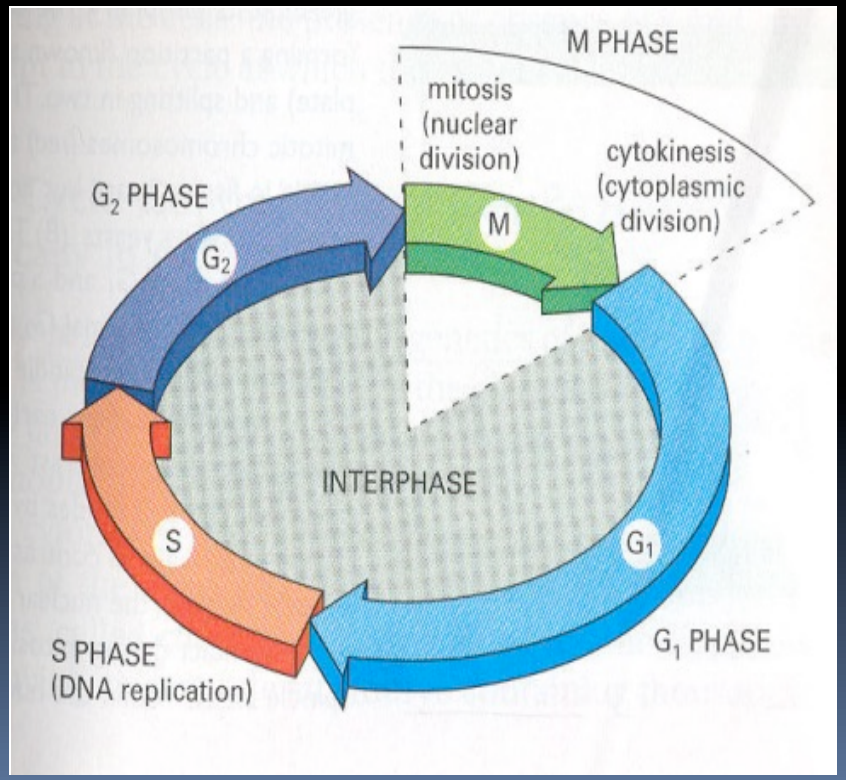
UNIVERSITY of
TERAMO

Biotechnology of Reproduction

MOLECULAR REGULATION OF MEIOSIS

Prof. Luisa Gioia



CSF-mediated arrest (corresponding to high MPF activity) is primarily induced by **APC inhibition**

High MPF activity may be maintained via separates pathways:
direct inhibition of APC/C and direct stabilization of MPF

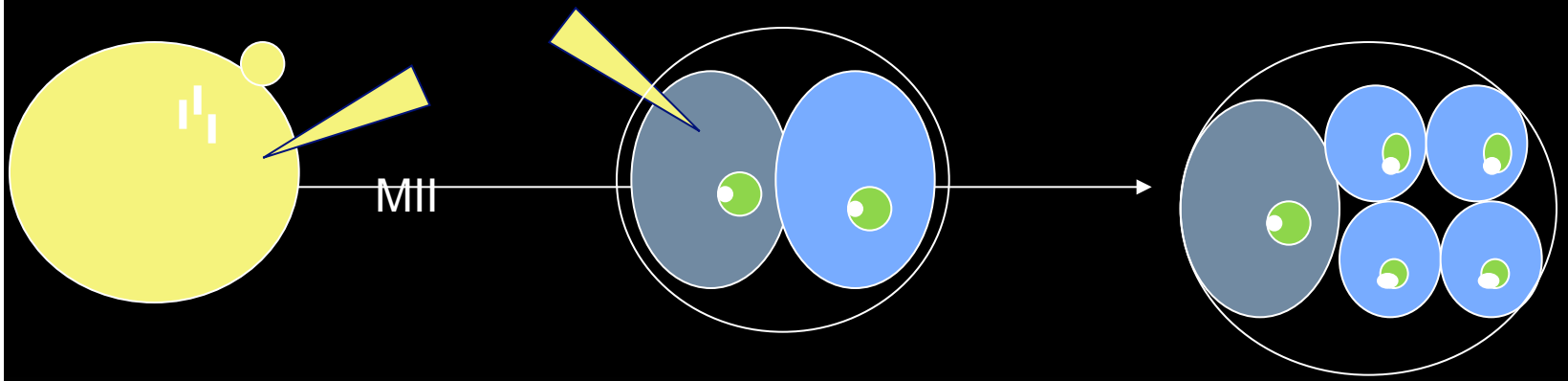
CSF pathways

Three pathways contribute to CSF-mediated arrest:

- **c-Mos/MEK/MAPK/p90**
- **Emi2**
- **Cyclin E/Cdk2**

Cytostatic factor (CSF) activity in the cytoplasm of MII oocyte causes oocyte MII arrest

Experimental evidence on CSF



- *Injection of active **protein kinasi p90** in one blastomer blocks its division*

CSF pathways

- **c-Mos/MEK/MAPK/p90**

This pathway has been shown to be involved in MPF stabilization (set the proper level of MPF activity)

Downstream components of this pathway are not completely known and can change accoring to the species

In frog (unlike mouse) this pathway involves proteins of SPINDLE ASSEMBLY CHECKPOINT** which act by inhibiting APC

****Bub1, Mad1, Mad2**: localize to the kinetochores of chromosomes generating inhibitory signal that delays the onset of Anaphase if chromosomes are not aligned or impaired tension with spidnle MT occurs

All appear to be required downstream of cMos pathway, therefore CSF arrest by cMos pathway is mediated by these proteins

QUALITY CONTROL OF THE CELL CYCLE

Checkpoint controls in mammalian oocytes

The cell has several systems for interrupting the cell cycle if something goes wrong

G2-M Transition

Influenced by:

- Cell size
- DNA damage
- DNA replication

Metaphase-Anaphase Transition

Influenced by:

- Chromosome attachments to spindle

M

G2

G1

S
(DNA synthesis)

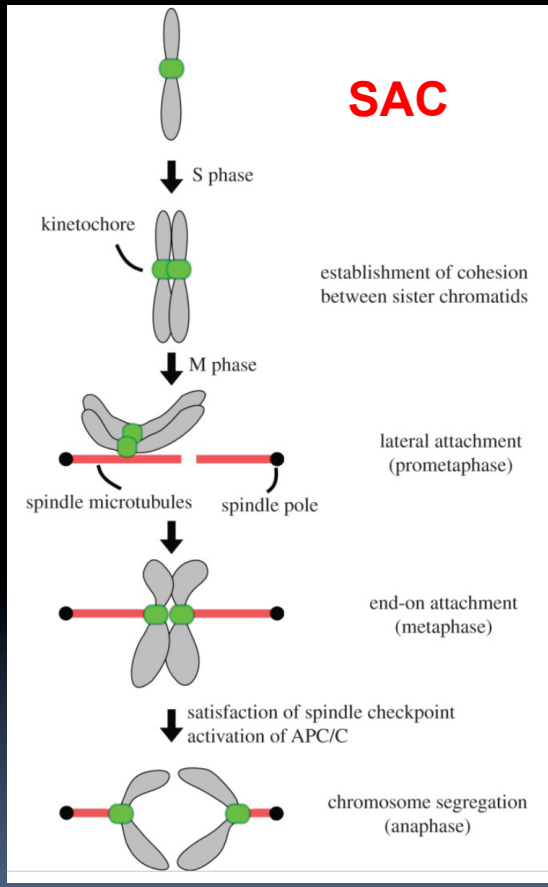
G0

Restriction Point (Start)

Influenced by:

- Growth factors
- Nutrients
- Cell size
- DNA damage

Spindle Assembly Checkpoint



The Spindle Assembly Checkpoint (SAC) ensures accurate chromosome segregation by delaying anaphase onset until all the chromosomes are correctly attached to the spindle through their kinetochores

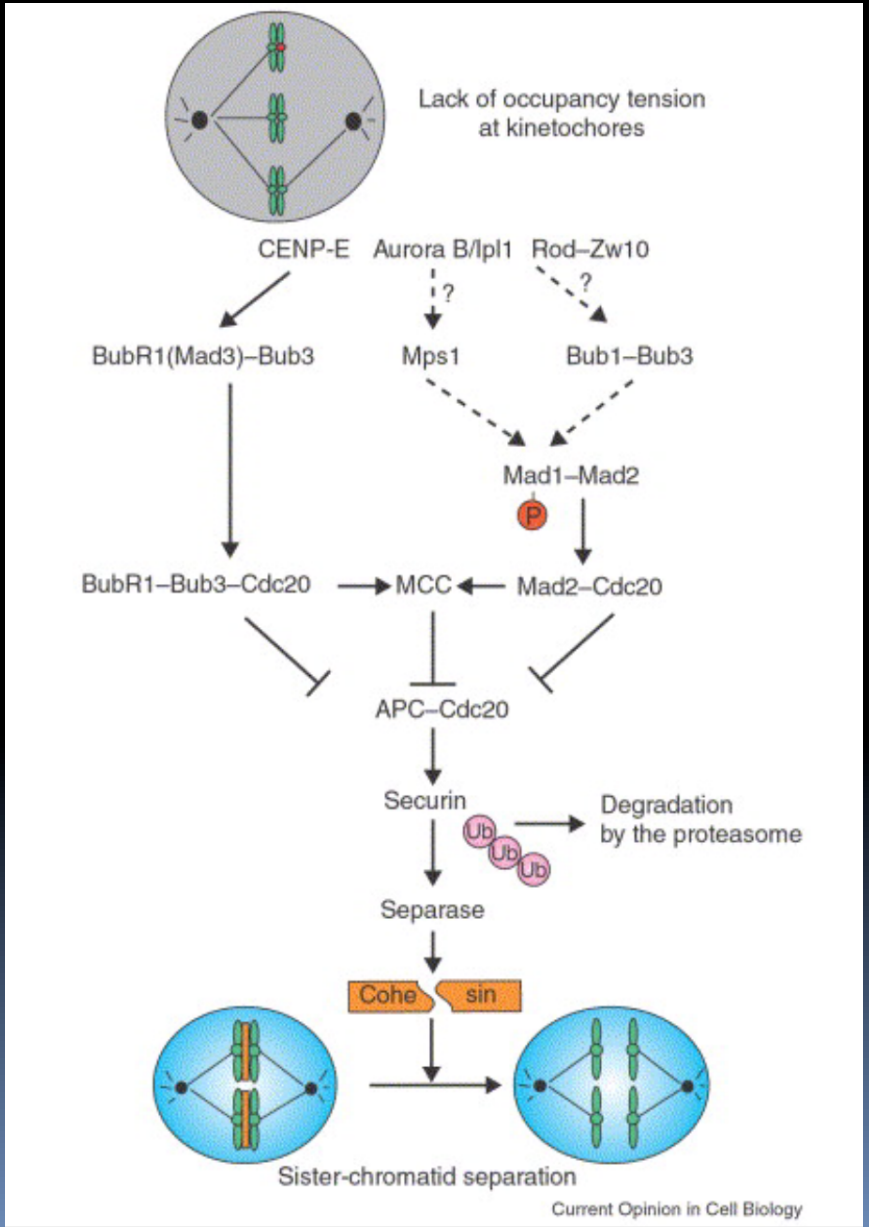
This checkpoint depends on the activity **kinetochore proteins:**

-Bub1 (Budding uninhibited by benzimidazole)

-Mad (Mitotic arrest-deficient)

(Hoyt et al. 1991, Li & Murray 1991)

Regulation of APC-Cdc20 by the spindle checkpoint



CSF pathways

- **Emi2**

CSF-mediated arrest may be primarily induced by Emi2 pathway which causes APC inhibition but also requires the **cMos/MEK/MAPK pathway to set MPF levels within physiological limits** (not too high to induce an arrest that cannot be broken, or too low to induce parthenogenetic activation)

CSF-mediated MII arrest

Emi2/CdK2 pathway partecipates in APC inhibition

- **Emi2 accumulates during egg maturation**
- **Emi2 is present in CSF-arrested egg extracts**

- **Emi2 is a substrate of a polo-like kinase (PlK)**
- **Emi2 is rapidly degradated on Ca addition**
- **At fertilization, Ca increase activates CaMKII, which phosphoyilates Emi2. PlK further phosphorylates Emi2 causing Emi2 ubiquitinilation and degradation**

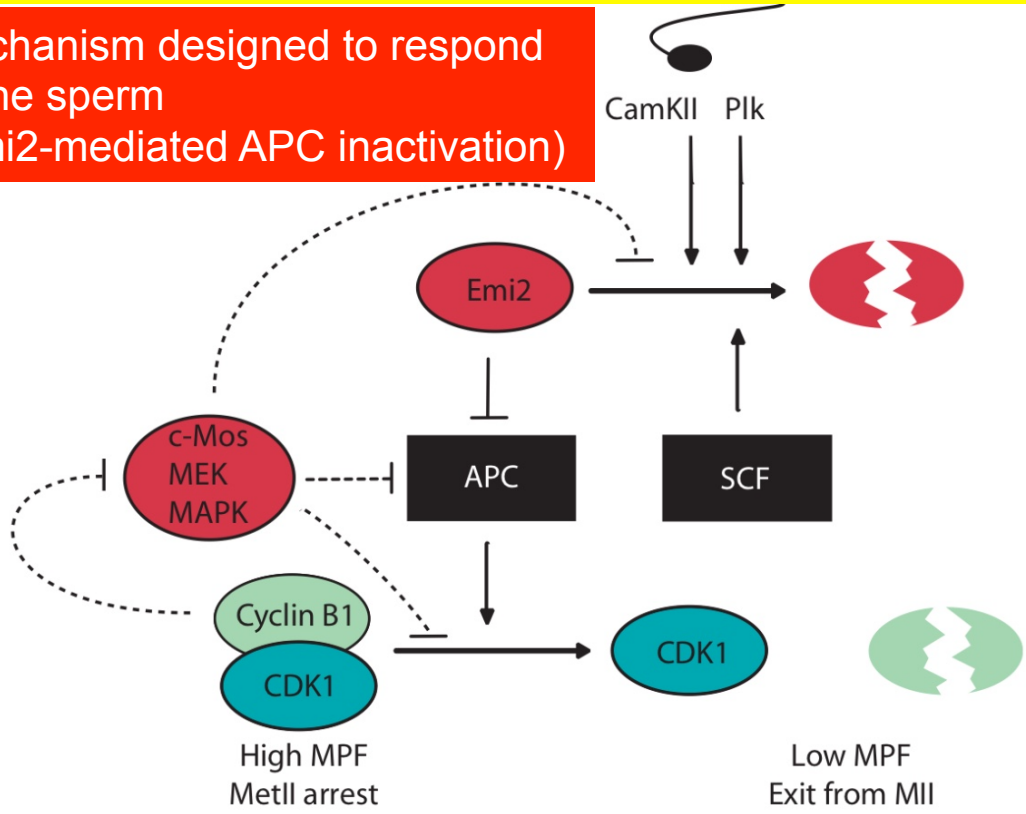
The levels of Emi2 remain low until their marked rise in MII

Model of CSF arrest by CSF:
MPF stabilization plus
APC inhibition

CSF-mediated MII arrest

Emi2/CdK2 pathway partecipates in APC inhibition

Mechanism designed to respond to the sperm (Emi2-mediated APC inactivation)



Keep MPF active until the time of fertilization (cMos pathway)

The levels of cyclin E/ Cdk2 and Emi2 remain low until their marked rise in MII

- **Emi2 accumulates during egg maturation**
- **Is present in CSF arrested egg extracts**
- **Emi2 is a substrate of a polo-like kinase (PIK)**
- **Is rapidly degradated on Ca addition**
- **At fertilization, Ca increase activates CaMKII, which phosphorilates Emi2. PIK further phosphorylates Emi2 causing its ubiquitinilation and degradation**

CSF pathways

- Cyclin E/Cdk2: **contributes to APC inhibition**

Downstream target for CyclinE/Cdk2 is **Mps1**, a spindle checkpoint protein

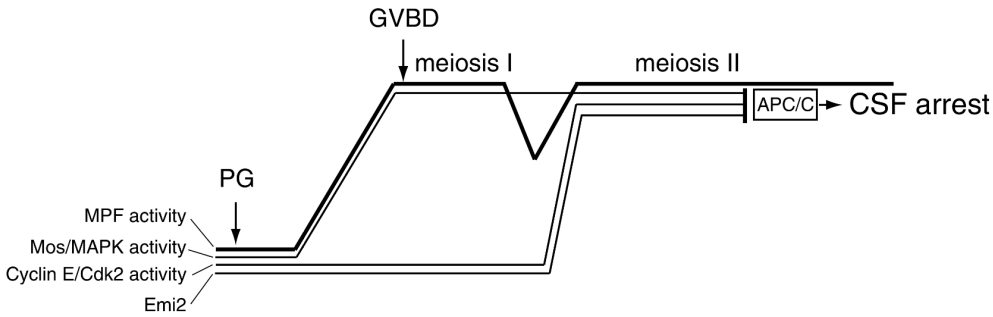


Figure 1 Pathways contributing to the establishment of CSF arrest. During the maturation of oocytes, Mos/MAPK activity is high after GVBD in M I and remains high throughout M II. The levels of cyclin E/Cdk2 and Emi2 remain low until their marked rise in M II. The combination of the Mos/MAPK pathway, cyclin E/Cdk2 pathway and Emi2 contributes to full inhibition of APC/C and establishment of CSF arrest. Both the Mos/MAPK pathway and Emi2 are required for the establishment of arrest, whereas the cyclin E/Cdk2 pathway contributes to APC/C inhibition but is not necessary for establishment of CSF arrest.

CSF pathways

Three pathways contribute to CSF-mediated arrest:

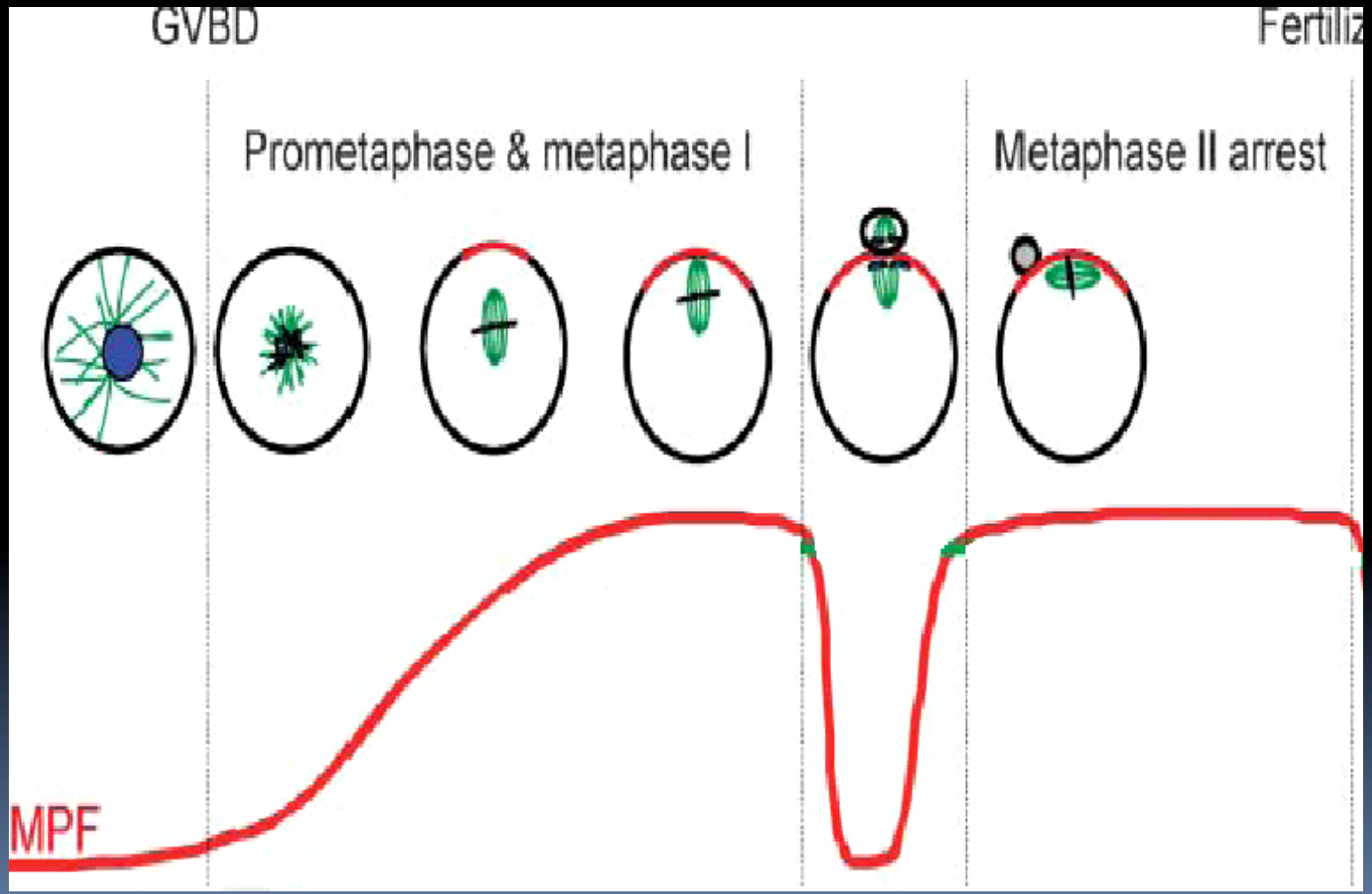
- **c-Mos/MEK/MAPK/p90**
- Emi2
- Cyclin E/Cdk2

Establishment of stable CSF arrest may occur only after full **APC/C inhibition** is promoted by the action of all three pathways

Oncogene (2007) 26, 1286–1289

High MPF acivity during MII

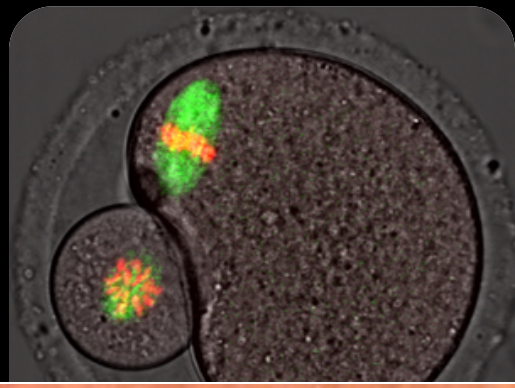
Chromatids are paired on equatorial plane of spindle



Second meiotic division

In contrast to the first meiotic division, the entry into the second one is similar to mitosis

- **MPF activity increases rapidly** and the spindle forms quickly.
- Moreover, the MII **chromosomes** are identical to mitotic chromosomes, **composed of sister chromatids** with active kinetochores.

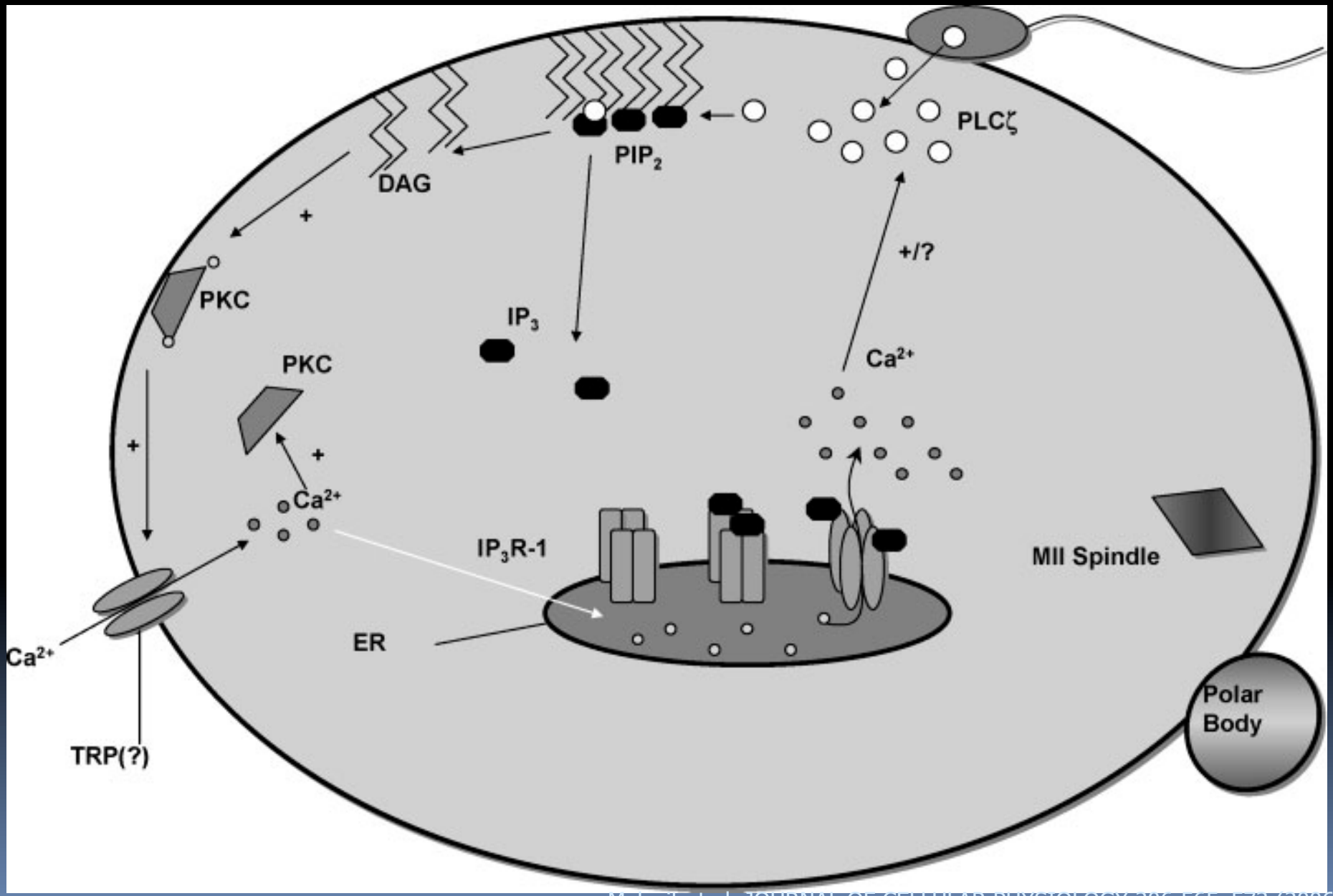


The **meiotic spindle** remains as a **stable structure** during the arrest with chromosomes perfectly aligned on the equator of the spindle

MISS (MAP kinase-interacting and spindle-stabilizing protein) and **DOC1R** are **two MAP kinase substrates** associated with the spindle in MII arrested oocytes (Lefebvre et al. 2002, Terret et al. 2003)

Role for both proteins in the maintenance of the spindle structure during the arrest at metaphase.

Mechanism of Ca^{++} increase in egg at fertilization



Malcuit et al. JOURNAL OF CELLULAR PHYSIOLOGY 206:565–573 (2006)

Fertilization: MII –AII transition

The oocyte leaves MII arrest and enters AII due to the activation of APC induced by intracellular Ca^{++} increase

Ubiquitination and **proteolysis** of APC substrates take place:

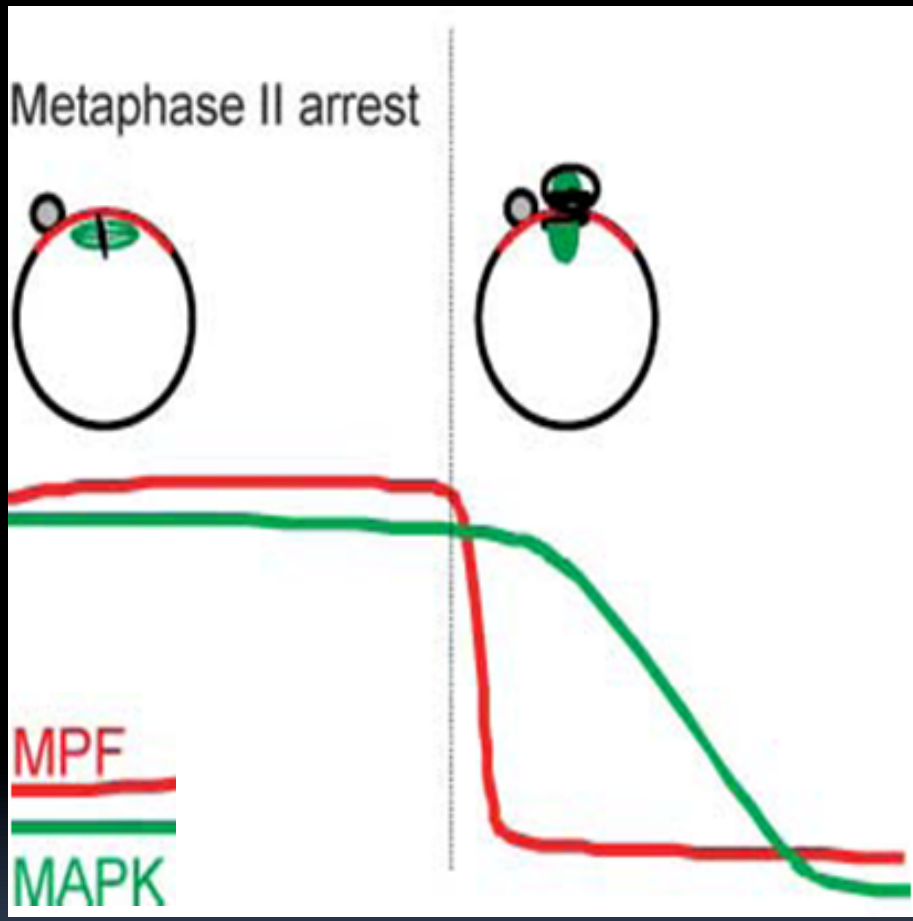
-**Cyclin** → **MPF activity decreases**
-**Securin** → separase is activated and chromatids are separated

Fertilization

MII ➔ AII

- At fertilization, due to high levels of Ca^{++}, APC is activated and consequently:

- **Cyclin is degradated and MPF is inactivated**



MPF activity is turned off at MII / AII transition

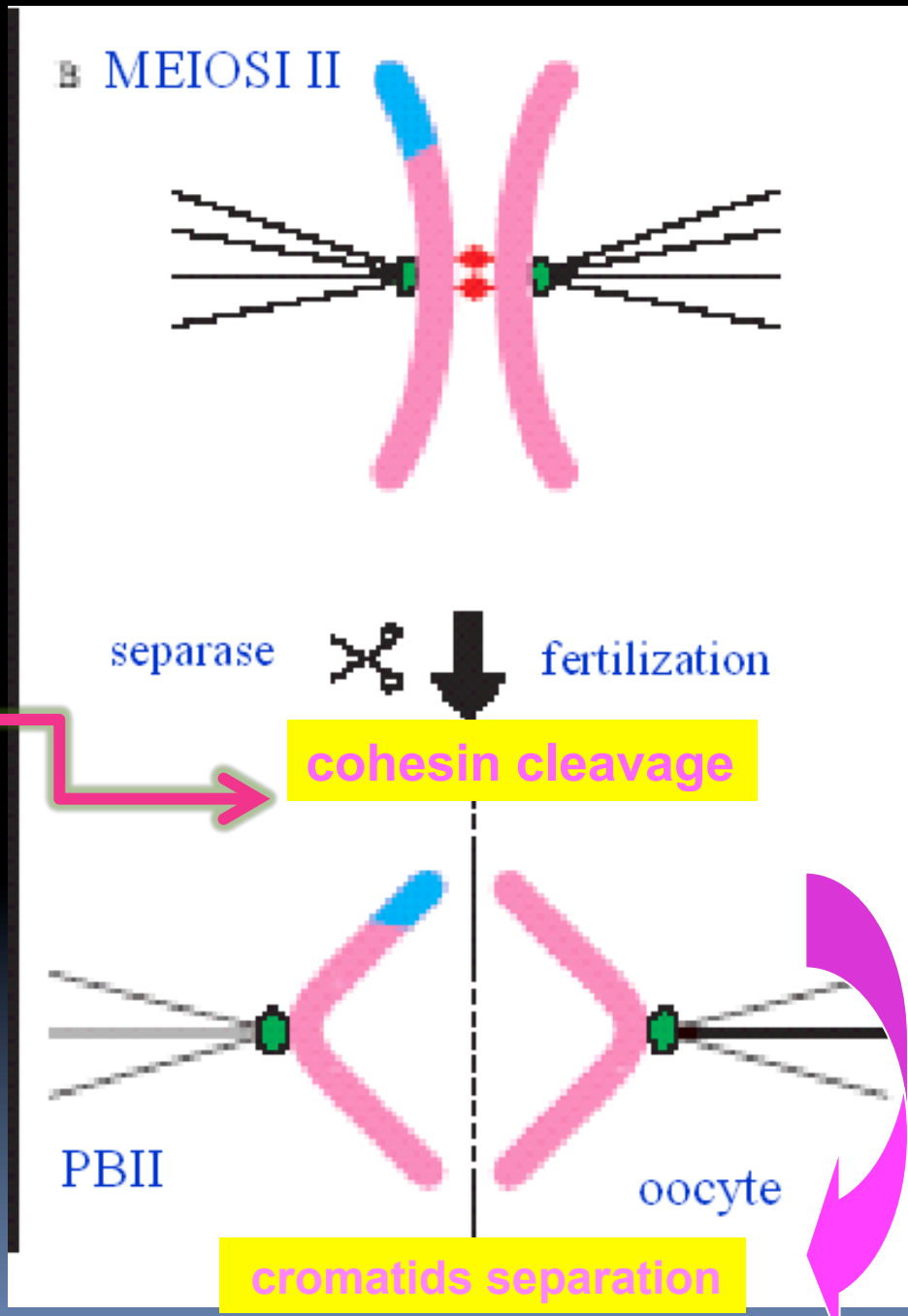
Activated **APC**

↓

Ubiquitination and proteolysis of SECURIN

↓

Activation of SEPARASE

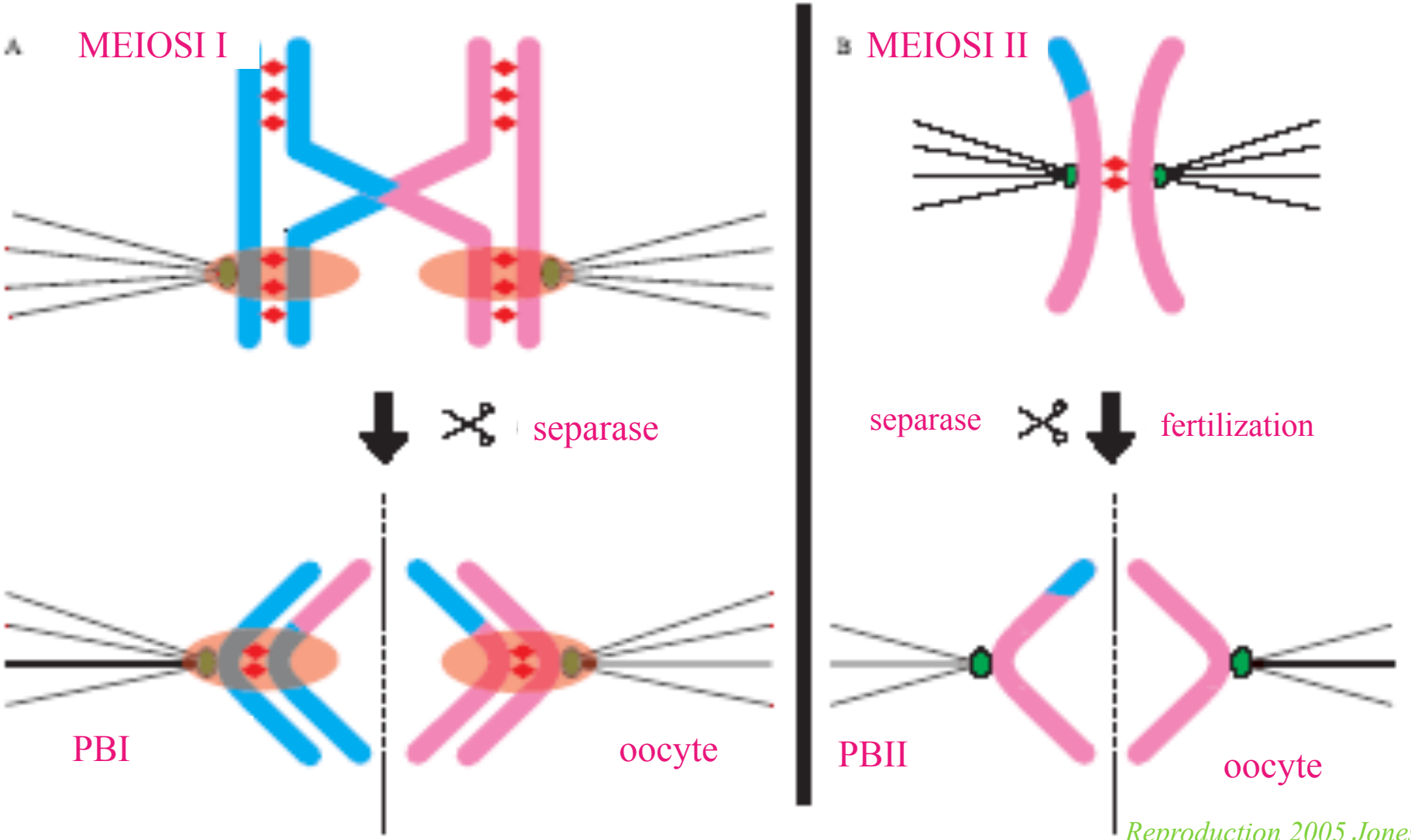


Schematic of the two meiotic divisions

During MI homologues remain attached by cohesin molecules (red) ;
cohesin at the centromeres is protected from degradation (red shaded oval).

At anaphase onset **activation of separase** is triggered and cohesin is proteolytically cleaved. Loss of cohesion in the arms allows the microtubule pulling forces to initiate poleward movement of homologues.

In the second meiotic division sister **chromatids** are kept attached by centromeric cohesin and at fertilization a sperm-derived **Ca2 signal activates separase** to allow sisters to separate. For this to occur **protection of centromeric cohesin is lost after meiosis I**

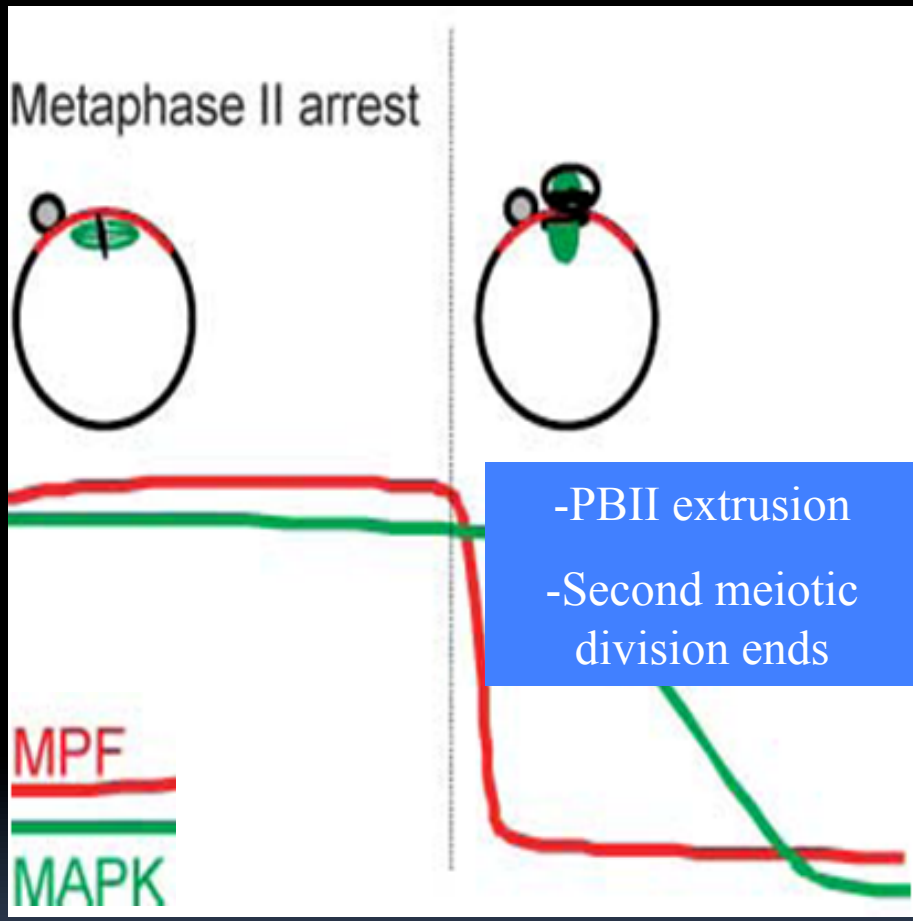


Reproduction 2005 Jones

Fertilization

AII - TII

- At TII the oocyte extruded the PBII. Meiosis ends
- Diploid condition will be re-established due to fusion with sperm genome

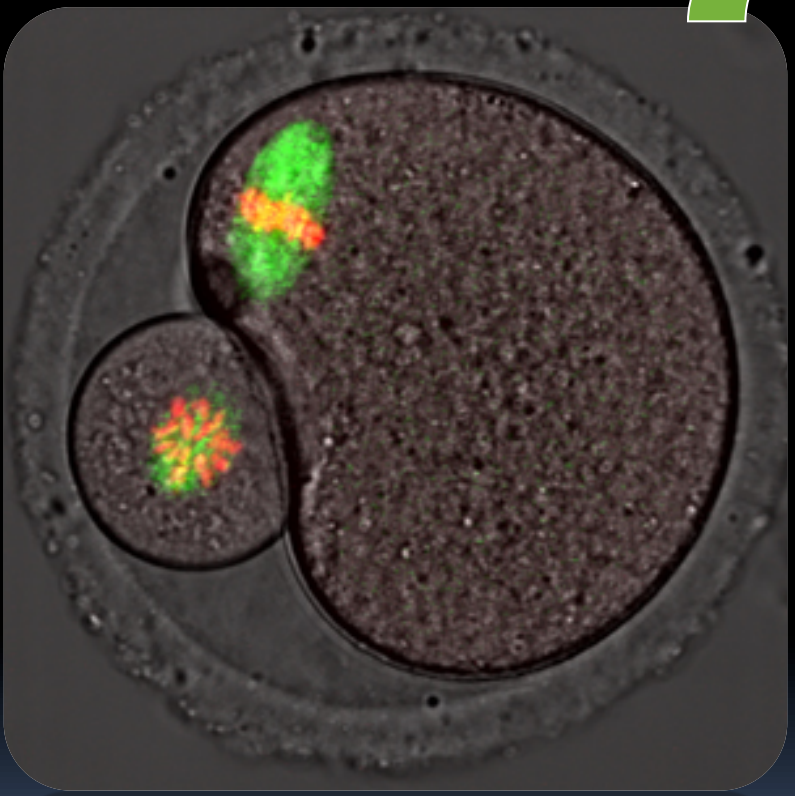


MPF activity is turned off at MII / AII transition

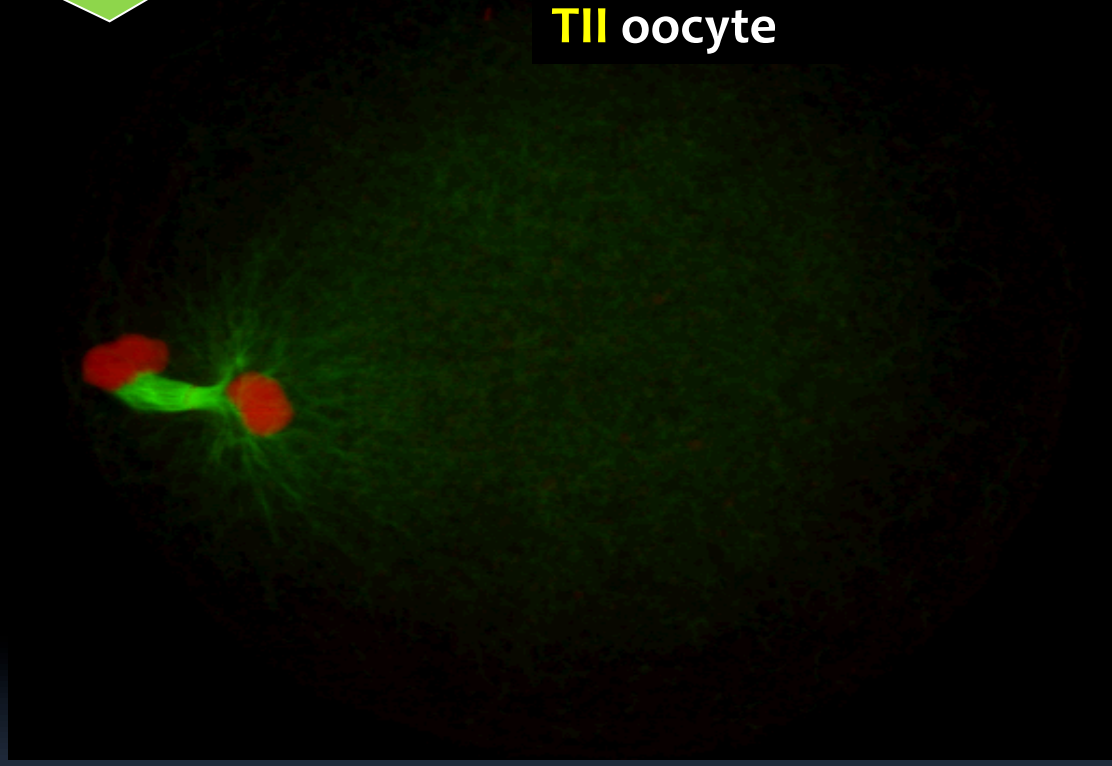
Completion of meiosis

The oocyte M-phase ends only after fertilization

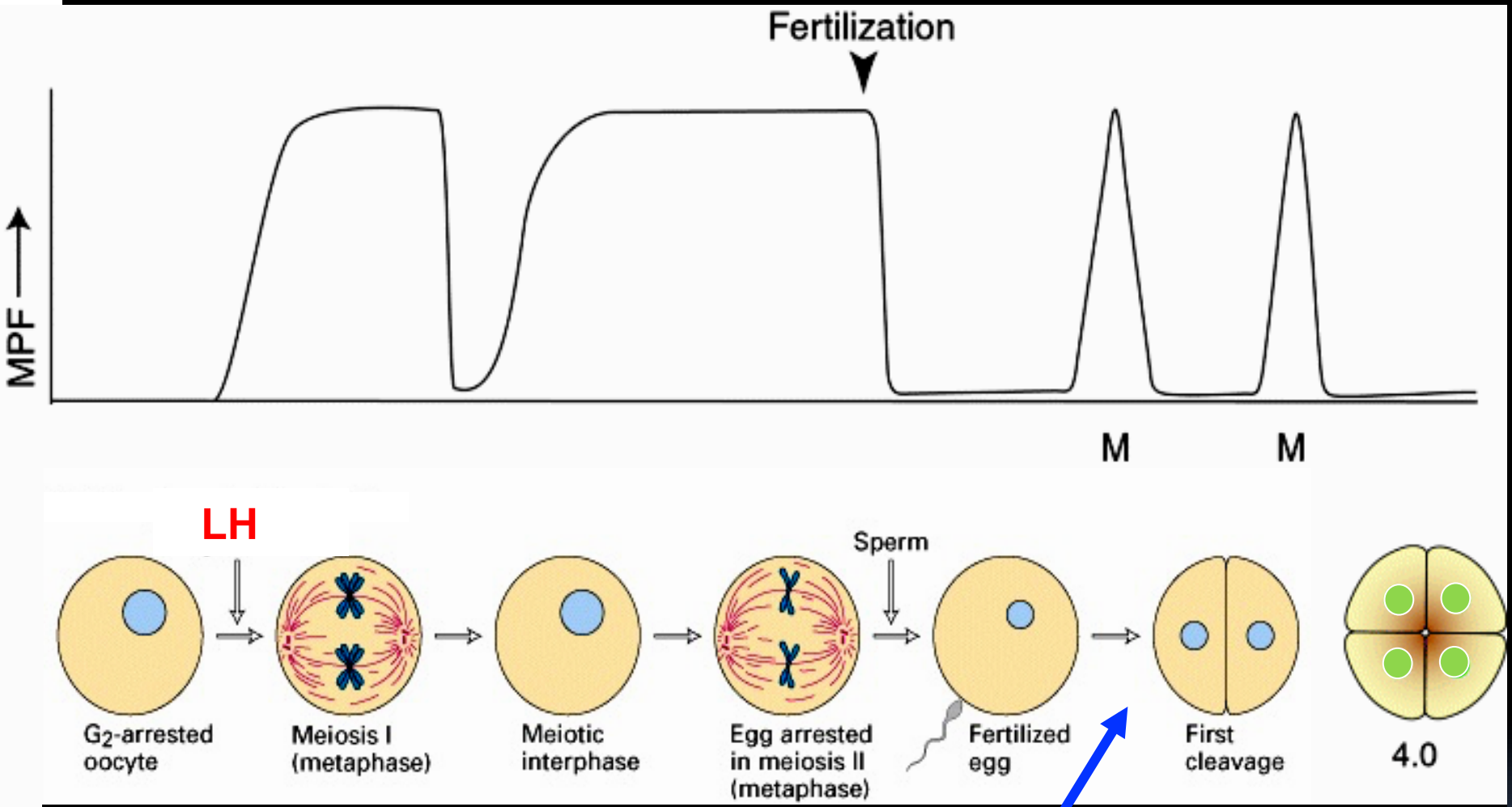
MII oocyte



TII oocyte



- Expulsion of the second polar body (PBII)
- The PBII contains sister chromatids



MPF Regulates Mitosis as well as Meiosis

Yoshio Masui, *Differentiation* (2001) 69:1-17

Lodish et al., Molecular Cell Biology