

Bi8940 Developmental Biology

Lesson 5

Vertebrate Organogenesis: Endo- and Mesodermal Derivatives

Jan Hejátko

Laboratory of Molecular Plant Physiology,
Department of Functional Genomics and Proteomics,
and
Functional Genomics and Proteomics of Plants
CEITEC

Masaryk University,
Brno, Czech Republic

hejatko@sci.muni.cz, www.ceitec.eu



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Outline of Lesson 5

Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis
 - formation of gonads
 - hematopoiesis and circular system development
 - limbs formation

- Endoderm derivatives development
 - alimentary canal and its derivatives formation



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Outline of Lesson 5

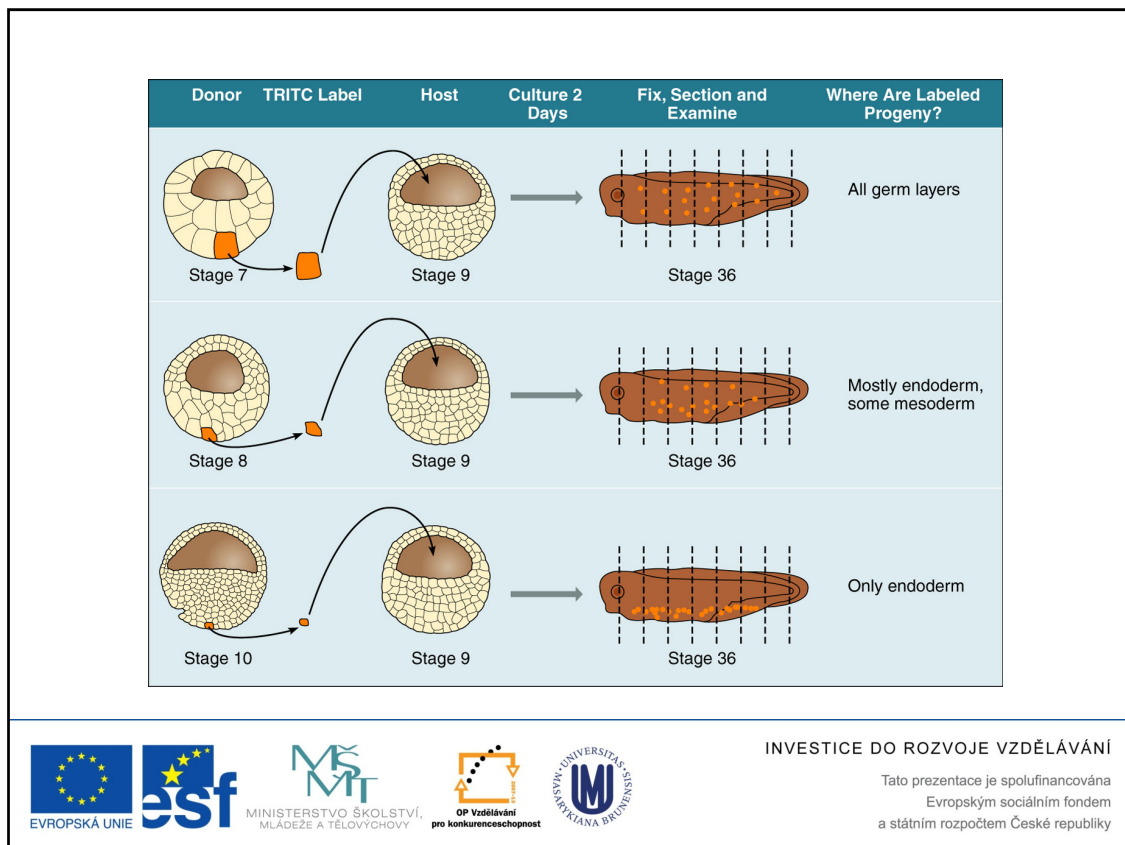
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

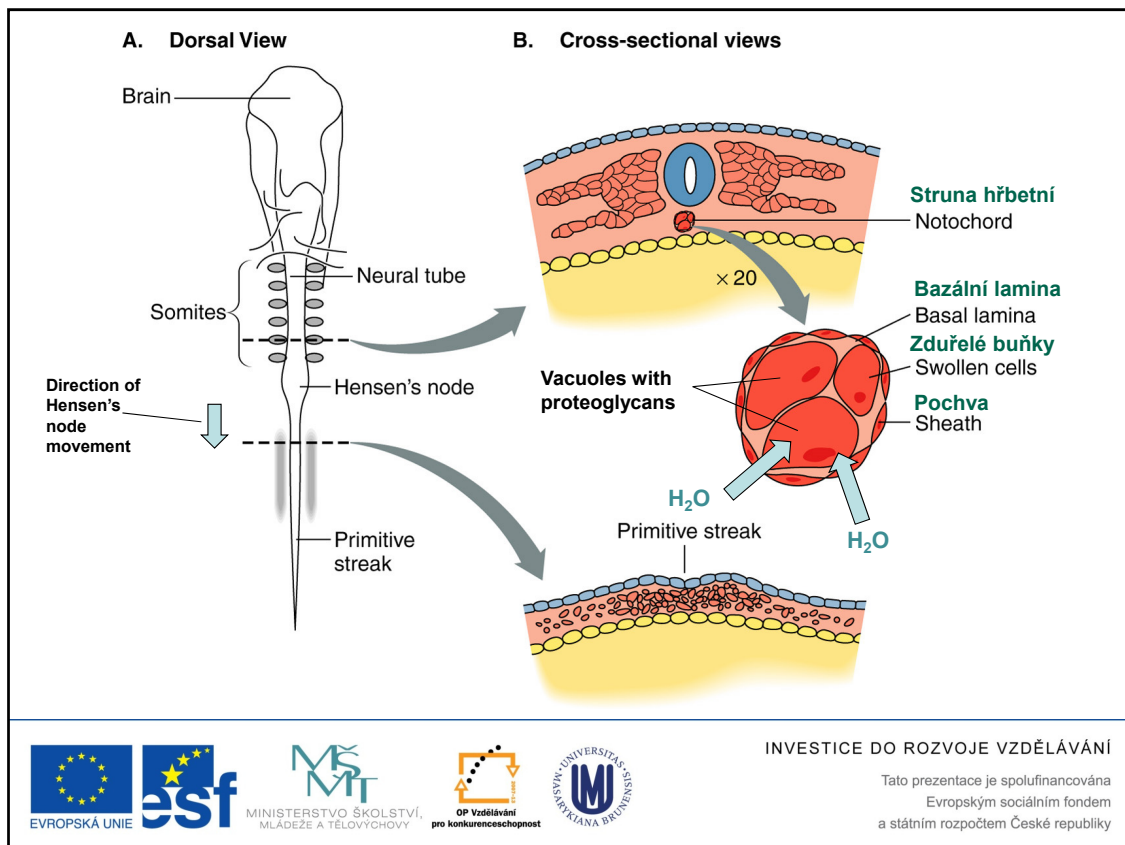
Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



The developmental potential might be estimated (here during gastrulation) using experiments, where donor cells are marked by staining with tetramethylrhodaminisothiocyanate (TRITC) or recently, more specific via marker gene expression.

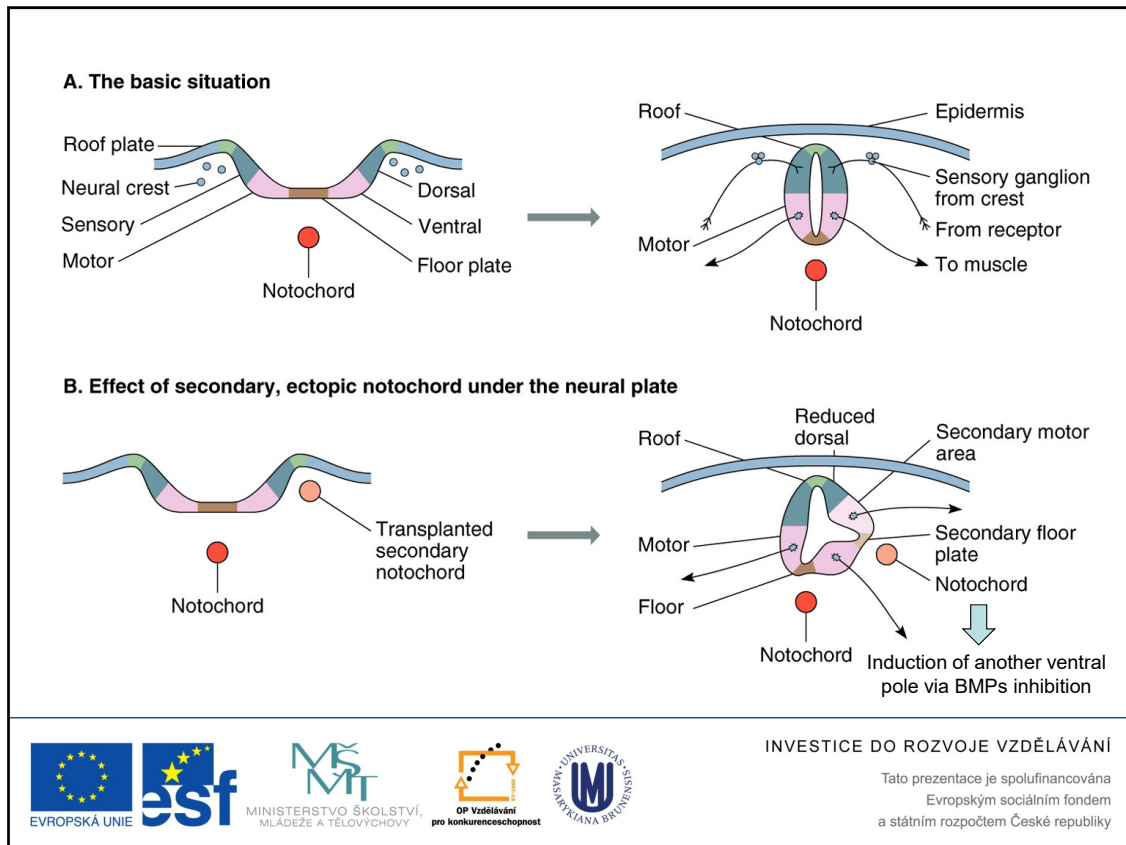
The developmental potential in vertebrates is becoming narrower during development.

Thus, though the cell is determined to form mesoderm, it is not determined at this time to form a certain type of mesoderm. For example, the prospective mesoderm cell may be capable of forming muscle, while its progeny may also be able to form cartilage or body-wall mesenchyme.



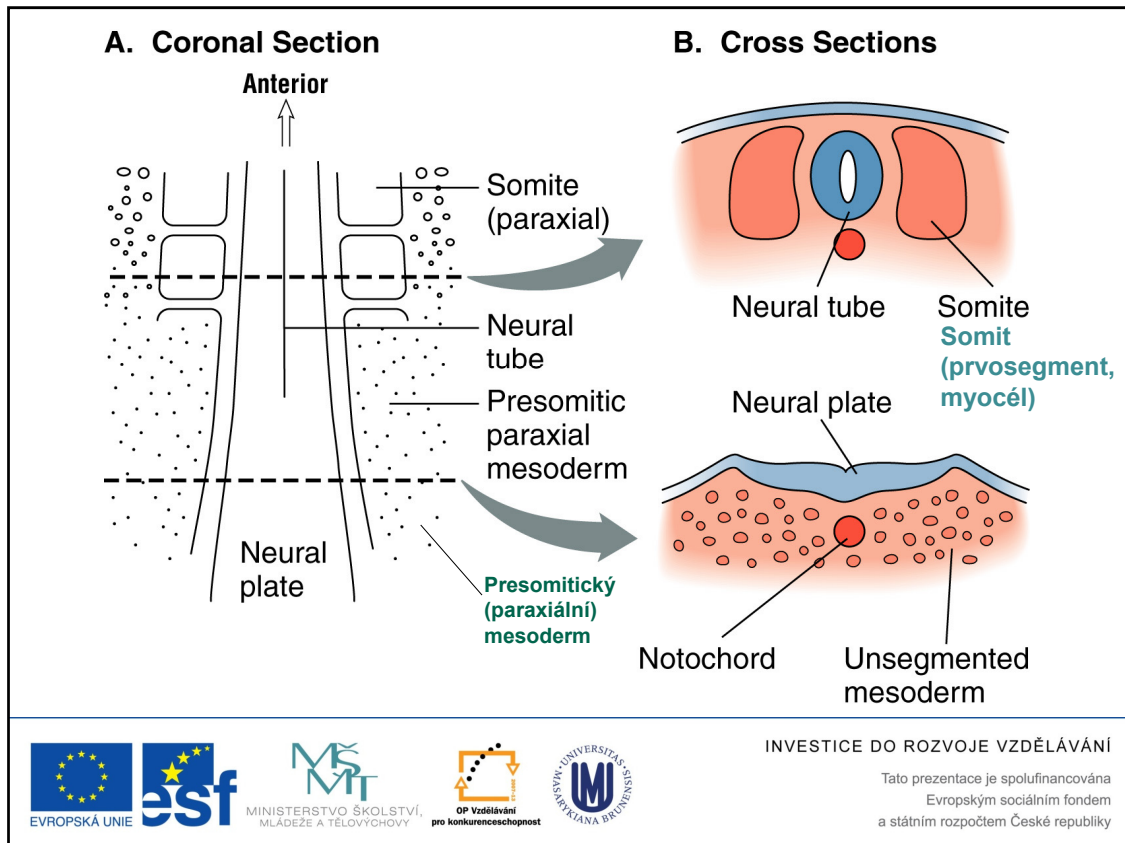
During gastrulation, as the Hensen's node moves along the primitive streak, the dorsal most mesoderm, adjacent to the forming neural tube, the midline mesoderm, forms notochord (posteriorly from the level of prospective hindbrain) that is only a few cell layers in diameter in amniotes.

At the surface, notochord is covered by cellular sheath and basal lamina. The notochord cells form vacuoles that accumulate proteoglycans. That causes that the water is imported there and the notochord becomes stiffer, forming thus a transient support to the developing embryo.



As discussed previously, the notochord originates from the Hensen's node cells and has the ability to induce another neural tube formation (its ventral pole), probably by blocking BMP signaling.

A gradient of BMPs is formed along the dorsoventral axis. High levels of BMP favor not only dorsal ectoderm, but also mesoderm (see slides 15, 16) while low BMP levels allow ventral ectoderm/mesoderm structures to develop.



At both sides of the notochord, the so called presomitic (paraxial) mesoderm is formed that will develop into somites, except the paraxial mesoderm in the head region that does not form somites.

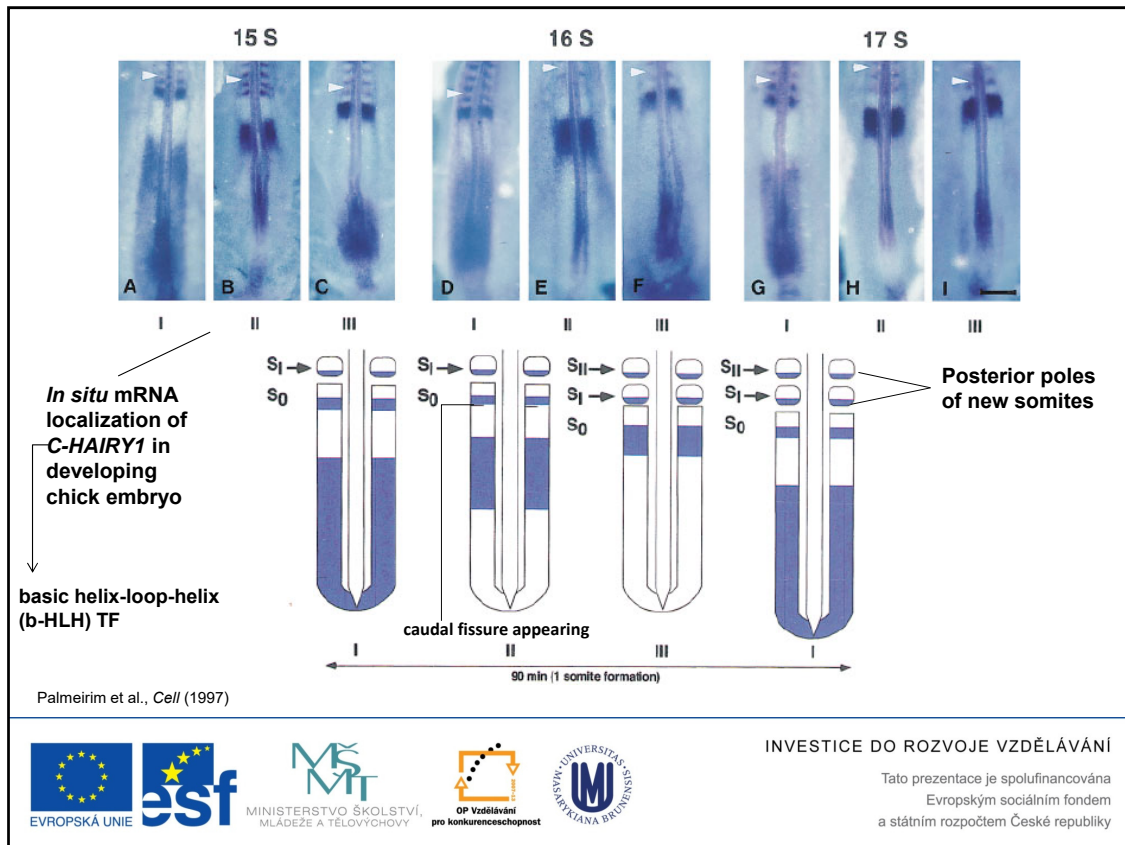
Beginning at the anterior end, the cells of presomitic mesoderm start to aggregate, forming thus individual somites.

Process of cell aggregation is common to the beginning of development of most of the mesodermal structures, as will be discussed further.

This process progresses towards the posterior end and it is accompanied by the expression of *C-HAIRY*, a mice homologue of *Drosophila's HAIRY* gene. In *Drosophila*, *HAIRY* expression coincides with segments formation (will be discussed later).

C-HAIRY is expressed in waves, every taking for about 90 minutes and allowing thus individual somites formation. High levels of *HAIRY* expression remains at the posterior portions of the newly formed somites.

Together with other genes, *C-HAIRY* is a part of internal molecular clock (see the next slide).



The rhythm of somite production is characteristic of the species at a given temperature (90 min in the chick embryo at 37°C and 20 min for the zebrafish embryo at 25°C). The total number of somites is constant within a given species. It is usually about 50, although in some animals, such as snakes, it can reach up to 400 (Pourquie, Science, 2003).

In vertebrates, segmentation involves a molecular oscillator—the segmentation clock—which acts in the presomitic mesoderm (PSM). Evidence for this oscillator was firstly provided by the observation of the regular pulses of expression in PSM cells of the mRNA coding for the basic helix-loop-helix (b-HLH) transcription factor *c-hairy1*, a vertebrate homolog of the protein encoded by the fly pair-rule gene *hairy* (Palmeirim et al., *Cell*, 1997, see the slide).

c-hairy1 mRNA expression in the PSM defines a highly dynamic caudal-to-rostral (posterior/anterior) expression sequence reiterated during formation of each somite.

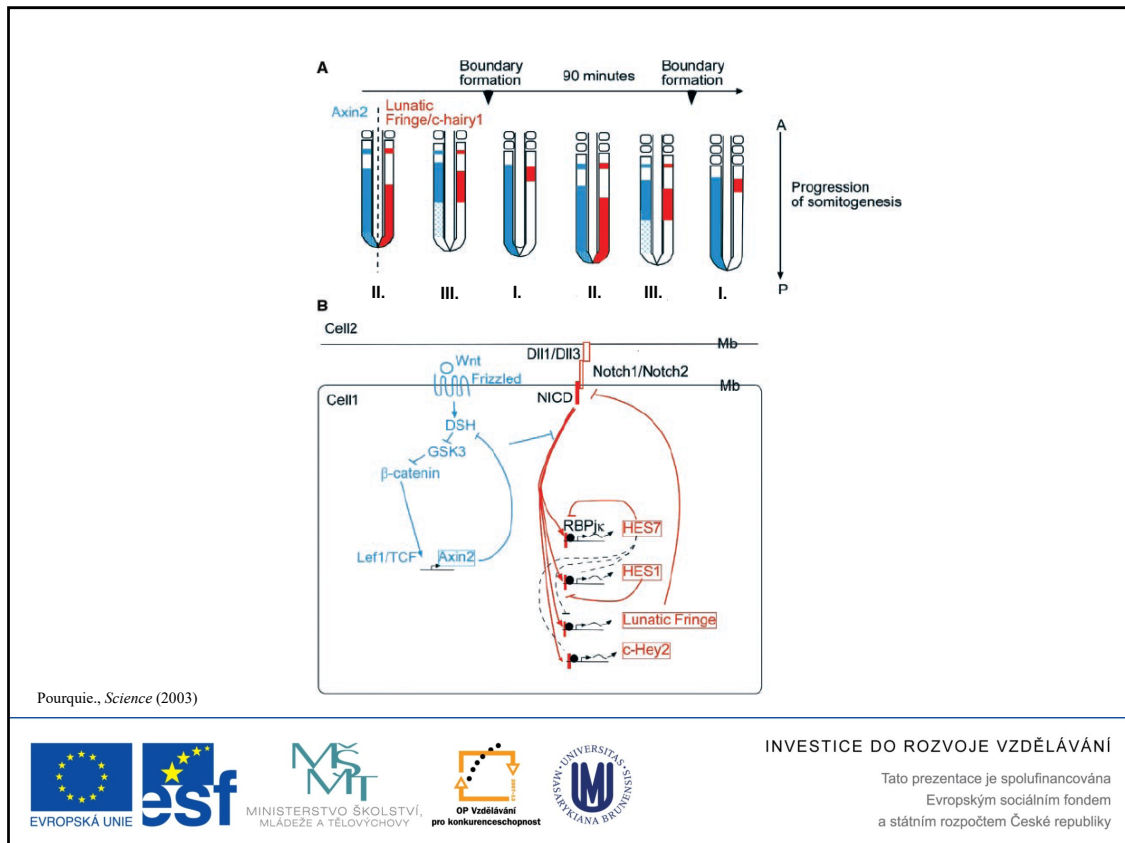
(Top) In situ hybridization with *c-hairy1* probe showing different categories of *c-hairy1* expression patterns in embryos aged of 15 (A, B, and C), 16 (D, E, and F), and 17 (G, H, and I) somites. Rostral to the top. Bar 5 200 μ m.

(Bottom) Schematic representation of the correlation between *c-hairy1* expression in the PSM with the progression of somite formation.

While a new somite is forming from the rostral-most PSM (somite 0, S₀), a narrow stripe of *c-hairy1* is observed in its caudal aspect, and a large caudal expression domain extends rostrally from the tail bud region (stage I; A, D, and G). As somite formation proceeds, as evidenced by the visualization of the appearing caudal fissure, the *c-hairy1* expression expands anteriorly, the caudal-most domain disappears, and *c-hairy1* appears as a broad stripe in the rostral PSM (stage II; B, E, and H).

When somite 0 is almost formed, the stripe has considerably narrowed, and *c-hairy1* is detected in the caudal part of the prospective somite (stage III; C, F, and I) while a new caudal expression domain arises from the tail bud region (in C can be seen the beginning of stage I of the next cycle).

This highly dynamic sequence of *c-hairy1* expression in the PSM was observed at all stages of somitogenesis examined (from 1 to 25 somites), suggesting a cyclic expression of the *c-hairy1* mRNA correlated with somite formation. Arrowheads point to the most recently completely formed somite (somite I, S_I).



Several additional genes, referred to as **cyclic genes**, that exhibit a dynamic behavior similar to that of *c-hairy1* have now been identified in fish, frog, chick, and mouse embryos, suggesting that the segmentation clock is conserved among vertebrates.

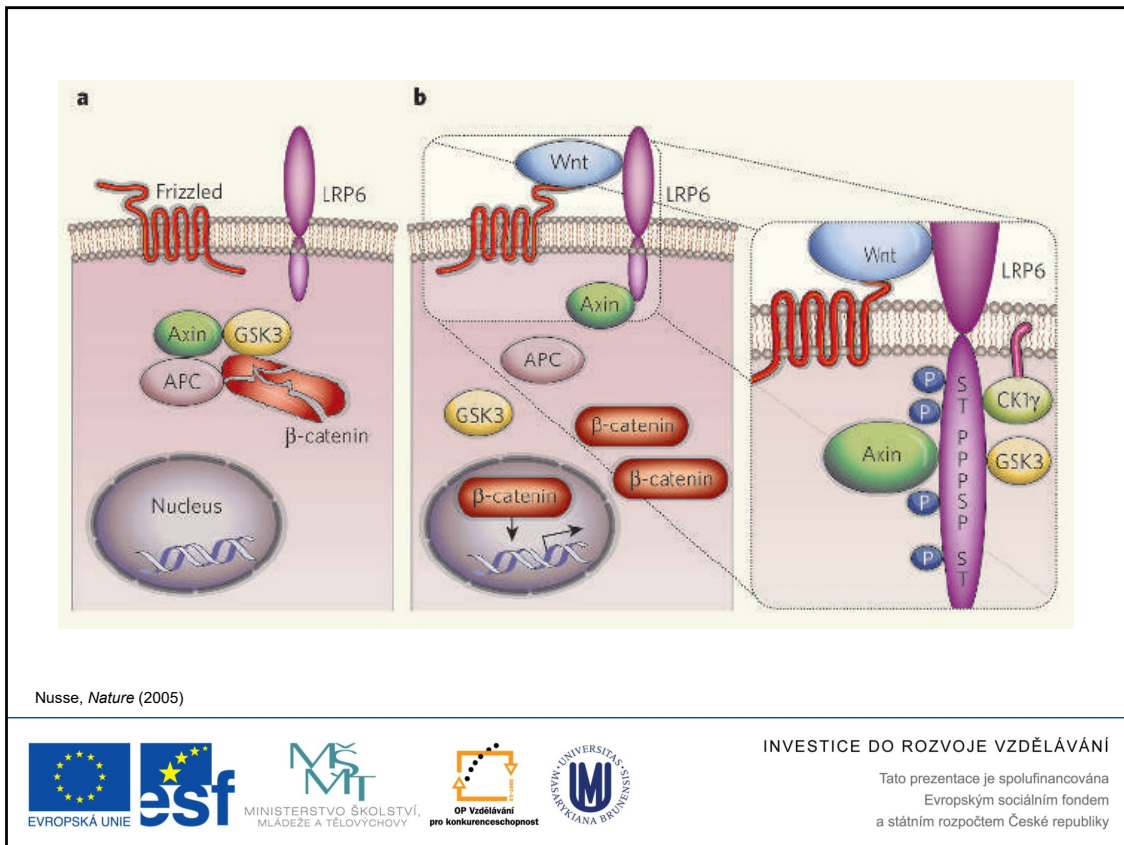
The best characterized set of cyclic genes is involved in **Notch signaling**, suggesting that Notch activation lies at the heart of the oscillator. Such genes encode **several transcription factors** of the **Hairy and Enhancer of Split (HES)** family, acting downstream of Notch signaling, as well as the **glycosyl transferase Lunatic Fringe** and the **Notch ligand deltaC**.

The NOTCH signalling pathway includes several negative feed-back loops mediated e.g. by Lunatic-Fridge or transcriptional repressors from the HES family (see the slide).

A second group of cyclic genes linked to the **Wnt signaling pathway** has recently been uncovered. Thus far, only one cycling gene in this class has been identified: the **inhibitor of Wnt signaling Axin2**.

In the mouse, *axin2* is expressed in a dynamic sequence similar to, but out of phase with, that of the Notch-related cyclic genes (Fig. A). *axin2* is directly regulated by Wnt signaling and could participate in the establishment of an autoregulatory negative feedback loop involved in its periodic expression. *axin2* oscillations persist in Notch pathway mutants, whereas both *axin2* and *lunatic fringe* oscillations are disrupted in *wnt3a* mutants, indicating that Wnt signaling acts upstream of the Notch-regulated cyclic genes (Fig. B).

Therefore, in the mouse, the segmentation clock appears to be composed of a Wnt-based regulatory loop entraining a series of Notch-based loops (Fig. B). The details of the interactions between these different loops are presently not understood. Also, the conservation of the Wnt-based loop across vertebrates remains to be examined. The exact role of this oscillator in the segmentation process remains unclear (Pourquie, *Science*, 2003).



The name Wnt was coined as a catenation of Wg (wingless) and Int and is pronounced 'wint'. The wingless gene had originally been identified as a recessive mutation affecting wing and haltere development in *Drosophila melanogaster*. It was subsequently characterized as segment polarity gene in *Drosophila melanogaster* that functions during embryogenesis and also during adult limb formation during metamorphosis.

The *INT* genes were originally identified as vertebrate genes near several integration sites of mouse mammary tumor virus (MMTV) in mouse breast cancers. The Int-1 gene and the wingless gene were found to be homologous, with a common evolutionary origin evidenced by similar amino acid sequences of their encoded proteins.

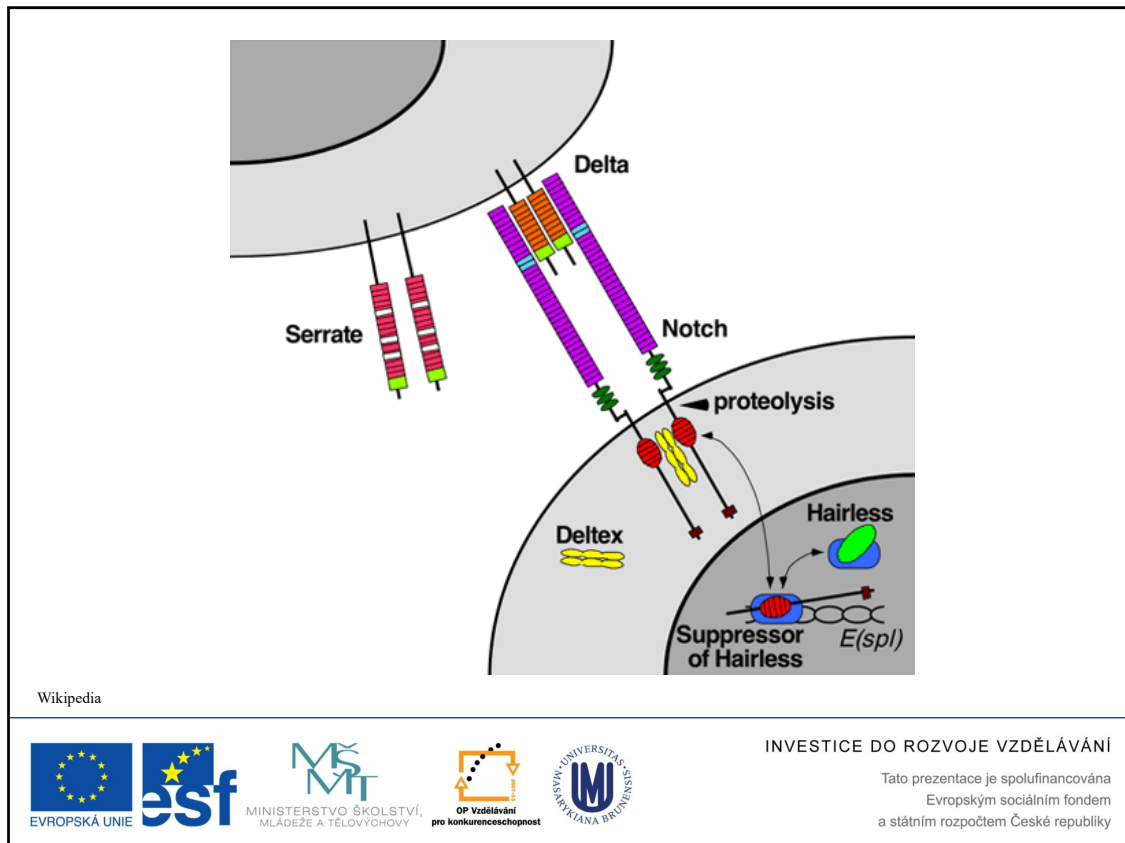
Mutations of the wingless gene in the fruit fly were found in wingless flies, while tumors caused by MMTV were found to have copies of the virus integrated into the genome forcing overproduction of one of several Wnt genes. The ensuing effort to understand how similar genes produce such different effects has revealed that Wnts are a major class of secreted morphogenic ligands of profound importance in establishing the pattern of development in the bodies of all multicellular organisms studied (Wikipedia).

The canonical Wnt signalling pathway is shown on the slide. Here, the Wnt protein binds to its receptor, the transmembrane protein Frizzled.

a. In cells not activated by Wnt, a complex between β -catenin, Axin, APC and GSK3 causes phosphorylation of β -catenin and its consequent destruction. The Wnt receptors LRP6 and Frizzled are unoccupied. **B.** Without Axin, β -catenin is stabilized and it enters the nucleus to control gene expression.

Inset, binding of Wnt to cells results in phosphorylation (P) of LRP6 residues in its cytoplasmic tail. Zeng et al. and Davidson et al. show that this is catalysed by the GSK3 and CK1 protein kinases. CK1 is attached to the membrane by a lipid anchor domain. Several other sites on LRP6 that become phosphorylated are not shown here. The phosphorylated LRP6 recruits Axin, removing it from the β -catenin destruction complex and stabilizing β -catenin.

Up-to-date and detailed information on these interactions and WNT signaling pathways will be presented during advanced lecture of Dr. V. Bryja (Bi9903 Developmental Animal Physiology I and Bi9906 Developmental Animal Physiology II).

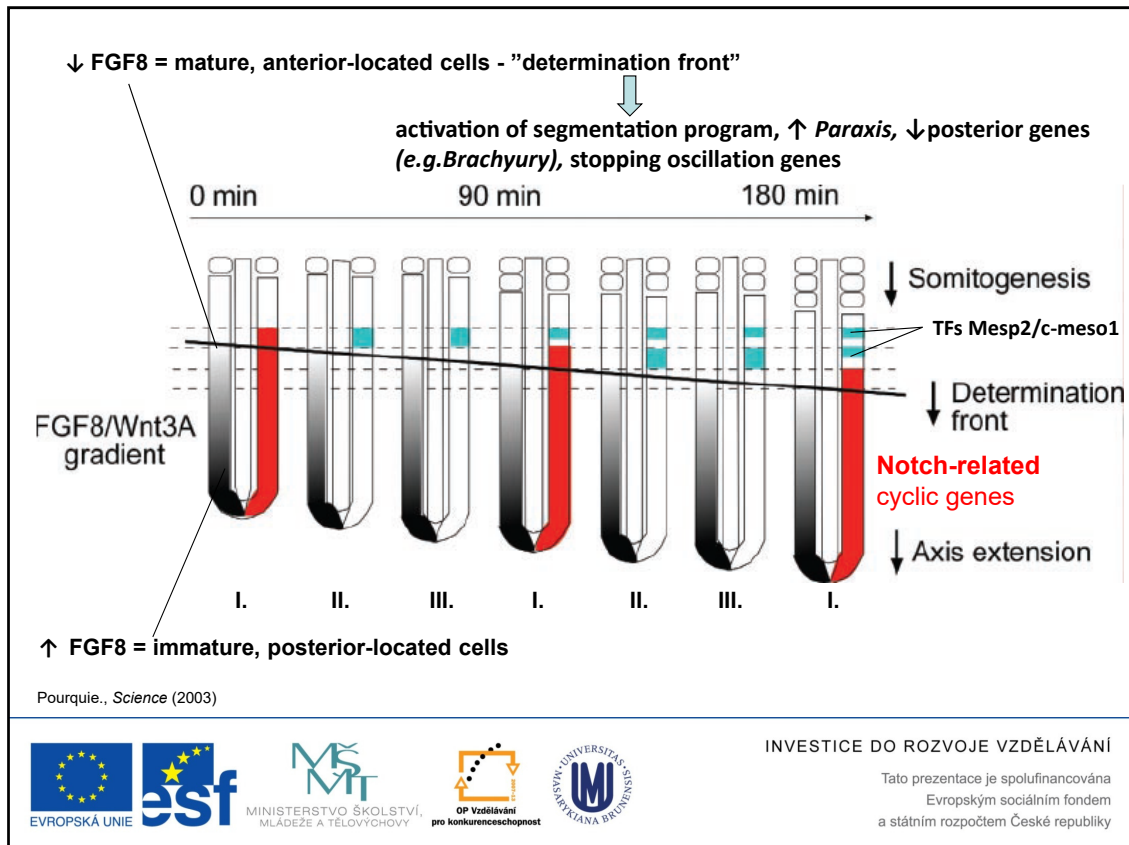


Notch signalling

In 1914, John S. Dexter noticed the appearance of a notch in the wings of the fruit fly *Drosophila melanogaster*. The alleles of the gene were identified in 1917 by Thomas Hunt Morgan. Its molecular analysis and sequencing was independently undertaken in the 1980s by Spyros Artavanis-Tsakonas and Michael W. Young.

The notch protein sits like a trigger spanning the cell membrane, with part of it inside and part outside. Ligand proteins binding to the extracellular domain induce proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to alter gene expression.

Because most ligands are also transmembrane proteins, the receptor is normally triggered only from direct cell-to-cell contact. In this way, groups of cells can organize themselves, such that, if one cell expresses a given trait, this may be switched off in neighbor cells by the intercellular notch signal. In this way, groups of cells influence one another to make large structures (Wikipedia, http://en.wikipedia.org/wiki/Notch_signaling_pathway).



FGF Signaling: Translation of the Clock Pulsation into Spatial Periodicity. Model for segment formation in vertebrates based on mouse and chick data.

The FGF8/Wnt3A gradient, which regresses posteriorly during somitogenesis, is shown in black. The anterior boundary of the gradient defines the determination front, which corresponds to the position of the wavefront (thick black line). The phase I expression of Notch-related cyclic genes is shown in red. The expression of *Mesp2/c-meso1* is shown in blue.

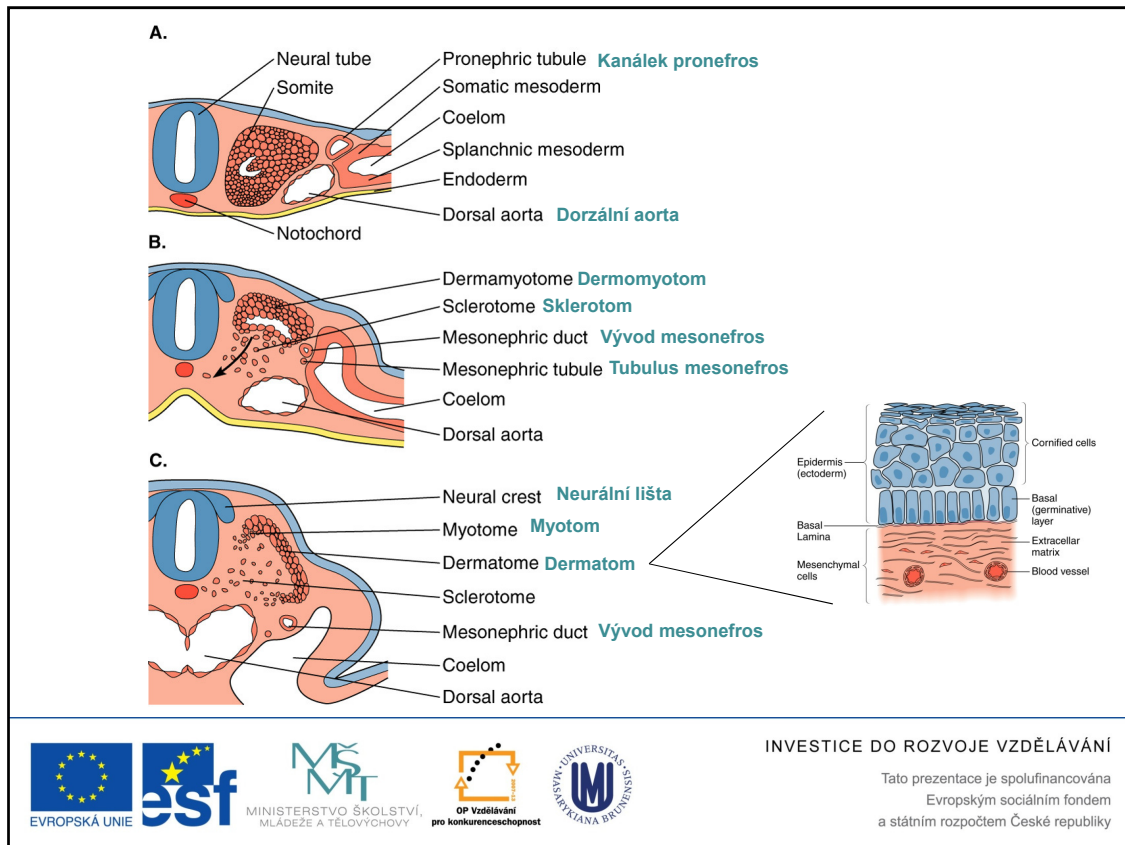
Recent studies have shown that the secreted growth factor FGF8 (fibroblast growth factor 8) could be implicated in converting the clock pulsation into the periodic arrangement of segment boundaries. *FGF8* mRNA is strongly expressed in mesenchymal presomitic mesoderm (PSM) precursors in the primitive streak and tail bud as well as in the posteriormost PSM, and its expression progressively decreases in more anterior cells, thus establishing a gradient over two-thirds of the PSM length (see the slide, black).

Overexpression of FGF8 in PSM cells can maintain their posterior identity and block segmentation, suggesting that high concentrations of FGF8 are required to actively maintain newly formed posterior PSM cells in an immature state. It was proposed that, because of the progressive decrease of *FGF8* expression during maturation of the PSM, when cells become located in the anterior PSM, they reach a threshold of FGF signaling allowing them to activate their segmentation program.

This threshold level, which was termed the "determination front" (see the slide), marks a transition in genetic regulation in PSM cells, as shown by the activation of new sets of genes such as *Paraxis*, the down-regulation of posterior genes such as *Brachyury*, and the slowing down and stopping of the oscillations of the clock genes.

Wnt3A was also recently proposed to assume a role similar to that of FGF8 by establishing a gradient-controlling segmentation in the PSM (20). However, because *Wnt3A* acts upstream of *FGF8* in the PSM, it could act together with or by way of the FGF8 gradient.

At the determination-front level, gene coding for the transcription factor *Mesp2/c-meso1* becomes periodically activated in a one-somite wide domain, providing the earliest evidence for segmentation in the PSM.



Somite is transient embryonal structure. Soon as the somites are formed, the medioventral portion of the somite disagregates. The released cells, called **sclerotome** abut the notochord and will differentiate into segmental cartilage, a precursor of the vertebrae and proximal portion of the ribs.

The dorsal portion of the somites remains of epithelial character. It is called dermamyotome, as it is comprised of dermatome and myotome.

Dermatome will give rise to dermis, the lower portion of the integuments that contains some migrated ectoderm cells forming pigment cells, as discussed in previous chapter.

Myotome will develop into muscles.

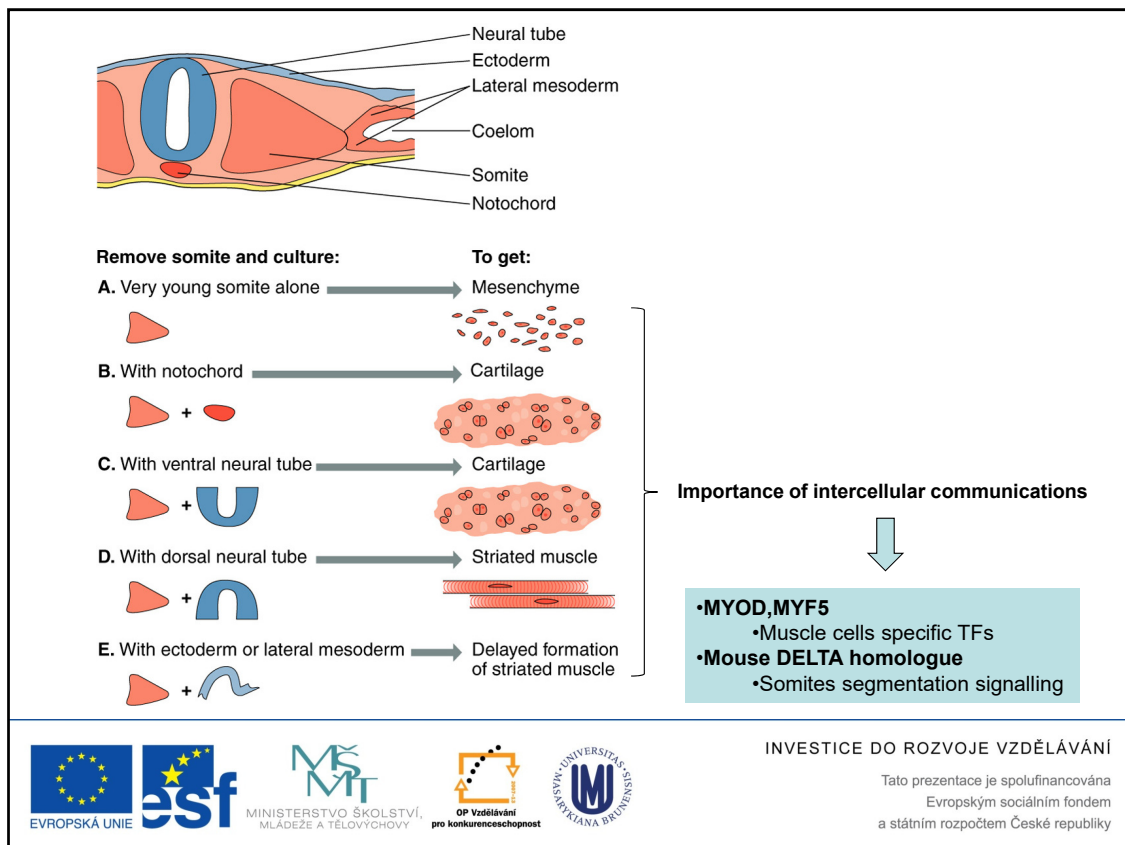
The relative positions of the forming kidney, pronephros and mesonephros, are shown and will be discussed later.

The lateral portion of the dermamyotome will provide cells that will develop into muscles of the body wall, limbs and distal portion of the ribs.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

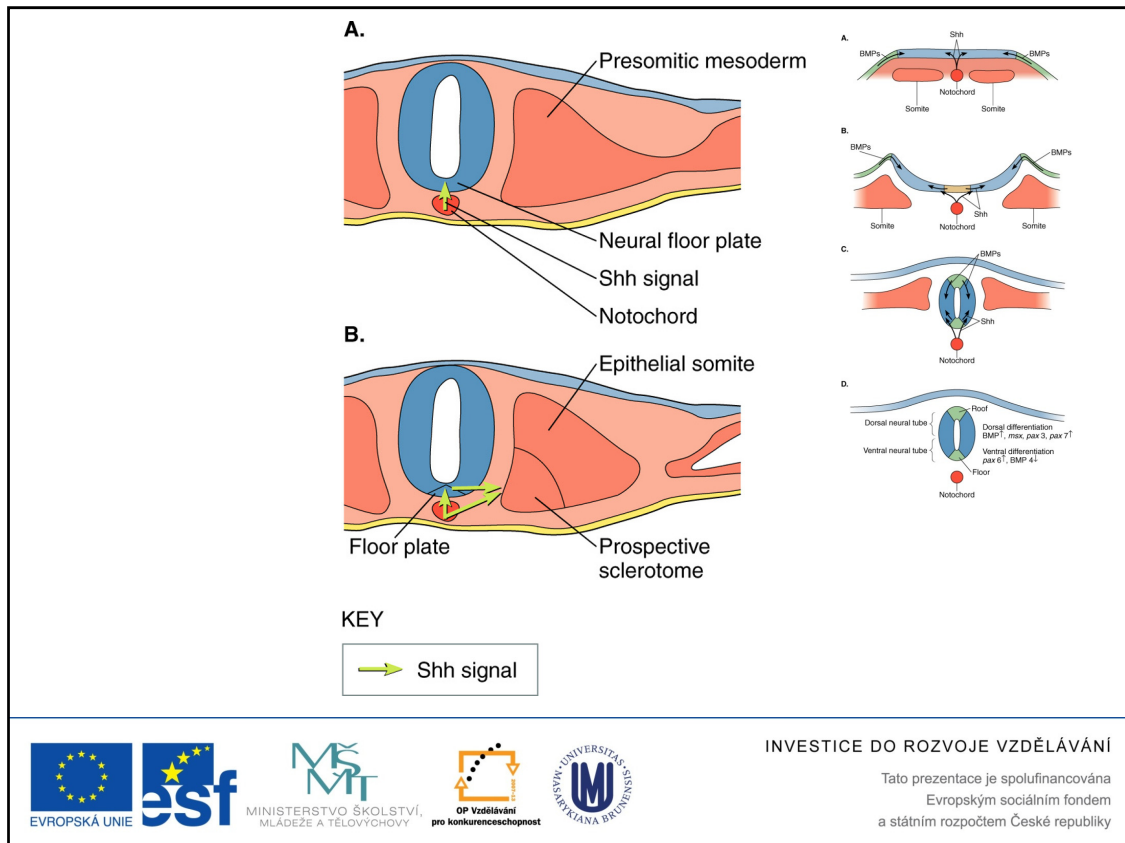


What is the molecular mechanism involved in the determination of the narrowing developmental potential of mesoderm?

The explantation experiments have provided experimental evidence about importance of the intercellular communications. As shown in the figure, the nature of developing tissue is determined by the presence of surrounding tissue, particularly neural tube (and its dorsal or ventral portion), lateral mesoderm and overlying ectoderm.

Recently, some of the molecular determinants involved in the tissue differentiation have been identified. These are e.g. **muscle cells specific TFs MYOD** (see later slides) and **MYF5**. When ectopically expressed, these TFs induce muscle cell differentiation and thus could be considered as **muscle marker genes**.

Also some of the signalling molecules involved in specific processes during mesoderm structures differentiation were identified. This is e.g. **homologue of the DELTA gene from Drosophila** that was identified in mouse. It is a signalling molecule necessary for the somites segmentation.

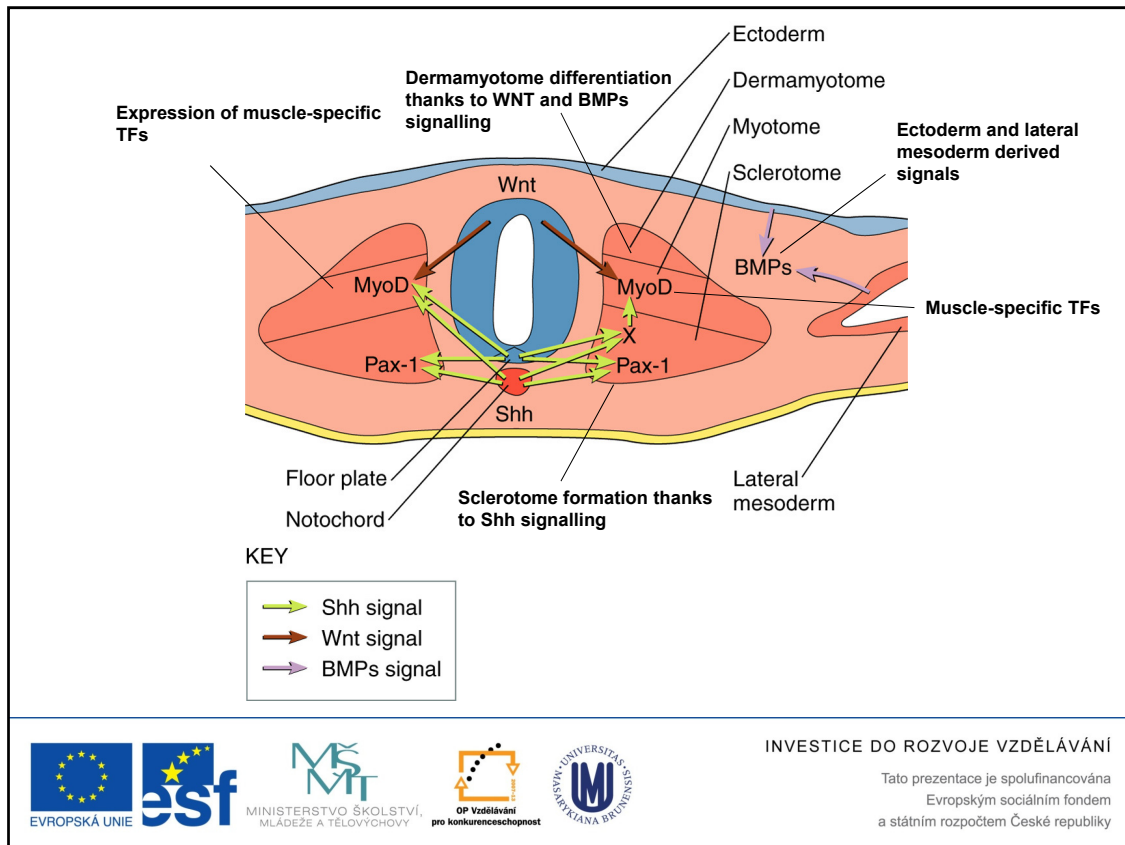


Another important signalling molecule is **SHH (SONIC HEDGHEOG)** that we have discussed previously (see Chapter 6 and the problem of the dorsoventral neural tube patterning, inset).

SHH is secreted by the neural floor plate and by the notochord.

SHH secreted from the notochord alone is not able to induce sclerotome formation.

However, notochord secreted SHH induces SHH production from the floor plate of the neural tube (see previous slide), which allows to reach sufficient amount of SHH to induce sclerotome differentiation and thus triggers the process of cartilage formation.



Complicate signalling networks are operating in the different developmental processes during embryogenesis.

For the proper development of early dorsal mesoderm, interaction of specific signals from different portions of neural tube, notochord, ectoderm and lateral mesoderm and their proper timing and position are necessary.

WNT, secreted from the dorsal portion of neural tube together with ectoderm and lateral mesoderm derived signals like BMPs is necessary for the dermamyotome differentiation.

SHH triggers sclerotome formation.

Both dorsal and ventral signals are necessary for the later myotome formation that is accompanied by the expression of muscle-specific TFs, e.g. MyoD (see the figure). SHH acts in the dorsal somite determination together with WNT either directly or via unknown intermediate (X, see figure).

More about the Wnt signalling in the specialized lecture of Assoc. Prof. Bryja, Developmental physiology of animals I and II (Bi9903 and Bi9906).



Outline of Lesson 5

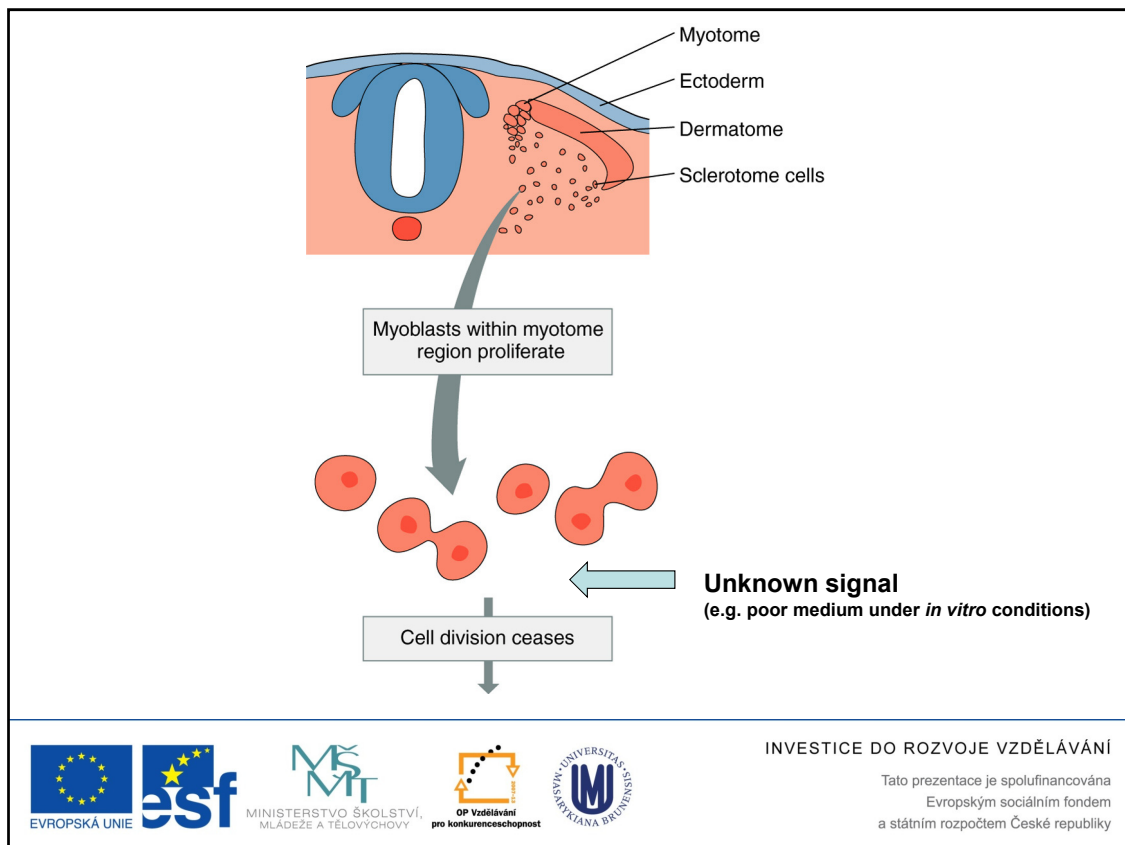
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles



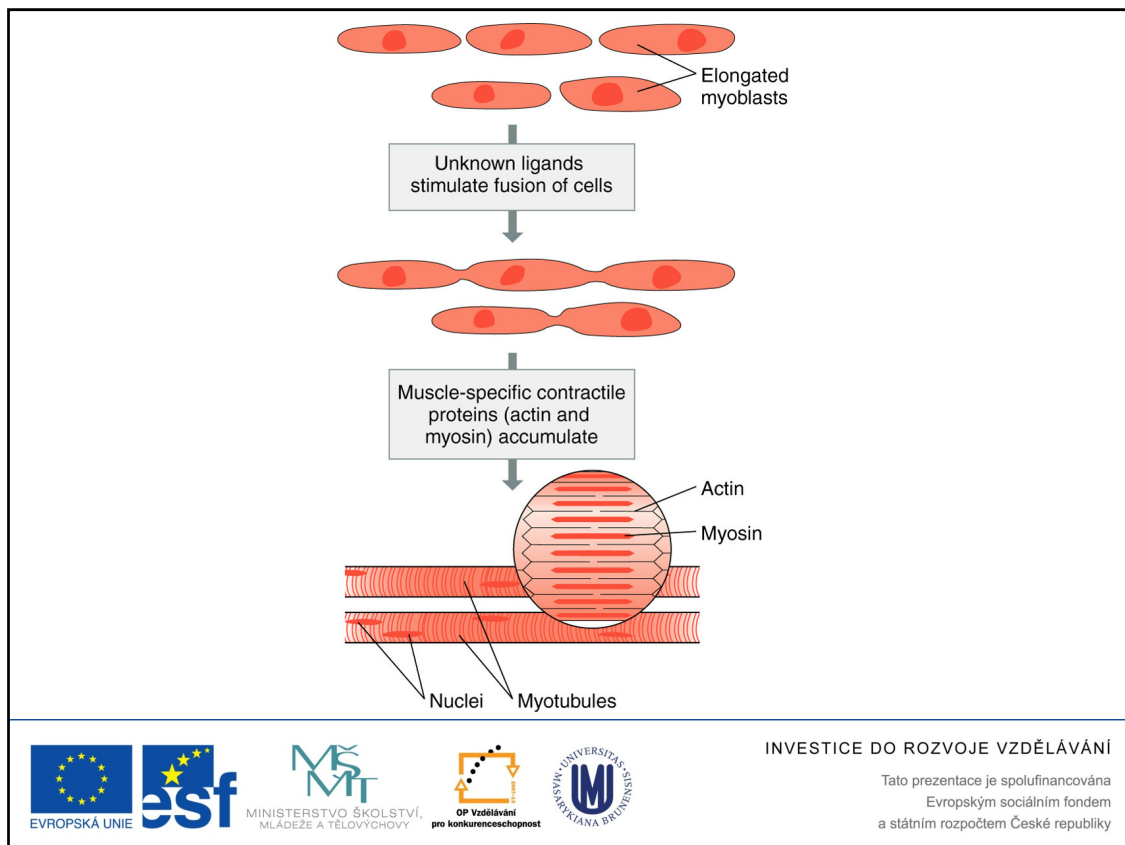
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Muscle cells differentiate from myoblasts, the prospective muscle cells located in myotome.

Myoblasts stop proliferation that could be induced e.g. by the replacement of cells from rich to poor medium and enter the G₀ phase of the cell cycle. The signal starting the development of muscle cells in embryos is still unknown.



Development of a given muscle differ and has its own timing and location and undergo specific developmental pathways.

Myoblasts elongate, fuse and form syncytia in case of striated muscle cells. The genes involved in the actin and myosin biosynthesis are activated.

There are differences of the developmental scheme based on the muscle type. Heart muscles are distinctive but similar to striated cells. Smooth muscles do not form syncytia and organization of actin and myosin is also different then in striated muscle cells.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Outline of Lesson 5

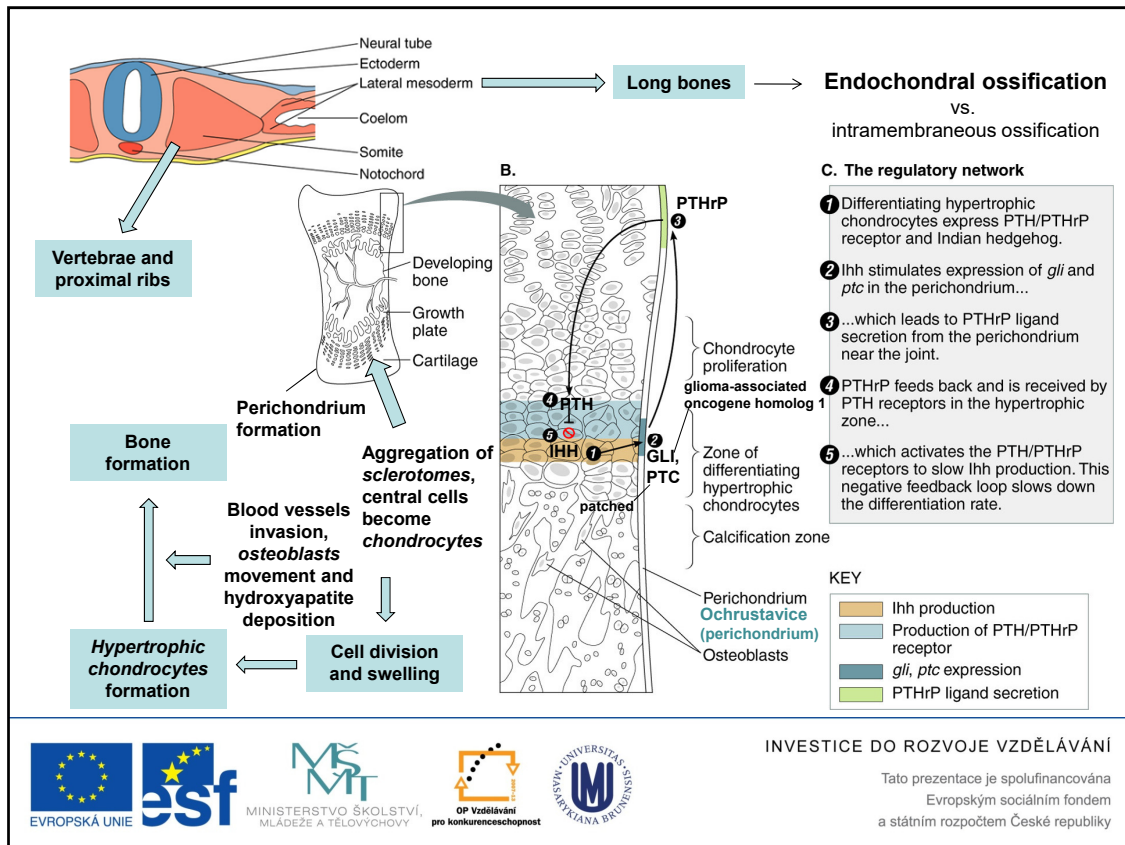
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Sclerotome develops into cartilage that later allows vertebrae and proximal portion of ribs formation. The cartilage for long bones of the limbs will develop from the lateral mesoderm.

Cartilage represents a template where the bone formation starts via a process called *endochondral ossification*. Only the skull bones form without cartilage directly in mesenchymal cells derived from the neural crest via a process called *intramembraneous ossification*.

Sclerotome cells aggregate and the central cells become chondrocytes (cartilage cells), other cells form a surrounding sheath, called *perichondrium*.

Chondrocytes divide and swell, forming thus *hypertrophic chondrocytes*.

The blood vessels invade the hypertrophic cartilage and bone forming *osteoblasts* move into the area. Osteoblasts deposit hydroxyapatite, a crystalline form of calcium phosphate.

Growth and morphogenesis of the bones depends on a carefully regulated processes of replacement of the cartilage by bone. If the process is too fast or *vice versa* too slow, it leads to shortening or elongation of the forming bone, respectively.

Complicated feed back of signalling molecules, e.g. parathyroid hormone (PTH), parathyroid hormone related peptide (PTHrP), INDIAN HEDGHEGOG (IHH) and other signalling molecules (see the scheme above) takes place in that type of growth regulation.

GLI stands for glioma-associated oncogene homolog 1 (zinc finger protein), PTC is PATCHED, a receptor for IHH.



Outline of Lesson 5

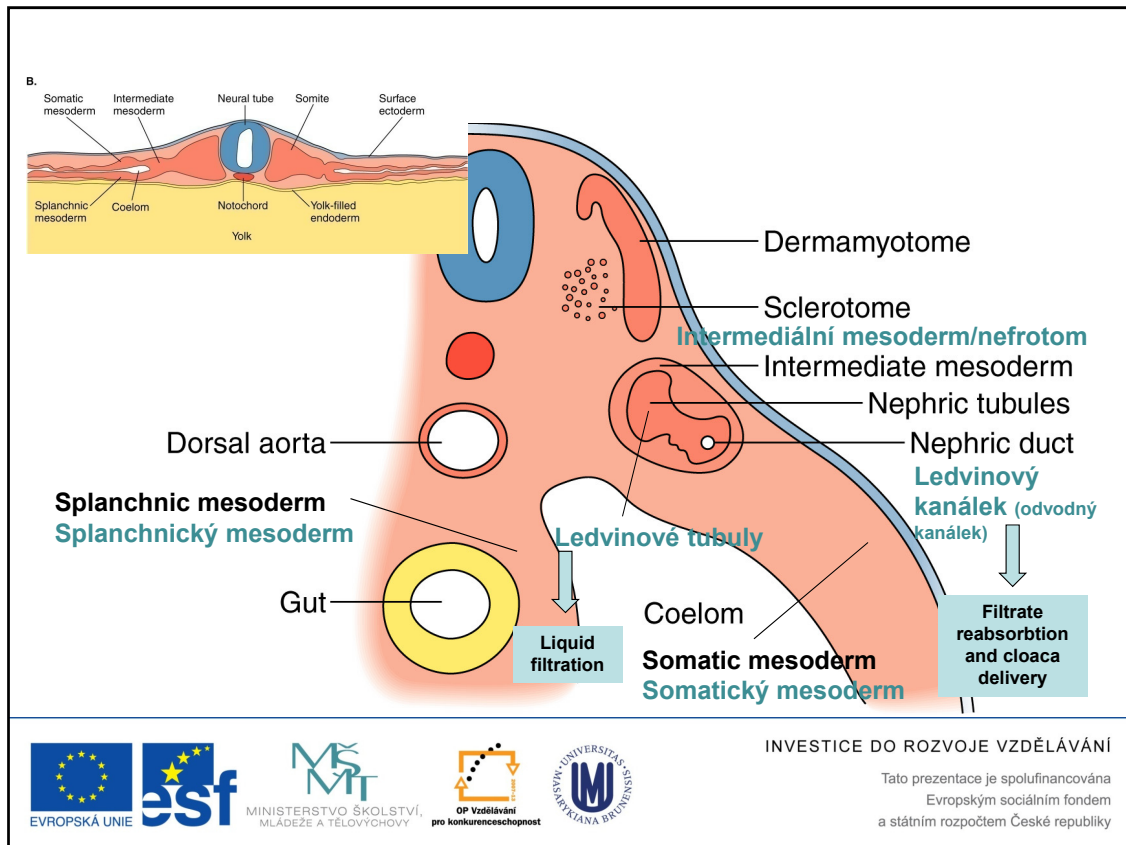
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

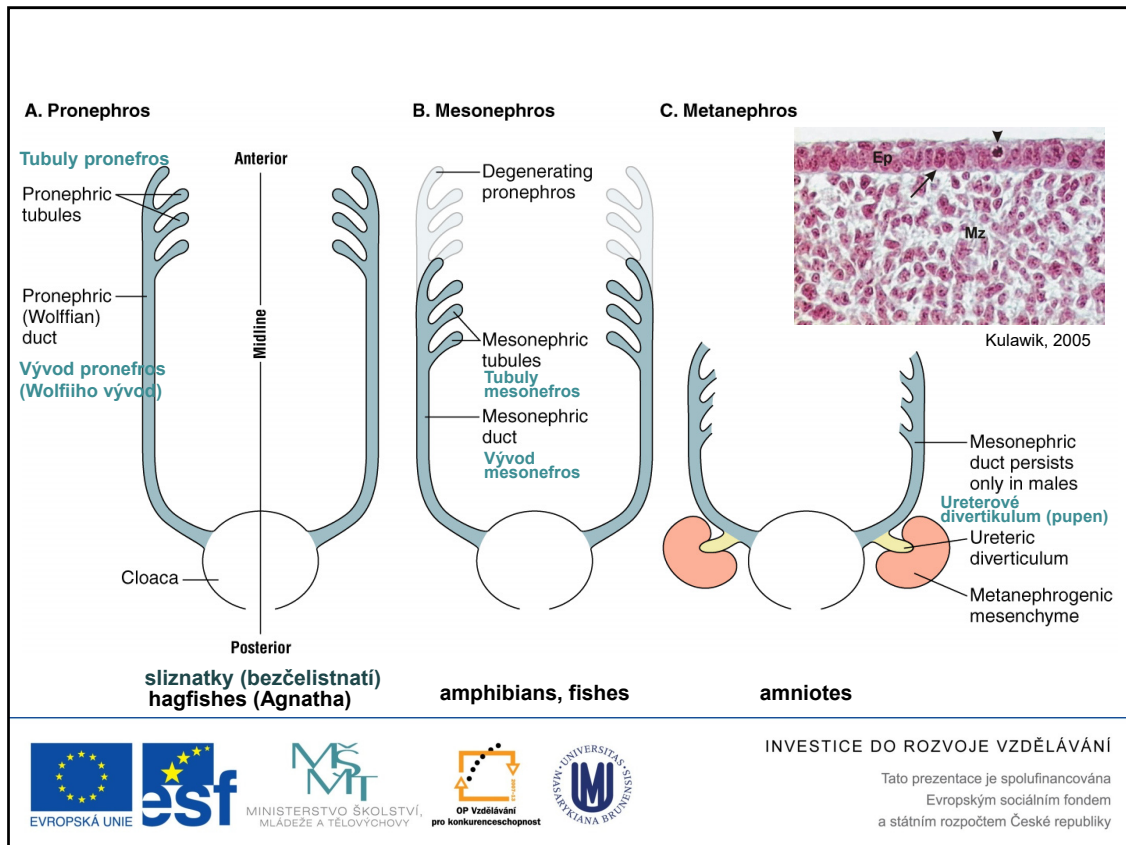
Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



The region between somite and splanchnic and somatic mesoderm is called *intermediate mesoderm* (see the inset above, showing the cross-section of the chicken embryo), or also *nephrotome*, as it develops into tubules and ducts that make up the kidney.

Some of these ducts will be co-opted or shared with the developing reproductive system.

The kidney consists of two major parts: *tubules* that filtrate liquid and small molecules from the blood and *ducts* that reabsorb some of the filtered materials and deliver the filtrate to the cloaca.



Tubules differentiate in an anteroposterior axis, the more posterior, the more complex structures develop.

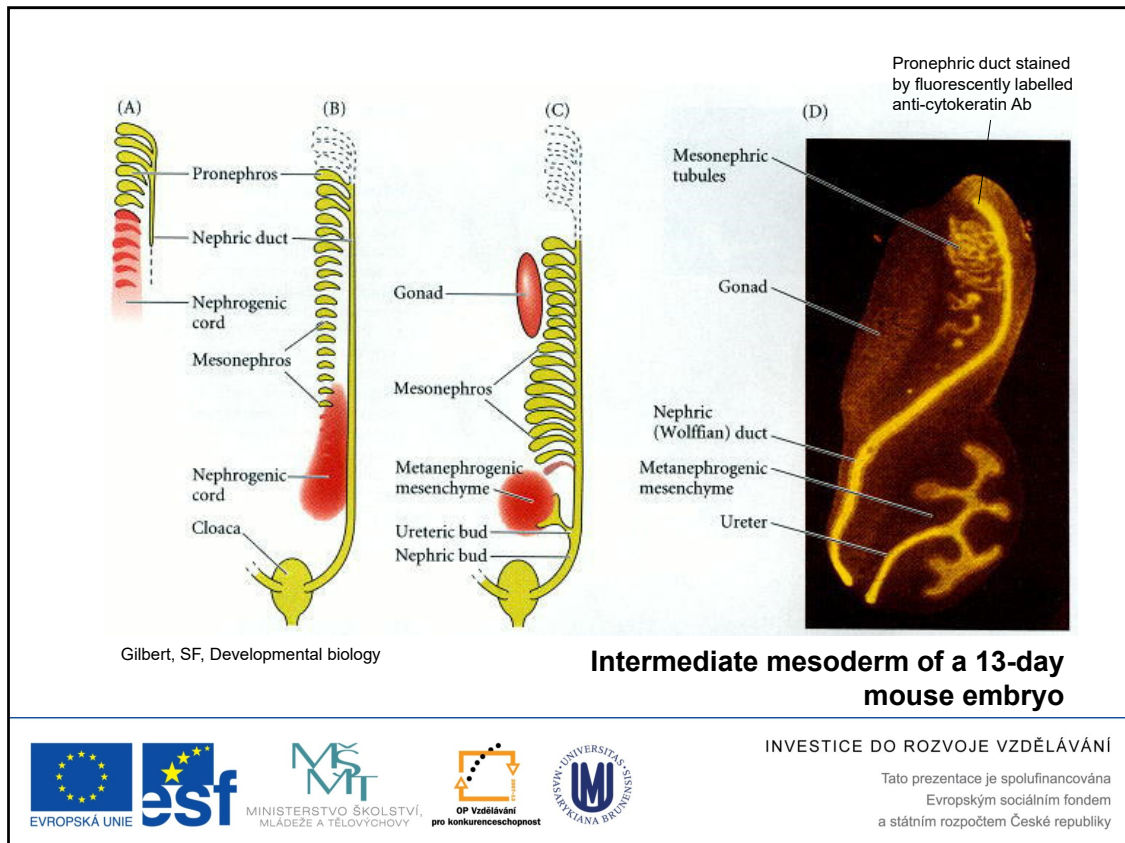
In more simple organisms, e.g. in hagfishes (Agnatha) only the most primitive, i.e. most anterior tubules develop, forming what is called **pronephros**.

During development of amphibians and fishes, the first developed pronephric-type anterior tubules degenerate and are replaced by more posterior and more complex structures of **mesonephros**.

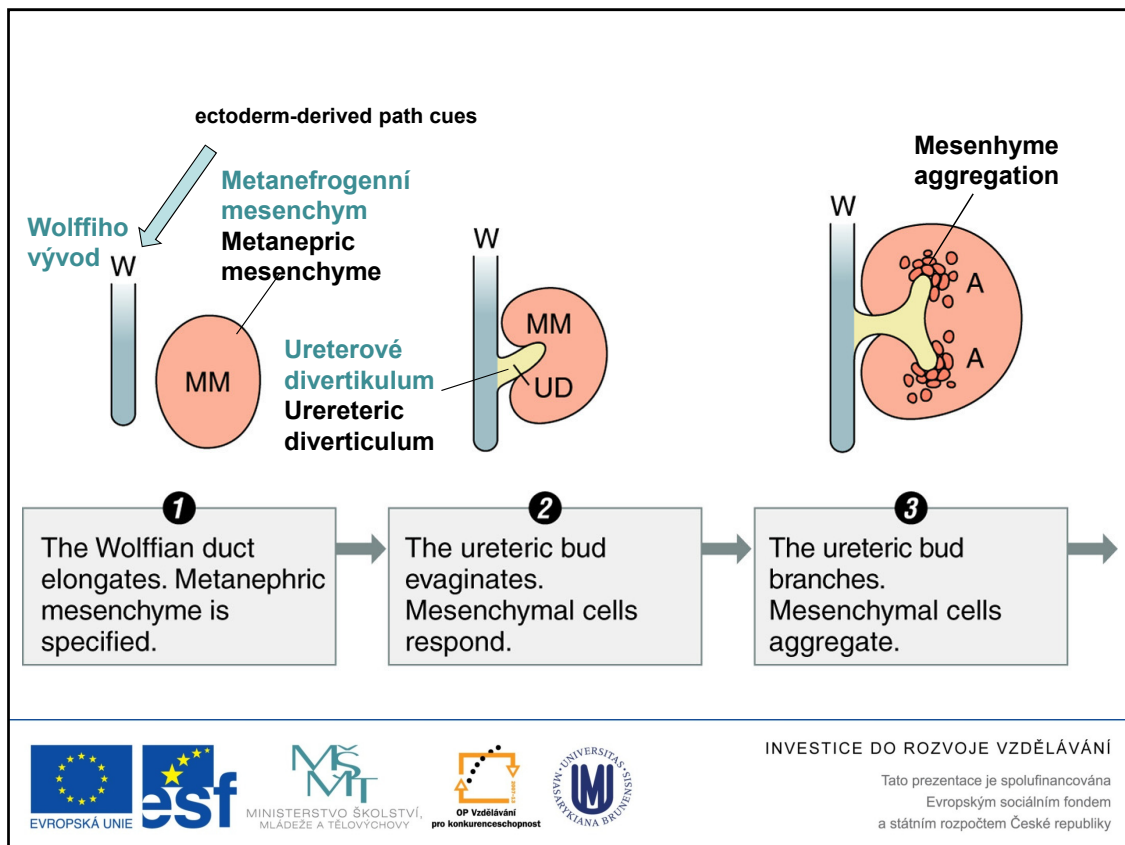
Finally, in amniotes, the anterior primitive tubules act only during embryonic development and the definitive kidney develop from a posterior located *metanephrogenic mesoderm* forming **metanephros**.

Inset: Mesenchym of rabbit tongue during prenatal development. Image of median cross-section of the dorsum of the body of the tongue at day 15 of prenatal life in the rabbit. Ep – epithelium, arrow – basement membrane, arrowhead – cell during mitosis, Mz – mesenchyme. LM, x 40, Masson-Goldner staining (Kulawik, 2005; <http://www.ejpau.media.pl/volume8/issue4/art-17.html>).

The image demonstrates the nature of mesenchyme as an 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 (Wikipedia).



General scheme of development in the vertebrate kidney. (A) The original tubules, constituting the pronephros, are induced from the nephrogenic mesenchyme by the pronephric duct as it migrates caudally. (B) As the pronephros degenerates, the mesonephric tubules form. (C) The final mammalian kidney, the metanephros, is induced by the ureteric bud, which branches from the nephric duct. (D) The intermediate mesoderm of a 13-day mouse embryo showing the initiation of the metanephric kidney (bottom) while the mesonephros is still apparent. The duct tissue is stained with a fluorescent antibody to a cytokeratin found in the pronephric duct and its derivatives. (A-C after [Saxén 1987](#); D courtesy of S. Vainio.)

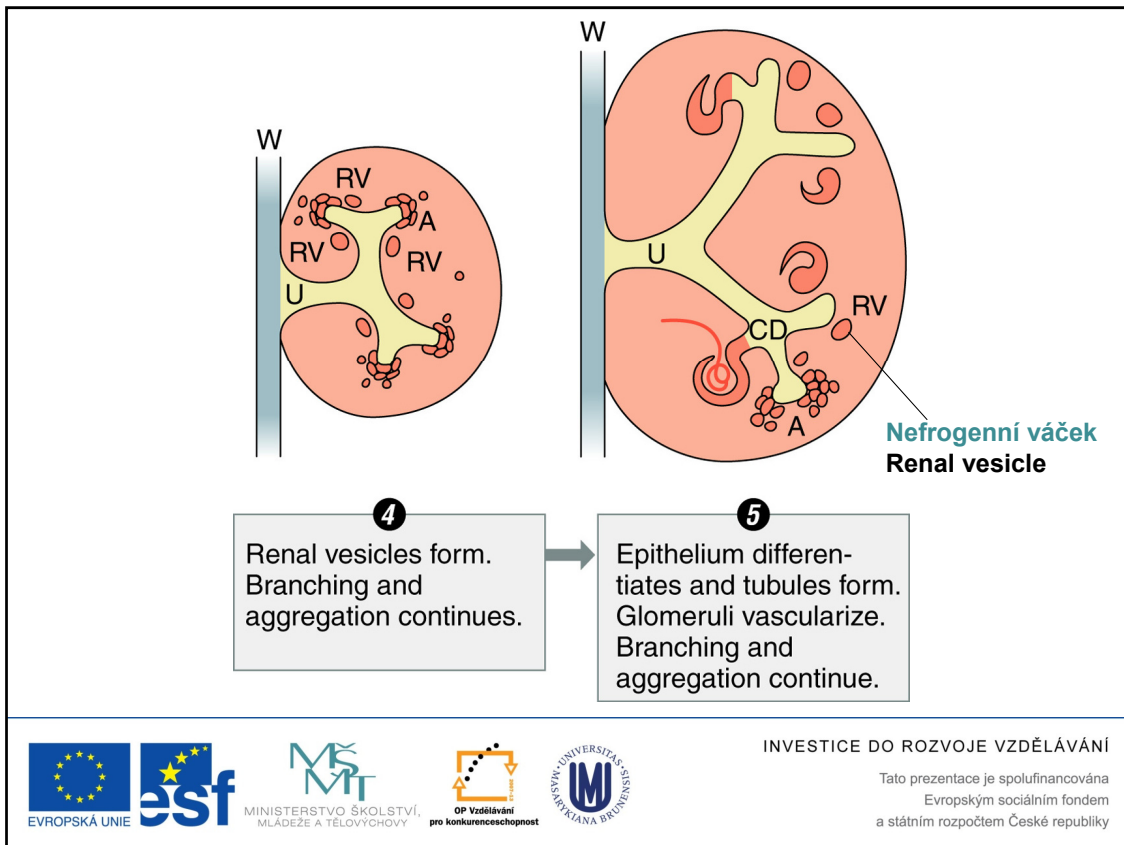


The main duct formed during nephros formation is called **Wolfian (pronephric) duct**. It elongates by migration mechanism via a path marked by cues from the overlying ectoderm.

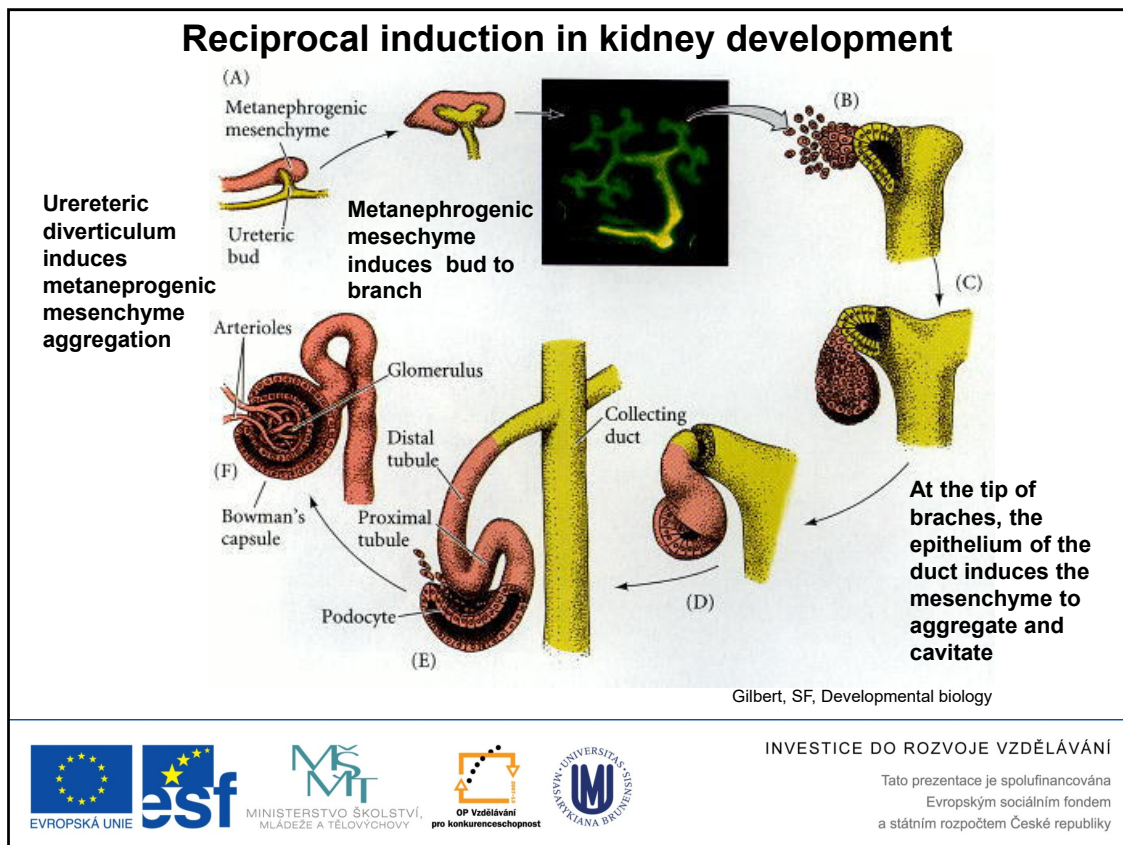
Near the connection of the duct to the cloaca, a bud diverts from the duct and extends to the posterior *metanephric mesenchyme*, which is stimulated to condense.

Wolfian duct persist only in males and degenerates in females (see further).

The developing kidney undergoes further development, see next slides.

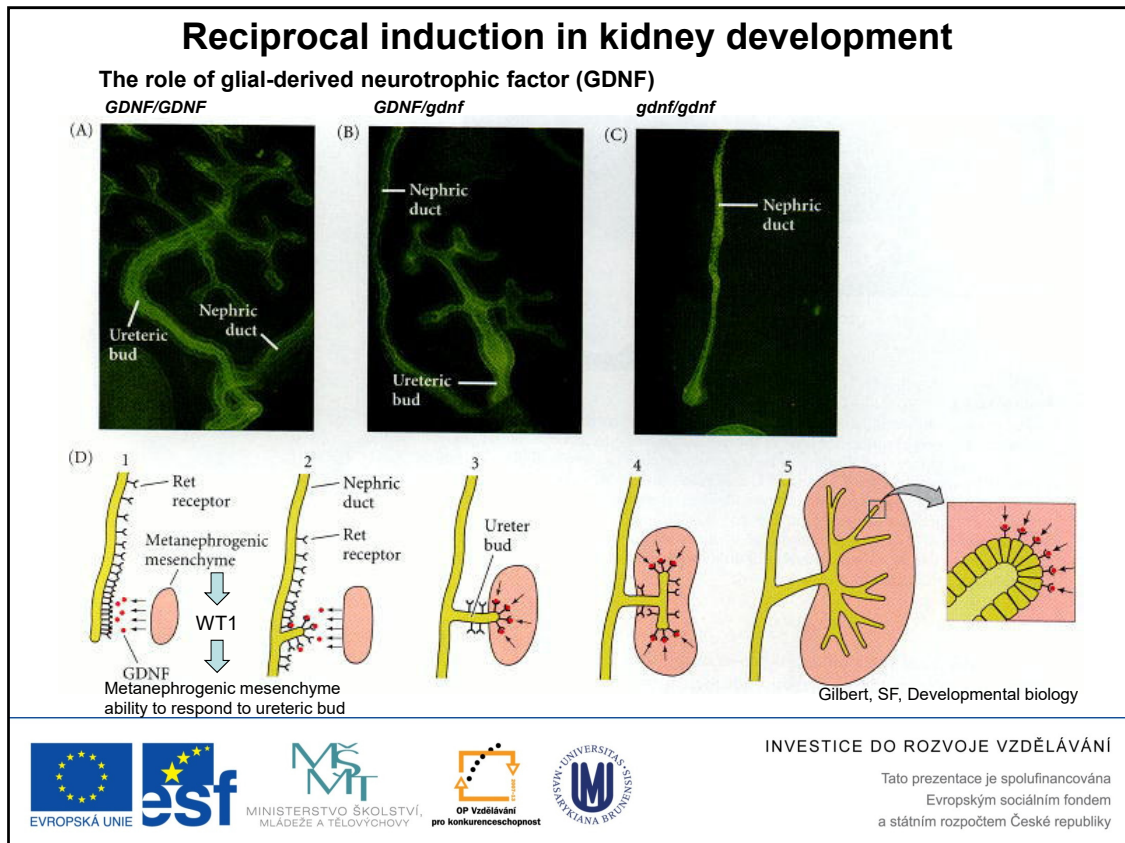


- A mesenchyme aggregation
- RV renal vesicle
- U uterus
- CD collecting duct



Reciprocal induction in the development of the mammalian kidney. (A) As the ureteric bud enters the metanephrogenic mesenchyme, the mesenchyme induces the bud to branch. (B-F) At the tips of the branches, the epithelium induces the mesenchyme to aggregate and cavitate to form the renal tubules and glomeruli (where the blood from the arteriole is filtered). When the mesenchyme has condensed into an epithelium, it digests the basement membrane of the ureteric bud cells that induced it and connects to the ureteric bud epithelium. The mesenchyme becomes the nephron (renal tubules and Bowman's capsule), while the ureteric bud becomes the collecting duct for the urine. (After [Saxén 1987.](#))

Reciprocal induction in kidney development



The mechanisms of reciprocal induction

The induction of the metanephros can be viewed as a dialogue between the ureteric bud and the metanephrogenic mesenchyme. As the dialogue continues, both tissues are altered. There appear to be at least eight sets of signals operating in the reciprocal induction of the metanephros.

Step 1: Formation of the metanephrogenic mesenchyme

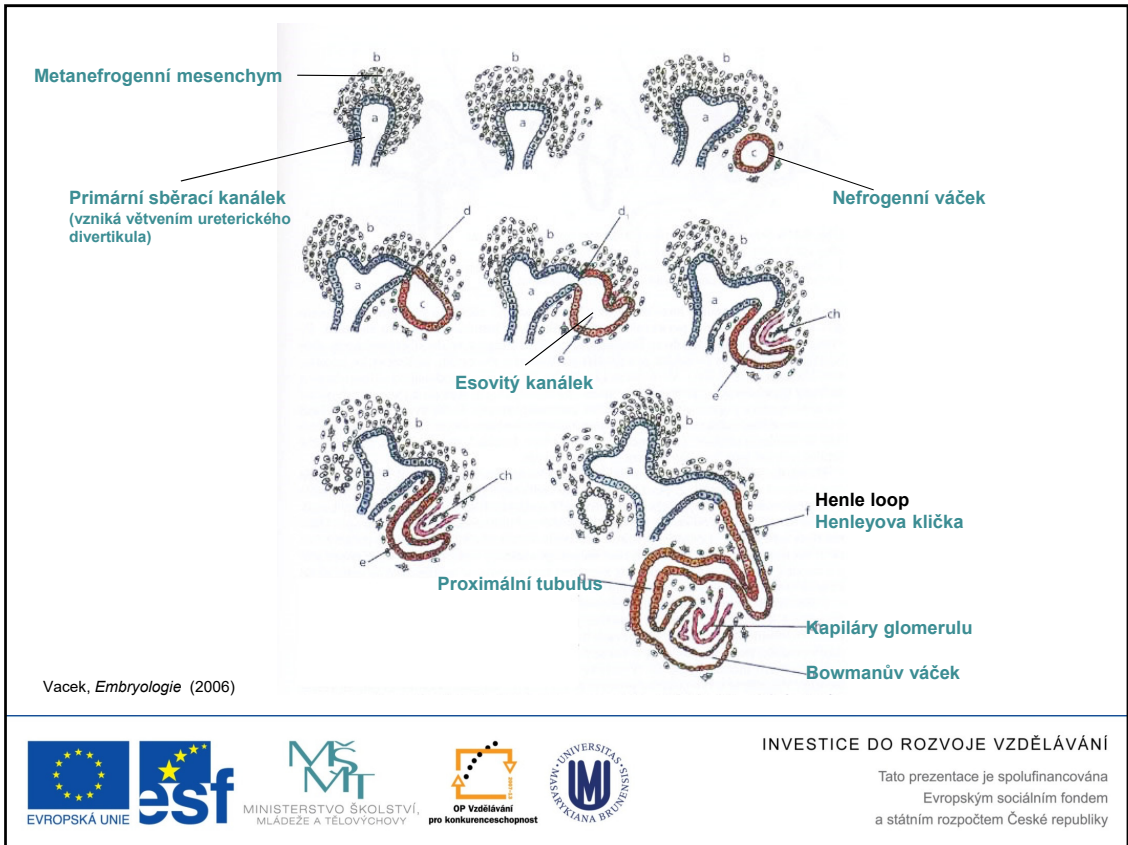
Only the metanephrogenic mesenchyme has the competence to respond to the ureteric bud to form kidney tubules, and if induced by other tissues (such as embryonic salivary gland or neural tube tissue), the metanephrogenic mesenchyme will respond by forming kidney tubules and no other structures (Saxén 1970; Sariola et al. 1982). Thus, the metanephrogenic mesenchyme cannot become any tissue other than nephrons. Its competence to respond to ureteric bud inducers is thought to be regulated by a transcription factor called WT1, and if the metanephrogenic mesenchyme lacks this factor, the uninduced cells die (Kriedberg et al. 1993). In situ hybridization shows that WT1 is normally first expressed in the intermediate mesoderm prior to kidney formation and is then expressed in the developing kidney, gonad, and mesothelium (Pritchard-Jones et al. 1990; van Heyningen et al. 1990; Armstrong et al. 1992). Although the metanephrogenic mesenchyme appears homogeneous, it may contain both mesodermally derived tissue and some cells of neural crest origin (Le Douarin and Tiellet 1974; Sariola et al. 1989; Sainio et al. 1994).

Step 2: The metanephrogenic mesenchyme secretes GDNF and HGF to induce and direct the ureteric bud

The second signal in kidney development is a set of diffusible molecules that cause the two ureteric buds to grow out from the nephric ducts. Recent research has shown that glial-derived neurotrophic factor (GDNF) is a critical component of this signal. GDNF is synthesized in the metanephrogenic mesenchyme, and mice whose *gdnf* genes were knocked out died soon after birth from their lack of kidneys (Moore et al. 1996; Pichel et al. 1996; Sánchez et al. 1996). The GDNF receptor (the c-Ret protein) is synthesized in the nephric ducts and later becomes concentrated in the growing ureteric buds (Figure 14.21; Schuchardt et al. 1996). Mice lacking the GDNF receptor also die of renal agenesis. Another protein synthesized by the metanephrogenic mesenchyme is hepatocyte growth factor (HGF; also known as scatter factor), and the receptor for HGF (the c-met protein) is made by the ureteric buds. Antibodies to HGF will block ureteric bud outgrowth in cultured kidney rudiments (Santos et al. 1994; Woolf et al. 1995). The synthesis of GDNF and HGF by the mesenchyme is thought to be regulated by the WT1 protein.

The image: Ureteric bud growth is dependent on GDNF and its receptor. (A) The ureteric bud from a 11.5-day wild-type mouse embryonic kidney cultured for 72 hours has a characteristic branching pattern. (B) In embryonic mice heterozygous for the genes encoding GDNF, the size of the ureteric bud and the number and length of its branches are reduced. (C) In mouse embryos missing both copies of the *GDNF* gene, the ureteric bud does not form. (Scale bars = 100 μ m.) (D) The receptors for GDNF are concentrated in the nephric ducts and later becomes concentrated in the growing ureteric buds. GDNF secreted by the metanephrogenic mesenchyme stimulates the growth of the ureteric bud from this duct. At later stages, the GDNF receptor is found exclusively at the tips of the ureteric buds. (A-C from Pichel et al. 1996, photographs courtesy of J. G. Pichel and H. Sariola; D after Schuchardt et al. 1996.)

Further steps follow, i.e. the ureteric bud secretes FGF2 and BMP7 to prevent mesenchymal apoptosis and LIF from the ureteric bud induces the mesenchyme cells to aggregate and to secrete wnt4 (for further reading see <http://www.ncbi.nlm.nih.gov/books/NBK10089/>).





Outline of Lesson 5

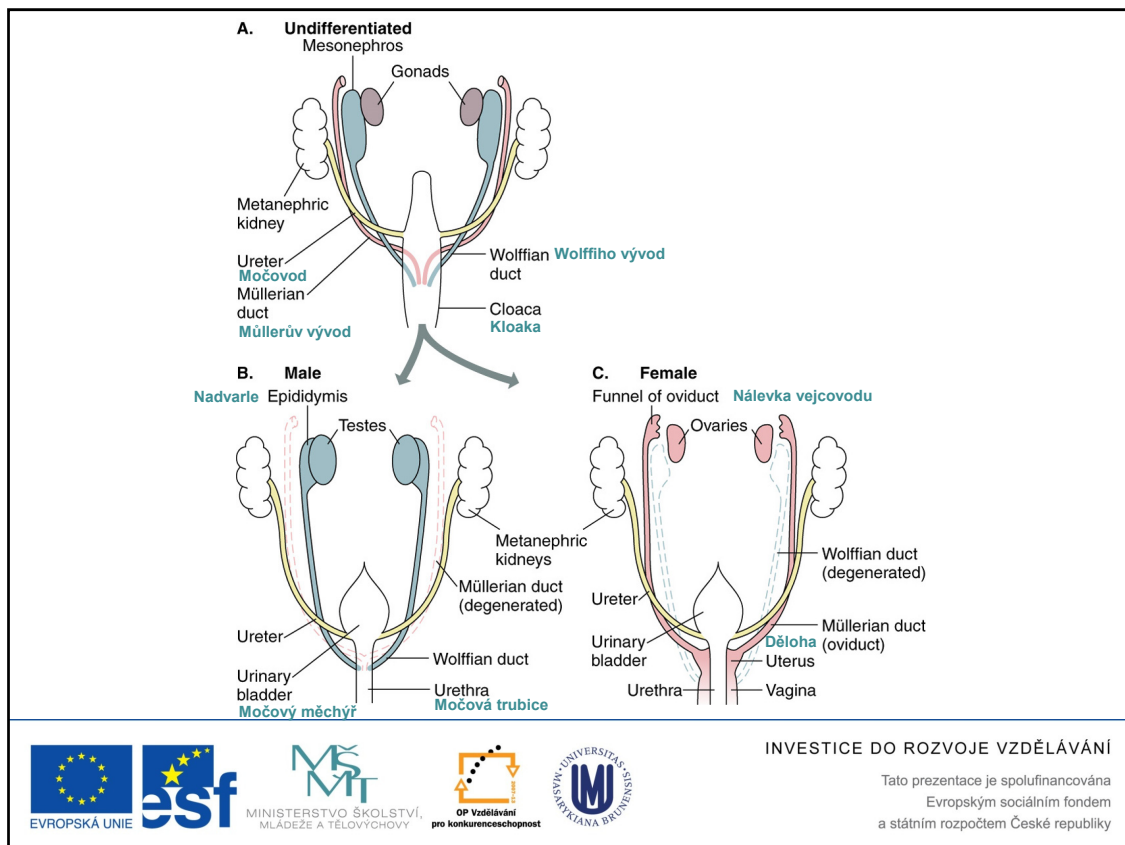
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis
 - formation of gonads



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

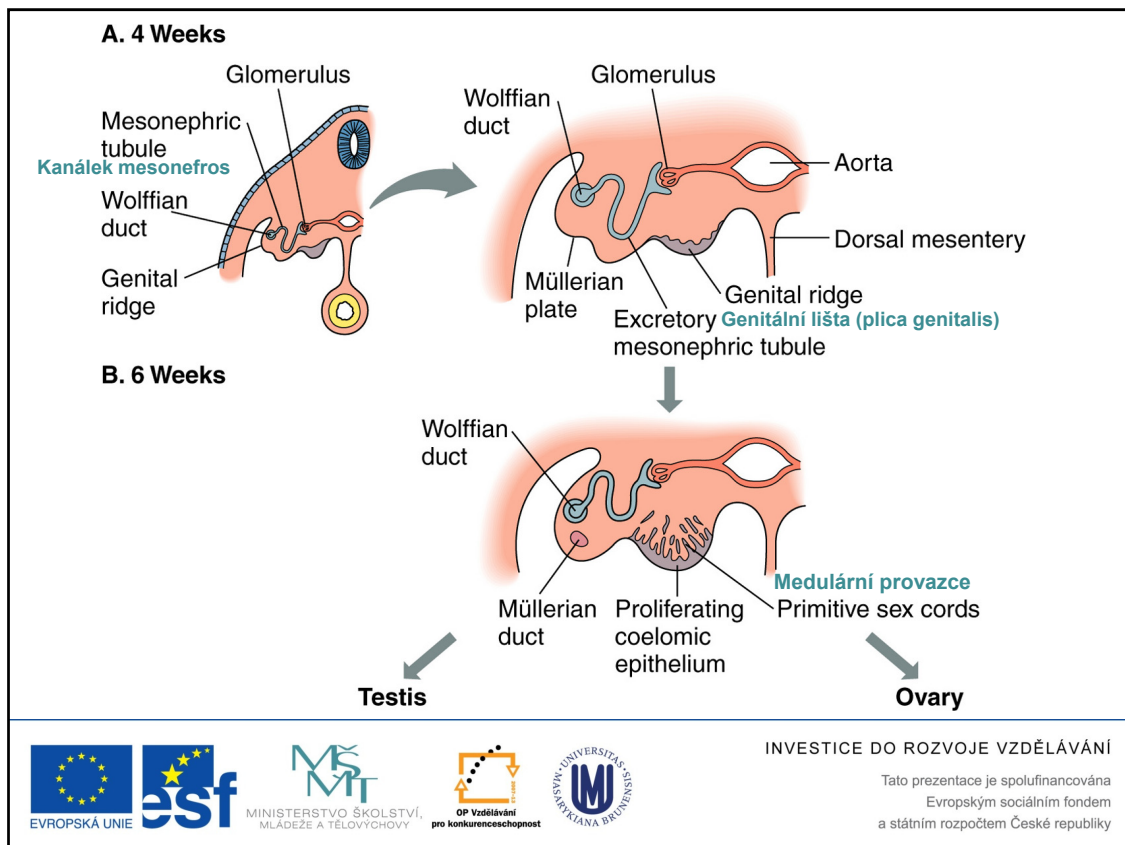
Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



In parallel with the Wolffian duct, another duct, what is called **Müllerian duct** is formed.

In males, the Müllerian duct degenerates due to anti-Müllerian duct factor production that induces apoptosis of its cells.

In females, the Müllerian duct persists to form the oviduct, the uterus and part of the cervix.

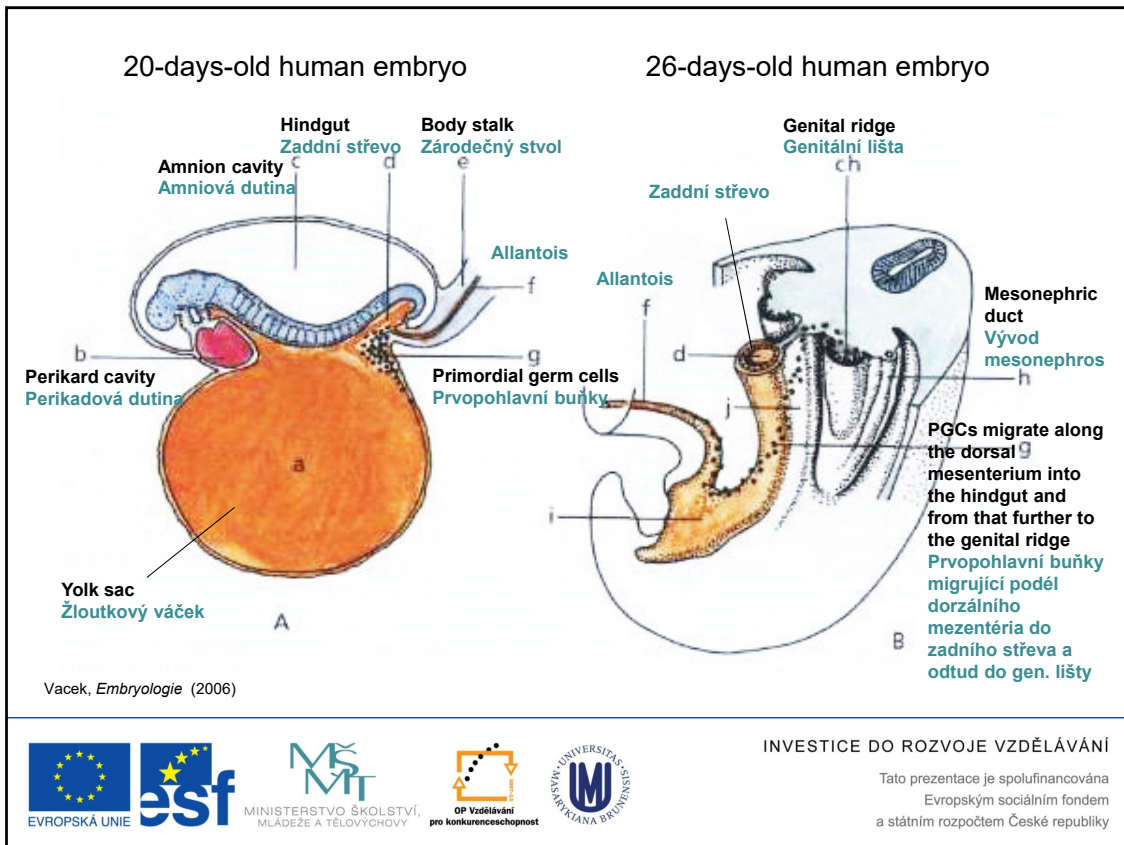


Gonads form from the so called genital ridge area.

The coelomic epithelium in the genital ridge region proliferates to form sex cords, which penetrate to the mesoderm and transiently associates with mesonephros and both Mullerian and Wolffian ducts.

At that time, the primordial germ cells (PGCs) migrate from elsewhere (in different vertebrates, the origin of the PGCs is different, e.g. in amphibians PGCs arise in the endoderm). In humans, PGS originate from the yolk sac (see the next slide).

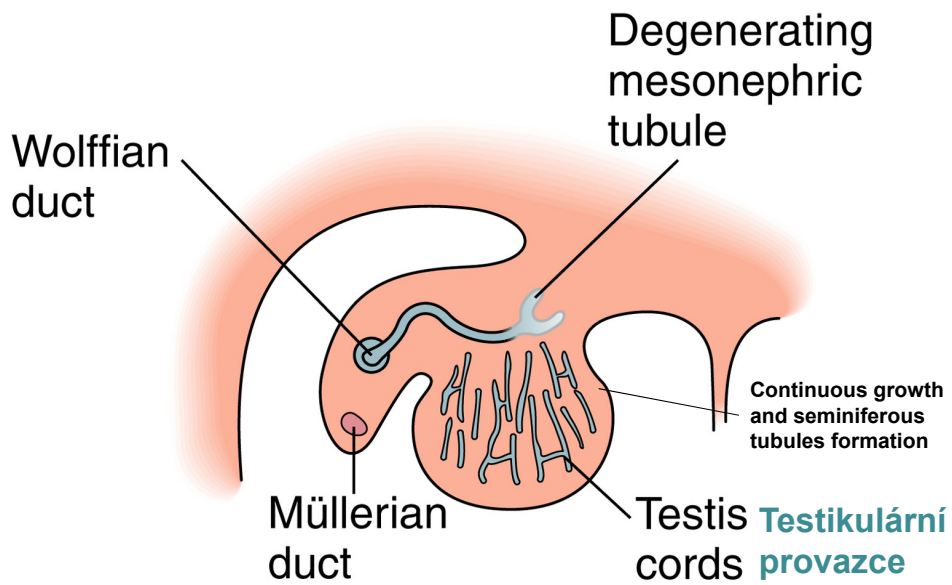
Upon the moment of primitive sex cord formation, the further development of males and females differs.



20 days old human embryo with PGC in the yolk sac (A, left).

Ventrolateral view into the trunk (coelom) cavity of the 3 mm large, 26 days old human embryo (9B, right), showing the PGCs migrating to the genital ridge.

C. 8 Weeks



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

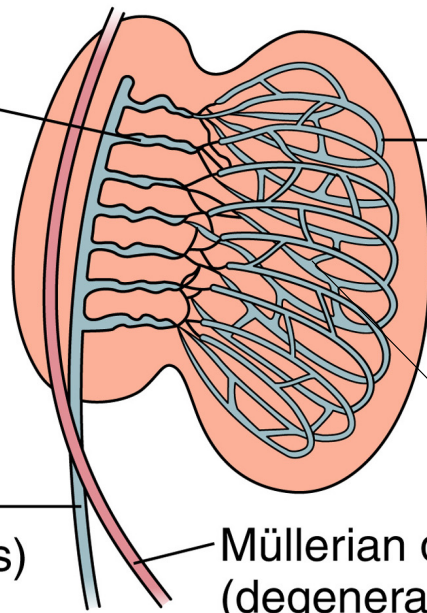
Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

In males, the coelomic epithelium (sex cords) continues proliferation, becoming the *seminiferous tubules*.

D. 16 Weeks

Excretory
mesonephric
tubules
(Efferent
ducts)
**Ductuli
efferentes**

Wolffian duct
(ductus deferens)



**Testikulární
provazce**

**Testis
cords**

**Produkce
Müllerovy inhibiční
látky**

**Anti-Müllerian
duct factor
production by
differentiated
Sertoli cells**

Müllerian duct
(degenerating)



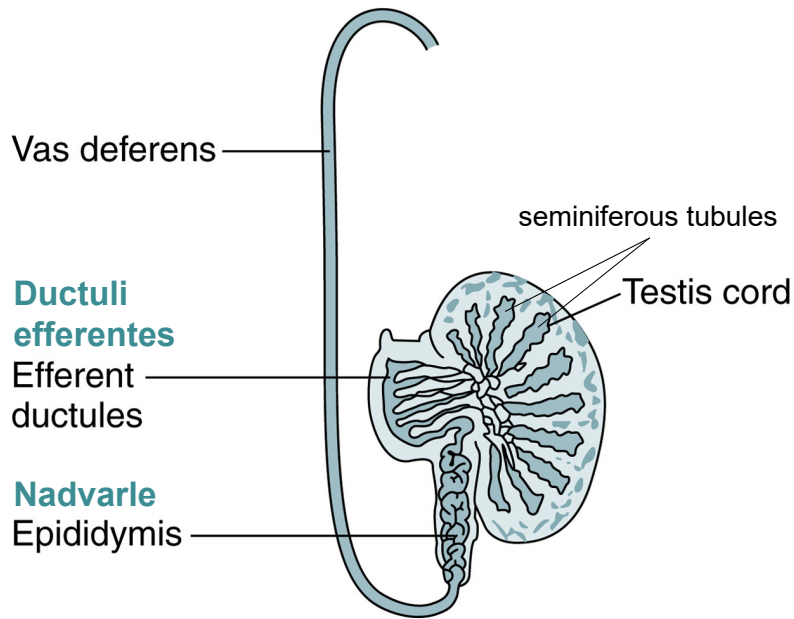
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

Sertoli cells differentiate in the tubules and secrete the anti-Müllerian duct factor.

PGCs come to inhabit the tubules (cords), forming thus spermatozoa, mesenchyme of the genital ridge becomes interstitial tissue of testis.

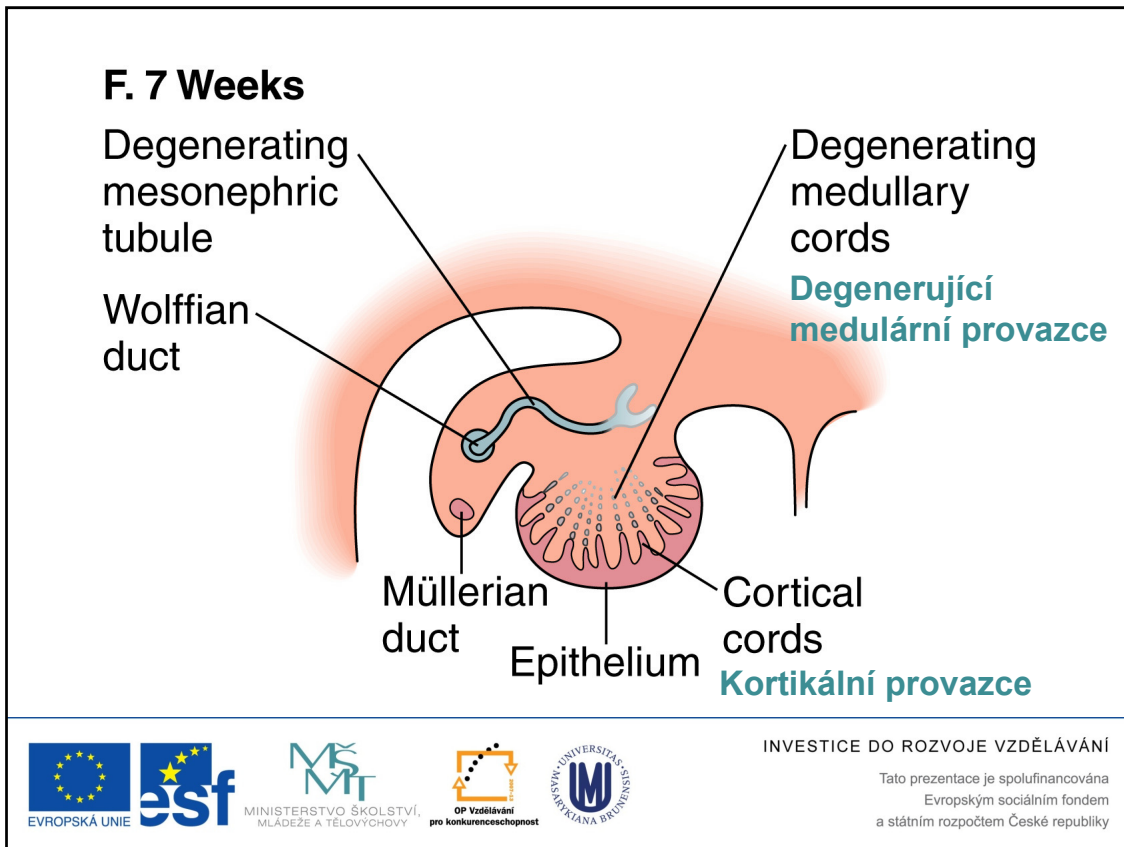
E. Adult



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

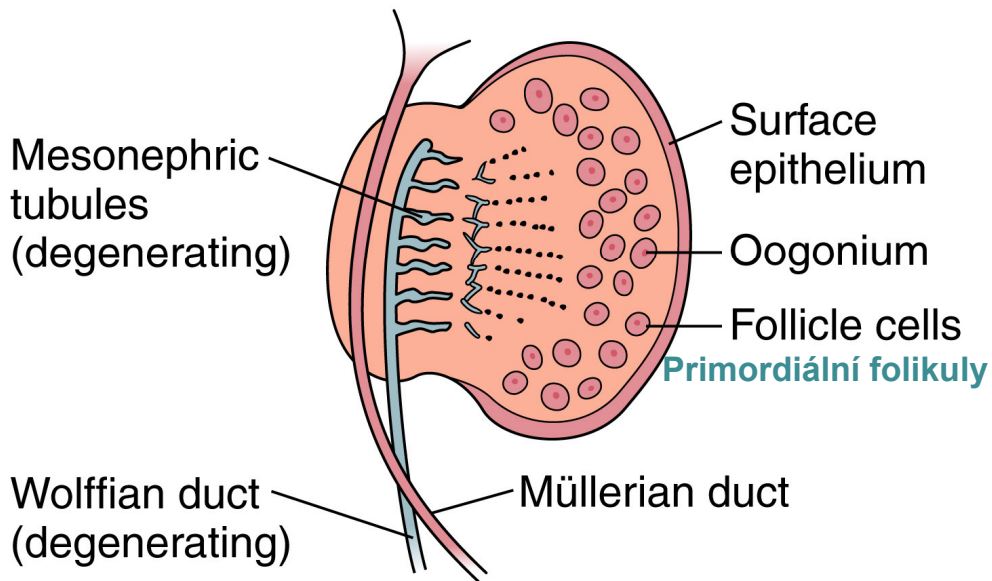
Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

The seminiferous tubules connect to the Wolfian duct, which becomes vas deferens of the male reproductive system.



In female genital ridge, the first epithelial cords degenerates and the secondary cords from the coelomic epithelium proliferate.

G. 21 Weeks



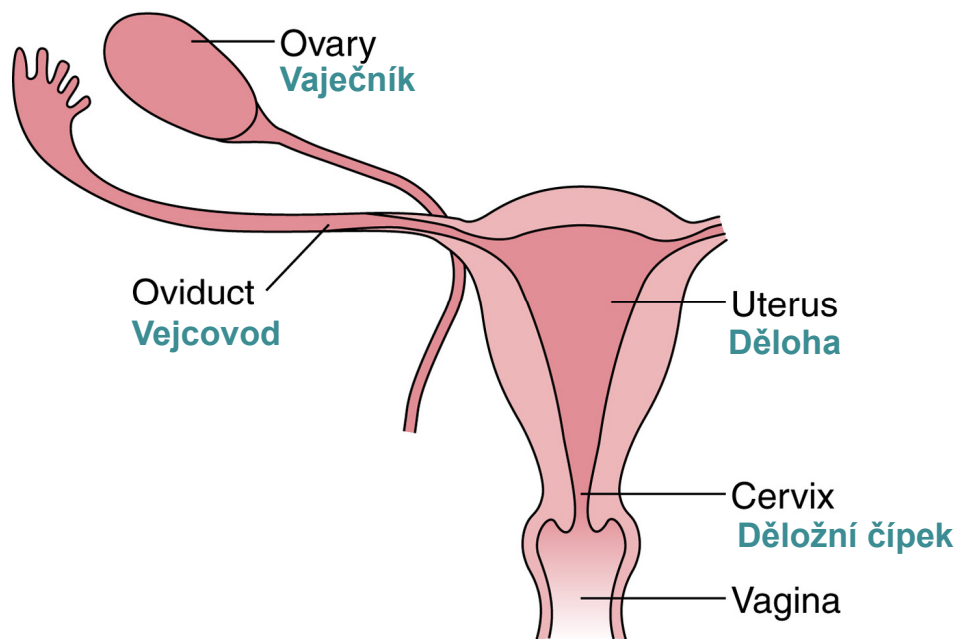
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

These secondary epithelial cords remain located near the surface and form the prospective follicle cells, in which the PGCs reside and form oocytes.

The Wolffian ducts degenerate while Müllerian ducts develop into oviducts.

H. Adult



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Outline of Lesson 5

Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis
 - formation of gonads
 - hematopoiesis and circular system development

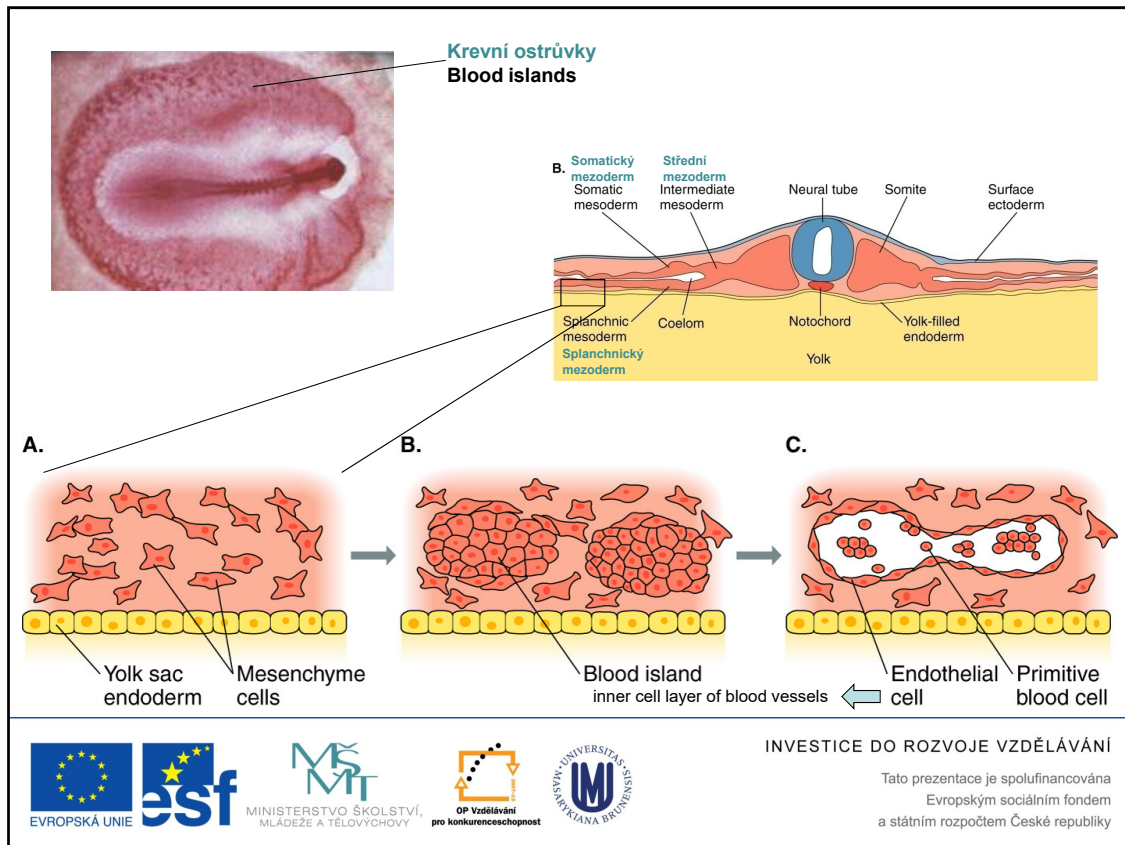


MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Hematopoiesis (blood formation) is a stepwise process. The hematopoiesis differs during development in terms of the location in the developing embryo and differs also among animal groups.

In birds and mammals, the earliest blood vessels and erythrocytes form in the splanchnic mesoderm, surrounding the yolk sac, thus outside the embryo proper. In the figure above (top left), there are shown blood islands forming in the splanchnopleura of chicken embryo.

Similarly to other mesodermal structures discussed previously, one of the first steps is mesodermal cell aggregation, leading to what is called *blood islands formation*.

In the centres of the blood islands, the cells differentiate into erythrocytes while the surrounding cells form *endothelium*, i.e. the inner cell layer of blood vessels. Note a certain parallel with osteocytes formation discussed before.

In embryonal mesoderm, similar process occurs leading to the formation of endothelial tubes arising from mesodermal aggregations.

The larger tubes, prospective arterioles and venules-will be surrounded by smooth muscle layers.

During early development most of the erythrocytes originates in the yolk sac while later on, the endothelial lining of the dorsal aorta and nearby blood vessels will proliferate, to provide primitive additional erythrocytes and stem cells.

The location of hematopoiesis changes further during development: The stem cells formed from the lining of dorsal aorta populate liver, which is the place of blood formation of fetus until late in gestation. The final place of erythropoiesis of adults becomes bone marrow populated by the stem cells. Lymphocytes appear in the second half of the embryonic development of mammals.

The quality of the hemoglobin also changes during development (see next slide).

Important process of vessel system development represents the angiogenic sprouting, i.e. formation of new vessels.

During embryogenesis, this process occurs via so called **vasculogenesis**, where most of the blood vessels develop by *in situ* formation of endothelial tubes.

Some of the vessels evolve thanks to invagination of existing endothelium into surrounding tissue, a kind of invasion process called **angiogenesis**.

The process of angiogenesis is very important also in the adult age, where new vessels are formed e.g. after injury or tumors vascularization. Vascular endothelial growth factor (VEGF) and angiopoietin are recently known factors involved in angiogenesis.

Interestingly, arteries, but not veins, in embryo often grow along nerves paths. Recent experiments show that nerves or surrounding Schwann cells stimulate the growth of blood vessels and their differentiation into arteries.

Embryonic hemoglobin $\epsilon\epsilon\zeta\zeta$
yolk sac

Fetal hemoglobin $\alpha\alpha\gamma\gamma$
liver

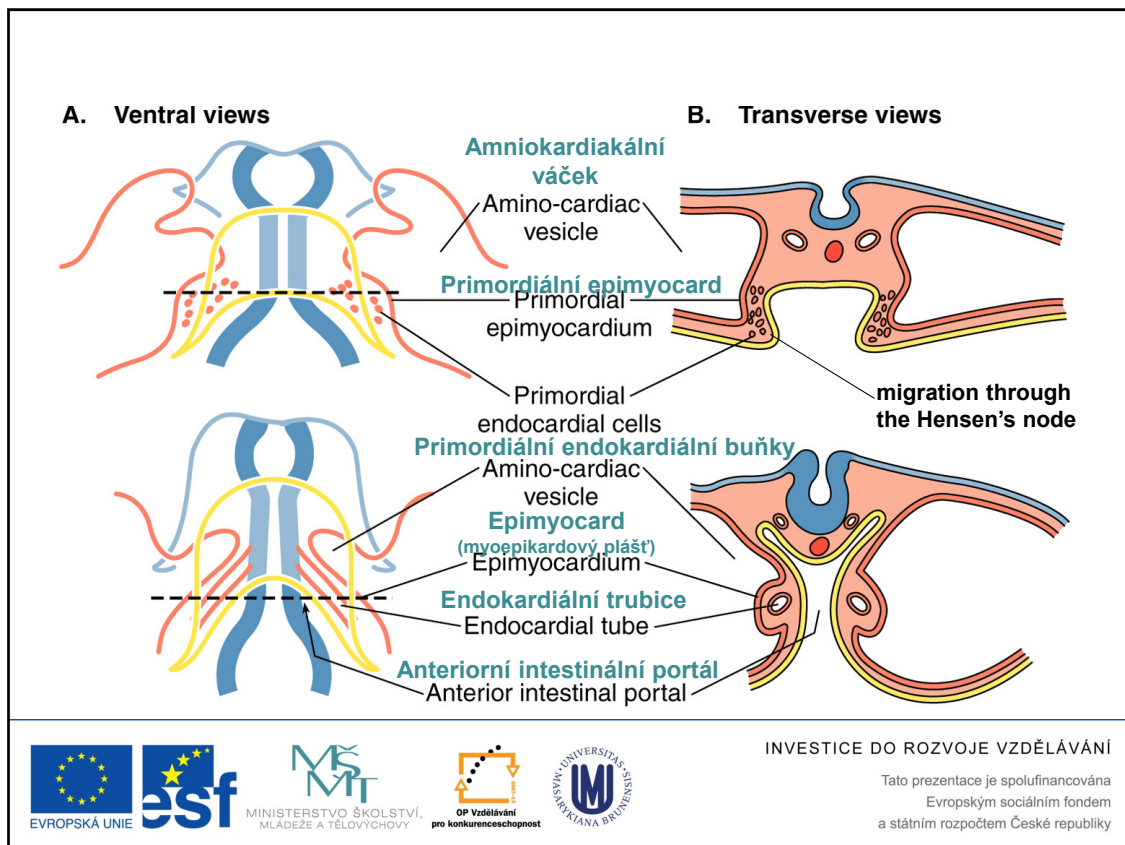
Adult hemoglobin $\alpha\alpha\beta\beta$
bone marrow

EVROPSKÁ UNIE INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

The yolk sac erythrocytes produce so called embryonic form of α and β globin, called ζ (zeta) and ϵ (epsilon), while later erythrocytes from the embryonic liver contain adult α chains but fetal form of β globin, called γ (gamma).

Finally, the bone marrow produces $\alpha\alpha\beta\beta$ tetramer of globins, forming the peptide portion of the hemoglobin in adults.



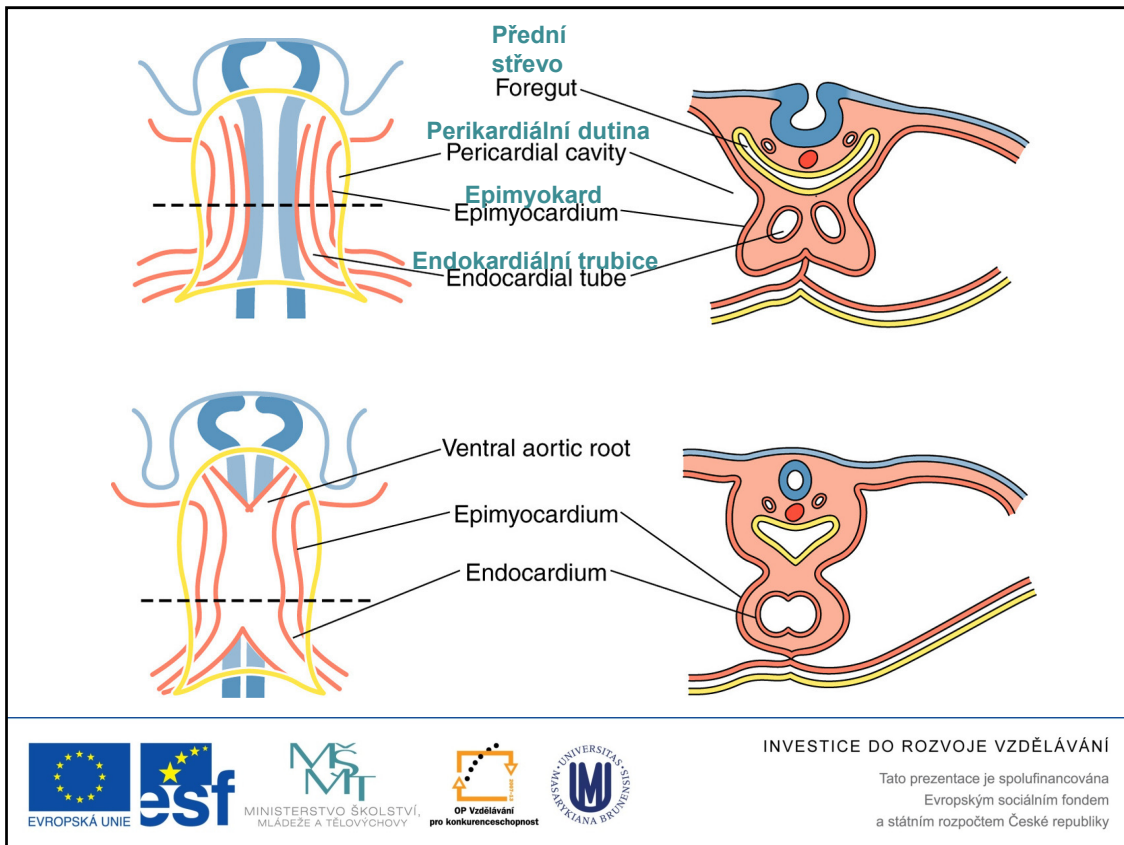
Cells forming the heart migrate through the Hansen's node and it is not clear up to know, whether they aggregate in the future heart or if they just differentiate there.

Heart forms as an endothelium in the splanchnic layer of the anterior ventral mesoderm. Here, the islands of mesodermal cells form endothelium that develops into a specialized muscular layer called *myocardium*.

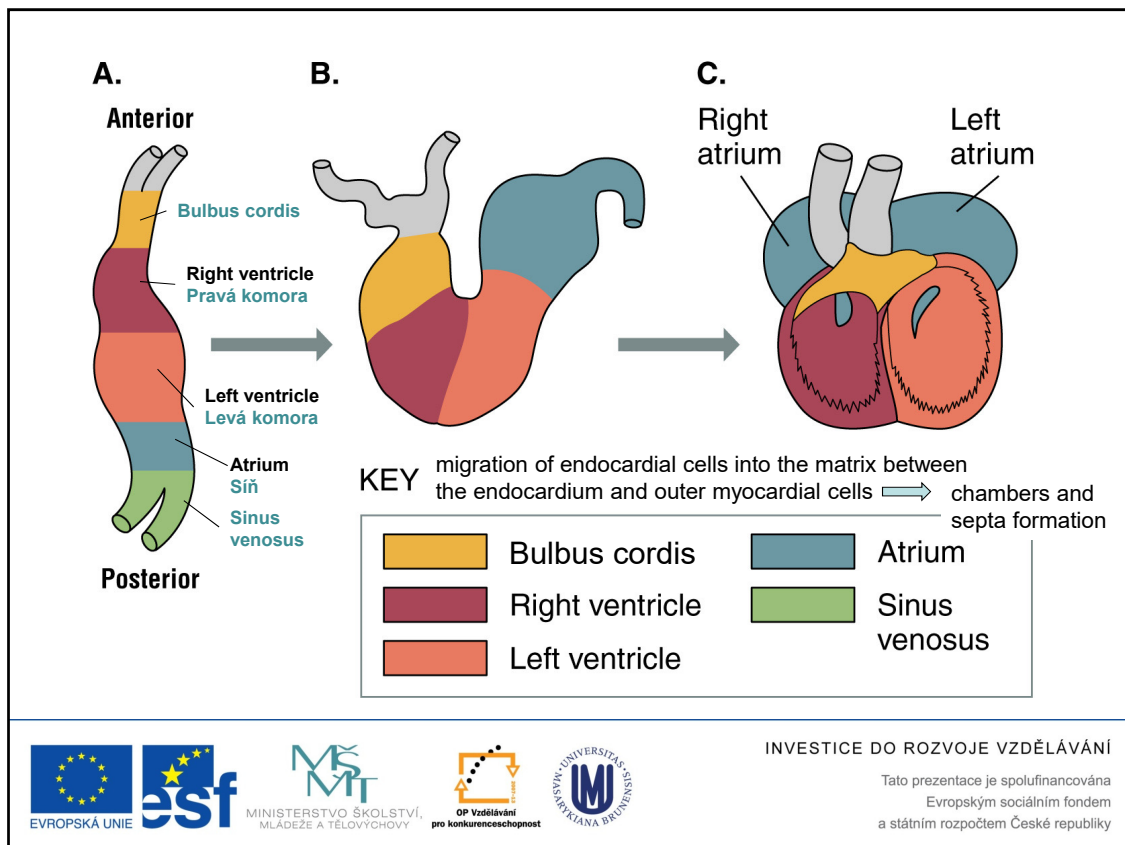
Myocardium develops from the *epimyocardium*, an undifferentiated splanchnic mesodermal layer of the embryonic heart that subsequently differentiates into *myocardium* and *epicardium*.

The muscle fibres of myocardium undergo unique developmental pathway and the tube forms heart.

In the figures, the ventral view is on the left-hand side (A) while cross section is on the right-hand side (B).



In amniotes, heart develops originally as a pair of endocardial tubes that fuse at the end, forming the single tube.



The heart starts functioning as a single tube, with blood entering posteriorly through the *sinus venosus*, then moving through the *atrium* and *ventricle* and finally passing anteriorly out from the *bulbus cordis*.

As the heart develops, it undergoes a series of turning, forming a large loop to the right side and then curls over itself, leading to the final shaping of the complex heart structure.

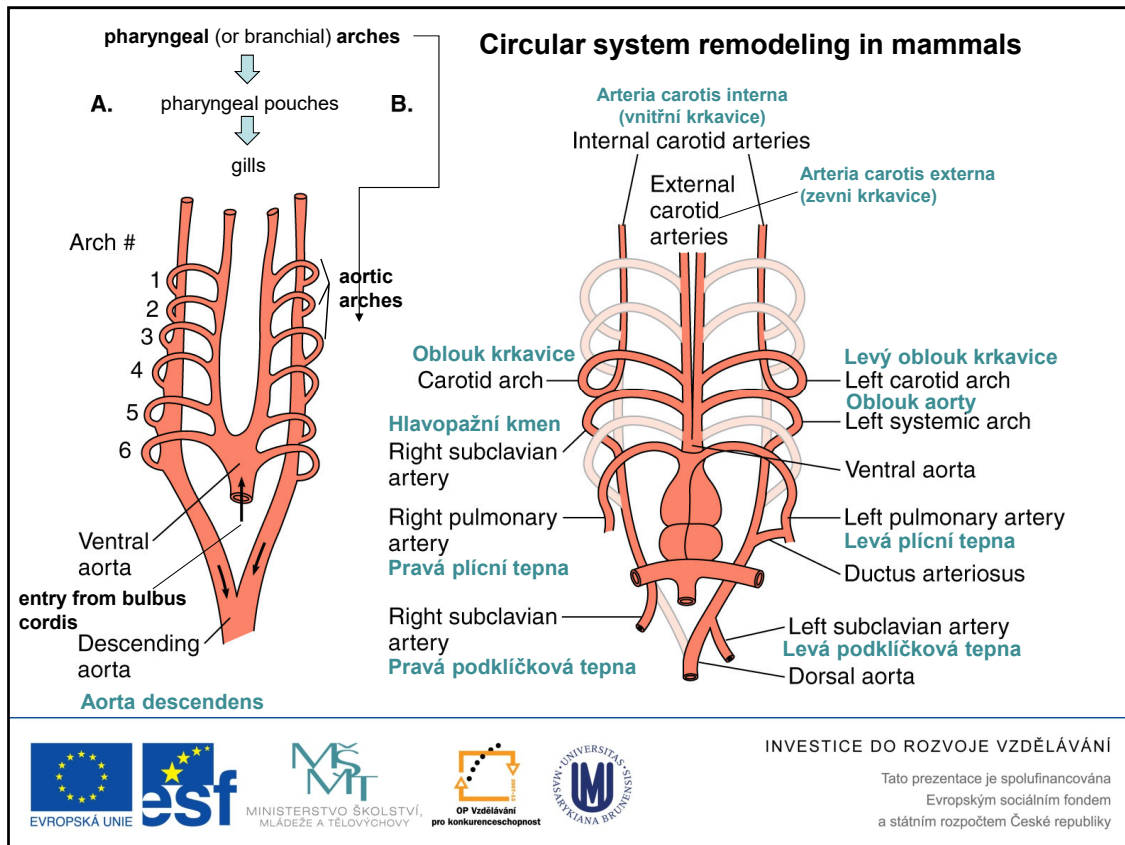
Thus, what was originally anterior ventricular portions, turns to be posterior.

Internal heart structures (chambers and septa) develop via migration of endocardial cells into the matrix that lies between the endocardium and outer myocardial cells.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



Early development of the vertebrate vessel system is common, leading to the basic plumbing, which, however, is remodeled during later development specifically in respective group of vertebrates.

In the region of prospective head and neck, the ridges form that are called **pharyngeal (or branchial) arches**. Between the arches, the ectoderm and endoderm are closely apposed and ectoderm invaginates forming thus **pharyngeal pouches**. In aquatic, nonamniote vertebrates these structures perforate and develop into gills.

Within the mesoderm of pharyngeal arches will develop vessels forming six pairs of *aortic arches*. These arches form in aquatic nonamniote vertebrates the capillary bed of the gills.

In the original anatomical arrangement, all blood is pumped out of the heart via bulbus cordis that connects to the ventral aorta. From there, the blood goes via aortic arches back to the descending aorta (see the figure A).

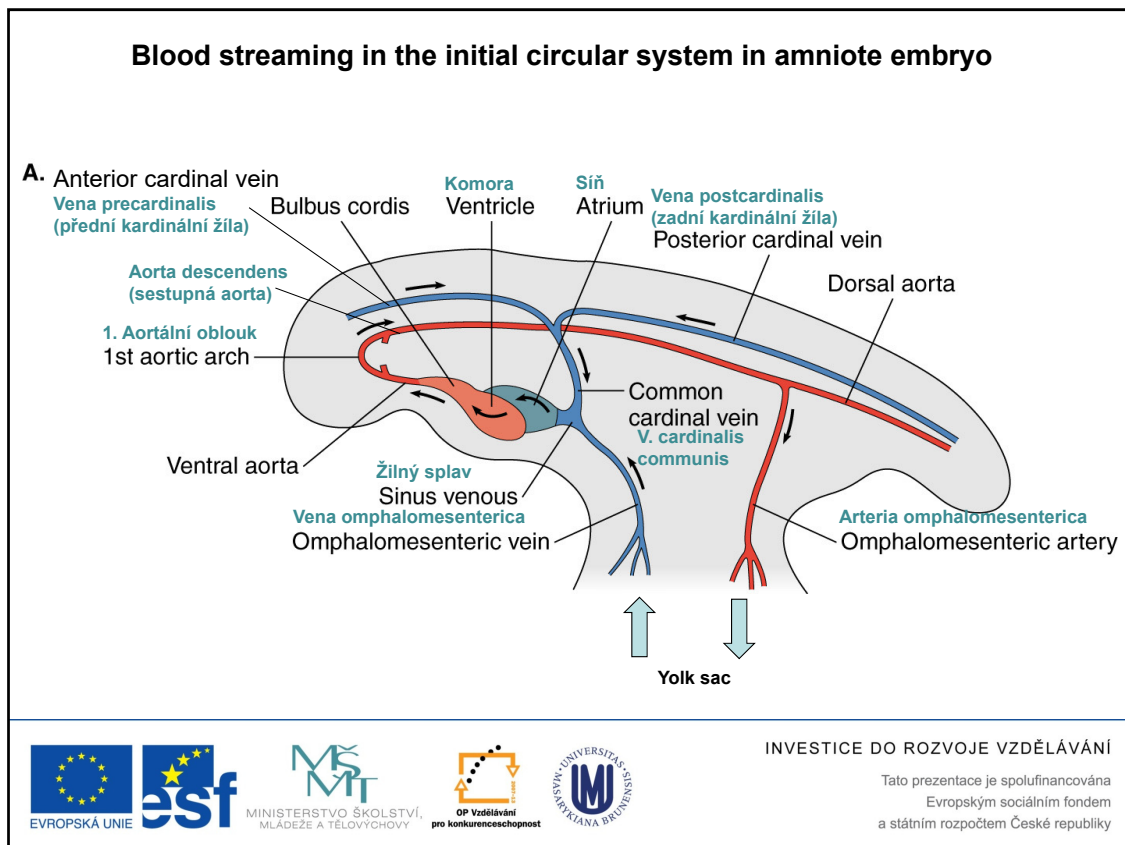
In amniotes, the above described anatomical arrangement gets extensively remodeled: Some of the aortic arches atrophy while some develop into major arteries of the head and neck.

In mammals, the first two and the fifth arch atrophy.

The **third arch** together with anterior part of the ventral aorta develops into **internal and external carotid arteries**, the major arteries of the head.

The **fourth arch** forms **dorsal aorta**, the major source of the blood in the posterior portion of the embryo. From the dorsal aorta, several branches develop that supply kidney, digestive tract, yolk sac and so on. Also the **subclavian artery** develops from the right fourth arch that supplies blood to the anterior limbs.

The **sixth arch** gives rise to **pulmonary arteries** to the lungs.

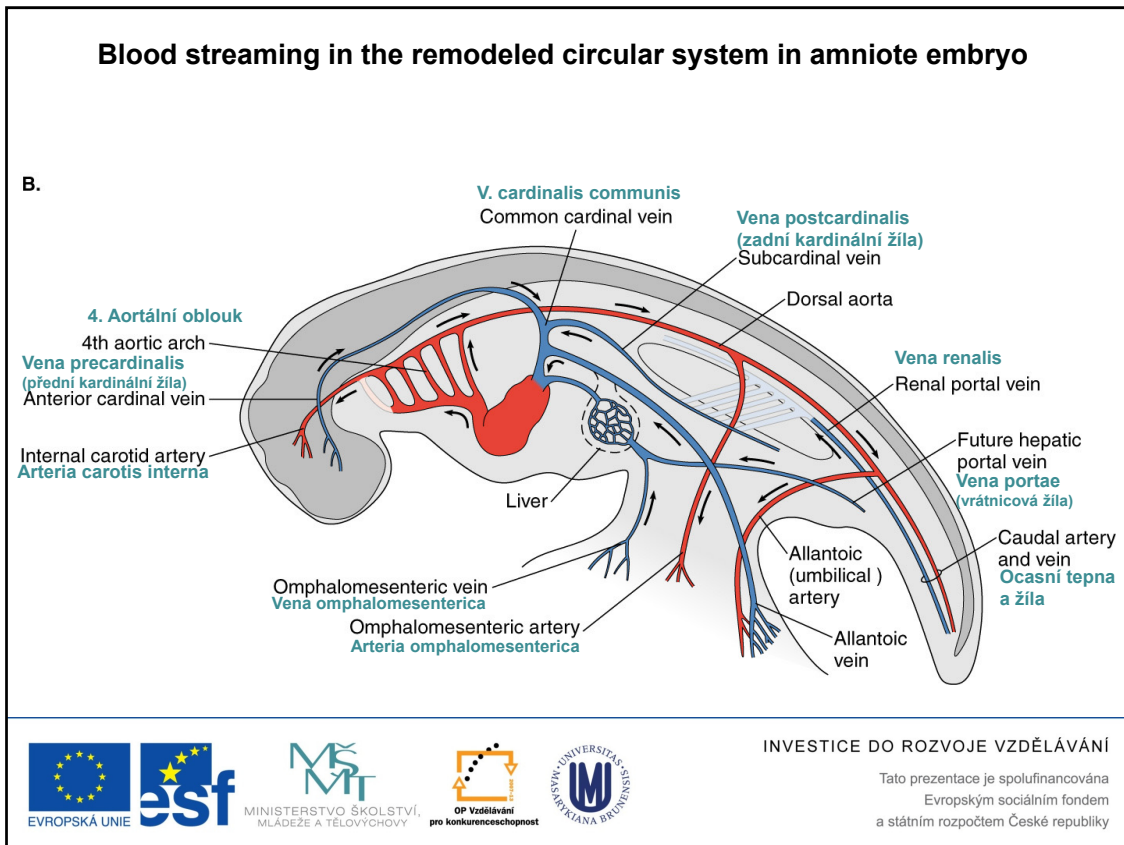


Streaming of the blood in the initial system of stylized amniote vertebrates embryo.

Blood is collected from capillary beds in all nascent tissues, empties into the **common cardinal vein** and then into the entry chamber of the heart, the **sinus venosus**.

Omphalomesenteric vein collects the blood from the yolk sac, while **omphalomesenteric artery** brings the blood there.

Then the blood enters chambers of the atrium, passes to the ventricle and moves out through the bulbus cordis once again.



In the later remodeled system, the venous blood gets diverted through the liver on its way to the common cardiac vein and back to the heart.

Extensive remodeling occurs including dramatic changes at the birth, when the lungs start to respire and the yolk sac or placenta are out of function.



Outline of Lesson 5

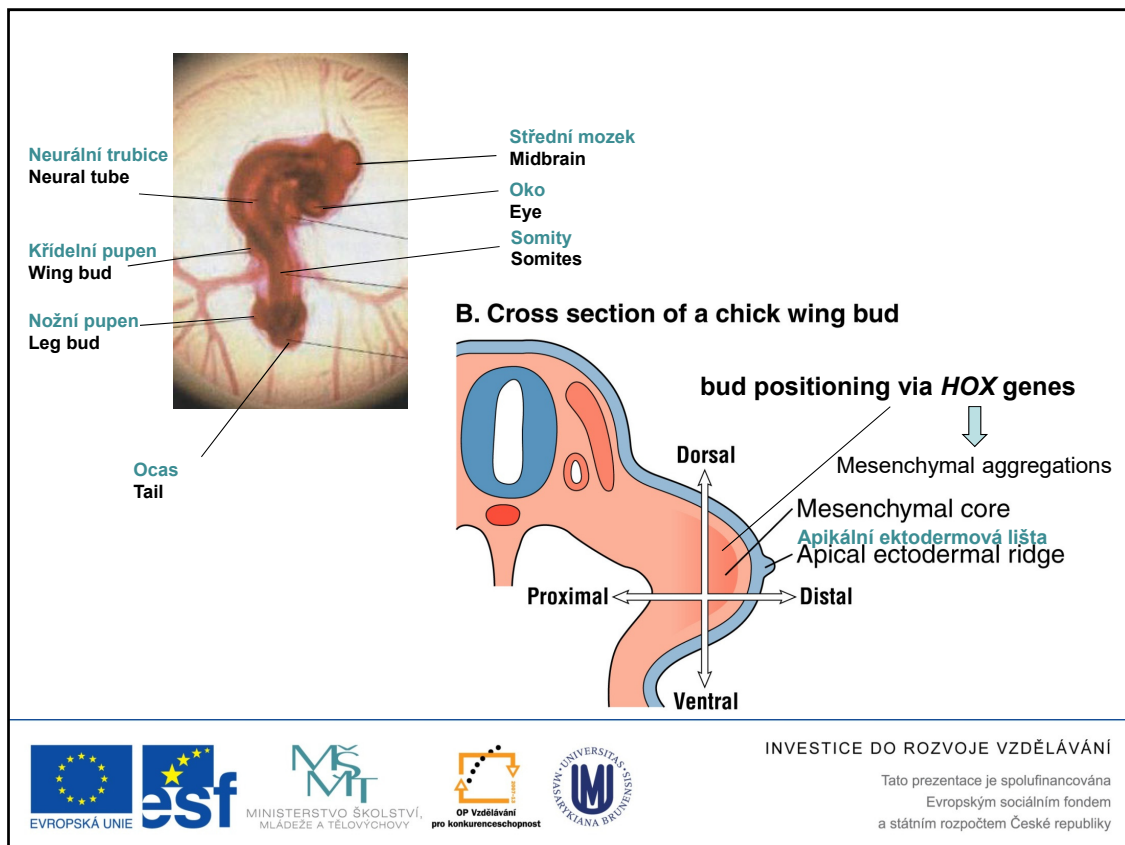
Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis
 - formation of gonads
 - hematopoiesis and circular system development
 - limbs formation



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

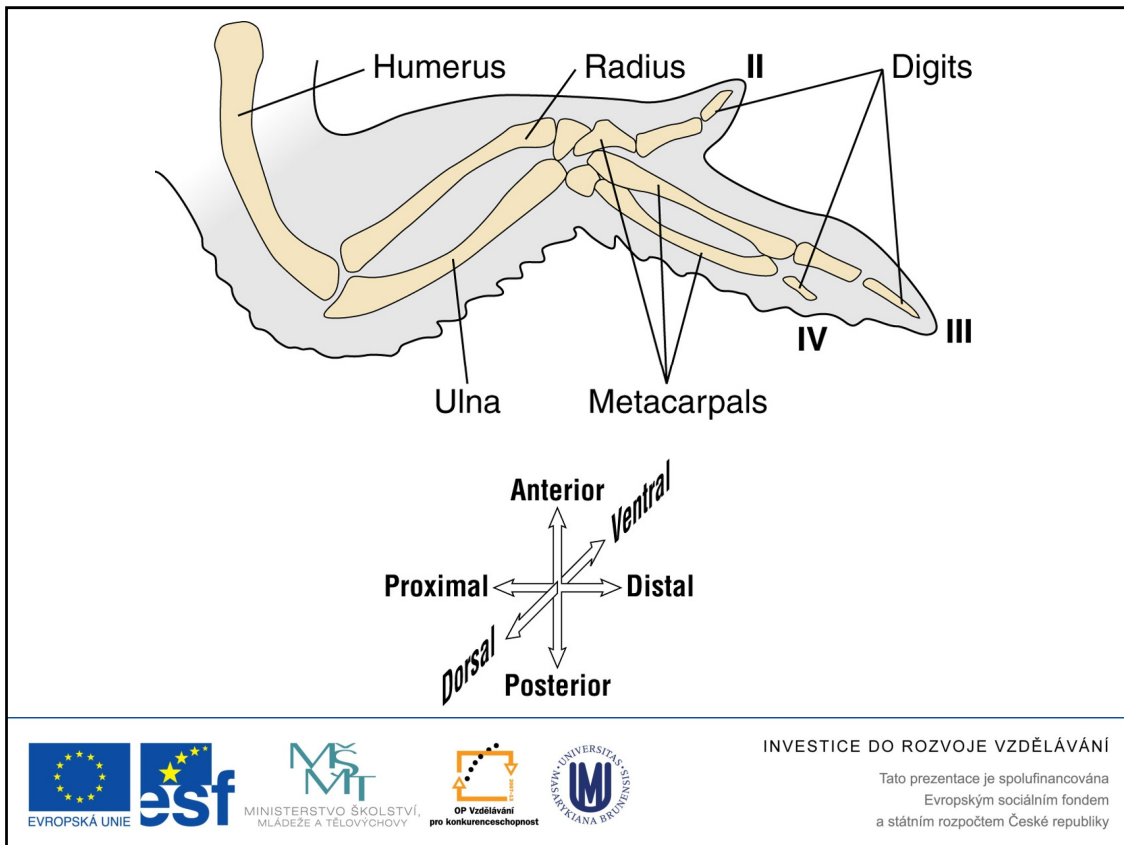


Limbs in vertebrates undergo specific developmental modifications, dependent on the final limb type, e.g. wings (different in birds and mammals), fins, flippers, hooves etc..

Limbs develop from the somatic mesoderm, where the what is called *limb buds* develop, both anteriorly and posteriorly. The positioning of the limb buds is determined by *HOX* genes (will be discussed later).

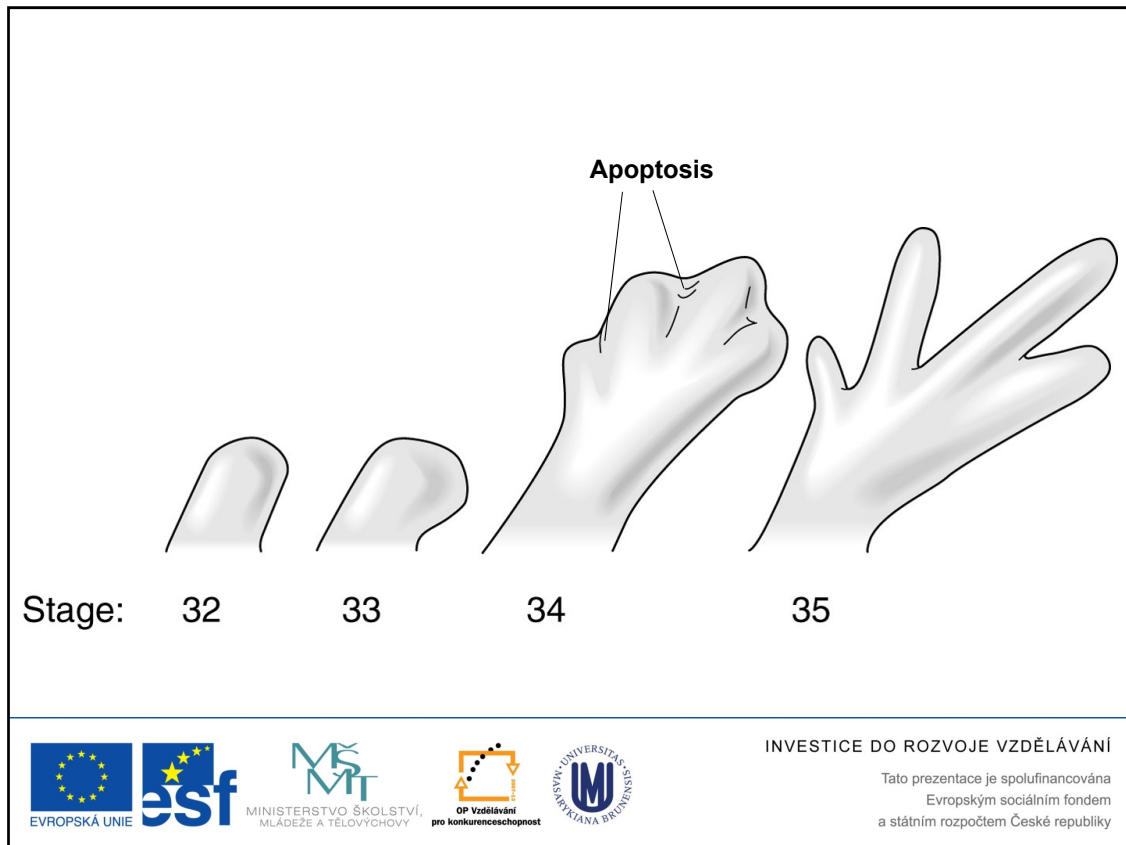
One of the most important structures that determines the proximodistal axis patterning of the developing limb is so called **apical ectodermal ridge (AER)**. The role of AER will be discussed later.

As the bud grows, again, the mesenchymal aggregations that will be a precursors of the cartilage appear. Ectoderm and mesoderm will differentiate into skin and surface structures (e.g. feathers or hairs).

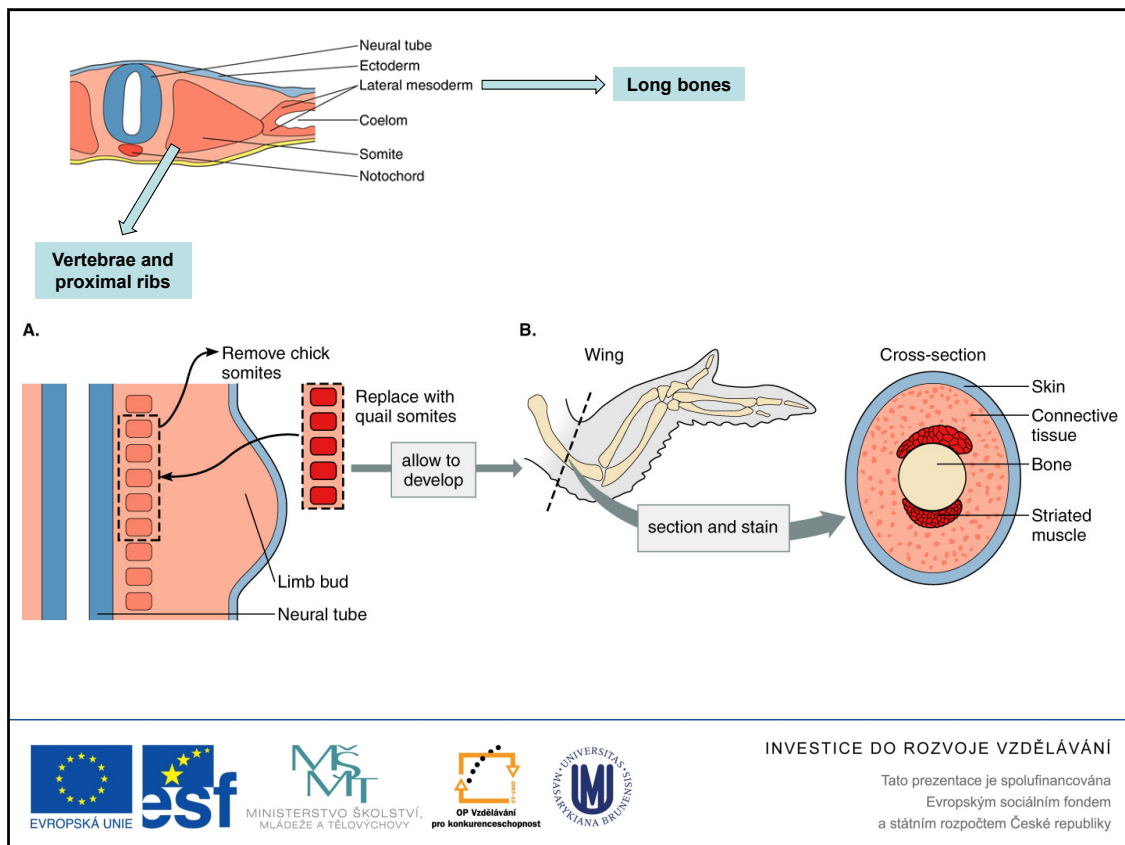


Limbs show complex organization in all (i.e. proximodistal, anteroposterior and dorsoventral axes).

As an example, the major skeletal elements of the chick embryo is shown here.



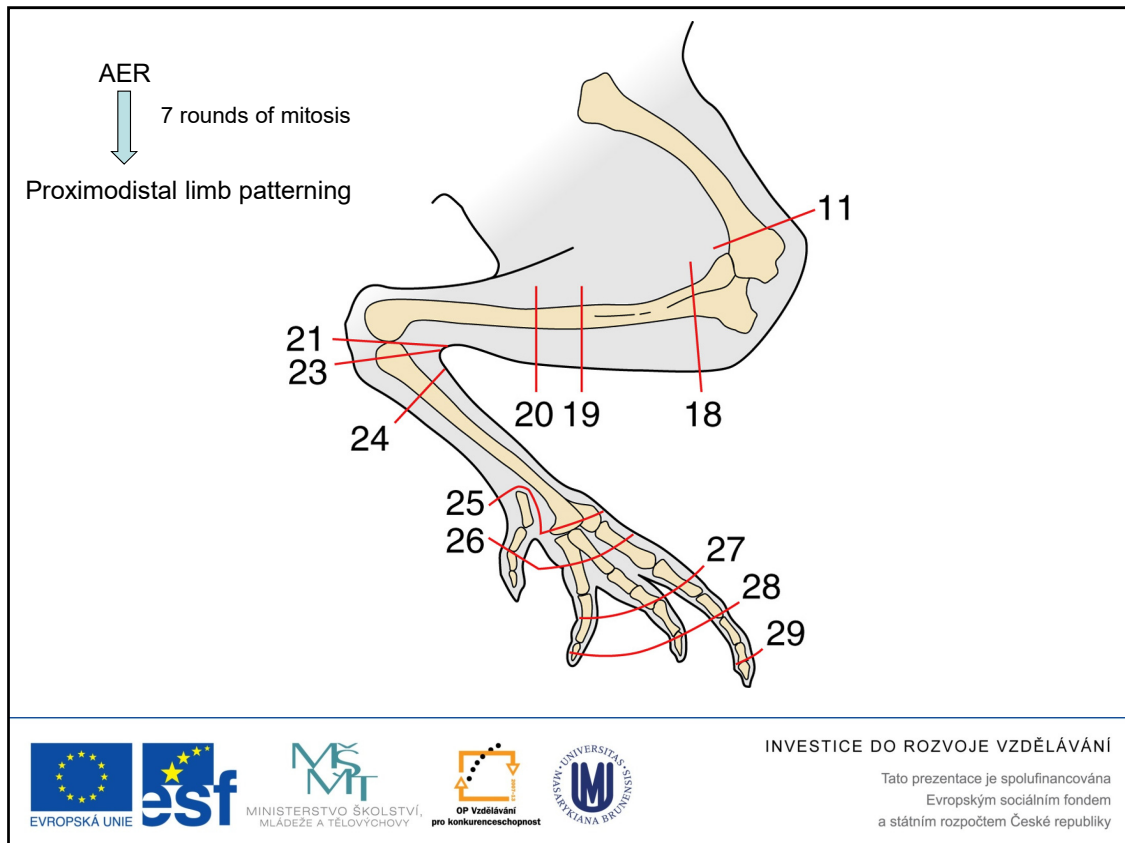
The tissue between the developing skeletal elements of digits might persist to form webbing or undergoes apoptosis. The figure shows developmental stages 32-35 (of chicken embryo, see slide 57), which correspond to 7.5-8.5 days of incubation.



The transplantation experiments using Japanese quail and chicken embryos have provided important experimental evidence about the origin of cells involved in the limb formation.

The nuclei of quail embryo might be easily distinguished by specific staining—they stain more intensely.

These experiments have shown that not all cells in the limb originate from lateral (somatic) mesoderm. When the chicken somites 15-20 were replaced with quail ones, the striated muscles of developed limbs had muscle cells that originated from quail somites.



As mentioned previously, AER plays a very important role in the proximodistal patterning of the developing limbs. The proximodistal pattern arise in the mitotically active region below the AER.

7 rounds of mitosis leading to the formation of successive cell layers is necessary to complete the final proximodistal limb patterning. The later the AER is removed before the process is completed, limbs that are defective in the more distal limb structures will develop.

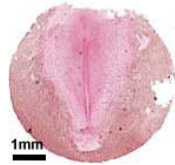
Numbers in the figure indicate the stage of development, when AER was removed and the lines depict the level of limb truncation.

Besides AER, there are two additional important organizing centers. These are i) the surrounding ectoderm and ii) the what is called **zone of polarizing activity (ZPA)**.

ZPA is located in the posterior mesoderm of the bud and its role will be discussed later.

Chicken embryo as a developmental model

Hamburger & Hamilton Stage 4 (15 hours)



HH Stage 9 (31 hours 8 somite)



HH Stage 10 (33 hours)



HH Stage 16-17 (56 hours)



HH Stage 38 (12 days)



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

Stages of chick development according to Hamburger & Hamilton, Series of Embryonic Chicken Growth. *J. Morphology*, 88 49 - 92 (1951).

The Hamburger and Hamilton dissected chicken development according to different morphologically identifiable traits.



Outline of Lesson 5

Organogenesis in Vertebrates: Endo- and Mesodermal Derivatives

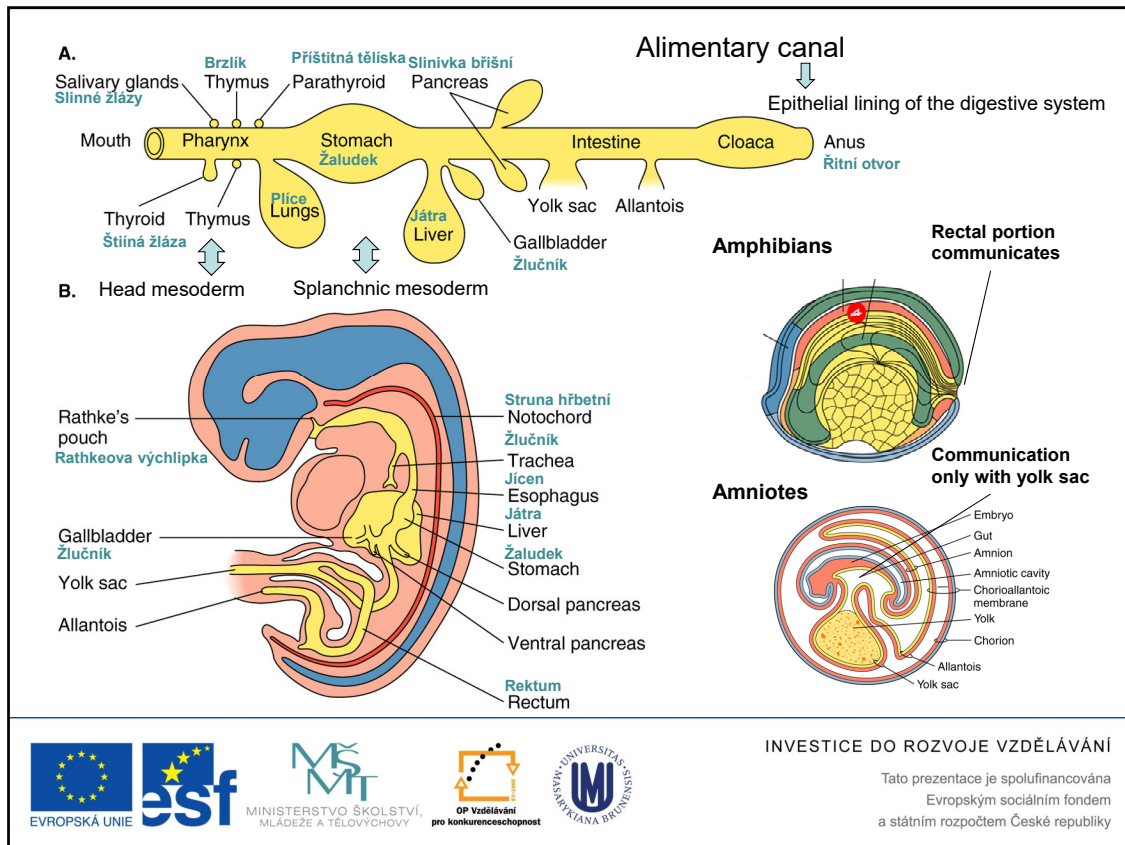
- Mesoderm derivatives development
 - somites formation and signalling
 - formation of muscles
 - endochordal ossification and signalling
 - nephrogenesis
 - formation of gonads
 - hematopoiesis and circular system development
 - limbs formation

- Endoderm derivatives development
 - alimentary canal and its derivatives formation



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky



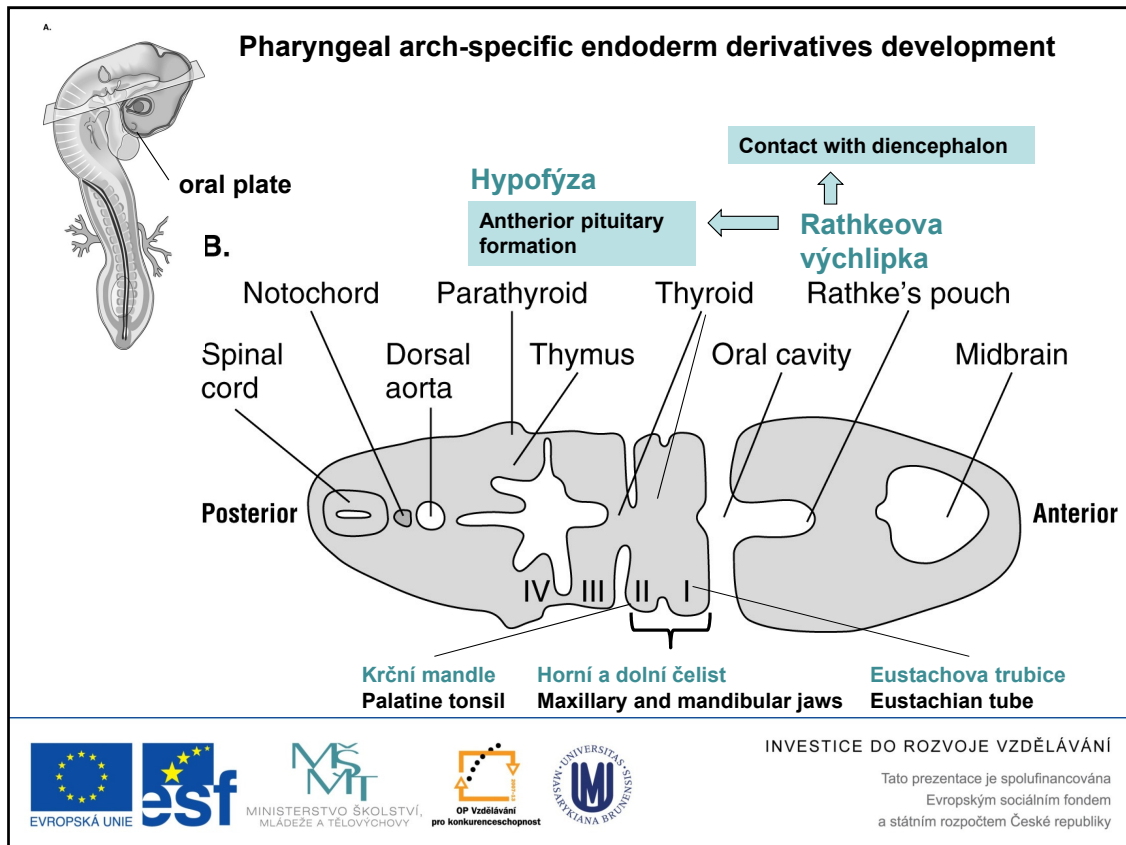
Endoderm forms what is called *alimentary canal*, i.e. the epithelial lining of the digestive system.

Development of the connection of the digestive system to the surrounding environment is specific for respective vertebrates groups.

E.g. in amphibians, the rectal portion of the digestive tube communicates with the outside, while the oral opening forms later. In comparison, amniotes have both ends blind with the midgut connected with the yolk sac and both cloacal and oral opening form later.

The epithelium lining in concert with local surrounding of mesenchyme forms outpocketings from the alimentary canal that develop into secretory and endocrine glands of the head and neck, lungs, liver, gallbladder, and the pancreas.

It is surrounded by the mesoderm – head mesoderm anteriorly, splanchnic mesoderm in the rest of the body. Reciprocal interactions between the surrounding mesoderm and endoderm participate in the final development of all of the endodermal structures.



As mentioned previously, all vertebrates form the so called arches in the wall of pharynx.

Endoderm will develop into many important structures here in an arch-specific manner.

At the anterior end, the archenteron fuses with ectoderm and forms oral plate that later perforates and forms mouth.

Posterior to the oral plate is an inpocketing of ectoderm called Rathke's pouch, which later contacts the diencephalon. Diencephalon induces Rathke's pouch to form the anterior pituitary.

In the region of individual arches, the endoderm cells associates with arches, proliferates and invaginate in association with the adjacent mesenchyme.

In aquatic vertebrates, gills develop from the arches and gill slits from the individual clefts of the pharynx.

However, in terrestrial vertebrates, the arches are only transient structures. However, the pouches between the arches give rise to the set of important organs.

Pouch I forms the Eustachian tube.

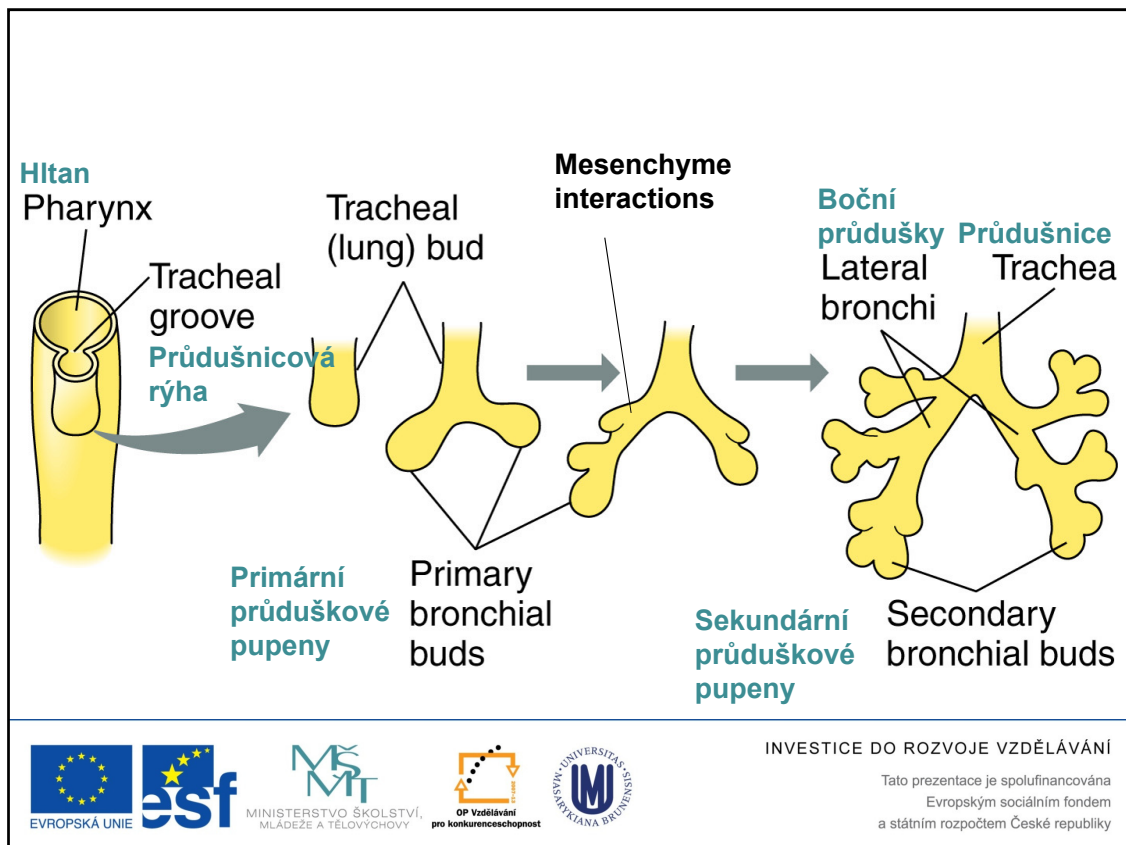
Pouch II the palatine tonsil.

The floor area of pouch I and II develop into the thyroid.

Pouches III and IV give rise to portions of the thymus and parathyroid gland.

Salivary glands develop also from pharyngeal endoderm. Interaction of the salivary endoderm and surrounding salivary mesenchyme results into complicated branching of the salivary glands.

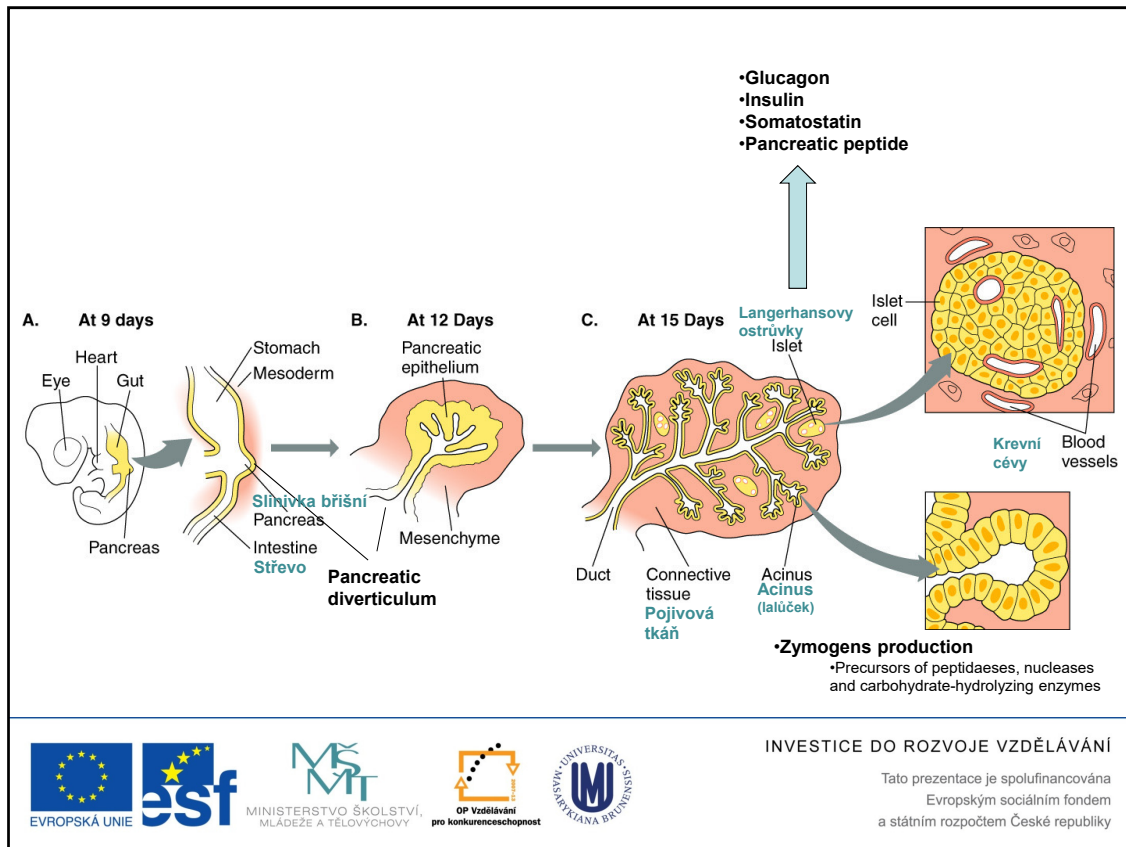
In a position of branchial arches I and II upper (maxillary) and lower (mandibular) jaws are formed. Teeth formation is a result of mutual interactions between the mesenchyme and epithelium. Teeth are of ectodermal origin (progeny of neural crest cells).



Lungs develop as an invagination that forms from the alimentary canal just posteriorly from the pharynx.

Trachea develops from the pharynx evagination. Trachea then further branches and forms bronchi and alveoli at their termini.

Again, similarly to the salivary glands branching, interaction of the endodermal epithelium with the surrounding mesenchyme drives the branching and some of the genes involved in the process were recently identified.



Posterior to the esophagus, the endodermal epithelium develops into specialized cell types that will form stomach.

Endodermal outpocketings of the epithelium in this region will result into liver, gallbladder and pancreas formation.

These organs start to develop via evaginations into surrounding mesodermal mesenchyme, called *diverticula*.

Liver start to develop as a *hepatic diverticulum*, that proliferates, branch and differentiates as a glandular part of the future liver. Its base forms hepatic duct and branch forms gallbladder.

Pancreas develops posteriorly from the dorsal and ventral pancreatic diverticula, which introgress into the mesodermal mesenchyme and again interact with it.

The pancreatic diverticula branch and form blind pockets, called *pancreatic acini*. Acini will differentiate into exocrine cells that will produce zymogens, i.e. precursors of peptidases, nucleases and carbohydrate-hydrolyzing enzymes.

Some of the epithelial endodermal cells aggregate and develop into endocrine *islets of Langerhans cells*. These islets contain at least four cell types and each of them will produce different pancreatic hormones: glucagon, insulin, somatostatin or pancreatic peptide.



Key Concepts

- **Developmental potential** is studied via e.g. **transplantation experiments**, while **developmental fate** is best studied using modern **molecular techniques** in a normal embryo.
- Vertebrate embryos have a **conservative axial organization** proceeding from the midline to the periphery: **notochord**, **somite**, **nephrotome**, or **gonad** and **lateral mesoderm** (limb buds).
- **Iterative oscillations** of the gene expression in a combination with **gradient of gene expression** allow **proper body segmentation** during embryonic development. **Underlying molecular mechanisms** are at least **partially conserved** in *Drosophila* and vertebrates.
- Normal tissue and organ formation depends not only on the **presence of signalling molecules**, but also on the **appropriate timing of ligand-receptor interactions**. Such communications can establish **feed-back loops**, as demonstrated e.g. in case of cartilage and bone formation.
- **Cell aggregation/disaggregation** is one of the **key developmental mechanisms** guiding new organ formation.
- **Some structures** are **transient during development**, e.g. pronephros, some, e.g. circular system are **extensively remodelled** during development.



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky