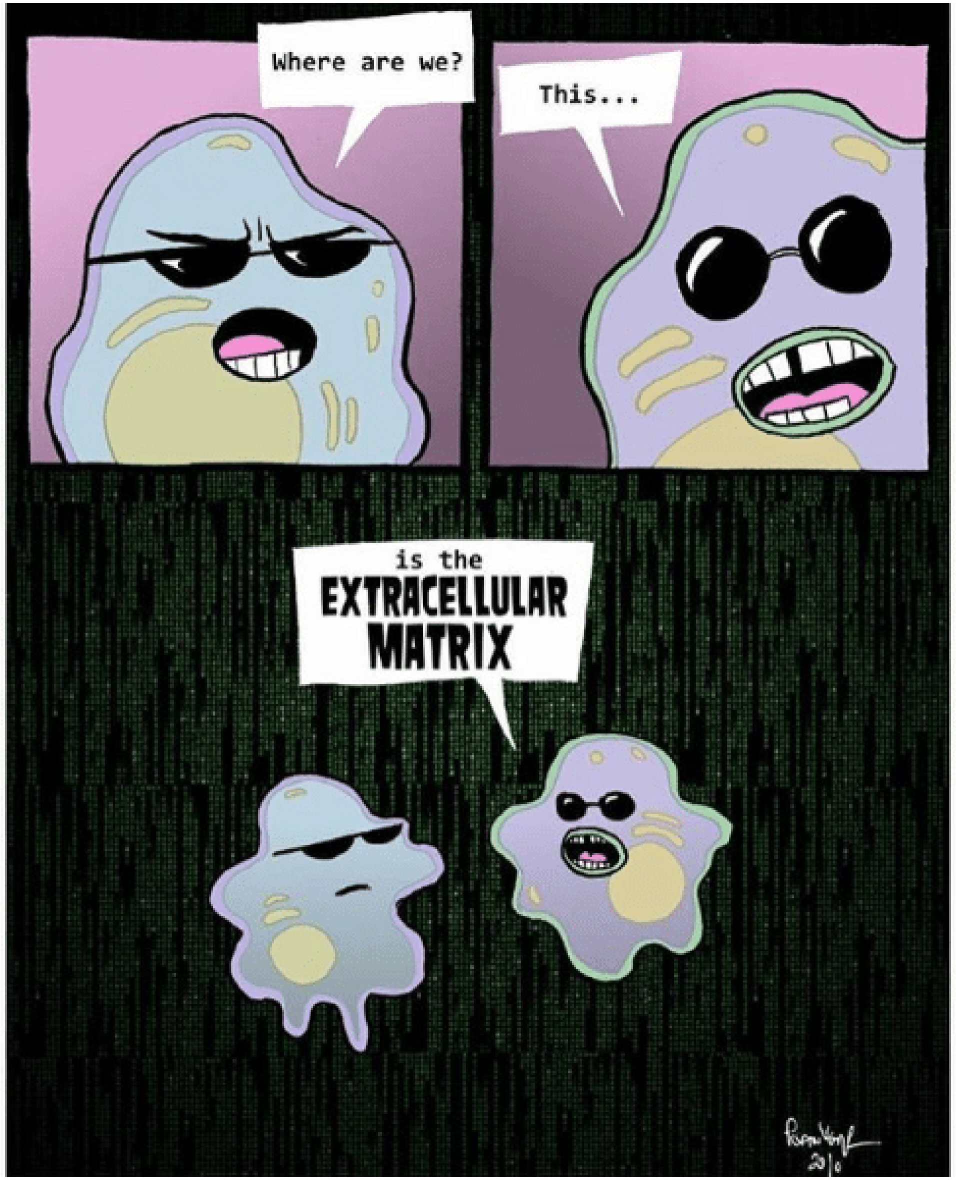
Vývojová biologie

The role of the extracellular matrix in development



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Vývojová biologie

Contents

Introduction - Extracellular matrix (ECM)

Cell → Tissue → Organ → Individual

- What holds cells together to form tissue?
- How do cells and tissues interact with each other?
- How do they coordinate to form the whole individual?

?

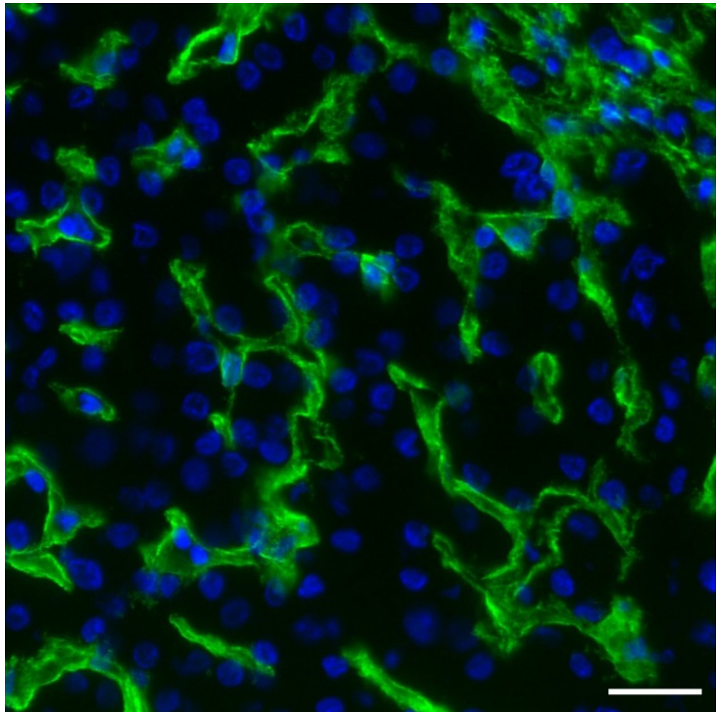
ECM – **E**xtra **C**ellular **M**atrix

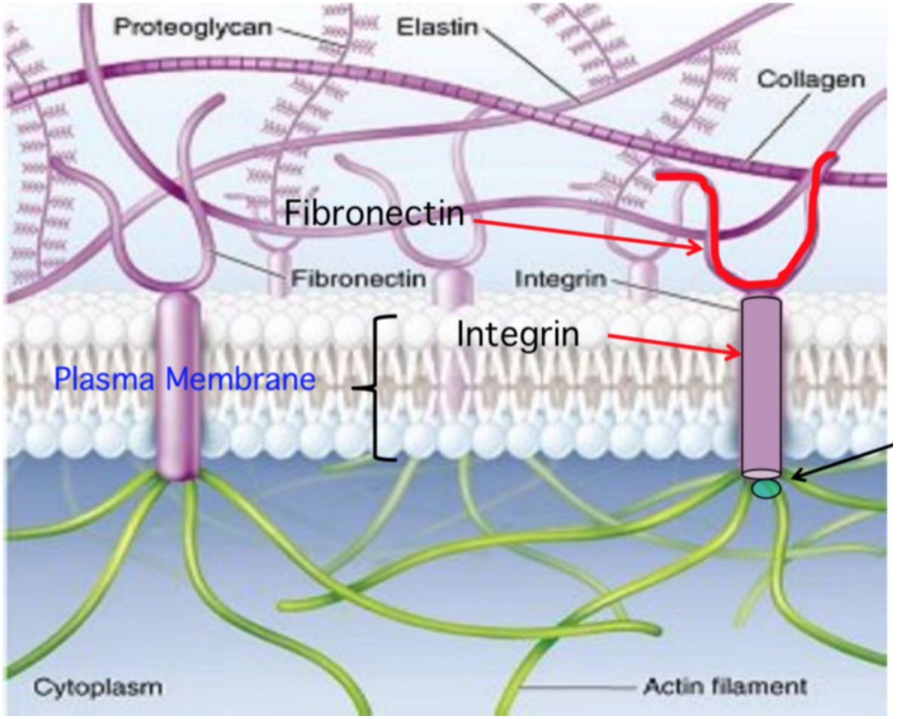
What is an extracellular matrix (ECM)?

- It is a three-dimensional insoluble network of extracellular macromolecules such as collagen, enzymes and glycoproteins, etc., which provide structural and biochemical support to surrounding cells.
- It mediates cell adhesion, communication between cells and differentiation.
- It instructs cells "when to grow, when to divide, when to produce different molecules, when to die."
- It consists of about 300 proteins, mainly: collagen, proteoglycans, elastin, glycoproteins (fibronectin and laminin). Each component has certain physical and biochemical properties.

Introduction - Extracellular matrix (ECM)

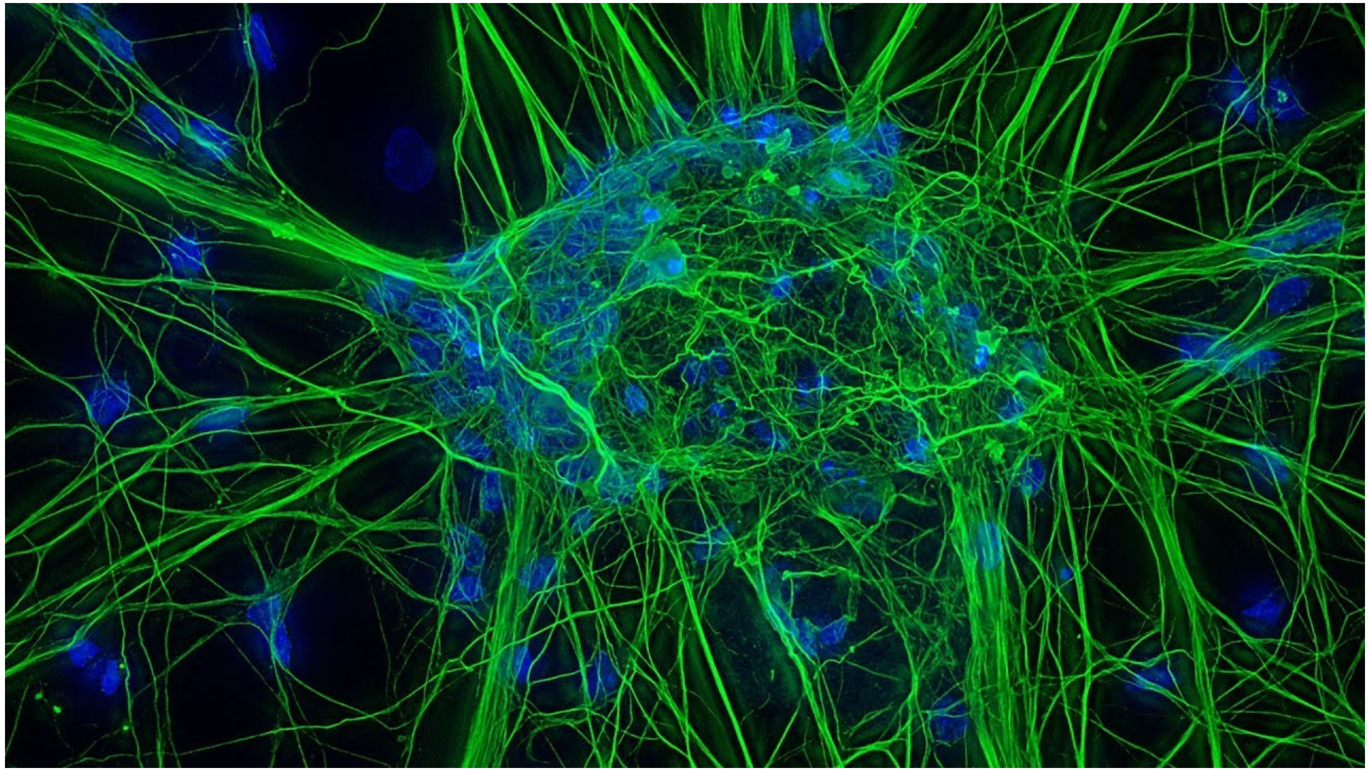
- There is a certain analogy between cytoskeleton and ECM
- Just as the cytoskeleton determines the internal structure of the cell, participates in movement, transport, etc., so the ECM has a similar function but outside the cell and at the level of tissues and organs.
- ECM is composed of many types of macromolecules, fibers, proteins, glycoproteins, but most collagen (about 30% of all proteins).



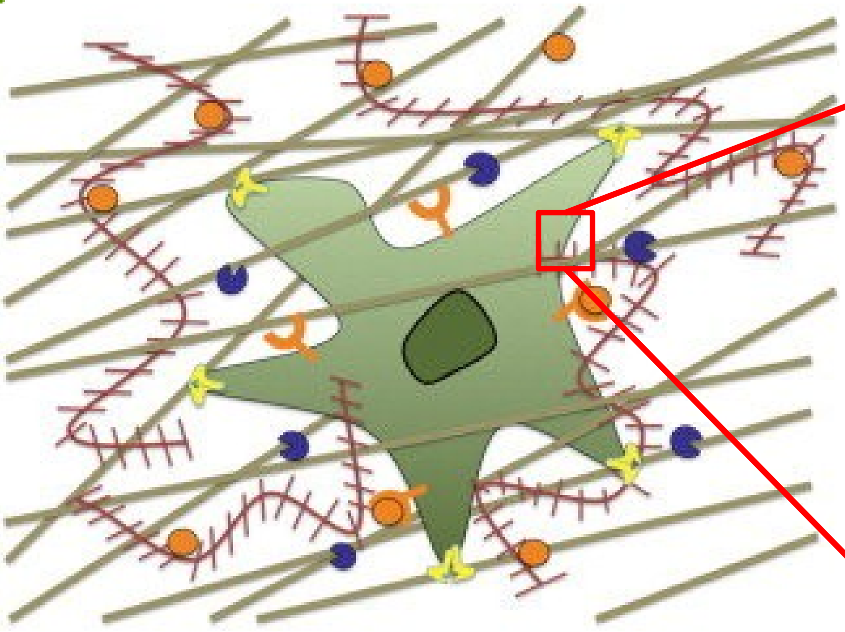


Introduction - Extracellular matrix (ECM)

- All ECM components help to attach cells and structure cells into tissues.
- **But they also give cells a signal of when to grow, when to divide, when to produce certain molecules, and even when to die.**



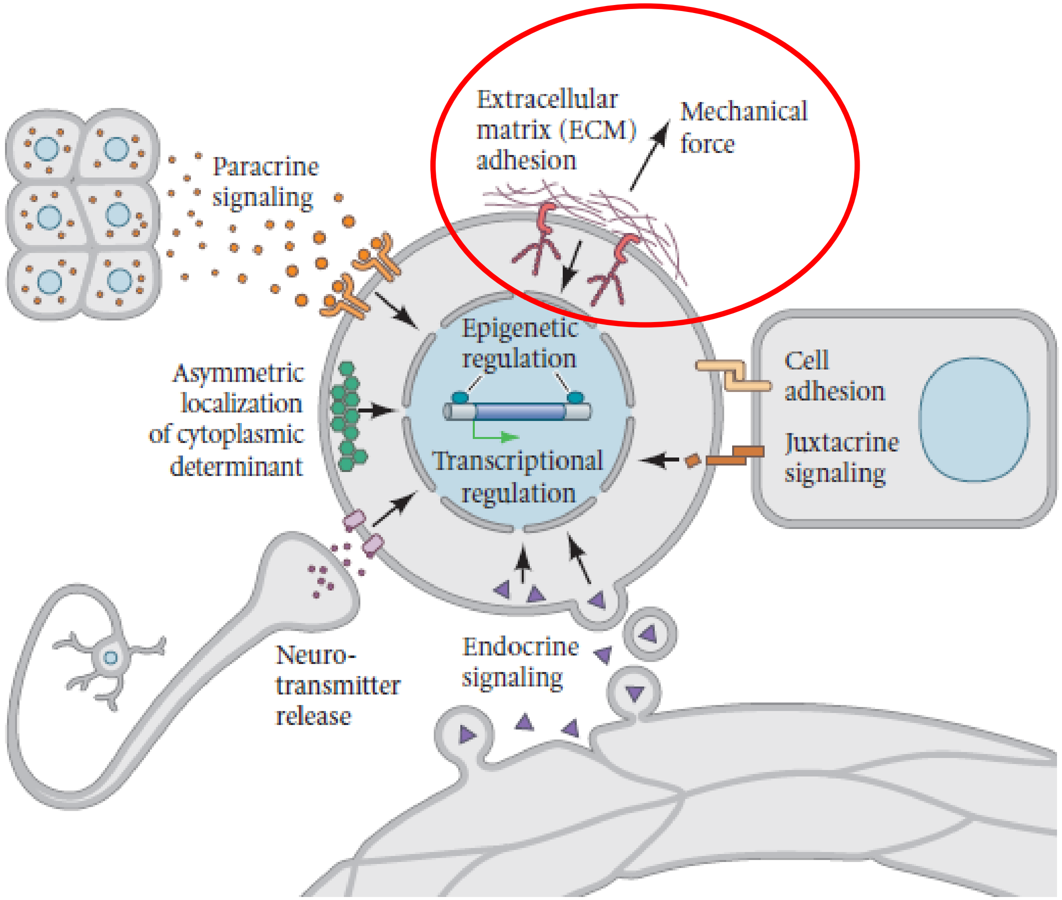
Extracellular matrix (ECM)

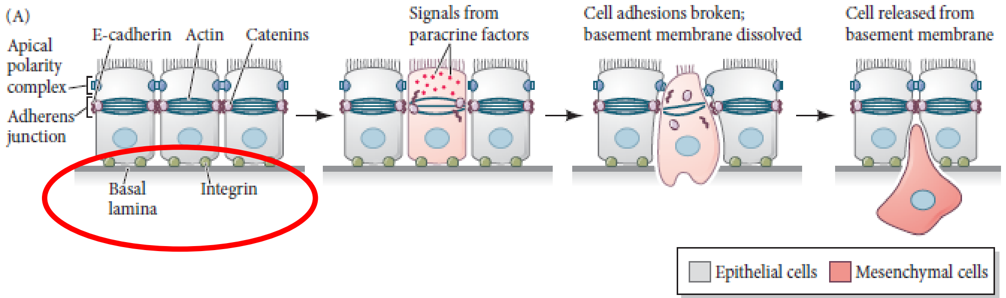


- Glycoproteins
- Proteoglycans
- Fibrous proteins
- Polysaccharides
-
-
- Collagen is the most represented (~30% of all proteins in mammals)

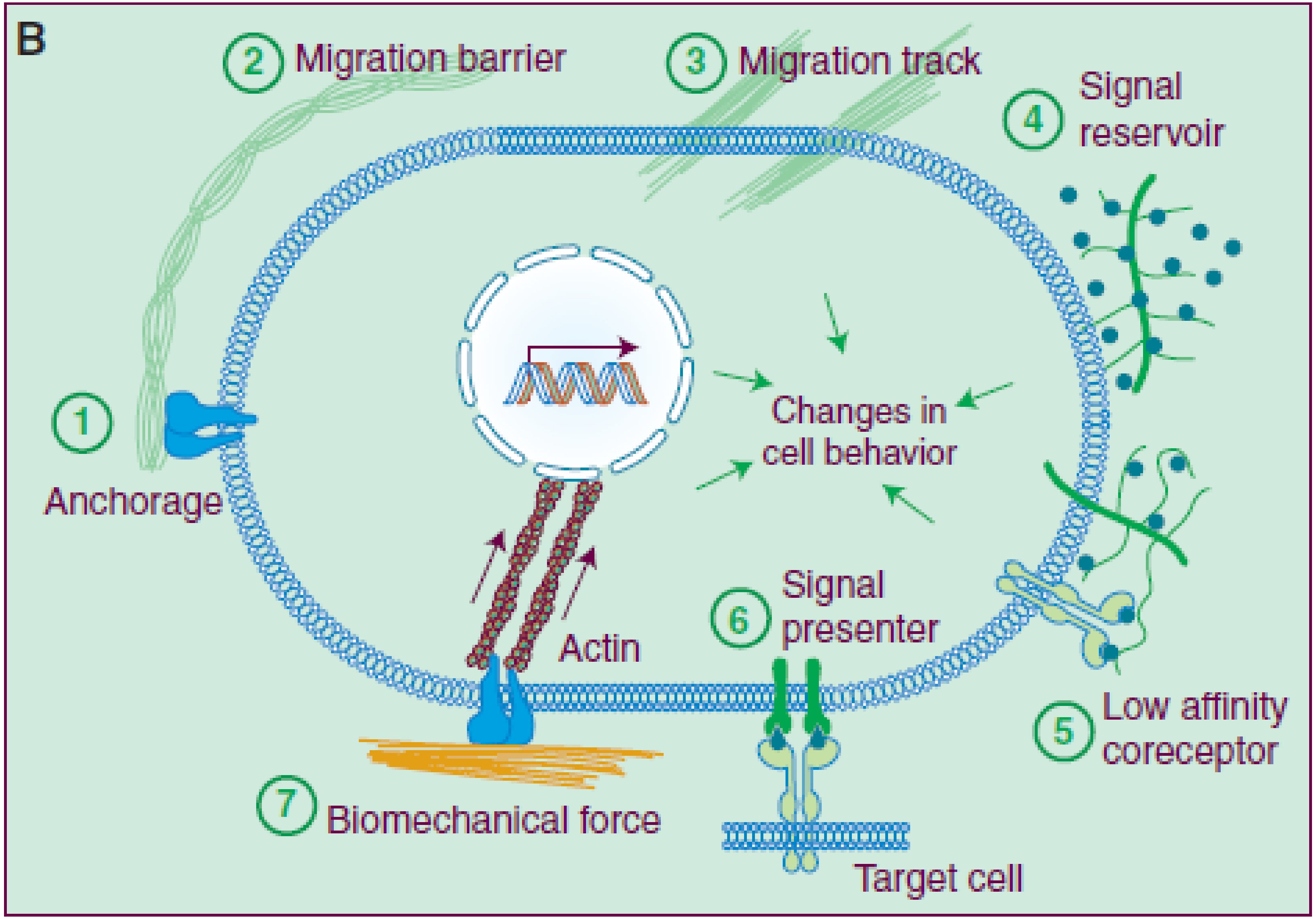
Integrins "connect" the cytoskeleton with the ECM

Link to the previous lecture – what you already know

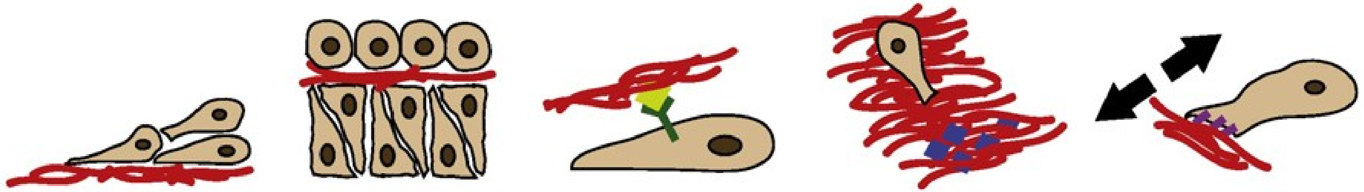




What is extracellular matrix (ECM) - Features



What is extracellular matrix (ECM) - Features



Functions as adhesive substrate

- *tracks to direct migratory cells*
- *concentration gradients for haptotactic migration*

Provides structure

- *defines tissue boundaries*
- *provides integrity and elasticity to developing organs*
- *degraded by invasive cells during development and disease*

Presents growth factors to their receptors

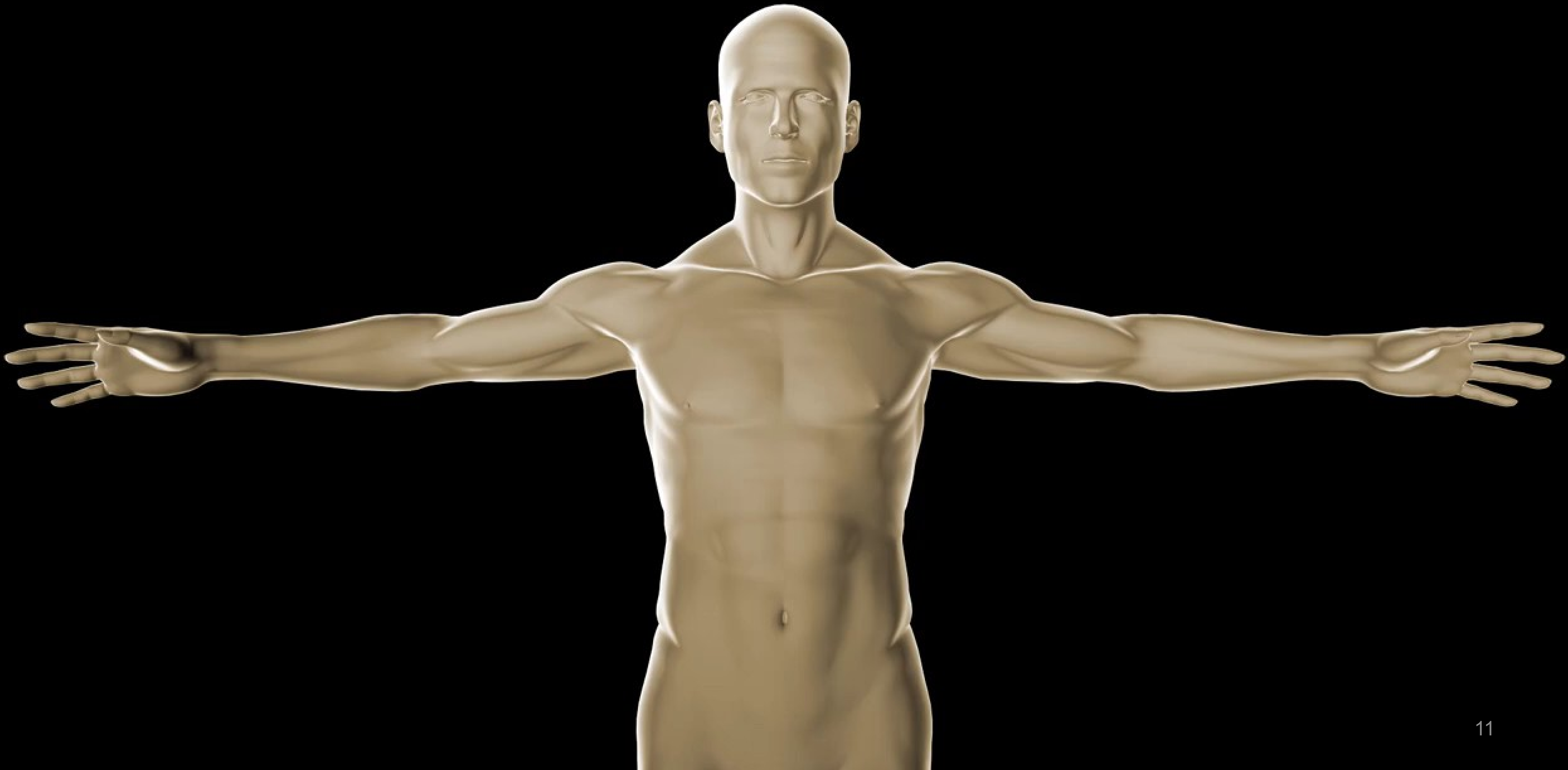
- *controls spatial distribution of ECM-bound surface molecules*
- *facilitates crosstalk between growth factor receptors and ECM receptors*

Sequesters and stores growth factors

- *allows for spatio-temporal regulation of factor release*
- *organizes morphogen gradients*
- *mediates release of factors in the presence of appropriate cell-mediated forces or proteolytic degradation*

Senses and transduces mechanical signals

- *defines mechanical properties permissive/ instructive to cell differentiation*
- *activates intracellular signaling through interaction with cell-surface receptors*
- *engages cytoskeletal machinery and synergizes with growth factor signaling*



Components of ECM – how to understand it?

Vývojová biologie

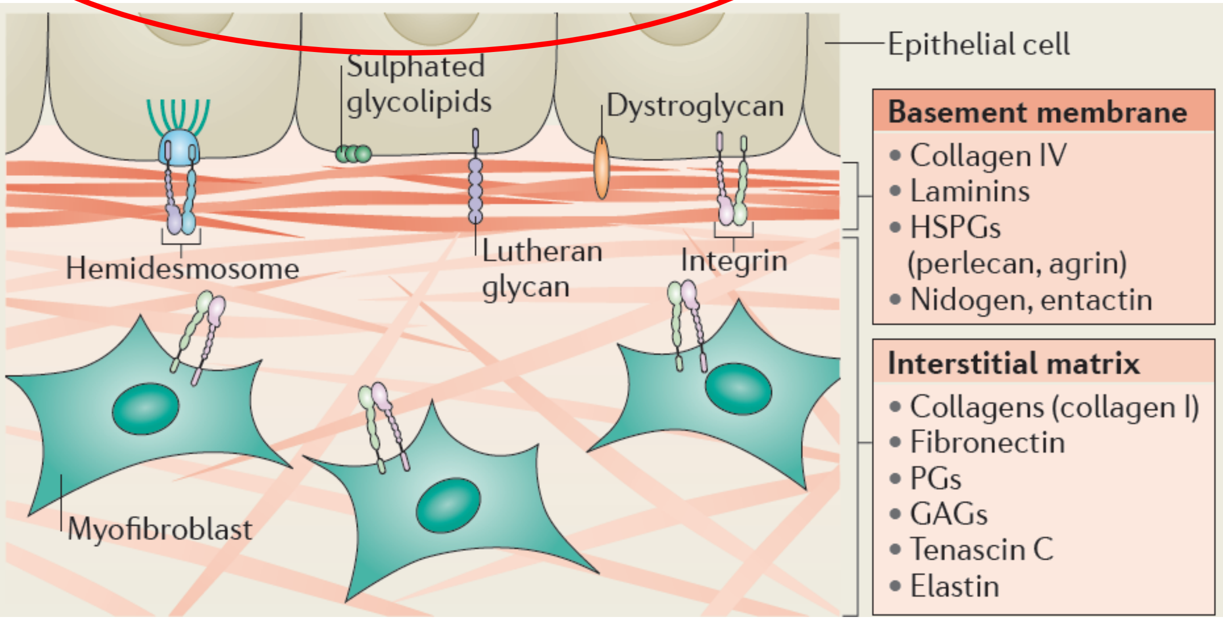
Components of the ECM

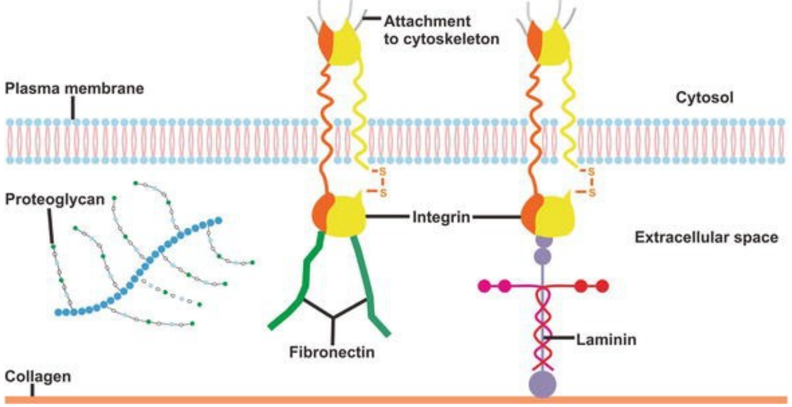
Proteoglycans — Heparan sulfate, Chondroitin sulfate

Glycoproteins — Laminin, Fibronectins

Proteins — Collagens

Polysaccharides — Hyaluronic acid.

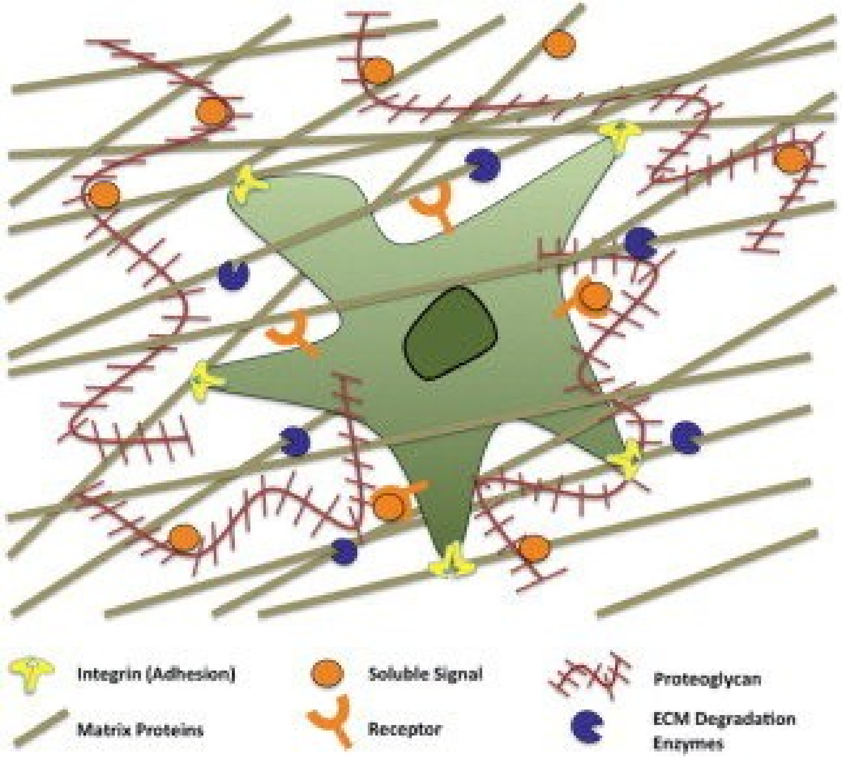


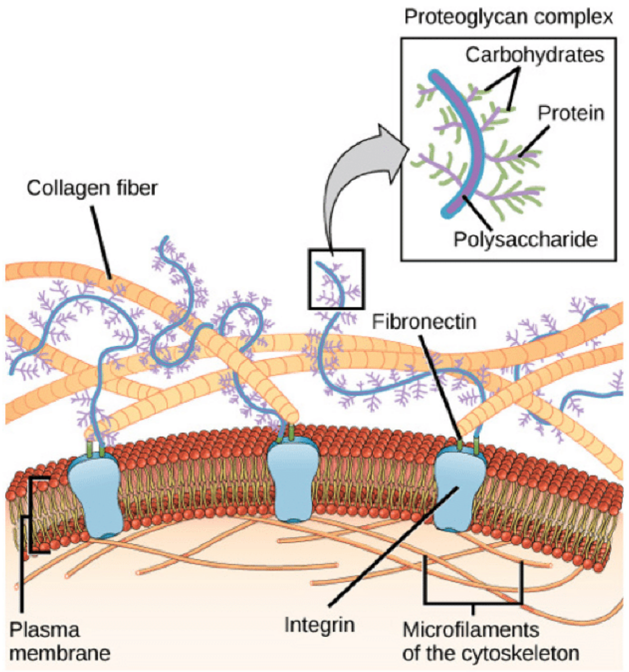


Kusindarta, 2018

Components of ECM - Proteoglycans

- A key role in the transport of paracrine signals.
- Large molecules that consist of a central protein and a covalently bound polysaccharide chain.
- Examples include: Heparan Sulfate, Chondroitin Sulfate.

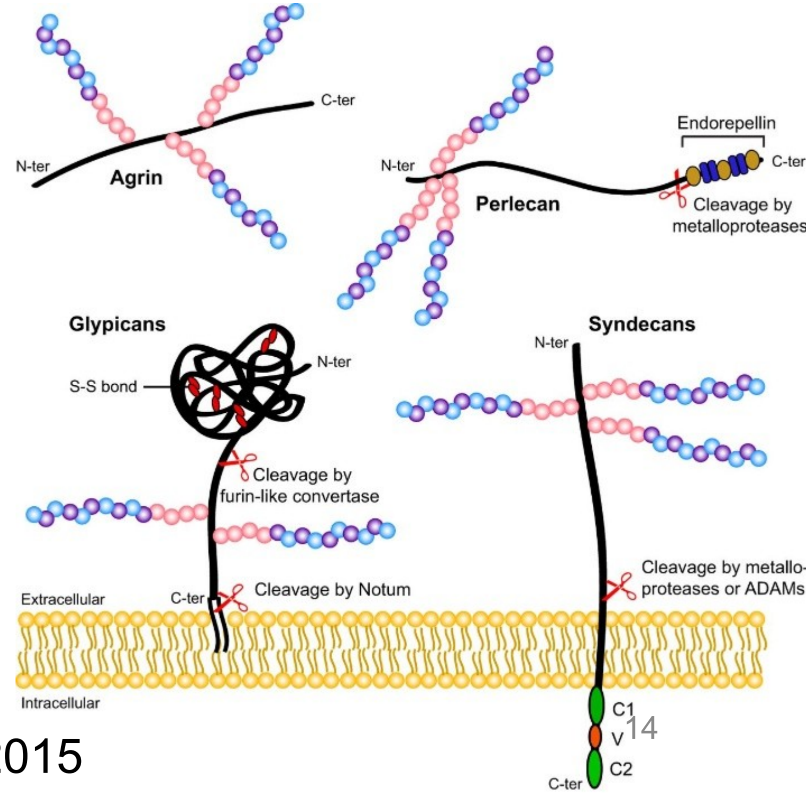




Components of ECM – Proteoglycans – Heparan sulfate (HS)

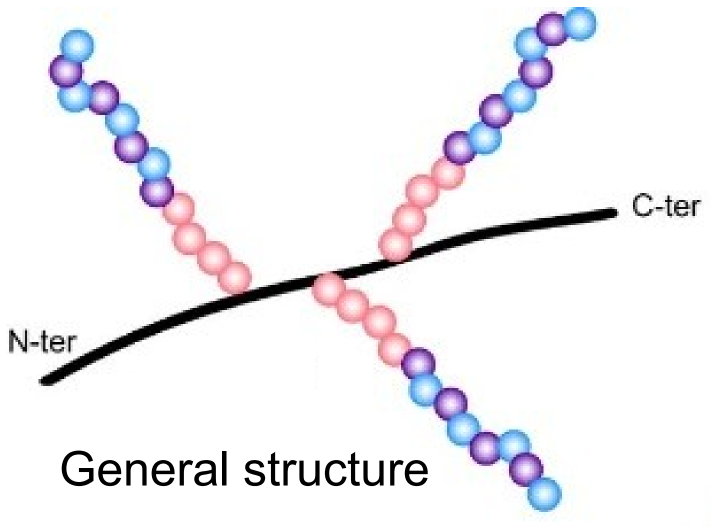
Heparan sulfate (HS)

- Linear polysaccharide, which occurs in the form of proteoglycan (a protein with covalently bound HS chains) – in almost all animal tissues
- A key role in ligand binding to -> receptors does not have to take place without it!
- Mutations that block the synthesis of Heparan sulfate cause various defects in cell migration, morphogenesis and differentiation.
- Different organs and tissues during development produce different compositions of HS
- HS changes during development => important for proper development.
- These findings led to the "sugar code" hypothesis – specific HS - modification controls individual events during development through interaction with signal pathways.
- many organs, including hematopoietic, skeletal systems and liver, the lungs and kidneys do not form properly if HS is absent.



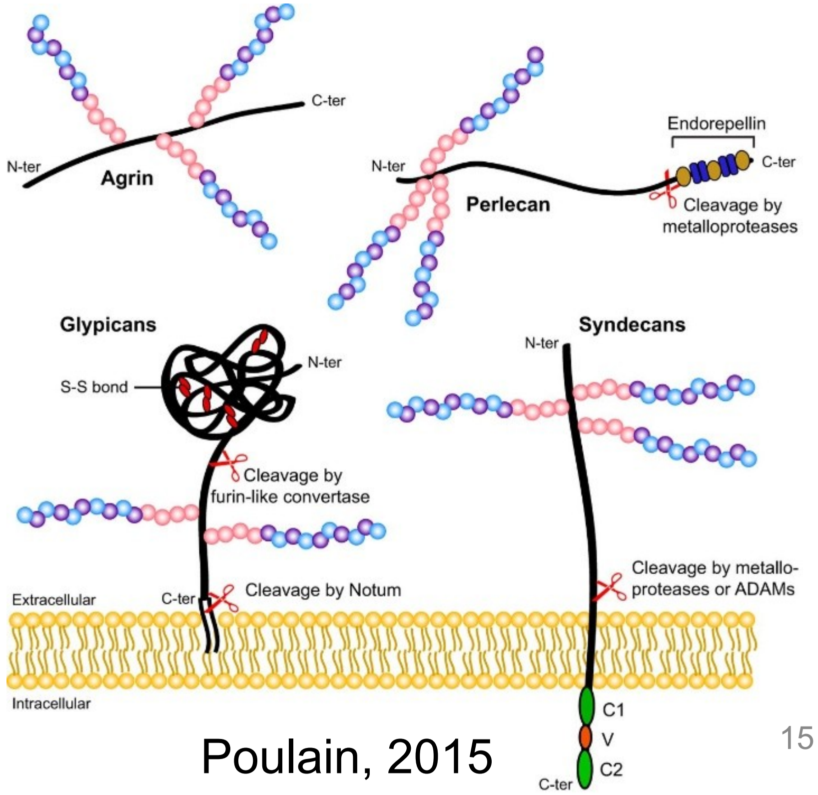
Poulain, 2015

Components of ECM – Proteoglycans – Heparan sulfate



General structure

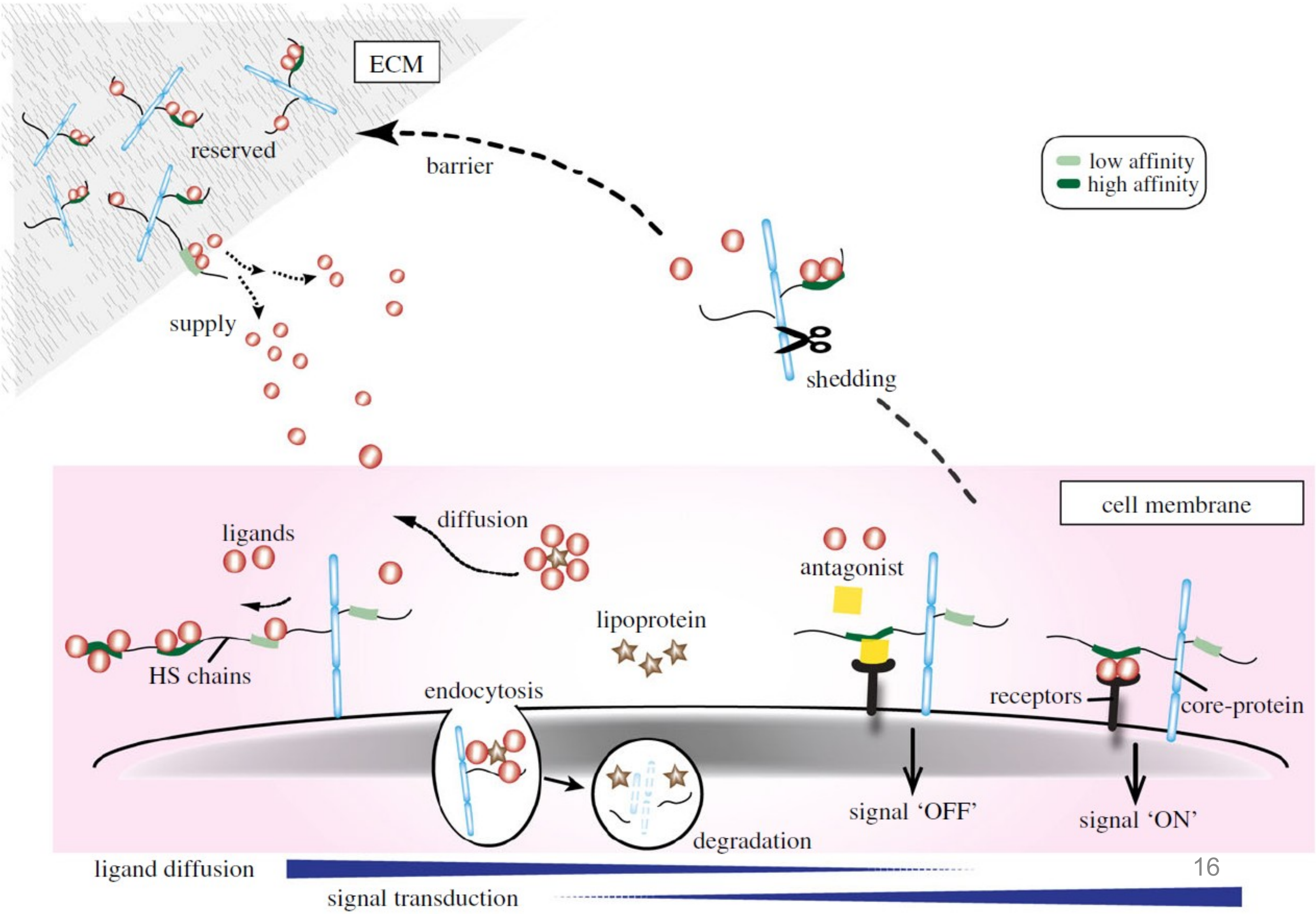
- Proteins have a different number of places for carbohydrate binding
- Some may also contain chondroitin sulfate
- HS is synthesized in the Golgi apparatus



Poulain, 2015

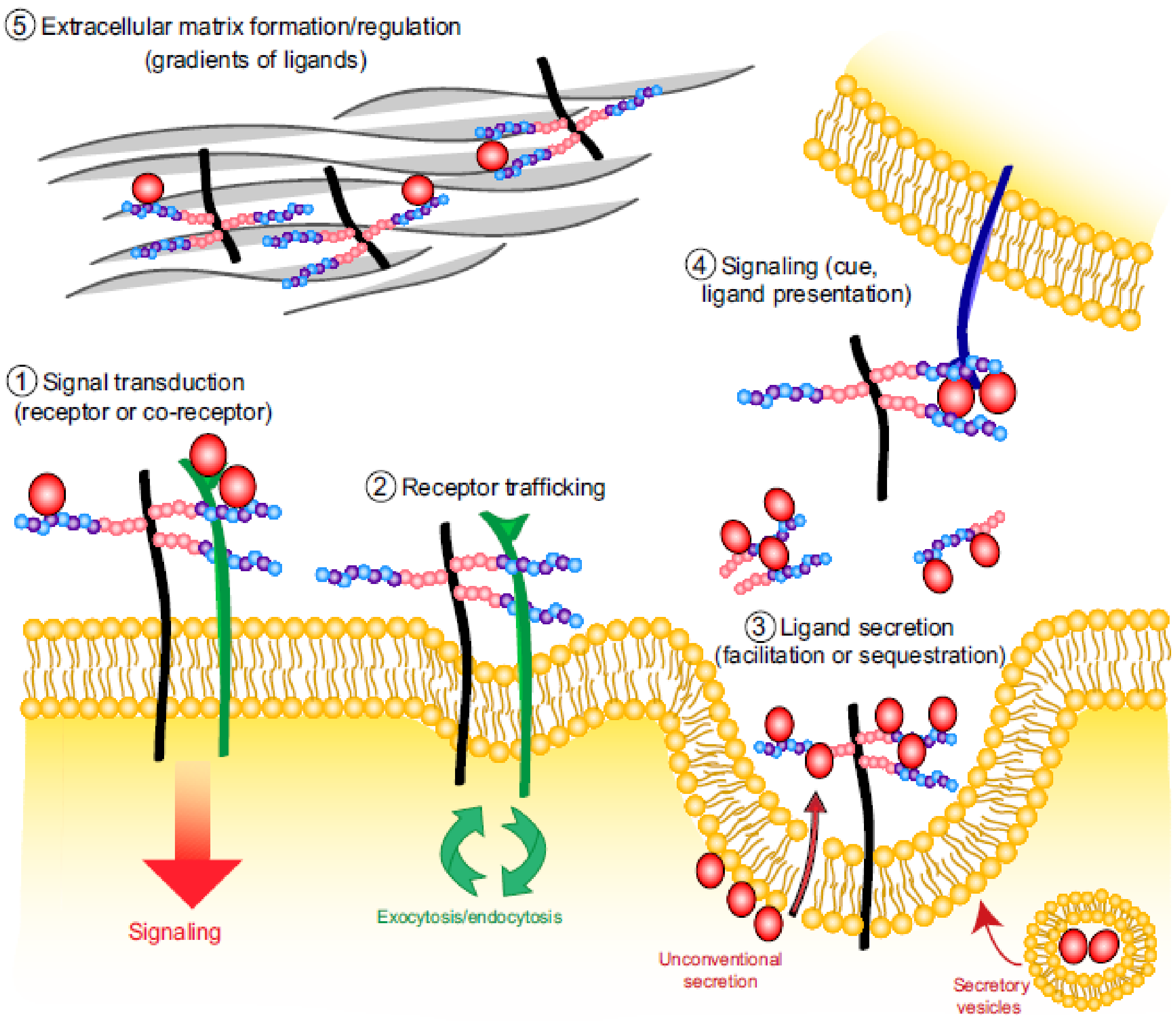
Components of ECM – Proteoglycans – Heparan sulfate – features

Function



Components of ECM – Proteoglycans – Heparan sulfate – features

Function

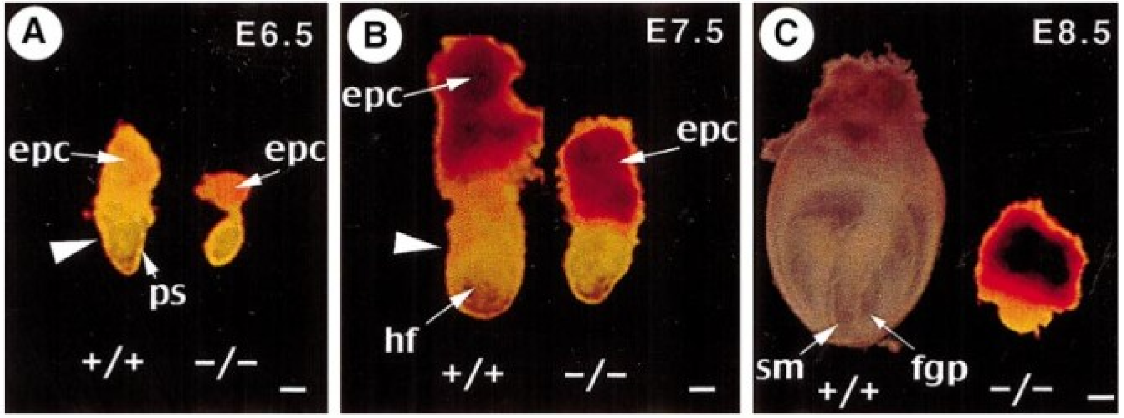


- As receptors/co-receptors (Wnt, FGF, Shh)
- Increase in the concentration of the ligand, or receptor on the surface of cells in lipid rafts
- Regulation of receptor signaling
- Control of ligand secretion
- Directly as signaling molecules
- Checking the distribution of signal gradients.
- ECM component

Components of ECM – Proteoglycans – Heparan sulfate – features

Exostosin Glycosyltransferase 1 Ext1 -/-

Inhibition of HS synthesis - > disruption of gastrulation

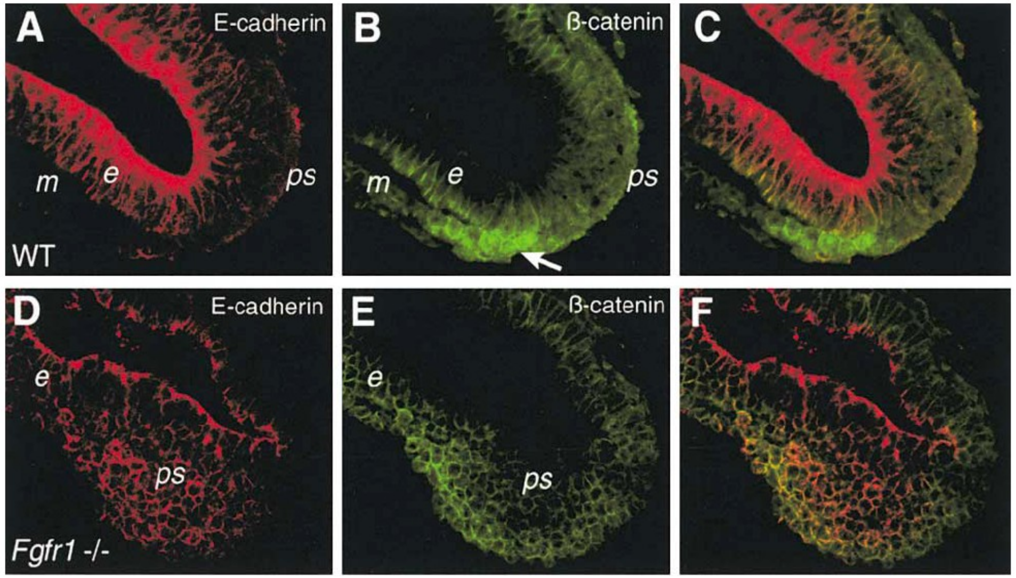


Lin et al., 2000

This defect is due to a failure in the production of heparan sulfate to which Hedgehog binds and uses as part of its diffusion mechanism

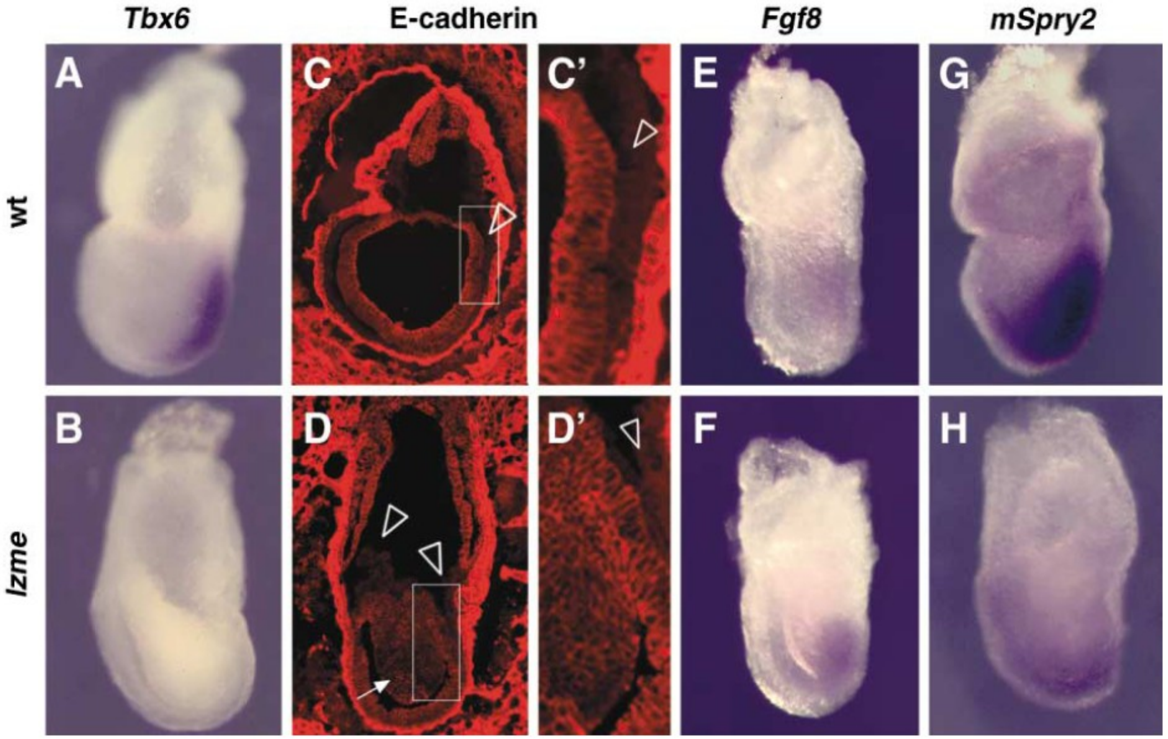
Components of ECM – Proteoglycans – Heparan sulfate

- Link to the previous lecture



Ciruna and Rossant, 2001

- => Heparan sulfate is key for FGF signaling

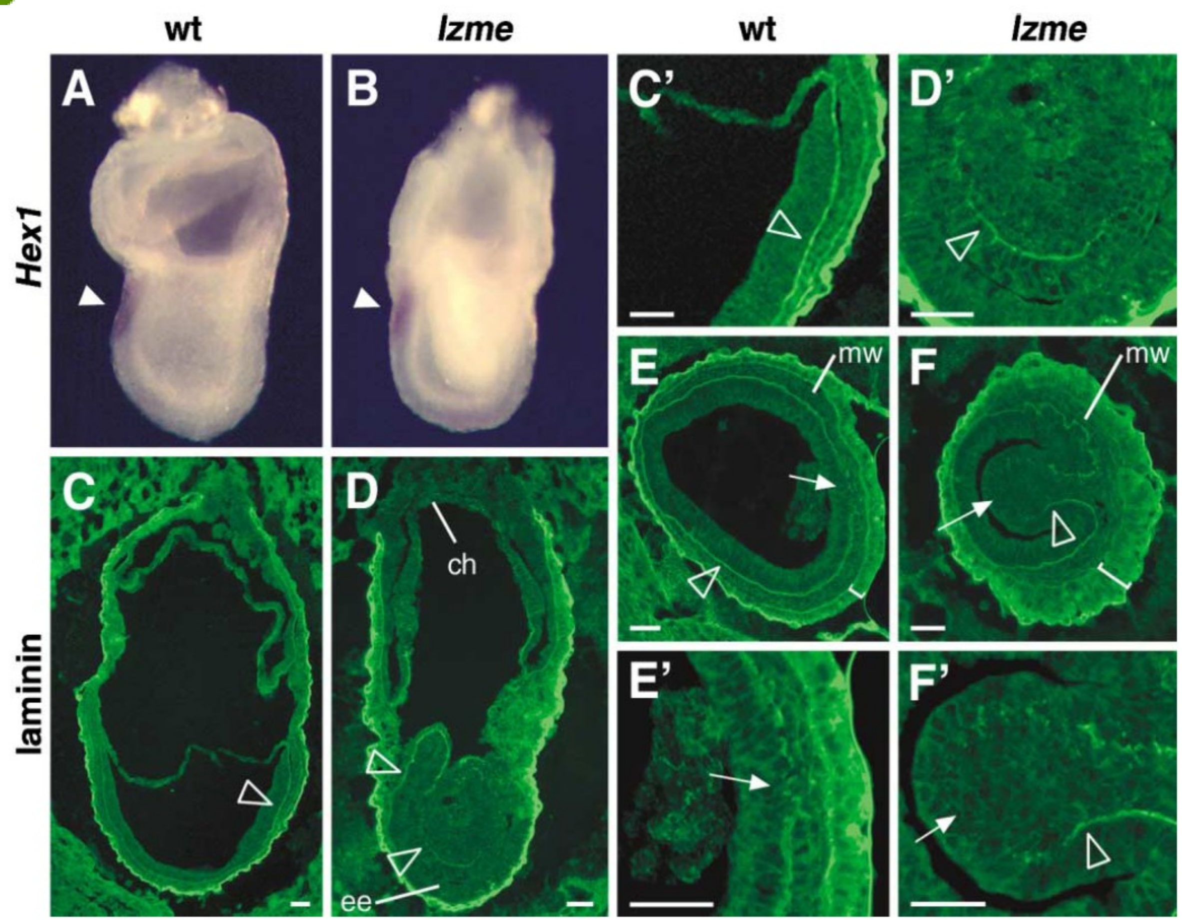


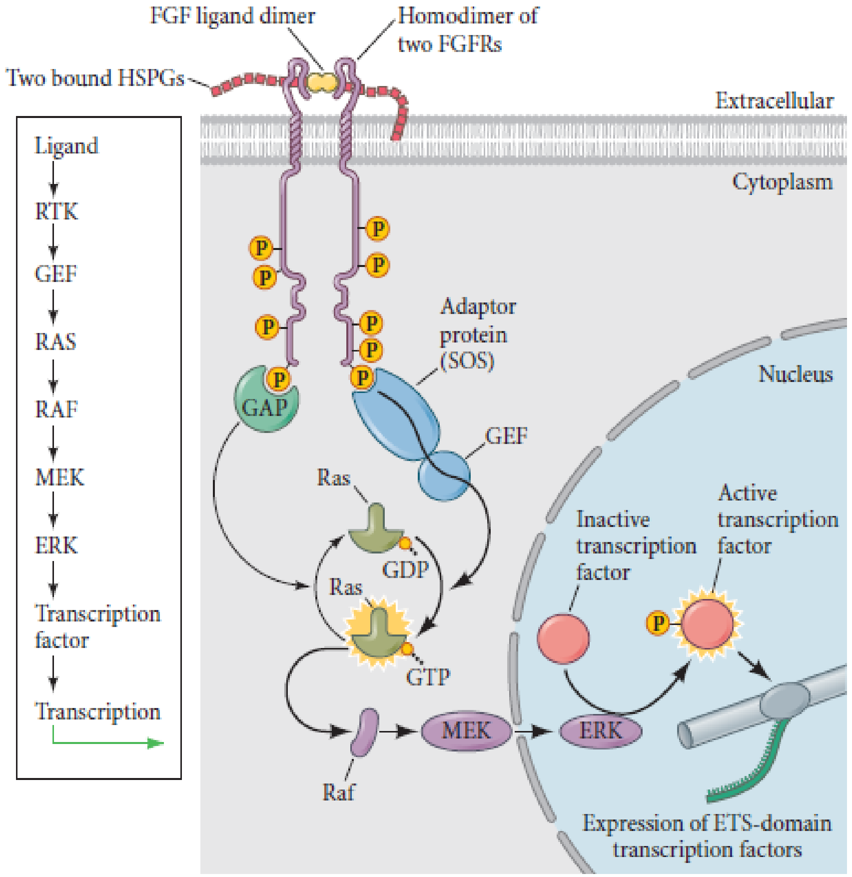
Garcia-Garcia and Anderson, 2003

- Izme = mutation in the gene Ugdh – UDP-glucose dehydrogenase, which is needed for the synthesis of lateral glycosaminoglycan (GAG) chains of proteoglycans.

Without Heparan sulfate or FGF signaling, the mesoderm does not migrate
Phenotypes of mutations in proteoglycan genes often mimic mutations in growth factor receptors

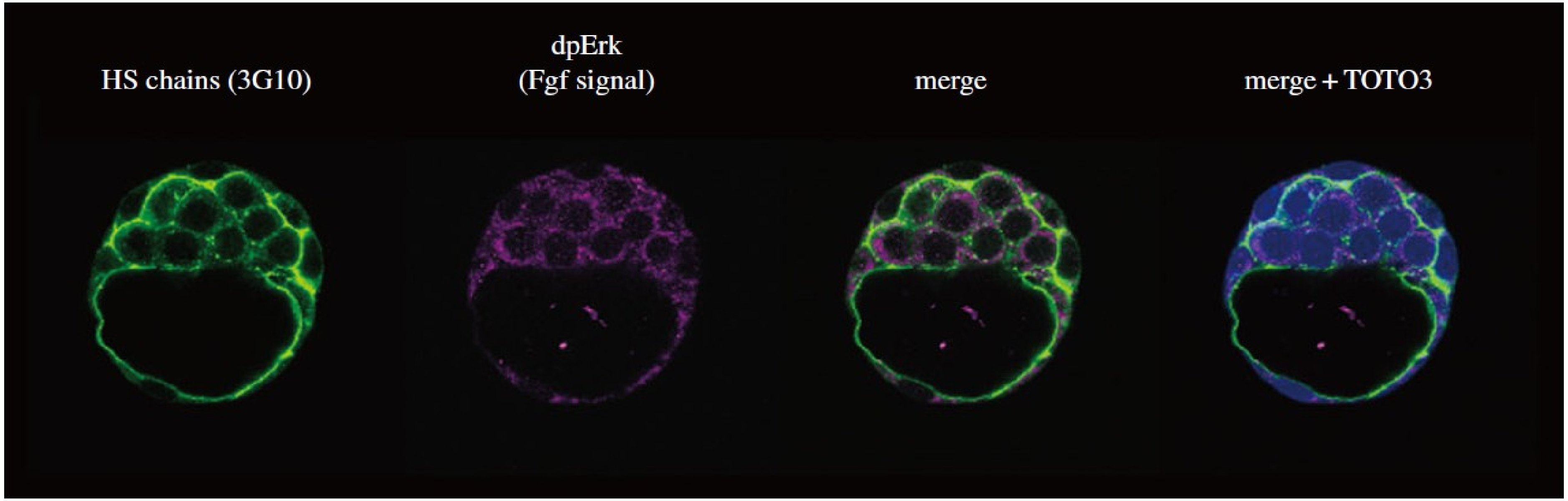
Components of ECM – Proteoglycans – Heparan sulfate





Vývojová biologie

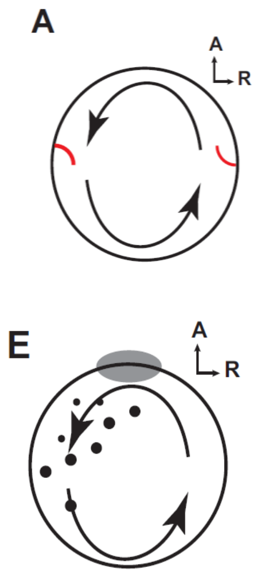
Components of ECM – Proteoglycans – Heparan sulfate

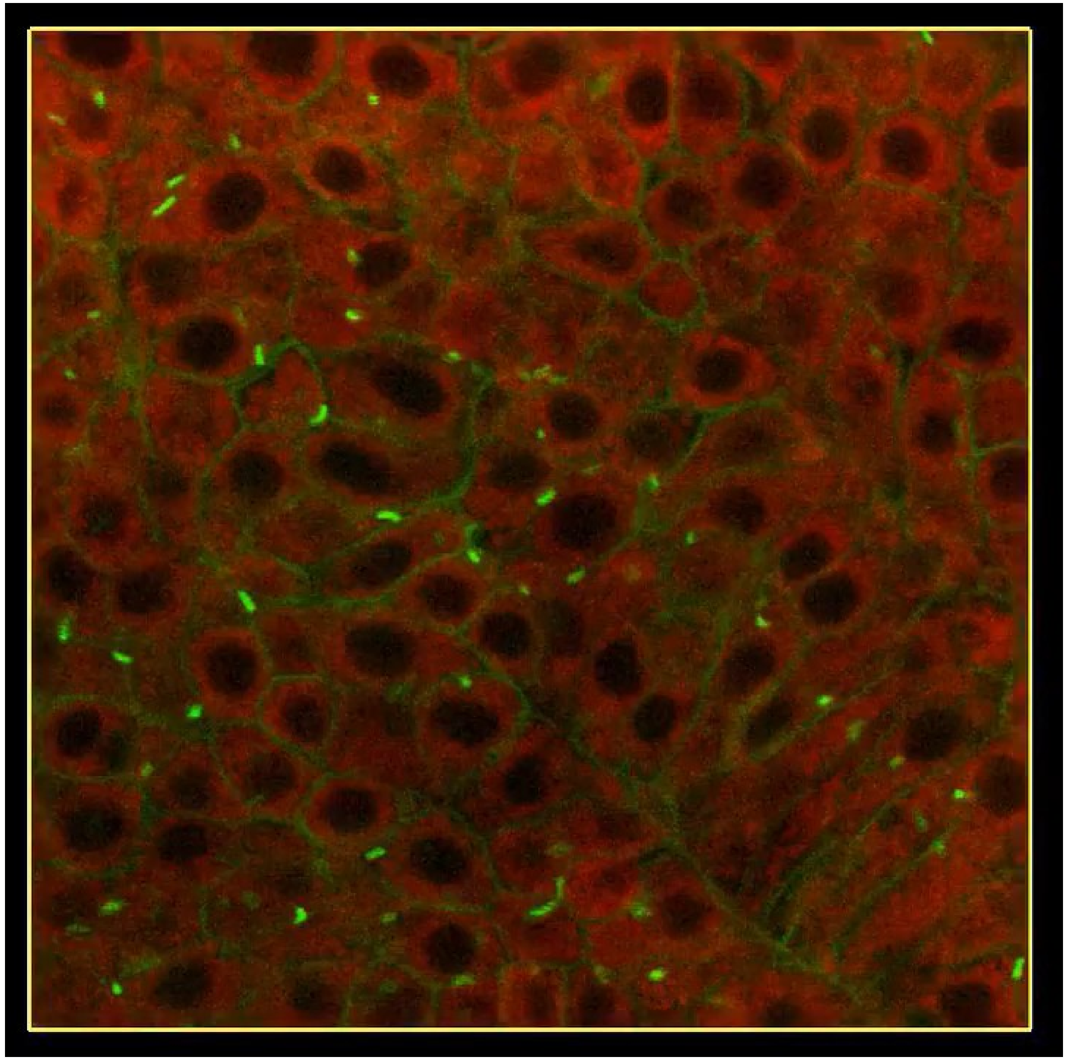


Components of ECM – Proteoglycans – Heparan sulfate

Right-left (a)symmetry

Kupffer's vesicle - is a ciliated organ that controls left-right (LR) patterning

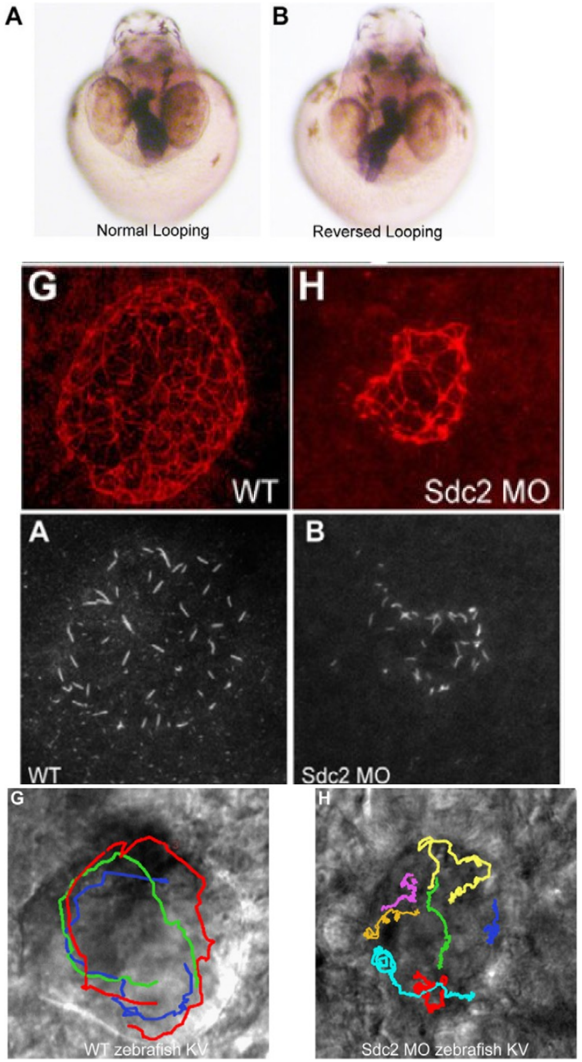


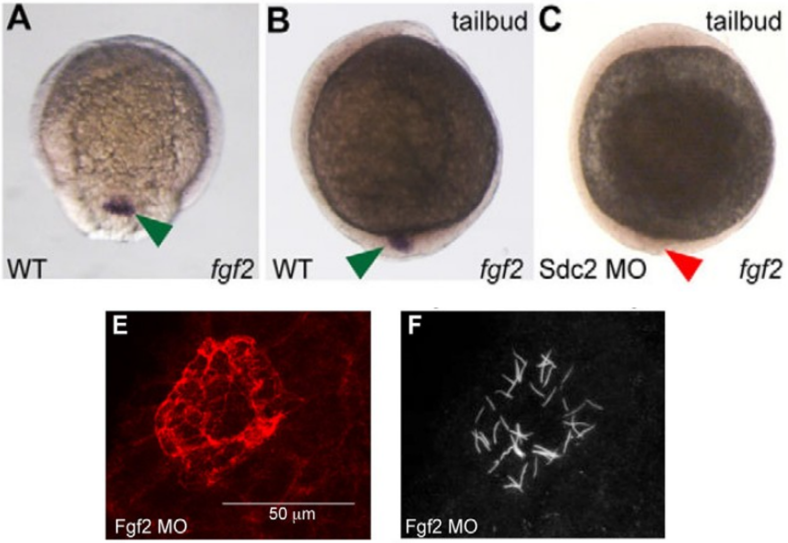


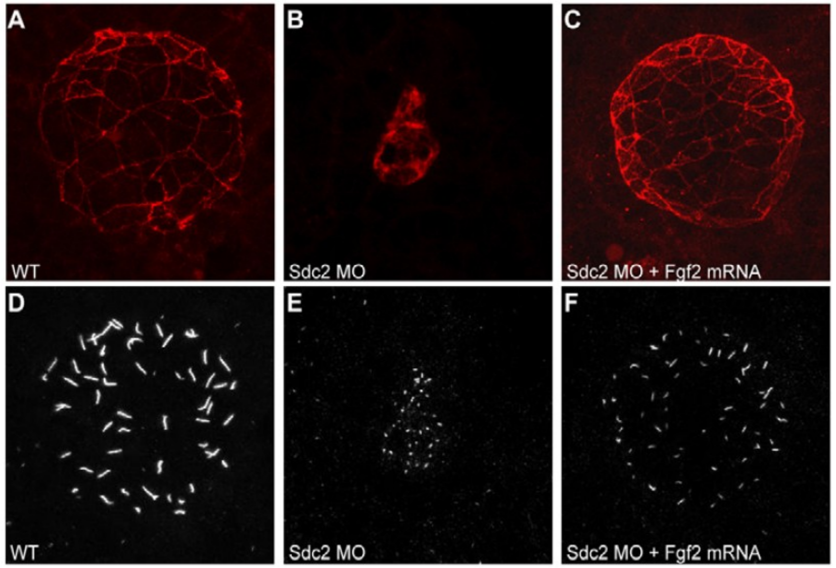
Ferreira et al., 2017

Components of ECM – Proteoglycans – Heparan sulfate

Right-left symmetry – Sdc2 MO

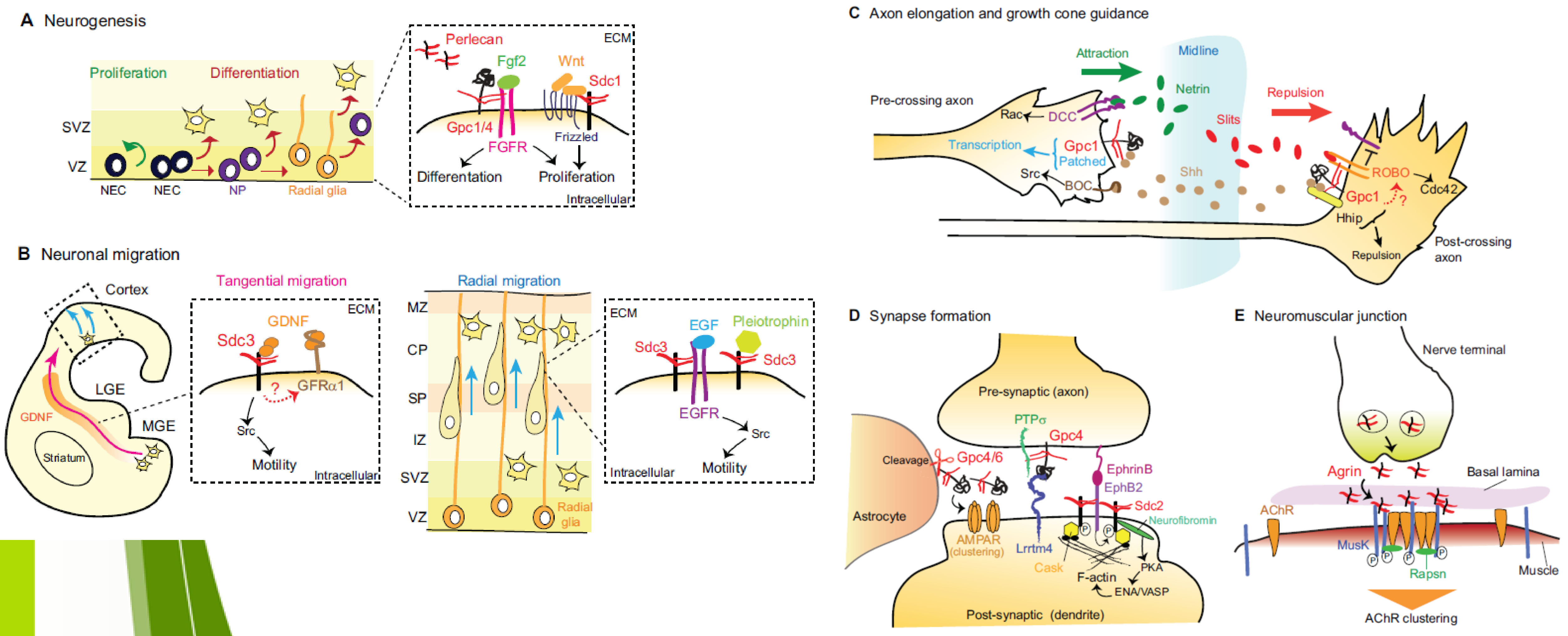






Arrington et al., 2013

Components of ECM – Proteoglycans – Heparan sulfate

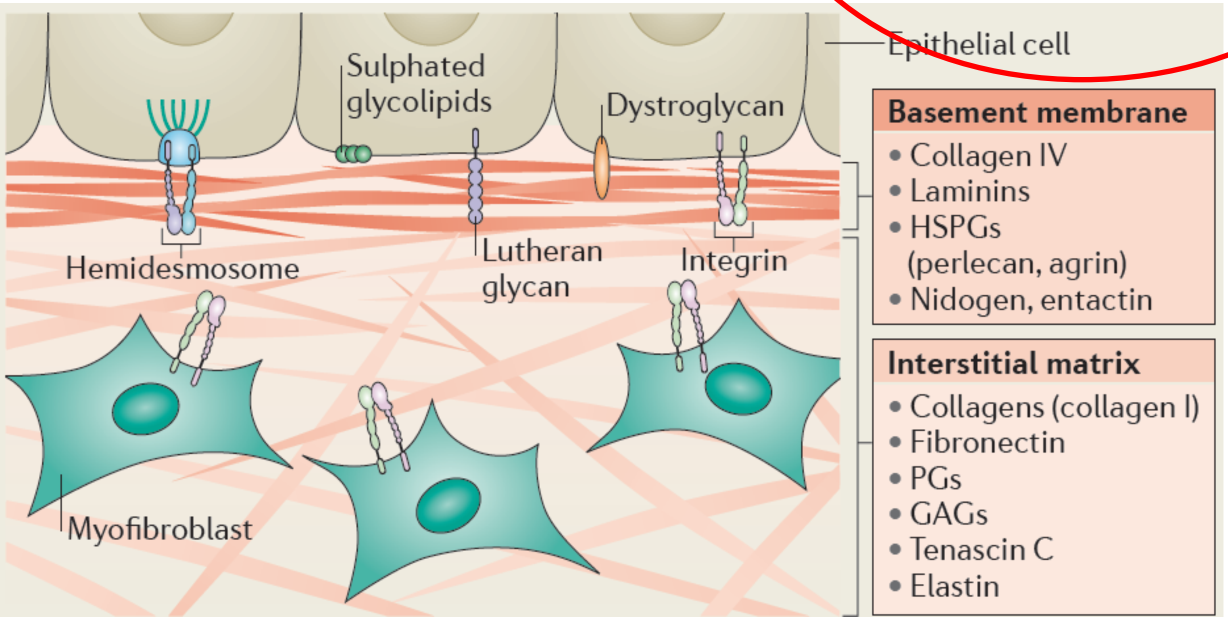


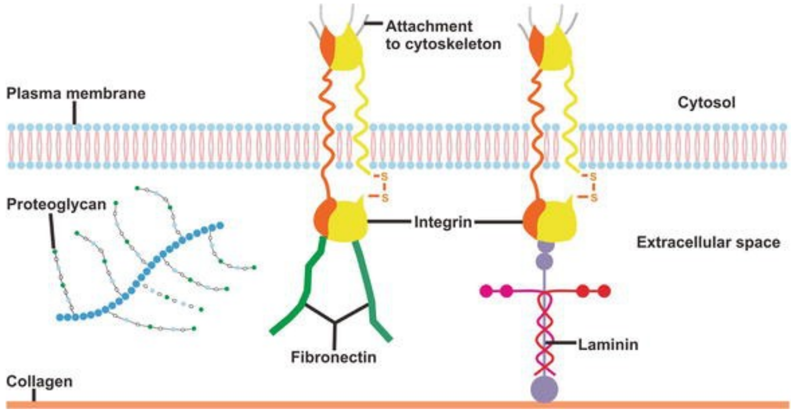
Components of ECM – how to understand it?

Components of the ECM

Proteoglycans | Glycoproteins | Proteins | Polysaccharides

Heparan sulfate | Chondroitin sulfate | Laminin | Fibronectins | Collagens | Hyaluronic acid.



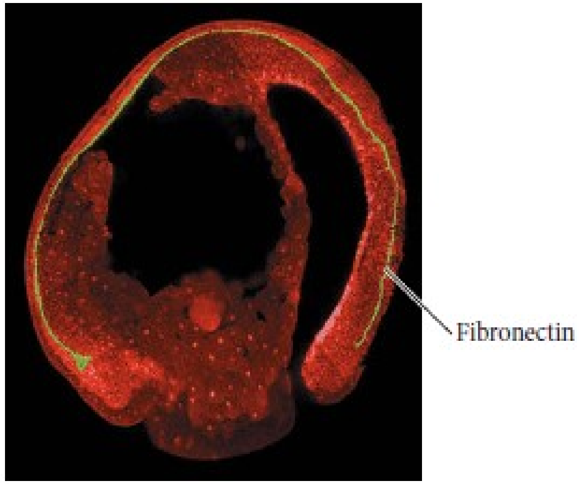


Kusindarta, 2018

Vývojová biologie

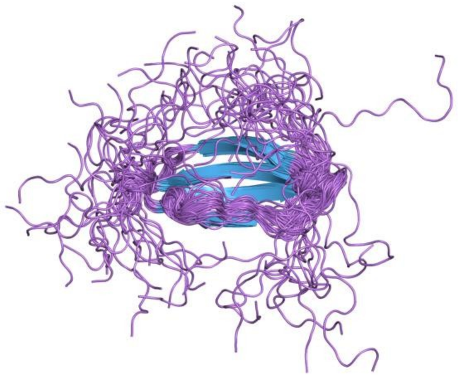
COMPONENTS OF ECM – Glycoproteins

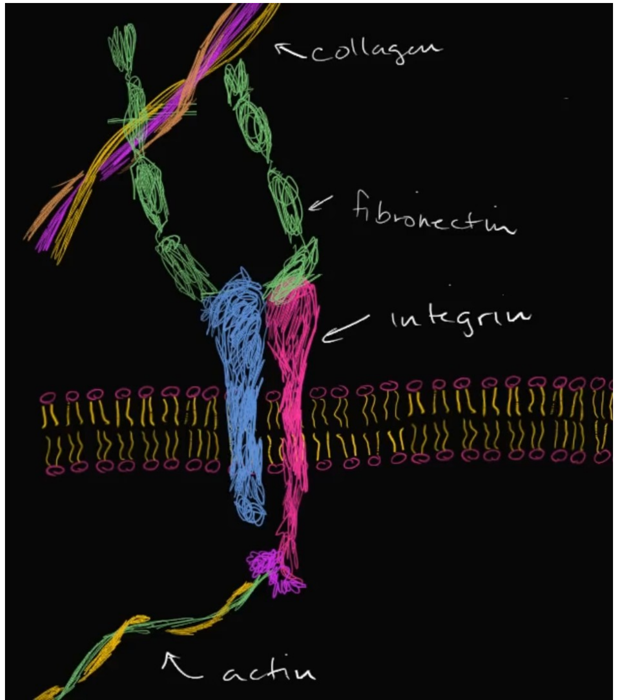
- Fibronectin, Laminin
- Responsible for the organization of the ECM and cells into ordered structures



Components of ECM – Glycoproteins - Fibronectins

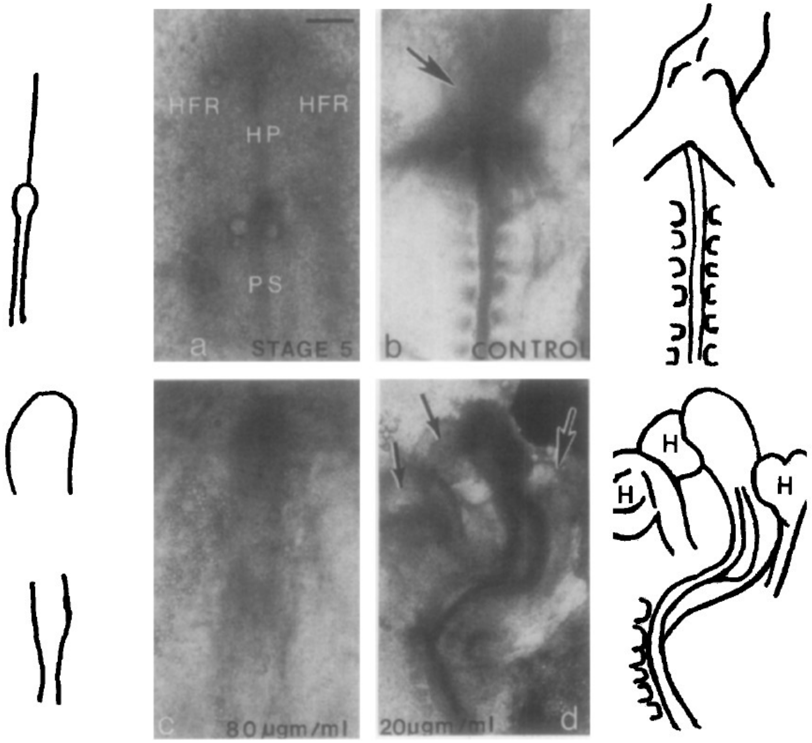
- Large glycoproteins (ca. 460 kDa) – heterodimers composed of 2 subjenotes connected by disulfide bond
- Two groups of fibronectins:
    - Soluble fibronectin (plasma – 300 µg/ml) – produced by hepatocytes
    - Insoluble, cellular fibronectin
- It binds to: collagen, fibrin, heparin, DNA, actin, integrin. **<u>"Link between cell (integrin) and ECM components"</u>**
- Function: cell adhesion (cell-to-cell contact and also mediates cell-collagen contact, proteoglycan), maintaining the shape of cells => development and morphogenesis
- It has several distinct binding sites and their interaction with the respective molecules leads to the correct arrangement of cells within the ECM.





Components of ECM – Glycoproteins - Fibronectins

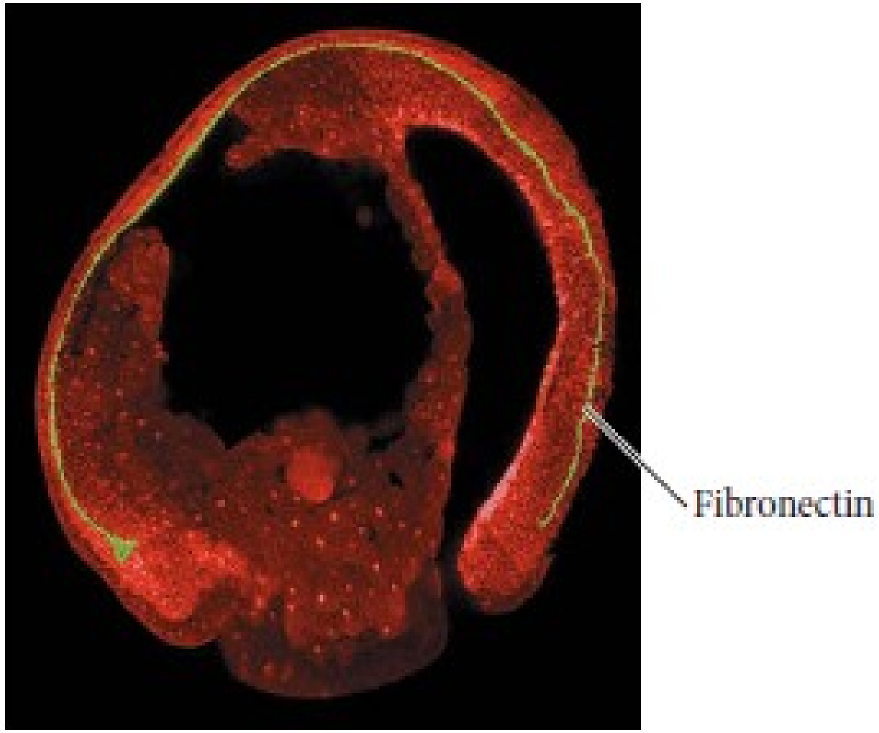
- Important role in cell migration
- Fibronectin refers to the pathways through which cells migrate – e.g. migration of germ cells into the gonads, migration of heart cells to the middle part of the embryo.

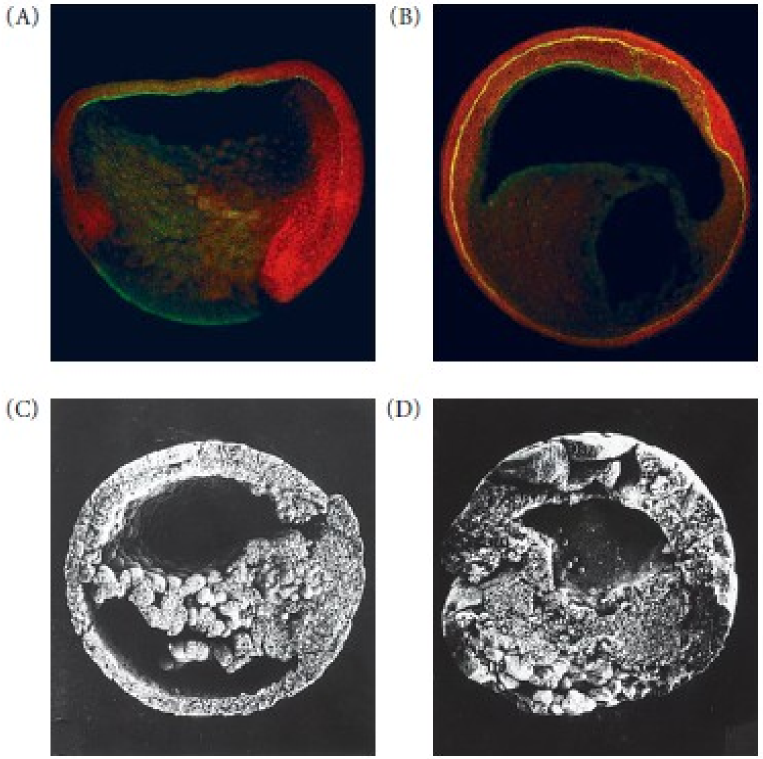


Linask, Lash, 1988

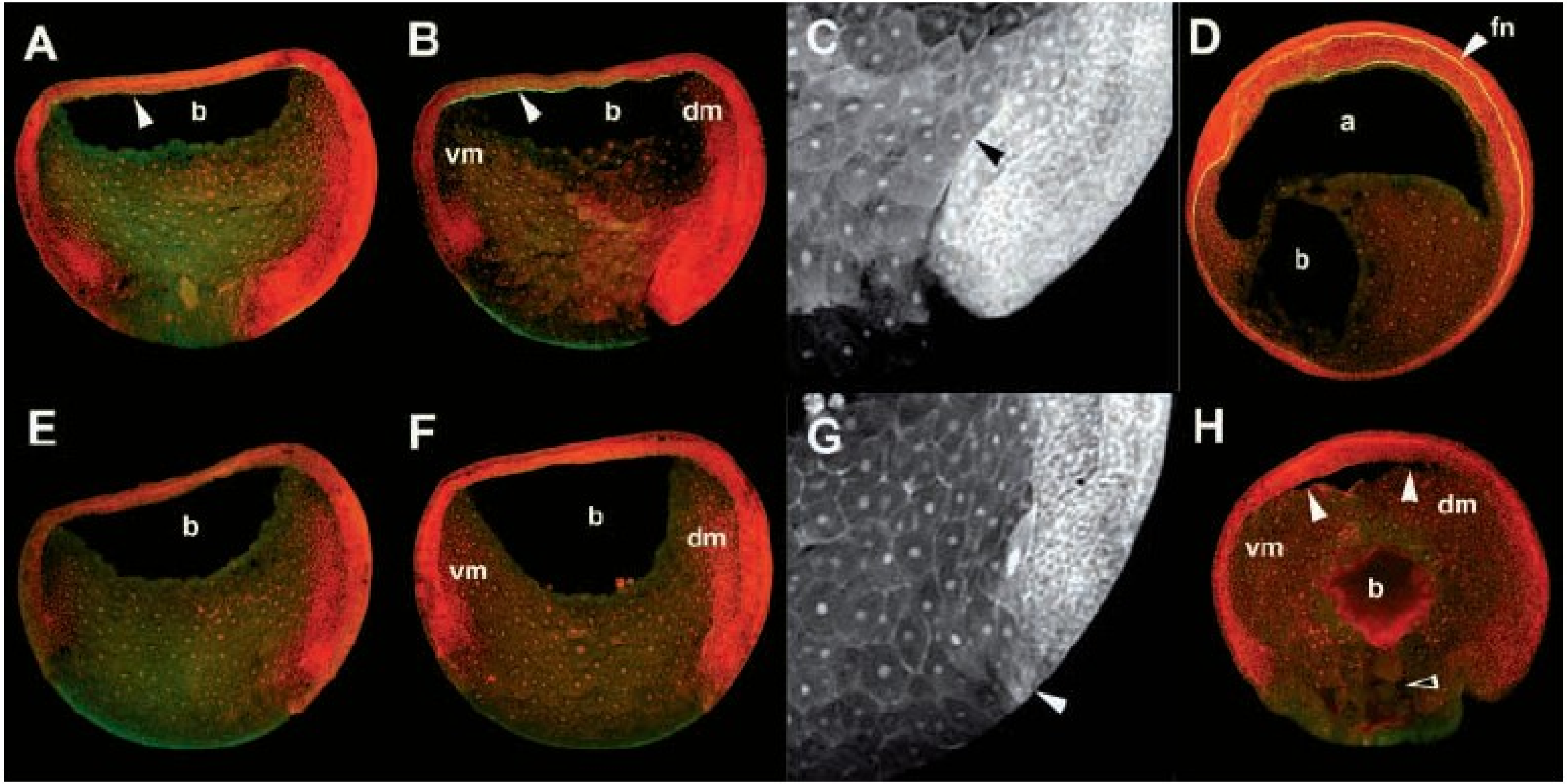
Components of ECM – Glycoproteins - Fibronectins

- Important role in cell migration
- Fibronectin paves the ways through which cells migrate – during gastrulation it is crucial for the migration of mesoderm cells.





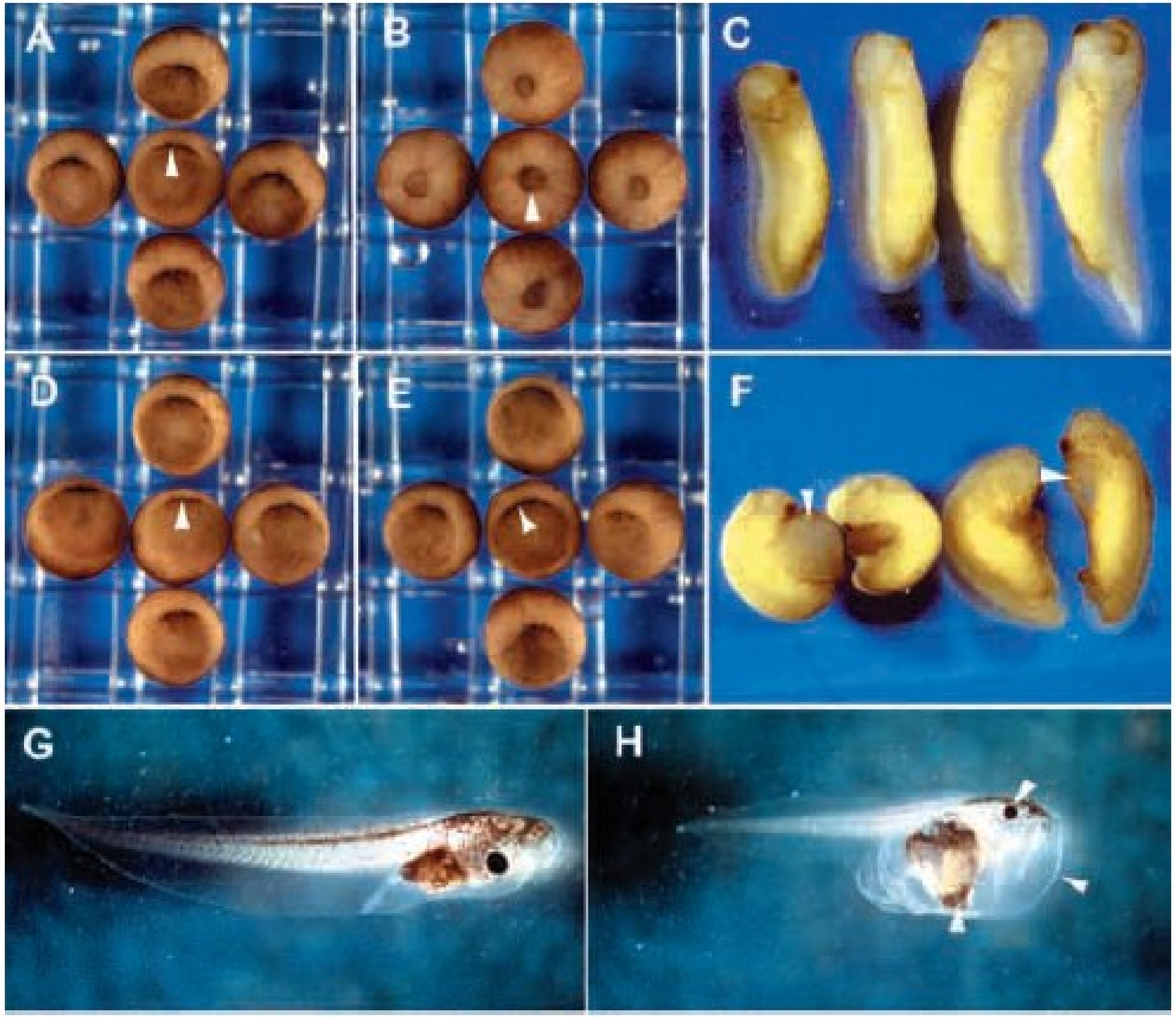
Components of ECM – Glycoproteins - Fibronectins



M. Marsden and D. W. DeSimone, 2001

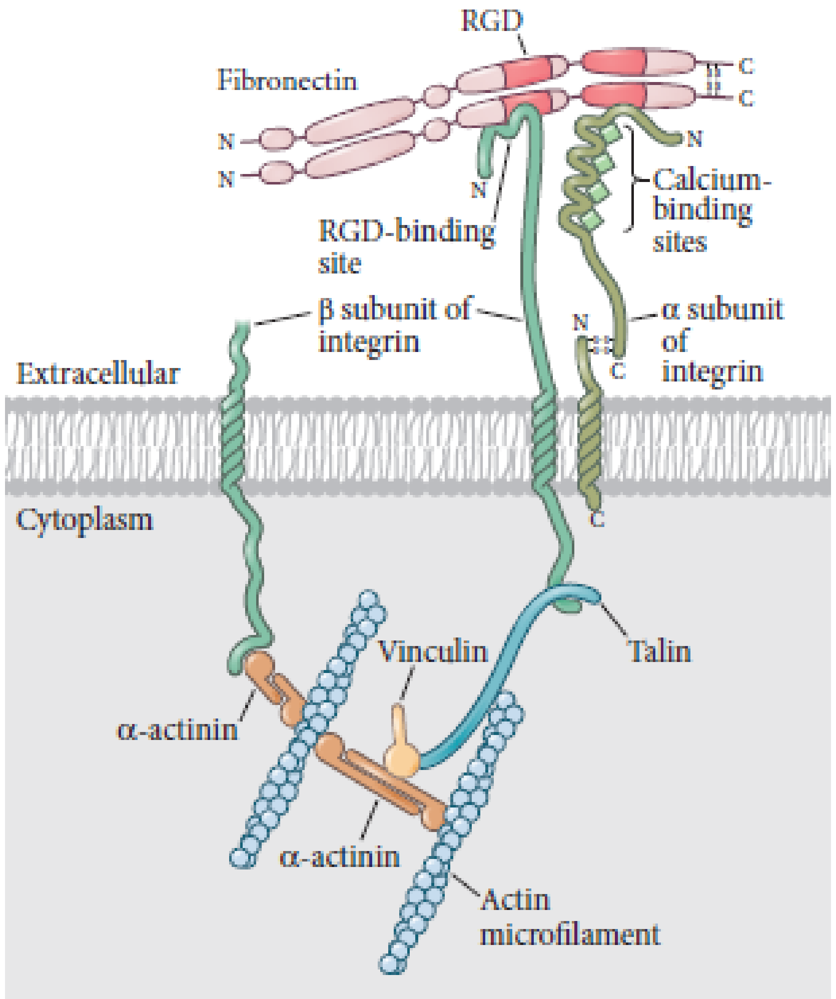
Components of ECM – Glycoproteins - Fibronectins

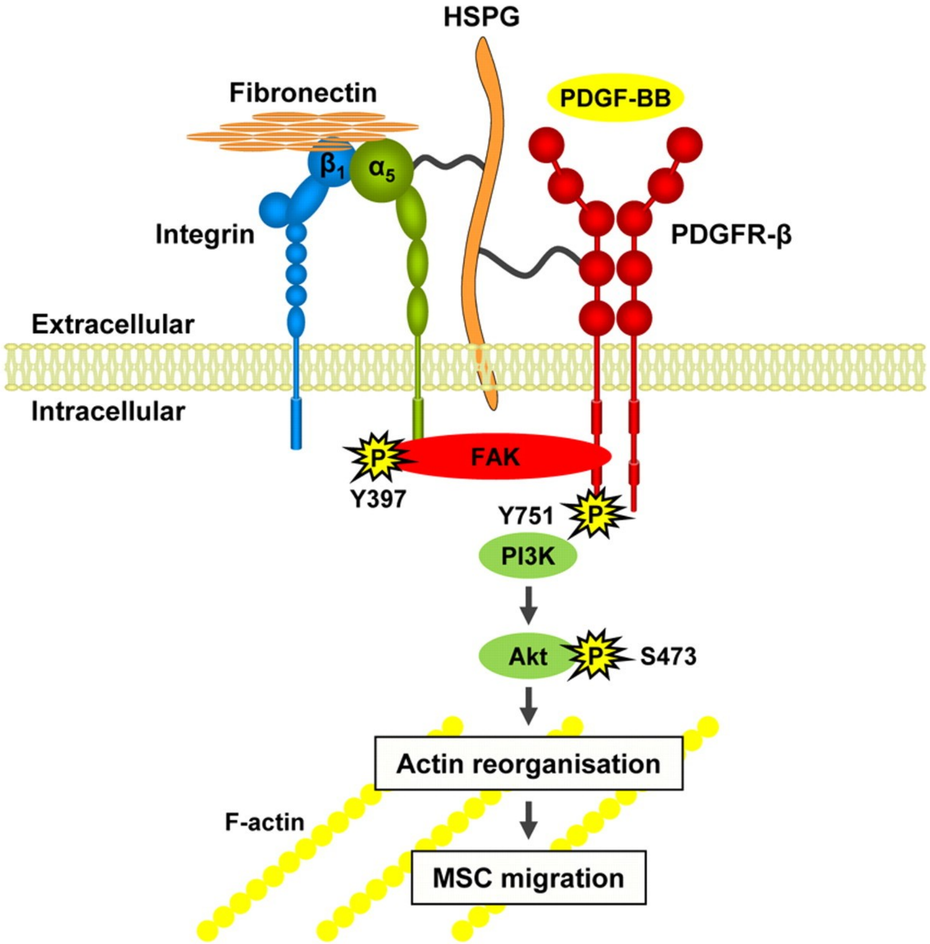
Intra-blastocoelar injection of anti-FN mAbs disrupts gastrulation. (A-C,G) Embryos injected at stage 9.5 with mAb 4H2 show normal development at stage 10.5, as indicated by dorsal lip formation (A, arrowhead). (B) At stage 11.5 the blastopore is almost closed (arrowhead) and embryos go onto develop into tadpoles (C,G). mAb 1F7-injected embryos (D-F,H) develop a normal blastopore at stage 10.5 (D, arrowhead). (E) By stage 11.5, there is a significant delay in blastopore closure and little movement of the blastopore lip is apparent (arrowhead). (F) mAb 1F7-injected embryos are truncated along the AP axis and bent ventrally. The blastocoel is retained and displaced ventrally (arrowhead). (H) Blastulae injected with mAb 1F7 develop into tadpoles that have small eyes, display head edema, and lack gut (arrowheads), heart, blood vessels and blood.



M. Marsden and D. W. DeSimone, 2001

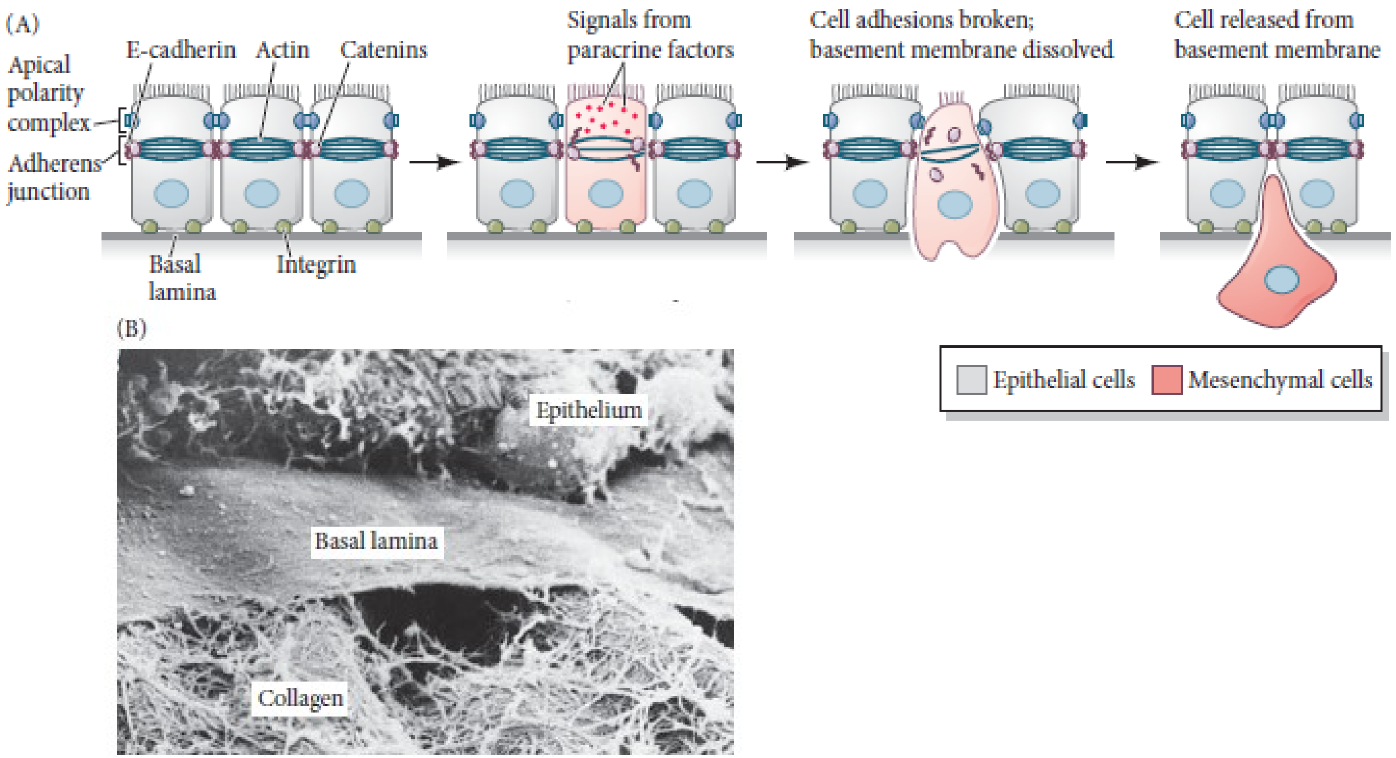
Components of ECM – Glycoproteins - Fibronectins



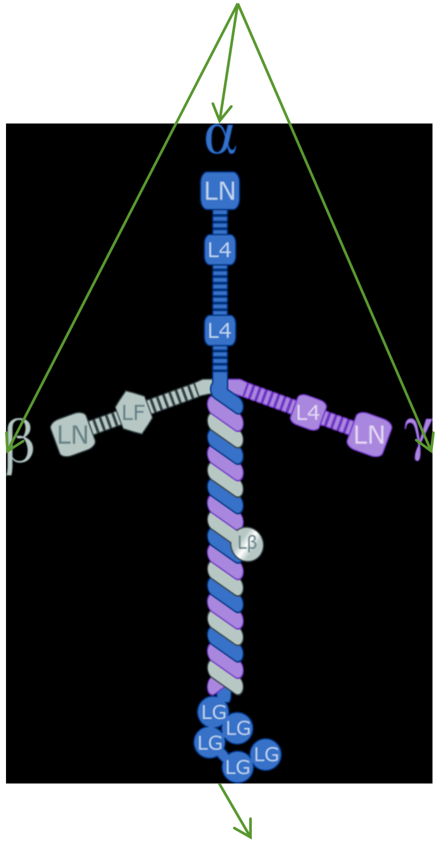


Components of ECM – Glycoproteins - Laminin

- Together with collagen, it represents an essential component of the basal lamina



Binding to other laminin and ECM components

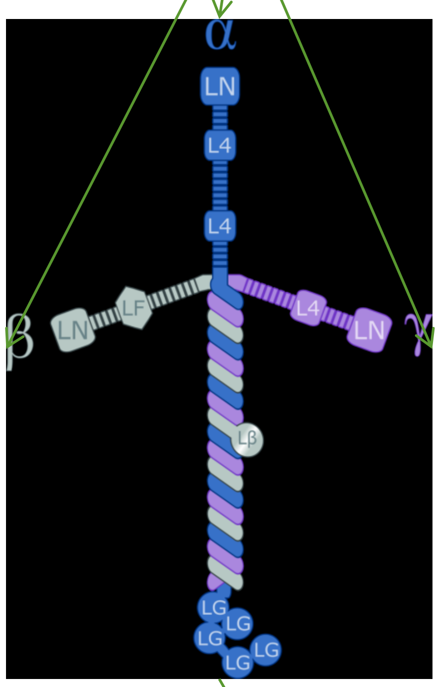


Vazba na buňku
Přes integriny, dystroglykan

Components of ECM – Glycoproteins - Laminin

- Nomenclature:
- Laminin-521 contains α5, β2, γ1 chains.

Binding to other laminin and ECM components



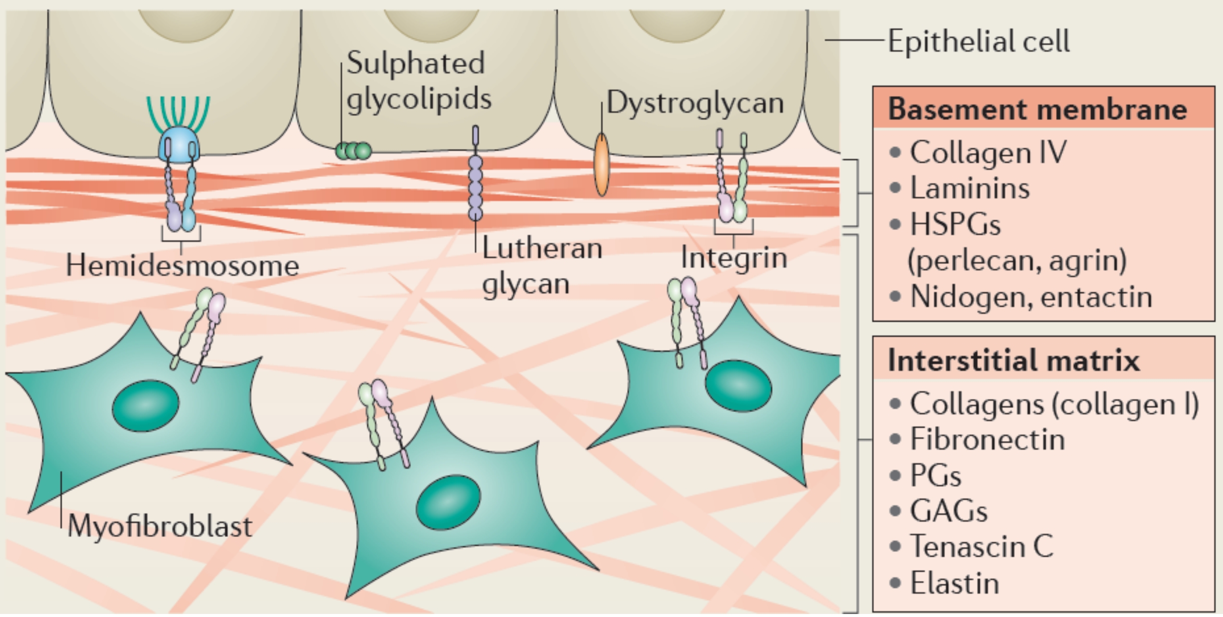
Cell binding
Through integrins, dystroglycan

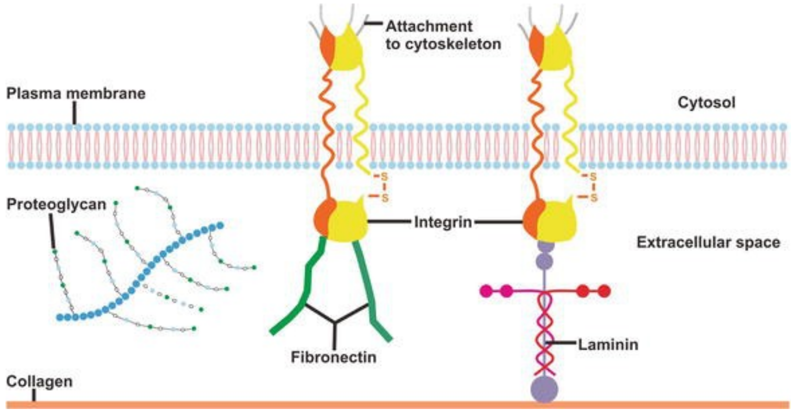
Components of ECM – how to understand it?

Components of the ECM

Proteoglycans — Glycoproteins — Proteins — Polysaccharides

Heparan sulfate, Chondroitin sulfate, Laminin, Fibronectins, Collagens, Hyaluronic acid.

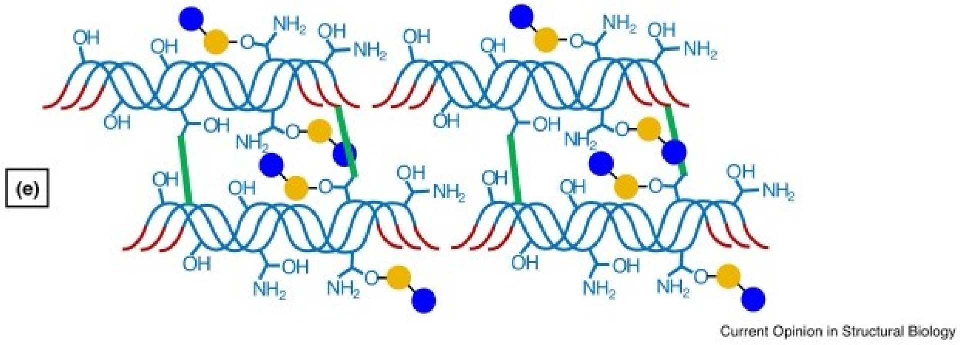




Kusindarta, 2018

Components of ECM – Proteins - Collagens

- The main structural protein of ECM (about 30% of all proteins in mammals)
- Produced mainly by fibroblasts
- Gelatin is essentially hydrolyzed collagen.
- 90% of collagen consists of Collagen I, a total of about 30 types of collagen
- Collagen is glycosiled through prolyl-4-hydroxylase and lysyl hydroxylase – I need Vitamin C as a cofactor. Vitamin C deficiency then leads to Scurvy
-



ECM - remodeling

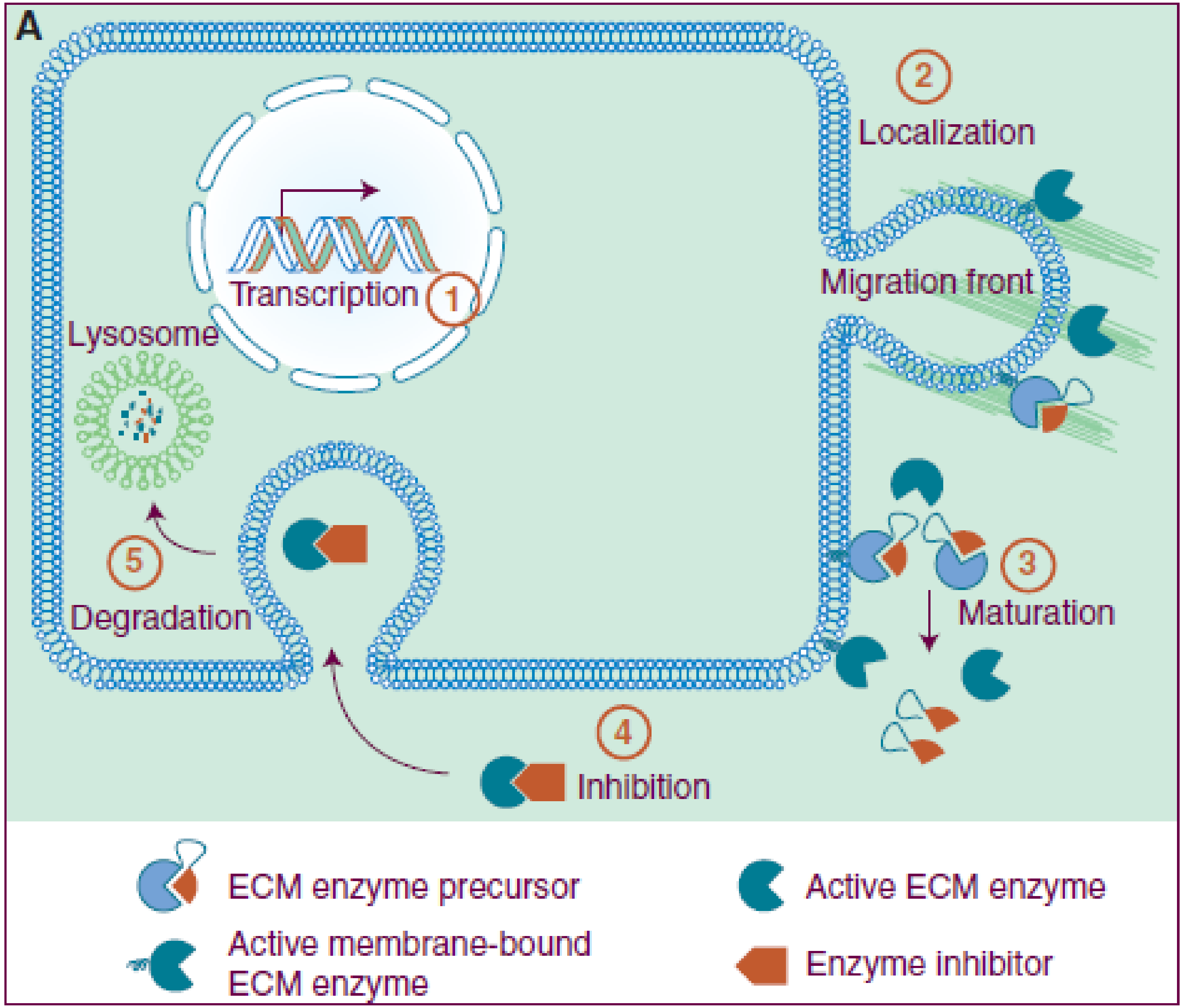
- The splitting of ECM components is a key process during ECM remodeling.
  - ECM quantity control
  - ECM composition regulation
  - Structure regulation
  - Release of growth factors (and other regulatory molecules)

- **Matrix metalloproteinase (MMP)**
  - The main enzymes that degrade ECM
  - Free (secreted) or bound to the cell membrane.
  - Secreted in the form of zymogene – activation up to ECM (other MMP or oxidation of thiol. group).

- **Adamalysins (ADAMTS), Meprins**

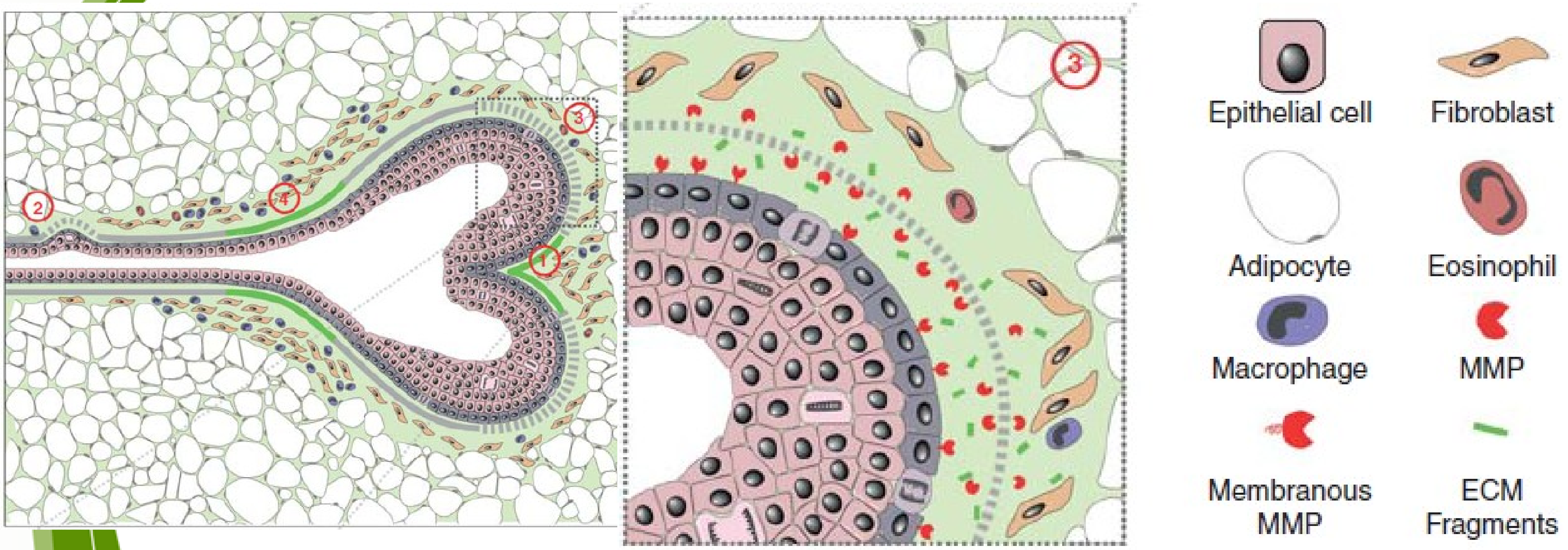
ECM - remodeling

Vývojová biologie



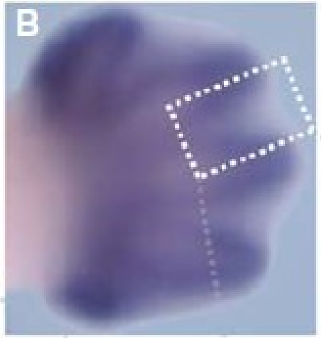
ECM - remodelace

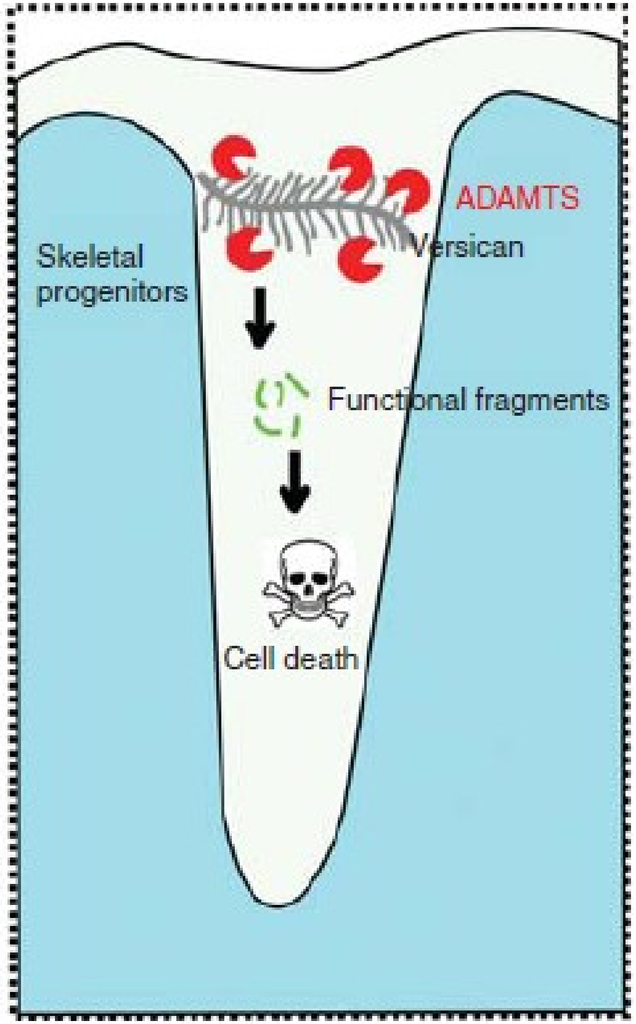
Vývojová biologie



ECM – remodelling - apoptosis

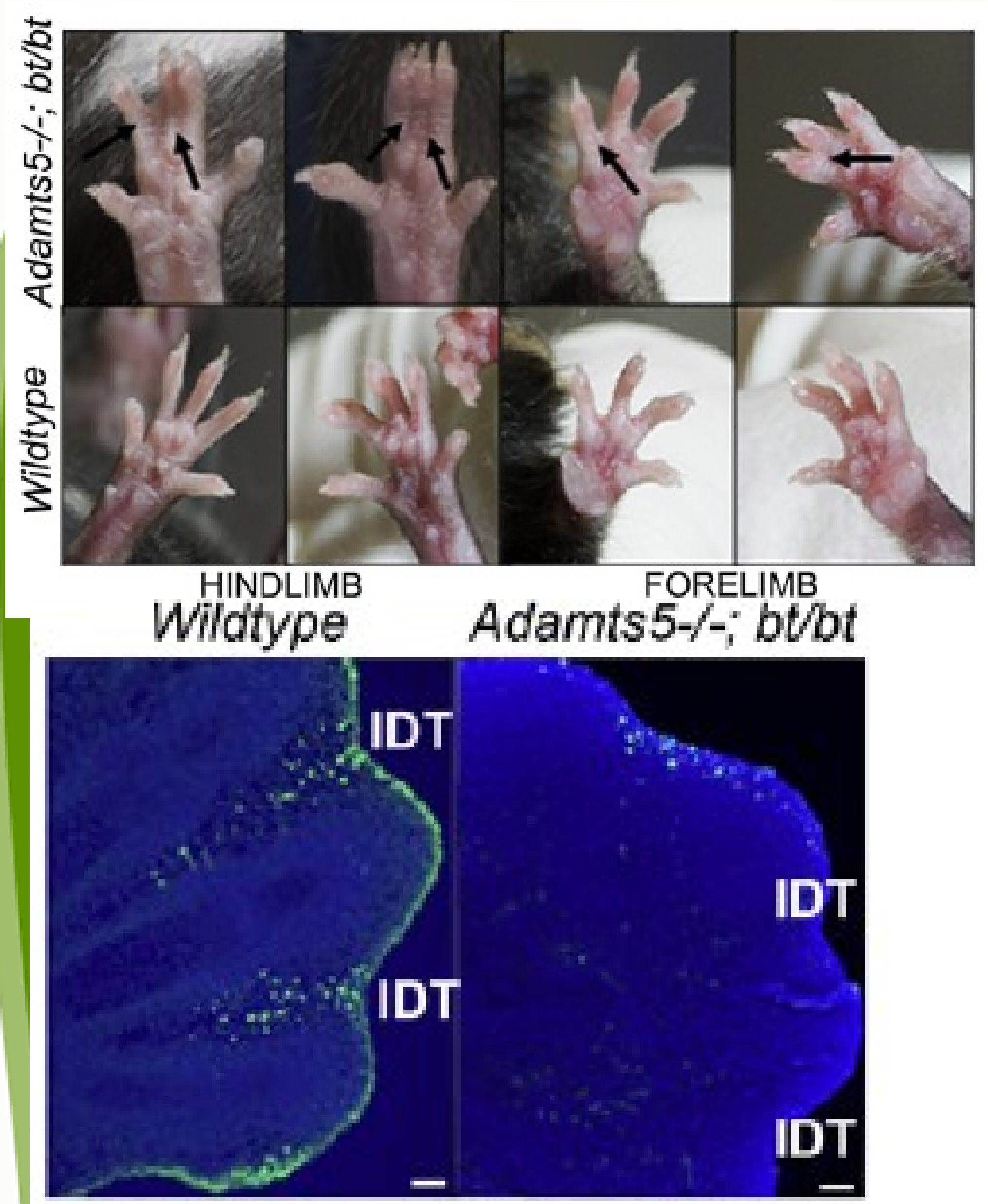
ADAMTS - **a disintegrin and metalloproteinase with thrombospondin motifs**





- Degradation of interdigital (between the fingers) tissue involves intensive remodeling of the ECM.
- Remodeling using ADAMTS5 and its substrate – a component of ECM versican.
- Versican cleavage product induces apoptosis
- In the absence of ADAMTS5, syndactyly occurs.
-

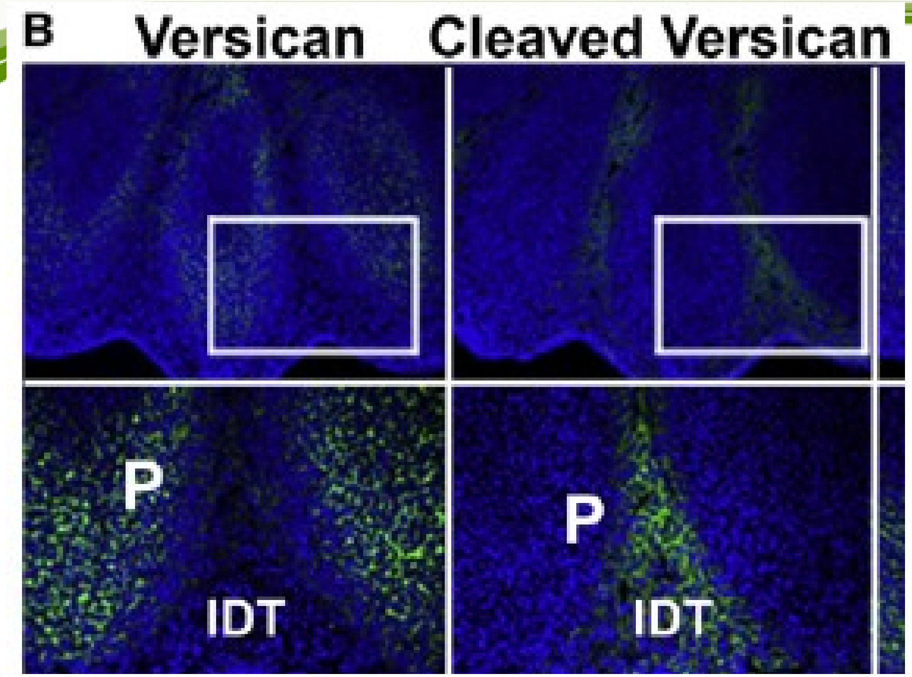
ECM – remodelling - apoptosis

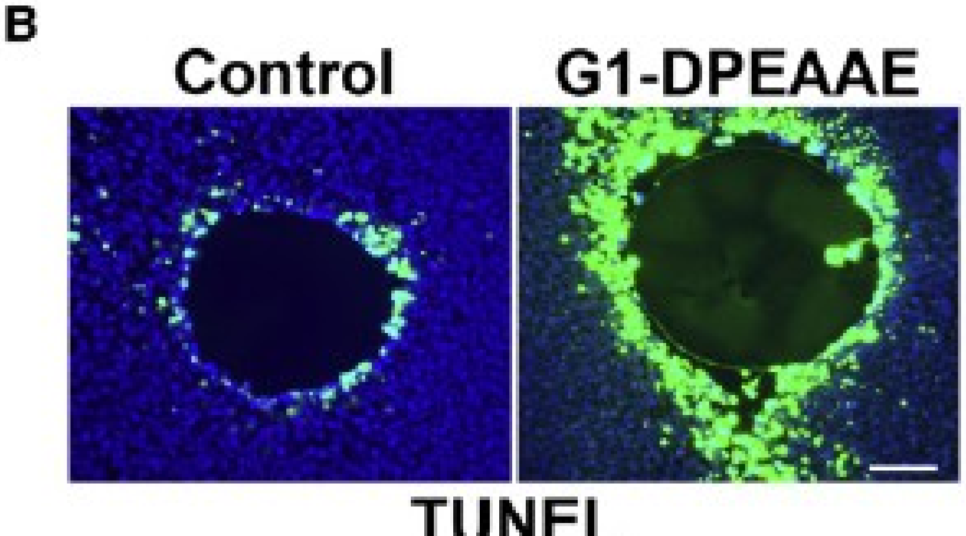


In the absence of ADAMTS5, syndactyly occurs...

....Because there is no apoptosis

ECM – remodelling - apoptosis

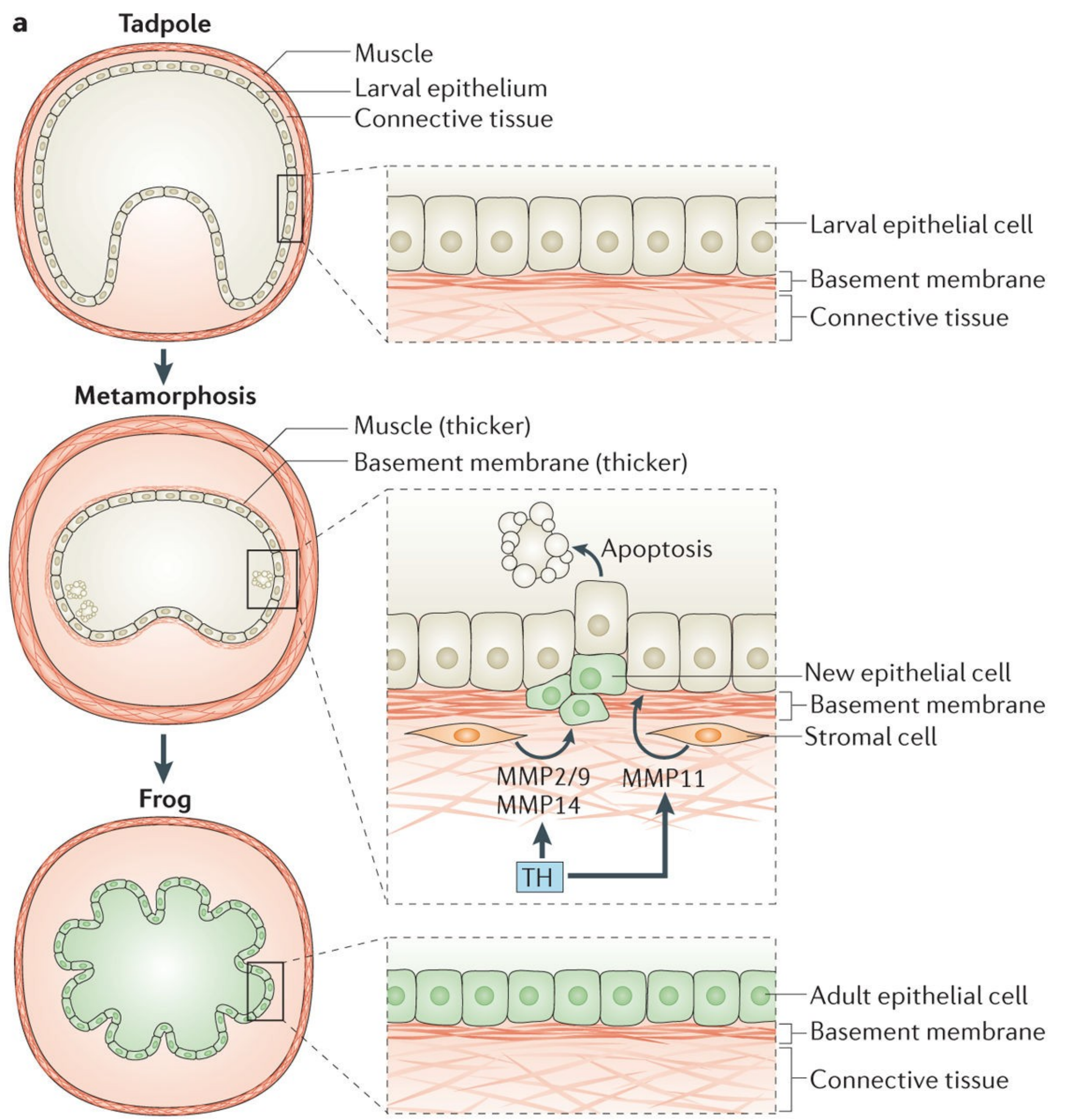




Even the presence of the cleaved form of Versican is capable of inducing apoptosis

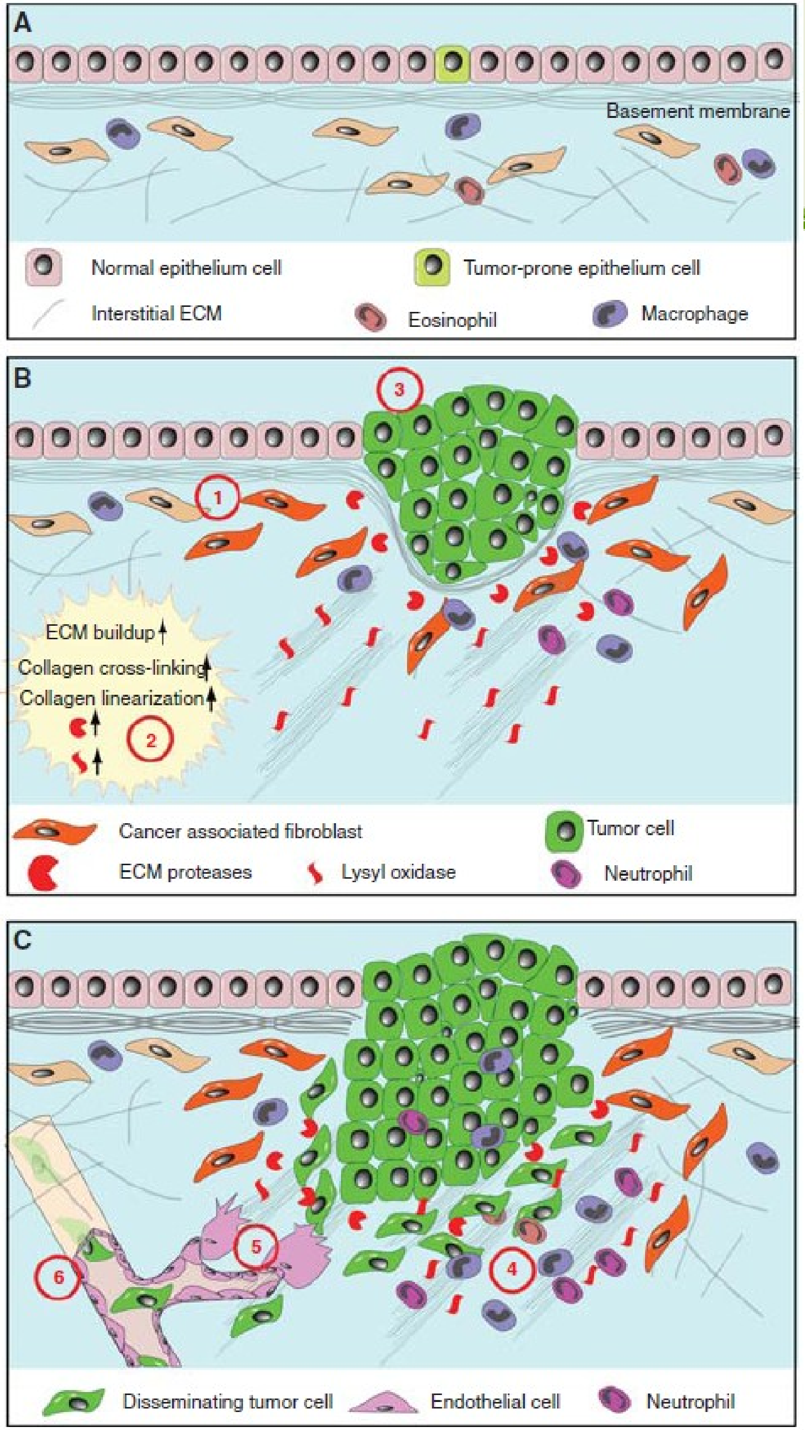
Vývojová biologie

ECM - remodeling



ECM - remodeling

Vývojová biologie



Components of the ECM — Conclusion

- Know the basic components of ECM, composition, functions + know examples
- Know the principles of ECM remodeling + know examples

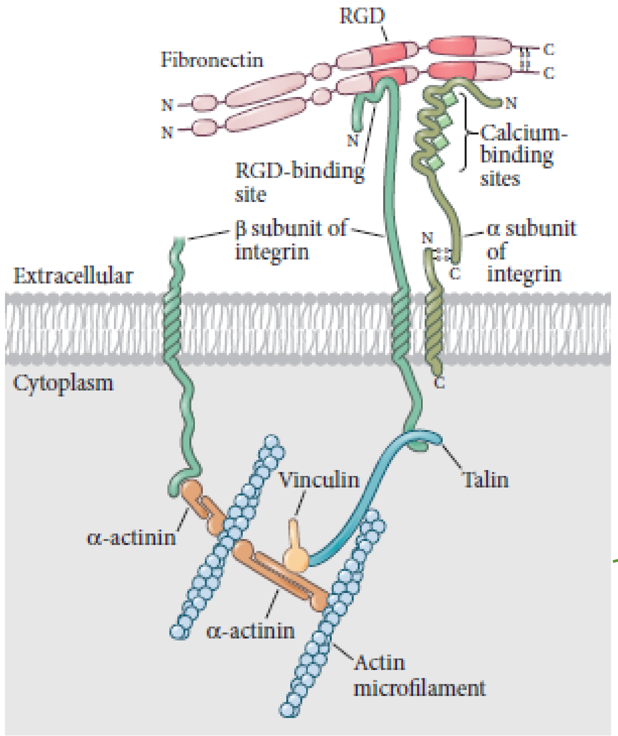
Components of ECM – Receptors

A cell must somehow "perceive" if and how it is anchored in the ECM = > it is an important source of signals and information for it.

Vývojová biologie

Components of ECM – Receptors - Integrins

- The ability of the cell to bind the adhesive glycoproteins ECM (laminin, fibronectin) depends on the expression of membrane receptors.
- Integrins = integrate the extracellular and intracellular matrix so that they work together.
- In the ECM, they bind to the arginine-glycine-aspartate (RGD) motif – present in the adhesive proteins of ecM – fibronectin, laminin, vitronectin.

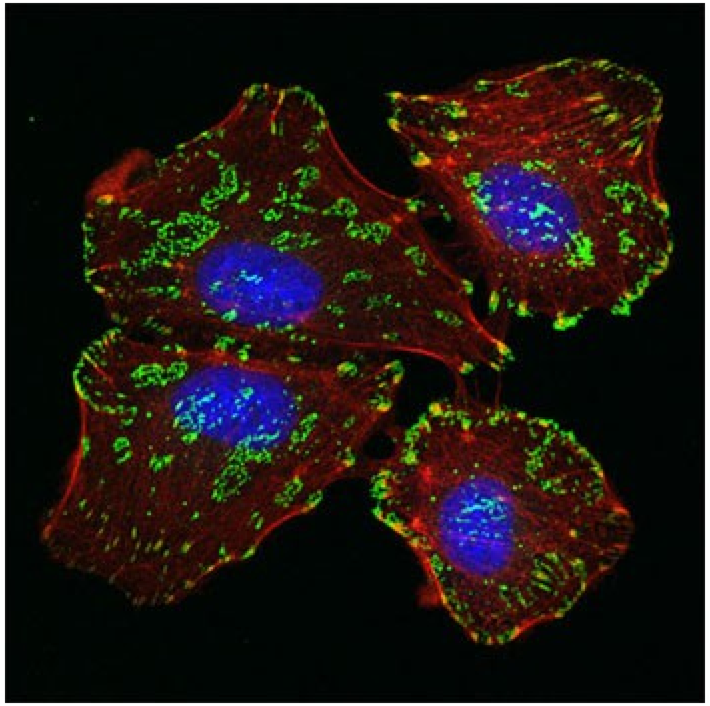


Inside the cell, it always binds to the cytoskeleton.

Vývojová biologie

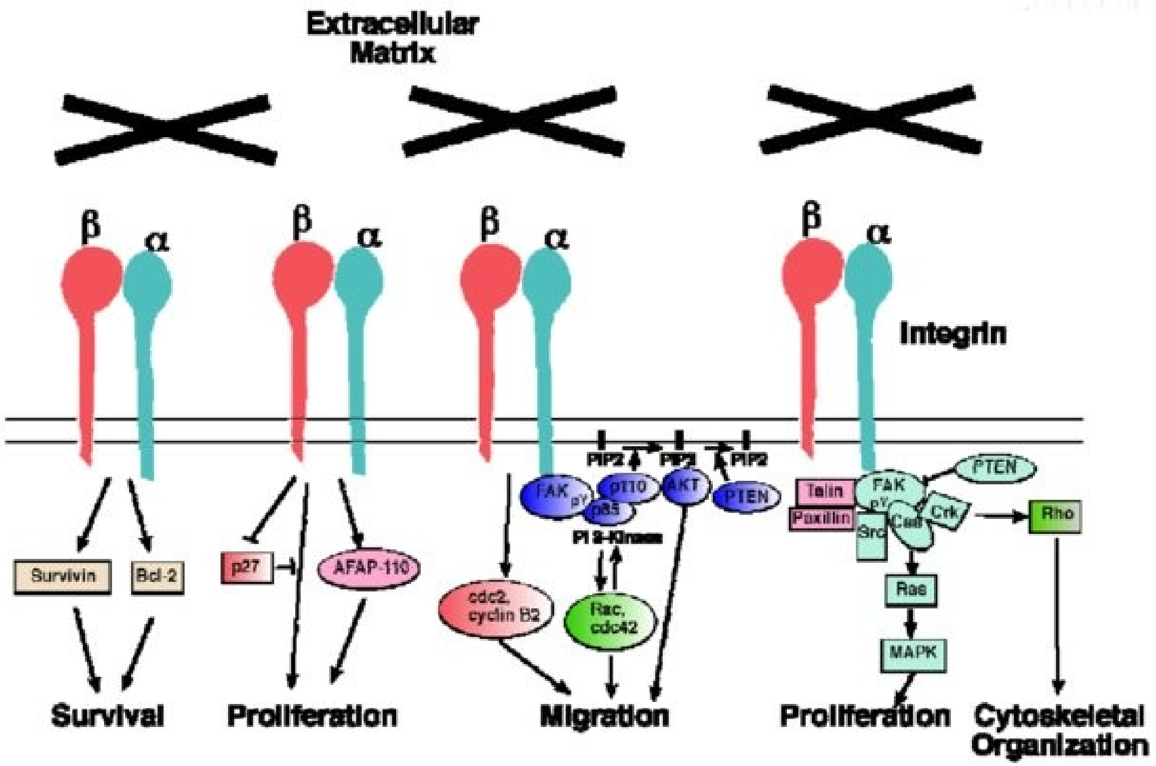
Components of ECM – Receptors - Integrins

- They have the ability to signal from the outside of the cell inward, which leads to a change in gene expression.
- Perceive the physical properties (pressure, stiffness) of the ECM - they activate/deactivate various signal pathways.
- Key to control gene expression during tissue development (mammary gland - able to signal the expression of estrogen receptor and casein protein genes)



Components of ECM – Receptors - Integrins

- **Activation of integrins can:**
  - Stimulate proliferation
  - Inhibit apoptosis
  - Activate the processes required to change cell shape or polarity
  - Change cell motility



Components of ECM – Receptors – Integrins

- Mouse embryos lacking the 1 integrin subunit arrest in development, shortly after implantation.
- The first observable defect is the inability of the inner cellmass to generate primitive endoderm and ectoderm layers. Further proliferation of the inner cellmass is also impaired.

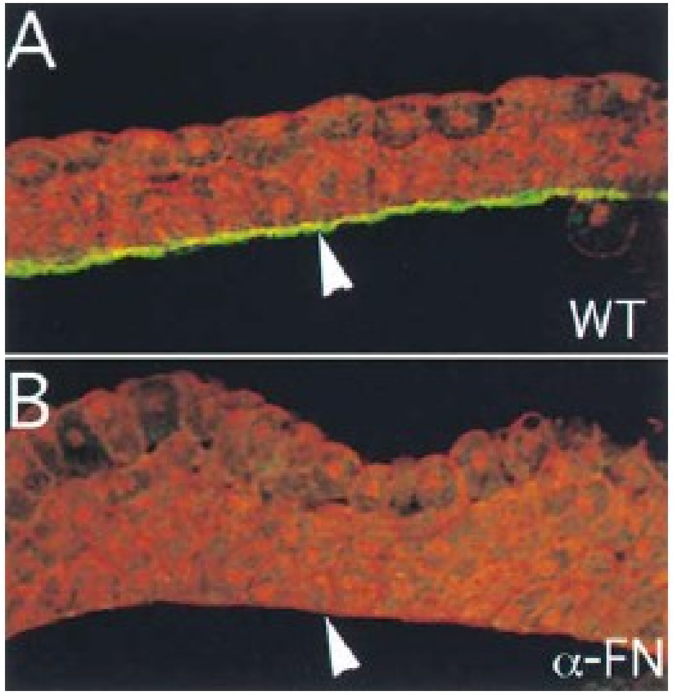
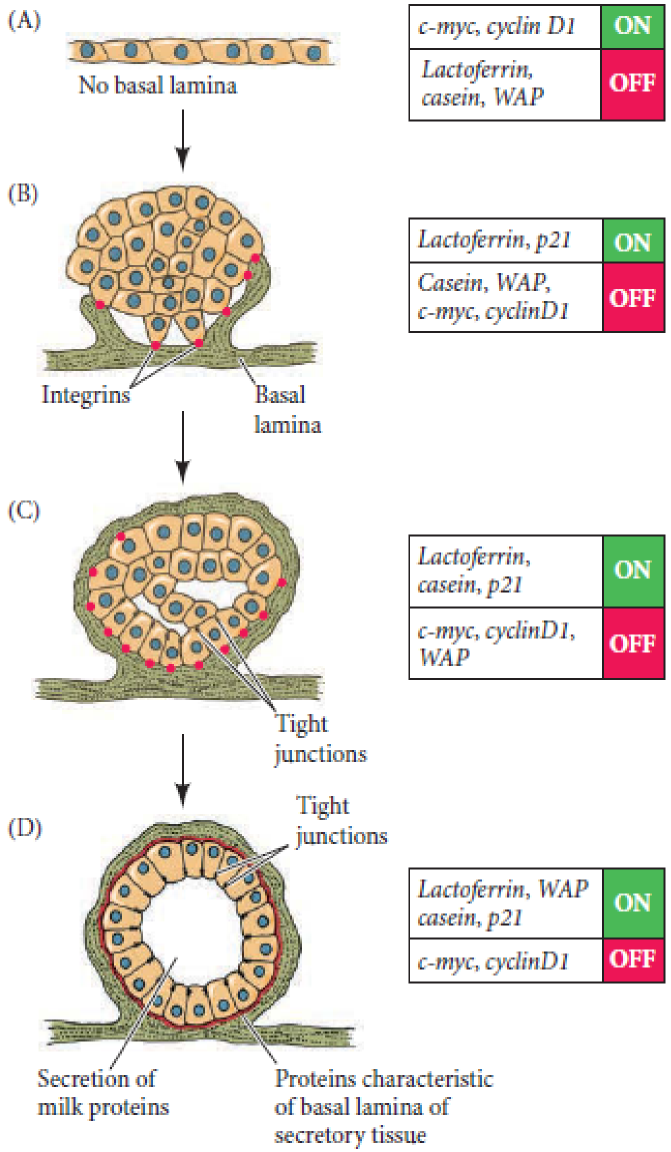


Figure 1. Integrins and Cell Rearrangements during Gastrulation

(A and B) The deposition of a fibronectin-containing ECM ([A], green) below the cells of the blastocoel roof (arrowhead) during *Xenopus* gastrulation is inhibited by antibodies that bind the integrin binding site on fibronectin ([B], arrowhead). The thinning of the blastocoel roof by radial intercalation is also blocked. Reproduced with permission from Marsden and DeSimone (2001).

Components of ECM – Receptors - Integrins

- Example:
- Which genes are regulated?



Components of ECM – Receptors - Integrins

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Integrins in Development: Moving on, Responding to, and Sticking to the Extracellular Matrix

Components of ECM – Receptors - Integrins

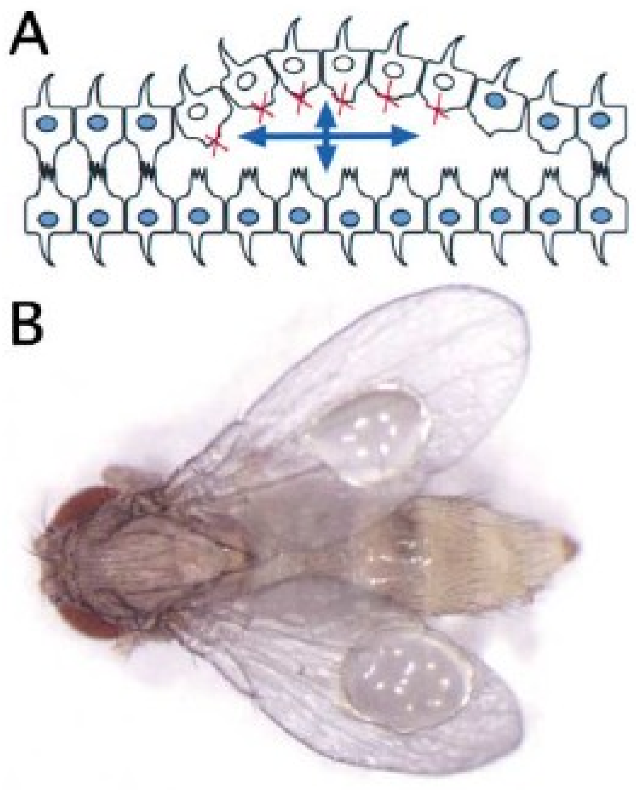
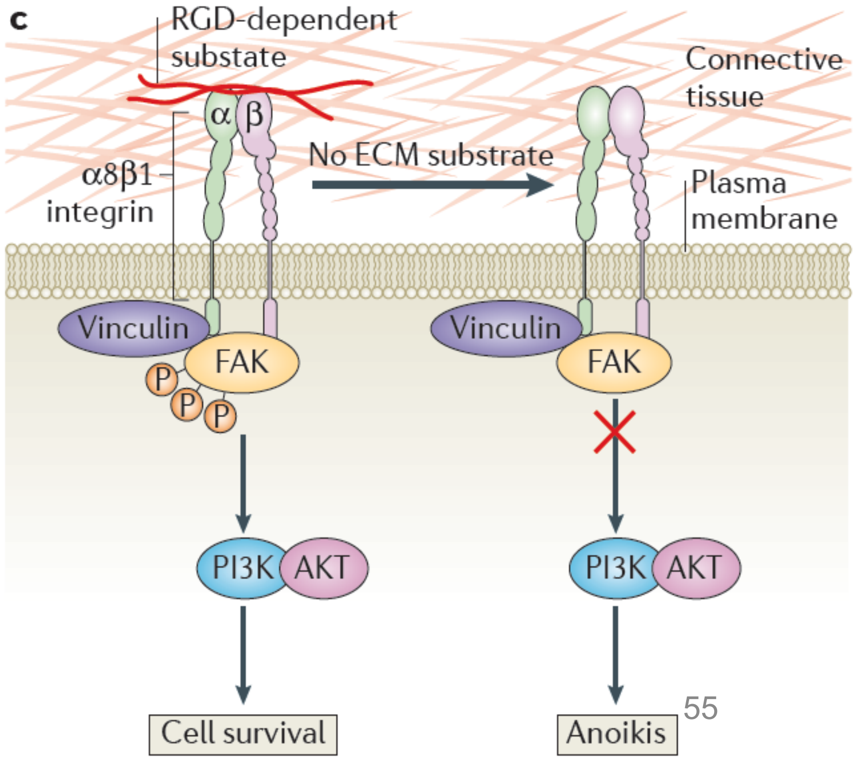


Figure 3. Integrins and Stable Adhesion between Epithelia

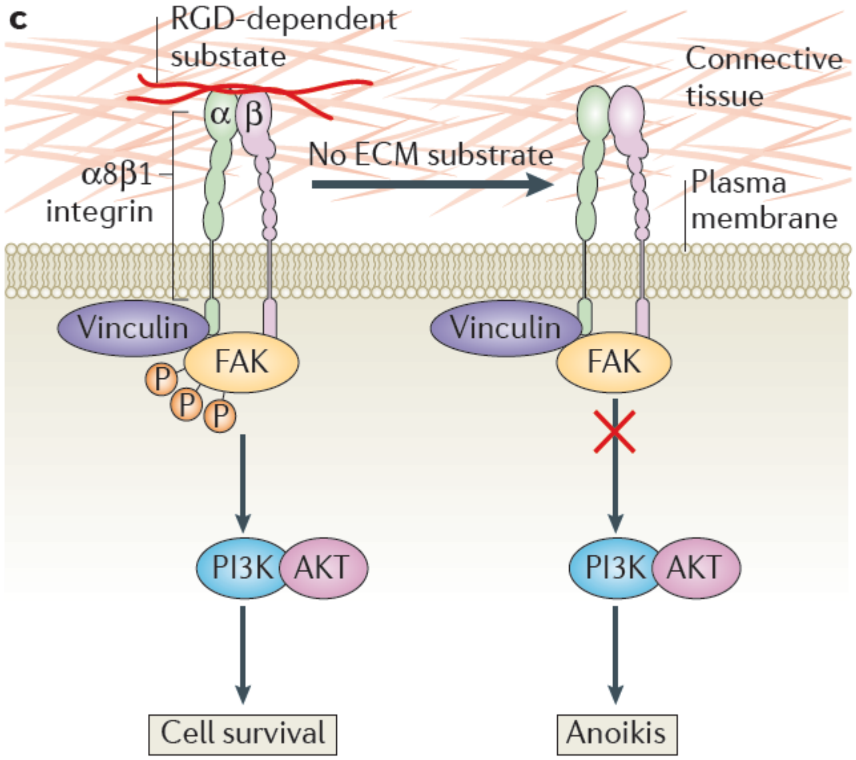
(A and B) The developing *Drosophila* wing consists of two epithelia that face each other with their basal sides. Loss of integrin function from clonal patches of cells leads to the formation of liquid-filled blisters (A). Other genes potentially involved in integrin function, such as *piopio*, shown in (B), were isolated in screens based on this easily scorable phenotype.

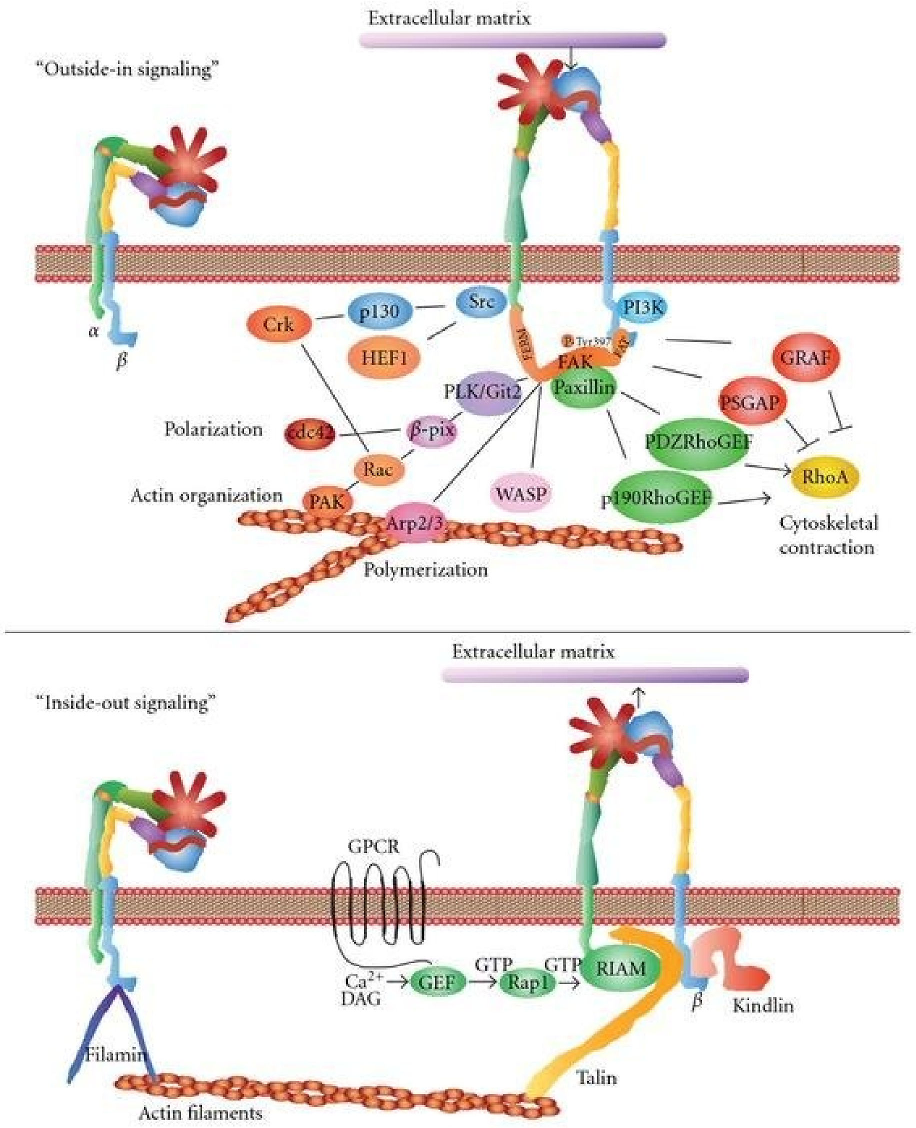
Anoikis

- If the cell separates from the epithelium (or ECM), it is potentially dangerous – it loses an important regulatory component (basal lamina/ECM).
- Therefore, there is an anoikis = programmed cell death, if the cell separates from the ECM, a signaling pathway is triggered that leads to programmed cell death.
- The binding of integrins to ECM components prevents Anoikis – a still unknown mechanism.



Integrins - Signalling





ECM receptors - conclusion

- Know the basic components of ECM
- What happens when ECM components is missing.
- To know what Integrins are, their structure and function.
- How the cell "perceives" contact with the ECM.
- ANOIKIS – even apoptosis is important in the course of development.

ECM – Dotazy?

Děkuji za pozornost

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