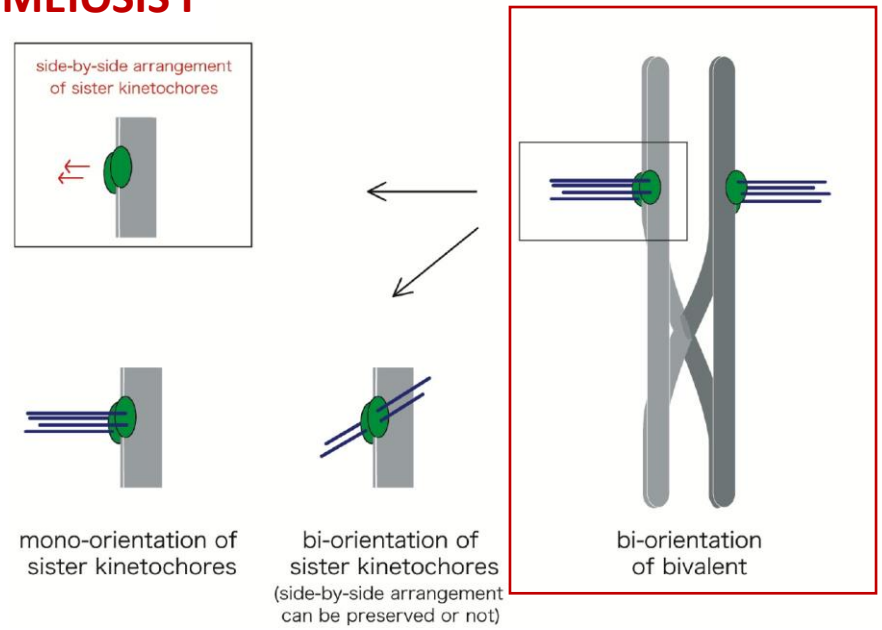
Kinetochore-microtubule attachment

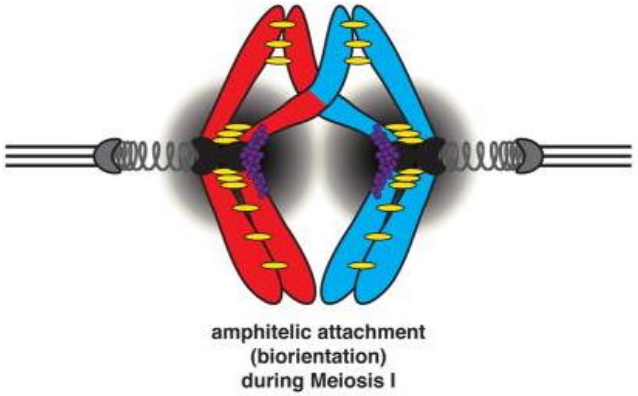
➢ MITOSIS/ MEIOSIS II





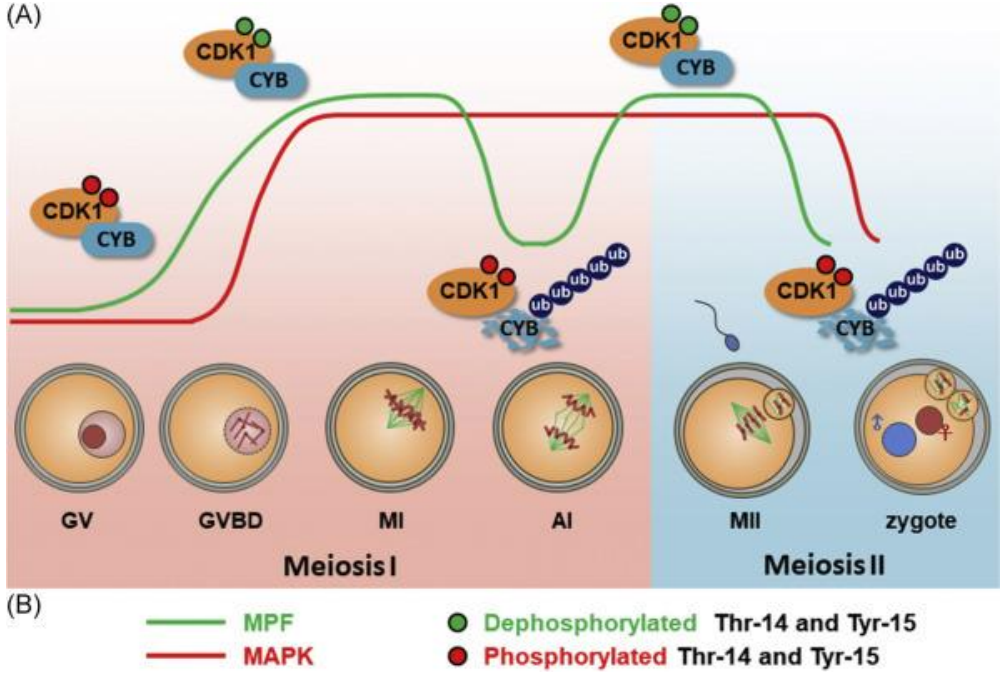
➢ MEIOSIS I





- sister chromatid monoorientation (behave like functional unit !)

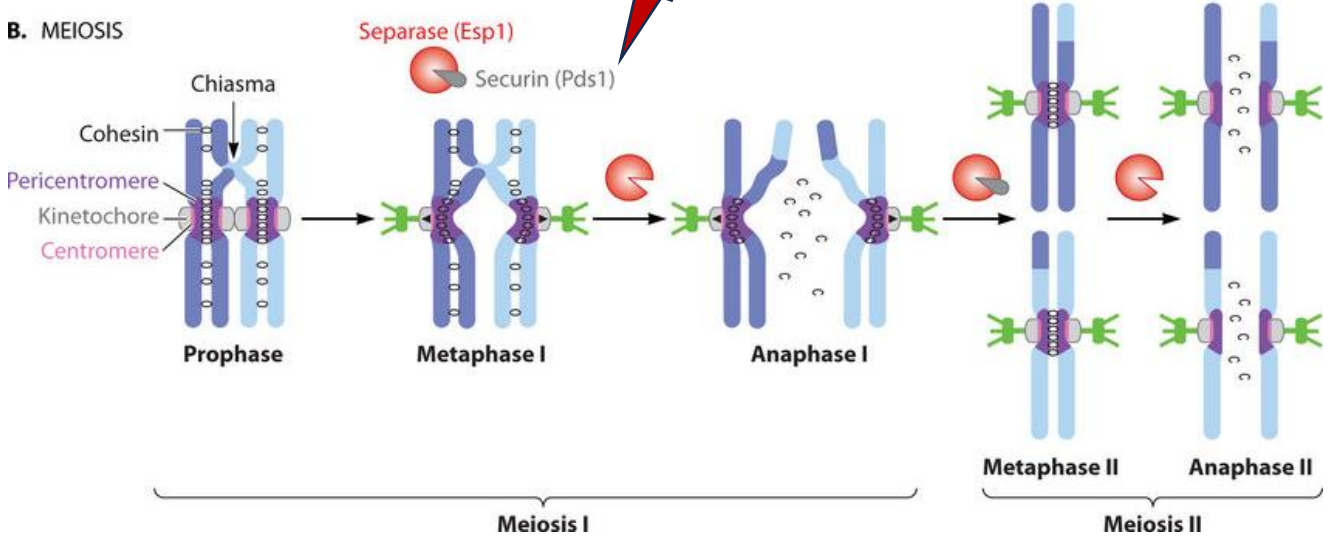
Anaphase entry



↓ CDK1 activity

Anaphase Promoting Complex (APC)

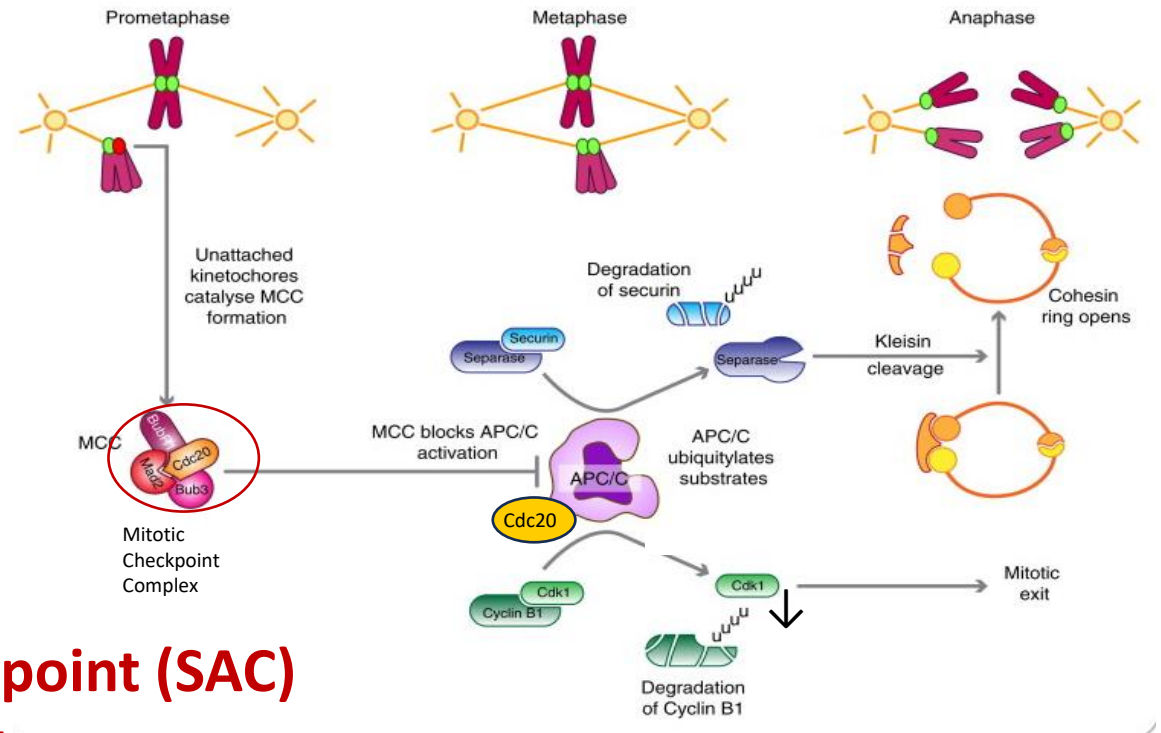
Separase-mediated cleavage of cohesins



Anaphase entry

➢ MITOSIS

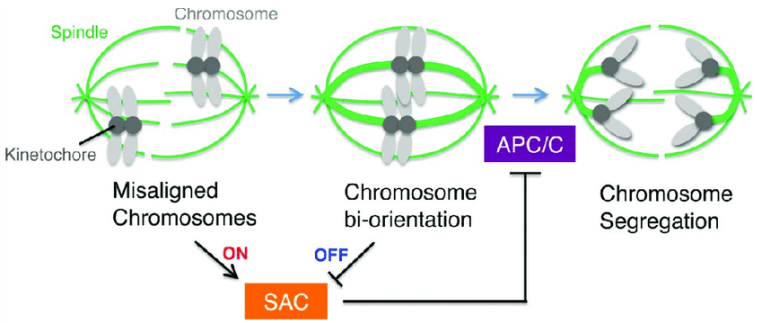
- Mitotic Checkpoint Complex (MCC) on unattached kinetochores prevent Anaphase Promoting Complex (APC) from cyclin B and securin destruction

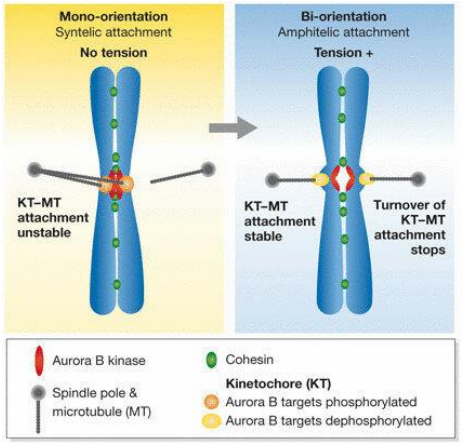


❖ Spindle assembly checkpoint (SAC)

- the pathway that delays mitosis until kinetochores are attached to microtubules

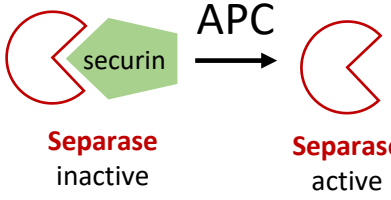
- **all kinetochores** must be occupied
- APC/C activation in minutes
- low aneuploidy rate
- role of interkinetochore tension





Anaphase entry

- **MEIOSIS I**



Rec8

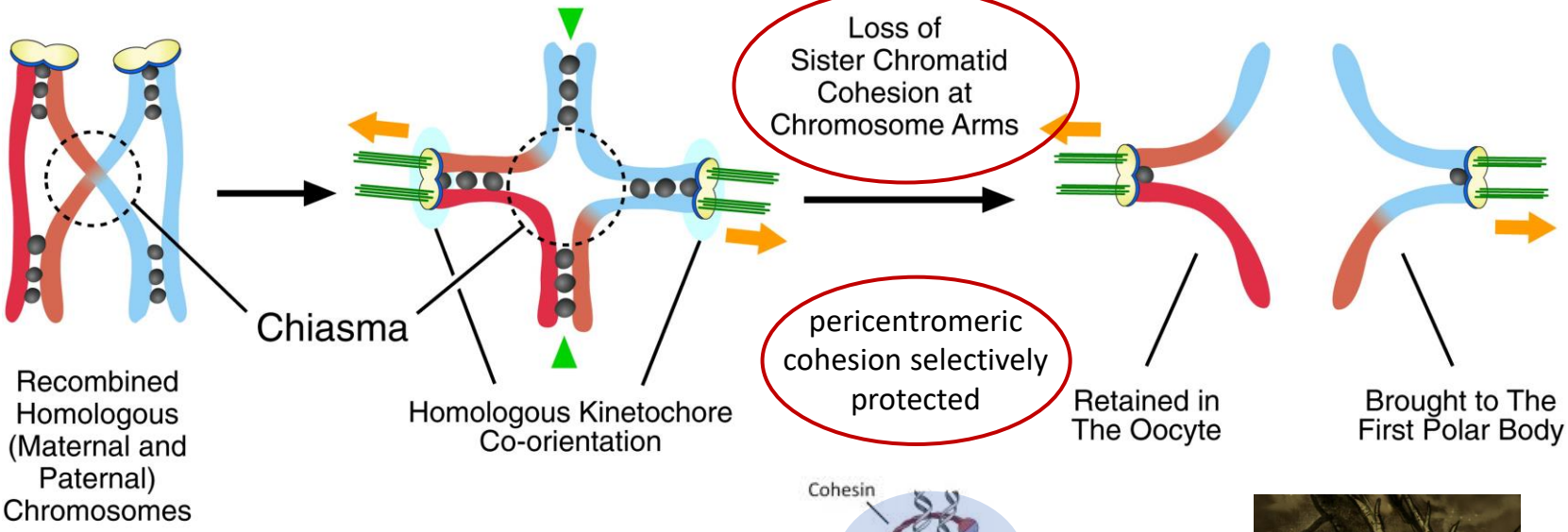
separase cleaves Rec8

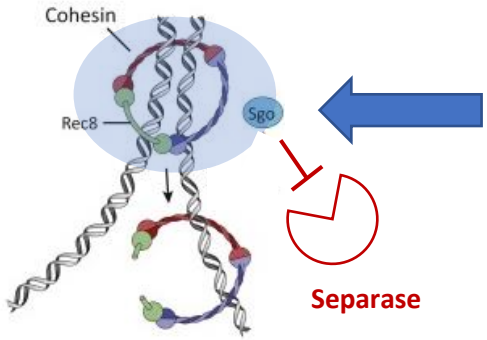
cohesion ring opened

Prophase I

Metaphase I

Anaphase I



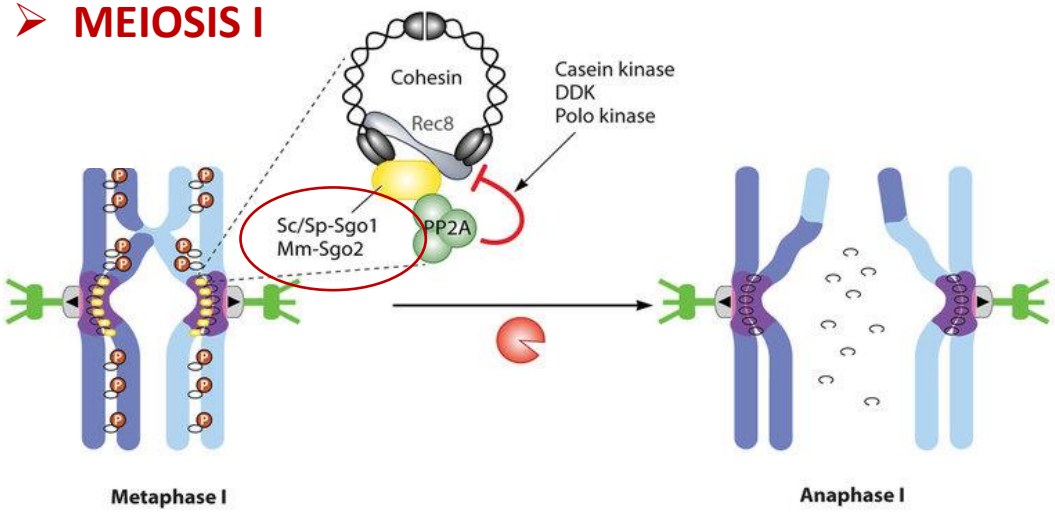


Shugoshin (Sgo)

- Japanese „guardian spirit“

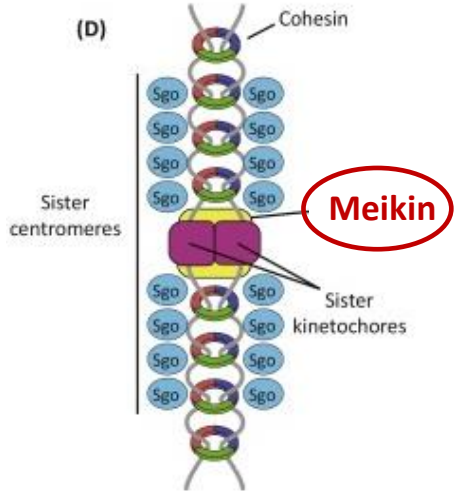
Loss of cohesion in anaphase I

➢ **MEIOSIS I**



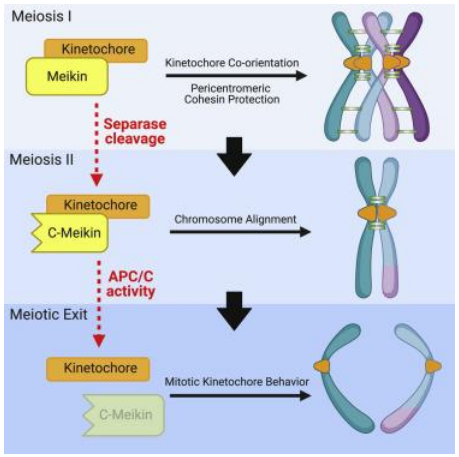
Shugoshin (Sgo2)

recruits PP2A, which removes Rec8 phosphorylation, making it a poor substrate for separase-dependent cleavage.

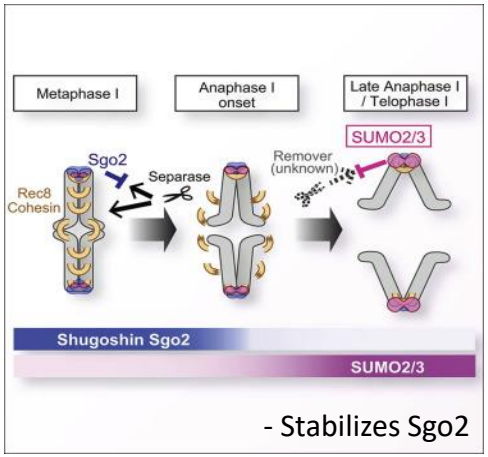


- Stabilizes location of Sgo
- Ensures chromosome alignment in MII

Kim et al 2015.



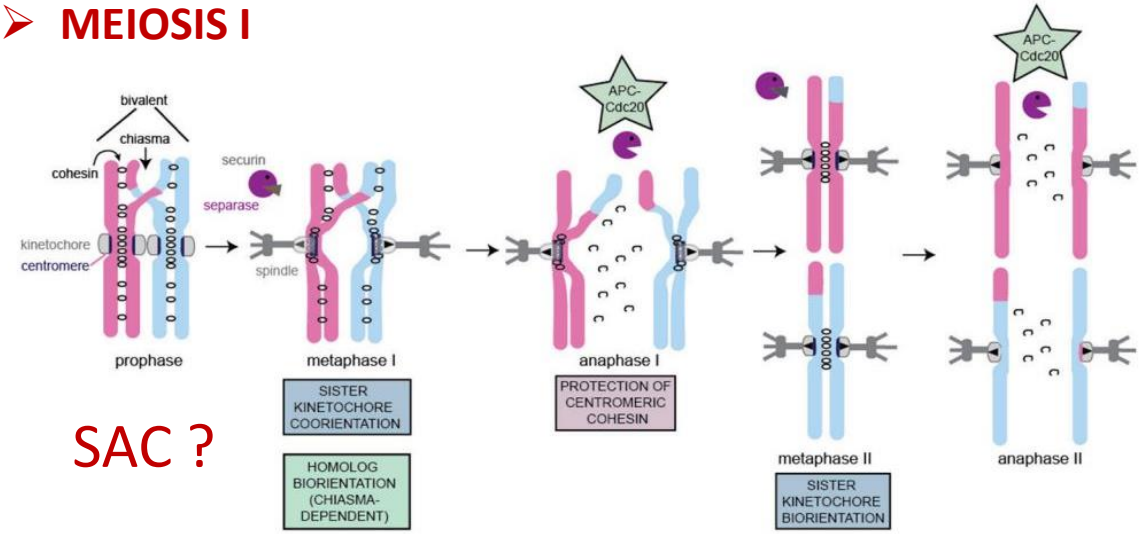
Maier et al, 2021



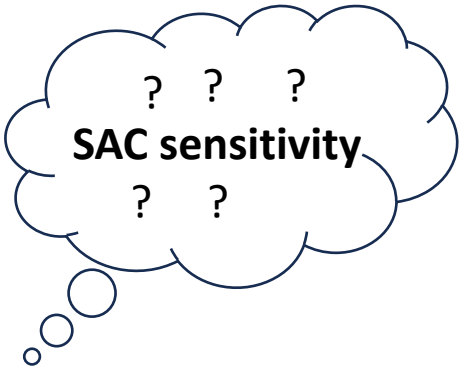
Ding et al, 2018

Anaphase entry

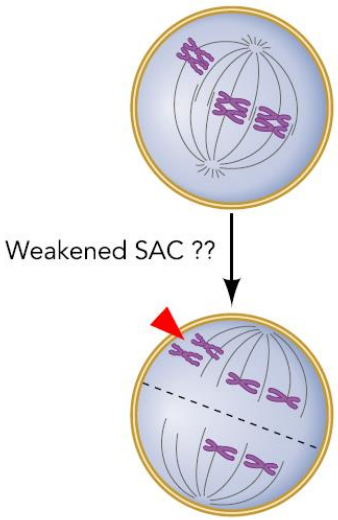
➢ **MEIOSIS I**

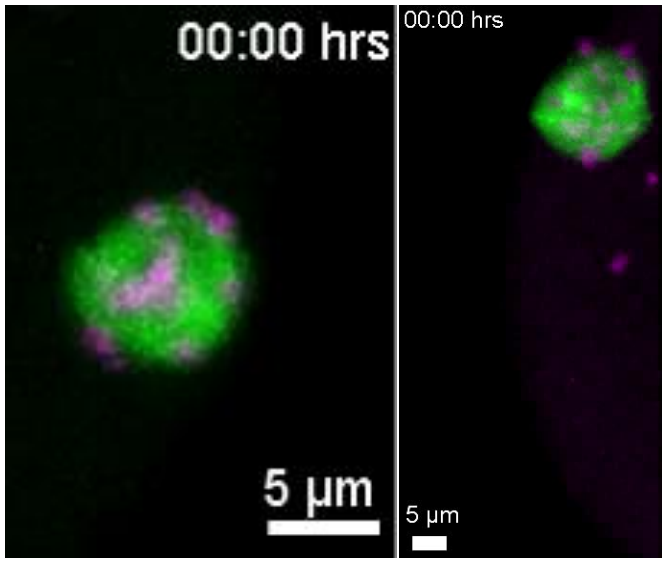


SAC ?



Marson and Wassmann 2017

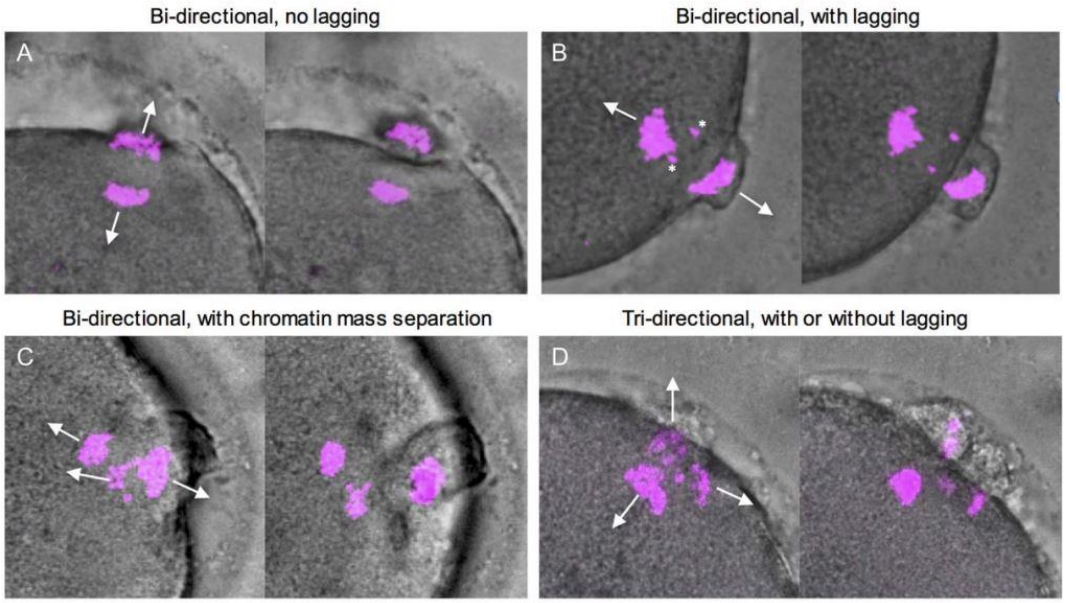




Chromosomes (H2B-mRFP)
Microtubules MAP4-EGFP)

Zielinska et al. eLife 2015.

SAC in human oocytes is permissive



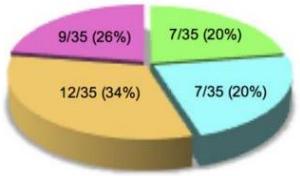
human reproduction ORIGINAL ARTICLE Reproductive biology

Tri-directional anaphases as a novel chromosome segregation defect in human oocytes

Jenna Haverfield[1,2], Nicola L. Dean[1,3], Diana Nöel[3], Gaudeline Rémillard-Labrosse[1], Veronique Paradis[3], Isaac-Jacques Kadoch[2,3], and Greg FitzHarris[1,2,*]

[1]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), University of Montréal, 900 Rue St Denis, Montréal, Québec, Canada H2X 0A9 [2]Department of Obstetrics and Gynaecology, University of Montréal, Montréal, Québec, Canada H3T 1J4 [3]Clinique de Procréation Assistée (CPA) du CHUM, Montréal, Québec, Canada H2L 4S8

Greg FitzHarris



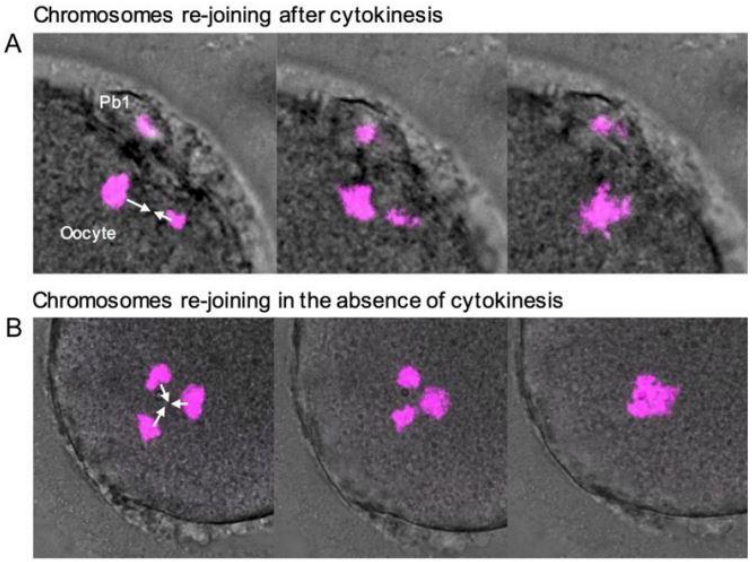
- Bi-directional, no lagging
- Bi-directional, with lagging
- Bi-directional, with chromatin separation
- Tri-directional

- misaligned chromosomes do not block anaphase I onset

- Tripolar anaphase may result in

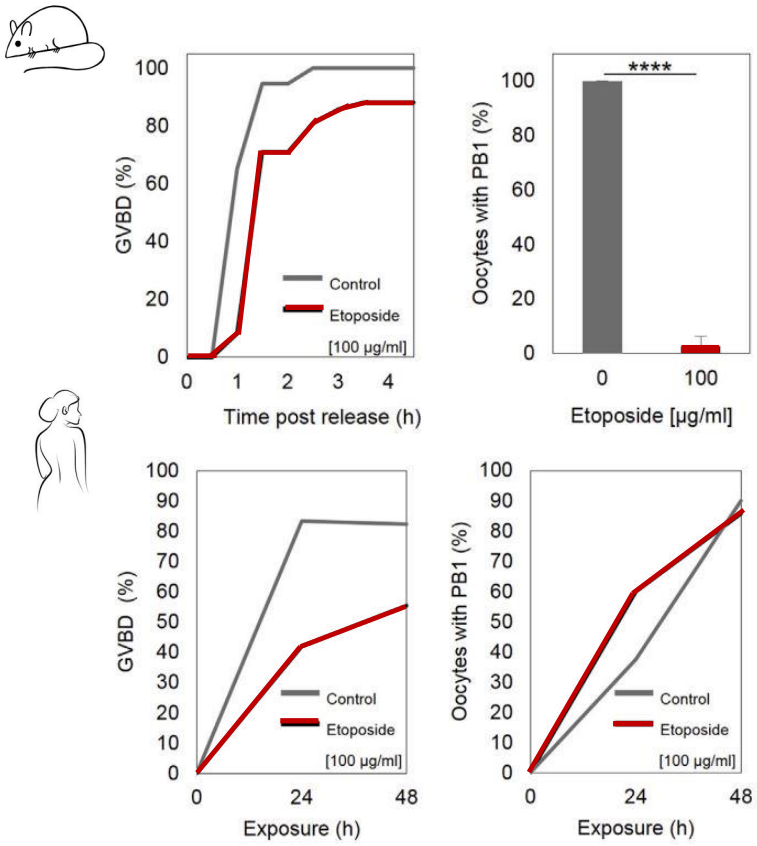
(1) reunion of 2 chromosomas masses in the aneuploid oocyte, 3rd mass extruded to the PB

(1) re-joining of chromatin and cytokinesis failure (seemingly non-maturing MI !)



Havrfield et al. 2017.

SAC in human oocytes is permissive



Induced DNA damage **does not prevent anaphase entry in human oocytes**

- Human oocyte with DNA damage harbour abnormal spindles but exhibit apparently normal morphology!

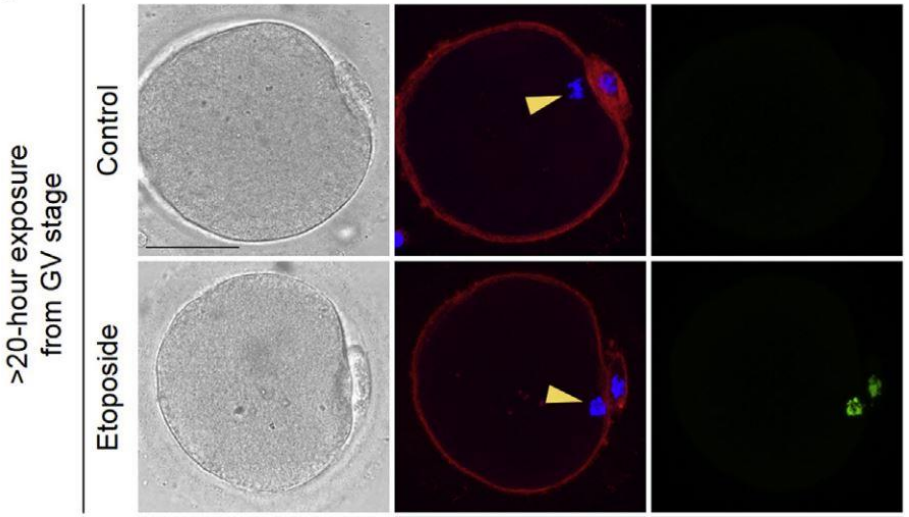
Remillard-Labrosse et al. 2019.

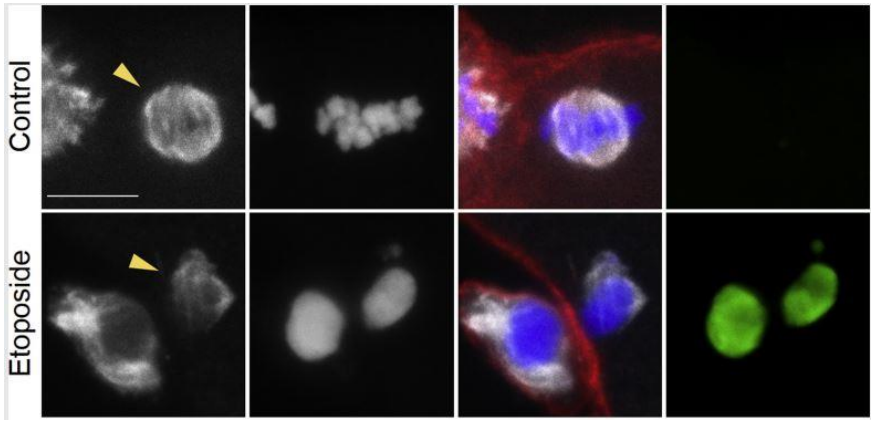
Human oocytes harboring damaged DNA can complete meiosis I

Gaudeline Rémillard-Labrosse, Ph.D.,[a] Nicola L. Dean, Ph.D.,[a] Adélaïde Allais, M.Sc.,[a] Aleksandar I. Mihajlović, Ph.D.,[a,b] Shao Guang Jin, M.D., Ph.D.,[c] Weon-Young Son, Ph.D.,[c] Jin-Tae Chung, M.Sc.,[c] Melissa Pansera, M.Sc.,[c] Sara Henderson, M.Sc.,[c] Alina Mahfoudh, M.Sc.,[c] Naama Steiner, M.D.,[c] Kristy Agapitou, B.Sc.,[d,e] Petros Marangos, Ph.D.,[d,e] William Buckett, M.D.,[c] Jacob Ligeti-Ruiter, M.D.,[c] and Greg FitzHarris, Ph.D.[a,b]

[a] Centre de Recherche du Centre Hospitalier de l'Université de Montréal; [b] Département d'Obstétrique-Gynécologie, Université de Montréal; and [c] Reproductive Centre, McGill University Health Centre, Montreal, Quebec, Canada; and [d] Department of Applications and Technology, University of Ioannina, [e] Department of Biomedical Research, Institute of Molecular Biology and Biotechnology–Foundation for Research and Technology, Ioannina; and [f] Institute of Life Fertility Unit, IASO Maternity Hospital, Athens, Greece

Greg FitzHarris





Modes of SAC functionality

- in somatic cells, one unattached kinetochore capble to activate SAC[1]
- DNA damage prevents entry to mitosis but not anaphase

- mouse oocytes with DNA damage undergo GVBD but arest in MI and fail to extrude PB[2]
- lack of response to unaligned chromosomes and lack of tension[3]

- **in human oocytes, neither misaligned chromosomes nor DNA damage activate robust SAC response[4,2]**
- only severe spindle disruption can prevent PB
- SAC signalling machinery is present[5]

BUT inefficient to prevent anaphase onset when one or a few chromosomes are not congressed and attached to kinetochores

[1] *Kuhn & Dumont, 2019; Rieder et al.,1994*
[2] *Remillard-Labrosse et al. 2019*
[3] *Gui & Homer, 2012; Kolano et al., 2012; Lane et al., 2012; Nagaoka et al., 2011; Sebestova et al., 2012*
[4] *Zielinska et al 2015, Havrfield et al. 2017*
[5] *Lagirand-Cantaloube et al, 2017*

Cytoplasmic volume affects SAC stringency

- cytoplasm scales the spindle and affects the timing of anaphase onset and efficiency of chromosome alignment

- oocytes with decreased cytoplasmic size have spindles with better-focused poles and higher SAC stringency

- large cytoplasmic volume dilutes the nuclear factors, including anaphase inhibitors, thus resulting in the failure of the spindle to induce a checkpoint arrest in response to a small number of misaligned chromosomes

Developmental Cell
Article

Large Cytoplasm Is Linked to the Error-Prone Nature of Oocytes

Hirohisa Kyogoku[1] and Tomoya S. Kitajima[1,2,*]
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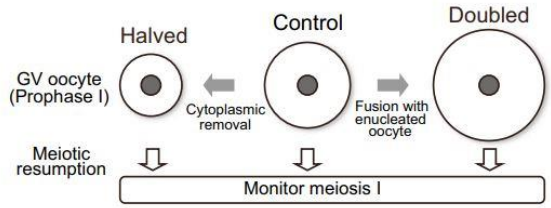
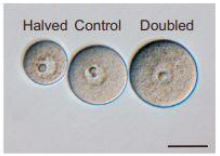
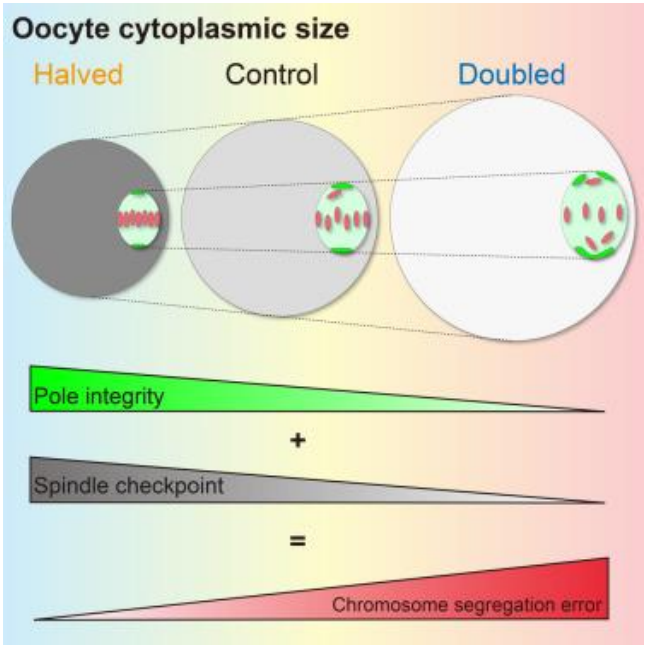
*Correspondence: tkitajima@cdb.riken.jp
http://dx.doi.org/10.1016/j.devcel.2017.04.009

Tomoya Kitajima

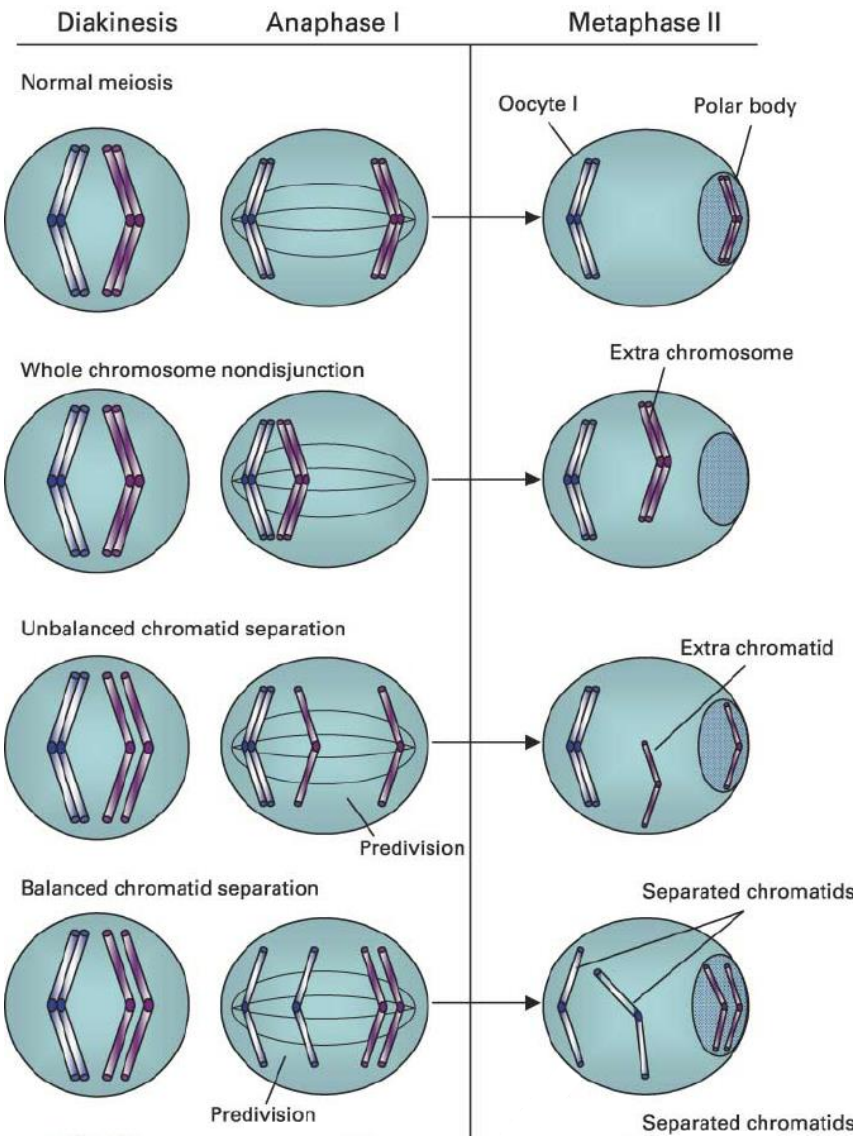






Kyogaku and Kitajima, Developmental Cell, 2017.

Chromosome segregation errors



Oocyte aneuploidy

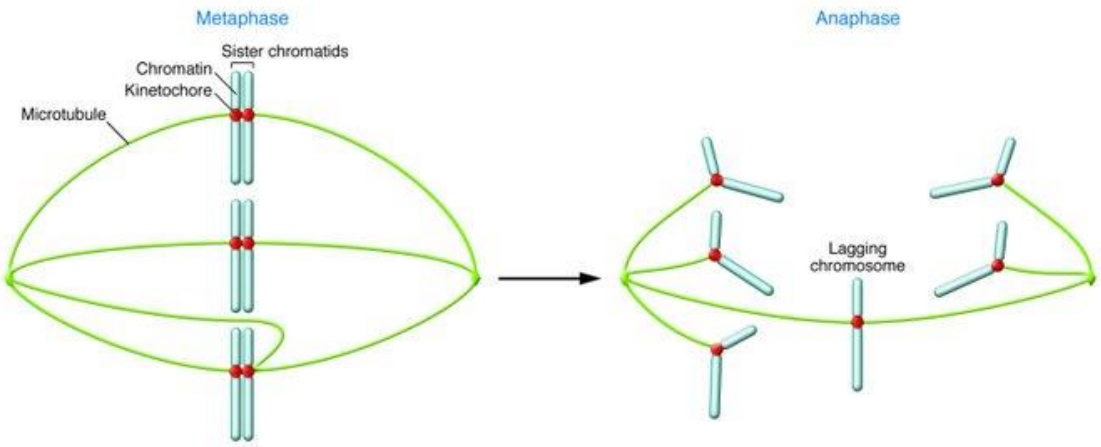
Chromosome nondisjunction (NDJ)

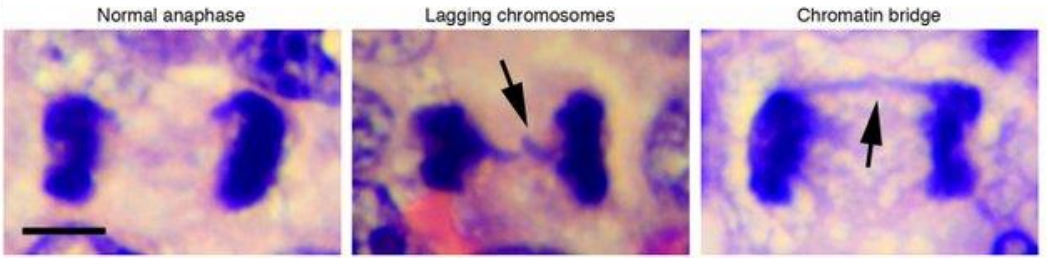
Precocious separation of sister chromatids (PSSC)

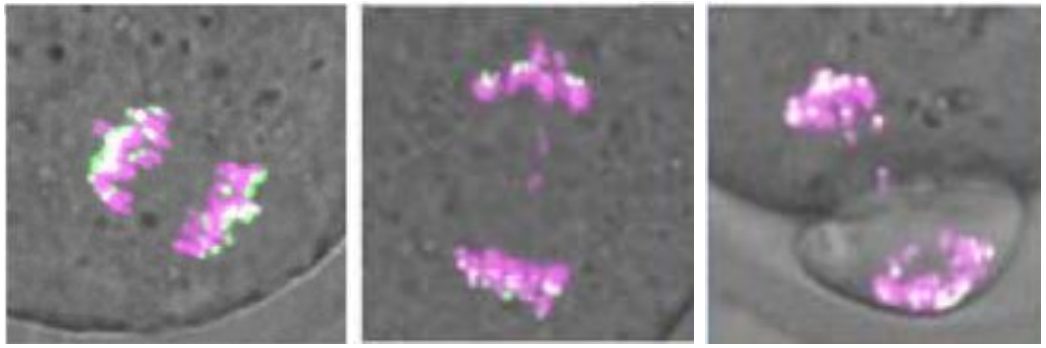
Chromosome lagging

- delayed chromosome/chromatid movement during anaphase

- risk of inaccurate segregation, chromosome loss/gain and aneuploidy



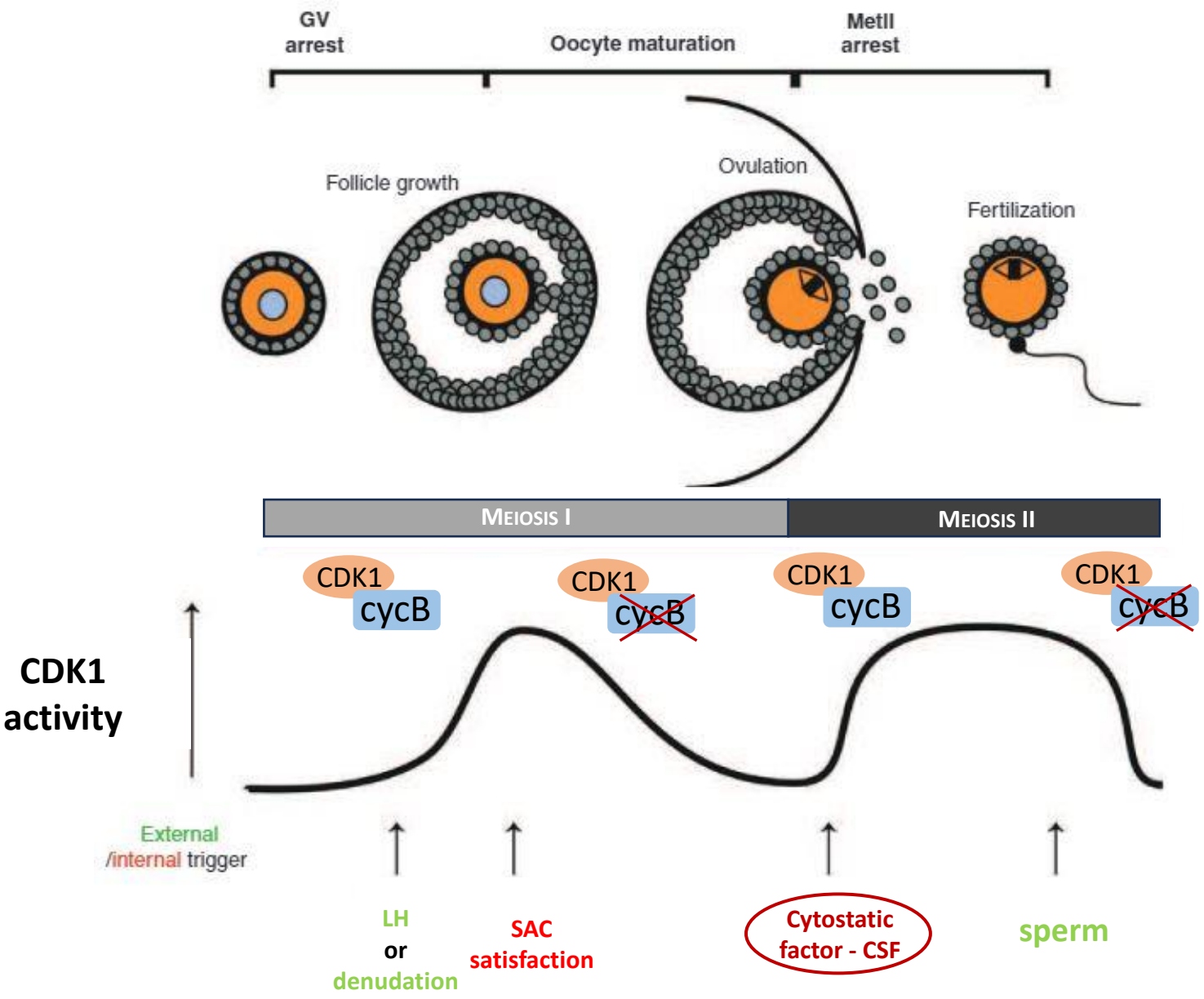




No lagging | Class I laggards | Class II laggards

Mihajlovic et al 2021

Transition from meosis I to meiosis II

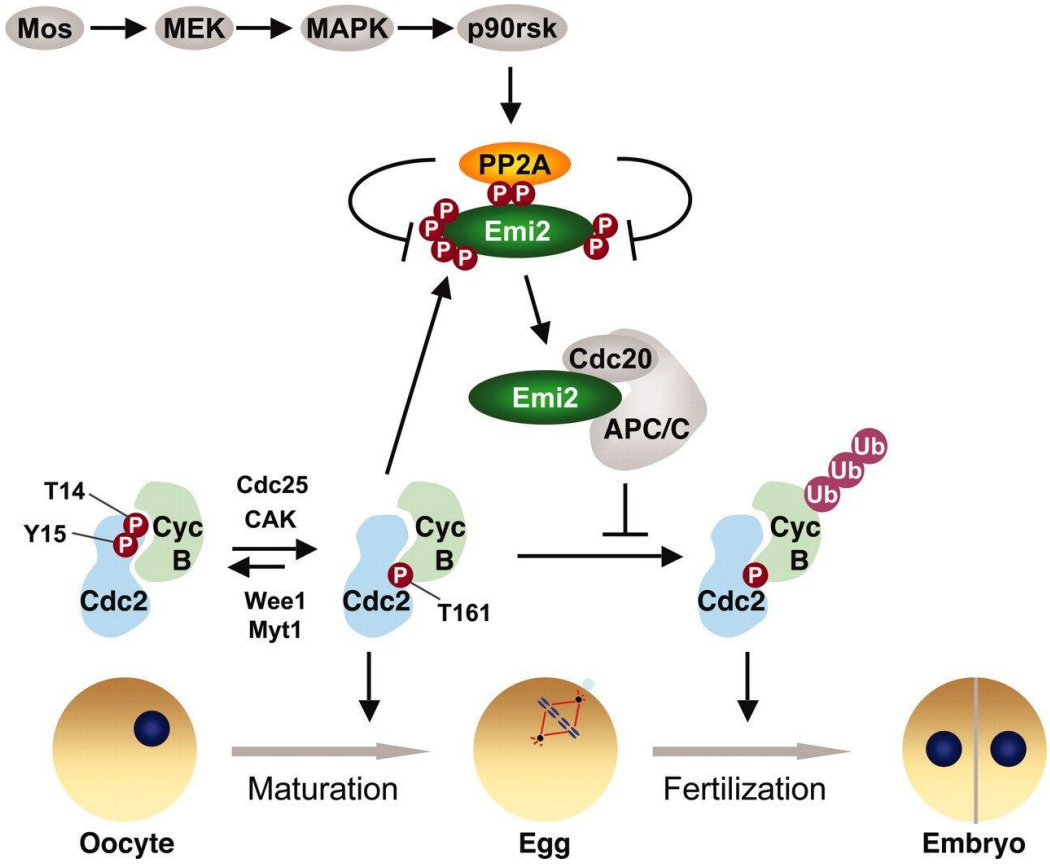


Metaphase II arrest

➢ Emi2 (Early Mitotic Inhibitor)

- meiosis specific inhibitor of APC (=„cytostatic factor“ - CSF)
- required for establishment and maintanance of MII arrest in mammalian oocytes
- phosphorylation needed to keep Emi2 stable

Magwick et al 2006



➢ Btg4

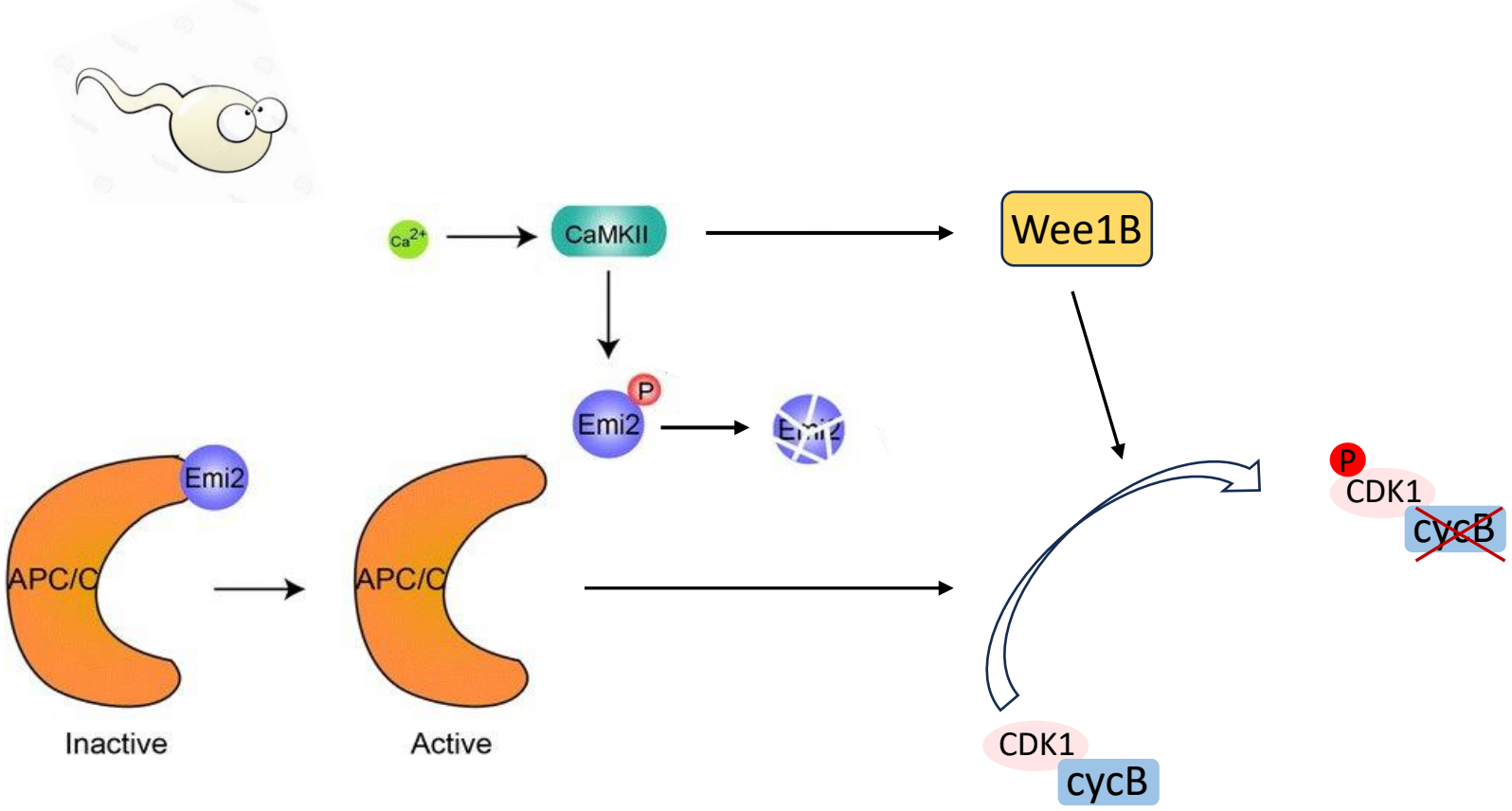
- contributes to APC/C inhibition by controling protein expression during MII arrest
- expression of Emi2 is perturbed when BTG is absent (RNAi - depletion)

Pasternak et al 2016

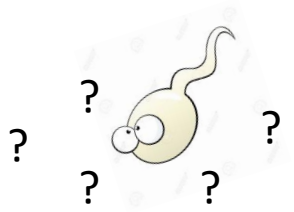
- MII arrest reached hours before ovulation and maitained for ~ 24 hours

MII arrest release

- **Calmodulin dependent protein kinase II (CAMKII)**
- activated by Ca2+ signal at fertilization
- phosphorylates Emi2 causing its degradation
- activates Week1B kinase that phosphorylates CDK1 contributing to its inactivation



Fertilization stage in different species



Primary diploid oocyte	First metaphase	First meiotic division	Second metaphase	Second meiotic division	Haploid egg
XX	XX		X X		– X
2n 4C	2n 4C		1n 2C		1n 1C
• Flatworms • Roundworms	• Molluscs • Insects		• Amphibians • Mammals		• Coelenterates • Echinoderms

Examples of organisms that are fertilized at the stage of meiosis indicated

https://is.muni.cz/do/med/el/ake/index.html

e-Atlas klinické embryologie

- CZE/ENG e-learningová pomůcka
- veřejně dostupné po zadání **hesla**

= datum narození Luise Brown (DDMMYYYY)