Embryologie I
OOGENESIS

autumn 2023

Oocyte meiosis

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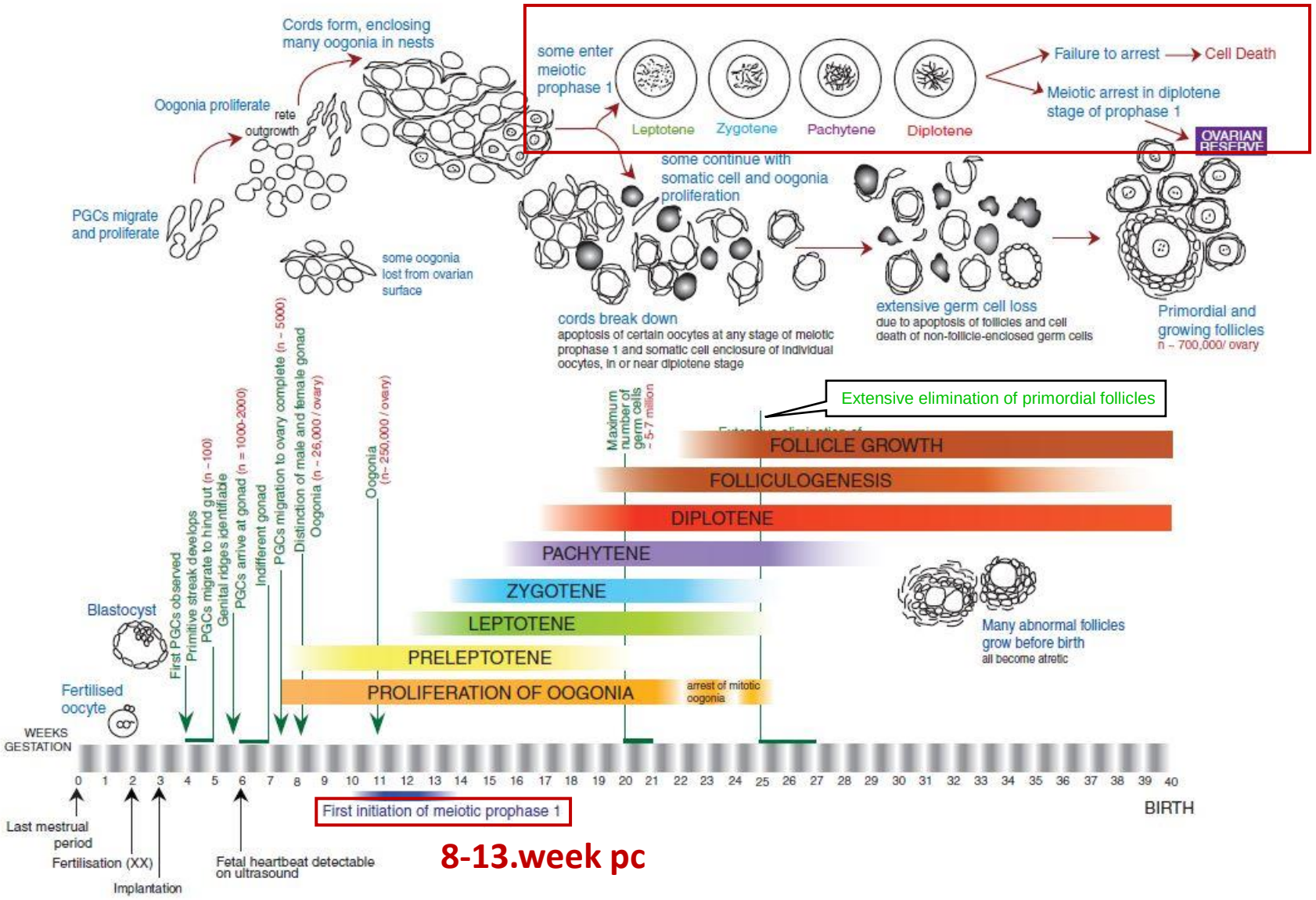
Prenatal stage of oogenesis



8-13.week pc

Entry to meiosis

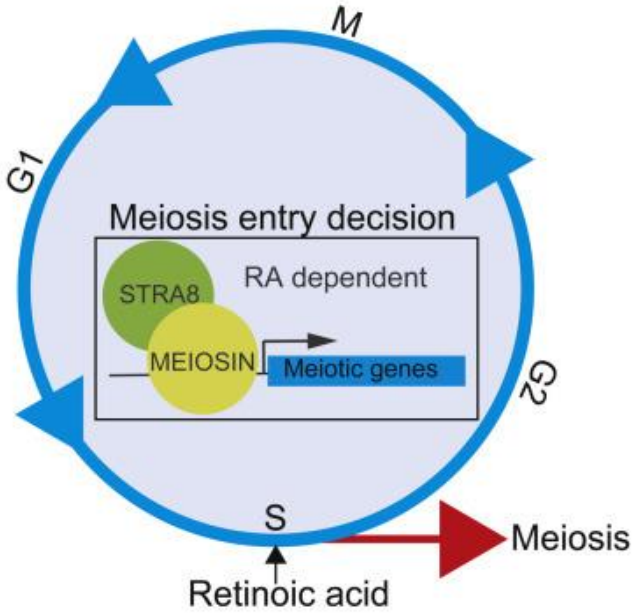
„MITOTIC-TO-MEIOTIC TRANSITION“

Retinoic acid (RA)

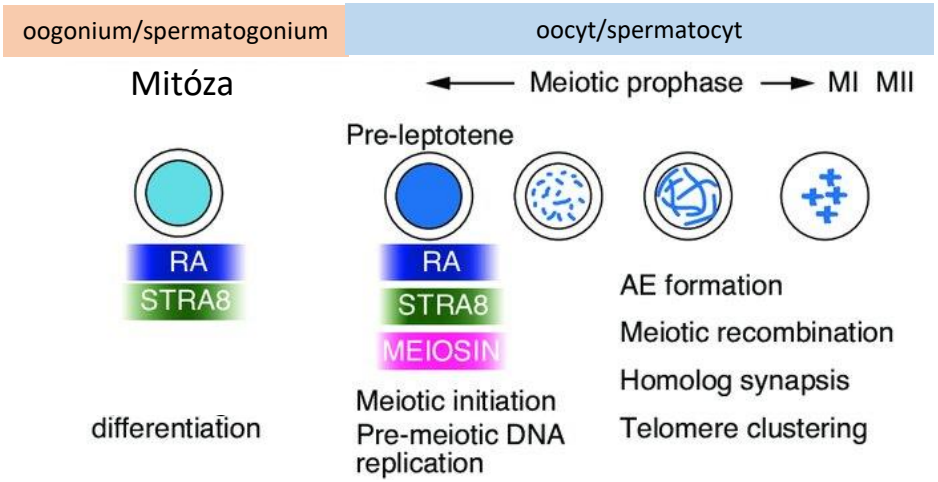
Stra8

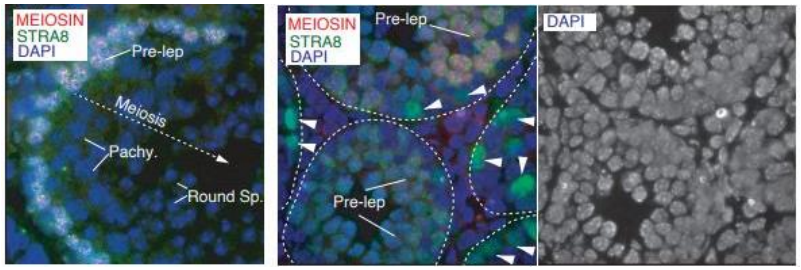
meiosin

= meiosis initiator



XX - prenatally
XY – postnatally





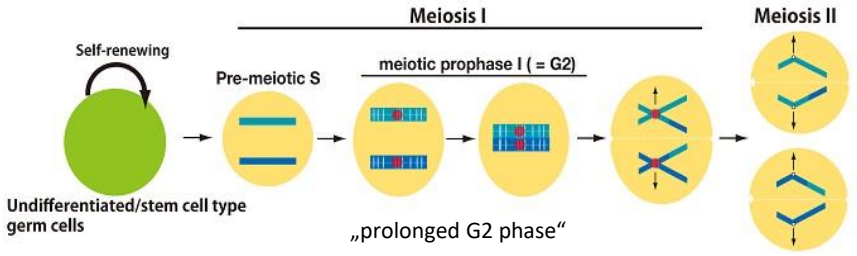
Ishiguro et al 2020

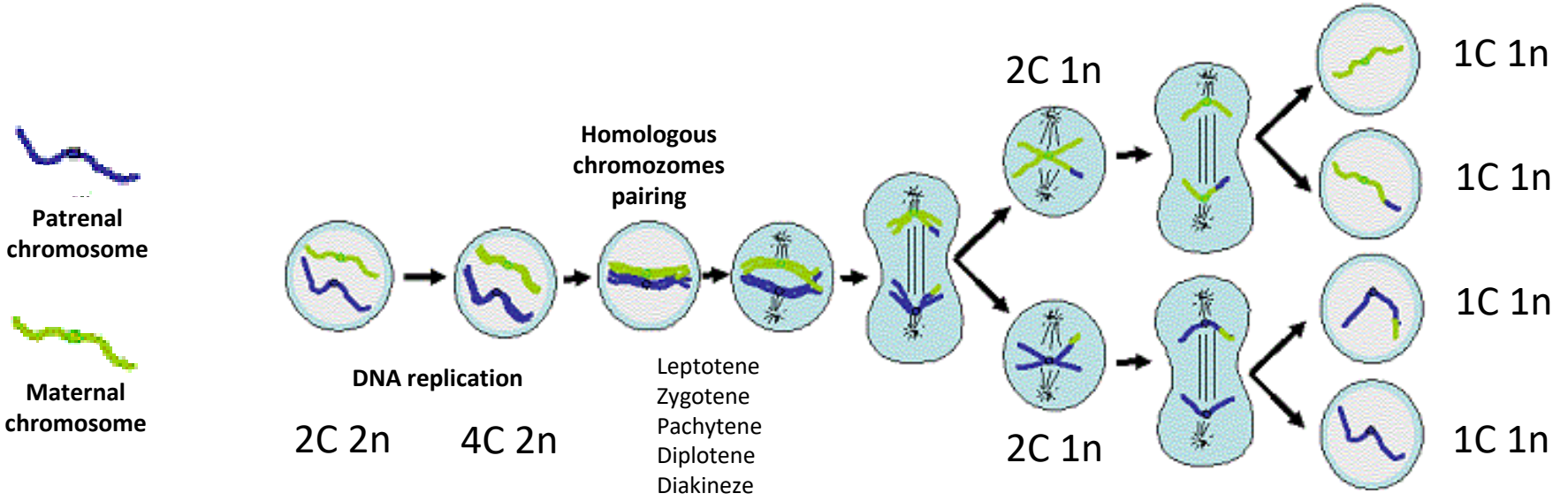
Meiosis

(1) Premeiotic S phase
- DNA replication

(2) Meiosis I
- separation of homologous chromosomes

(3) Meiosis II
- separation of sister chromatids

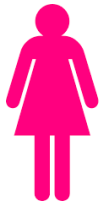




Meióza

SPERMATOGENEZE

OOGENEZE



Primordiální zárodečná buňka
(2C 2n)

Mitóza

Spermatogonie
(2C 2n)

Meióza I

Primární
spermatocyt
(4C 2n)

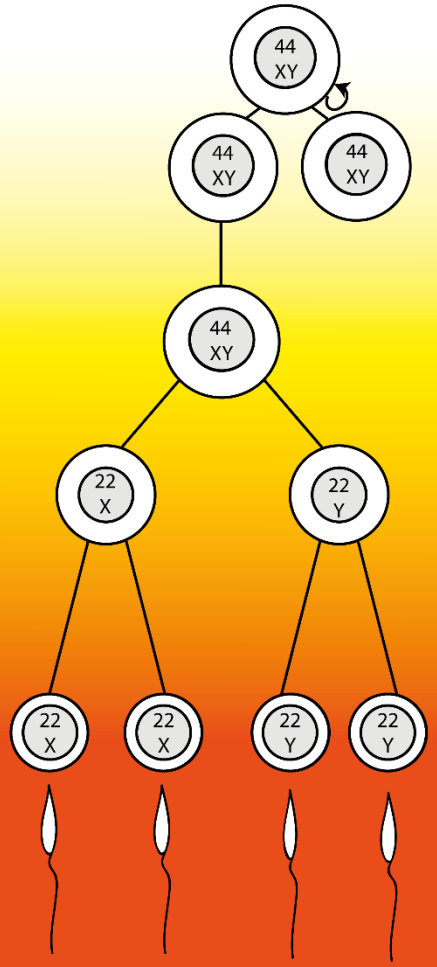
Sekundární
spermatocyt
(2C 1n)

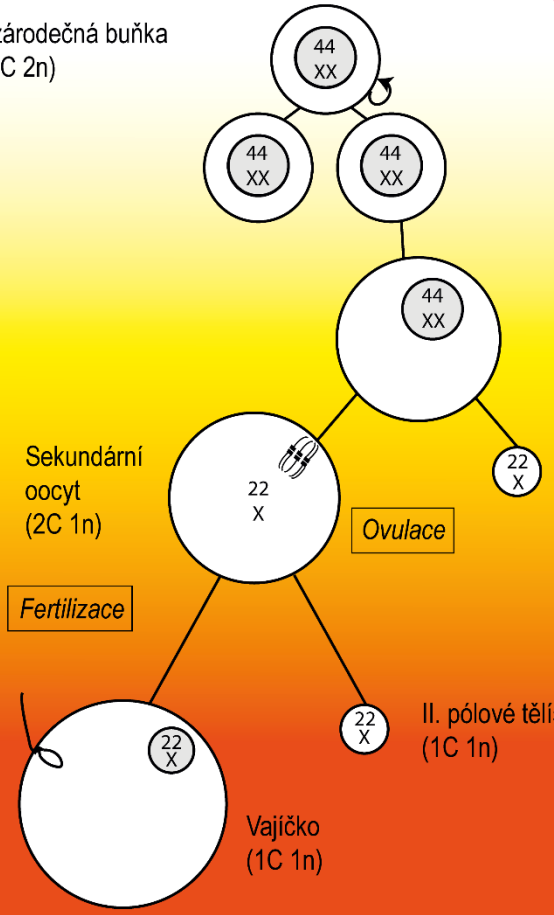
Meióza II

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)

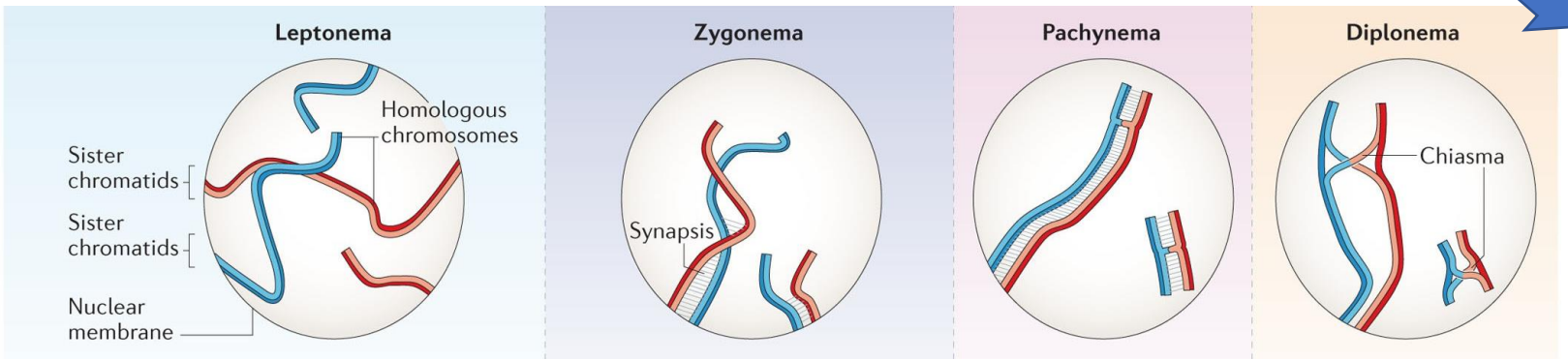
Vajíčko
(1C 1n)

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

Meiosis

➢ Prophase I

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



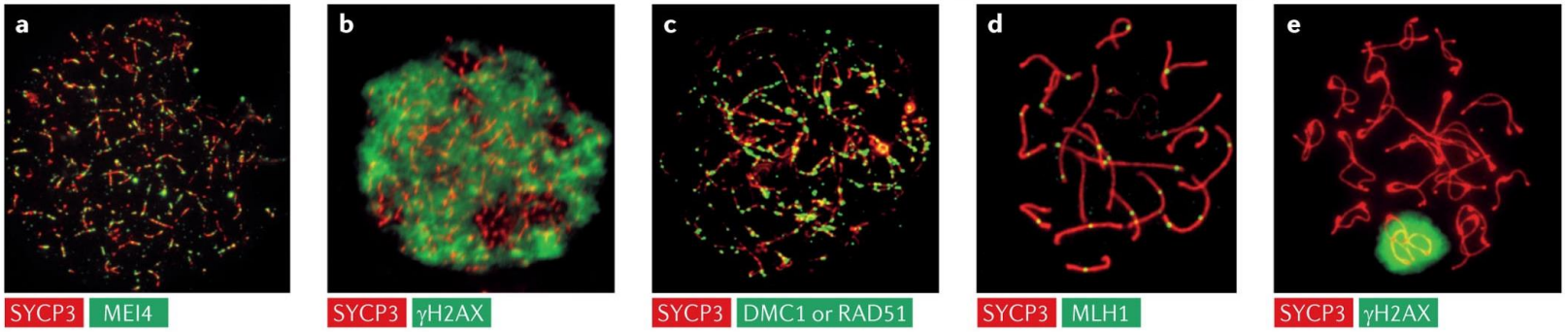
Prolonged arrest
= dictyatene

Condensation of replicated DNA

Synapsis of homologous chromosomes

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

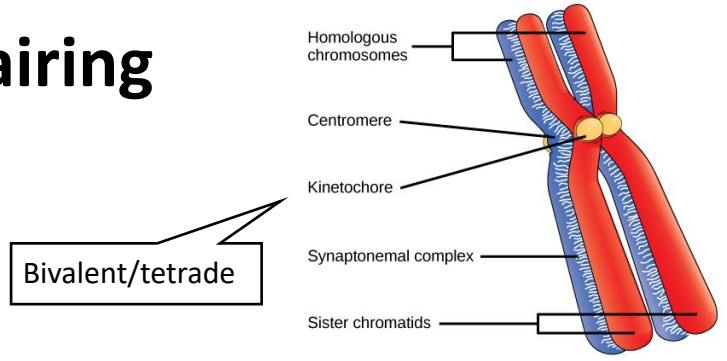
Synapsis disolution → bivalents with chiasmata



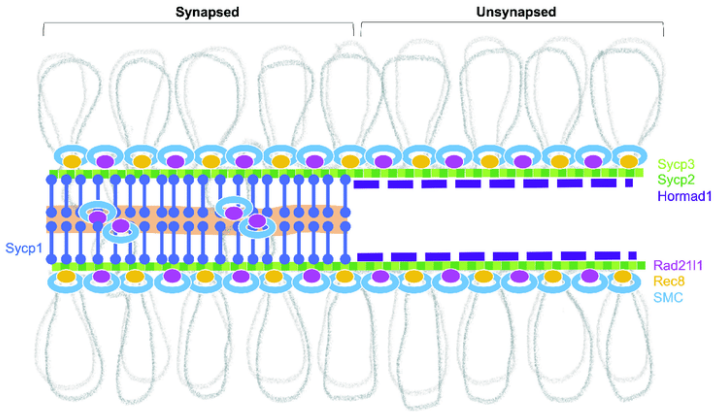
Meiosis

➢ Homologous chromosomes pairing

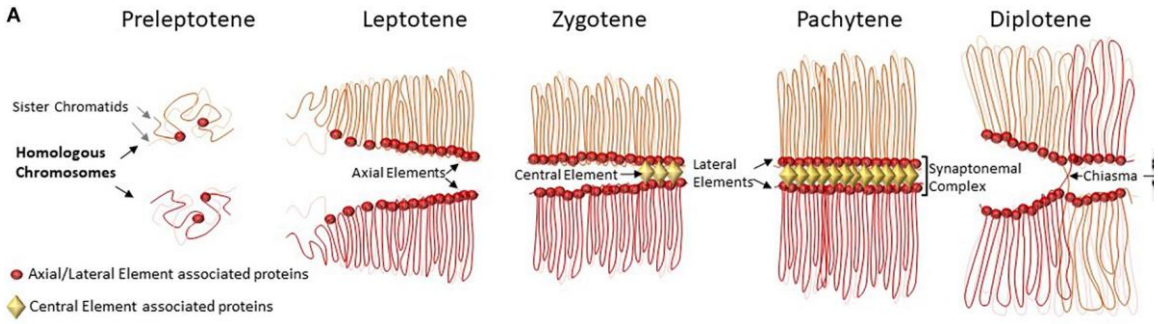
- **synapsis** = physical association of homologous chromosomes
- homolous sequence pairing

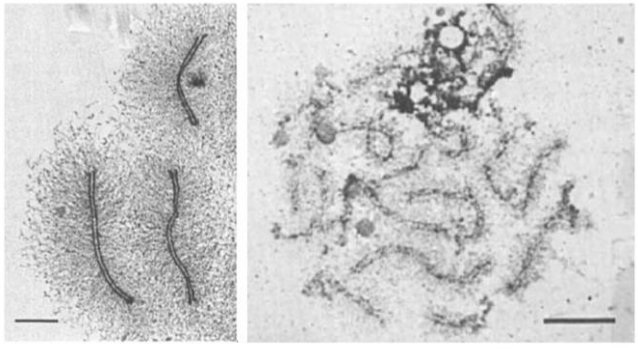


- **Synaptonemal complex**
  - axial element - SYCP3+SYCP2
  - central element - dimer SYCP1



- 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





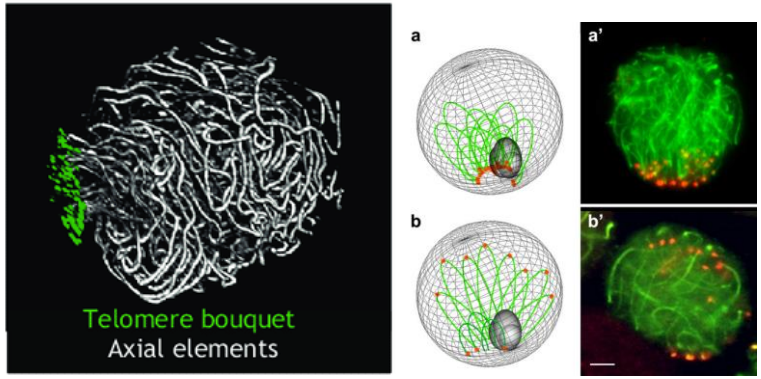
from Moens and Pearlman 1988

Meiosis

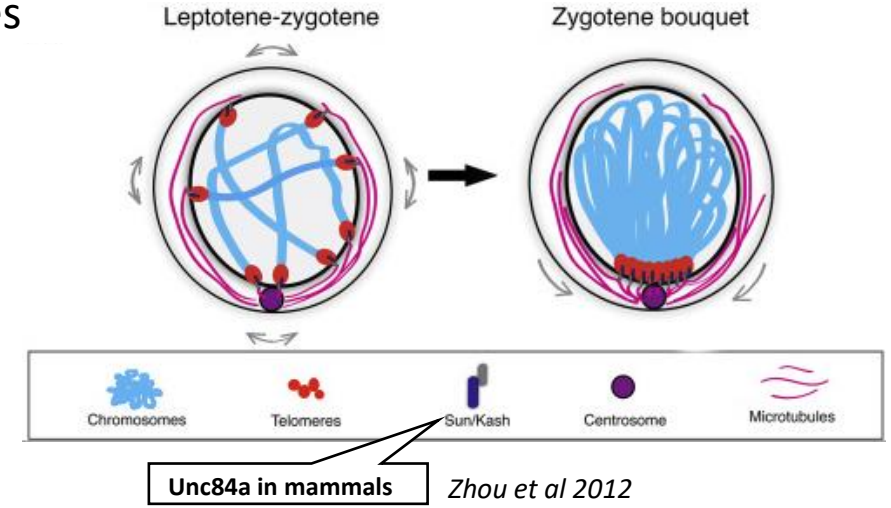
➢ Homologous chromosomes pairing

Elkouby and Mullins 2017

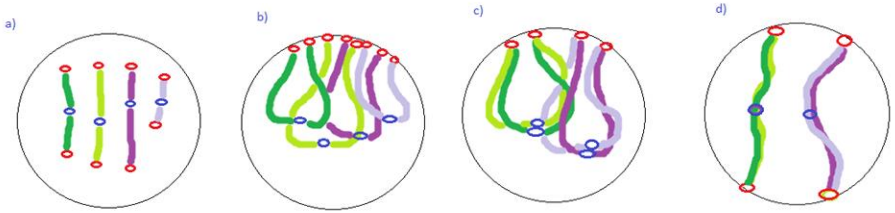
- pairing of distant homologous chromosomes accomplished by congregation of telomeres attached to nuclear membrane
  → tzv. „telomere bouqouet“

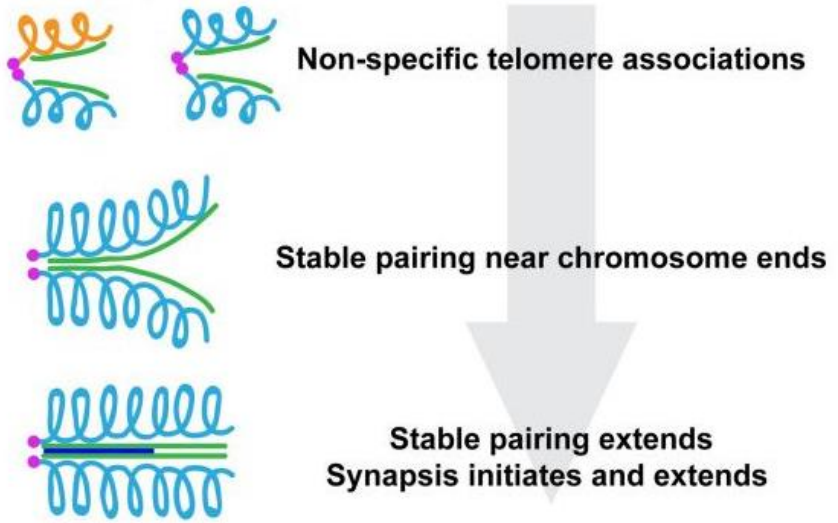


Berrios et al 2013



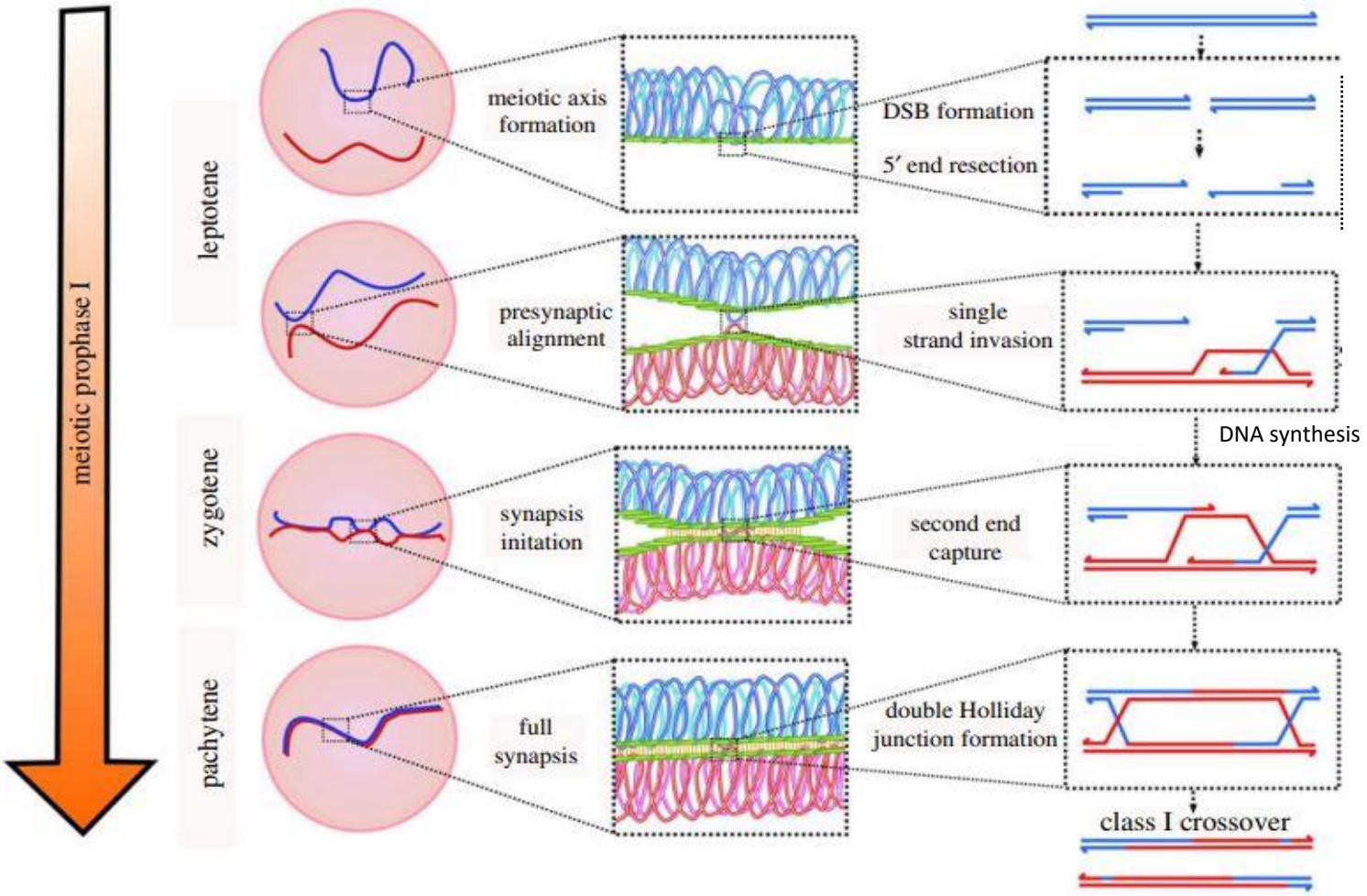
Zhou et al 2012





Meiosis

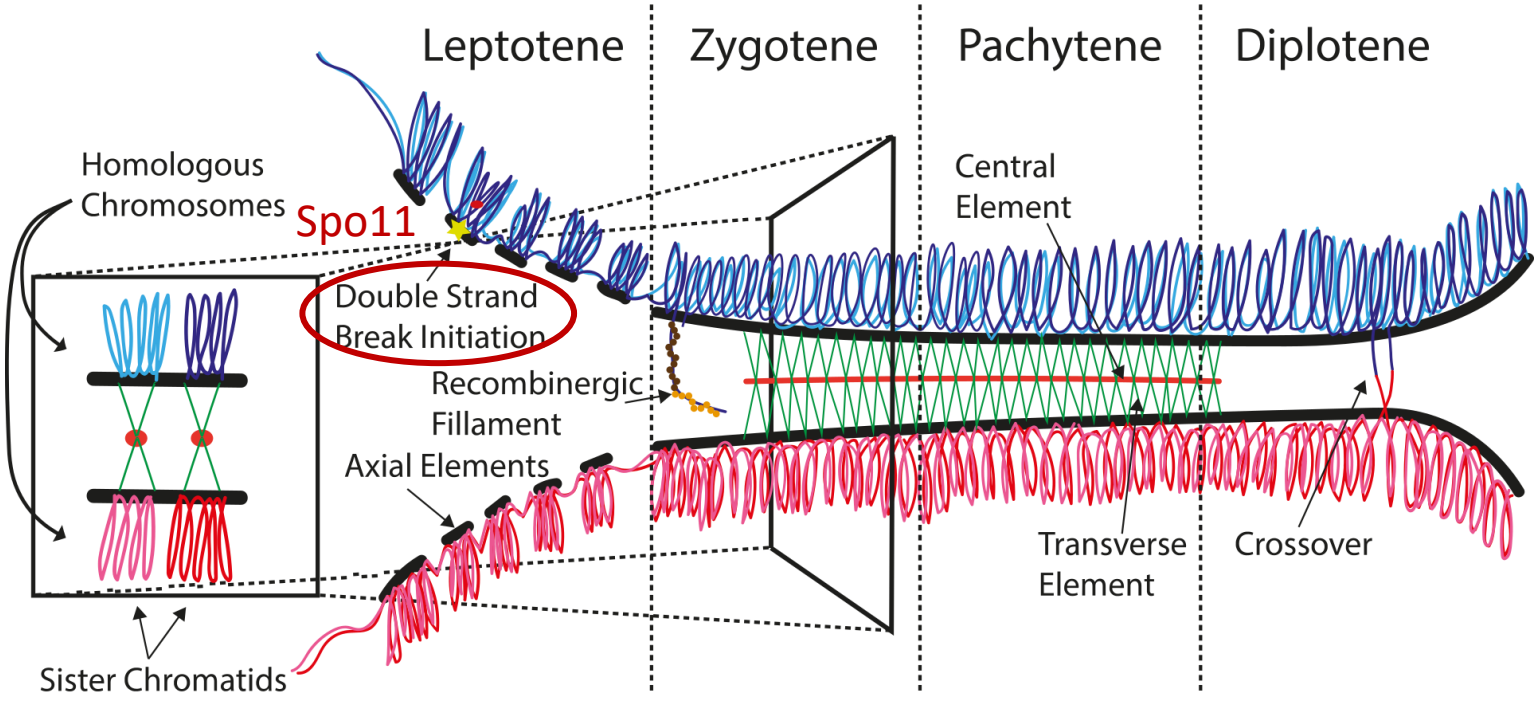
➢ Homologous recombination



Meiosis

➢ Homologous recombination

- DNA double strand breaks (DSB) precede synapsis formation



Meiosis

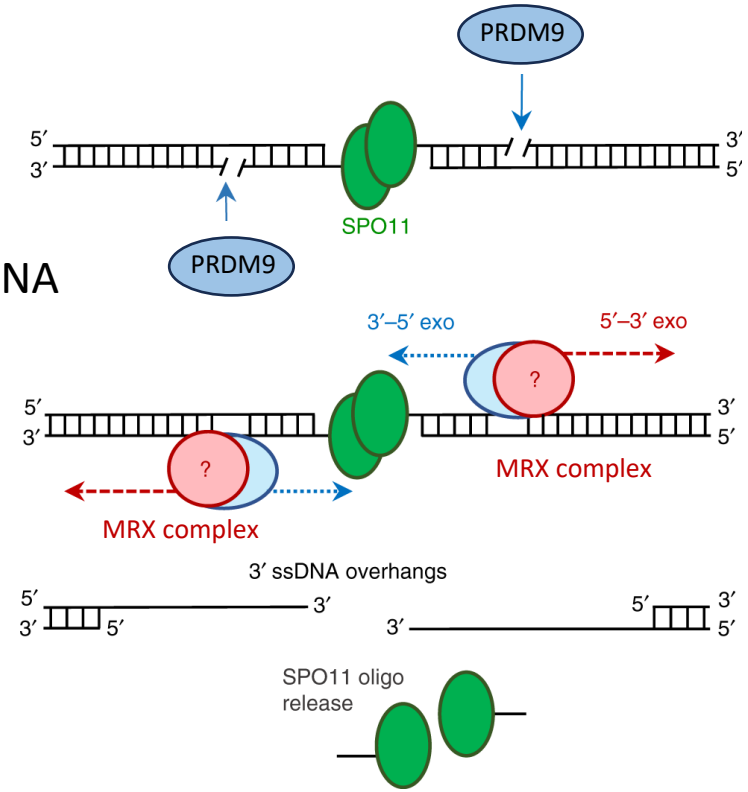
➢ Homologous recombination

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

- Spo11
  - DNA endonuklease
  - 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

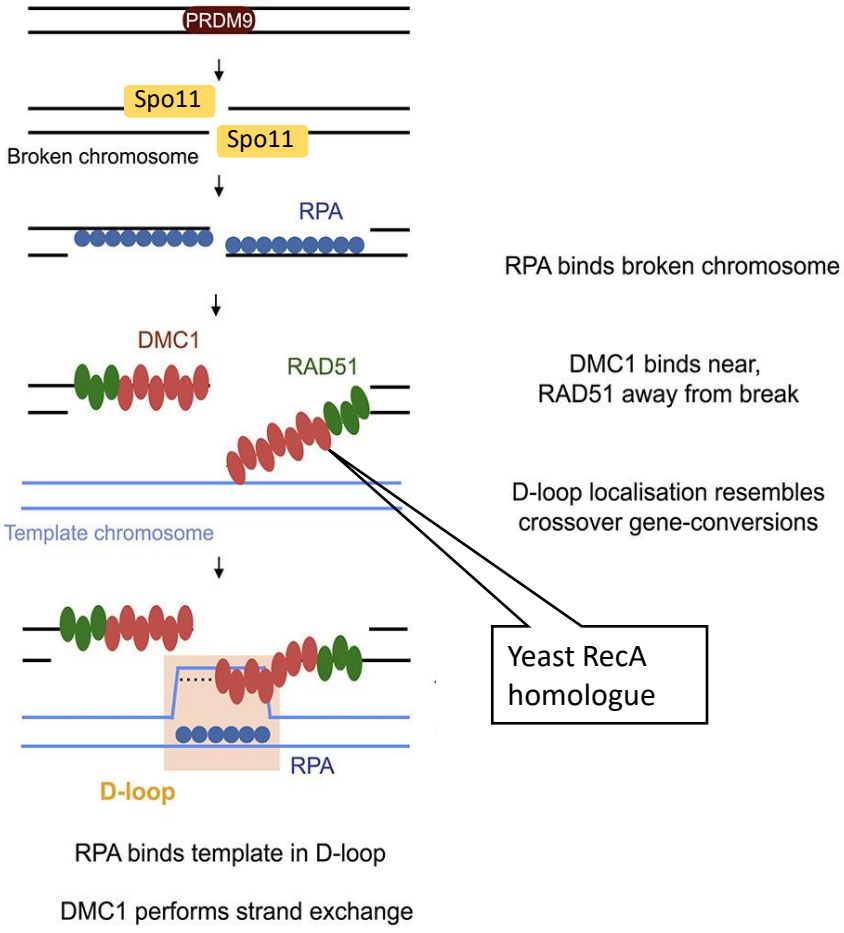


Meiosis

➢ 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



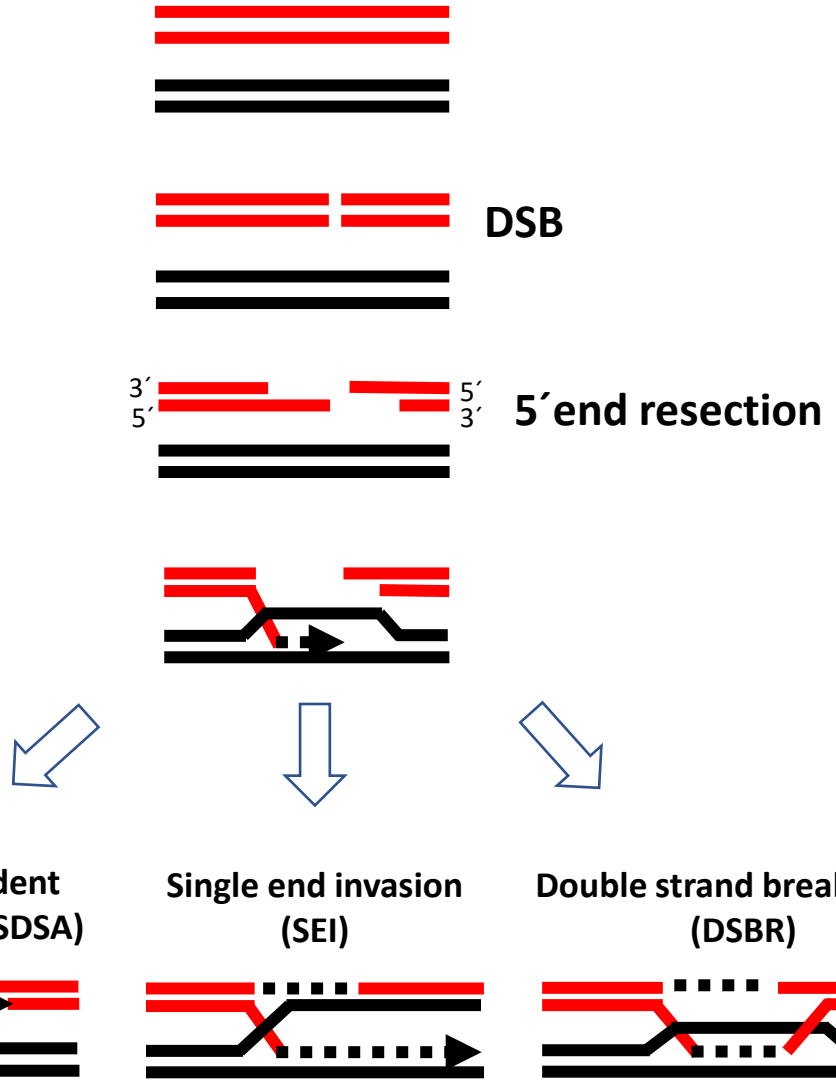
Hinch et al 2020

Meiosis

➢ Homologous recombination

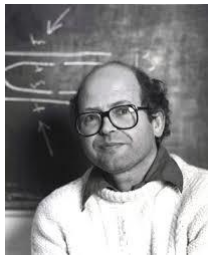
- DNA syntesis of DNA 3´end using non-sister chromatid as a template

+ strand ligation



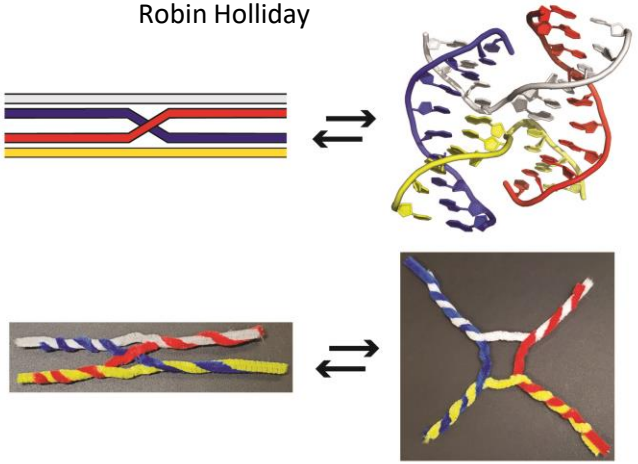
Meiosis

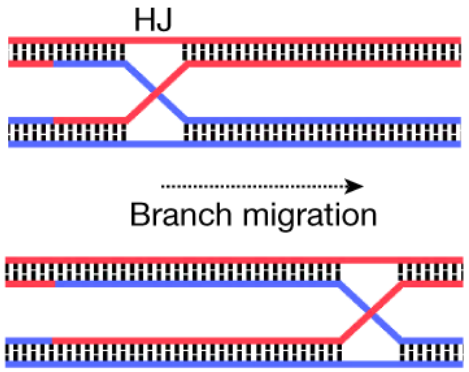
➢ Homologous recombination

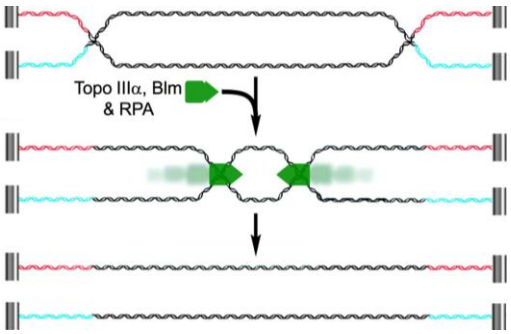


Robin Holliday

- **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

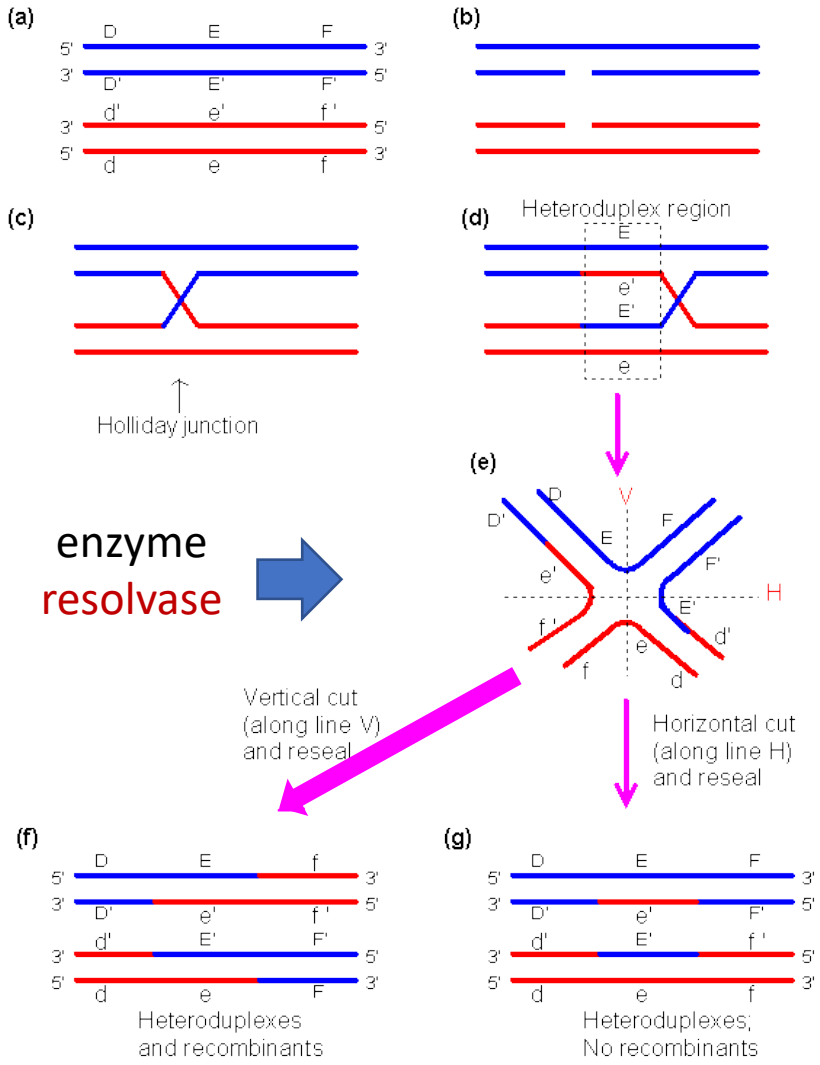
(a) D-loop resolution
→ DNA synthesis according to complementary strand

(b) convergent branch migration resulting in resolution of HJs

(c) strand exchange and HJ resolution without gene conversion

crossover ~10%

- gene conversion ocurrs 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

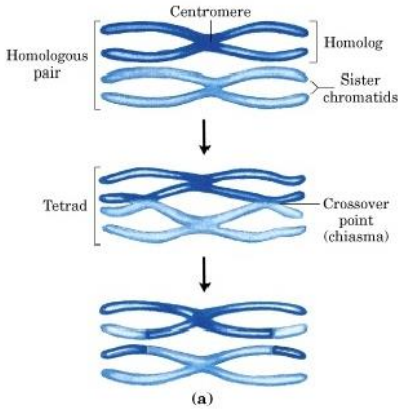
- visible after synaptonemal complex disolution during diplotene

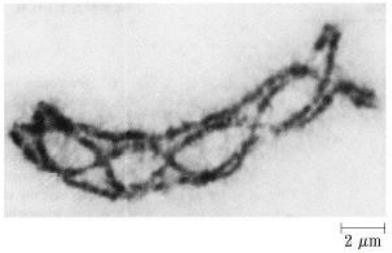
- links homologous chromosomes together in the form of bivalents (tetrades)

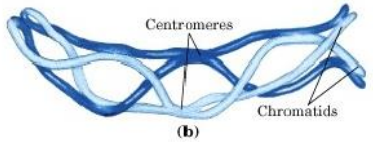
- disapprear as homologous chromosomes separate during anaphase I

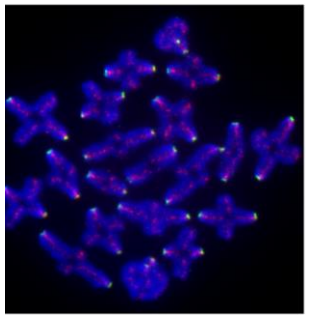
- sex differences in location and number of chiasmata (more distal in males)

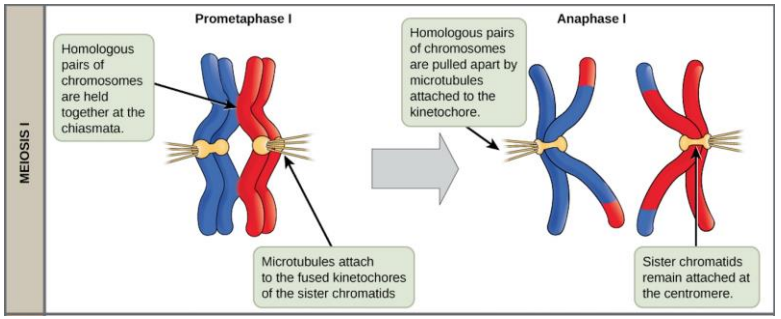
- altered number of chiasmata and location of chiasmata associated with aneuploidy



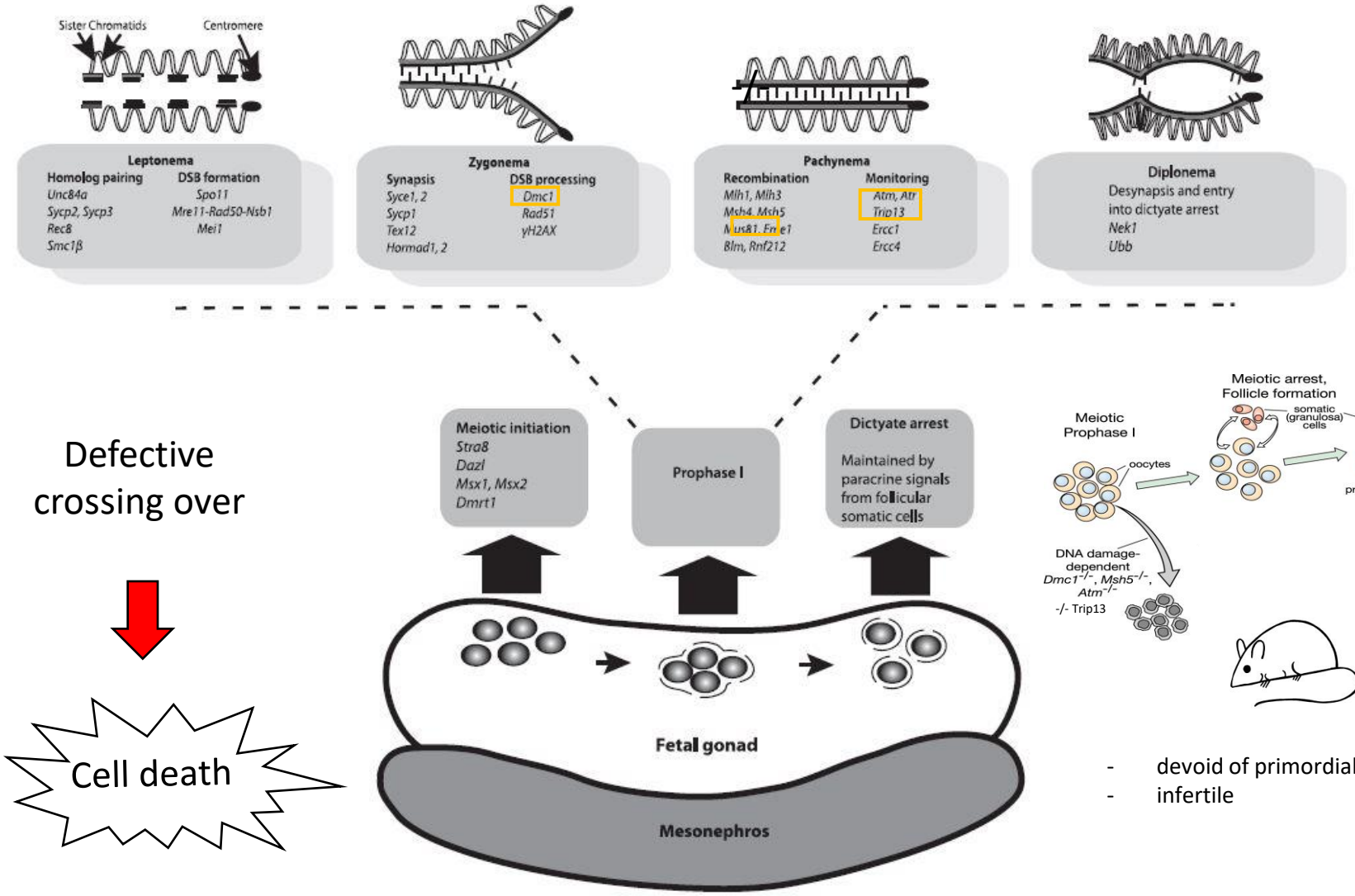








Regulation of meiotic prophase overview



- devoid of primordial follicles
- infertile

Chromatin configuration during diplotene arrest

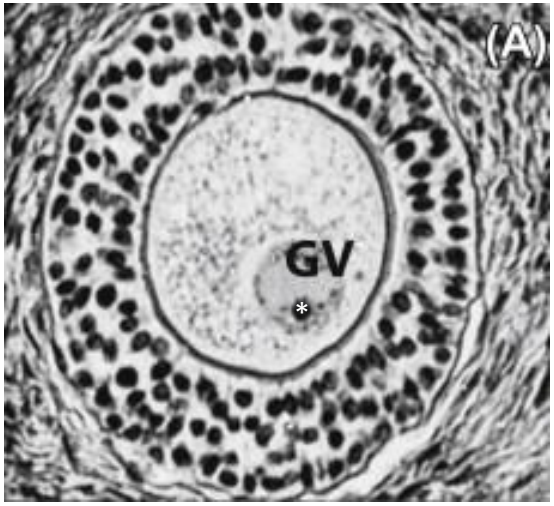
- **dictyate** stage = prolonged diplotene arrest

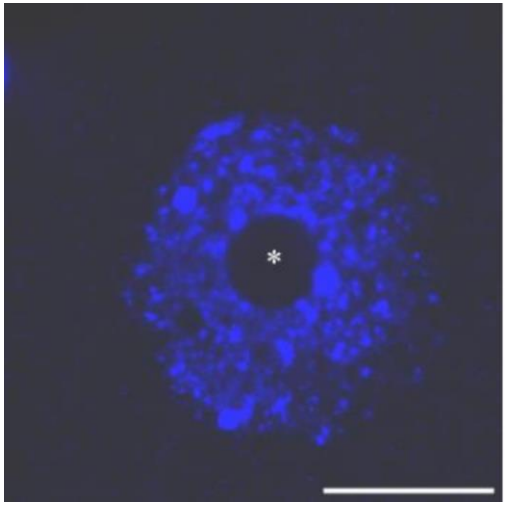
- chromosomes become dispersed, less distinct and form faint network

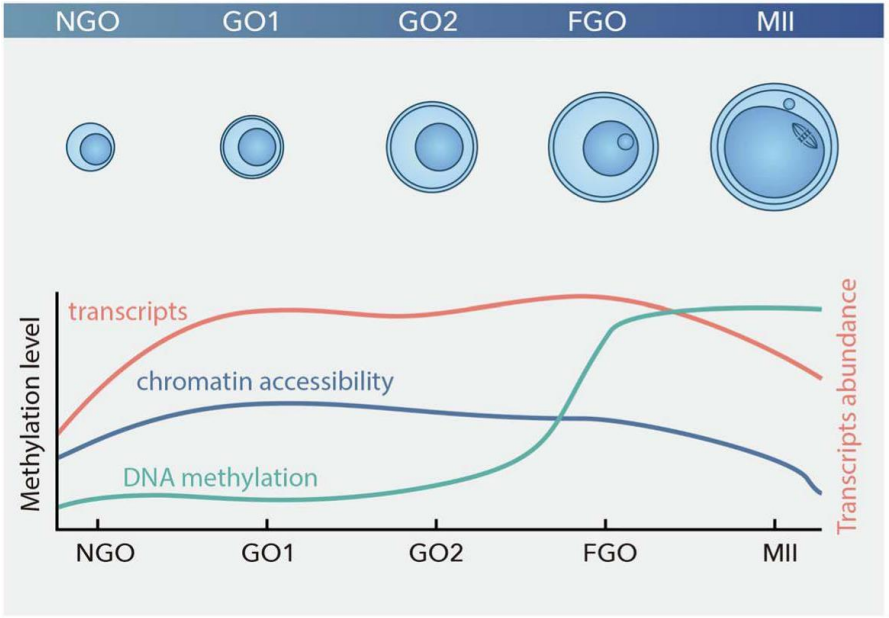
- germinal vesicle (GV)
  = prophase nucleus
  - ~30-40 µm

- 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

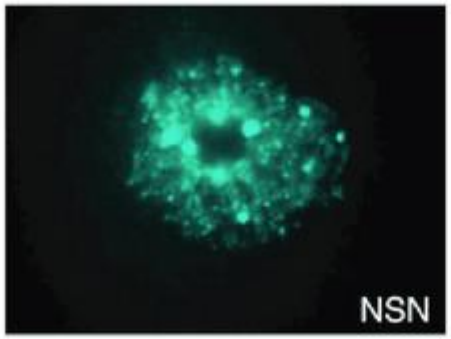
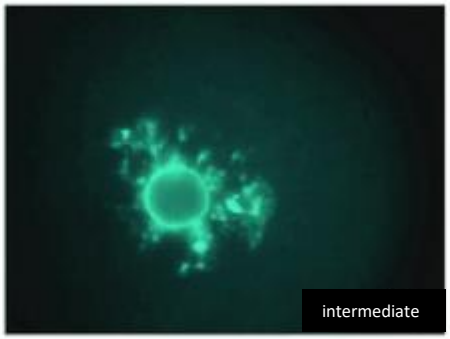
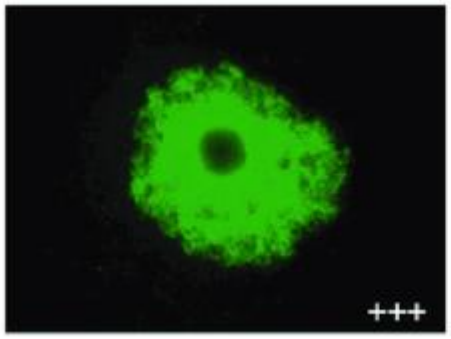
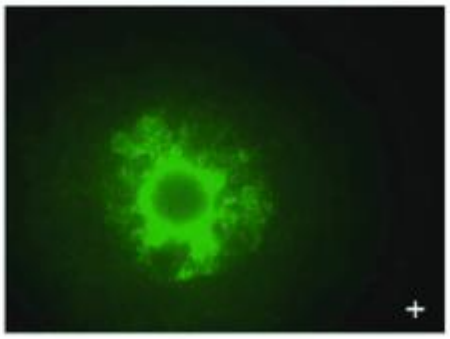
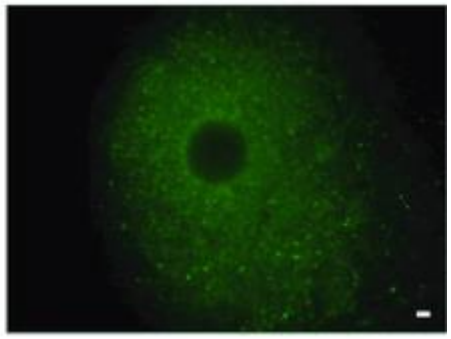






Qian and Guo 2022

Large scale chromatin remodelling

	Nonsurrounded nucleolus (NSN)	Partly-surrounded nucleolus (partly-NSN)	Surrounded nucleolus (SN)
Chromatin *Hœchst 33342*			
Transcription *BrUTP*			
folicules	*preantral/antral*	*antral*	*antral*

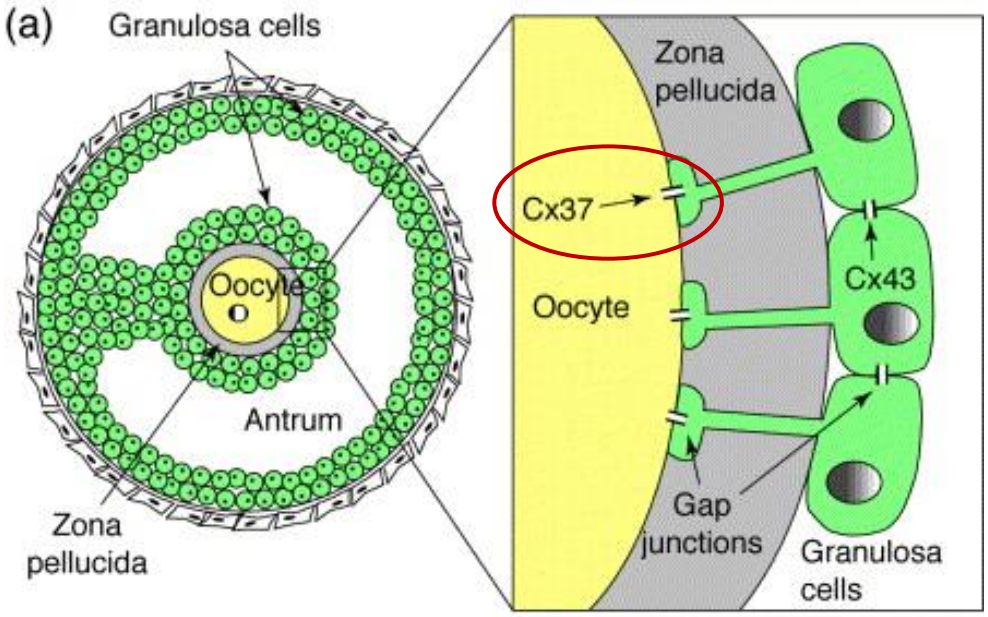
perinuclear rim of heterochromatin = karyoplast

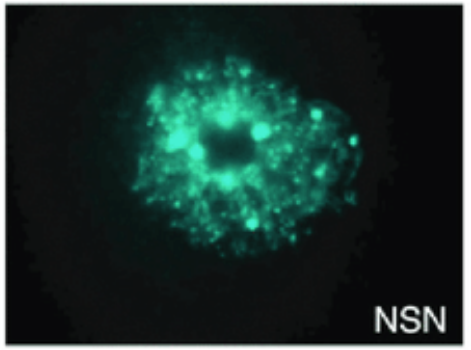
Pesty et al 2007

Chromatin condensation

Transcription activity

Large scale chromatin remodelling

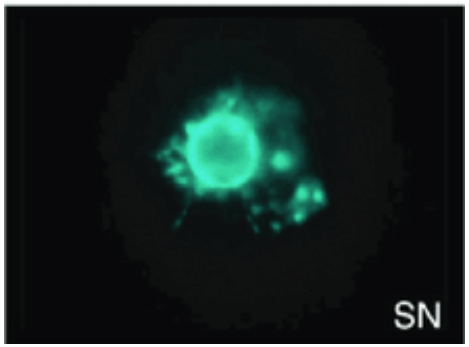




FSH

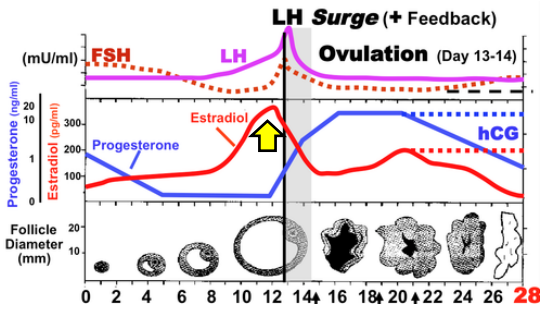
(+COC in IVM)

-/- Connexin37



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

Resumption of meiosis

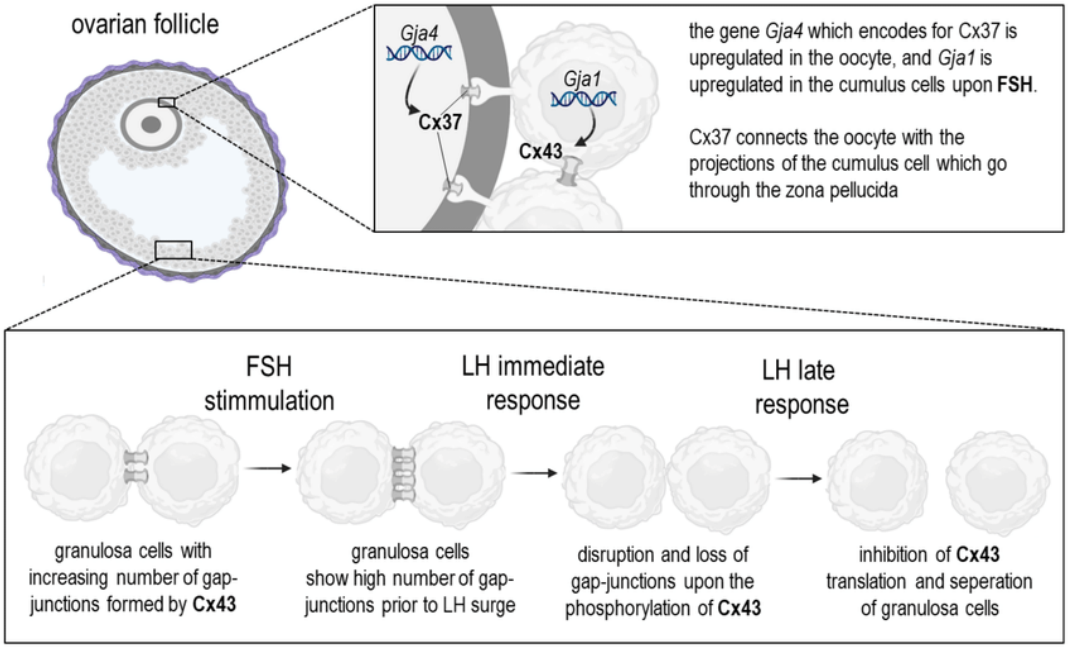


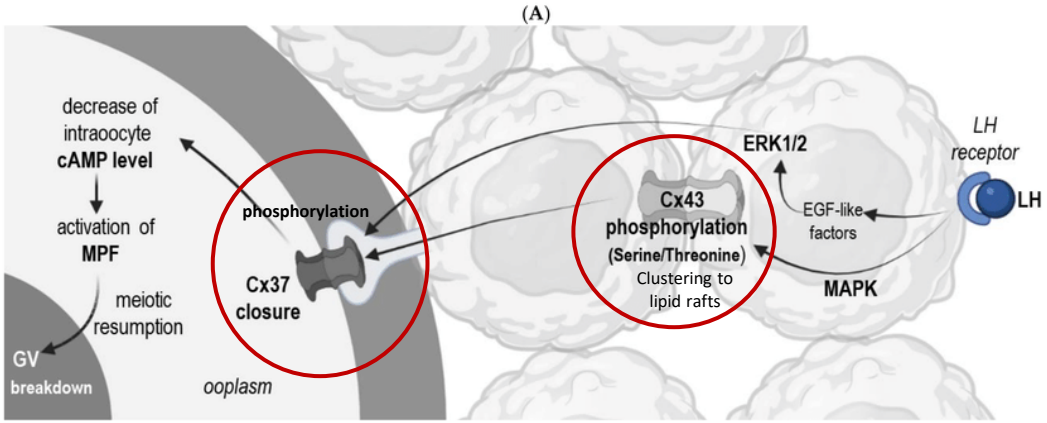
↑ estrogen

LH surge

oocyte-cGCs uncoupling

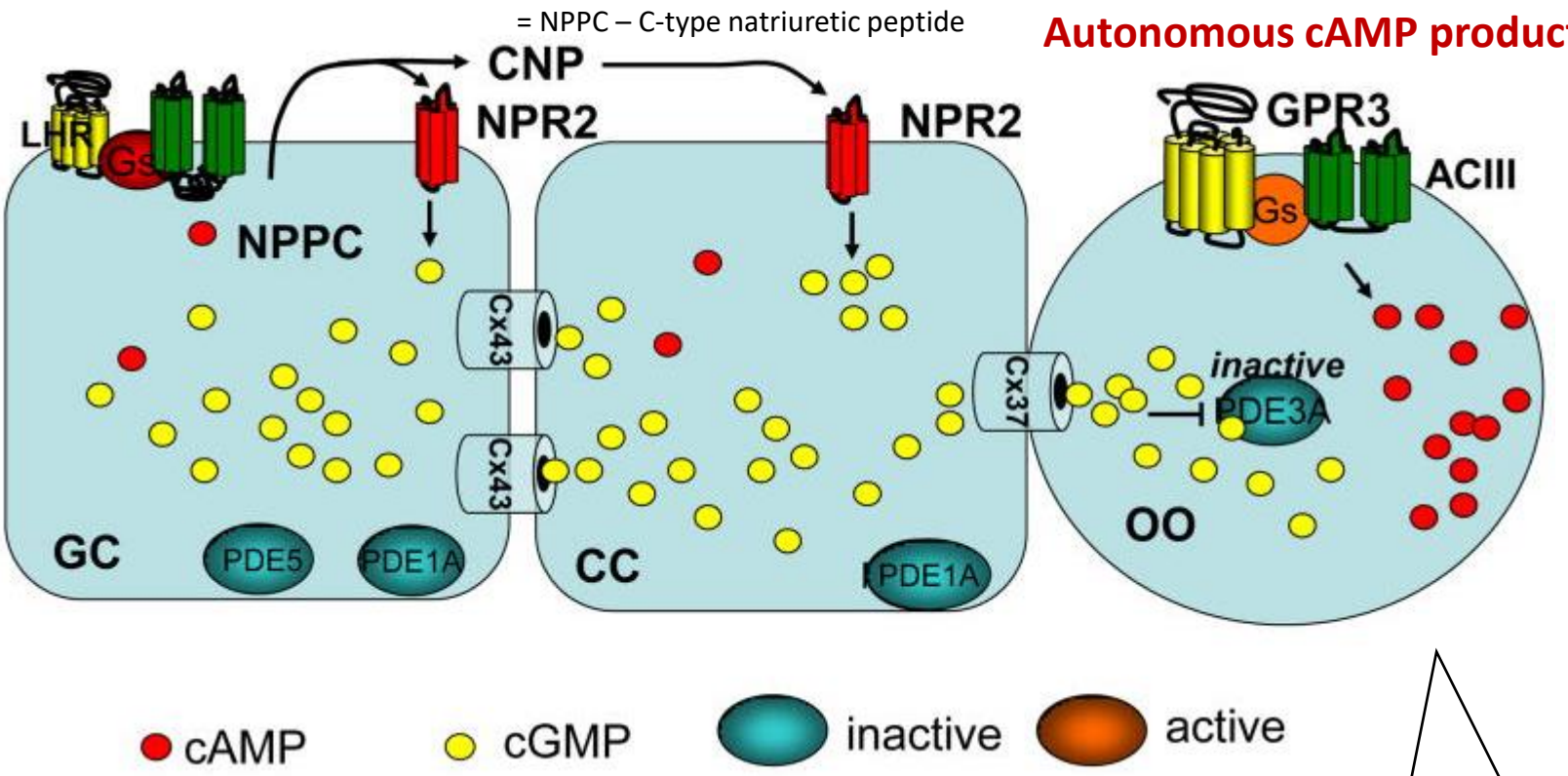
release from GV arrest





Kordowitzki et al 2021

Control of prophase arrest



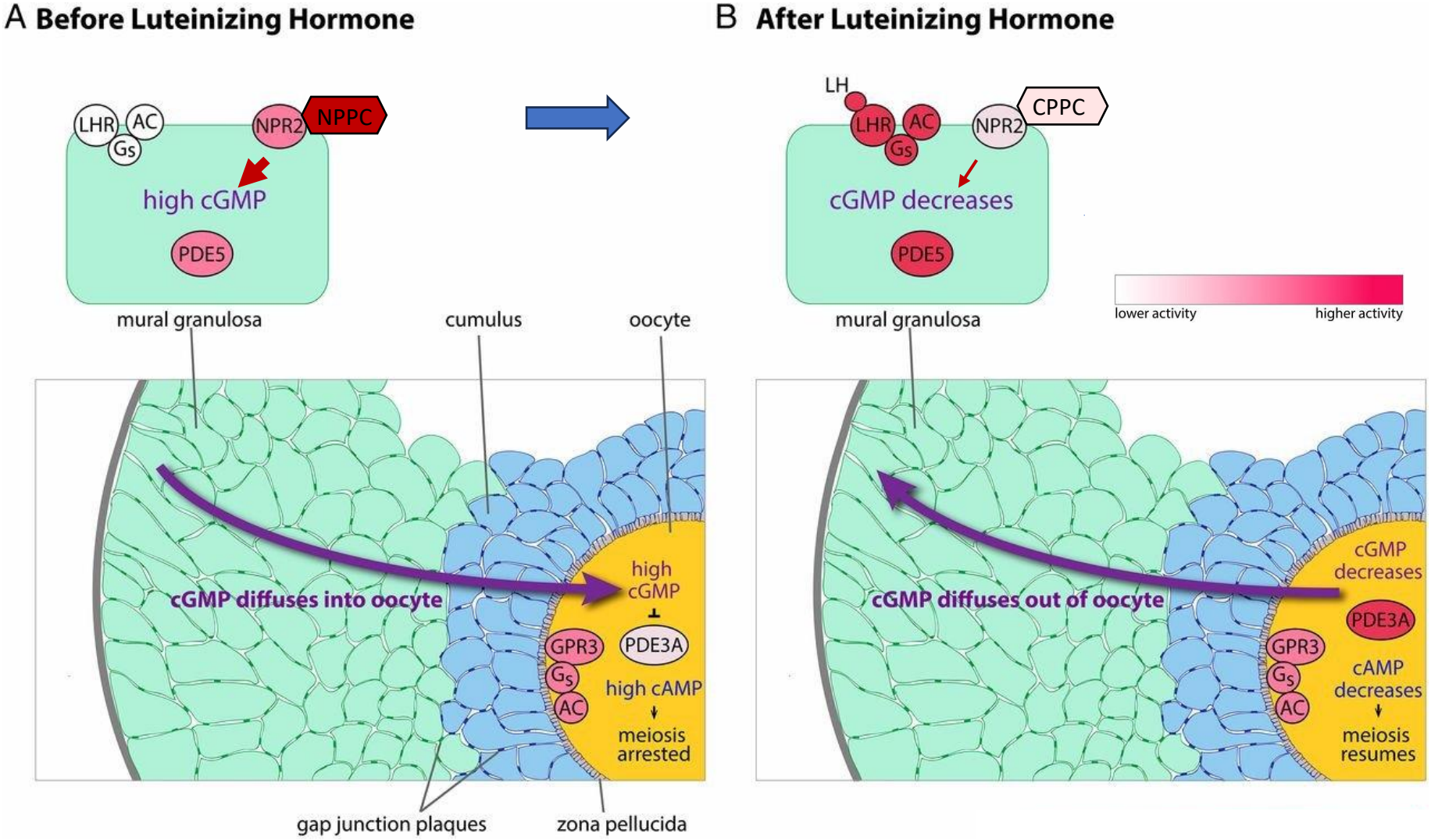
Conti et al 2011

Supplementation of cGMP by cGCs balances cAMP degradation by PDE

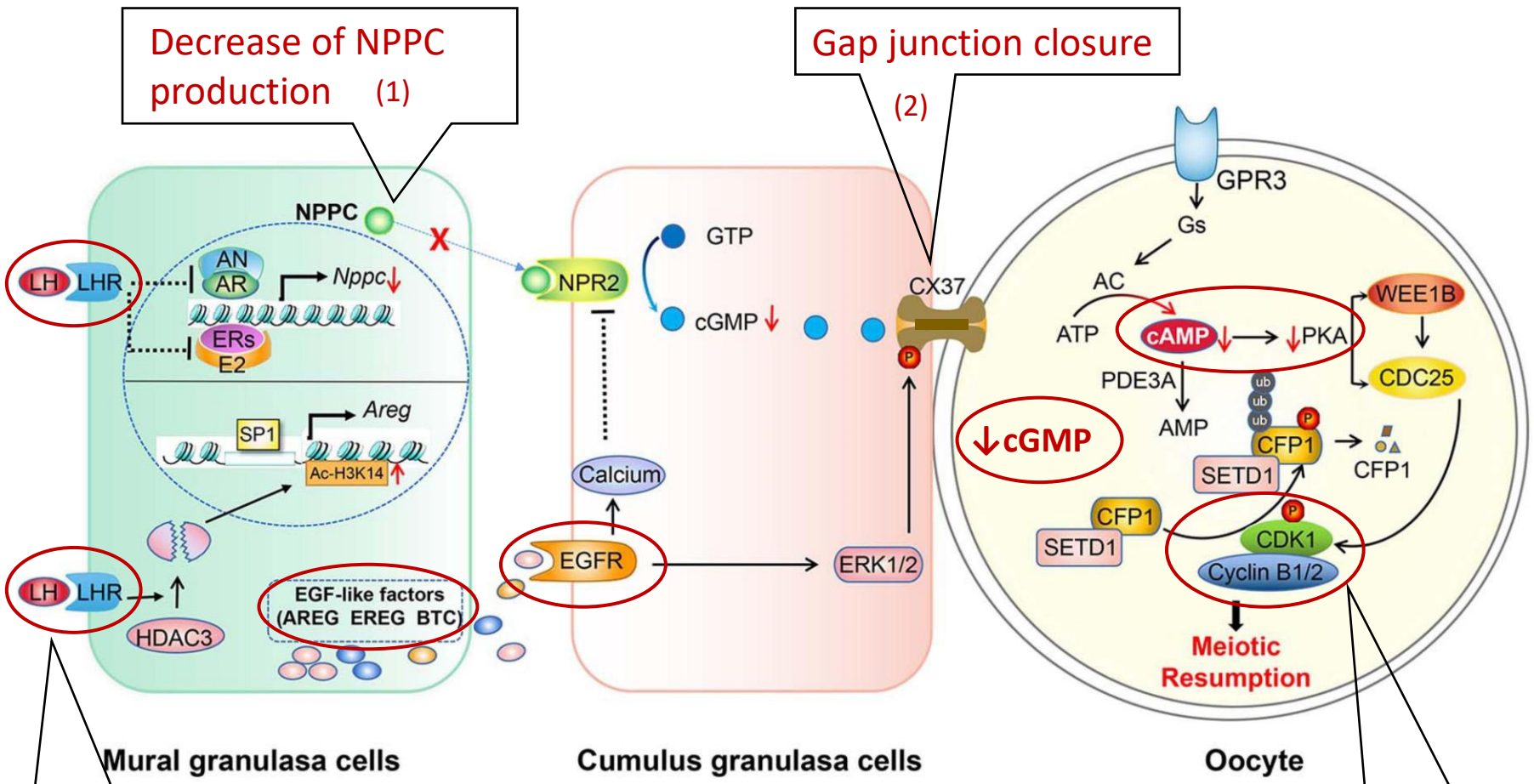
Prophase arrest is dependent on high levels of cAMP in ooplasm

* 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

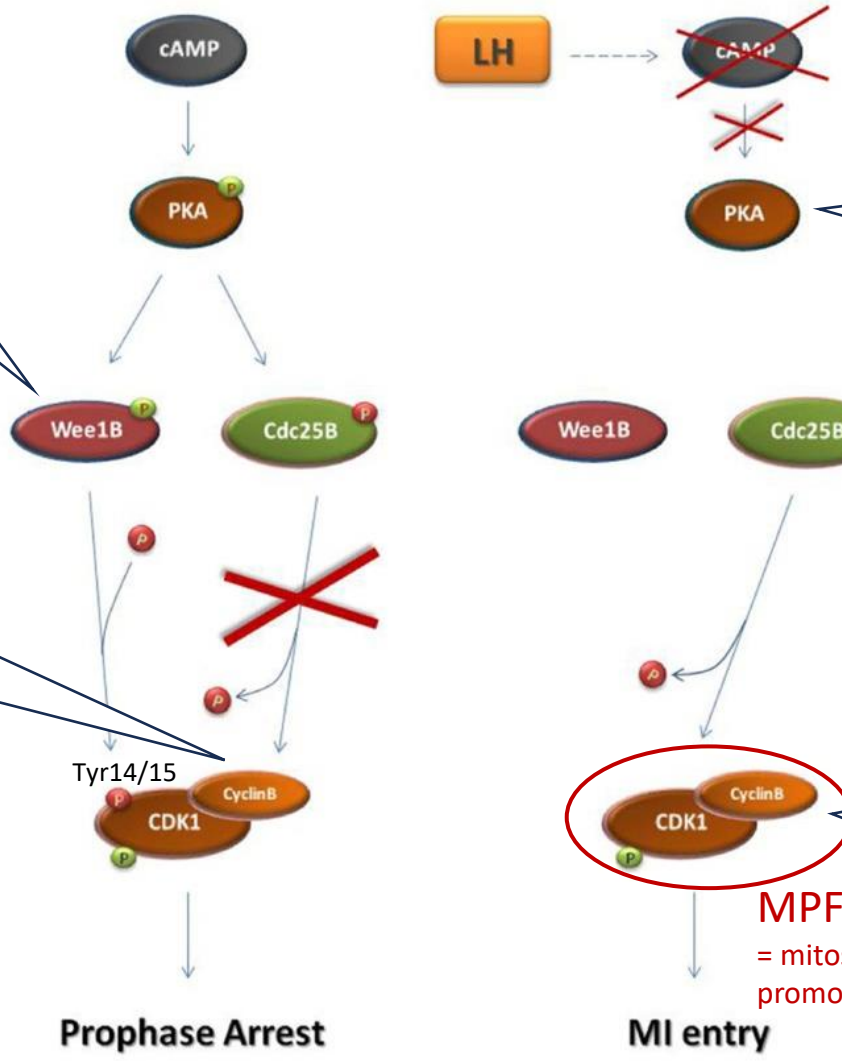
Decrease of cAMP and PKA activity leads to activation of meiosis promoting complex (**MPF**) and resumption of meiosis

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



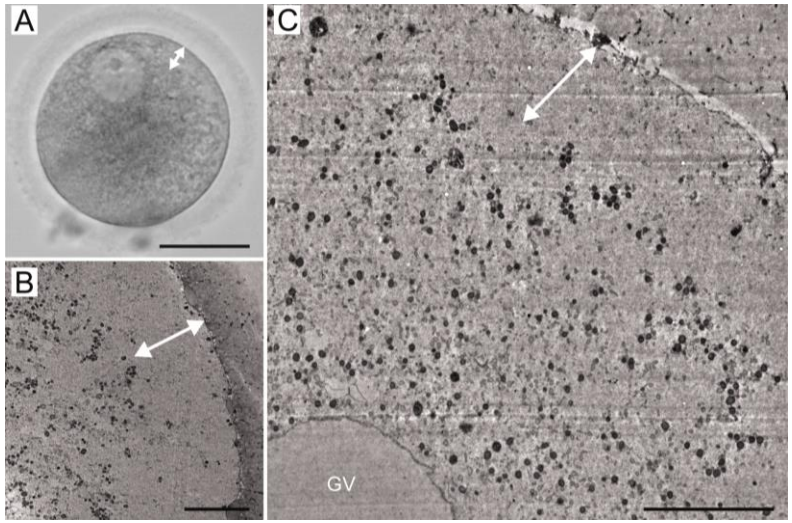

Visible sign of meiotic reactivation is

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

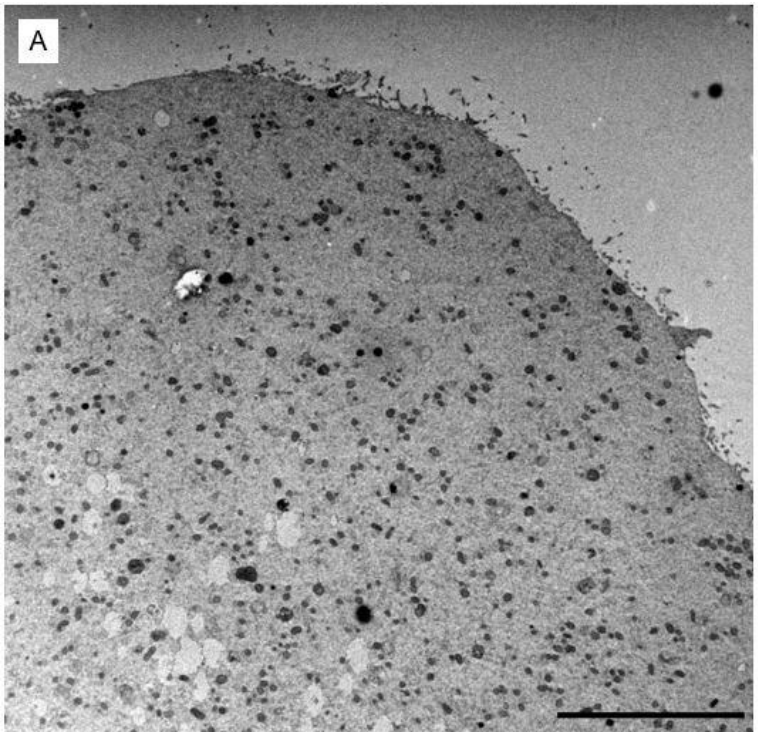
Preceded by:
- chromatin congression
- relocation of nucleus towards oolema
- GV belt disapperance

Resumption of meiosis

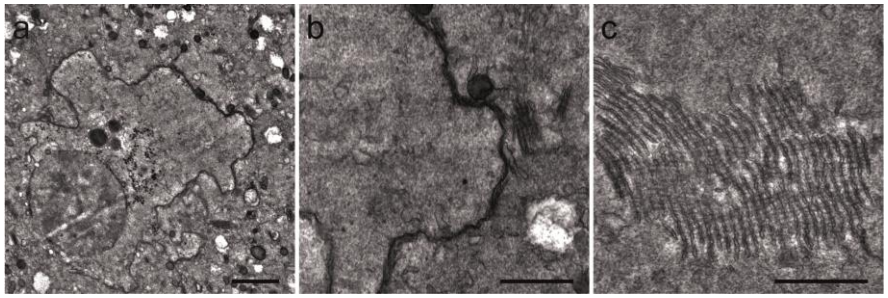
GV



GV belt – subcortical region depleted from cellular organelles



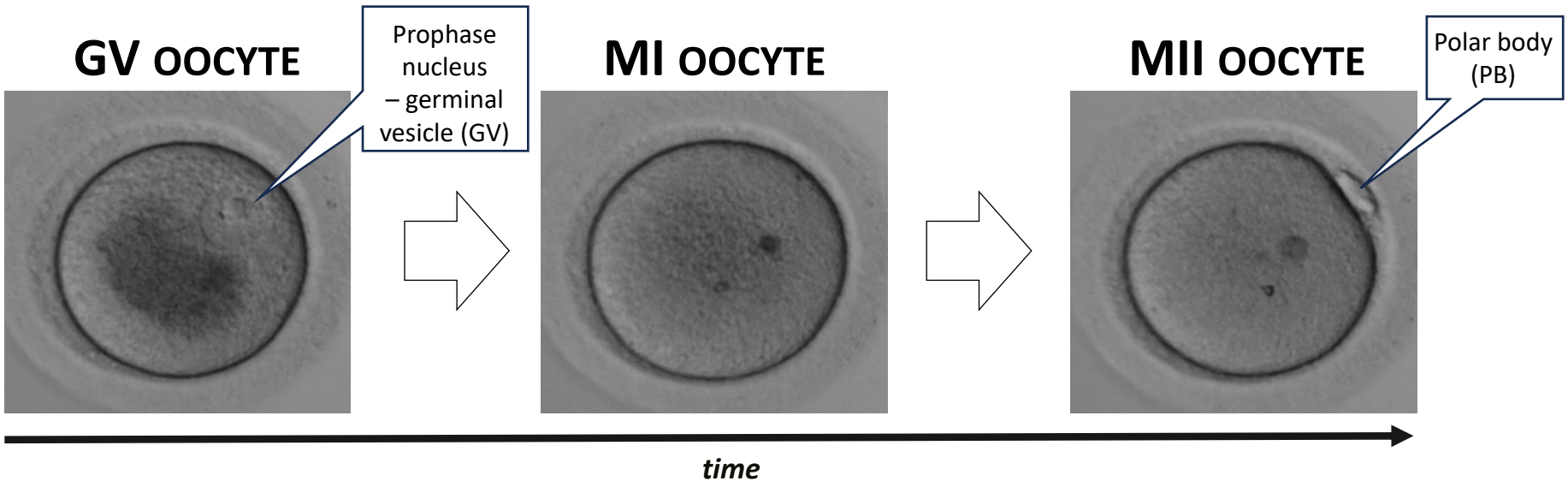
GVBD



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

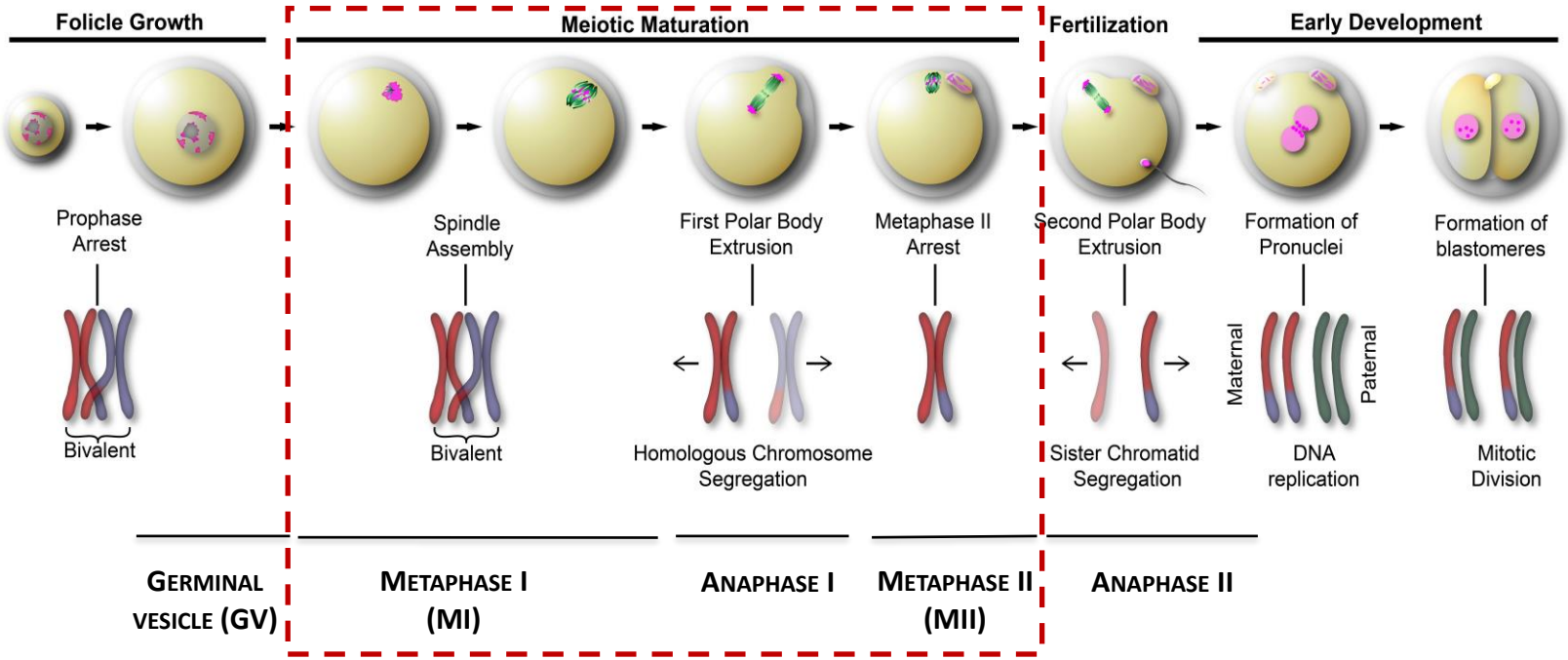
Trebichalska et al 2021

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

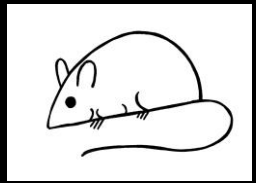
- structural and functional modification of organelles
- global changes in organelle arrangement
- mRNA translation and posttranslational modifications
- syntesis/degradation of maternal factors

Acquisition of fertilization and developmental competence

Nuclear maturation in mouse oocytes

(1) Initiation of microtubule nucleation
(2) Nuclear envelope break-down
(3) Chromatin condensation and chromosome individualisation
(4) Spindle build-up and bipolarization
(5) Chromosome alignment
(6) Spindle relocation to cortex
(7) Homologous chromosome segregation (asymmetic cytokinesis)
(8) MII spindle formation – MII arrest

MAP4 – microtubules
H2B - DNA



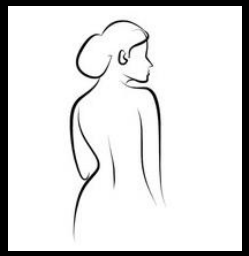
~ 12 hours

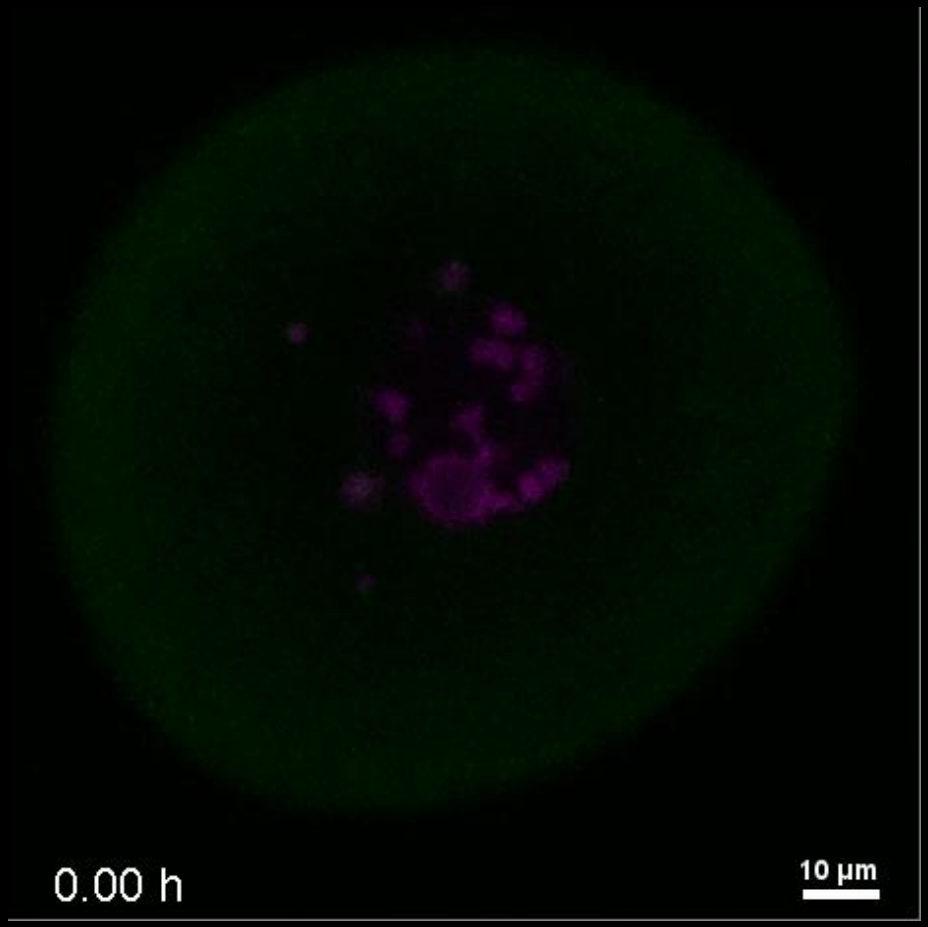
Schuh and Ellenberg, Cell 2007

Nuclear maturation in human oocyte

(1) Nuclear envelope break-down
(2) Chromatin condensation and clustering
(3) Initiation of microtubule nucleation
(4) Chromosome individualisation
(5) Spindle build-up and remodelling
(6) Spindle bipolarization and chromosome alignment
(7) Homologous chromosome segregation (asymmetic cytokinesis)
(8) MII spindle formation – MII arrest

MAP4 – microtubules
H2B - DNA

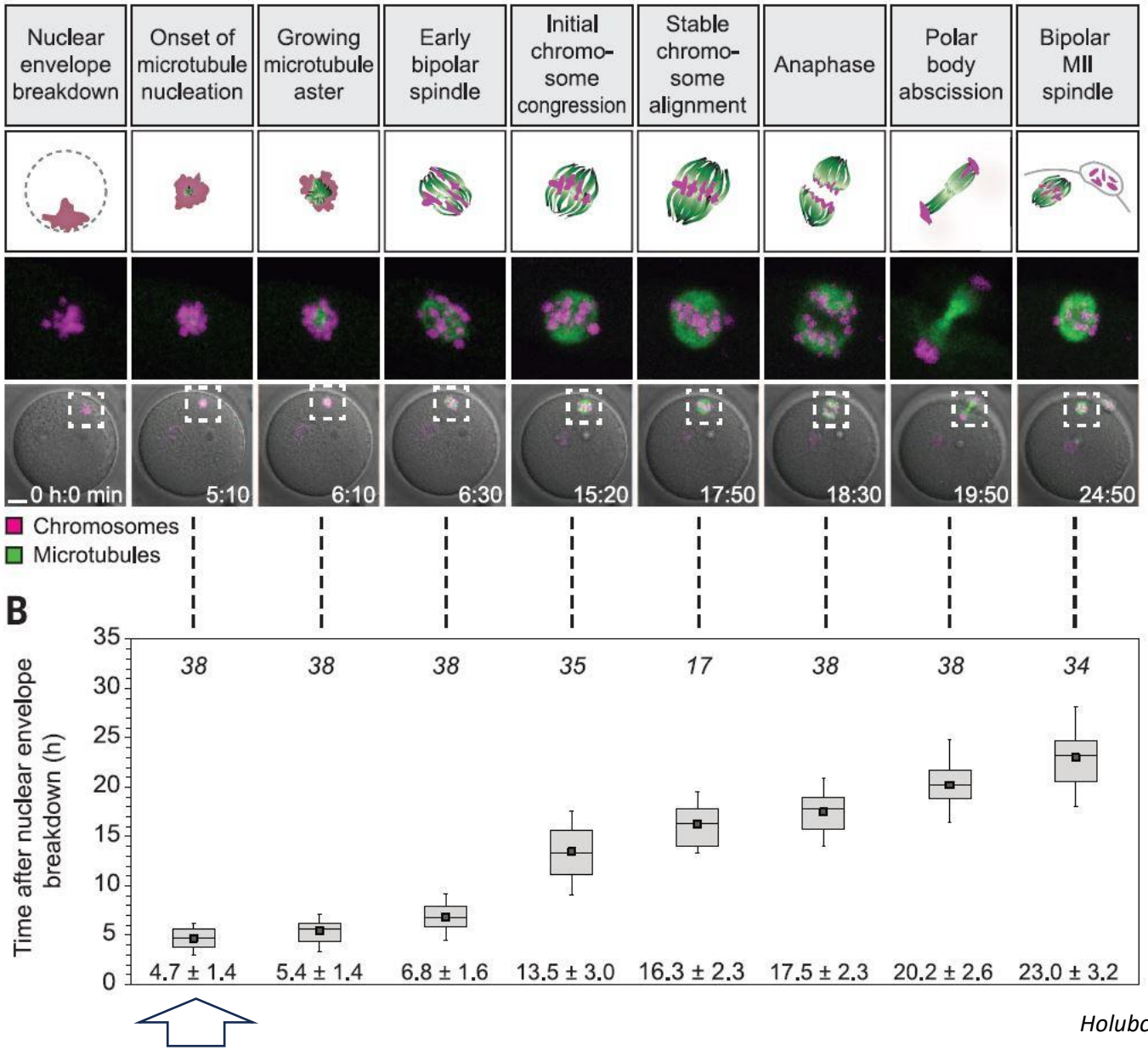




~ 24 hours

Holubcova et al Science 2015

Nuclear maturation in human oocyte

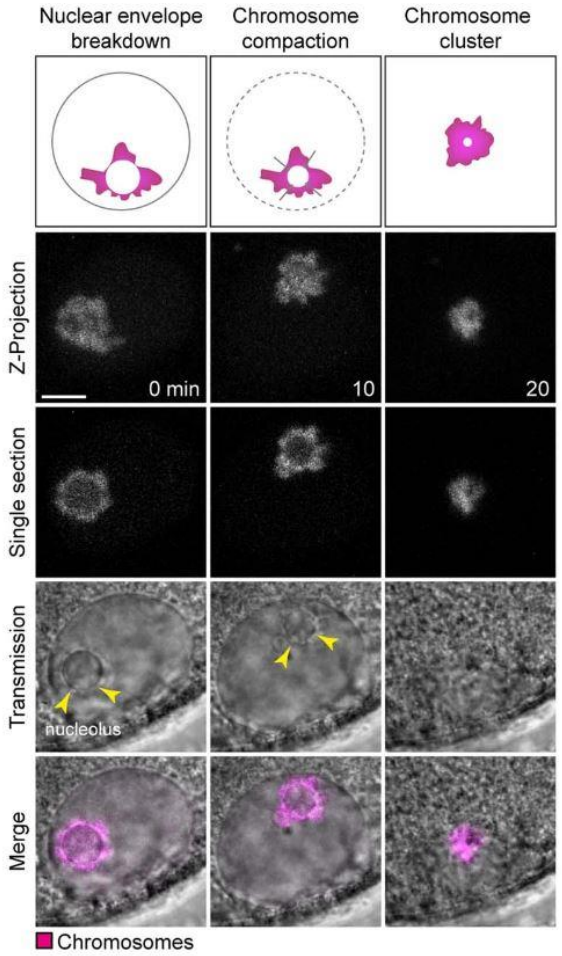


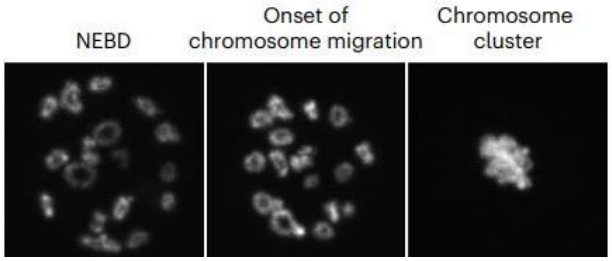
Holubcova et al Science 2015

Nuclear maturation in human oocyte

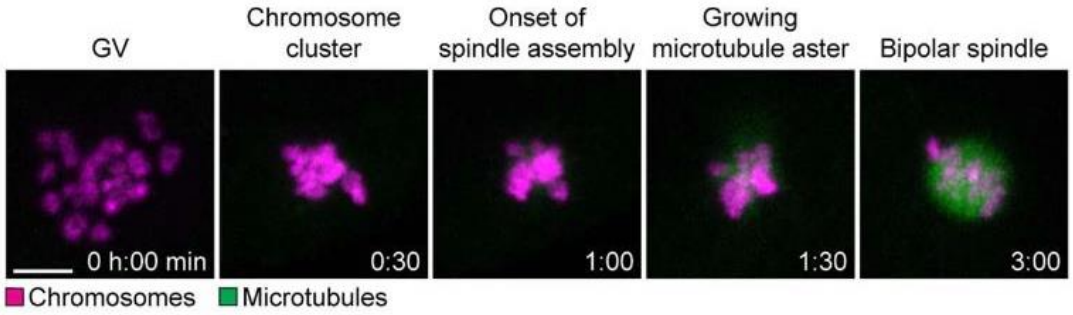
❖ Chromosome clustering

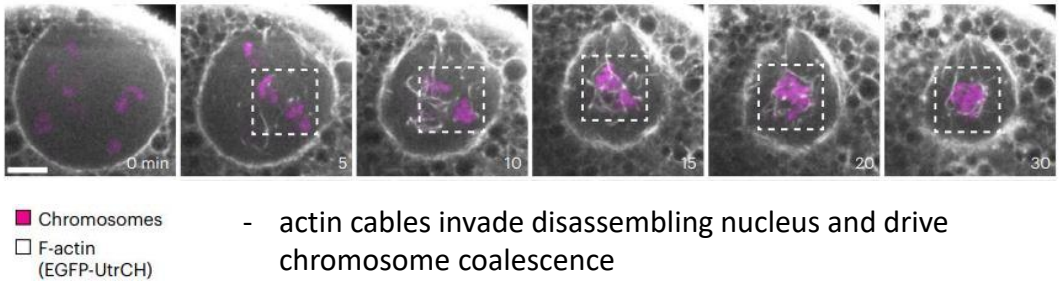
- transient stage of chromatin aggregation
- after NEBD and before onset of spindle assembly





Melina Schuh





- actin cables invade disassembling nucleus and drive chromosome coalescence

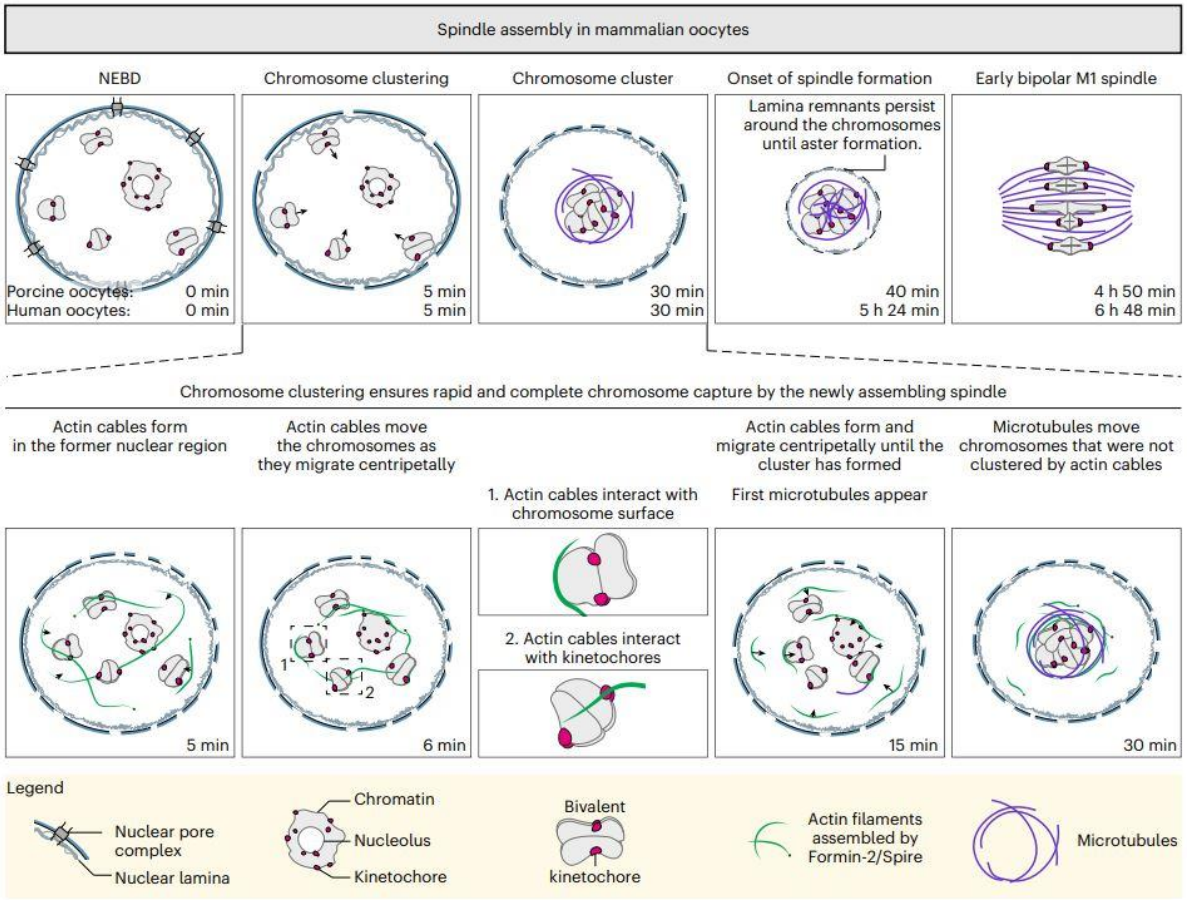
Harasimov et al, Nature Cell Biology 2023

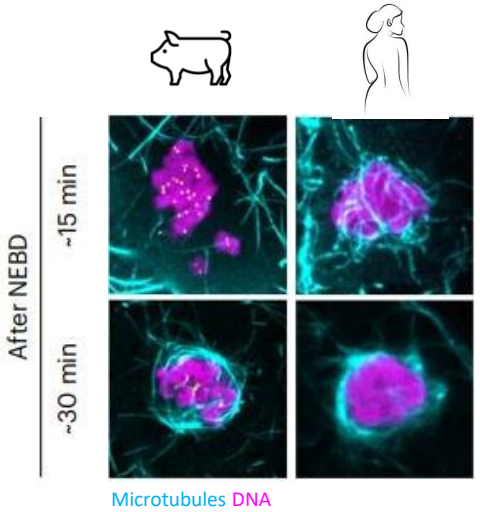
Nuclear maturation in human oocyte

❖ 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

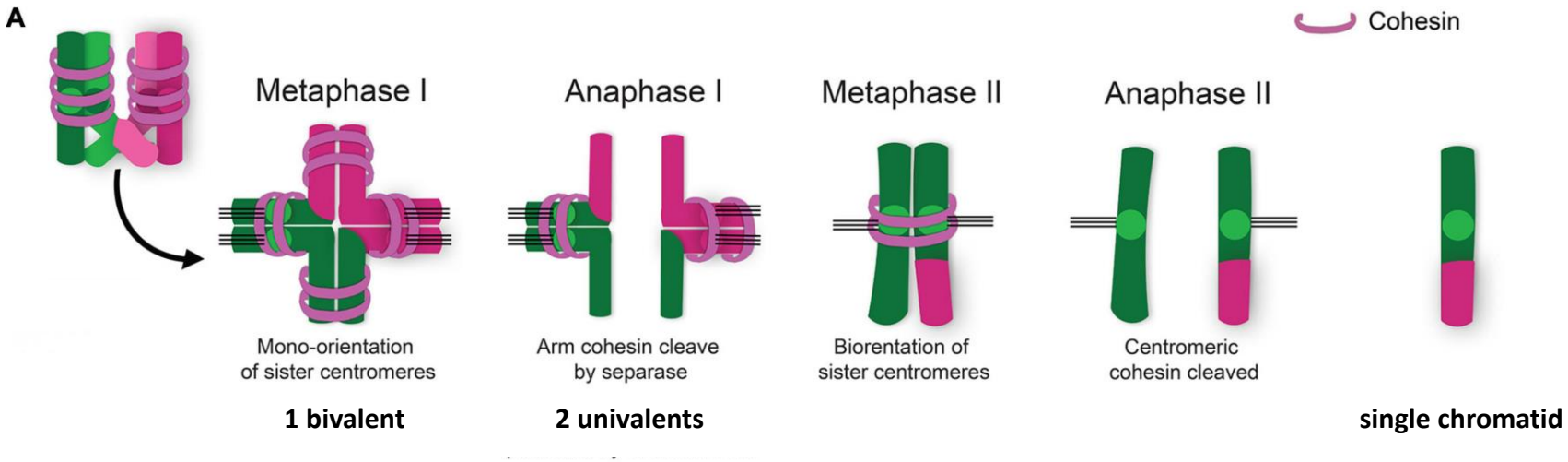


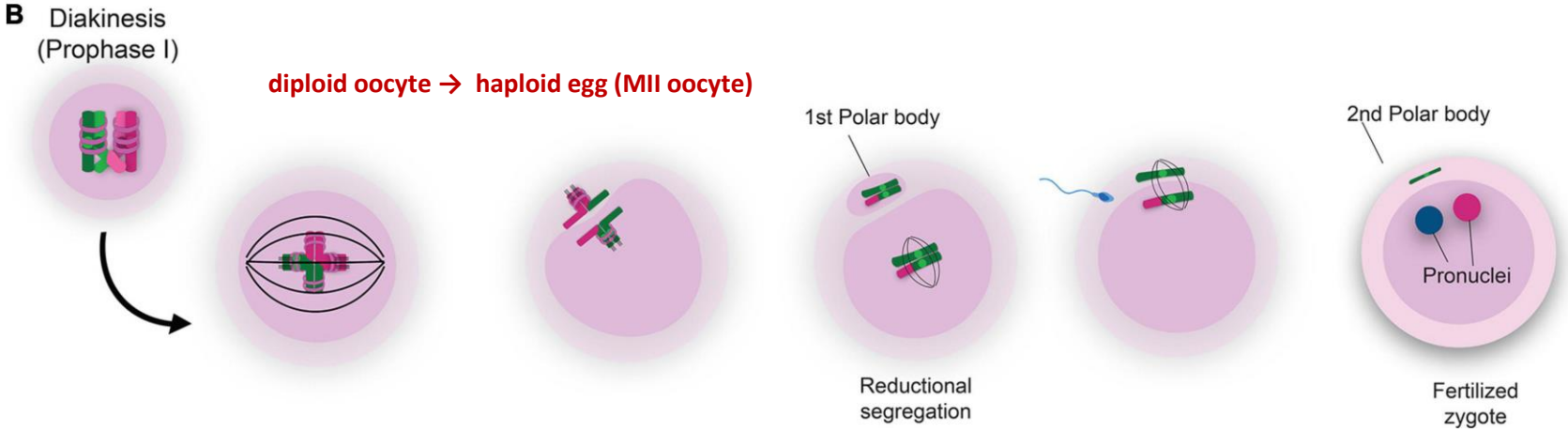


Microtubule „cage“

Harasimov et al, et al. 2023

Chromosome segregation



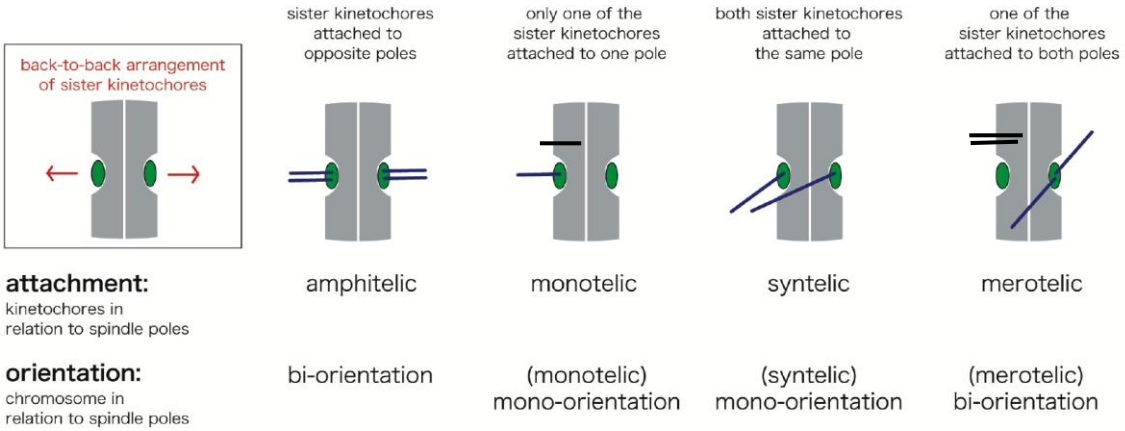


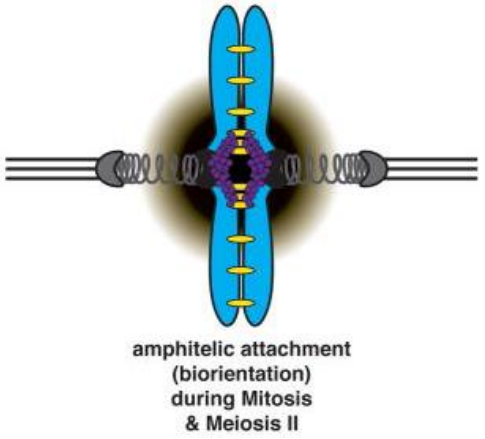
- **Meiosis I**
  - separation of homologous chromosomes
  - haploid set of chromosomes eliminates to 1st PB
  - 2n 4C → 1n 2C

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

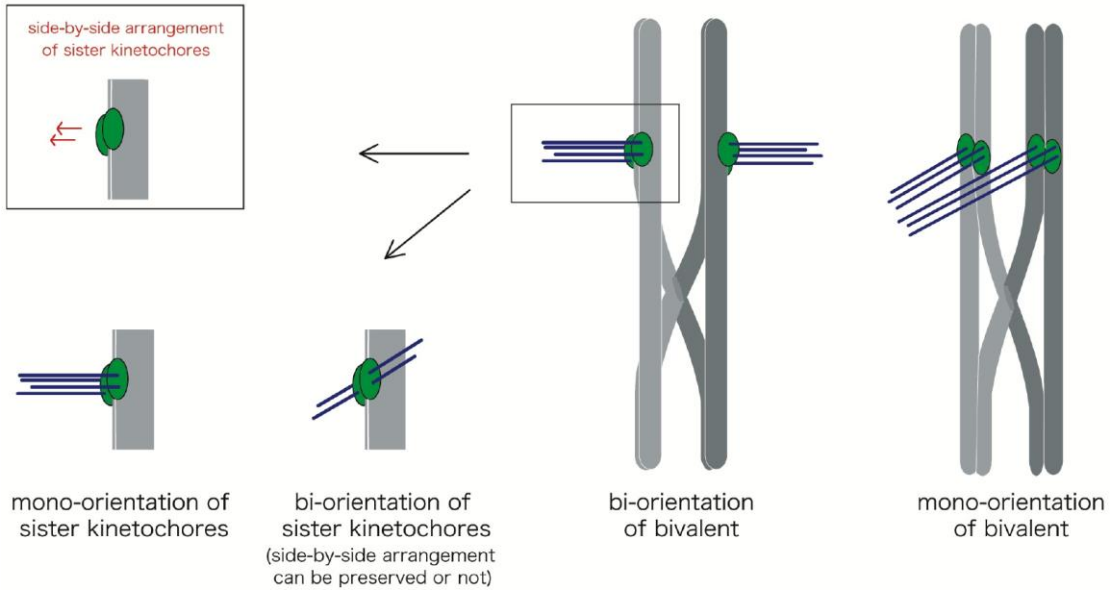
Kinetochore-microtubule attachment

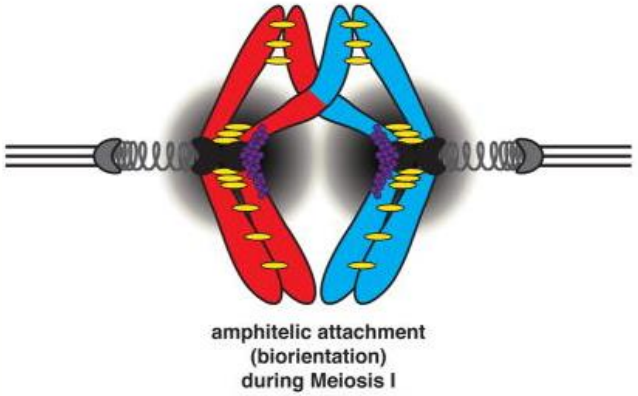
➢ MITOSIS/ MEIOSIS II





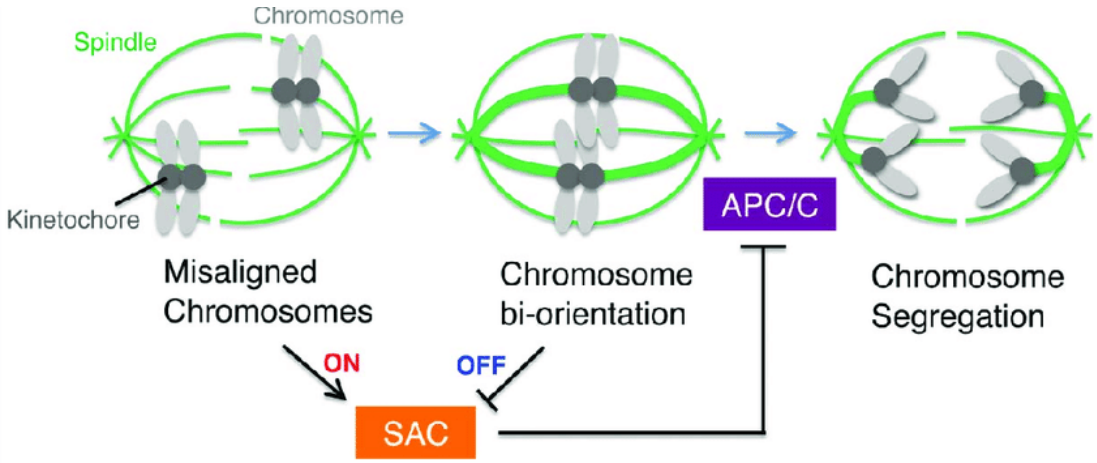
➢ MEIOSIS I





- sister chromatid monoorientation (behave like functional unit !)

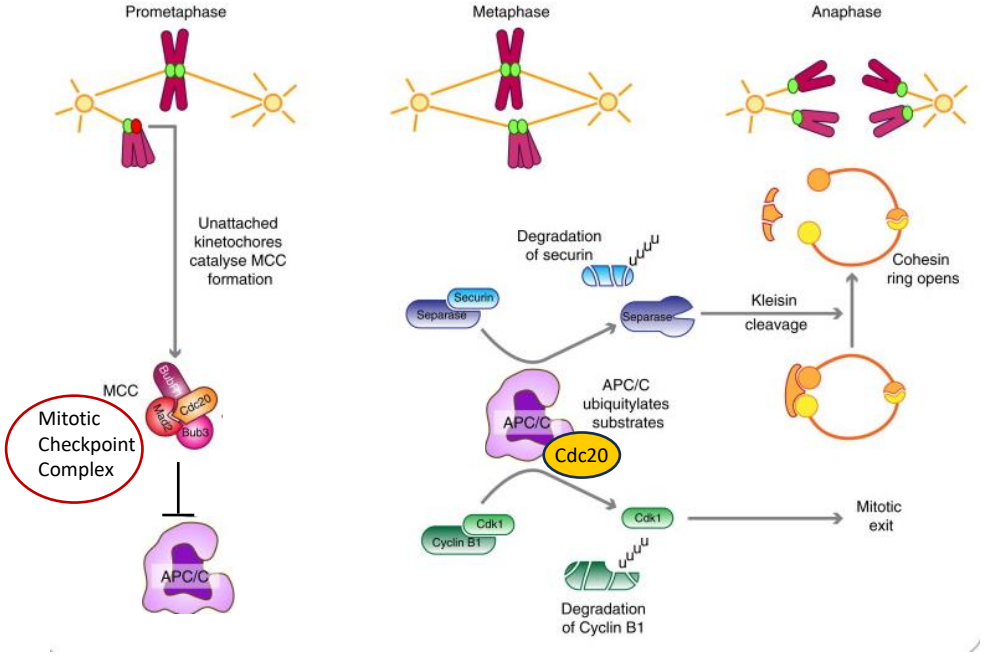
Spindle asembly checkpoint

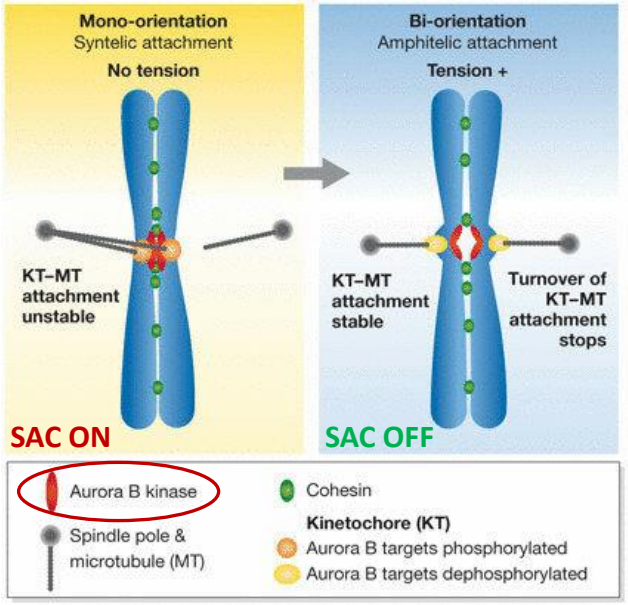


- the pathway that delays mitosis until **all kinetochores** are attached to microtubules

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

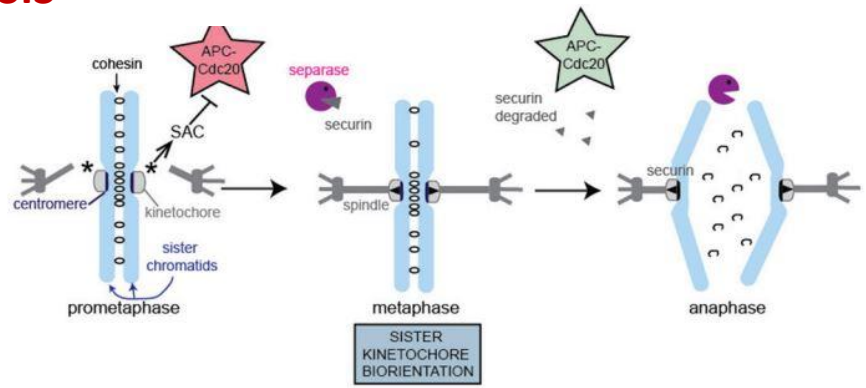
- SAC responds to interkinetochore tension





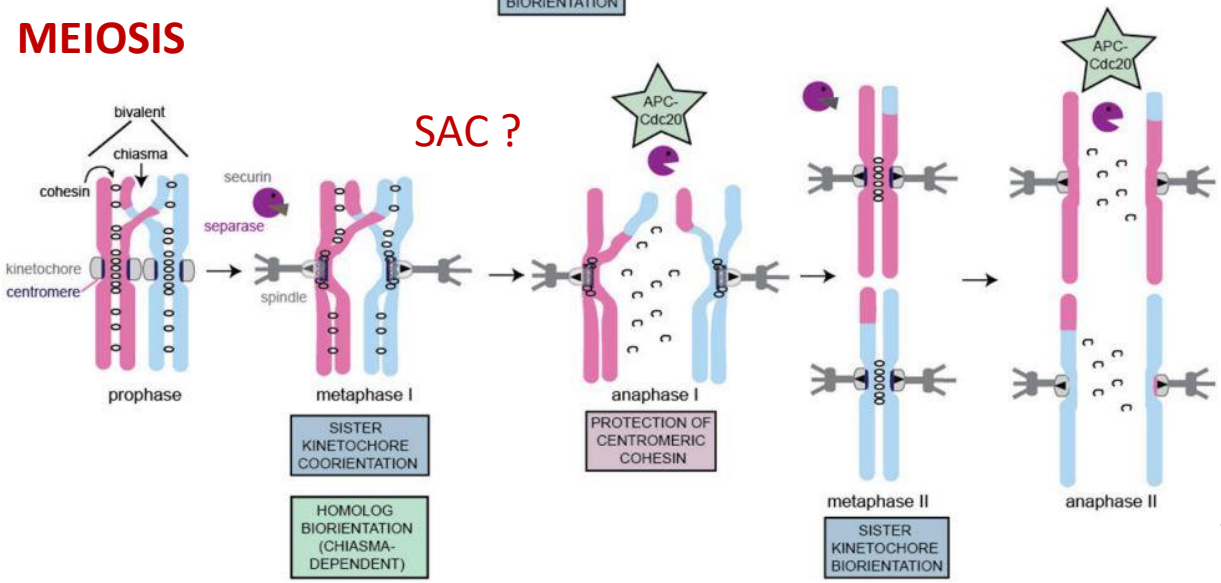
Spindle asembly checkpoint

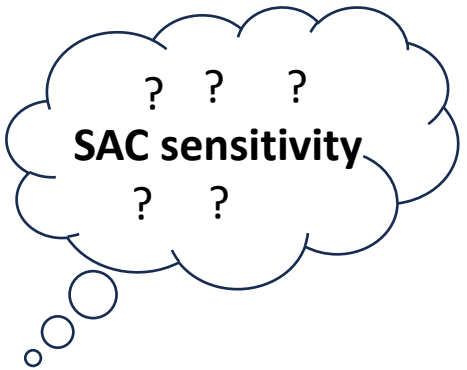
➢ MITOSIS



- all kinetochores must be occupied
- APC/C activation in minutes
- low aneuploidy rate

➢ MEIOSIS

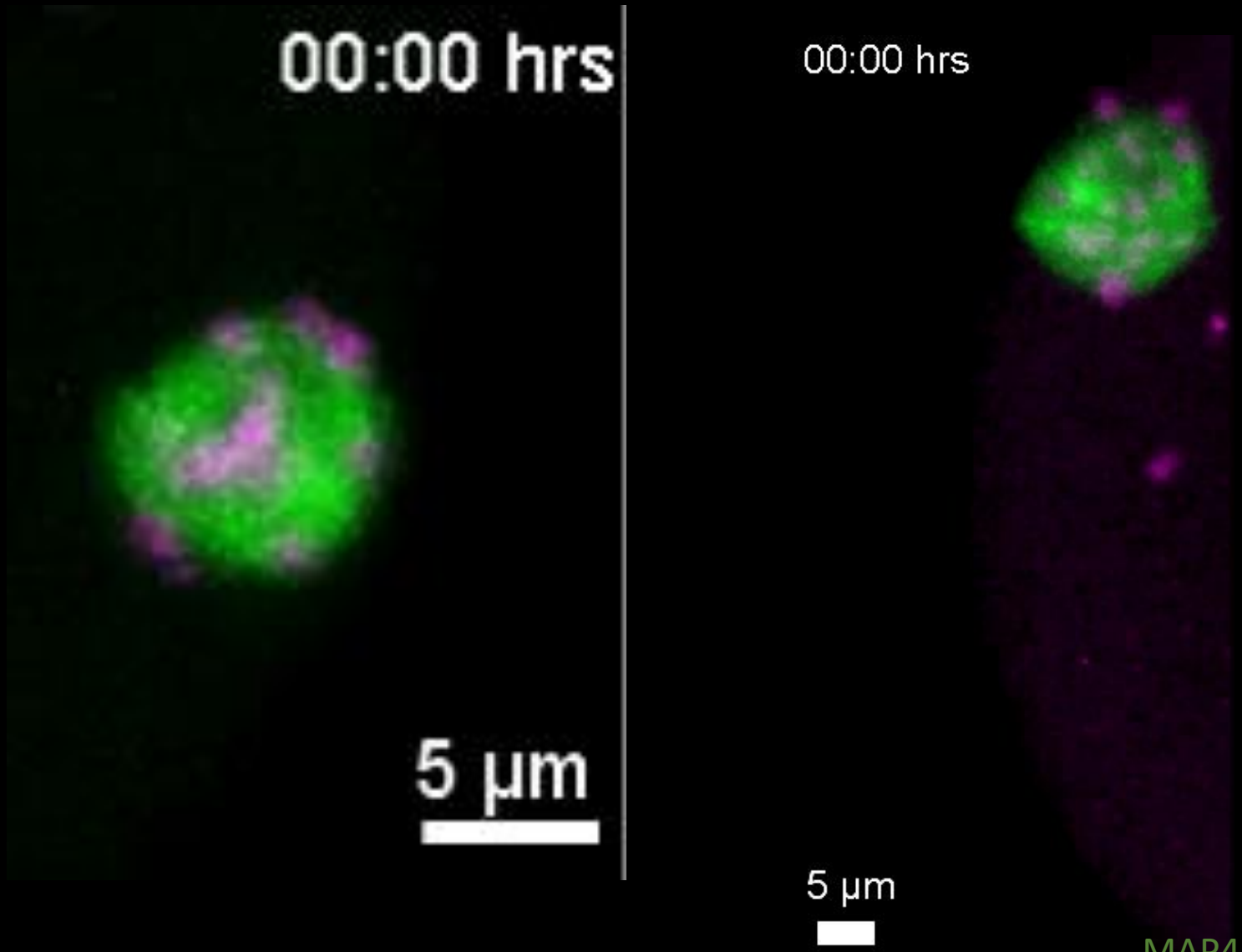




- only a majority of kinetochores must be occupied
- APC/C activation in hours
- high rate of aneuploidy

Marson and Wassmann 2017

Spindle asembly checkpoint

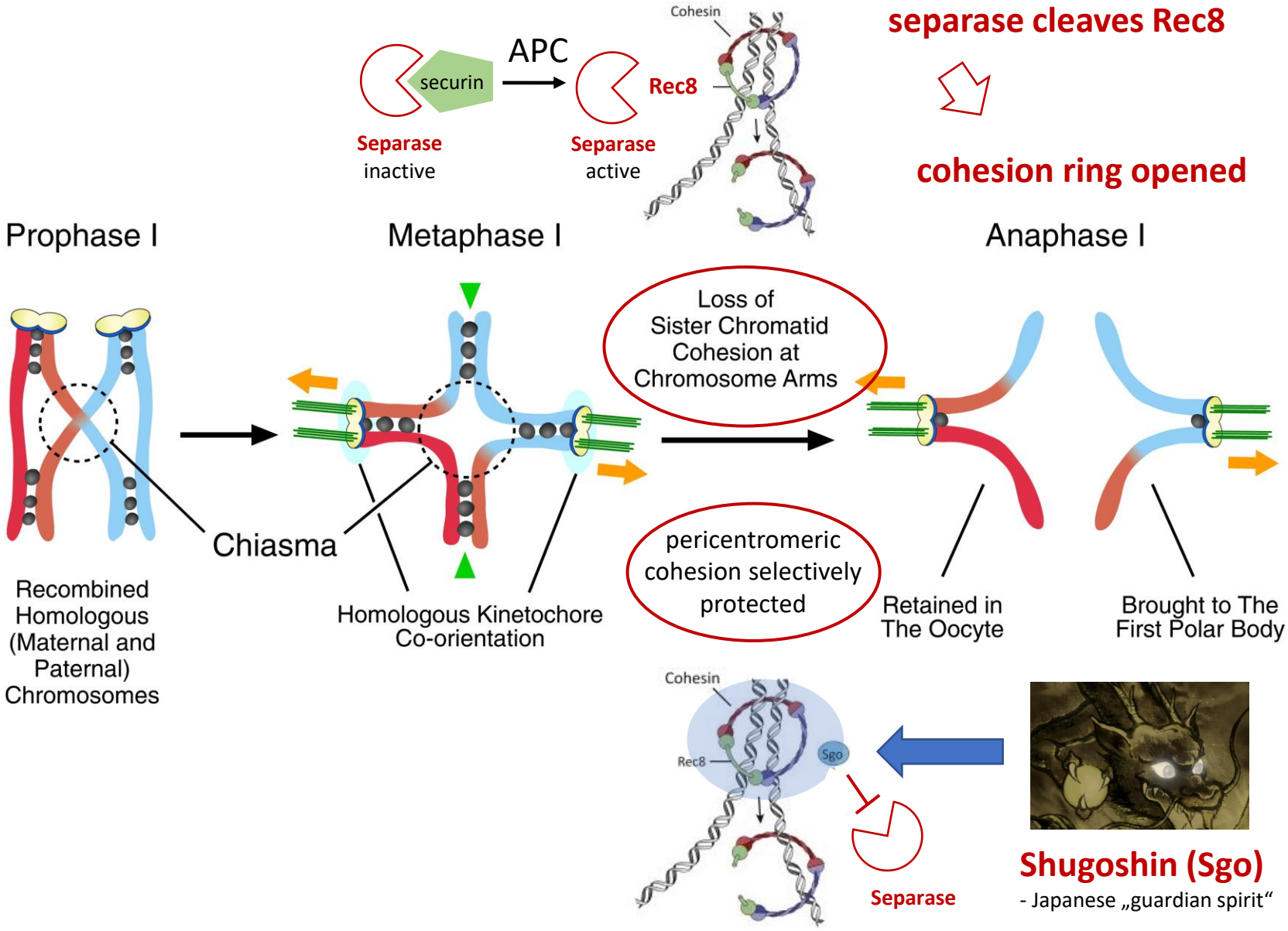


MAP4 – microtubules

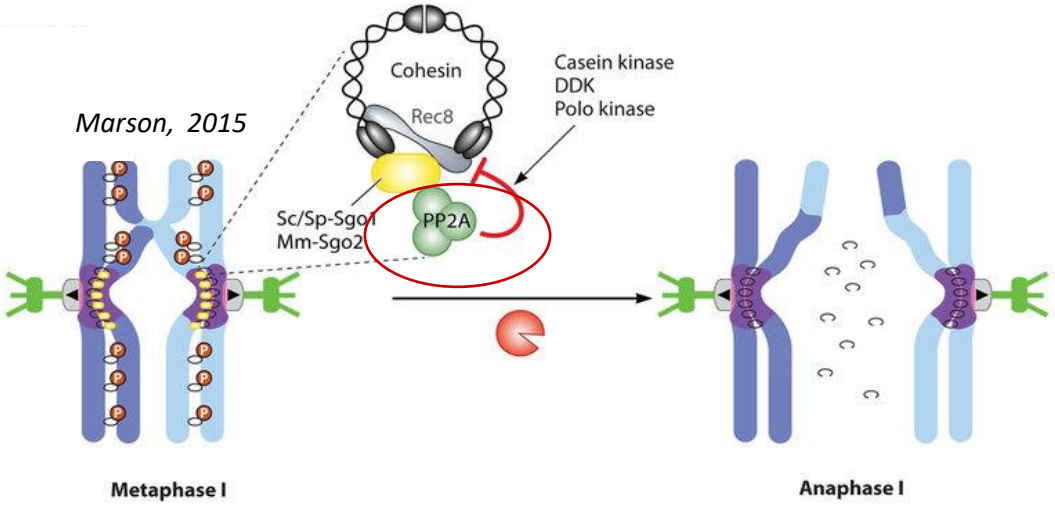
H2B - DNA

Holubcova et al Science 2015

Loss of cohesion in anaphase I

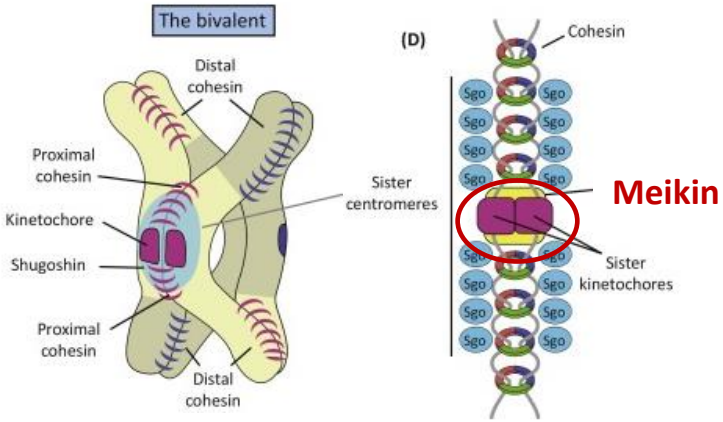


Loss of cohesion in anaphase I



Marson, 2015

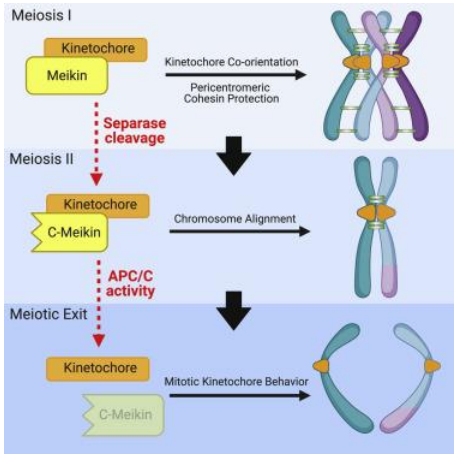
Sgo recruits **PP2A**, which removes Rec8 phosphorylation, making it a poor substrate for separase-dependent cleavage.



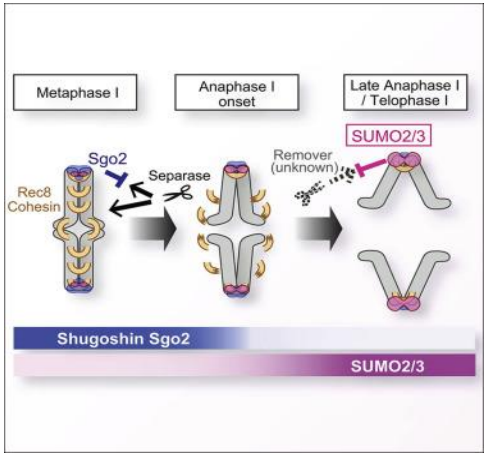
- Stabilizes location of Sgo
- Ensures chromosome alignment in MII

Webster and Schuh, 2017

Kim et al 2015.

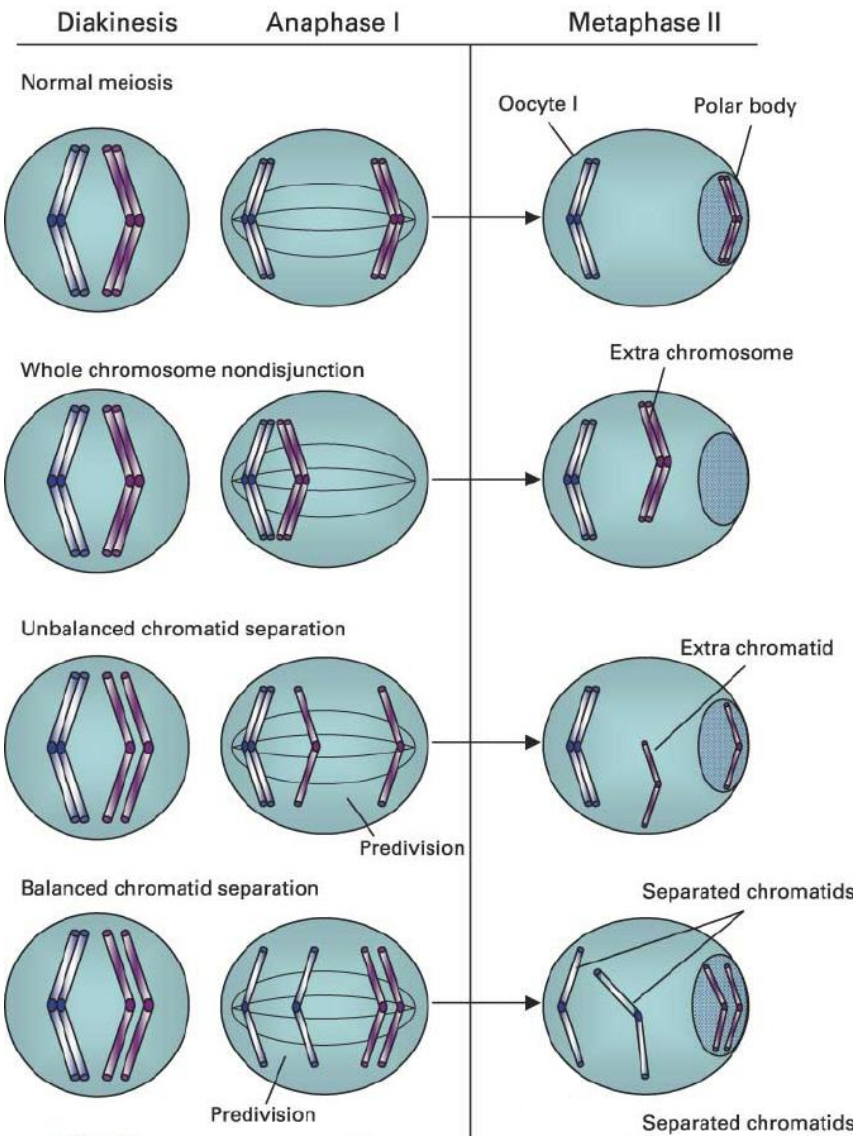


Maier et al, 2021



Ding et al, 2018

Chromosome segregation errors



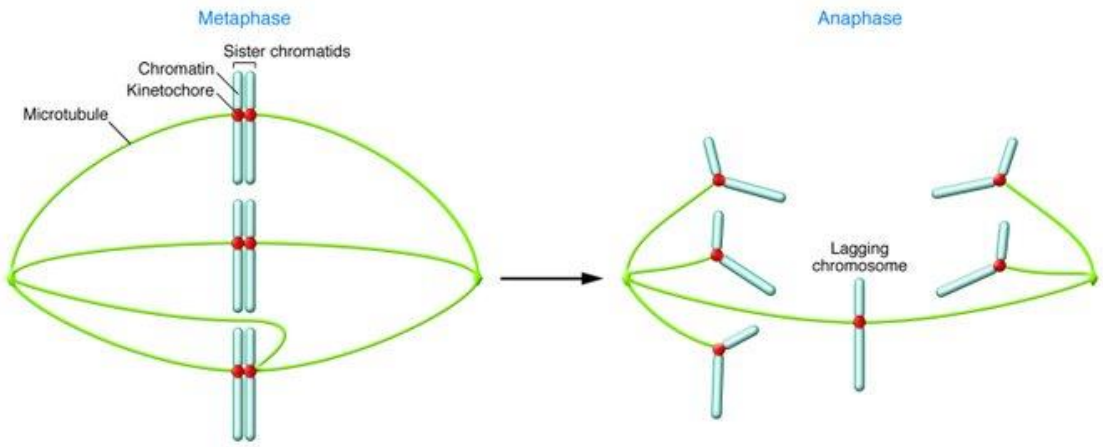
Oocyte aneuploidy

Chromosome nondisjunction (NDJ)

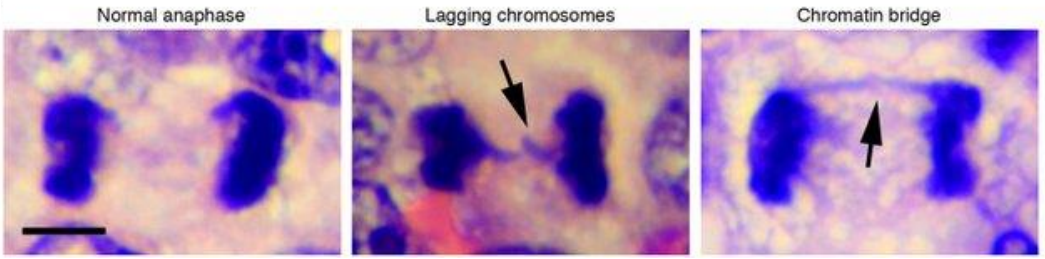
Precautious separation of sister chromatids (PSSC)

Chromosome lagging

- delayed chromosome/chromatid movement during anaphase

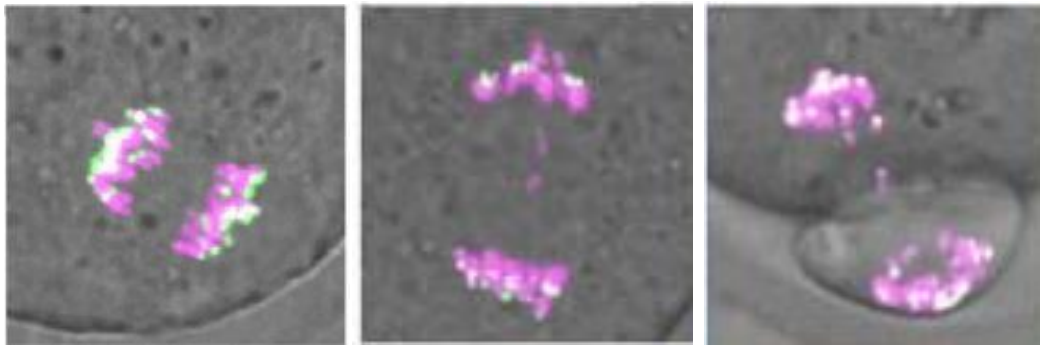


- risk of inaccurate segregation, chromosome loss/gain and aneuploidy



oocytes

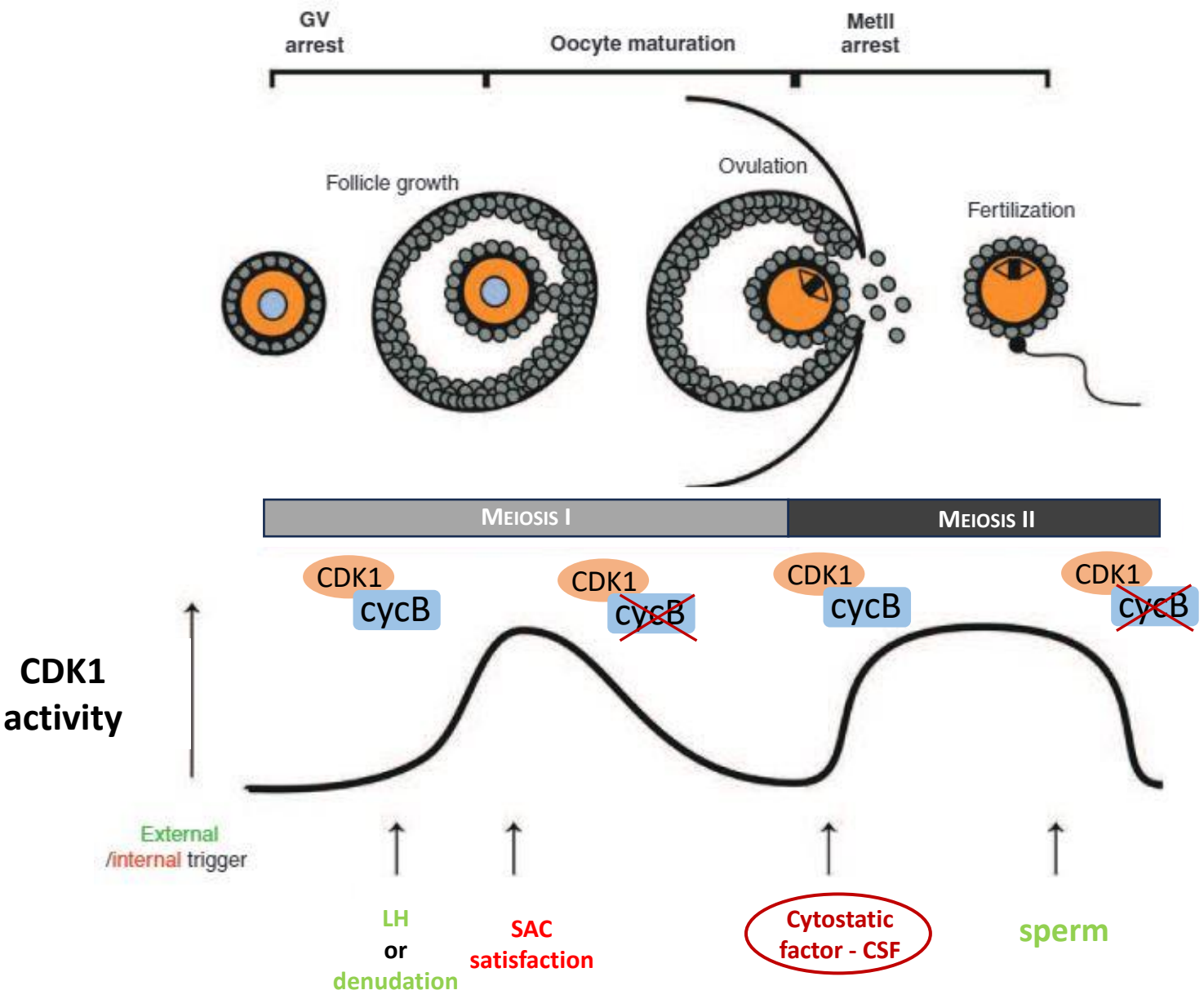
- class I – laggards
  - aligned at anaphase
  - reduced velocity
  - aneuploidy producing
- class II – laggards
  - misaligned at anaphase
  - normal velocity
  - benign



No lagging | Class I laggards | Class II laggards

Mihajlovic et al 2021

Transition from meosis I to meiosis II

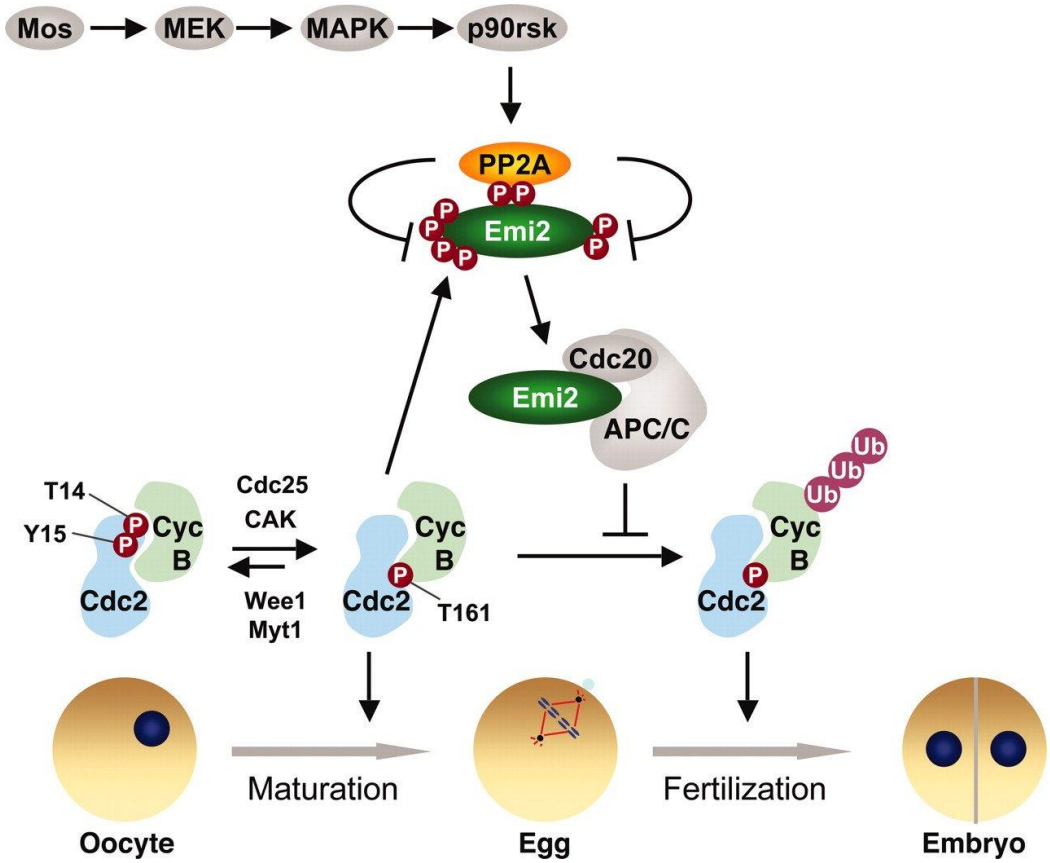


Metaphase II arrest

➢ 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

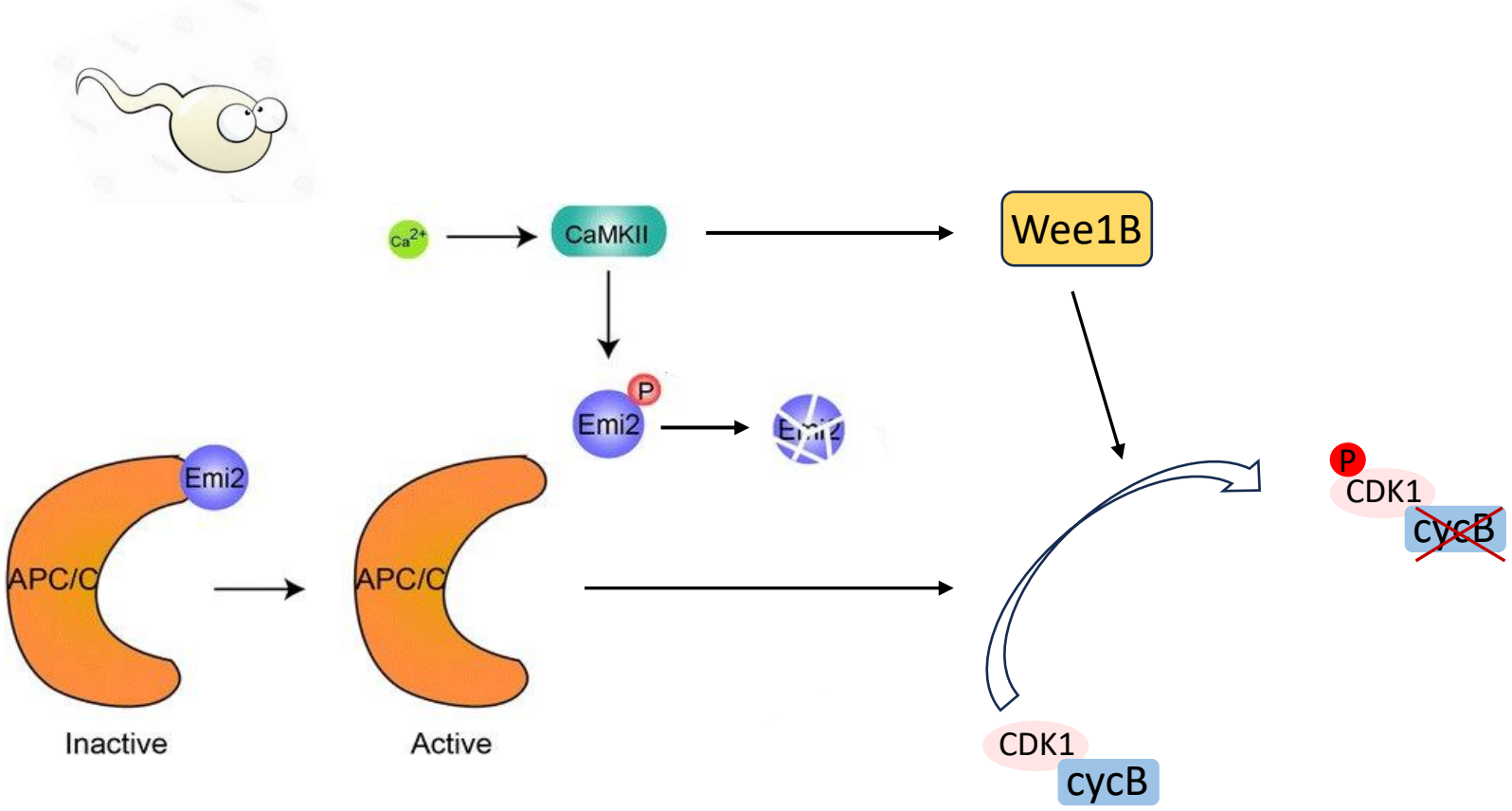
- contributes to APC/C inhibition by controling protein expression during MII arrest
- expression of Emi2 is perturbed when BTG is absent (RNAi - depletion)

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

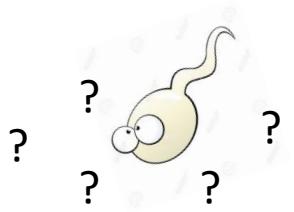
Pasternak et al 2016

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