MPF and *Xenopus* oocyte maturation -
Old story, new concepts
**Prophase I**

- GVBD
  - MPF
  - 3-5 hrs

- Metaphase I
  - 0.5 hr
  - 1.5 hr

- Interkinesis

- Metaphase II
  - 0.5 hr

**Progesterone**

- G2 arrest

- G2-M transition

- M-phase arrest
crucial for sexual reproduction

crucial for embryonic development (ploidy of the embryo, proper localization of maternal determinants, nutritive store...)

interesting for understanding the function of cell cycle regulators

interesting for the understanding of steroid transduction pathways
prophase I or G2

GVBD

1.2mm in diameter

- microinjections (anti-sense oligos, proteins or mRNA -active, dominant-negative-, pharmacological inhibitors, etc…)

- biochemistry (thousands of oocytes, 30 μg of soluble proteins par cell)

- oocyte extracts recapitulating *in vitro* MPF activation (addition or depletion of proteins without limits)
1. How is Cdc2 activated?

2. How is DNA replication inhibited?

3. Fate of an unfertilized oocyte?

I: Transduction

II: Transition

III: Arrest in MII

Cyc B
Cdc2
Cdc2
Cyc B

Myt1

Cyc B
pre-MPF

Inactive
Active

MPF

Progesterone

Metaphase I

Metaphase II

Prophase I

GVBD
Which proteins?
1. Newly synthesized proteins

Protein synthesis

in intracellular membranes

\[ \text{cAMP} \]

\[ \text{PKA} \]

Protein synthesis

\[ \text{N} \text{os} \]

\[ \text{MEK} \]

\[ \text{MAPK} \]

\[ p90^{\text{Rsk}} \]

\[ \text{cdc2} \]

\[ \text{Cyclin B2} \]

Pre-MPF

inactive

MPF

active

\[ \text{Morpholinos} \]

\[ \text{GVBD} \]

Dupré et al. EMBO J. 2002
A: 25 mer Traditional Antisense

M: 25 mer Morpholino Antisense

Prophase I

RNase H

+ Pg?
GVBD time-course induced by progesterone in the presence of Mos antisense

- **Pg**
- **Pg/A⁻ (traditional antisense)**
- **Pg/M⁻ (morpholinos)**

Dupré et al. EMBO J. 2002
Mos morpholinos antisense prevent Mos synthesis and MAPK activation

1. Newly synthesized proteins

Dupré et al. EMBO J. 2002
Which proteins?

1. Newly synthesized proteins
1. Newly synthesized proteins

Myt1

inactive

active

Cyclin B2

inactive

active

cdc2

active

inactive

cdc25

active

inactive

cdc25
1. Newly synthesized proteins

- Intracellular membranes
  - cAMP
  - PKA
- Protein synthesis

- Cyclin B1
  - cdc2
  - Myt1
  - cdc25
- Cyclin B2
  - cdc2
  - Myt1
  - cdc25
- GVBD

- cdc2
- Myt1
- cdc25

Accumulation of cyclin B1 in response to progesterone is independent of Cdc2 activation

Frank-Vaillant et al. Mol. Biol. Cell 1999
Cdc2 activation does not require cyclin B synthesis

Hochegger et al. Development 2001
Haccard & Jessus EMBO Rep. 2006
1. Newly synthesized proteins

intracellular membranes

- cAMP
- PKA

protein synthesis

- Cyclin B1
- Mos
- MEK
- MAPK
- Rsk

inactive

CDC2

active

Cyclin B2
intracellular membranes

\[ \text{cAMP} \]

PKA

protein synthesis

\[ \text{Cyclin B1}, \text{Mos}, \text{MEK}, \text{MAPK}, \text{Rsk} \]

inactive

\[ \text{cdc2}^{\text{P}, \text{P}} \]

active

\[ \text{Cyclin B2} \]
1. Newly synthesized proteins

antisense oligos against B1, B2, B4 and B5 cyclins

protein synthesis

\( \text{cAMP} \) \( \rightarrow \) PKA

\( \rightarrow \) intracellular membranes

\( \rightarrow \) ?

\( \text{Mos} \) antisense morpholinos

\( \text{U0126} \)

\( \text{Rsk} \) MAPK MEK

\( \text{inactive} \) \( \text{Cyclin B2} \) \( \rightarrow \) \( \text{active} \) \( \text{Cyclin B2} \)
Activation of Cdc2 induced by progesterone requires the synthesis of either cyclin B or Mos

Haccard & Jessus EMBO Rep. 2006
Haccard & Jessus Cell Cycle 2006
Jessus & Haccard Nature 2007
Control CycB Oligos + Mos Morpholinos

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% of GVBD</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
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<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
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</tbody>
</table>

Cyclin B1 + Pg

Haccard & Jessus EMBO Rep. 2006
Haccard & Jessus Cell Cycle 2006
Jessus & Haccard Nature 2007

Cyclin restores Cdc2 activation

1. Newly synthesized proteins

- Histone H1
- P-Tyr Cdc2
- Cyclin B2
- Cyclin B1
- P-MAPK

○ prophase

+ Pg

- Control
- CycB Oligos + Mos Morpholinos

+ Cyclin A

- Control
- CycB Oligos + Mos Morpholinos
Mos restores Cdc2 activation

Haccard & Jessus EMBO Rep. 2006
Haccard & Jessus Cell Cycle 2006
Jessus & Haccard Nature 2007
Cyclin B1

protein synthesis

intracellular membranes

PKA

protein synthesis

Cyclin B1

High rate

cdc2

Cyclin B1

Myt1/cdc25

Cyclin B2

pre-MPF

Cyclin B2

MPF

Mos

MEK

MAPK

Rsk
1. Newly synthesized proteins

Stimulation by progesterone in the absence of MAPK activation

MAPK is not necessary for both hyperphosphorylation and inhibition of Myt1

Karaiskou *et al.* Development, in revision
Progesterone does not lead to Myt1 hyperphosphorylation in the absence of Cdc2 activity

Karaiskou et al. Development, in revision
Injection of cyclin B in the presence of an inhibitor of protein synthesis. Cdc25, Myt1, P-Tyr-Cdc2, P-MAPK, P-Plk1, Cyc B2, cycB.

Cdc2 kinase activity is sufficient for Myt1 hyperphosphorylation.

1. Newly synthesized proteins

Karaiskou et al. Development, in revision
Critical roles of Cyclin B2 in prophase arrest

- **Prophase arrest**
- **Synthesis (Slow rate)**

**Cyclin B2**

- **cdc2**
- **cdc2**

**inactive**

**pre-MPF**

**Myt1**

**Cdc25**

Karaiskou et al. Development, in revision
Cyclin B2

cdc2

pre-MPF

Cyclin B2

Cdc25

Cdc25

Myc1

Mos

MEK

MAPK

Rsk

PKA

protein synthesis

intracellular membranes


Karaiskou et al. Development in revision
2. How is DNA replication inhibited?
Initiation of DNA replication in somatic cells

G1

Replication origins

Pre-RC formation

Pre-RC activation

Initiation

ORC

Cdc6

MCMs

Cdc45

Cdc45

Cdt1

Cdc6

Cdc45

MCMs (helicase)
2. Inhibition of DNA replication

Control of DNA replication during *Xenopus* oogenesis

Prophase I: No replication

- ORC
- MCMs
- Cdt1

Metaphase I: Synthesis

- Cdc6

Metaphase II: Functional preRC

- Cdc6
- ORC
- MCMs

Inhibitory mechanism
2. Inhibition of DNA replication

In the absence of Mos, DNA replication occurs after GVBD.
2. Inhibition of DNA replication

Control of DNA replication during *Xenopus* oogenesis

- **Prophase I**: ORC, MCMs, Cdt1
  - No replication

- **Metaphase I**: Cdc6
  - Synthesis

- **Metaphase II**: Cdc6, MCMs, Cdt1
  - Functional preRC

Inhibitory mechanism:
- Mos
- MEK
- MAPK
- Rsk
Mos inhibits DNA replication in interphasic extracts
2. Inhibition of DNA replication

Mos prevents Cdc6 loading onto chromatin
Control of DNA replication during *Xenopus* oogenesis

**Prophase I**
- No replication
  - ORC
  - MCMs
  - Cdt1

**Metaphase I**
- Synthesis
  - Cdc6

**Metaphase II**
- No replication
  - ORC
  - MCMs

**2. Inhibition of DNA replication**
In the ovary

**Fate of the unfertilized oocyte?**

**Meiotic maturation**

3. Committing suicide

**In the ovary**

1. **prophase I**
2. **GVBD**
3. **metaphase I**
4. **metaphase II**
5. **block**

**Progesterone**

**Cyclin B**

**cdc2**
Morphological changes of the spontaneous death of unfertilized oocytes

- progesterone

+ progesterone

The mitochondrial pathway of apoptosis

DNA damage

Developmental program

Stress

Pro-apoptotic members of the Bcl-2 family

Anti-apoptotic members of the Bcl-2 family

Targets (>300)

Cytochrome C

APOPTOSOME

Cell death

Mitochondrial- and caspase-dependent apoptosis

Overexpression of Bcl-X\textsubscript{L} and Mcl-1 inhibit the oocyte death.

3. Committing suicide
Caspase inhibitors delay the oocyte death

Absence of fertilization

Anti-apoptotic members of the Bcl-2 family?

Pro-apoptotic members of the Bcl-2 family?

3. Committing suicide

Cytochrome C

APOPTOSOME

C2 C3 C6 C7 C8 C9

Targets

Oocyte death

The role of MPF and MAPK in the induction of egg apoptosis

The apoptotic death of unfertilized eggs depends on Cdk1.
Inhibition of JNK activity delays apoptosis

Bad is phosphorylated on Ser128 in a Cdc2- and JNK-dependent manner during meiotic maturation. Bad is kept inactive in prophase oocytes and acquires its pro-apoptotic capacity during meiotic maturation.

Bad and JNK play a positive role in the egg death process.

What could be the biological significance of this process?
Apoptosis of ovulated eggs in the *Xenopus* female body

3. Committing suicide

Team « Biology of the oocyte »

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