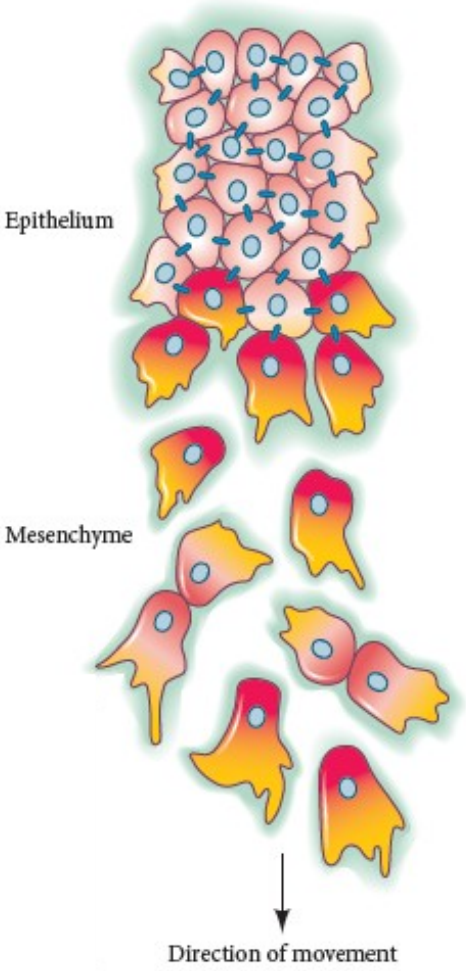
Epithelial-to-mesenchymal transition in development



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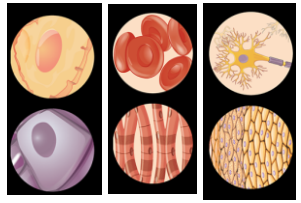
Duševní vlastnictví a poskytované studijní materiály

Outline

- Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET)
- Mechanis
- EMT and MET in development
- **BONUS:** The role of EMT in cancer metastasis

Link to the previous lecture



Are all cells in differentiated status?

NO!

The previous concept that terminally differentiated cells just execute their function(s) and they are more or less „static“ is not valid anymore.

Terminally differentiated epithelial cells are capable to change their phenotype using an activation of EMT „programme“ that allows to switch epithelial cell into a mesenchymal cell during development and adulthood.

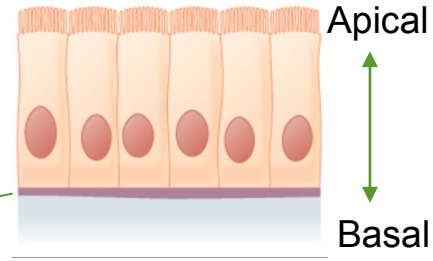
EMT vs MET

Epithelial-to-mesenchymal transition

EMT

- Series of events that lead to transformation of epihelial cells into mesenchymal cells

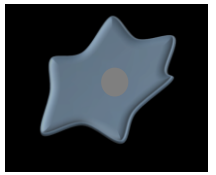
- A polarized, stationary epithelial cell, which interacts with a basal membrane, becomes a mesenchymal cell with an increased migration potential that is capable to invade tissues.
- During embryogenesis this transformation is critical for organ formation.

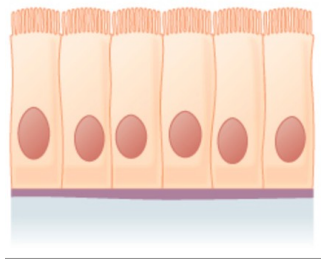


Mesenchymal-to-epithelial transition

MET

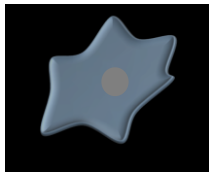
- Series of events leading to transformation of mesenchymal cells into epithelial cells.

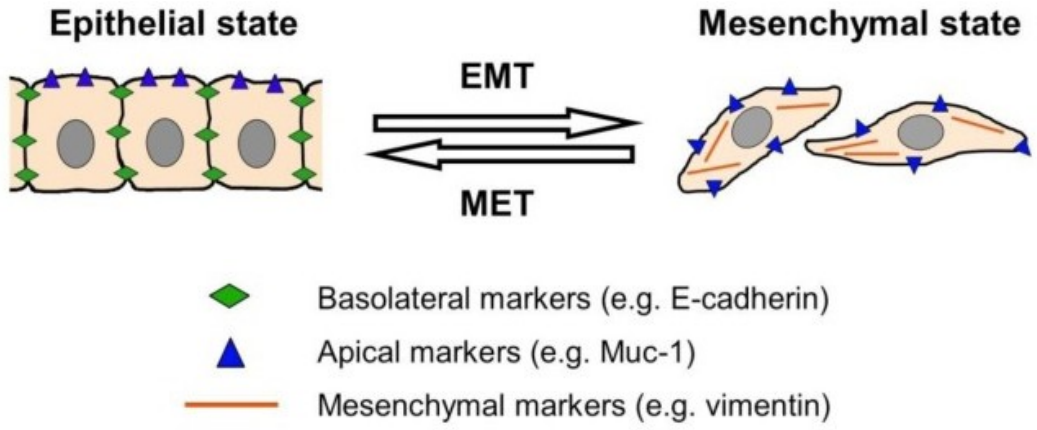




EMT

MET





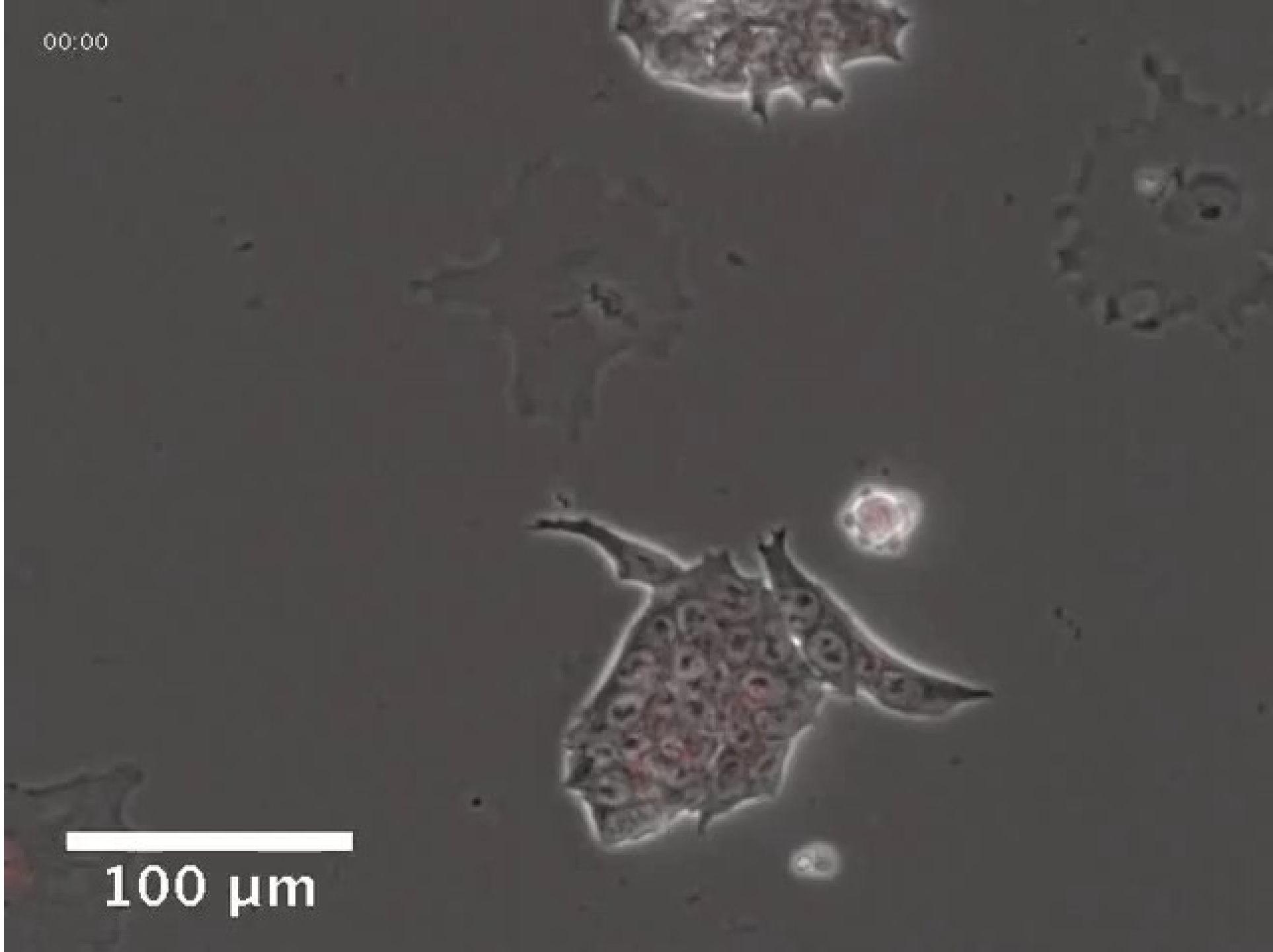
- Polygonal/column cell shape
- Apico-basal polarisation
- Strong cell-to-cell interction
- Migration potential is limited
- Markers (expressed genes):
- E-cadherin, Cytokeratins, Occludin, Claudin

- Spindle-shaped cell morphology
- Anterior-posterior polarization
- Focal interaction between cells
- Strong migration potential
- Markers (expressed genes):
- N-cadherin, Vimentin, Fibronectin

Typically, epithelial cells execute some function(s) in a tissue, while mesenchymal cells have rather supportive function.

EMT

These are mouse embryonic stem cells expressing a fluorescent reporter of Wnt/Beta-Catenin activity. As they are differentiated towards mesoderm, there is a huge increase in reporter activity and cells begin the process of an epithelial to mesenchymal transition (EMT).



MET

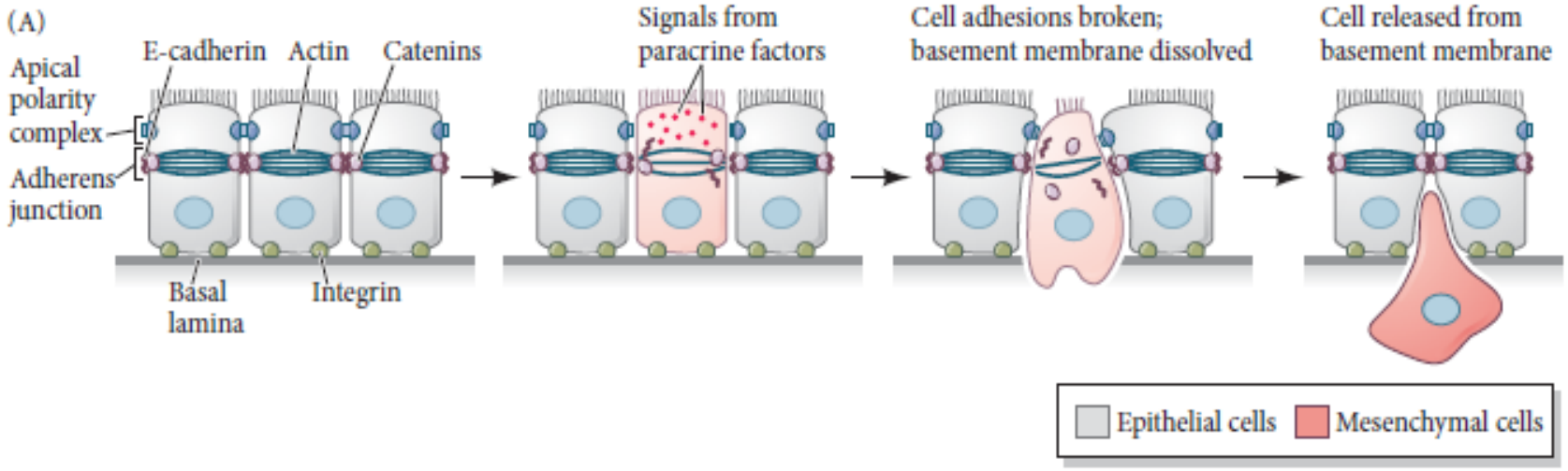
Reversible interconversion and maintenance of mammary epithelial cell characteristics by the ligand-regulated EGFR system

EMT vs MET – sum up

- You must be able to discrimine epithelial vs mesenchymal cells
- to describe EMT and MET and know the difference between them

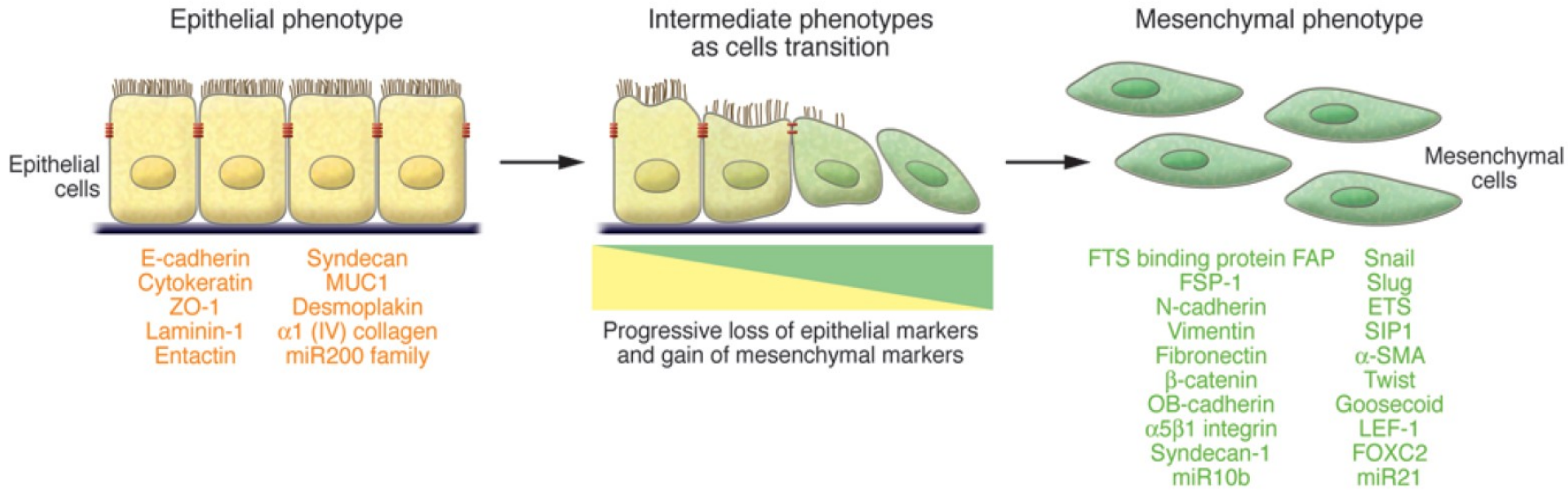
EMT vs MET – questions?

EMT - mechanism



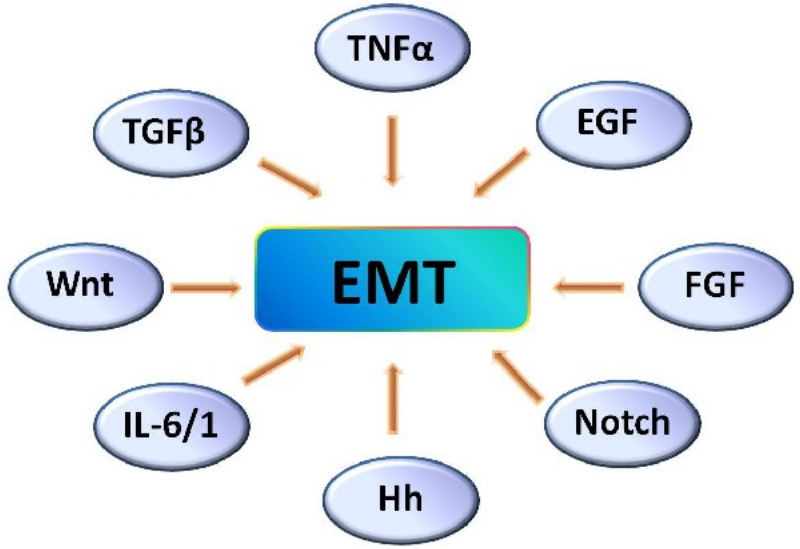
There is no „master regulator“ of EMT or MET!

EMT - mechanism

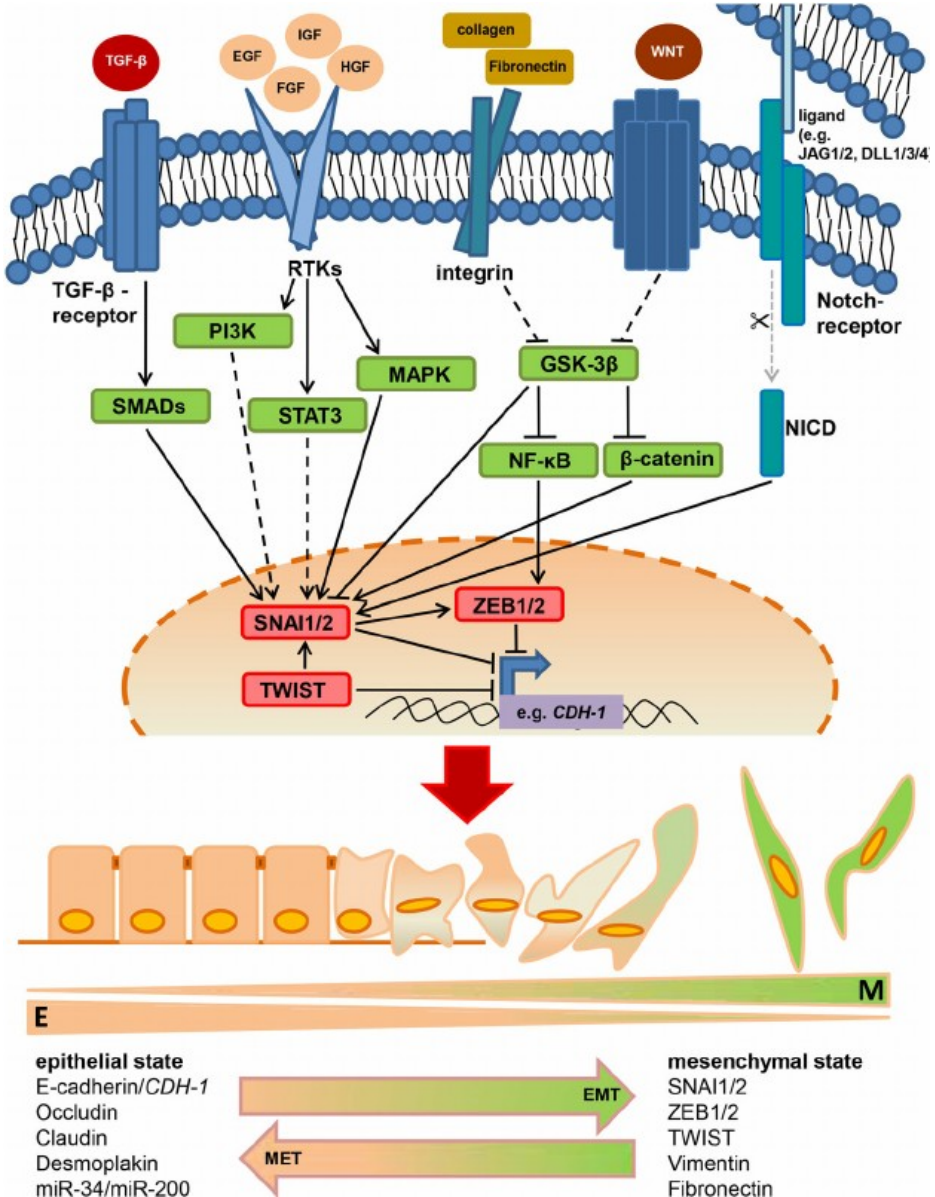


- Downregulation of Cadherins expression
- Complete rebuilding/reorganization of cytoskeletal actin
- Production of enzymes that are capable to degrade the basal membrane
- Cell proliferation

EMT - mechanism

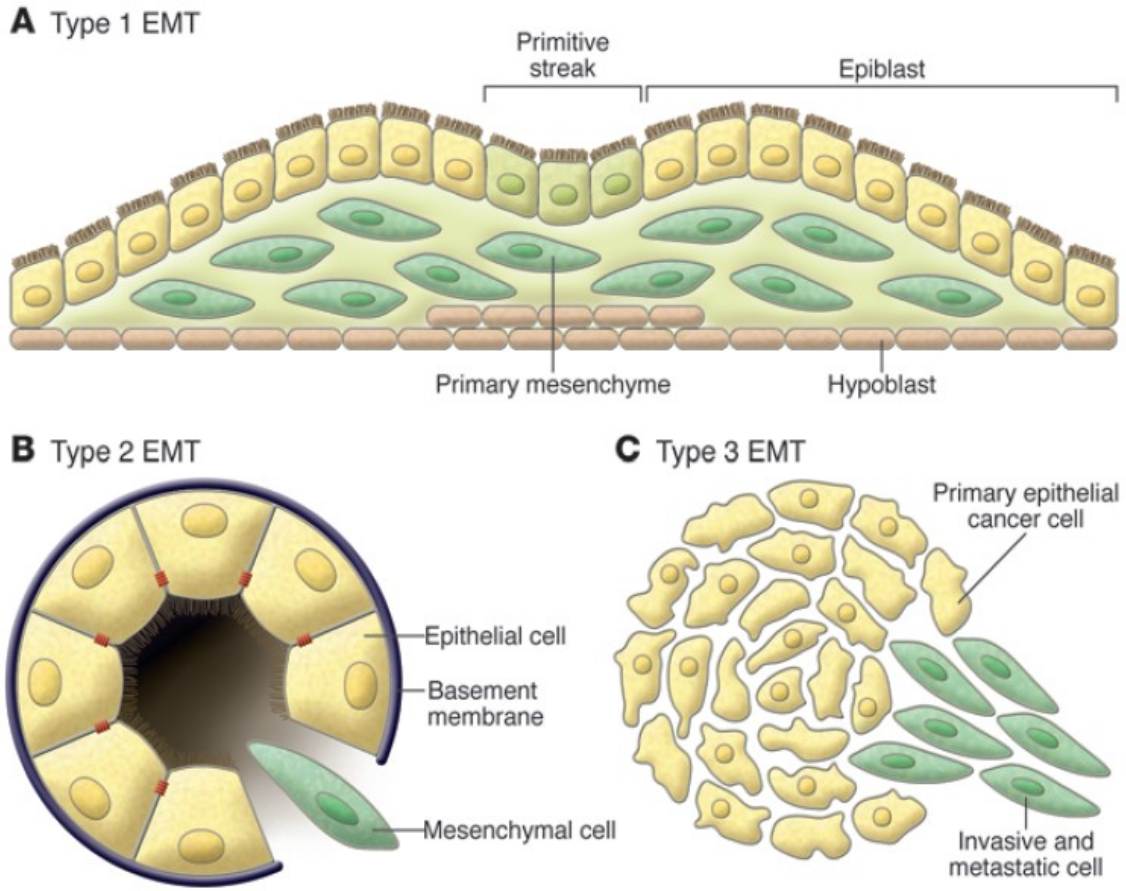


Remember 5 major pathways



EMT - mechanism

Where/When the EMT take place:



EMT in

- Embryonic development
- Cancer (metastasis)
- Inflamation and fibrosis

EMT – in embryonic development

- During the development some epithelial cells are more „plastic“ – are capable of transition epithel <–> mesenchym using EMT and/or MET processes.

  - **Embryo implantation**
  - **Embryogenesis**
  - **Organ development**
  - **Regeneration and homeostasis maintenance**

EMT – in development

Key role of EMT in development – it would not be possible without it

We will explain using 4 examples:

- Embryo implantation
- Gastrulation and mesoderm generation
- Neural crest formation
- Formation of vertebrae

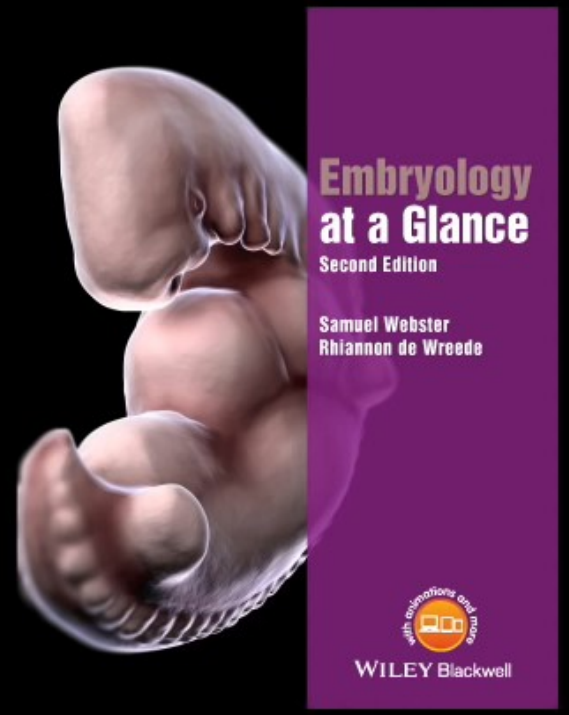
EMT – in development

Key role of EMT in development – it would not be possible without it

We will explain using 4 examples:

- Embryo implantation
- Gastrulation and mesoderm generation
- Neural crest formation
- Formation of vertebrae

EMT – implantation of embryo



EMT – in development

Key role of EMT in development – it would not be possible without it

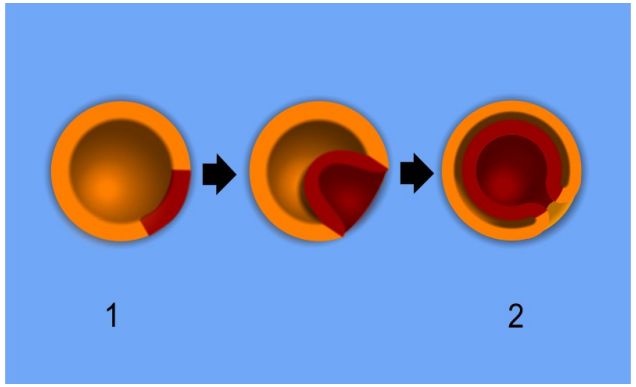
We will explain using 4 examples:

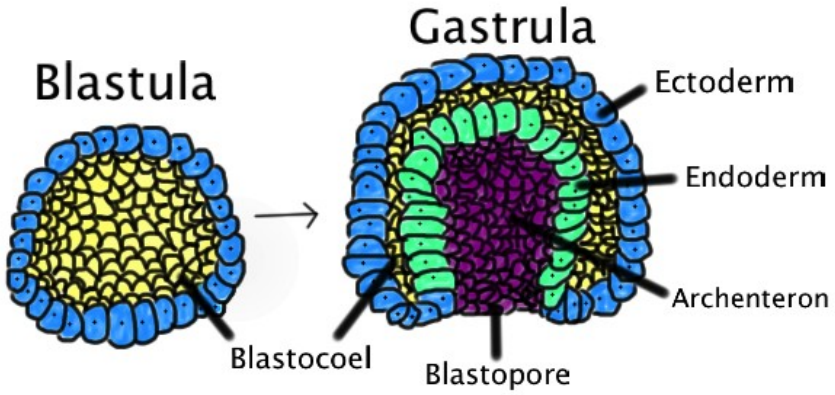
- Embryo implantation
- Gastrulation and mesoderm generation
- Neural crest formation
- Formation of vertebrae

EMT – during gastrulation

Gastrulation:

- Process of transition from blastula/blastocyst to gastrula
- Before the gastrulation an embryo is just a layer of epithelial cells
- Individual layers of the gastrula are transformed into germ layers ecto-, endo-, and mesoderm -> therefore EMT is critical here
- Before the gastrulation an embryo fully rely on maternal mRNA, only after the gastrulation is capable of synthetize own mRNA

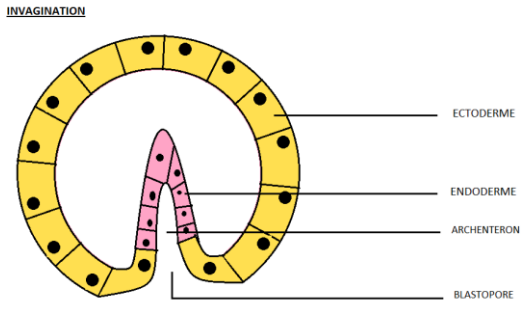


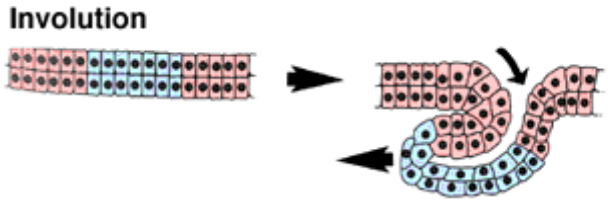


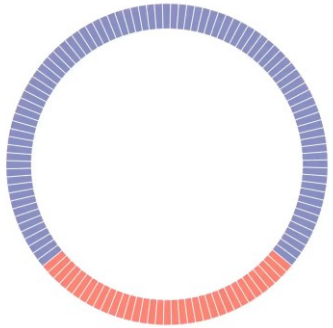
EMT – during gastrulation

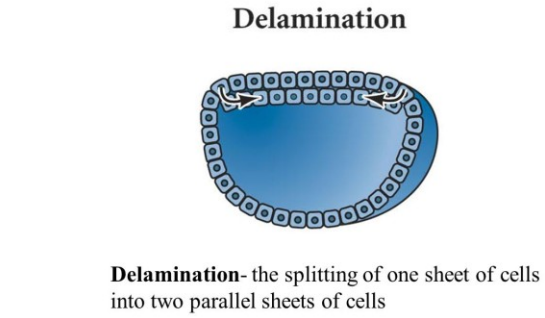
There are 5 major kinds of the gastrulation process:

Invagination – invagination of a group of cells
Involution – involution of the outer cell layer, so it covers cell layer beneath it.
Ingression – migration of cells into the inner part
Delamination – division of a cell layer into two paraler cell layers
Epiboly – the outer epithelial layer overgrow the prospective endoderm

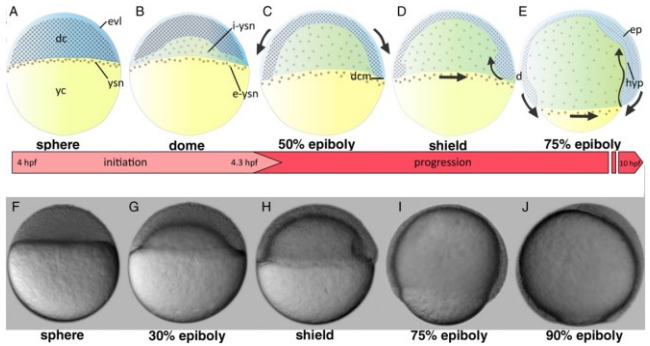




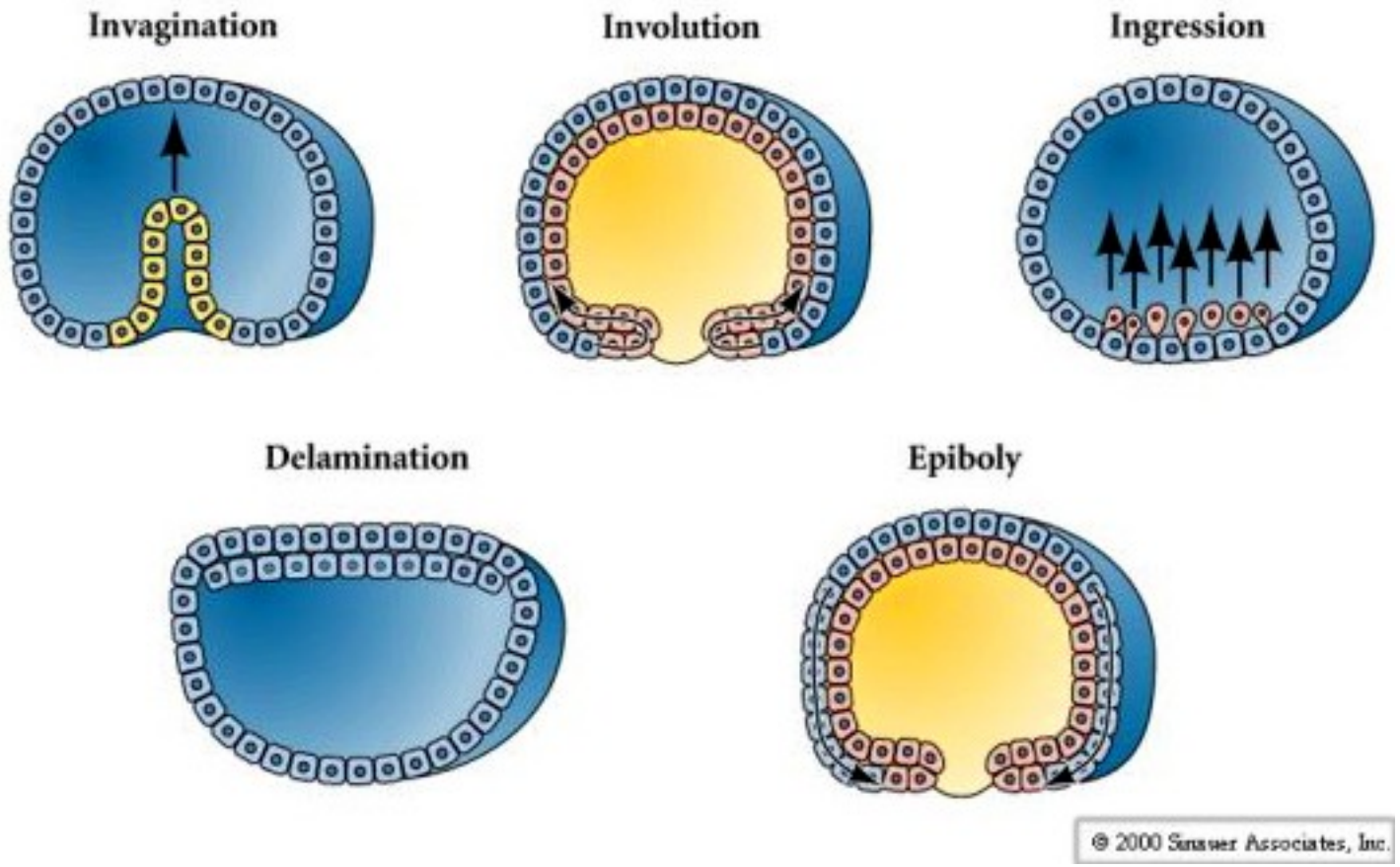




Delamination- the splitting of one sheet of cells into two parallel sheets of cells



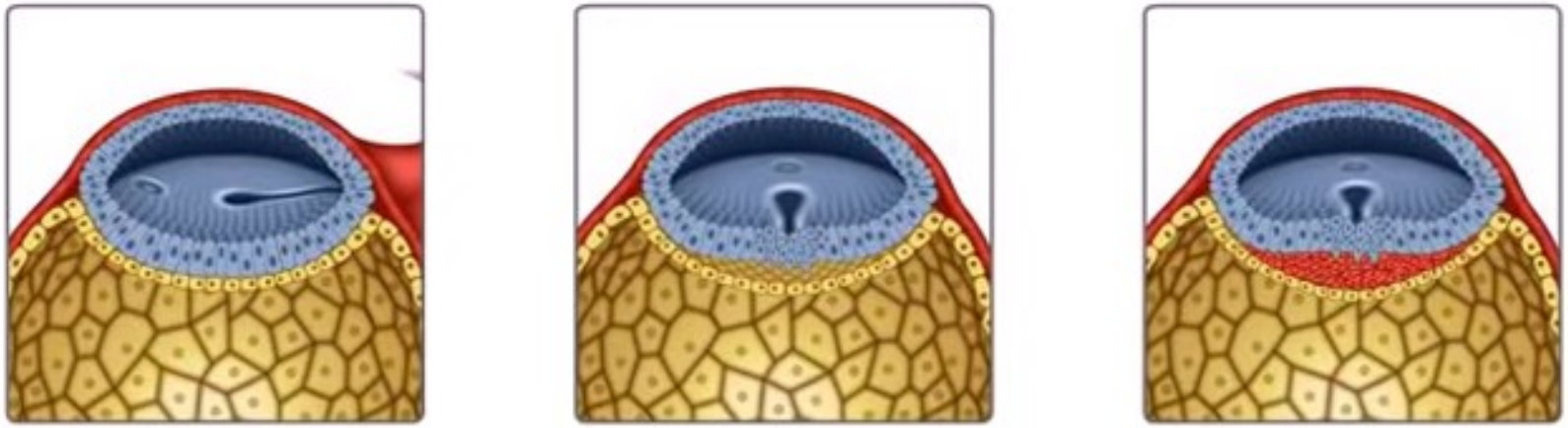
EMT – during gastrulation



EMT – during gastrulation

Gastrulation

Gastrulation is the process whereby the bilaminar embryonic disc undergoes reorganization to form a trilaminar disc.



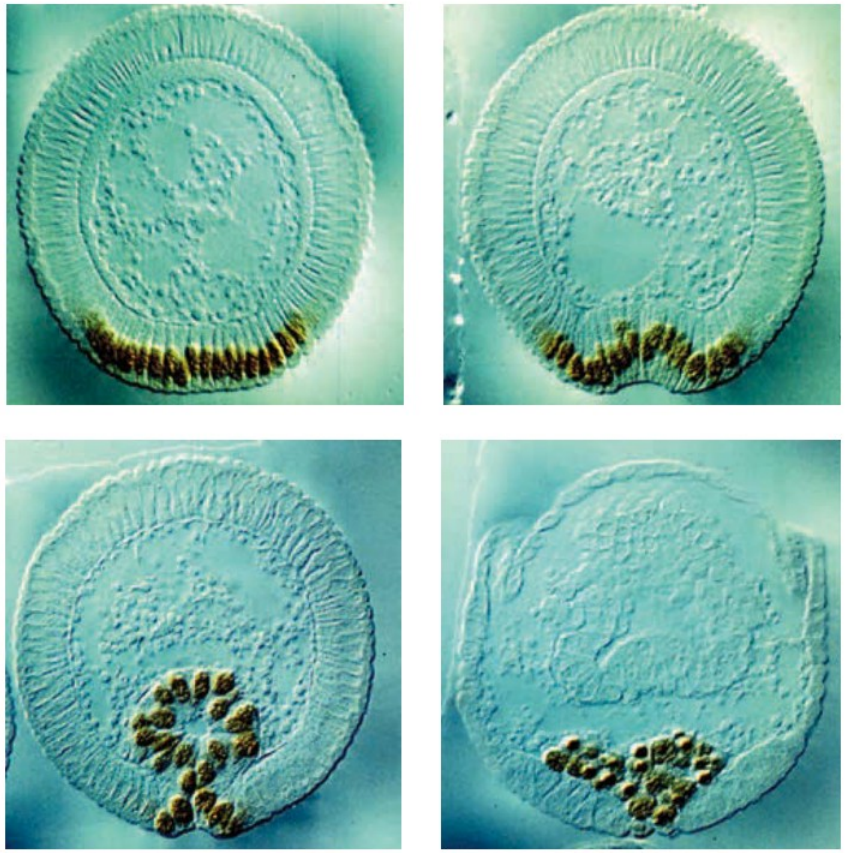
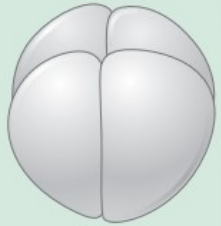
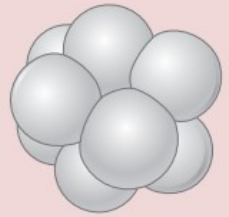
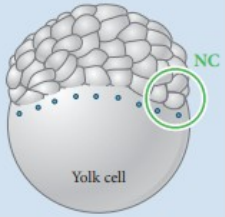
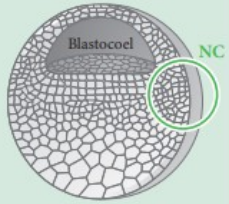
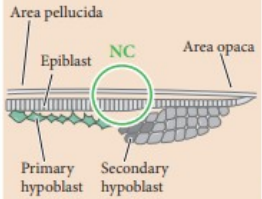
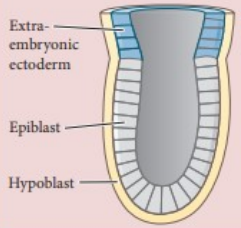
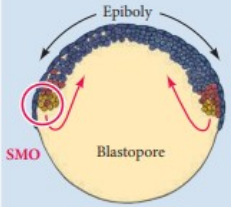
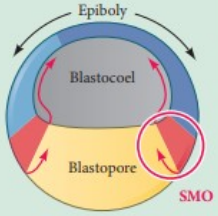
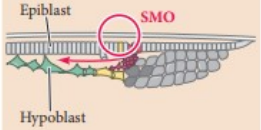
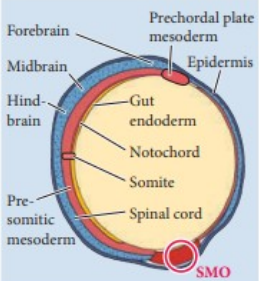
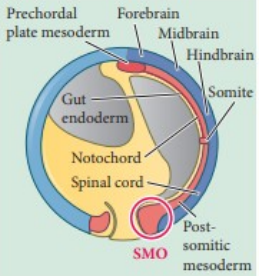
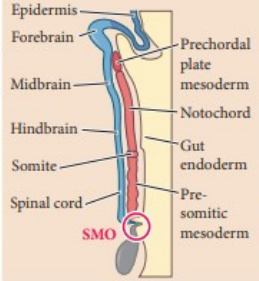
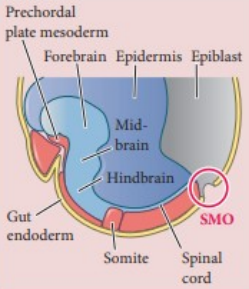
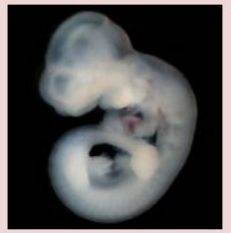
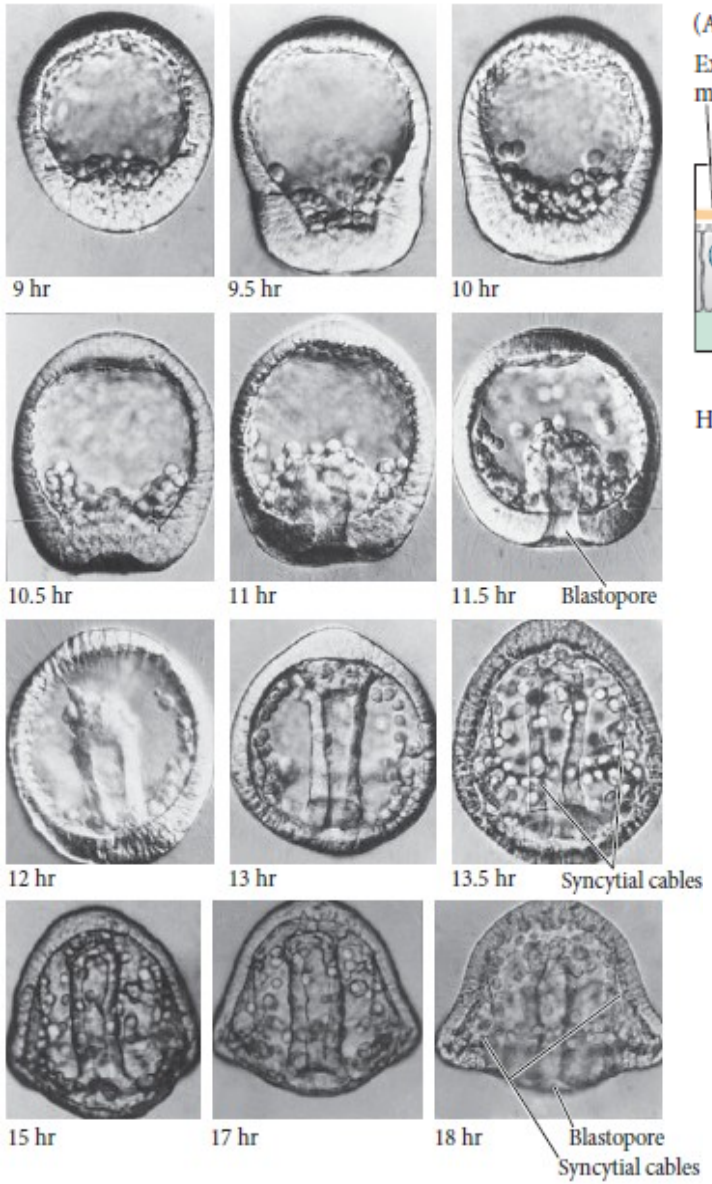


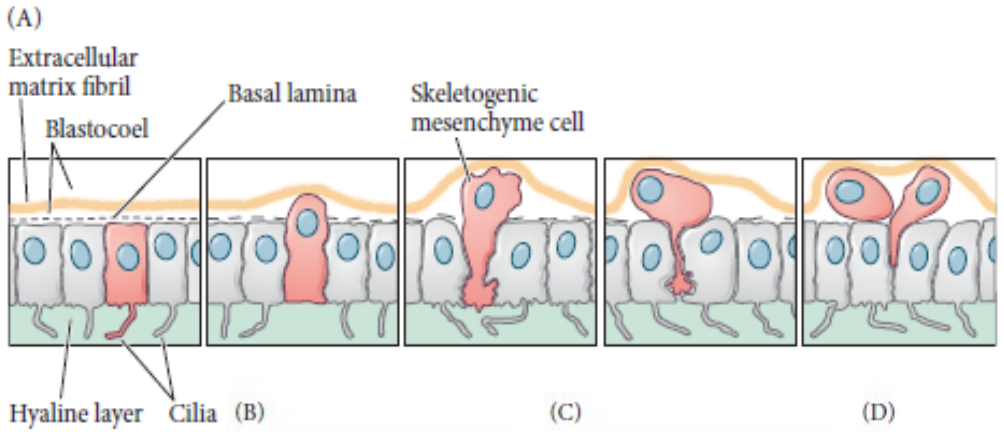
FIGURE 9.30 Gastrulation in *Drosophila*. In this cross section, the mesodermal cells at the ventral portion of the embryo buckle inward, forming the ventral furrow (see Figure 9.5A,B). This furrow becomes a tube that invaginates into the embryo and then flattens and generates the mesodermal organs. The nuclei are stained with antibody to the Twist protein, a marker for the mesoderm. (From Leptin 1991a, courtesy of M. Leptin.)

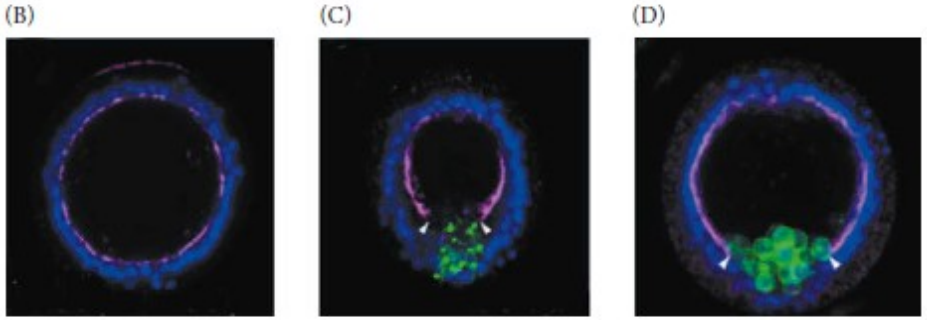
Vývojová biologie

STAGE	Zebrafish *Danio rerio*	Frog *Xenopus laevis*	Chicken *Gallus gallus*	Mouse *Mus musculus*
Early cleavage				
Late cleavage				
Early gastrula				
Late gastrula				
Pharyngula				

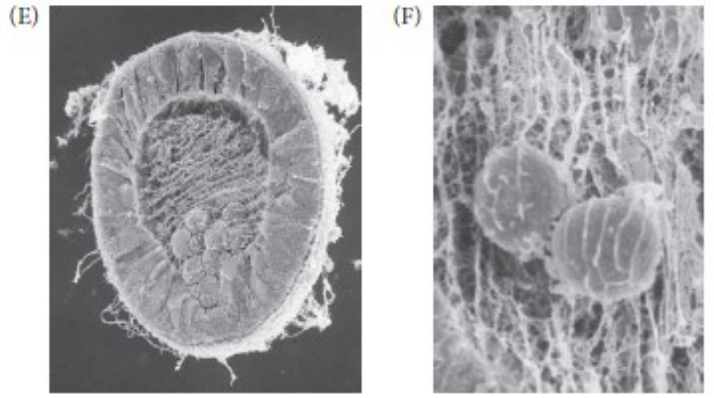
EMT – during gastrulation





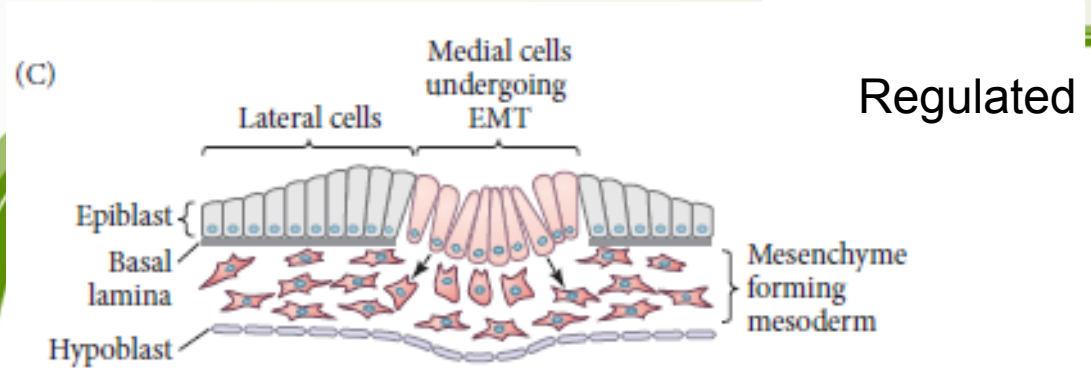


Skeletogenic mesenchyme cells breaking through extracellular matrix. The matrix laminin is stained pink, the mesenchyme cells are green and cell nuclei are blue. (B) Laminin matrix is uniformly spread throughout the lining of the blastocoel. (C) Hole is made in blastocoel laminin above the vegetal cells, and the mesenchyme begins to pass through it into the blastocoel. (D) Within an hour, cells are in the blastocoel.

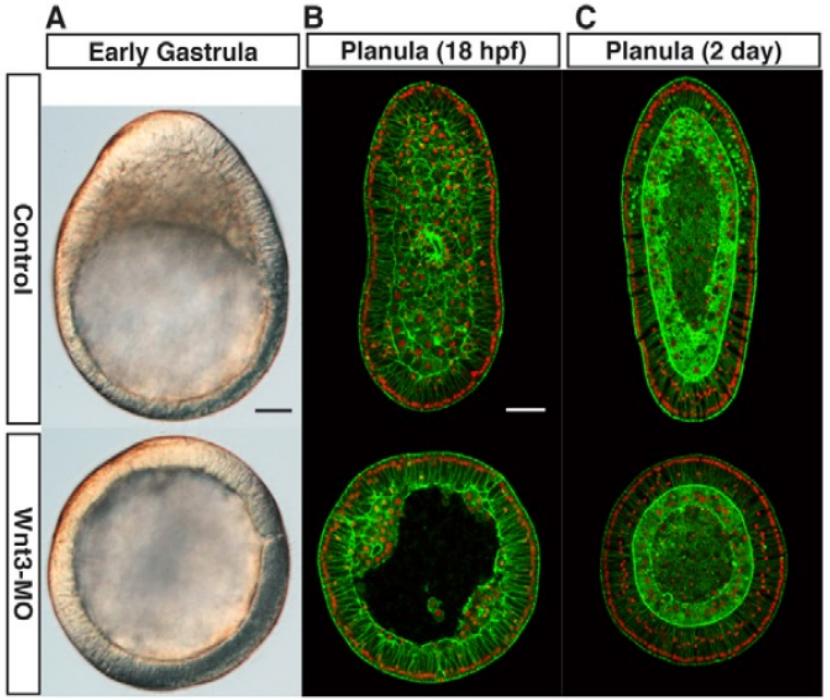


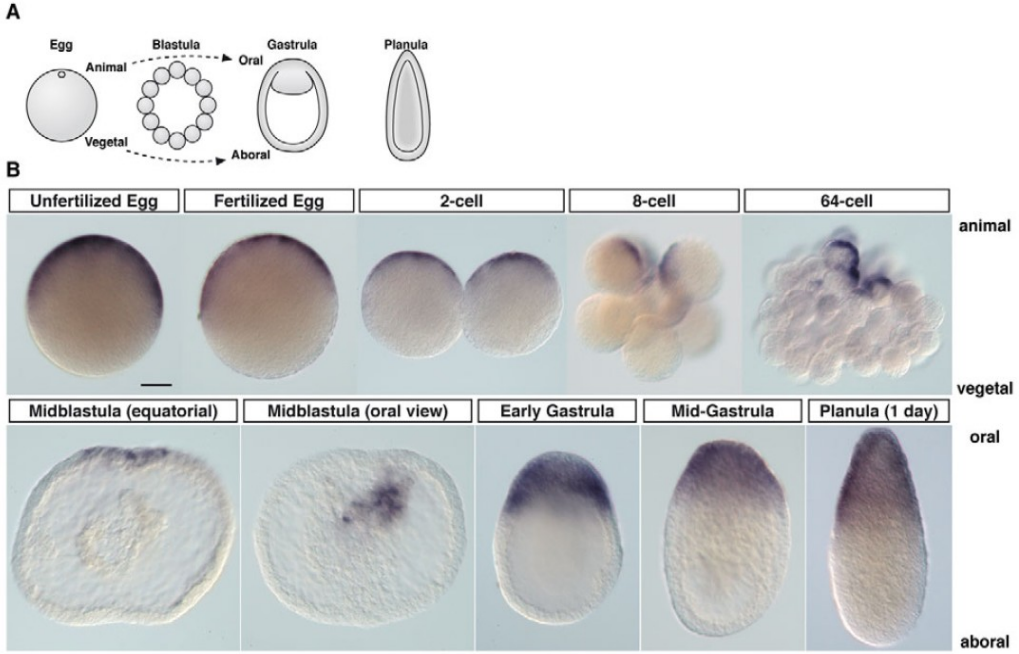
(E) Scanning electron micrograph of skeletogenic mesenchyme cells enmeshed in the extracellular matrix of an early Strongylocentrotus gastrula. (F) Gastrula-stage mesenchyme cell migration. The extracellular matrix fibrils of the blastocoel lie parallel to the animal-vegetal axis and are intimately associated with the skeletogenic mesenchyme cells.

EMT – during gastrulation



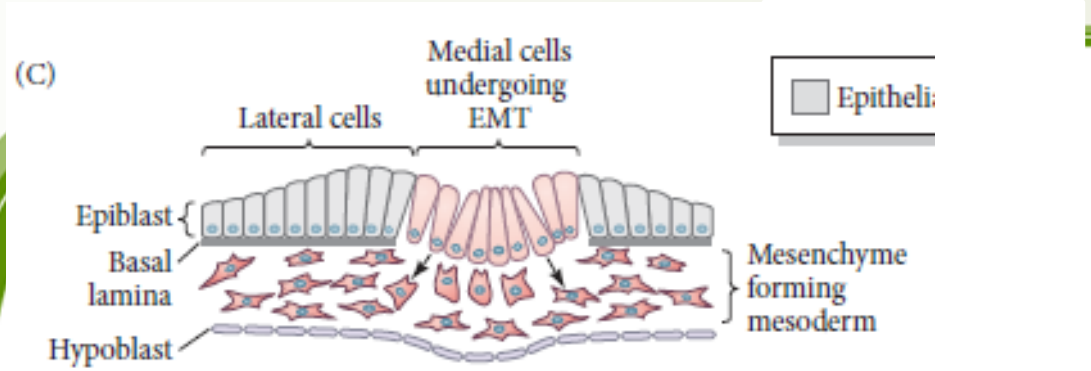
Regulated by canonical Wnt – Wnt3, TGF-β (Nodal, Vg1) a FGF



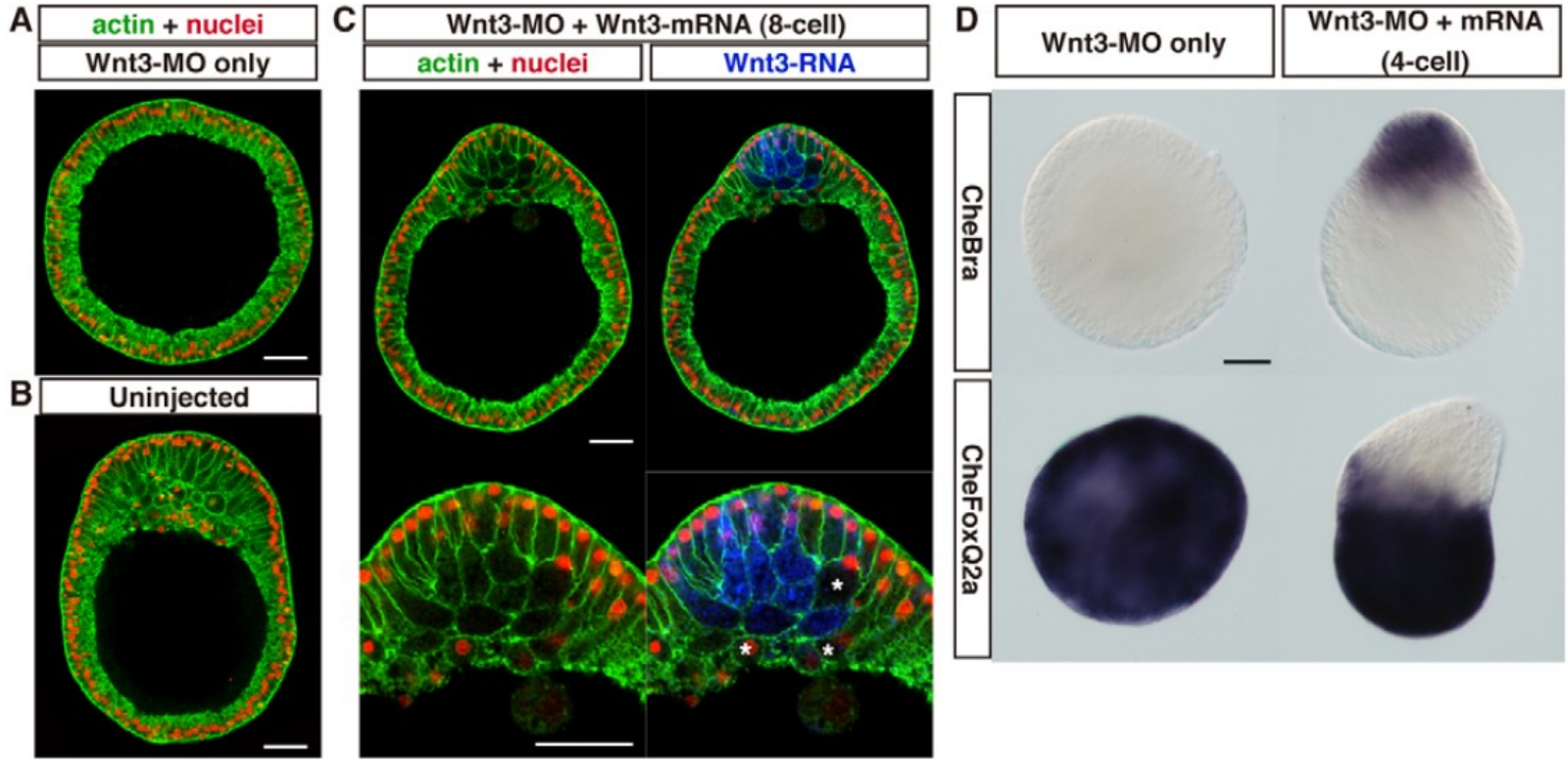


Momose, Development

EMT – during gastrulation

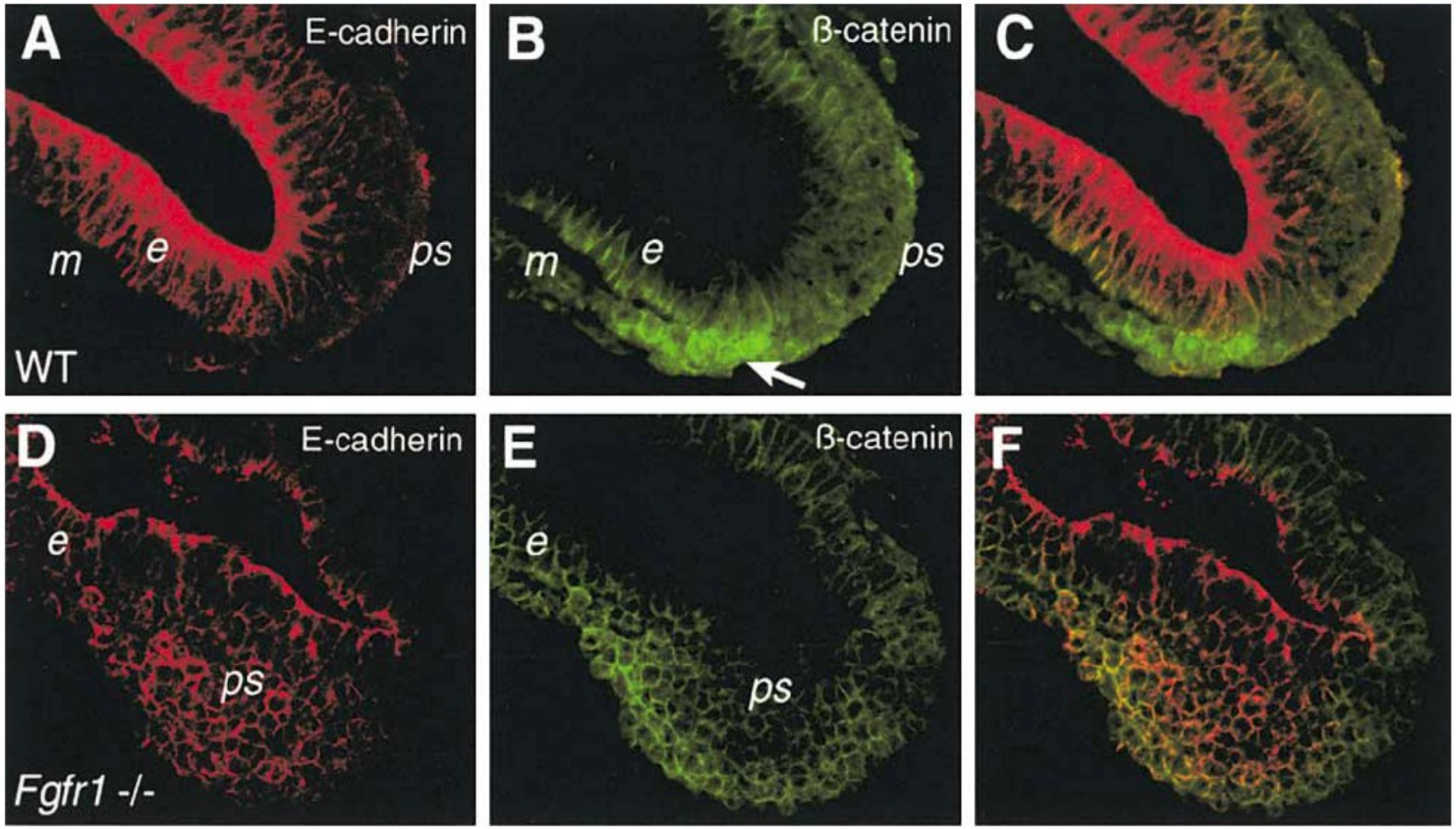


Rescue of the phenotype by injecting Wnt3-mRNA



EMT – during gastrulation

FGF



Mesoderm is not formed – failure of EMT (accumulation of E-cad positive cells)

Ciruna, 2001

EMT – in development

Key role of EMT in development – it would not be possible without it

We will explain using 4 examples:

- Embryo implantation
- Gastrulation and mesoderm generation
- Neural crest formation
- Formation of vertebrae

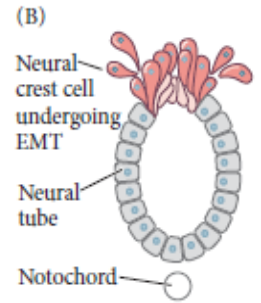
- **EMT – Neural crest formation**

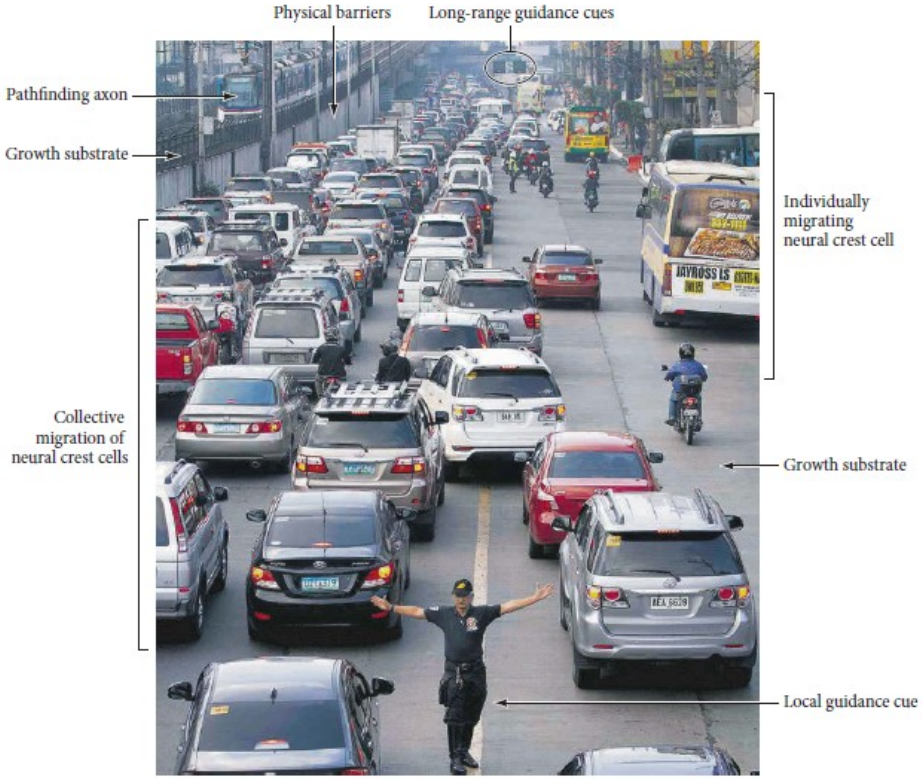
What is the neural crest?

- „Fourth germ layer“ - “the only interesting thing about vertebrates is the neural crest” (Thorogood 1989)
- Ectodermal origin
- Transient – is absent after embryo development
- Is generated from the neural tube by EMT. Cells are migrating alongside the anterior-posterior axis and are differentiating (changes of the cell environment leads to th generation of different cell types).

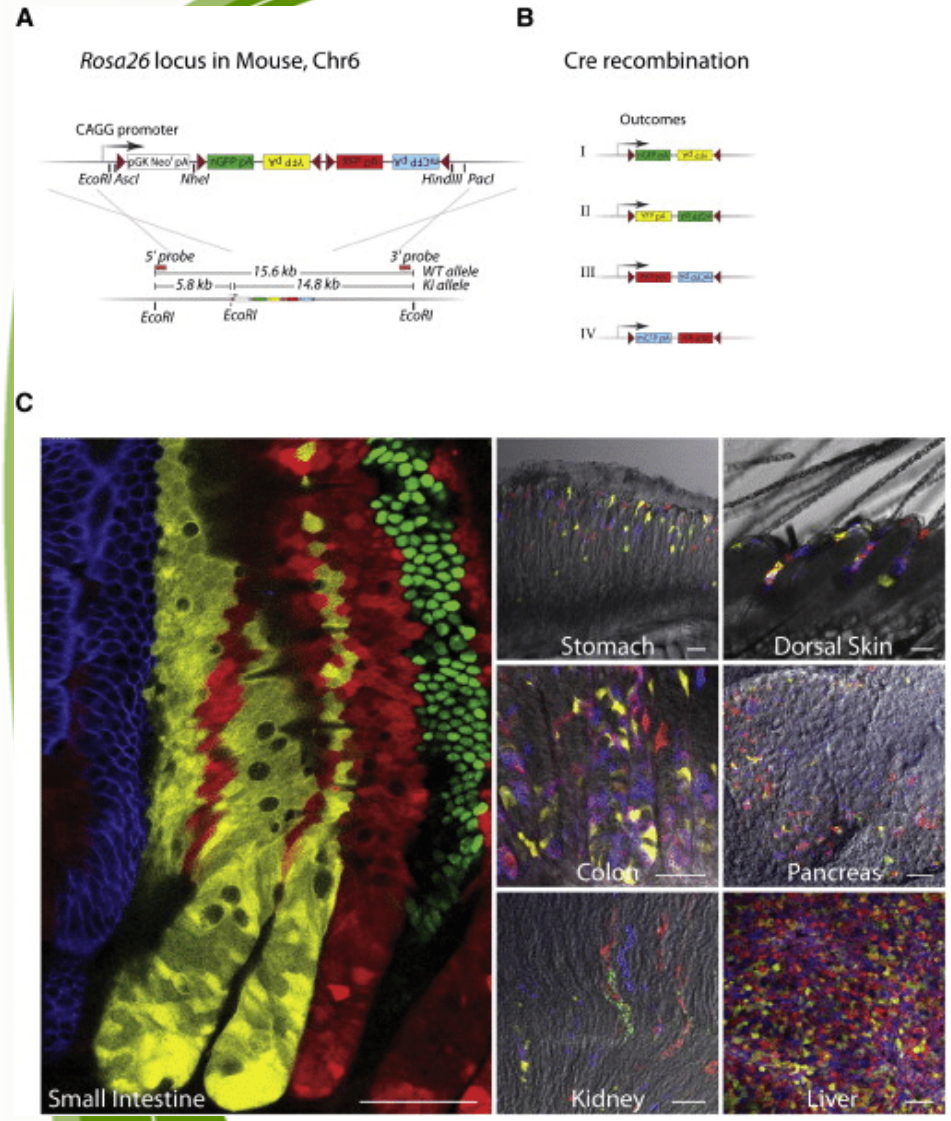
TABLE 15.1 Some derivatives of the neural crest

Derivative	Cell type or structure derived
Peripheral nervous system (PNS)	Neurons, including sensory ganglia, sympathetic and parasympathetic ganglia, and plexuses Neuroglial cells Schwann cells and other glial cells
Endocrine and paraendocrine derivatives	Adrenal medulla Calcitonin-secreting cells Carotid body type I cells
Pigment cells	Epidermal pigment cells
Facial cartilage and bones	Facial and anterior ventral skull cartilage and bones
Connective tissue	Corneal endothelium and stroma Tooth papillae Dermis, smooth muscle, and adipose tissue of skin, head, and neck Connective tissue of salivary, lachrymal, thymus, thyroid, and pituitary glands Connective tissue and smooth muscle in arteries of aortic arch origin





- **EMT – Neural crest formation**



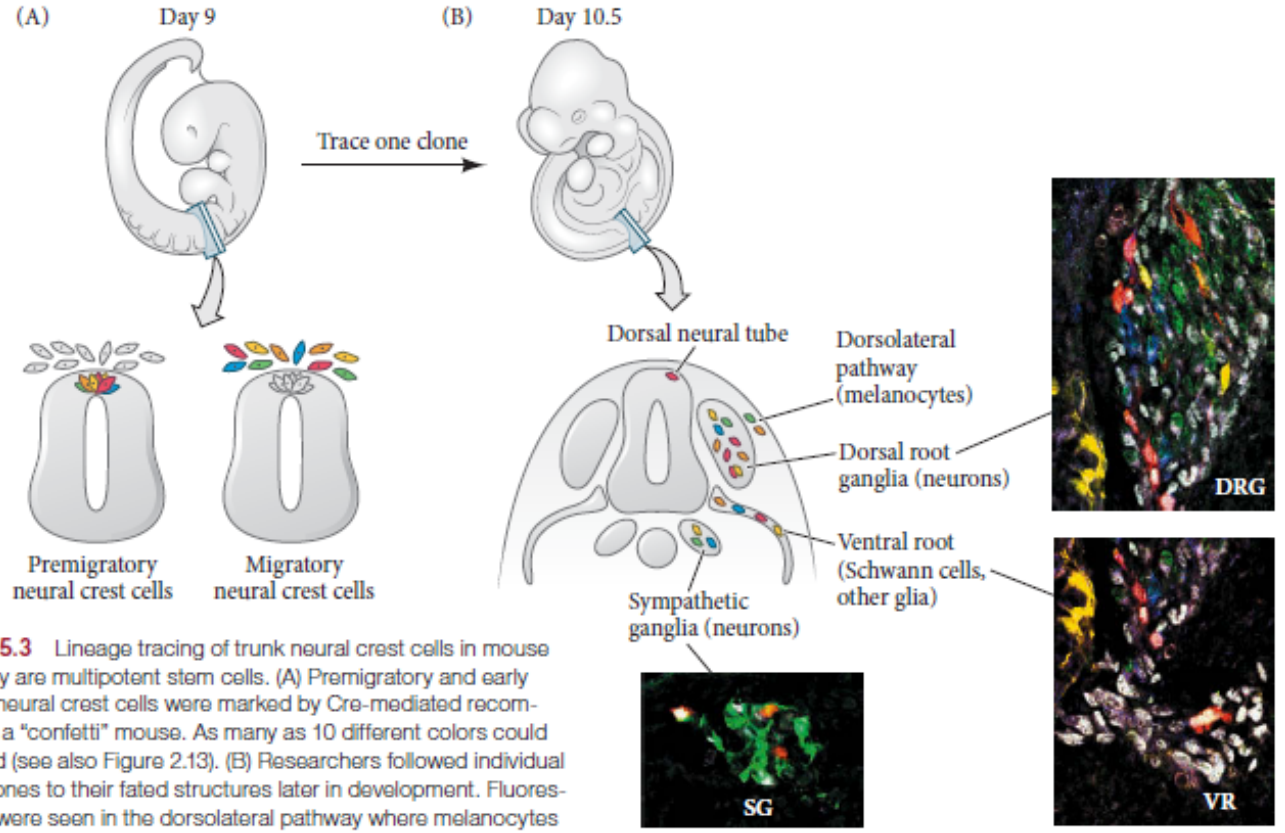


FIGURE 15.3 Lineage tracing of trunk neural crest cells in mouse proves they are multipotent stem cells. (A) Premigratory and early migratory neural crest cells were marked by Cre-mediated recombination in a "confetti" mouse. As many as 10 different colors could be induced (see also Figure 2.13). (B) Researchers followed individual colored clones to their fated structures later in development. Fluorescent cells were seen in the dorsolateral pathway where melanocytes differentiate; in the dorsal root ganglia (DRG), as part of the Schwann cell population on the ventral root, and in the sympathetic ganglia (SG). Micrographs show the tracing of premigratory cells labeled by the Wnt1-CreERT driver, which shows a unique YFP/RFP fluorescent combination in three different peripheral structures visualized with cell type-specific markers. (After Baggiolini et al. 2015.)

- **EMT – Neural crest formation**

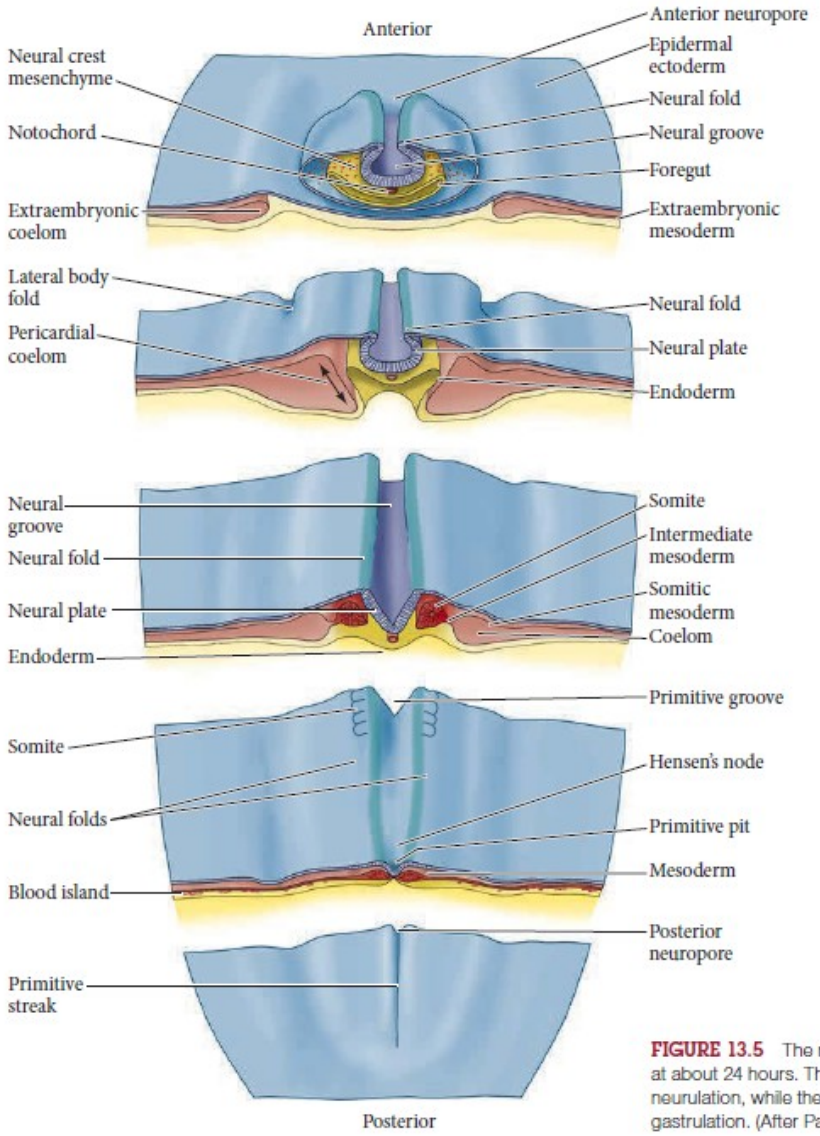
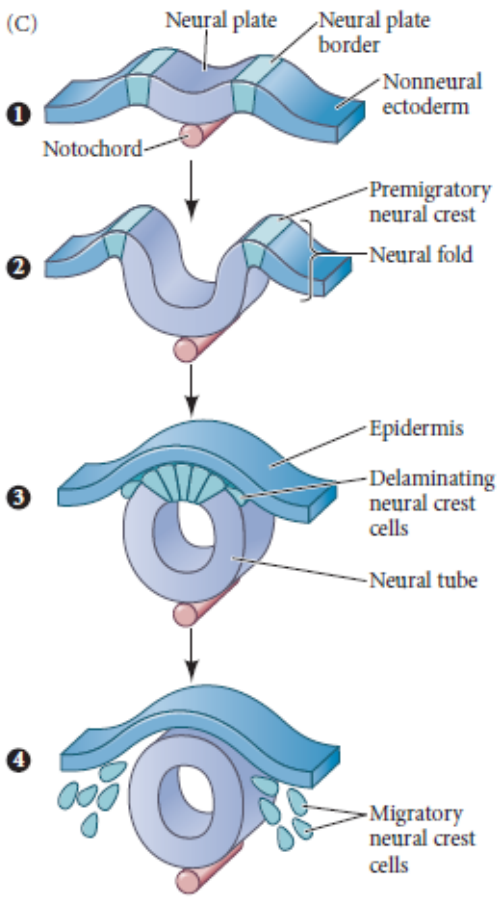
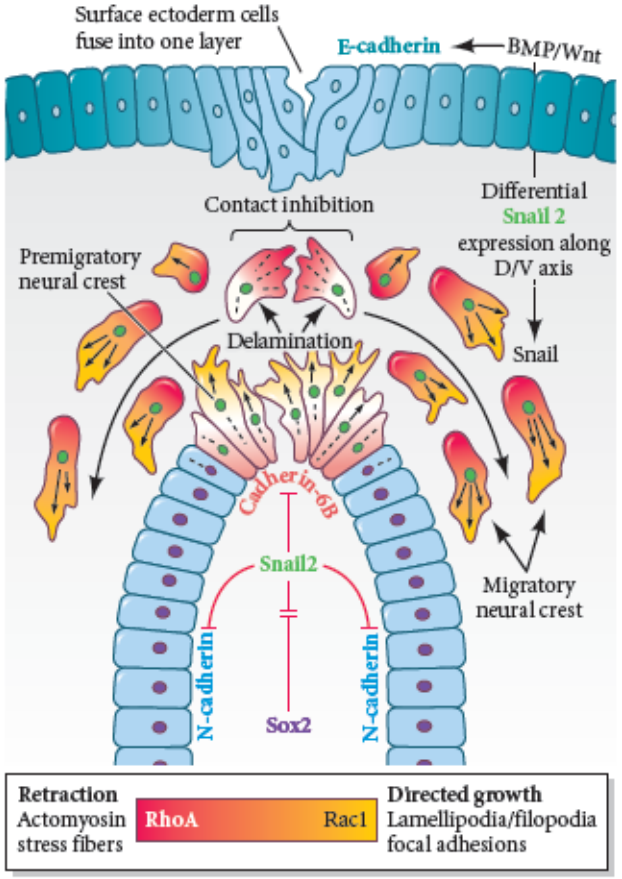


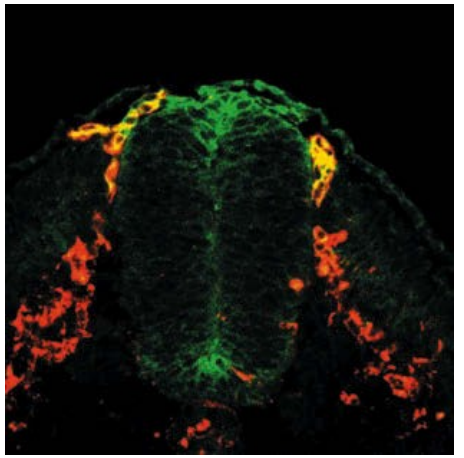
FIGURE 13.5 The neurulating chick embryo (dorsal view) at about 24 hours. The cephalic (head) region has undergone neurulation, while the caudal (tail) region is still undergoing gastrulation. (After Patten 1971.)

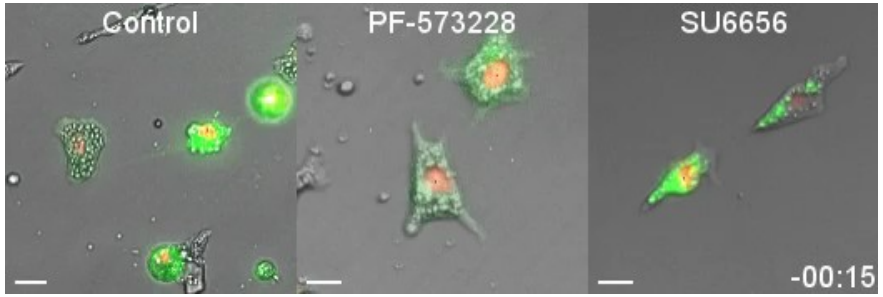
• EMT – Neural crest formation

Prospective neural crest cells are loosing adhesive junctions and are released from epithel – this process is called **delamination**

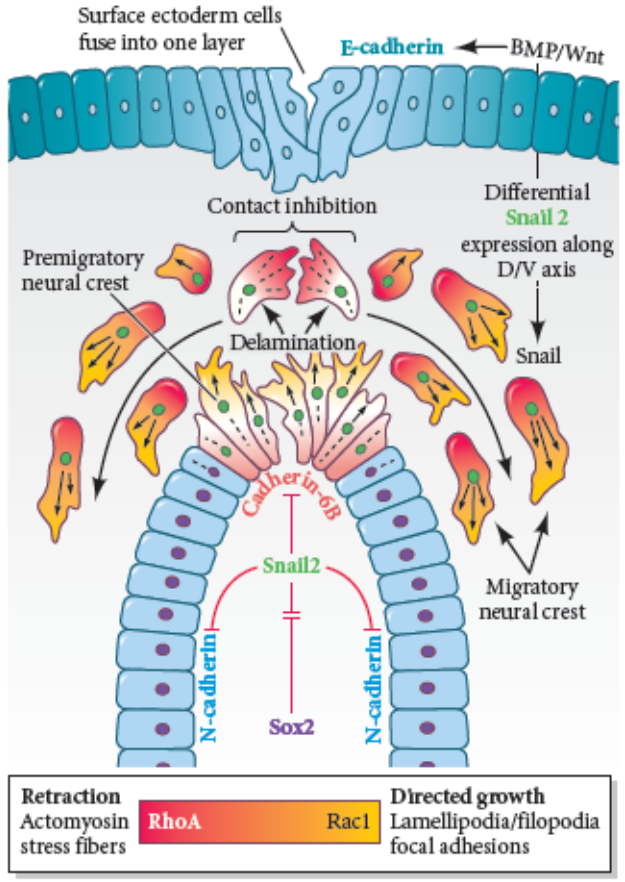








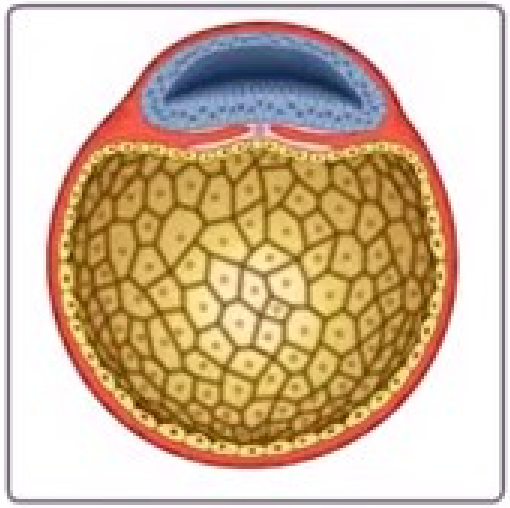
• EMT – Neural crest formation

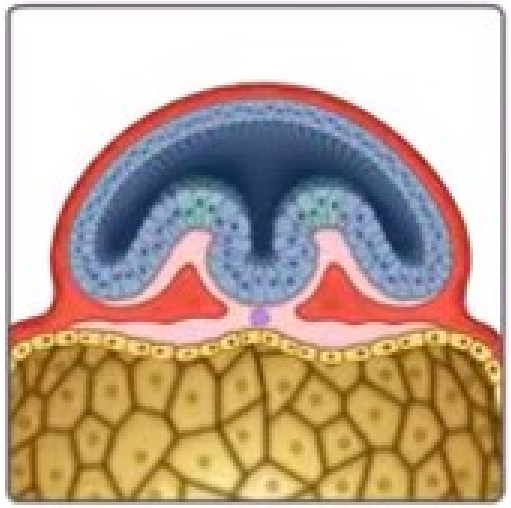


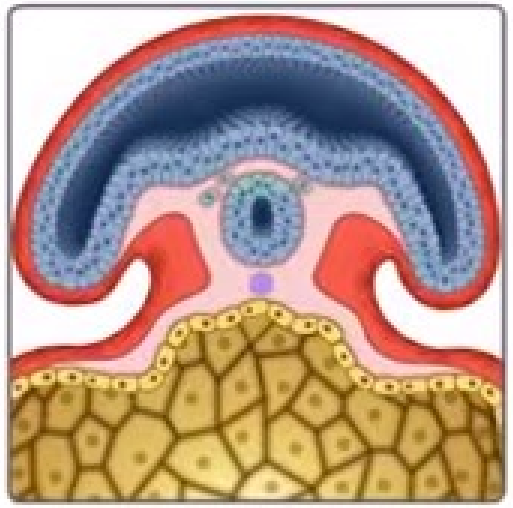
Neural crest delamination and migration by contact inhibition. The process of neural crest delamination is shown here at the time when the neural and surface ectoderms have separated and are both in the process of fusing at the midline into the neural tube and epidermis respectively. BMP and Wnt signals specify the three major regions of the neuroepithelium, which are distinguished by their expression of unique adhesion proteins: surface ectoderm (E-cadherin), neural tube (N-cadherin), and the premigratory neural crest (cadherin-6B). In the premigratory domain, BMP levels are the highest, with Wnt at intermediate amounts; this situation supports the upregulation of *Snail-2* (and *Zeb-2)* in these cells. Snail-2 proteins repress N-cadherin and E-cadherin in this domain. Cadherin-6B is upregulated only in the apical half of premigratory neural crest cells, and functions to activate RhoA and actomyosin contractile fibers for apical constriction and the initiation of delamination. Noncanonical Wnt signaling (not shown) establishes the polar activity of RhoA (red) and Rac1 (yellow) along the migratory axis of migrating neural crest cells. When neural crest cells contact one another, they experience contact inhibition, during which they will stop, turn, and migrate away in the opposite direction.

Neurulation

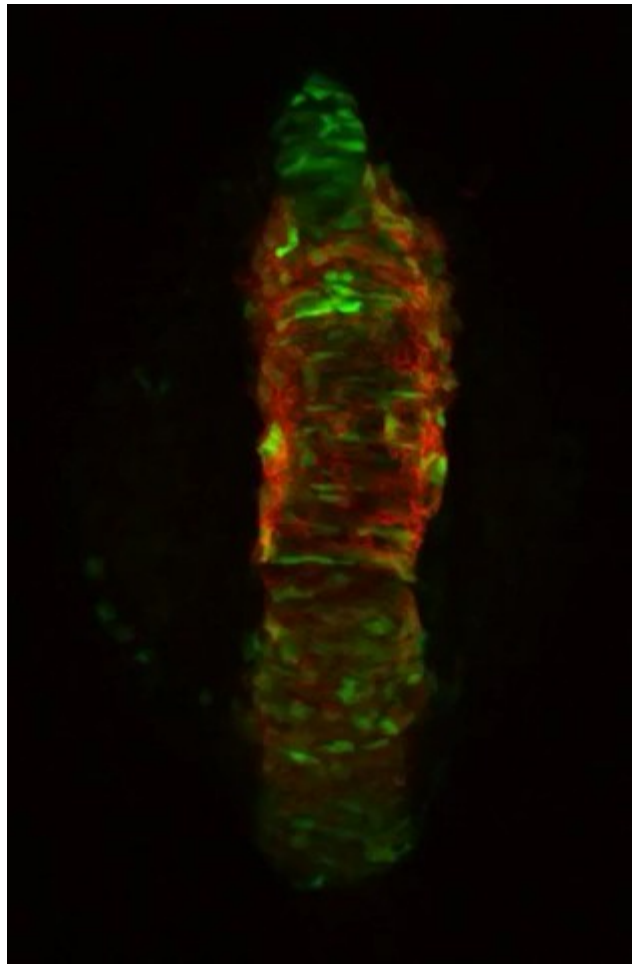
Neurulation marks the beginning of the formation of the central nervous system and is the process whereby the neural plate forms into a neural tube.



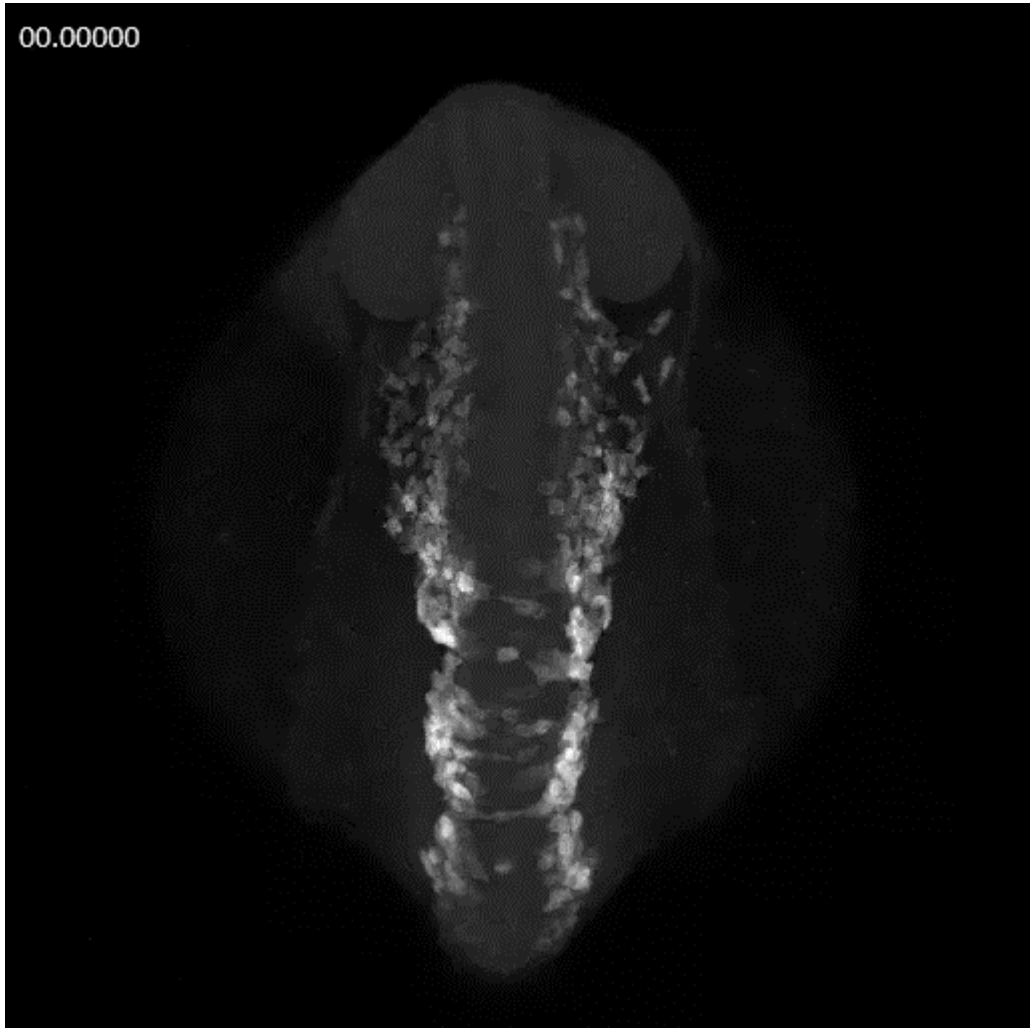




- **EMT – Neural crest formation**



- **EMT – Neural crest formation**



EMT – in development

Key role of EMT in development – it would not be possible without it

We will explain using 4 examples:

- Embryo implantation
- Gastrulation and mesoderm generation
- Neural crest formation
- Formation of vertebrae

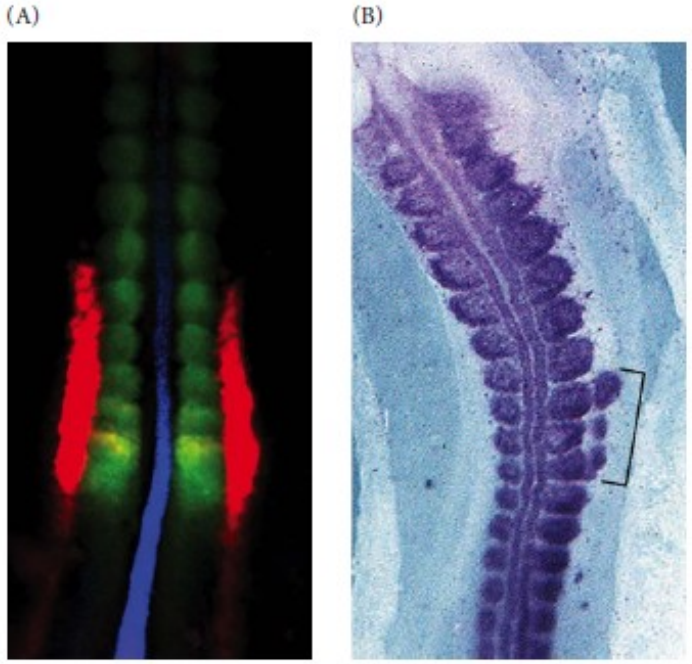
EMT – formation of vertebrae from somites

Somites and body segmentation

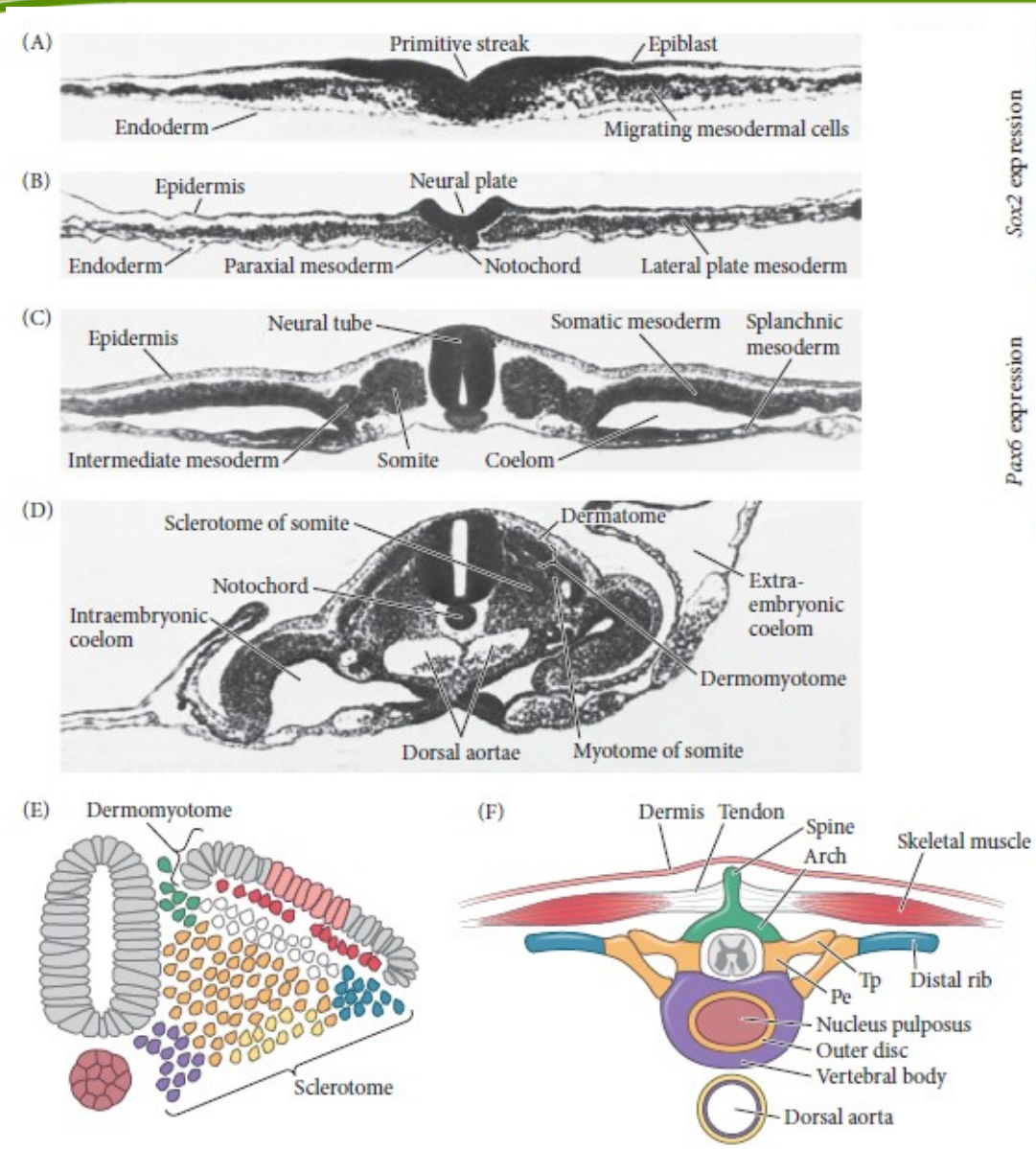
„How can a tissue be developmentally cut up into precisely sized segments?
How can snakes have some 300 segments while humans have only about 35?“
– Gilbert and Barresi

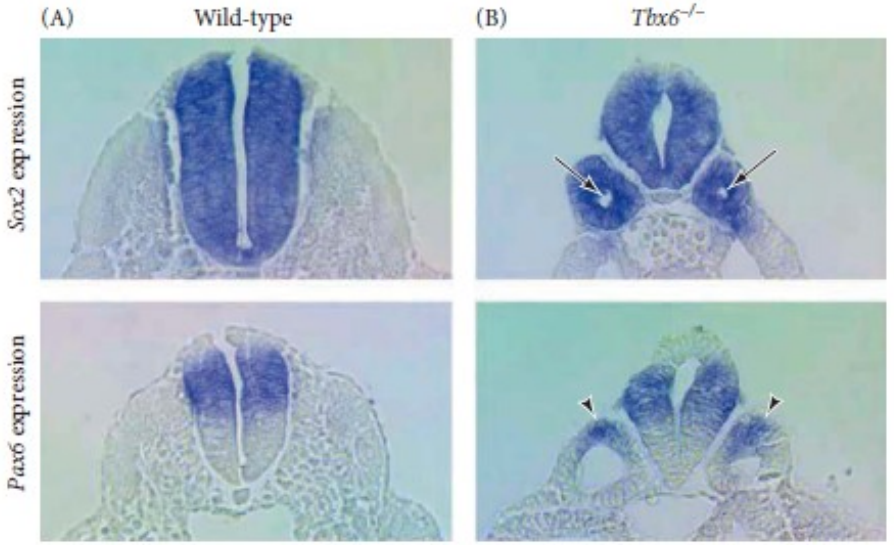
Somites are epthelial blocks (clusters) of cells that are localized in a close proximity of the neural tube

- Body segmentation - vertebrae



EMT – formation of vertebrae from somites





Three neural tubes: loss of the *Tbx6* gene transforms the paraxial mesoderm into neural tubes. In situ hybridization for the mRNA expression (blue) of the neural specification markers *Sox2* and *Pax6* in wildtype mice (A) and *Tbx6* knockout mice (B). *Sox2* is ectopically expressed throughout the presumptive paraxial mesoderm in the *Tbx6*$^{-/-}$ embryo, which has also taken on a neural tube-like morphology, even displaying a central lumen (arrows). Similarly, the dorsal neural tube marker *Pax6* shows regional cell specification within these ectopic neural tubes (arrowheads). (From Takemoto et al. 2011.)

MET

MET – during development – formation of somites

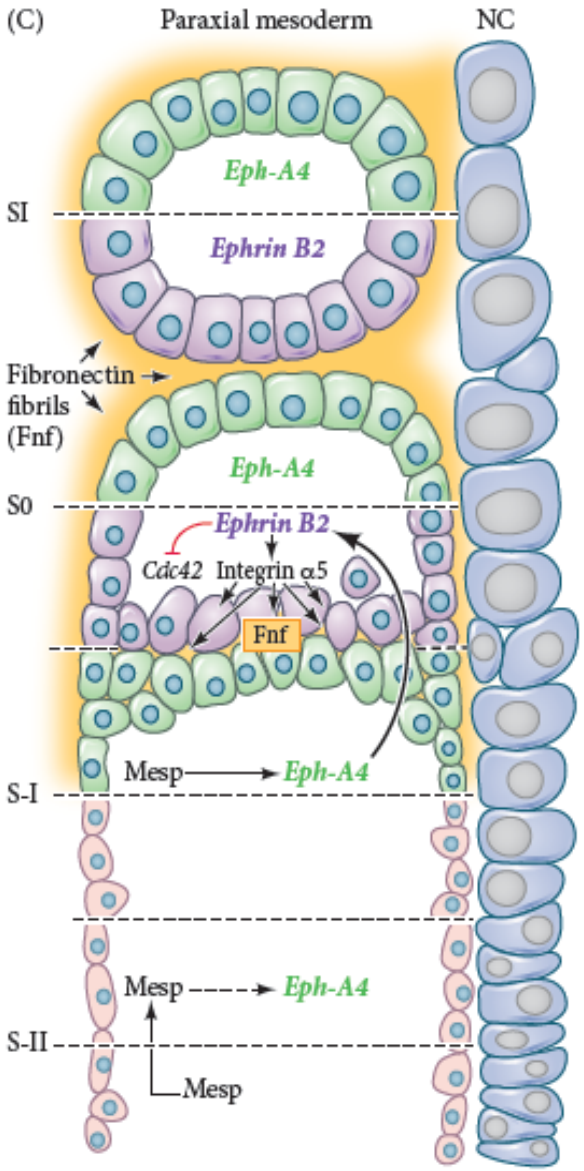
- Archtecture of somites is formed by epithelial blocks, but presomitic (paraxial) mesoderm is formed by mesenchymal cells.

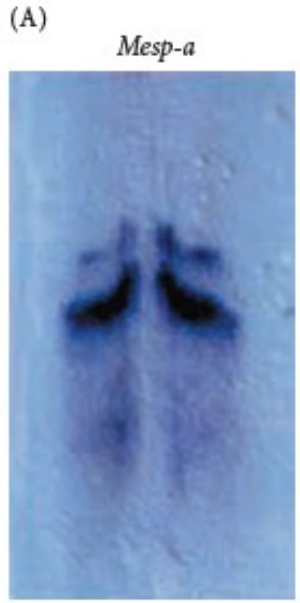
- Therefore, mesenchymal cells must be transformed into epithelial cells => MET

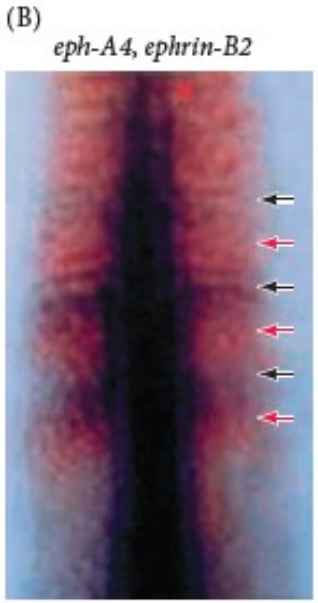
↑ Mesp (Mesodermal posterior)

MET – during development – formation of somites

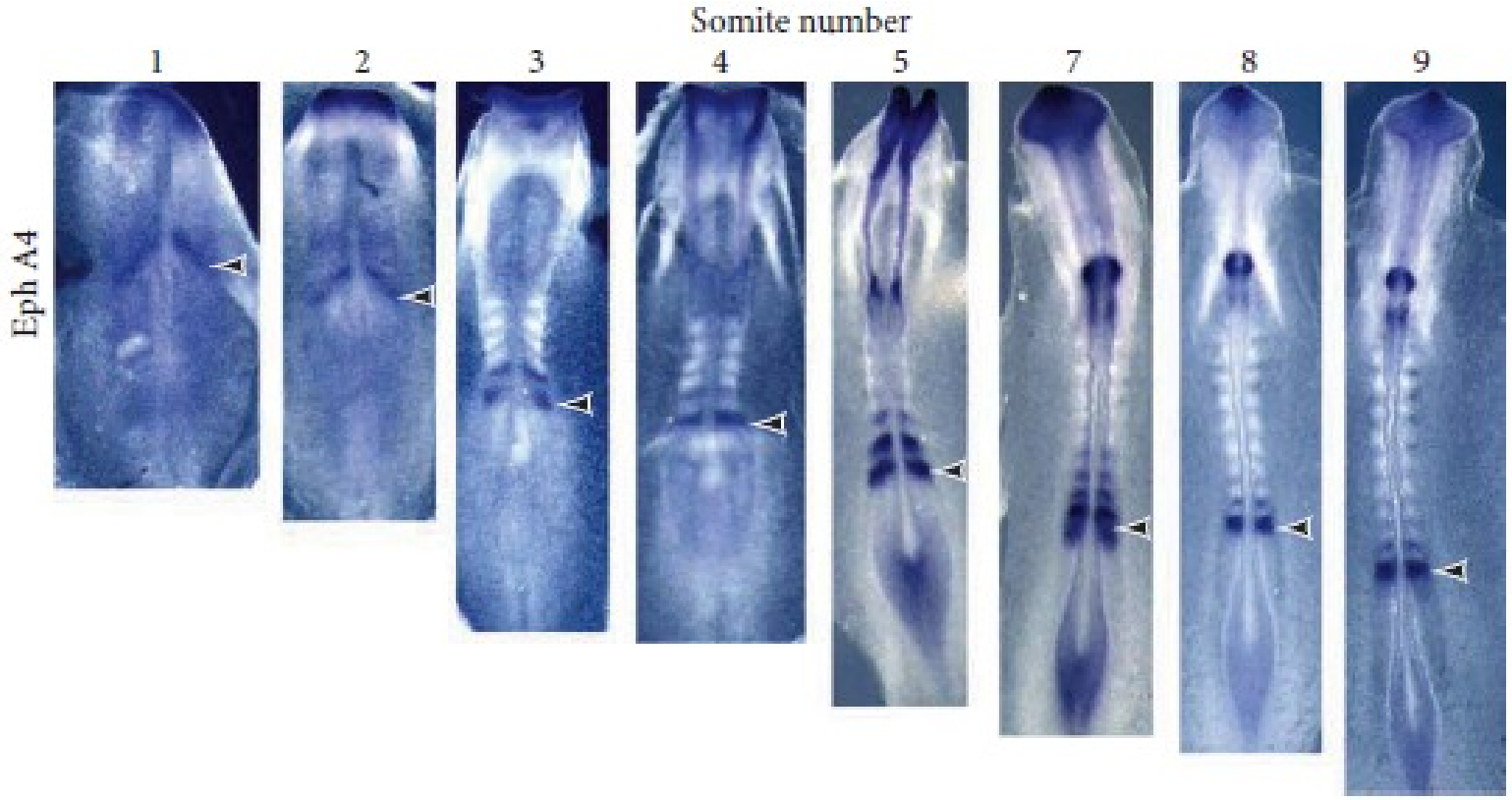
Eph-Ephrin signaling regulates epithelialization during somite boundary formation. Expression of (A) Mesodermal posterior-a (*Mesp-a*; dark purple) and (B) *Eph-A4* (black arrows) and *ephrin-B2* (red arrows) in the paraxial mesoderm of zebrafish embryos (dorsal views). (C) Model of Mesp-a and Eph-Ephrin signaling fostering mesenchymal-to-epithelial transitions that define the apposing cells of a somite boundary. *Mesp-a* becomes restricted to the anterior half of the S-I somitomere, which upregulates *Eph-A4* within this domain. In turn, *Eph-A4* upregulates its binding partner ephrin-B2 in the cells of the presumptive posterior S-0 somitomere, which triggers epithelialization and formation of a boundary. Fissure formation is facilitated by repression of *Cdc42* and activation of integrin ⟨5-fibronectin interactions downstream of *ephrin-B2*.



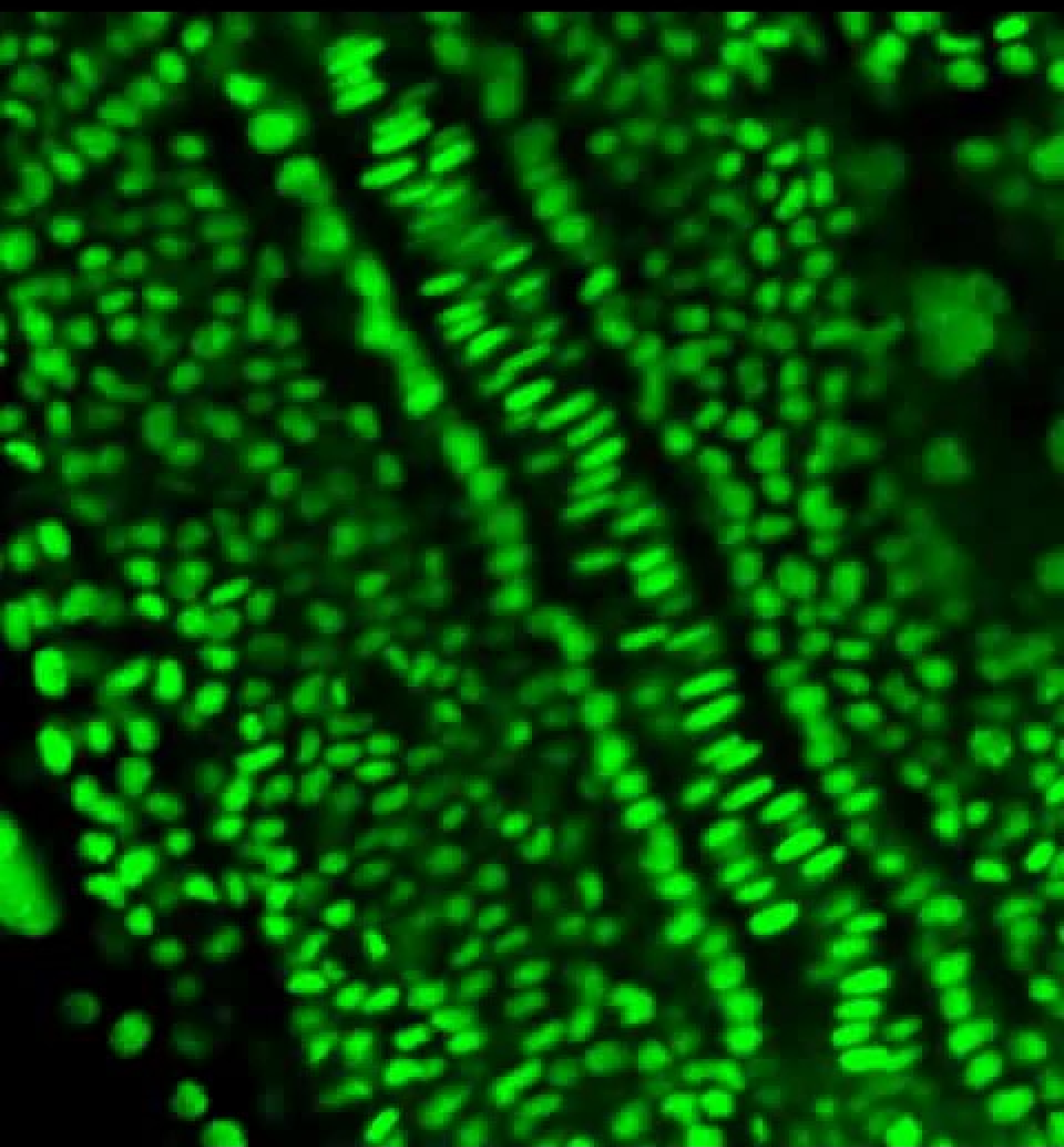




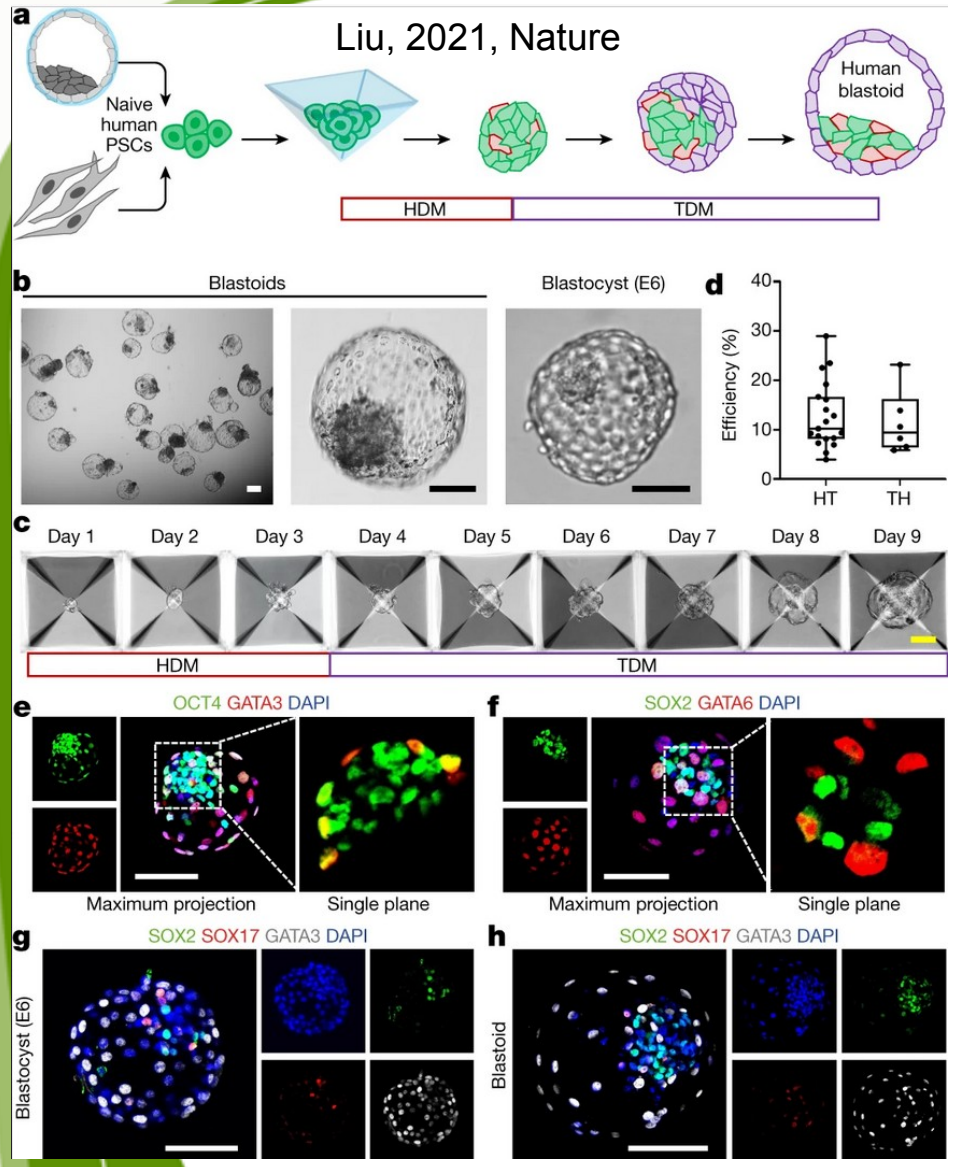
MET – during development – formation of somites



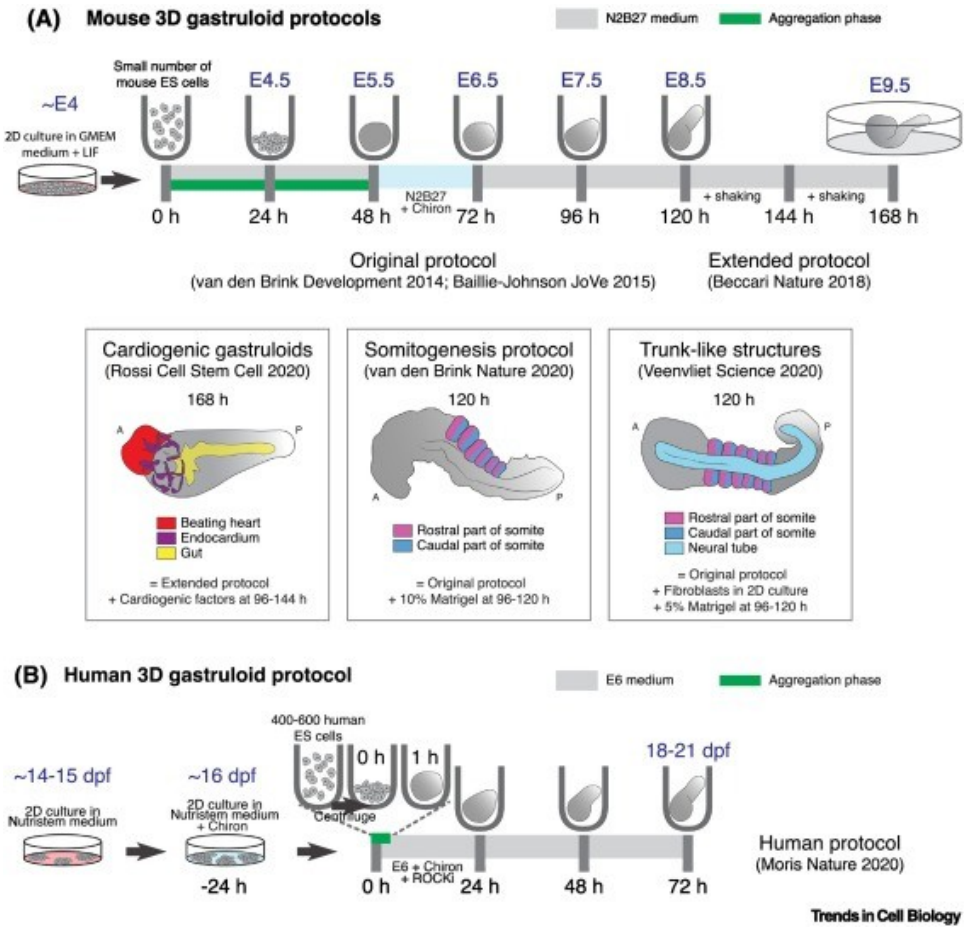
Vývojová biologie



Link to the previous lecture: Do you rember blastoids?

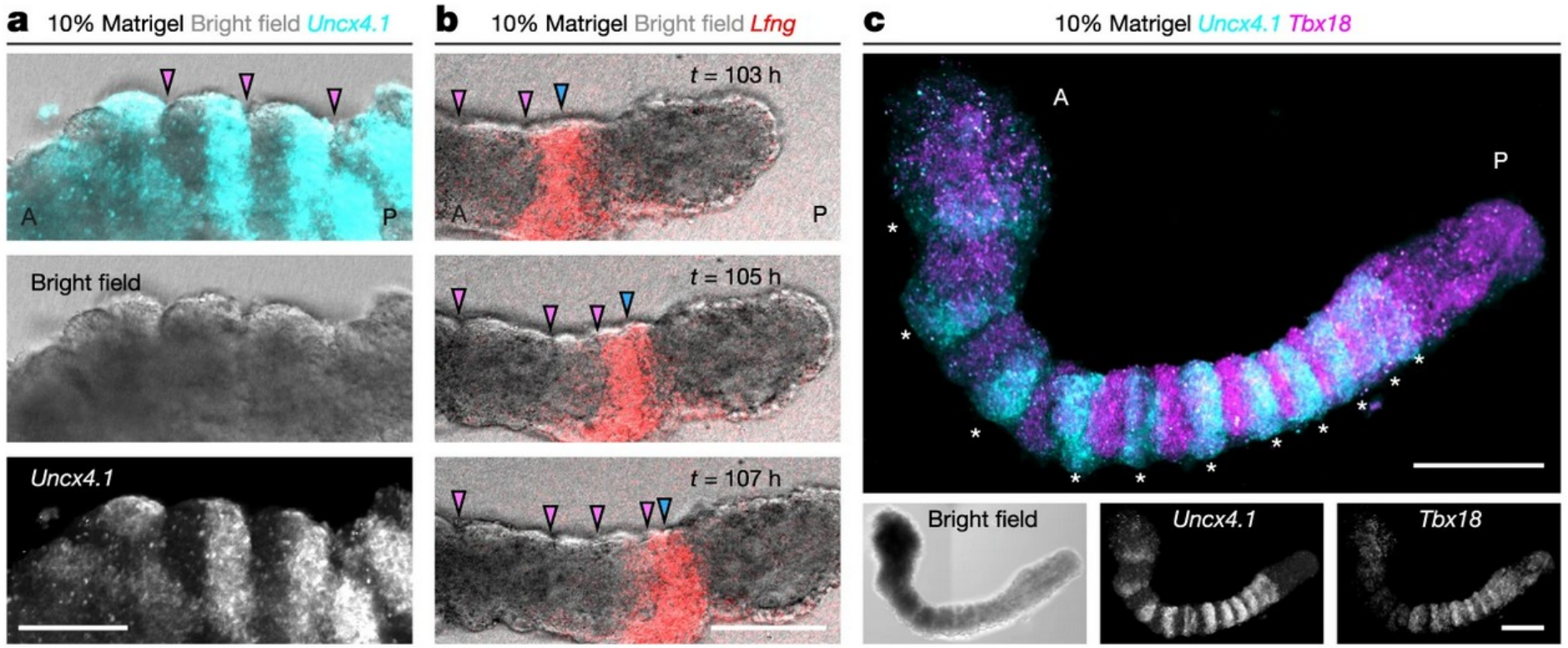


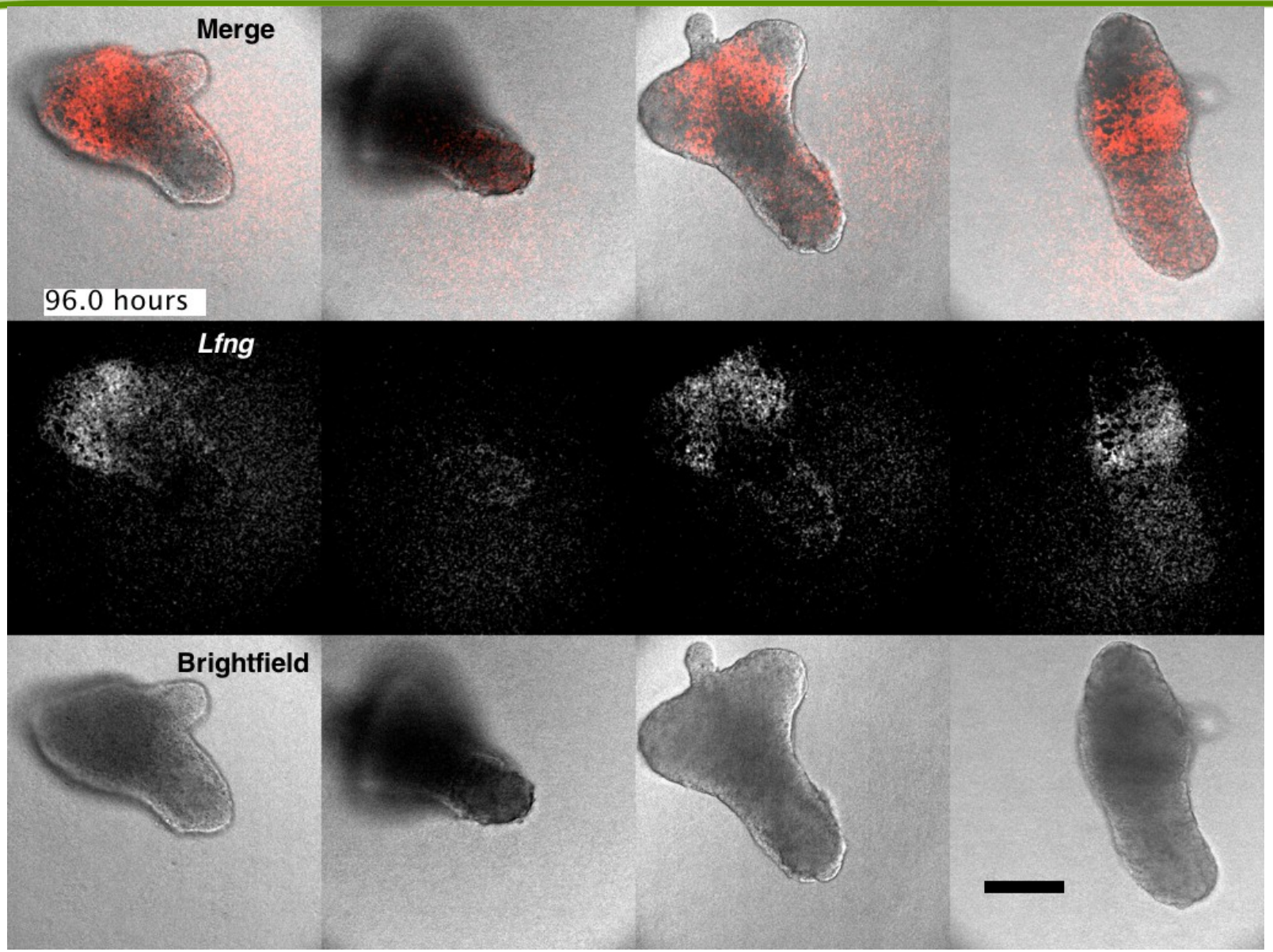
There are gastruloids as well!



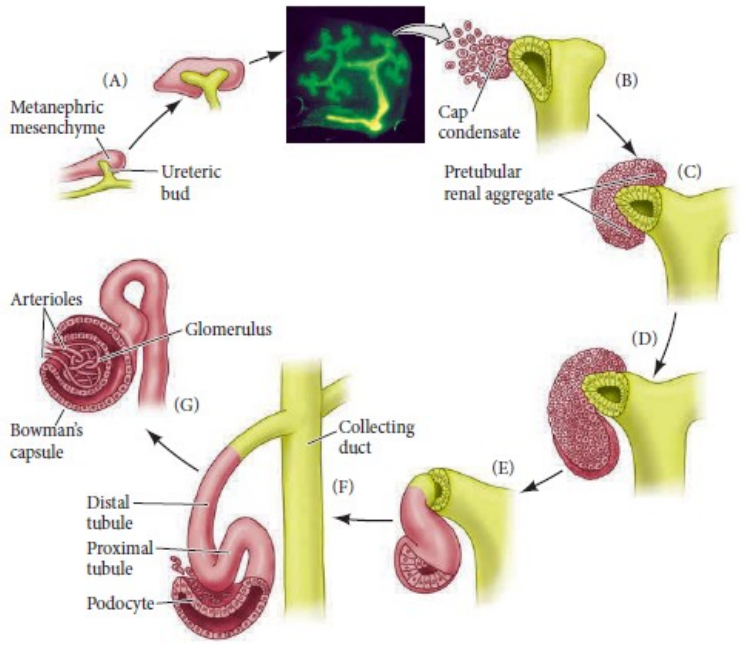
Van der Brink, 2021

Stainings and time-lapse imaging of somite formation in gastruloids

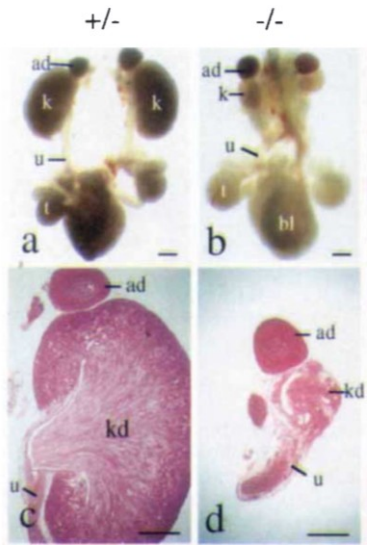


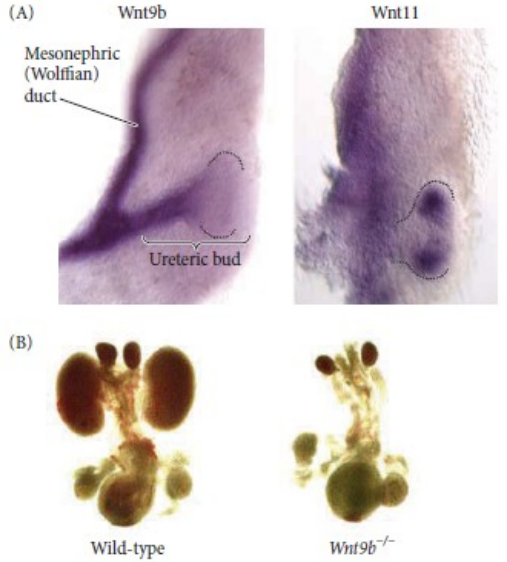

Merge
96.0 hours
Lfng
Brightfield

Another example of MET – Development of nephrons



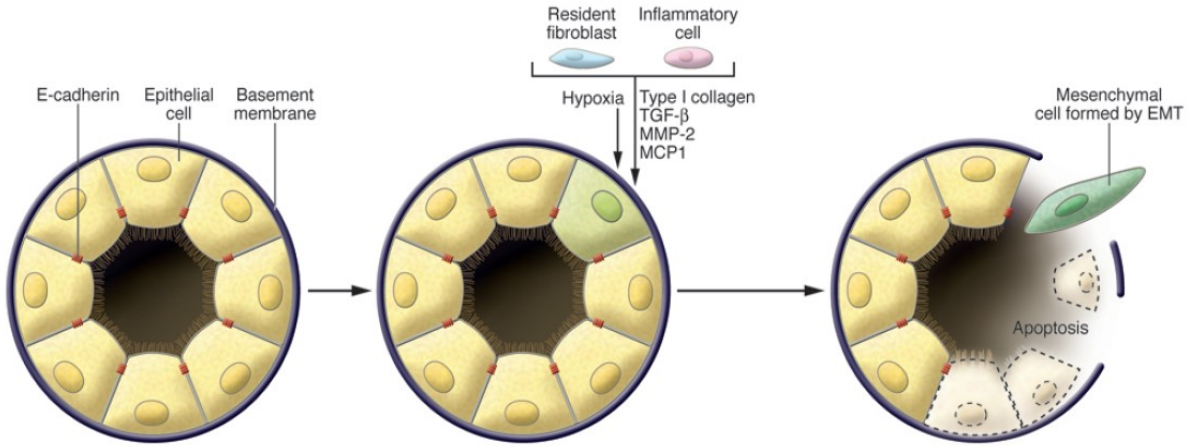
- Cells of mesenchymal origin are able to differentiate into progenitor cells of a nefron.
- They are responsive to Wnt9b and Wnt6 that is produced from uretetic bud
- Wnt9b and 6 are crucial for the transformation of metanefric mesenchyme into tubular epithel
- Mesenchyme has receptors for these Wnt molecules, leading to production of Wnt4 that finishes the transformation.
- In the absence of Wnt5 the mesenchyme is condensed, but epithelium is not formed.

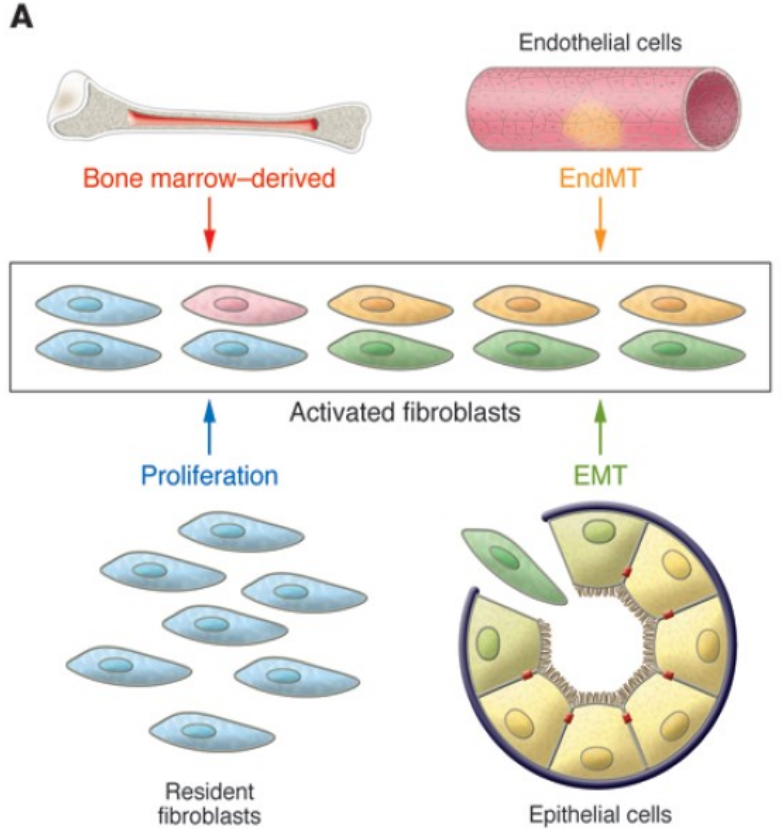




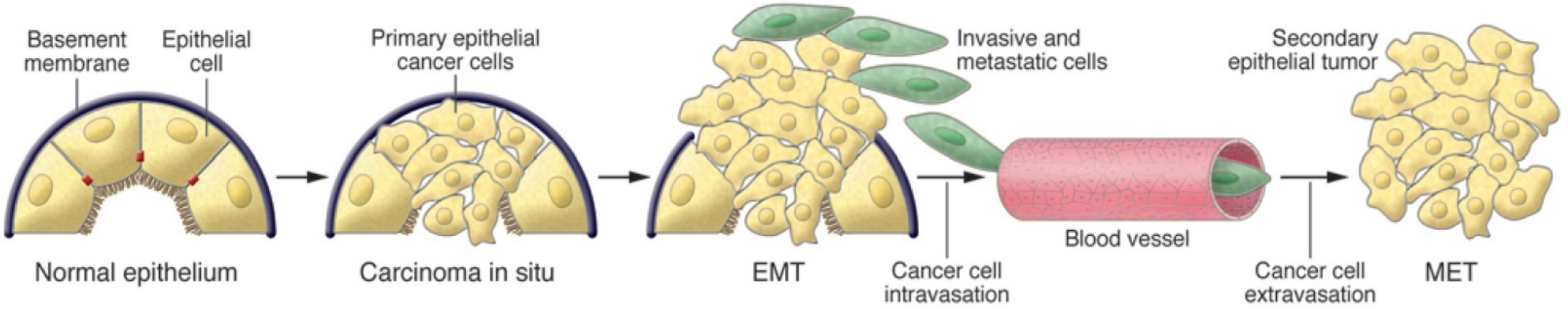
Questions?

EMT – inflamation and fibrosis

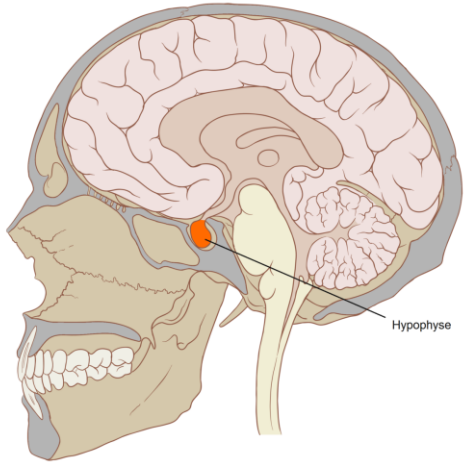


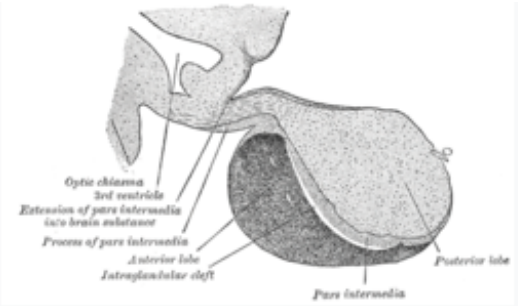


BONUS: EMT - cancer



BONUS: Embryonic origin of pituitary gland (hypophysis)





Median sagittal through the hypophysis of an adult monkey. Semidiagrammatic.

Details	
Precursor	neural and oral ectoderm, including Rathke's pouch
Artery	superior hypophyseal artery, infundibular artery, prechiasmal artery, inferior hypophyseal artery, capsular artery, artery of the inferior cavernous sinus[1]
Identifiers	
Latin	*hypophysis, glandula pituitaria*
MeSH	D010902
NeuroLex ID	birnlex_1353
TA98	A11.1.00.001
TA2	3853
FMA	13889

Anatomical terms of neuroanatomy

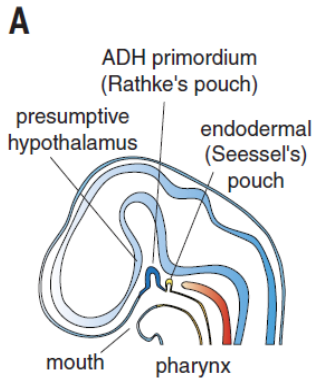
BONUS: Embryonic origin of pituitary gland (hypophysis)

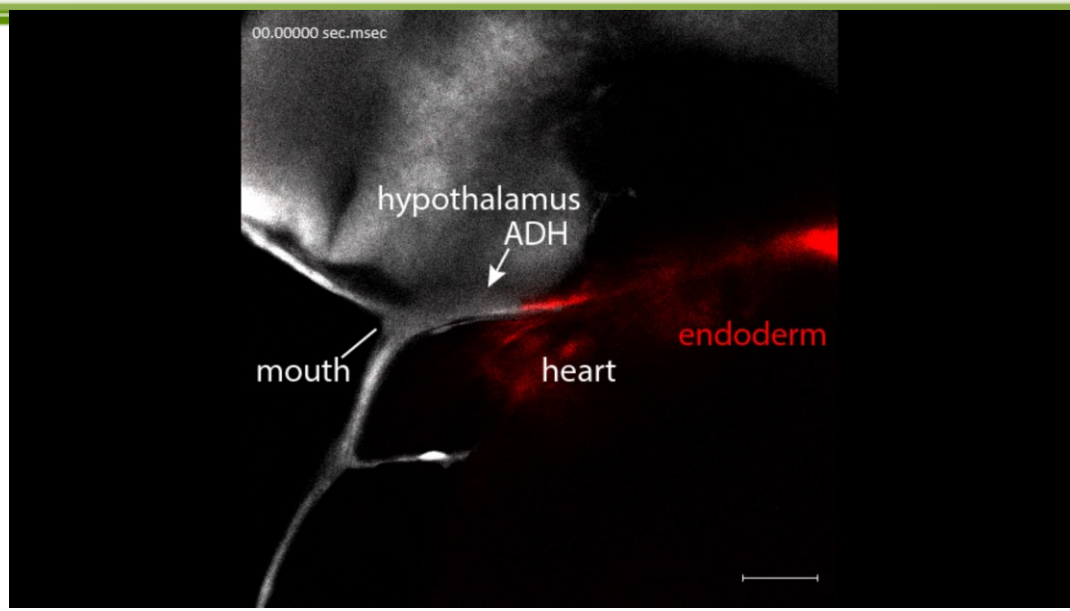
RESEARCH

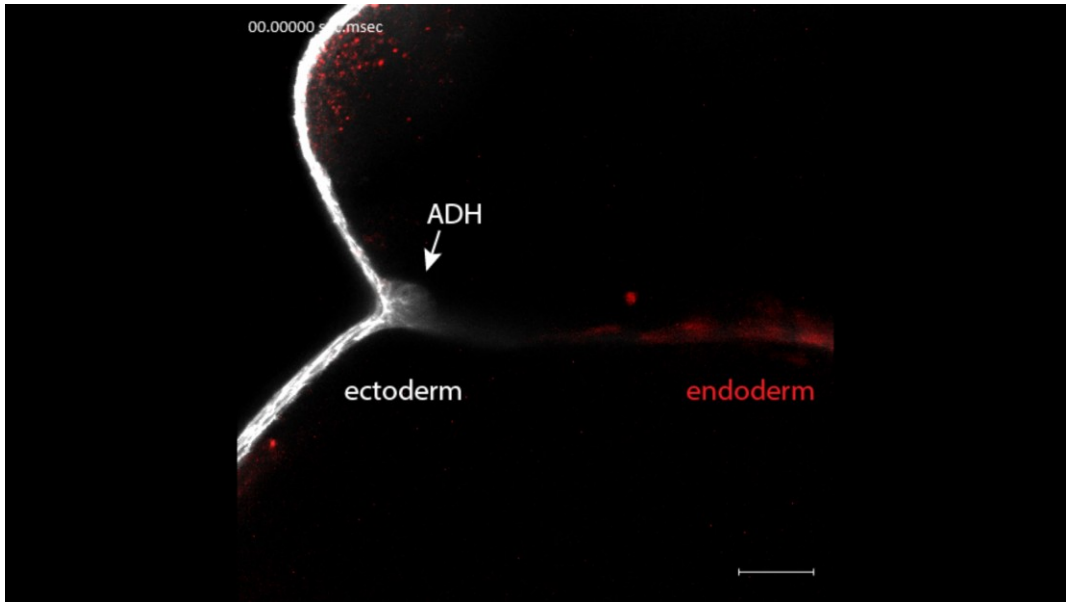
DEVELOPMENTAL BIOLOGY

Lineage analysis reveals an endodermal contribution to the vertebrate pituitary

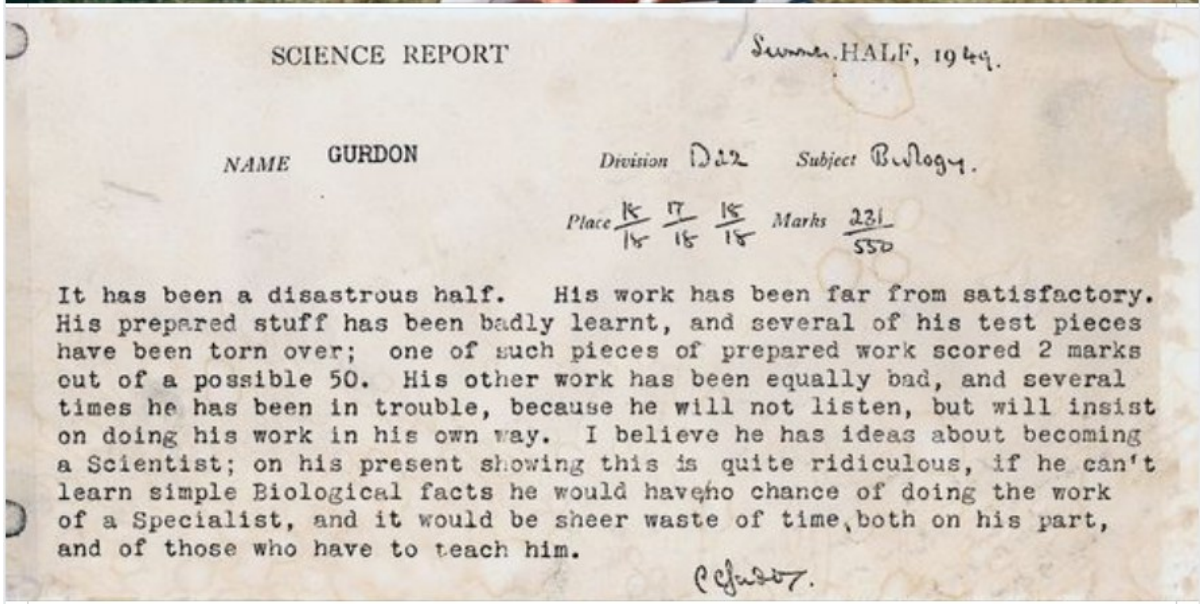
Peter Fabian[1], Kuo-Chang Tseng[1], Joanna Smeeton[1,2], Joseph J. Lancman[3], P. Duc Si Dong[3,4], Robert Cerny[5], J. Gage Crump[1]*







Thank you for your attention



SCIENCE REPORT

Summer HALF, 1949.

NAME GURDON Division D22 Subject Biology.

Place 14/18 17/18 15/18 Marks 231/550

It has been a disastrous half. His work has been far from satisfactory. His prepared stuff has been badly learnt, and several of his test pieces have been torn over; one of such pieces of prepared work scored 2 marks out of a possible 50. His other work has been equally bad, and several times he has been in trouble, because he will not listen, but will insist on doing his work in his own way. I believe he has ideas about becoming a Scientist; on his present showing this is quite ridiculous, if he can't learn simple Biological facts he would have no chance of doing the work of a Specialist, and it would be sheer waste of time, both on his part, and of those who have to teach him.

Tomáš Bárta
tbarta@med.muni.cz