C8545 Developmental Biology

Lesson 4

Vertebrate Organogenesis: Ectodermal Derivatives

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Outline of Lesson 4

Organogenesis in Vertebrates: Ectodermal Derivatives

- Early development of mammals
  - oogenesis and blastula formation
  - placental tissue differentiation
  - extraembryonic tissue formation
  - use of embryonal cells in mammalian transgenosis
- Differentiation of neural tissue
  - mechanisms of neural tissue specification
  - signaling in the spinal cord development
  - spatial-specific differentiation of neural crest derivatives
  - stratification of neural tube
- Development of brain and its derivatives
  - brain vesicles formation and development
  - eye development
  - cranial ganglia and sensory organ epithelia
- Integument

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Organogenesis in Vertebrates: Ectodermal Derivatives

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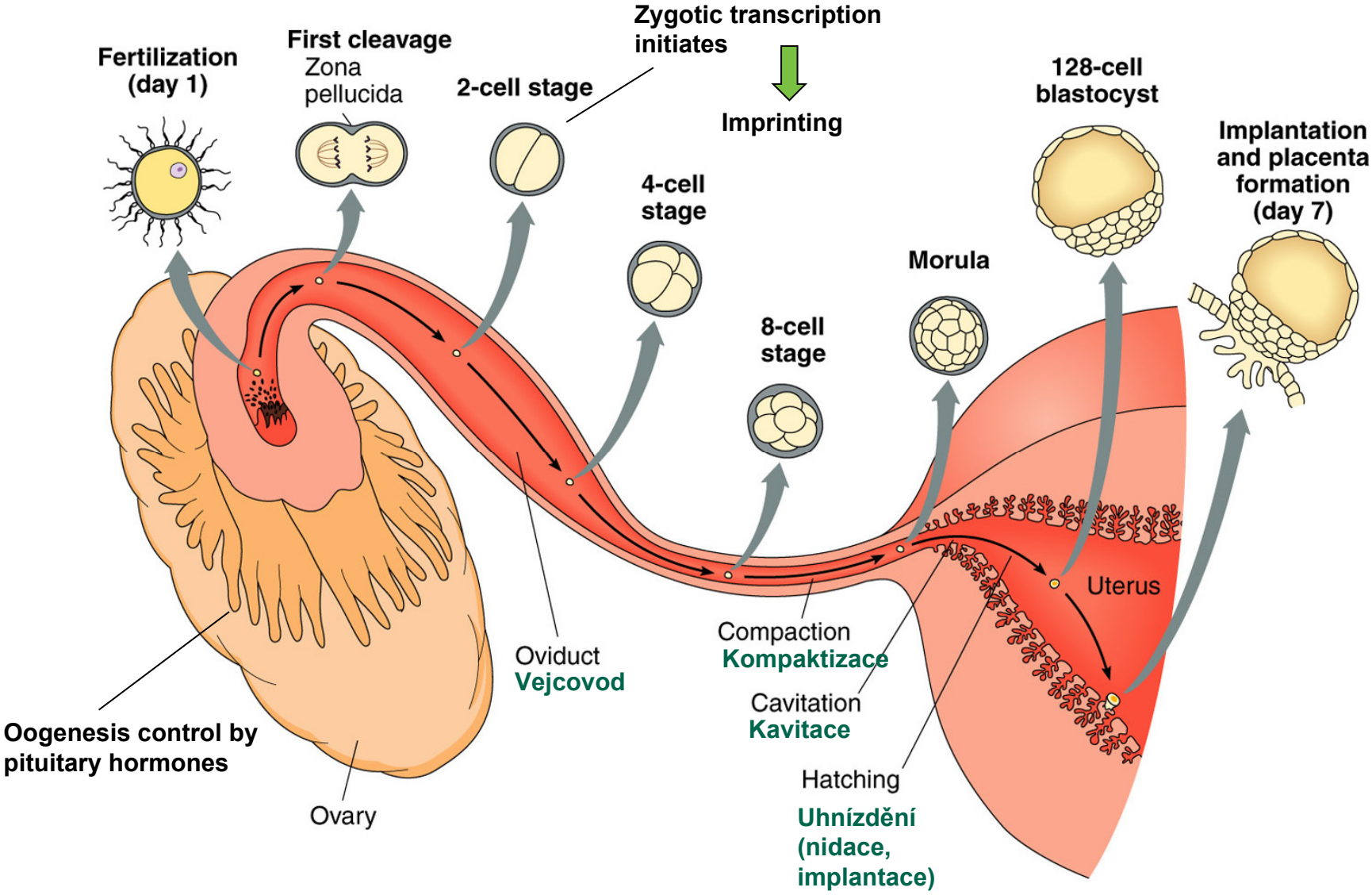
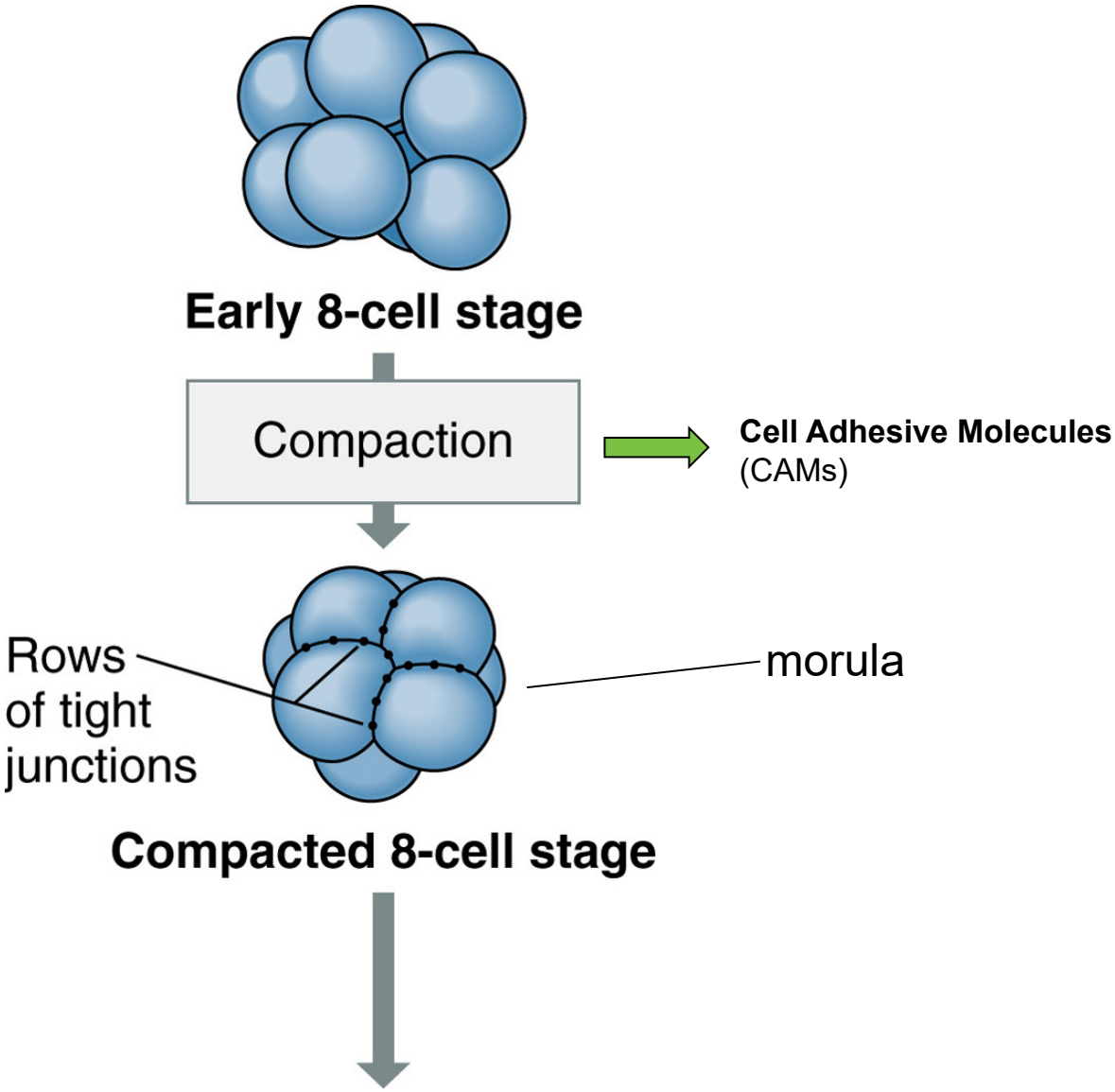
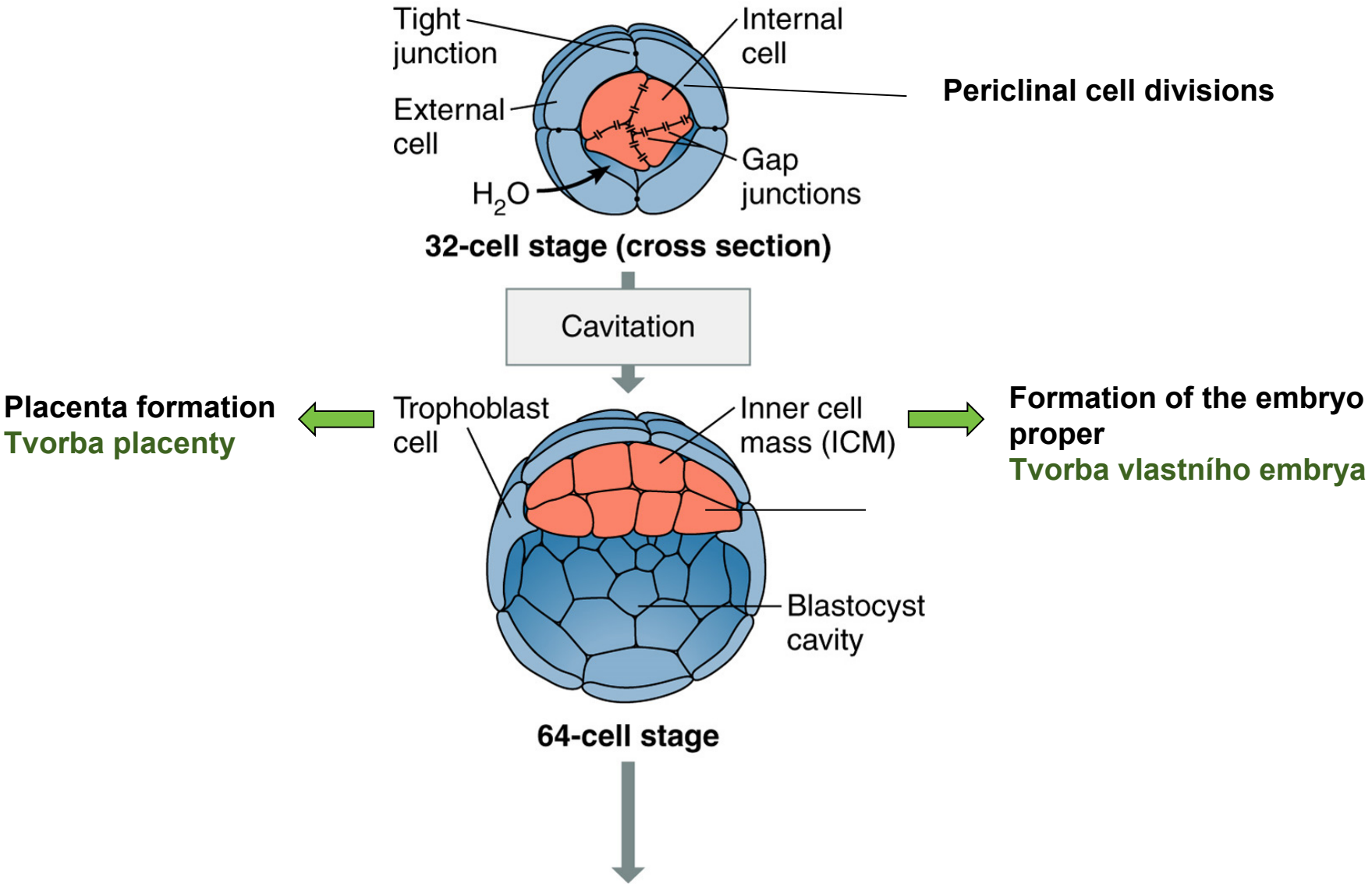
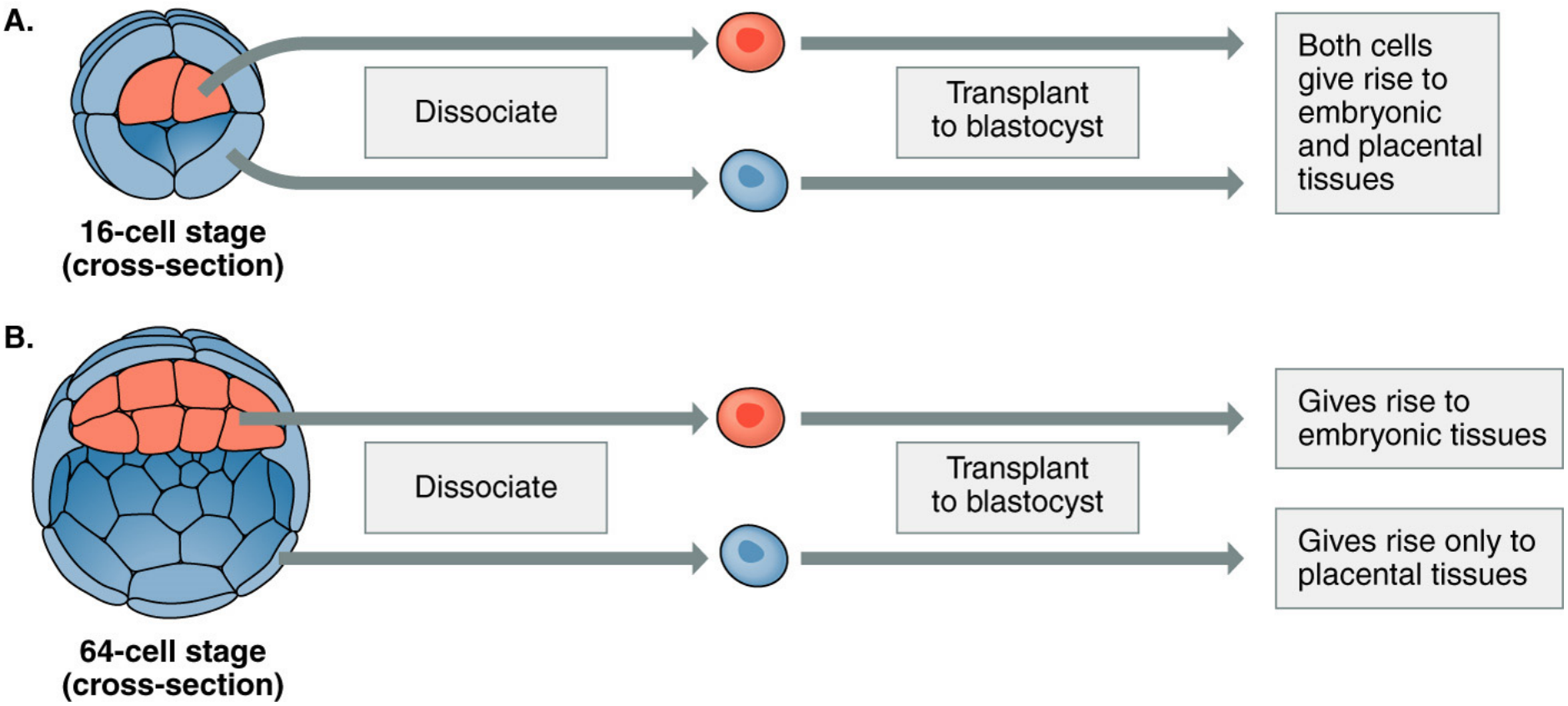
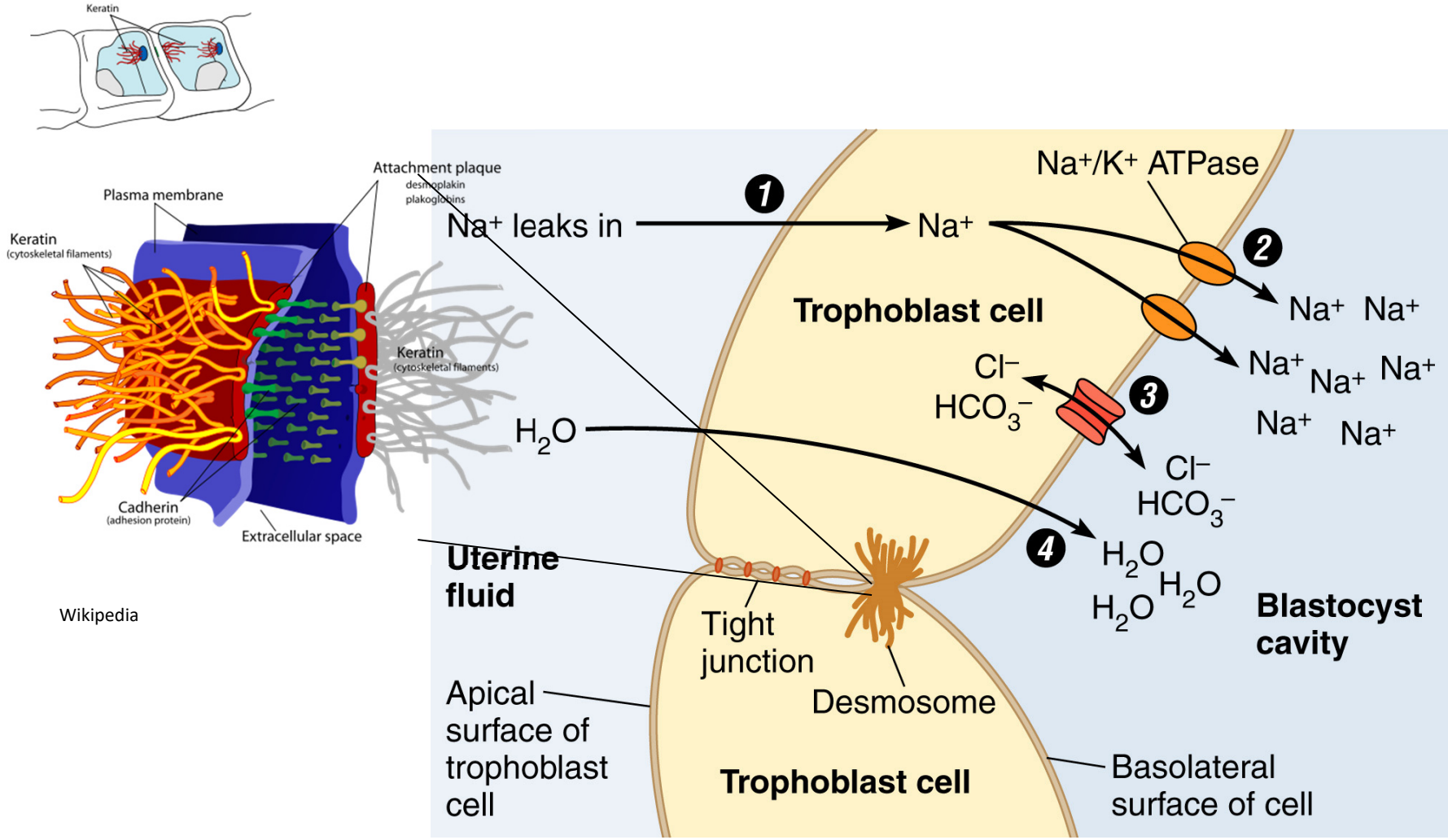
  - oogenesis and blastula formation


Fertilization (day 1)
First cleavage
Zona pellucida
2-cell stage
Zygotic transcription initiates
Imprinting
4-cell stage
8-cell stage
Morula
128-cell blastocyst
Implantation and placenta formation (day 7)
Uterus
Oviduct
Vejcovod
Compaction
Kompaktizace
Cavitation
Kavitace
Hatching
Uhnízdění (nidace, implantace)
Oogenesis control by pituitary hormones
Ovary

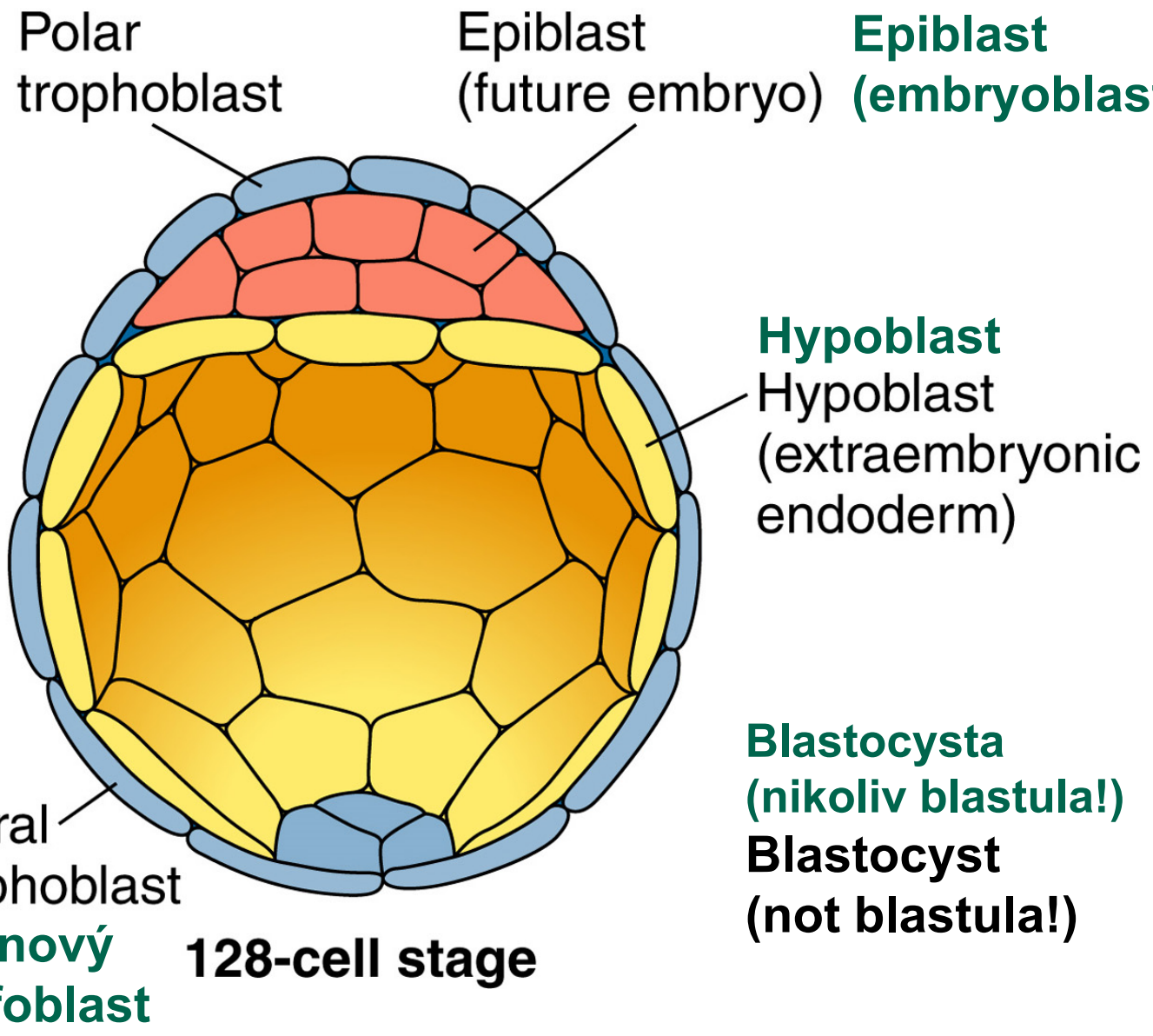

Early 8-cell stage
Compaction
Cell Adhesive Molecules
(CAMs)
Rows
of tight
junctions
morula
Compacted 8-cell stage


Tight junction
Internal cell
External cell
Gap junctions
H_2O
32-cell stage (cross section)
Periclinal cell divisions
Cavitation
Placenta formation
Tvorba placenty
Trophoblast cell
Inner cell mass (ICM)
Blastocyst cavity
64-cell stage
Formation of the embryo proper
Tvorba vlastního embrya


A.
Dissociate
Transplant to blastocyst
Both cells give rise to embryonic and placental tissues
16-cell stage (cross-section)
B.
Dissociate
Transplant to blastocyst
Gives rise to embryonic tissues
Gives rise only to placental tissues
64-cell stage (cross-section)


Keratin
Attachment plaque
desmoplakin
plakoglobins
Plasma membrane
Keratin
(cytoskeletal filaments)
Keratin
(cytoskeletal filaments)
Cadherin
(adhesion protein)
Extracellular space
Wikipedia
Na+/K+ ATPase
1
Na+ leaks in
Na+
2
Trophoblast cell
Na+ Na+
Na+ Na+ Na+
Na+ Na+
Cl−
HCO3−
3
H2O
Cl−
HCO3−
4
H2O
H2O H2O
H2O
Uterine
fluid
Tight
junction
Desmosome
Blastocyst
cavity
Apical
surface of
trophoblast
cell
Trophoblast cell
Basolateral
surface of cell

Polární trofoblast



Epiblast (embryoblast)

Hypoblast

Stěnový trofoblast

Blastocysta (nikoliv blastula!)

Blastocyst (not blastula!)

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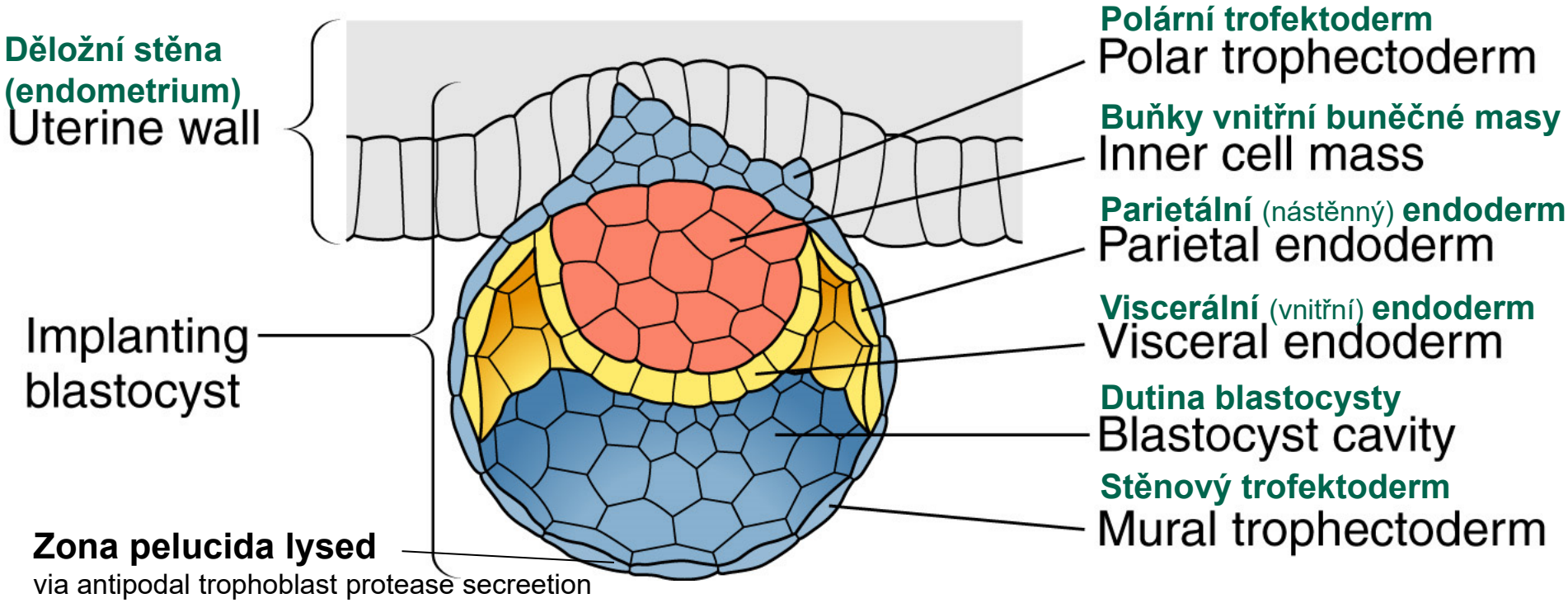
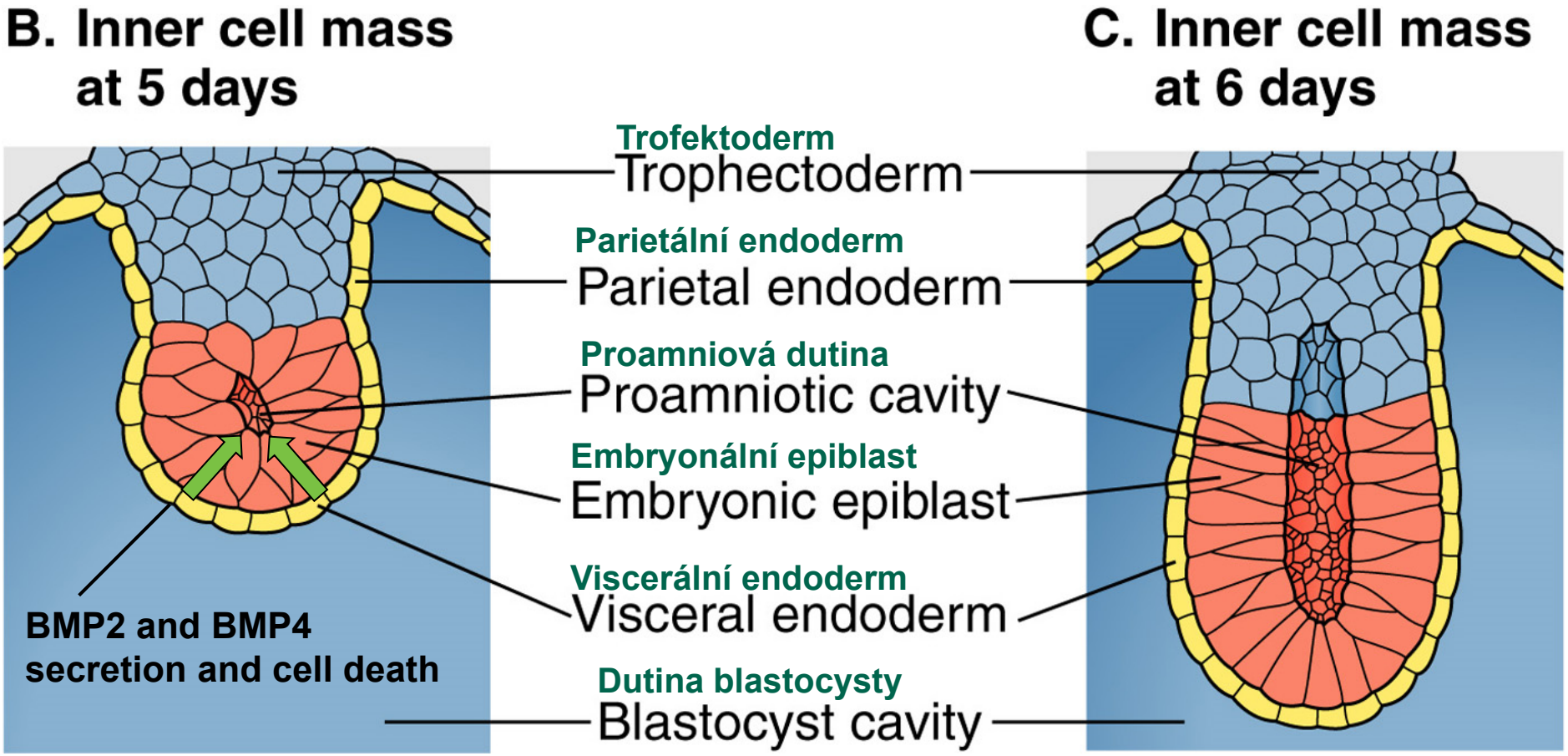
Organogenesis in Vertebrates: Ectodermal Derivatives

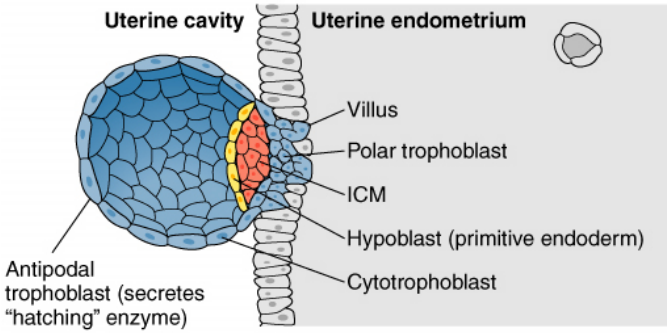
- Early development of mammals
  - oogenesis and blastula formation
  - placental tissue differentiation

A. Early blastocyst at time of implantation (4 days)

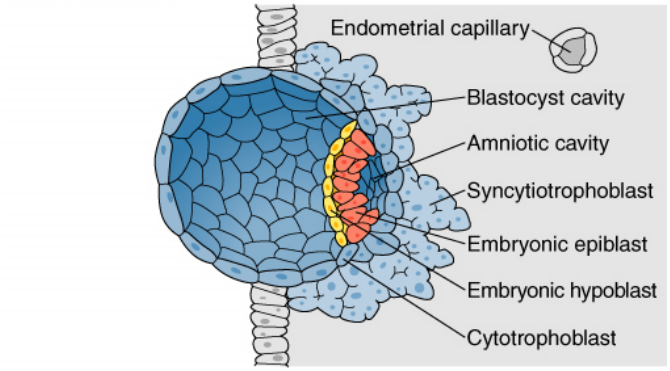



B. Inner cell mass at 5 days
C. Inner cell mass at 6 days
Trofektoderm
Trophectoderm
Parietální endoderm
Parietal endoderm
Proamniová dutina
Proamniotic cavity
Embryonální epiblast
Embryonic epiblast
Viscerální endoderm
Visceral endoderm
Dutina blastocysty
Blastocyst cavity
BMP2 and BMP4 secretion and cell death

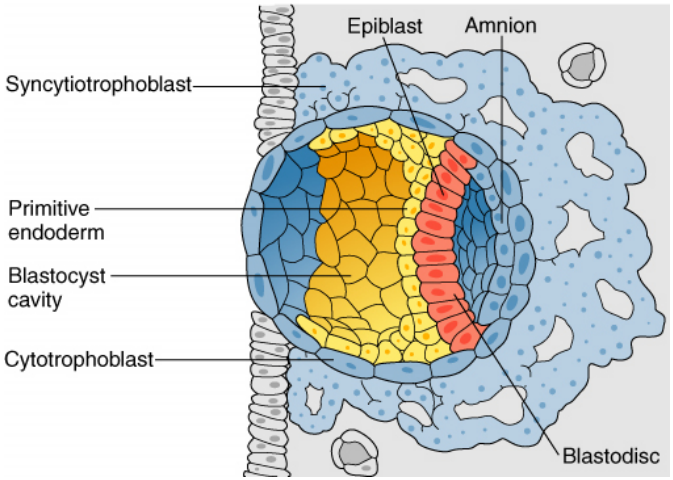
A. Day 7



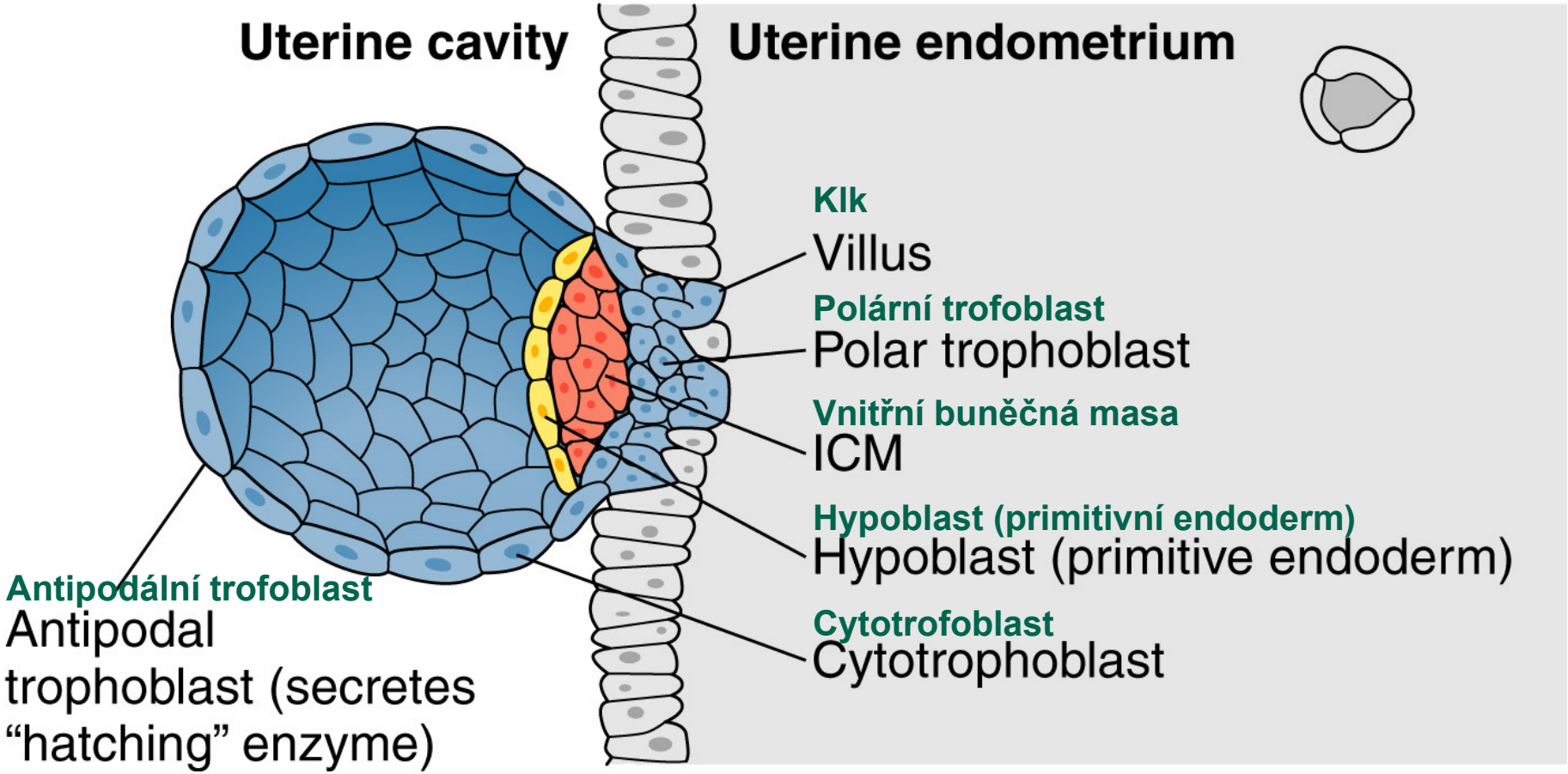
B. Early on Day 8



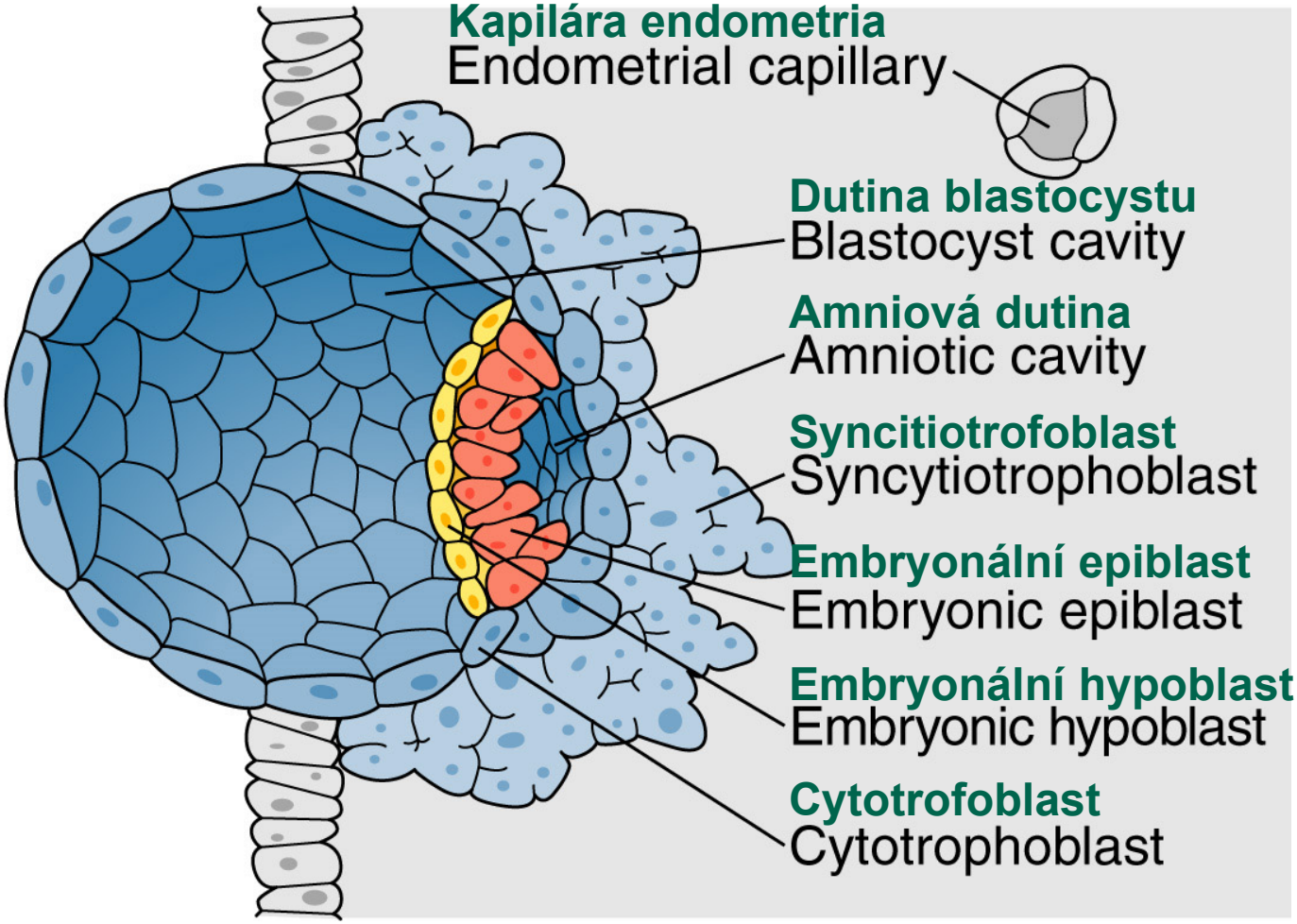
C. Late on Day 8



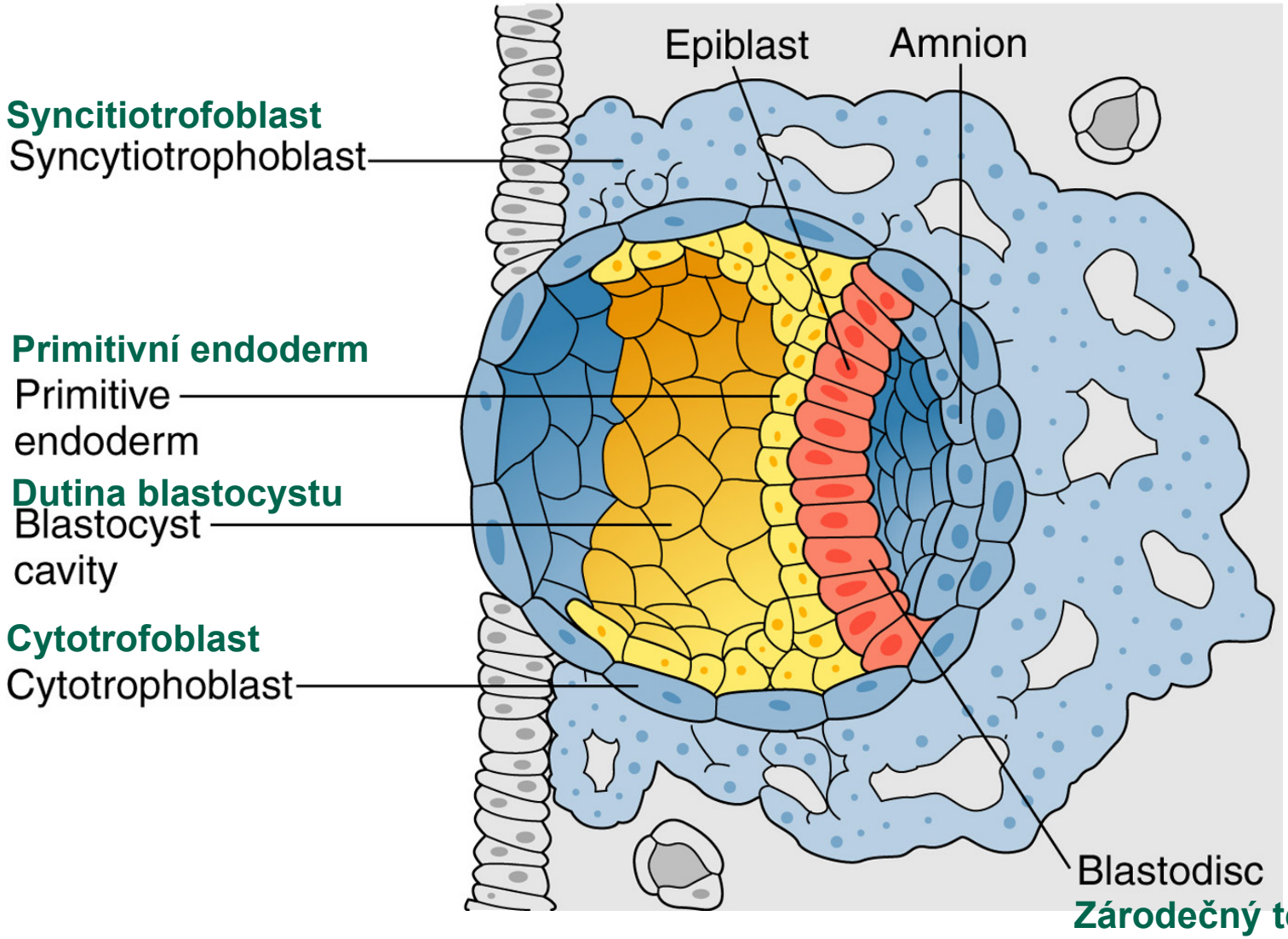
A. Day 7

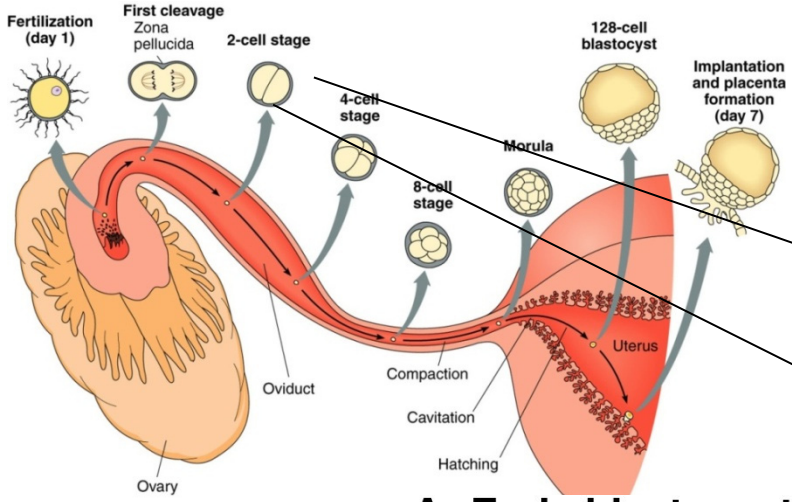

Uterine cavity
Uterine endometrium
Klk
Villus
Polární trofoblast
Polar trophoblast
Vnitřní buněčná masa
ICM
Hypoblast (primitivní endoderm)
Hypoblast (primitive endoderm)
Cytotrofoblast
Cytotrophoblast
Antipodální trofoblast
Antipodal trophoblast (secretes "hatching" enzyme)

B. Early on Day 8



C. Late on Day 8

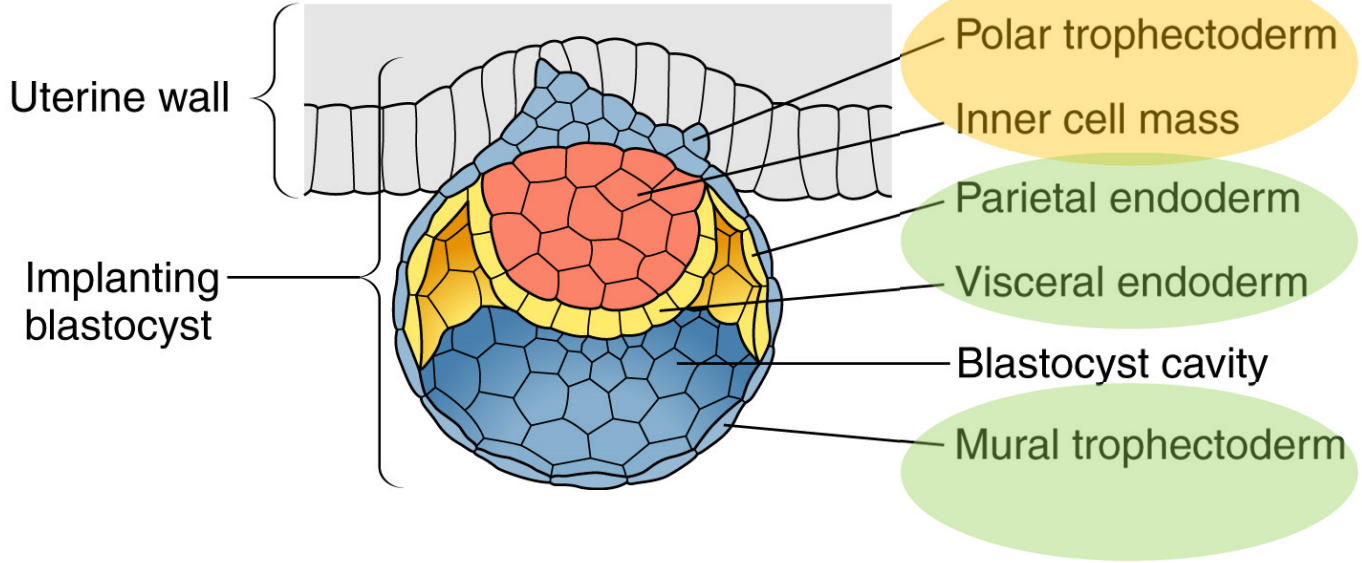




There's very early cell fate specification during mammals embryogenesis at the stage of two-celled embryo!

2-cell stage

A. Early blastocyst at time of implantation (4 days)



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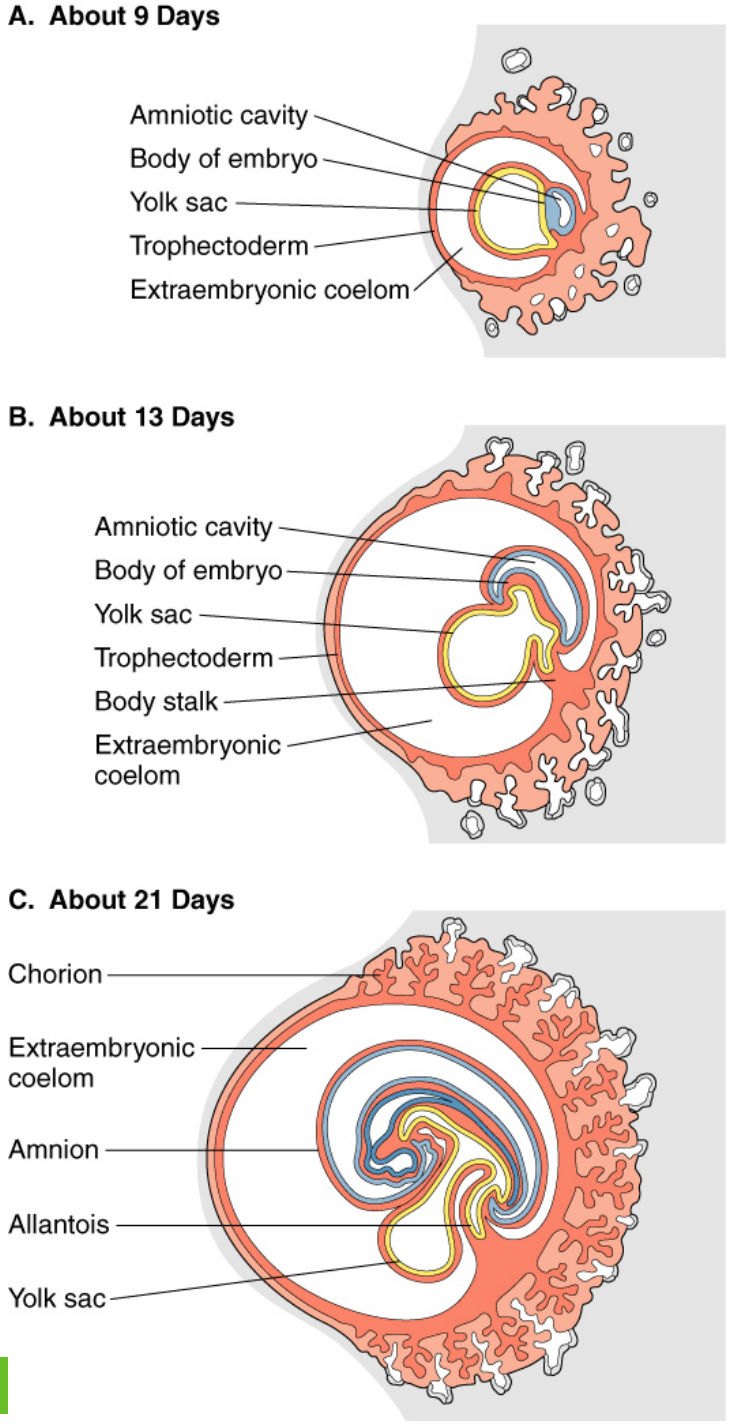
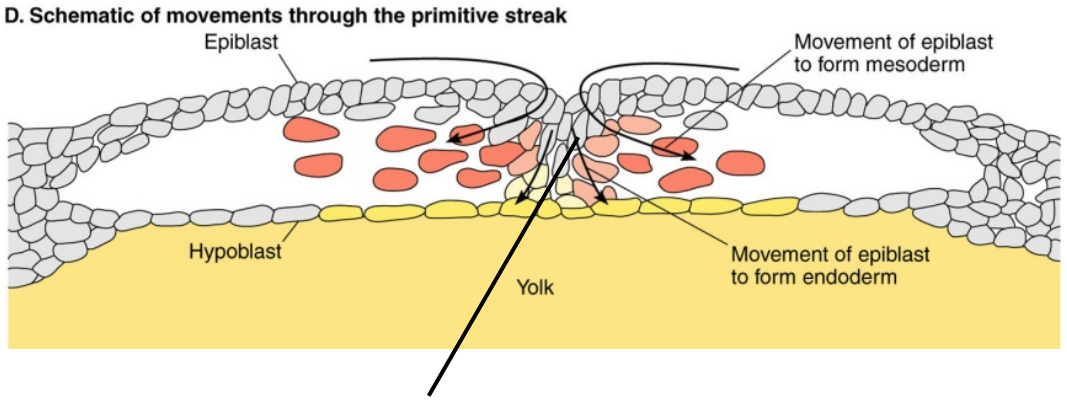
  - extraembryonic tissue formation





- **Processes of the primitive streak and Hensen's node formation are conserved between chicken and human embryos**
- **the first genes involved are being identified (e.g. *HEX, CERBERUS, ARCADIA*, etc.].**
- **However, the head organizing centre seems to be specific and necessary for human embryos.**

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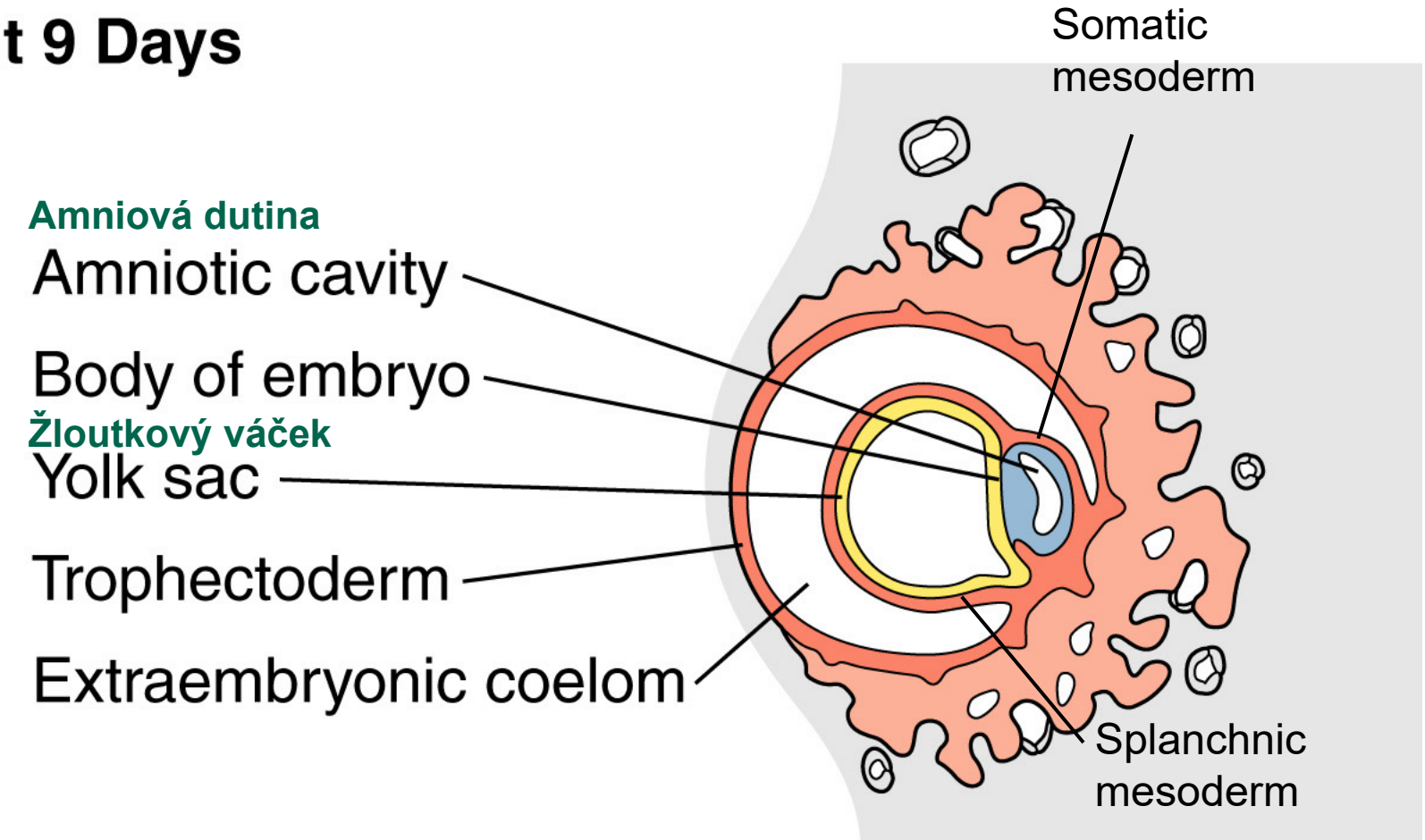
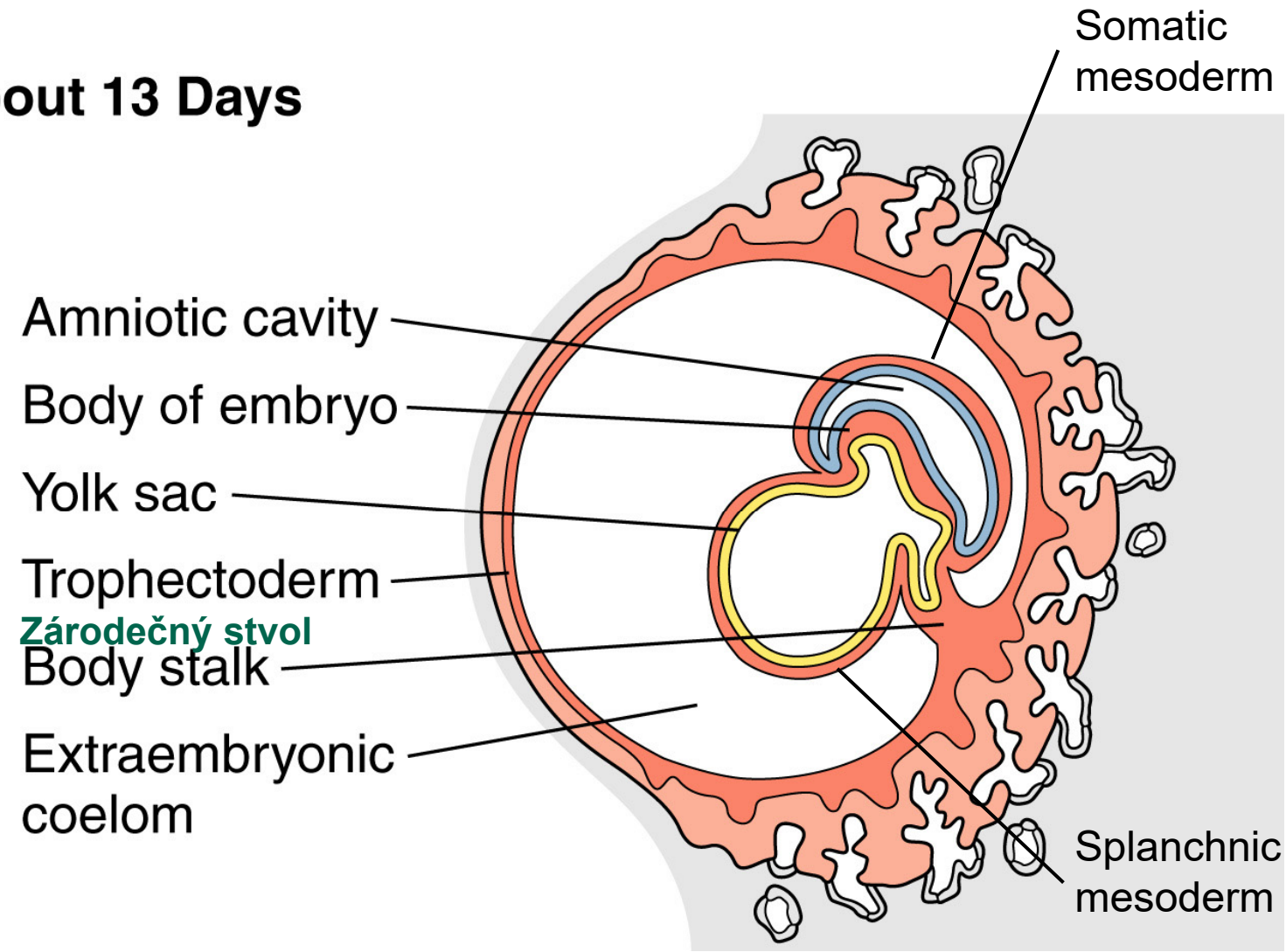
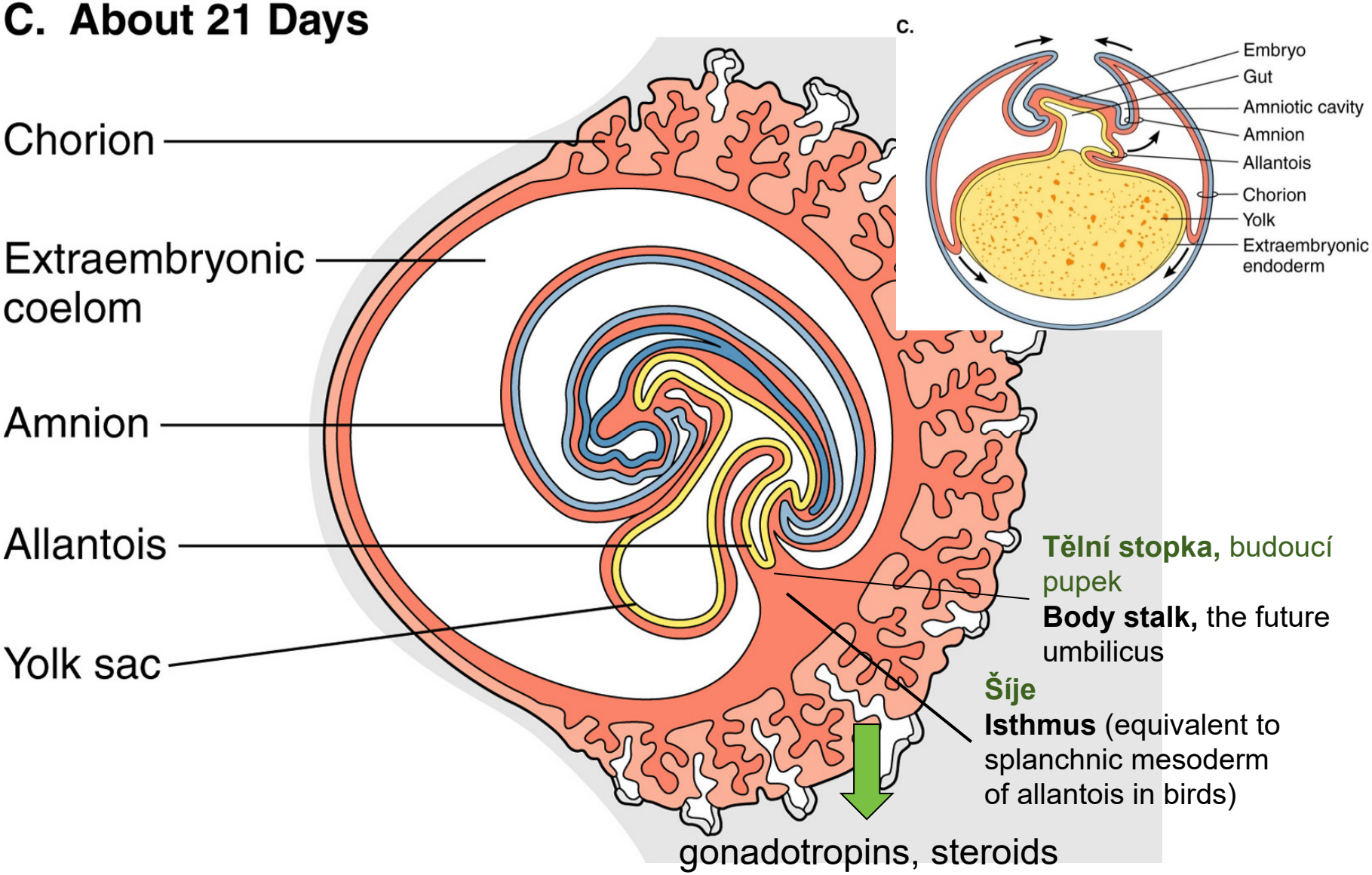
A. About 9 Days



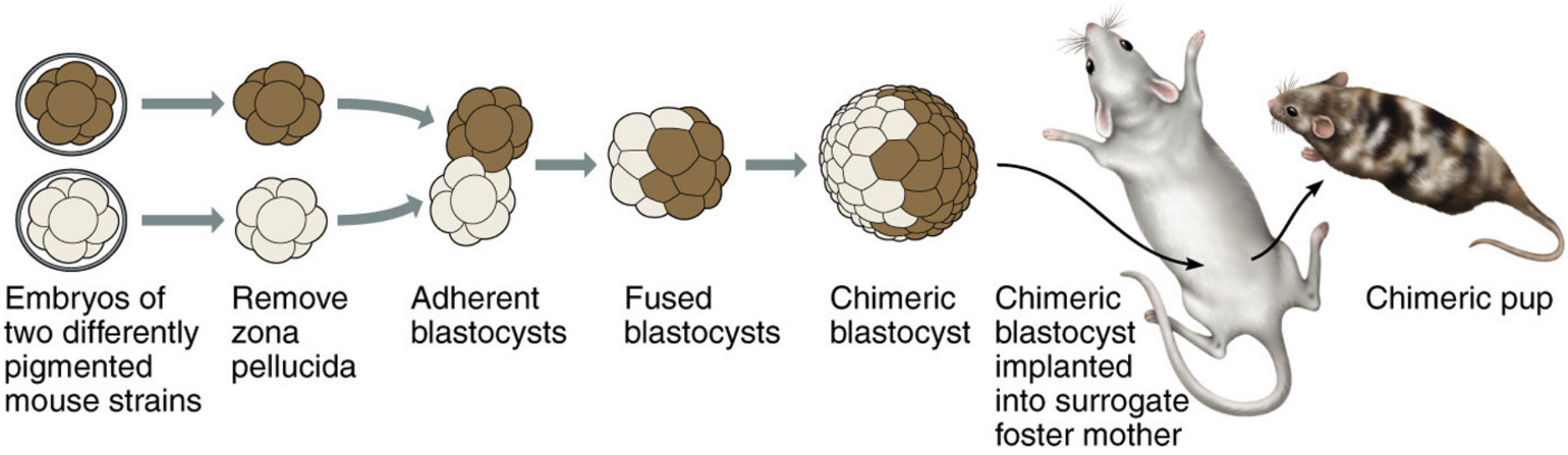
B. About 13 Days



C. About 21 Days



How many cells are necessary for the embryo proper formation?



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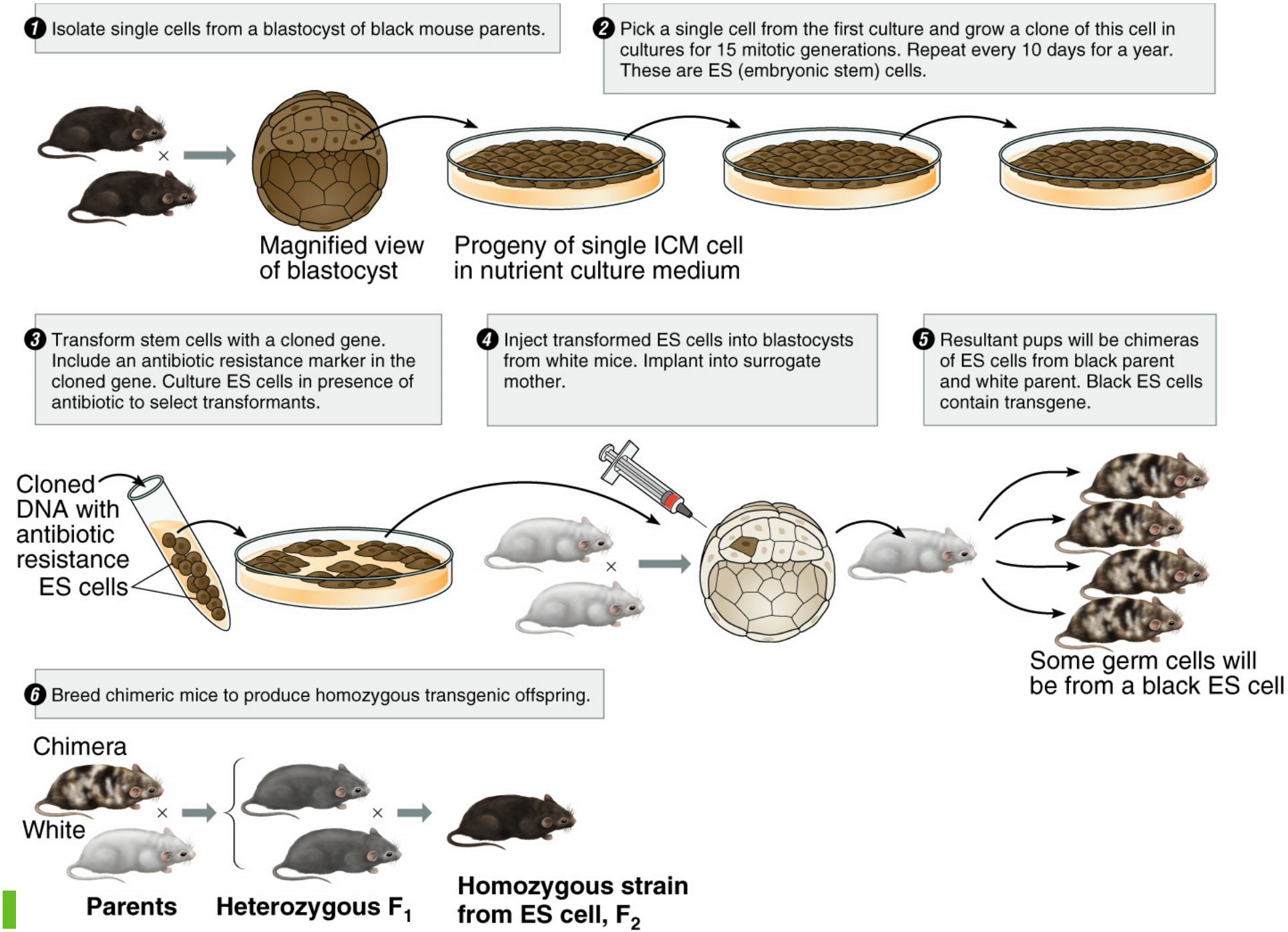
1 Isolate single cells from a blastocyst of black mouse parents.
2 Pick a single cell from the first culture and grow a clone of this cell in cultures for 15 mitotic generations. Repeat every 10 days for a year. These are ES (embryonic stem) cells.
Magnified view of blastocyst
Progeny of single ICM cell in nutrient culture medium
3 Transform stem cells with a cloned gene. Include an antibiotic resistance marker in the cloned gene. Culture ES cells in presence of antibiotic to select transformants.
4 Inject transformed ES cells into blastocysts from white mice. Implant into surrogate mother.
5 Resultant pups will be chimeras of ES cells from black parent and white parent. Black ES cells contain transgene.
Cloned DNA with antibiotic resistance ES cells
Some germ cells will be from a black ES cell
6 Breed chimeric mice to produce homozygous transgenic offspring.
Chimera
White
Parents
Heterozygous F1
Homozygous strain from ES cell, F2

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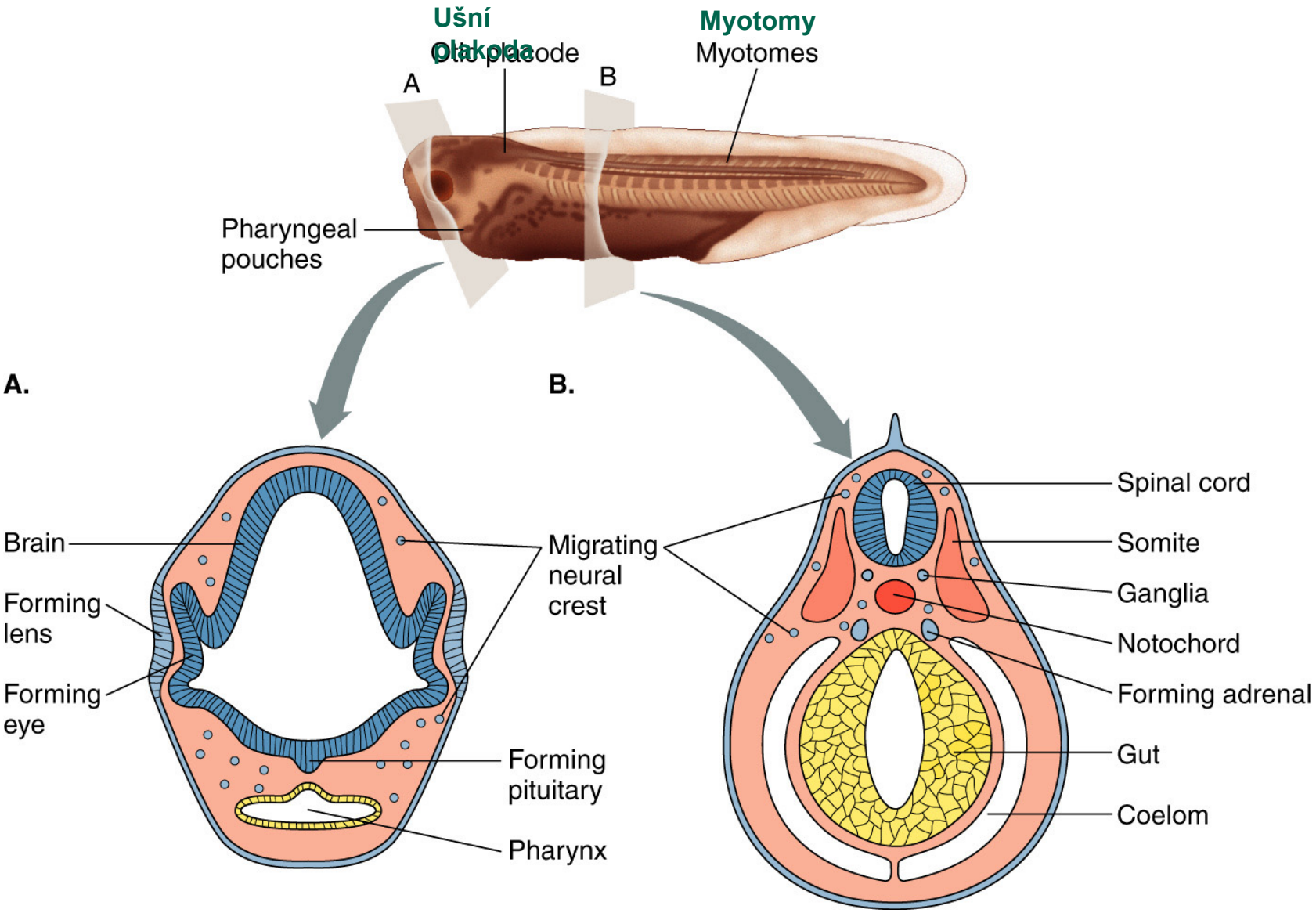
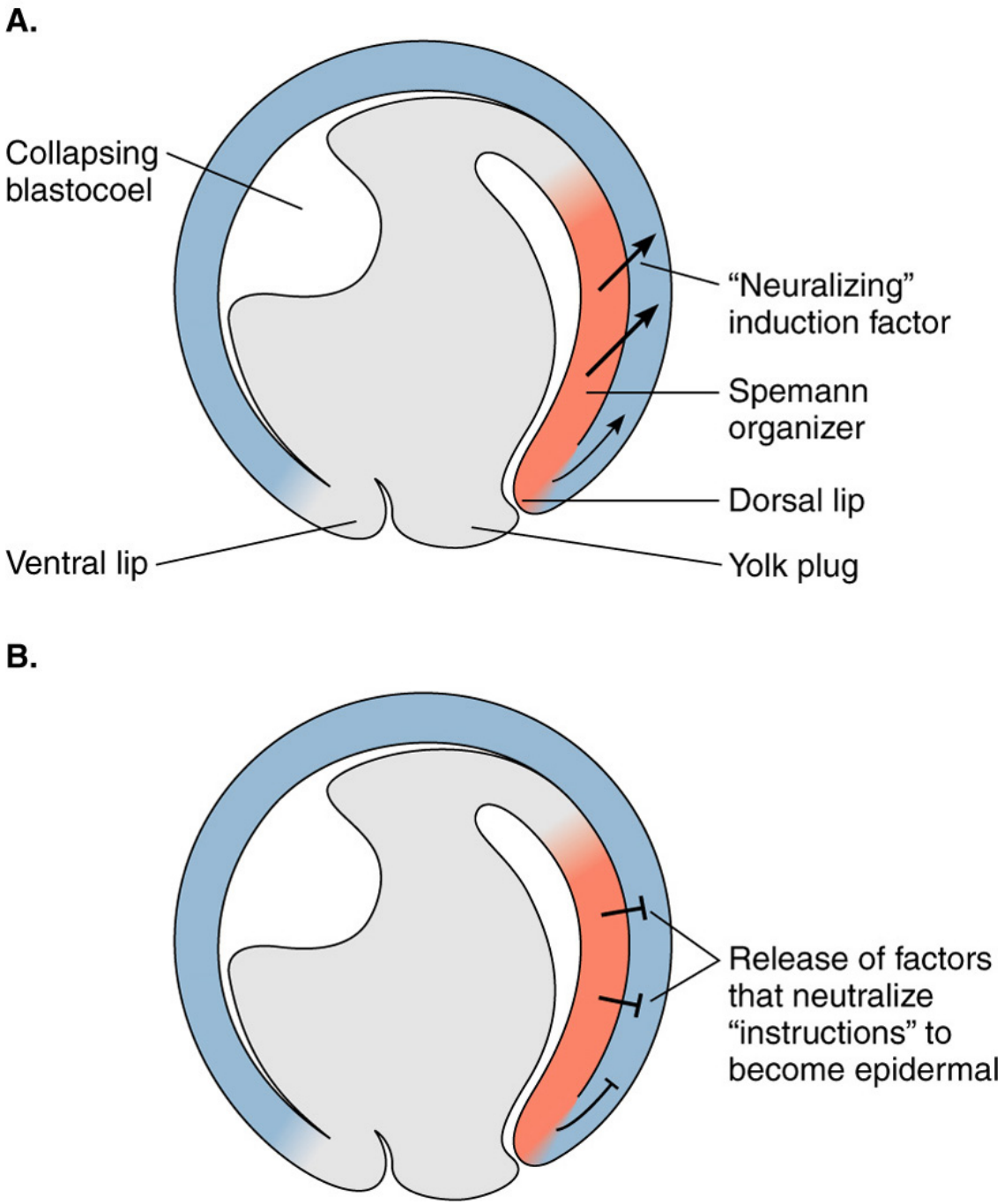
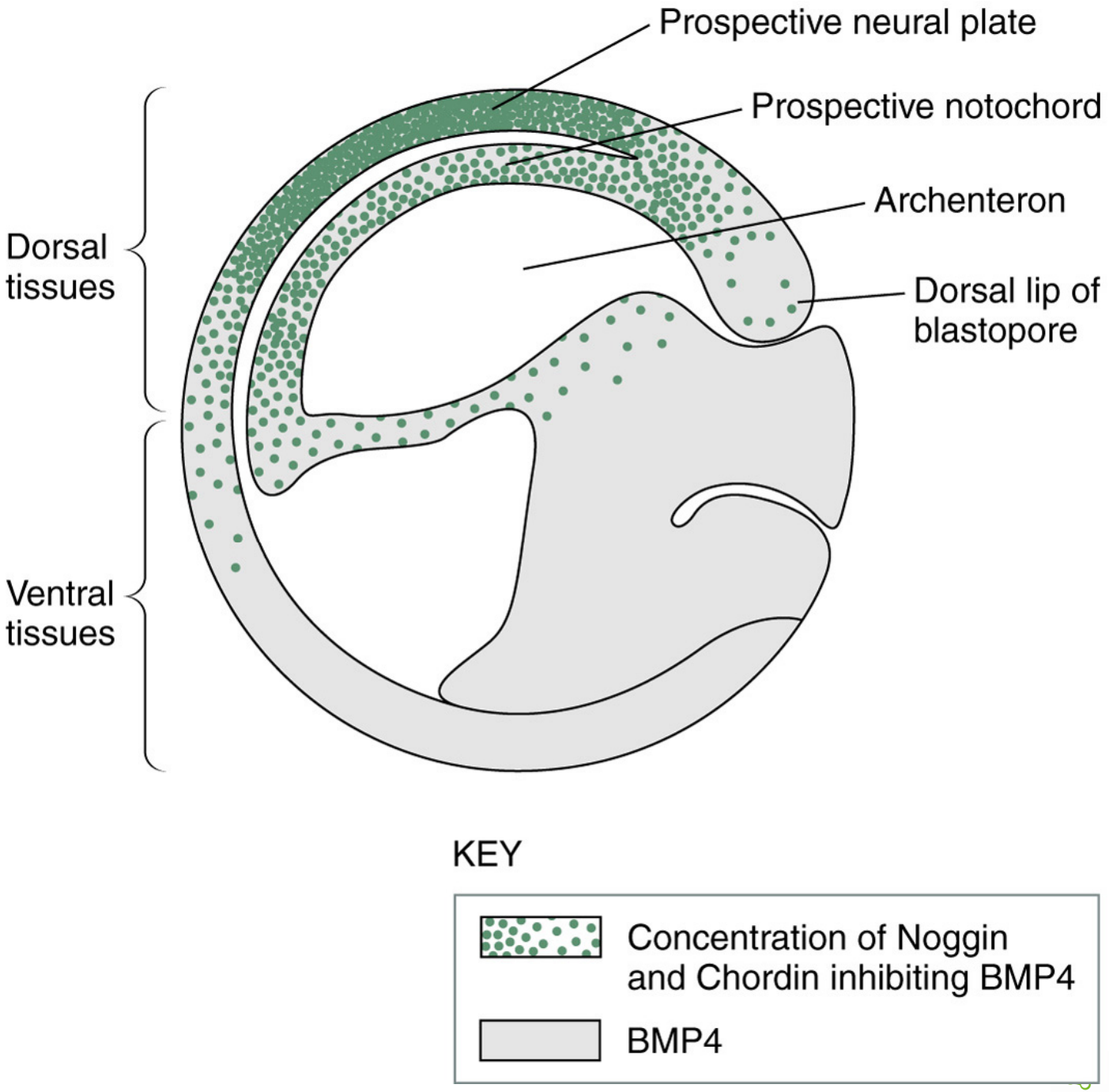
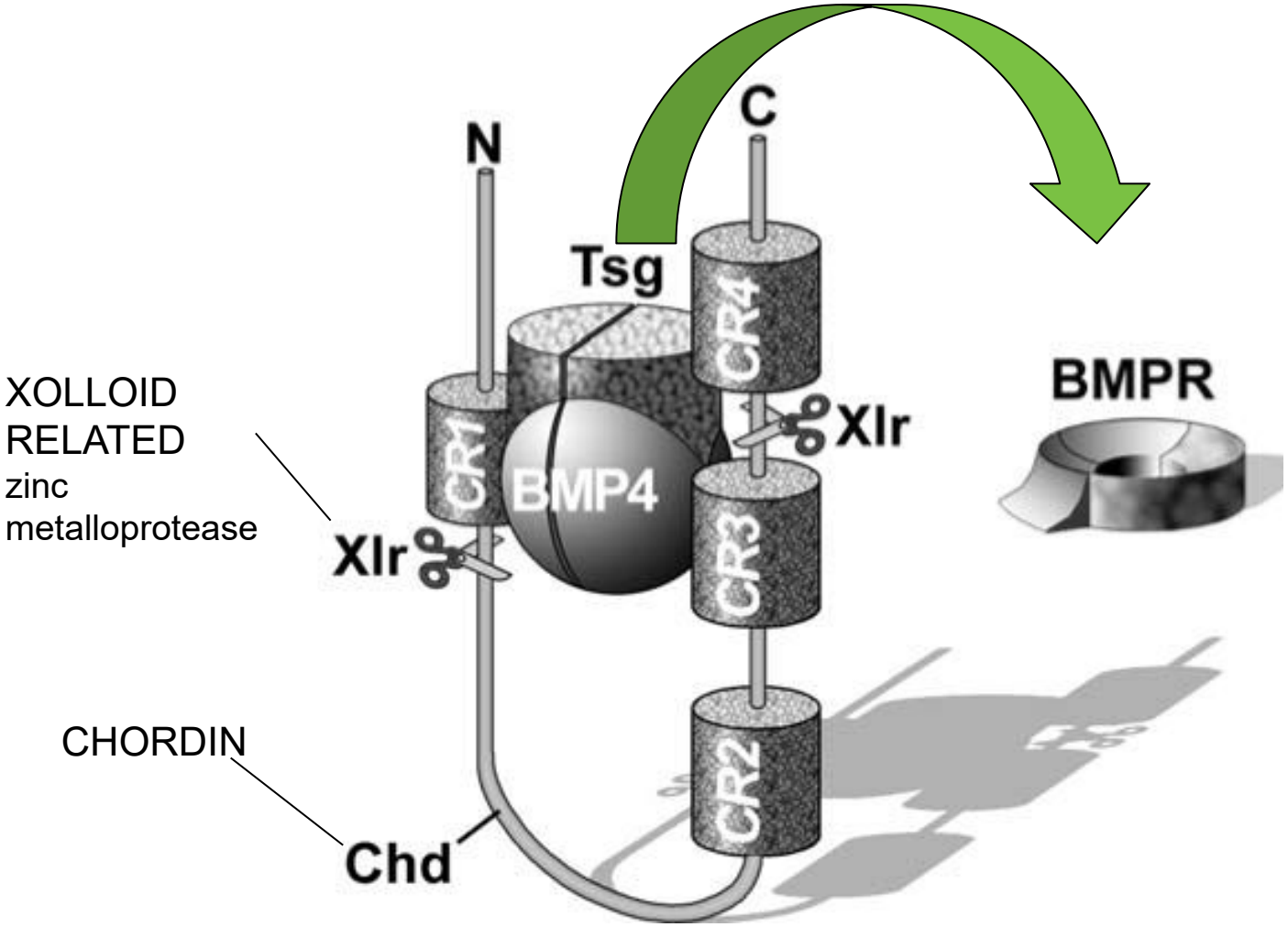
- Differentiation of neural tissue
  - mechanisms of neural tissue specification

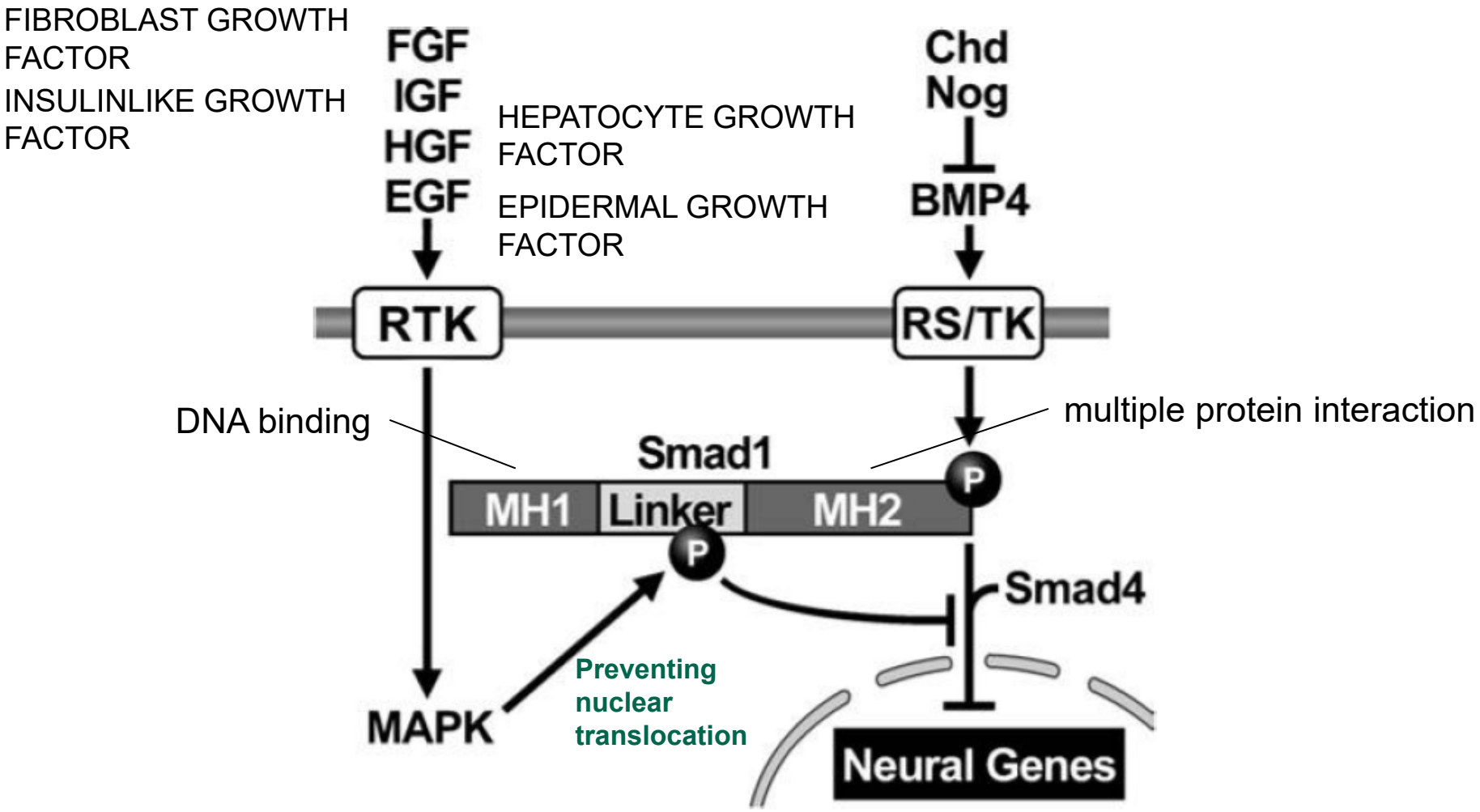

Ušní
Otic placode
Myotomy
Myotomes
A
B
Pharyngeal
pouches
A.
B.
Brain
Forming
lens
Forming
eye
Migrating
neural
crest
Forming
pituitary
Pharynx
Spinal cord
Somite
Ganglia
Notochord
Forming adrenal
Gut
Coelom


A.
Collapsing blastocoel
"Neuralizing" induction factor
Spemann organizer
Dorsal lip
Ventral lip
Yolk plug
B.
Release of factors that neutralize "instructions" to become epidermal


Prospective neural plate
Prospective notochord
Archenteron
Dorsal lip of blastopore
Dorsal tissues
Ventral tissues
KEY
Concentration of Noggin and Chordin inhibiting BMP4
BMP4



De Robertis and Kuroda, *Annu Rev Cell Dev Biol* (2004)



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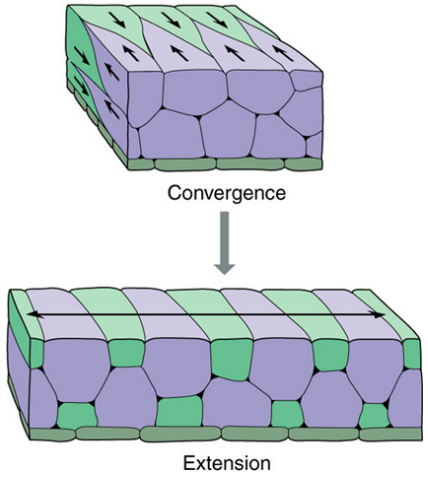
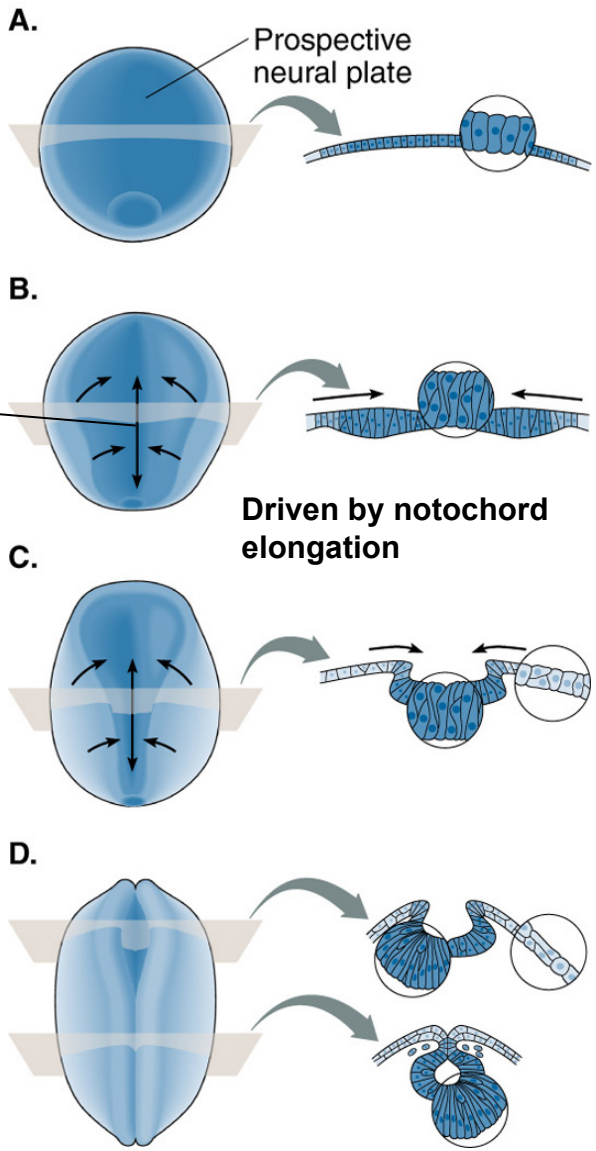
  - mechanisms of neural tissue specification
  - signaling in the spinal cord development



Covergent extension
Konvergentní extenze

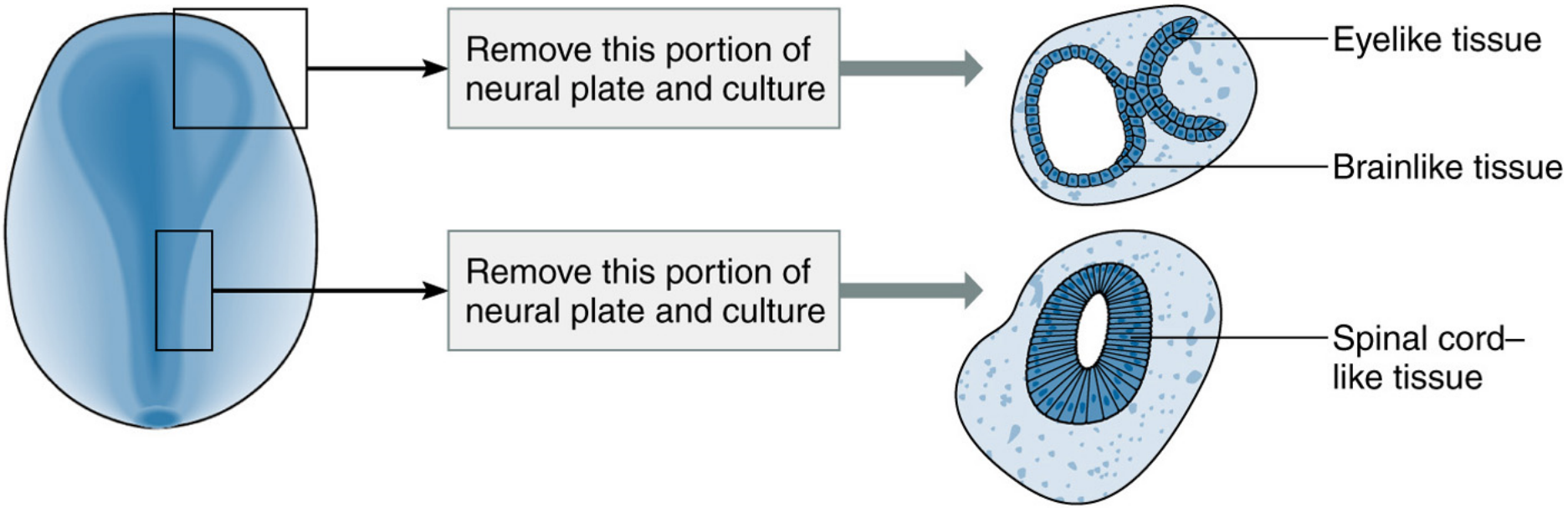


Driven by notochord elongation

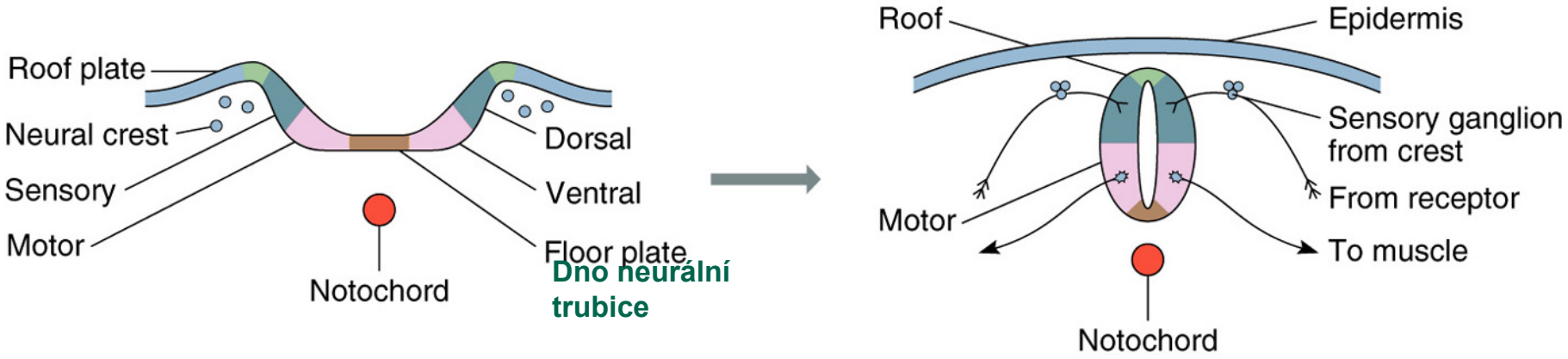
late gastrula

early-to-late neurula

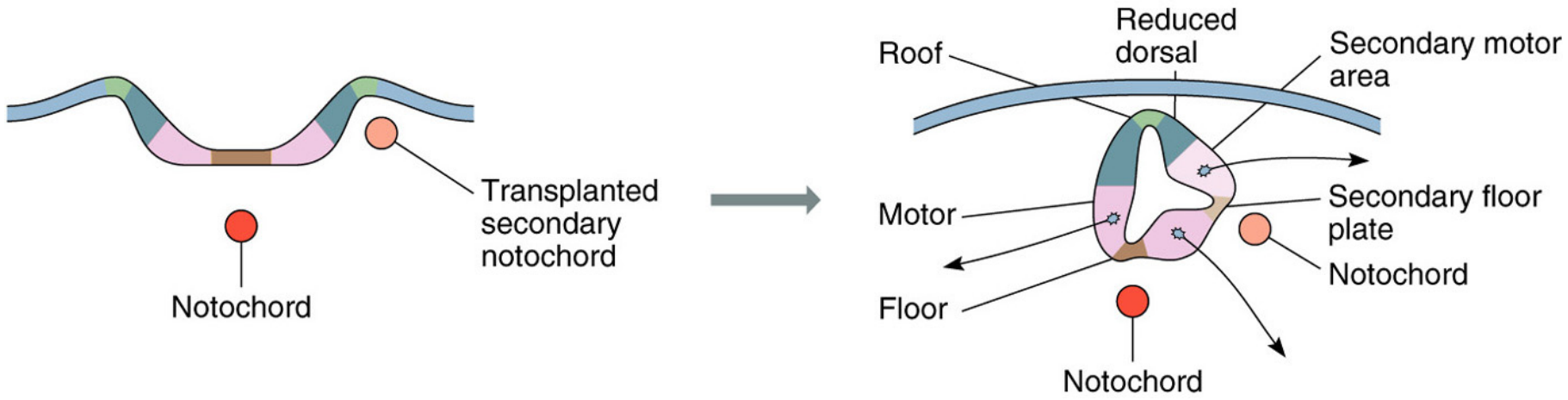
Homologues of the *Drosophila's HOM* genes are involved in the anteroposterior axis formation

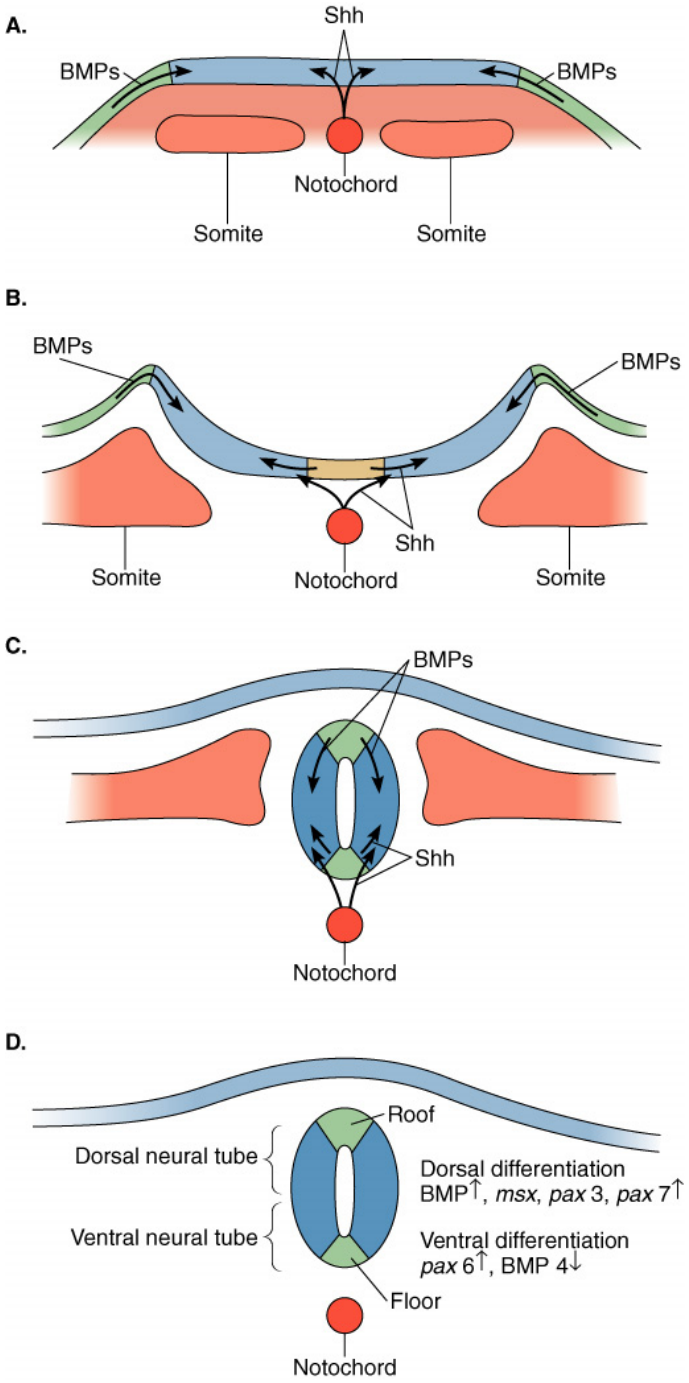


A. The basic situation



B. Effect of secondary, ectopic notochord under the neural plate





Valeria Marigo

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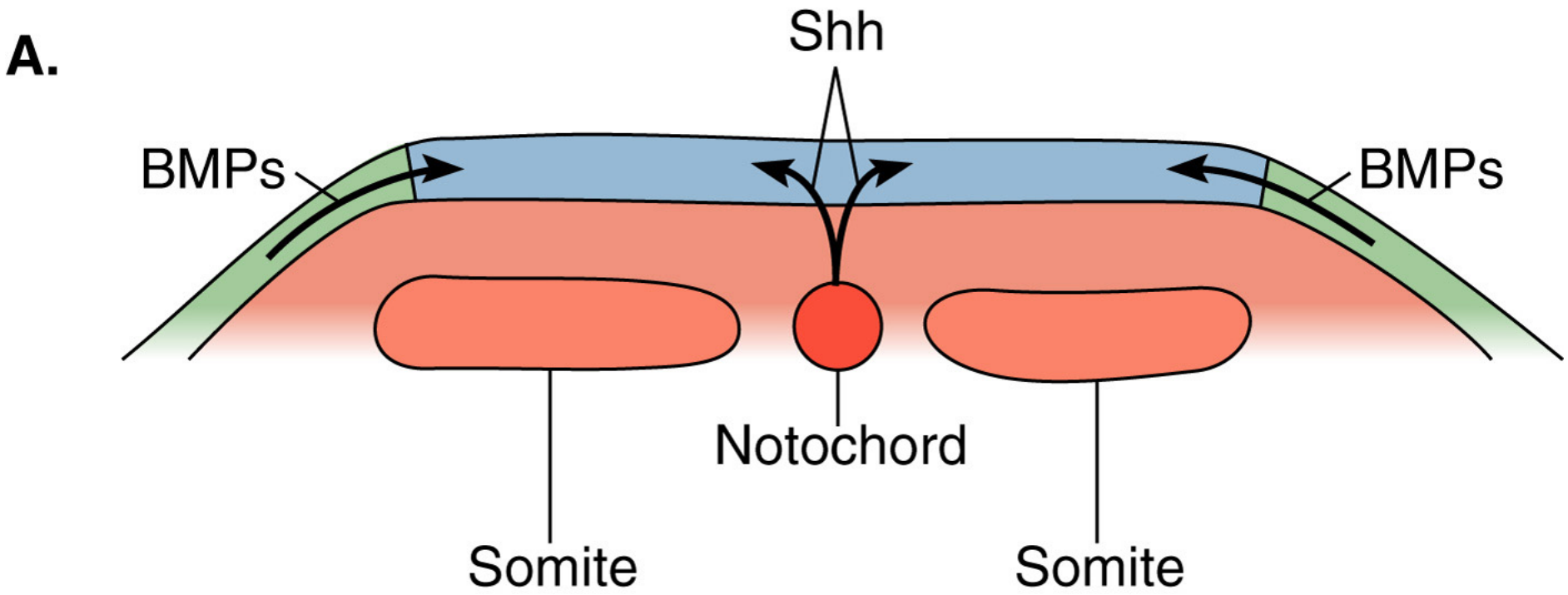
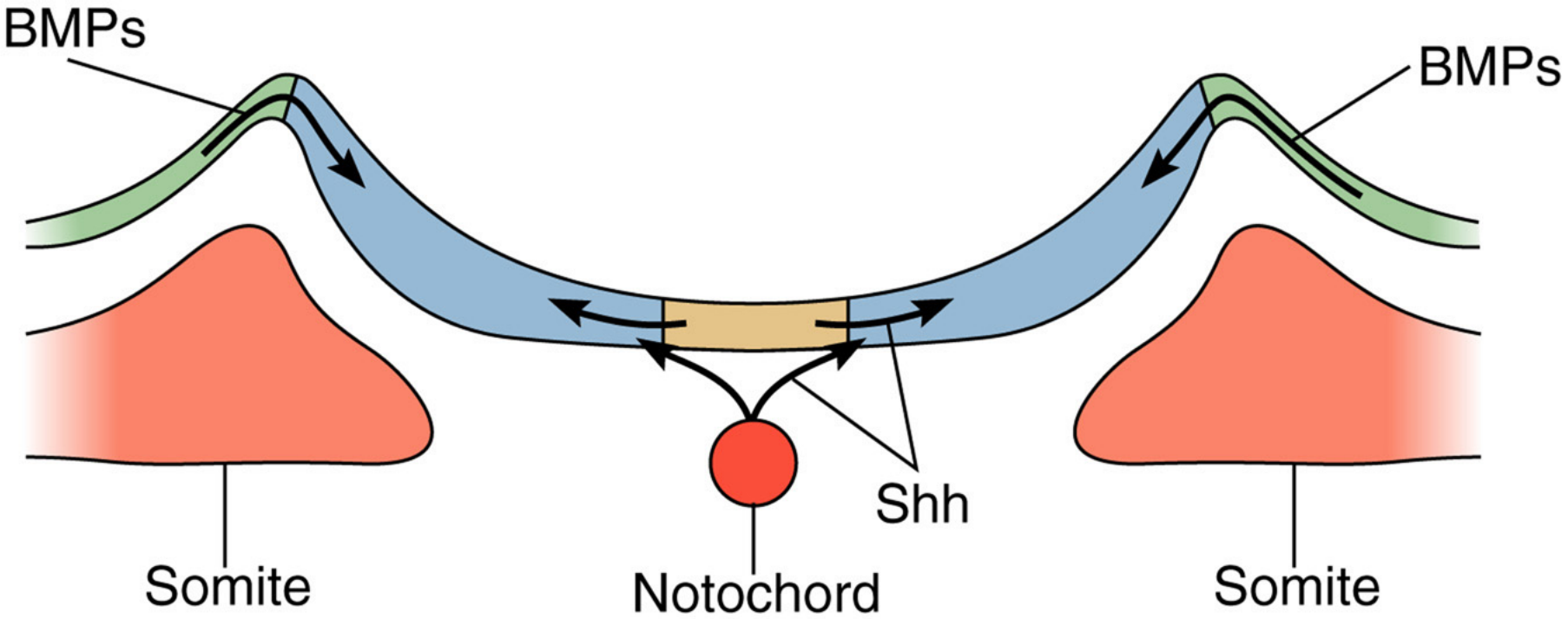
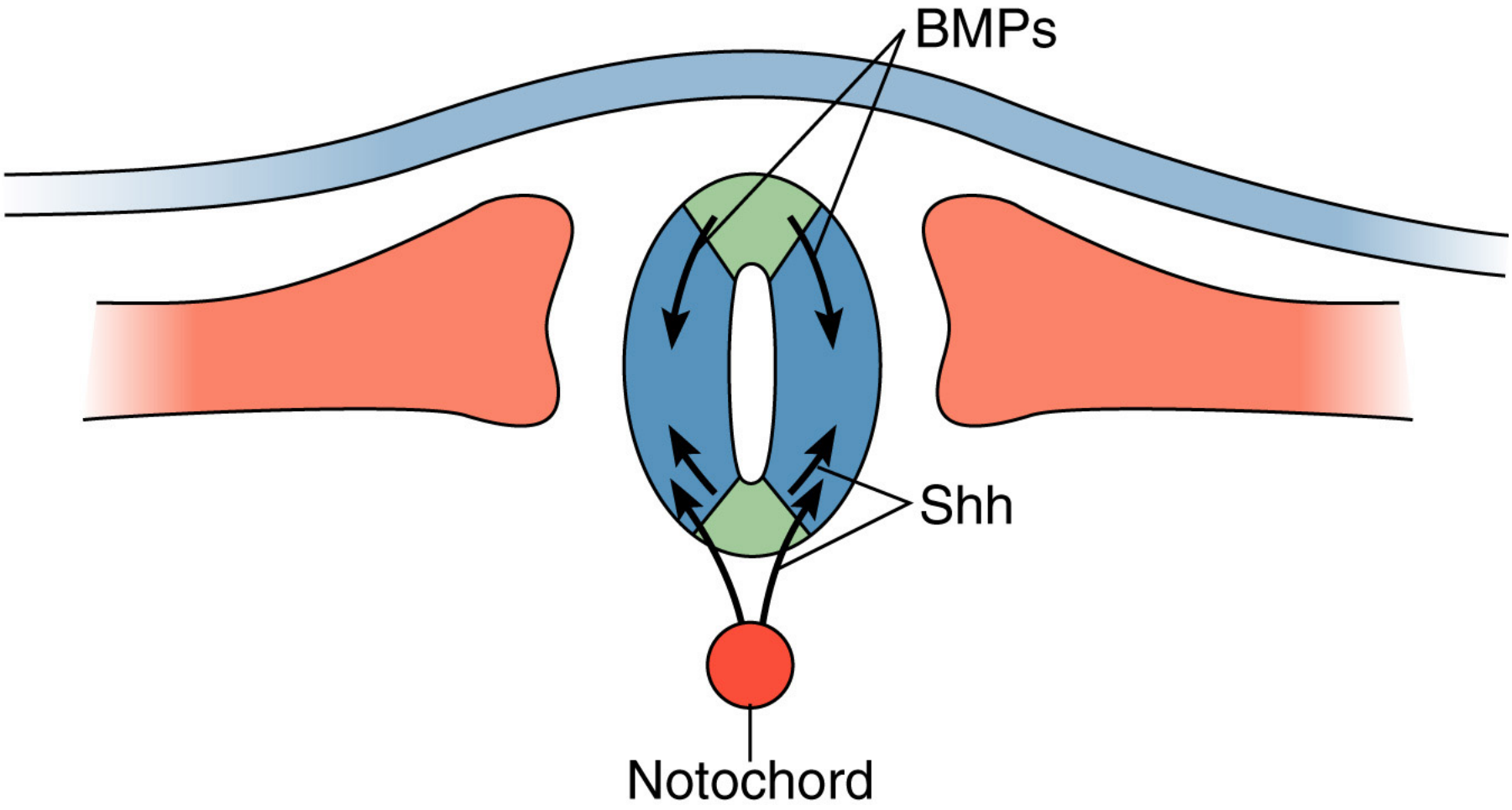
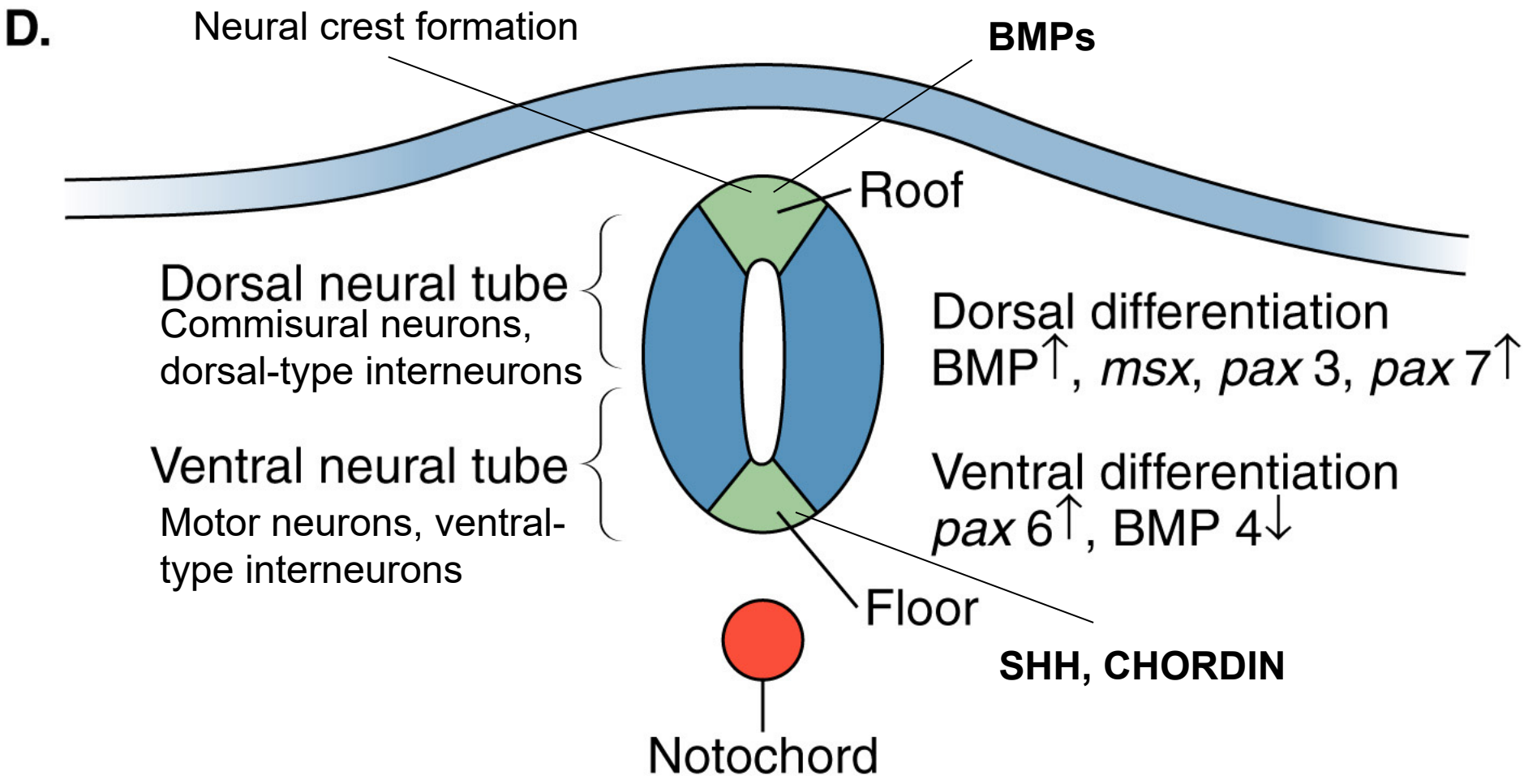

A.
Shh
BMPs
BMPs
Notochord
Somite
Somite

B.



C.




D.
Neural crest formation
BMPs
Roof
Dorsal neural tube
Commisural neurons,
dorsal-type interneurons
Dorsal differentiation
BMP↑, msx, pax 3, pax 7↑
Ventral neural tube
Motor neurons, ventral-
type interneurons
Ventral differentiation
pax 6↑, BMP 4↓
Floor
SHH, CHORDIN
Notochord

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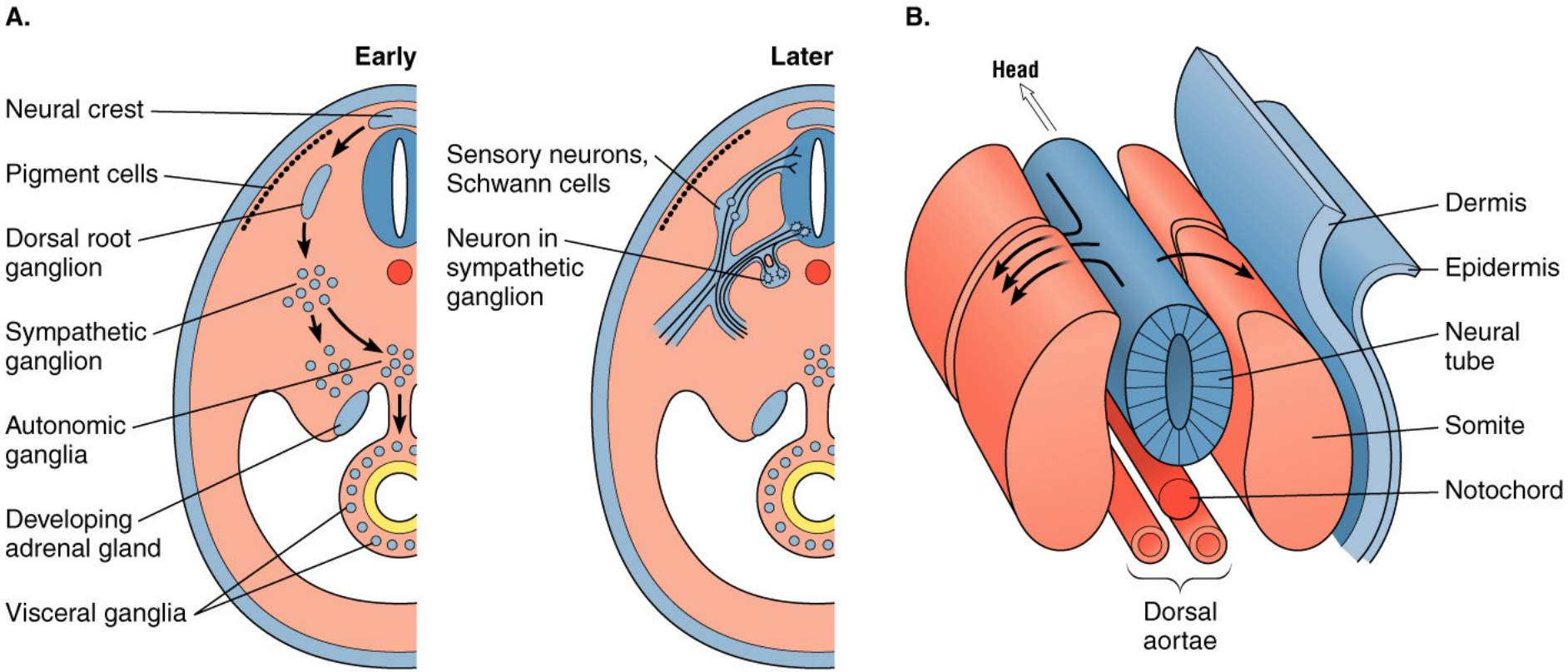
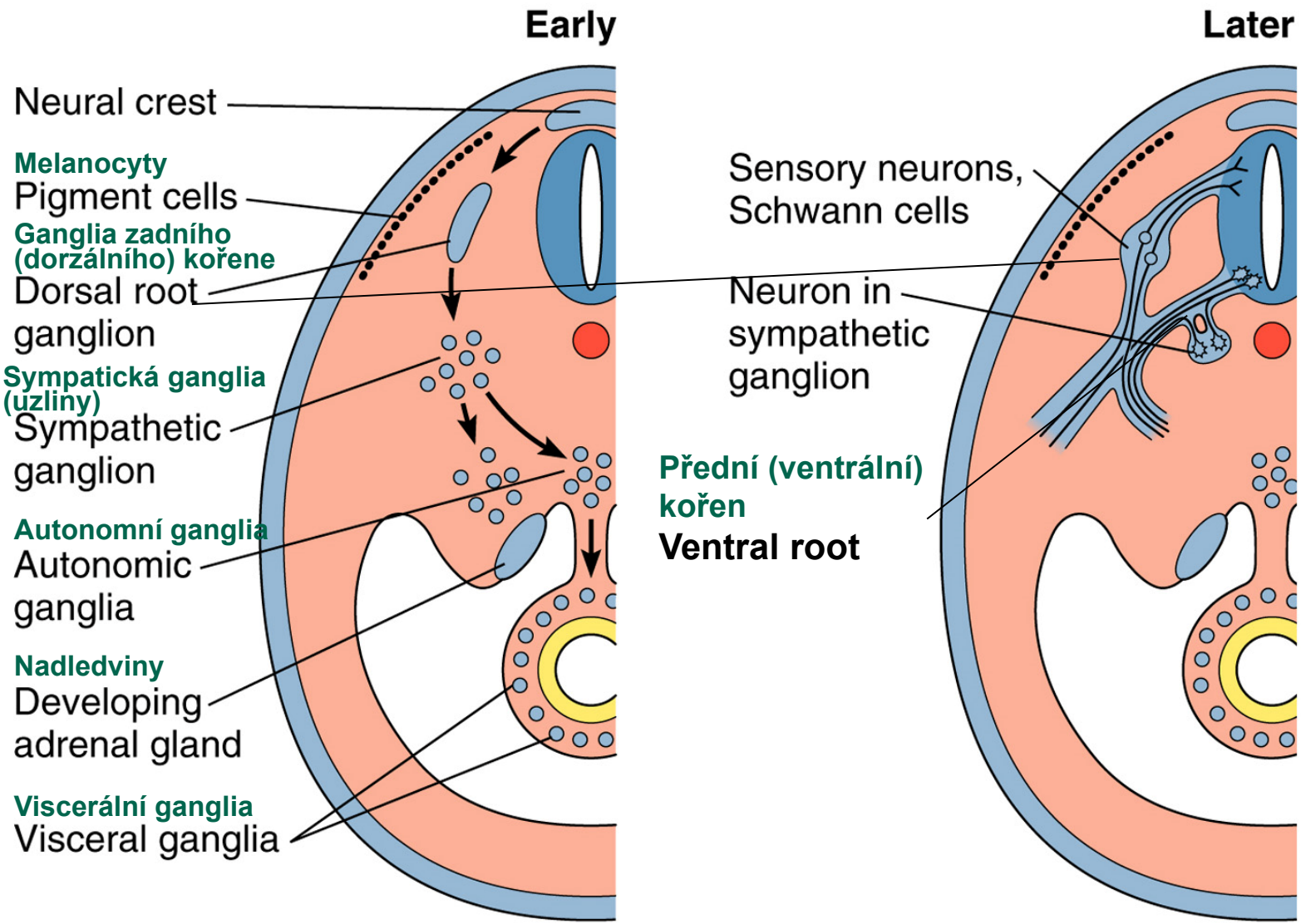
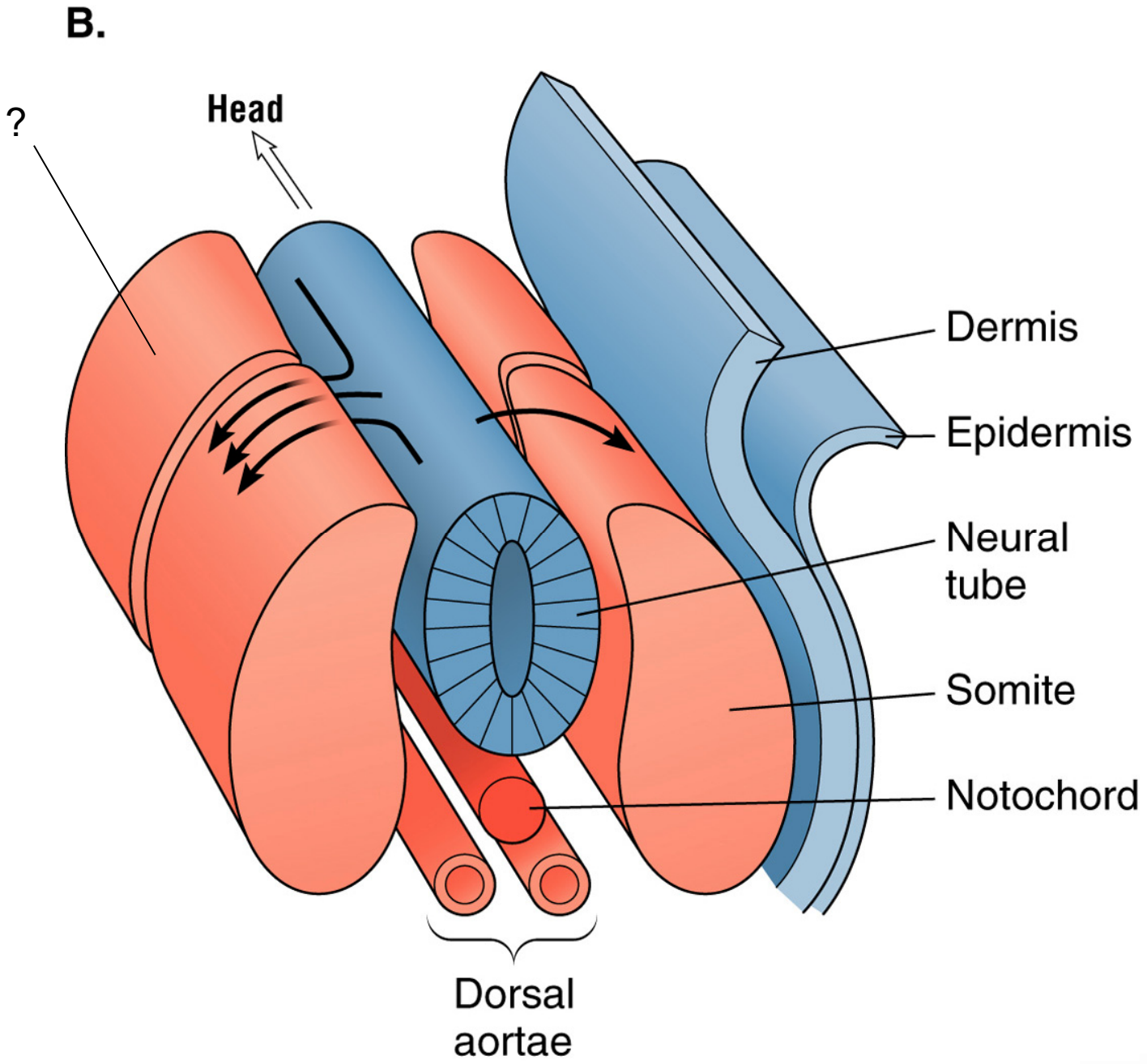
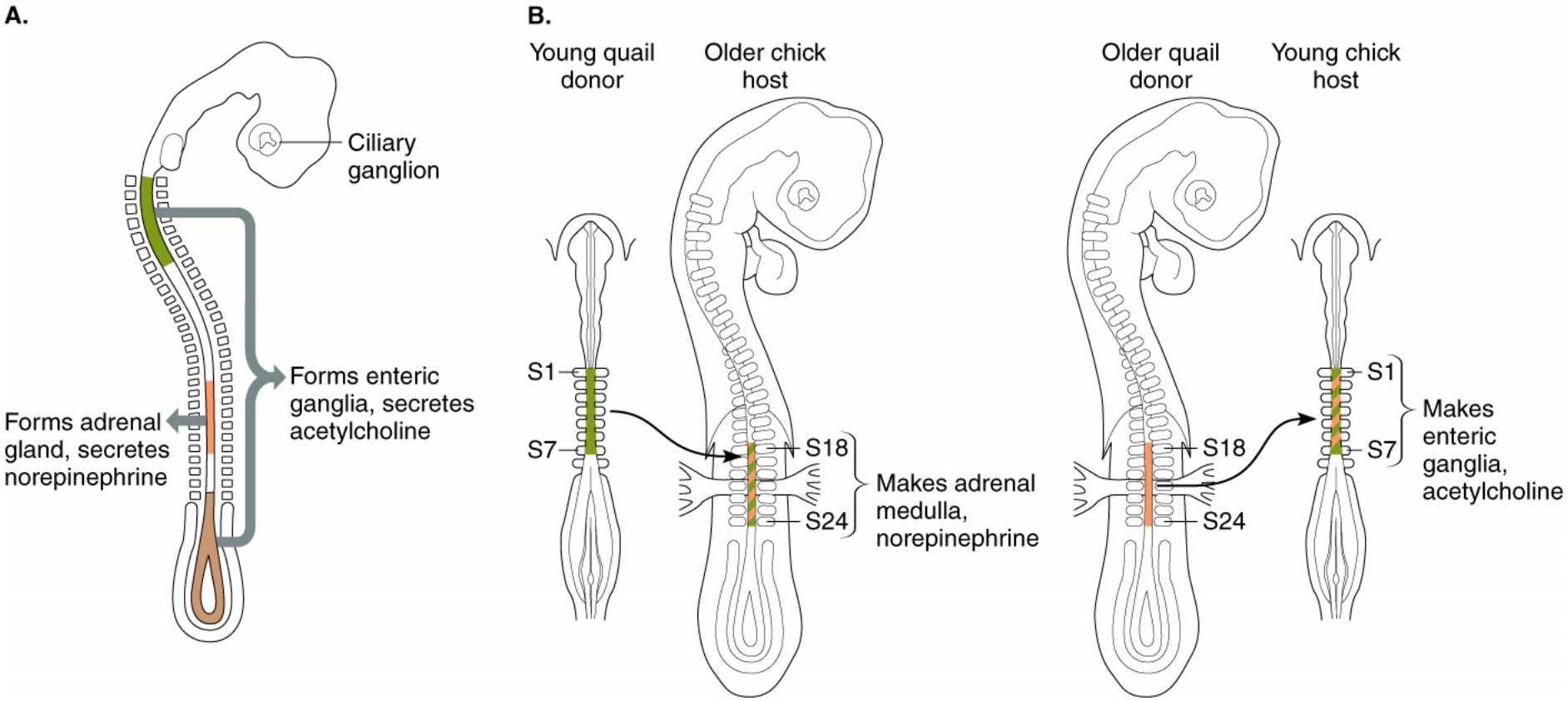
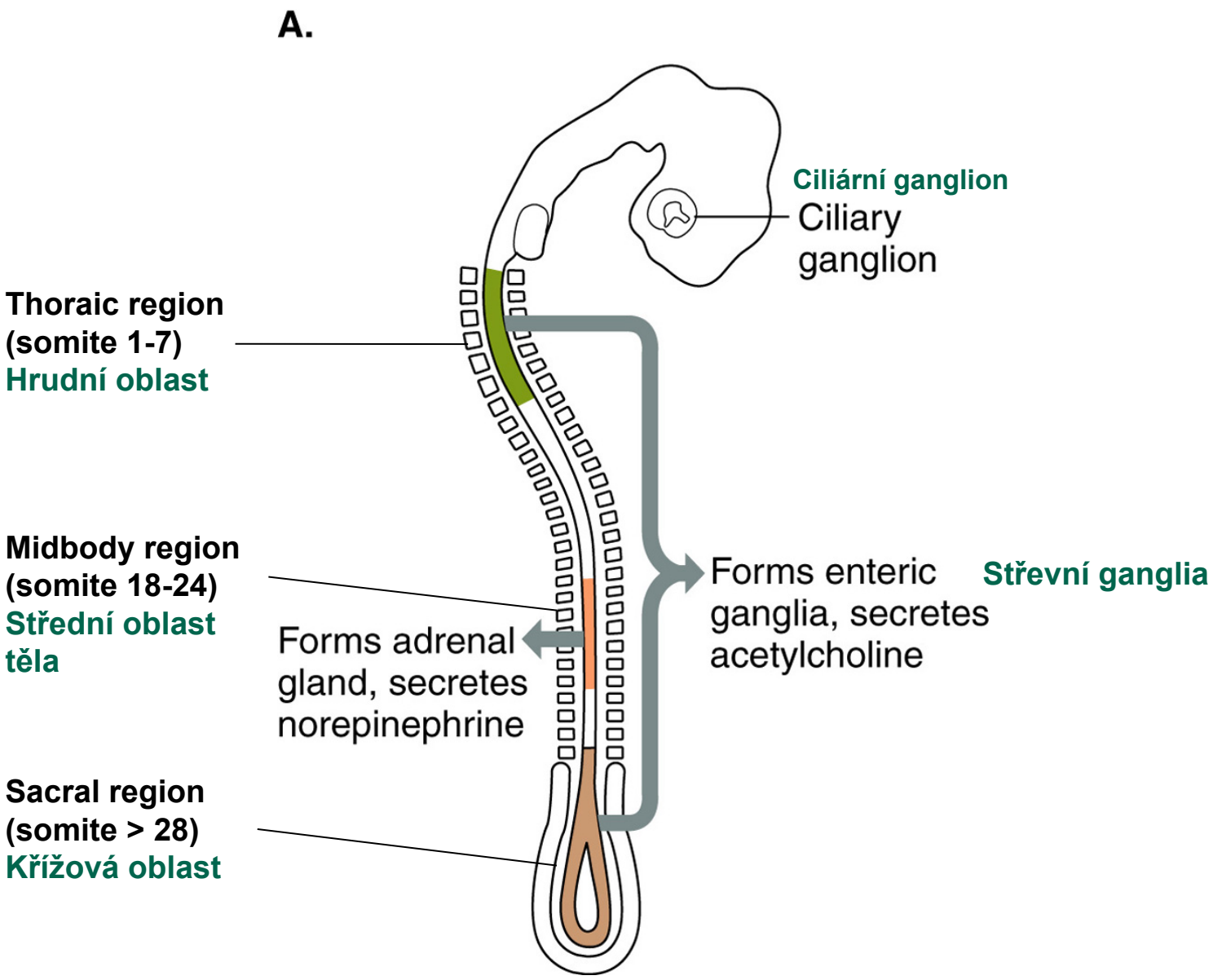
  - signaling in the spinal cord development
  - spatial-specific differentiation of neural crest derivatives


A.
Early
Later
Neural crest
Pigment cells
Dorsal root ganglion
Sympathetic ganglion
Autonomic ganglia
Developing adrenal gland
Visceral ganglia
Sensory neurons, Schwann cells
Neuron in sympathetic ganglion
B.
Head
Dermis
Epidermis
Neural tube
Somite
Notochord
Dorsal aortae

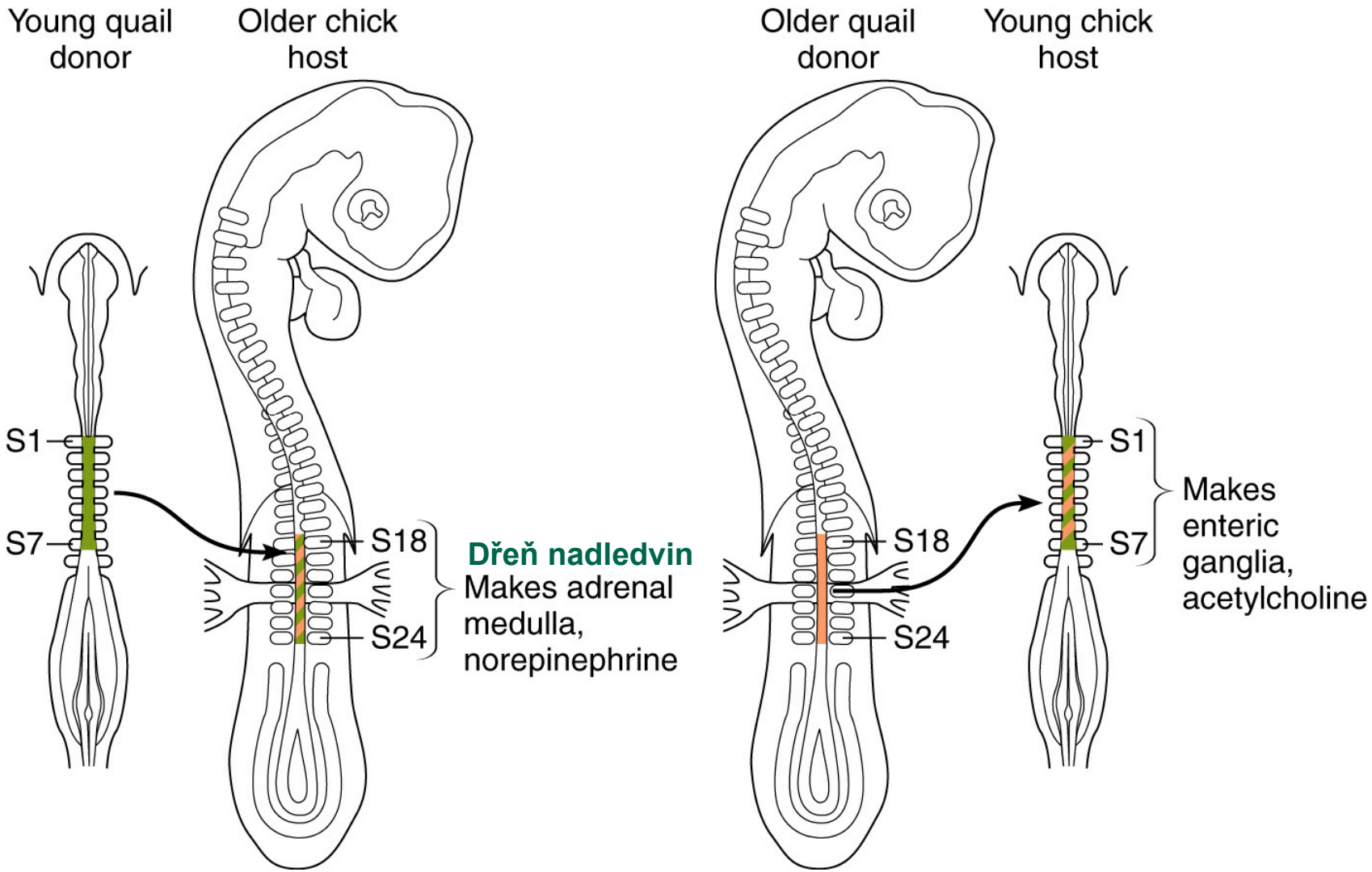

A.
Early
Later
Neural crest
Melanocyty
Pigment cells
Ganglia zadního (dorzálního) kořene
Dorsal root ganglion
Sympatická ganglia (uzliny)
Sympathetic ganglion
Autonomní ganglia
Autonomic ganglia
Nadledviny
Developing adrenal gland
Viscerální ganglia
Visceral ganglia
Sensory neurons, Schwann cells
Neuron in sympathetic ganglion
Přední (ventrální) kořen
Ventral root


B.
?
Head
Dermis
Epidermis
Neural tube
Somite
Notochord
Dorsal aortae


A.
Ciliary ganglion
Forms enteric ganglia, secretes acetylcholine
Forms adrenal gland, secretes norepinephrine
B.
Young quail donor
Older chick host
S1
S7
S18
S24
Makes adrenal medulla, norepinephrine
Older quail donor
Young chick host
S1
S7
S18
S24
Makes enteric ganglia, acetylcholine


A.
Ciliární ganglion
Ciliary ganglion
Thoraic region (somite 1-7)
Hrudní oblast
Midbody region (somite 18-24)
Střední oblast těla
Sacral region (somite > 28)
Křížová oblast
Forms adrenal gland, secretes norepinephrine
Forms enteric ganglia, secretes acetylcholine
Střevní ganglia

B.


Young quail donor
Older chick host
S1
S7
S18
S24
Dřeň nadledvin
Makes adrenal medulla, norepinephrine
Older quail donor
Young chick host
S1
S7
S18
S24
Makes enteric ganglia, acetylcholine

Outline of Lesson 4

Organogenesis in Vertebrates: Ectodermal Derivatives

- Early development of mammals
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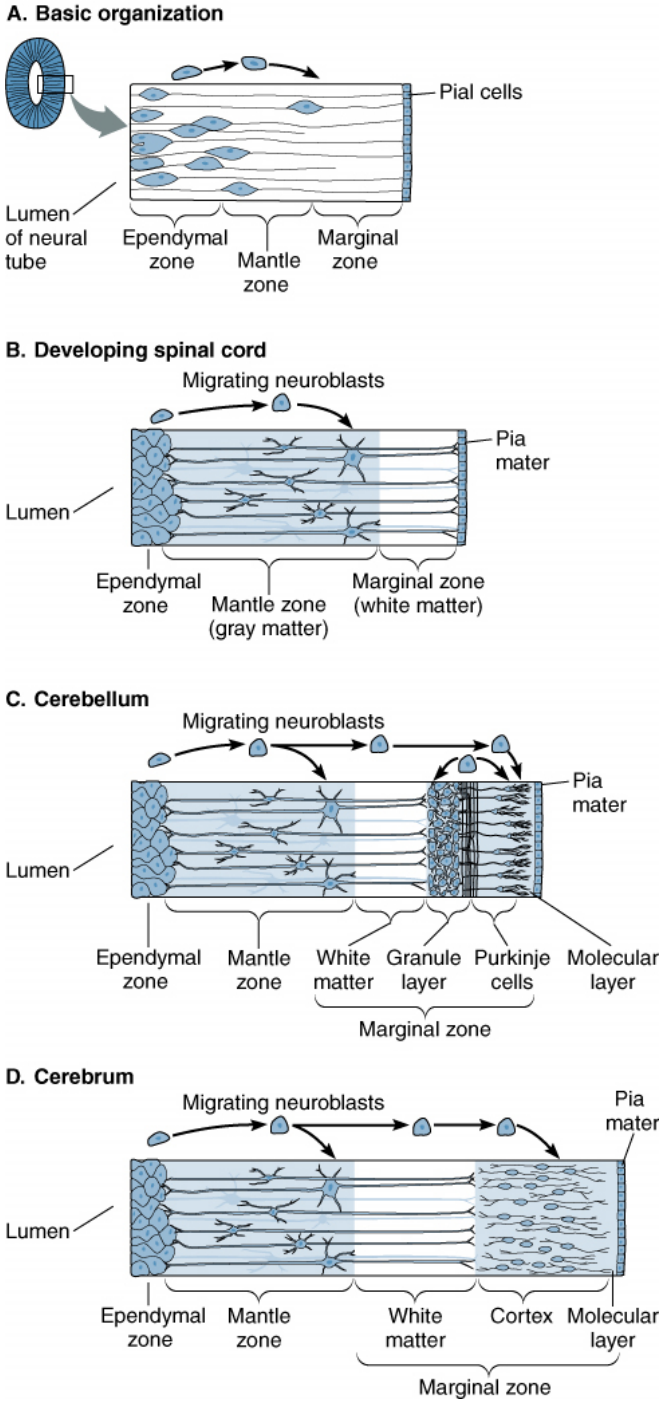
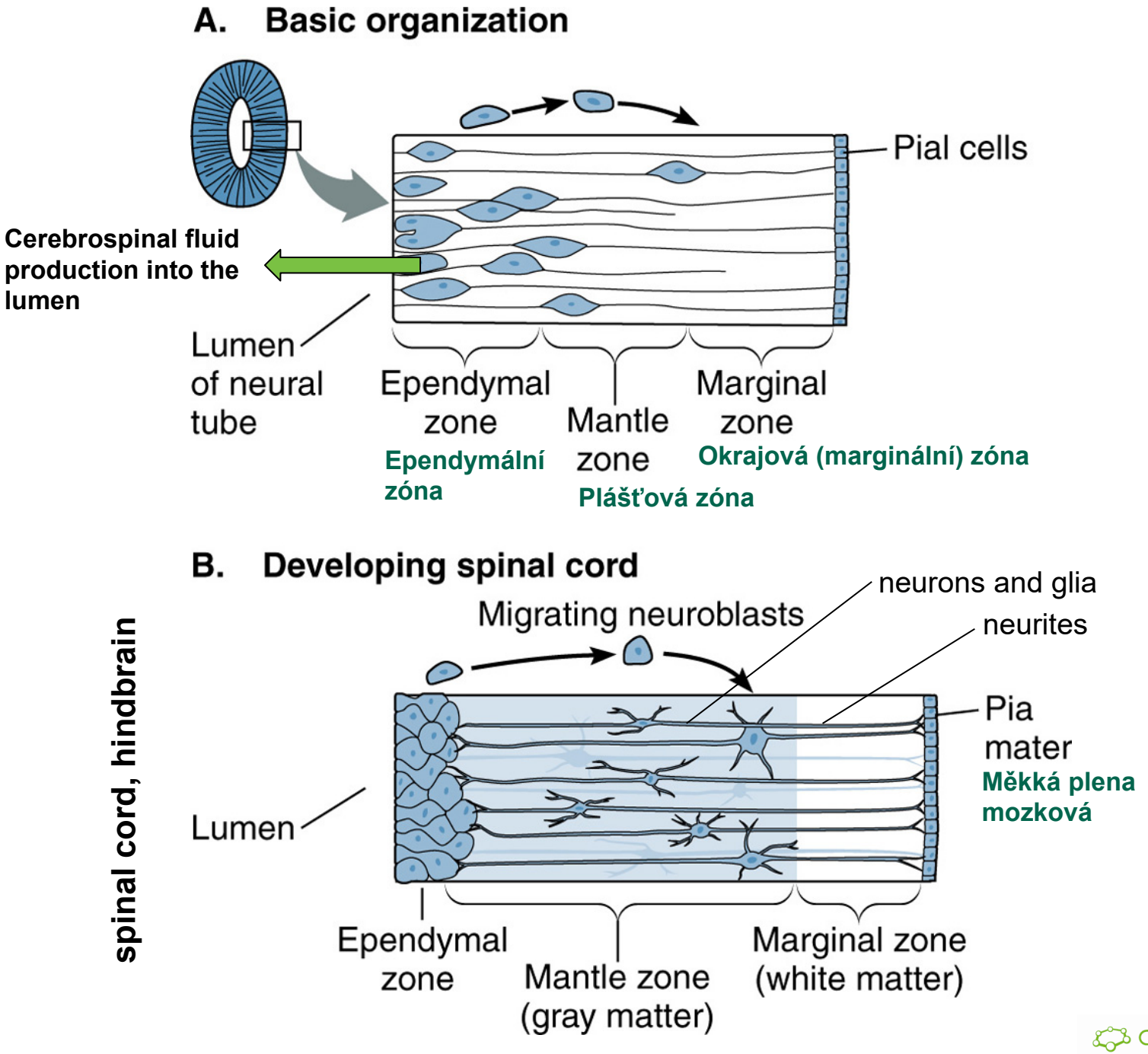
  - extraembryonic tissue formation
  - use of embryonal cells in mammals transgenosis
- Differentiation of neural tissue
  - mechanisms of neural tissue specification
  - signaling in the spinal cord development
  - spatial-specific differentiation of neural crest derivatives
  - stratification of neural tube

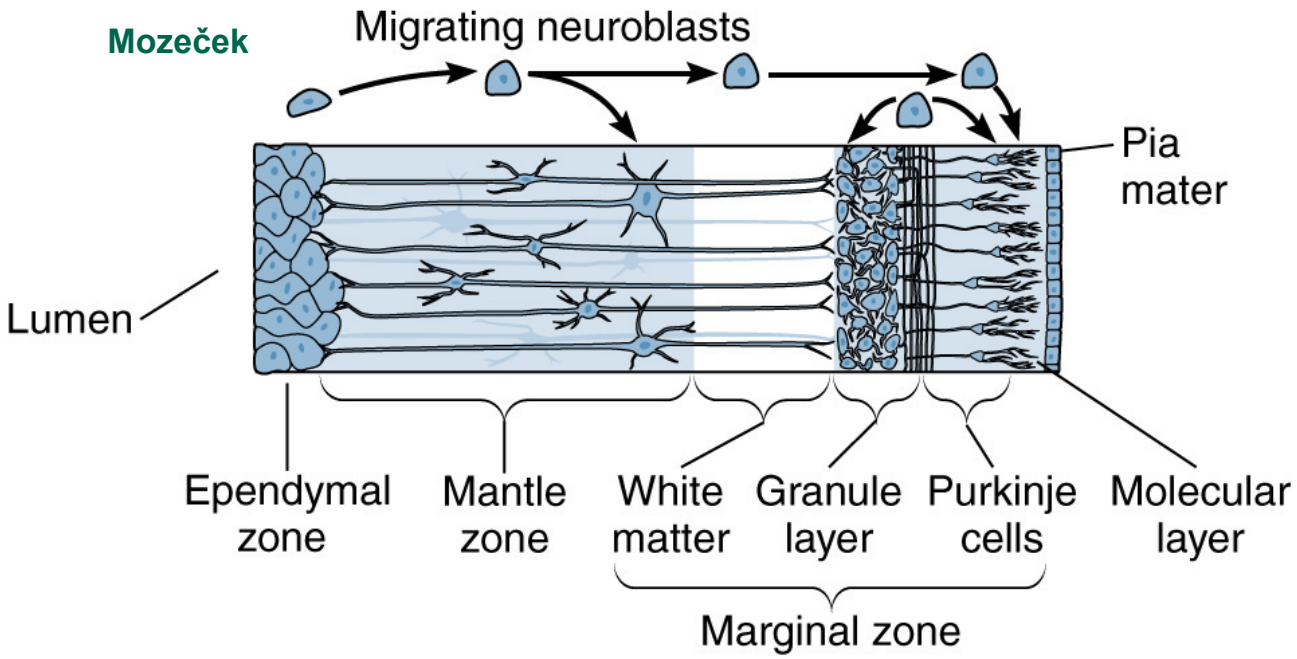

A. Basic organization
Pial cells
Lumen of neural tube
Ependymal zone
Mantle zone
Marginal zone
B. Developing spinal cord
Migrating neuroblasts
Pia mater
Lumen
Ependymal zone
Mantle zone (gray matter)
Marginal zone (white matter)
C. Cerebellum
Migrating neuroblasts
Pia mater
Lumen
Ependymal zone
Mantle zone
White matter
Granule layer
Purkinje cells
Molecular layer
Marginal zone
D. Cerebrum
Migrating neuroblasts
Pia mater
Lumen
Ependymal zone
Mantle zone
White matter
Cortex
Molecular layer
Marginal zone


A. Basic organization
Pial cells
Cerebrospinal fluid production into the lumen
Lumen of neural tube
Ependymal zone
Ependymální zóna
Mantle zone
Plášťová zóna
Marginal zone
Okrajová (marginální) zóna
B. Developing spinal cord
spinal cord, hindbrain
Migrating neuroblasts
neurons and glia
neurites
Pia mater
Měkká plena mozková
Lumen
Ependymal zone
Mantle zone (gray matter)
Marginal zone (white matter)

midbrain, forebrain

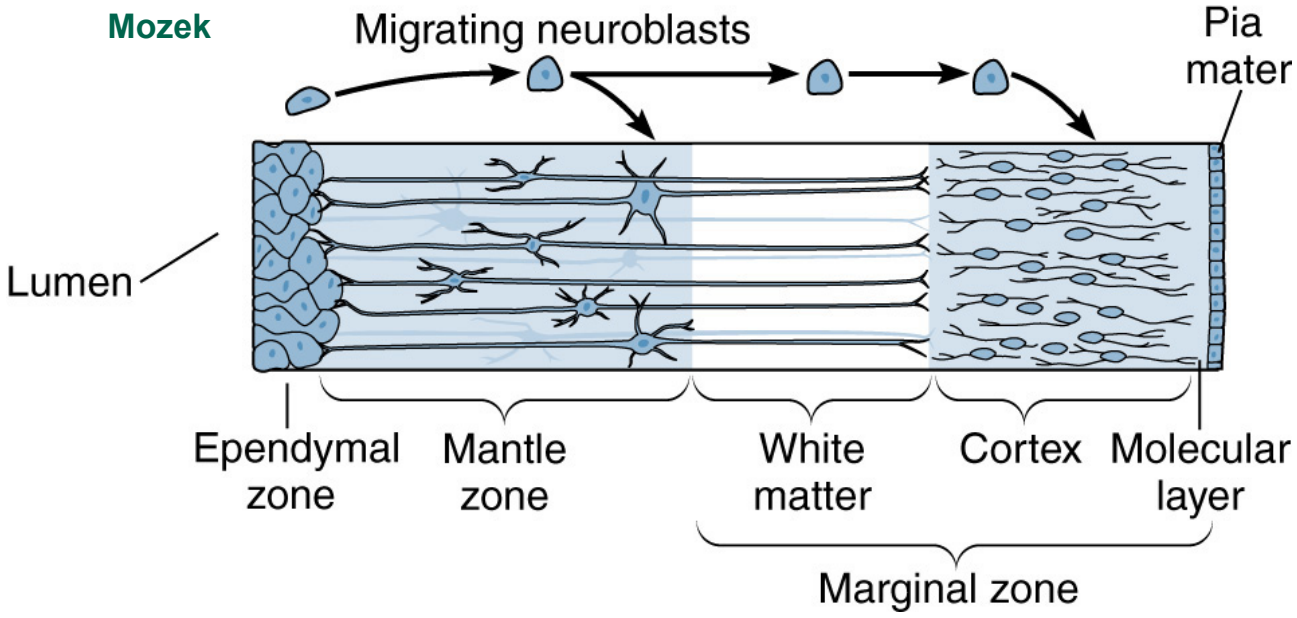
C. Cerebellum

Mozeček



D. Cerebrum

Mozek



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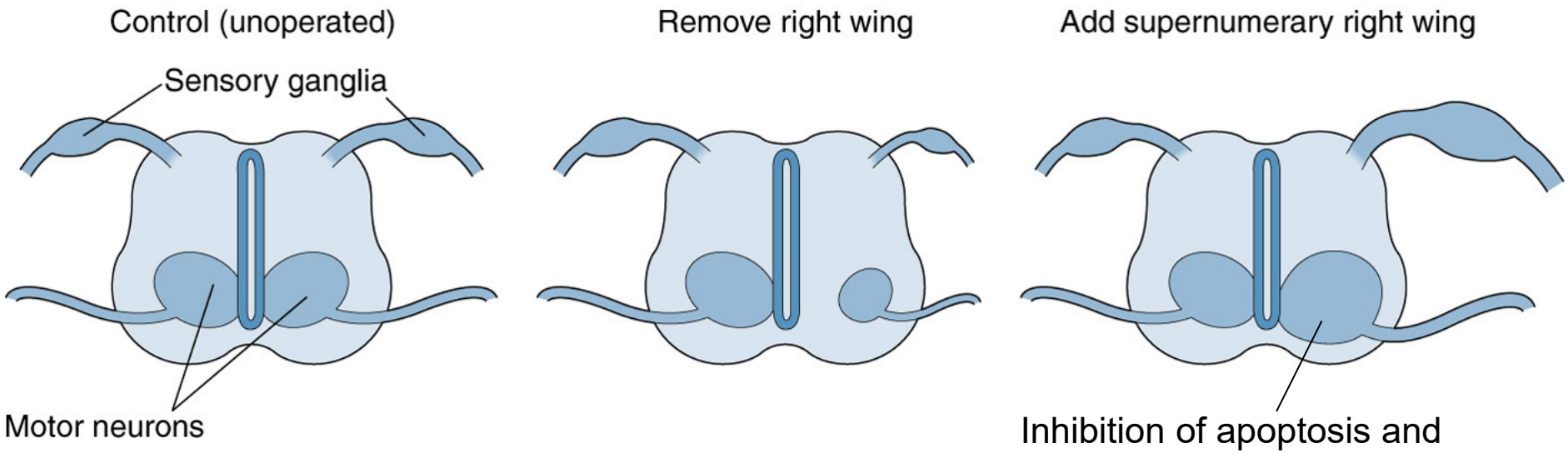
Control (unoperated)
Sensory ganglia
Motor neurons
Remove right wing
Add supernumerary right wing
Inhibition of apoptosis and presence of NGF
Activation of RTK

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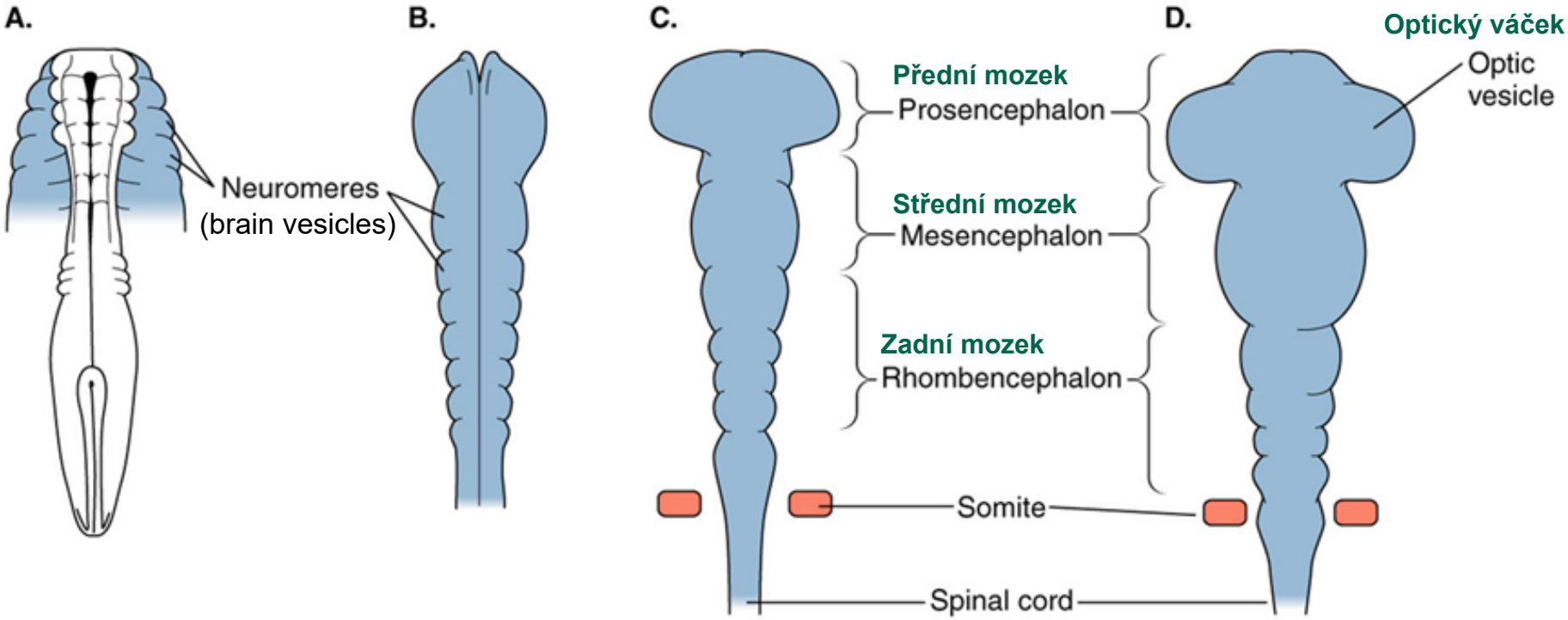
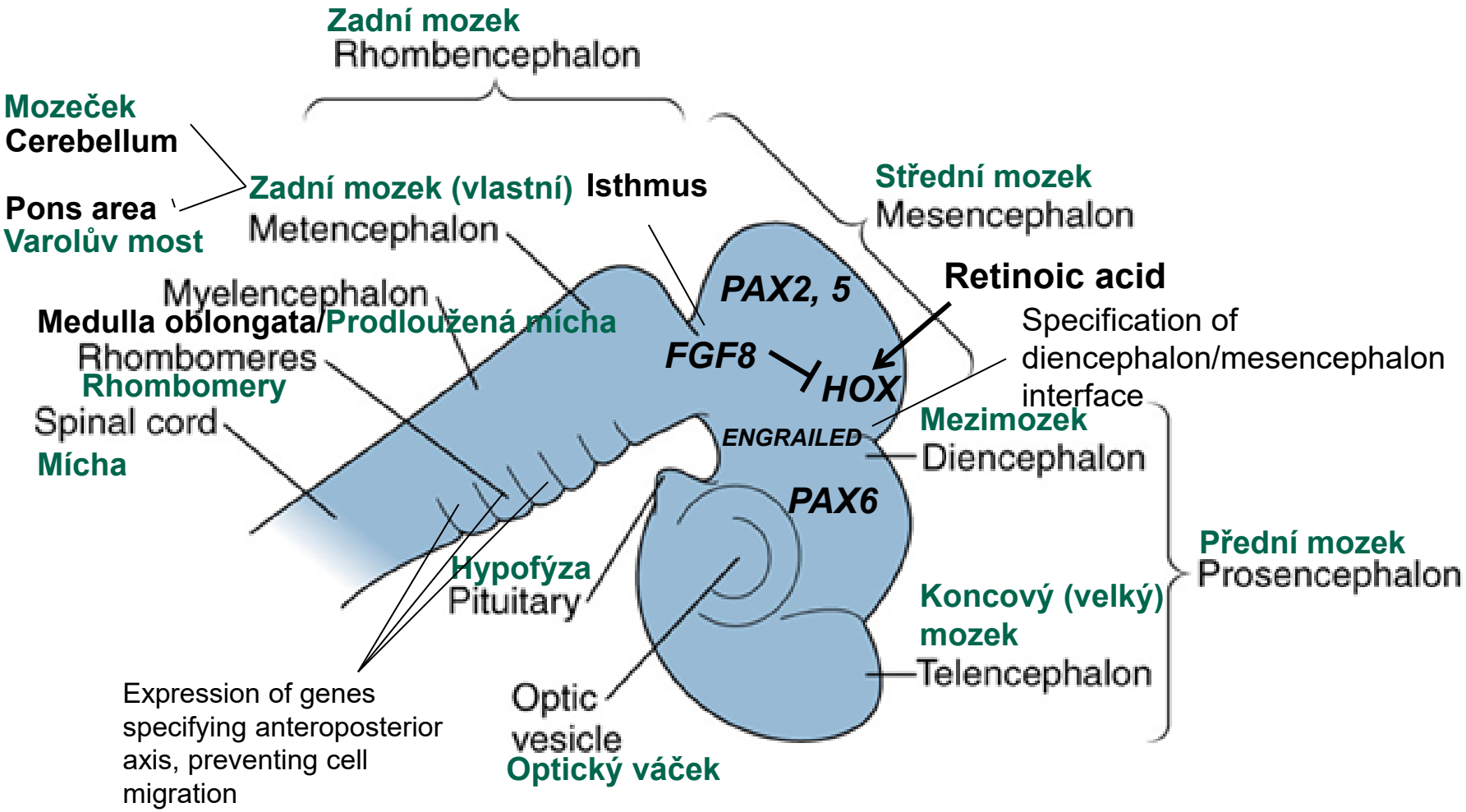
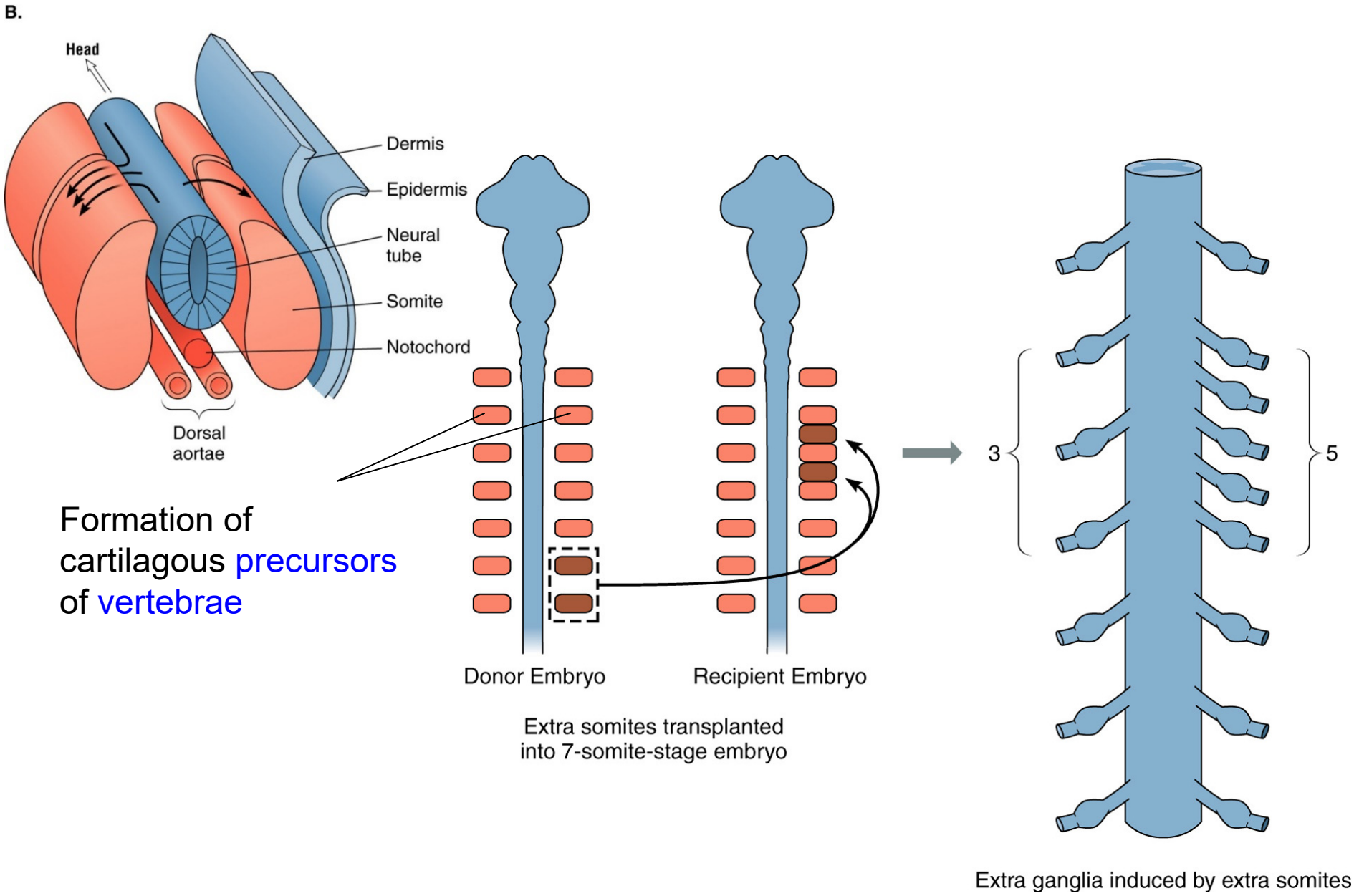
  - signaling in the spinal cord development
  - spatial-specific differentiation of neural crest derivatives
  - stratification of neural tube
- Development of brain and its derivatives
  - brain vesicles formation and development



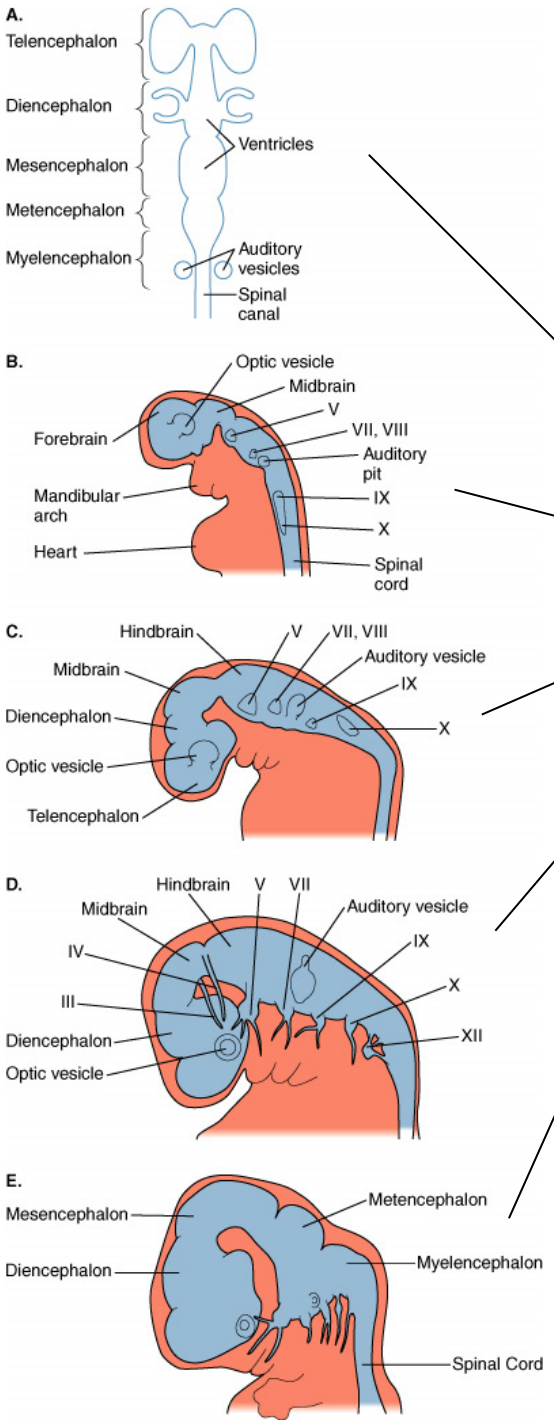
4-somites stage **7-somites stage** **11-somites stage** **14-somites stage**

Lateral view of three-days old chick embryo





Formation of cartilagous precursors of vertebrae



Segmental brain development, characterized by differential stratification of neural tube, *nuclei* and *tracts* anatomy.

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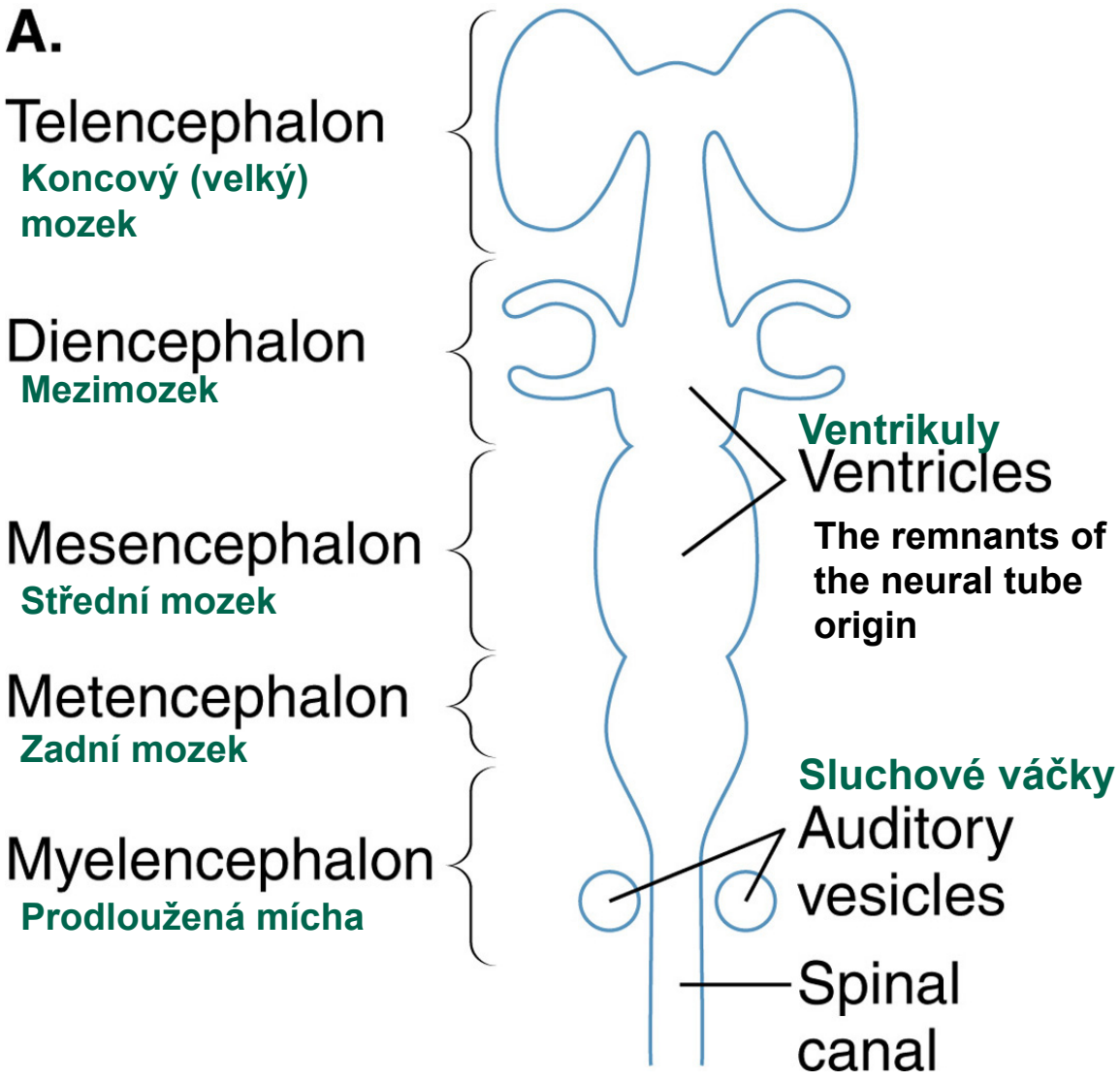
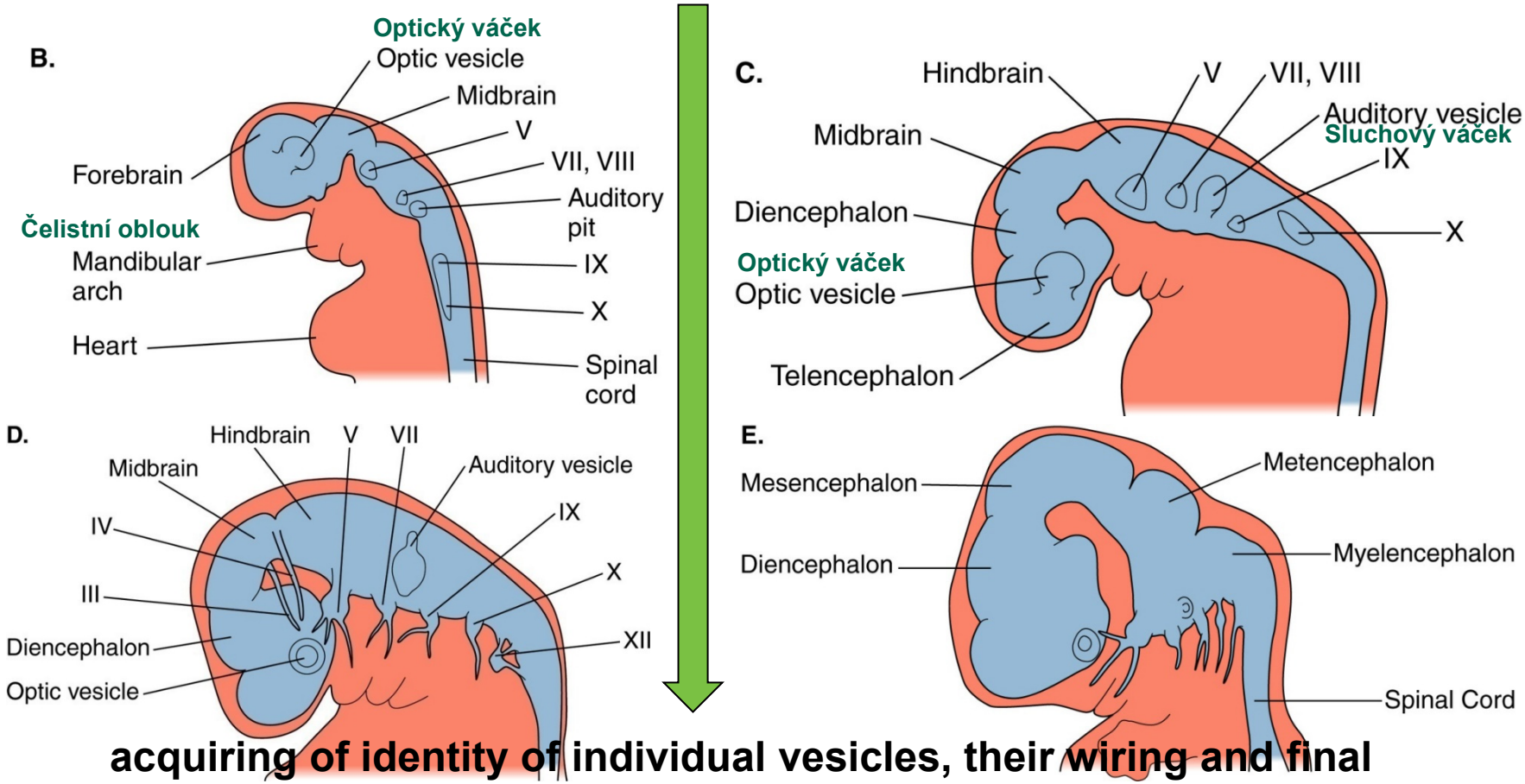

A.
Telencephalon
Koncový (velký) mozek
Diencephalon
Mezimozek
Mesencephalon
Střední mozek
Metencephalon
Zadní mozek
Myelencephalon
Prodloužená mícha
Ventrikuly
Ventricles
The remnants of the neural tube origin
Sluchové váčky
Auditory vesicles
Spinal canal

Changes in motility, cell division and apoptosis results into further segmental brain differentiation and vesicles formation



acquiring of identity of individual vesicles, their wiring and final sculpting of the brain

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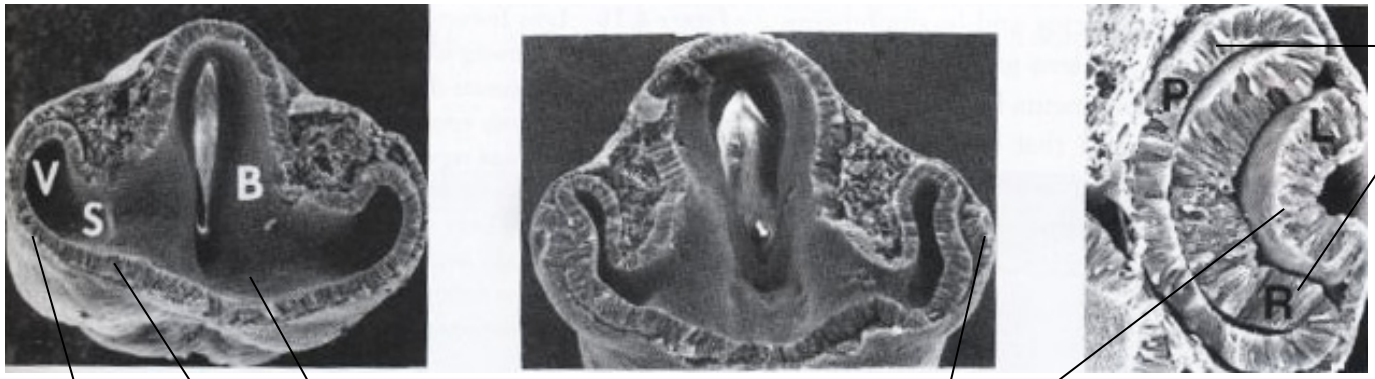
  - stratification of neural tube
- Development of brain and its derivatives
  - brain vesicles formation and development
  - eye development



Optical vesicle
Optický váček

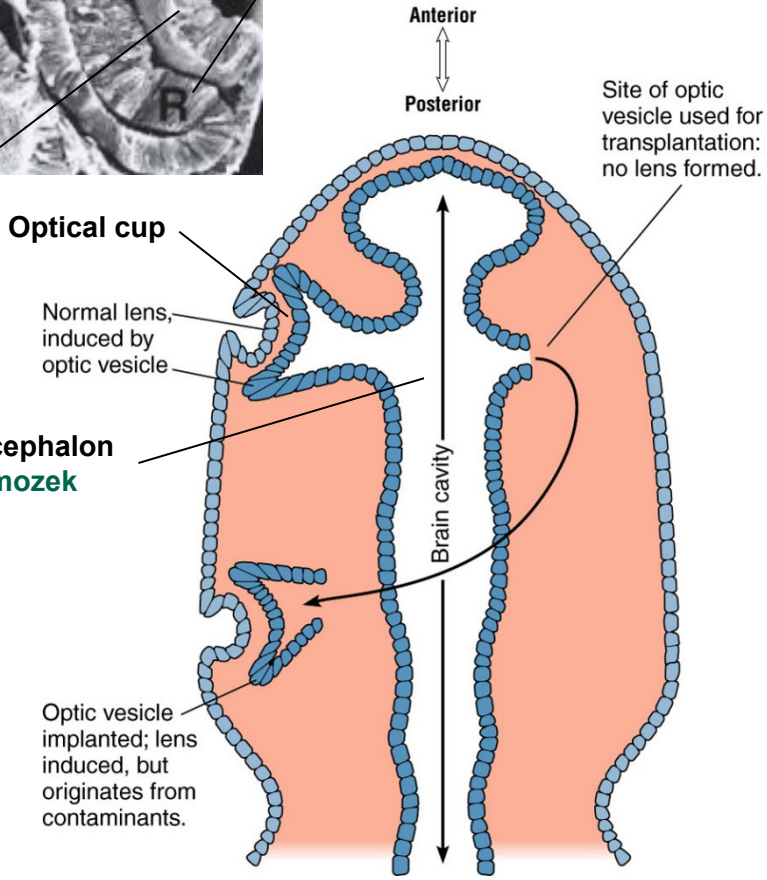
Brain
Mozek

Stalk of optical vesicle
Stopka optického váčku

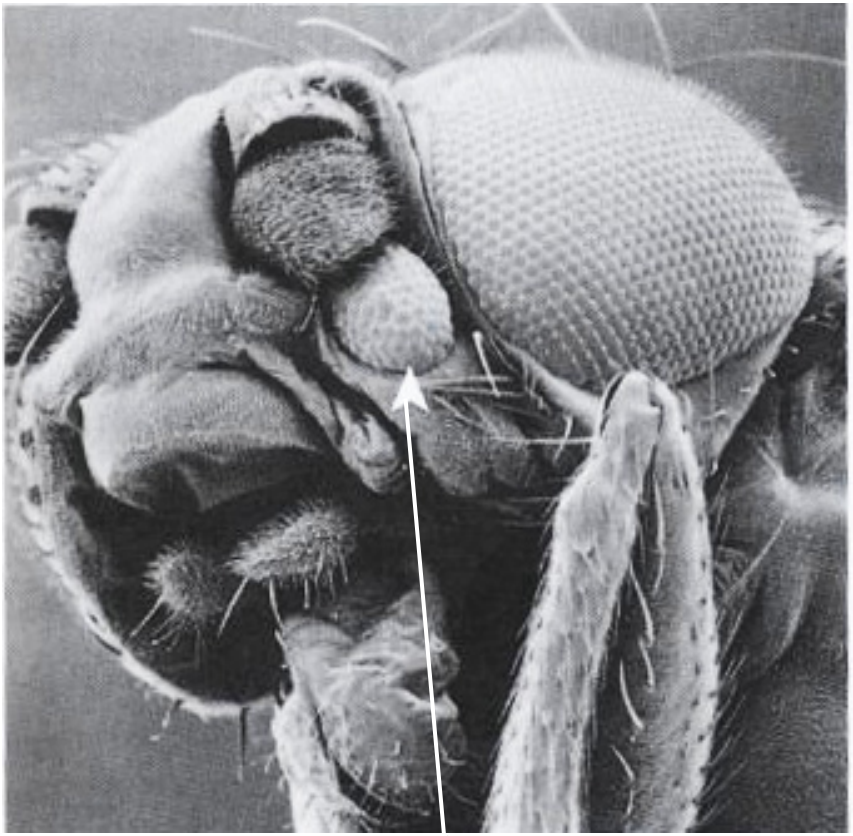
Lens placode
Čočková plakoda

Pigmented retina
Pigmentovaná sítnice

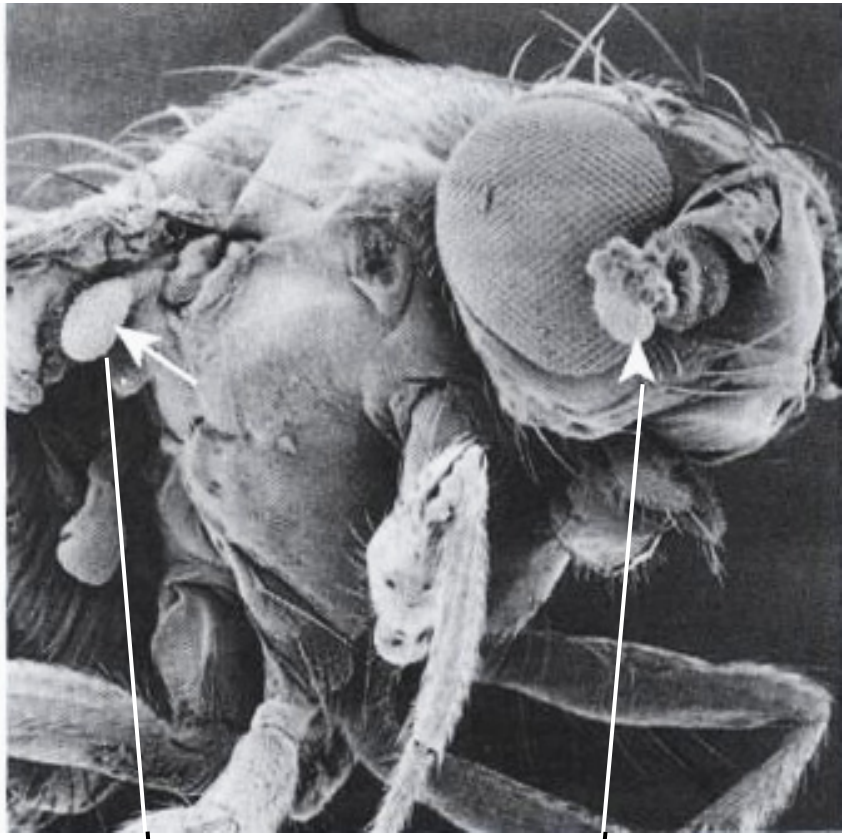
Neural retina – photoreceptors (rods, cons)
Neurální sítnice



Ectopic overexpression of *EYLESS* results into ectopic eye formation in *Drosophilla* and mouse *PAX6* is able to complement *eyless* mutation

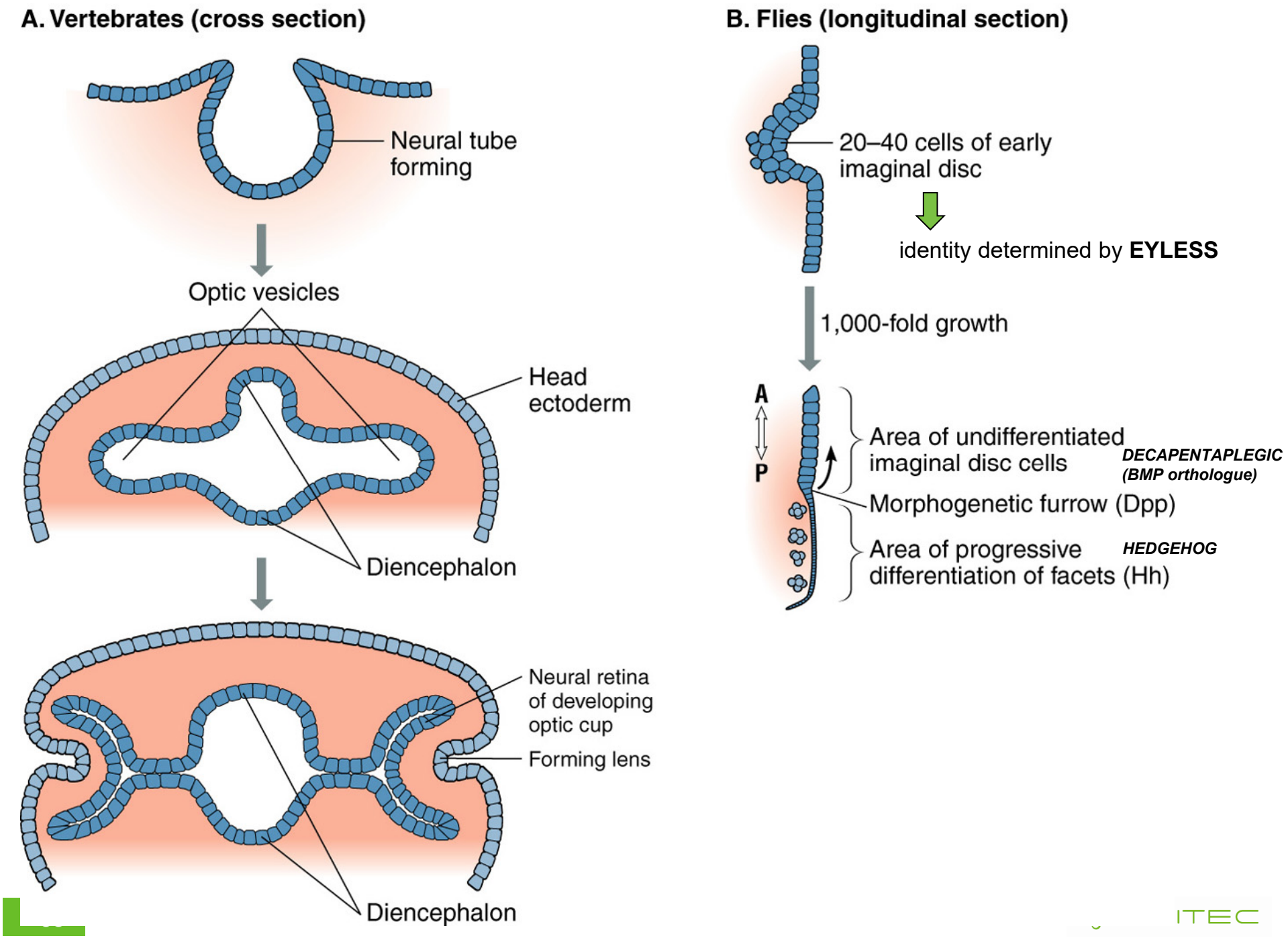


Ectopic eye in the head region



Ectopic eye below the wing

Ectopic eye on the antena


A. Vertebrates (cross section)
Neural tube forming
Optic vesicles
Head ectoderm
Diencephalon
Neural retina of developing optic cup
Forming lens
Diencephalon
B. Flies (longitudinal section)
20–40 cells of early imaginal disc
identity determined by EYLESS
1,000-fold growth
A
P
Area of undifferentiated imaginal disc cells
DECAPENTAPLEGIC (BMP orthologue)
Morphogenetic furrow (Dpp)
Area of progressive differentiation of facets (Hh)
HEDGEHOG

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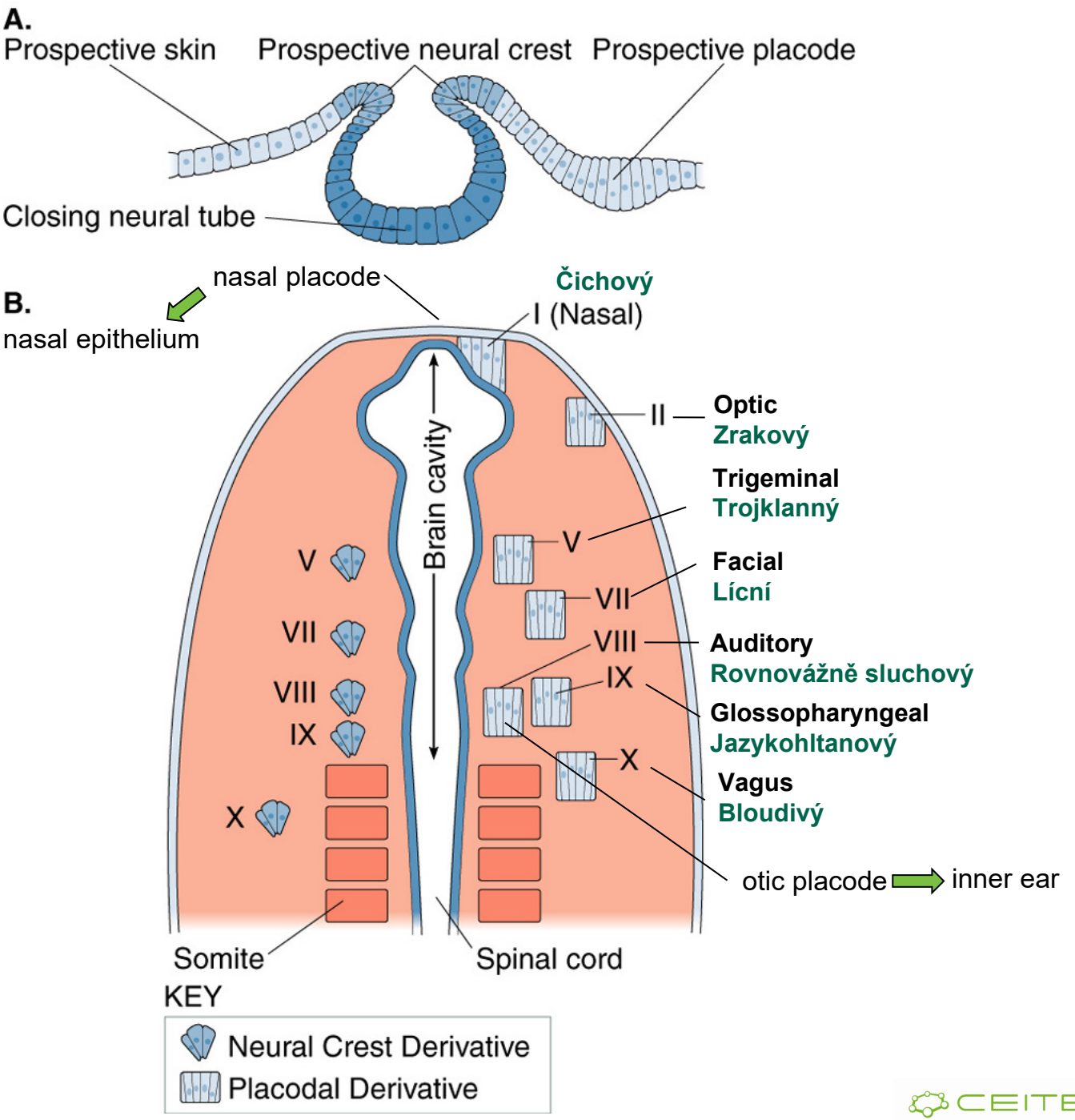
  - eye development
  - cranial ganglia and sensory organ epithelia


A.
Prospective skin
Prospective neural crest
Prospective placode
Closing neural tube
B.
nasal epithelium
nasal placode
Čichový
I (Nasal)
II
Optic
Zrakový
Trigeminal
Trojklanný
V
Facial
Lícní
VII
Auditory
Rovnovážně sluchový
VIII
IX
Glossopharyngeal
Jazykohltanový
X
Vagus
Bloudivý
otic placode
inner ear
Brain cavity
V
VII
VIII
IX
X
Somite
Spinal cord
KEY
Neural Crest Derivative
Placodal Derivative

I. Čichový (nervus olfactorius)

II.Zrakový (n. olfactorius)

III. Okulomotorický (n. oculomotoricus)

IV. Kladkový (n. trochlearis)

V. Trojklanný (n. trigeminus)

VI. Odtažný (n. abducens)

VII.Lícní (n. facialis)

VIII. Rovnovážně sluchový (n. statoacusticus)

IX. Jazykohltanový (n. glossopharyngicus)

X. Bloudivý (n. vagus)

XI. Přídatný (n. accesoriccus)

XII. Podjazykový (n. hypoglossus)

TABLE 6.2 A SUMMARY OF THE FUNCTION AND DERIVATION OF CRANIAL NERVES

Name	Number	Function	Contributions From:	
			Placode	Neural Crest
Olfactory	I	Smell	+	
Optic	II	Sight	+	
Oculomotor	III	Eye muscle (motor)		
Trochlear	IV	Eye muscle (motor)		
Trigeminal	V	Sensory	+	+
Abducens	VI	Eye muscle (motor)		
Facial	VII	Mainly motor		+
Auditory	VIII	Hearing	+	
Glossopharyngeal	IX	Mixed	+	+
Vagus	X	Mixed	+	+
Accessory	XI	Mainly motor		
Hypoglossal	XII	Tongue (motor)		

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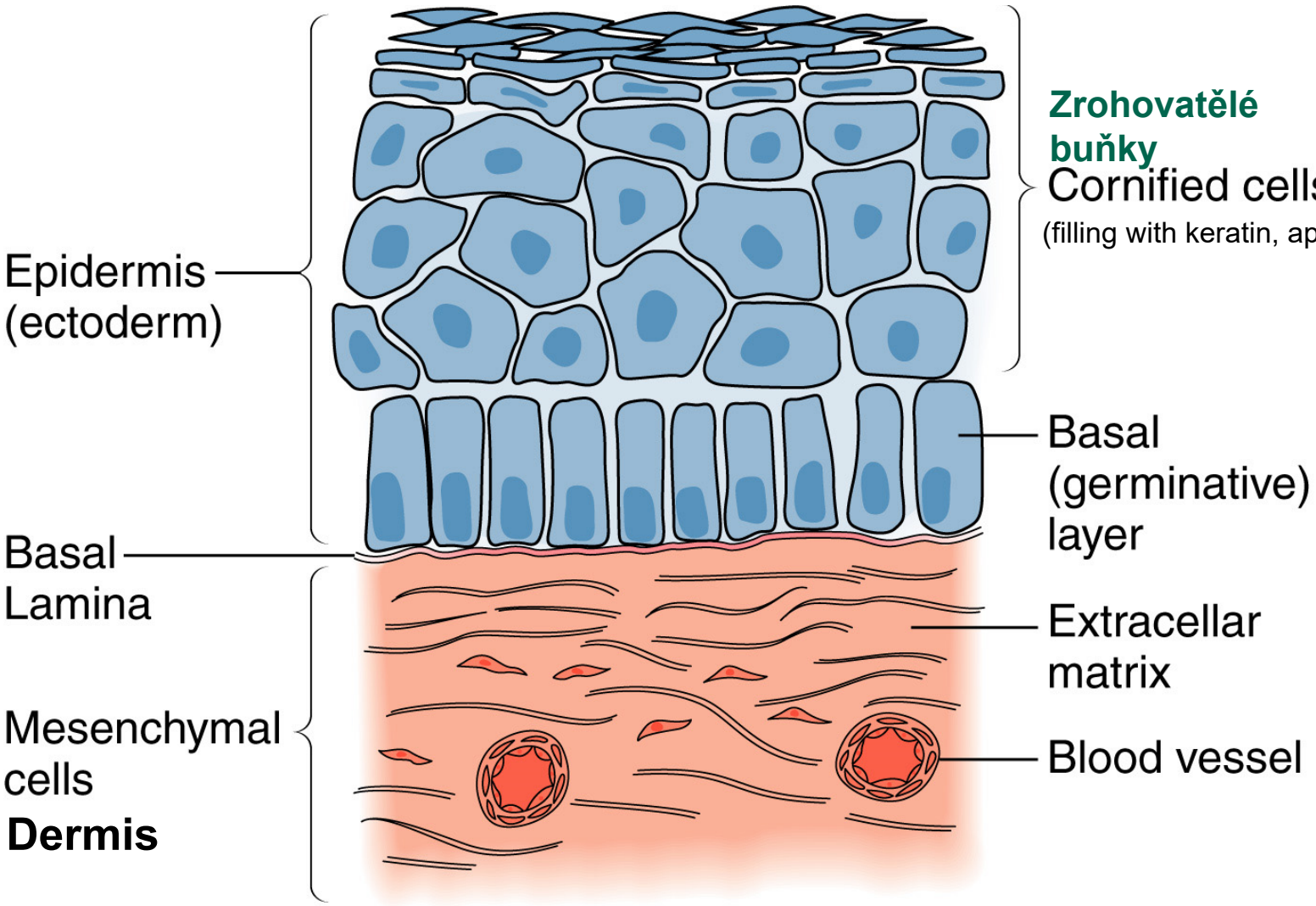
- Integument


Zrohovatělé buňky
Cornified cells
(filling with keratin, apoptosis)
Epidermis (ectoderm)
Basal (germinative) layer
Basal Lamina
Extracellar matrix
Mesenchymal cells
Blood vessel
Dermis

Key Concepts

- The **first zygote division** in the mammal development is **highly asymmetric**
- Mammals developed **placenta as a terrestrial life adaptation** that is different from terrestrial adaptations of birds and amphibians and allows **intrauteral embryo development**.
- There is **intense tissue communication** during **neural tube developmen**t allowing its **differentiation in both anteroposterior and dorsoventral axis** via formation of **morphogen gradient**.
- Multipoptent neural crest undergo complex **targeted cell movements** that allows their **spatial-specific differentiation**.
- Eye development is **highly conserved**.
- Both neural cord and eye development employ **common mechanism** of **morphogenic gradient** formation: **BMP/HGG**

Discussion