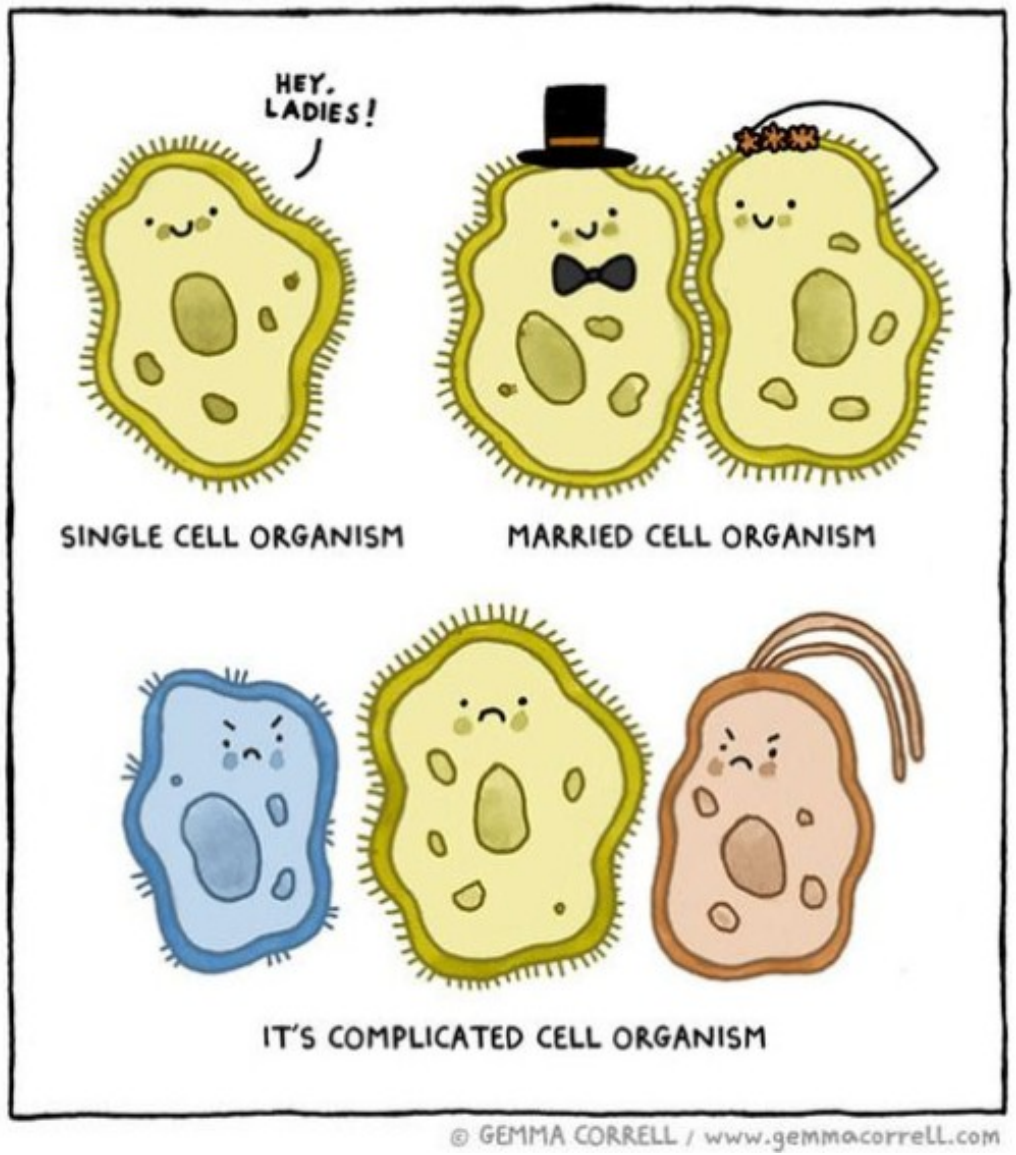
Vývojová biologie

# The Role of Cell Adhesions in Development

Tomáš Bárta
tbarta@med.muni.cz



# Obsah přednášky

- Introduction
- General principles of cell-to-cell contacts
- Cadherins
- Nectins
- Selectins
- Ephrins
- Summary

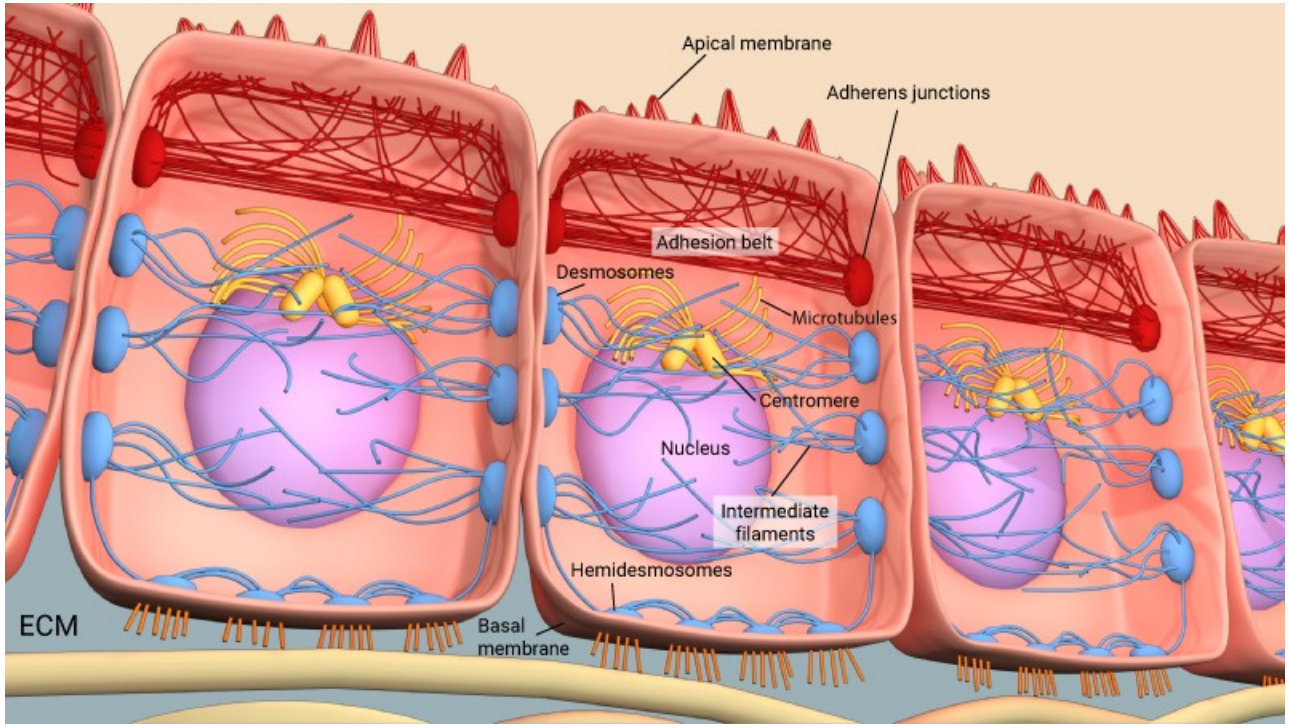
# Cell adhesion

- How separated tissues are generated from cell populations?
- How organs are generated in particular positions and how migrating cells reach their destinations?
- What separates mezoderm from ectoderm, so skin has dermis and epidermis?
- How some cells – e.g. precursors of pigmented and germ cells are migrating, so they can reach the final destinations?


Can we apply this thermodynamic model for cells in an embryo?

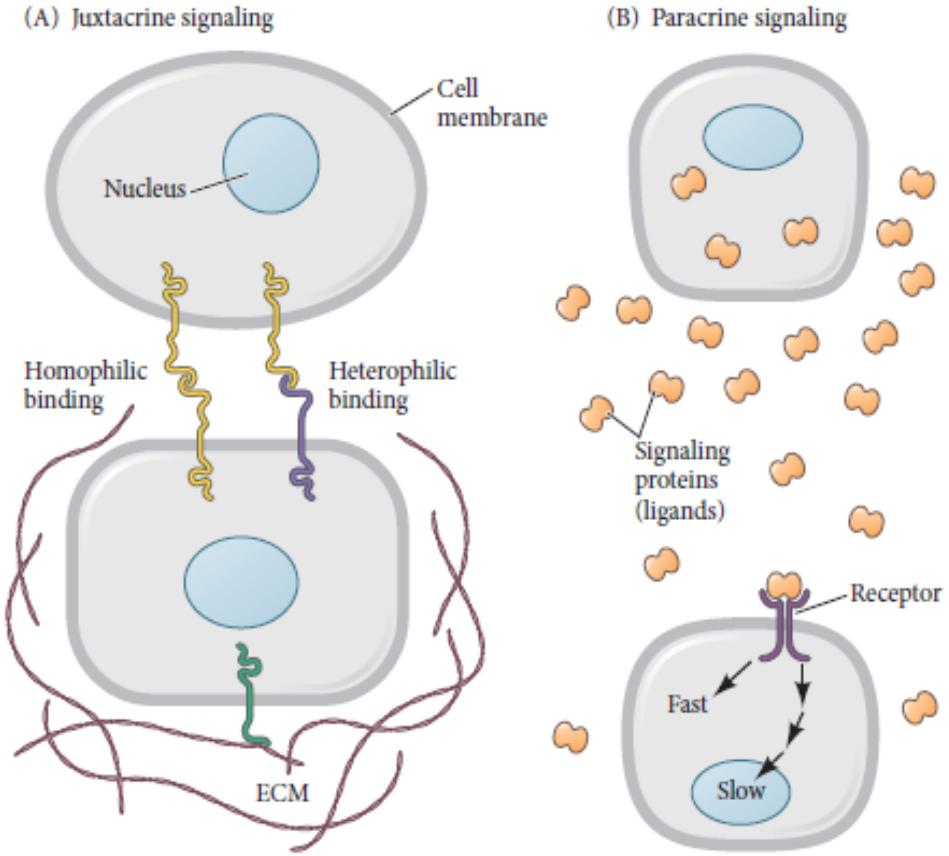
# Cell adhesion

- Evolutionary associated with multicellular organisms.
- Spatio-temporal regulation of cell adhesion is critical for the proper embryonic development (**Epidermolysis bullosa**)
- Adhesion molecules are not only proteins that stick cells together, but they also execute some function(s) – cell signaling. They contain extracellular, membrane a intracellular domain – they are capable of signaling (a cell „knows“ about neighboring cells and interactions with ECM)



# Cell adhesion in communication

- V embryo the cells are communicating for a short range:
  - Direct contact (juxtacrinne signaling)
  - Secretion into ECM (paracrine signaling)

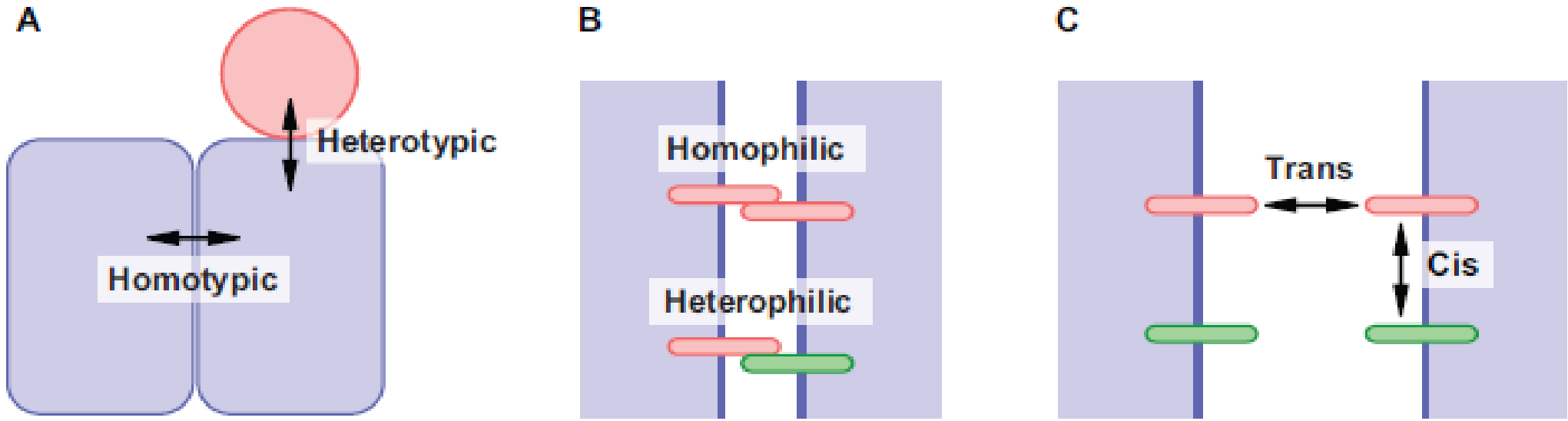



- Proteins that are secreted by a cell (or on a cell surface) and mediates communication are the signaling molecules (ligands)
- Proteins built in membrane that bind the ligands (imobilized or free) are receptors.
  - Homophilic vs. Heterophilic binding (@juxtacrine signaling)
- A conformation change happens after binding leading to change of properties of intracellular domain of the receptor.

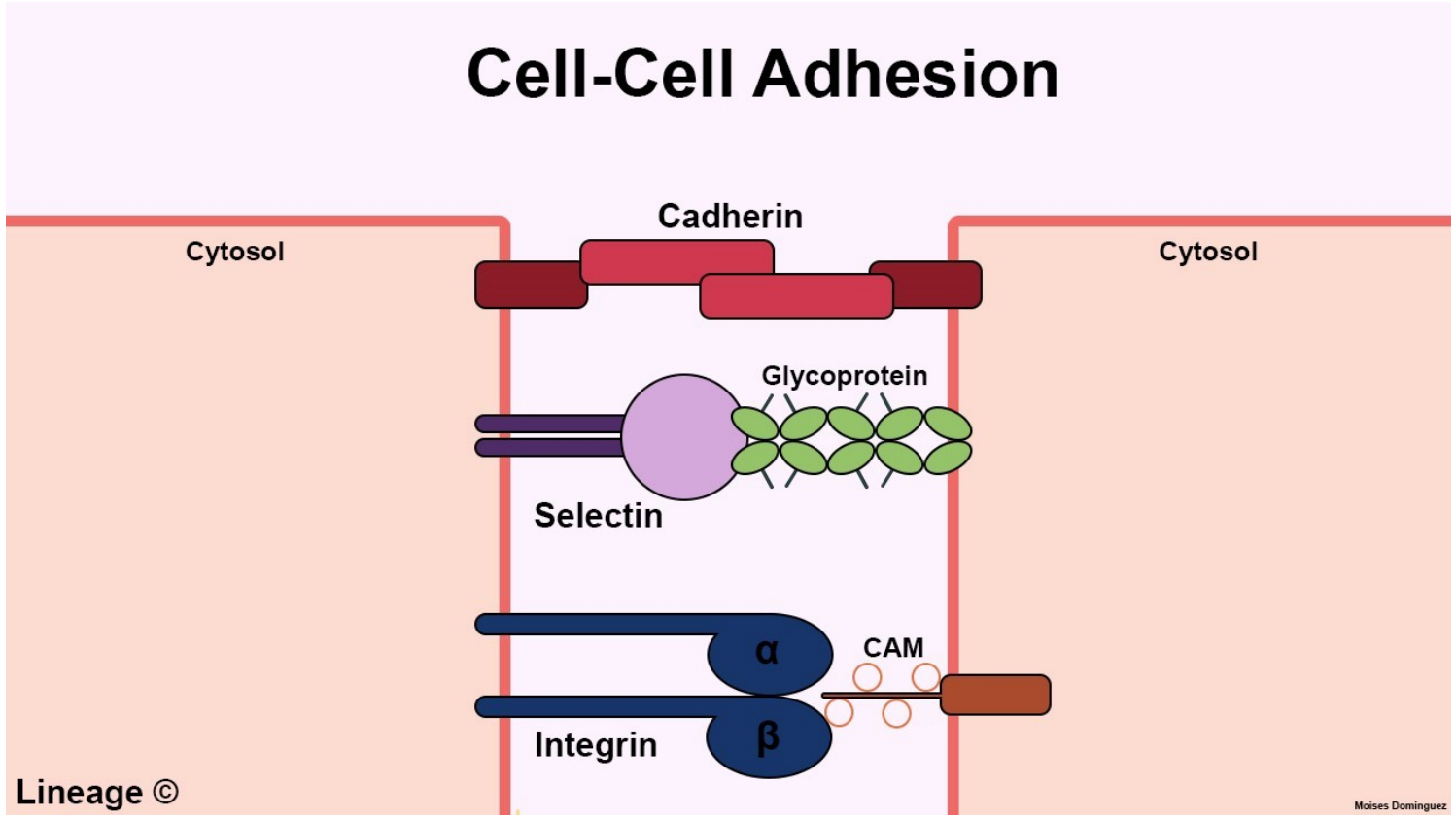
- Important for cell-to-cell communication

# Cell adhesion – expressions we will need





# Molecules mediating cell-cell adhesion



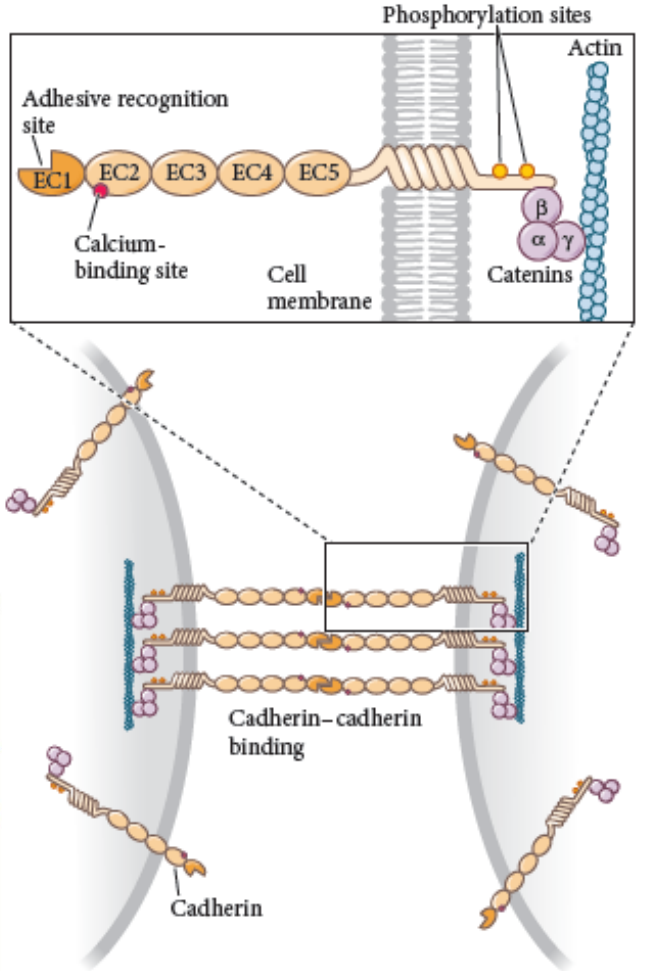

+Nectin
+Efrin

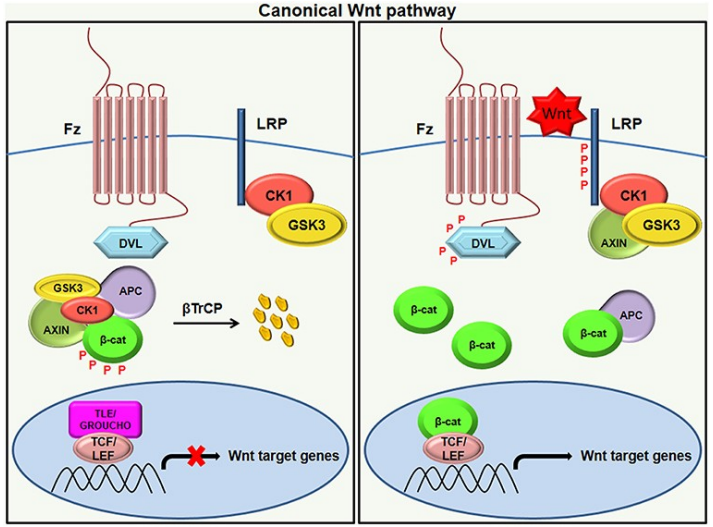
# Cadherins

# Cell adhesions - Cadherins

Cadherins

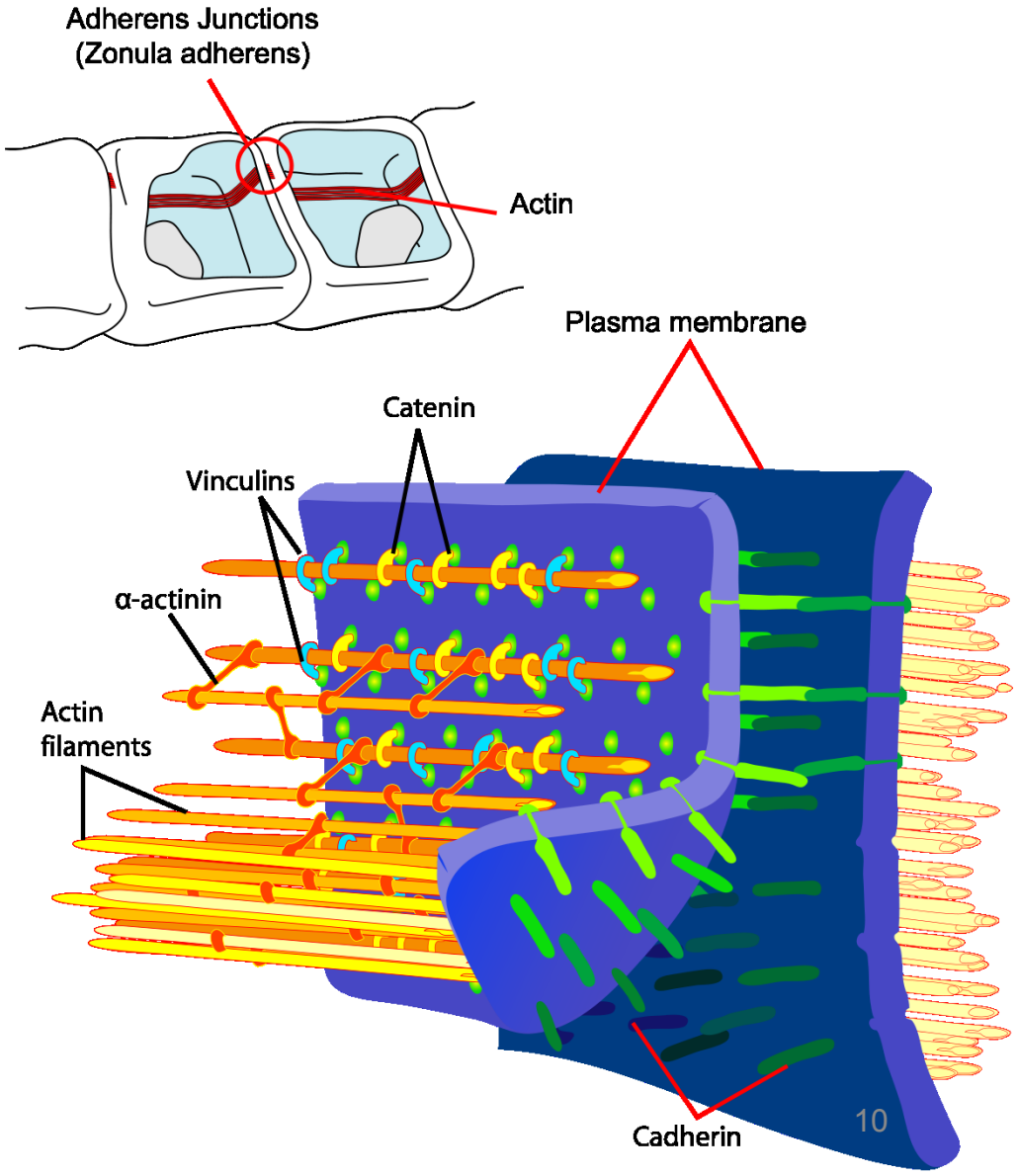
- ***ca***lcium-*d*ependent ***adhe***sion molecules
- Crucial for cell-cell adhesions
- Intracellular - They bind to cytoskeleton (Catenins)
- Complex of Cadherins and Catenins represents adhesion connections (adherens junctions) – typical for epithelium.
- Catenins may act as signaling molecules into the do nucleus (canonical Wnt signaling)
- Cadherin blocking (antibodies, RNAi) leads to disintegration of epithelium.



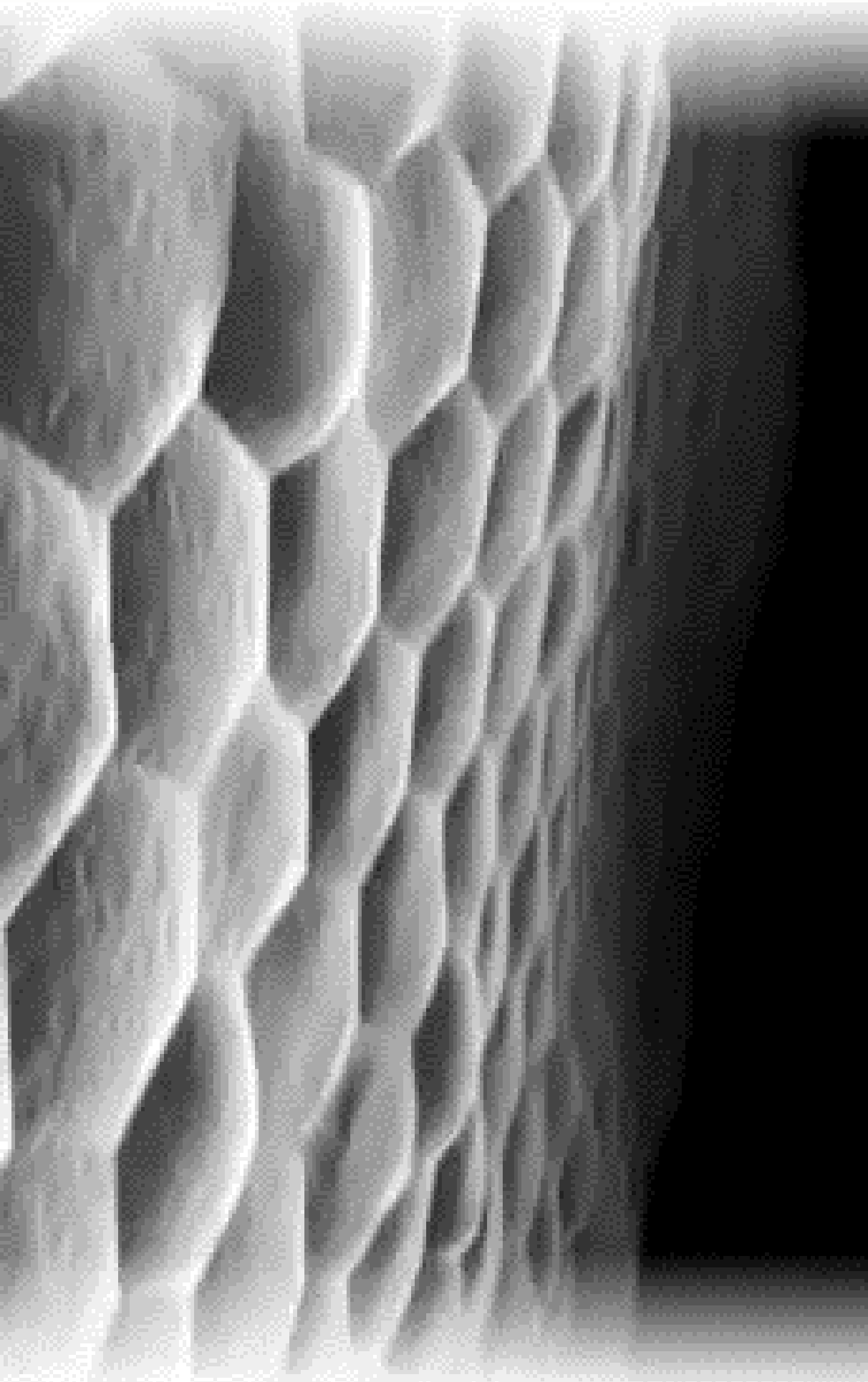



# Cell adhesion – Cadherins - Function

- Extracellular domain of Cadherins is for cell adhesion
- Cadherins bind to cytoskeletal actin -> contributing to cytoskelet -> providing mechanical force for forming of tissues
- Are capable of signaling into the nucleus -> leading to change of gene expression



# Vývojová biologie



# Cell adhesion – Cadherins - Types

**E-Cadherin**
- Expressed in epithelium

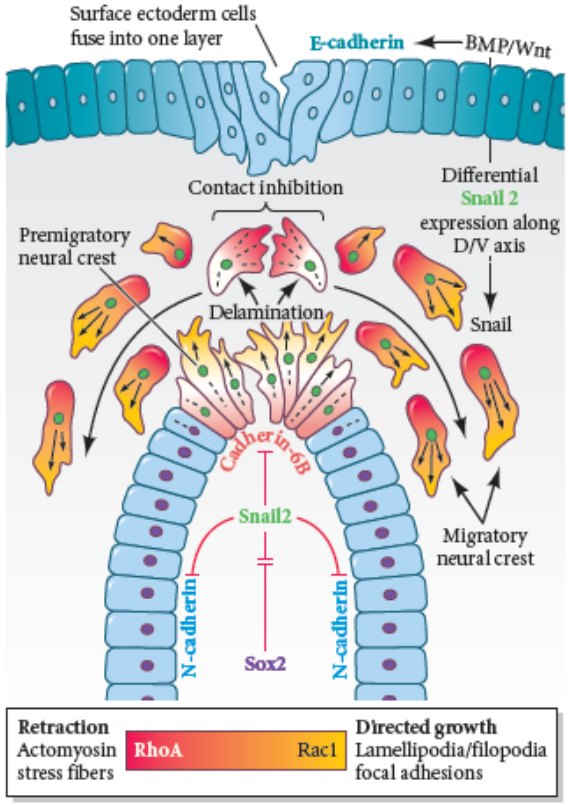
Link to the previous lecture:

**N-Cadherin**
- Expressed during the development of nervous system.
- Neural Cadherin

**R-Cadherin**
- Expressed in the retina
- Retinal Cadherin

**P-Cadherin**
- Expressed in placenta
- Placental Cadherin

**Protocadheriny**
- Lack binding to cytoskelet.

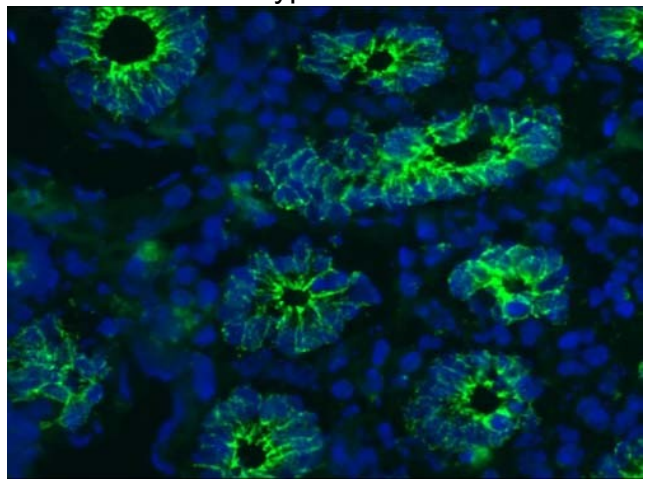


# Cell adhesion – E-Cadherin

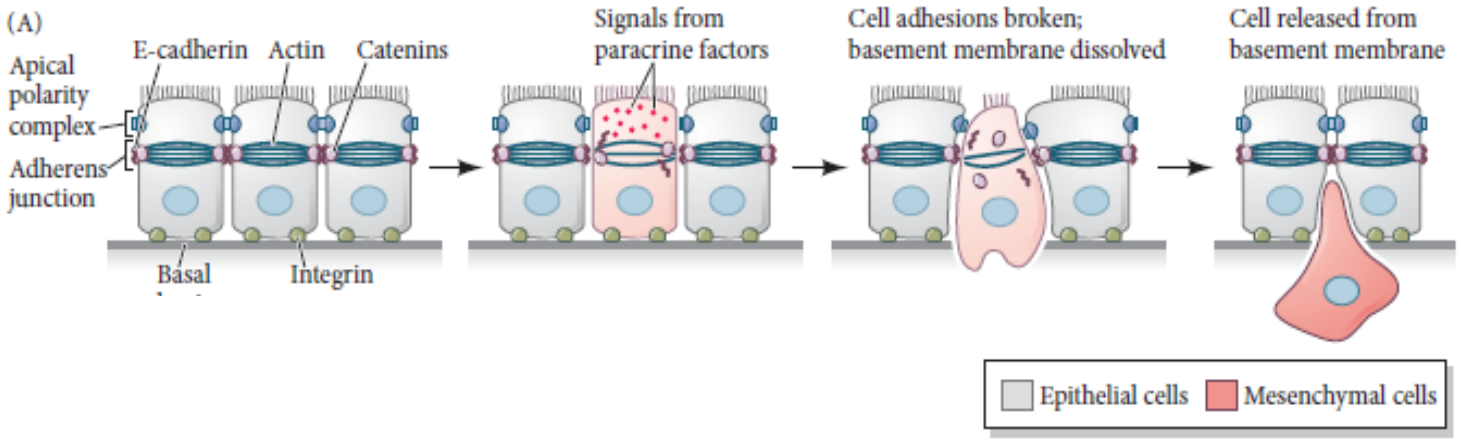
E-Cadherin (E-cadherin, Cadherin-1(CDH1), L-CAM, ARC-1, uvomorulin)

- Expressed on all mamalian embryonic cells, later only in epithel
- Evolutionary conserved
- Essential glycoprotein in development, cell differentiation and in tissue homeostasis mantenance.
- Important for establishment and maintenance of epithelial polarity,
- Mediates homophilic cell-cell interactions.

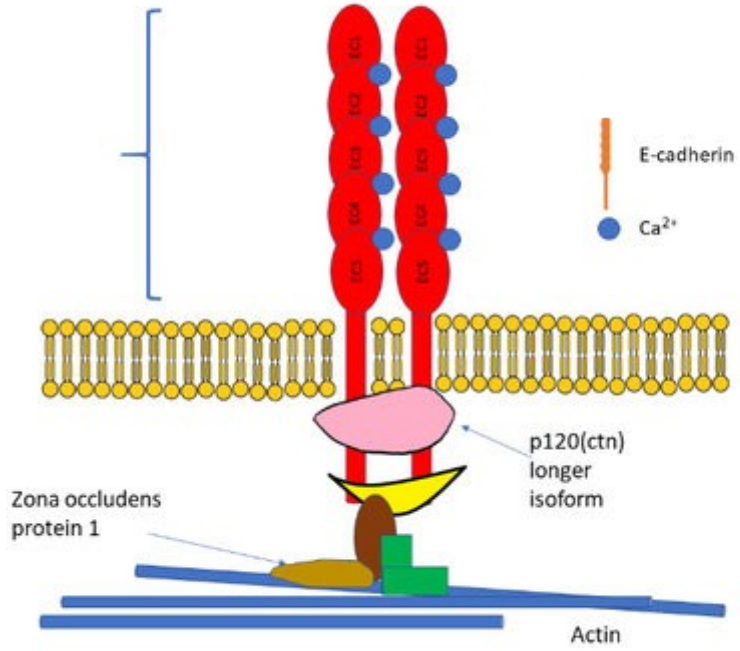
Crypts



Link to the previous lecture:



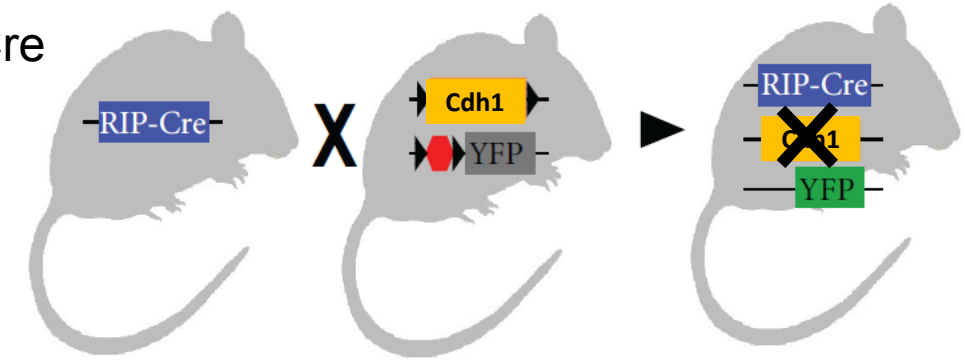
# Cell adhesion – E-Cadherin

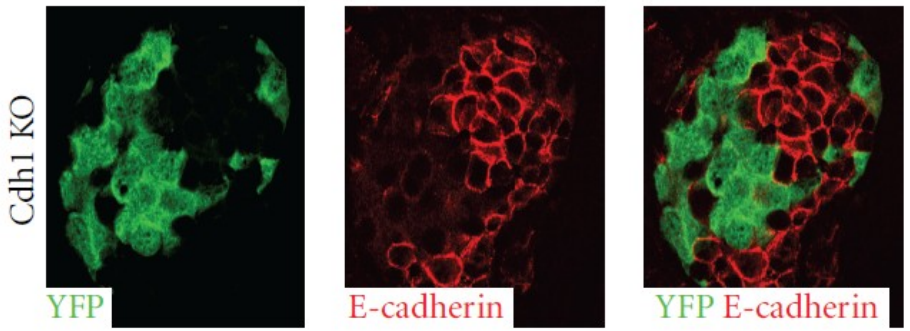


# Cell adhesion – E-Cadherin

- Mice knock-outs for CDH1: lethal before implantation, morula is dissociating – no adhesion. Embryo does not form epithel. e.g. trophectoderm
- Therefore in order to study the role of CDH1, you have to use tissue-specific KO (i.e. CRE recombinase)

- RIP-Cre - **R**at **i**nsulin **p**romoter -> Cre recombinase is expressed only in pancreas.



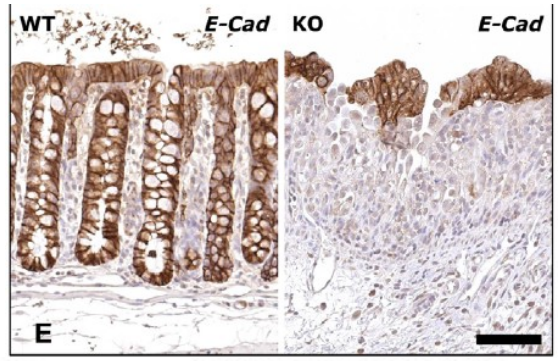



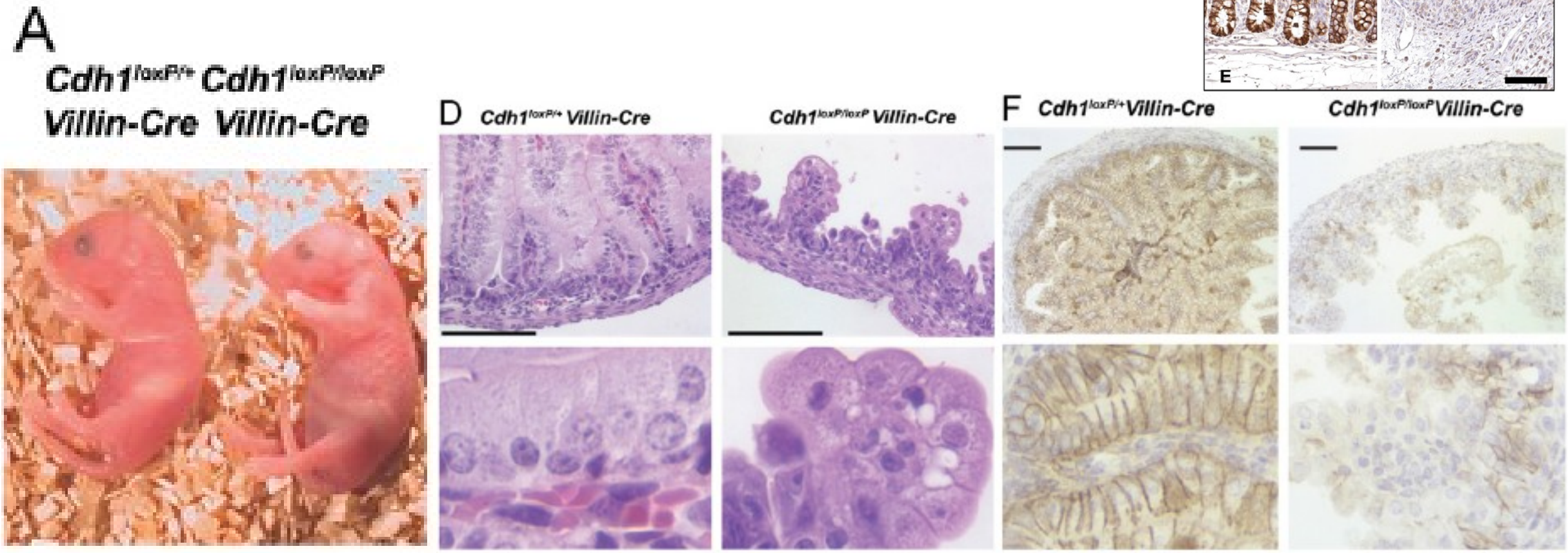



Mandelbaum et al., 2012

# Cell adhesion – E-Cadherin

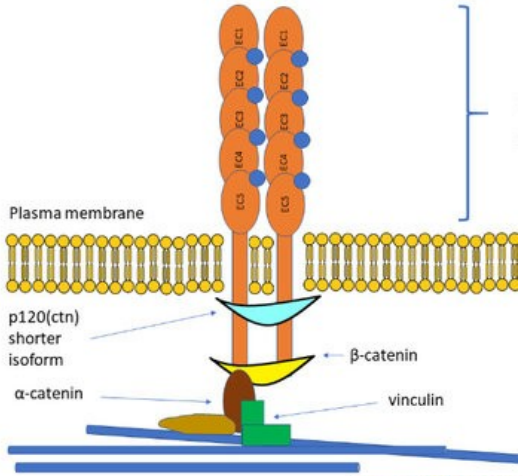
- Tissue-specific KO of E-cadherin in intestine.

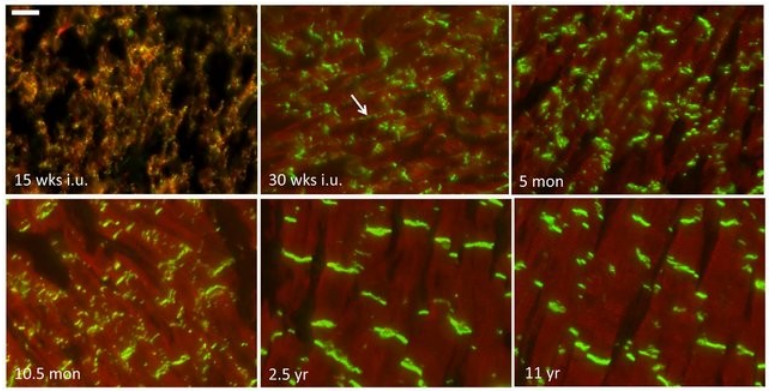


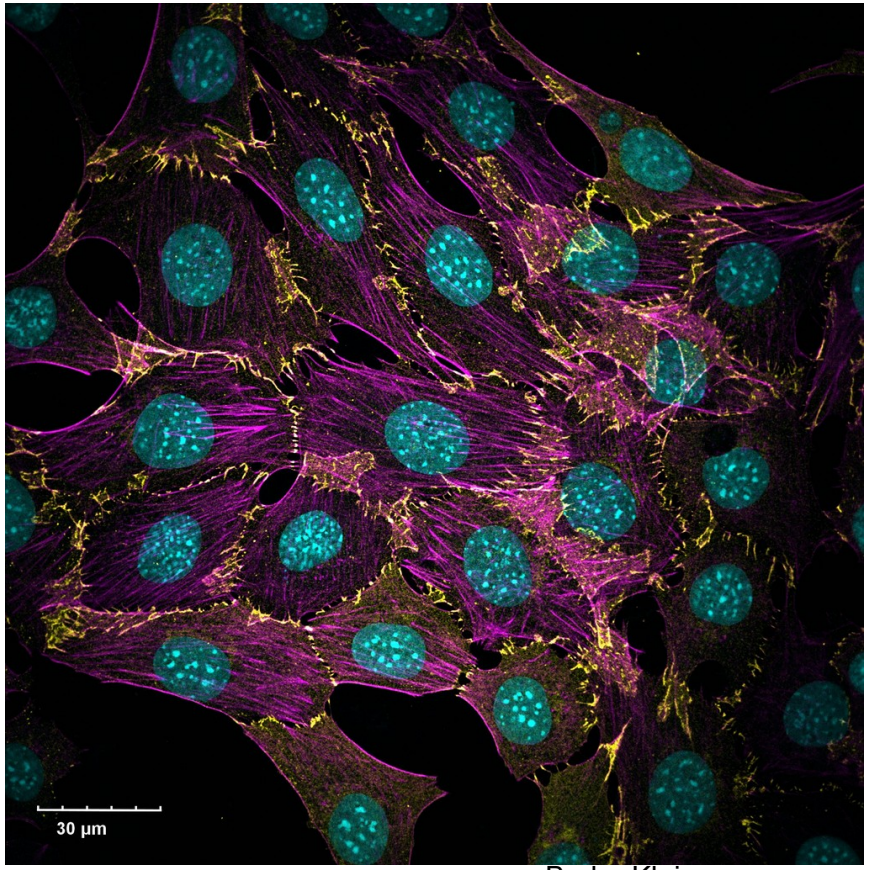
# Cell adhesion – N-Cadherin

- **N-Cadherin, Cadherin-2** (**CDH2**) or **neural cadherin** (**NCAD**)
- In cardiac muscle, N-cadherin is an integral component in the adhesive junctions located at intercalation discs that function to mechanically and electrically connect adjacent cardiomyocytes.




Smooth muscle cells

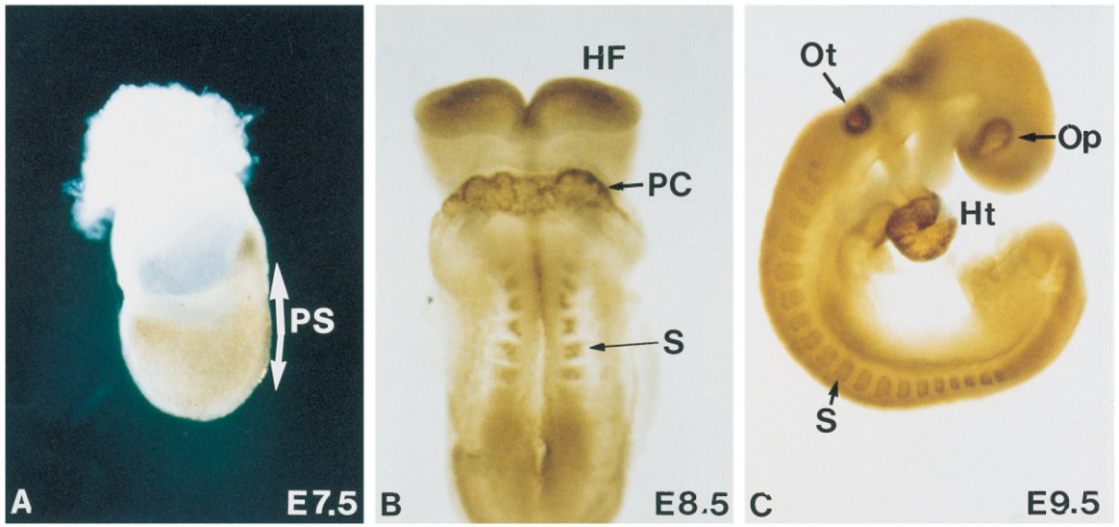



Burke-Kleinman

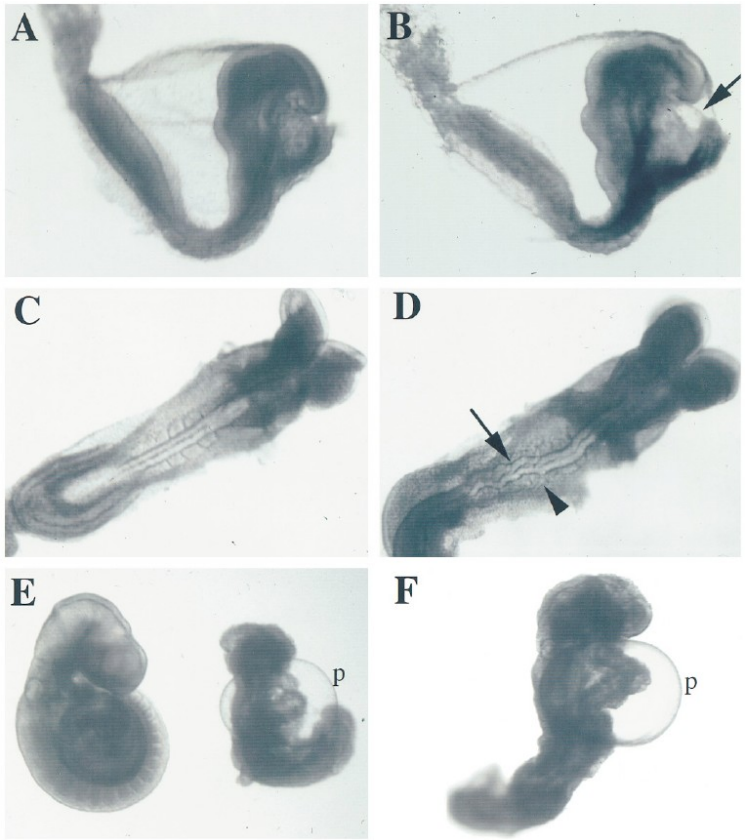
# Cell adhesion – N-Cadherin

- Mice KO: lethal at E10, abnormal shape of somites, heart abnormalities

Staining of wild type for N-Cad

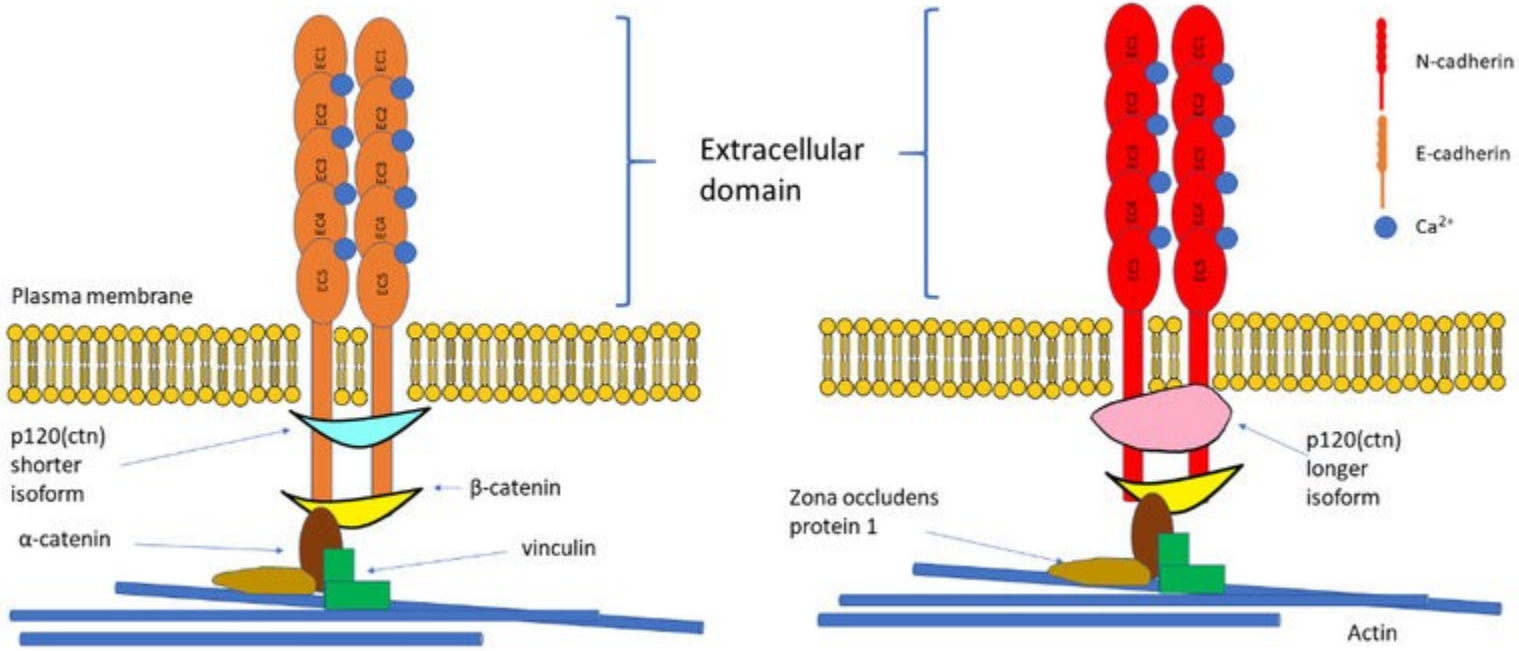



N-Cad -/-



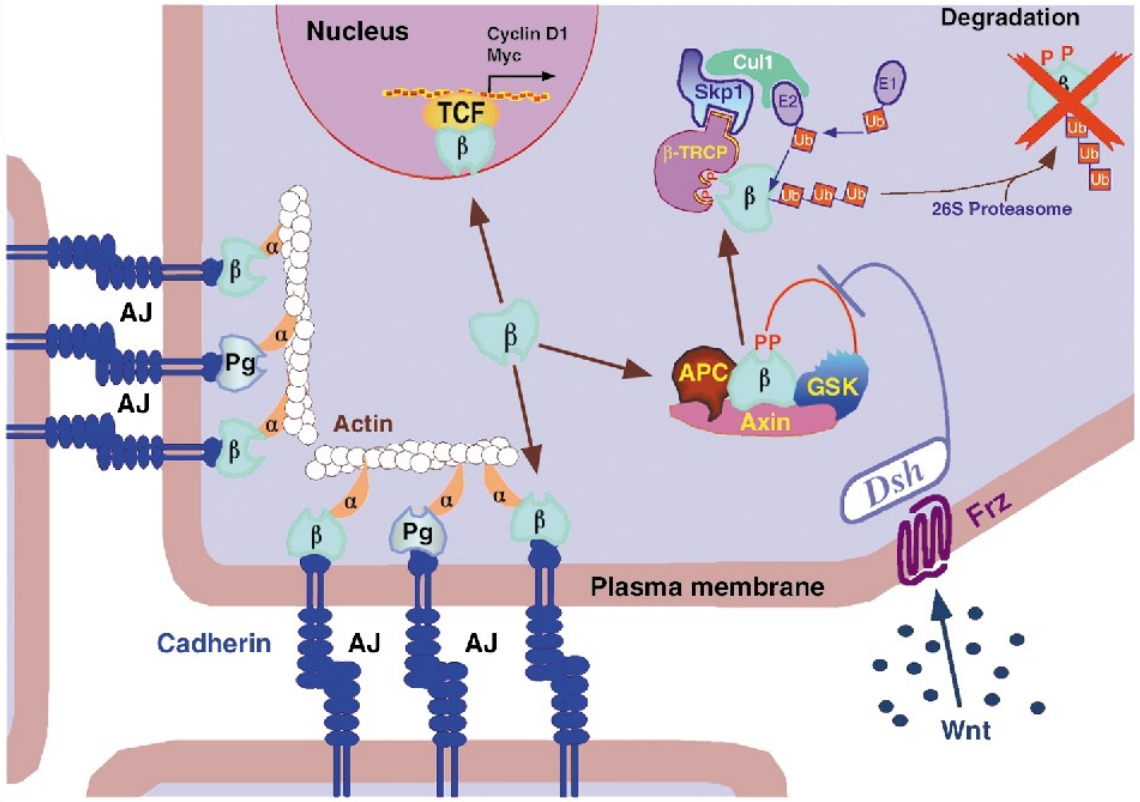

Radice et al., 1997

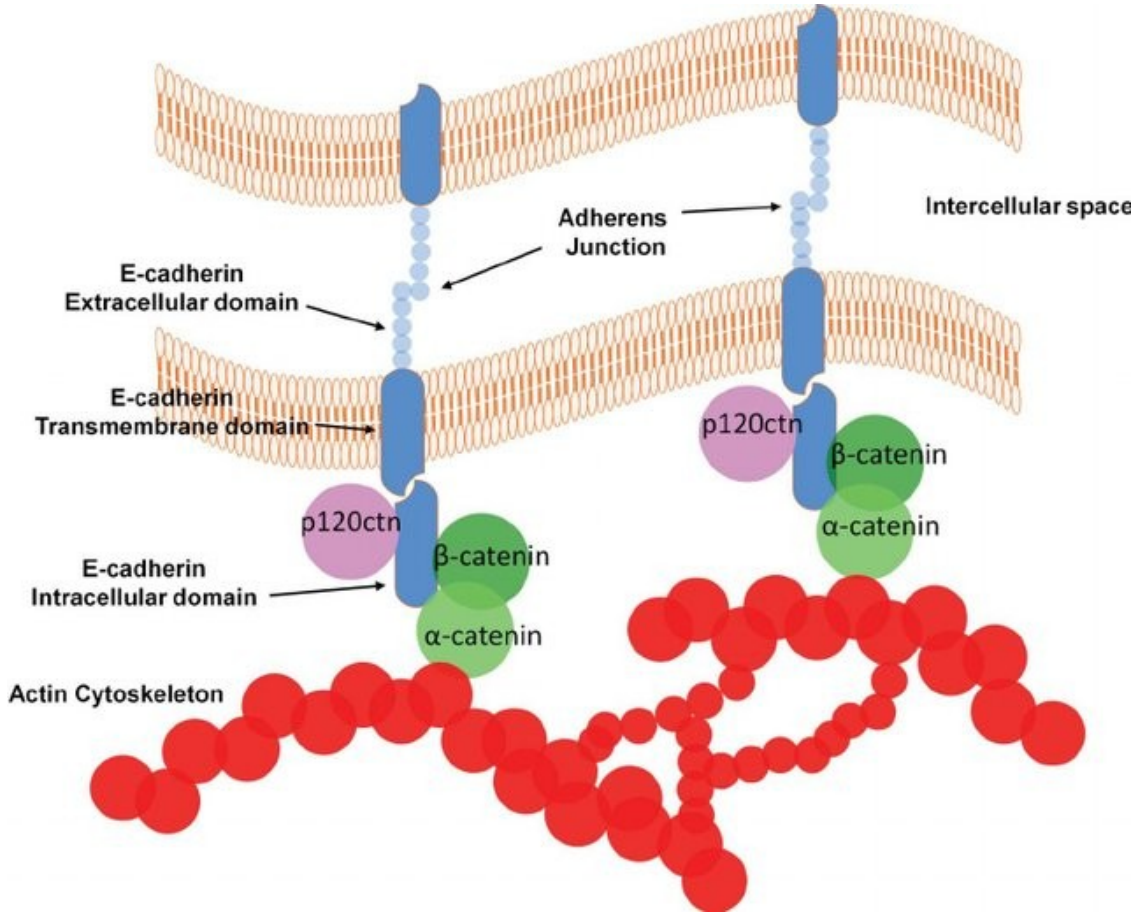
# N-Cadherin vs E-Cadherin



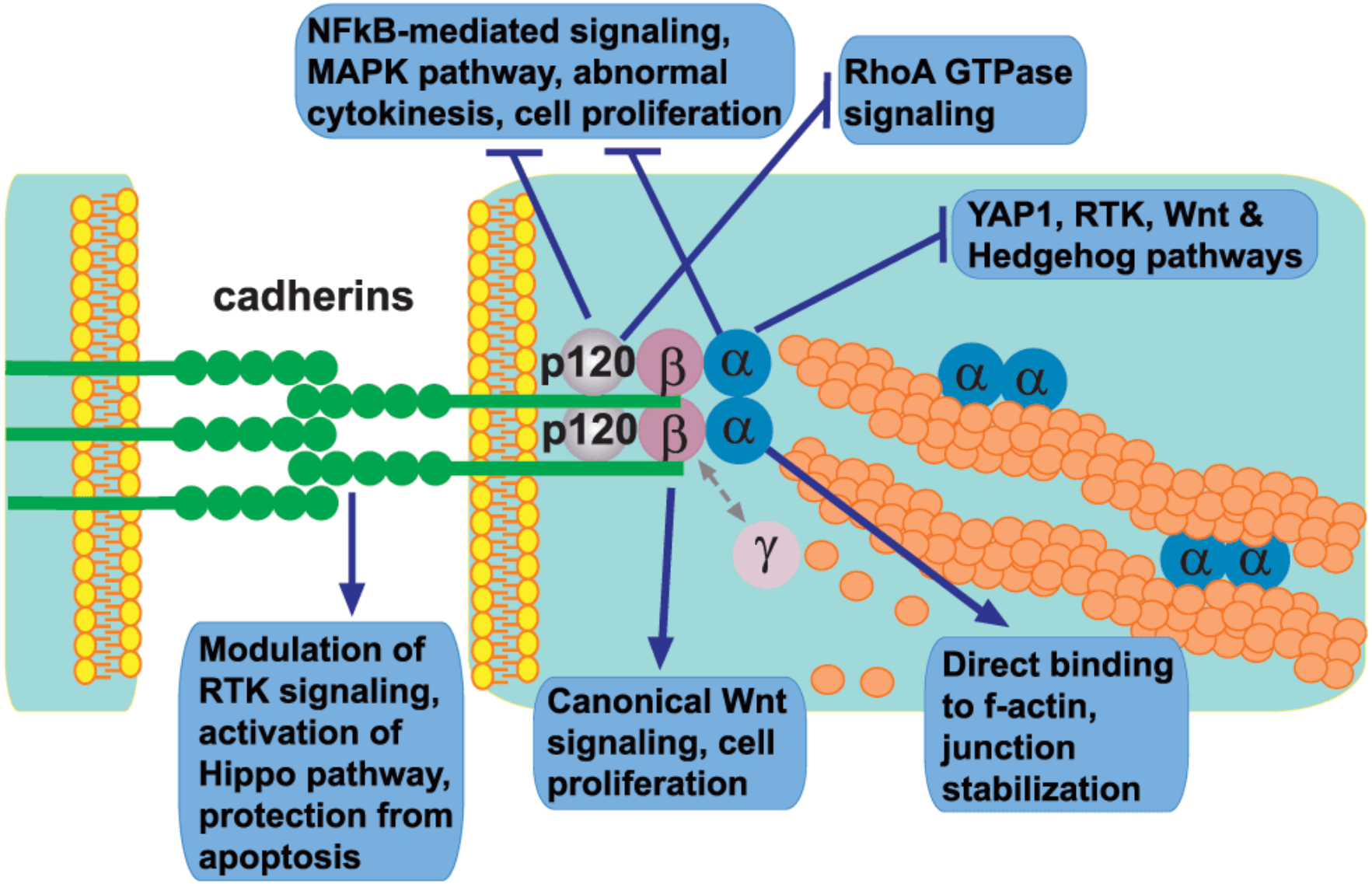
# Cell adhesions – Cadherins - Signalling

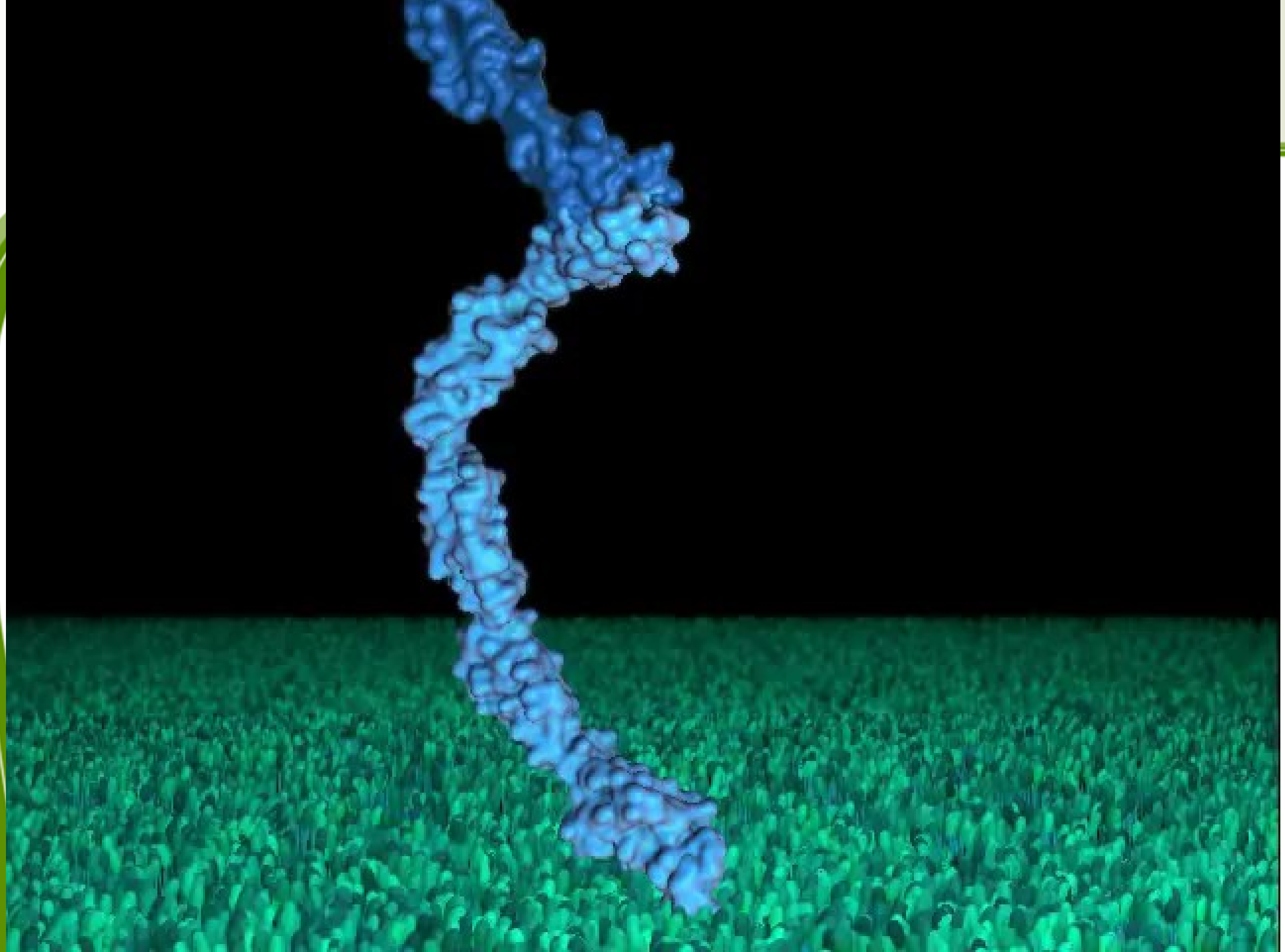
- They mediate not only physical contact between cells, but also signaling to the nucleus. E.g. through catenins - crosstalk with Wnt signaling.



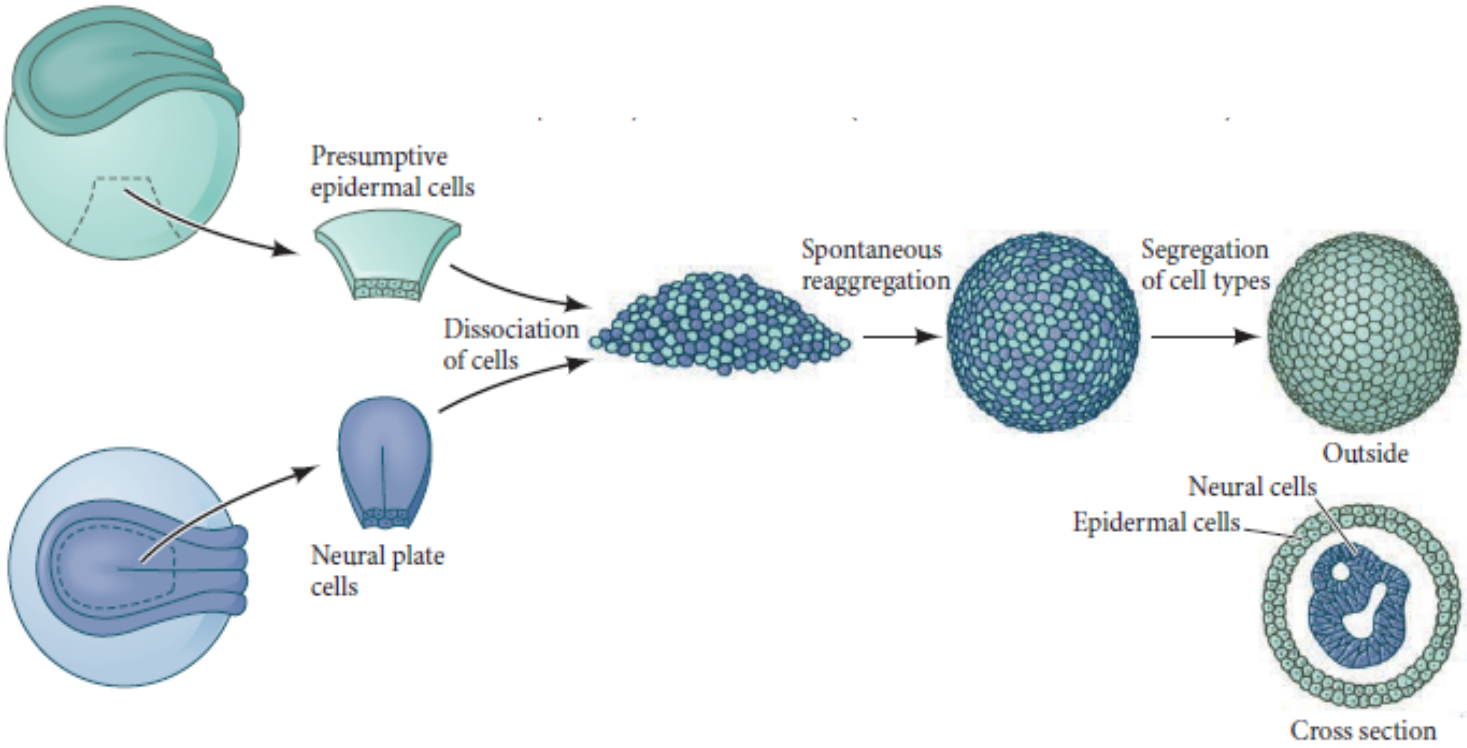



# Cell adhesion – Cadherins - Signaling





# Cell adhesion – selective cell affinity




Epidermal cells from pigmented embryos and neural plate cells from non-pigmented embryos were dissociated and mixed together. The cells reaggregated so that the epidermis covered the nerve tissue.

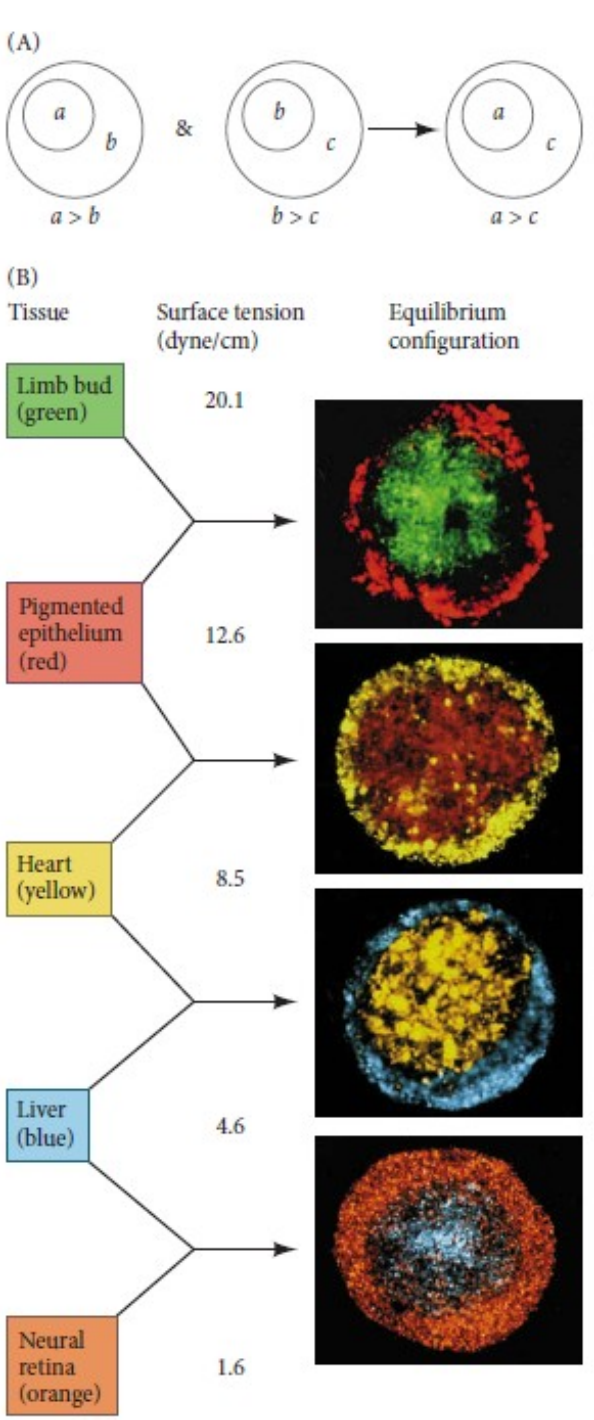
Townes and Holtfreter, 1955

# Cell adhesion

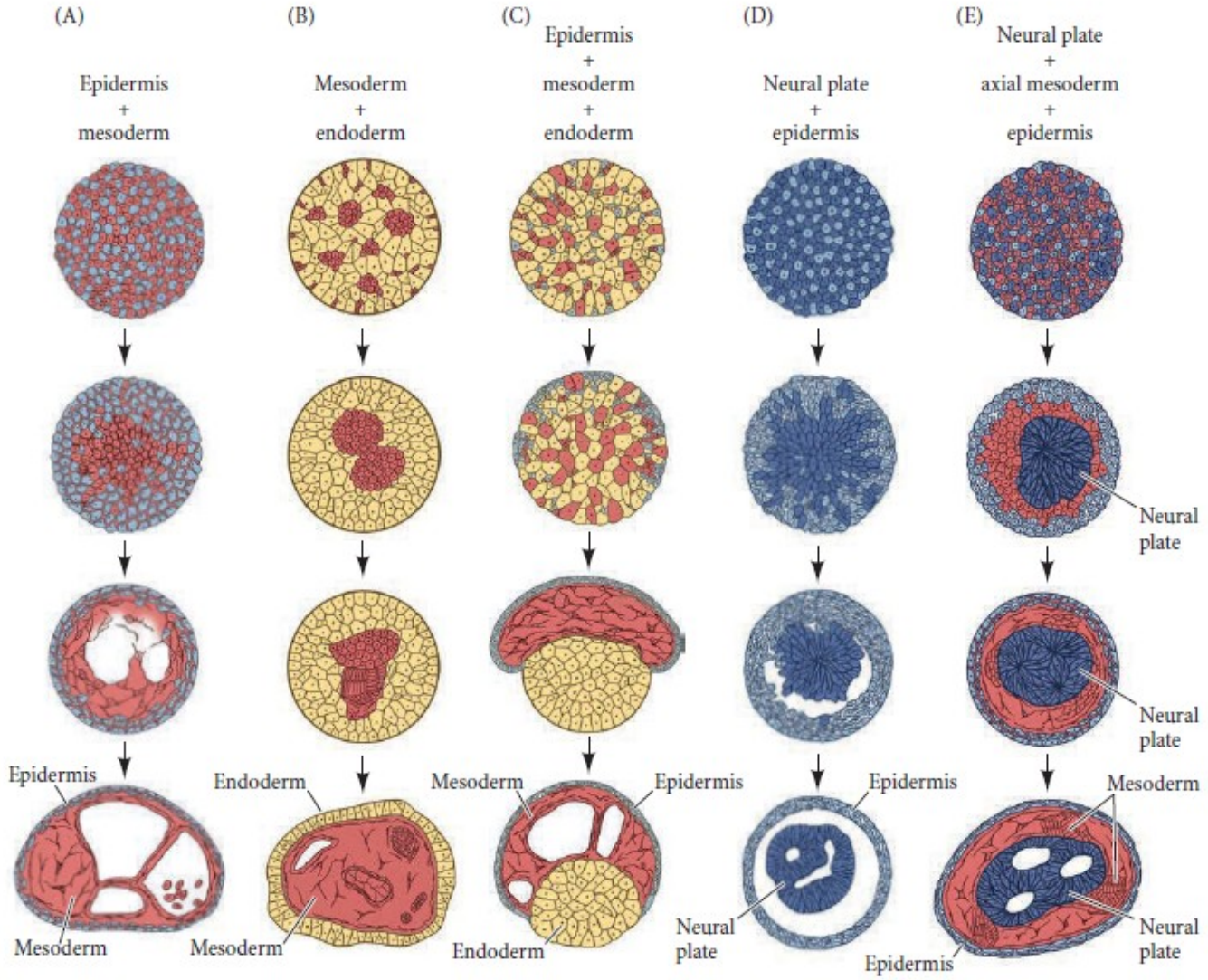
differential adhesion hypothesis

„According to this hypothesis, the early embryo can be viewed as existing in an equilibrium state until some change in the adhesive properties of the cell's plasma membrane changes. The movements that result seek to restore the cells to a new equilibrium configuration."

The boundary between tissues is thus formed by different types of cells, which have different adhesive molecules in different amounts on their surface



# Cell adhesion




## Selective affinity

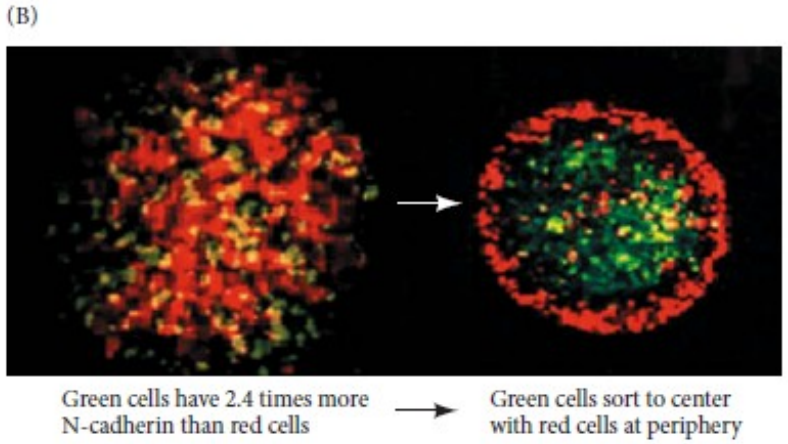
- The inner surface of the ectoderm has a positive affinity for the mesoderm and a negative affinity for the endoderm. Mesoderm has a positive affinity for both endo- and ectoderm.

*„Somehow, the cells are able to sort out into their proper embryonic positions.“*
Townes and Holtfreter, 1955

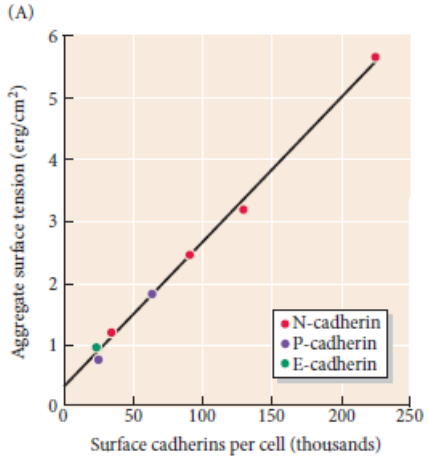
- Selective affinity changes during development.
- Cells must interact differently with other cells/tissues at specific times and changing conditions.
- Crucial for morphogenesis.

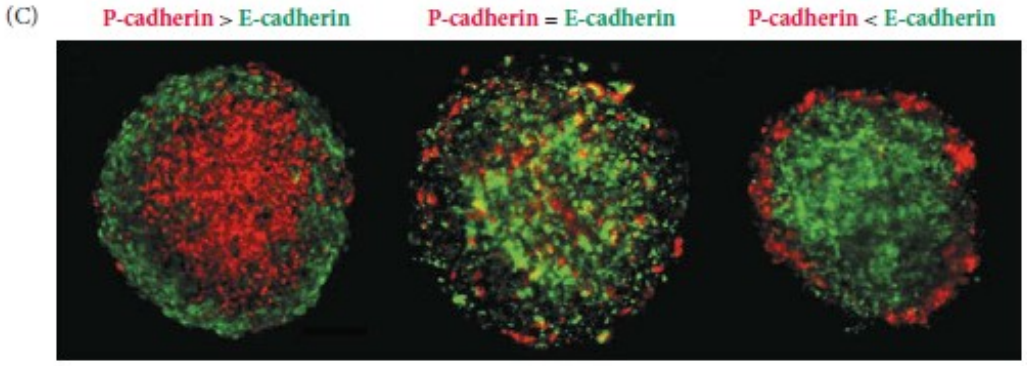
# Cell adhesion – Cadherins

**Cell adhesion is determined by the amount of Cadherin on the cell membrane.**

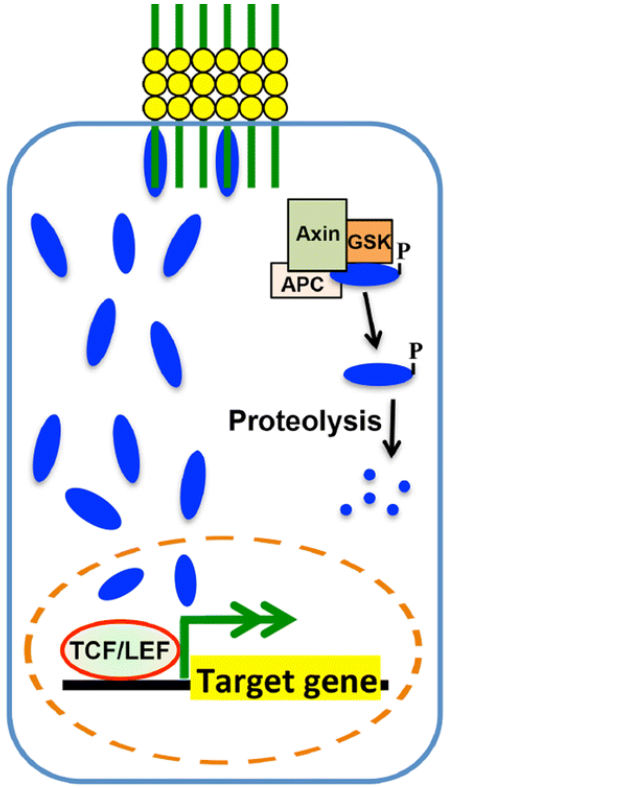



**Even when cells express different Cadherins**



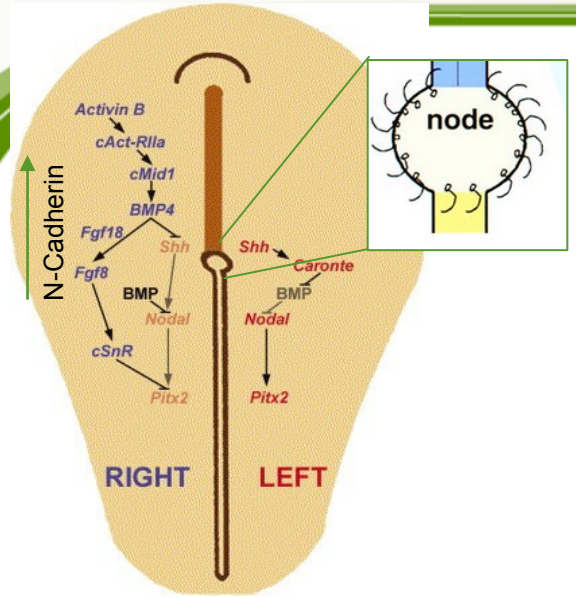



# Cadherins in L/R asymetry

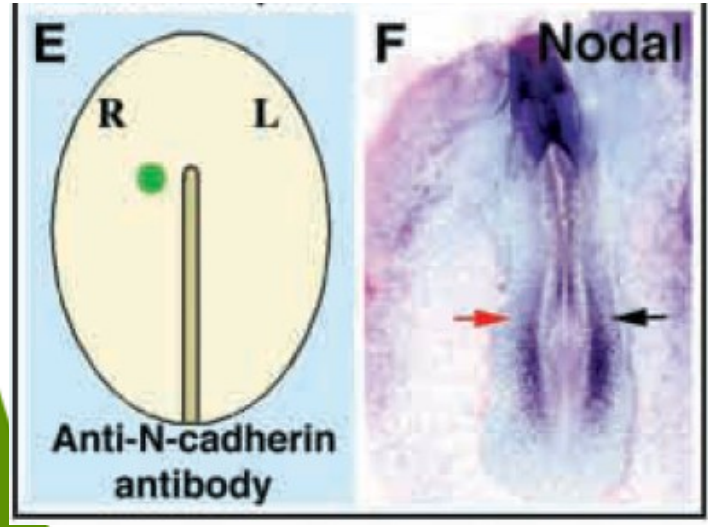



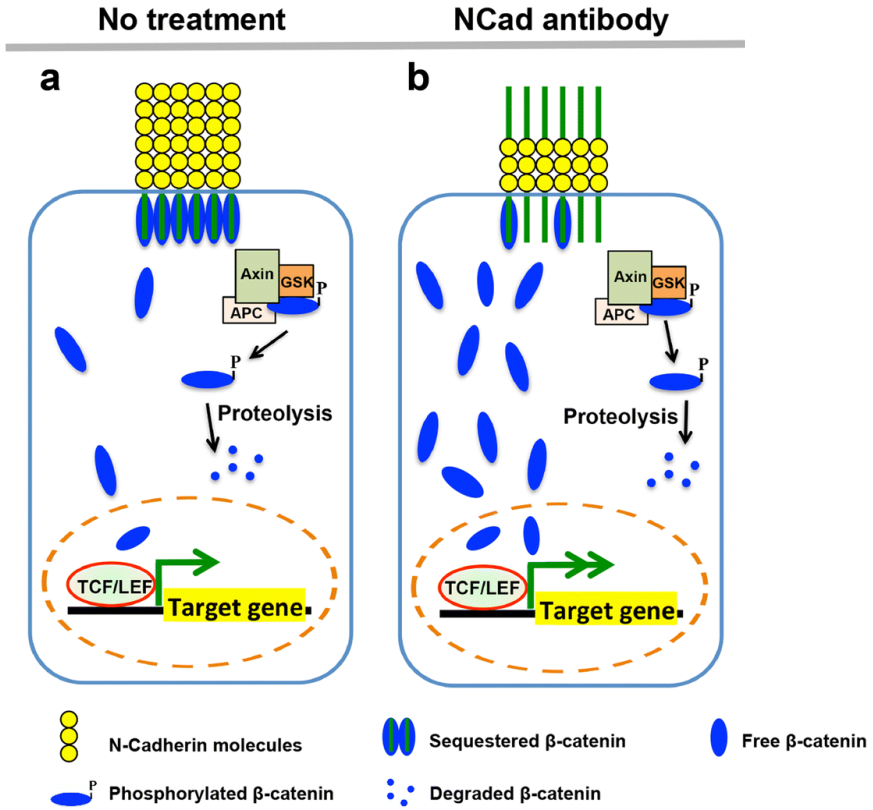

N-Cadherin molecules
Sequestered β-catenin
Free β-catenin
P
Phosphorylated β-catenin
Degraded β-catenin

# Cadherins in L/R asymetry

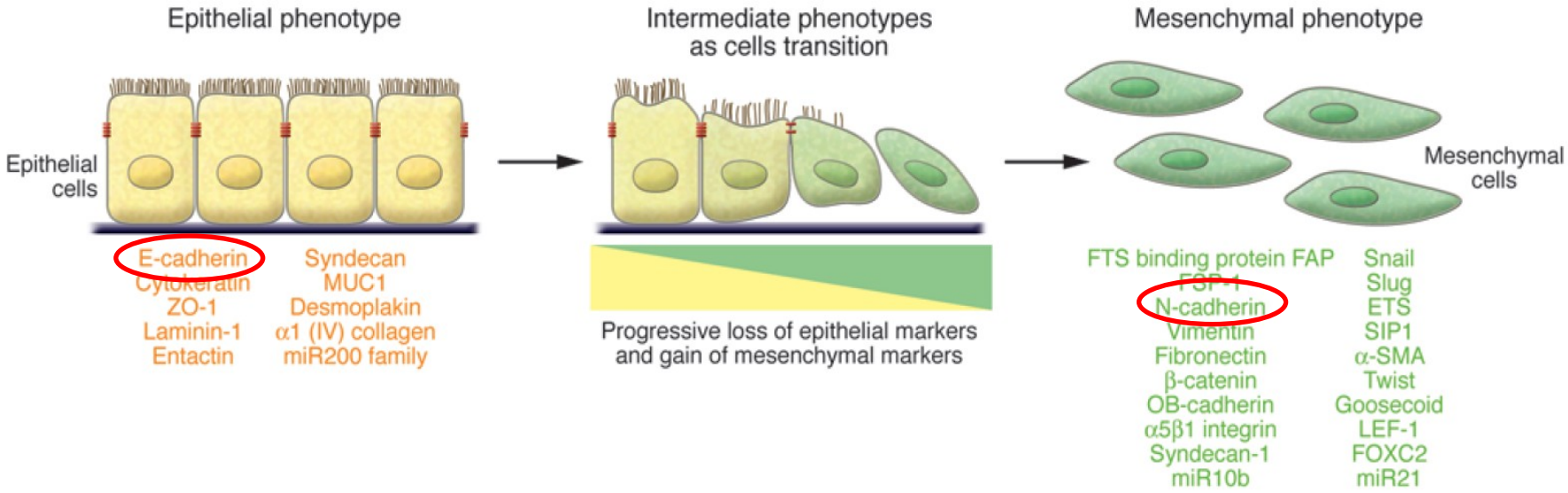



- High expression of N-Cadherin in the right half leads to the sequestration of β-catenin, which would otherwise be accessible for Wnt signaling, thus Wnt is inhibited in the right half.

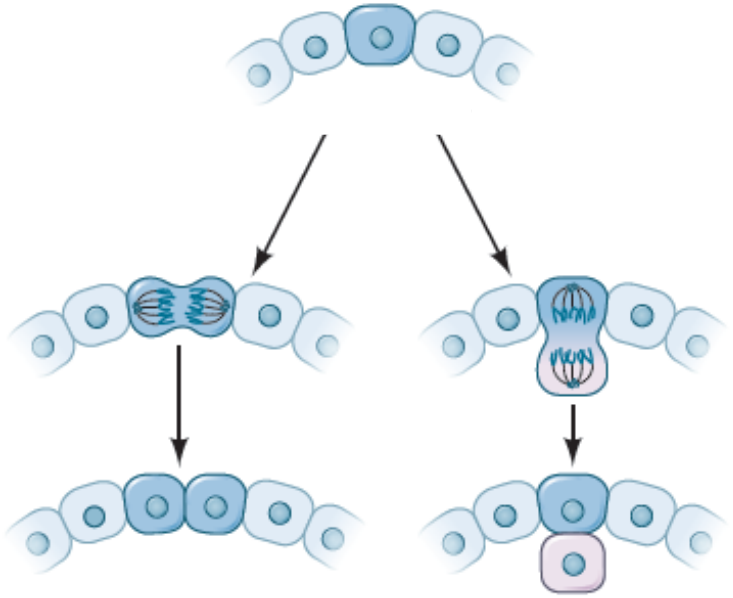


# Cadherins in EMT and gastrulation: link to the previous lecture



# Cadherins and asymmetric division: link to the first lecture



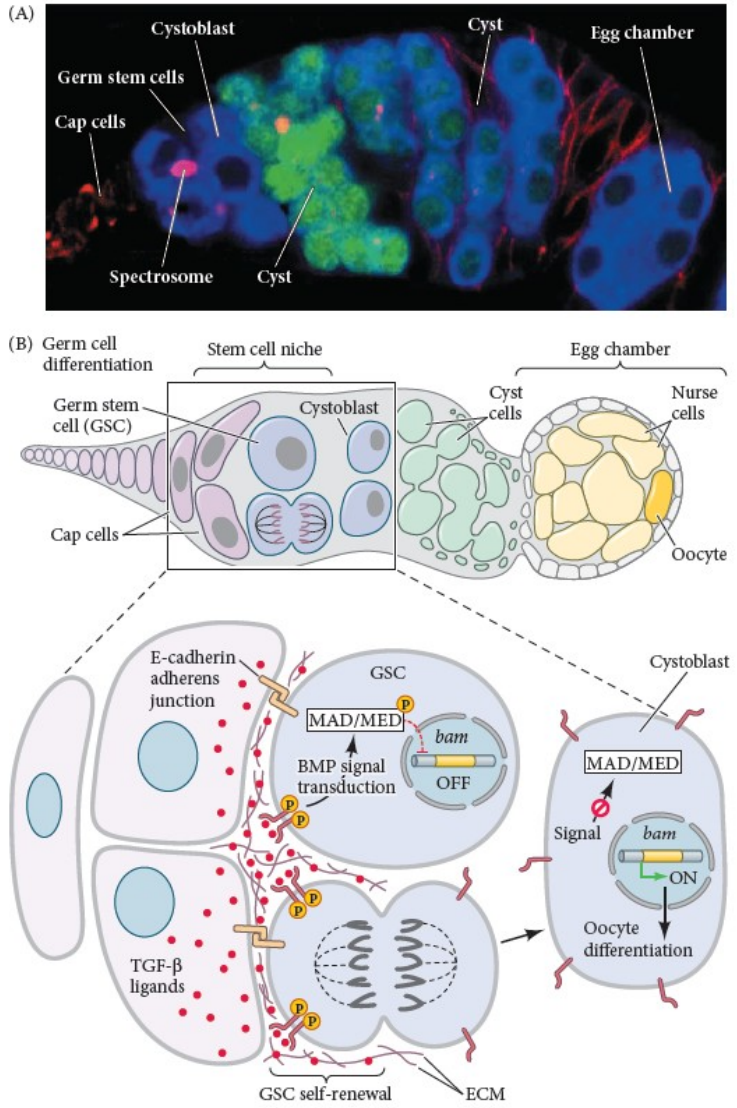
## Symetrical cell division

Cytoplasmatic determinants distributed evenly to both daughter cells, generating the same cells

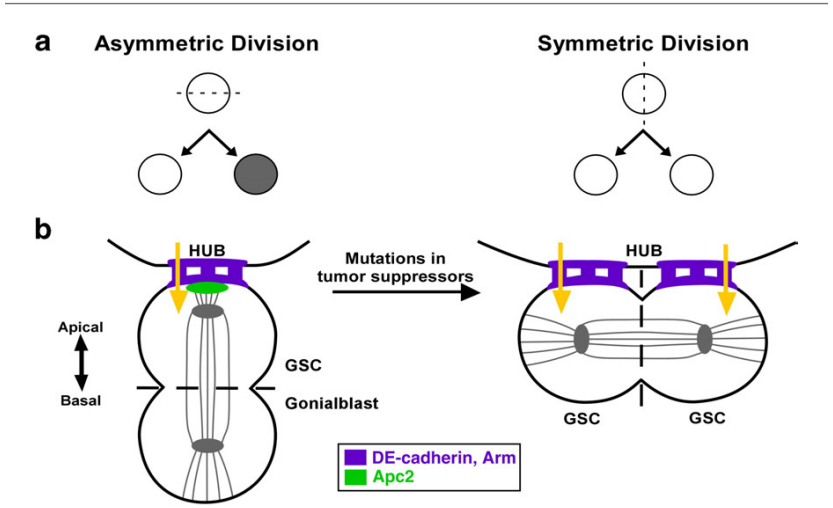
## Asymetrical cell division

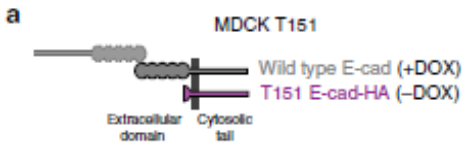
Cytoplasmatic determinants distributed unevenly, generating two different cells

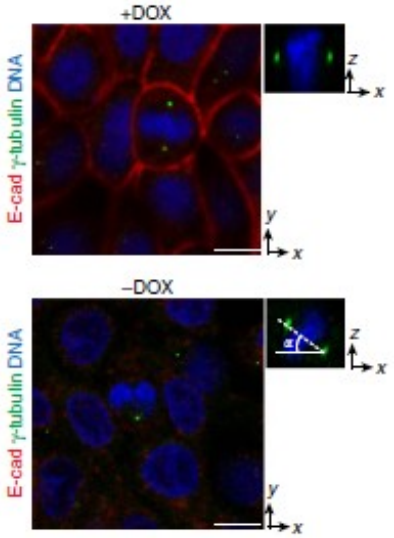
Drosophila ovarian stem cell niche

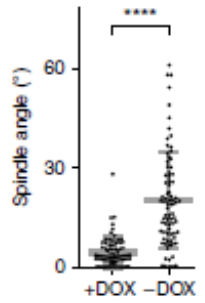


# Cadherins and asymmetric division: link to the first lecture




- A truncated variant of E-cadherin led to the induction of asymmetric division

Gloerich et al., 2016

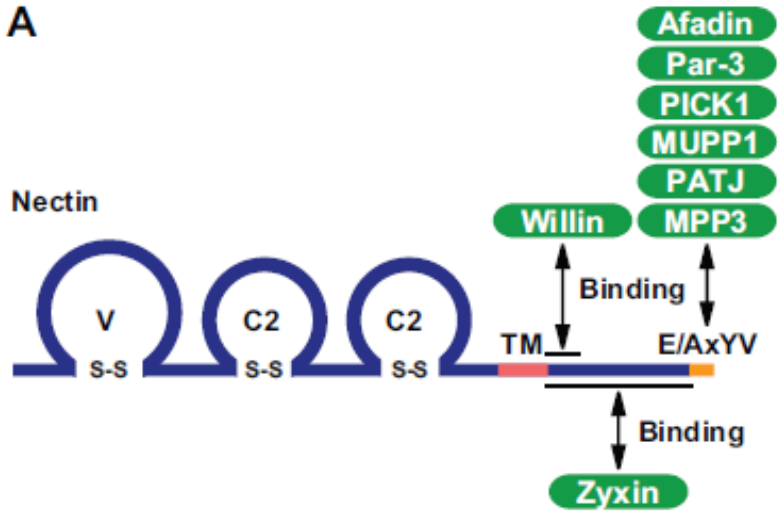
# Cadherins sumup:

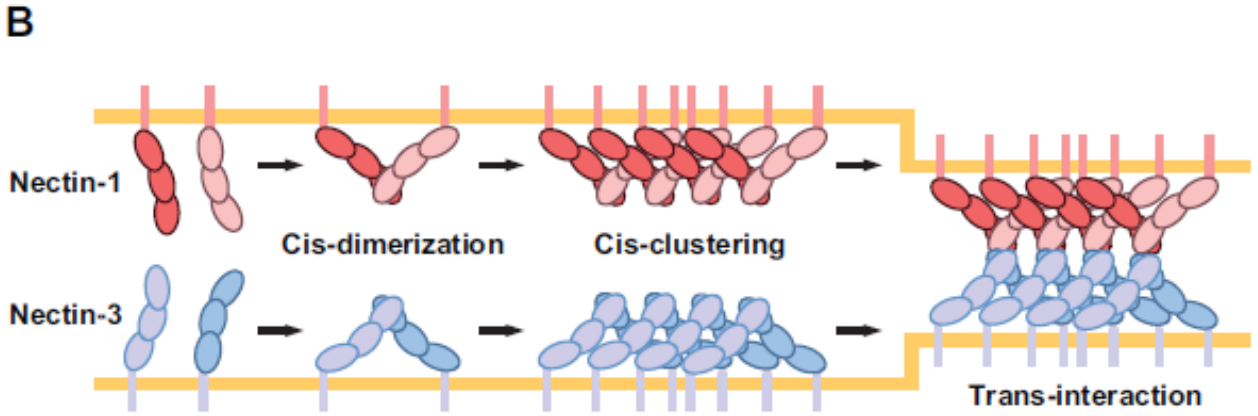
- Ca-dependent binding
- They bind to actin - binding to the cytoskeleton
- Signaling via catenins (canonical Wnt signaling)
- N- and E-cadherin link to EMT and MET
- KO of N- and E-Cadherin

# Nectins

# Nectins

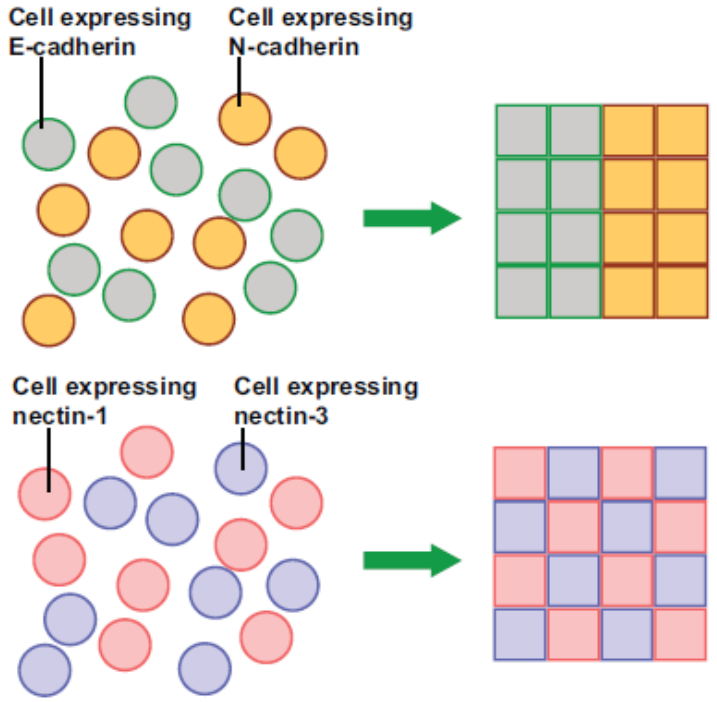
- Protein family (4 members - nectin-1 to 4)
- The genes for nectins are PVRL1-4 (Poliovirus receptor-like), it also binds alphahepesviruses
- Ig-like proteins, Ca2+ independent adhesion
- They mediate homo but mainly heterophilic intercellular trans interactions



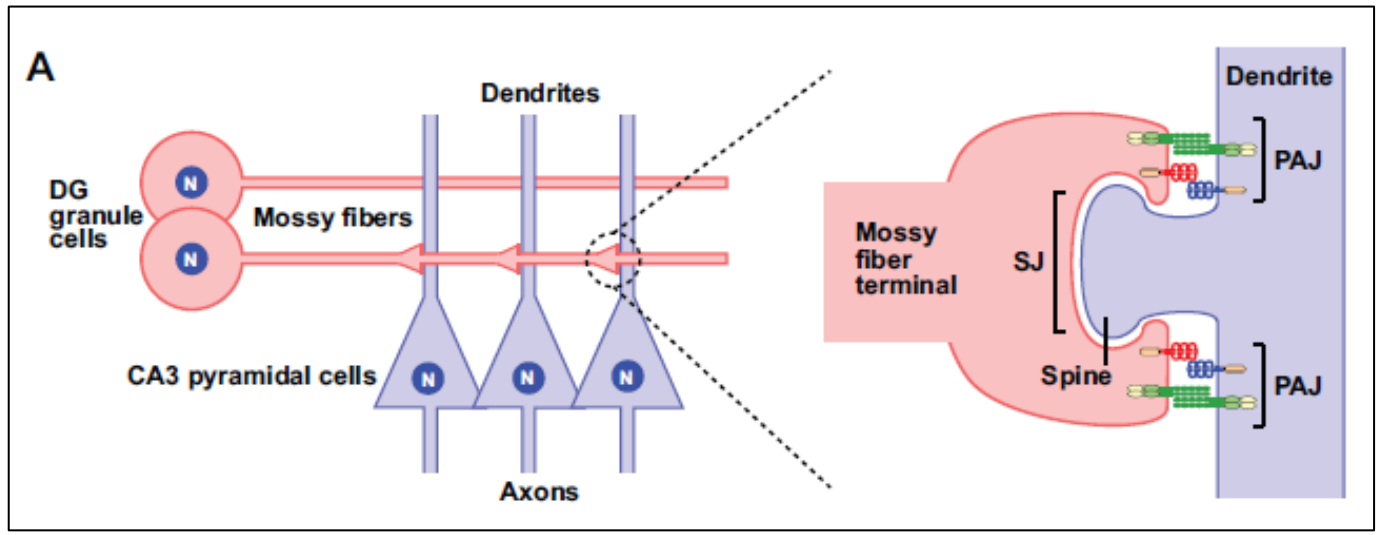
# Nectins

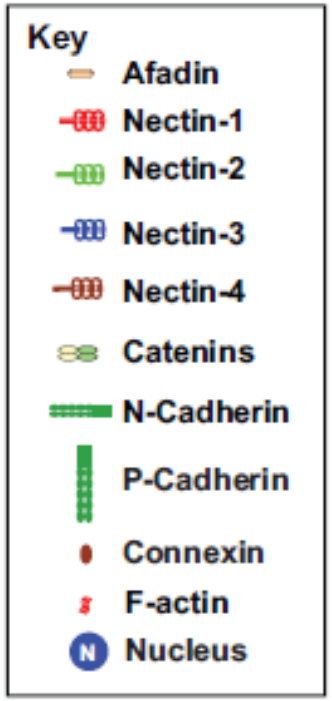
- Cadherins favor homophilic interactions
- Nectins favor heterophilic trans interactions -> causes mosaic, we often find them where mosaic of cells is needed (e.g. auditory system - combination of supporting cells and hair cells)
- They can participate in adhesion with Cadherins or independently



# Nectins

- Nectins are where a heterotypic connection, or mosaic, is needed
- For example, in nerve synapses

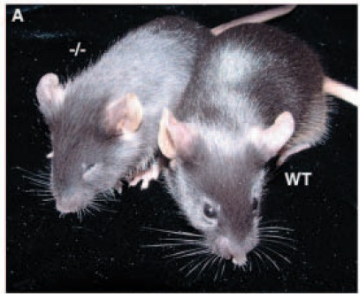


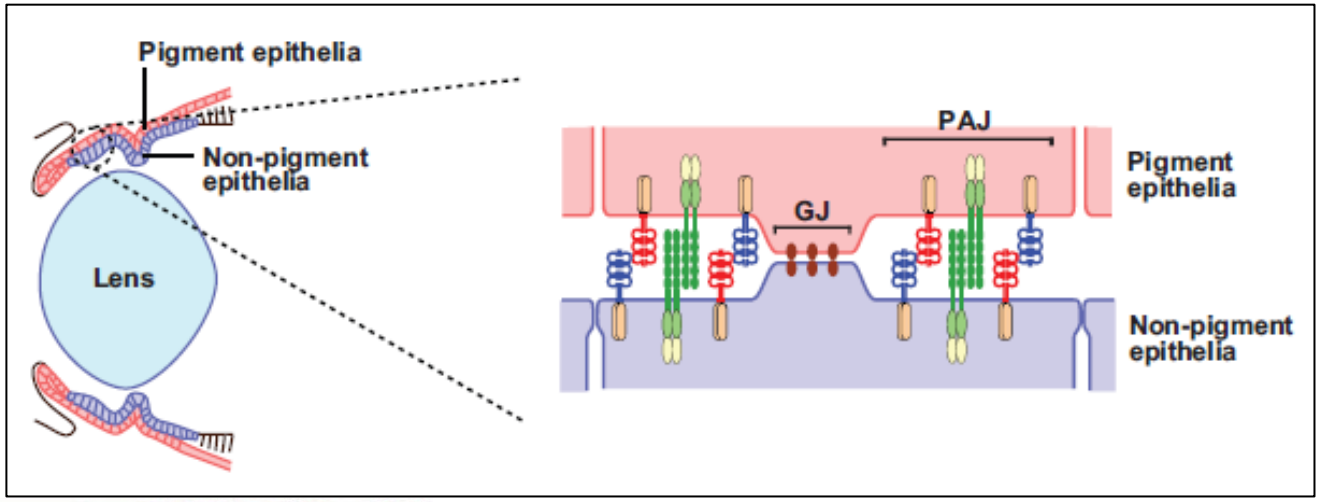



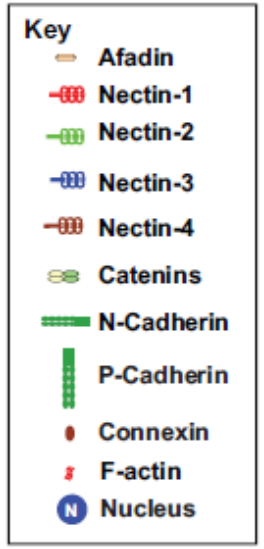
# Nectins in eye development

- Nectins are where a heterotypic connection, or mosaic, is needed
- For example, in contact between epithelia in the eye

Nectin-1 mice KO - mikroophthalmy

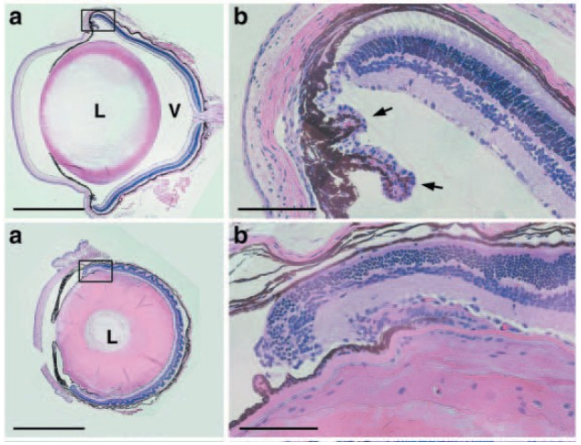


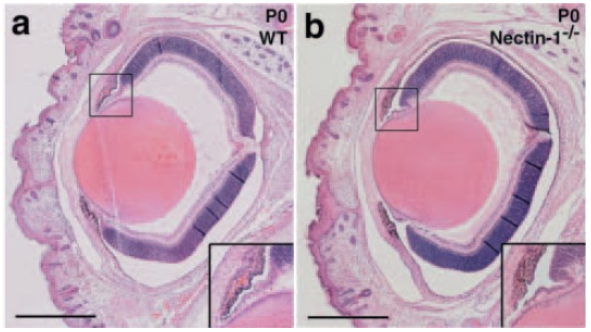



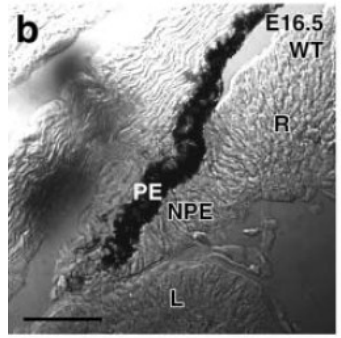



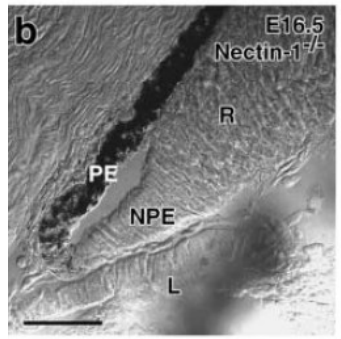

wt

Nectin1 -/-



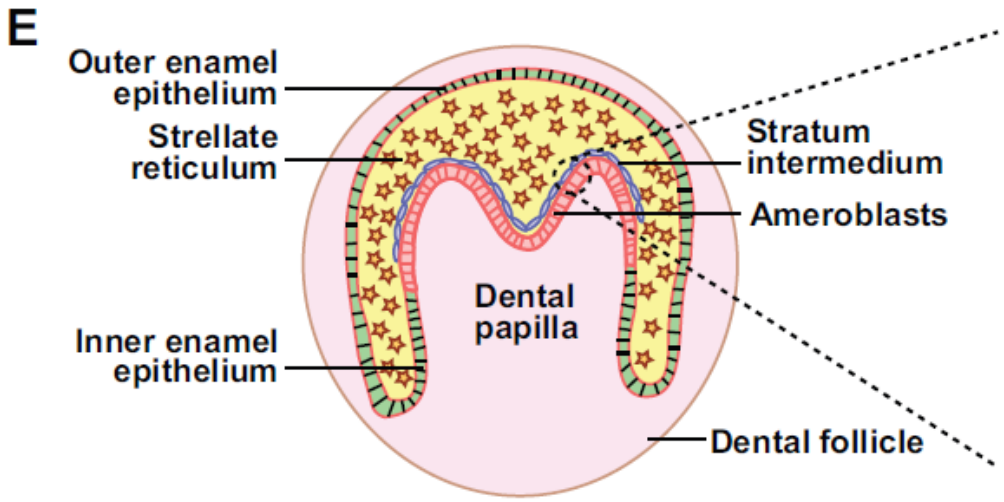



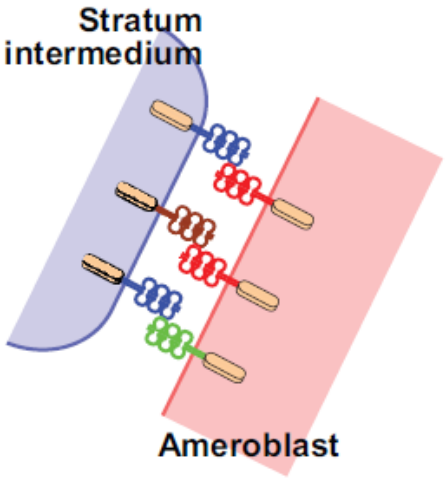



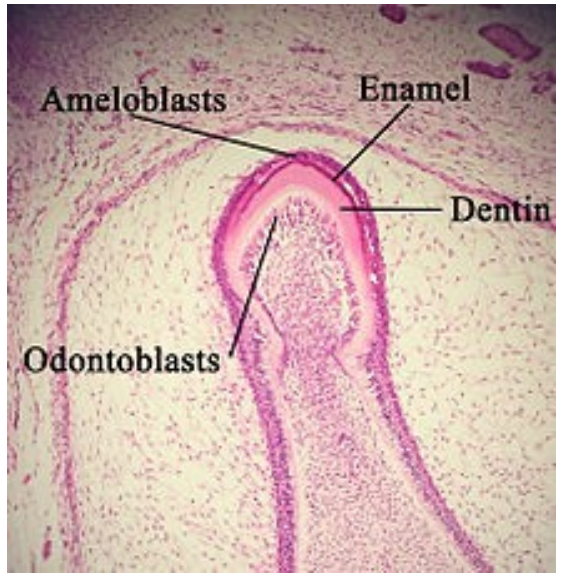

Inagaki et al., 2005

# Nectins

- In tooth

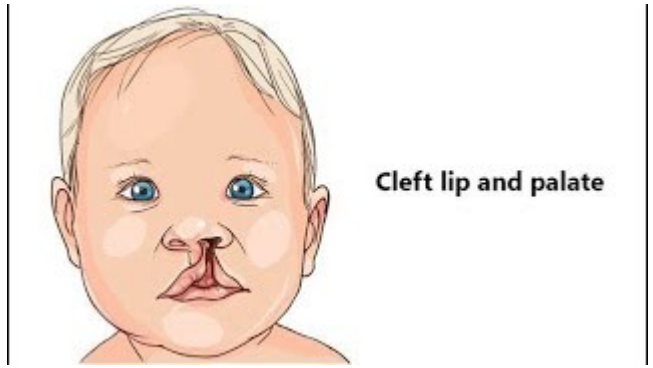


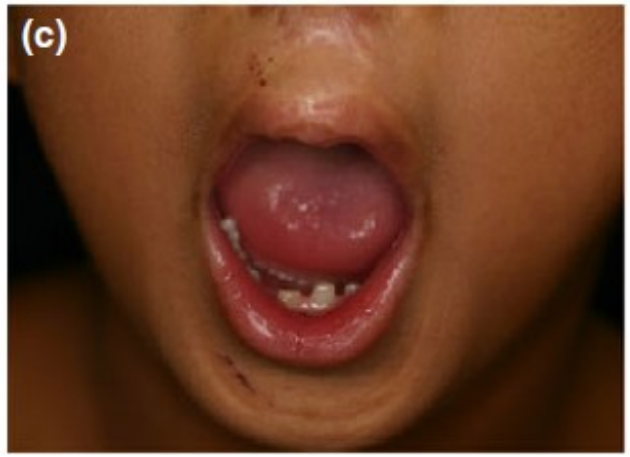



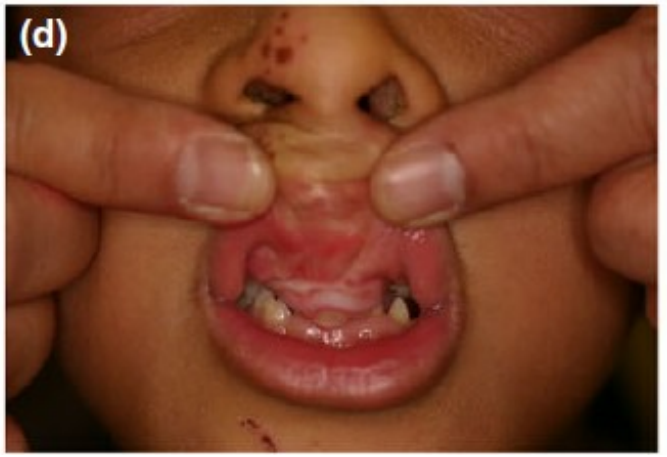



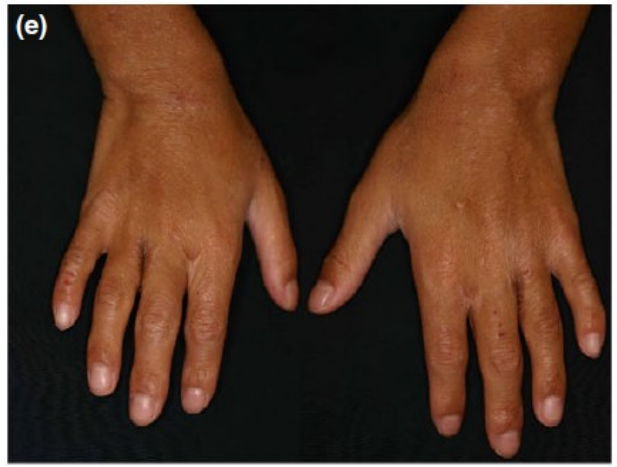
# Nectins in development

- Ectodermal dysplasia cleft palate mutations in the PVRL1 (nectin-1) gene
- It results in a truncated version of the protein that lacks the intracellular and transmembrane domains

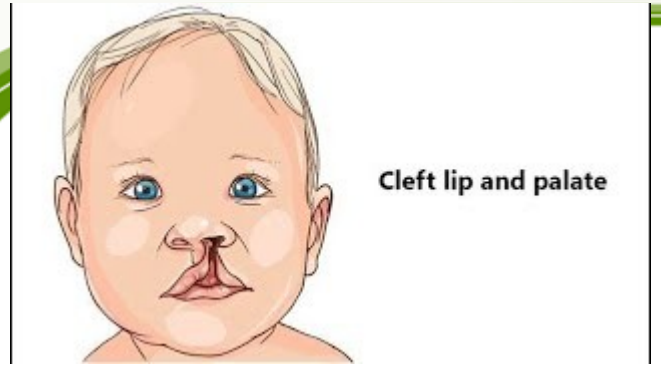


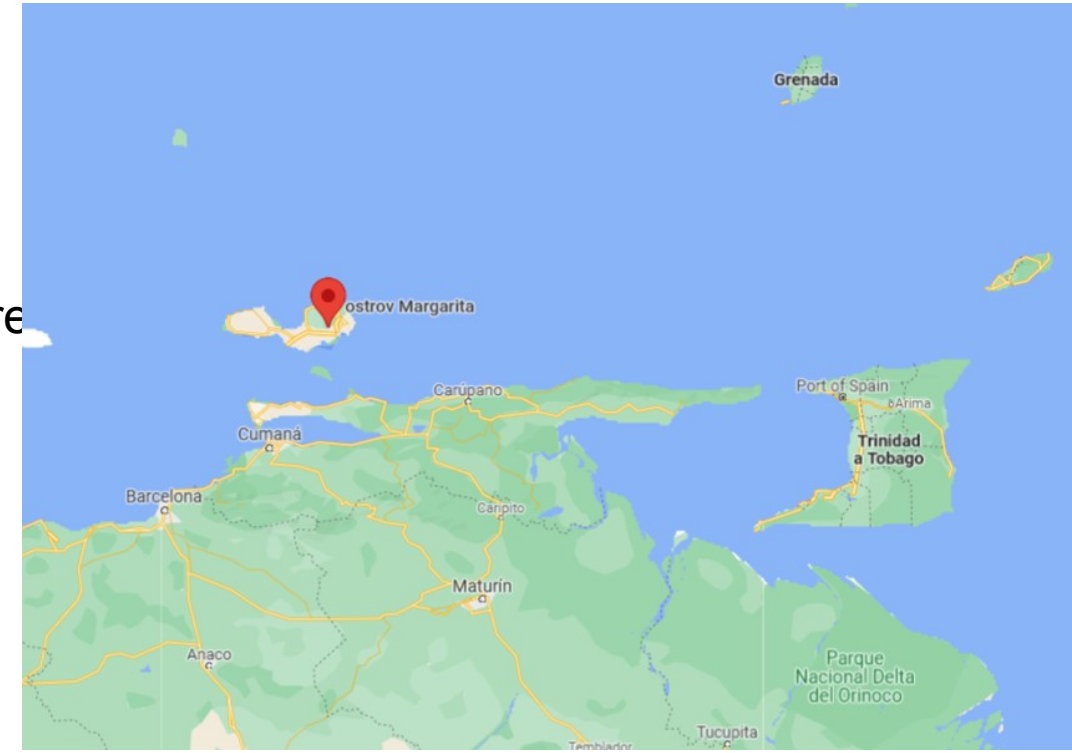



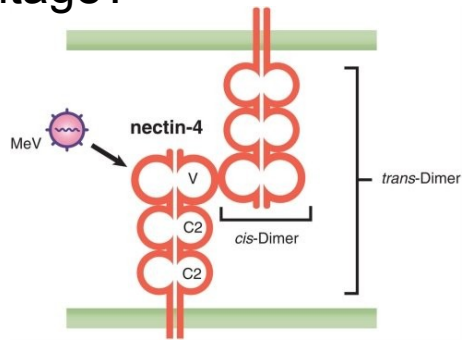
# Nectins in development




- The disease is autosomal recessive, parents are mostly heterozygotes
- High incidence on Margarita Island..WHY?

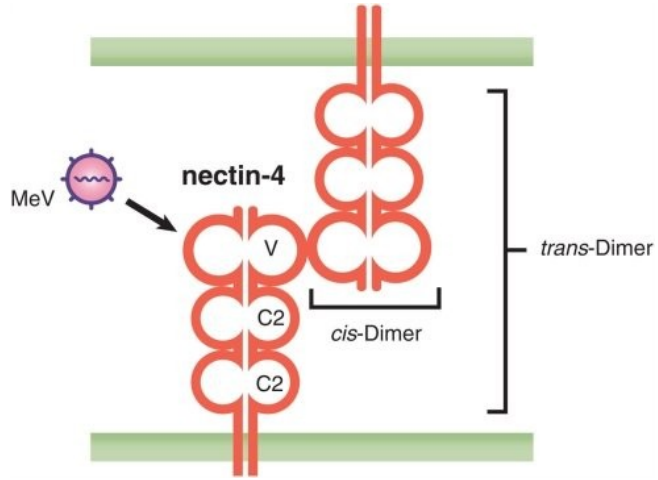



- Resistance of heterozygotes to alpha herpesviruses (chicken pox) -> evolutionary advantage?

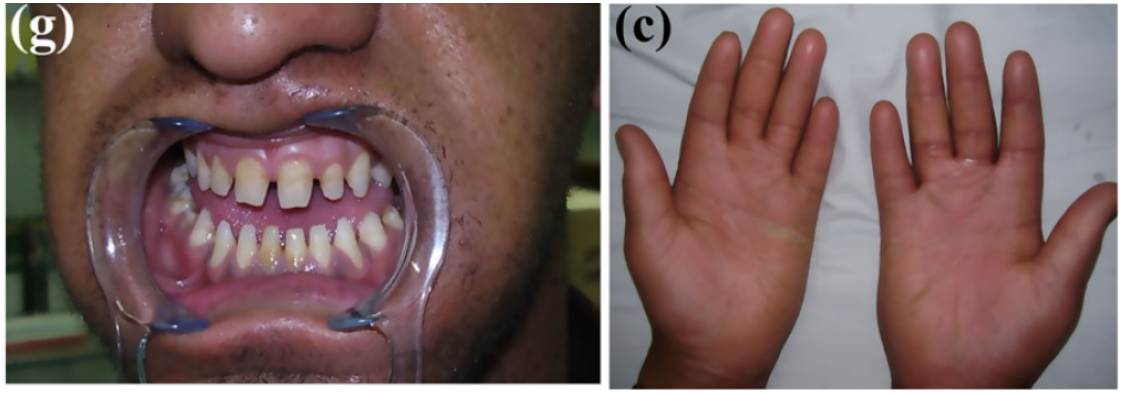


# Nectins in development

- Because nectin-1 and nectin-4 often form a heterophilic bond, mutations in nectin-4 often phenocopy mutations in nectin-1

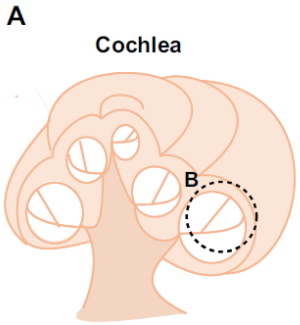


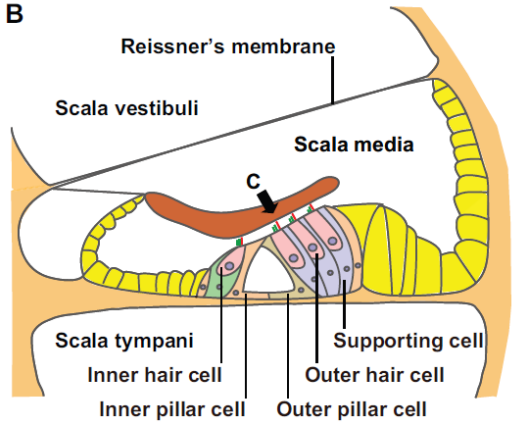

Mutations in nectin-4

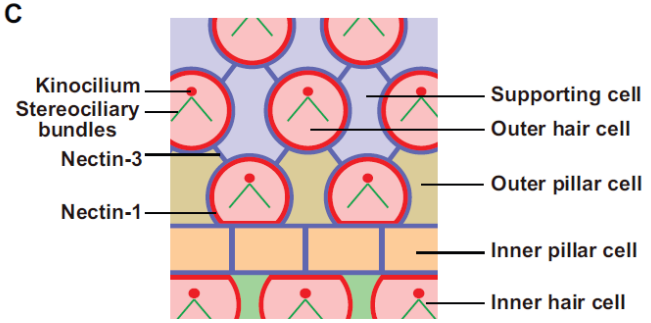


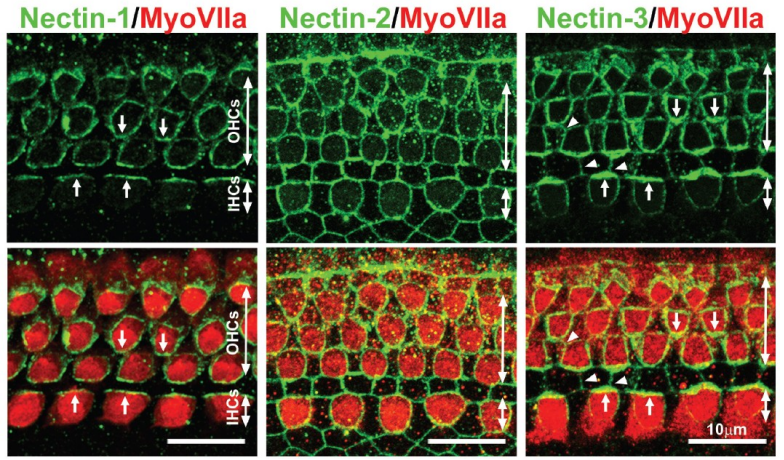
# Nectins in the development of the auditory system

The auditory system is made up of a "mosaic" of cells - several different cell types

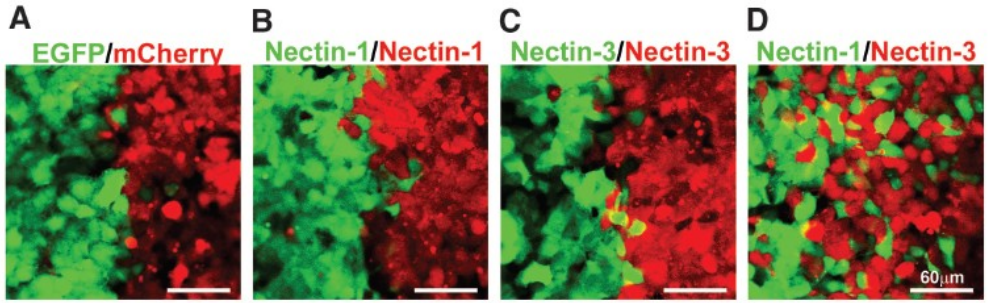


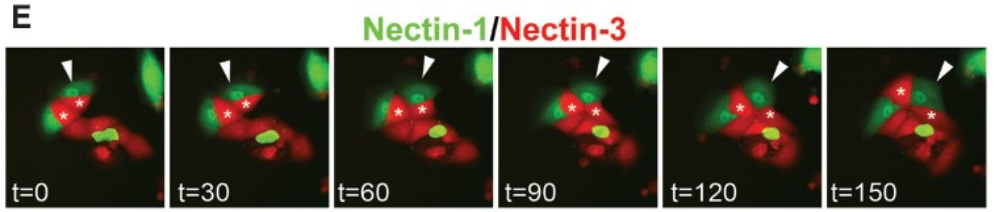




Cell suspensions that express various nectins

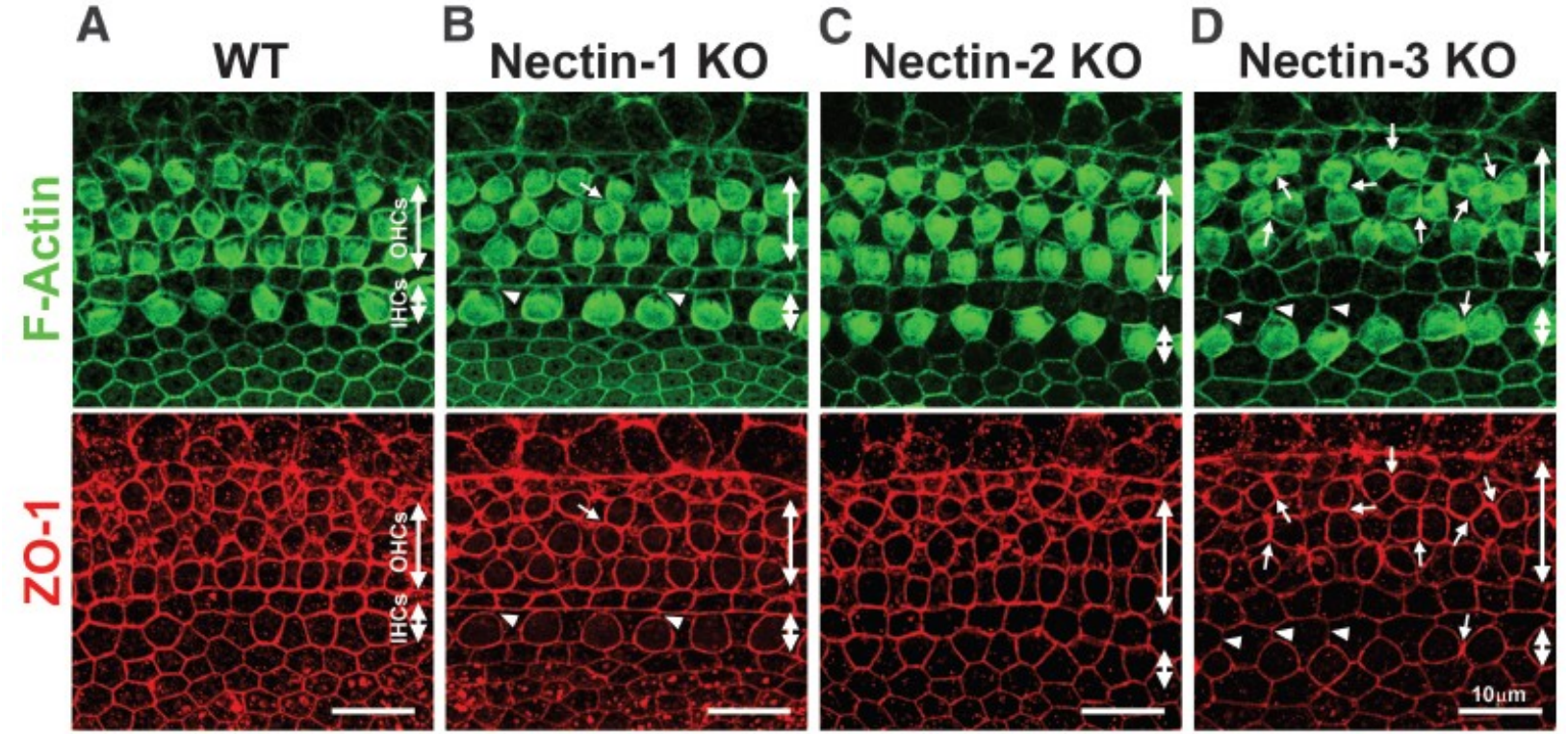



Togashi et al., 2011

# Nectins in the development of the auditory system

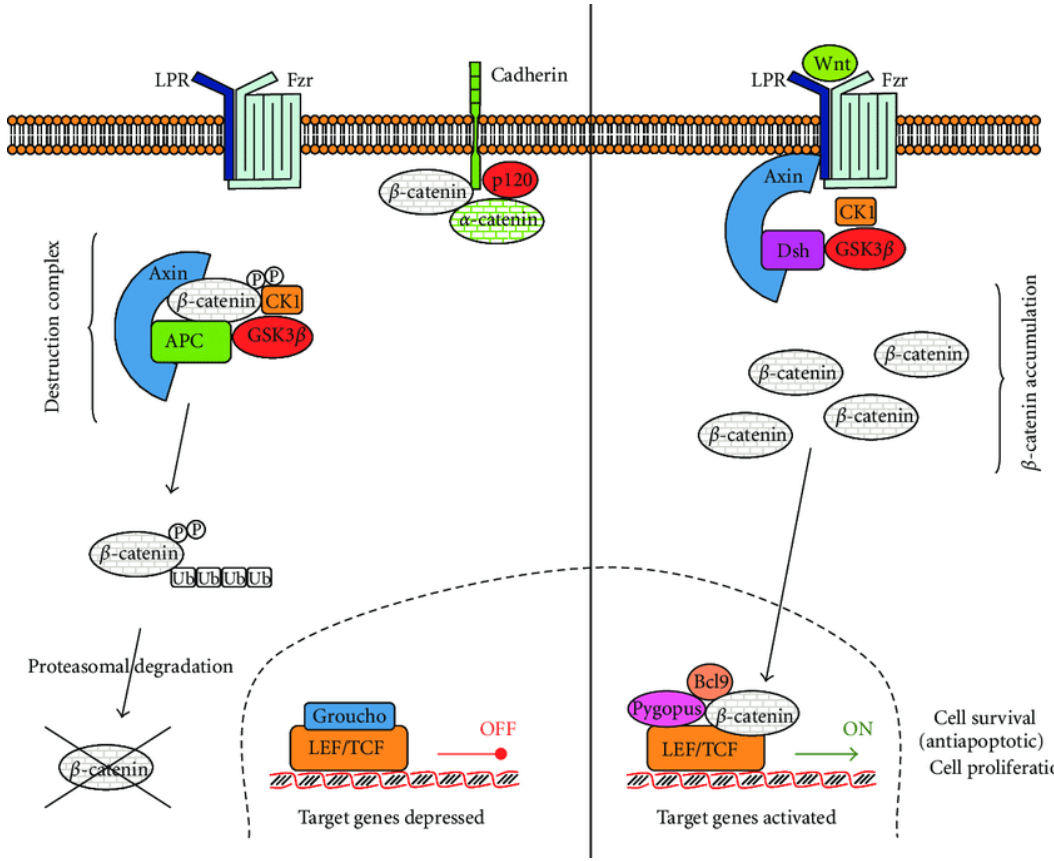
Deficiency of nectins leads to disruption of the mosaic

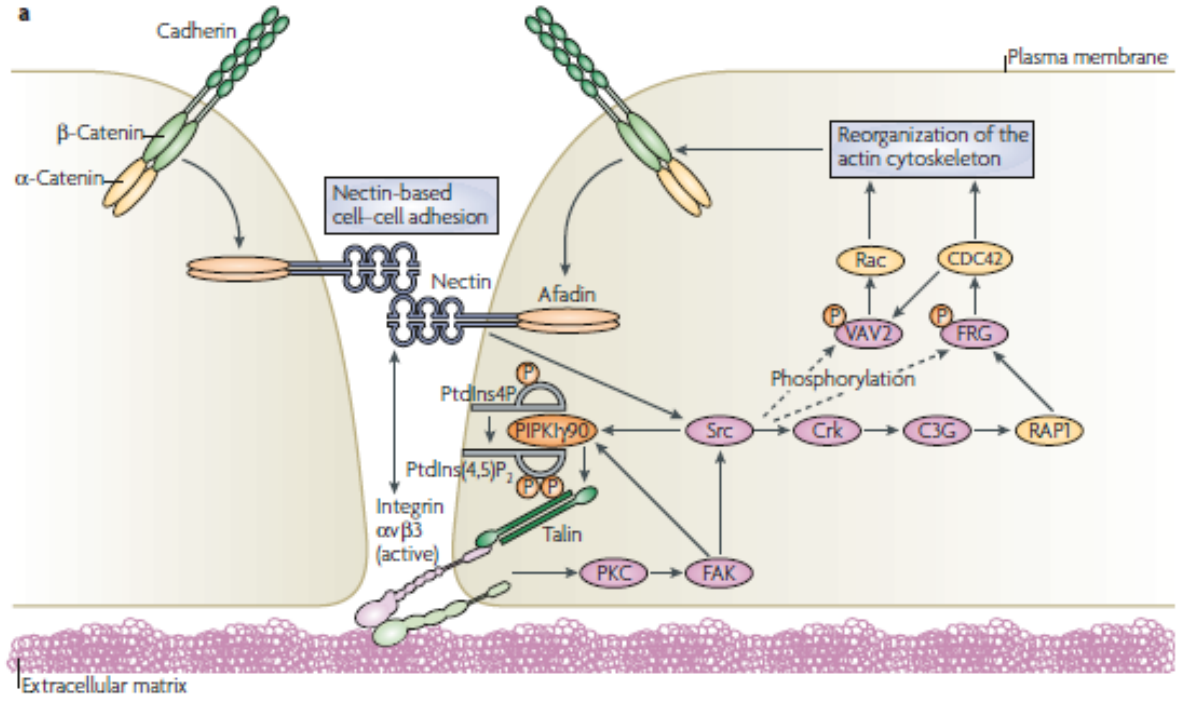


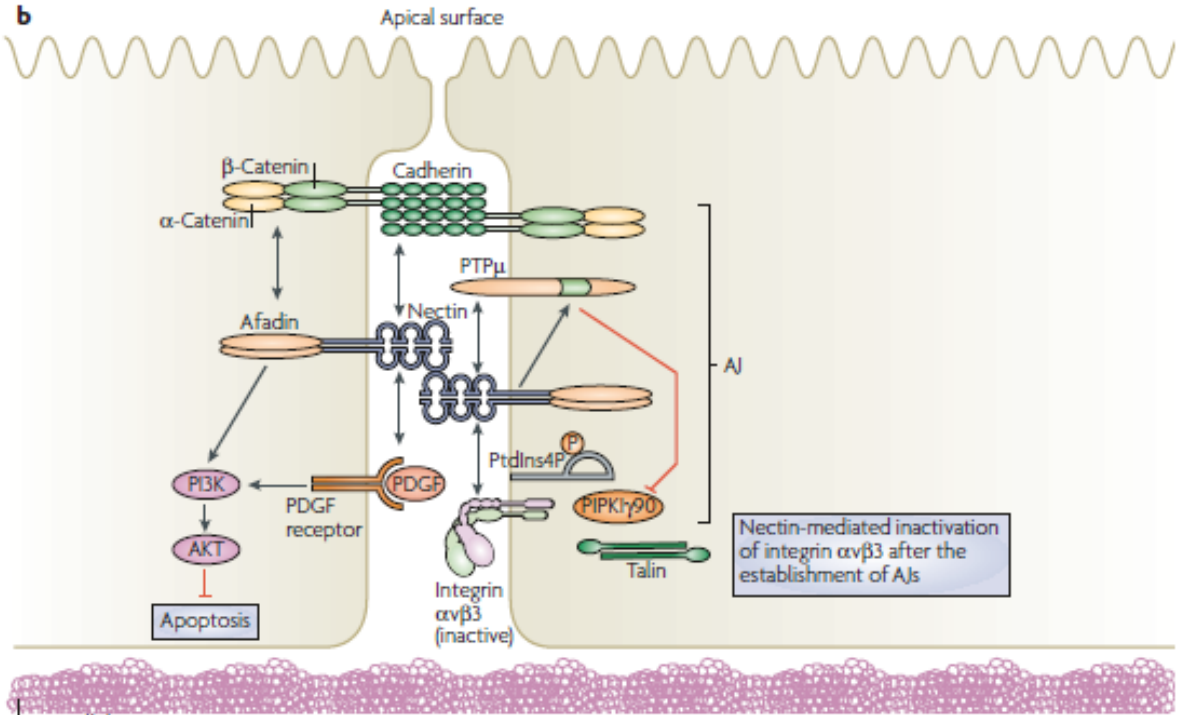

Togashi et al., 2011

# Nectins and signaling

- Nectins work together with cadherins. Nectins initiate cell-cell connections and subsequently recruit cadherins - key to the formation of adherens junctions
- By cooperating with Cadherins, they also regulate canonical Wnt signaling



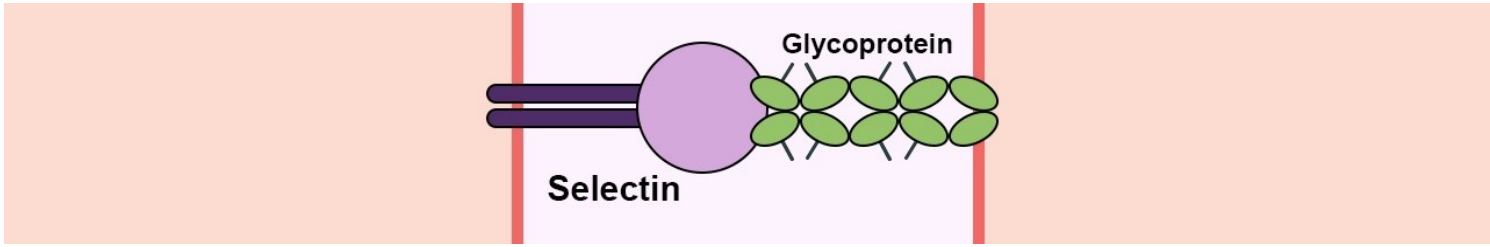
# Nectins sumup

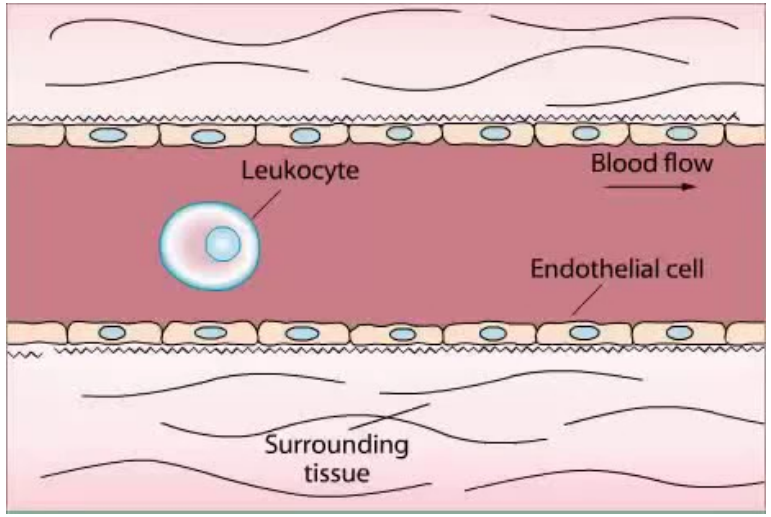
- Ig-like proteins, Ca2+-independent binding
- Important for heterophilic trans interaction between cells
- Often essential for mosaic

# Selectins

# Selectins

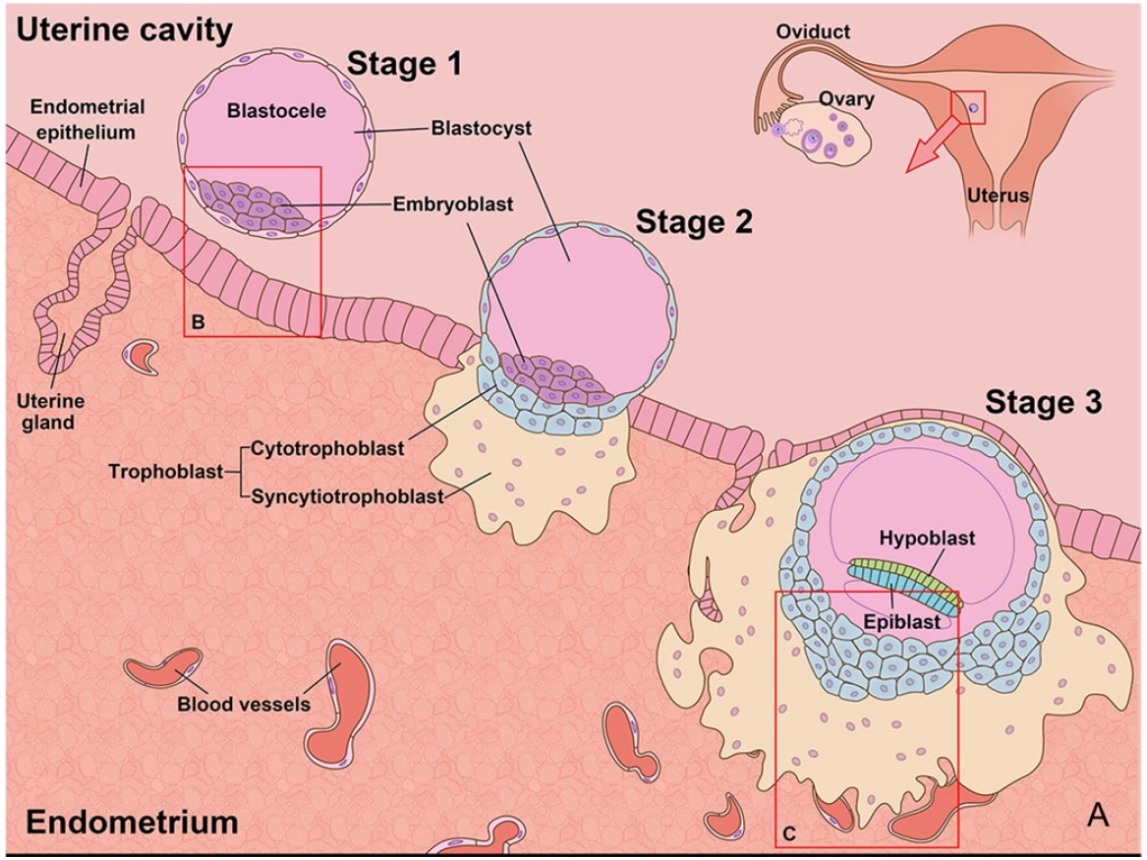
- Membrane glycoproteins
- They recognize carbohydrate molecules on the surface of a neighboring cell - so it is not an interaction of two identical types of molecules, as in the case of cadherins or nectins.
- Binding is dependent on the presence of Ca2+
- They are used in the binding of immune cells to the endothelium



# Selectins during embryo implantation

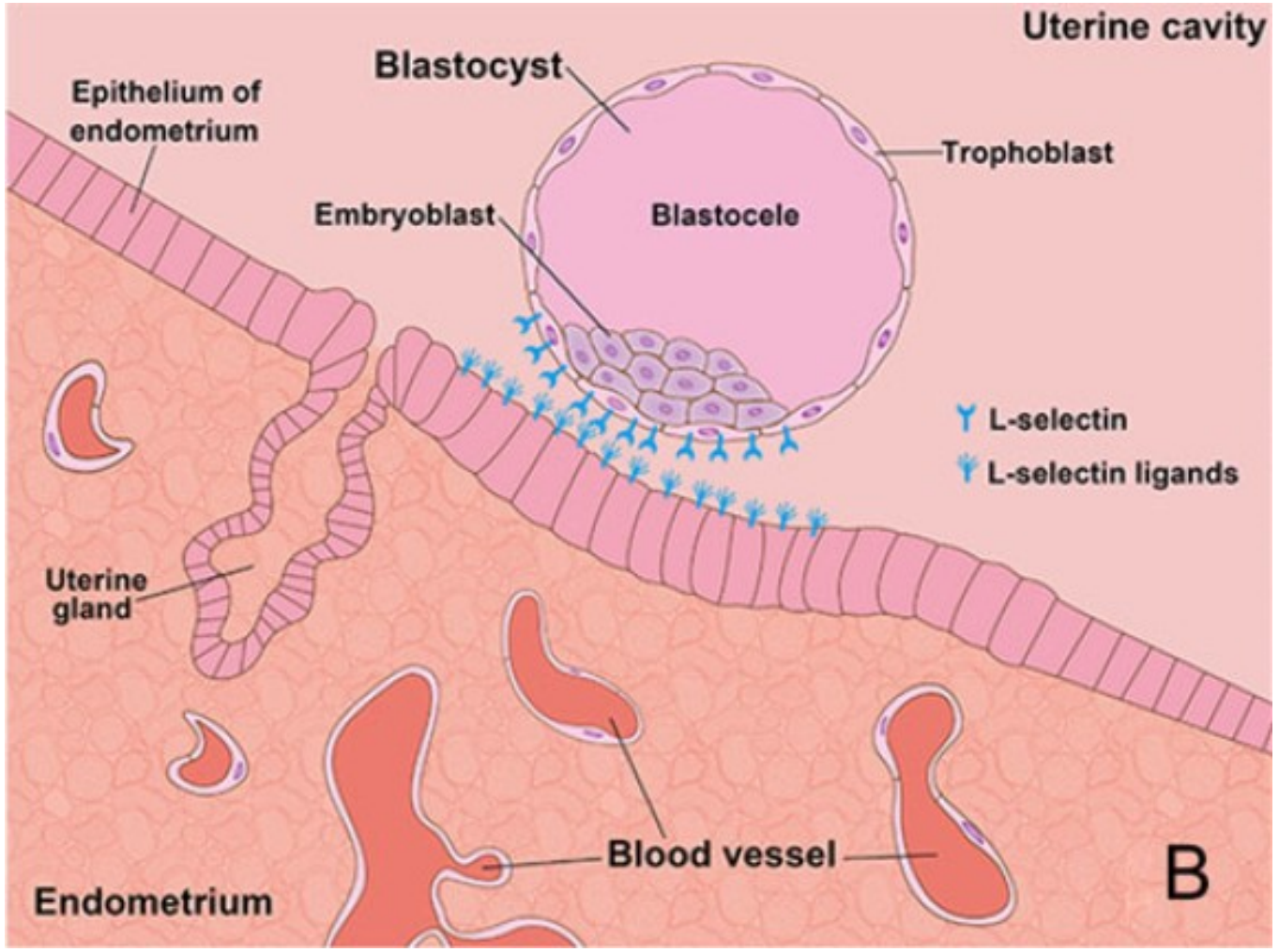
- They play a role not only in "leukocyte rolling/homing", but also during embryo implantation.
- The mechanism of implantation involves a transient interaction between the blastocyst and the uterine surface epithelium before trophoblast epithelial cells penetrate the uterine wall.



# Selectins during embryo implantation

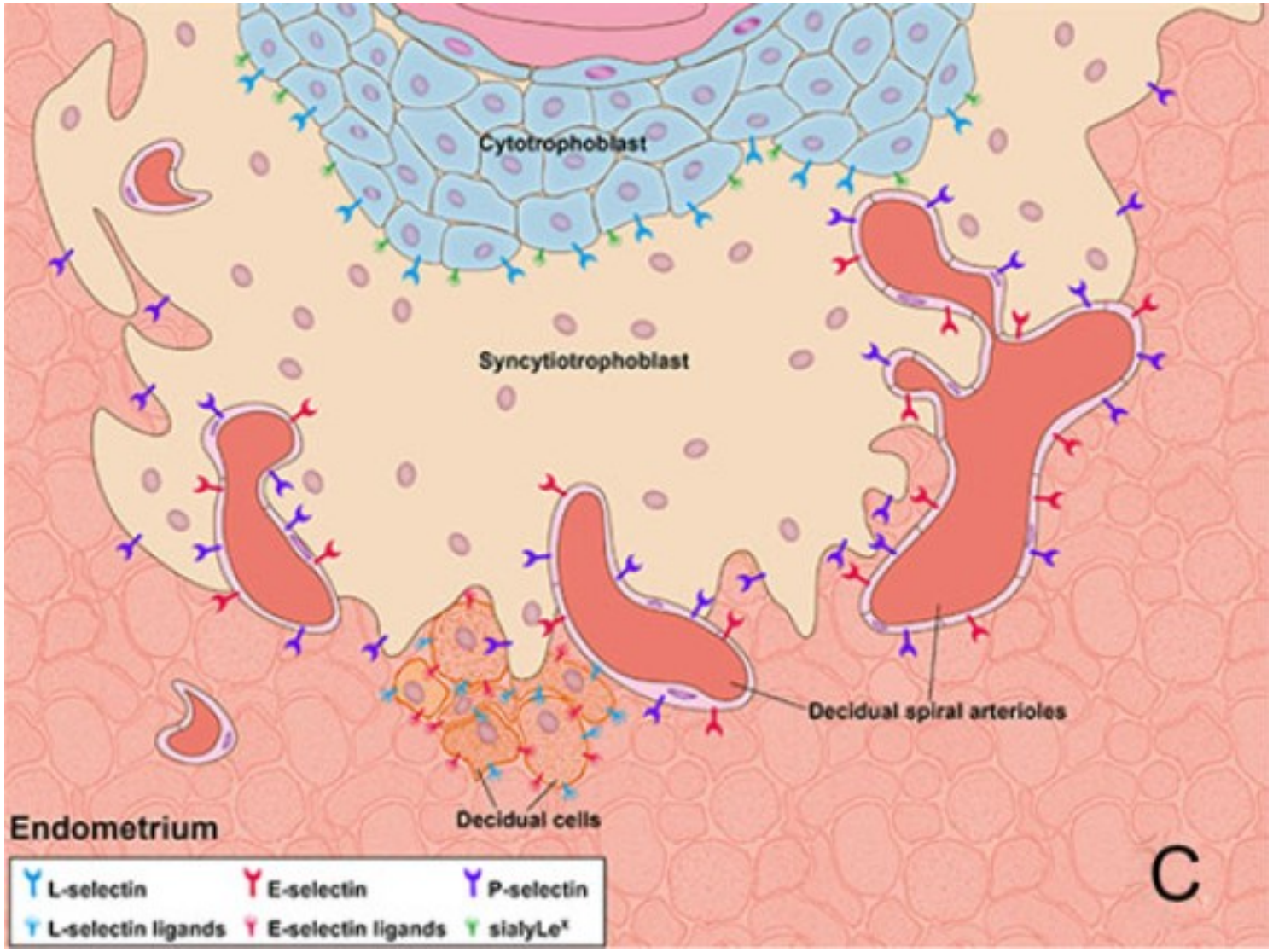
Embryo implantation initiation

- L-selectin is expressed on the trophoblast surface
- Its ligands are expressed on the surface of the endometrium
- A mechanism for infertility?



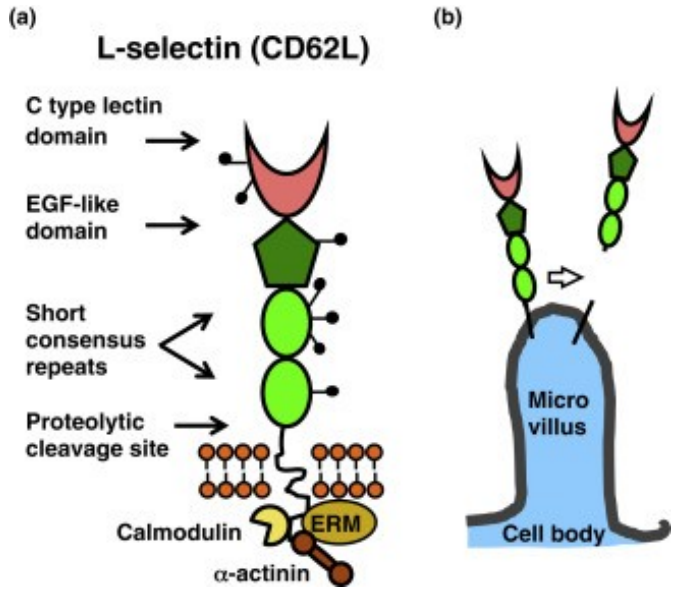
# Selectins during embryo implantation

- After implantation, the trophoblast grows into the endometrium
- Interaction with the endothelium is ensured through P- and E-selectin



# Selectins and signalling

Cytoplasmic domain interacts with Calmodulin - Ca2+ dependent signaling and with actin



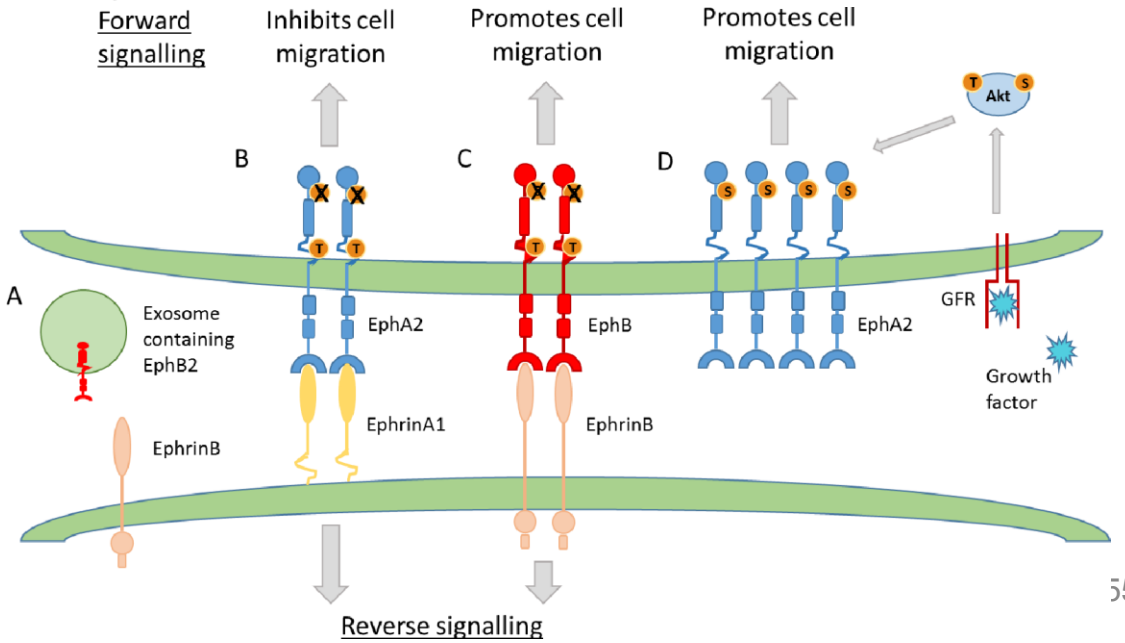
# Selectins Sumup

- Ca-dependent binding to polysaccharide
- Described in the binding of immune cells to the endothelium
- Important during embryo implantation - the interaction of the trophectoderm with the endothelium of the mother
- They signal to the nucleus via calmodulin

# Ephrins

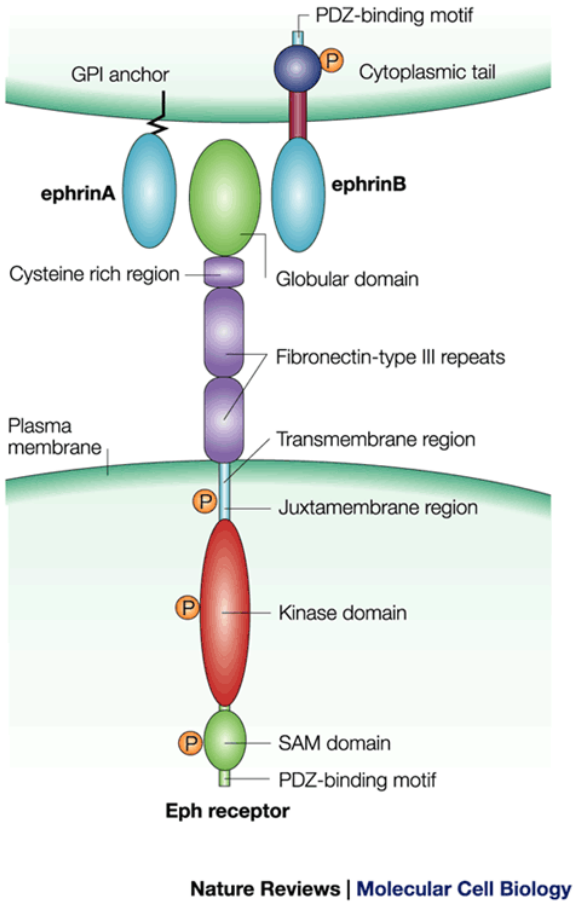
# Ephrins

- Ephrins (Eph receptor-interacting proteins) are ligands of Eph receptors (erythropoietin-producing human hepatocellular receptors).
- Both Eph receptors and ephrins are cell-bound membrane proteins.
- Cell-cell interaction is required for receptor activation
- Eph receptors: belong to tyrosine kinase receptors -> signaling
- They play a role in development, guidance axons, tissue formation, migration cells and body segmentation.



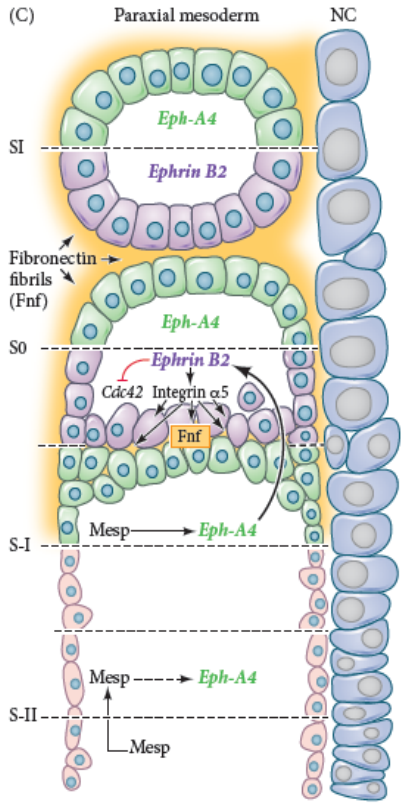
# Ephrins - signaling

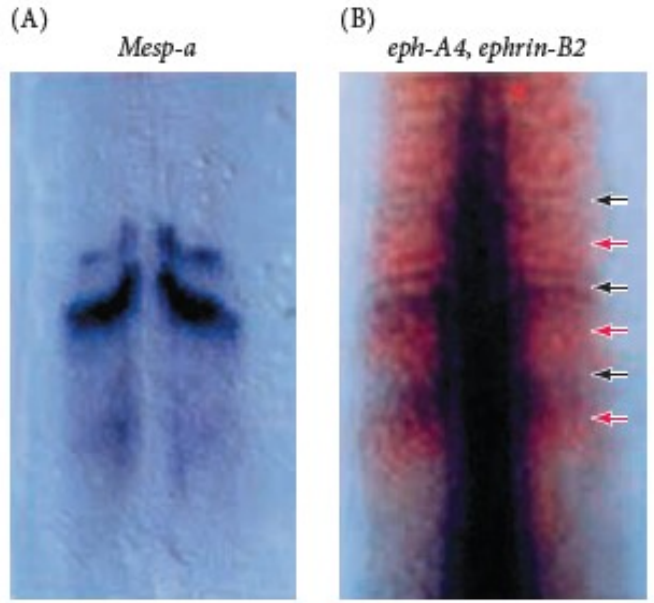
- In mammals: 16 types of receptors (2 groups EphA EphB), 14 ligands (again in two groups EphA/B).
- Interesting fact: two-way signaling - classically, the receptor can signal ("forward signaling"), but also the ligand ("reverse signaling").



# Ephrins and segmentation

- It is the basic process of embryogenesis occurring in most invertebrates and all vertebrates, by which the body is initially divided into functional units.
- What is the segmentation for?
- The segmented areas of the embryo represent boundaries for different biochemical and morphological processes - drastically different cell behavior - vital for future differentiation and function of the organ/organism.
- Link to the EMT and MET lecture:

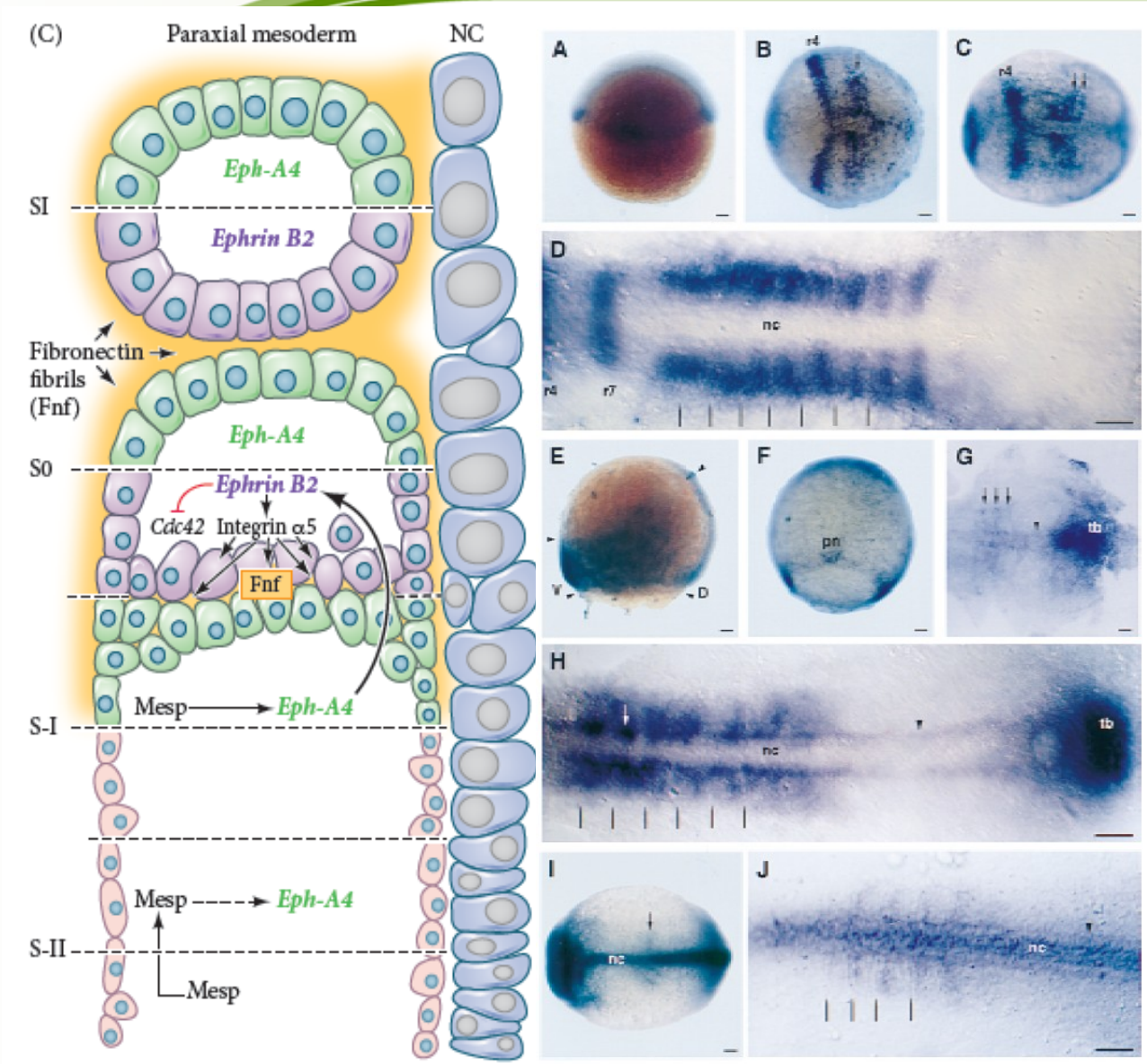


# Ephrins and segmentation

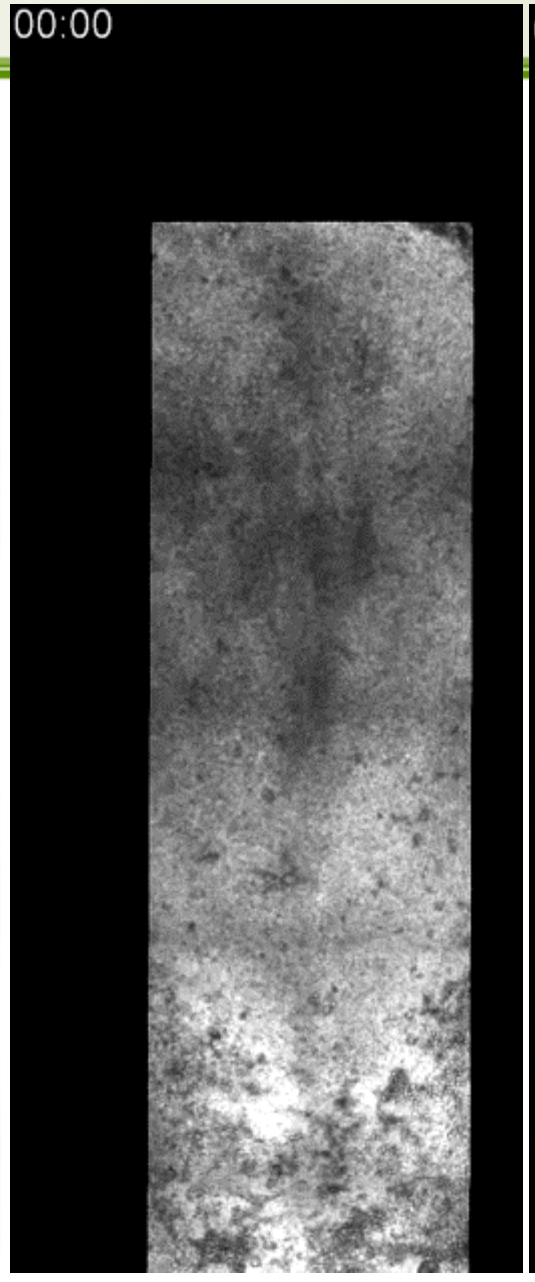
Denans et al., 2015

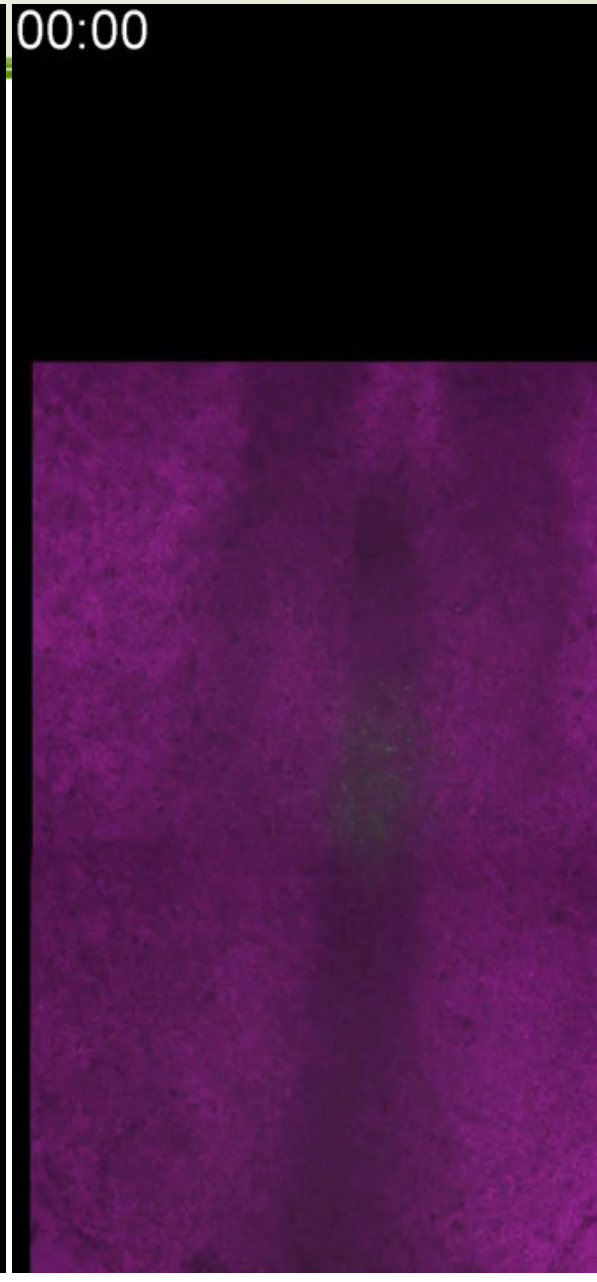






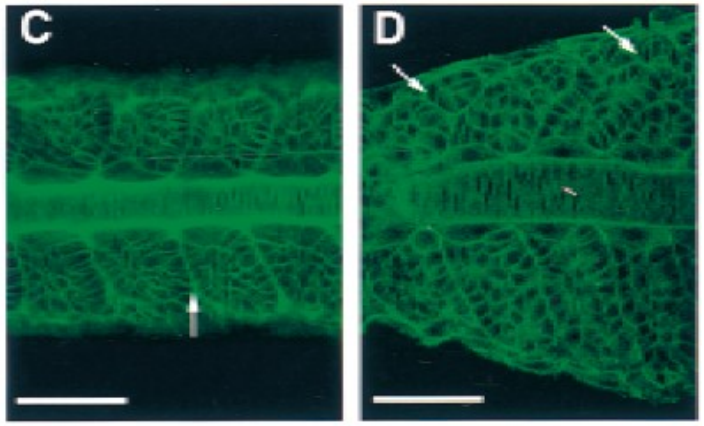




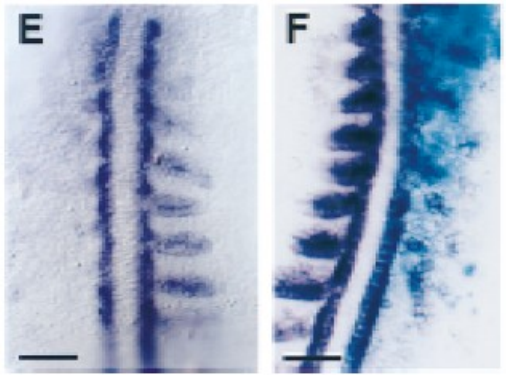



# Ephrins and segmentation

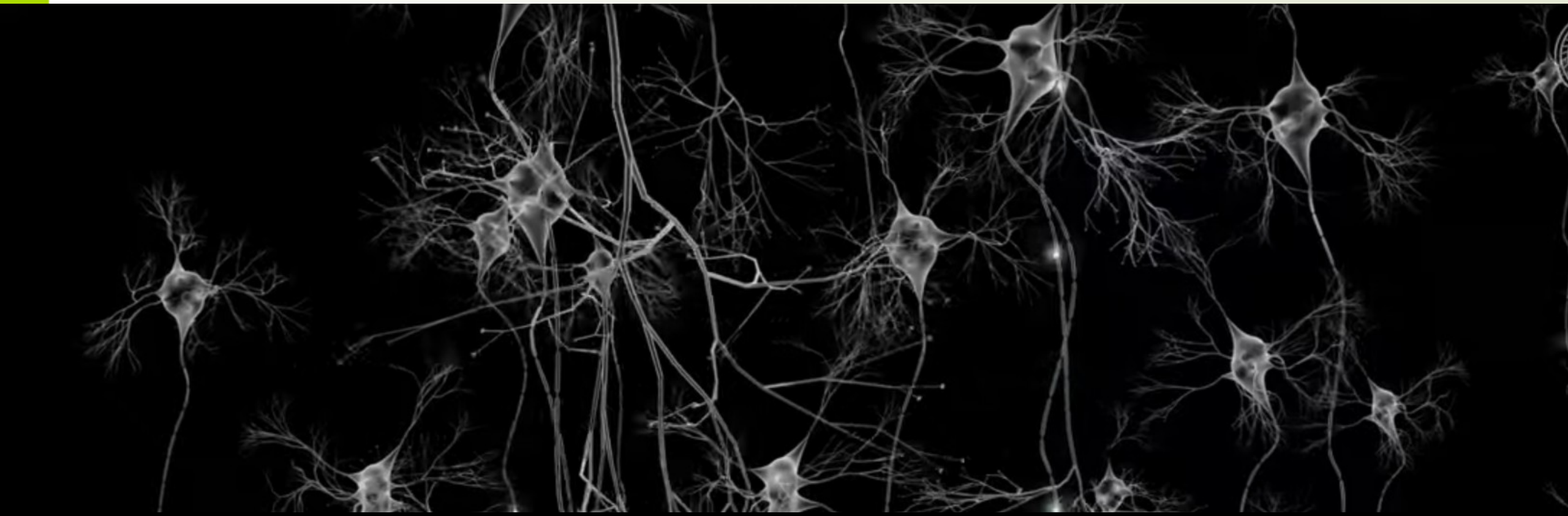
wt ephrin-B2 overexpression




Overexpression of DN forms of ephrins and their receptors leads to errors in somite formation

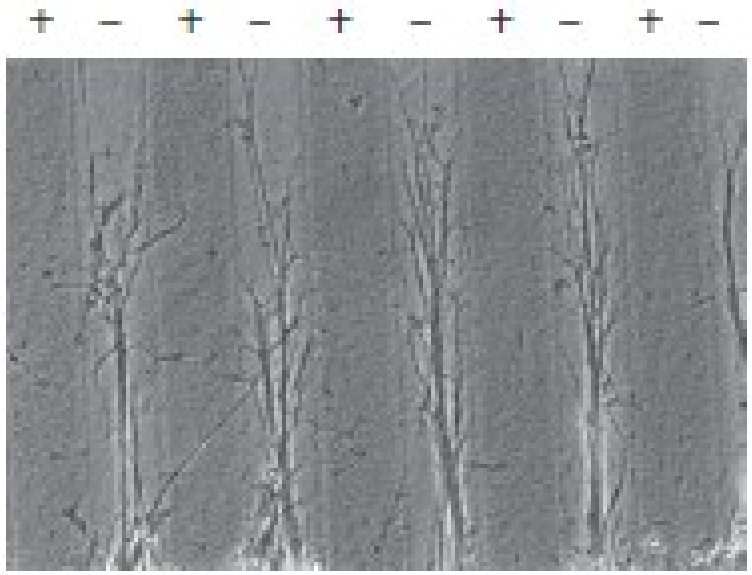


# Ephrins and axon guidance



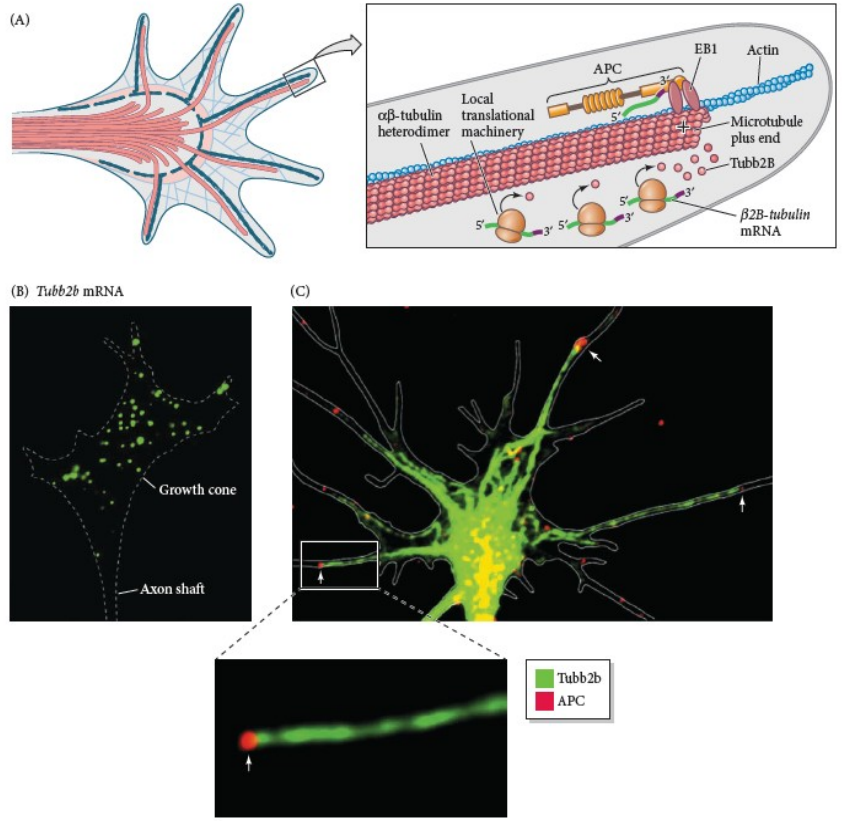
- Billions of neurons need to communicate with each other through synapses.
- Neuron connections are formed not only during embryonic development, but also after birth.
- An incorrect connection or number of neurons leads to serious problems in the development of the nervous system.
- How can billions of cells communicate properly with each other?
- How does each cell know where and which cell to connect to?

# Ephrins and axon guidance



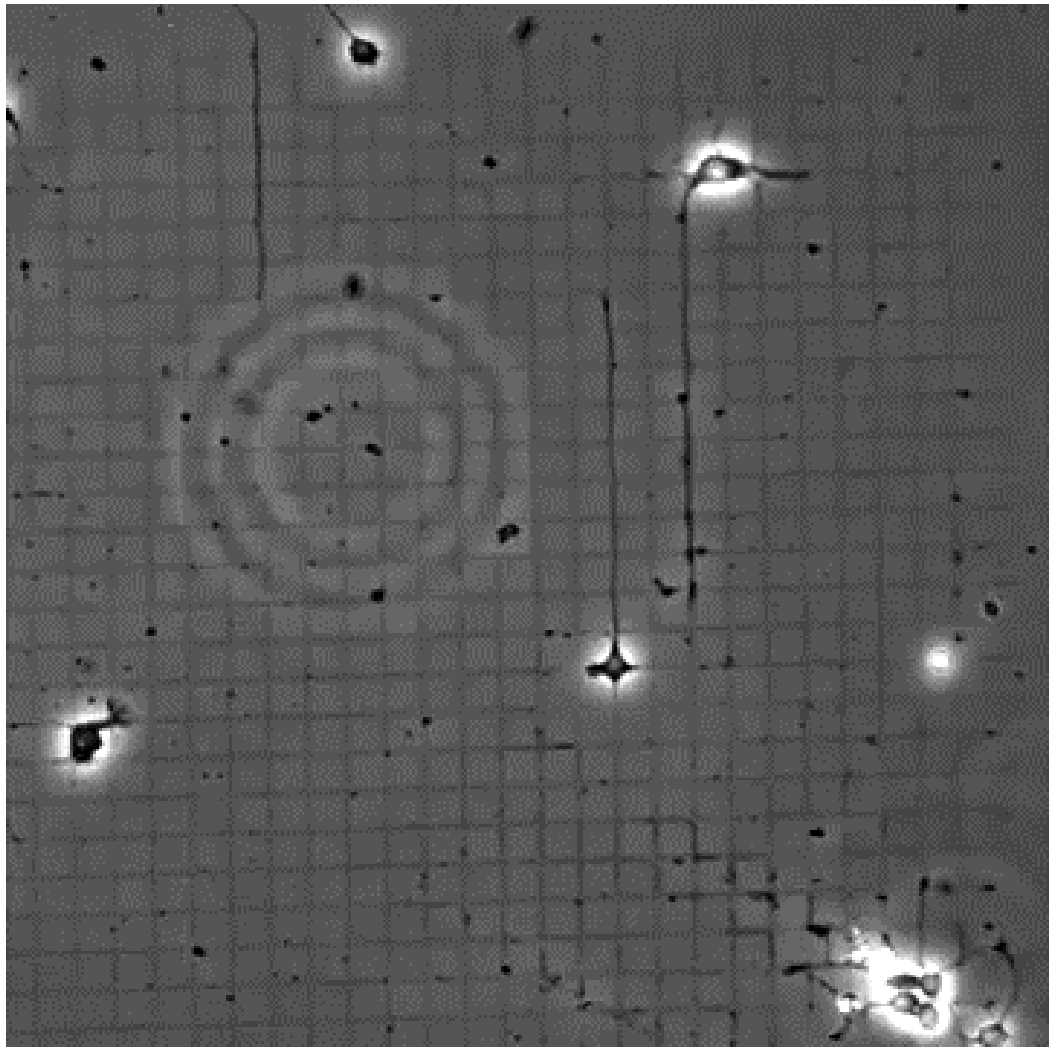
# Ephrins and axon guidance

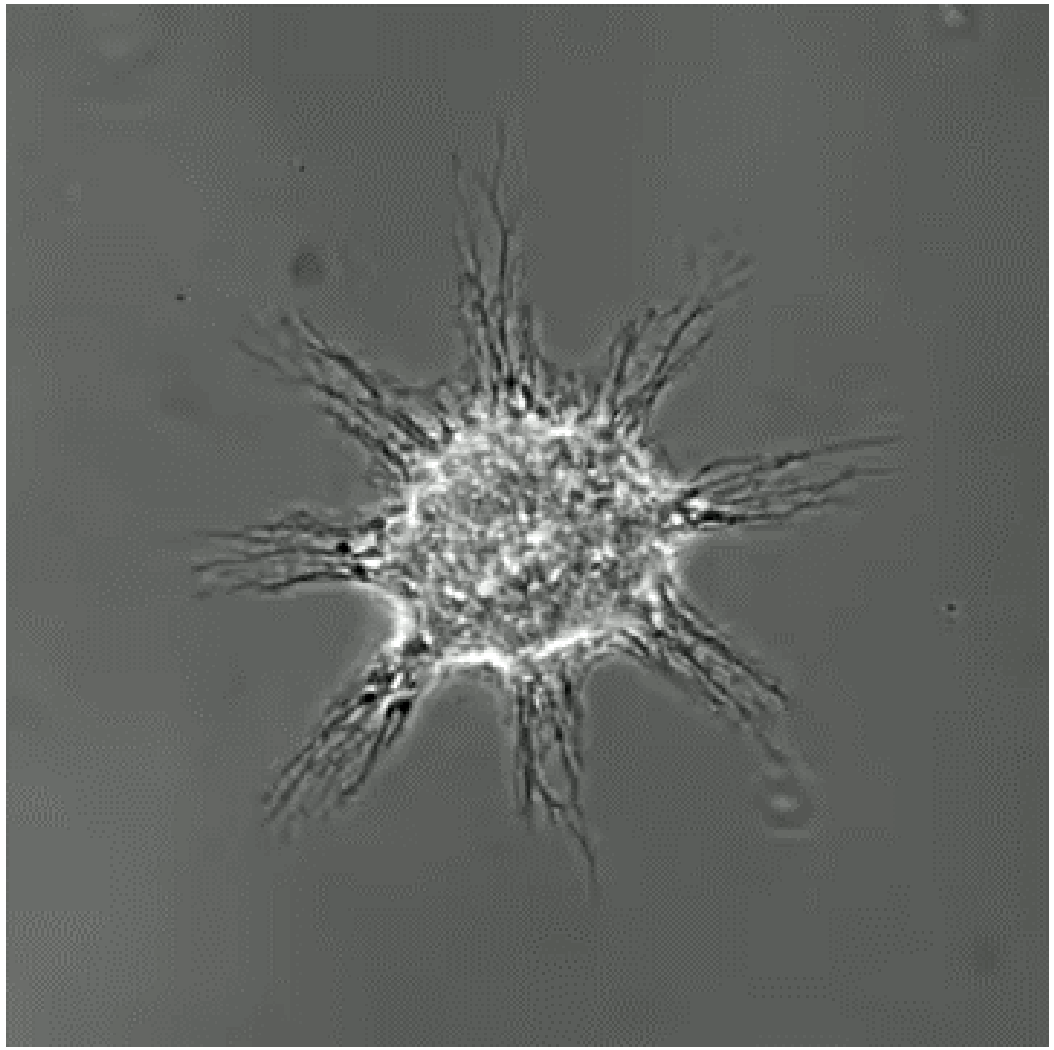
- Neuronal connections are mediated by molecular guides that guide axons (axon guidance). Eph/ephrin signaling regulates the migration of axons to their target destinations largely by inhibiting the growth of axonal growth cones and repelling the migrating axon away from the site of Eph/ephrin activation.
- This mechanism depends on the relative levels of Eph and ephrin expression and allows gradients of Eph and ephrin expression in target cells to direct the migration of axonal growth cones based on their own relative levels of Eph and ephrin expression.
- E.g. "forward signaling" by EphA and EphB receptors mediates growth tip collapse, while "reverse signaling" via ephrin-A and ephrin-B induces growth tip survival.



# Ephrins and axon guidance

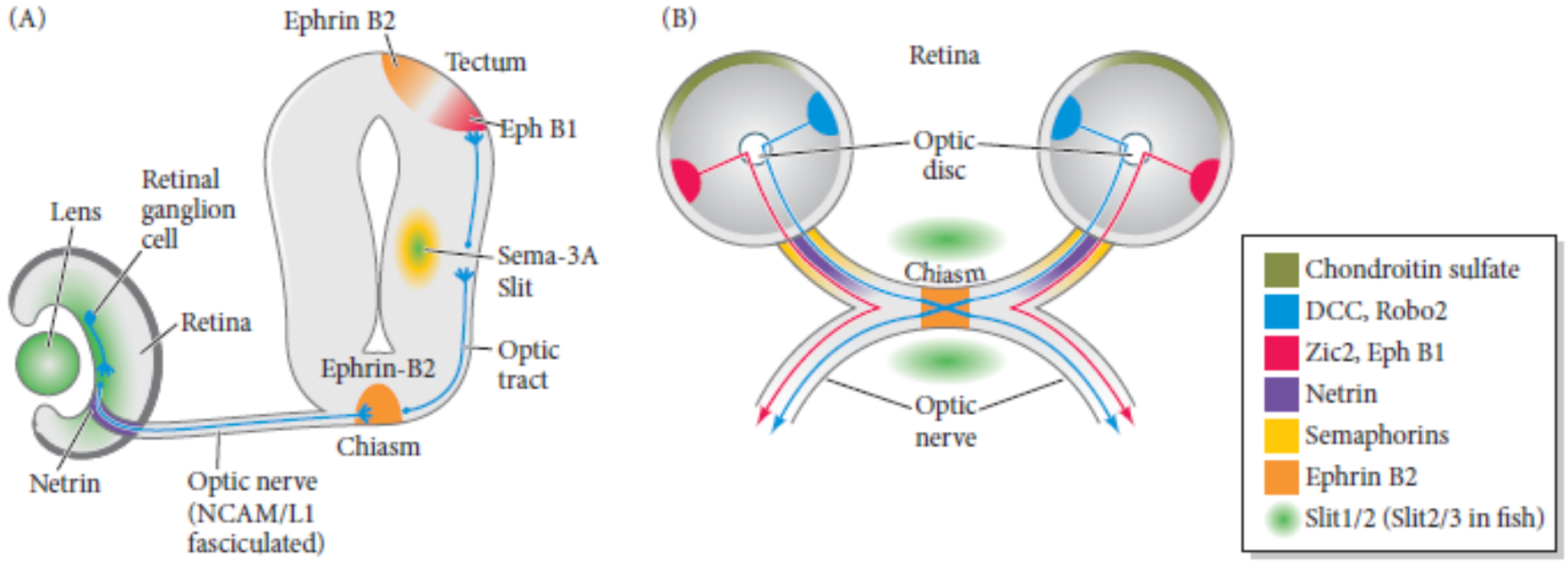
How can it be studied? E.g. micropatterning.





# Ephrins and axon guidance: example Chiasma opticum

- Individual neurons of the retina (about millions of retinal ganglion cells) send their axons to the areas of the brain where visual perception is processed (optic tectum).
- The crossing point of the optic nerves is defined by the repulsion between individual Ephrins and their receptors (+semaphorins).
- Eph receptors are on growing axons, ligands on surrounding cells.



# Ephrins and axon guidance: example Chiasma opticum

Demonstration of optic nerve crossing using colors implanted into the retina

How is an intact image created?

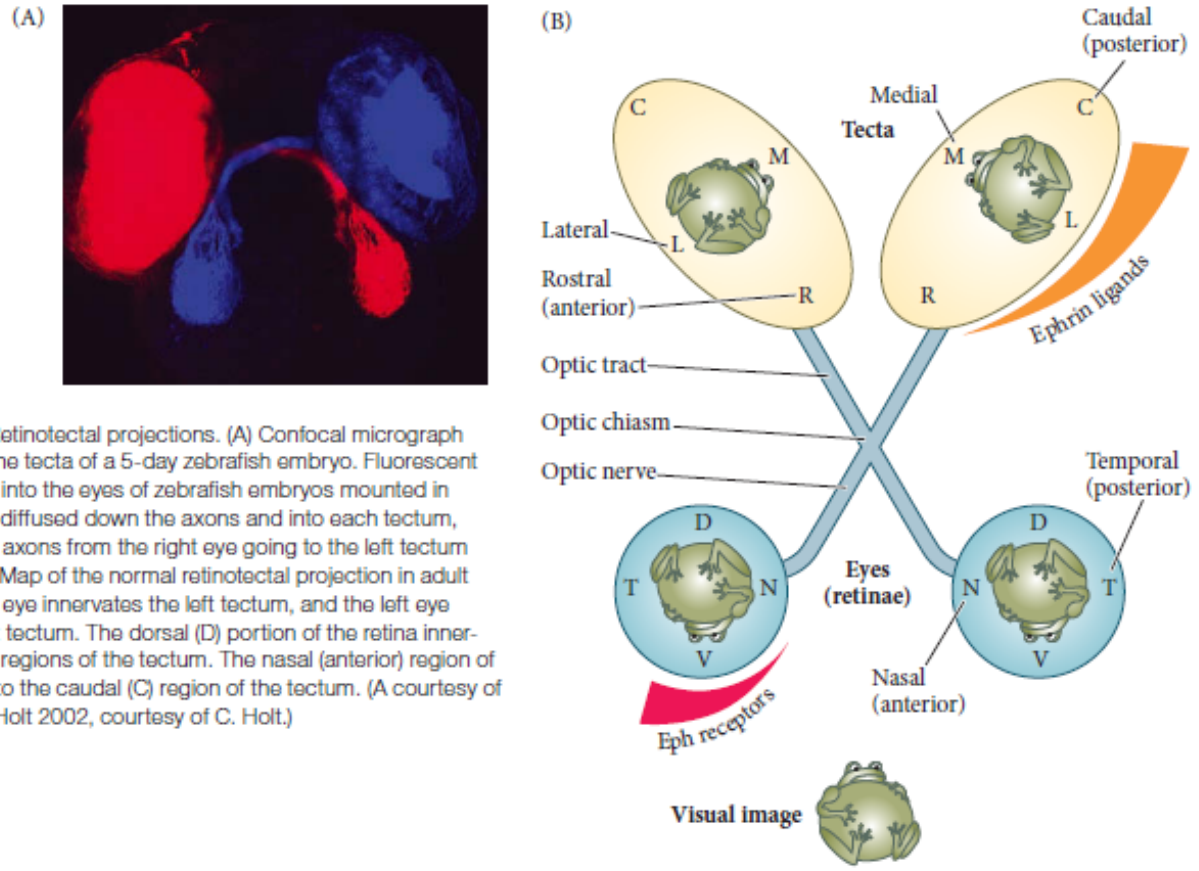
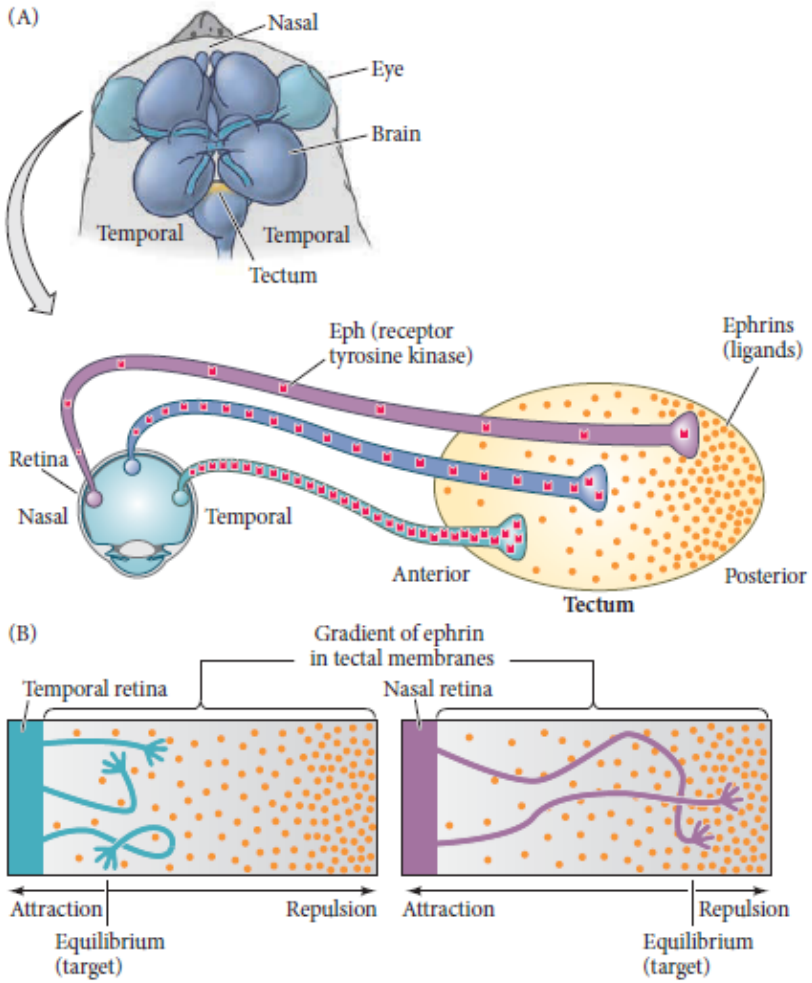



**FIGURE 15.38** Retinotectal projections. (A) Confocal micrograph of axons entering the tecta of a 5-day zebrafish embryo. Fluorescent dyes were injected into the eyes of zebrafish embryos mounted in agarose. The dyes diffused down the axons and into each tectum, showing the retinal axons from the right eye going to the left tectum and vice versa. (B) Map of the normal retinotectal projection in adult *Xenopus*. The right eye innervates the left tectum, and the left eye innervates the right tectum. The dorsal (D) portion of the retina innervates the lateral (L) regions of the tectum. The nasal (anterior) region of the retina projects to the caudal (C) region of the tectum. (A courtesy of M. Wilson; B after Holt 2002, courtesy of C. Holt.)

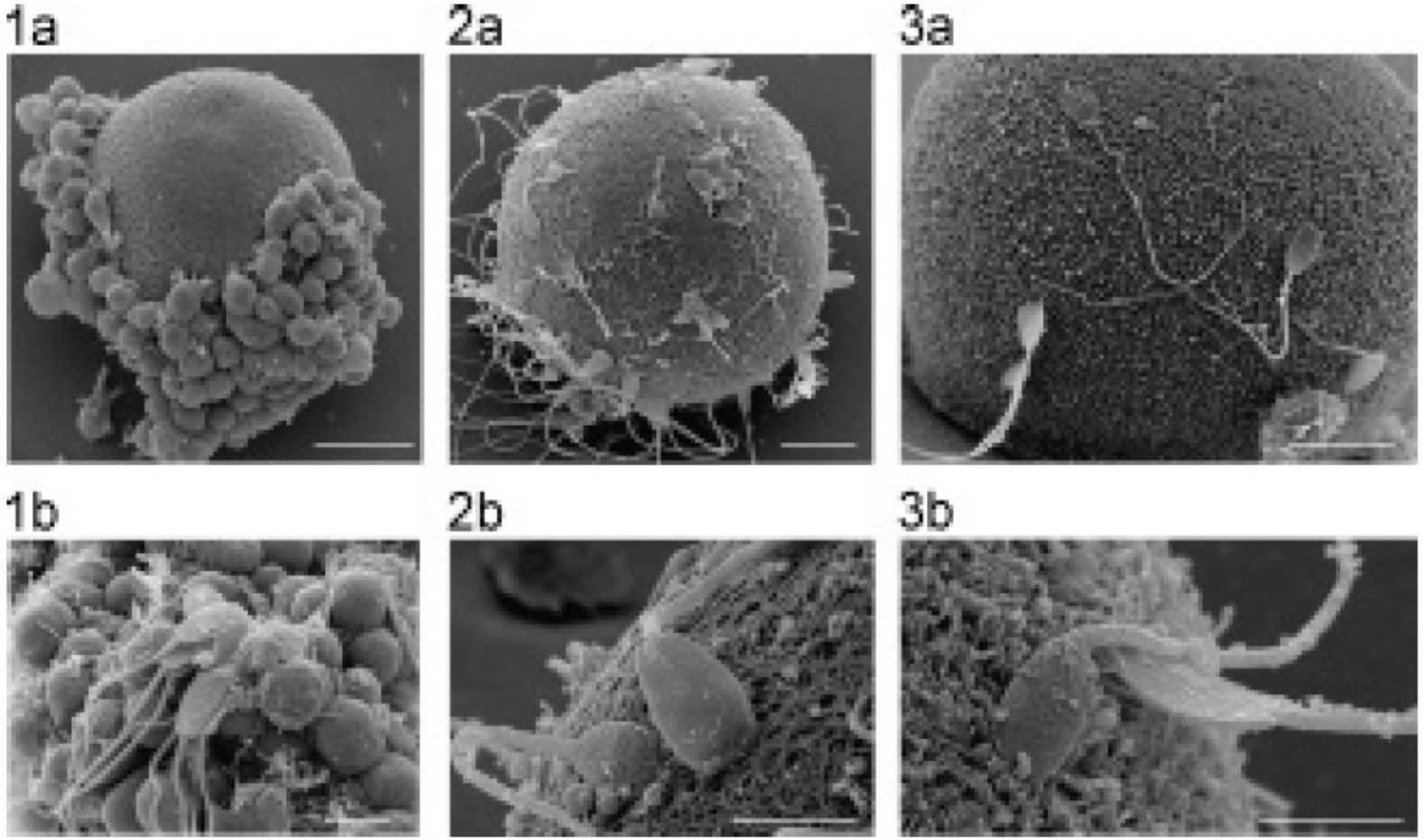
- When a group of cells in the retina is activated (eg, the posterior part of the retina), a similar group in the optic tectum (eg, the caudal) is also activated.
- This demonstrates the connection of individual retinal cells to their corresponding cells in the tectum.
- But how exactly is it connected?

# Ephrins and axon guidance: example Chiasma opticum

By the gradient of Eprins (in the tectum) and their receptors (on the guided axon)



# Cell adhesion – Sperm and oocyte



# Cell adhesion – Sperm and oocyte

Examples of **cell–cell** adhesion proteins found in spermatozoa and oocytes and evidence of their participation in fertilization-related events.

| Adhesion molecules | Gamete expression | Involvement in fertilization-related events | References |
|---|---|---|---|
| **Immunoglobulins**[a] | | | |
| Basigin | S and O | Sperm–*zona pellucida* interaction | Ding et al. (2002), Saxena et al. (2002), Saxena and Toshimori (2004) |
| CD4 | S and O | Homologous fertilization | Gobert et al. (1990), Mori et al. (1991), Guo et al. (1995) |
| ICAM-1, NCAM, VCAM-1 | O | Embryo development | Kimber et al. (1994), Campbell et al. (1995) |
| EWI-2 | O | Sperm–oolemma interaction | Glazar and Evans (2009) |
| Growth factor receptors | S and O | Sperm capacitation, acrosomal exocytosis<br>Oocyte maturation | Yoshida et al. (1998), Einspanier et al. (2002), Prochazka et al. (2003), Cotton et al. (2006), Breitbart and Etkovitz (2011) |
| Izumo | S | Sperm–oolemma fusion | Inoue et al. (2005), Hayasaka et al. (2007), Kim et al. (2013) |
| JAM-A | S | Sperm motility | Shao et al. (2008) |
| MHC class II | S | Homologous fertilization | Mori et al. (1990) |
| **Selectins** | | | |
| L-selectin | S and O | Sperm–*zona pellucida* interaction | Campbell et al. (1995), Lucas et al. (1995) |
| P-selectin | S | Sperm–*zona pellucida* interaction<br>Sperm–oolemma interaction | Fusi et al. (1996a), Geng et al. (1997) |
| **Integrins** | | | |
| Different α and β subunits | S and O | Sperm–oolemma interaction<br>Oocyte activation | Tarone et al. (1993), Fusi et al. (1993, 1996b), Almeida et al. (1995), Campbell et al. (1995), Takahashi et al. (2000), Tatone and Carbone (2006), Barraud-Lange et al. (2007), Vjugina et al. (2009) |
| **Cadherins** | | | |
| E-cadherin | S and O | Sperm–oviduct interaction<br>Sperm–*zona pellucida* interaction<br>Sperm–oolemma interaction<br>Homologous fertilization | Campbell et al. (1995), Rufas et al. (2000), Ziv et al. (2002), Purohit et al. (2004), Marín-Briggiler et al. (2008), Takezawa et al. (2011), Caballero et al. (2014) |
| N-cadherin | S and O | Sperm–oolemma interaction | Goodwin et al. (2000), Rufas et al. (2000), Ziv et al. (2002), Marín-Briggiler et al. (2010) |
| P-cadherin | S and O | – | Rufas et al. (2000), Ziv et al. (2002) |
| Others | S | – | Johnson et al. (2004) |

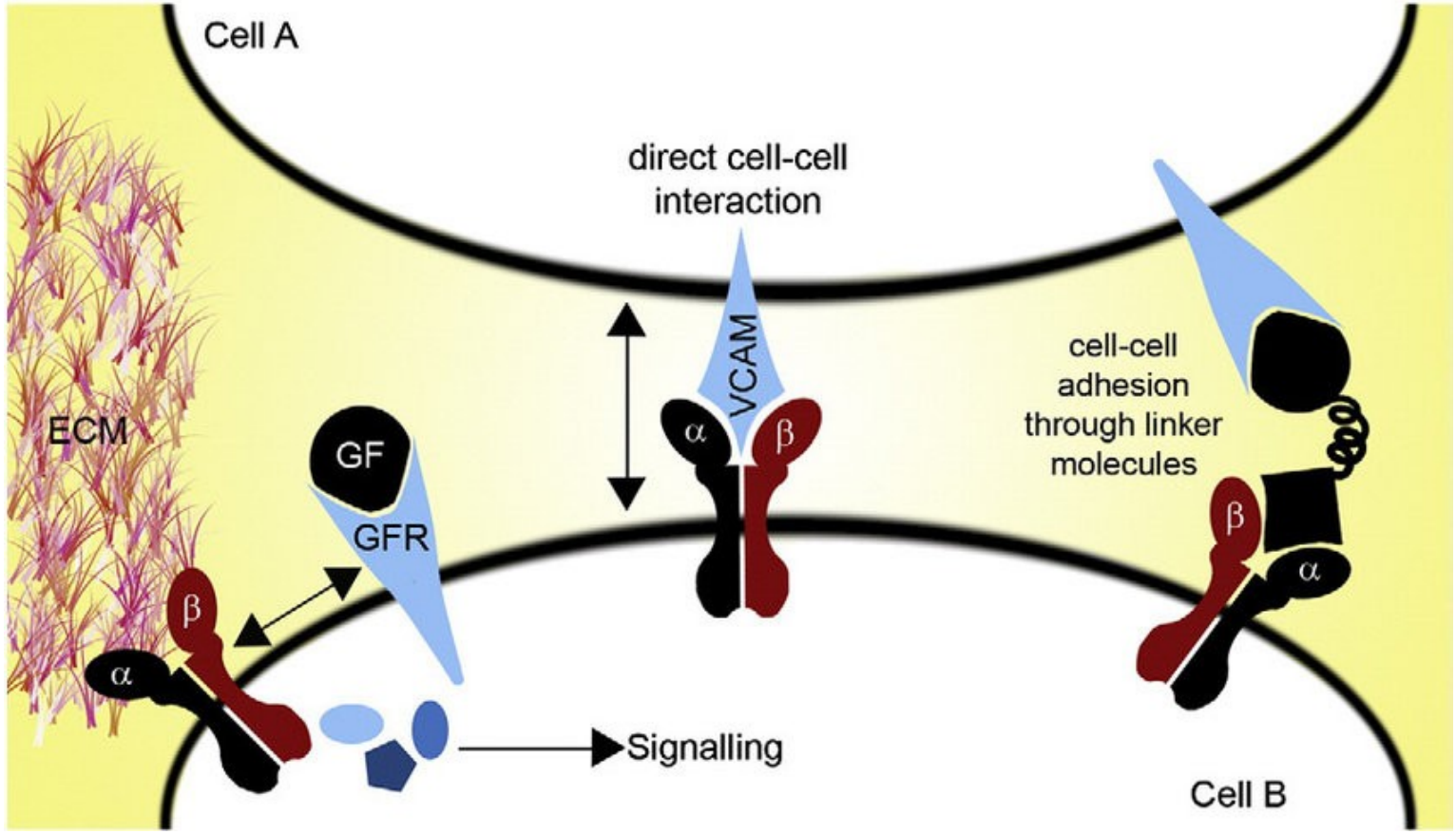
S=spermatozoa; O=oocyte.

[a] This superfamily comprises more than 700 known proteins, examples of some members of this superfamily are provided.

# Integrins

# Integrins

- They mostly have a function in the cell-ECM interaction - we will discuss in the next lecture
- But also in cell-cell interaction



# Cell adhesion - sumup

- Juxtacrine signaling
- Principles of adhesion What are cadherins, binding principle, basics of cadherin signaling
- The strength of adhesion depends on the amount of canderins on the cell surface
- Differential adhesion hypothesis
- Other molecules that participate in intercellular contact

Tomáš Bárta
tbarta@med.muni.cz