11th Postgraduate Course for Training in
Reproductive Medicine and Reproductive Biology

Fertilization

Françoise Urner 2001

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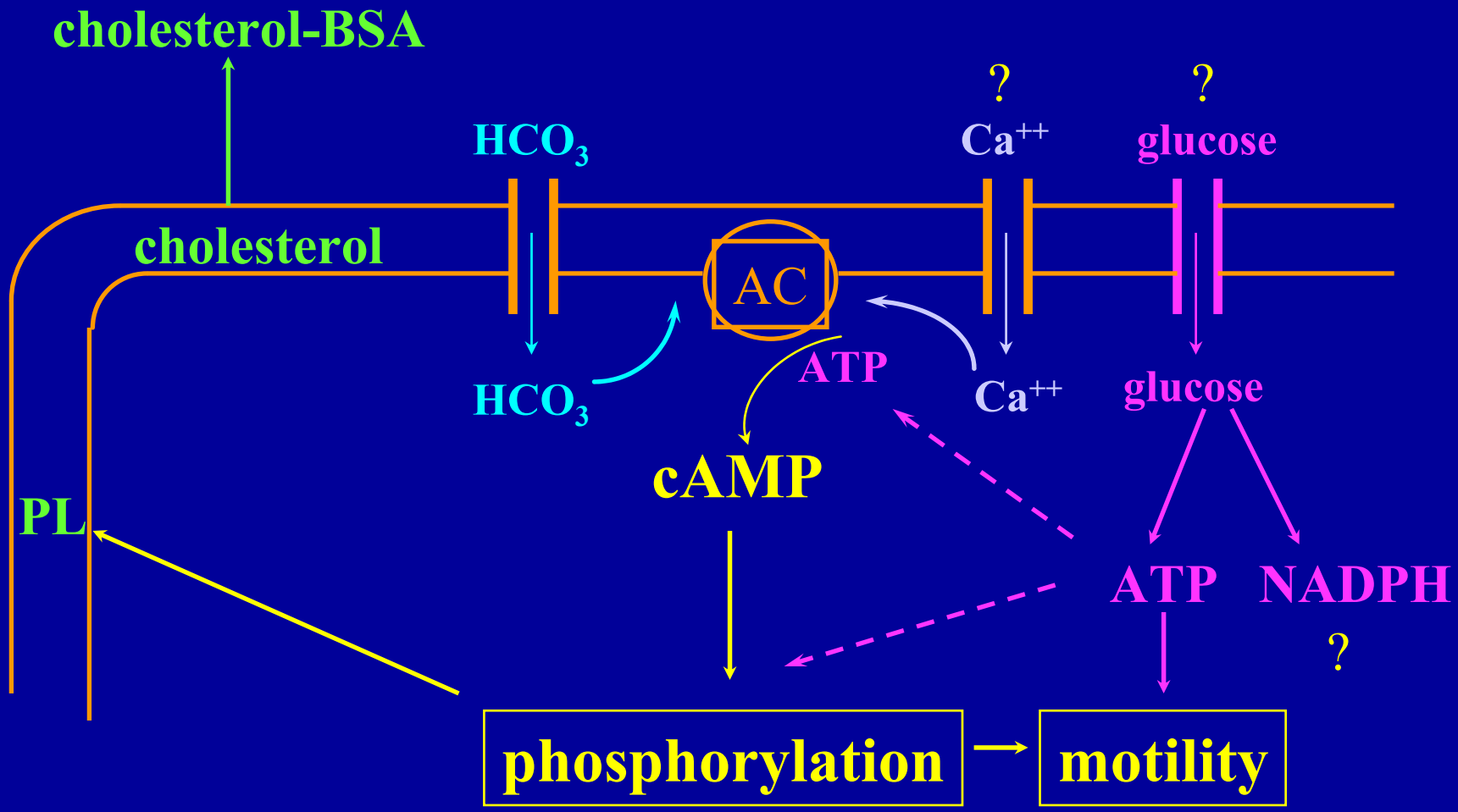
GAMETE MATURATION

Sperm capacitation

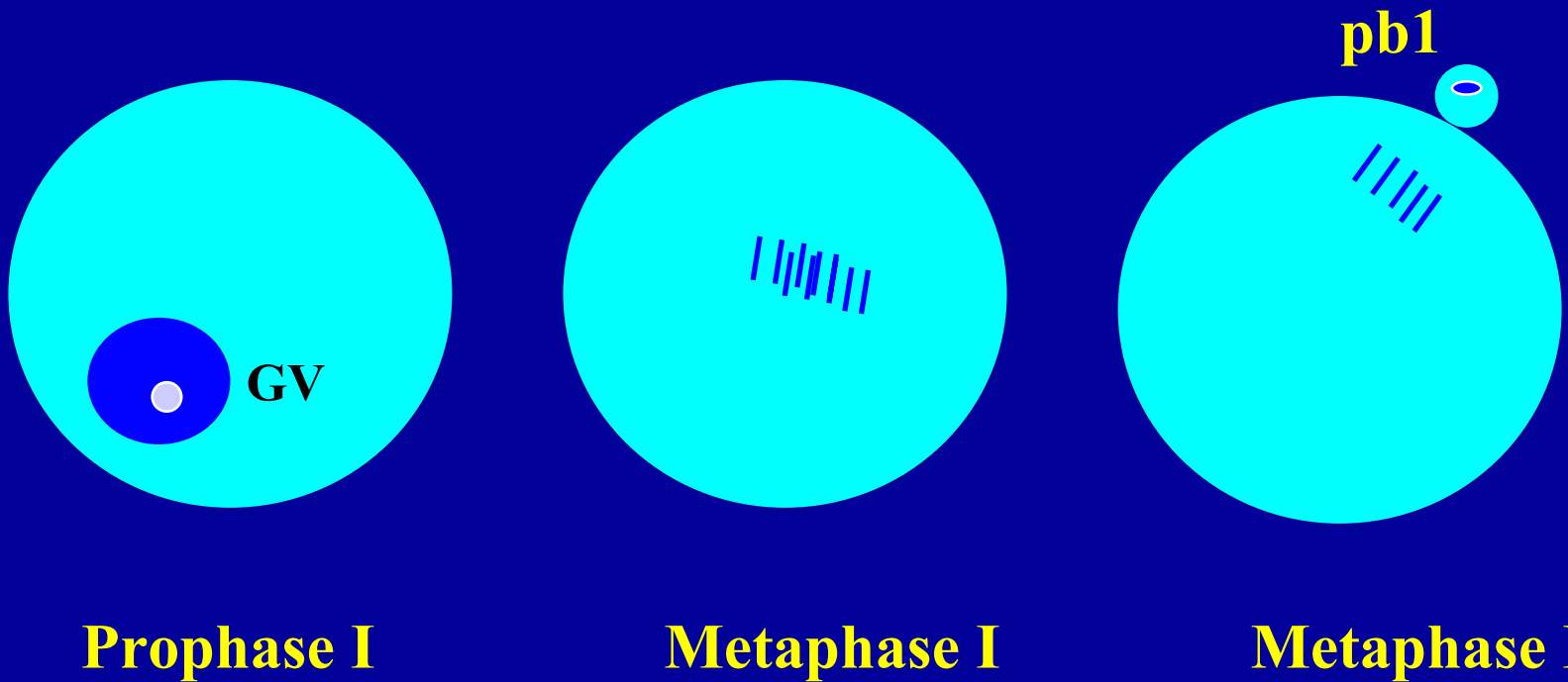
- Modifications of the sperm plasma membrane
- Metabolic changes
- Post-translational modifications of proteins
- Activation of motility

Signaling pathway involved in capacitation



Oocyte maturation

- Reinitiation of meiosis from prophase I to metaphase II
- Depends on the preovulatory surge of LH



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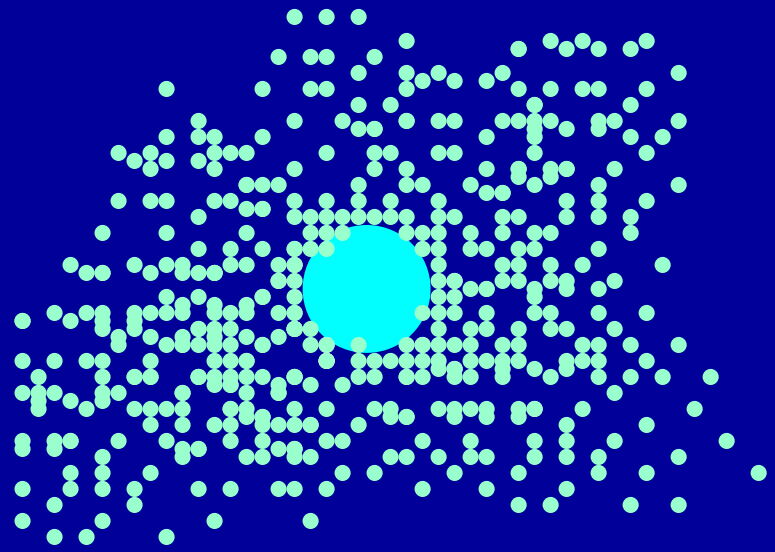
SPERM PENETRATION OF CUMULUS CELL COMPLEX

Cumulus cell complex

- Cumulus cells : somatic cells surronding the oocyte and embedded in a extracellular matrix containing hyaluronic acid



- Only capacitated and acrosome-intact sperm penetrate the cumulus complex to reach the oocyte

- Forward mobility is required for penetration

- Hyaluronidase activity of sperm surface molecules may participate in sperm progression

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SPERM INTERACTION WITH THE ZONA PELLUCIDA

Zona pellucida

- Glycoprotein coat surronding the oocyte
- Initial binding of sperm to the oocyte
- Barrier to interspecies fertilization
- Barrier to polyspermy

Binding of sperm to the zona pellucida

Primary binding

- **Binding of capacitated and acrosome-intact sperm to ZP3 (oligosaccharide moiety)**
- **Specifics receptors are present in the sperm plasma membrane, but many different molecules have been proposed as candidate receptors**

Secondary binding

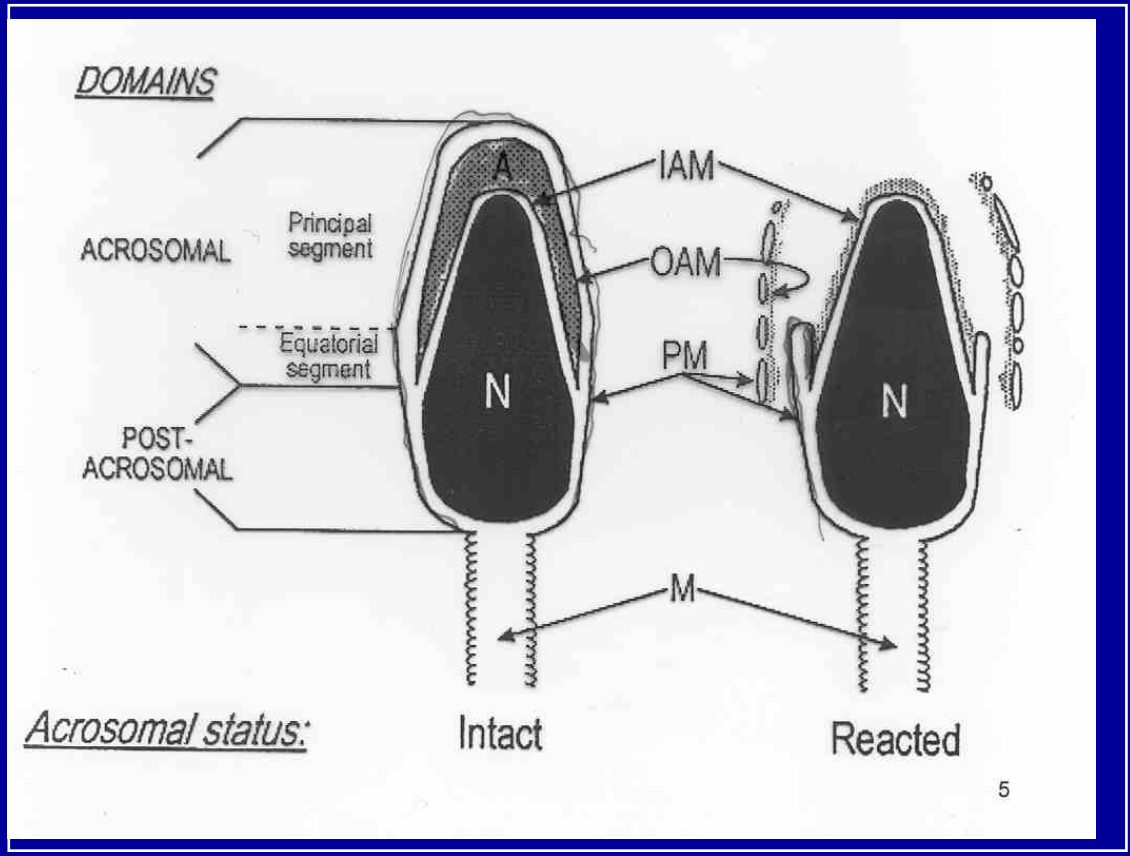
- **Binding of sperm to ZP2 following binding to ZP3 and acrosome reaction**
- **Specific receptors are probably present onthe inner acrosomal membrane of the acrosome-reacted sperm**

Acrosome reaction

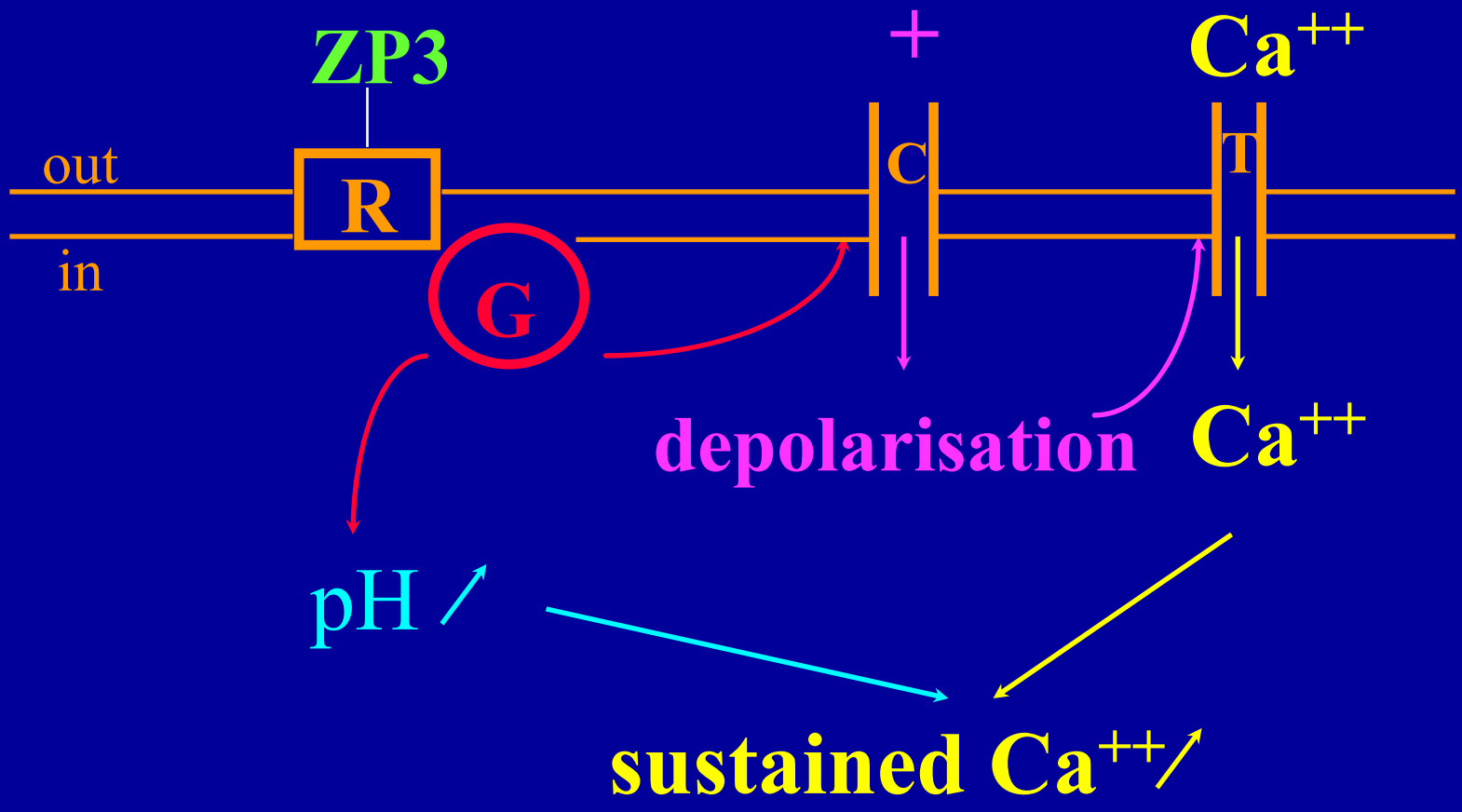
- Triggered in sperm bound to ZP3
- Calcium-dependent event
- Essential for sperm penetration of the zona pellucida and sperm fusion with the oocyte plasma membrane

Acrosomal exocytosis

- Fusion between the plasma membrane (PM) and the outer acrosomal membrane (OAM)
- Exposure of the inner acrosomal membrane (IAM)
- Release of the acrosomal content (acrosin)
- Appearance of the equatorial segment (ES)



Calcium signaling pathway involved in acrosome reaction



Penetration of the zona pellucida

- Strong motility is required (hyperactivated motility)
- Enzymatic digestion of the zona pellucida by acrosin facilitates penetration

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SPERM PENETRATION INTO THE OOCYTE

Sperm binding to the oolemma and fusion

- Gamete binding occurs between the equatorial segment of the sperm and the microvilli of the oocyte surface

- Specific surface molecules have been proposed to be involved in binding of the gametes

- Following binding, sperm fusogenic molecules promote fusion between the plasma membranes of the equatorial segment and the microvilli

- Subsequent incorporation of the whole sperm occurs as a phagocytosis-like process

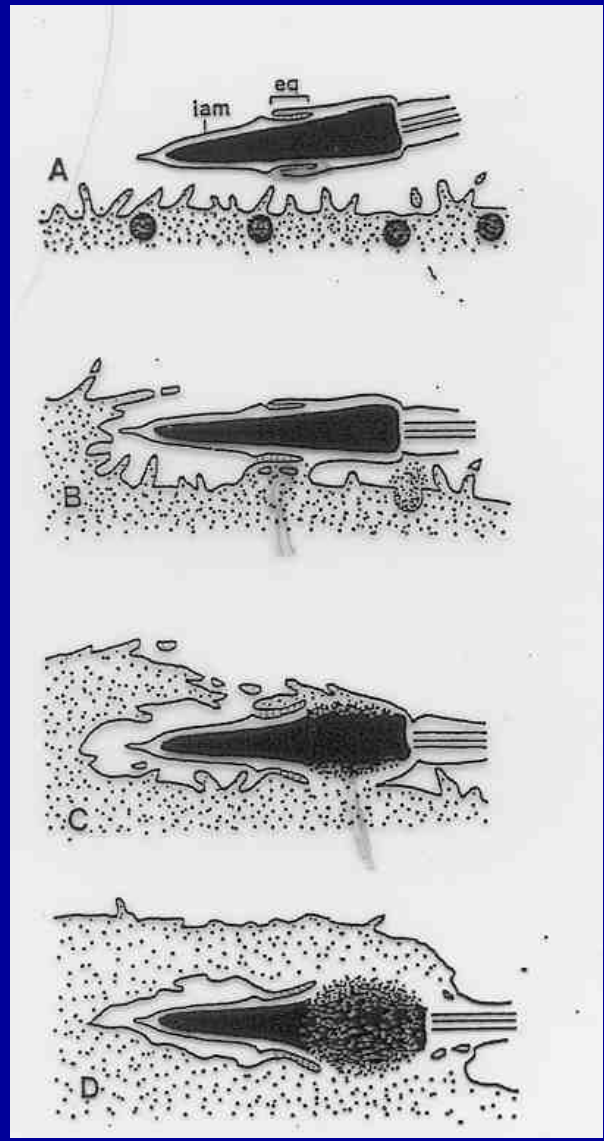
Gamete fusion

A: gamete binding

B: membrane fusion and pore formation

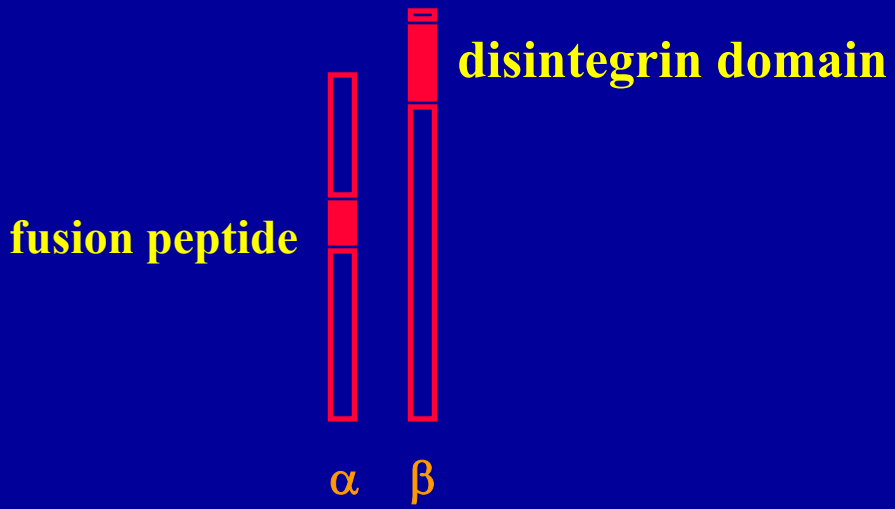
C: initiation of chromatin decondensation

D: incorporation of the head by phagocytosis

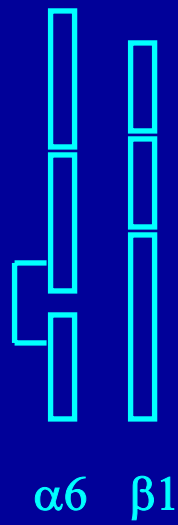


Fusion mediated by fertilin-integrin interaction

sperm: fertilin



oocyte: integrin



- Binding of fertilin (disintegrin domain) to integrin
- Conformational change of the α and β subunits
- Exposure of the fusion peptide (α subunit)
- Insertion of the fusion peptide in the oocyte plasma membrane
- Fusion of the plasma membranes

Sperm incorporation in the oocyte

- Nucleus → paternal genome
- Centrosome → migration of the pronuclei + organisation of the mitotic spindle of the first cleavage
- Cytoplasmcontains factors involved in oocyte activation (?)
- Mitochondria are present during the first embryo cleavages
- Tail (axonemal microtubules, fibrous sheat) is present during the first embryo cleavages

Sperm chromatin remodeling in the oocyte

- Removal of sperm nuclear envelope
- Reduction of disulfide bonds of protamines by glutathione
- Initiation of sperm chromatin decondensation
- Removal of protamines and replacement with maternal histones
- Chromatin recondensation
- Chromatin decondensation ⇨ male pronucleus formation

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OOCYTE ACTIVATION

Oocyte activation

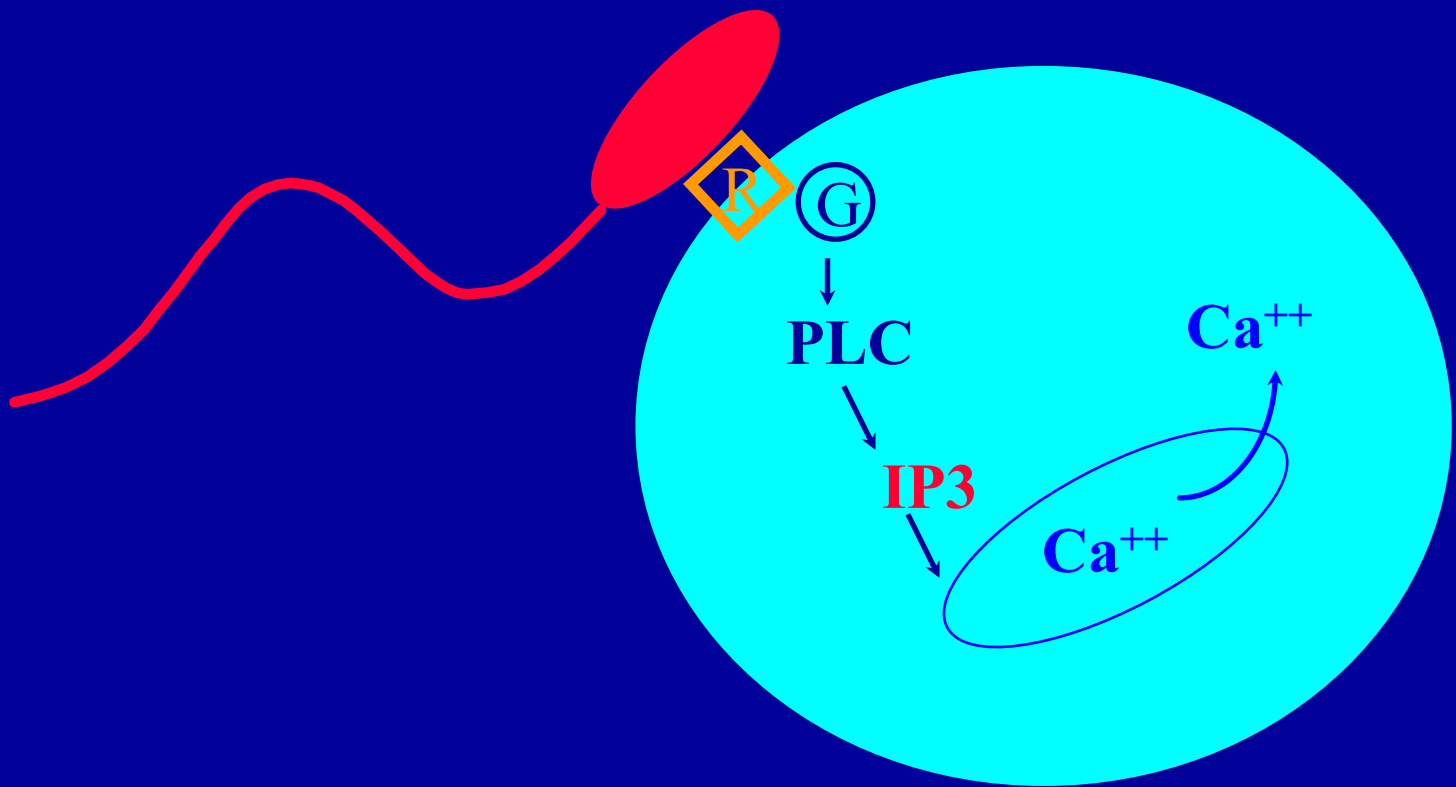
- Sequence of events triggered by the fertilizing sperm in the oocyte and that are required to initiate embryonic development

- Repetitive calcium rises in the oocyte plays a central role in triggering activation

- Activation events include: cortical granules exocytosis, resumption of meiosis, pronucleus formation, DNA synthesis, first cleavage

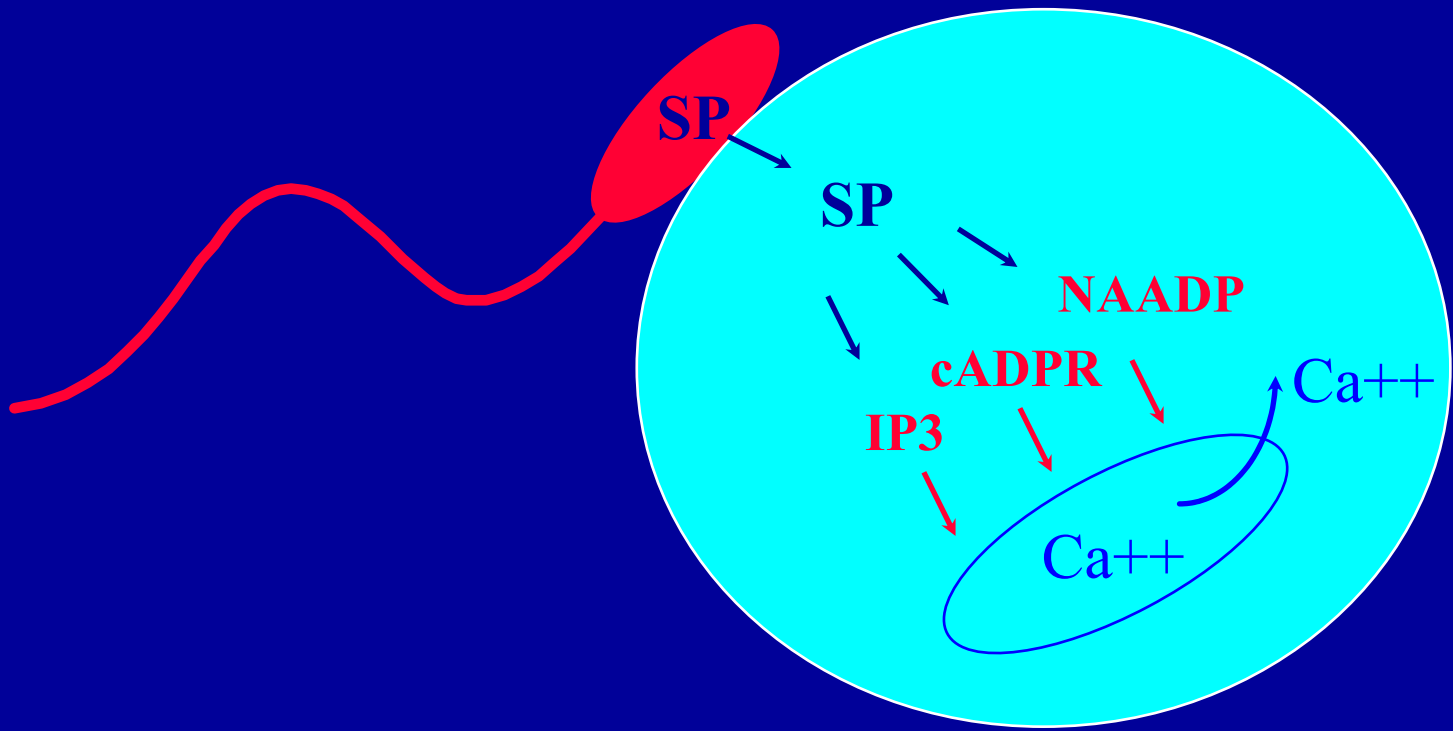
Calcium signaling pathway: receptor hypothesis

- The sperm bound to the oolemma activates a signal transduction pathway leading to a rise in calcium
- Activation is triggered prior to gamete fusion



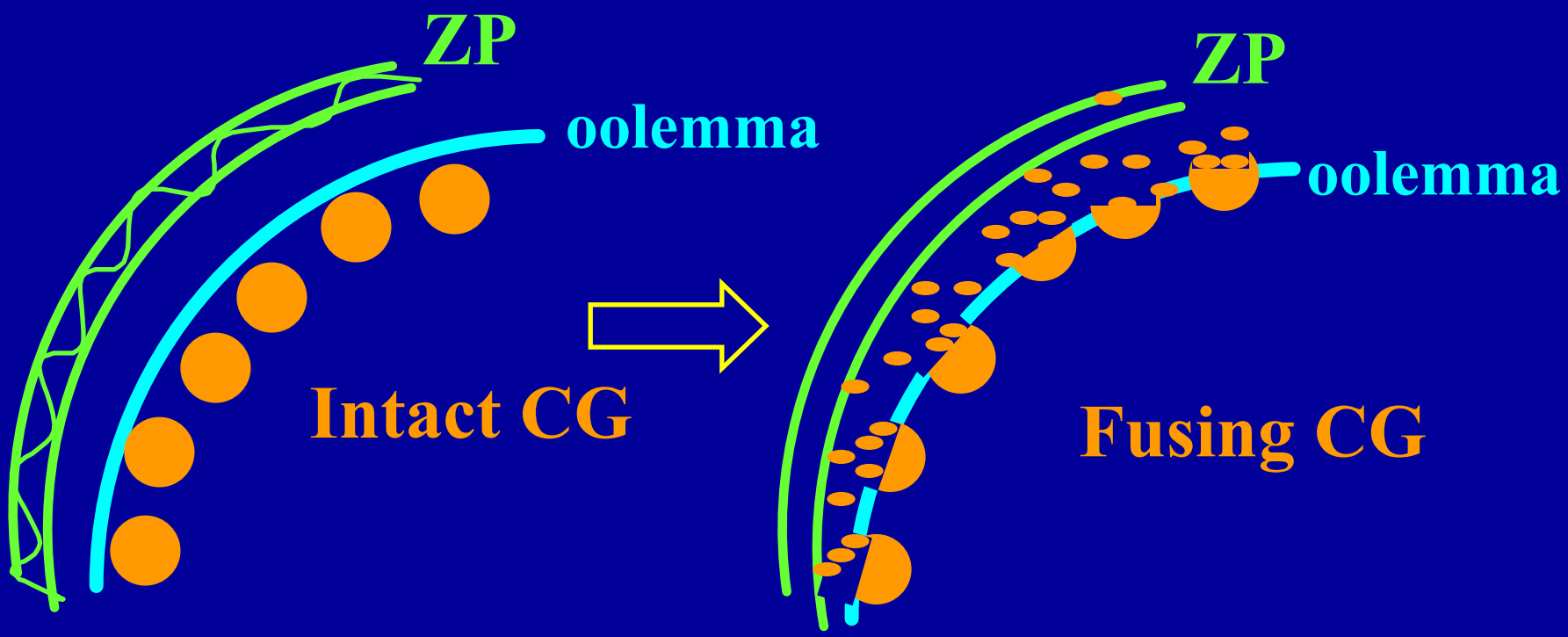
Calcium signaling pathway: sperm factor hypothesis

- Activation is initiated by a sperm factor introduced into the ooplasm following gamete fusion and responsible for a rise in calcium
- Sperm factor: oscillin, Phospholipase C, SOAF ??



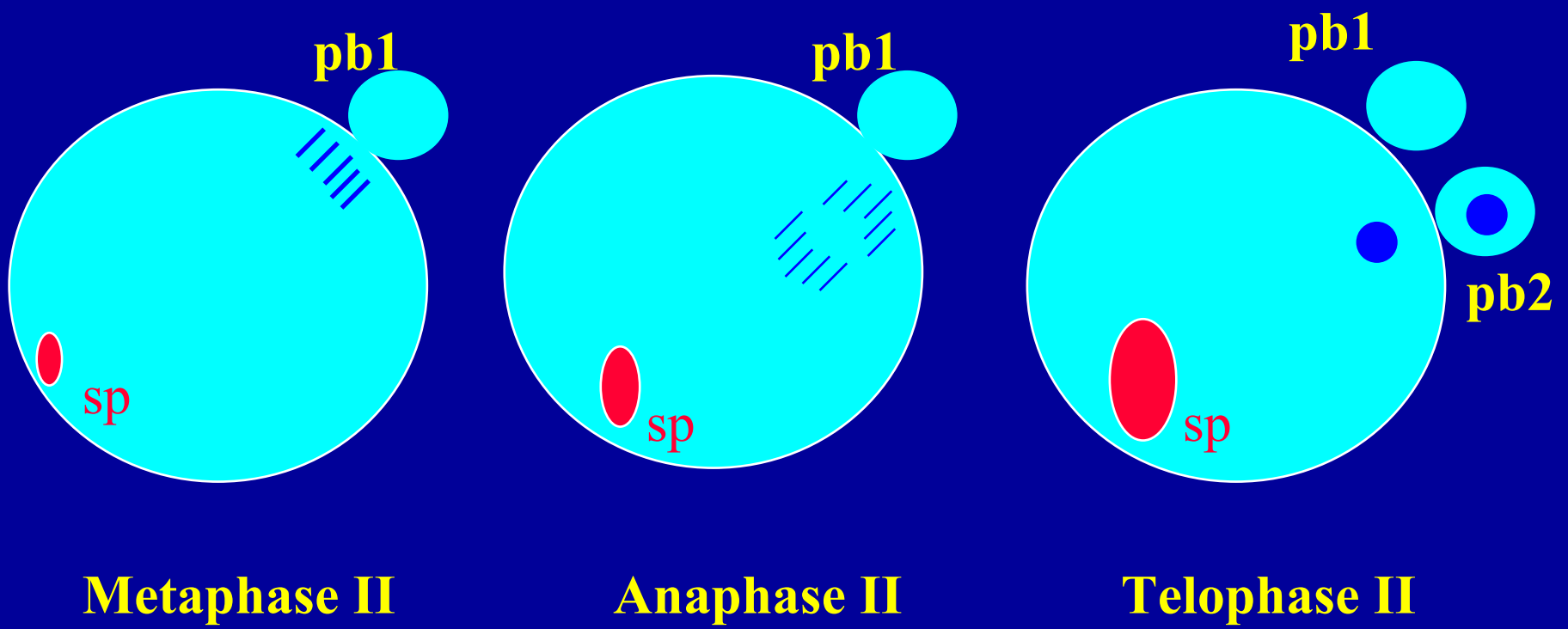
Exocytosis of cortical granules

- The cortical granules fuse with the oolemma and release their content into the perivitelline space to modify the ZP
- Modification of the zona pellucida prevents polyspermia



Resumption of meiosis

- Occurs during sperm chromatin decondensation
- Meiosis arrested at the metaphase II stage is reinitiated to the telophase II stage



Pronuclei

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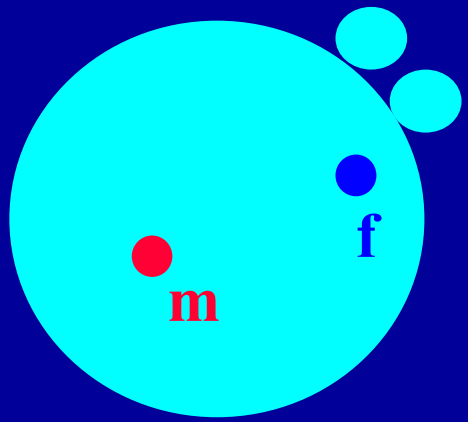

• Pronuclear envelope

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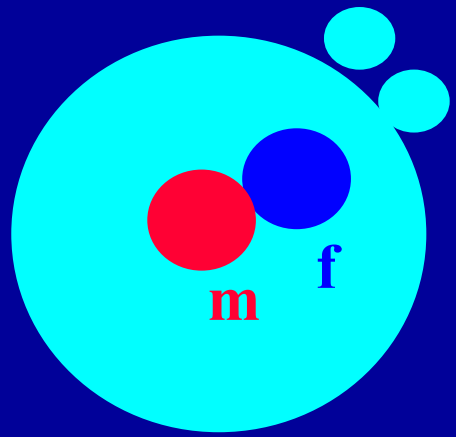

•Apposition of the pronuclei

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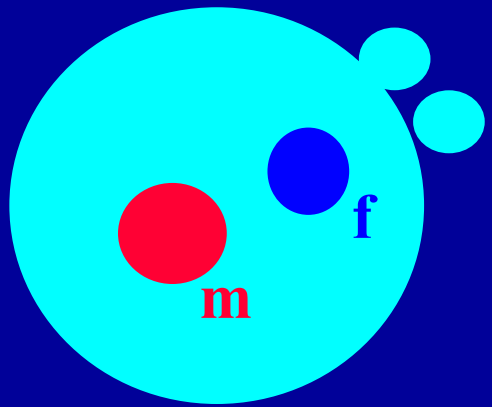

• Enlargement + migration of the pronuclei
•DNA replication

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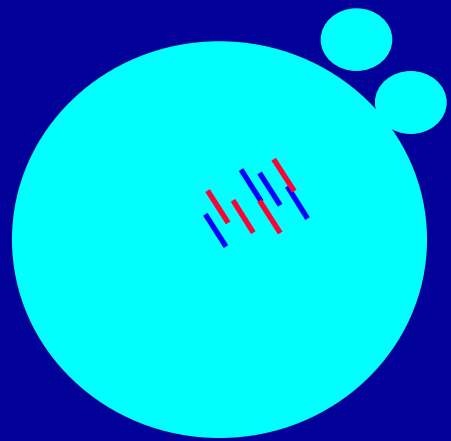

• Chromosomes condensation

Summary

- Maturation of the gametes (capacitation, meiotic maturation)
- Sperm penetration of the cumulus cell complex
- Binding of sperm to the zona pellucida
- Sperm acrosome reaction
- Zona pellucida penetration
- Binding of sperm to the oolemma (oocyte activation ?)
- Sperm fusion with the oolemma (oocyte activation?)
- Sperm chromatin decondensation
- Resumption of meiosis
- Pronuclei formation
- DNA synthesis in both pronuclei
- Association of paternal and maternal chromosomes
- First cleavage