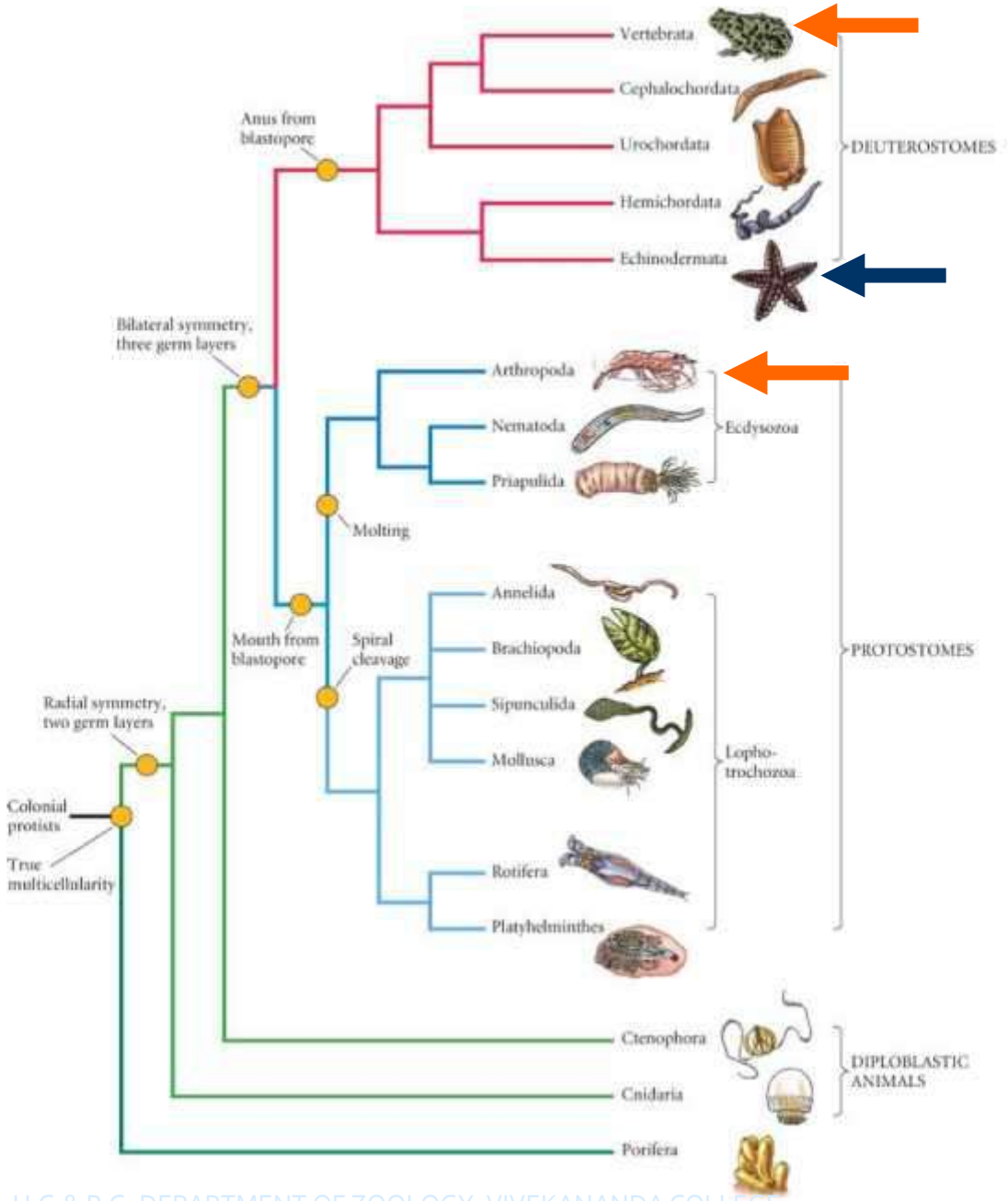
VIEVKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED A GRADE

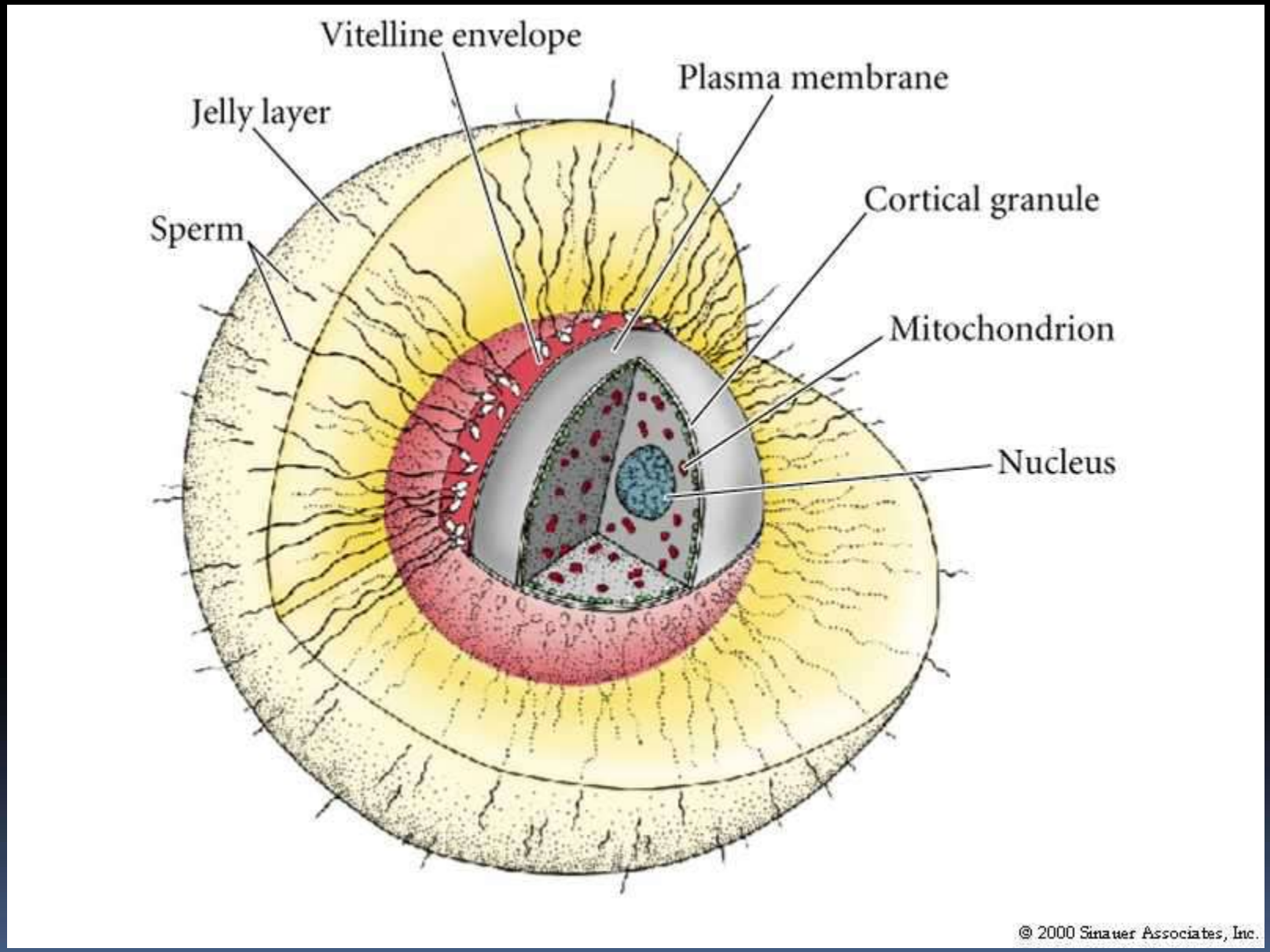
- TOPIC :- Early Embryonic Development
- COURSE TITLE:- CC2 Comp anatomy Devbio
- PAPER :- ZOOG CC2-2- TH
- SEMESTER :- 2 CC2 GE-2
- UNIT:- 6 FERTILIZATION
- NAME OF THE TEACHER:- DR. TRIJIT NANDA
- NAME OF THE DEPARTMENT:- ZOOLOGY

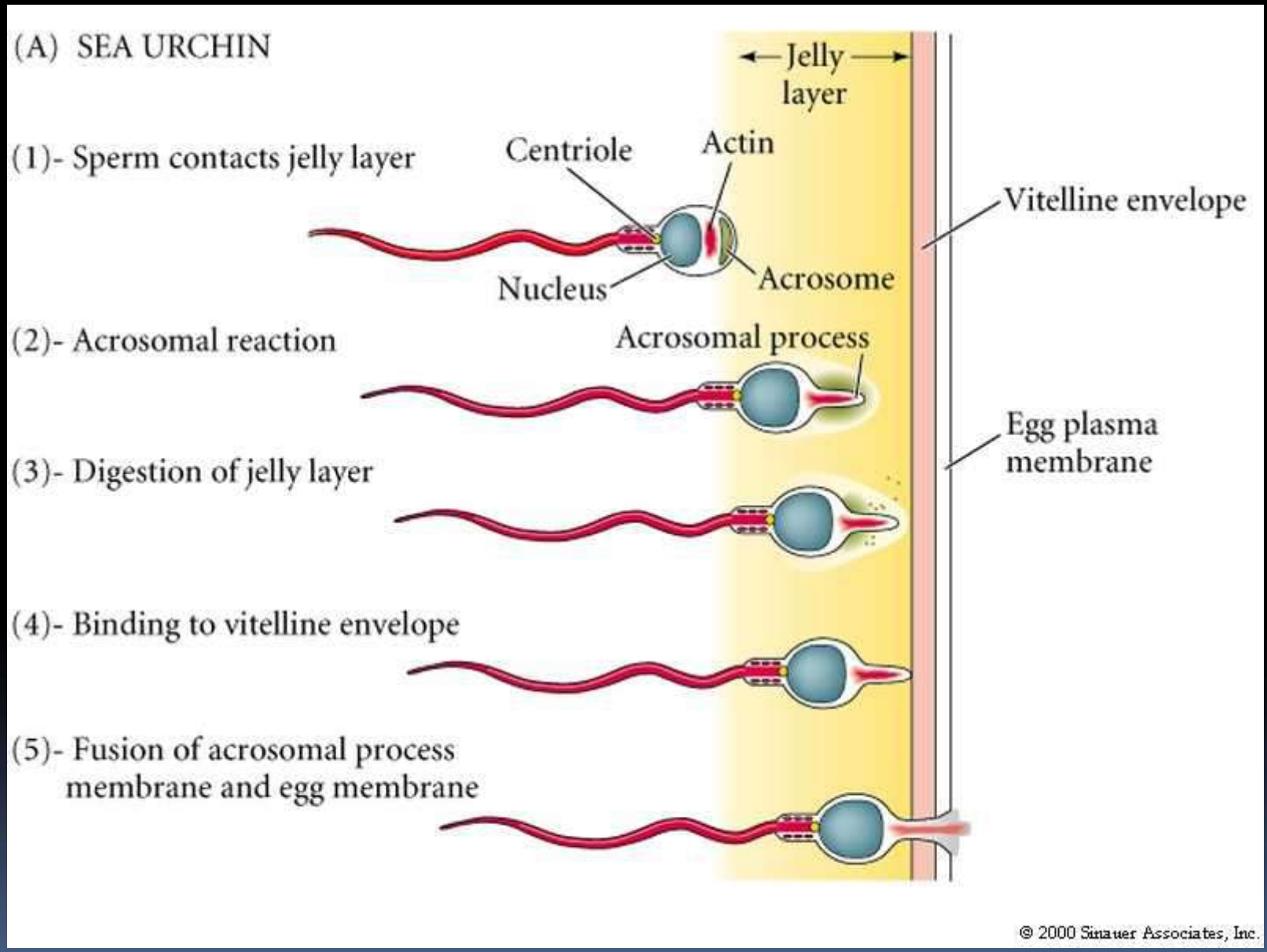
Animals



Attraction of sea urchin sperm to egg in Sea water:

Swimming along the gradient of the *RESACT* peptide


Vitelline envelope
Plasma membrane
Jelly layer
Cortical granule
Sperm
Mitochondrion
Nucleus
© 2000 Sinauer Associates, Inc.


(A) SEA URCHIN
Jelly layer
(1)- Sperm contacts jelly layer
Centriole
Actin
Vitelline envelope
Nucleus
Acrosome
(2)- Acrosomal reaction
Acrosomal process
Egg plasma membrane
(3)- Digestion of jelly layer
(4)- Binding to vitelline envelope
(5)- Fusion of acrosomal process membrane and egg membrane
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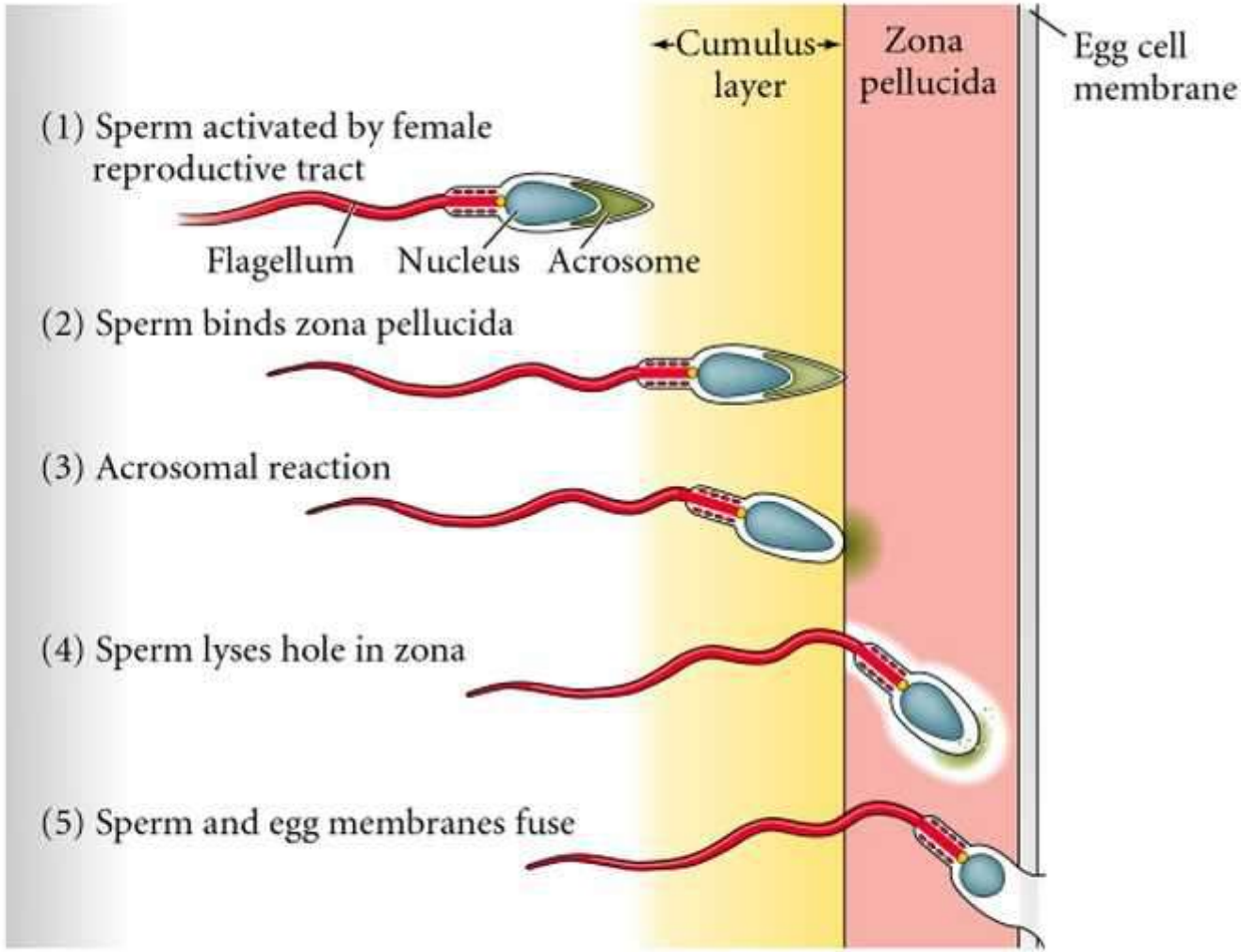
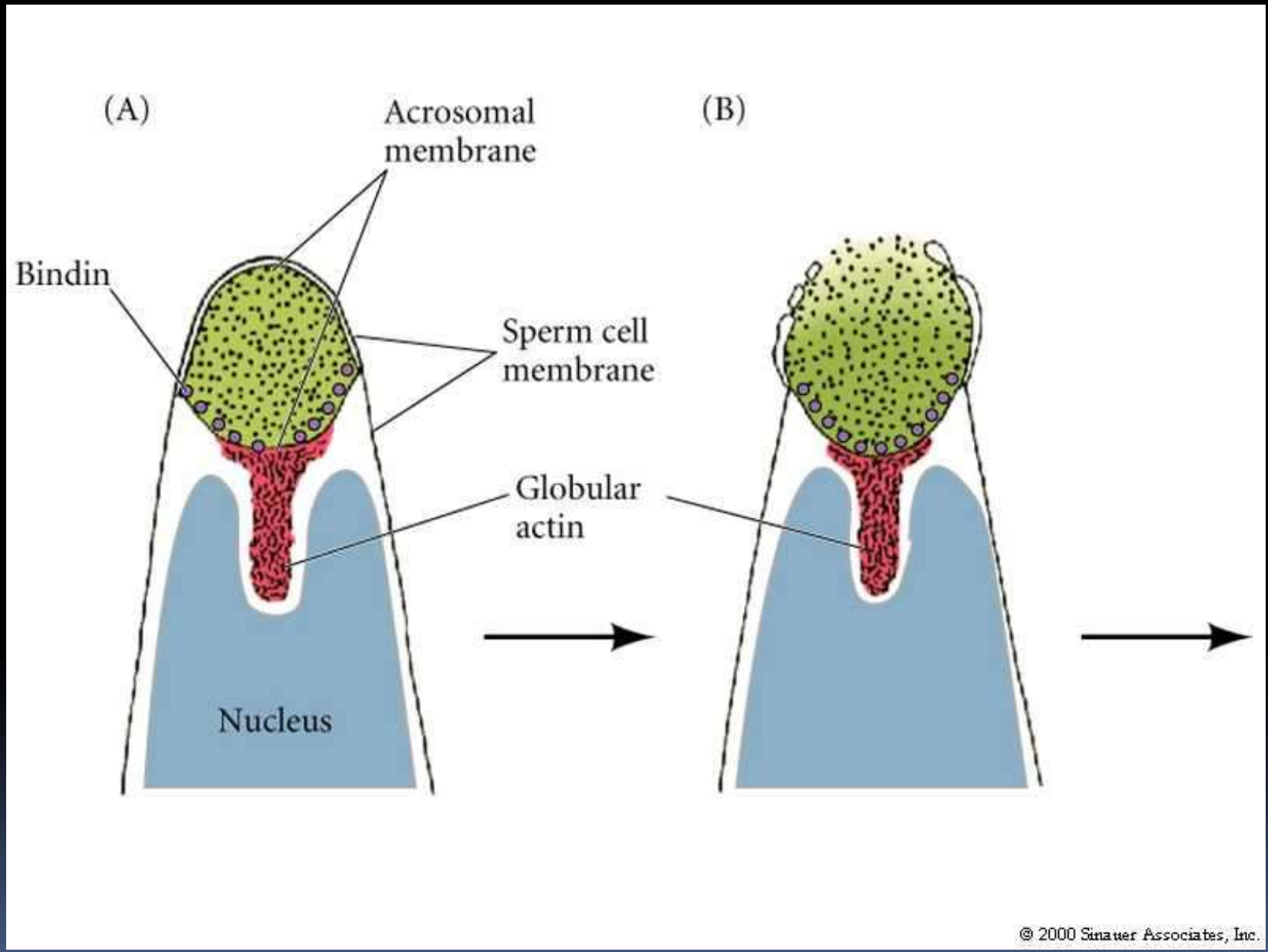
(B) MOUSE




(A)
Acrosomal membrane
(B)
Bindin
Sperm cell membrane
Globular actin
Nucleus
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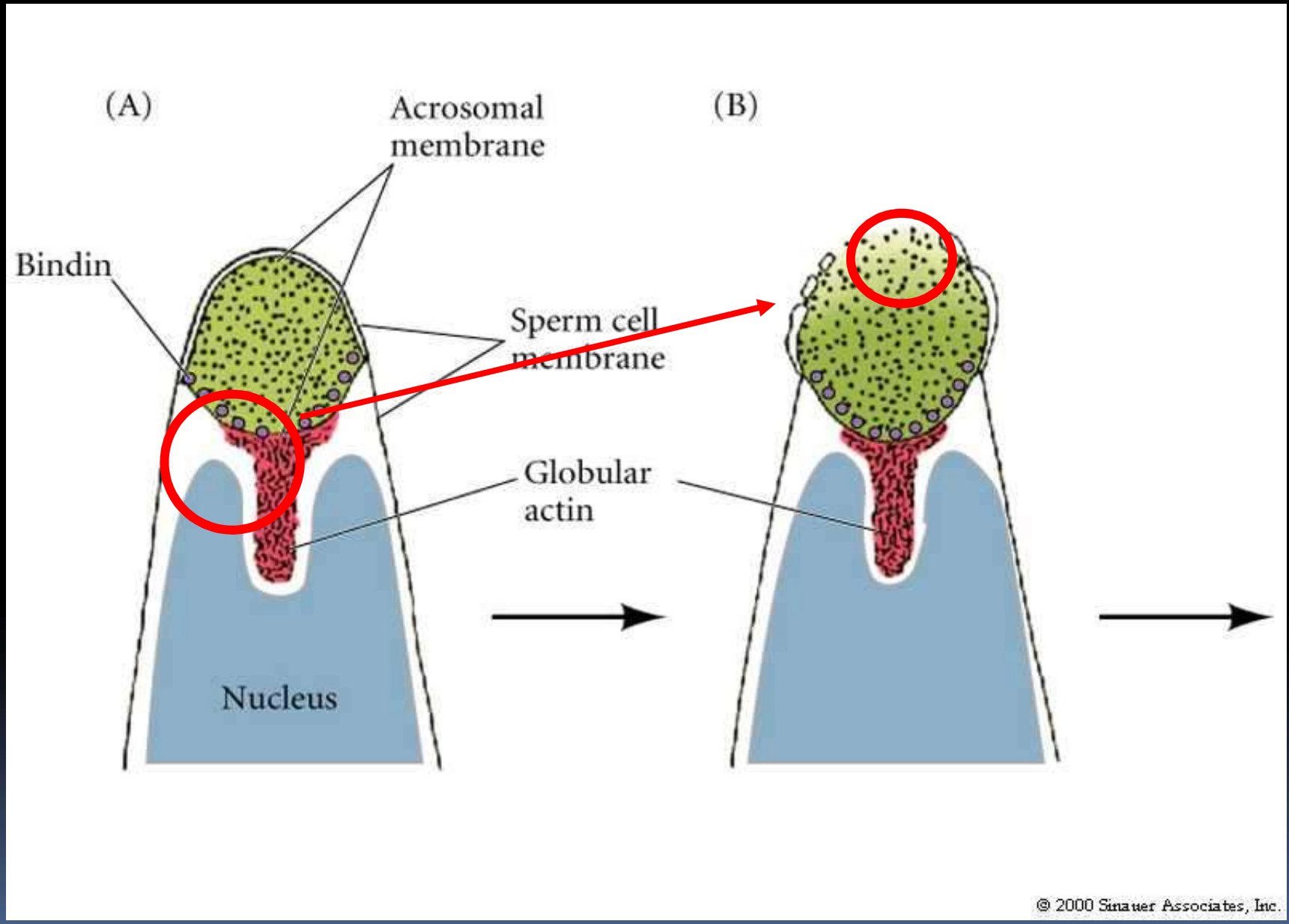
(A)
Acrosomal membrane
(B)
Bindin
Sperm cell membrane
Globular actin
Nucleus
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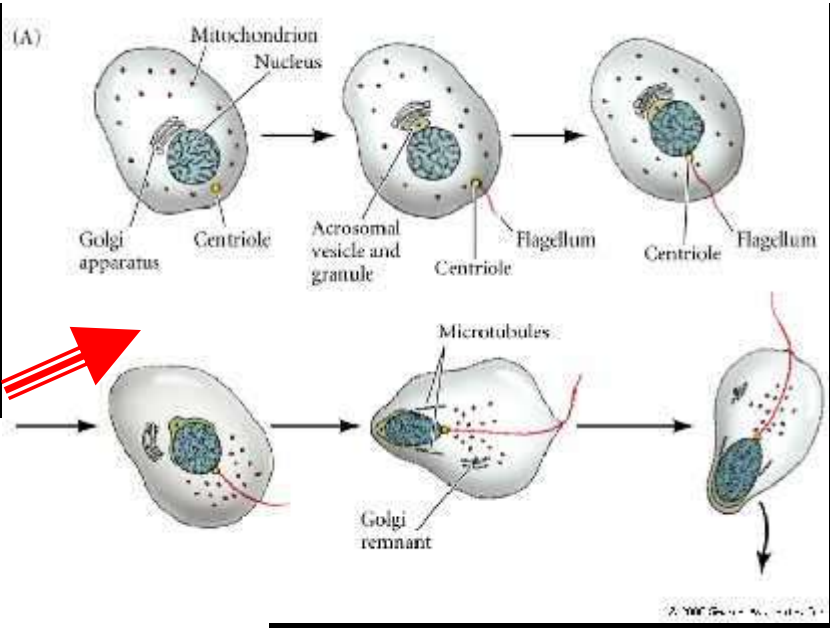
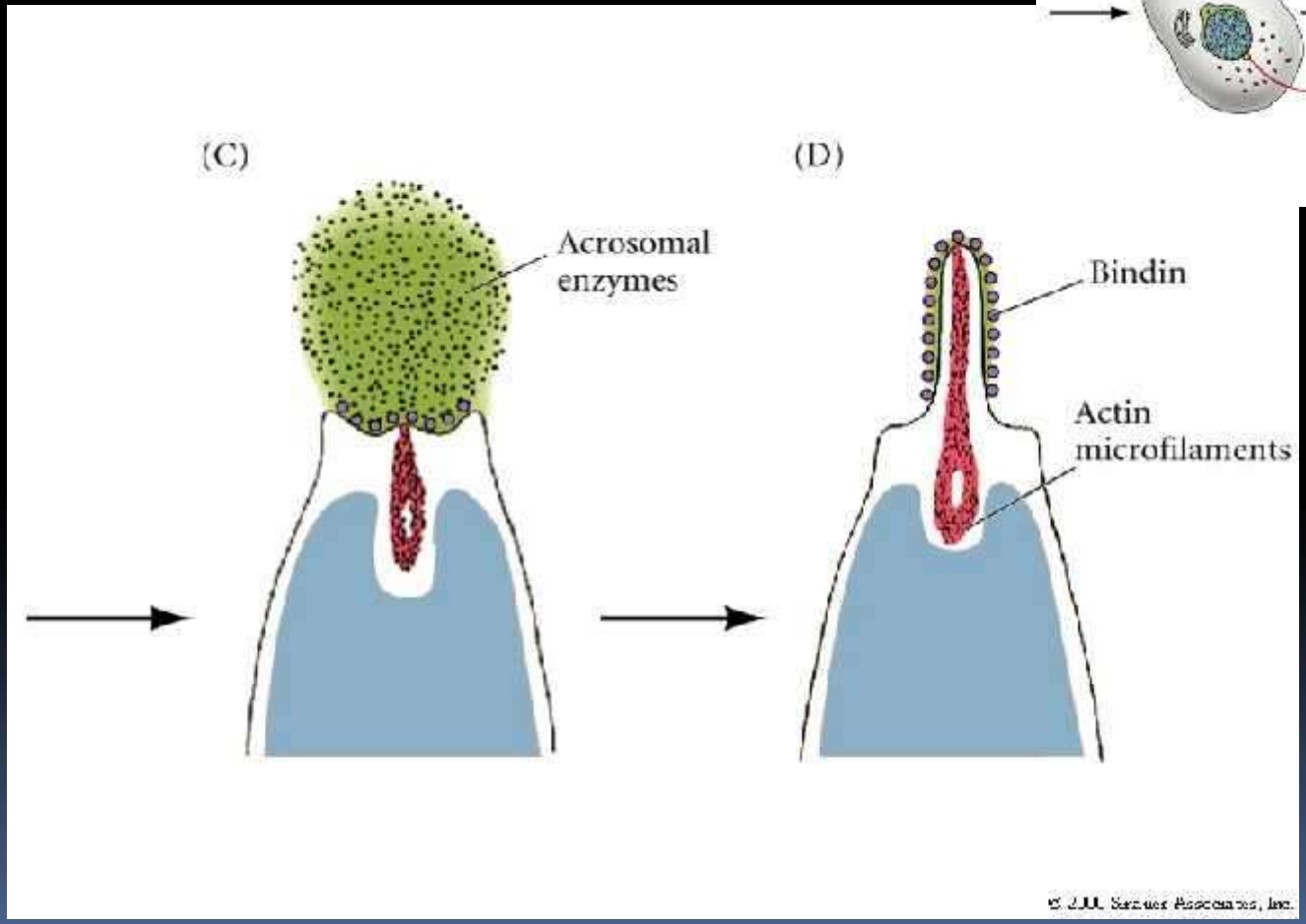
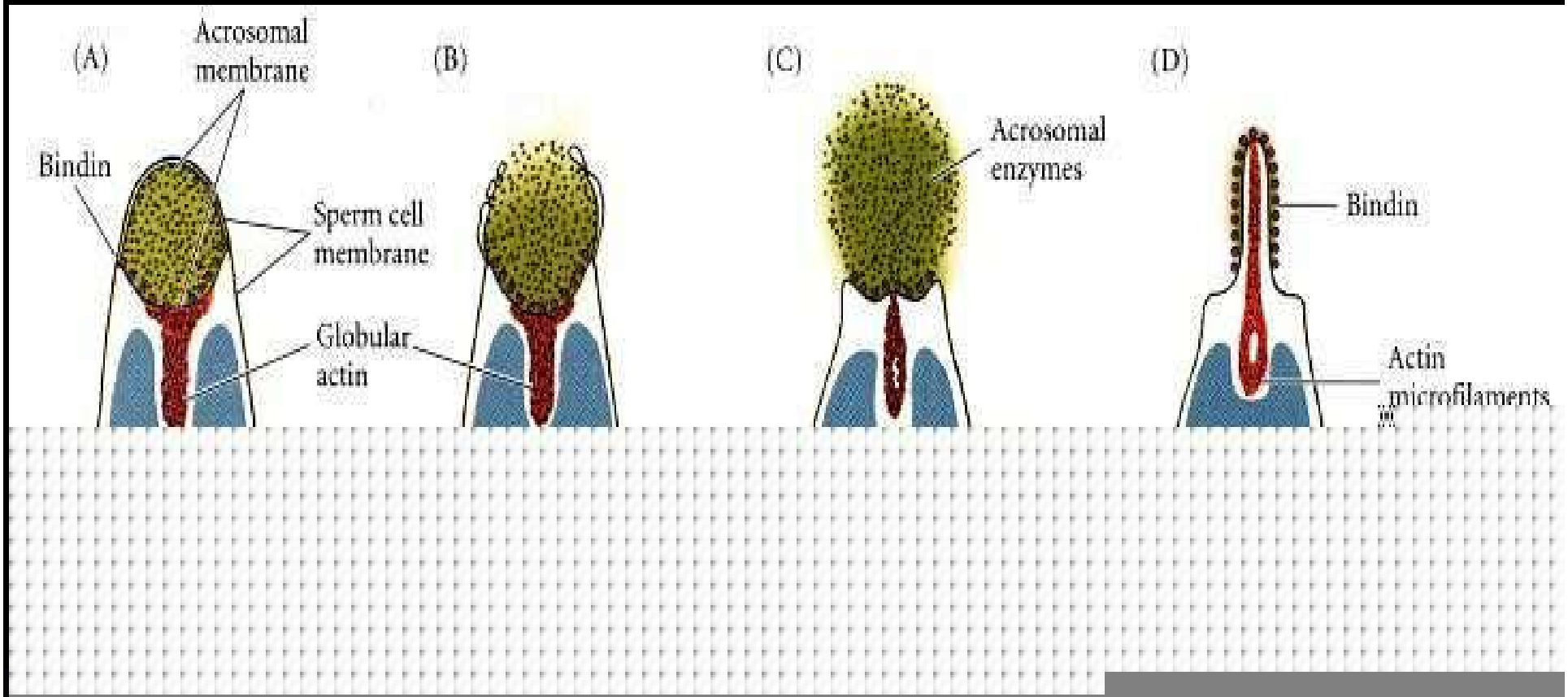
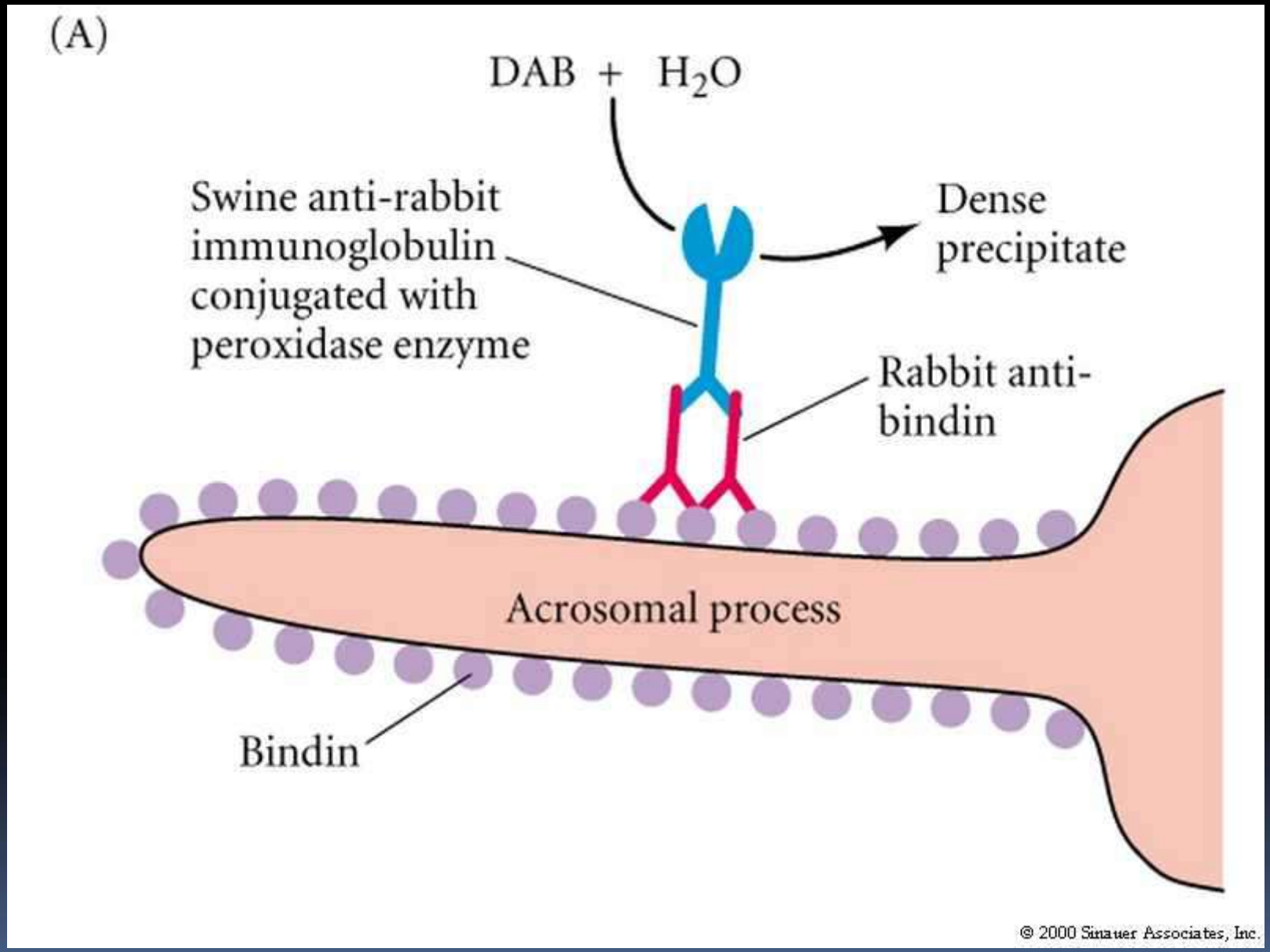

(A)
Mitochondrion
Nucleus
Golgi apparatus
Centriole
Acrosomal vesicle and granule
Centriole
Flagellum
Centriole
Flagellum
Microtubules
Golgi remnant


(C)
Acrosomal enzymes
(D)
Bindin
Actin microfilaments


(A)
Acrosomal membrane
Bindin
Sperm cell membrane
Globular actin
(B)
(C)
Acrosomal enzymes
(D)
Bindin
Actin microfilaments


(A)
DAB + H_2O
Swine anti-rabbit immunoglobulin conjugated with peroxidase enzyme
Dense precipitate
Rabbit anti-bindin
Acrosomal process
Bindin
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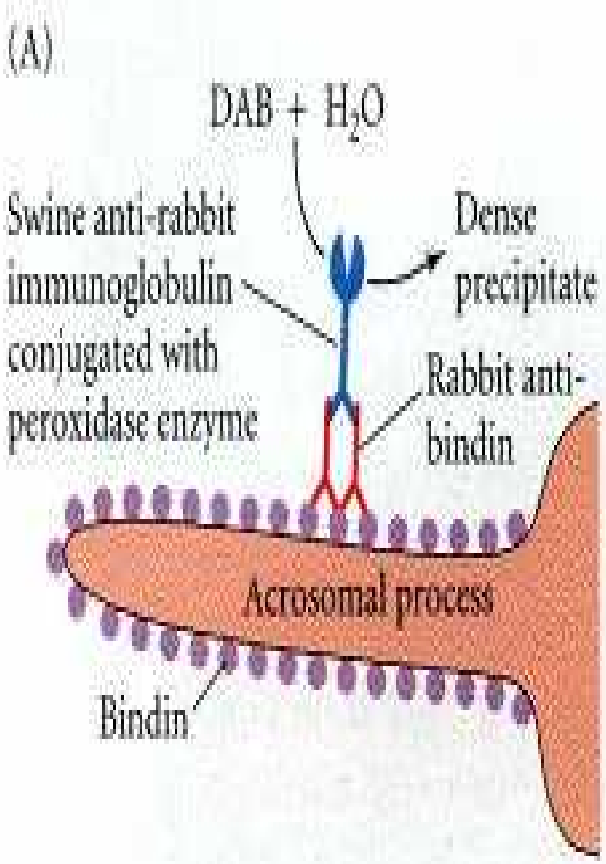
(A)
DAB + H_2O
Swine anti-rabbit immunoglobulin conjugated with peroxidase enzyme
Dense precipitate
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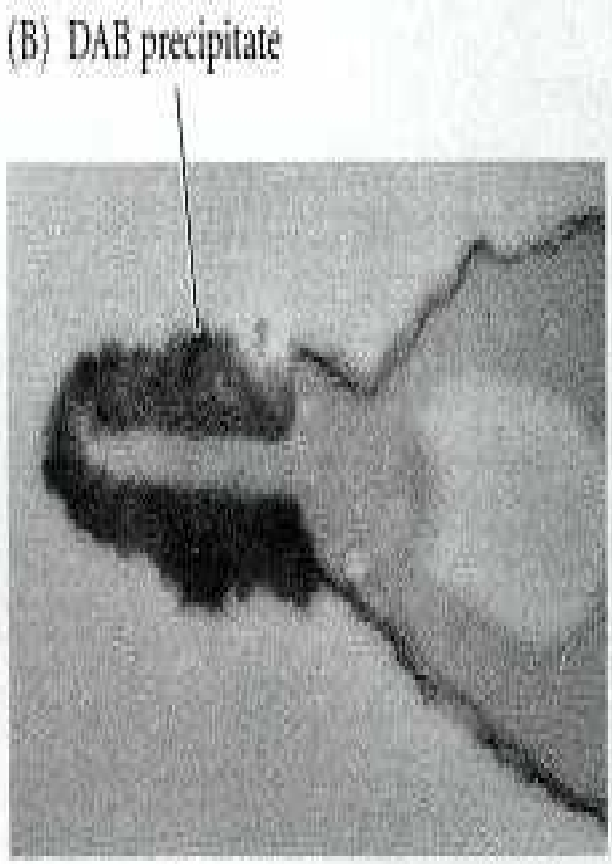
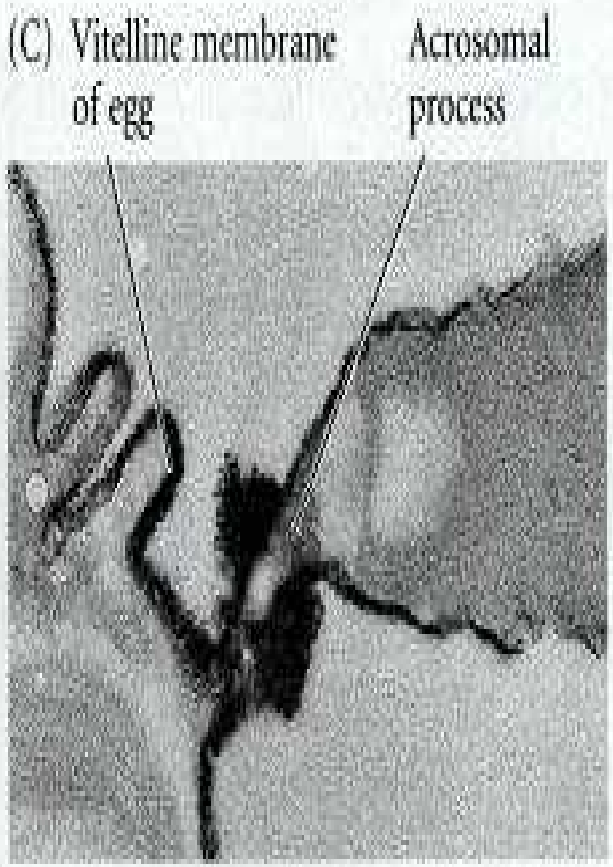
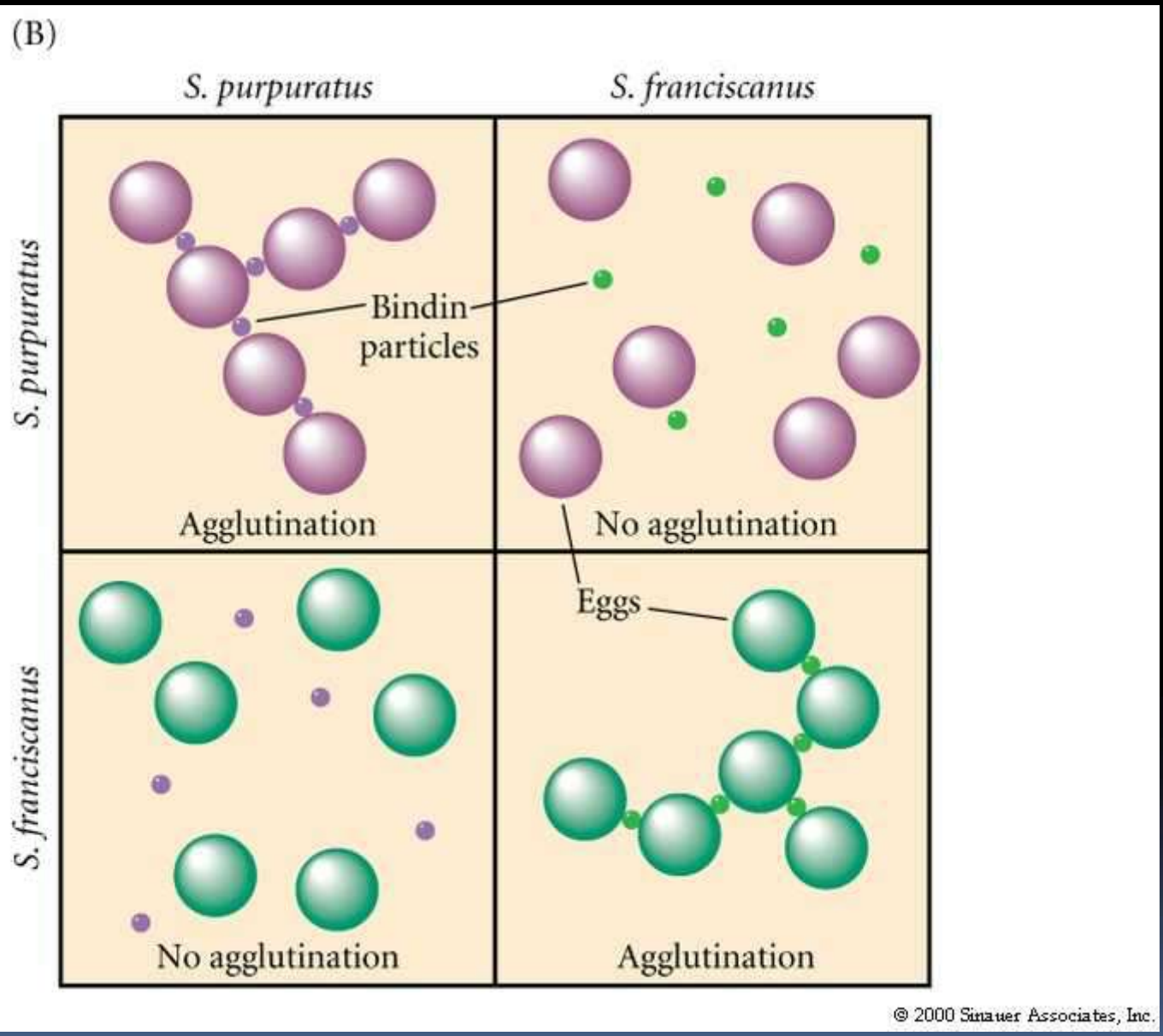

(B) DAB precipitate


(C) Vitelline membrane of egg
Acrosomal process


(B)
S. purpuratus
S. franciscanus
S. purpuratus
S. franciscanus
Bindin particles
Agglutination
No agglutination
Eggs
No agglutination
Agglutination
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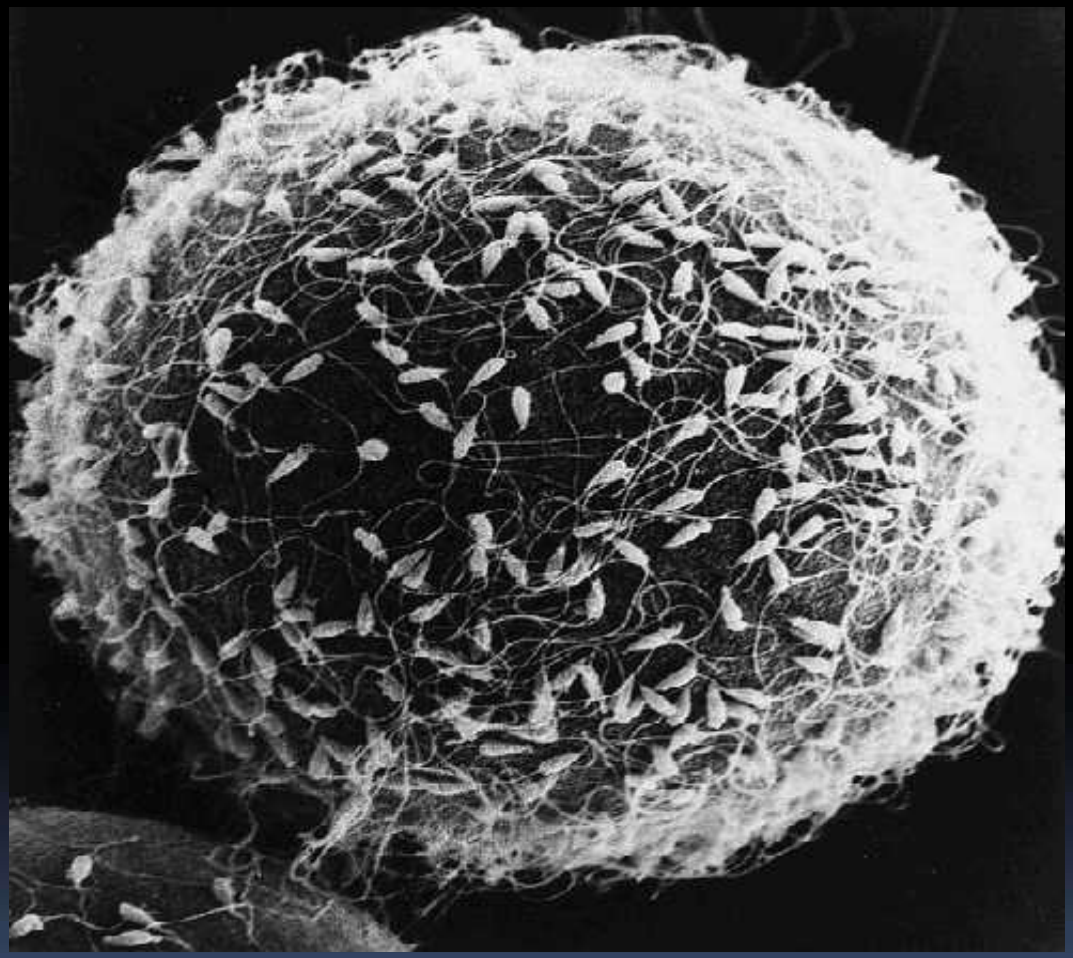
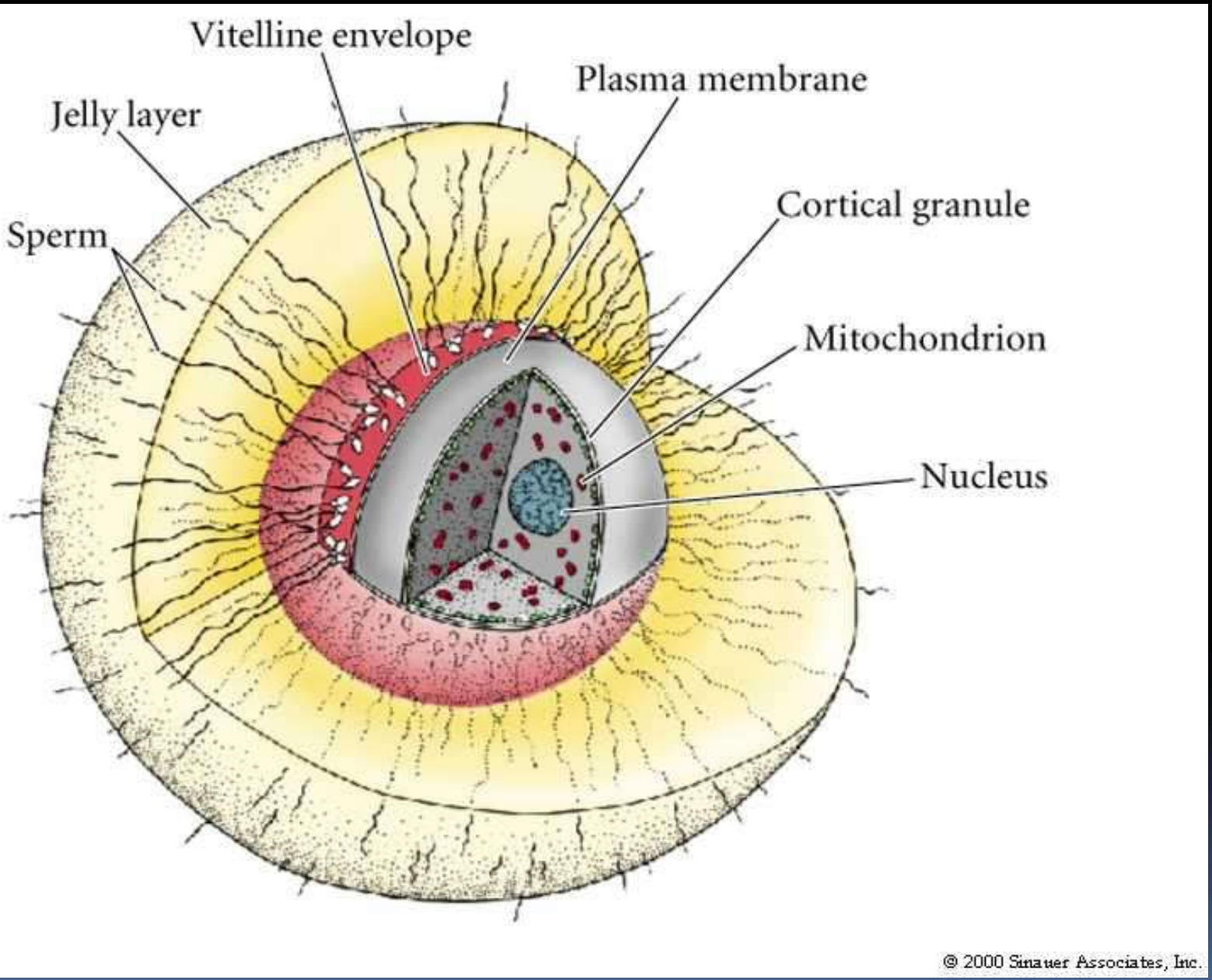
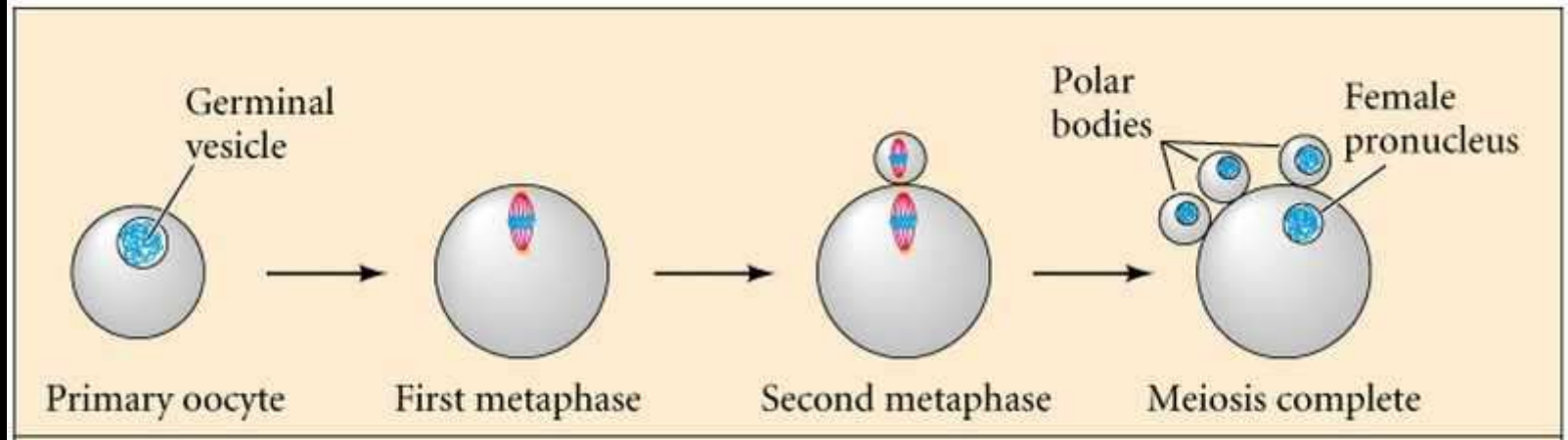
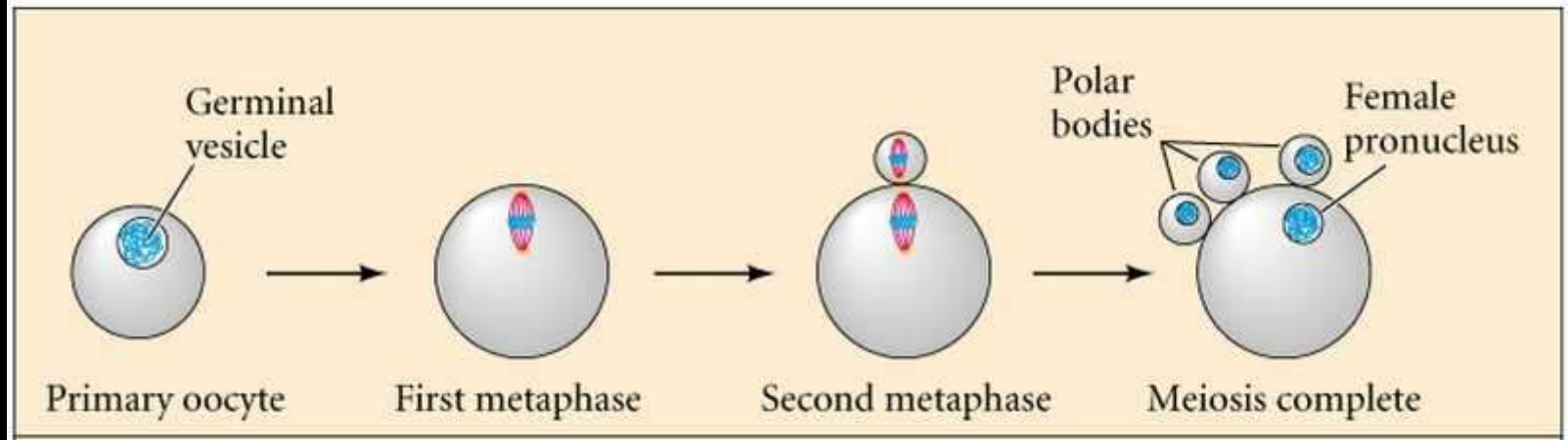


DR. TRIJIT NANADA, U.G.& P.G. DEPARTMENT OF ZOOLOGY, VIVEKANANDA COLLEGE


Vitelline envelope
Plasma membrane
Jelly layer
Cortical granule
Sperm
Mitochondrion
Nucleus
© 2000 Sinauer Associates, Inc.



Primary oocyte	First metaphase	Second metaphase	Meiosis complete
The roundworm	The nemertean worm	The lancelet	Cnidarians (e. g., anemones)
The mesozoan	The polychaete worm	Amphibians	Sea urchins
The sponge	The mollusc	Most mammals	
The polychaete worm	The core worm	Fish	
The clam worm	Many insects		
The clam	Starfish		
The echiuroid worm			
Dogs and foxes			



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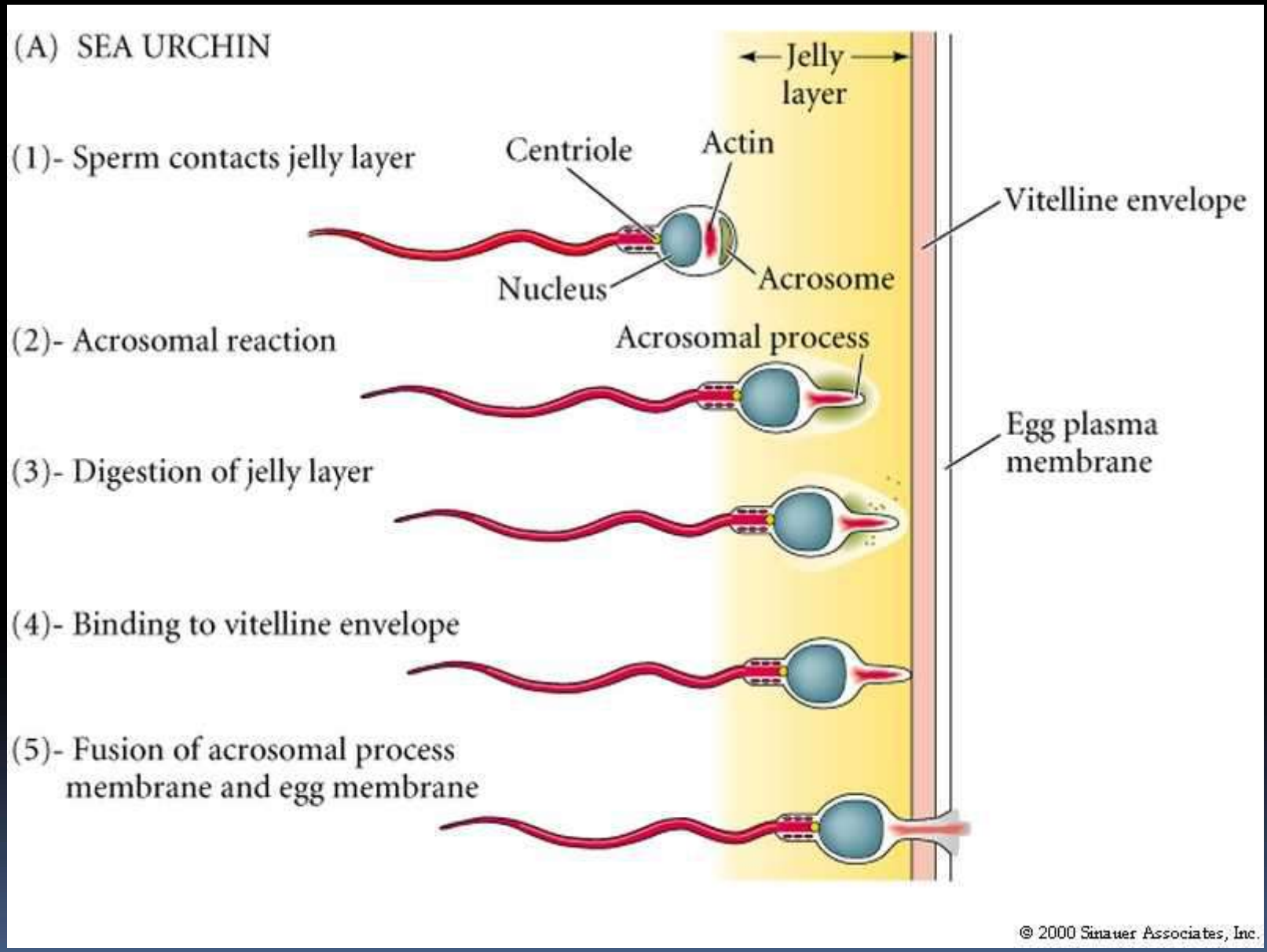
The clam worm	Many insects		
The clam	Starfish		
The echiuroid worm			
Dogs and foxes			


(A) SEA URCHIN
Jelly layer
(1)- Sperm contacts jelly layer
Centriole
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GAMETOGENESIS

AND FERTILIZATION UNIT

OPPORTUNITY: MICROTUBULES AND MOTORS

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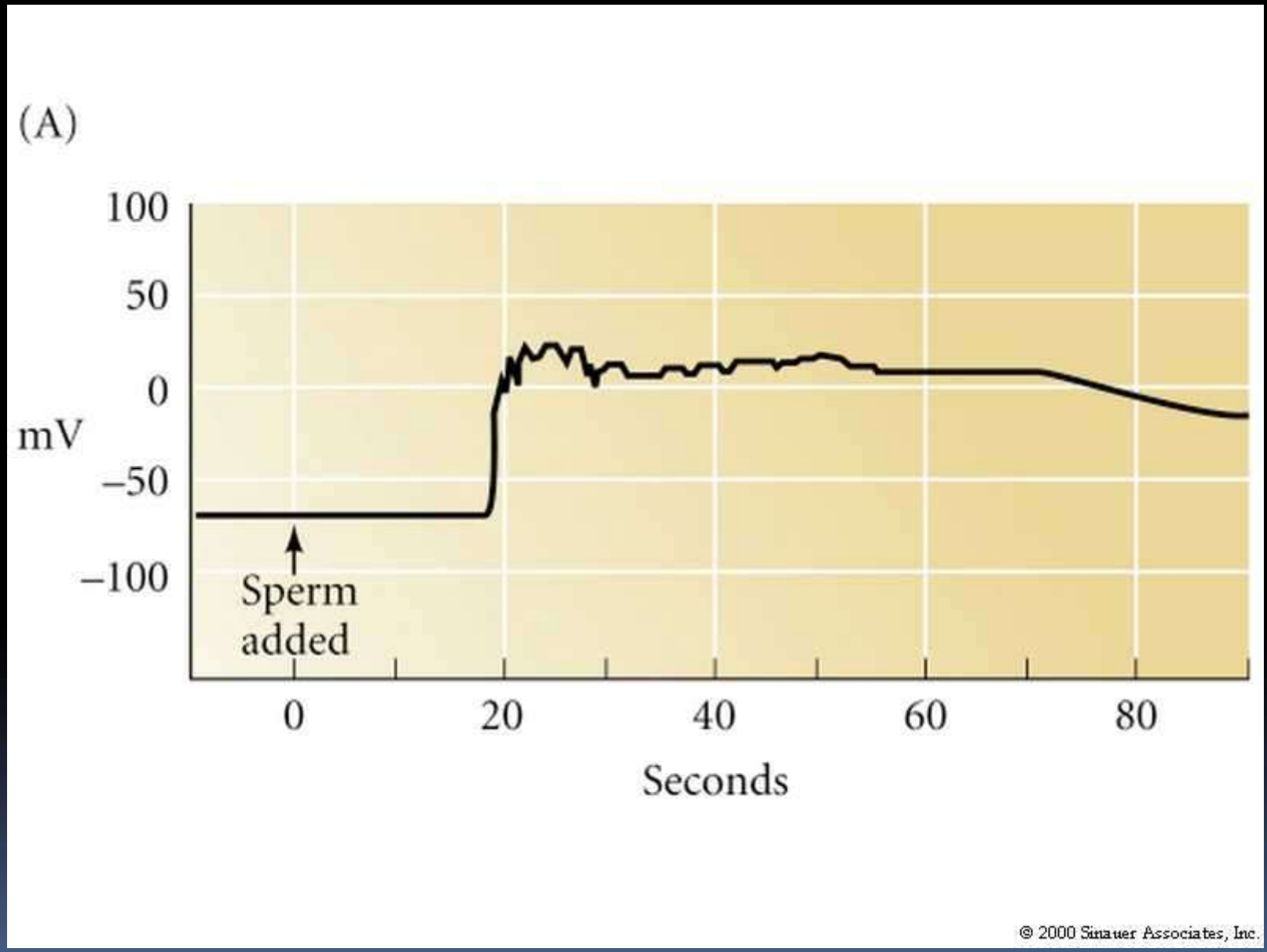
Table 7.1. Events of sea urchin fertilization

Event	Approximat
EARLY RESPONSES	
Sperm-egg binding	0 seconds
Fertilization potential rise (fast block to polyspermy)	within 1 sec
Sperm-egg membrane fusion	within 6 sec
Calcium increase first detected	6 sec
Cortical vesicle exocytosis (slow block to polyspermy)	15–60 sec
LATE RESPONSES	

PubMed

Increase in pH (reaches high)	1–5 min
Sperm chromatin decondensation	2–12 min
Sperm nucleus migration to egg center	2–12 min
Egg nucleus migration to sperm nucleus	5–10 min
Activation of protein synthesis	starts at 5–15 min
Activation of amino acid transport	starts at 5–15 min
Initiation of DNA synthesis	20–40 min
Mitosis	60–80 min
First cleavage	85–95 min


(A)
100
50
0
mV
−50
−100
Sperm
added
0
20
40
60
80
Seconds
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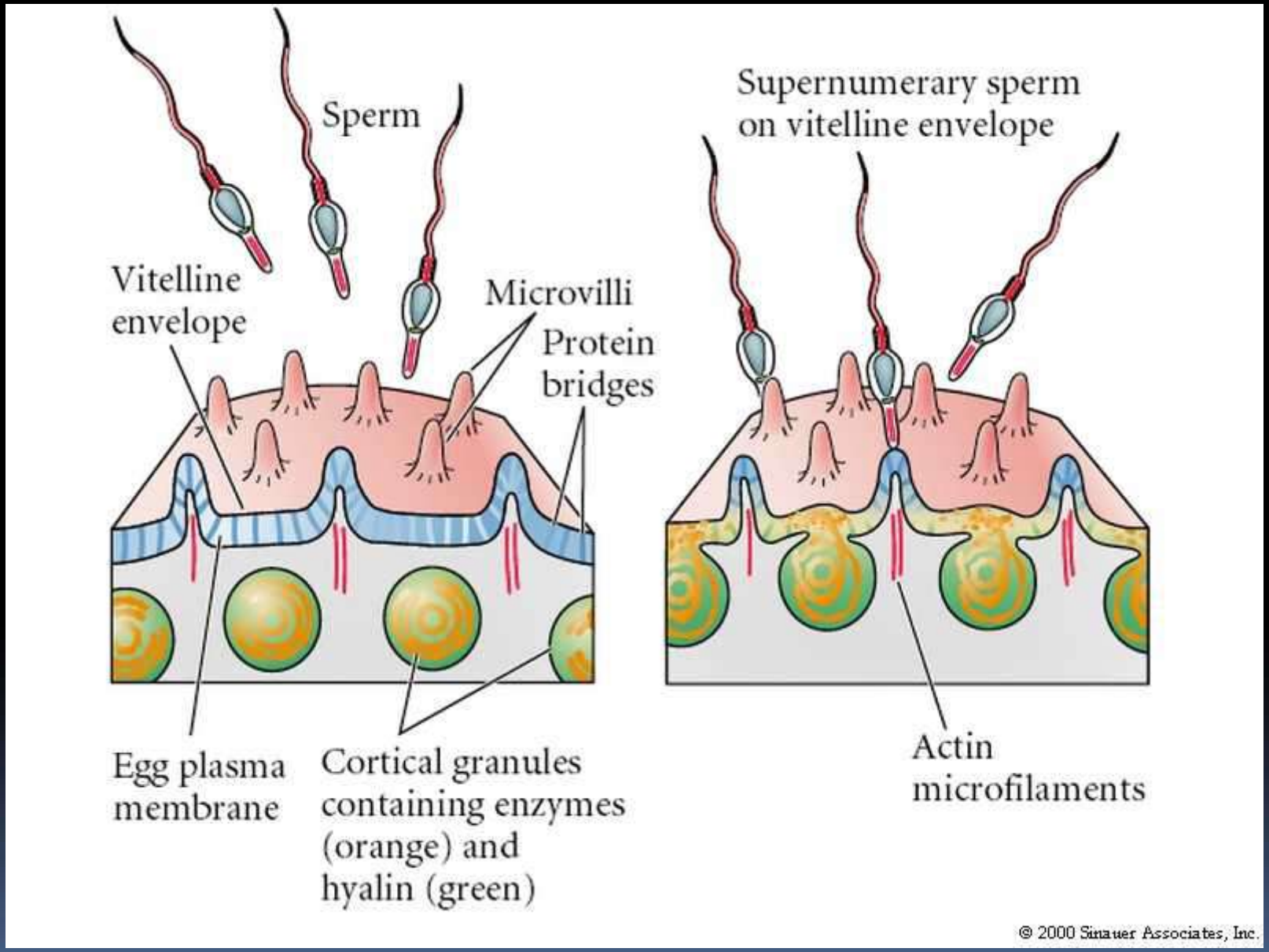
Sperm
Vitelline envelope
Microvilli
Protein bridges
Egg plasma membrane
Cortical granules containing enzymes (orange) and hyalin (green)
Supernumerary sperm on vitelline envelope
Actin microfilaments
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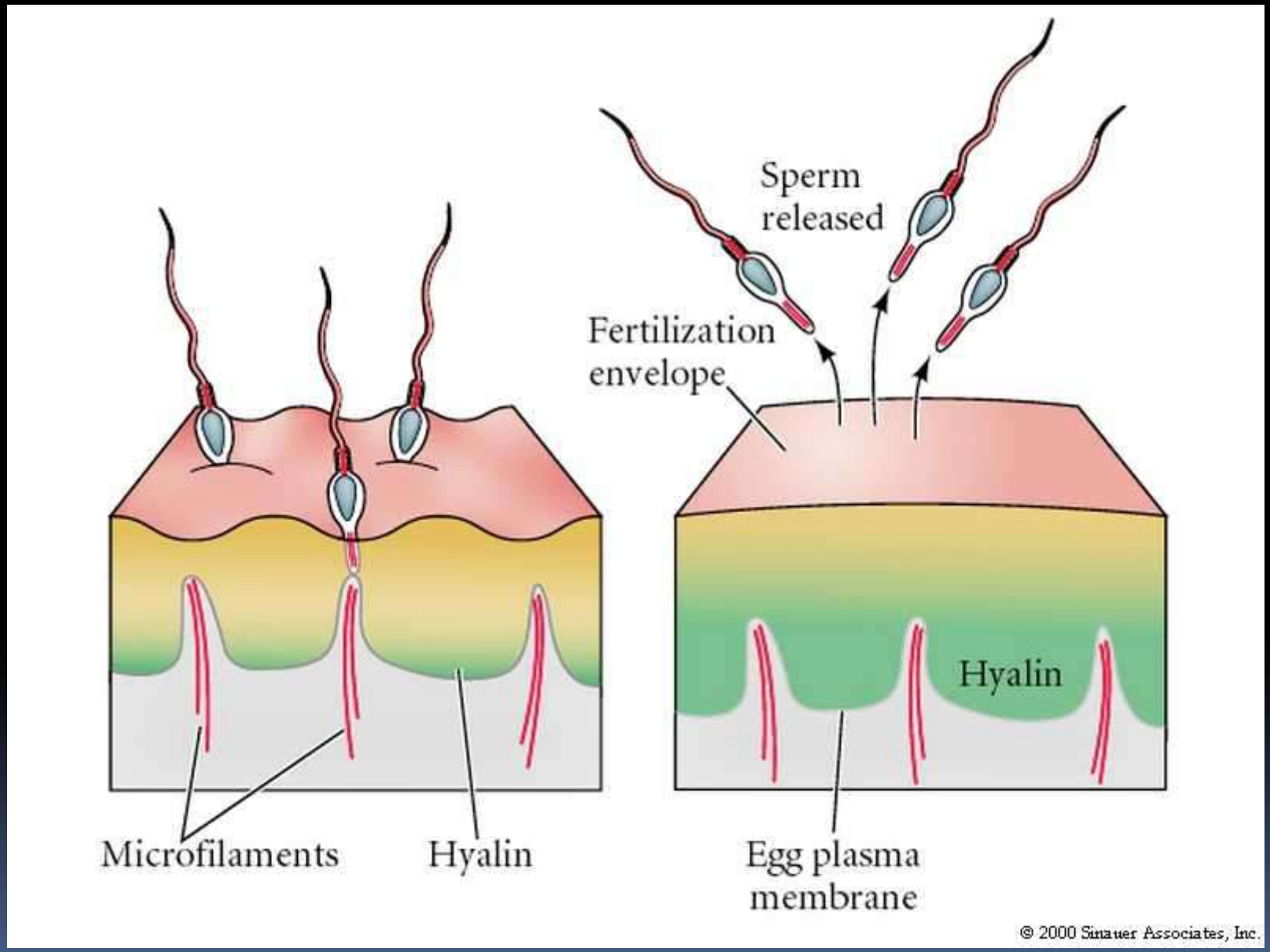
Sperm released
Fertilization envelope
Hyalin
Microfilaments
Hyalin
Egg plasma membrane
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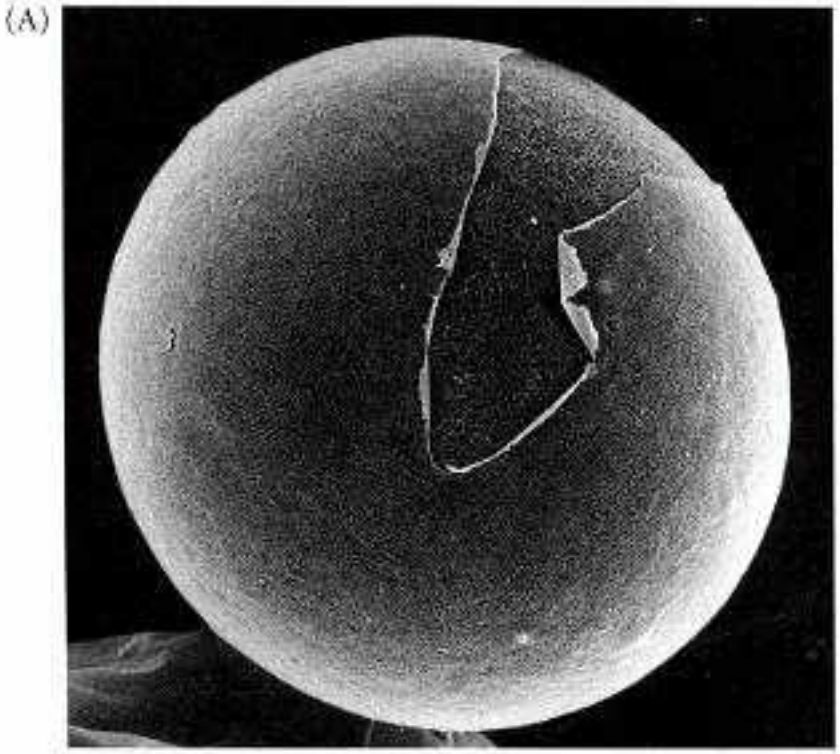
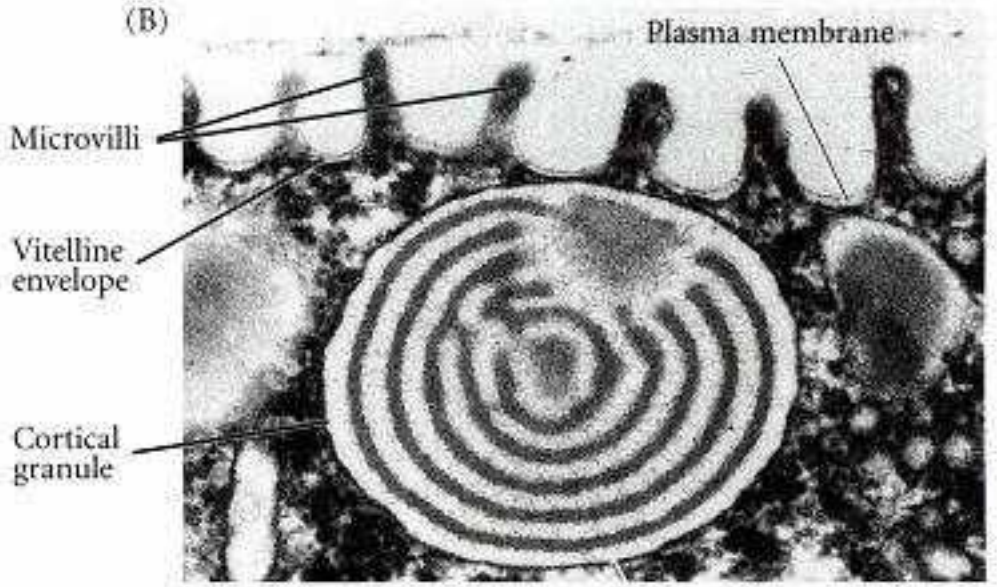

(A)


(B)
Plasma membrane
Microvilli
Vitelline envelope
Cortical granule

Vade Mecum Sea Urchin Fertilization

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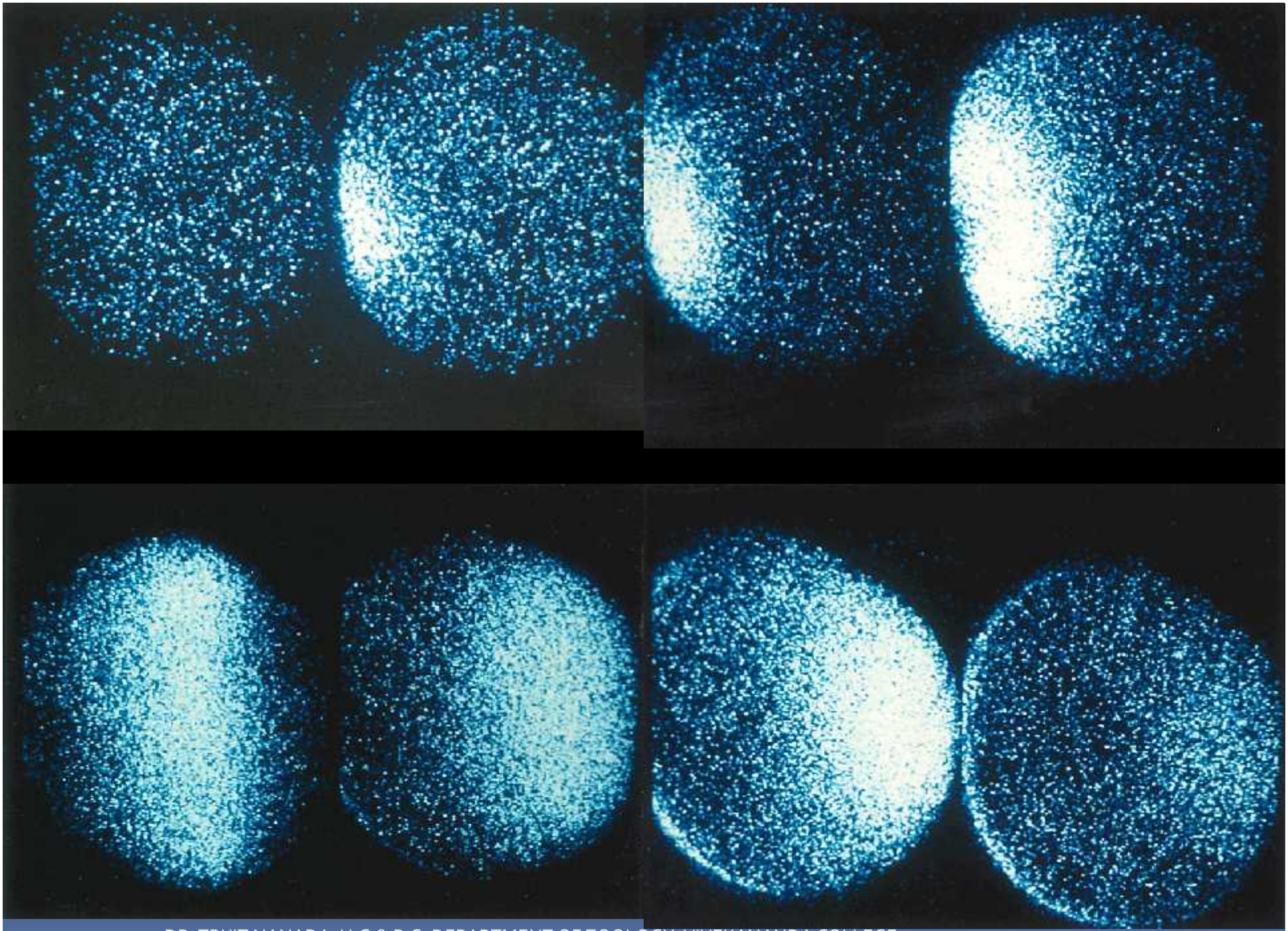

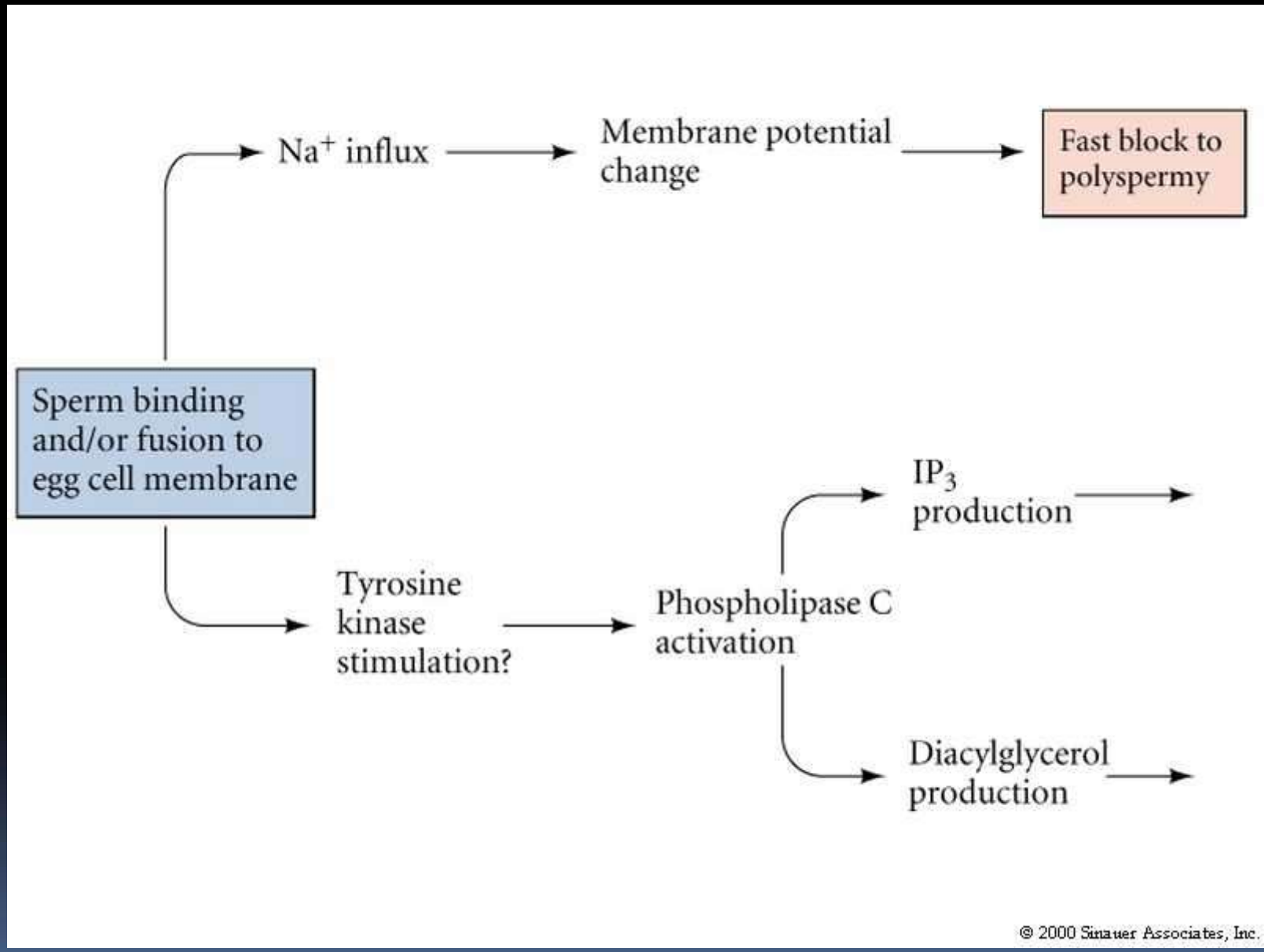
Sperm binding and/or fusion to egg cell membrane
Na+ influx
Membrane potential change
Fast block to polyspermy
Tyrosine kinase stimulation?
Phospholipase C activation
IP3 production
Diacylglycerol production
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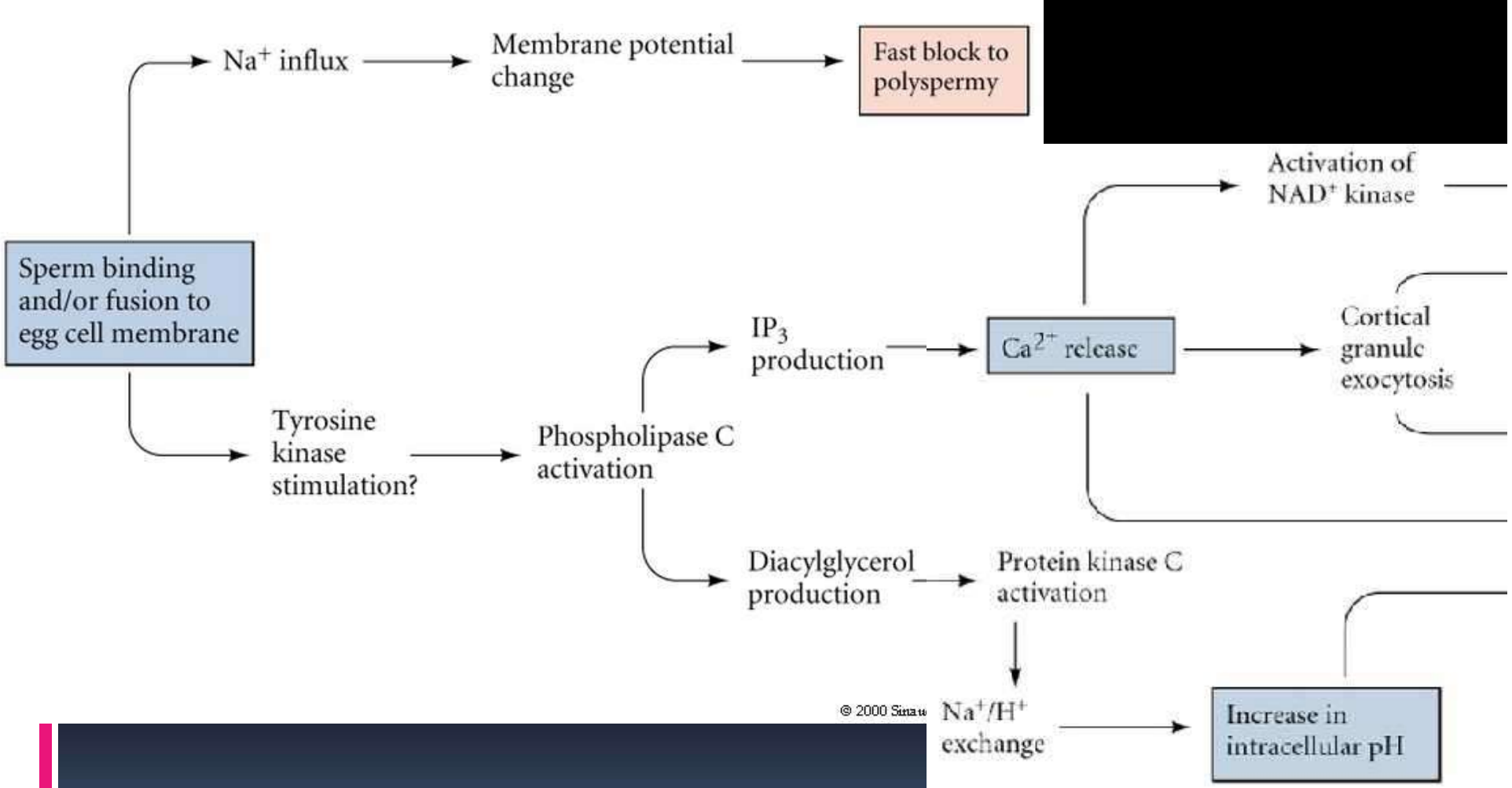
Sperm binding and/or fusion to egg cell membrane
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Membrane potential change
Fast block to polyspermy
Tyrosine kinase stimulation?
Phospholipase C activation
IP3 production
Ca2+ release
Activation of NAD+ kinase
Cortical granule exocytosis
Diacylglycerol production
Protein kinase C activation
Na+/H+ exchange
Increase in intracellular pH
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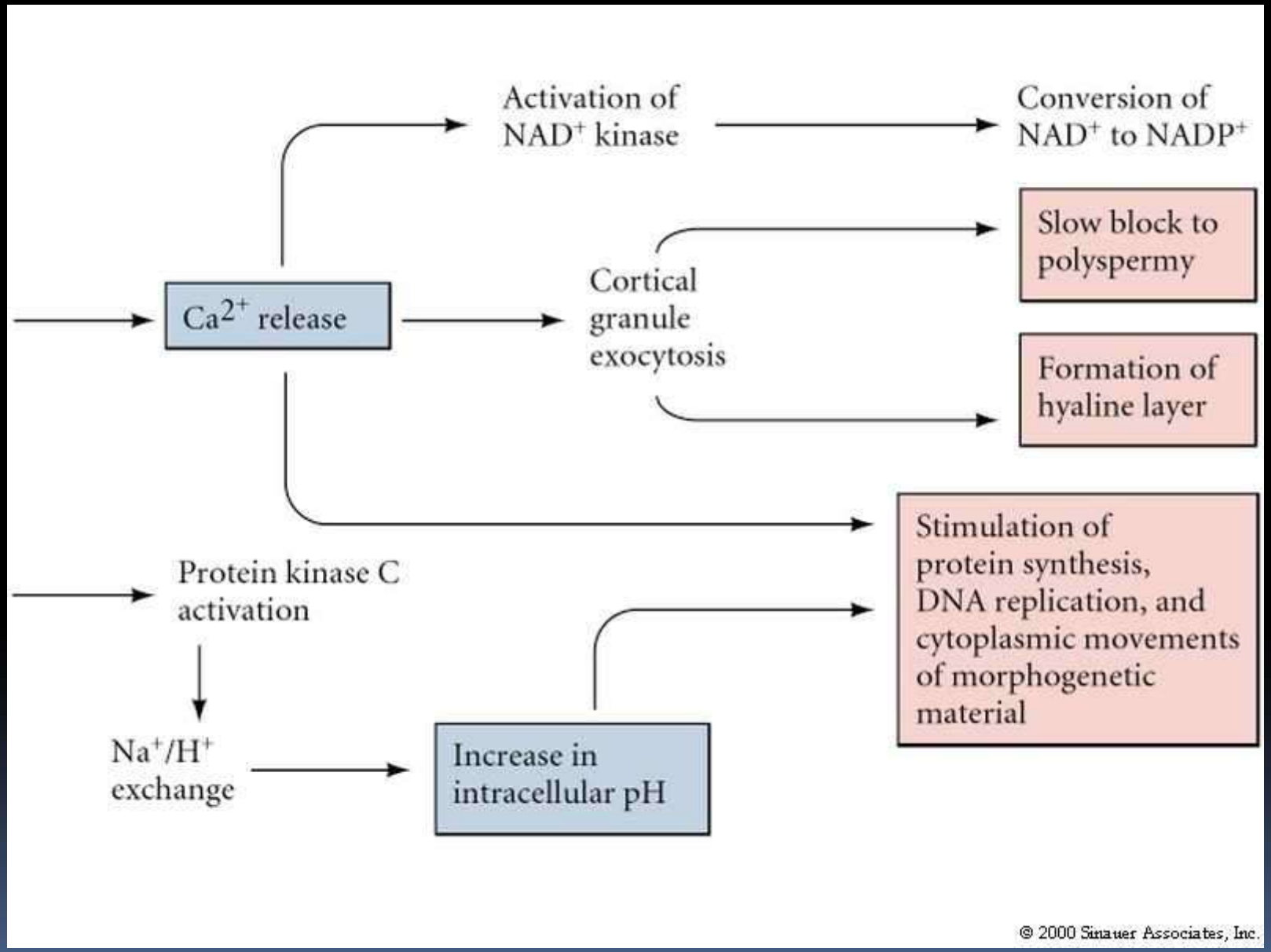
Activation of NAD+ kinase
Conversion of NAD+ to NADP+
Slow block to polyspermy
Cortical granule exocytosis
Ca2+ release
Formation of hyaline layer
Stimulation of protein synthesis, DNA replication, and cytoplasmic movements of morphogenetic material
Protein kinase C activation
Na+/H+ exchange
Increase in intracellular pH
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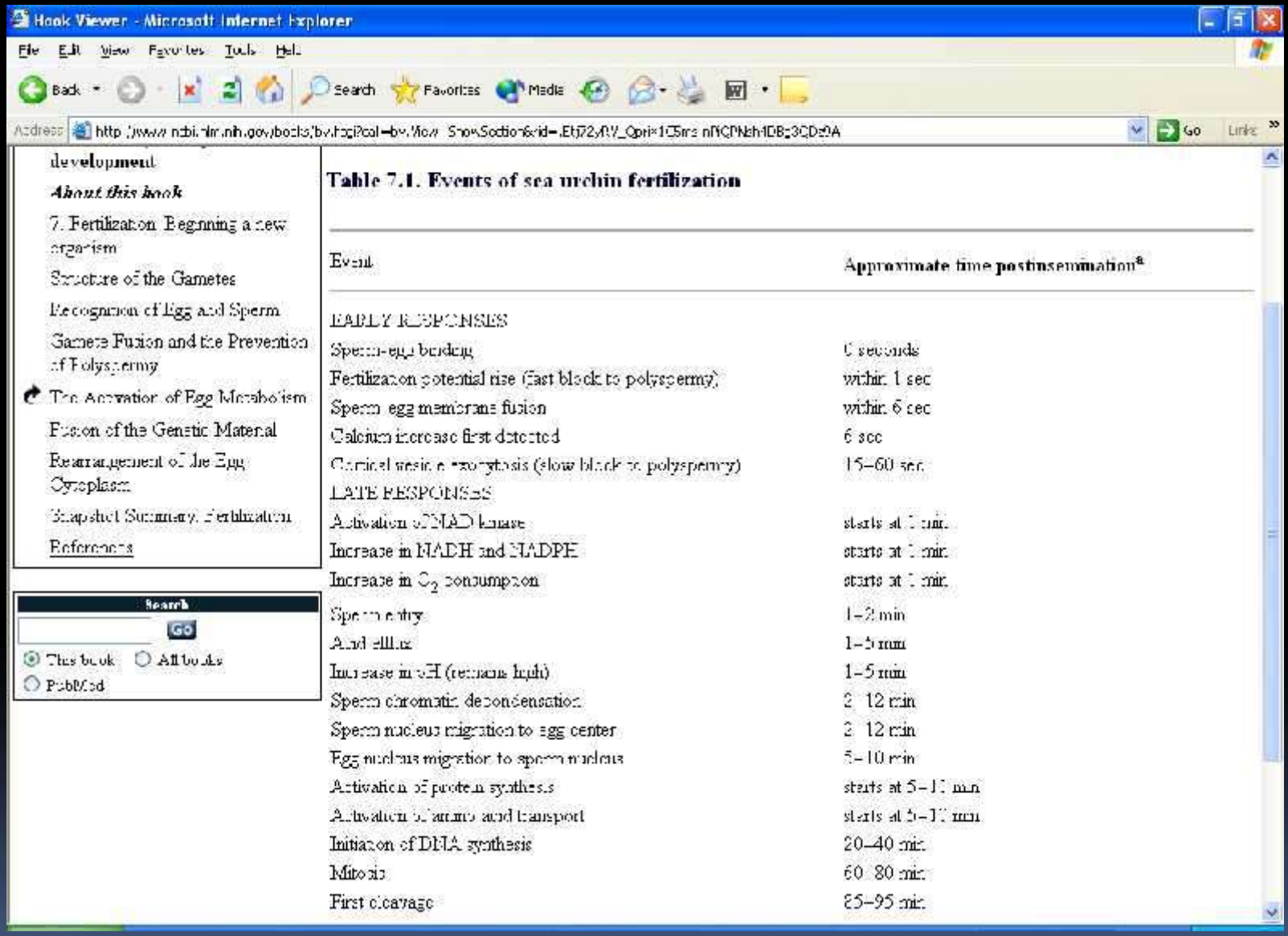

Table 7.1. Events of sea urchin fertilization

Event	Approximate time postinsemination[a]
EARLY RESPONSES	
Sperm-egg binding	0 seconds
Fertilization potential rise (fast block to polyspermy)	within 1 sec
Sperm-egg membrane fusion	within 6 sec
Calcium increase first detected	6 sec
Cortical vesicle exocytosis (slow block to polyspermy)	15–60 sec
LATE RESPONSES	
Activation of NAD kinase	starts at 1 min
Increase in NADH and NADPH	starts at 1 min
Increase in O_2 consumption	starts at 1 min
Sperm entry	1–2 min
Amphiasters	1–5 min
Increase in pH (reaches high)	1–5 min
Sperm chromatin decondensation	2–12 min
Sperm nucleus migration to egg center	2–12 min
Egg nucleus migration to sperm nucleus	5–10 min
Activation of protein synthesis	starts at 5–10 min
Activation of amino acid transport	starts at 5–10 min
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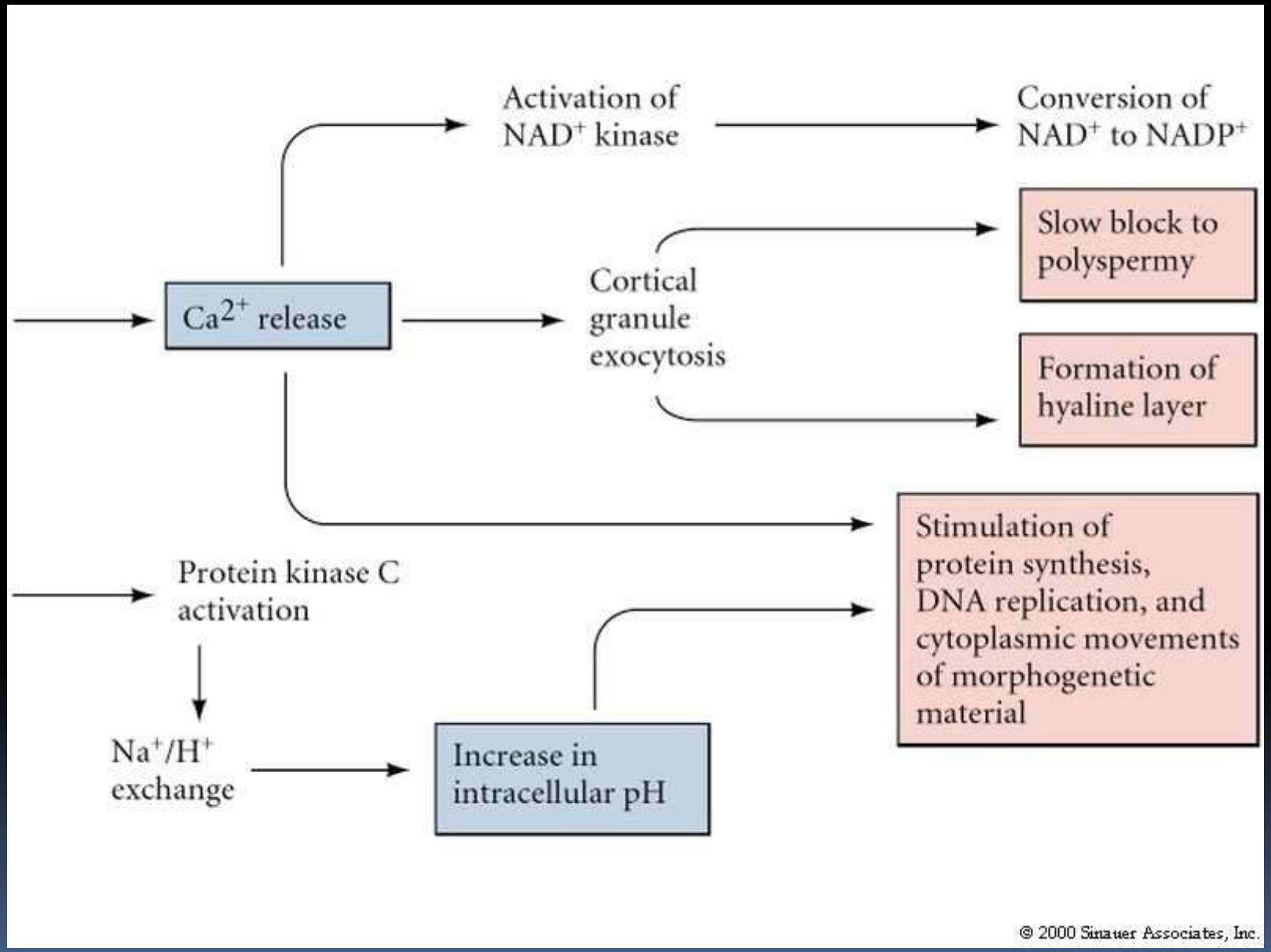
Activation of NAD+ kinase
Conversion of NAD+ to NADP+
Slow block to polyspermy
Cortical granule exocytosis
Ca2+ release
Formation of hyaline layer
Stimulation of protein synthesis, DNA replication, and cytoplasmic movements of morphogenetic material
Protein kinase C activation
Na+/H+ exchange
Increase in intracellular pH
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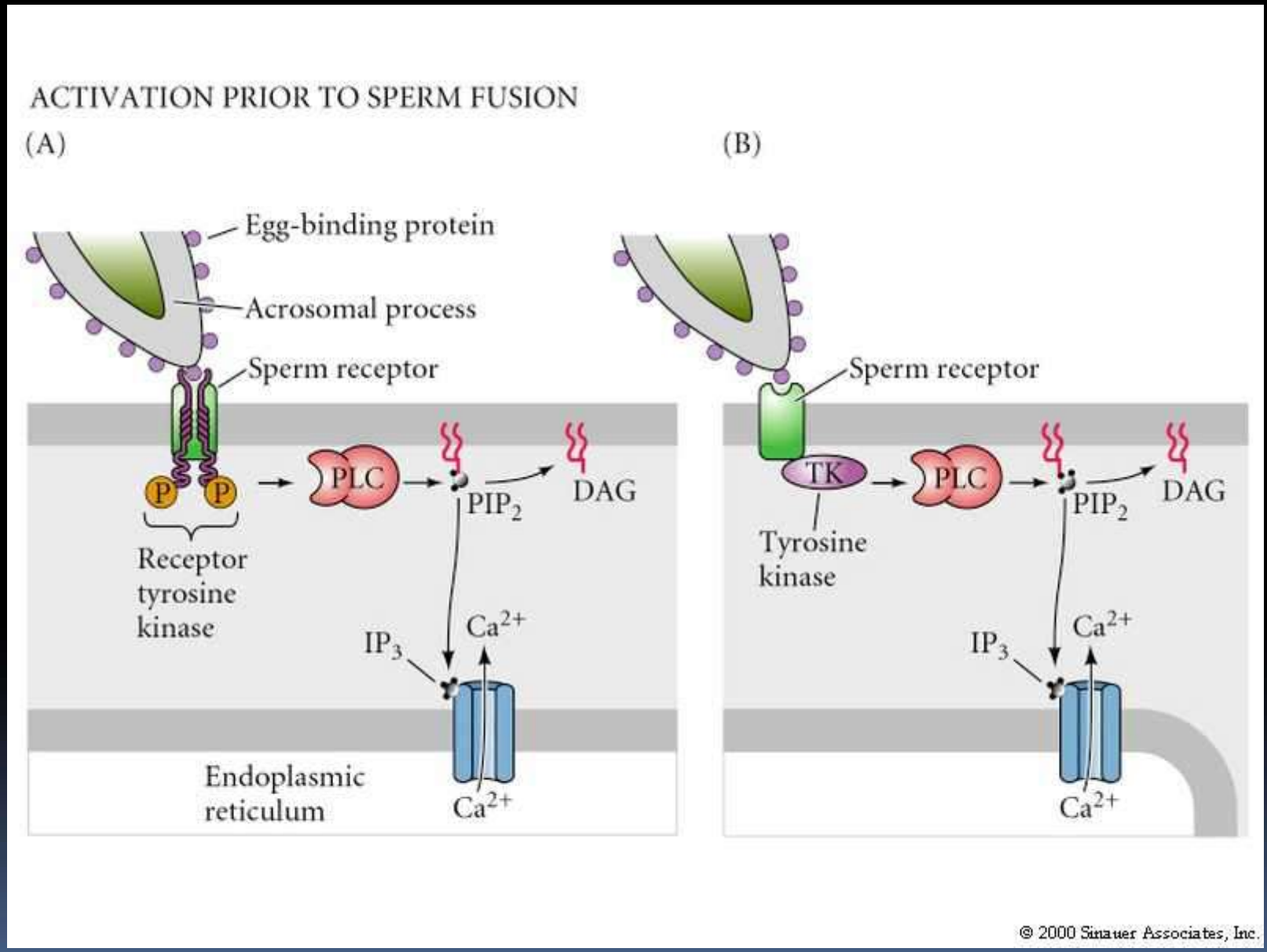
ACTIVATION PRIOR TO SPERM FUSION
(A)
(B)
Egg-binding protein
Acrosomal process
Sperm receptor
P
P
Receptor tyrosine kinase
PLC
PIP2
DAG
IP3
Ca2+
Endoplasmic reticulum
Ca2+
Sperm receptor
TK
Tyrosine kinase
PLC
PIP2
DAG
IP3
Ca2+
Ca2+
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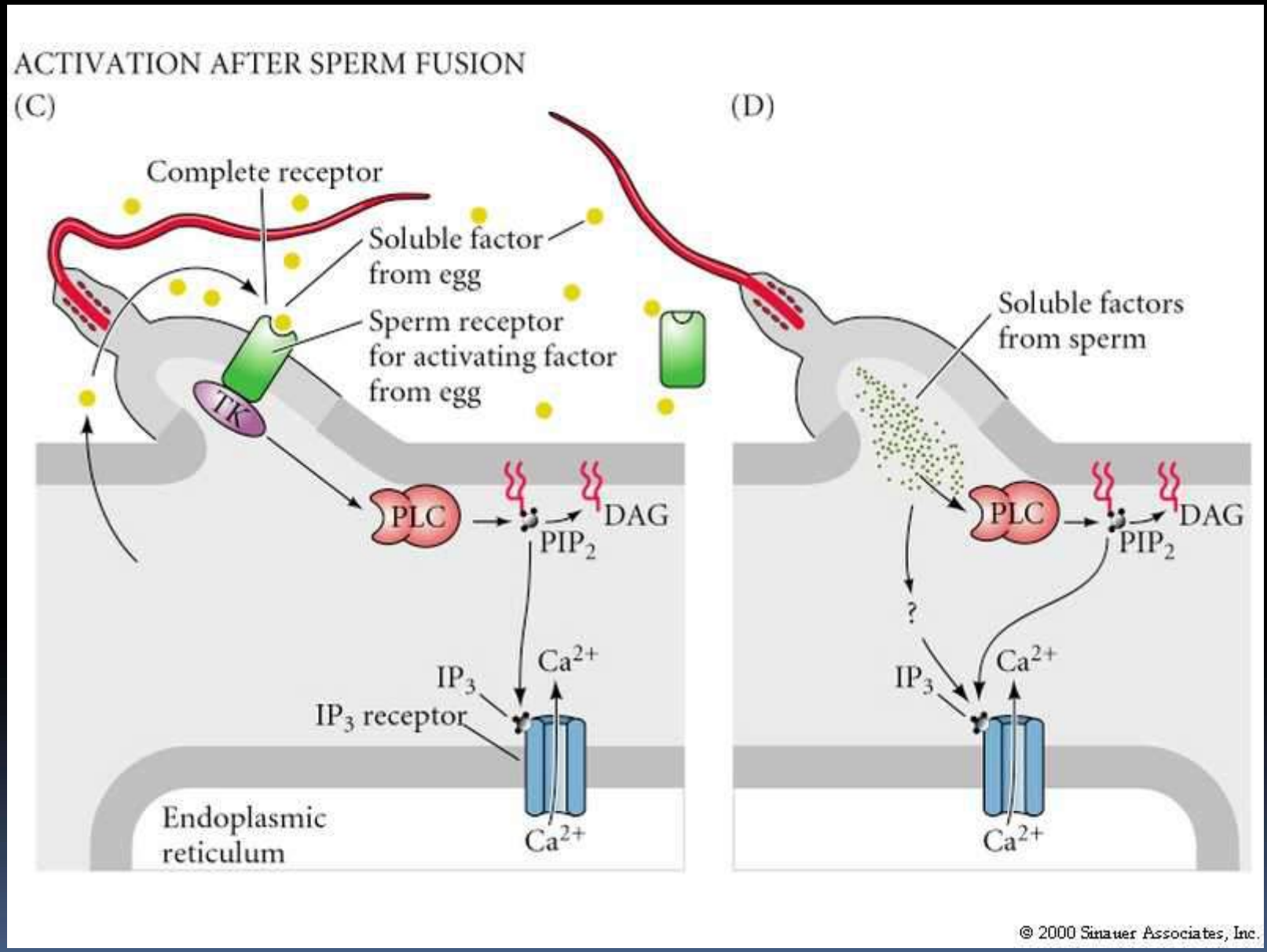
ACTIVATION AFTER SPERM FUSION
(C)
(D)
Complete receptor
Soluble factor from egg
Sperm receptor for activating factor from egg
TK
PLC
DAG
PIP2
IP3
IP3 receptor
Ca2+
Endoplasmic reticulum
Soluble factors from sperm
?
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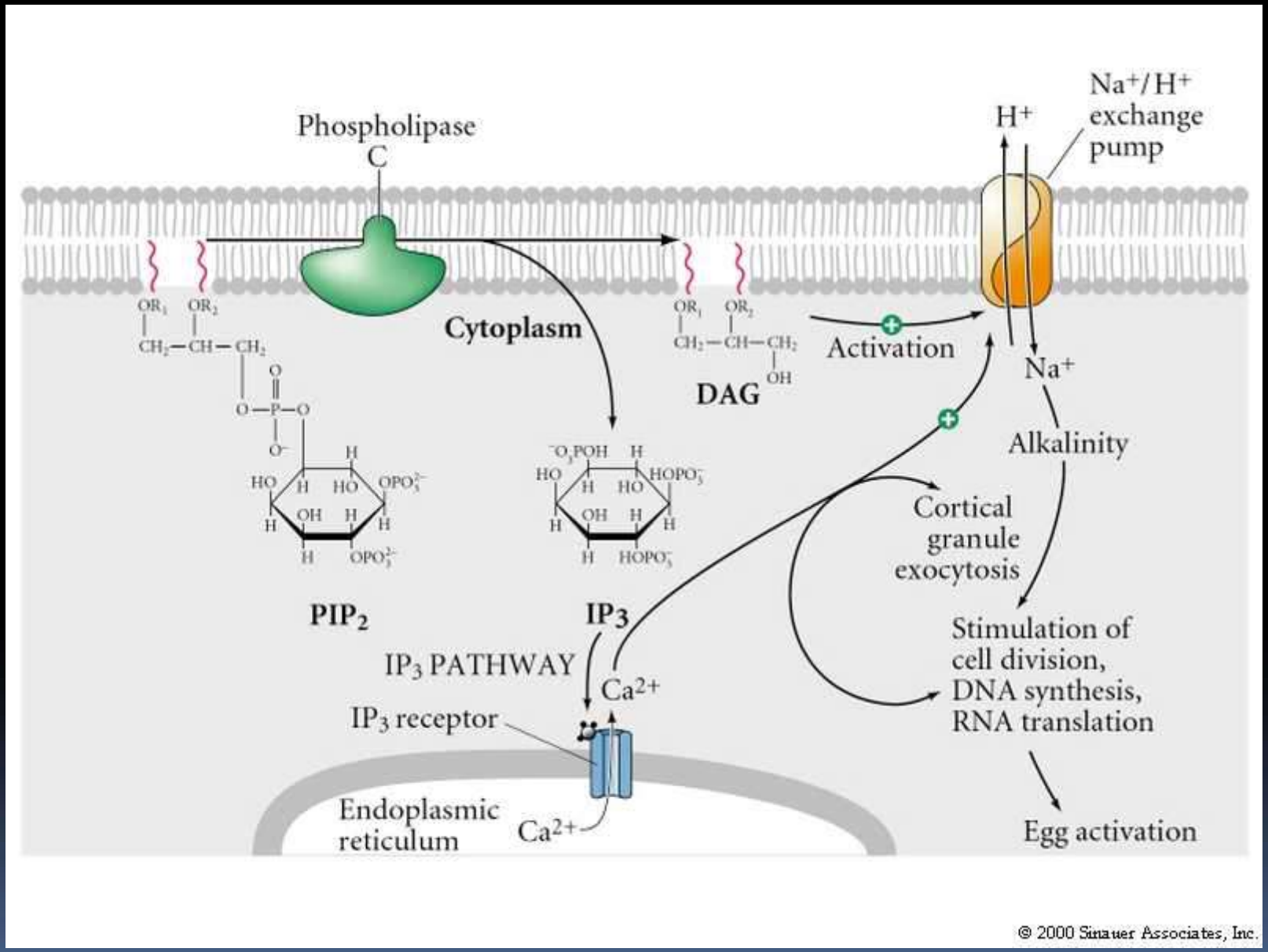
Phospholipase C
Cytoplasm
Na^+/H^+ exchange pump
H^+
Na^+
Activation
DAG
Alkalinity
Cortical granule exocytosis
Stimulation of cell division, DNA synthesis, RNA translation
Egg activation
PIP_2
IP_3
IP_3 PATHWAY
Ca^{2+}
IP_3 receptor
Endoplasmic reticulum
Ca^{2+}
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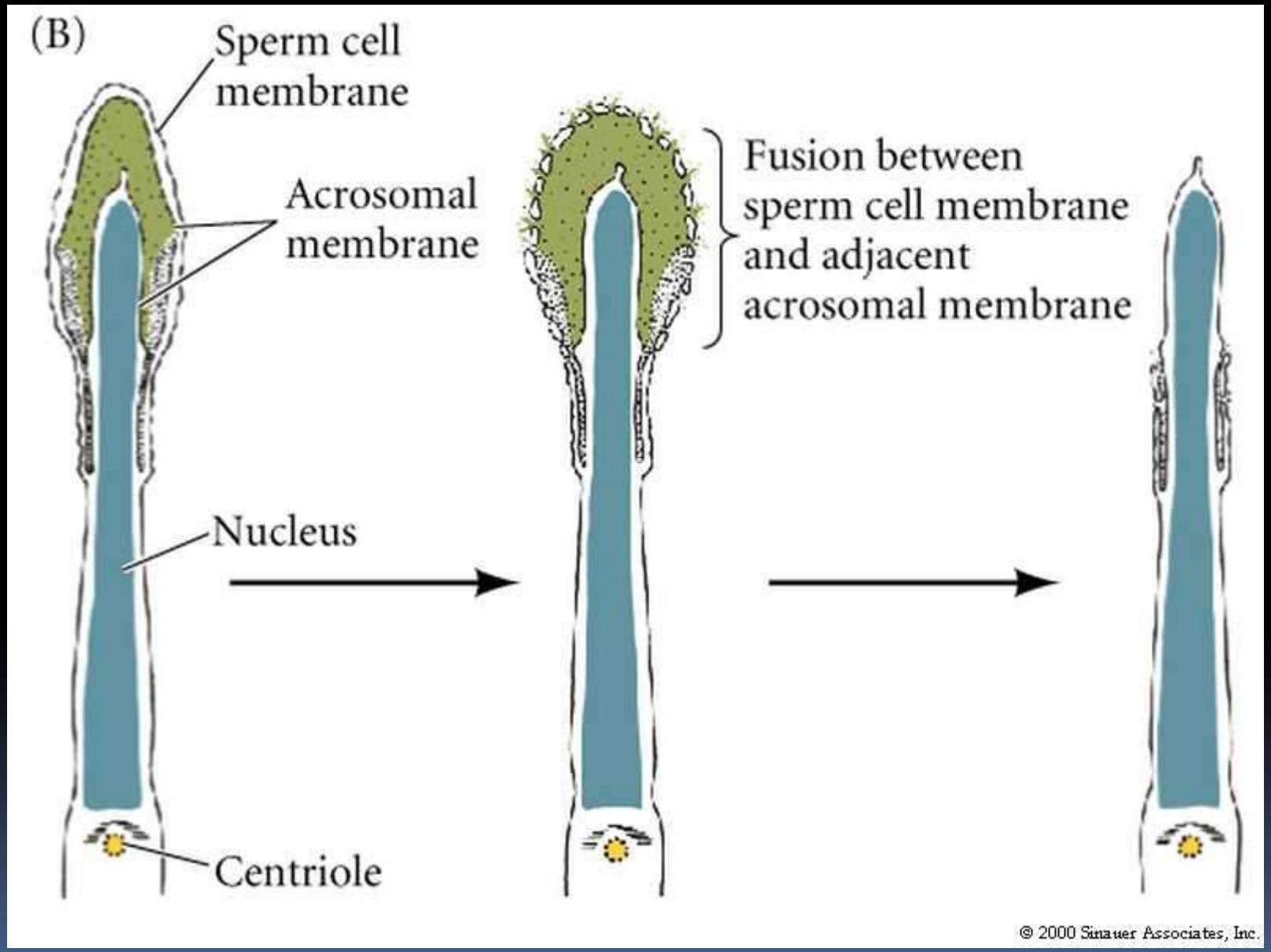
(B)
Sperm cell membrane
Acrosomal membrane
Nucleus
Centriole
Fusion between sperm cell membrane and adjacent acrosomal membrane
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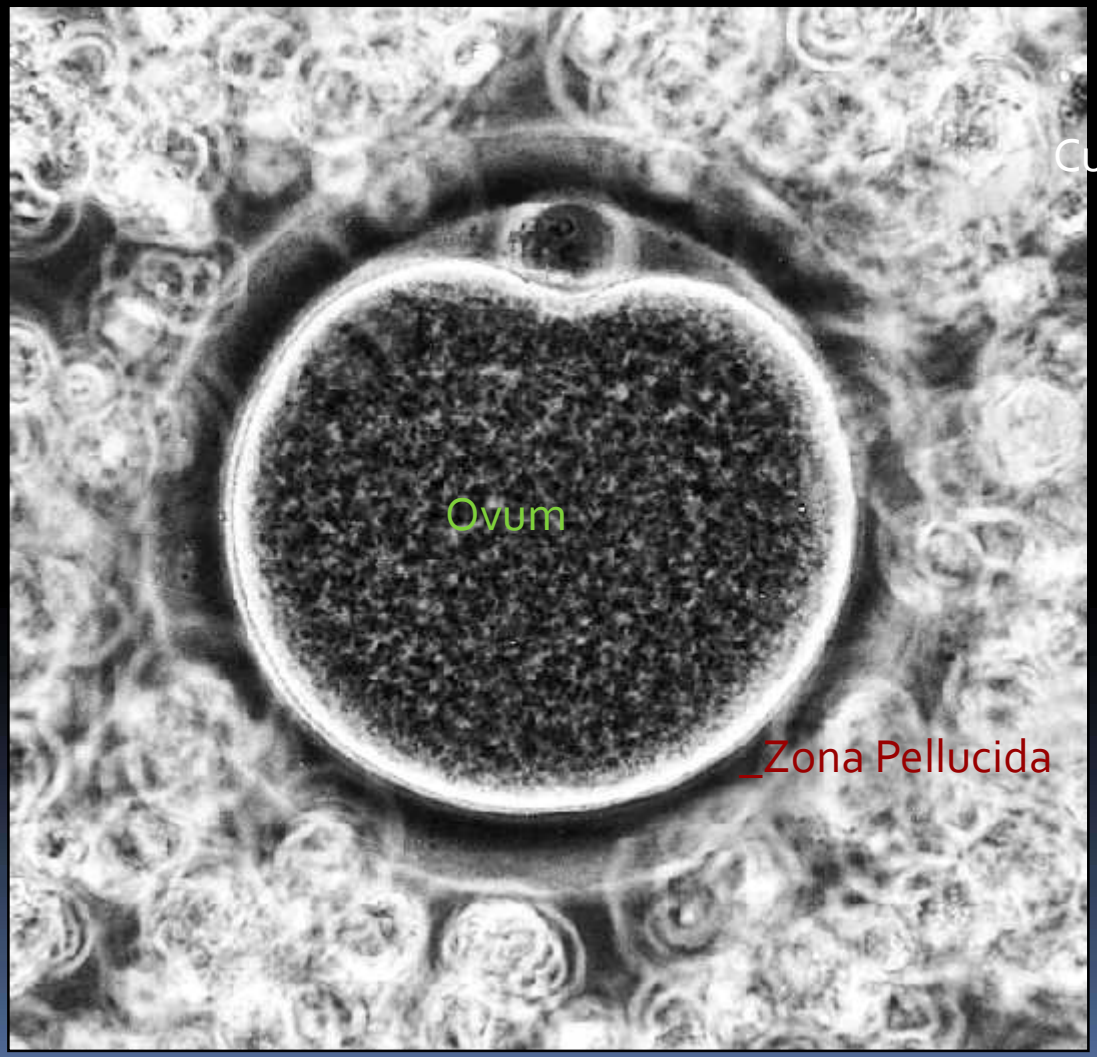
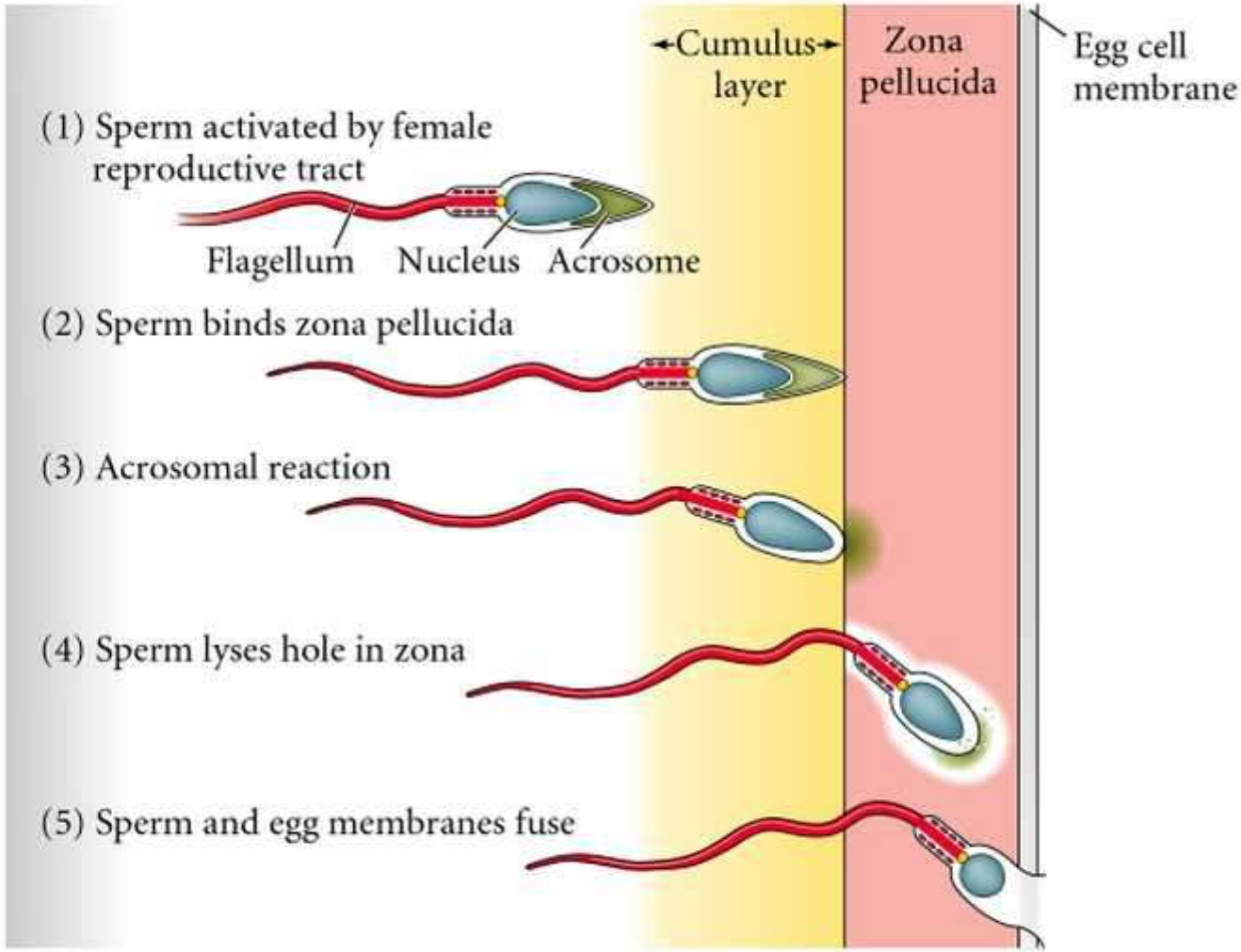
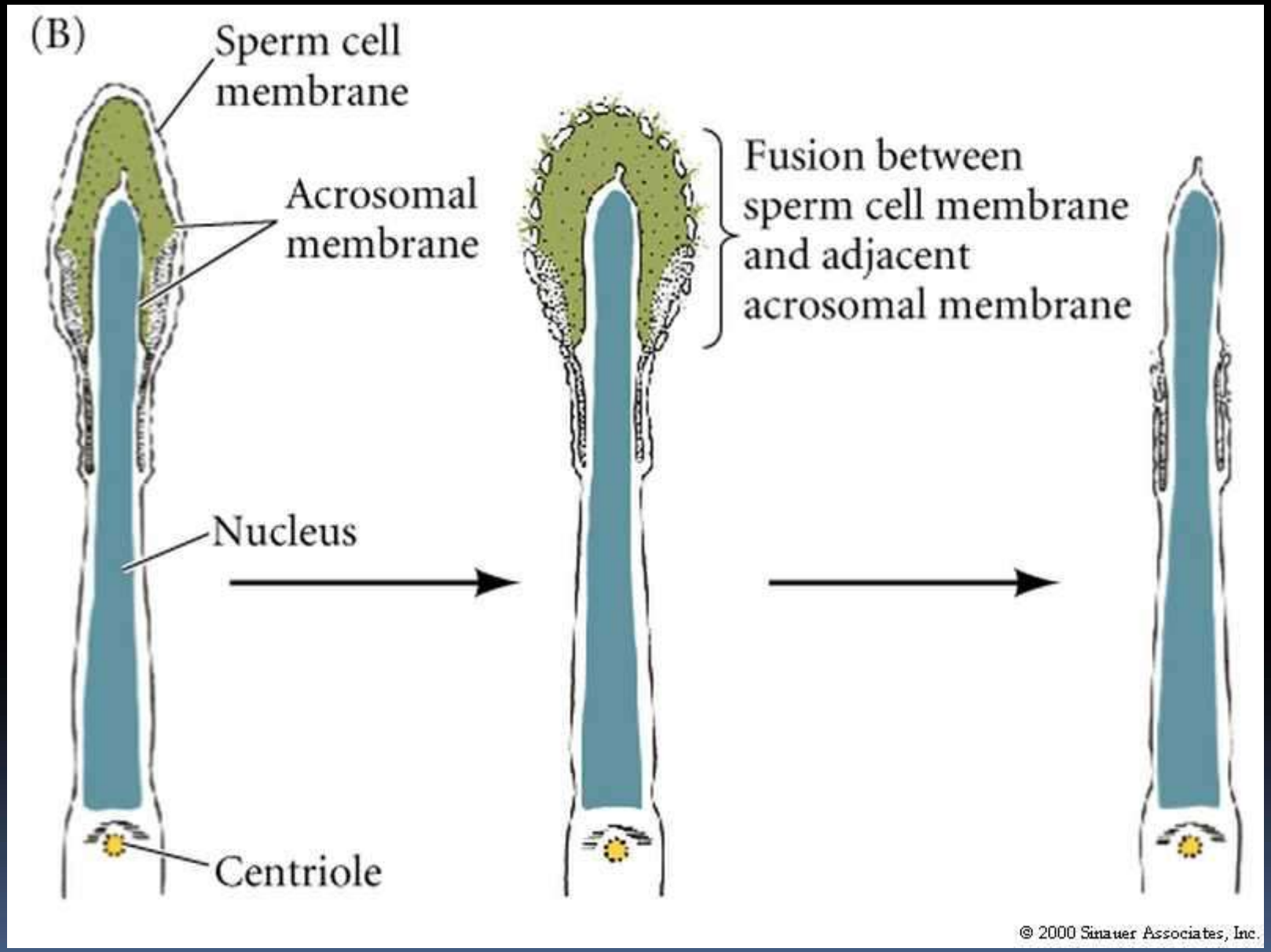

Cumulus
Ovum
_Zona Pellucida

(B) MOUSE




(B)
Sperm cell membrane
Acrosomal membrane
Nucleus
Centriole
Fusion between sperm cell membrane and adjacent acrosomal membrane
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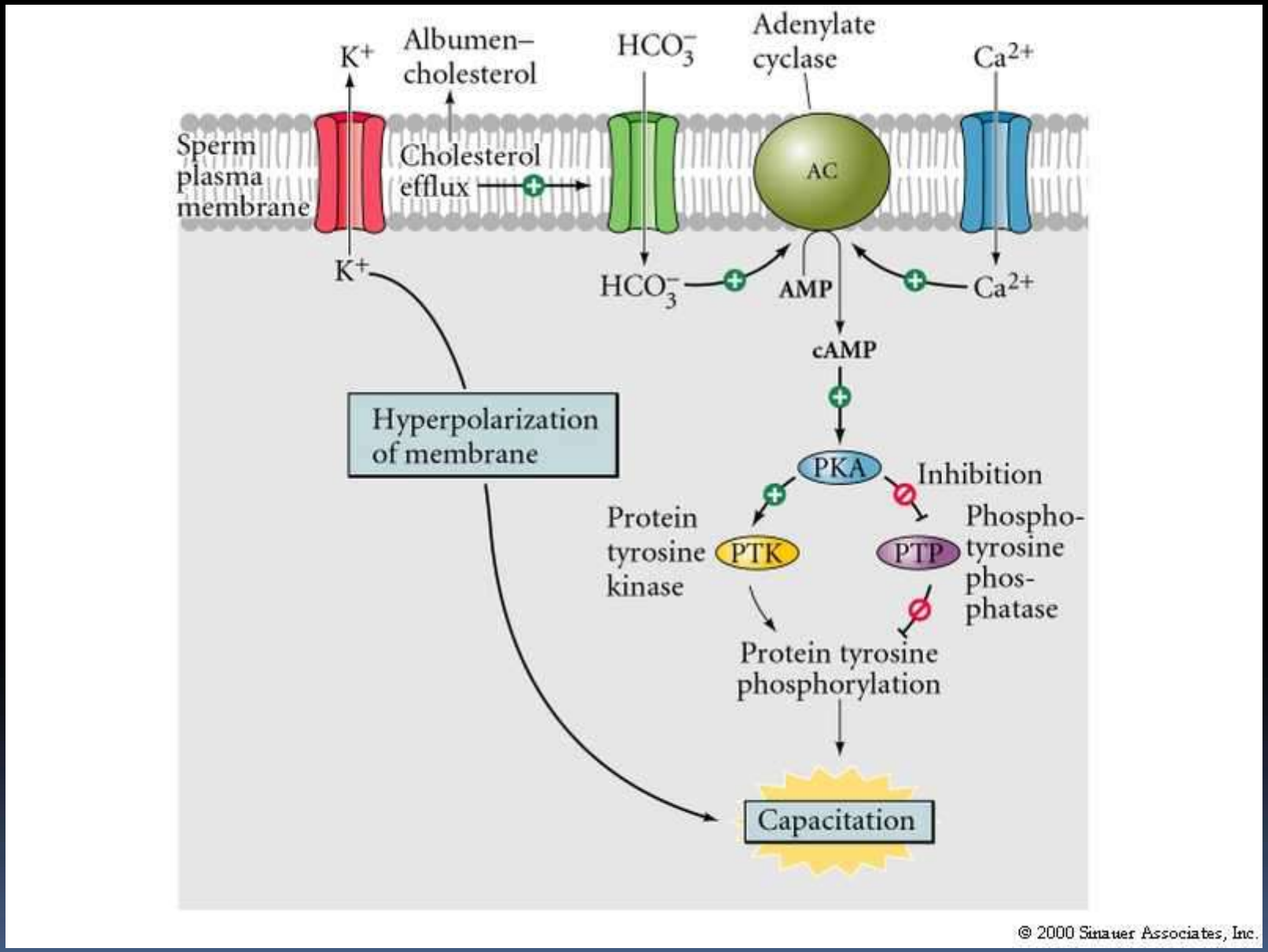
K+
Albumen–
cholesterol
HCO3−
Adenylate
cyclase
Ca2+
Sperm
plasma
membrane
Cholesterol
efflux
AC
K+
HCO3−
AMP
Ca2+
cAMP
Hyperpolarization
of membrane
PKA
Inhibition
Protein
tyrosine
kinase
PTK
PTP
Phospho-
tyrosine
phos-
phatase
Protein tyrosine
phosphorylation
Capacitation
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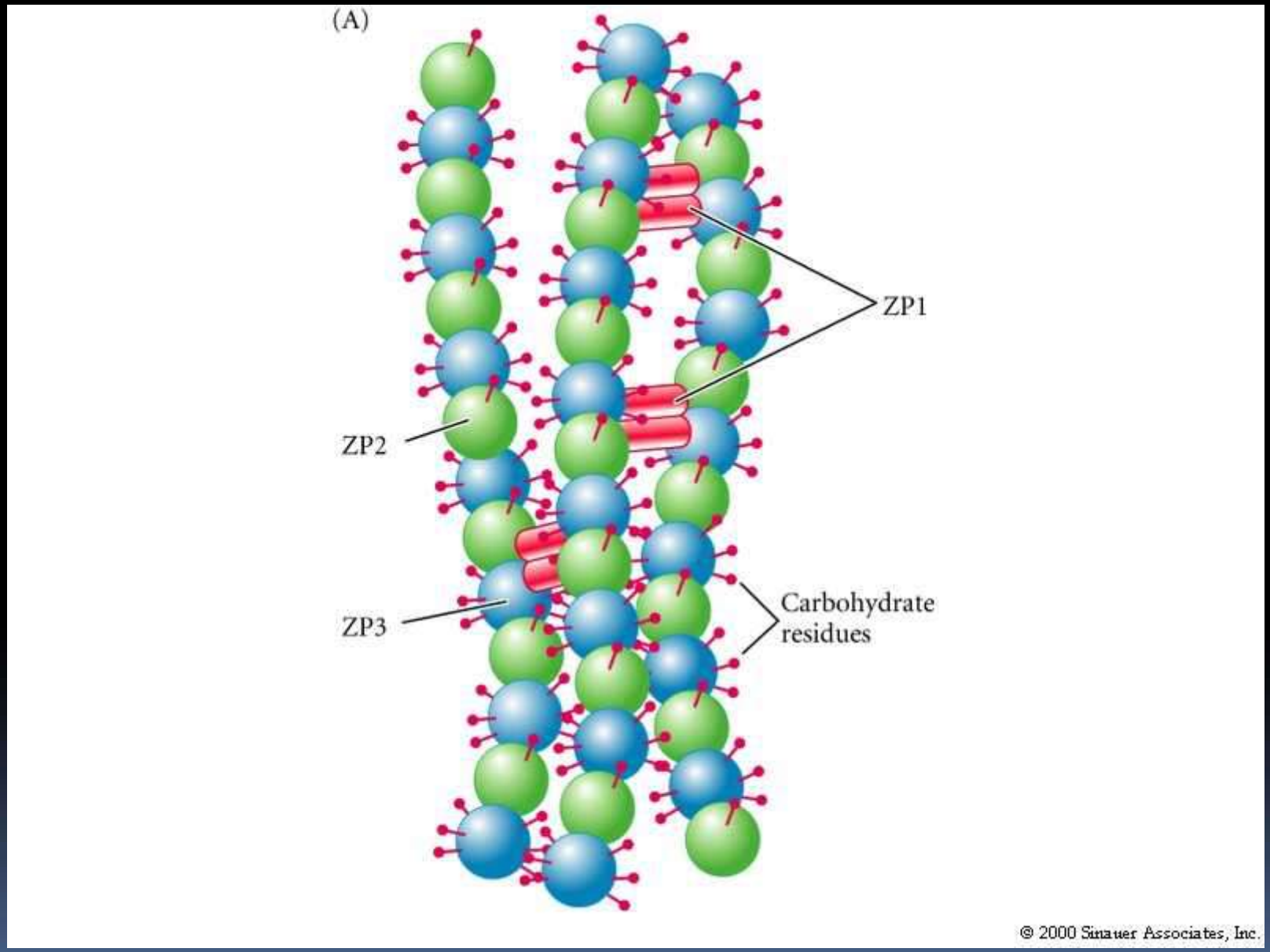
(A)
ZP1
ZP2
ZP3
Carbohydrate residues
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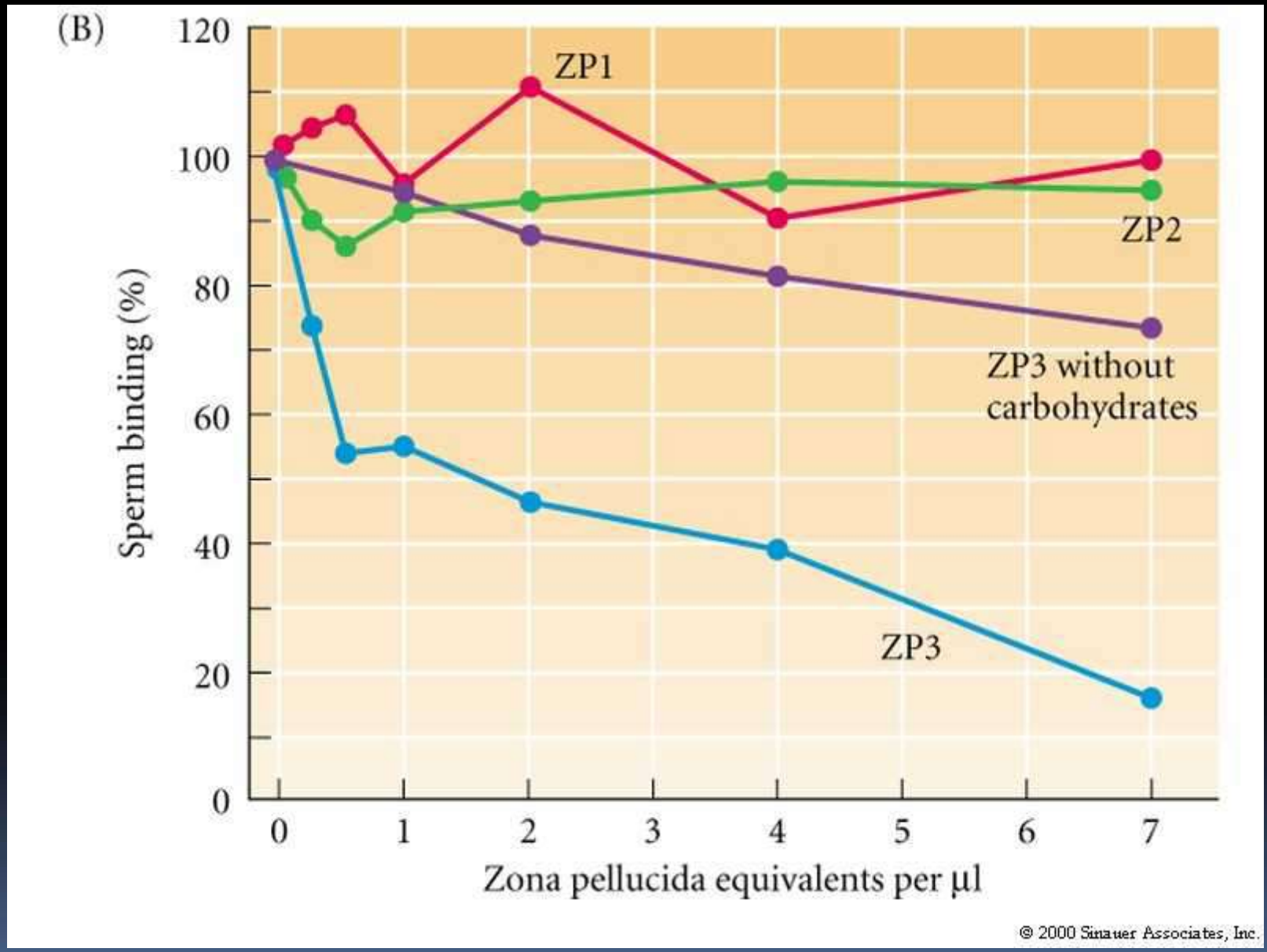
(B)
120
100
80
60
40
20
0
Sperm binding (%)
ZP1
ZP2
ZP3 without carbohydrates
ZP3
0
1
2
3
4
5
6
7
Zona pellucida equivalents per µl
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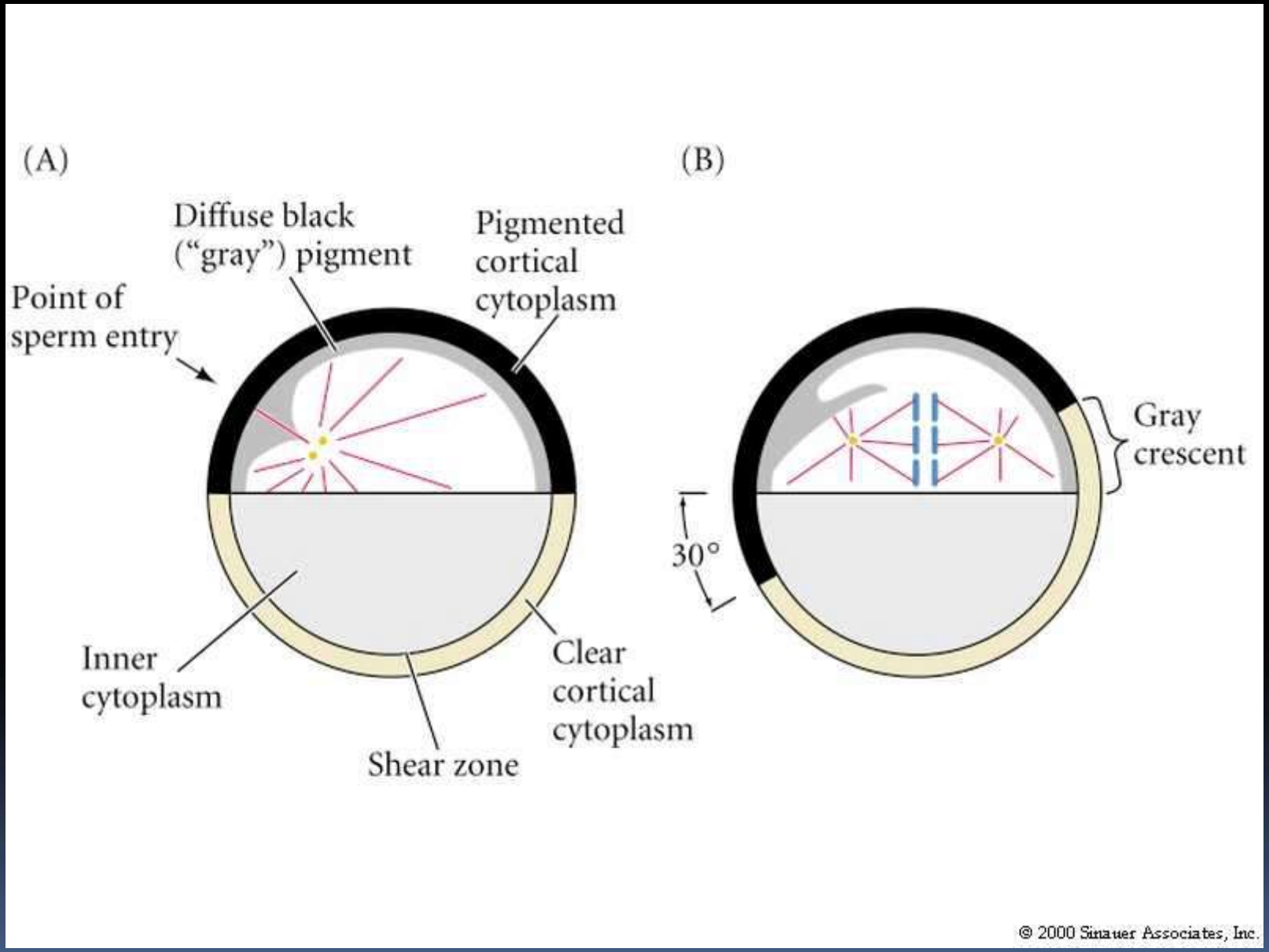
(A)
Diffuse black
("gray") pigment
Pigmented
cortical
cytoplasm
Point of
sperm entry
Inner
cytoplasm
Clear
cortical
cytoplasm
Shear zone
(B)
Gray
crescent
30°
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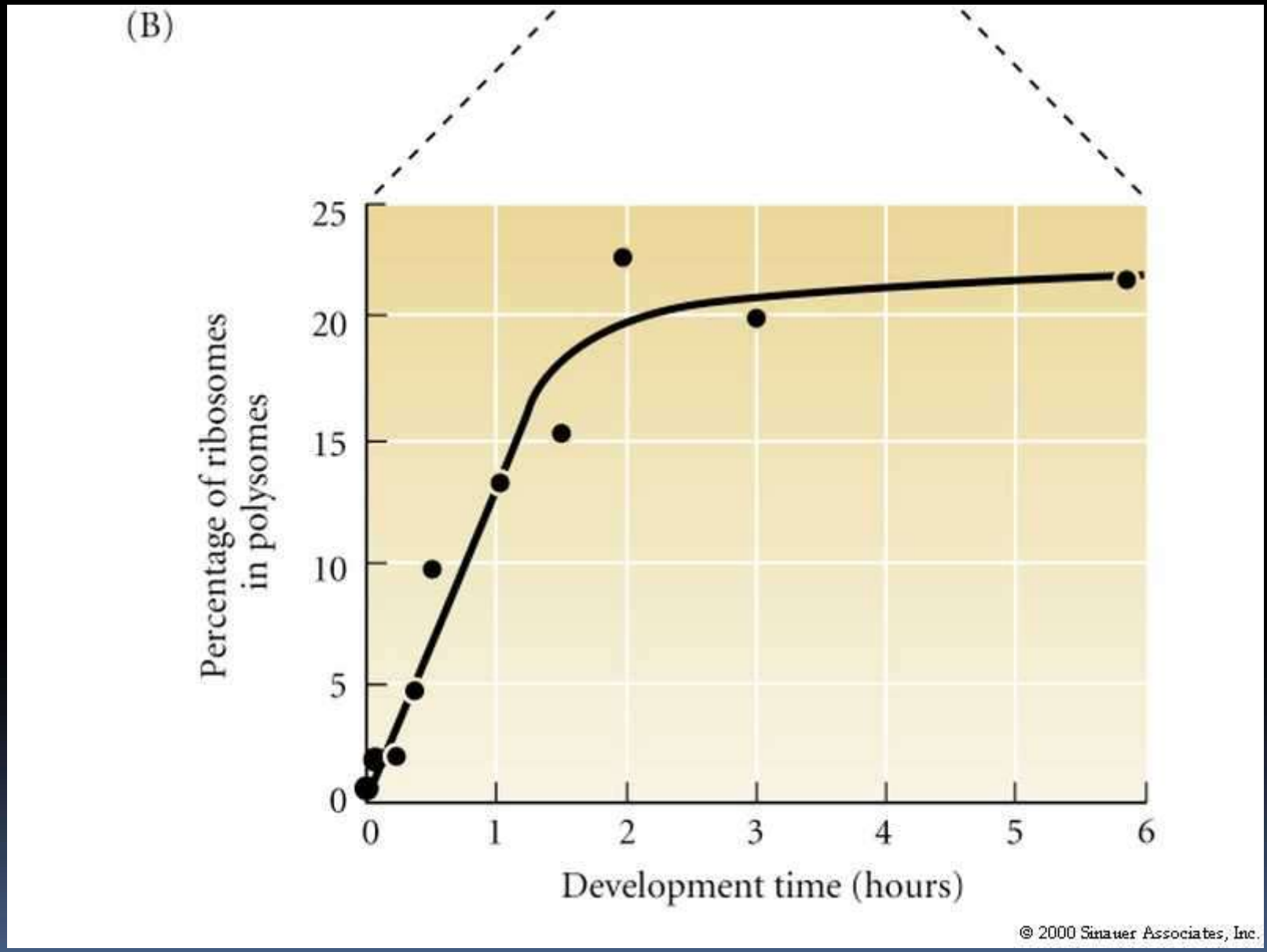
(B)
Percentage of ribosomes in polysomes
25
20
15
10
5
0
0
1
2
3
4
5
6
Development time (hours)
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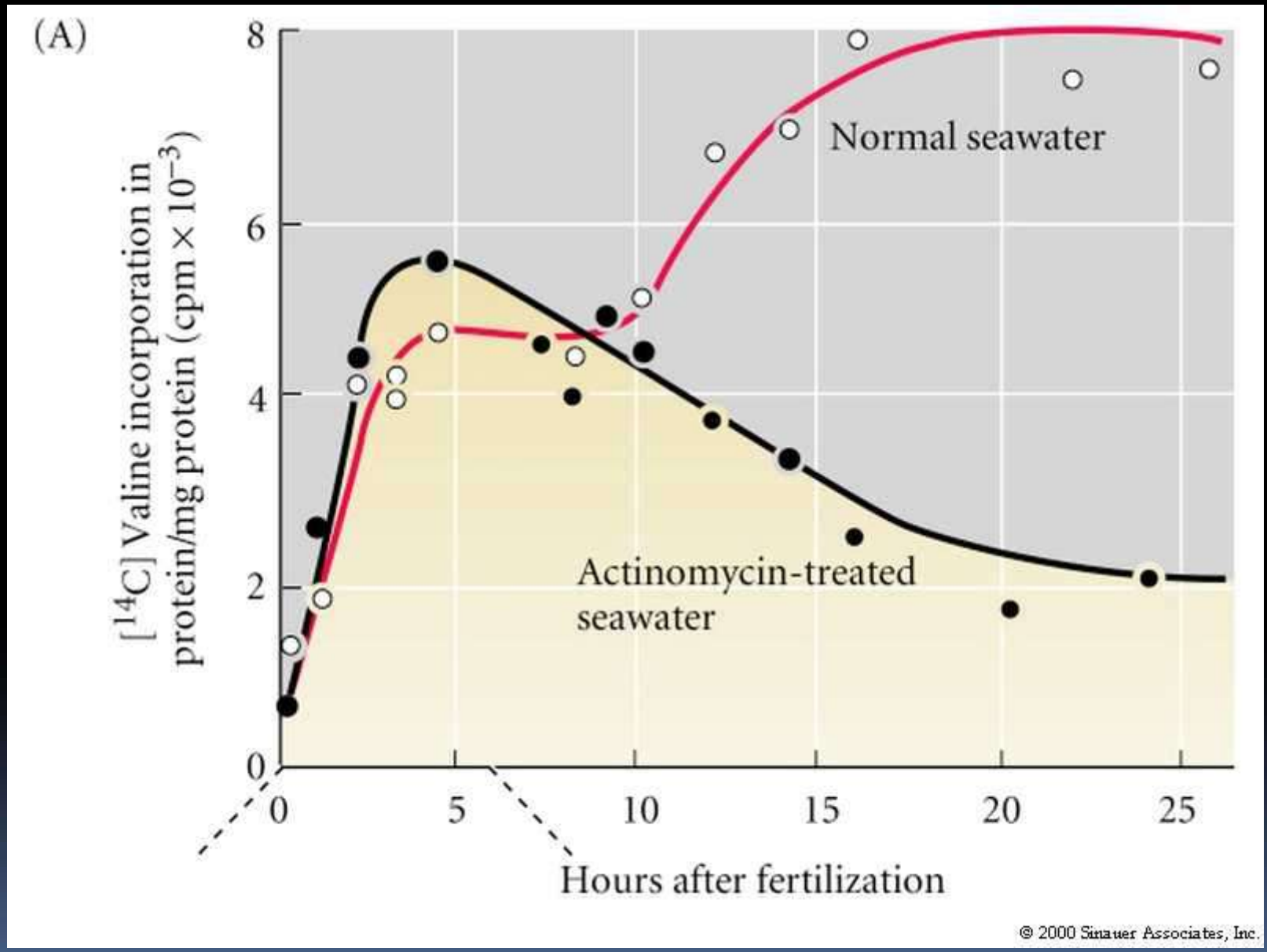
(A)
[14C] Valine incorporation in protein/mg protein (cpm × 10−3)
8
6
4
2
0
0
5
10
15
20
25
Hours after fertilization
Normal seawater
Actinomycin-treated seawater
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Organizer Concept

Male + Female ♀

Fertilization

Zygote

Repeated cleavage

Blastomeres

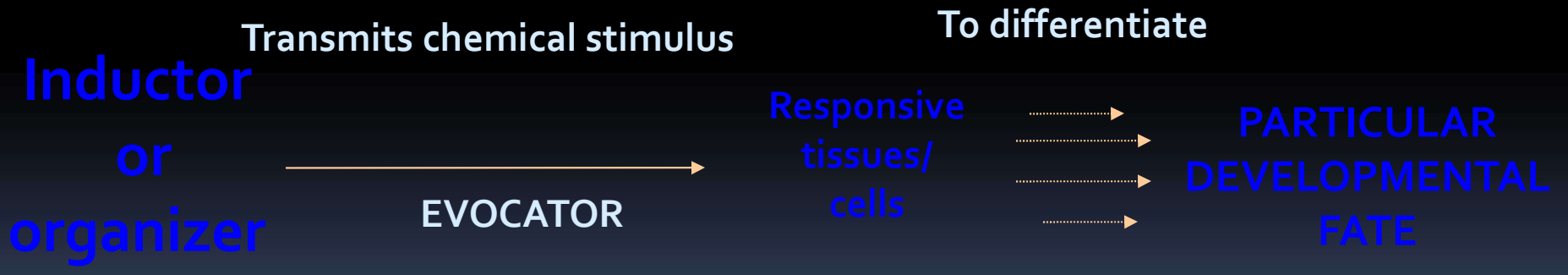
Grow Divide Differentiate

Complex Organism

Early gastrulation blue print of the future organ system established

Depending upon

Specific relationships of a particular (assigned) group of tissues of the developing embryo



MORPHOGENETIC EFFECT PRODUCED IS COINED AS EMBRYONIC INDUCTION

INDUCTION AND COMPETENCE

The kind of interaction at close range between two or more cells/tissues of different histories and properties - *Induction*

INDUCTION

INDUCER

Tissue A → Tissue B

RESPONDER

Induced tissue

Changes the cellular behaviour

The ability to respond to a specific inductive signal - Competence – *Actively acquired condition*

PAX 6 protein in the developing mammalian eye competent to respond to the inductive signal coined as *Competence factor*

INDUCTIVE INTERACTION

INSTRUCTIVE

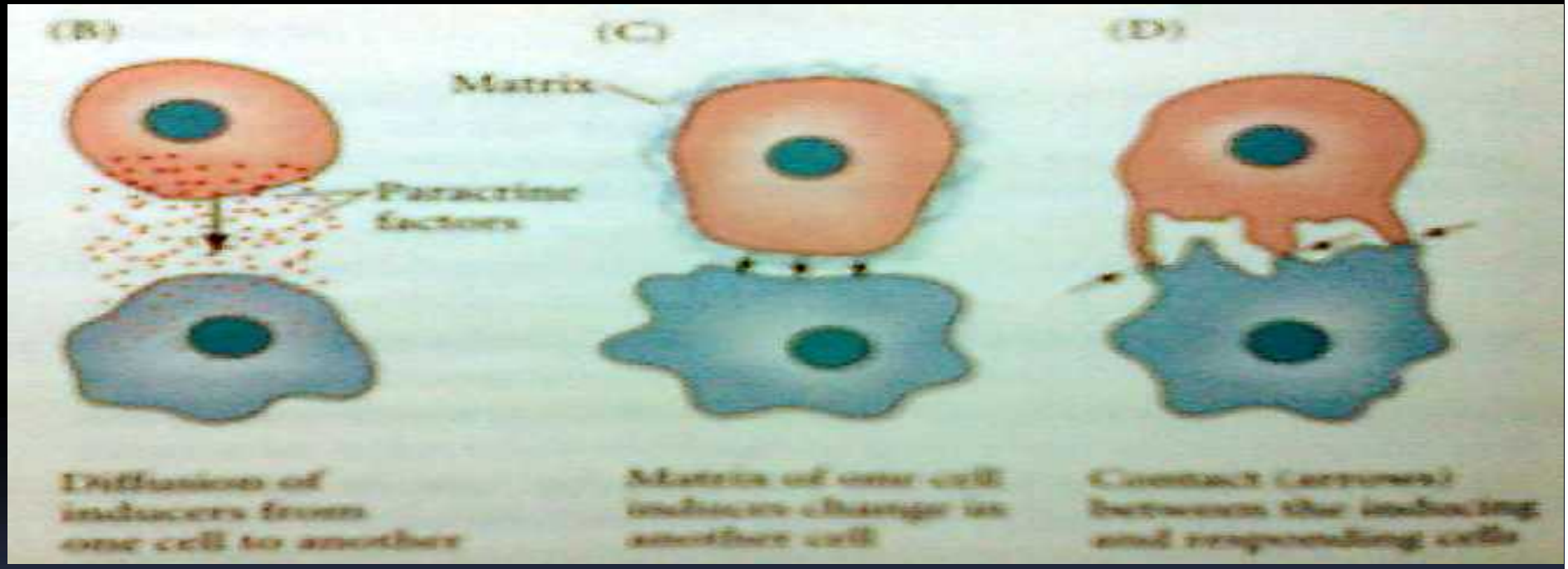
Responsive tissue has the option of forming more than one type of tissues depending upon the nature of inductive stimulus

PERMISSIVE

Responding tissue contains all the potentials that are to be expressed and needs only an environment that allows the expression of these traits

PARACRINE FACTORS

How are the signals between inducer and responder transmitted?



Diffusion of inducers from one cell to other

Matrix of one cell induces change in other cell

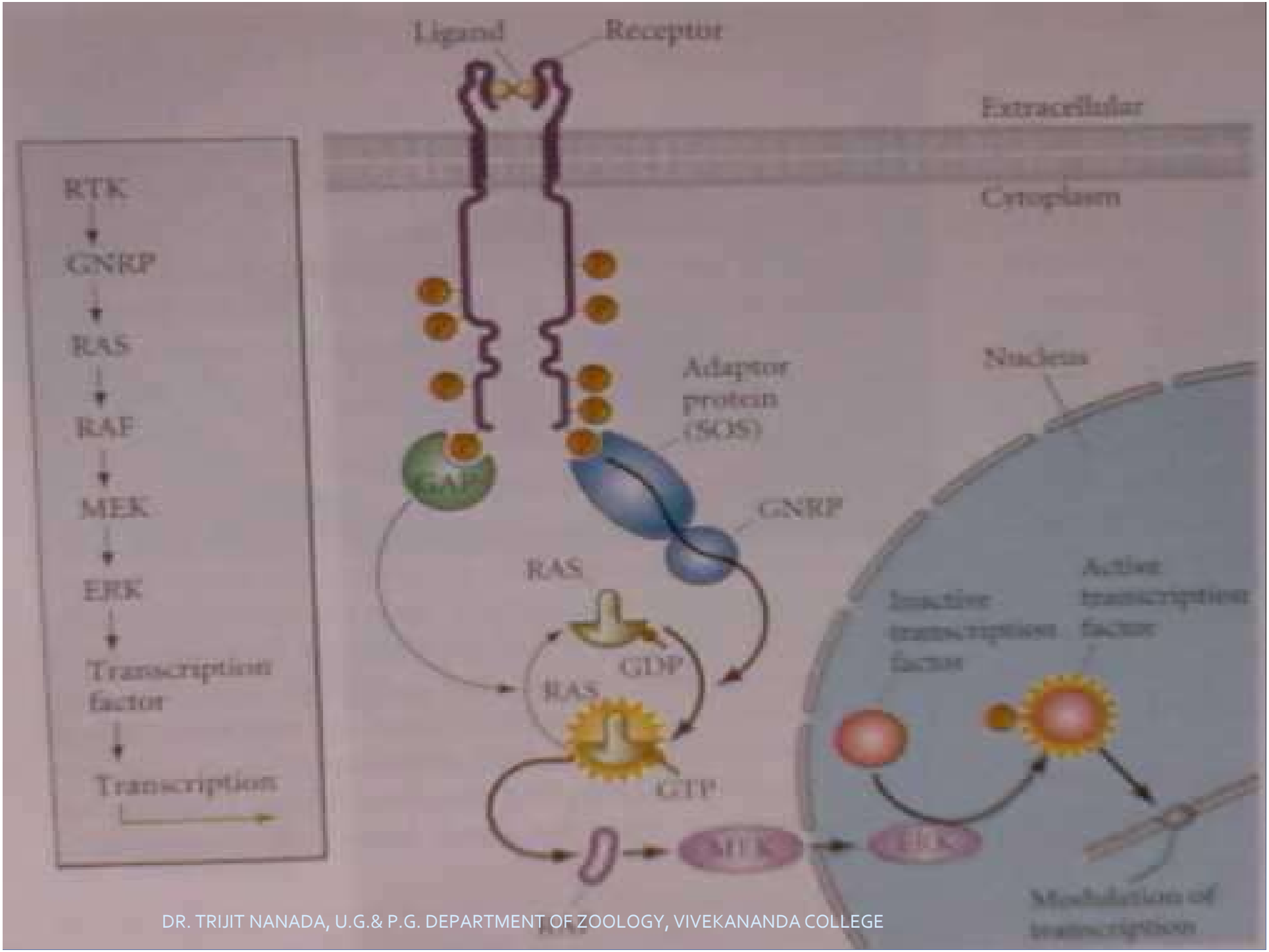
Contact (arrows) between inducing and responding cells

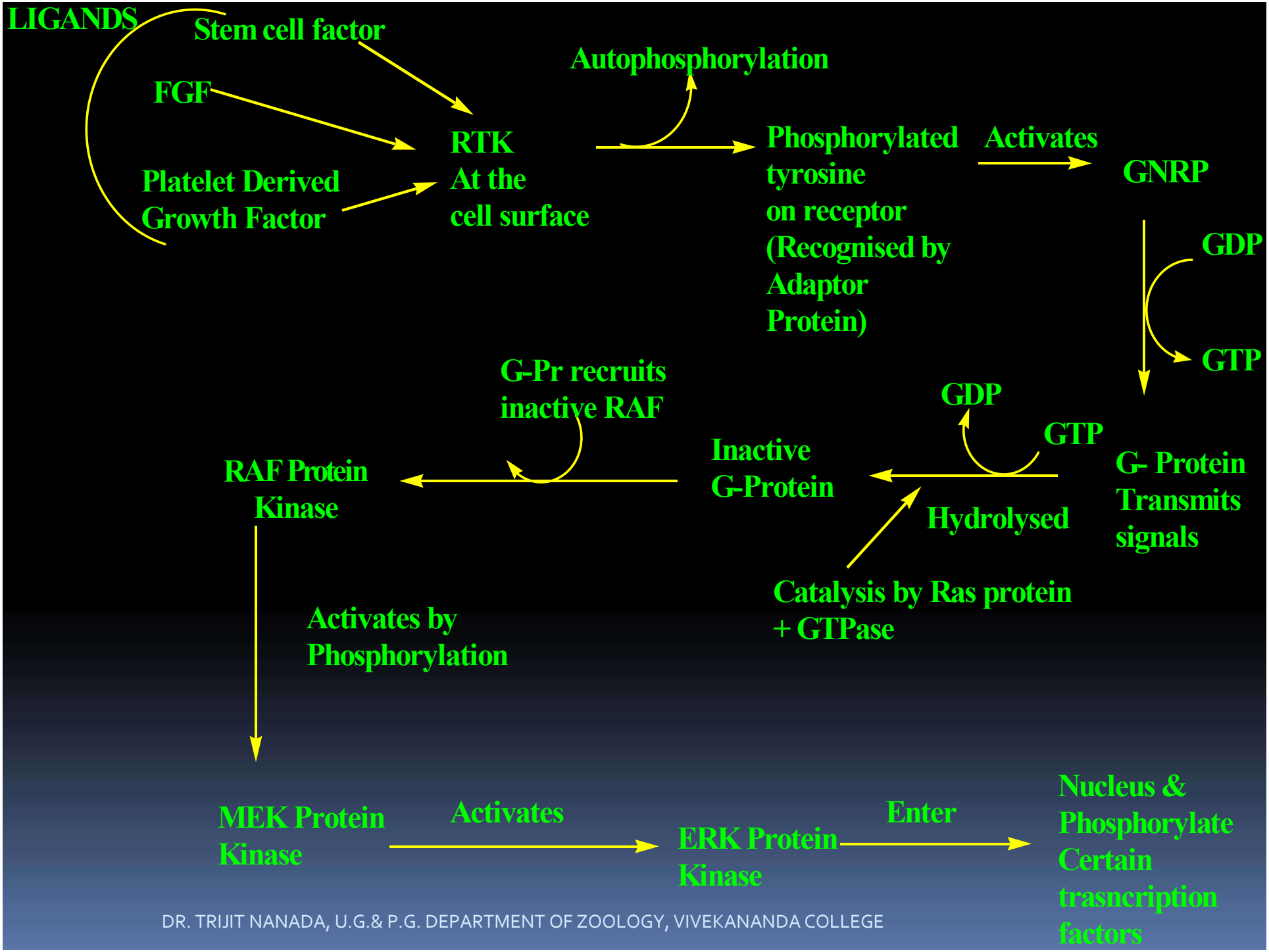
CELL SURFACE RECEPTORS AND THEIR SIGNAL TRANSDUCTION PATHWAYS

The pathways between cell membrane and genome

Steps involved:

- Ligand (paracrine factor) binds its receptor in the ECS
- Ligand induces conformational changes in the receptor structure
- Transmission of transmembrane information to the cytoplasmic domain
- The conformational change in the cytoplasmic domain-induces enzymatic activities.


Ligand
Receptor
Extracellular
Cytoplasm
RTK
GNRP
RAS
RAF
MEK
ERK
Transcription factor
Transcription
Adaptor protein (SOS)
GNRP
RAS
GDP
RAS
GTP
Nucleus
Inactive transcription factor
Active transcription factor
Modulation of transcription


LIGANDS
Stem cell factor
FGF
Platelet Derived Growth Factor
RTK At the cell surface
Autophosphorylation
Phosphorylated tyrosine on receptor (Recognised by Adaptor Protein)
Activates
GNRP
GDP
GTP
G-Pr recruits inactive RAF
RAF Protein Kinase
Inactive G-Protein
GDP
GTP
Hydrolysed
G- Protein Transmits signals
Catalysis by Ras protein + GTPase
Activates by Phosphorylation
MEK Protein Kinase
Activates
ERK Protein Kinase
Enter
Nucleus & Phosphorylate Certain trasncription factors

"A TRIBUTE TO THE TRUE ORGANIZERS"

A piece of the upper blastopore lip of an amphibian embryo undergoing gastrulation exerts an organizing effect on its environment in such a way that, if transplanted to an indifferent region of another embryo, it causes there the formation of a secondary embryonic anlage. Such a piece can therefore be designated as a Organizer.

Hans Spemann

Hilde Mangold

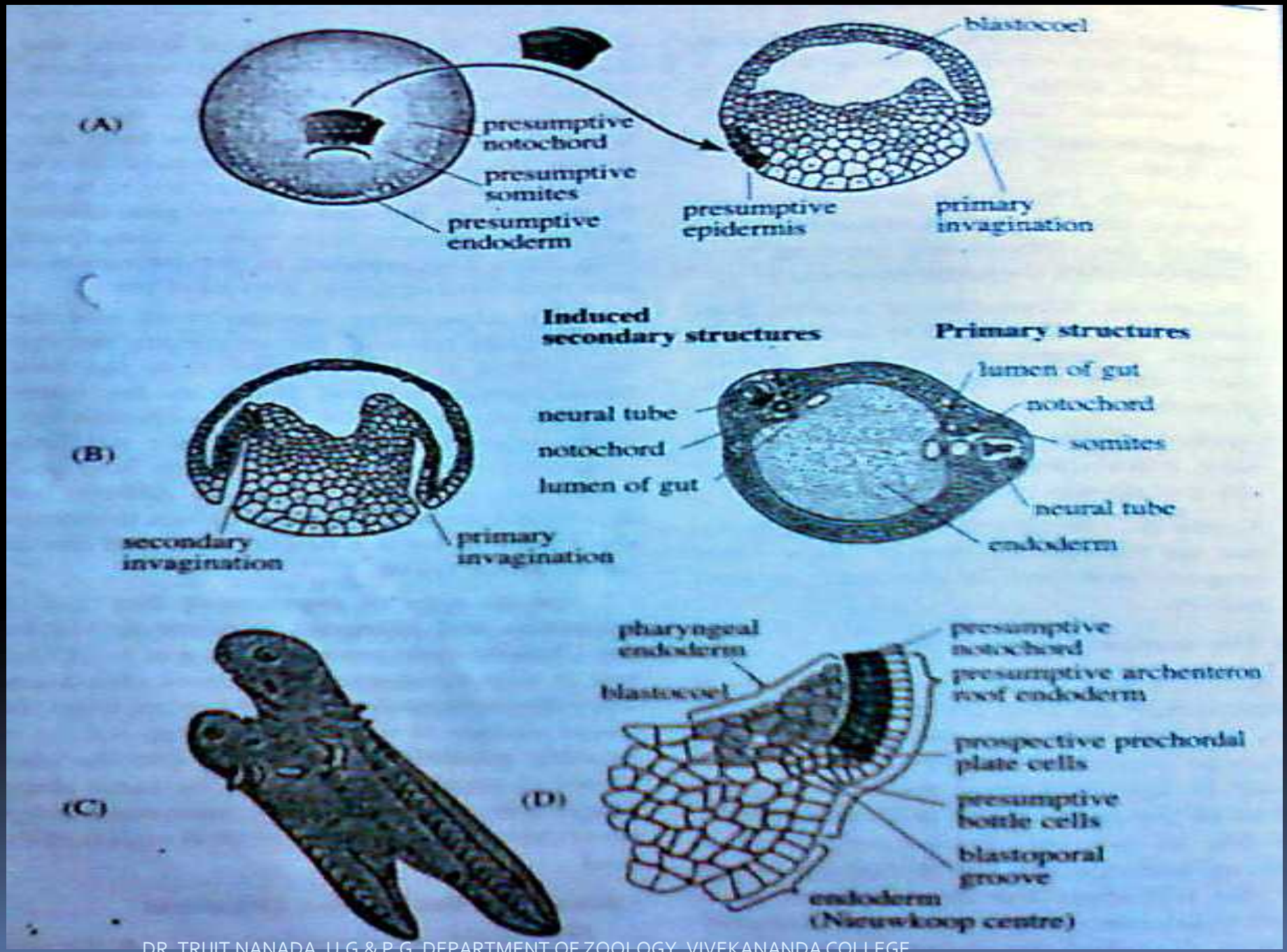
The Primary Embryonic Induction

The transplantation experiment conducted in 1924 by Hans Spemann and his pupil Hilde Mangold

- Showed that, of all the tissues in the early gastrula, only one has its fate determined - the dorsal lip of the blastopore, which was derived from the gray crescent cytoplasm.

- When this dorsal lip material was taken from pigmented *Triturus taeniatus* and transplanted into the presumptive belly skin region of the non-pigmented *T. cristatus* of the same age, then it not only continued to be the blastopore lip, but also initiated gastrulation and embryogenesis in the surrounding tissue

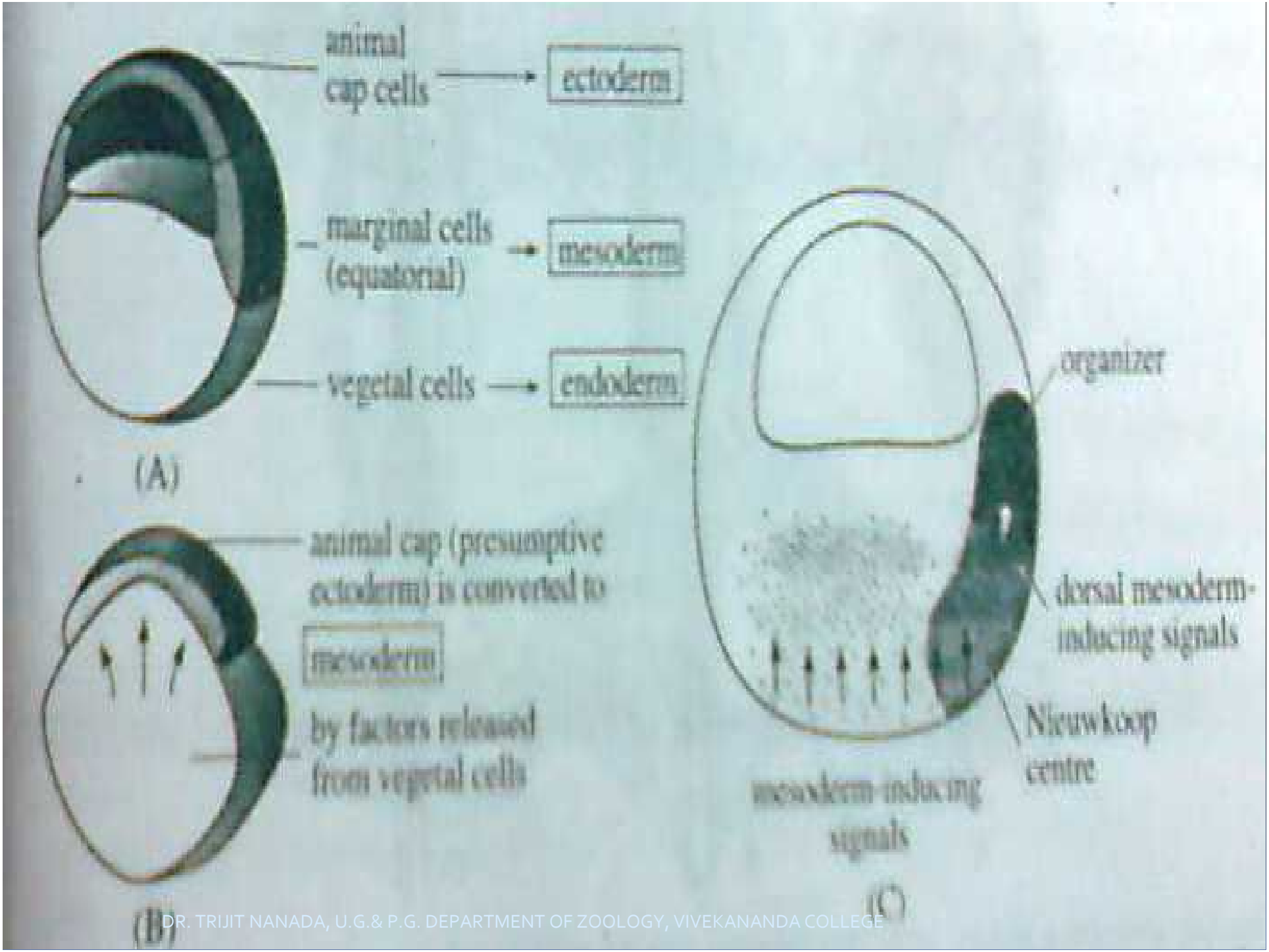
  This resulted in the formation of two conjoined embryos instead of one.


(A)
presumptive notochord
presumptive somites
presumptive endoderm
blastocoel
presumptive epidermis
primary invagination
(B)
Induced secondary structures
Primary structures
neural tube
notochord
lumen of gut
secondary invagination
primary invagination
lumen of gut
notochord
somites
neural tube
endoderm
(C)
(D)
pharyngeal endoderm
blastocoel
presumptive notochord
presumptive archenteron roof endoderm
prospective prechordal plate cells
presumptive bottle cells
blastoporal groove
endoderm (Nieuwkoop centre)

The Nieuwkoop center

Peter Nieuwkoop **demonstrated how the dorsal blastoporal lip obtained its properties.**

- Demonstrated that the properties of the newly formed mesoderm were induced by vegetal (presumptive endoderm) cells lying beneath it.
- Nieuwkoop removed the equatorial cells (presumptive mesoderm) from a blastula and showed that neither the animal cap (presumptive ectoderm) nor the vegetal cap (presumptive endoderm) produced any mesodermal tissue.
- When the two caps were applied one against the other, the animal cap cells were induced to form mesodermal structures such as notochord, muscles, kidney cells and blood cells.
- The dorsal most vegetal cells of the blastula are capable of inducing the organizer. These cells are called the Nieuwkoop center.


animal cap cells
ectoderm
marginal cells (equatorial)
mesoderm
vegetal cells
endoderm
(A)
animal cap (presumptive ectoderm) is converted to
mesoderm
by factors released from vegetal cells
(B)
organizer
dorsal mesoderm-inducing signals
Nieuwkoop centre
mesoderm-inducing signals
(C)

Molecular setup of the Nieuwkoop center

1. The endoderm in *Xenopus*, induces the formation of mesoderm due to the presence of *Xenopus brachyury* (Xbra) gene.
2. The Xbra protein is a transcription factor that activates the genes that produce mesoderm specific proteins.
3. All the cells of the vegetal area are able to induce the overlying marginal cells to become mesoderm.
4. Only the dorsal most marginal cells become the organizer.
5. The factor that forms the Nieuwkoop center at this region is a multifunctional protein, called β-catenin, that acts as a nuclear transcription factor.
6. β-catenin in *Xenopus* embryos, begin to accumulate at the dorsal region of the egg during the cytoplasmic movements of fertilization.
7. Initially the cells containing β-catenin is present both in the Nieuwkoop center and the organizer regions, but by late cleavage it comes to lie specifically in the Nieuwkoop center.
8. β-catenin is essential for forming the dorsal axis.

Conclusion:

The organizer tissue serves the following five major functions

It has the ability to become dorsal mesoderm (pre – chordal plate, chordamesoderm etc.)

It has the ability to dorsalize the surrounding mesoderm into lateral mesoderm.

It has the ability to induce the dorsal ectoderm into neural ectoderm.

It has the ability to subsequently transform the neural ectoderm into the neural tube.

It initiates the movements of gastrulation.

Recently it is found that the endoderm releases a signal protein called activin that induce the formation of mesoderm prior to gastrulation. This is evidenced through experimentation - when isolated ectoderm cultured with activin and consequently ectoderm turned mesoderm in gastrulation. The ingracing notochordal cells later release another signal protein follistatin which otherwise inactivating activin available in the endoderm. Therefore a mechanism is being pursued wherein notochord has the ability to maintain follistatin secretion and surmounts the effects of activin. There after an inducer coming out from the notochord itself which is known as noggin to produce neural plate only.

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