

# C8545 Developmental Biology

## Lesson 4

### Vertebrate Organogenesis: Ectodermal Derivatives

Jan Hejátko

**Functional Genomics and Proteomics of Plants**  
CEITEC  
and  
**National Centre for the Biomolecular Research,**  
Faculty of Science

**MUNI**  
**SCI**

Masaryk University, Brno  
[hejatko@sci.muni.cz](mailto:hejatko@sci.muni.cz), [www.ceitec.eu](http://www.ceitec.eu)



# Outline of Lesson 4

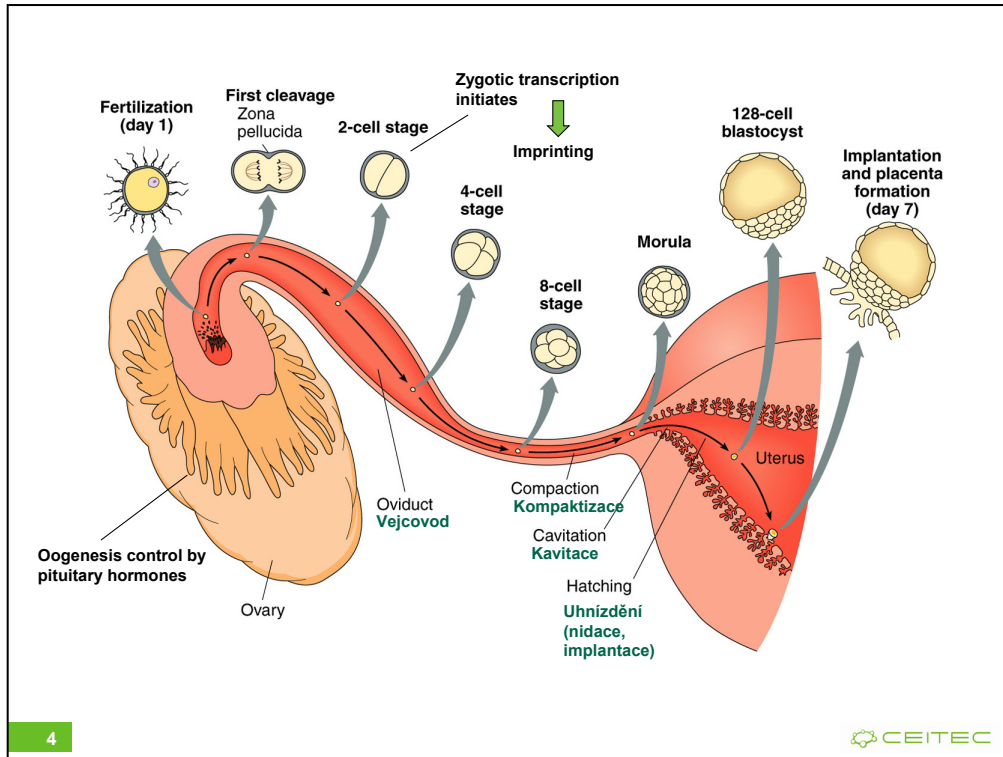
## Organogenesis in Vertebrates: Ectodermal Derivatives

- Early development of mammals
  - oogenesis and blastula formation
  - placental tissue differentiation
  - extraembryonic tissue formation
  - use of embryonal cells in mammalian transgenesis
- 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

- Early development of mammals
  - oogenesis and blastula formation



In contrast to amphibians, mammals have developed different type of terrestrial adaptations:

1. Mammals do not accumulate huge amount of yolk. Instead, embryo resides in the specialized portion of the oviduct, **uterus**.
2. In the uterus, the nutrition and gas/metabolite exchange is ensured via communication of the embryo with the maternal circulation via **placenta**.

Oogenesis comprises following steps: growth of the oocyte, meiosis and formation of membranes by the follicle cells surrounding the egg.

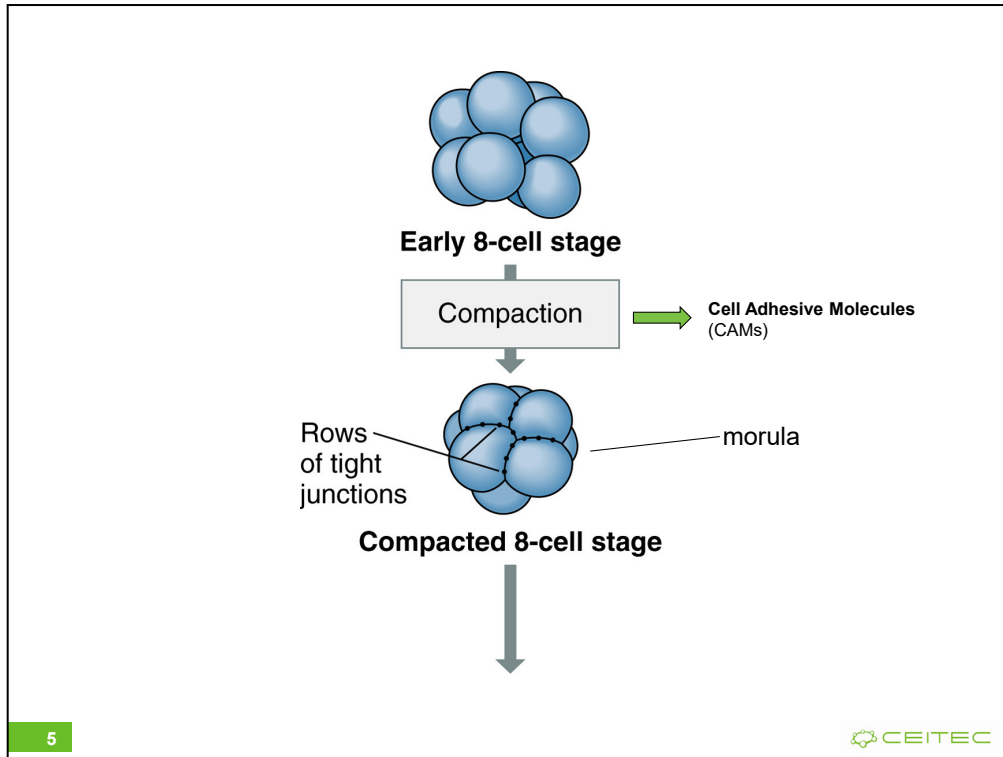
The process of oogenesis and ovulation is regulated by *pituitary hormones*.

Fertilization occurs in the upper portion of the mammalian oviduct, the **Fallopian tubes**.

There are further differences in comparison to the amphibian early embryogenesis:

1. After fertilization, the pace of cell division is much slower in comparison to amphibians. In mice and humans, the first division takes approximately a day and the next division occur every 10-12 hours. The morula reaches the uterus after 5-7 days, consisting thus from approximately 100 cells.
2. On the other way, zygotic transcription initiates at the very early, 2 cell stage.
3. There is limited maternal control of egg or embryo organization.
4. Very early, the formation of extraembryonic membranes begins.
5. There might be differentially expressed genes in the males and females due to differential methylation, *imprinting*.

Recent cell staining experiments, however, suggest that different portions of egg develop into different portions of the embryo. Thus, even mammals might be affected by egg organization.

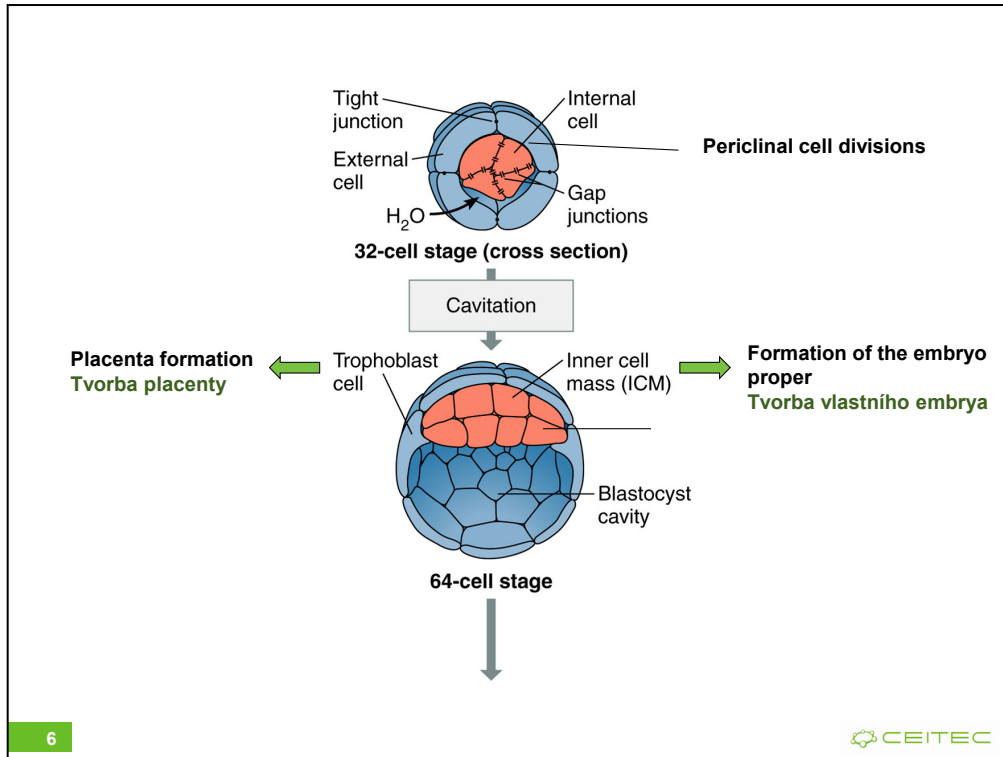


In most of the mammals, early embryogenesis events are conserved.

At the 8-cell stage, *blastomeres*, the individual cells of the early cleaving embryo, the *morula*, become a true polarized epithelium, the cells are flatten and become tightly adherent-this is called **compaction**.

The glycoproteins called *cell adhesive molecules* (CAMs) are responsible for the contact of individual blastomeres.

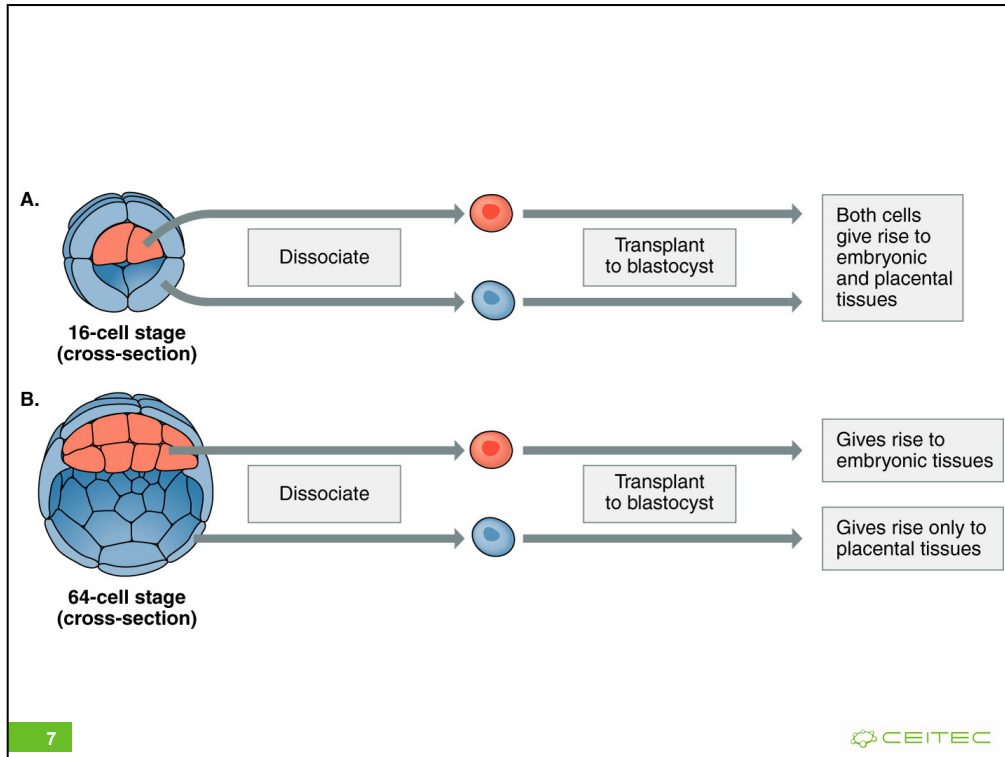
The early cleaved embryo is called **morula**.



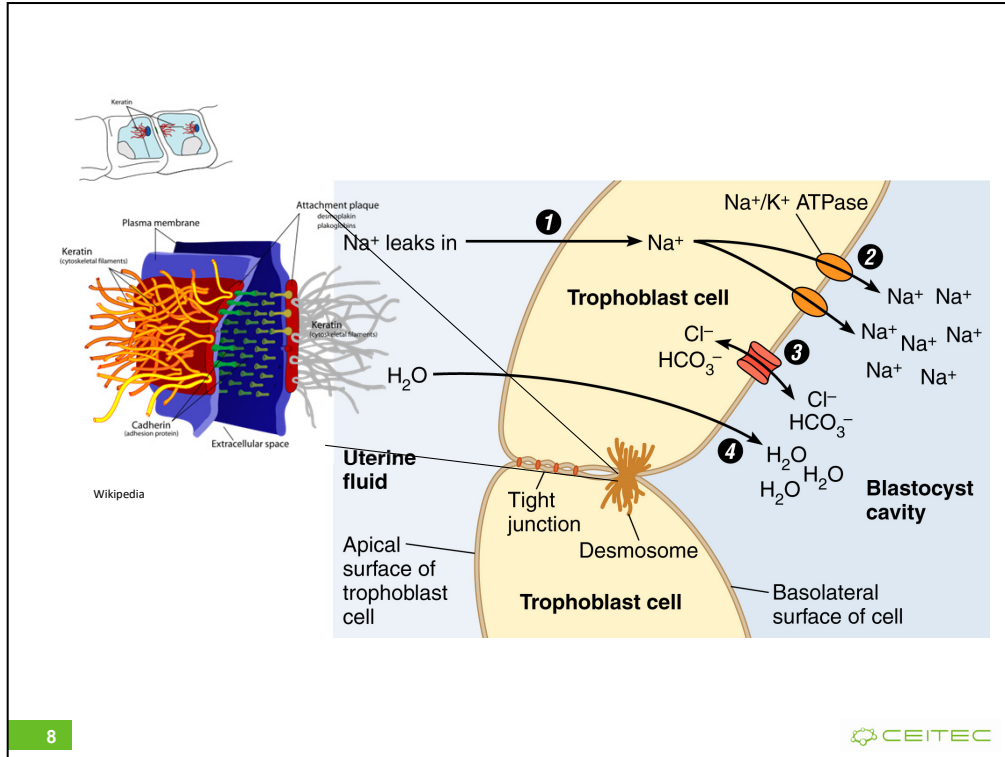
Besides the *anticlinal divisions* (with plane of division perpendicular to the cell layer surface) that adds new cells to the surface, *periclinal divisions* (parallel to the cell layer surface) produce internal cells, the **inner cell mass (ICM)**.

ICM will produce the embryo proper and the amnion.

The outer cells are called **trophoblast** and these will develop into placenta.



After the sixth round of cell divisions (at stage of 64 cells), the cells of trophoblast and ICM become specialized-as shown by surgical experiments of cell separation, ICM cells are no longer capable of giving rise to trophoblast, nor trophoblast to ICM.

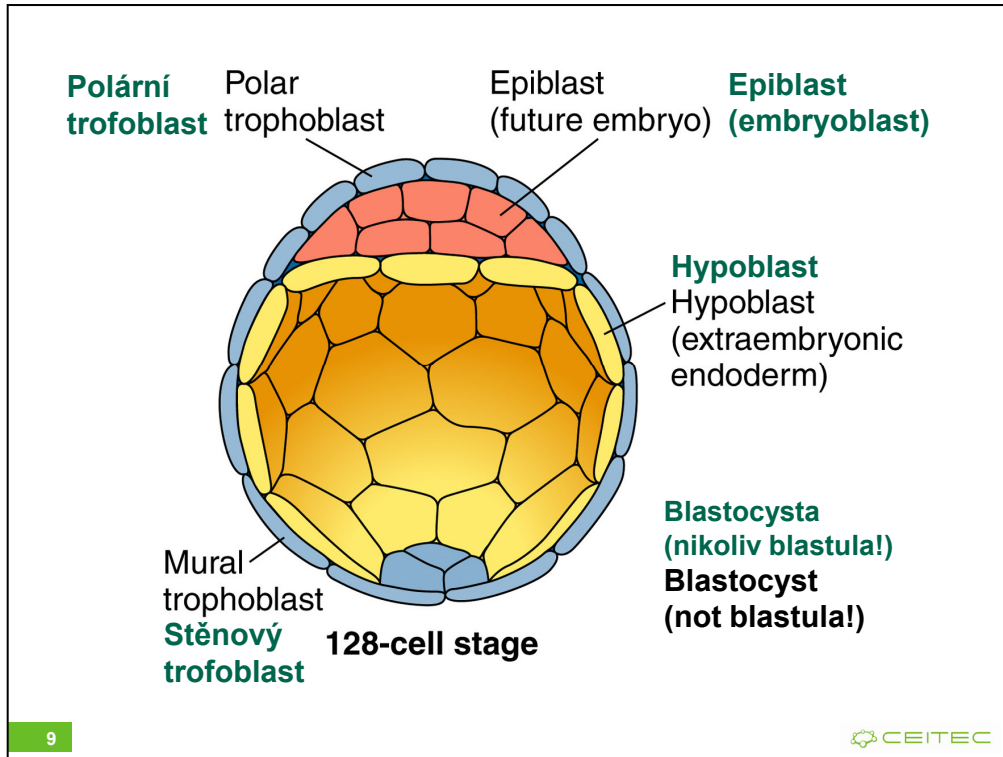


Polarized epithelium of the trophoblast has assymmetrically distributed sodium pumps on the apical surface.

Activity of these pumps leads to the accumulation of sodium ions in the internal space, which causes increase of the internal turgor (osmotic pressure) due to water import. Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange occurs in order to maintain the electrical neutrality.

That leads to the filling of the inner space by water and its swelling, a process called **cavitation**.





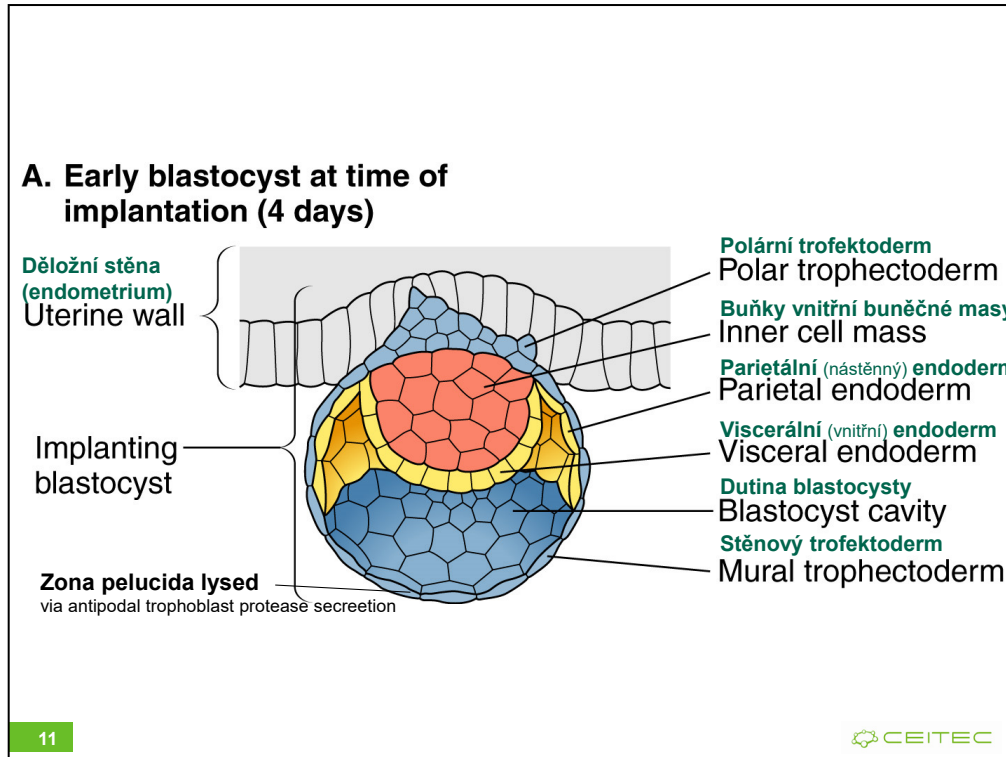
The embryo at the stage of 128 cells is called **blastocyst** (NOT blastula).

The blastocyst is unique structure that forms exclusively in mammals and it is incomparable to blastula.

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  - oogenesis and blastula formation
  - placental tissue differentiation

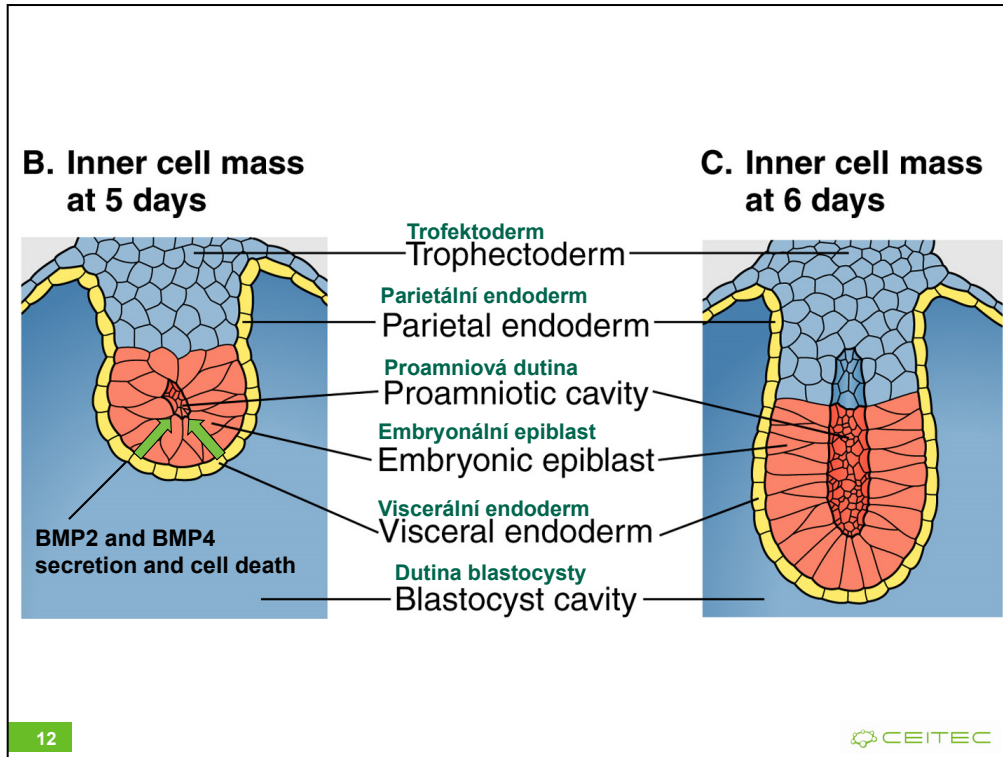


Near the end of the day four, the surface cell layer called *zona pelucida*, produced by follicle cells in the ovary, is lysed due to the protease secretion by trophoblast located opposite ICM (*antipodal trophoblast*) and the embryo **implantates** into the highly vascularized uterus.

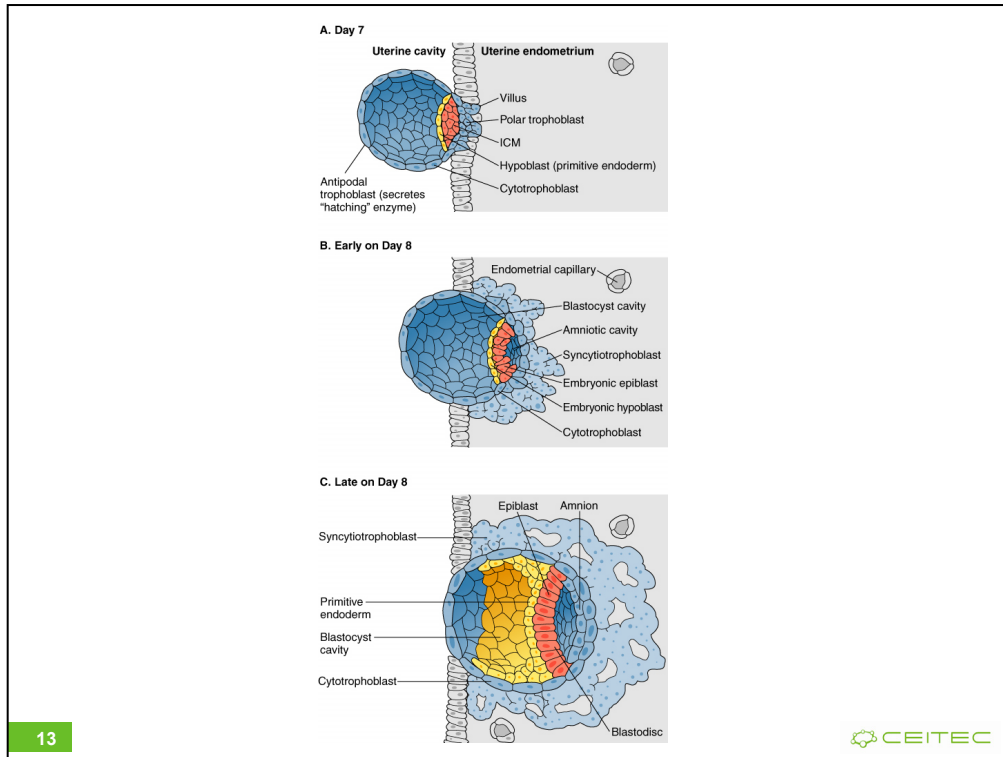
The ICM is covered with a layer of *visceral endoderm*, the blastocyst cavity with *parietal endoderm*.

The trophoblast cells proliferate and invade into the uterine wall.

The anatomical designation *trophectoderm* equals to trophoblast-that might lead to confusion.



Visceral endoderm secretes signalling ligands (BMP2 and BMP4, BONE MORPHOGENIC PROTEINS, involved in the bone formation), which are crucial for the initiation of complex processes of cell death, leading to the cavity formation inside the ICM.

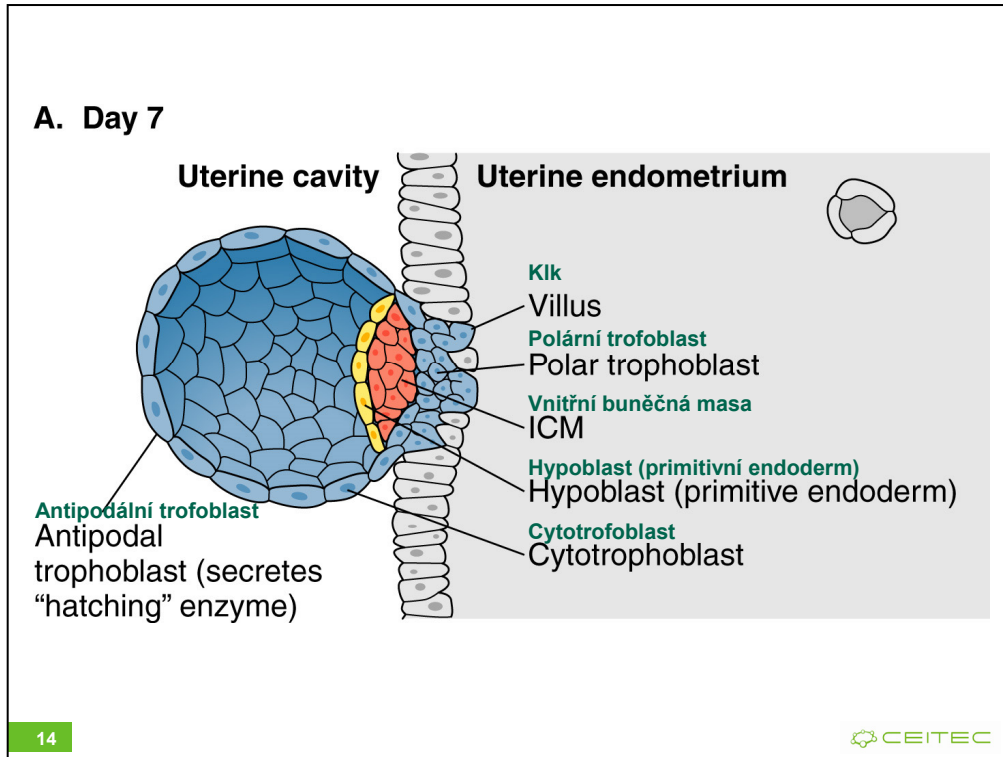


Process of the human embryo invagination. The process of the blastocyst invagination slightly differs from that of mice (discussed before), particularly in the shape of ICM (blastoderm).

The shape of blastoderm (blastodisc) in human embryos is more resembling the situation in chicken "flat" blastoderm.

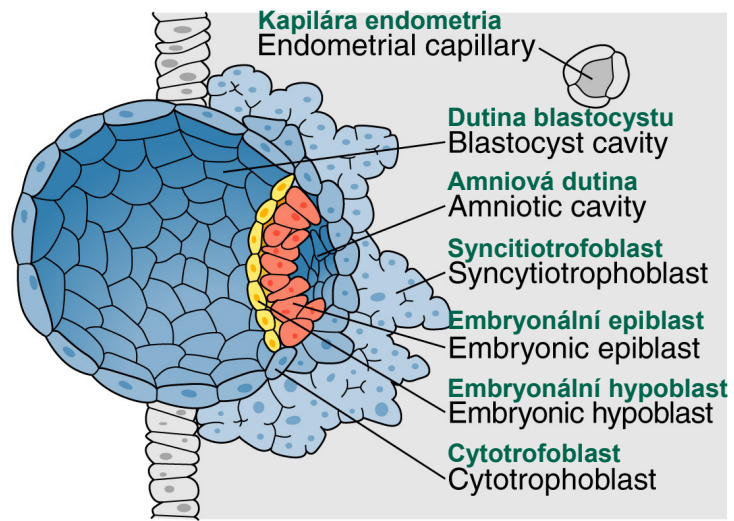
In mammals, the visceral endoderm forms what is called yolk sac, a fluid filled sac, partially resembling yolk in the birds. However, there is no yolk as such in mammals.

However most of the major events are fully comparable.

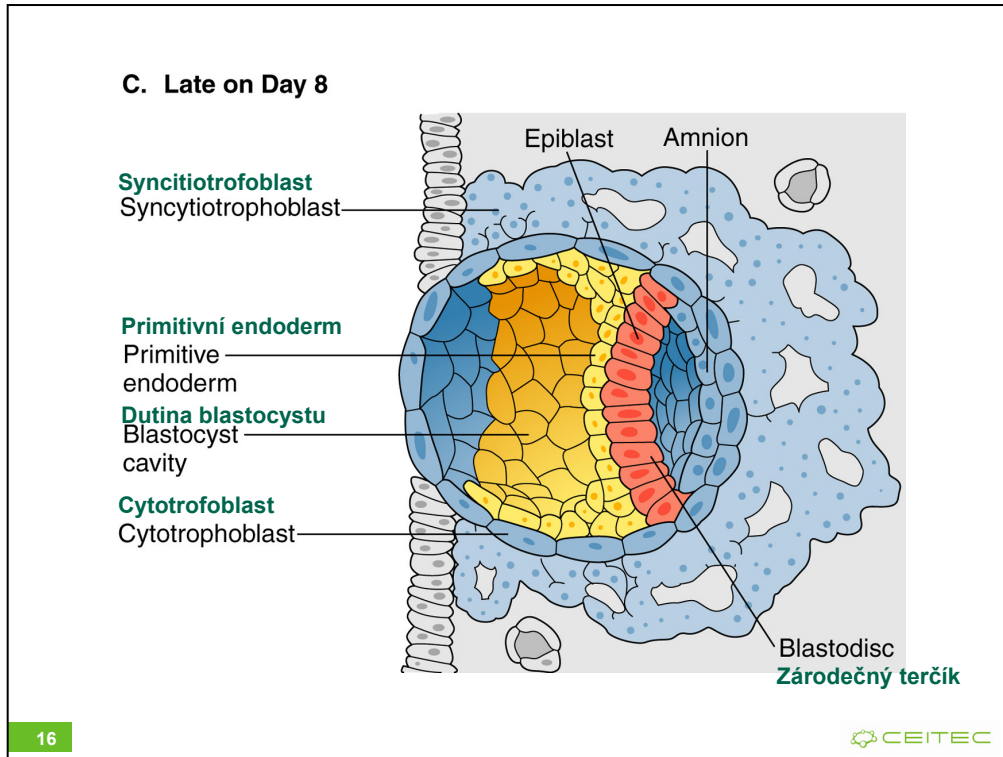


The seventh-day old embryo in the process of implantation. The polar trophoblast cells are invading uterine endometrium.

## B. Early on Day 8

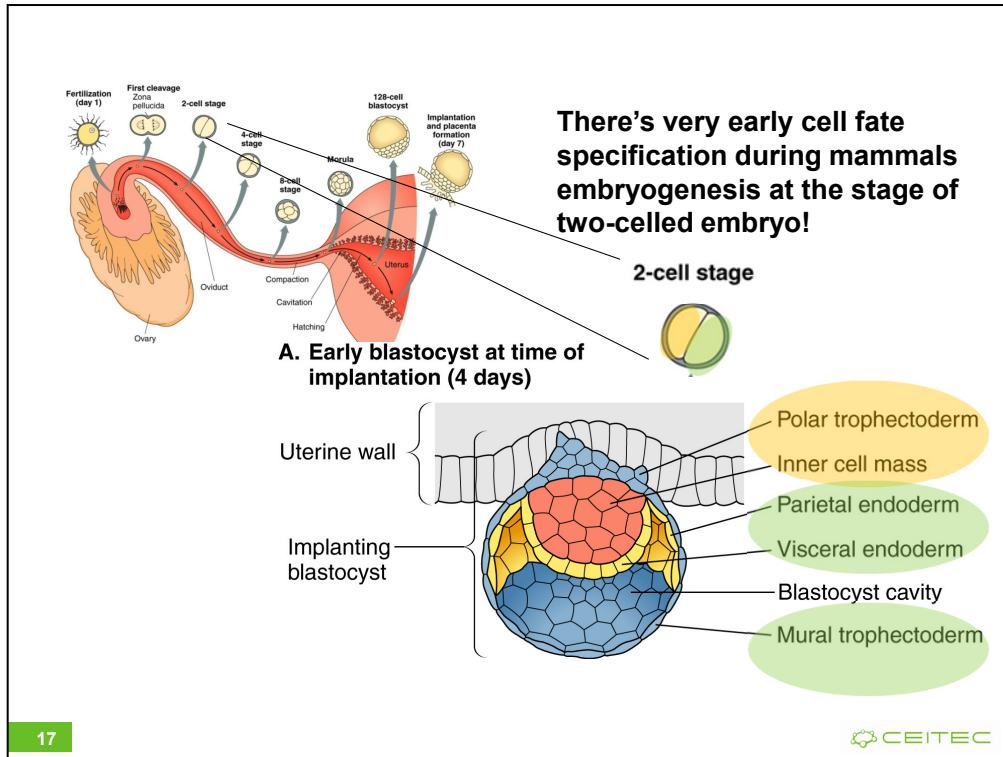


On the eight day, trophoblast is more proliferated and is composed of both, cellular and outer syncitial portion. The amniotic cavity started to form in ICM.



Late on the eight day, the amniotic cavity is well formed, embryo is completely implanted and the epiblast and primitive endoderm is apparent. The primitive endoderm later proliferates, giving rise to parietal endoderm adjoining mural trophoblast and visceral endoderm that covers ICM.





Formation of ICM is unique to mammals and it is connected with a very early cell specification. Recently it has been shown that the cell polarity occurs already at the two-cell stage of cleaving embryo (!).

Richard Gardner in Oxford and Karolina Piotrowska in Cambridge have shown that one of the first two cells give rise to the upper portion of the embryo, including ICM and polar trophectoderm while the other one to the lower embryo portion, consisting of mural trophectoderm and primitive endoderm.

Hence, the plane that is orthogonal to the plane of the ICM is approximately orthogonal to the plane of the first mitotic spindle.

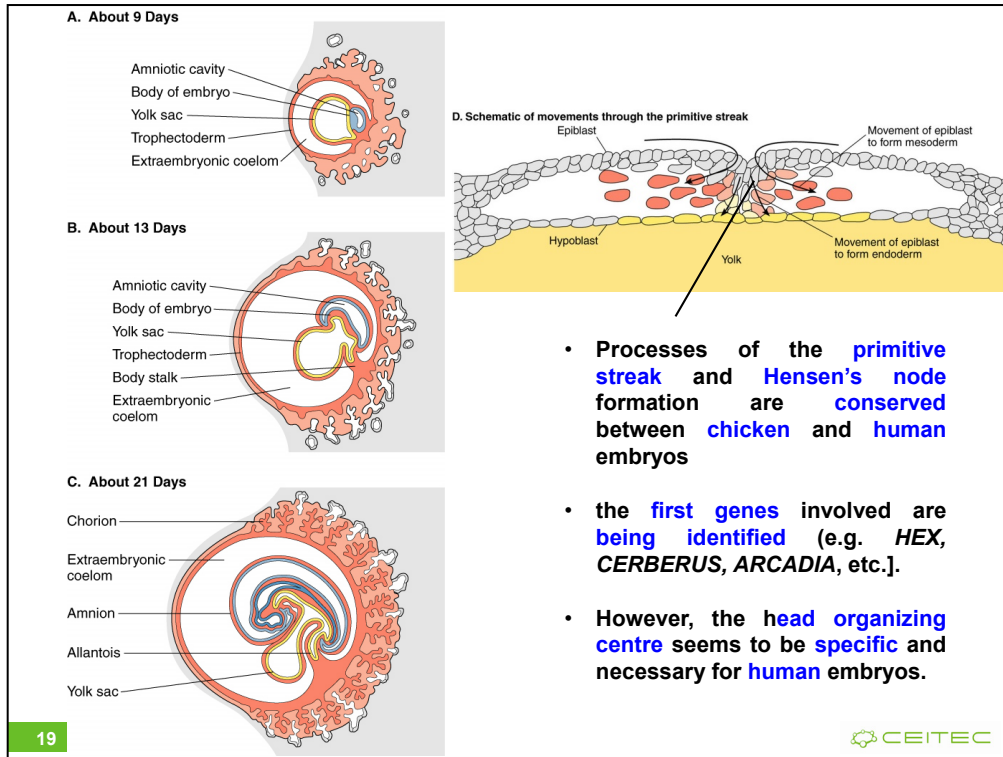
Very probably, neither maternal mRNA or protein localization, nor gravitational influence or sperm entry do affect this process.

The molecular mechanism of the asymmetric cells division that might be underlying this early cell speciation will be discussed later.

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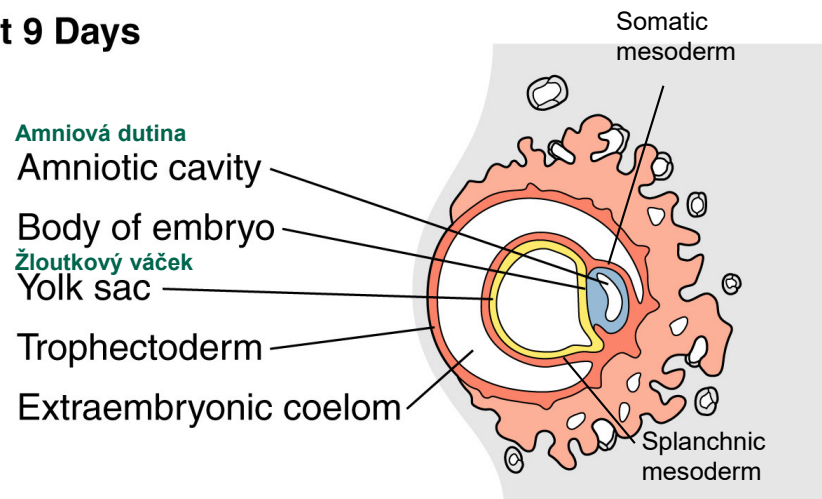
Formation of individual germ layers is similar as those in chicken. I.e., the cells from the posterior epiblast migrate to the midline, forming thus a **primitive streak** that poses an **anteriorly localized organizing centre** (equivalent to Hensen's node, see the small inset above). In contrast to mice, the human embryos are more similar to flatten chick embryo and its gastrulation discussed in the previous lesson.

The function of the node is conserved, as shown in experiments, where the node from mammals was transplanted to the area pellucida in chicken and newly formed axis was apparent. Similarly transplantation to other mammal embryo will cause another neural axis formation, except that anterior brain regions are missing in the newly formed axis. Thus, very probably there are other factors and/or second organizing centre necessary for the head development in mammals.

Recently, genes expressed in the node and/or necessary for the node function or secreted from other tissues, e.g. visceral endoderm and necessary for specific developmental events, e.g. head formation are of interest and are being identified (e.g. *HEX*, *CERBERUS*, *ARCADIA*, etc.).

Finally, the processes of the germ layers, i.e. ectoderm, endoderm and mesoderm formation during gastrulation, i.e. the cell migration and invagination through the node are similar as in the chicken.

## A. About 9 Days



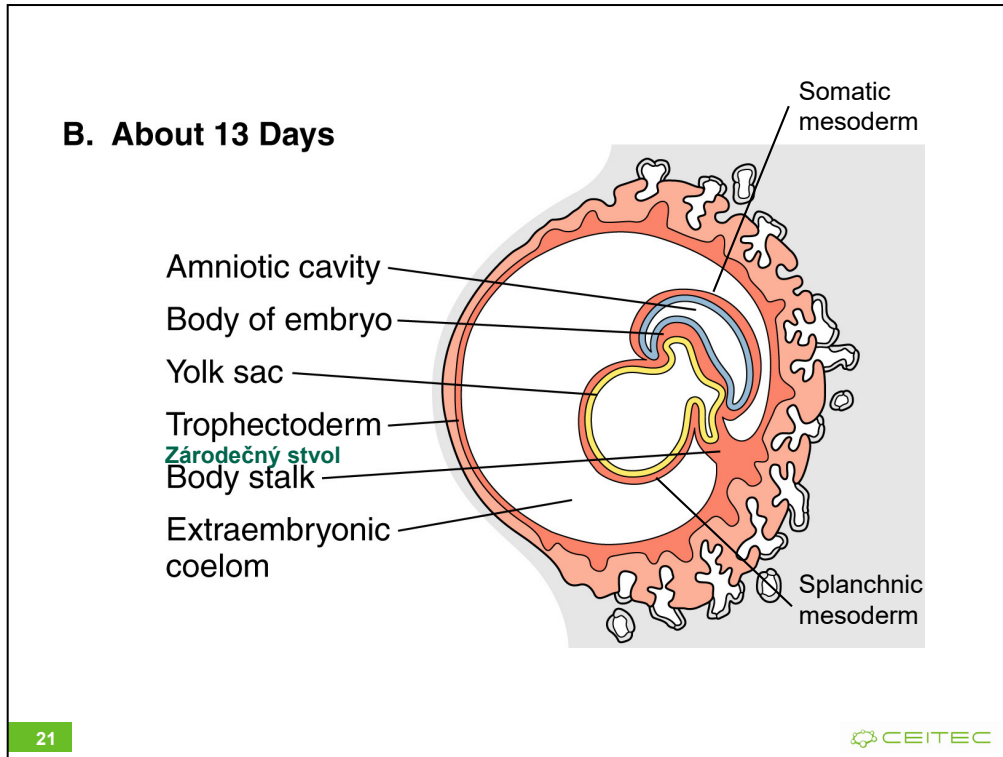
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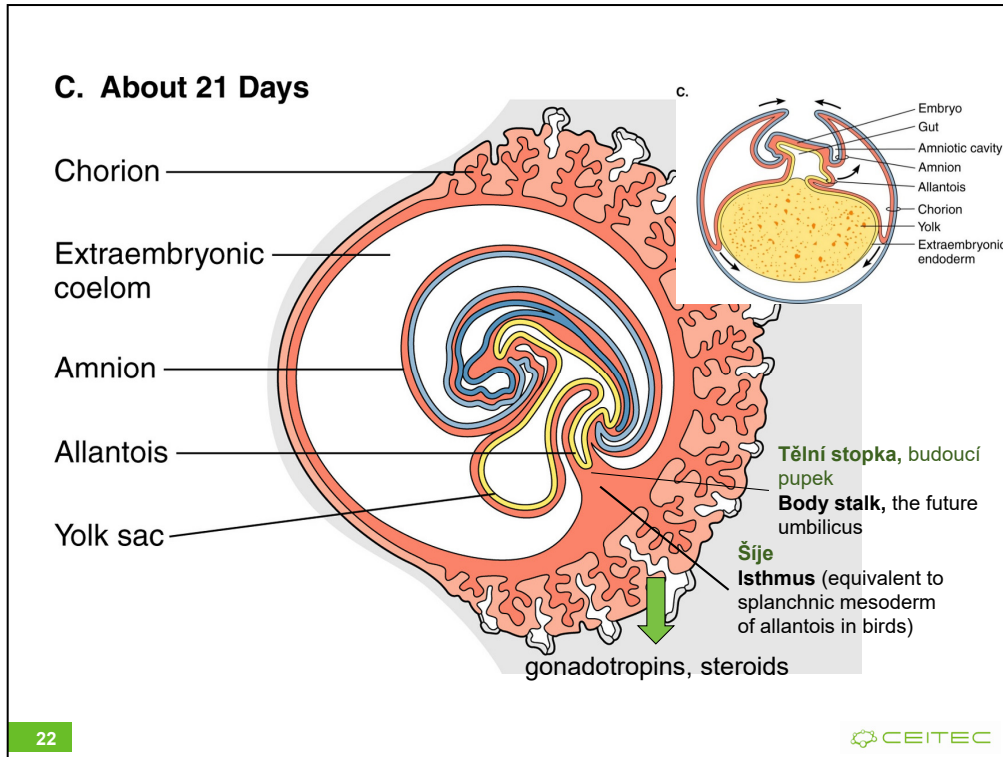
Similarly to other vertebrates, invaginations along the midline of the node and cell migrations form neural plate and lateral portions, where epidermis will form.

There is somatic and splanchnic mesoderm separated by a coelom (could be better observed on later slides). The visceral endoderm (old hypoderm) is pushed aside to become extraembryonic endoderm.

There is no substantial amount of yolk in mammals, the yolk sac enclosed by the (mostly extraembryonal) endoderm and contains fluid.



The extraembryonic somatic mesoderm covers together with the extraembryonic ectoderm the amniotic cavity and forms amnion. The inner extraembryonic ectoderm of the amnion was produced by the ICM (see before).



Extraembryonic tissue forms membranes enclosing the embryo and secretes amniotic fluid, in which the embryo is bathed. Occasionally, cells shed from amnion that could be a target of specific procedure, *amniocentesis*, which allows e.g. genetic diagnosis of the developing fetus.

Some mesoderm near the developing posterior end remains connected to the trophoblast, creating thus an isthmus that allows vascularization of the embryo portion of the placenta. This is equivalent to the splanchnic mesoderm of the allantois in birds (compare with chicken embryo, inset).

Trophoblast invades into the maternal tissue and forms the placenta. Trophoblast forms, as previously mentioned, syncytial form (*syncytiotrophoblast*) and cellular form (*cytotrophoblast*), that is more proximal to the embryo.

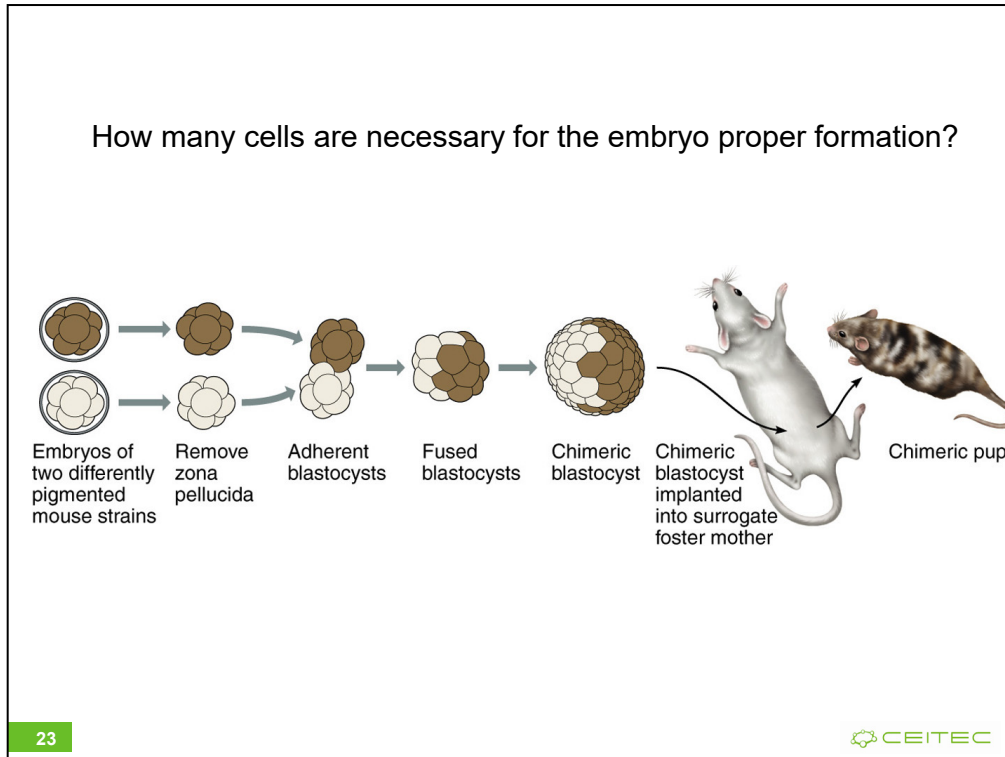
Developing placenta remains connected to the embryo via mesodermal stalk, the *body stalk*, that will become the *umbilicus*.

The degree of intimacy of the anatomical association between embryo and maternal tissue varies in different organisms.

In humans and in mice, the maternal tissue of uterus erodes, the maternal capillaries open to form a system of sinuses and maternal blood gets in the direct contact with endothelial layers of the embryo. This allows direct exchange of metabolites and nutrients between embryo and its mother.

Placenta consist of both maternal and embryonal tissue and has important functions. It allows nutrition, respiration and excretion of the embryo and overtakes the function of pituitary and ovary before pregnancy, i.e. hormone production (chorionic gonadotropins and steroids).

Placenta also provides immunological protection for both mother and fetus, as the fetus carries paternally inherited antigens. However, the mechanism of this protection is not known.



Scheme of the experiments that allowed to specify the number of cells necessary for the embryo proper formation.

Two embryos from two, distinctly genotyped (i.e. coloured) mice are fused (after the zona pellucida is removed). If the only one cell would be sufficient, then the mice would be of one of the originally used genotypes. If two cells would be involved, one quarter of the progeny would be of either genotype and one half would be mixed i.e. they will become chimeras (if both genotypes would become the precursor of the embryo proper with the same probability).

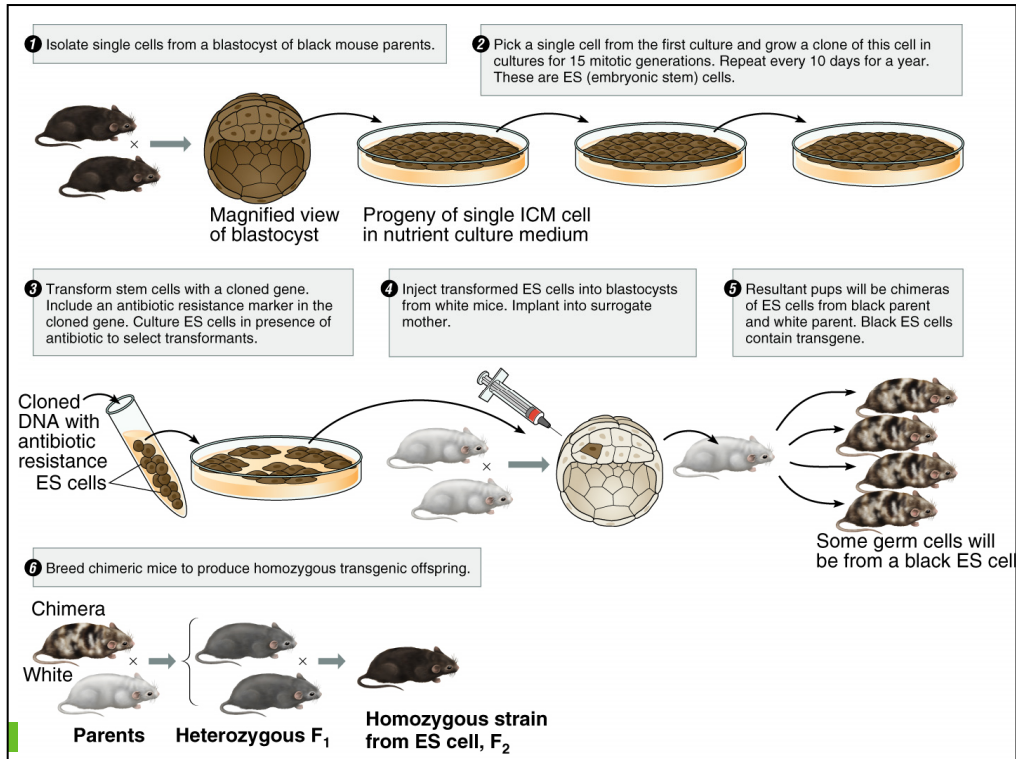
Beatriz Minz has found that the number of chimeras is about 75%, which corresponds to the number of three precursor cells.

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Individual ICM cells of the embryo could be isolated and later re-introduced into the new embryo. These ICM cells are called **embryonic stem (ES) cells**. It is a very important technique that allows production of transgenic mice.

The isolated ES cells are transformed via foreign DNA construct and it is injected within the embryo. The transformed cell becomes a part of the embryo and might result in the formation of different tissue types, among them the spermatogonia or oogonia. i.e. the tissue that provides progenitor for sperm or egg cells in the resulting chimera. Thus, the progeny of those chimeras will inherit the modified cell with certain probability and these individuals will carry the transgene in every cell of their body. Thus, the transgenic mice will be produced.

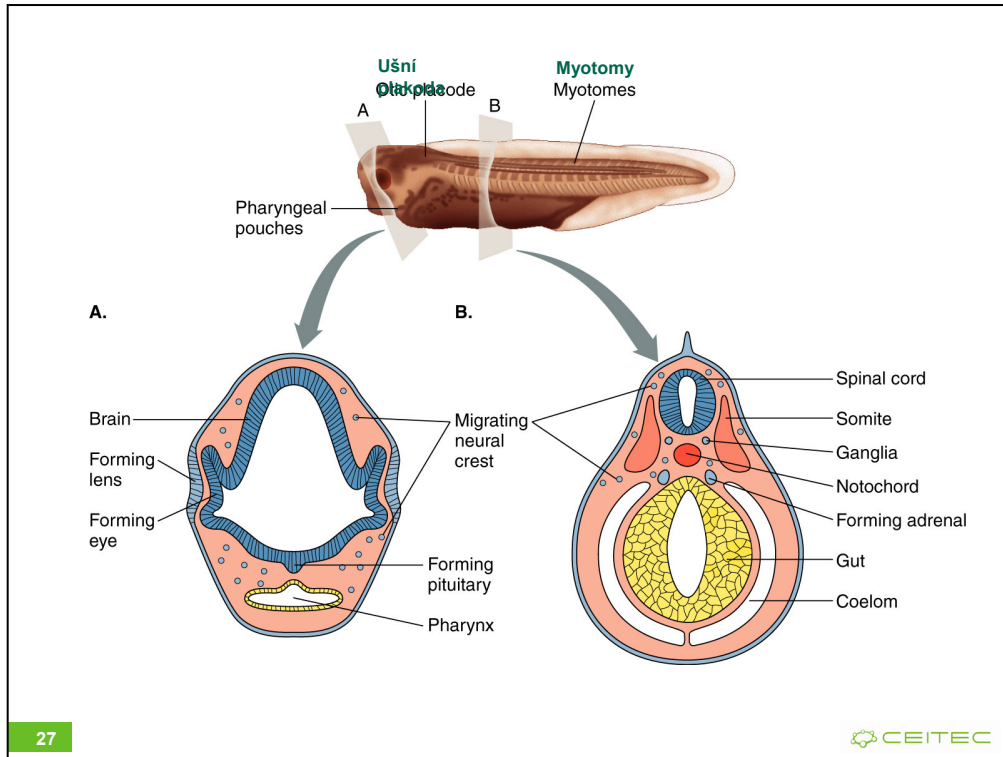
This is very important mainly with regard to the knockout mutant (K.O.) production. In the modified ES, the genes might be specifically eliminated via DNA recombination. In that way, the function of many of the mouse genes was identified.

E.g. the gene *NODAL* is expressed in the anterior portion of the primitive streak that is equivalent to the Hensen's node. *nodal/nodal* embryos are lethal, they do not undergo gastrulation and form almost no mesoderm.

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- Differentiation of neural tissue
  - mechanisms of neural tissue specification

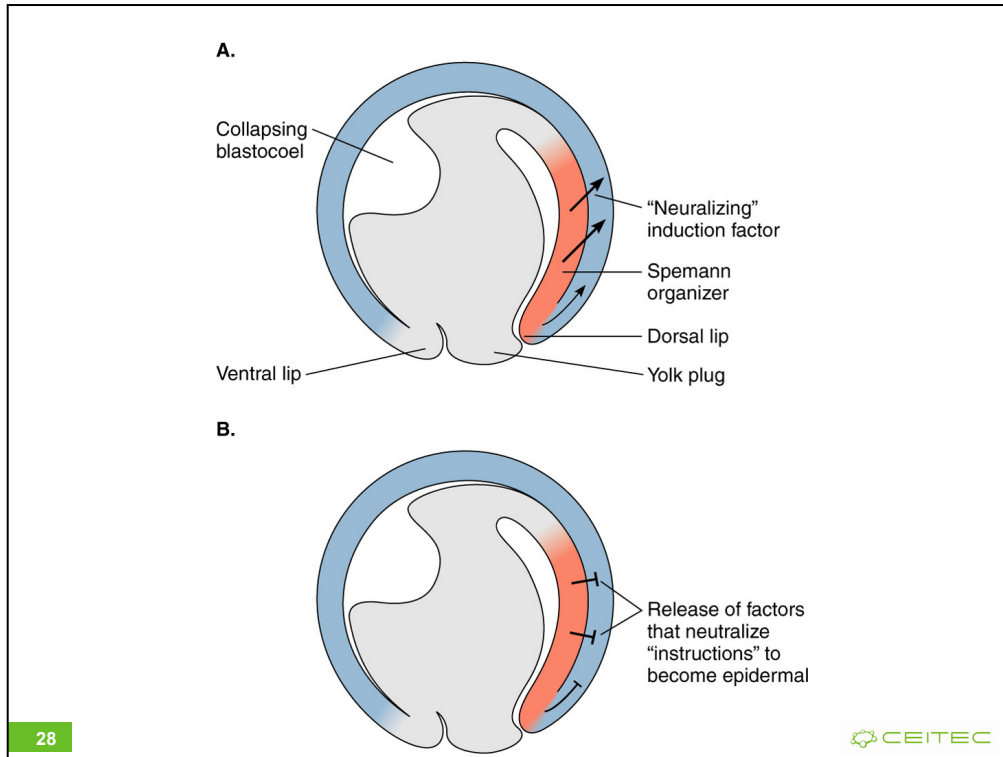


Sections through the young larva of amphibians showing the ectoderm derivatives:

In the head region, there is brain, forming eye and migrating neural crest cells, in the trunk region, there is apparent neural tube and neural crest cells, forming ganglia and adrenal.

In both sections, there is epidermal portion of the integument.

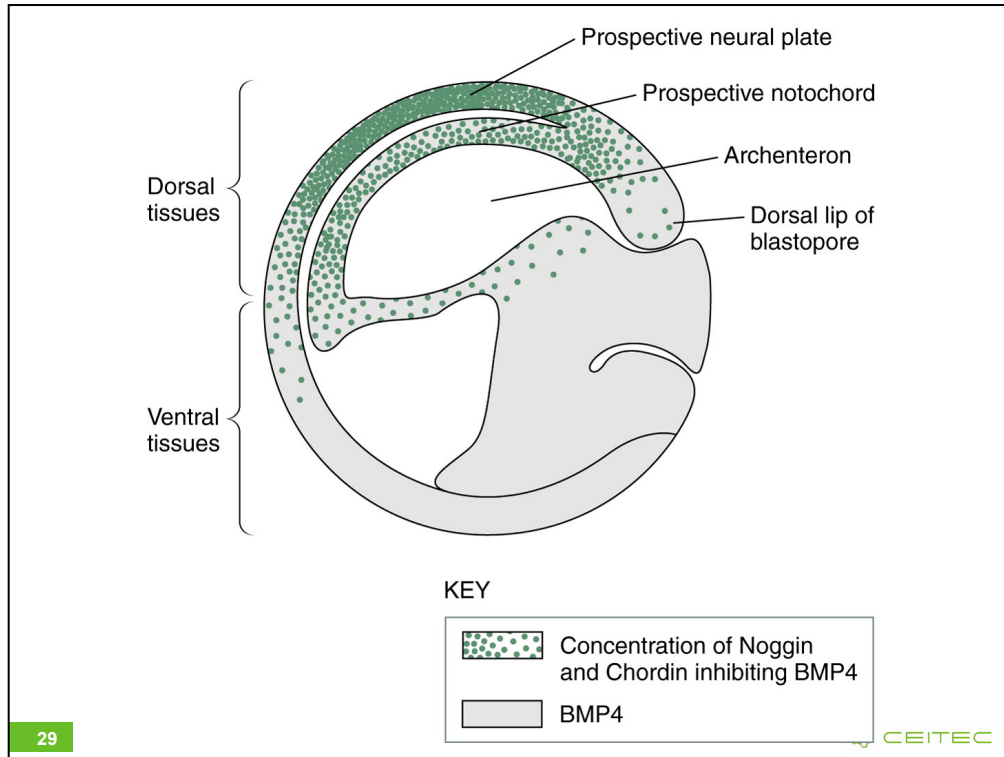
*Myotomes* refer to upper (dorsal) portion of the somites that will develop into muscles (will be discussed in the next lecture).



What are the factors that induce neural plate formation during early gastrulation?

Plenty of substances, among them even pH or different ions concentration were identified. Recent hypothesis suggests that neural development is a default state.

There are in principal two possible ways of the neural tissue induction: *(i)* induction of epidermis to form neural tissue (upper figure) or, *(ii) vice versa*, factors inhibiting default developmental programme that is epidermis formation by ectoderm (lower figure).

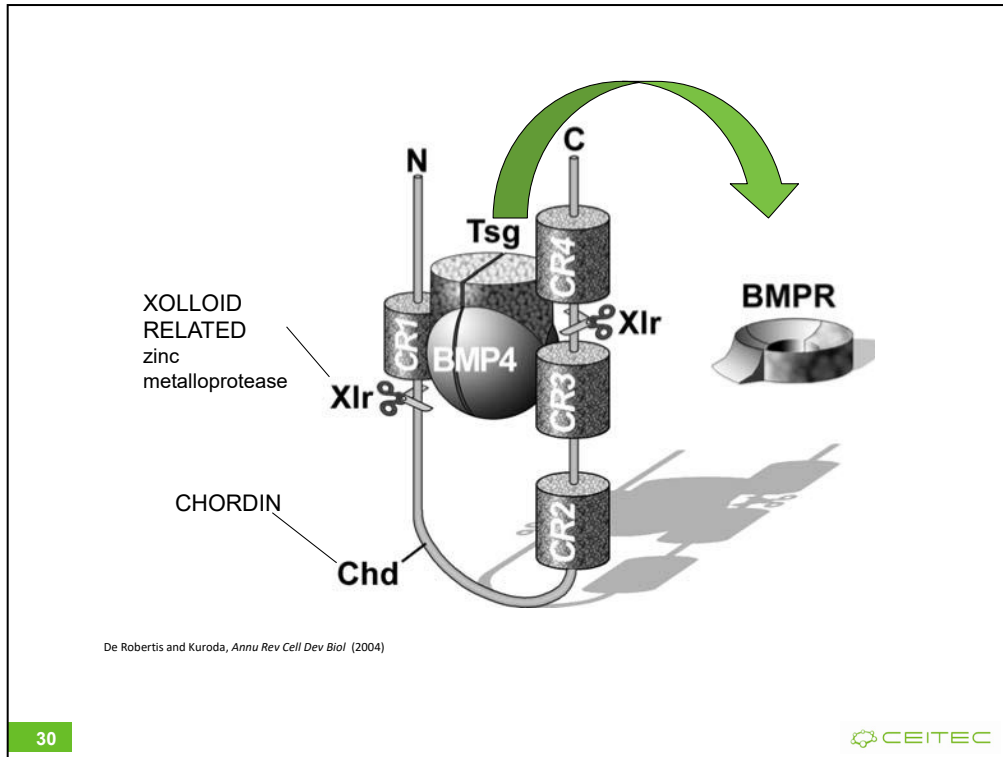


It has been found that by default, the neural cell development occurs. Thus, to allow epithelial cell development, this default developmental program must be inhibited .

Recently, factors inhibiting neural tissue and thus promoting epidermis formation were identified. These are BMPs (BONE MORPHOGENIC PROTEINS, also mentioned previously in the fifth chapter as inducers of the cell death secreted by endodermis) and allowing cavity formation on the ICM).

BMPs are secreted by the prospective ventral and marginal portions of the embryo and form a gradient. They interact with their receptors and induce epithelial fate formation.

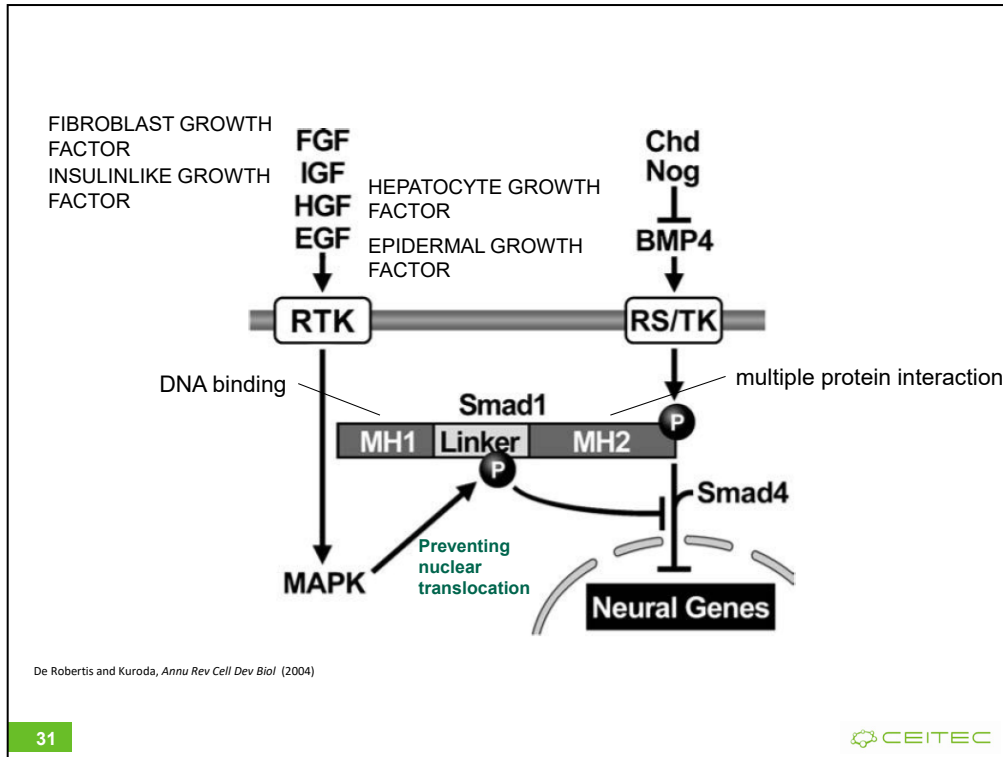
In the prospective neural plate region, *NOGGIN* and *CHORDIN* are expressed. Their protein products inhibit the interaction of BMPs with their receptors and this allow running the default, neural developmental program.



A molecular complex involving BMP, CHORDIN, and TSG regulates the D-V activity gradient of BMP4 in *Xenopus*.

CHORDIN binds BMP4 through cysteine-rich domains CR1 and CR3.

After cleavage of CHORDIN by the XOLLOID-RELATED (XLR) zinc metalloprotease, the affinity between CR modules and BMP4 is greatly reduced, and BMP4 protein is able to bind to and activate BMP receptor (BMPR) on the cell surface (De Robertis and Kuroda, 2004).



However, as recently found, both inhibition of BMP4 and activation via FGF (fibroblast growth factor) and IGF (insulin-like growth factor) are necessary for the neural tissue induction.

Integration of multiple signaling pathways at the level of Smad1 phosphorylation during neural induction. Neural induction requires extremely low levels of Smad1 activity that are reached through the combination of two signaling systems.

One is inhibition of binding between BMP4 and its serine/threonine kinase (RS/TK) receptor by anti-BMP molecules such as Chordin and Noggin. In the absence of those inhibitors, the Smad proteins are phosphorylated at the carboxy-terminal SXS sequences, thus triggering nuclear translocation. In the nucleus, Smad proteins inhibit neural gene expression.

The other is inhibitory phosphorylation of Smad1 in the linker region by receptor tyrosine kinase (RTK) signals such as FGF, IGF, HGF, and EGF mediated by activation of MAPK. That phosphorylation prevents nuclear translocation of Smad1 and has an inhibitory effect on Smad activity. That allows neural gene expression and thus acquisition of neural tissue identity.

Thus, both of these regulations, i.e. inhibition of the BMPs via CHORDIN and NOGGIN and negative regulation of Smads via MAPK activity is necessary for the neural tissue initiation.

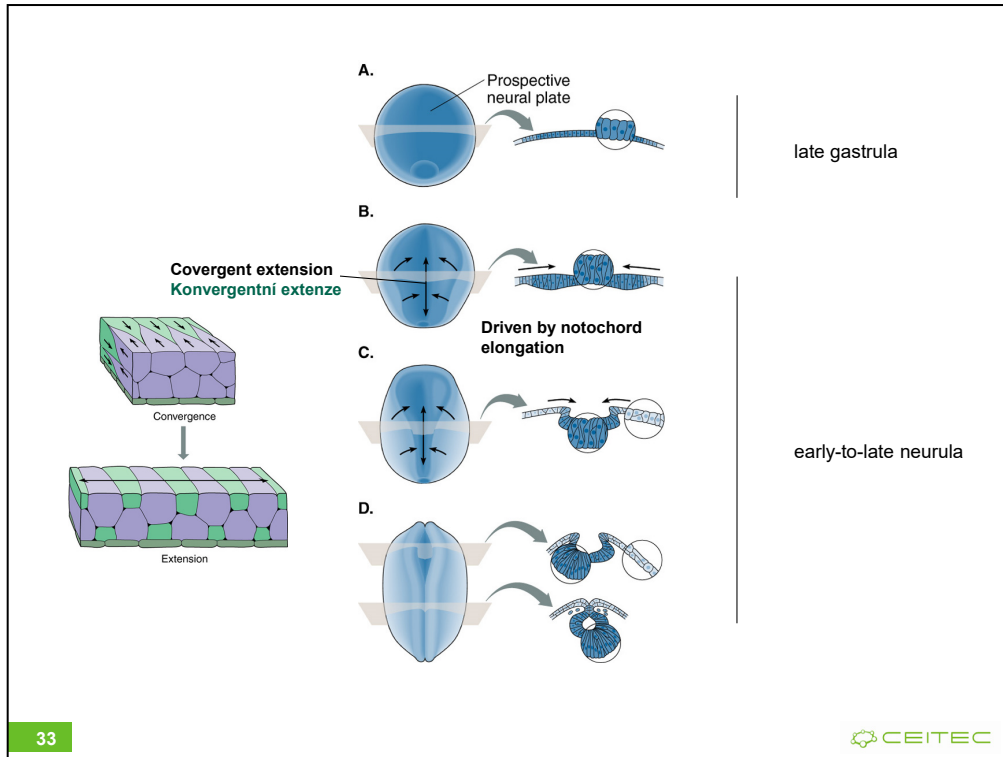
MH1 and MH2 are evolutionarily conserved globular Mad-homology domains; MH1 contains the DNA-binding domain and MH2 multiple protein interaction sites.

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  - **signaling in the spinal cord development**



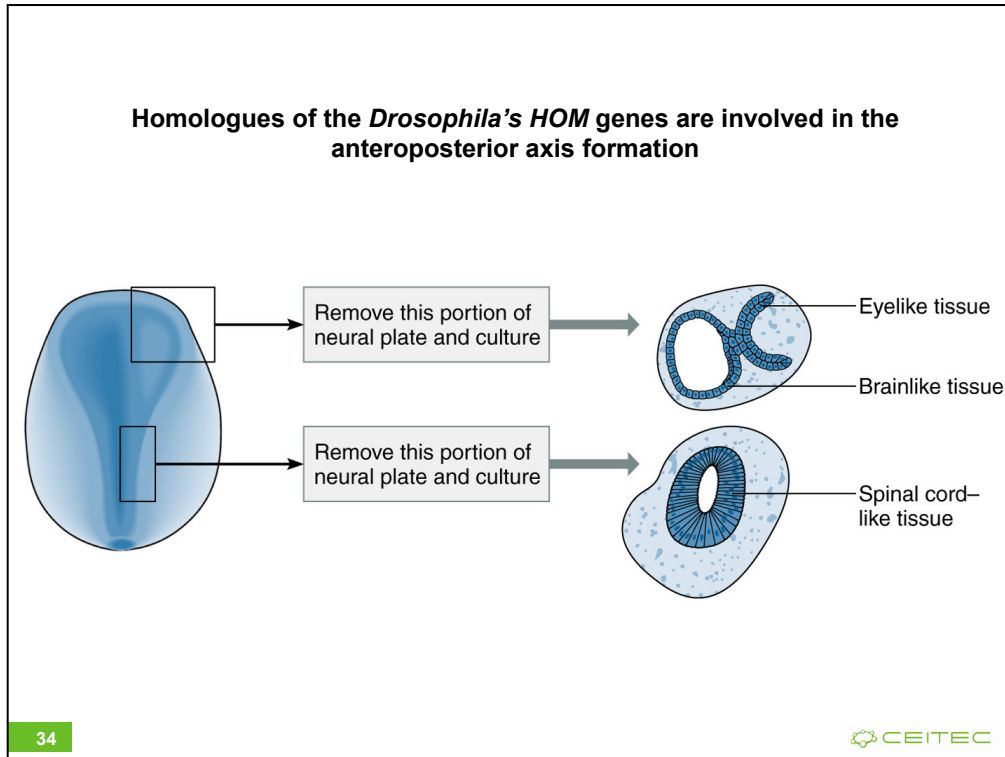


During development of the neural plate, the epidermal cells first elongate in the animal-vegetal axis, forming a “pavement” of columnar cells.

The entire region of the prospective neural plate lengthens as the cells rearrange (see the arrows in the figures) that is called *convergent extension*.

The neural plate elongation is at least partially driven by the elongation of the notochord. When the notochord is surgically removed, the neural plate does not elongate.

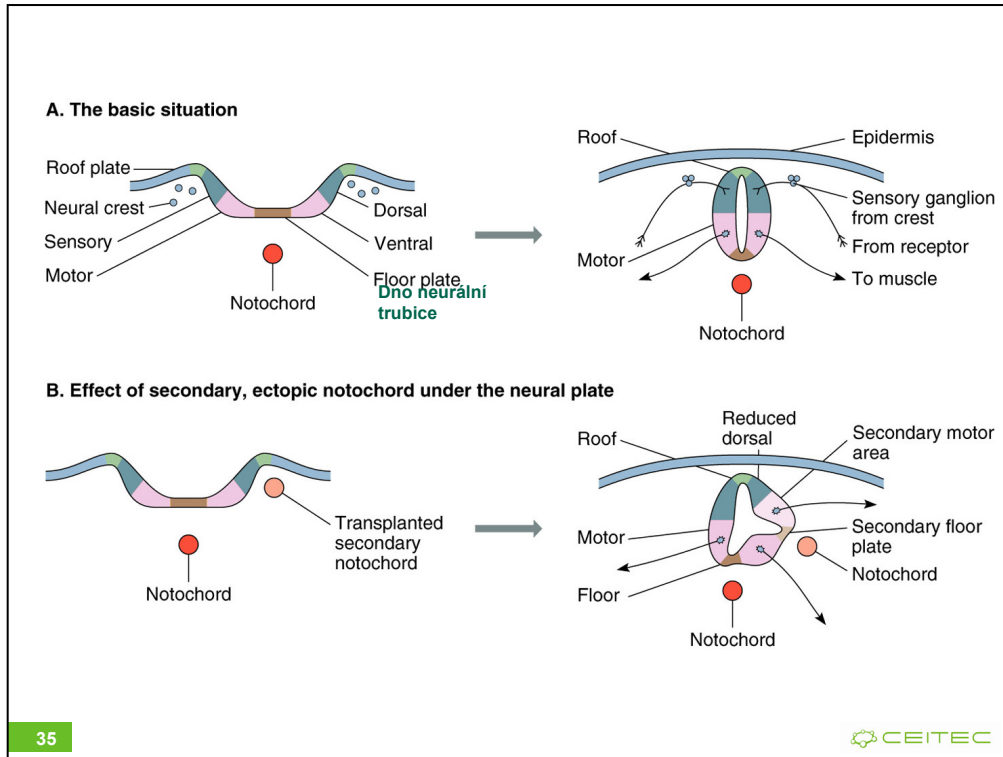
As the cell columnarization is completed, the neural plate forms tear-like structure leading finally to the neural tube formation.



Early in the neural plate formation, the developmental fate of the cells becomes predetermined in the anteroposterior axis.

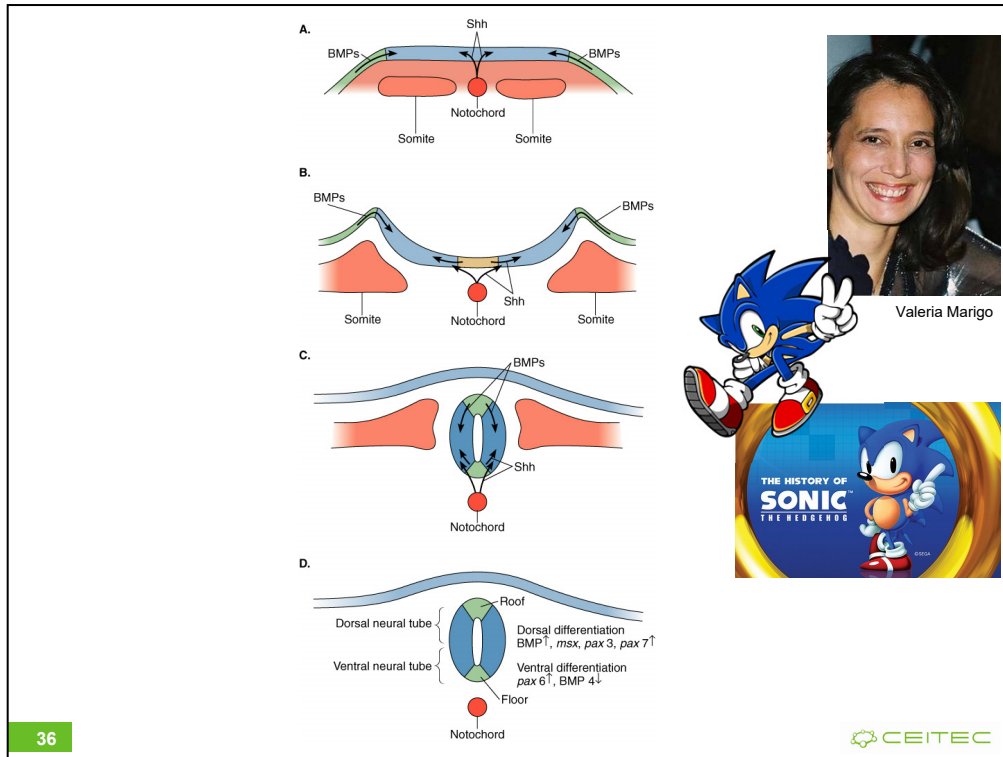
If the cells from the anterior portion of the neural plate are explanted, eye-like tissue forms. However, this will never happen when the posterior portion of the neural plate is removed and cultivated in the cell culture medium.

Homologues of *Drosophila*'s *HOM* genes, involved in the specification of *Drosophila* segments, were identified along the anteroposterior axis in the neural plate region. The molecular nature of the process will be discussed later in more detail.



There are two organizing centers, important for the dorsoventral patterning of the neural plate: **Notochord** and **floor plate**, the ventral portion of the neural plate.

When another notochord from donor embryo is ectopically placed close to the existing neural plate, secondary ventral region is formed leading to severely disturbed developmental aberrations with two “bottoms” of the resulting embryo.



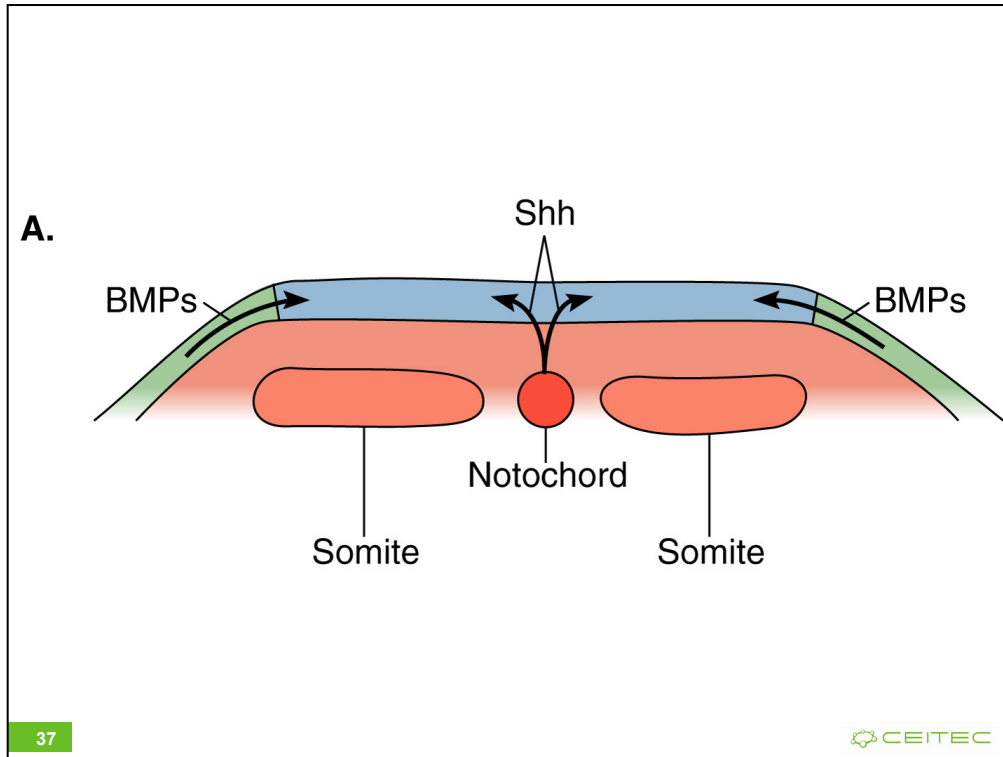
Both notochord and floor plate produce SHH, protein (homologous to the *Drosophila*'s HEDGHEHOG, see later).

The SHH protein is autocatalytically cleaved and cholesterol binds to the C-terminal portion of the N-terminal peptide. This peptide then acts as a signalling molecule. Binding of the cholesterol allows tethering of the peptide to the cell membranes, thus restricting its diffusion.

The underlying molecular mechanisms of the SHH action is not completely known, however, there is experimental evidence suggesting that:

1. SHH is necessary for the proper dorsoventral patterning of the neural plate
2. SHH activates expression of ventral-specific genes
3. Vice versa, SHH suppresses activity of BMP4 in the ventral portion of the neural tube
4. SHH induces formation of interneurons and motor neurons

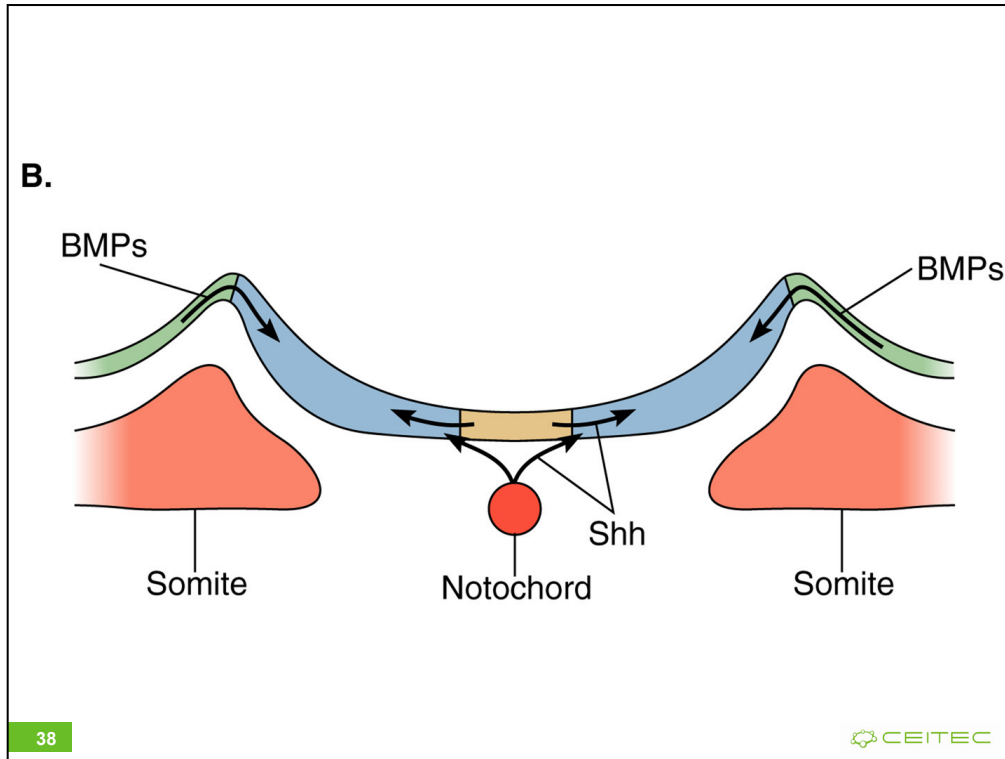
SHH was cloned by Italian researcher Valeria Marigo in 1995 (Marigo et al., Genomics, 1995) and designated according to the popular videogame.



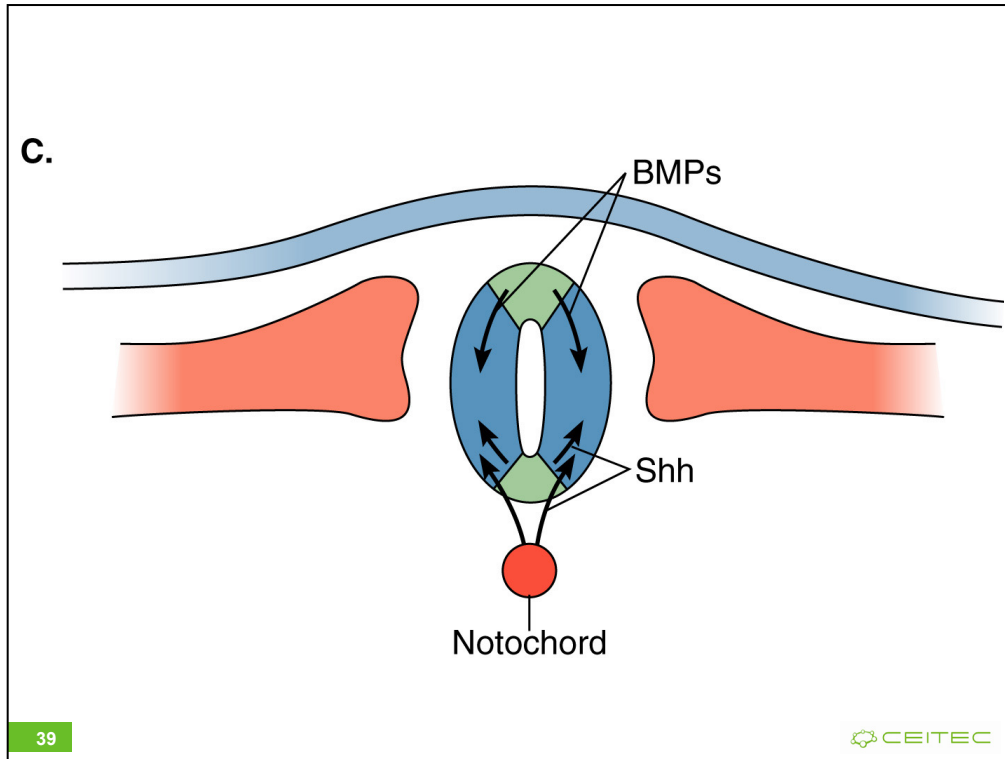
SHH seems to induce different types of neurons in the ventral spinal cord based on its concentration. That is, SHH probably acts as a **morphogen**.

Different gene expression domains mutually interact and are of spatial interaction during neural tube formation.

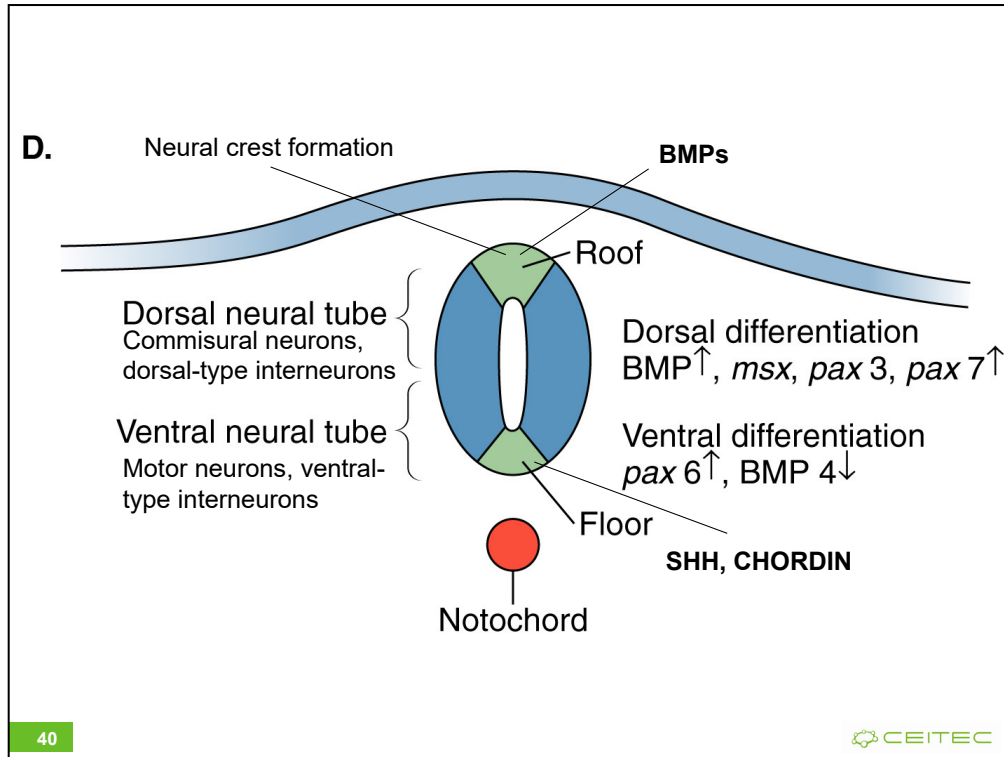
*BMPs* are expressed in the lateral portion of the neural plate that will give rise to the dorsal spinal cord, while *SHH* is expressed by notochord.



As the neural folds are formed and the neural groove gets closed, the neural plate is still under the influence of BMPs, emitted by the non neural ectoderm adjacent to the lateral portion of the neural plate and SCC, emitted by both notochord and floor plate.



Finally, as the neural tube closes, the dorsal portion (roof of the neural plate) is characterized by the expression of *BMPs*, while the ventral part by the expression of *SHH*.



Because of its origin, the most dorsal portion of the spinal chord express high level of *BMPs* that subsequently activate expression of specific TFs (e.g. *MSX*, *PAX3*, *PAX7*).

In the region of the dorsal spinal chord where the expression of *SHH* is low or no, commissural (neurons with neurites across the midline) and dorsal-type interneurons are formed here.

The high activity of *SHH* suppresses *BMP4* and induces ventral differentiation. *CHORDIN*, an inhibitor of *BMP4* action in the neural tissue initiation (see before) is also involved in the *SHH*-mediated inhibition of *BMPs* in the neural tube differentiation.

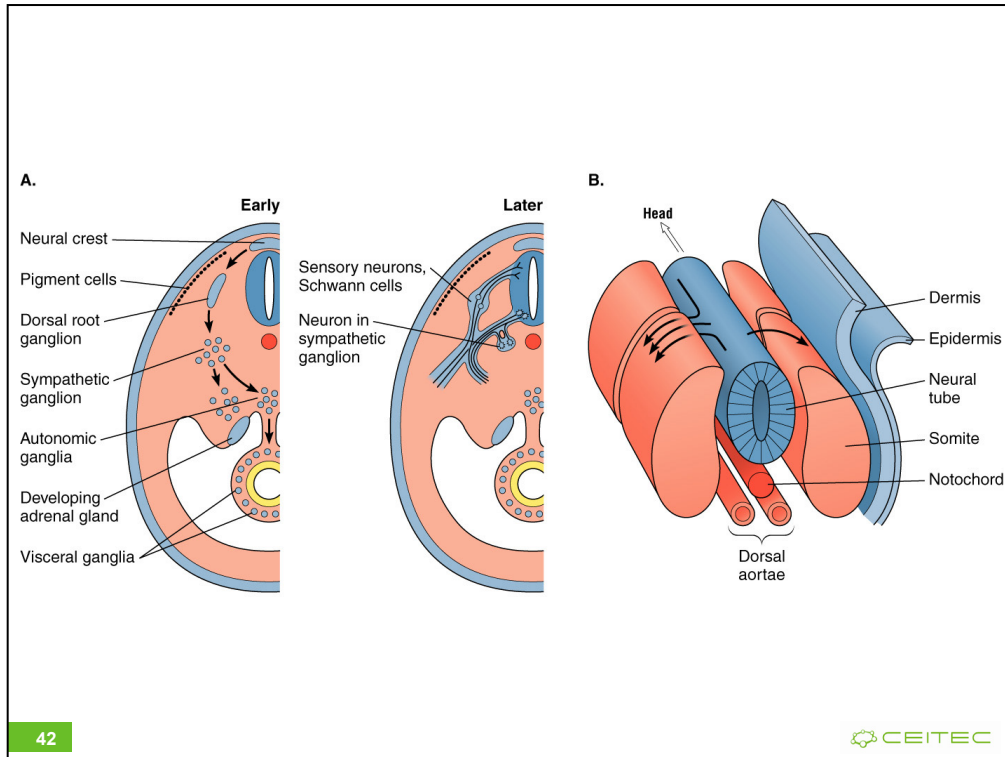
The above described lateral portion of the neural plate give rise to subpopulation of cells called *neural crest*, which will be discussed in the following slides.



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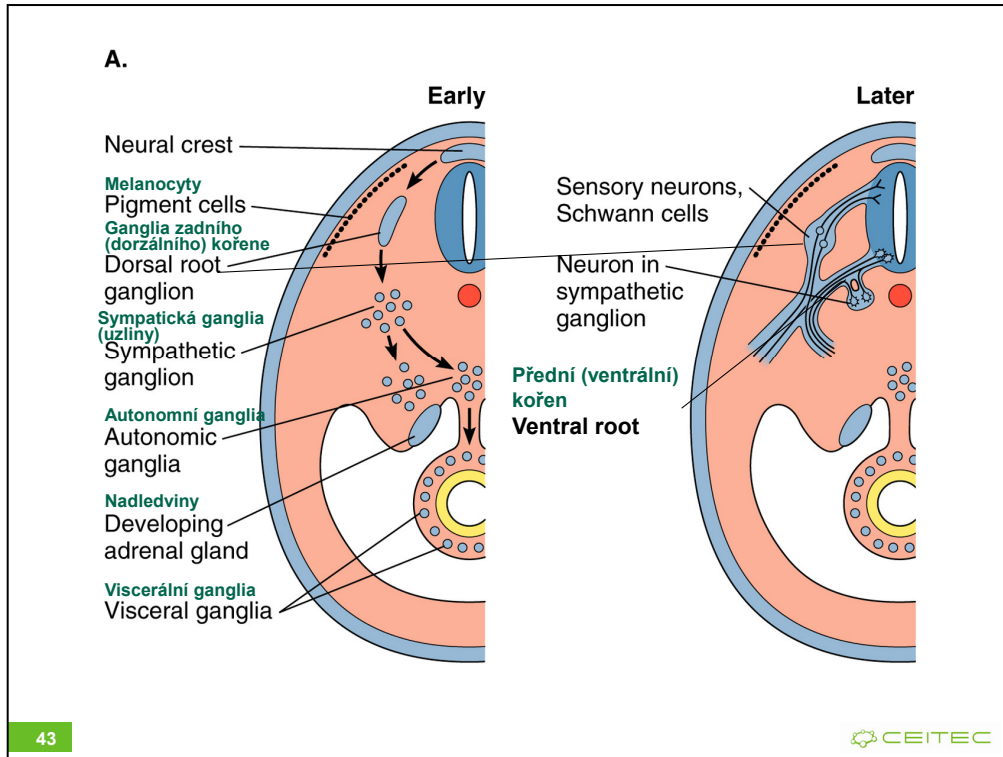
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  - mechanisms of neural tissue specification
  - signaling in the spinal cord development
  - **spatial-specific differentiation of neural crest derivatives**



Neural crest cells are a specific cell subpopulation that originates in the lateral portion of the neural plate during closure of the neural tube. These cells migrate through specific paths to specific locations in the embryo, where they will develop into a variety of tissue types (see following slides).

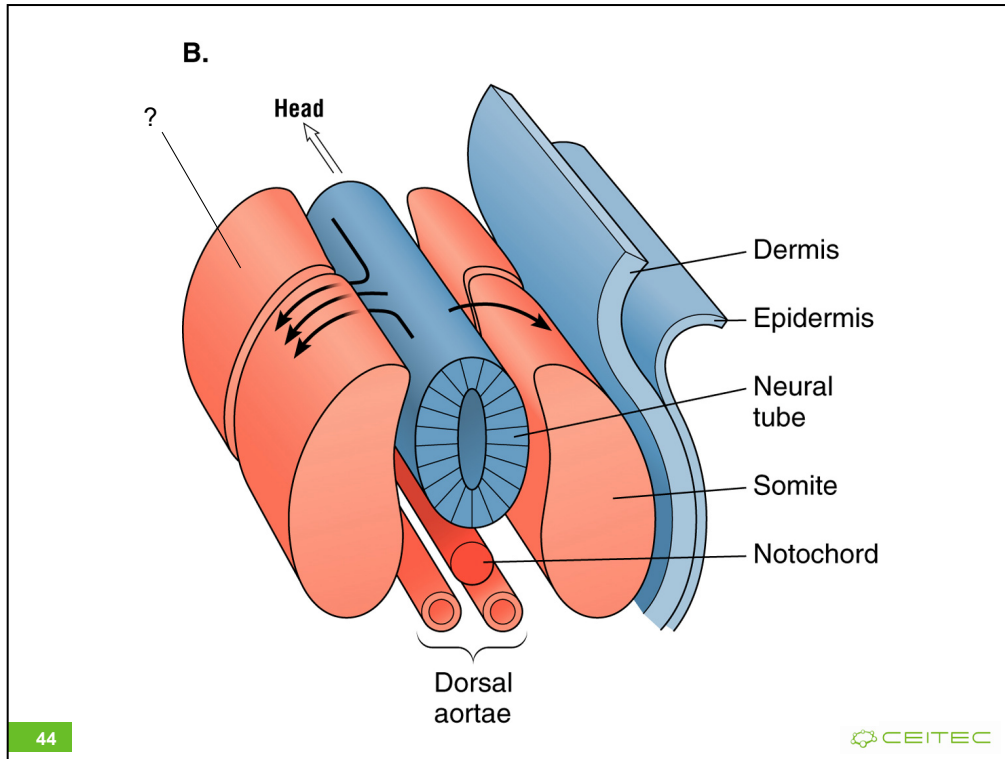
The molecular mechanisms of cell movement and their path specification are one of the fundamental questions of developmental biology and will be discussed in more detail in following chapters.



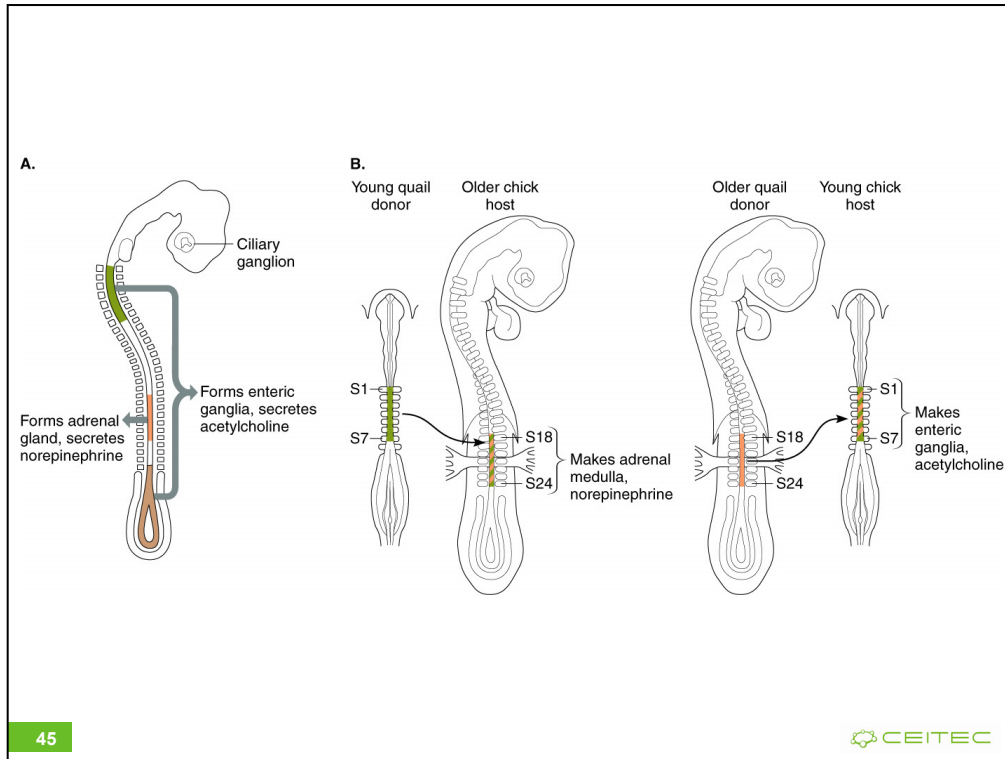
The cells that migrate under epidermis will develop into pigment cells (melanocytes), others migrate more ventrally, giving rise to sensory and autonomic ganglia and adrenal medulla (see the figures).

The neural crest of the brain region contributes to sensory ganglia of cranial nerves, cartilage, bones, teeth and other mesenchymal tissue.

Mesenchyme, or mesenchymal connective tissue, is a type of undifferentiated loose connective tissue that is derived mostly from mesoderm, although some is derived from other germ layers; e.g. neural crest cells and thus originates from the ectoderm. Most embryologists use the term mesenchyme only for those cells that develop from the mesoderm (Wiki).



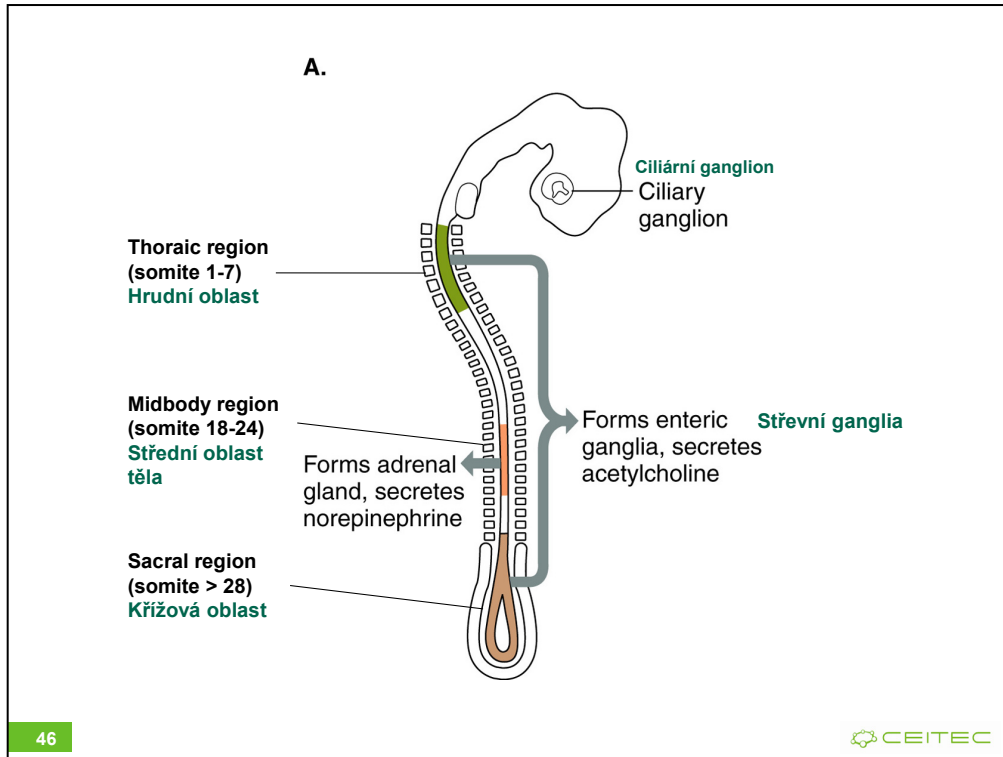
In the trunk, the neural crest cells migrate through the anterior portion of somite. Molecular nature of this spatial preference is not known.



The developmental fate of neural crest cells is largely specified by their final localization in developing embryo.

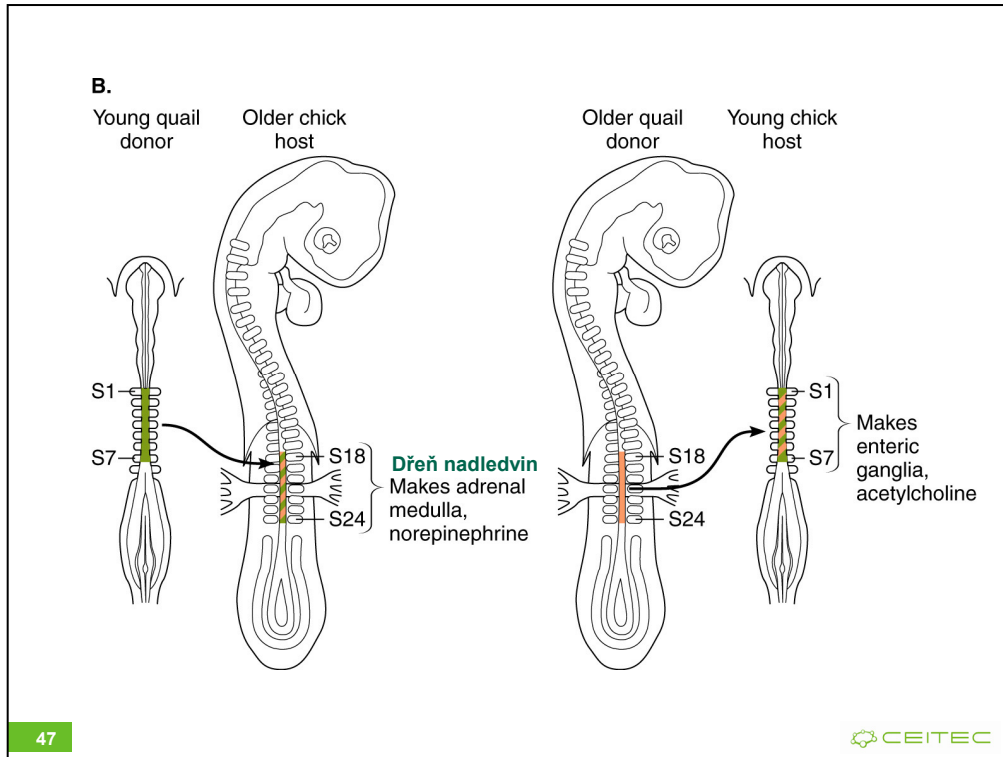
That could be demonstrated by the grafting experiments using quail and chicken embryonic tissue.

The nuclei of these two species are differentially stained, allowing thus identification of their origin.



In normal development, neural crest cells migrating through the area of thoracic region (somite 1-7) and the sacral region (somite after 28) form sympathetic ganglia that secrete acetylcholin.

While the neural crest cell from the midbody region (somite 18-24) form adrenal medulla, which secretes norepinephrine.



Crest cells from the thoracic region of young quail donor transplanted to the older (more differentiated) chick midbody region, produce adrenal medulla, making norepinephrine.

*Vice versa*, the midbody from older chicken graft transplanted to the younger thoracic quail embryo region will result into enteric ganglia producing acetylcholine.

Enteric ganglia occur in the gut and are part of the so called **enteric nervous system** (ENS). The ENS is a subdivision of the peripheral nervous system (PNS), that directly controls the gastrointestinal system

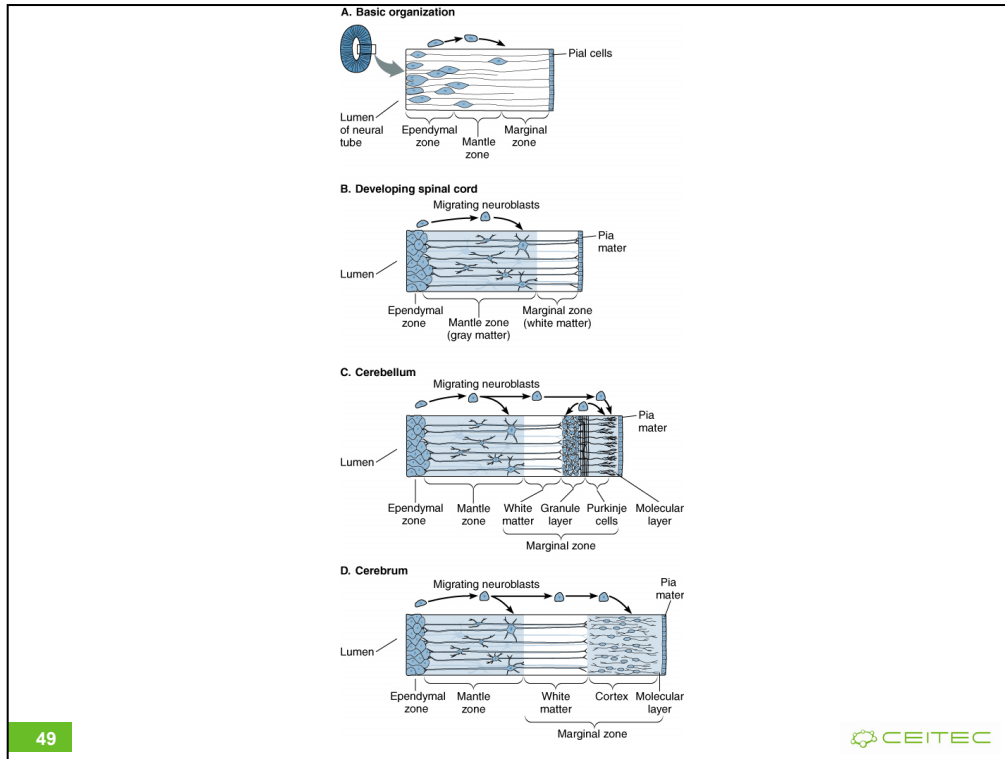
However, the multipotenciality of the neural crest cells is not absolute. Posteriorly transplanted cells from the head region form sensory and autonomy neurons in accordance to their new location while the cells from the trunk implanted to the head fail to form skeletal structures.

# Outline of Lesson 4

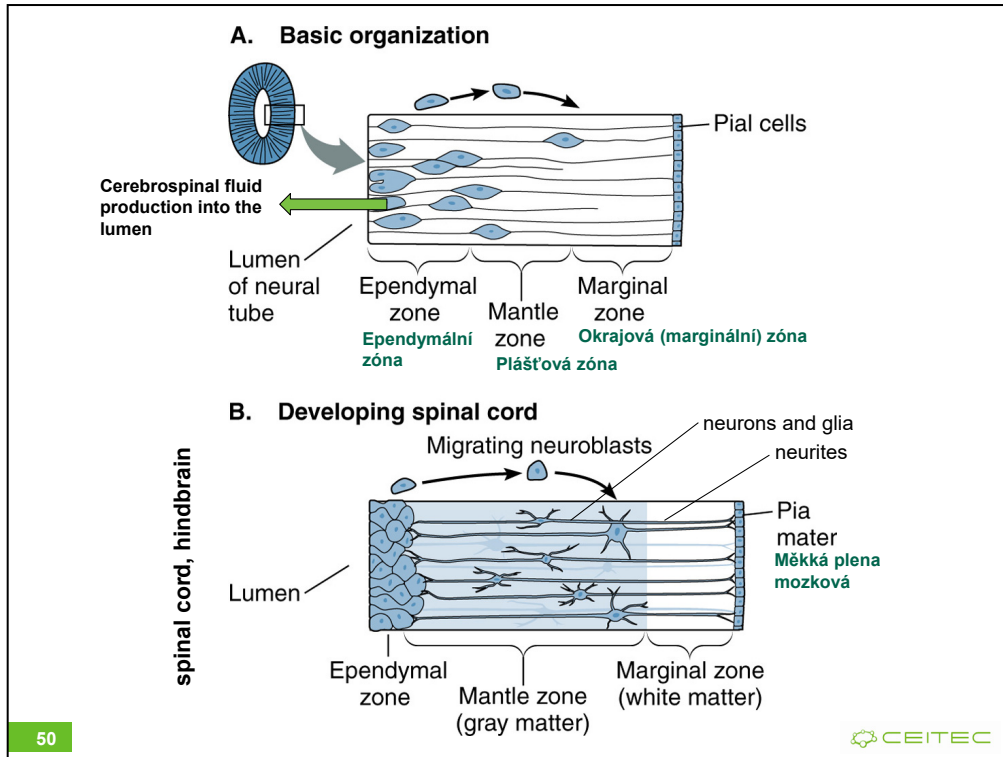
## Organogenesis in Vertebrates: Ectodermal Derivatives

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  - placental tissue differentiation
  - extraembryonic tissue formation
  - use of embryonal cells in mammals transgenesis
  
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  - signaling in the spinal cord development
  - spatial-specific differentiation of neural crest derivatives
  - **stratification of neural tube**





During development of the CNS, the epithelial cell layer forming the neural tube gets stratified. That is reflected in the morphological and functional diversifications in individual layers.



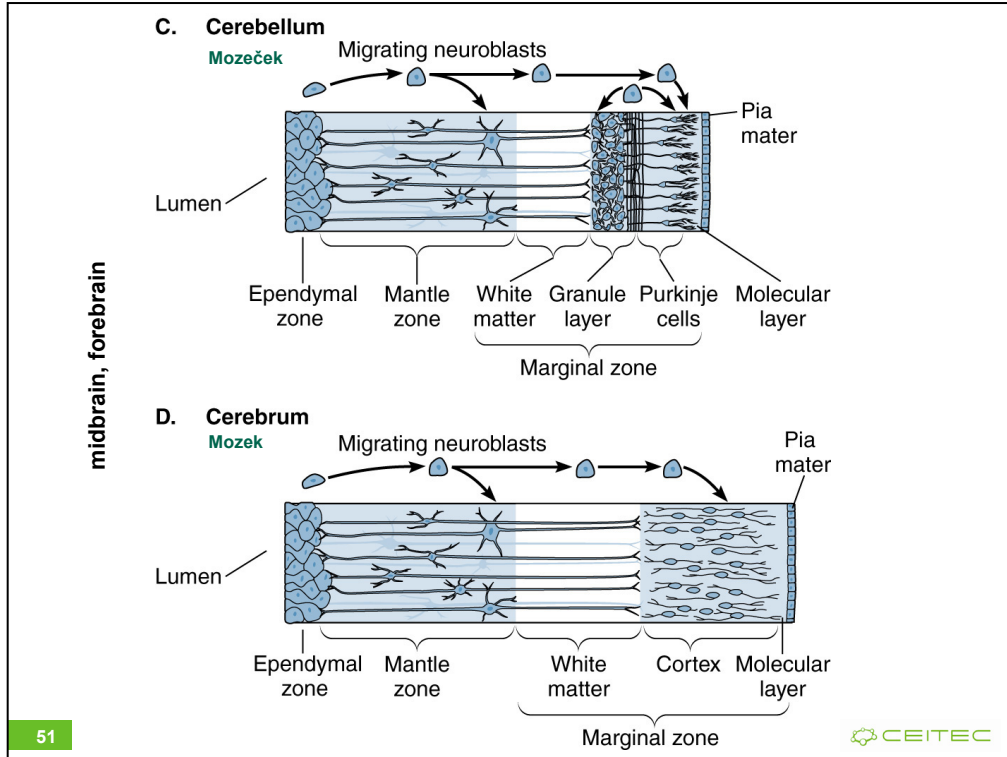
Closest to the lumen of neural tube, **ependymal zone** is formed. This zone is formed of cuboidal cells and these cells participate on the production of *cerebrospinal fluid* inside the lumen.

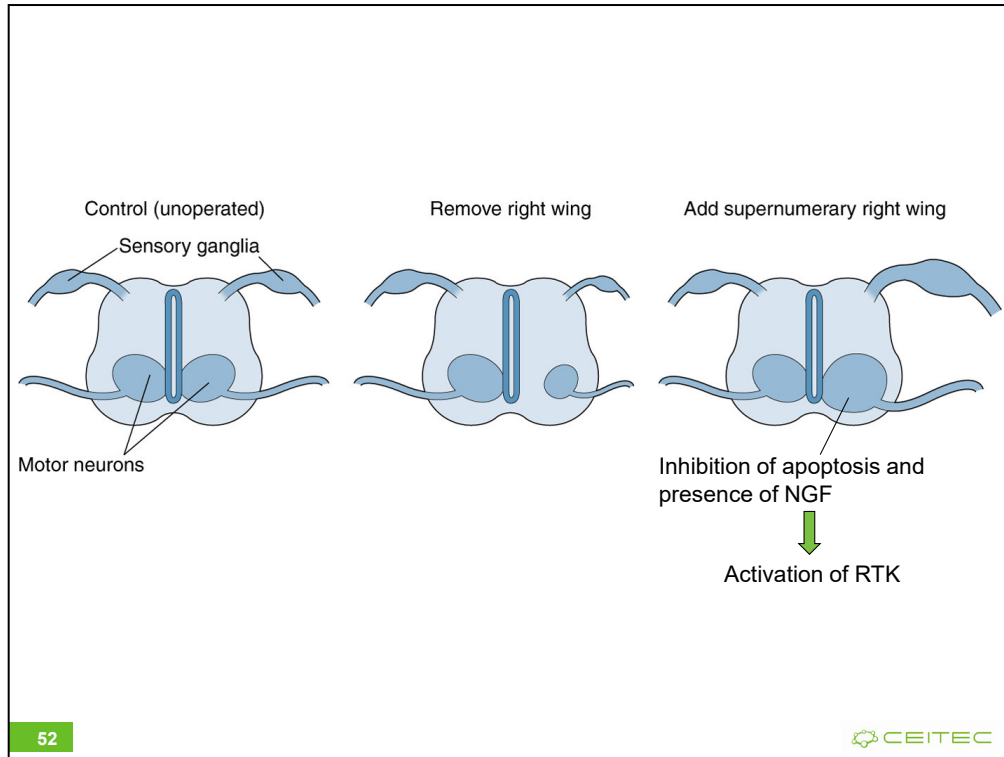
The cells stretch to the periphery, the so called **marginal zone**. When the cell enters G2 phase, their nuclei migrate to the ependymal zone, undergo mitosis and the daughter cells stretch again.

As the development proceeds, cells tend to inhabit the **mantel zone** and form neurons and supporting *neuroglial cells* (glia).

Cell bodies accumulate in the mantel zone while neurites extend through the peripheral (marginal) zone, which has fewer cell bodies and more neurites (gray vs. white matter, see figure).

This anatomical arrangement is largely preserved in spinal cord and hindbrain, in the midbrain and forebrain, there are characteristic further rearrangements due to further cell migrations and divisions (see the next slide).





The size of the spinal cord varies along the body axis, with extended number of neural cell bodies in the region of limbs. This is due to increased number of the peripheral targets innervations, which has been clearly shown by the limb removal and/or transplantation experiments.

When the limb is removed, the number of motor neurons was not increased. However, after supernumerary limb transplantation, the number of motor neurons extended in this position (see the figures).

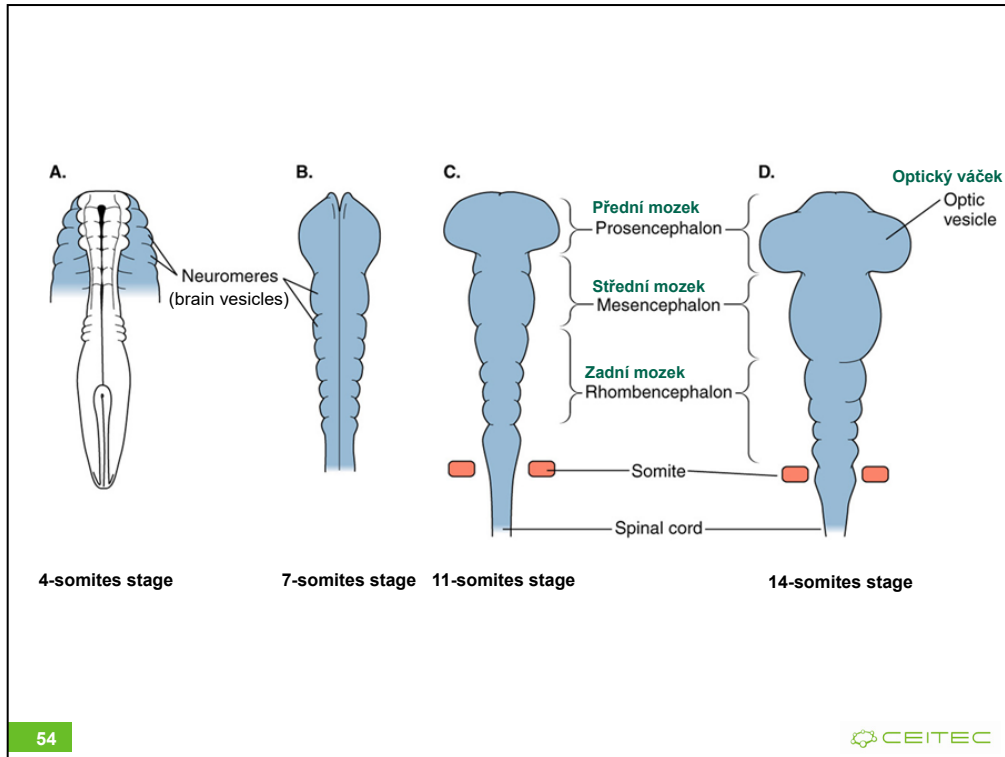
At the cellular level, this is due to inhibition of apoptosis (programmed cell death) that occurs in the developing spinal cord. In the regions where the limbs are developing, the newly formed connections with their muscle cells protect neurons from the apoptosis, extending thus their number in that region.

For the growth and proper development of neurons, the so called NEURAL GROWTH FACTORS (NGFs) are necessary. There is a whole family of these factors, specific for different neuron types. The first NGF was identified in the chicken tumors and it was identified as a small dimeric glycoprotein. This glycoprotein is recognized by the receptor tyrosine kinase and stimulates neurons proliferation and outgrowth of neurites from somatic sensory and sympathetic neurons.

# Outline of Lesson 4

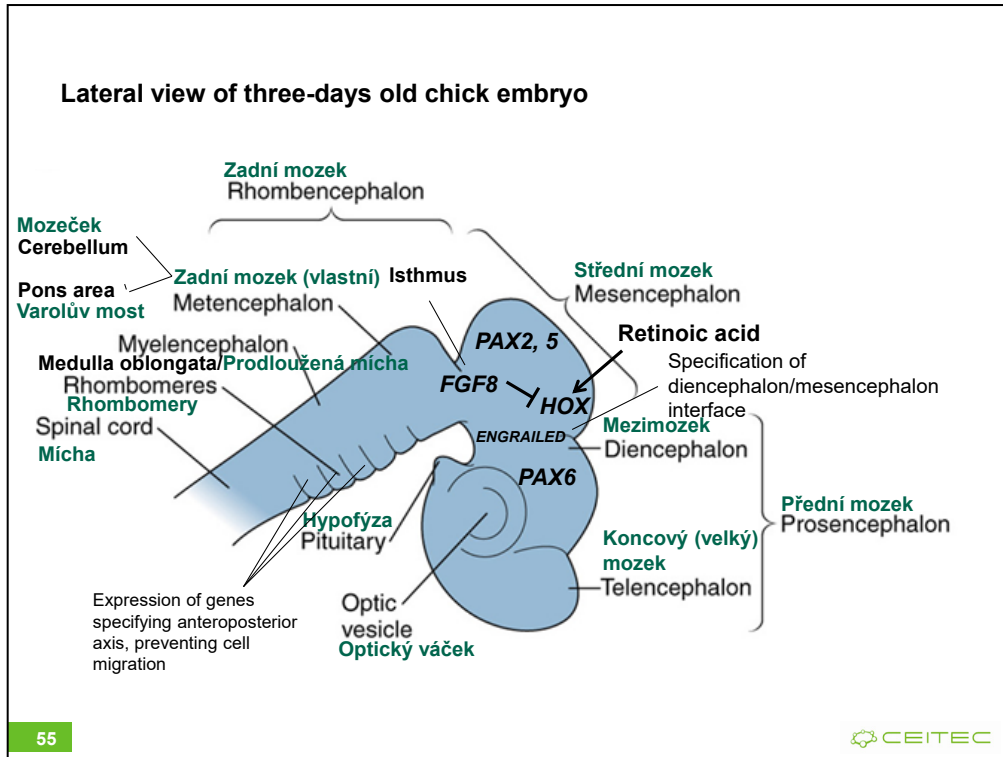
## Organogenesis in Vertebrates: Ectodermal Derivatives

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



During development of the CNS, segmental dilatations are formed along the anteropostreior axis. These are called *neuromeres* or sometimes also *brain vesicles*.

These will develop in to for- mid- and hindbrain, called *prosencephalon*, *mesencephalon* and *rhombencephalon*, respectively.



*Prosencephalon* forms the forebrain (cerebral hemispheres) and the diencephalon (hypothalamus).

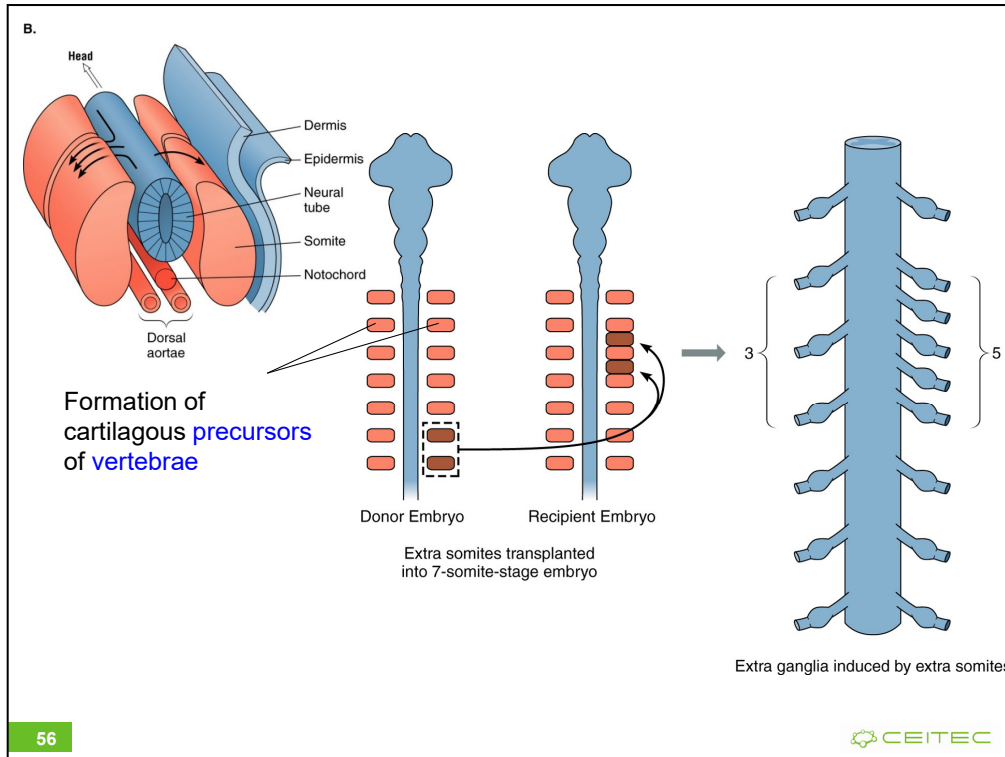
*Mesencephalon* forms midbrain.

*Rhombencephalon* forms metencephalon (cerebellum and pons area) and the myelencephalon, which forms the hindbrain (brain stem).

The different brain vesicles reveal specific gene expression very early in the development. E.g. *PAX6* (discussed previously in connection with the ventral neural cord formation, see slide # 40) is expressed in diencephalon while *PAX2* and *PAX5* in mesencephalon.

Isthmus, the strip of tissue between mesencephalon and rhombencephalon, produces FIBROBLAST GROWTH FACTOR 8 (FGF8), which inhibits expression of *HOX* gene in the anterior rhombencephalon. *Vice versa*, retinoic acid, possibly produced by the surrounding mesoderm tissue, induces *HOX* expression. Thus, the mutual interplay of two counteracting factors establishes *HOX* expression pattern.

Some vertebrates form the very distal vesicles, called *rhombomeres*. Rhombomeres are borders of specific gene expression, as will be discussed later. Rhombomeres also represent developmental boundaries, that prevent cell migration.



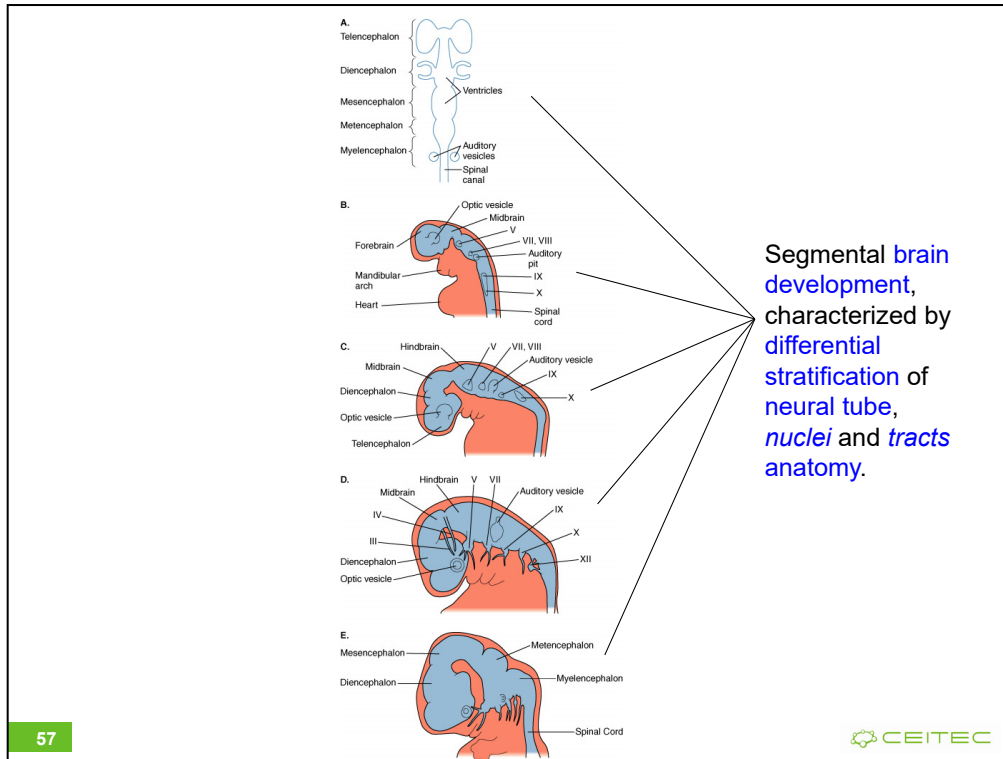
The spinal cord has a segmental organization, too, consisting of individual neuromeres. Each neuromere is connected with two somites, each of them on the opposite lateral side.

The crest cells migrate, as discussed before (see the inset on the left-hand side), through the anterior portions of the somites and some of them form dorsal root sensory ganglia, one on each side of the neuromere.

The somites form cartilaginous precursors of vertebrae, which are segmented in register with ganglia and neuromeres, too.

The somites are crucial for the segmental development of the spinal cord. Removal or transplantation of somites leads to absence or ectopic ganglia formation, respectively. This suggests communication between somites and developing spinal cord.

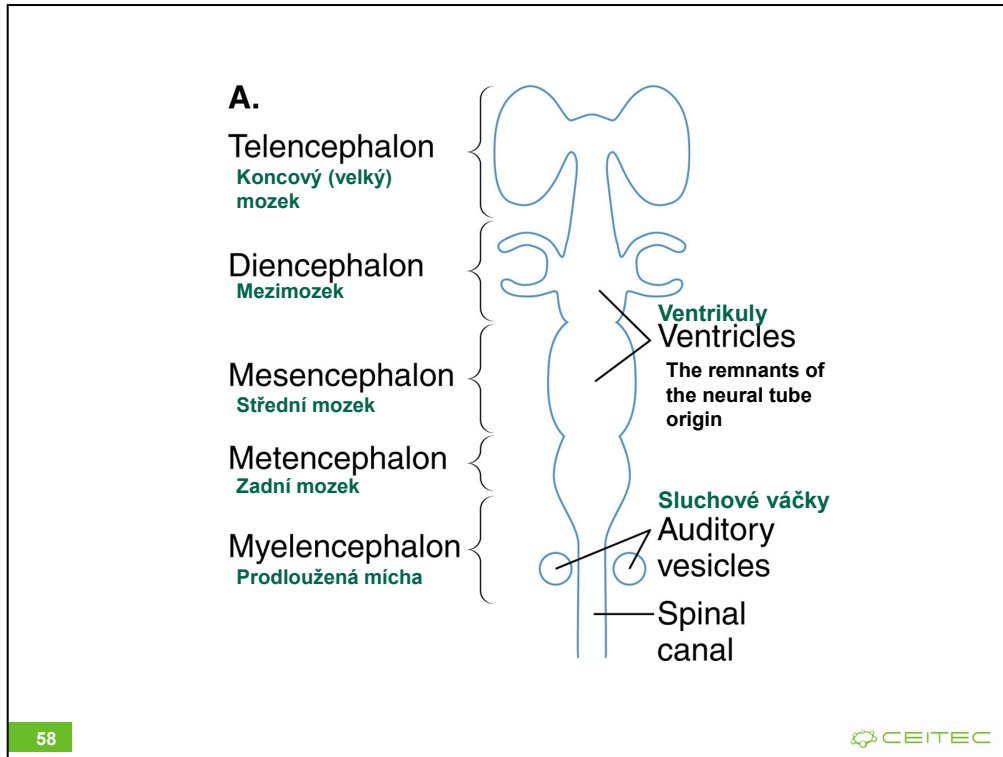




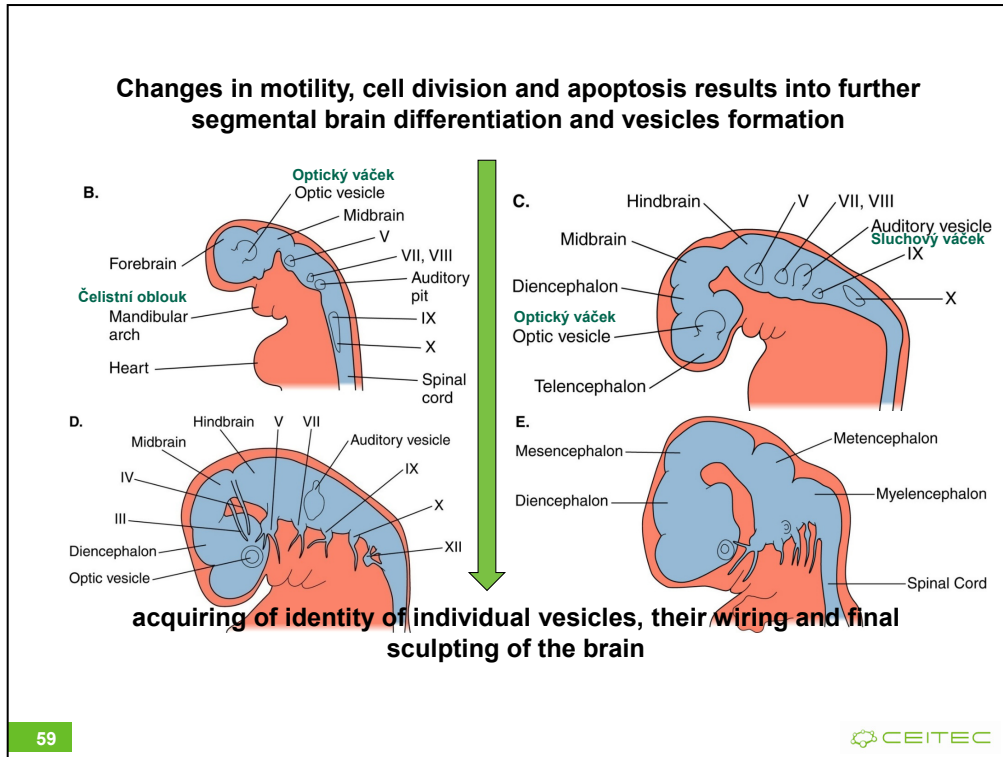
During development, brain develops in a segmental way, i.e. the migratorial behavior of the neurons originating at the ependymal border varies according to the area of developing CNS.

That results in a different anatomy of formed layers or congregations of cell bodies, called *nuclei*.

Neurites connecting different nuclei form *tracts*.



As a remnant of the brain formation from the neural tube, hollow multipocketed chambers are formed inside the brain, which are called *ventricles*. These ventricles communicate with the spinal canal that goes through the spinal cord, another remnant of the neural tube.



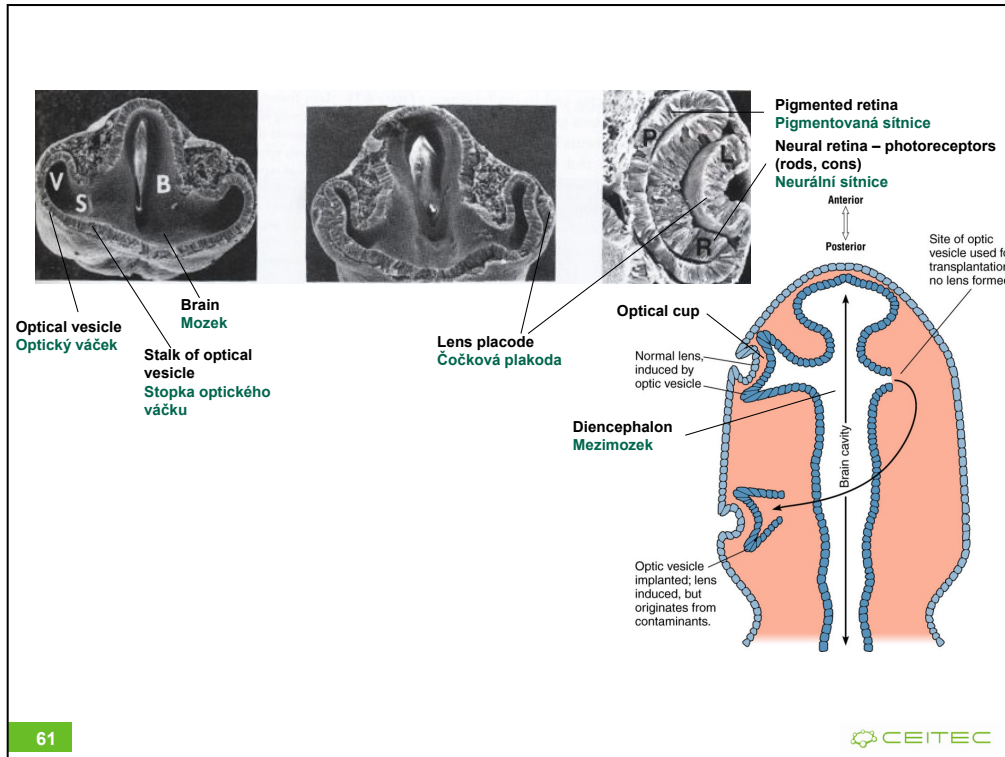
Along the anterioposterior axis, the changes in motility, cell division and apoptosis result into segmental differentiation of individual brain portions, called *vesicles*.

Formation of individual vesicles is a result of complex events, leading to acquiring of identity of individual vesicles, their wiring and final sculpting of the brain. The molecular mechanisms driving those events will be discussed later.

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  - **eye development**



The eyes develop from diencephalon, which forms two outpocketings on each side. These outpocketings develop into optic vesicles, which further proliferate and evaginate through the head mesenchyme (that is mostly of the neural crest origin (see previous slide # 43) and abut the surface ectoderm.

Then the optical vesicle invaginates, forming thus *optical cup*. As the optical cup forms, the overlying ectoderm is induced and changes from cuboidal to columnar and invaginates, allowing thus *lens placode*, a future lens, formation.

The outer layer of the optical cup develops photoreceptors (rods and cones). The efferent neurons of the retina project back to the brain via the optic nerve that develops from the stalk of the optical vesicle.

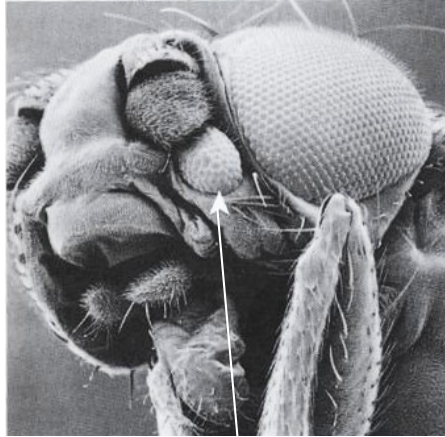
The inner surface of the optic cup becomes pigmented retina, while the outer surface becomes the photoreceptors and neurons of the neural retina and also generates an epithelial iris.

The surrounding head mesenchyme derived from mesoderm develops into hard scleral covering of the eye ball and eye muscles.

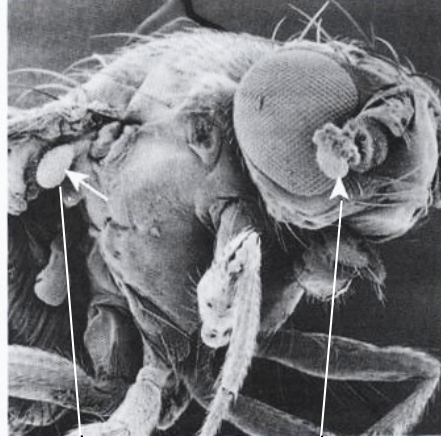
The early transplantation experiments (see above) suggested that lens cup is able (similarly to Spemann organizer) to induce ectopically lens formation. However, more recent experiments have shown that the newly induced lens originates from the contaminant ectoderm of the donor, adjacent to the lens cup.

Recent model proposes that the lens-induction signals of the optical cup interact with multiple signals produced earlier in the development by surrounding tissue, e.g. from prospective head mesoderm and from anterior endoderm. The nature of individual receptors and signals remains to be identified.

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



Ectopic eye in the head region



Ectopic eye below the wing

Ectopic eye on the antenna

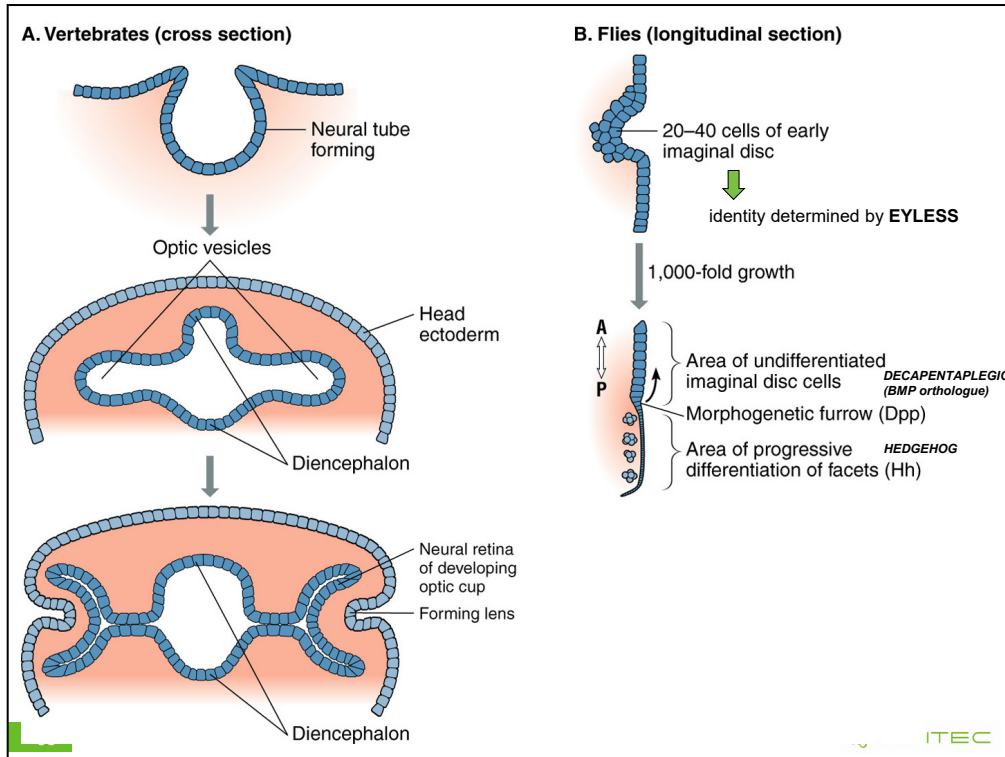
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The what is called **master genes** were identified to be critical for eye formation. In *Drosophila*, mutants in *EYELESS* gene are unable to form eye. When ectopically expressed, *EYELESS* is able to induce eye formation (see the images).

*EYELESS* encodes TF that very probably triggers the downstream cascade leading to the eye formation. These genes are called “master” or “executive” genes.

In mouse, homologue of the *EYELESS* is *PAX6* (see previous slides # 40). *PAX6* is able to complement *eyeless* mutation, suggesting thus functional conservation of the whole developmental cascade which had to evolve approximately 500 mil. years ago, before the arthropods separated from the vertebrate lineage.



The development of the eye in vertebrates and *Drosophila* differs tremendously. Thus, it is very surprising that the eyeless mutation could be complemented with the mouse PAX6.

In vertebrates, the eye development was just discussed.

In *Drosophila*, the eye develops from the specific imaginal disc, one of the patches of stem cells in the embryo that were discussed in the second lesson. The *Drosophila* eye (what is called “compound eye”) is composed of approx. 800 identical facets, each of them having its own lens and a set of eight photoreceptors. Each facet sends an efferent neurite to the ventrally located anterior ganglion of the fly’s nervous system.

Just before and during metamorphosis, the differentiation of the imaginal disc cells is apparent as a wave (morphogenetic furrow) moving from the posterior towards the anterior pole. The homologue of vertebrate BMPs, *DECAPENTAPLEGIC (DPP)* a *Drosophila* TGF-beta homologue, is secreted in the morphogenetic furrow, while *HEDGEHOG (HH)* is expressed in cells posterior to the furrow.

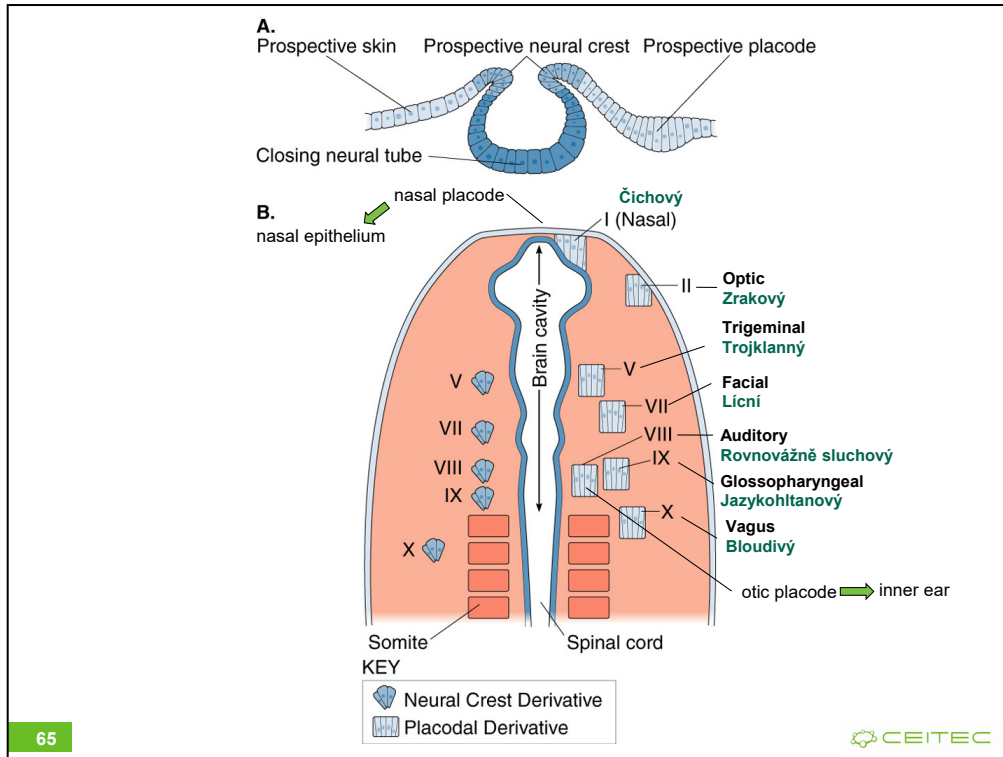
The identity of the disc is established by the action of EYELESS.

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  - **cranial ganglia and sensory organ epithelia**





Multiple autonomous and sensory neurons in cranial ganglia and sensory organ epithelia (e.g. nasal epithelium) are formed from the what is called *placodes*. These are patches of ectoderm that, similarly to neural plate, elongates to form columnar epithelium.

The major placodes include the nasal and otic placodes that form portions of the nose and inner ear, respectively. Several ganglia receive neural crest as well as placodal contributions.

**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)		

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. LičnÍ (n. facialis)

VIII. Rovnovážně sluchový (n. statoacusticus)

IX. Jazykohltanový (n. glossopharyngicus)

X. Bloudivý (n. vagus)

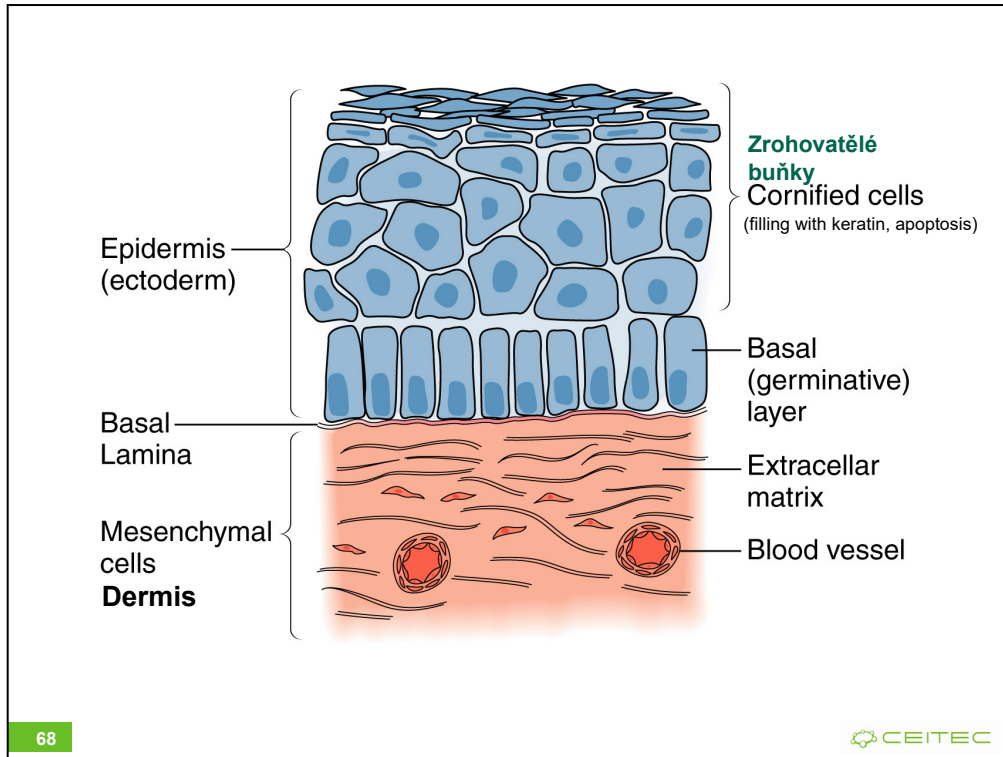
XI. Přídavný (n. accessorius)

XII. Podjazykový (n. hypoglossus)

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  - cranial ganglia and sensory organ epithelia
- Integument



Ectoderm and mesoderm form integument.

Ectoderm forms basal germinative layer, where the ongoing mitosis ensures supply of new cells. Epidermal cells filling with keratin undergo apoptosis, resulting in formation of outer, cornified layer that is several cells thick.

The ectoderm also forms specialized structures of the skin, e.g. hairs, feathers, sweat and sebaceous glands, gills and mammary glands.

Mesoderm gives rise to the underlying dermis, comprised of mesenchyme and a vascular supply.

# 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 **intrauterine embryo development**.
- There is **intense tissue communication** during **neural tube development** allowing its **differentiation in both anteroposterior and dorsoventral axis** via formation of **morphogen gradient**.
- Multipotent 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 **morphogenetic gradient** formation: **BMP/HGG**

# Discussion