Bi8940 Developmental biology
Lesson 9
Morphogenesis

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Outline of Lesson 9

Morphogenesis

- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres
  - Changes in the cell motility and tissue interactions during organogenesis
- Morphogenesis in plants
  - Introducing leaf development as an example of morphogenesis in plants
  - The role of oriented cell division and its relative distribution
    - Regulation of cell division by TCP and boundary genes
    - Auxin-regulated positional information for cell division
    - *KNOX* and boundary genes in the leaf complexity

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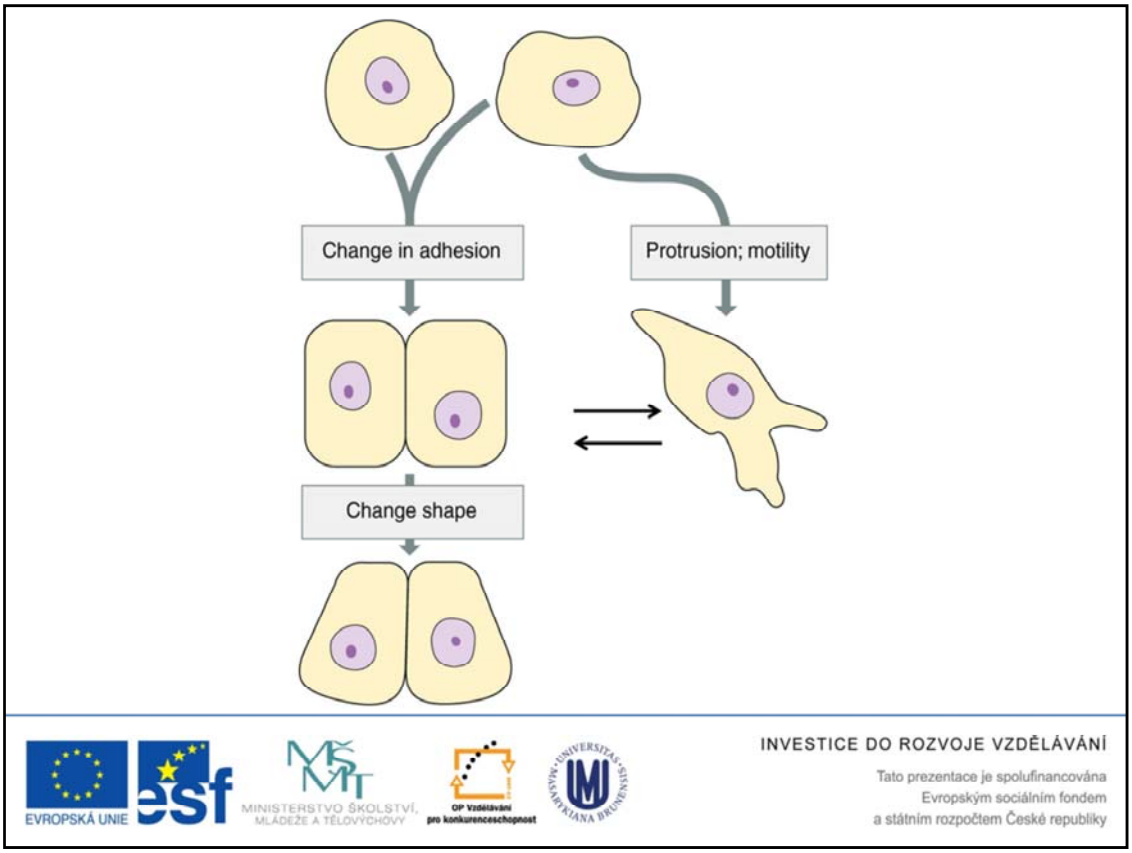
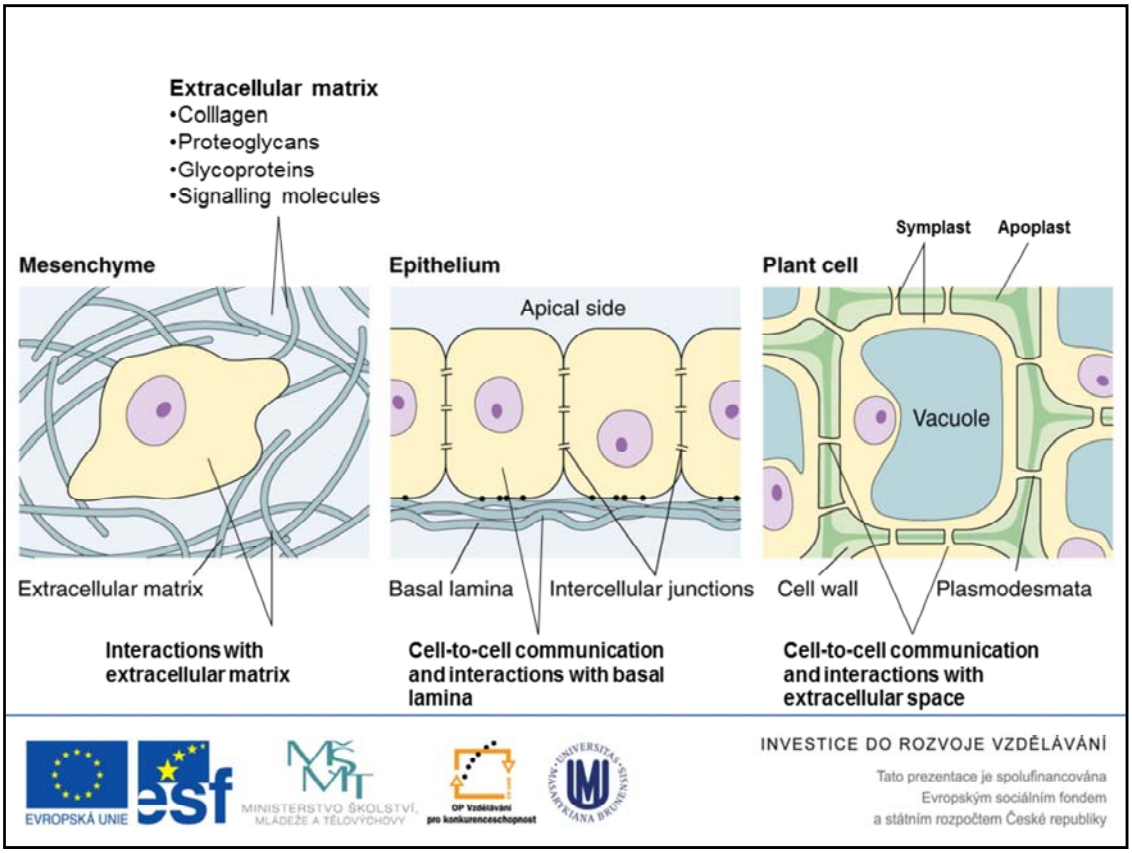


Morphogenesis is triggered by the action of upstream acting factors, the morphogens.

In animals, the morphogenesis is accompanied by several changes in cells or their groups, e.g. changes in adhesion, motility (or protrusion), shape and rate of proliferation.

For those changes, the communication among the cells and their interaction with the surrounding cellular environment is critical. In the following part, we will discuss the major factors involved in those aspects of the cellular life.



In contrast to epithelial cells, the cells of mesenchyme are not in a tight contact with each other. The intercellular spaces are filled with what is called **extracellular matrix**.

The extracellular matrix consists of *collagen, proteoglycans, glycoproteins* and *signalling molecules* and is highly hydrated.

The figure above demonstrates the differences between mesenchymal, epithelial and plant cells. In plant morphogenesis (see later), the cell wall plays a role, too.

Some of the important proteins that are part of the extracellular matrix will be described in the following slides.

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  - Extracellular matrix regulators of morphogenesis

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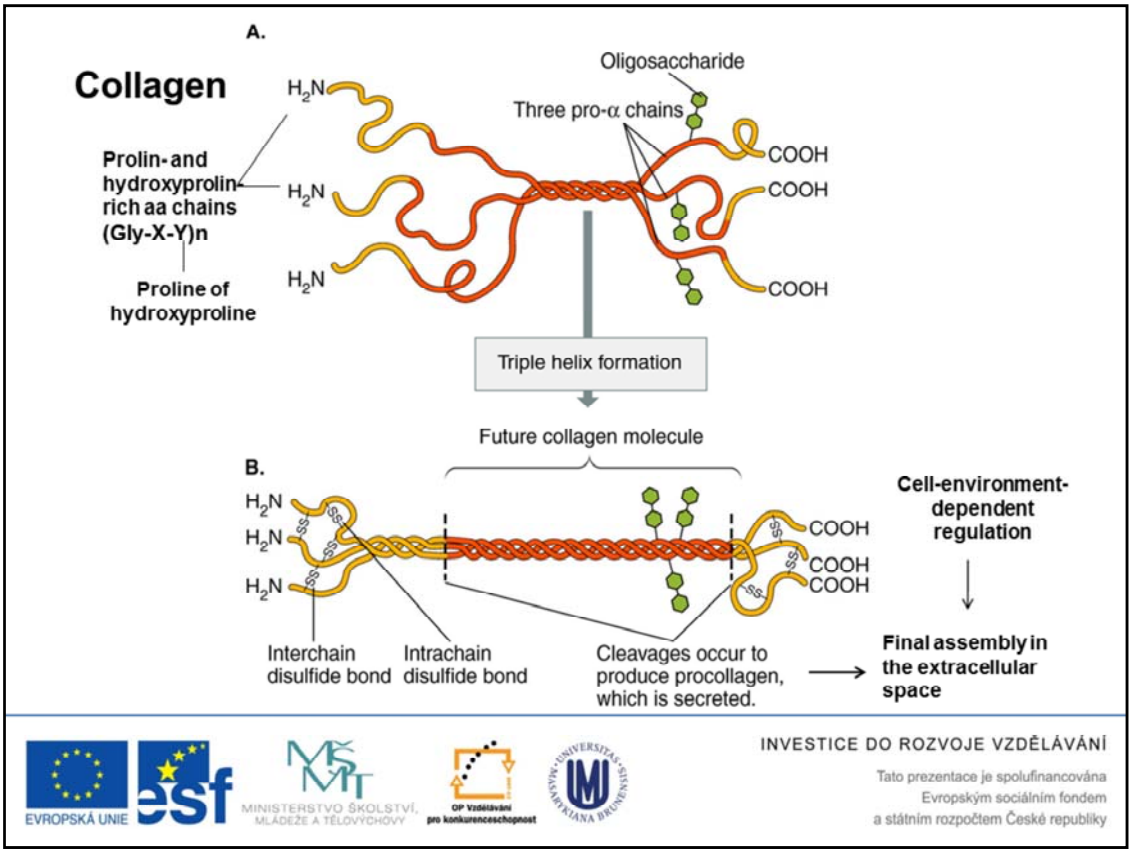


Collagen is formed from the proline or hydroxyproline-rich chains of amino acids. There are at least 18 types of collagen in vertebrates, all of them being composed of tandem three-amino-acid repeats of the form (Gly-X-Y)n, in which Y is proline or its hydroxylated form, the hydroxylproline.

There are several important posttranslational modifications of the collagen. These are e.g. hydroxylation of some prolines to hydroxyprolines, glycosylations and protease-mediated cleavage of peptides at both N- and C-terminal ends. Further there are intramolecular disulfide bonds being formed at both N- and C-termini.

Three of the protein chain subunits are joined together into triple helix via intermolecular disulfide bonds that occur along the length of the whole subunit chain.

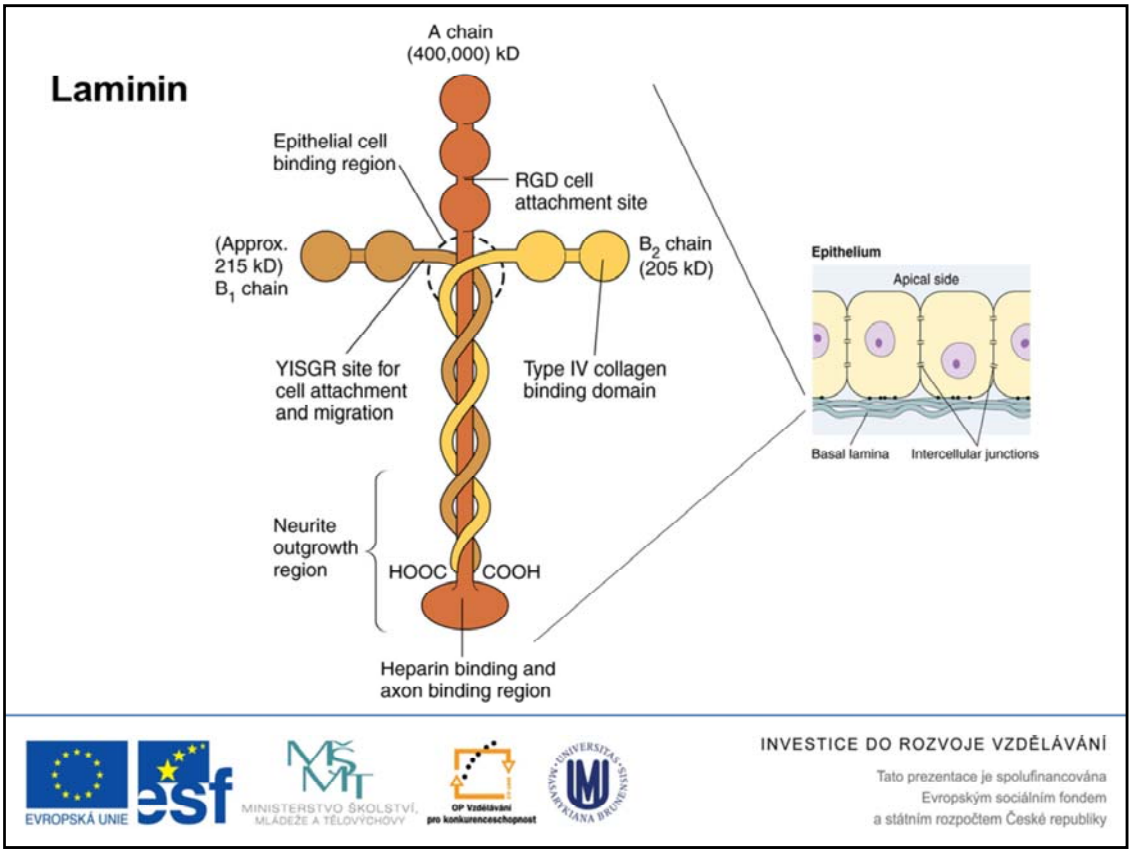
Collagen synthesis, glycosylation, hydroxylation, helix formation and other modifications occur still in a cell, while the final assembly takes place in the extracellular space.

Importantly, the extracellular environment regulates the final assembly of collagen. For example, salivary glands are composed of branched epithelial ducts ending in the secretory acini. During their development, the epithelial cells secrete the enzymes that is necessary for the final assembly of the collagen.

TABLE 12.1 THE MAJOR TYPES OF COLLAGEN

Type	Class	Chain Composition[a]	Kinds of Tissues
I	Fibrillar	$2[\alpha_1(I)] + 1[\alpha_2(I)]$	90% of total: skin, bone, cornea, ligaments
II	Fibrillar	$3[\alpha_1(II)]$	Cartilage
III	Fibrillar	$3[\alpha_1(III)]$	Skin, blood vessels, found with type I
IV	Network	$2[\alpha_1(IV)] + 1[\alpha_2(IV)]$	Basal lamina

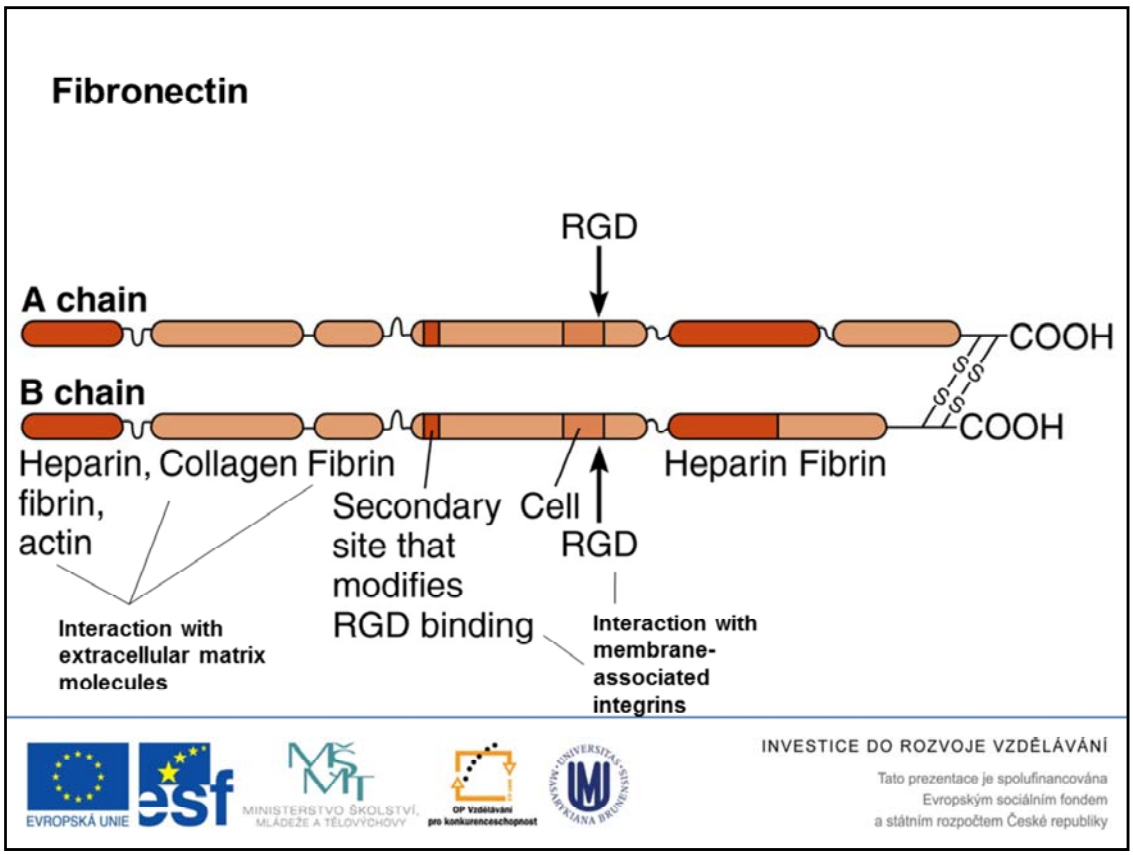
The different collagen types differ in the composition of individual procollagen subunits and sometimes, the individual subunits are not identical in the final triple helix (see the table above).



Laminins are another proteins that occur mostly in *basal lamina* between mesenchyme and epithelial cells.

It is composed of three different protein chains that wound around each other at their carboxyl ends. Different regions of the lamininin molecule include sites recognized by other proteins.

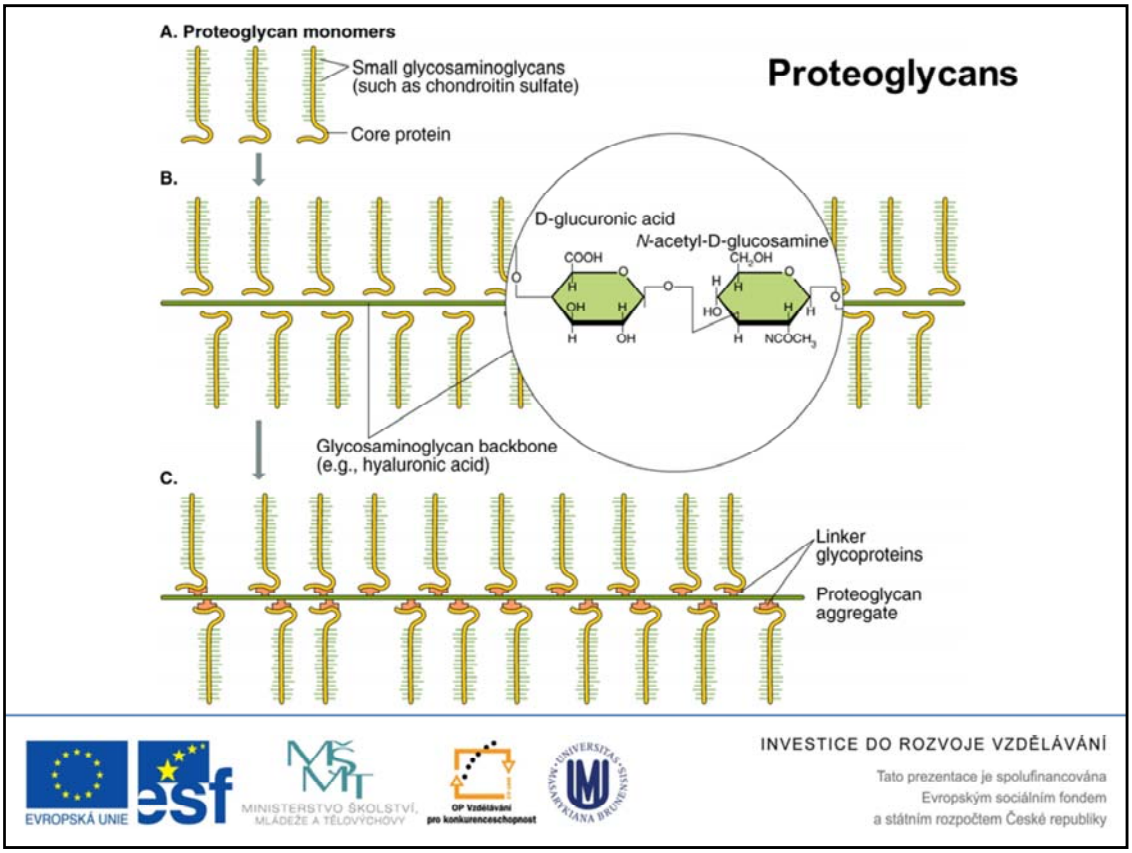
B2 chain has a domain that interacts with type IV collagen, another important component of the basal lamina; the chain A binds to a proteoglycan *heparin*.



Fibronectin is a heterodimer glycoprotein that serves as a bridge between extracellular matrix and cellular integral proteins (see also slide #14).

Fibronectin contains cell binding motifs “RGD” and “RGDS”. These sequences are important for the interaction of fibronectin with cell mebrane-associated molecules called **integrins**.

The above described glycoproteins (collagen, laminin and fibronectin) form much of the extracellular matrix.



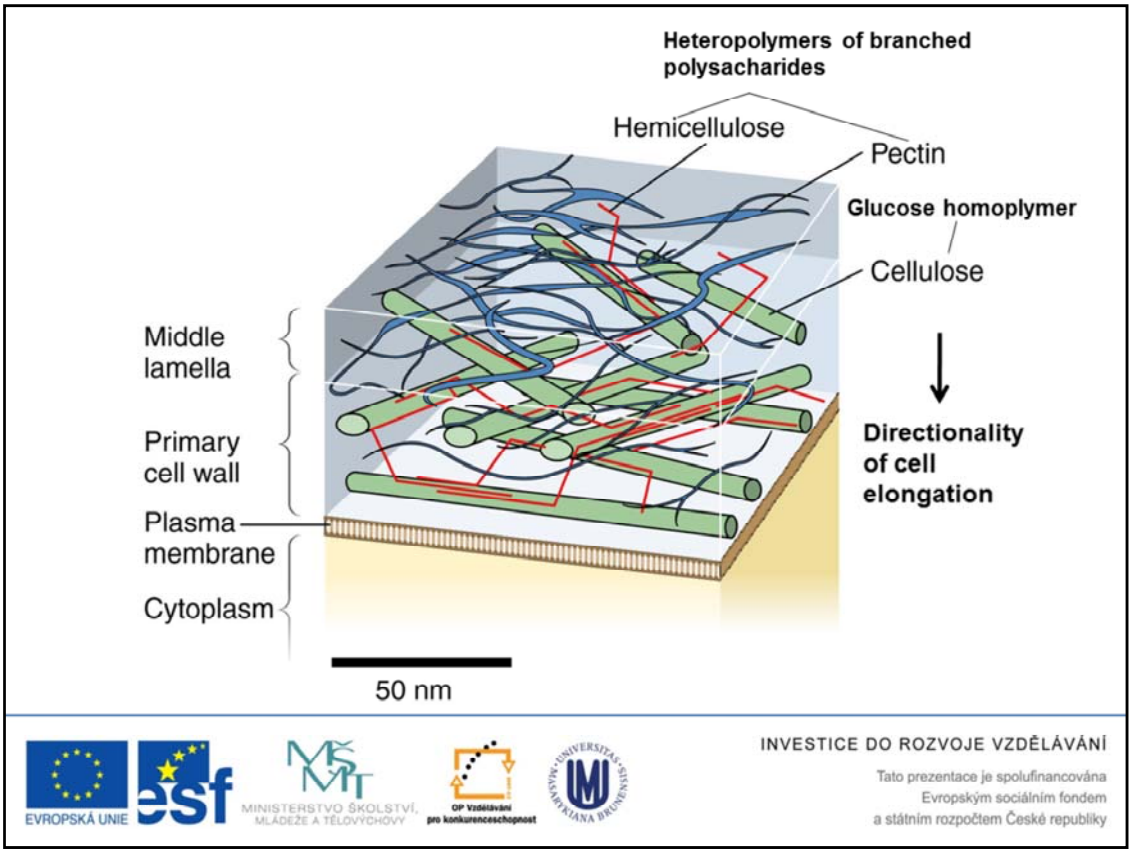
A specific portion of the extracellular matrix macromolecules is formed by **proteoglycans**.

Proteoglycans are formed of proteins and large amount of unusual polysacharides called **glycosaminoglycans (GAGs).**

These macromolecules are composed of repeating disaccharide subunits containing acidic sugars, usually glucuronic acid and the sugars usually have sulfonated hydroxyl groups.

Long GAG polymers are very hydrophilic and bind huge amount of water, leading to the huge volume relative to their mass. Recall the involvemnt of proteoglycans-rich vacuoles in the water absorbance during notochord formation (swelling of its cells, see Lesson 5).

Long GAG molecule, e.g. *hyaluronic acid*, which is connected via linker glycoproteins to the small GAGs, e.g. *chondroitin sulfate*, forming thus typical "brush" structure of glycoproteins that have important regulatory functions.

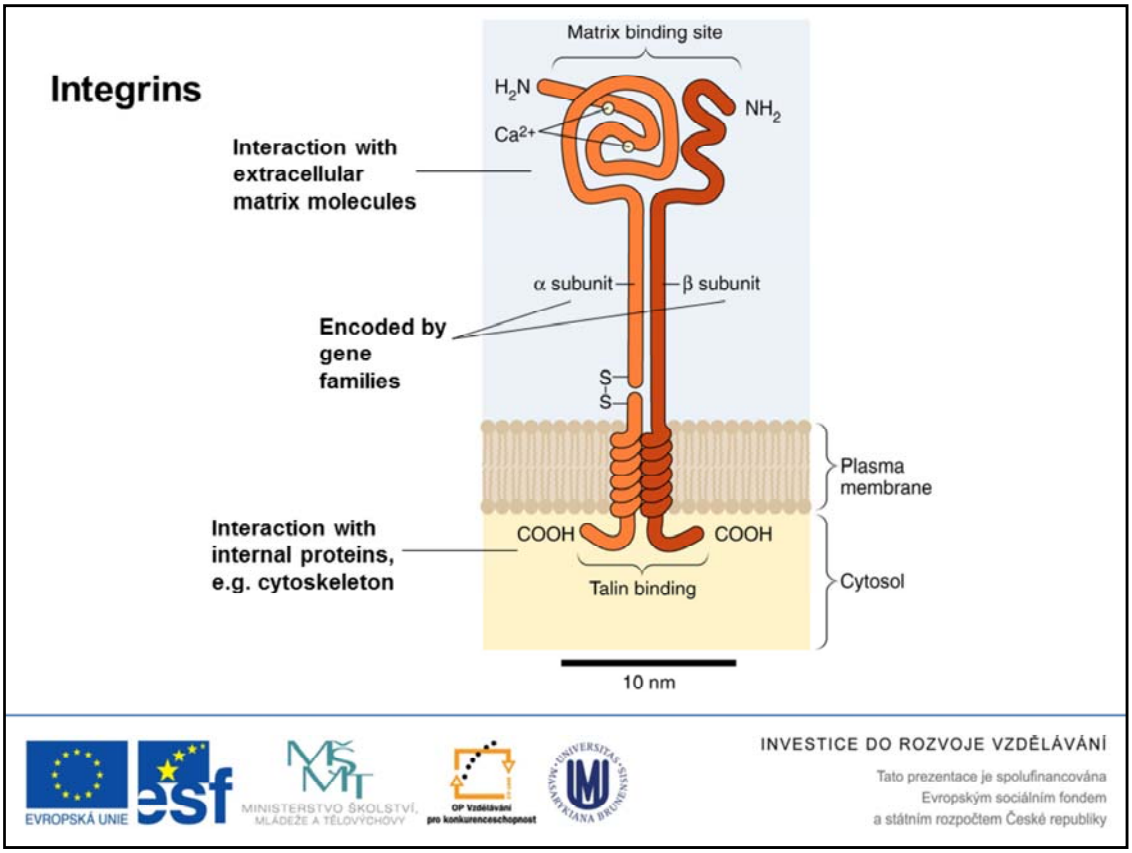


In contrast to mobile animal cells, the plant cells are engaged in the rigid cell walls that precludes their movement during development. However, the plant cells can expand during development in a process that is called *elongation* (or elongation growth).

Directionality of the elongation is determined by the arrangement of **cellulose** fibrils in the primary cell wall that are embedded in a matrix of **hemicellulose** and **pectins.**

Elongation occurs in the young tissues where the cell walls are elastic, i.e. the cellulose microfibrils are not cross-linked yet.

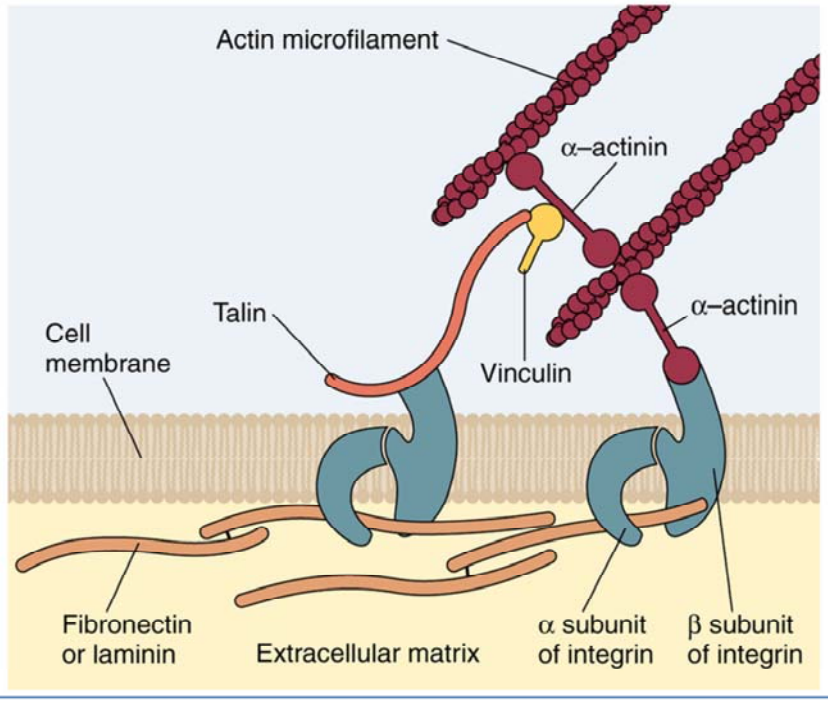
Cellulose is glucose homopolymer, while hemicellulose and pectin are heteropolymeric molecules formed by branch-chained polysacharides. Cellulose, hemicellulose and pectins are hold together via both covalent and non-covalent bonds.



Integrins are specific molecules that allow interactions of cells with the individual components of the extracellular matrix.

Extracellular portion of the heterodimer might interact with extracellular matrix molecules. The intracellular portion might be connected to internal proteins and transfers the signal to the cell via e.g. interaction with cytoskeleton (see the next slide) or via regulation of the secondary messenger pathways.

Integrins are heterodimers consisting of two subunits called α and β. Each of the subunits is encoded by the gene family, leading thus into many possible combinations of formed heterodimers (see the table on the next slide).



Integrins might directly interact with cytoskeleton.

The interaction of integrins with cytoskeleton is complex and is mediated by other proteins, e.g. *talin*, *vinculin* or *α-actinin*.

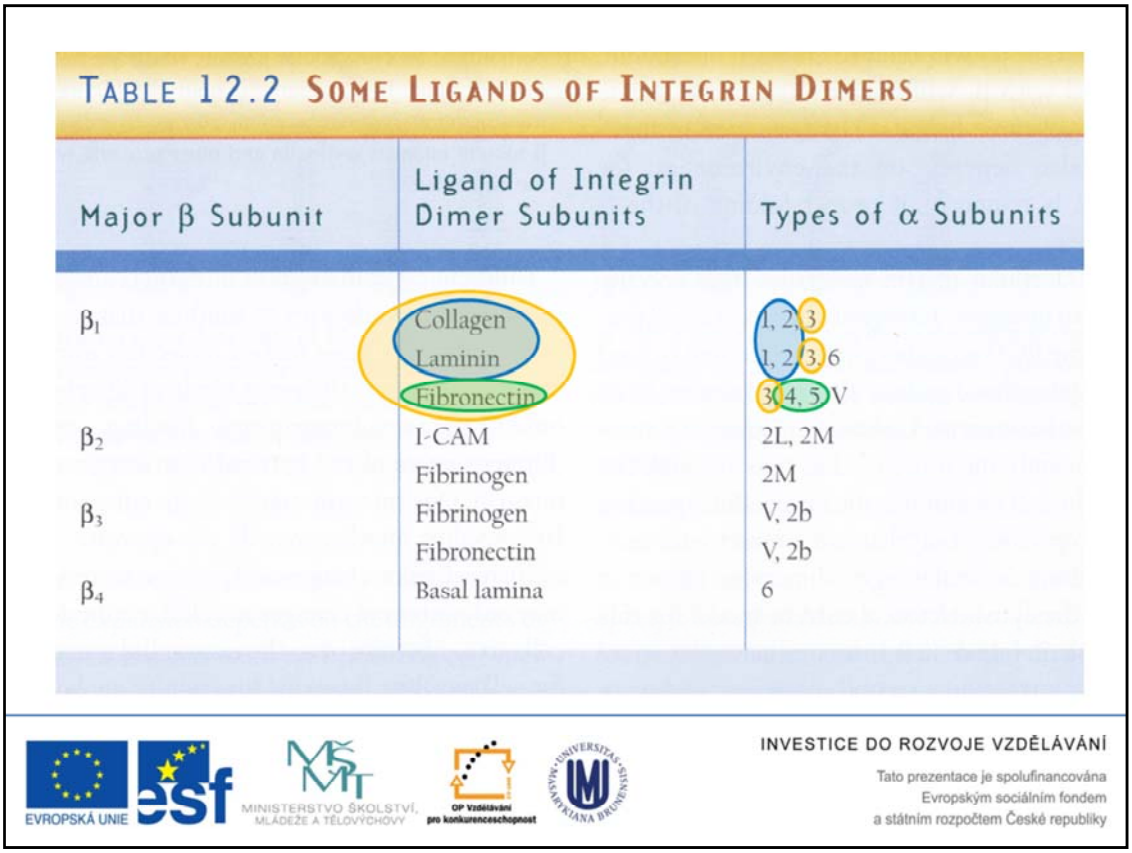


TABLE 12.2 SOME LIGANDS OF INTEGRIN DIMERS

Major β Subunit	Ligand of Integrin Dimer Subunits	Types of α Subunits
β_1	Collagen	1, 2, 3
	Laminin	1, 2, 3, 6
	Fibronectin	3, 4, 5, V
β_2	I-CAM	2L, 2M
	Fibrinogen	2M
β_3	Fibrinogen	V, 2b
	Fibronectin	V, 2b
β_4	Basal lamina	6

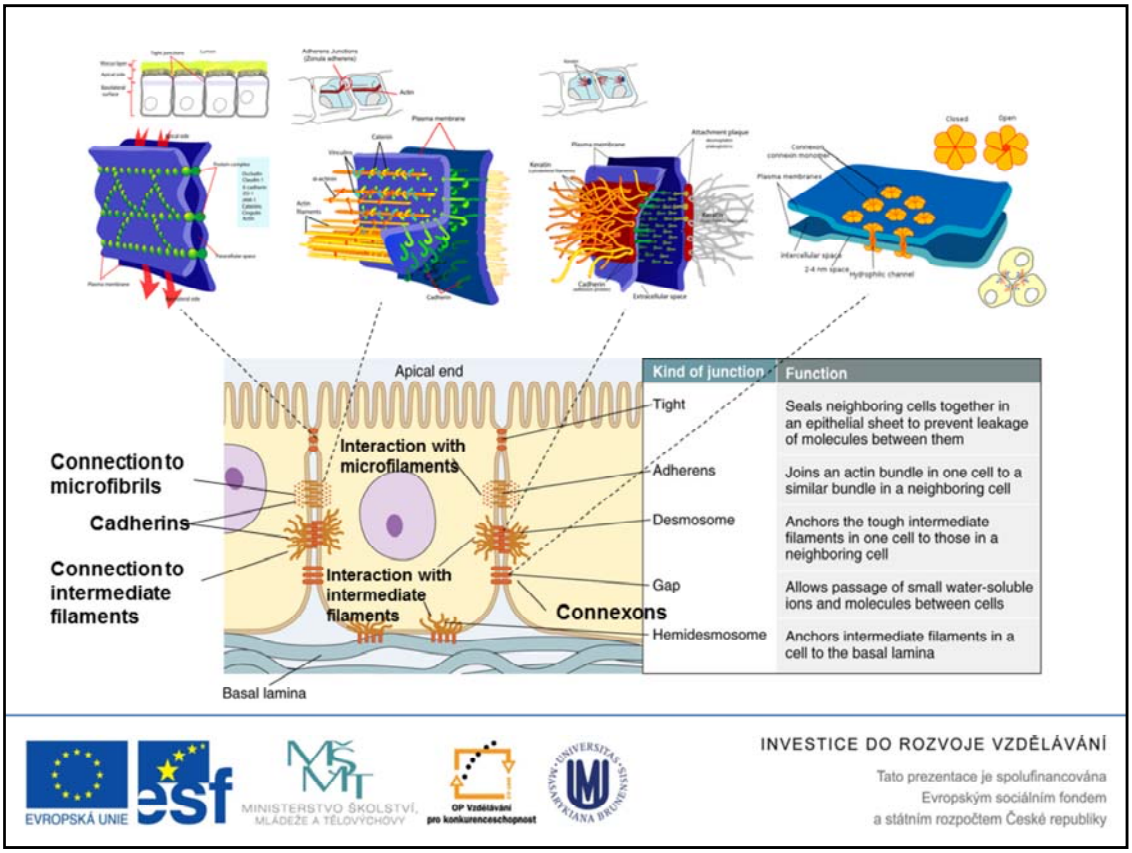
Different combinations of α and β subunits allow different binding specificity of integrins.

However, the specificity is not complete, as could be seen e.g. in case of integrin interactions with collagen or laminin. E.g. integrins consisting of β1 in combination with α1 or α2 will bind to collagen and laminin, α4 or α5 combined with β1 will bind to fibronectin while α3 β1 will bind to all three ligands.

Both collagen and fibronectin posses RGD binding motif, which mediates the interaction with integrins.

The binding integrin specificity is reflected in the functional specificity, too. Some integrins mediate cell adhesion while do not stimulate cell motility, the other ones do the opposite.

Cell adhesion is critical for the cell movement (see later).



There are several types of intercellular connections in epithelial cells.

Tight junctions form a circumferential seal in the lateral plasma membranes. That results into functional isolation of the epithelial surface (the lumen) from the lateral plasma membranes and the basal lamina.

Adherens junctions are often basal located to the tight junctions and besides connecting the cells to each other, they connect the cell surface to the intracellular microfibrils, forming thus intracellular belt circumventing the epithelial cell.

Desmosomes are specialized connections forming "spot welds". The integral membrane proteins of desmosomes connect to the intermediate filaments of the cytoplasm (e.g. keratins, see the figure). Both desmosomes and adherens junctions utilize "gluer" proteins called *cadherins* (see later, slide # 25).

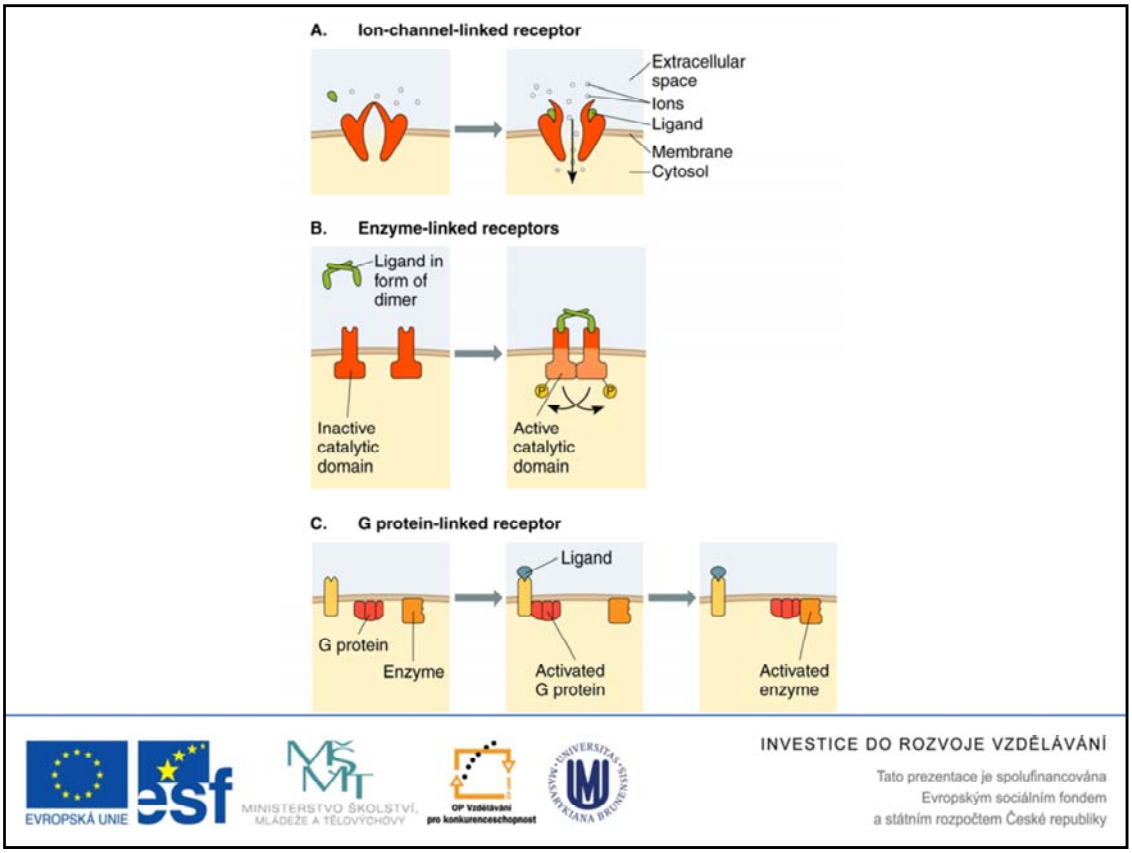
Recall the different cell junctions discussed in case of internal and external cell subpopulation and later trophoblast cell connection during blastocyst formation in mammals (see Lesson 4).

Hemidesmosomes are similar to desmosomes except that they connect intermediate filaments to the basal lamina, not to other cells.

Gap junctions consist of specific structures called *connexons* that are formed from membrane-spanning proteins. When two connexons are connected together, that form an aqueous pore between the two cells.

In plants, the term epidermis is being used instead of epithelial cells. In the plat cells, plasmodesmata allow intercellular communications (see the slide #5).

The "welding" of the epithelial cells and thus formation of different junctions is a dynamic process. E.g. during ectodermal organogenesis, the neural crest cell are derived from the originally tightly associated cells of the neural epidermis. These cells re-associate again in the target destinations, e.g. in the adrenal medulla.



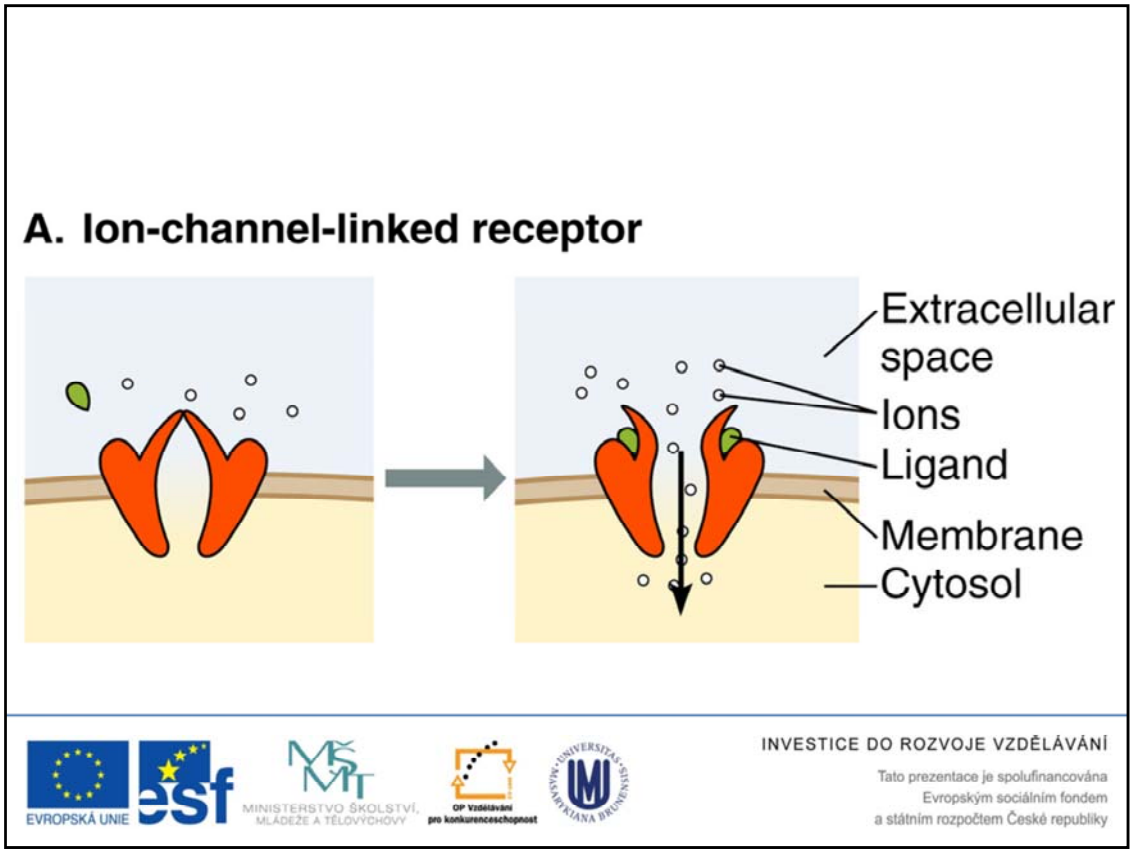
There are several receptor types involved in the communication between extracellular space and the cell environment.

These are

A. ion-channel-linked receptors,

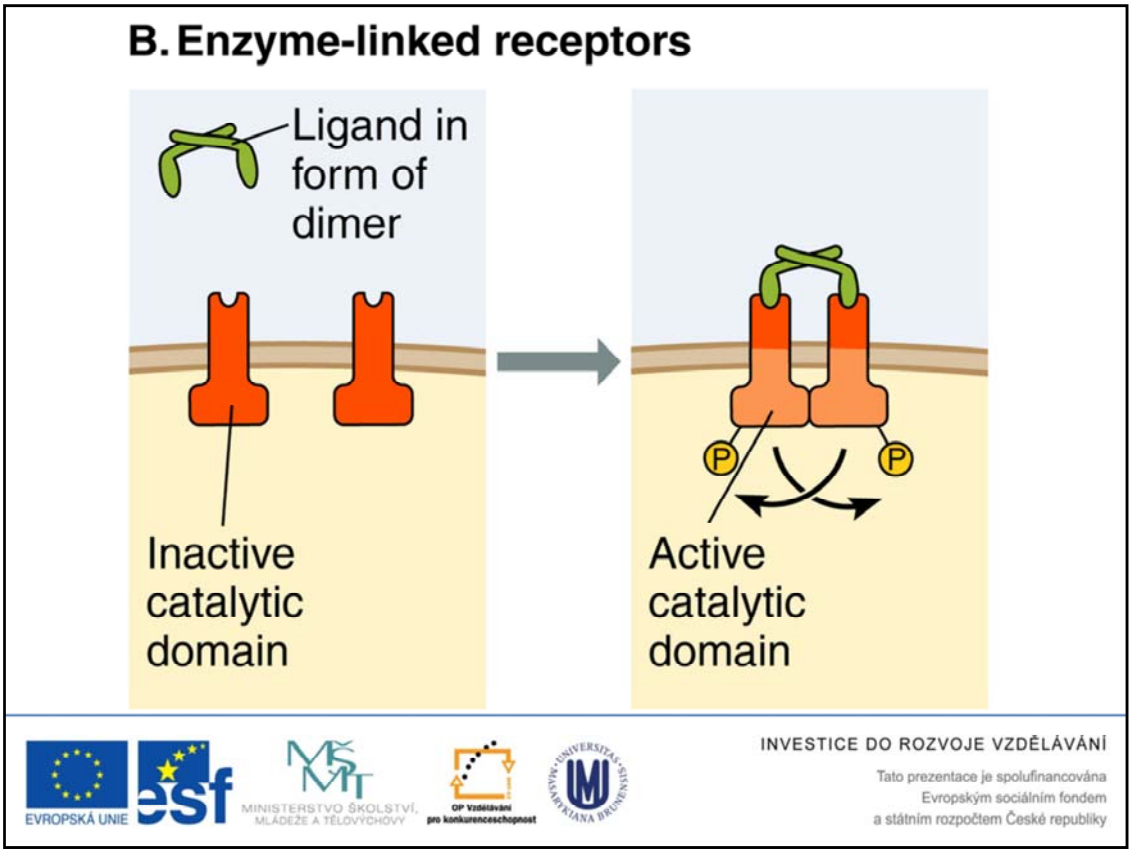
B. enzyme-linked receptors and

C. G protein-linked receptors (see next slides).



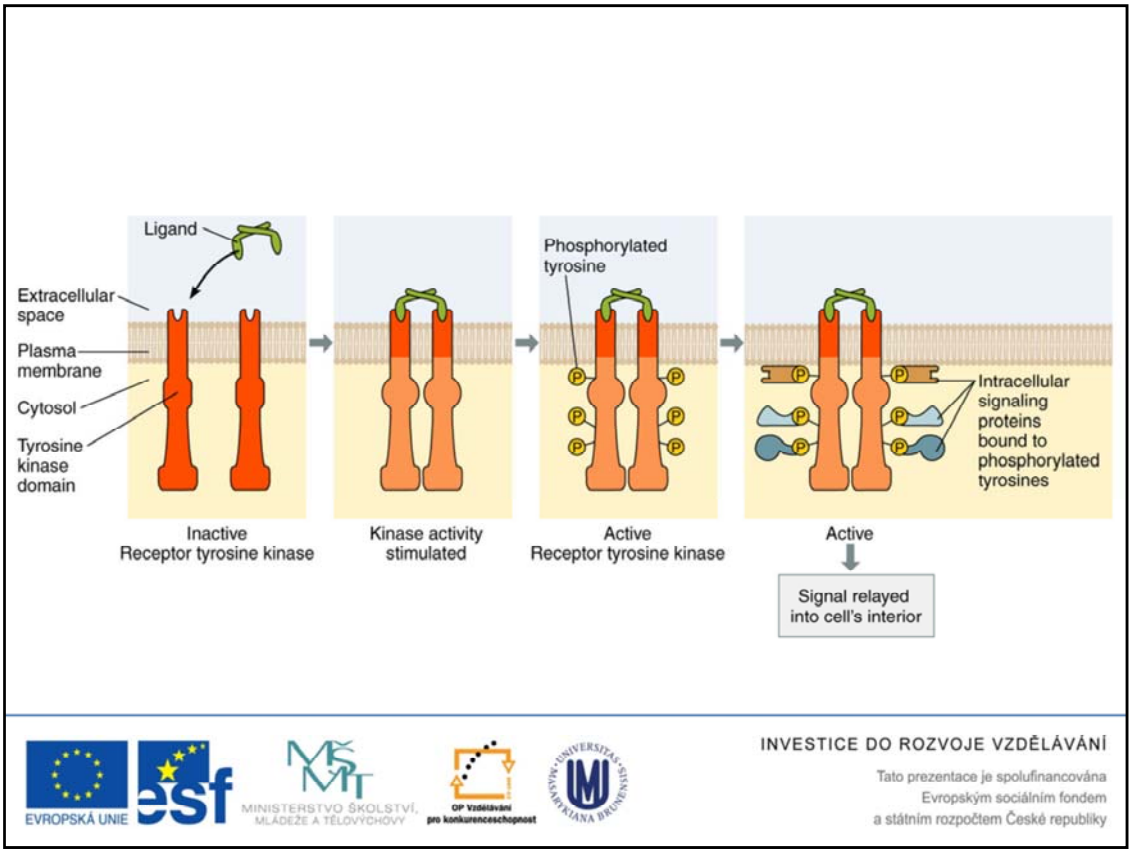
Ad A.

The ion-channel-linked receptors changes its conformation in a response to the ligand binding, leading to the open/close status of the ion channel.



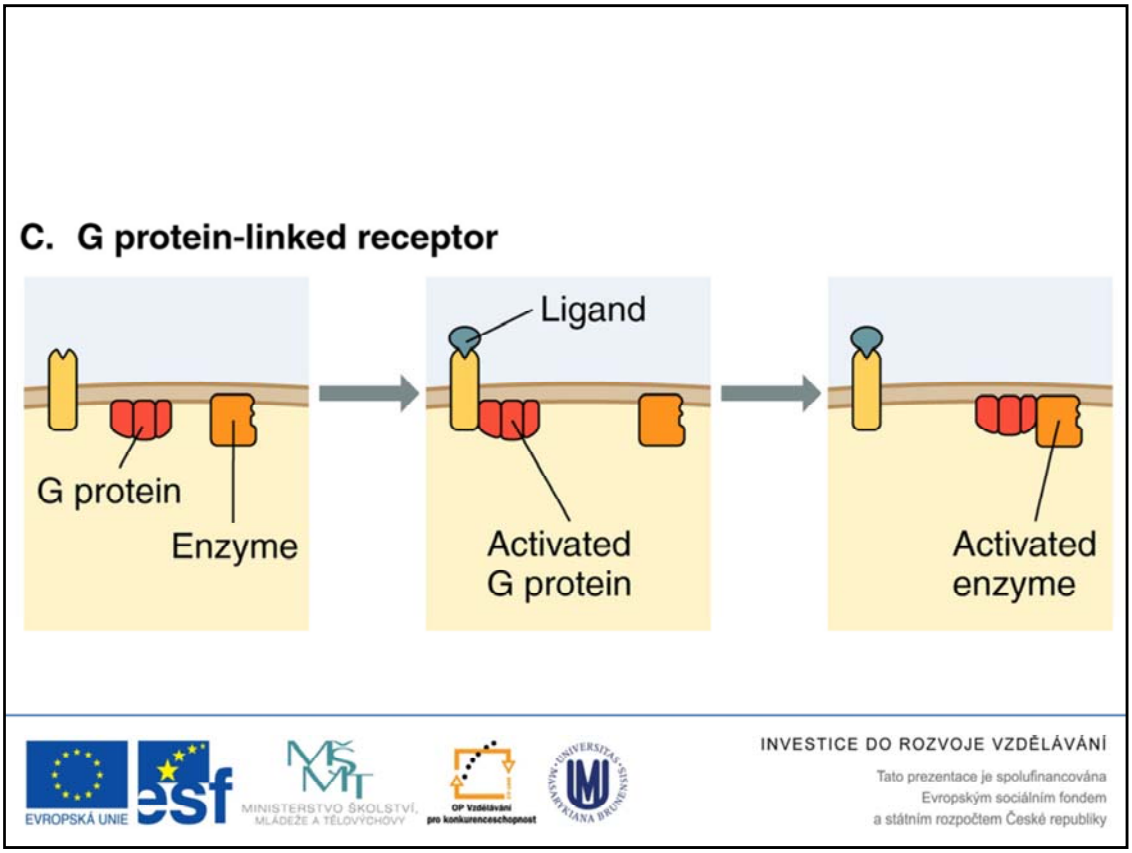
Ad B.

Enzyme-linked receptors interact with ligand and dimerize upon its recognition. That leads to the autophosphorylation of the intracellular domain. Dependent on the substrate of the kinase, there are recognized serin/theronin or tyrosin kinase receptors.



Detailed schematic representation of the series of events after activation of the receptor tyrosin kinase (RTK).

The interaction with ligand leads to the cross-phosphorylation of the dimer that allows interaction with proteins possessing domains (called SH2 or SH3) that specifically interact with the phosphorylated tyrosine.



Ad C.

Interaction of the G protein-linked receptor activates G protein that binds GTP (its α subunit). That results into dissociation of the β and γ subunits, which further activate other downstream targets, e.g. phospholipase C (recall the phospholipase C-mediated signaling leading to Ca2+ release after fertilization discussed in lesson 1).

The different receptor types could be combined in frame of one signalling pathway, e.g. G protein-coupled receptors are combined with ion-channel-linked receptors in case of phospholipase C-mediated signalling regulating the egg response after fertilization (see Lesson 1).

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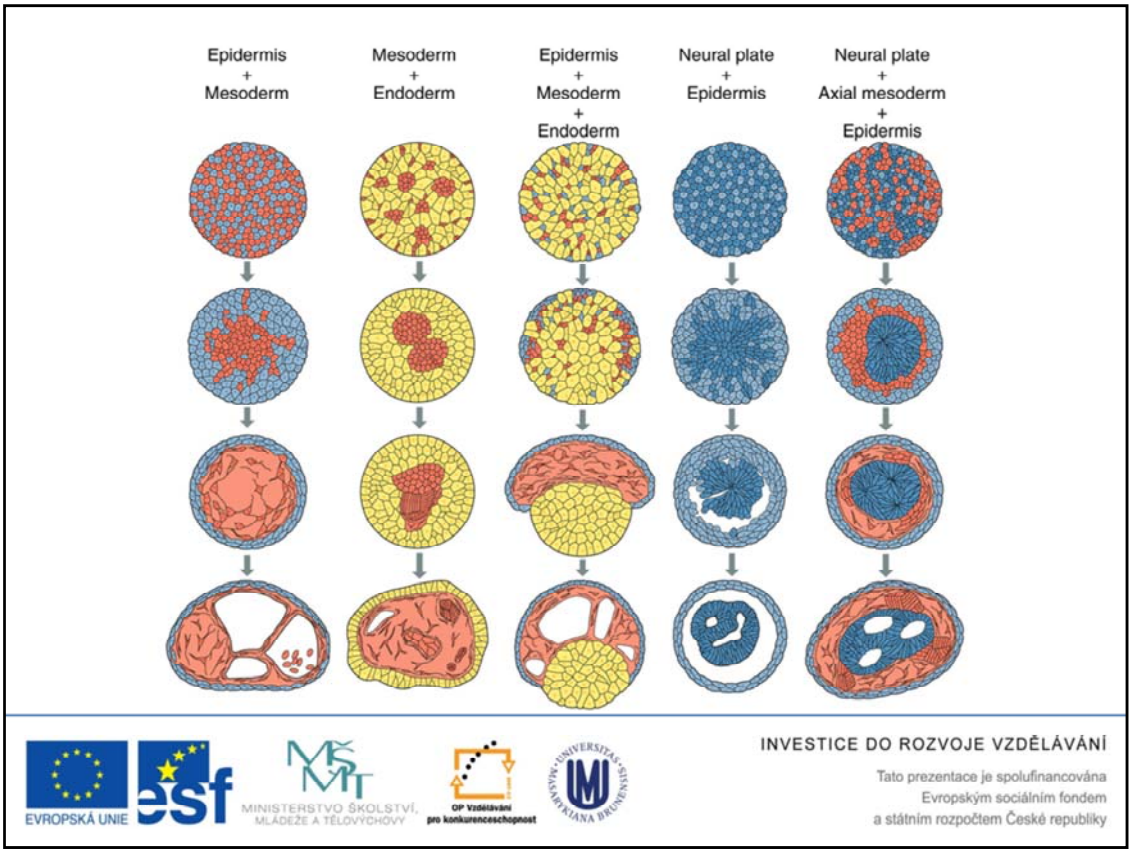


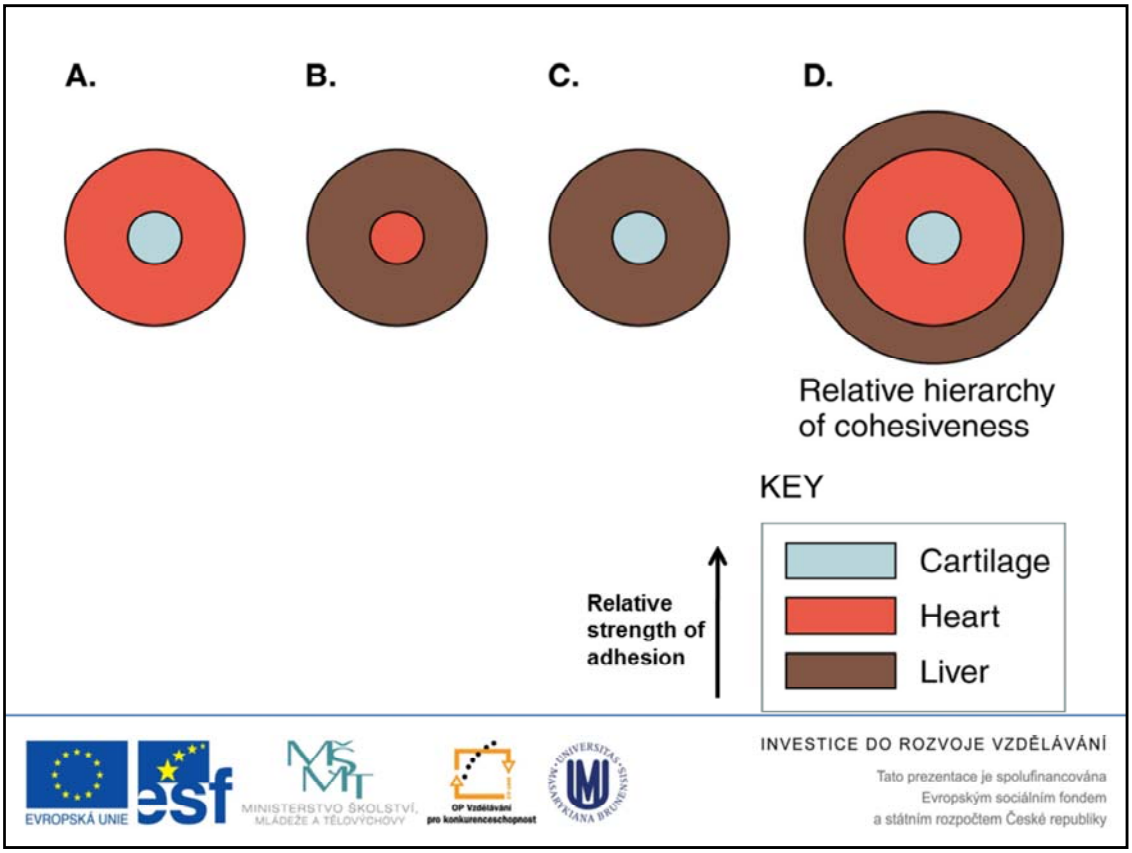
Classical experiments by the Johannes Holtfreter showing the specificity of cell aggregations.

Cells of the amphibian gastrula were dissected using pipette in the solution lacking Ca2+. The cells were then cultivated in the medium with Ca2+, which led to the cell re-aggregation.

Interestingly, when cells derived from different germ layers were mixed together, the cells were able to sort-out according to their origin, i.e. ectodermal cells associated with ectodermal, endodermal with endodermal and so on.

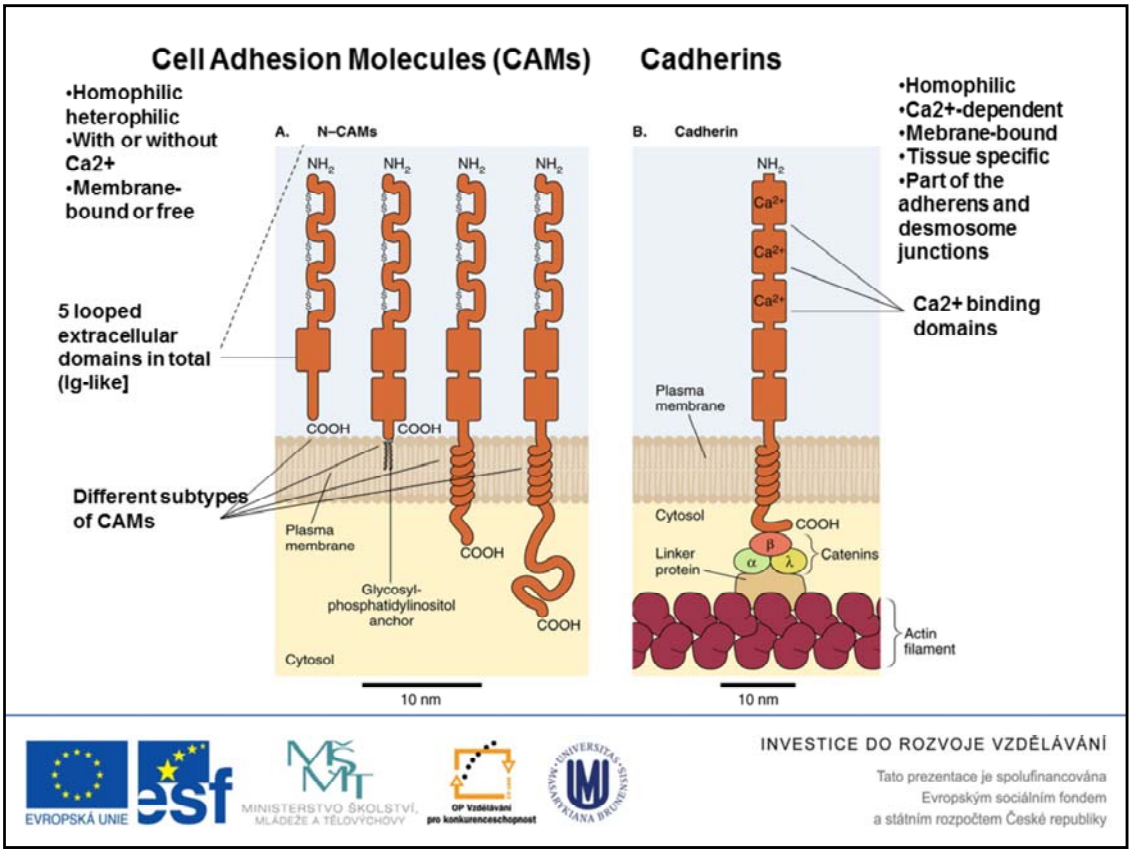
Further, the cells were oriented to each other in specific arrangements. E.g. when the prospective epidermis and mesoderm were mixed together, the epidermal cells were always oriented on the surface and mesodermal cell in the inner space.

This suggests that there is a certain **specificity of cell-to-cell aggregations**.



Malcolm Steinberg at the Princeton University find out that the relative strength of the adhesion leads to the specific cellular pattern. The more adhering cells tend to locate to the more central positions.

Here, the re-association of cartilage, heart and liver progenitors is shown with cartilage cells having the strongest adhesion and locating in the centre.



There are plenty of cell surface molecules involved in the specific cell adhesion. Two classes of these molecules are shown in the figure above.

First class is called **cell adhesion molecules (CAMs)**.

The extracellular portion of CAMs consists of 5 looped domains (in the figure above, only three of them are shown for simplicity) that bear similarity with immunoglobulins; therefore, they are considered as Ig-like molecules.

There are different types of CAMs that are either free or associated with the membrane via glycolipid anchor or have a hydrophobic domain that passes through the membrane.

Usually, the interactions allowing specific cell adhesion require Ca2+ and are *homophilic*, i.e. the cells expressing the same CAM type adhere. However, there are also cell interactions that are heterophilic and/or do not require Ca2+.

The other class are **cadherins**.

Cadherins consist of extracellular portion that contains several Ca2+ domains and typically have hydrophobic transmembrane domain. Cadherins always require Ca2+ and the mediated interactions are homophilic.

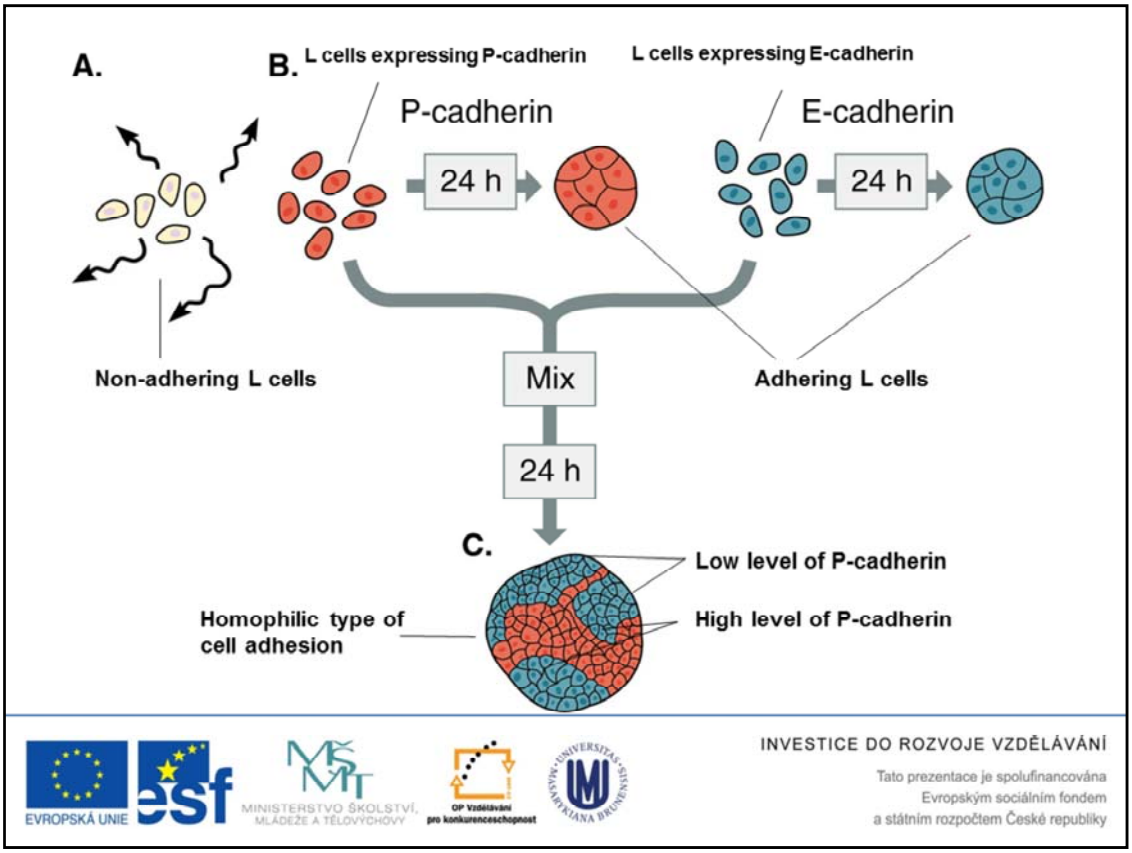
Different tissues are characterized by different cadherin expression. E.g. in the placenta there are P-cadherins, in the epithelial tissue are E-cadherins and N-cadherins are typical for nervous tissues.

Cadherins are also part of the adherens and desmosome junctions, where cadherins interact at the cytoplasmic portion of these junctions via linker proteins called *catenins* with actin or intermediate filaments.

TABLE 12.3 TYPES OF CELL ADHESION MOLECULES

Class of Molecule (Synonyms)	Binding Mechanism	Ion Dependence	Examples
N-CAM	Homophilic	No	Neural plate
Ng-CAM	Heterophilic	No	Nervous system
I-CAM	Heterophilic	No	Endothelial cells
L-CAM (E-cadherin, uvomorulin)	Homophilic	Ca^{2+}	Blastomeres
A-CAM	Homophilic	Ca^{2+}	Mesoderm, lens, muscle
P-cadherin	Homophilic	Ca^{2+}	Endoderm, placenta
N-cadherin	Homophilic	Ca^{2+}	Central nervous system
EP-cadherin (C-cadherin)	Homophilic	Ca^{2+}	Cleavage stage blastomeres
Integrins	Heterophilic	Varies	Extracellular matrix

The table shows examples of cell adhesion molecules and types of their interactions.



Scheme of the experiment performed by Masatoshi Takeichi in Kyoto.

Takeichi and his colleagues expressed P- or E-cadherin in mouse L cells that do not adhere at all. The transformed cells acquired the ability to adhere and when mixed, the cells expressing the same type of cadherins aggregated (homophilic type of interaction).

Furthermore, the level of P-cadherins was distinctive for the sorting-out of the cells and their relative position. I.e. the cells expressing higher amount aggregated together and located centrally in comparison to cells expressing lower amounts of P-cadherin.

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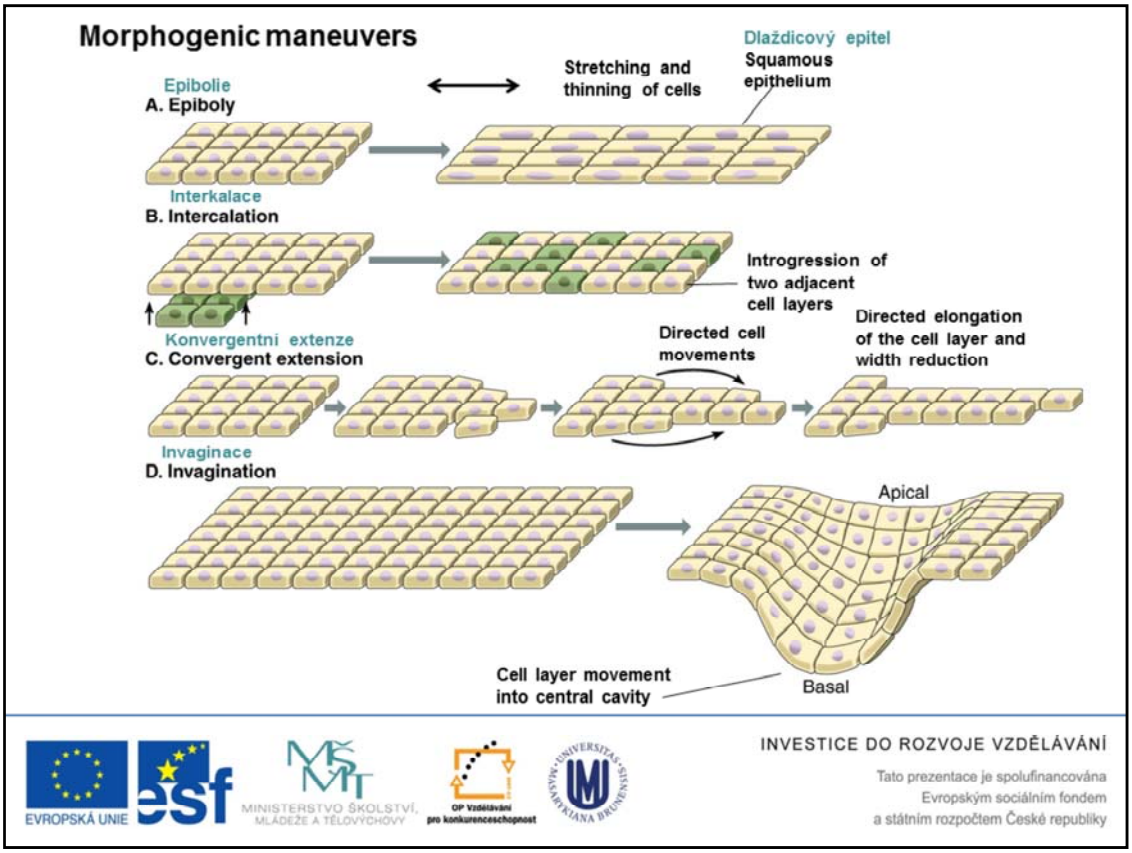


There are several of cellular movements or what is called "morphogenic maneuvers" taking place during morphogenesis. The individual processes listed here are usually rather complex and it is not easy to discriminate among them during embryogenesis.

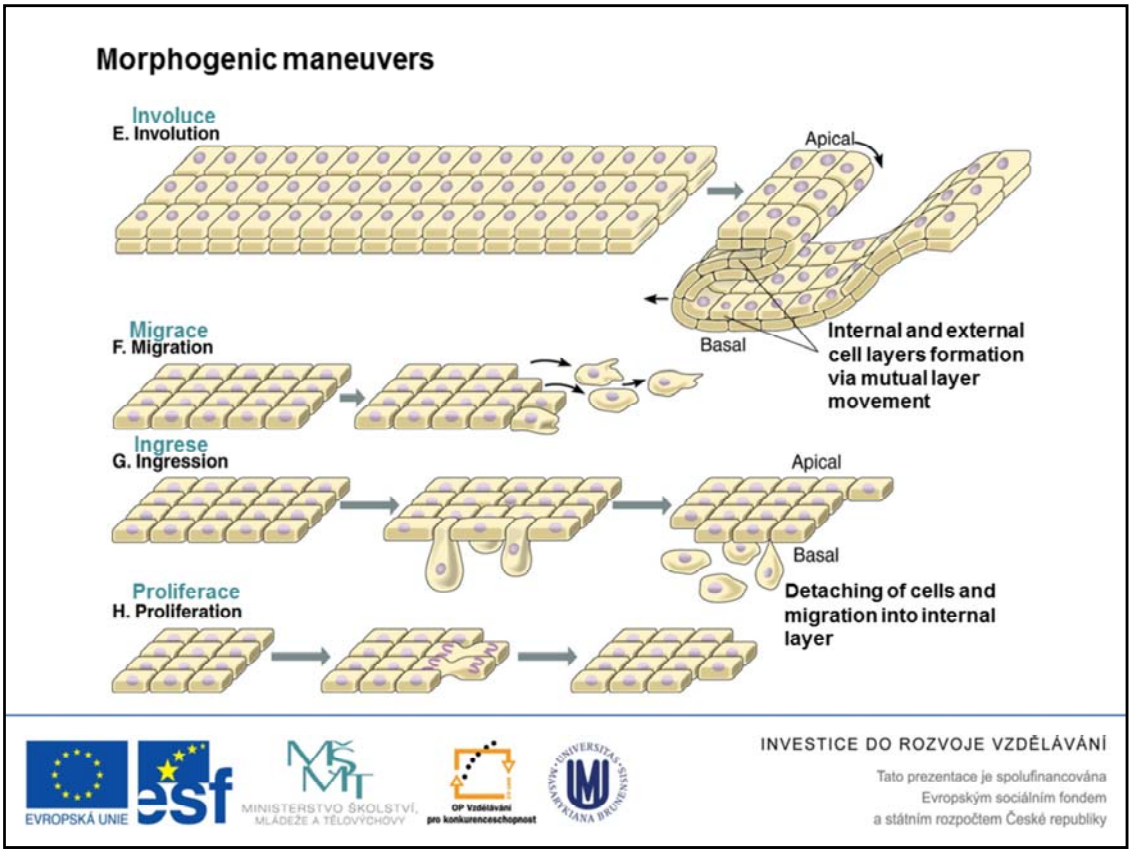
However, the basic classification is useful.

A. **Epiboly**, i.e. stretching of cells in one direction leads to the elongation of the cell layer in one direction and thinning of the cells. The resulting epithelium is sometimes called *squamous*. The epiboly occurs during gastrulation in amniotes (discussed in the Lesson 3) in the region of the prospective dorsal posterior portion of the embryo.

B. **Intercalation** is reduction of the number of cell layers via introgression of the two adjacent cell layers. Radial and mediolateral intercalation was also discussed in case of the amniotes' gastrulation (Lesson 3).

C. **Convergent extension** is directional movement of the cells in one direction leading to the reduction of the cell layer width and its directed elongation.

D. **Invagination** is movement of the whole portion of the cell layer into the internal cavity, as observed e.g. in the blastopore formation in amniotes or ventral furrow formation in *Drosophila*.

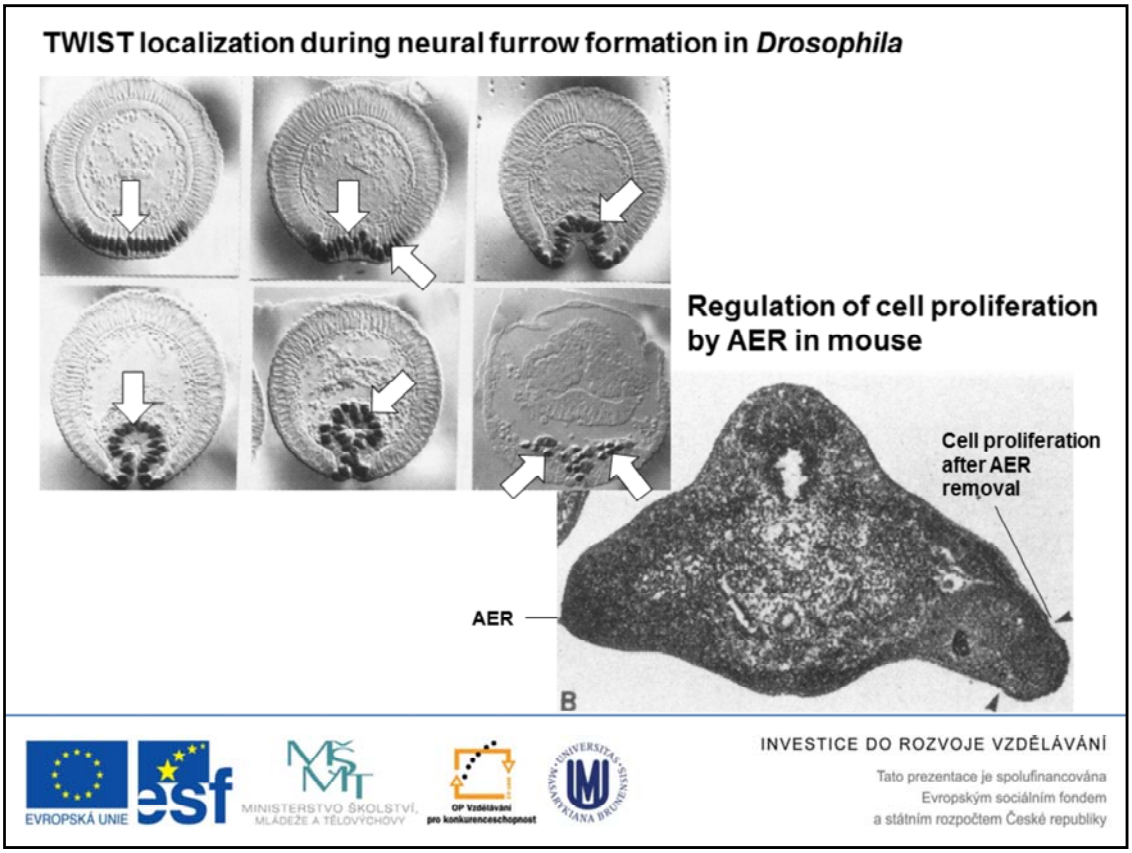


E. **Involution** is the movement of cellular layers to each other, leading into formation of internal and external cell layers. That could be observed e.g. during gastrulation in amniotes during involution of the blastopore lip (see Lesson 3).

F. **Migration** is the change of the cell motility and adhesion that leads to the change of its position. This process take place e.g. during neural crest cells migration (see Lesson 4).

G. **Ingression** is detaching of the individual cells from the cellular layer and their migration in the internal cavity. That occurs e.g. in the formation of neuroblasts from ectoderm during gastrulation of *Drosphila* (see Lesson 2).

H. **Proliferation** is change of the shape and position of cellular layers via cell division that could be targeted (mostly in plants) or not.



There are several examples of the above mentioned processes, taking place in different organisms and in different stages of development.

E.g. invagination could be observed in the neural furrow formation during gastrulation of *Drosophila*. The process is driven by expression of two genes, *SNAIL* and *TWIST*. The figure above shows individual stages of the neural furrow formation and staining of cells that express *TWIST* using antibodies against TWIST protein (see figure on the left-hand side, arrows).

Regulation of morphogenesis could be also demonstrated by the regulation of proliferation in the limb formation in mouse embryo. The apical ectodermal ridge (AER, discussed in Lesson 5) regulates the proliferation of the adjacent mesoderm via growth factor production. If the AER is removed and the embryo is cultured in the medium with fibroblast growth factor FGF4, that leads to the deregulation of the proliferation and ectopic formation of additional tissues (see the figure above).

Outline of Lesson 9

Morphogenesis

- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres
  - **Changes in the cell motility and tissue interactions during organogenesis**

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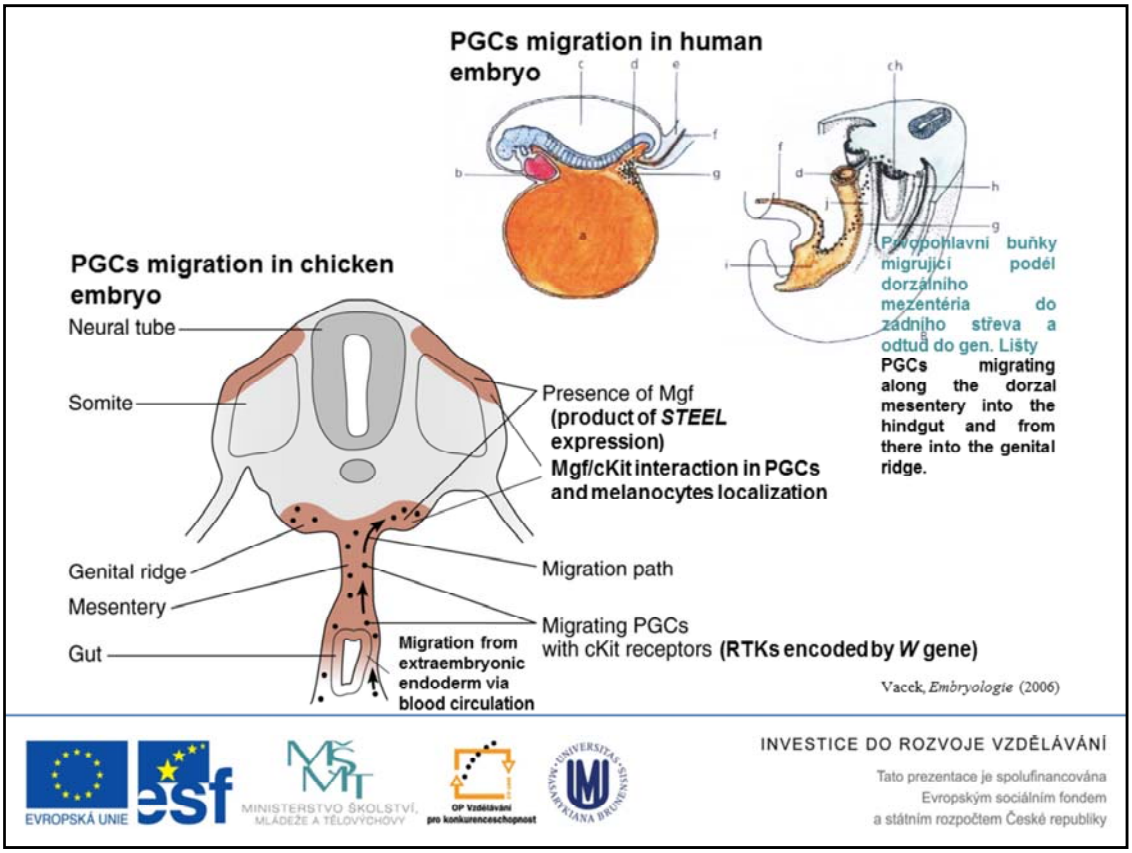


Changes in motile behavior is involved the regulation of the targeted migration of the primordial germ cells (PGCs).

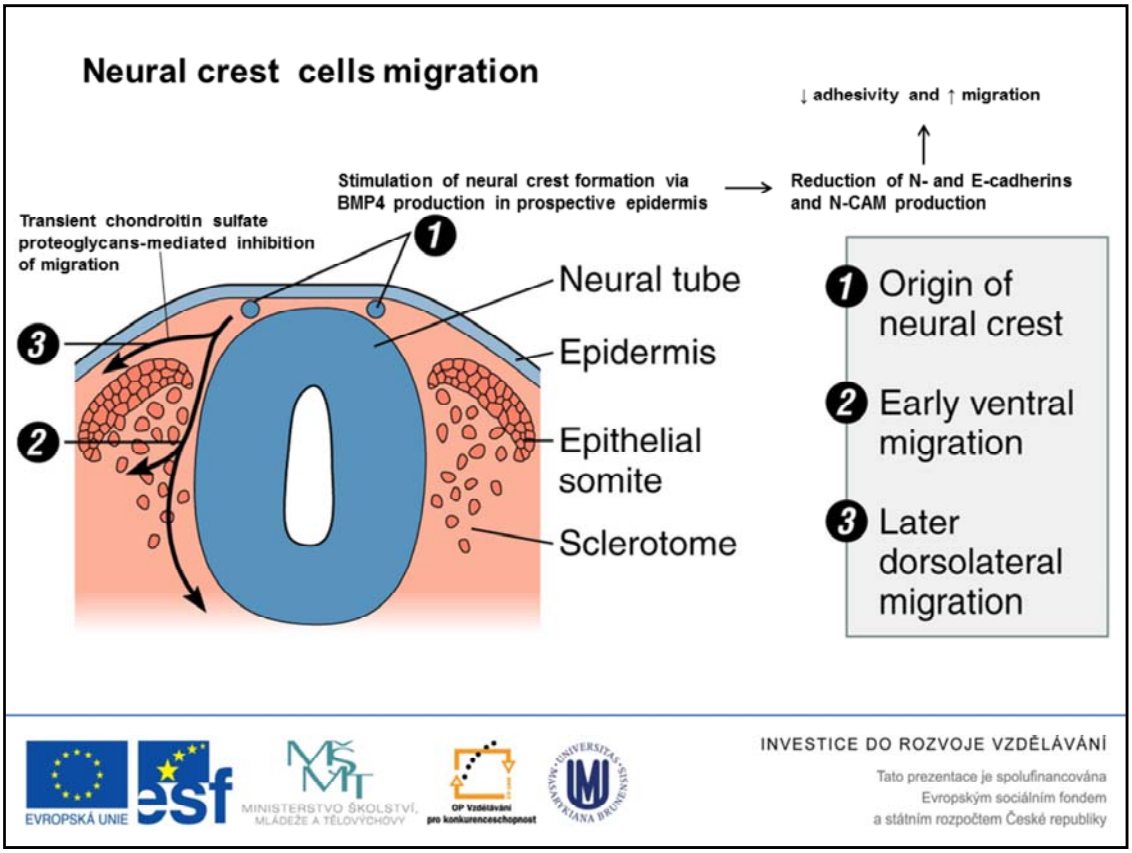
In the chicken embryo, PGCs migrate from the extraembryonic endoderm via blood circulation to the genital ridge. In mammals, the PGCs migrate from the yolk sac endoderm to the germ ridge via “overland” route through the mesenchyme. In mammals, it is not clear if the migration occurs also through the circulation and if yes, to which extent.

The interaction of the RTK cKit, encoded by the *W* gene with its ligand Mgf, encoded by the *STEEL* gene is involved in the complex process of the PGCs migration.

The migrating PGCs express the cKit and the presence of Mgf is congruent with the migration pathway (see the figure above). The cKit/Mgf interaction ensures that the PBCs remain attached in the germinal ridge and do not travel outside.

Mutations in genes for either the receptor or the ligand lead to the defects in the directed migrations of PGCs, as well as defective migration of melanoblasts arising from the neural crest and of hematopoiesis.

Accordingly, Mgf /cKit colocalization is present in the migrating population of other cell types, e.g. melanoblasts of the neural crest (see the dorsolateral localization of Mgf expression in the figure above).



Another example of the specificity in the motile behavior is directed migration of neural crest cells (for the basics of the neural crest migration see Lesson 4).

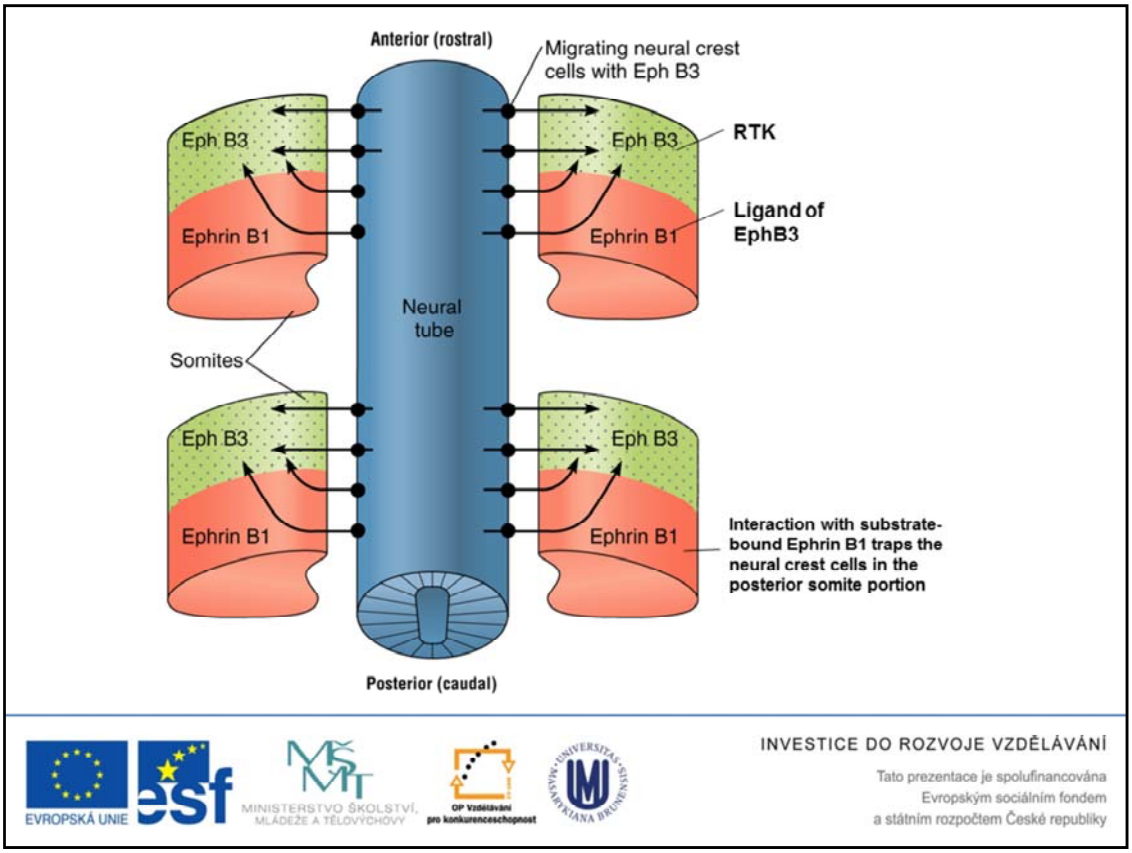
Neural crest forms from the dorsal portion of the neural tube. The necessary preposition of the induction of neural crest formation is that the edge of the neural crest must be adjacent to the prospective epidermis. Production of BMP4 by the epidermal cells was shown to stimulate neural crest formation.

There are two major routes of the neural crest cells, the earlier route, leading ventrally along the lateral portion of the neural tube (2 in the figure above) and the later, leading more dorsal (3).

In the dorsal route, the extracellular matrix contains chondroitin sulfate proteoglycans that probably hinder the migration during early stages. Later, the inhibitory activity of the matrix lessens in the dorsolateral route, which allows neural crest migration through that path during later stages.

Neural crest cells must migrate through long distances and arrive at spatiotemporally precisely defined locations. That behavior requires complex regulations of the cell adhesion, i.e. the adhesion is first reduced and later acquired again.

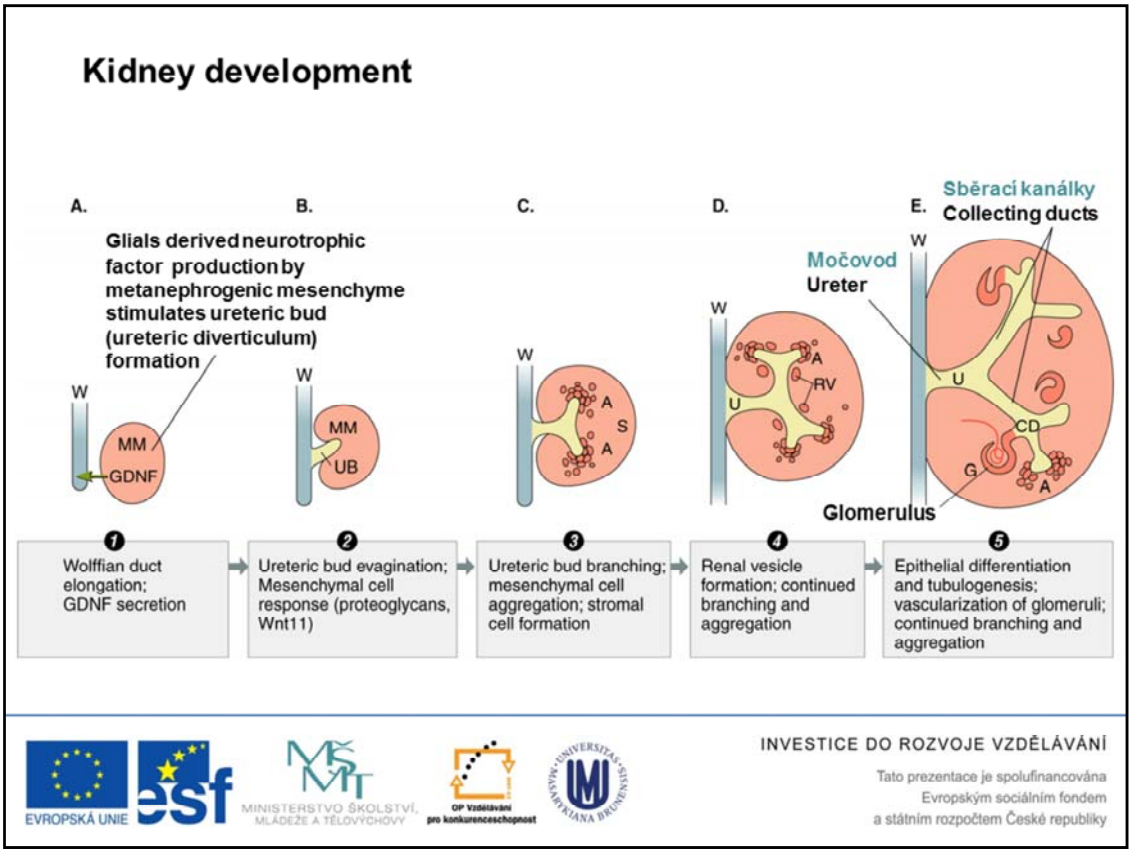
Migrating neural crest cells show reduced levels of N- and E-cadherins and later reduced levels of N-CAM that were discussed before. That is consistent with their temporary reduced cell adhesivity.



As discussed previously (see Lesson 4), migrating neural crest cells prefer transition through the antherior (nostral) portion of somites. Interaction of Ephrin B3 receptor (RTK) with its ligand, Ephrin B1, is probably involved in that type of specific cell migration.

Neural crest cells as well as anterior portion of somites express Eph3 receptor, while the posterior somites portion express Ephrin B1 that is tightly bound to them. In the experiments, where the migrating crest cells were flooded with soluble Eph B1, the neural cells migrated through both posterior and anterior portions of somites.

That suggests that if the migrating cell tries top migrate through the posterior portion, it become “trapped” by the substrate-bound Eph B1. That is supported by the findings that the neural crest cells can migrate to the area expressing substrate-bound Eph B1, but their leading protrusions collapse soon.



As an example of the complex tissue interactions affecting the morphogenesis in animals, the kidney formation is shown here.

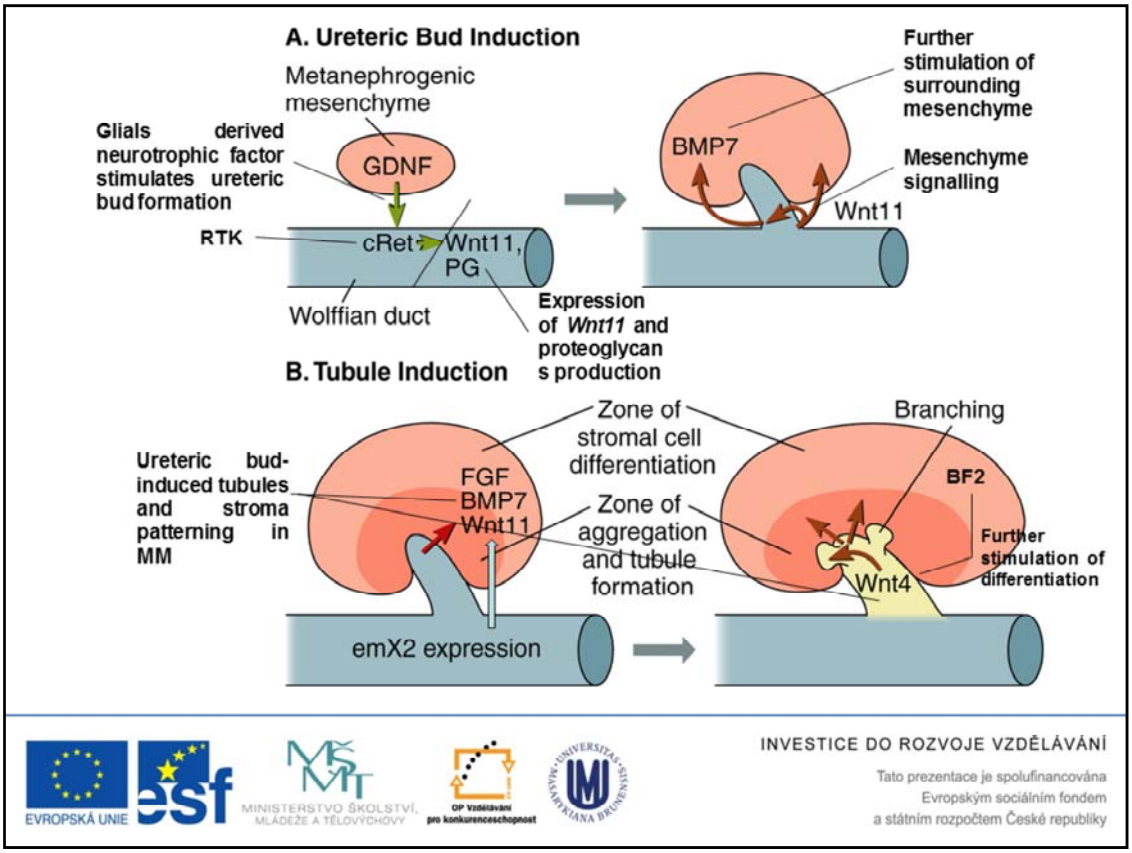
A. Kidney forms from the mesenchyme that is invaginated by the Wolfian duct. The mesenchyme produces glials derived neurotrophic factor (GDNF) that induces formation of ureteric bud (UB), growing from the Wolfian duct into the metanephrogenic mesenchyme (MM).

B. Ureteric bud evaginates and mesenchyme cells response by the production of proteoglycans; the bud produces signalling molecule from the Wnt family, Wnt11 (see also next slide).

C. The interaction of growing bud then induces formation of epithelial cells from the surrounding mesenchyme, leading to mesenchymal cell agregations (A) and epithelium formation of the tubular kidney system. Remaining mesenchymal cells form the stroma (S).

D. The ureteric bud further growths and branches that is associated with further aggregation and mesenchyme production of what is called renal vesicles.

E. Finally, the ureteric bud forms ureter (U) and collecting ducts (CD), which connect to the nephrons forming from the various kinds of epithelial aggregates in the mesenchyme. G, glomerulus.



The molecular interactions during induction of kidney tubules.

A. As just discussed (see the previous slide), metanephrogenic mesenchyme induces nephrogenic bud outgrowth via production of GDNF. That signal is received via heterodimeric RTK cRet. That leads to the expression of Wnt11 and production of proteoglycans (PG). This stimulates its own growth and also signals the mesenchyme. BMP7 from the duct further stimulates the surrounding mesenchyme.

B. The ureteric bud induces formation of tubules in the surrounding mesenchyme. This is done via expression of *emX2* expression that further stimulates production of other signalling molecules, e.g. Wnt11, BMP7 and various FGFs. These signals help pattern of the formation of tubules and stroma in the metanephrogenic mesenchyme. Signals are produced by both, ureteric bud and the mesenchyme (e.g. Wnt4 or BF2 that is produced by the stroma cells of the developing kidney). These signals further stimulate differentiation of the tubules in the mesenchyme and in the bud.

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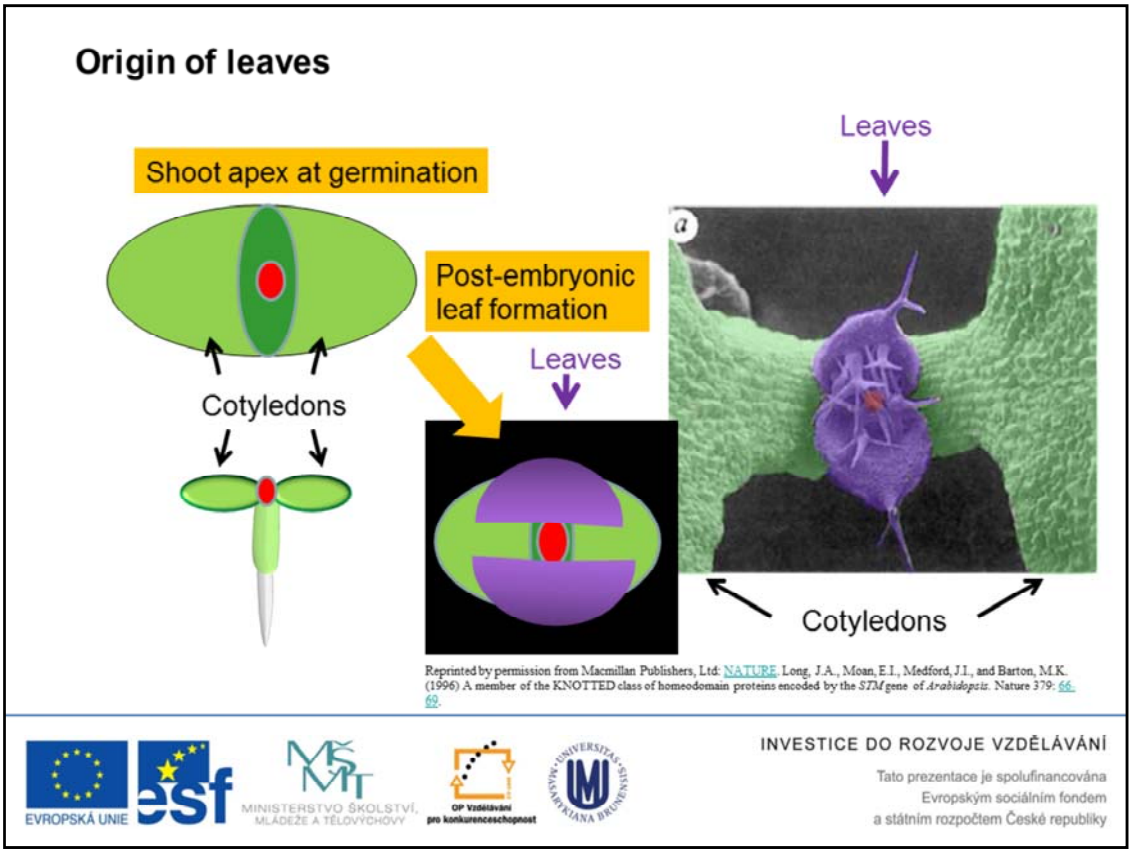
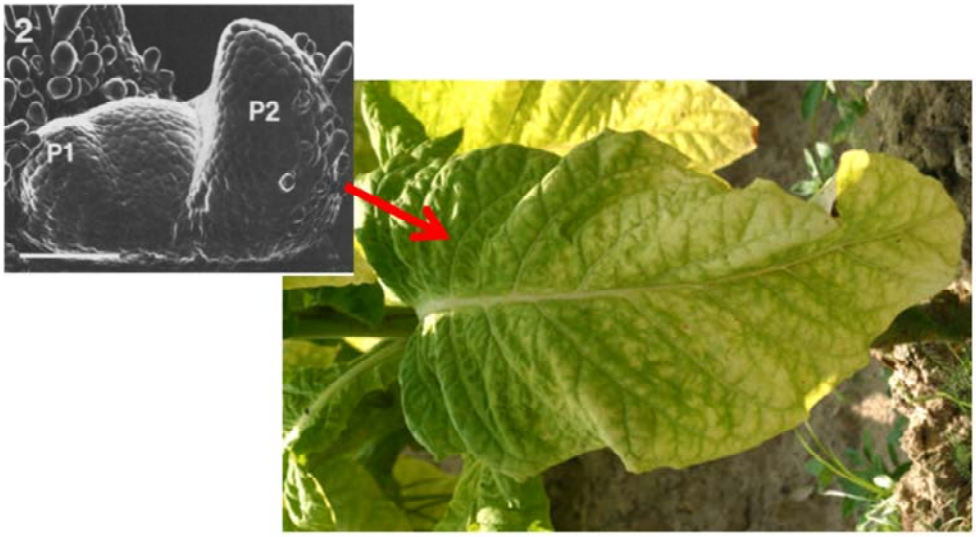


Leaves are formed by the shoot apical meristem (SAM) as previously discussed in the lesson on the plant embryogenesis (see Lesson 7).

How does a leaf primordium become a leaf?



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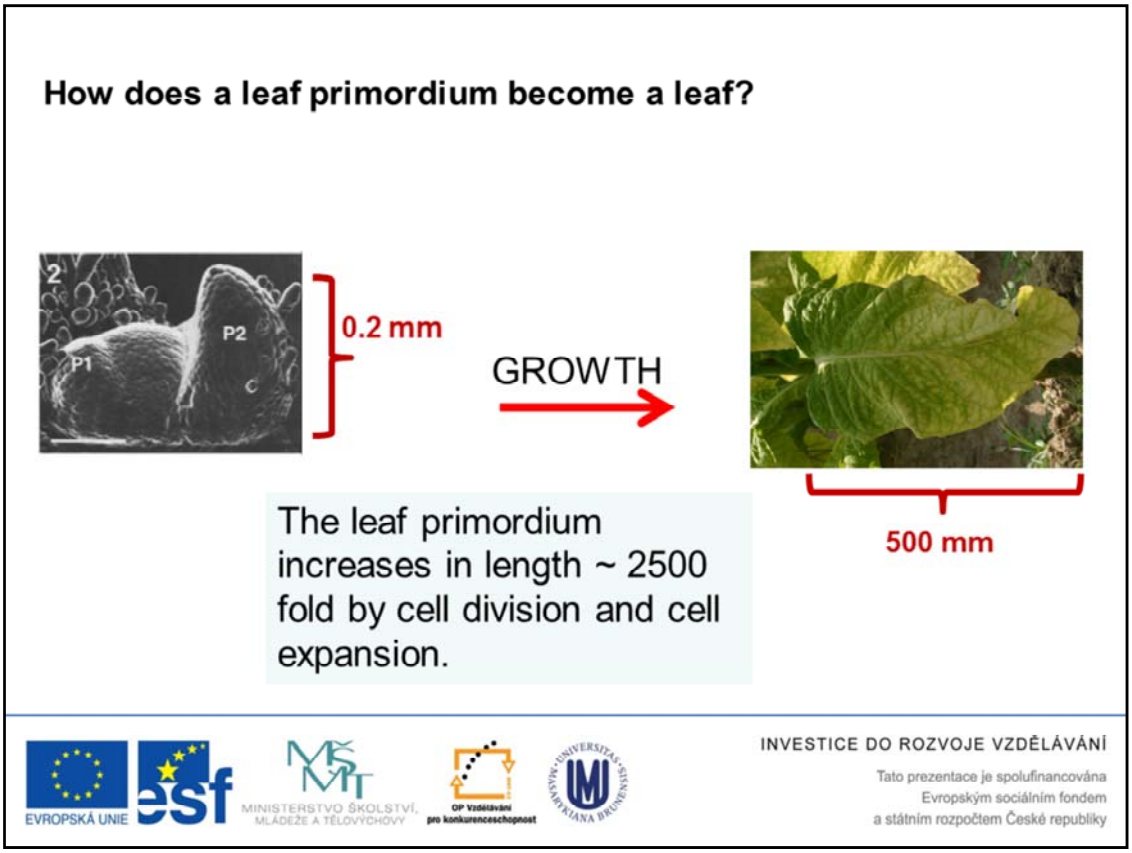
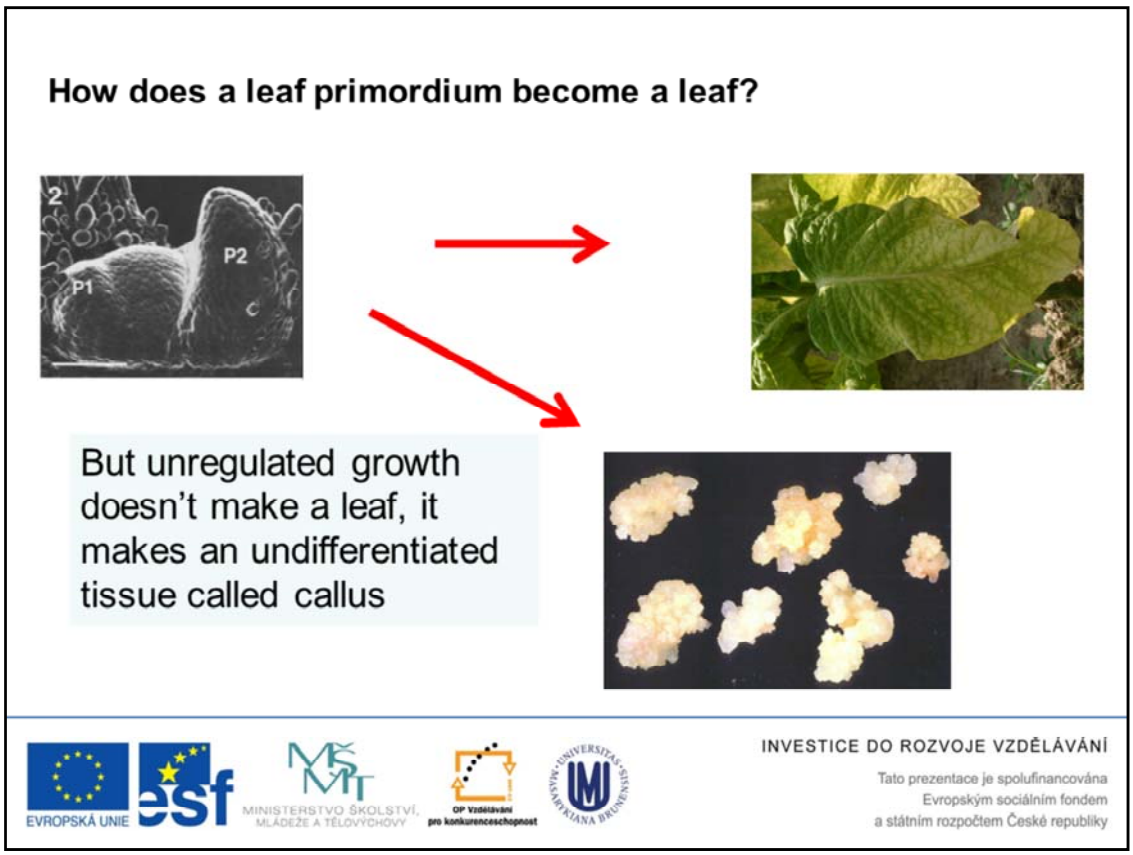
Here, we will discuss the mechanisms of leaf primordium development, i.e. how does a leaf primordium become a leaf.

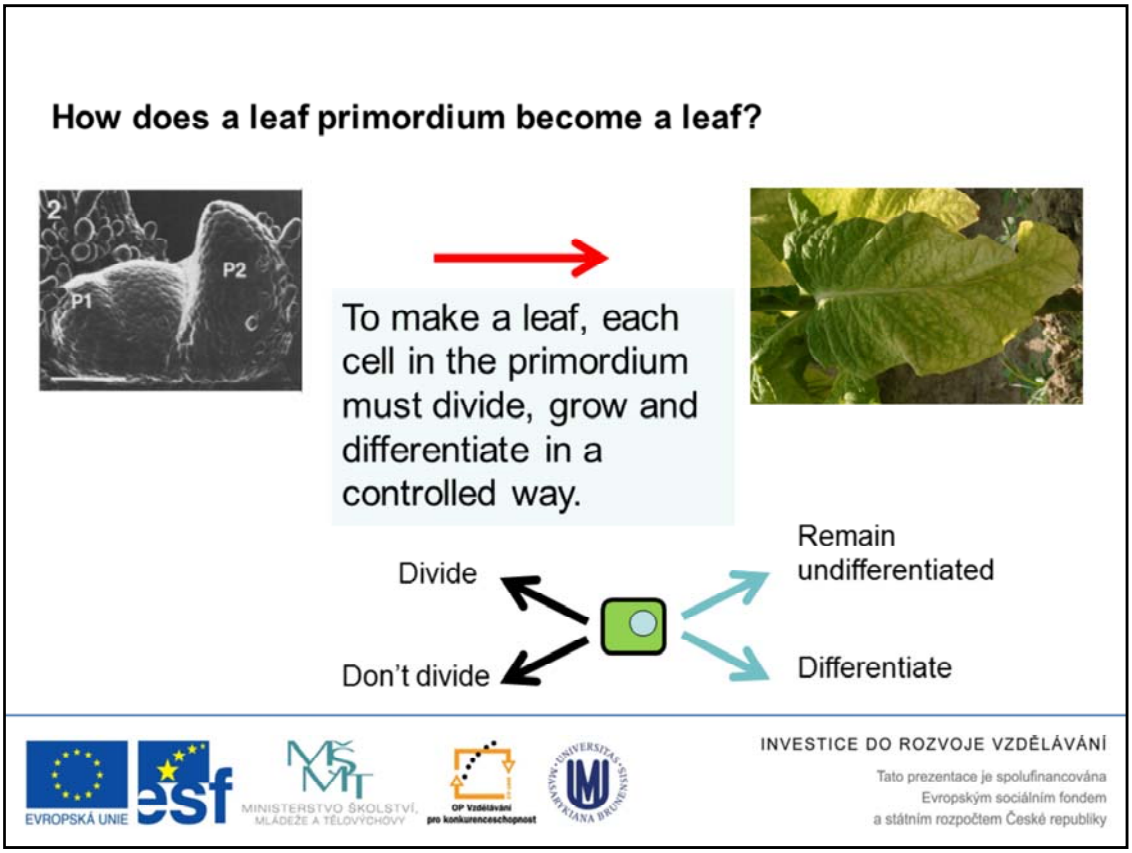


During the leaf formation, the primordium dramatically increases in size. There is approximately 2500 fold increase in the primordium size during leaf development.



The increase in size is achieved via both *cell division* (i.e. cell proliferation, thus similarly as in animals, see above) and *cell expansion*.

The cell division, however, must be precisely controlled. The unregulated cell division in plants leads to the formation of undifferentiated and disorganized mass of plant tissue called **callus**.



In contrast to animals, there is **no cell movement** during plant development and organogenesis.

The morphogenetic processes relay mostly on the tight spatiotemporal regulation of **cell division** and **cell differentiation.**

- Leaf diversity
- What determines leaf size and shape?
- What determines if a leaf is simple or compound?
- What controls cell differentiation?

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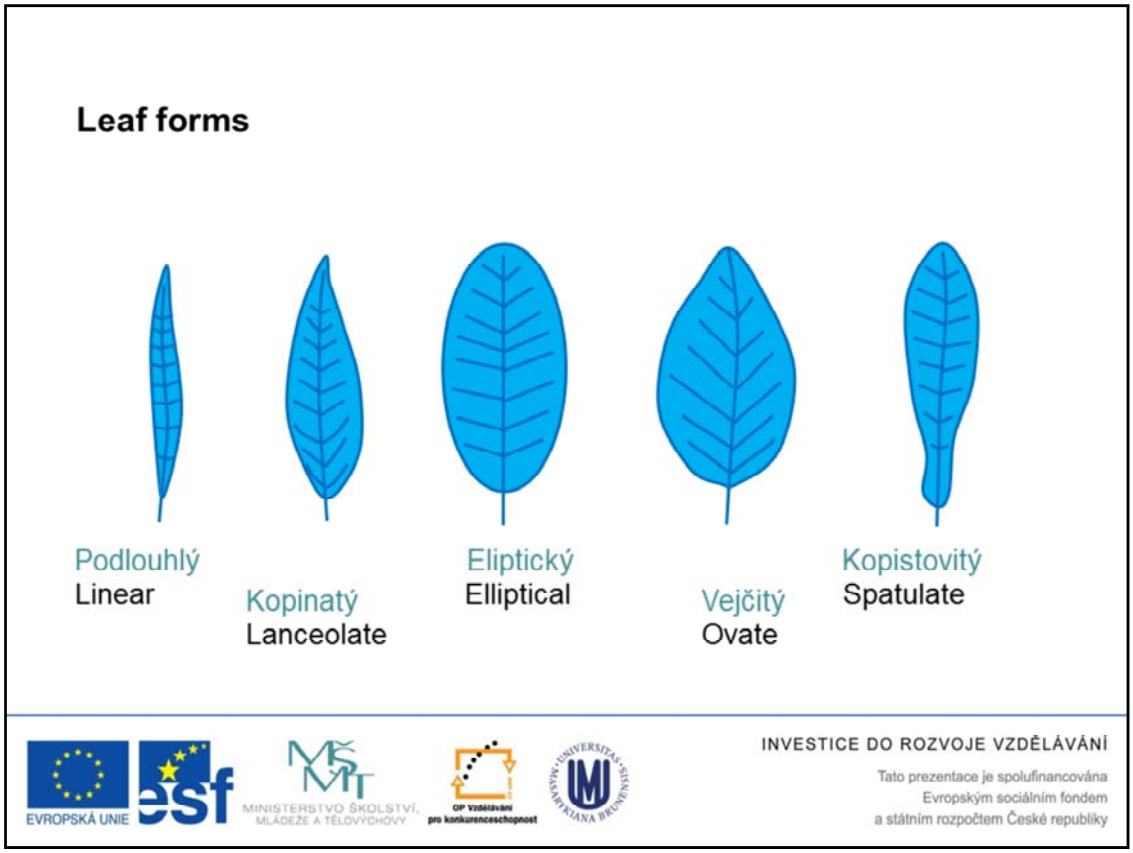
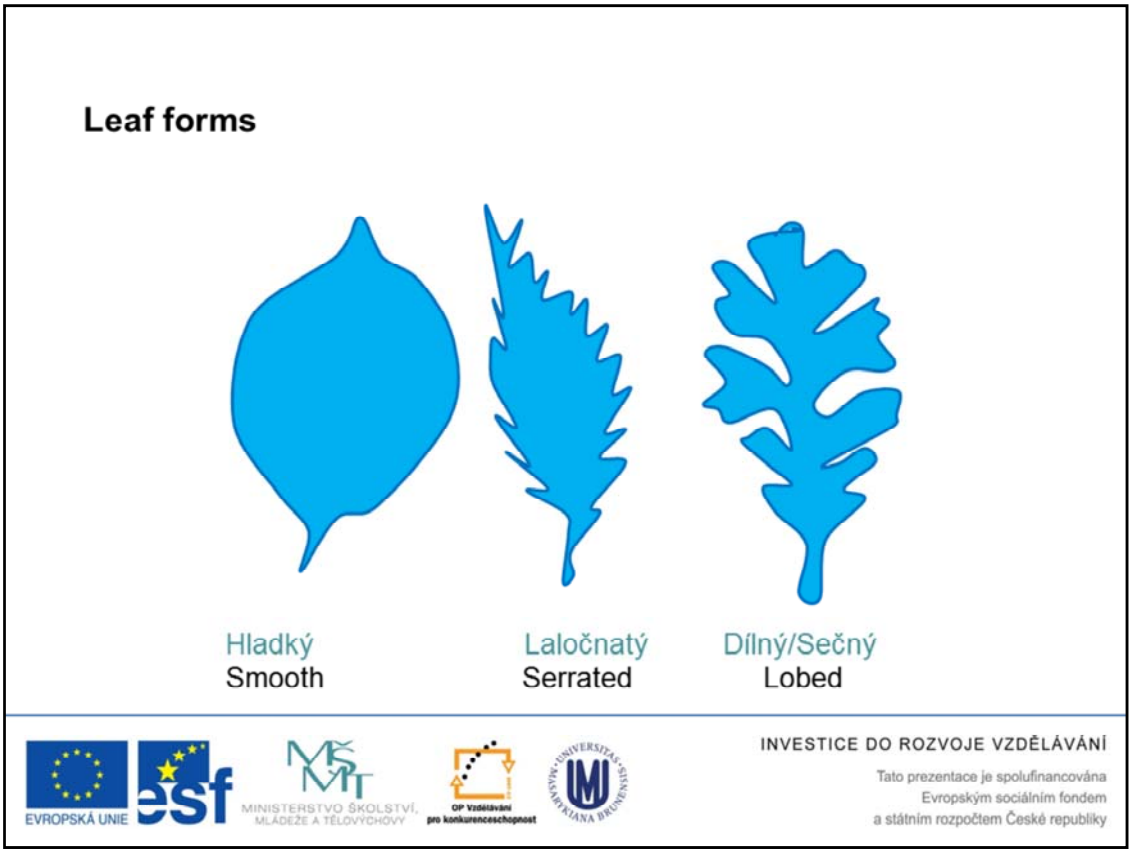
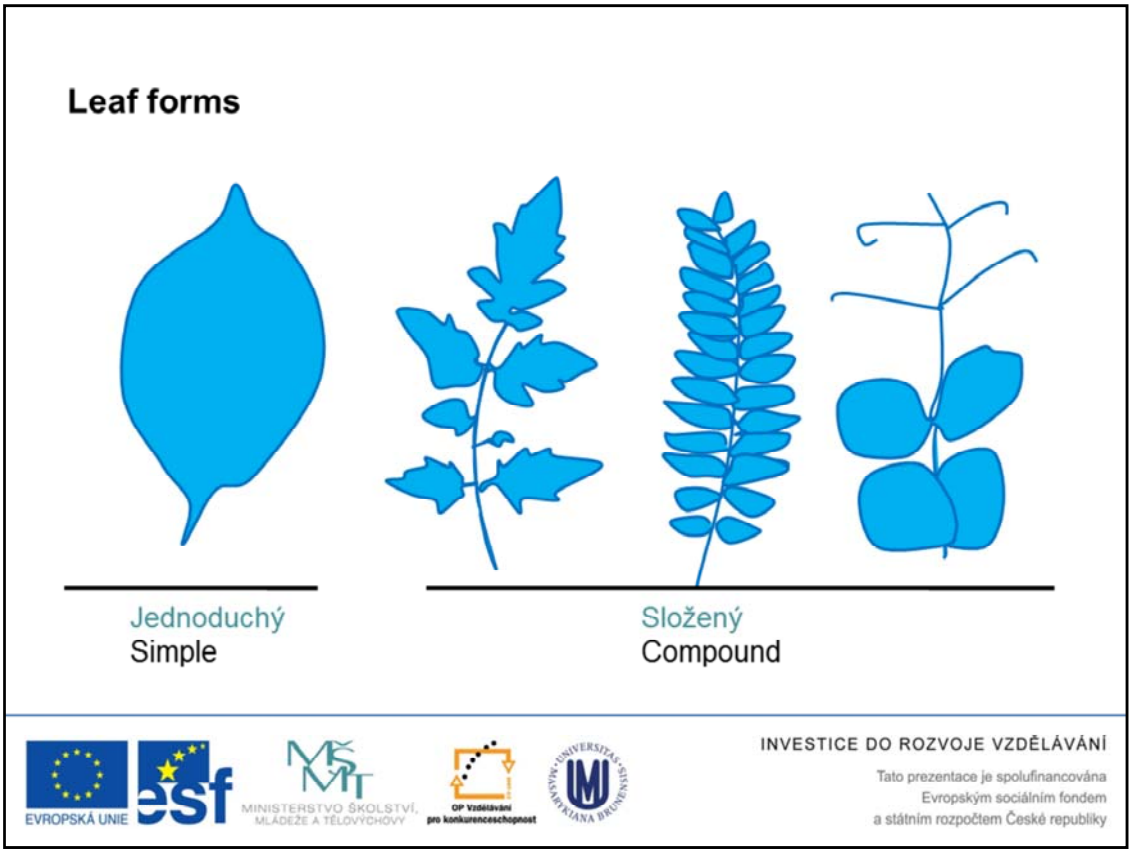


Plants can be identified by the shape of their leaves. Field guides contain classification keys based on leaf **shape**.



Plants can be identified by the shape of their leaves. Field guides contain classification keys based on leaf **shape, margins.**



Plants can be identified by the shape of their leaves. Field guides contain classification keys based on leaf **shape, margins, and complexity.**

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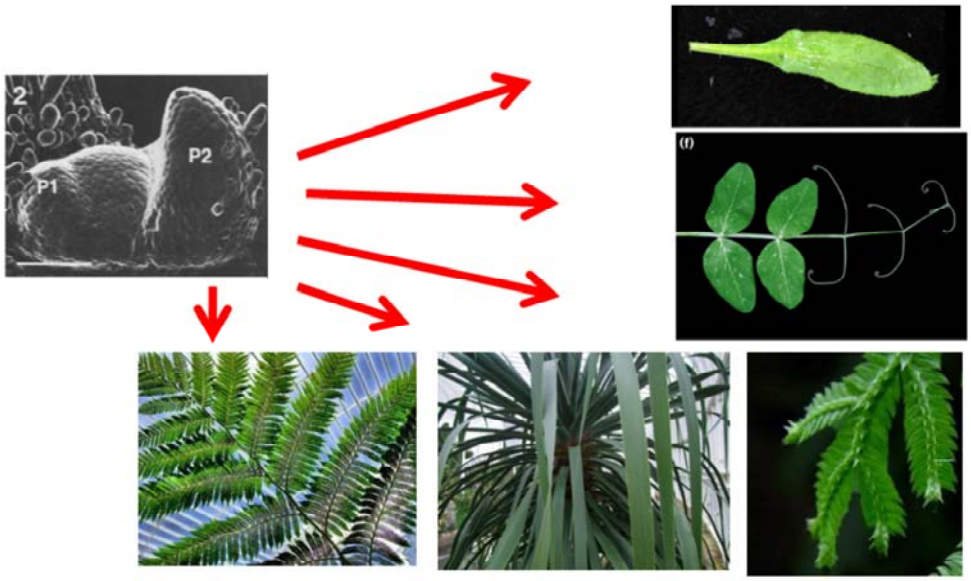
What determines the size and shape of a leaf?



Size is determined by growth. Shape is determined by differential growth

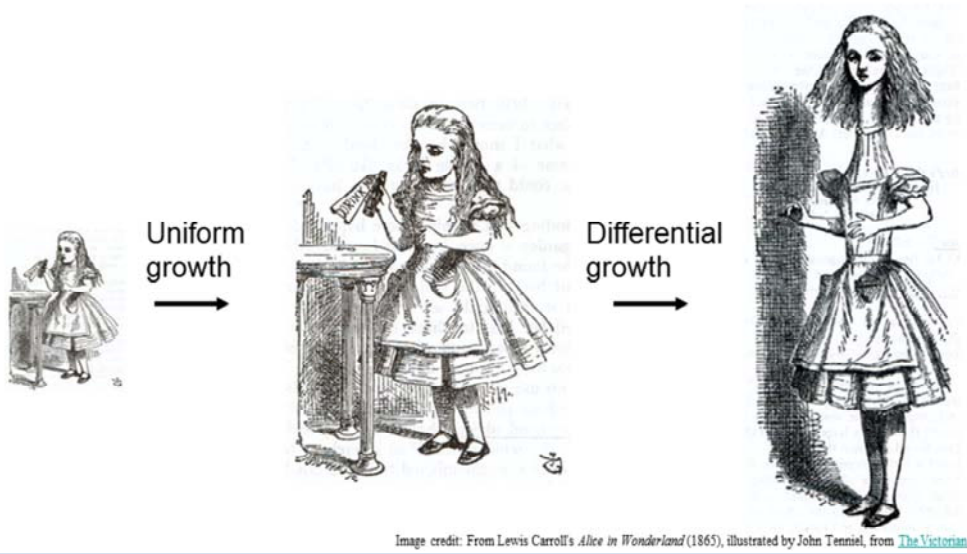


Image credit: From Lewis Carroll's *Alice in Wonderland* (1865), illustrated by John Tenniel, from The Victorian Web.

As mentioned previously, the size and the shape of the leaf is determined by the differential cell division and cell expansion.

What determines the size and shape of a leaf?

- Total number of cell division cycles
- Relative distribution of cell divisions
- Relative timing of cell cycle arrest
- Presence or absence of leaflets

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Increasing the number of cell divisions increases leaf size

ant-1 WT ANT-OX

Increasing number of cell cycles in developing leaf

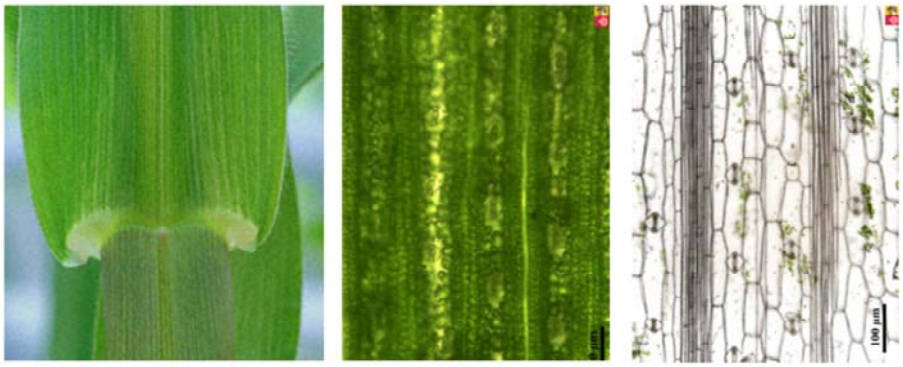
ANT → CYCD3 → Cell division

Mizukami, Y., and Fischer, R.L. Plant organ size control: *AINTEGUMENTA* regulates growth and cell numbers during organogenesis. PNAS 97:942-947. Copyright (2000) National Academy of Sciences, U.S.A.

The *AINTEGUMENTA* (*ANT*) gene controls organ size by promoting expression of cyclin D3. Higher levels of ANT mean more cell cycles and larger leaves.

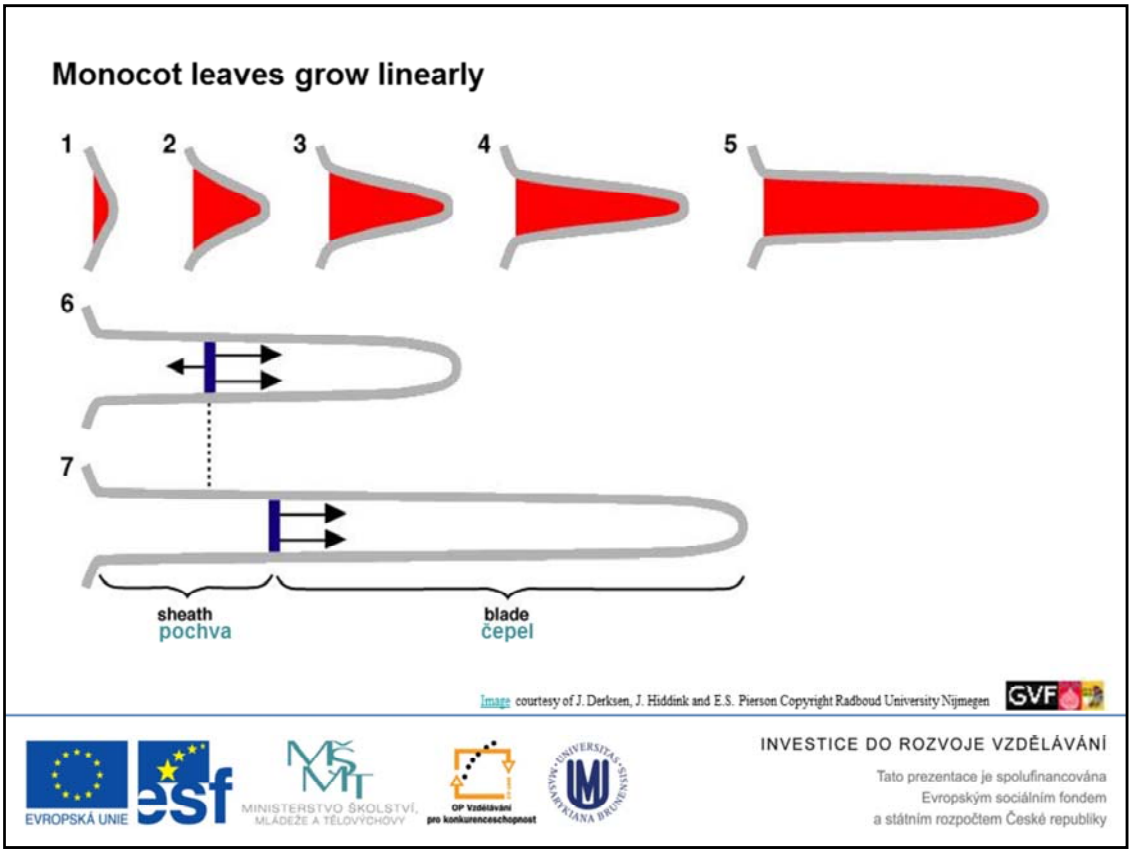
ANT is required for control of cell proliferation and encodes a putative transcriptional regulator similar to AP2. Loss of function alleles have reduced fertility, abnormal ovules and abnormal lateral organs. Expressed specifically in the chalaza and in floral organ primordia.

Patterns of cell divisions (and expansion) determine leaf shape



Monocot leaves are elongated and strap-like, with parallel sides and veins

The imporatance of the cell divison patterns might be demonstrated on the differences between the shape of monocotyledonous and dicotyledonous plant leaves.

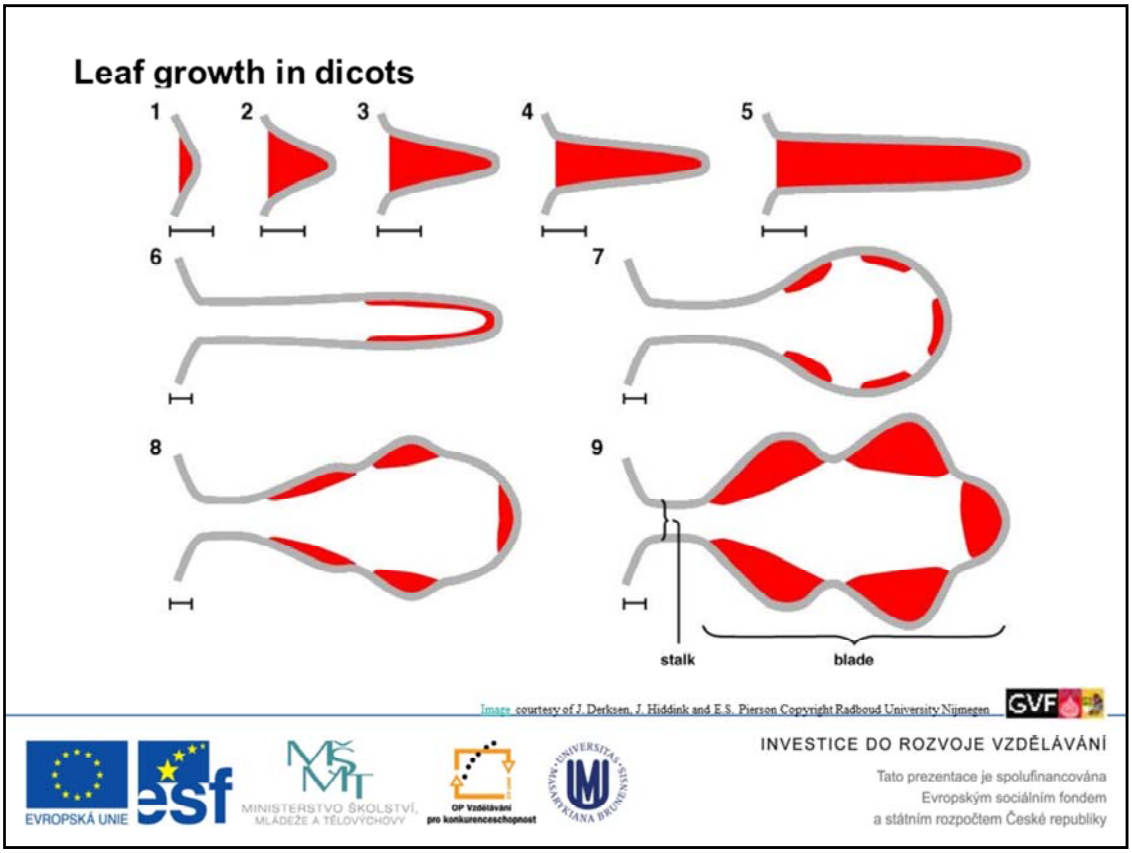


In long leaves of monocotyledonous plants, the cell division and expansion occurs mostly in unidirectional orientation.

Initially the primordium expands isodiametrically (in all directions).

At later stages, the leaf grows by uni- or bi-directional cell division and expansion.

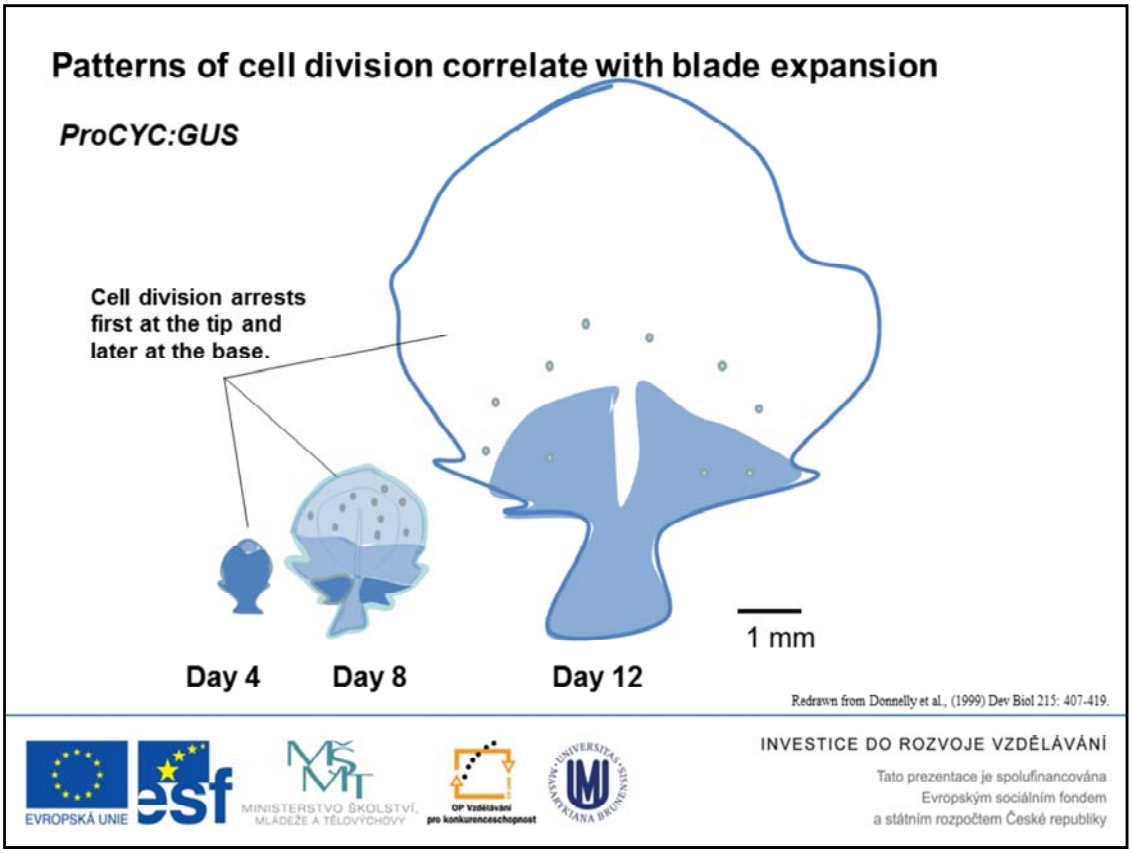
An animated video of this slide (leaf development over time) is available at: http://www.vcbio.science.ru.nl/en/virtuallessons/leaf/formation/.



In contrast to that, in leaves of dicotyledonous plants, the cell division patterns are more complex and cell divisions contribute to the width of the blade. Leaf shapes are formed by persistent growth in isolated regions of the developing blade.

E.g. In case of what are called serrated or lobbed leaves, the cell divisions occur in specific positions at the leaf margin. Factors regulating that process (e.g. auxin maxima formation) will be discussed later.

An animated video of this slide (leaf development over time) is available at: http://www.vcbio.science.ru.nl/en/virtuallessons/leaf/formation/



The spatiotemporal distribution of cell division might be estimated using transgenic lines carrying transcriptional fusion of *CYCLIN* gene promoter with reporter gene, e.g. *GUS*.

In those plants, the blue staining corresponds to dividing cells. Darker blue indicates higher levels of gene expression.

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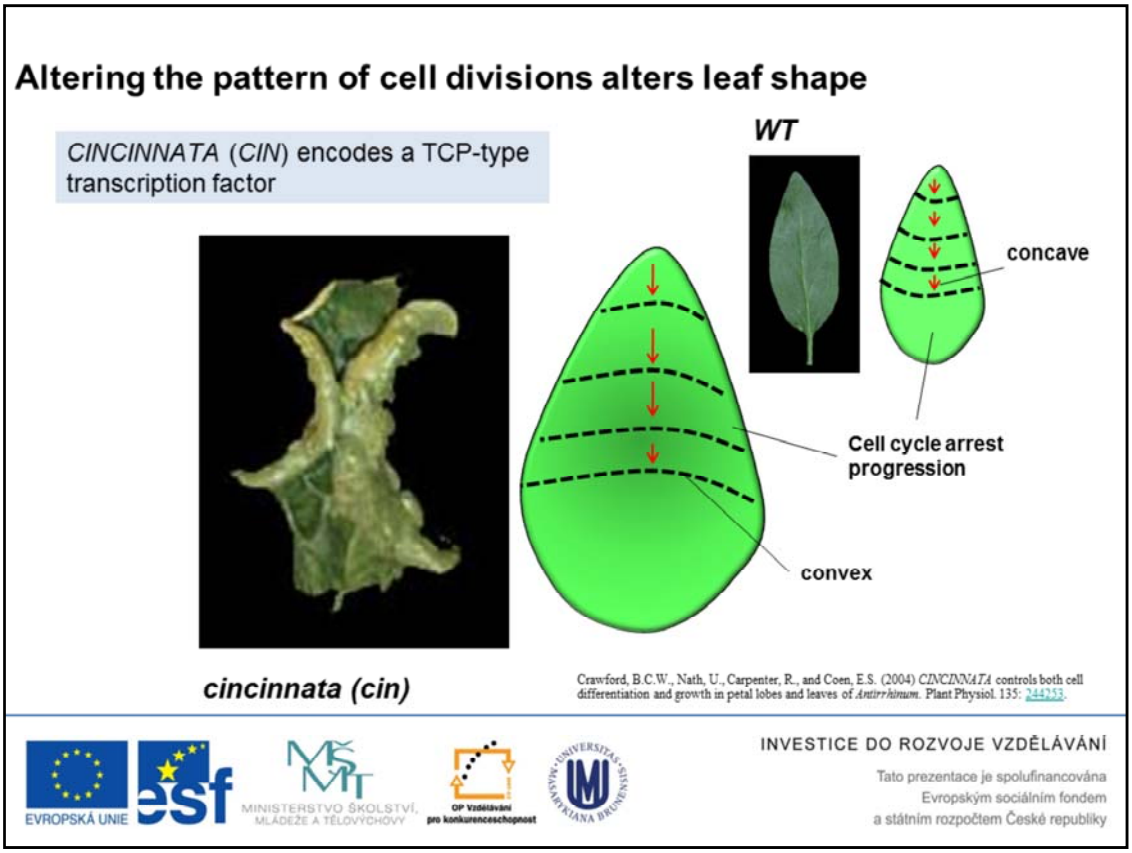
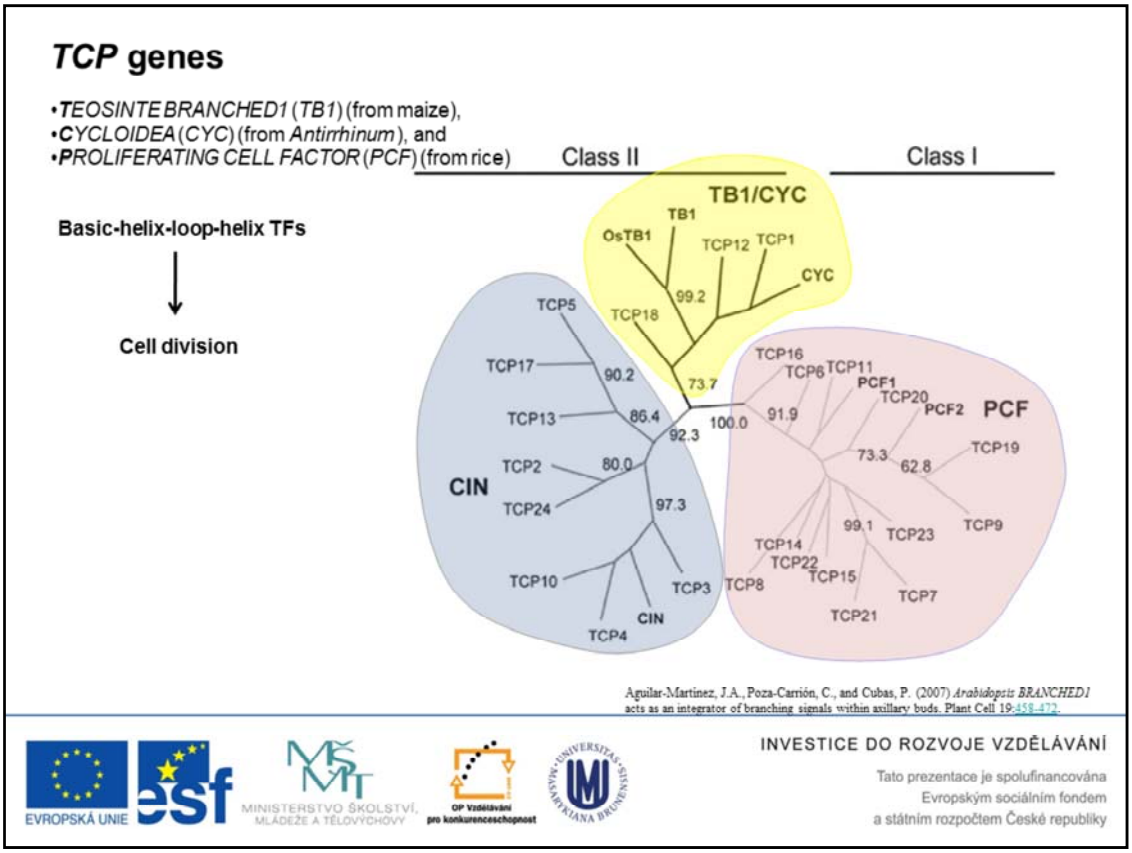


Factors driving the coordinated cell divisions across the leaf blade are mostly not known.

However, mutations in TCP genes encoding for TF are affected in the coordinated cell divisions.

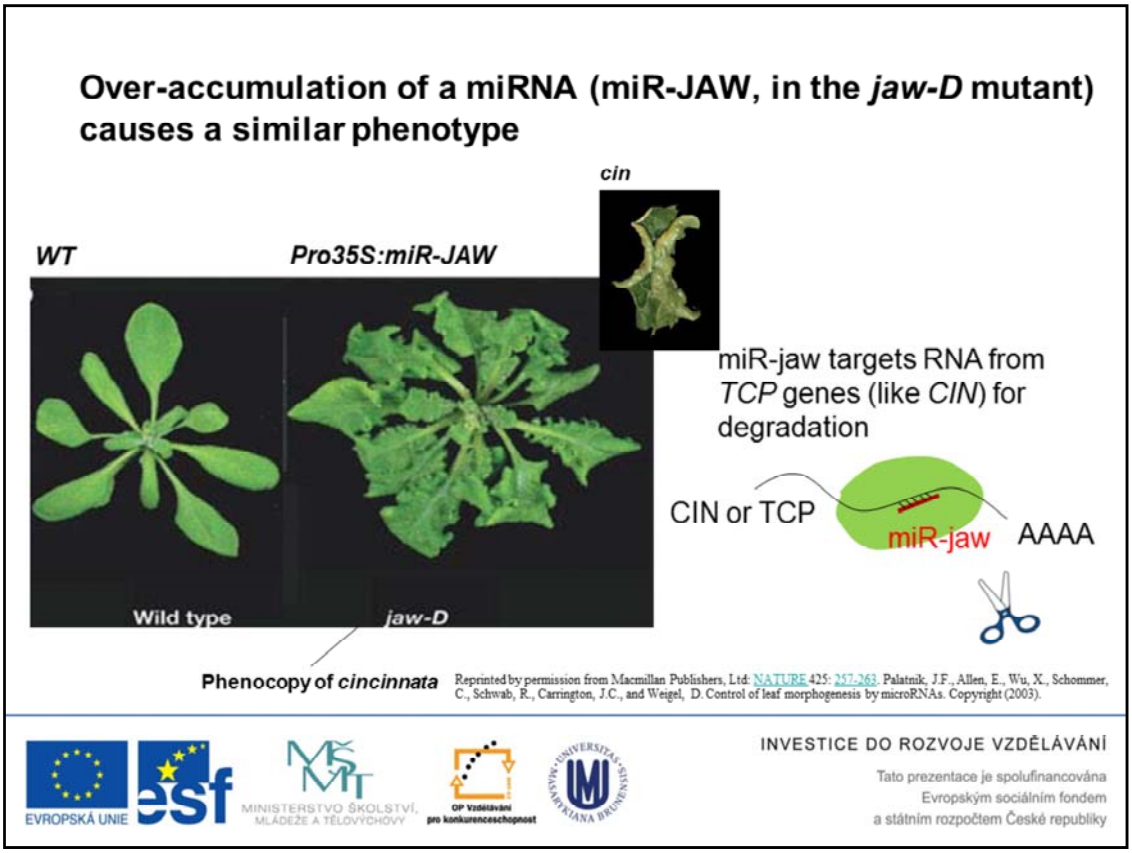
In wt of *Antirrhinum,* growth arrests occurs in a concave wave from tip to base, resulting in a flat blade (see the inset on the right-hand side).

However, in the mutant in the gene from the TCP family, *CINCINNATA* (*CIN*), the cell cycle progression is affected, with growth arrest occurring in a convex wave. Too much cell proliferation at margins causes leaves leads to formation of extra tissue at the leaf margins that causes the leaf to curl.

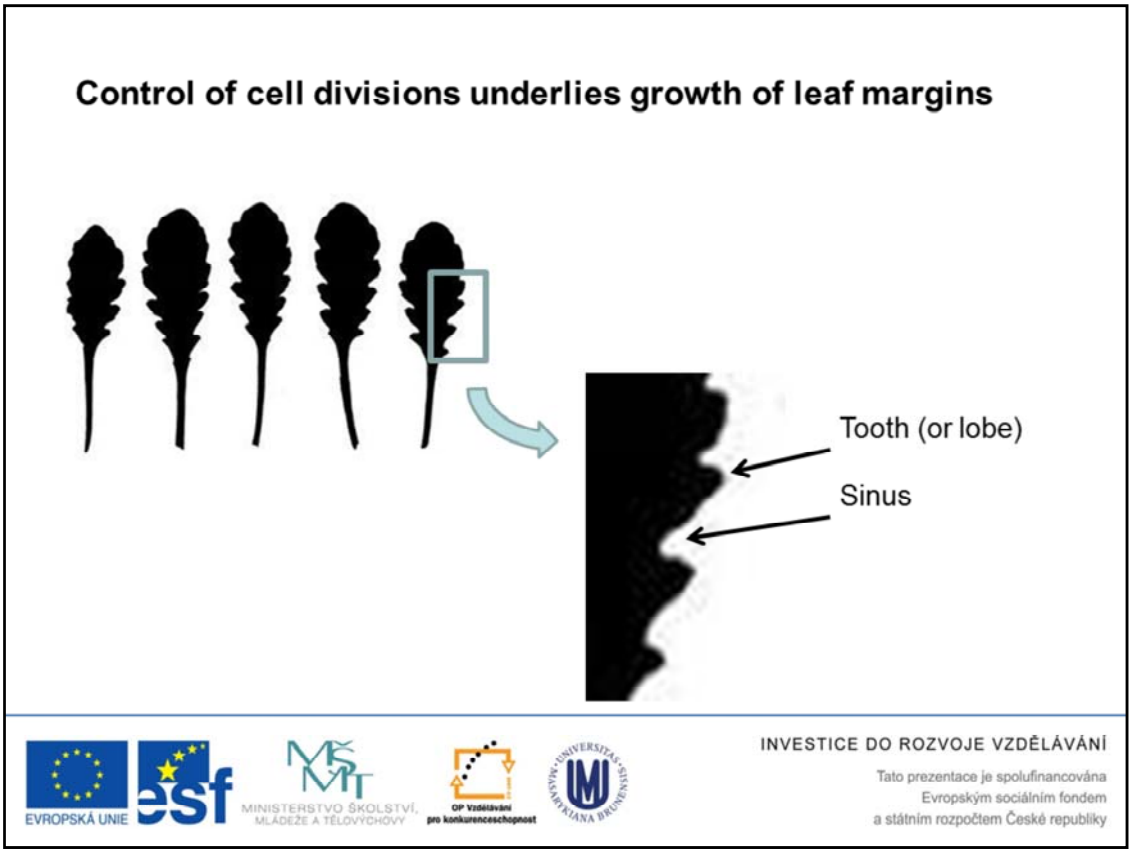


TCP gene family is named for identified members of the family: *TEOSINTE BRANCHED1* (***TB1***) (from maize), *CYCLOIDEA* (***CYC***) (from *Antirrhinum*), and *PROLIFERATING CELL FACTOR* (***PCF***) (from rice).

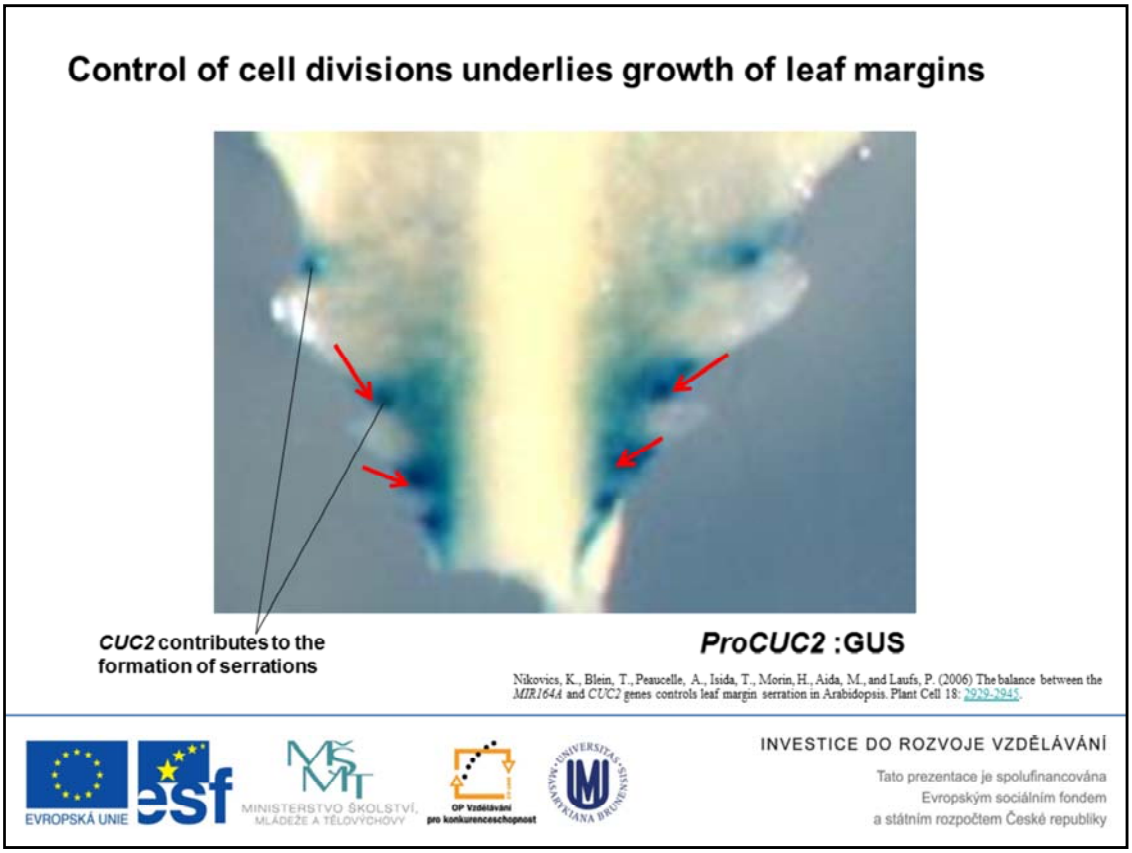
TCP genes encode basic-helix-loop-helix transcription factors, some of which can regulate cell proliferation.



Overexpression of miR-JAW lessens the amount of transcription factor made, resulting in a phenotype that is similar to the *cincinnata* loss of function mutant phenotype (for details on miRNAs production and function see the Lesson 10).



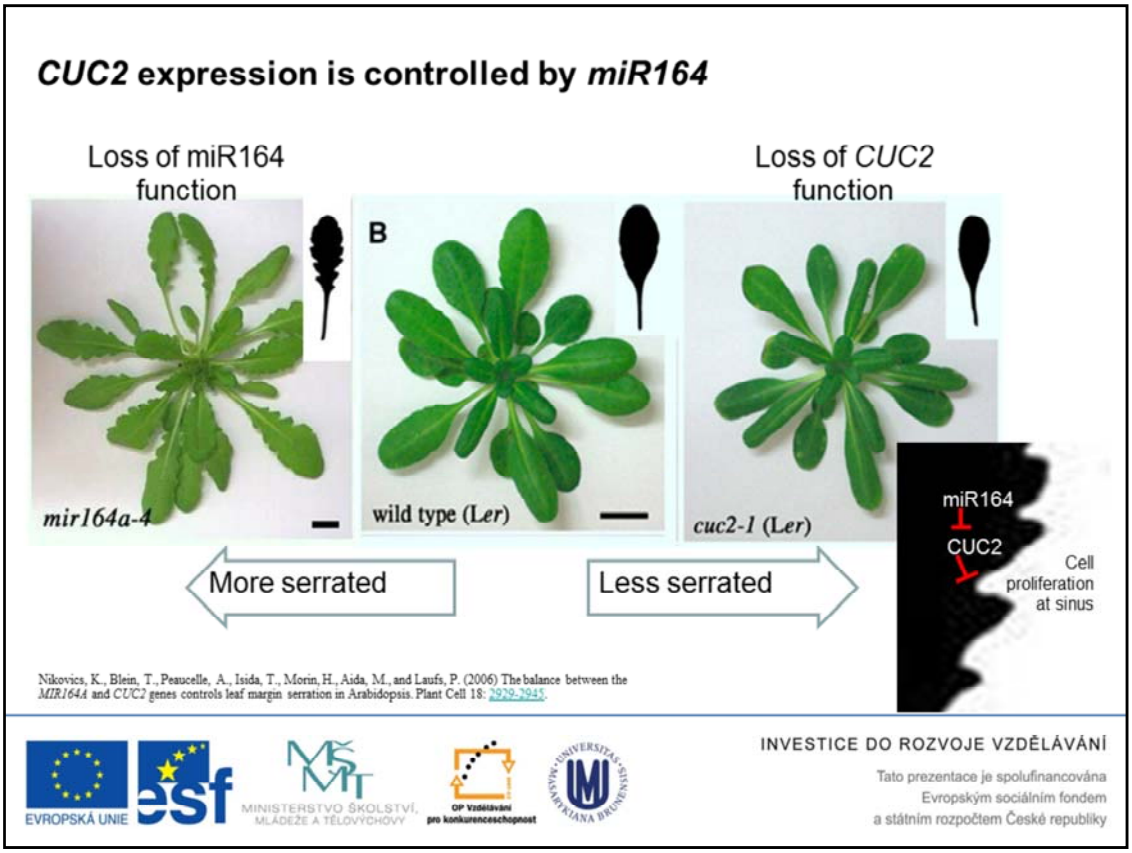
Arabidopsis leaves are serrated. Cell divisions in sinuses arrest before those in teeth.



The boundary gene *CUP-SHAPED COTYLEDONS2 (CUC2)* contributes to the formation of serrations.

As shown in the transgenic lines carrying transcriptional fusion of the *CUC2* promoter with *GUS*, *CUC2* is expressed in leaf sinuses.

CUC2 is part of the large NAM/CUC3 family of plant-specific transcription factors, some of which specify organ boundaries.



Loss-of CUC2 function causes smooth margins. CUC2 is necessary for division arrest in sinuses.

In contrast to that, the ectopic expression of *CUC2* due to mutation of the negative regulator of CUC2 expresion, miR164, results into more serrated leaves.

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    - Auxin-regulated positional information for cell division

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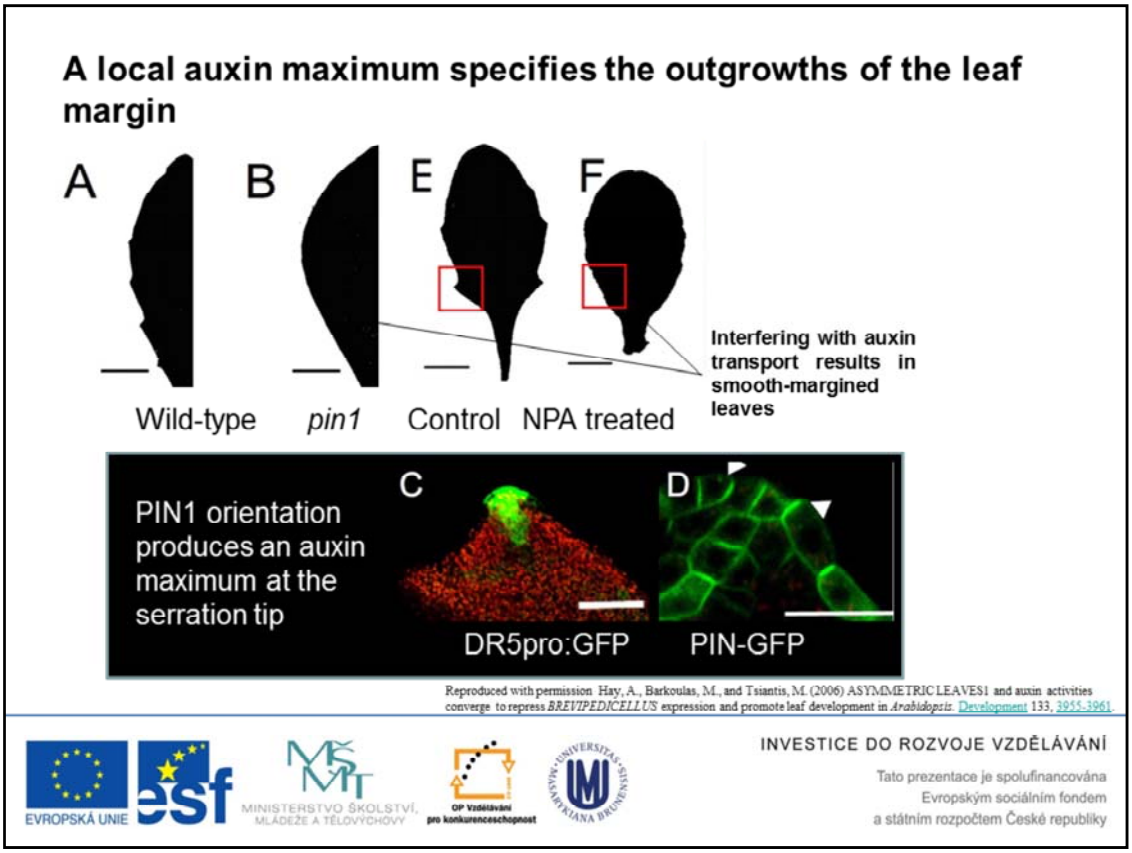
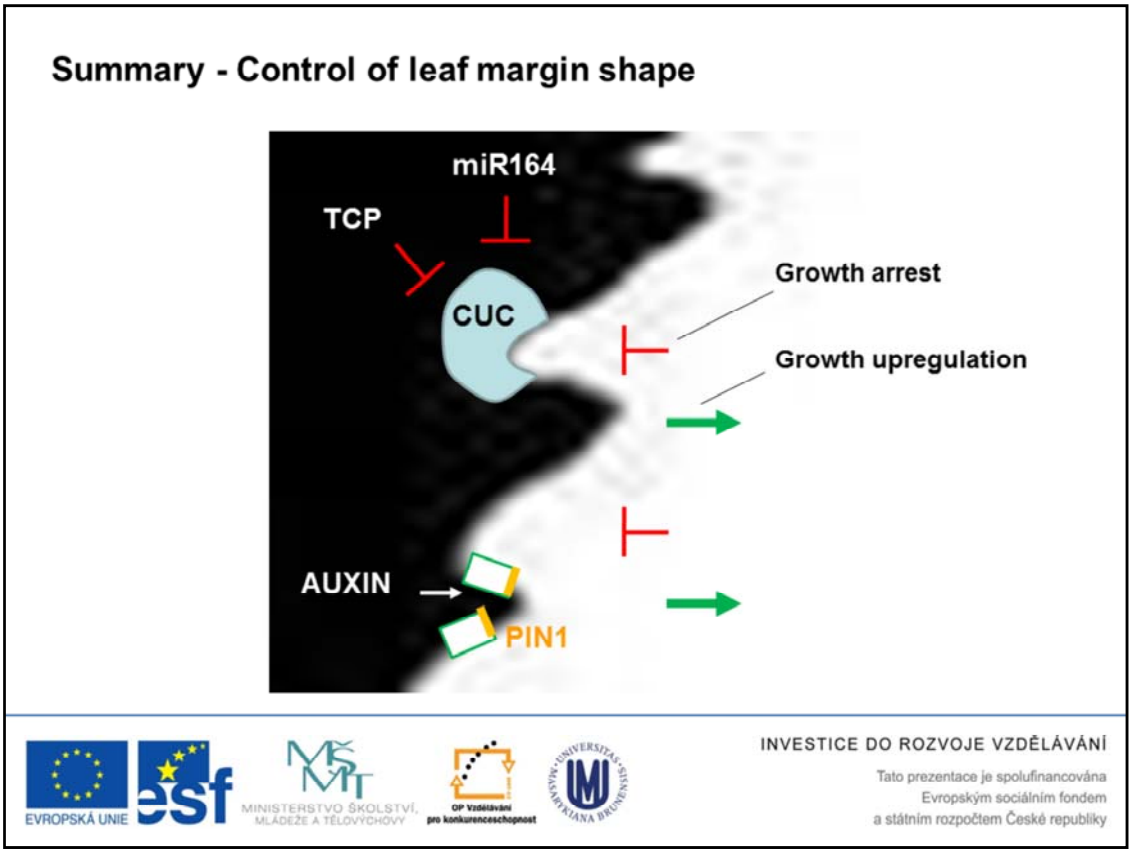


NPA is a specific inhibitor of polar auxin transport; PIN1 is an auxin efflux carrier (discussed previously, see Lesson 7 and 8).



Summary of the complex regulations affecting the cell division pattern at the leaf margins in *Arabidopsis*.

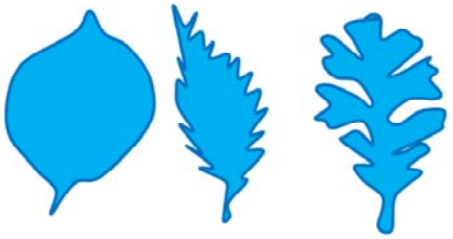
At the sinus, cell cycle arrest by *CUC* genes is controlled by *TCP* and *MIR164* genes.

Outgrowth of the tip of the serration is specified by polar auxin transport, i.e, by the formation of auxin concentration maxima.

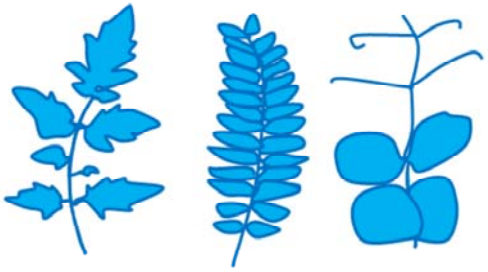
As a result, the cell proliferation controls leaf shape. Genes that promote or restrict division are precisely regulated through interactions with each other, miRNAs and auxin.

What determines if a leaf is simple or compound?

Simple



Compound



Redrawn from Champagne, C., and Sinha, N. (2004). Development 131:4401-4412

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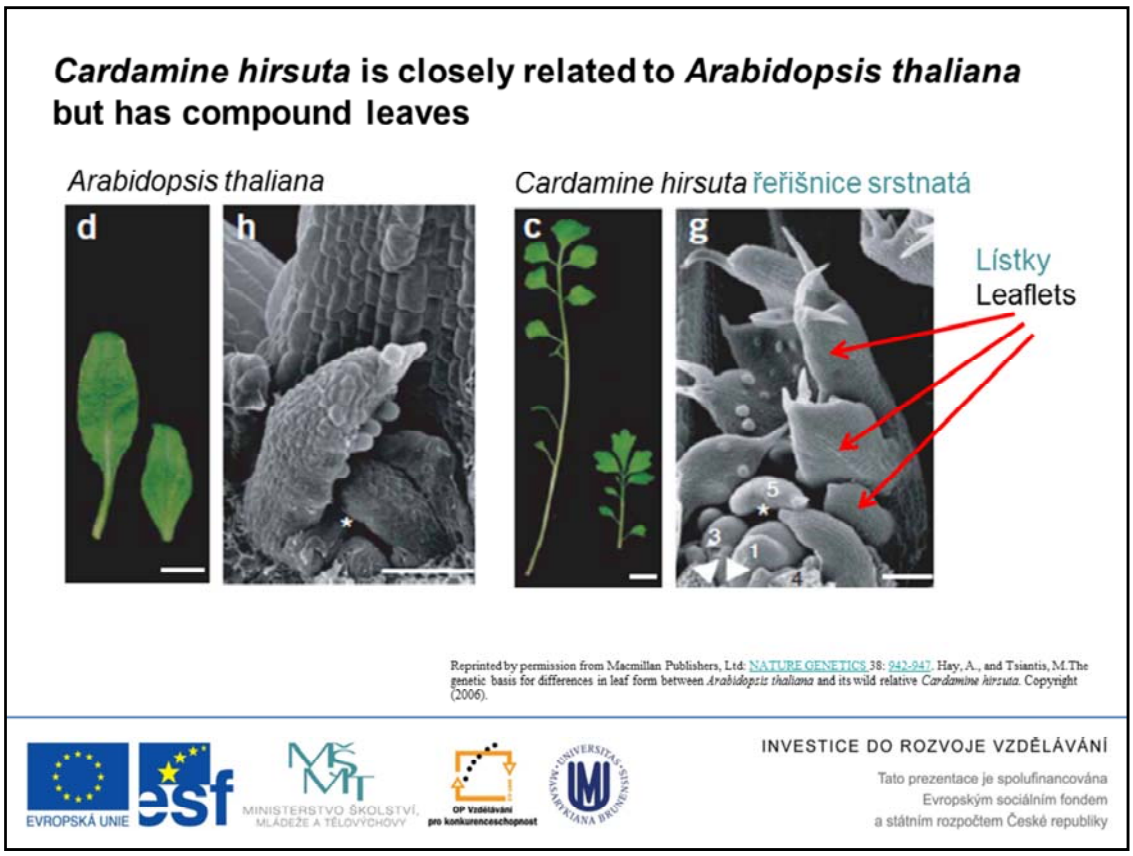
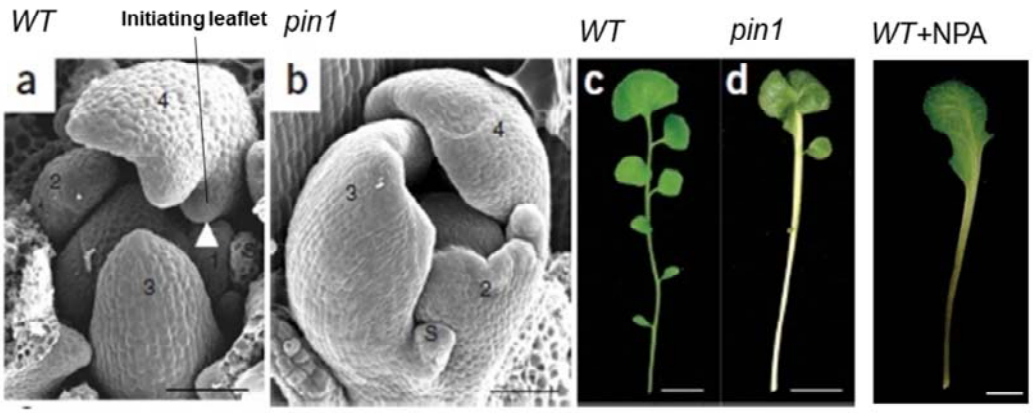


Compound leaves in the *Cardamine hirsuta* occurs via induction of what is called leaflets during early stages of the leaf development.

White arrowheads in panel g indicate leaflets initiating on leaf primordia 3 and 4.

Polar auxin transport is necessary for compound leaf formation

Cardamine hirsuta

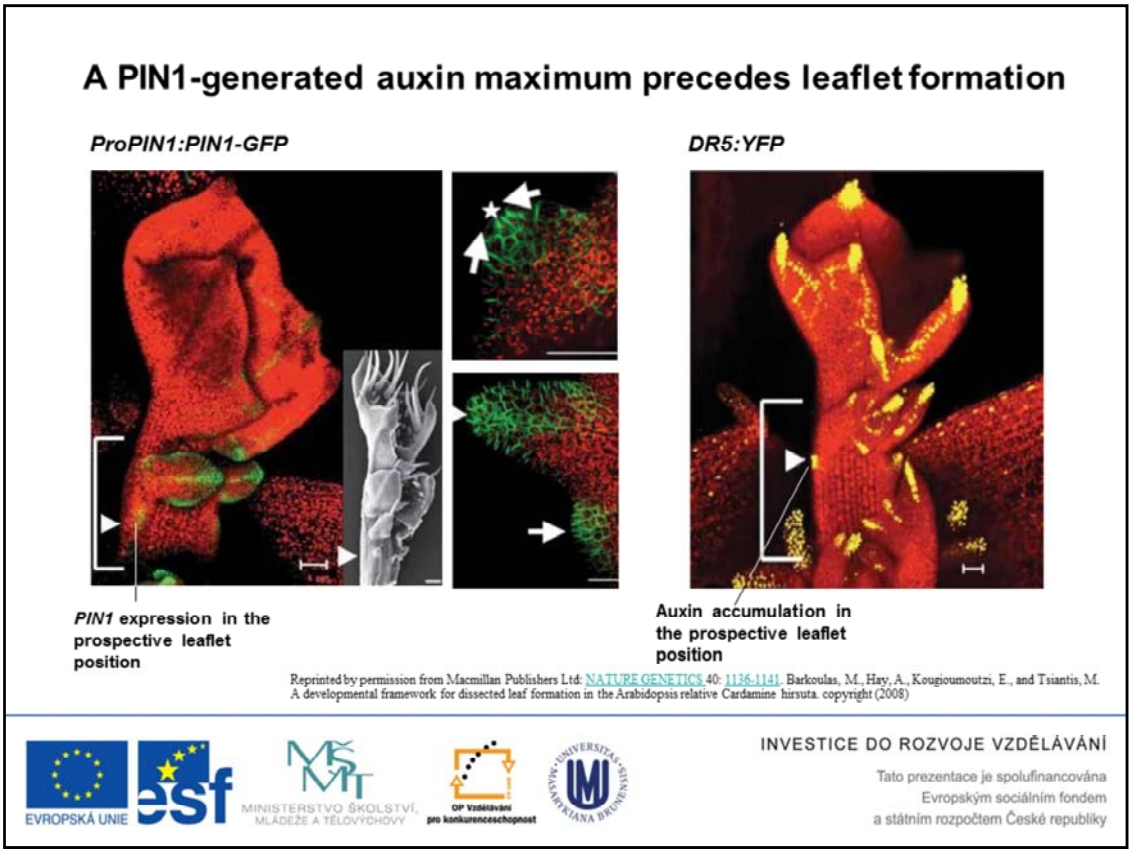


Reprinted by permission from Macmillan Publishers Ltd: NATURE GENETICS 40: 1136-1141. Barkoulas, M., Hay, A., Kougioumoutzi, E., and Tsiantis, M. A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. copyright (2008)

Polar auxin transport mediated by the efflux carriers from the PIN family is necessary for the leaflet induction and thus the compound leaf formation.

In the loss-of-function *pin1* mutant of *Cardamine hirsuta*, or in leaves treated with an inhibitor of polar auxin transport (NPA), leaflet formation is suppressed.

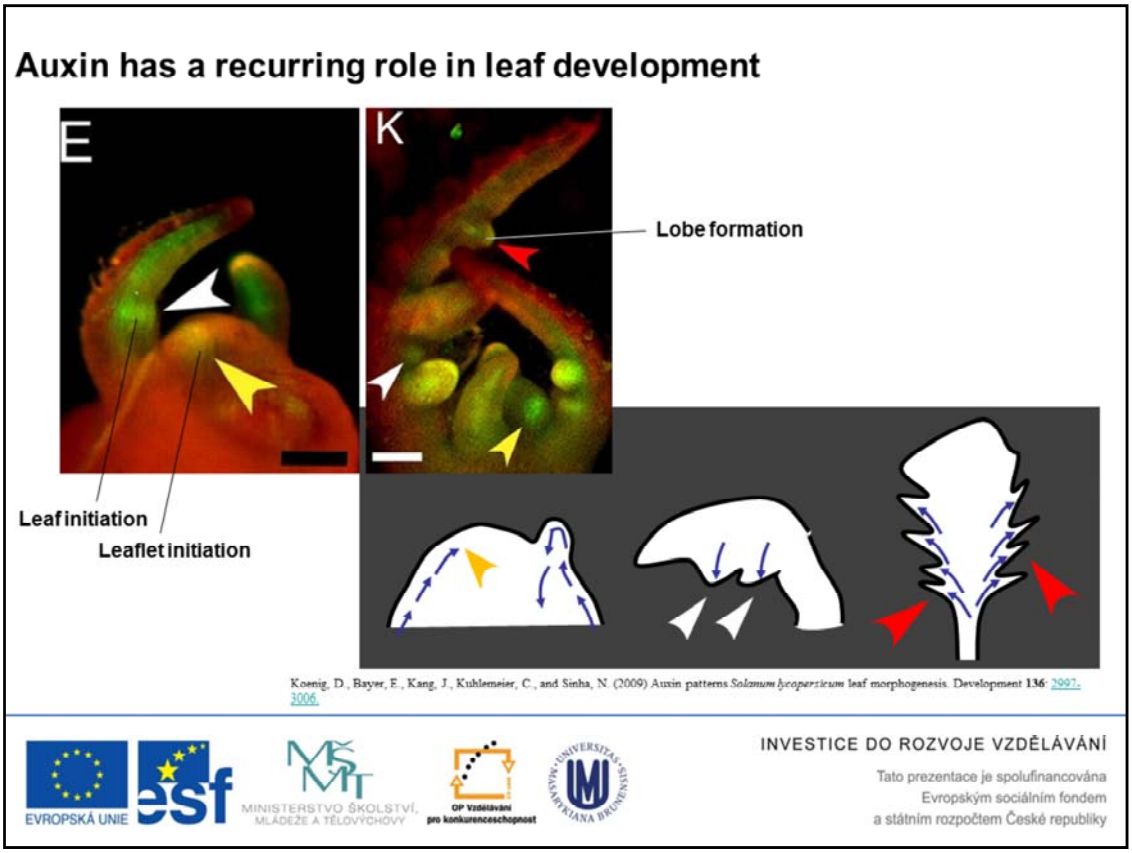
Arrowhead in (a) indicates initiating leaflet which is absent in a leaf of similar age in the pin1 mutant (b).



PIN1 gene is expressed and PIN1 protein polar localized in the developing leaf in the position of the prospective leaflet formation. The polar localization of PIN1 results into auxin maxima formation that could be visualized via DR5 activity (see the right-hand panel).

Up to now it is not completely clear whether auxin accumulation or enhanced auxin transport is triggering novel organ formation.

The arrowheads point to initiating leaflets. Arrows point to larger leaflets in which PIN1 is oriented towards the tip of the leaflet. Auxin accumulation is determined by expression of the auxin-responsive DR5 promoter fused to YFP (right-hand figure).



Auxin maxima precede leaf initiation (yellow arrow), leaflet initiation (white) and lobe initiation (red).

Auxin flow is indicated by red arrows.

Outline of Lesson 9

Morphogenesis

- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres
  - Changes in the cell motility and tissue interactions during organogenesis
- Morphogenesis in plants
  - Introducing leaf development as an example of morphogenesis in plants
  - The role of oriented cell division and its relative distribution
    - Regulation of cell division by TCP and boundary genes
    - Auxin-regulated positional information for cell division
    - *KNOX* and boundary genes in the leaf complexity

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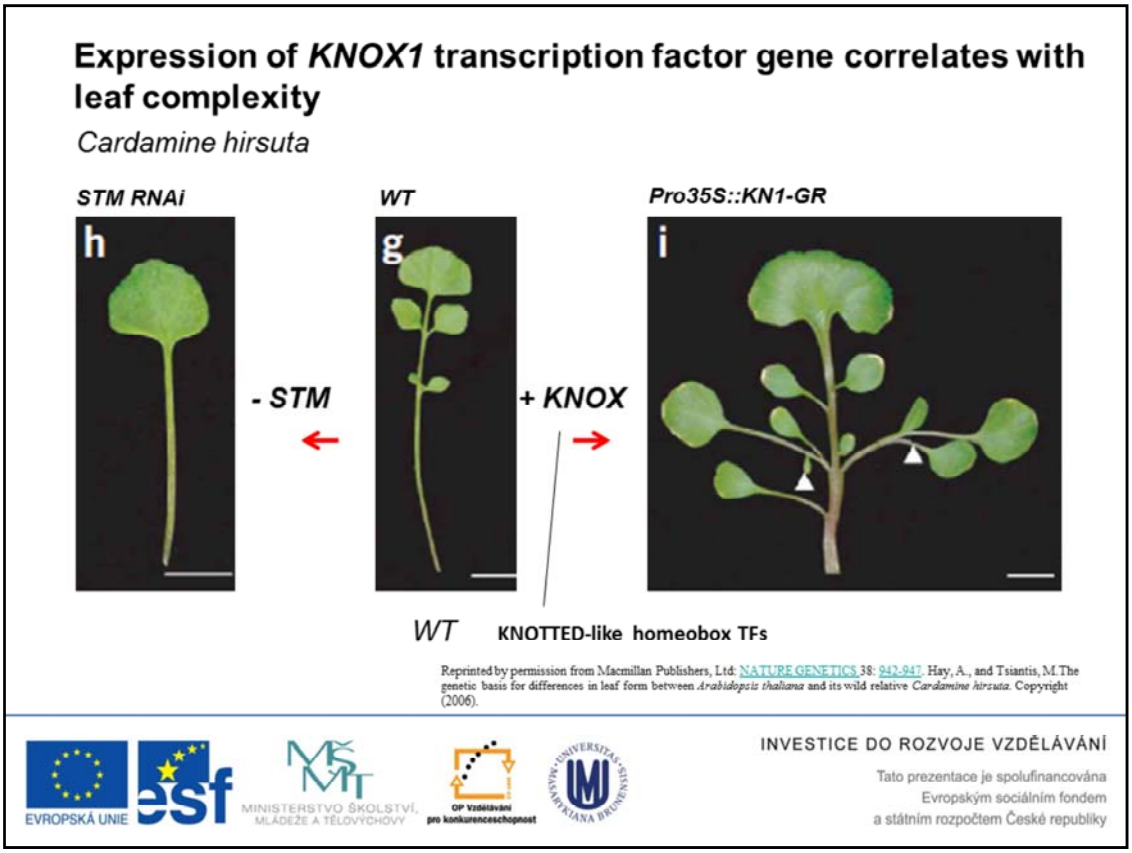
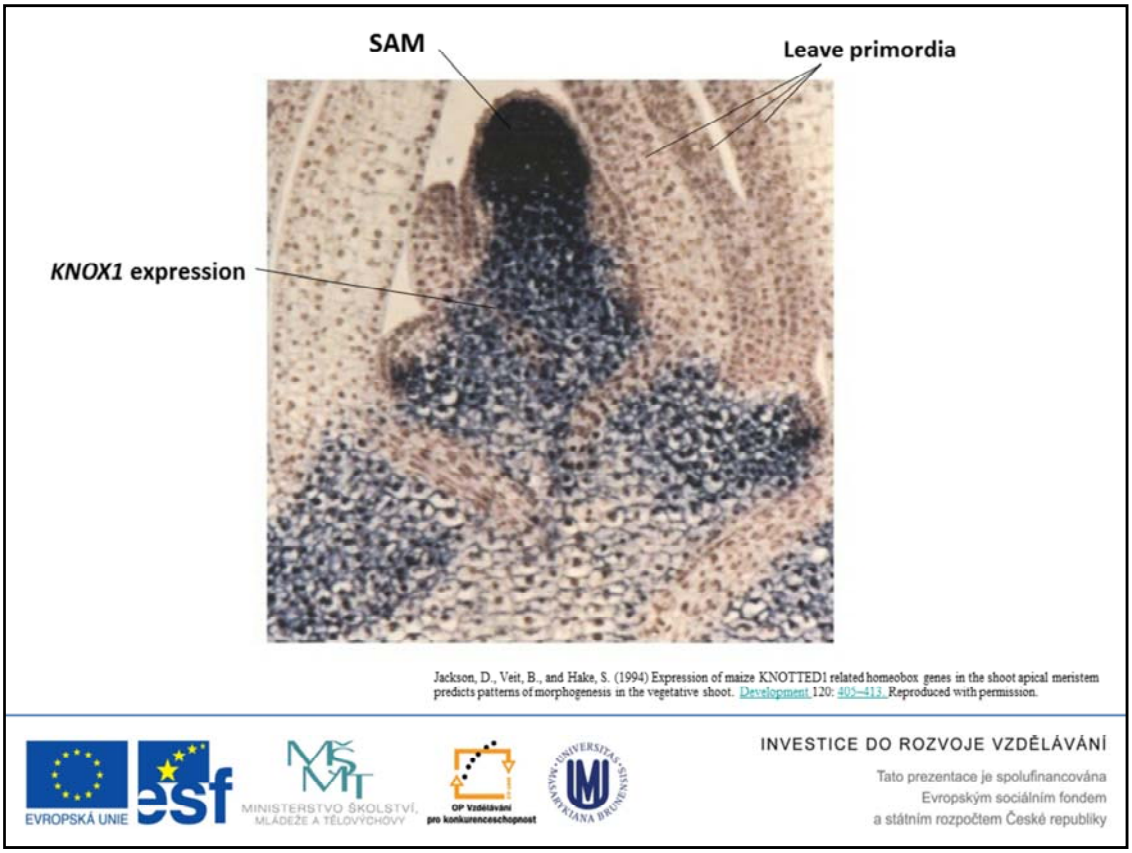


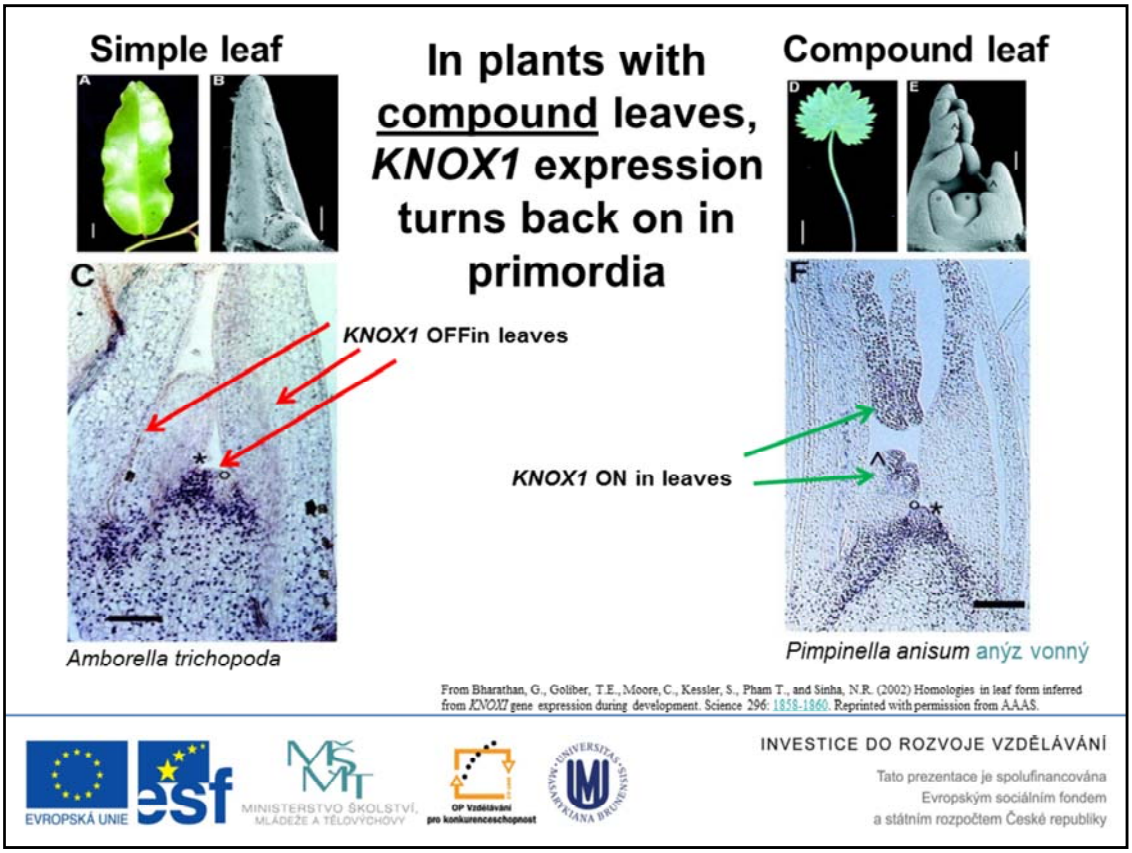
Besides the role of auxin in the leaflet initiation, members of the what is called *KNOX* genes, encoding for KNOTTED1-like homeobox TF in *Arabidopsis* and *Cardamine hirsuta*, is important regulator of leaflet growth.

Downregulation of the *SHOOTMERSTEMLESS (STM*) gene lead to absence of 4 parallel leaflets typical for WT *Cardamine* and to the formation of single leaves (left-handed figure).

In contrast, the ectopic overexpression of *KNOTTED1 (KN1),* another member of the homeobox genes, resulted into increased number of leaflet formation suggesting positive role of *KNOX* genes in the leaflet formation.

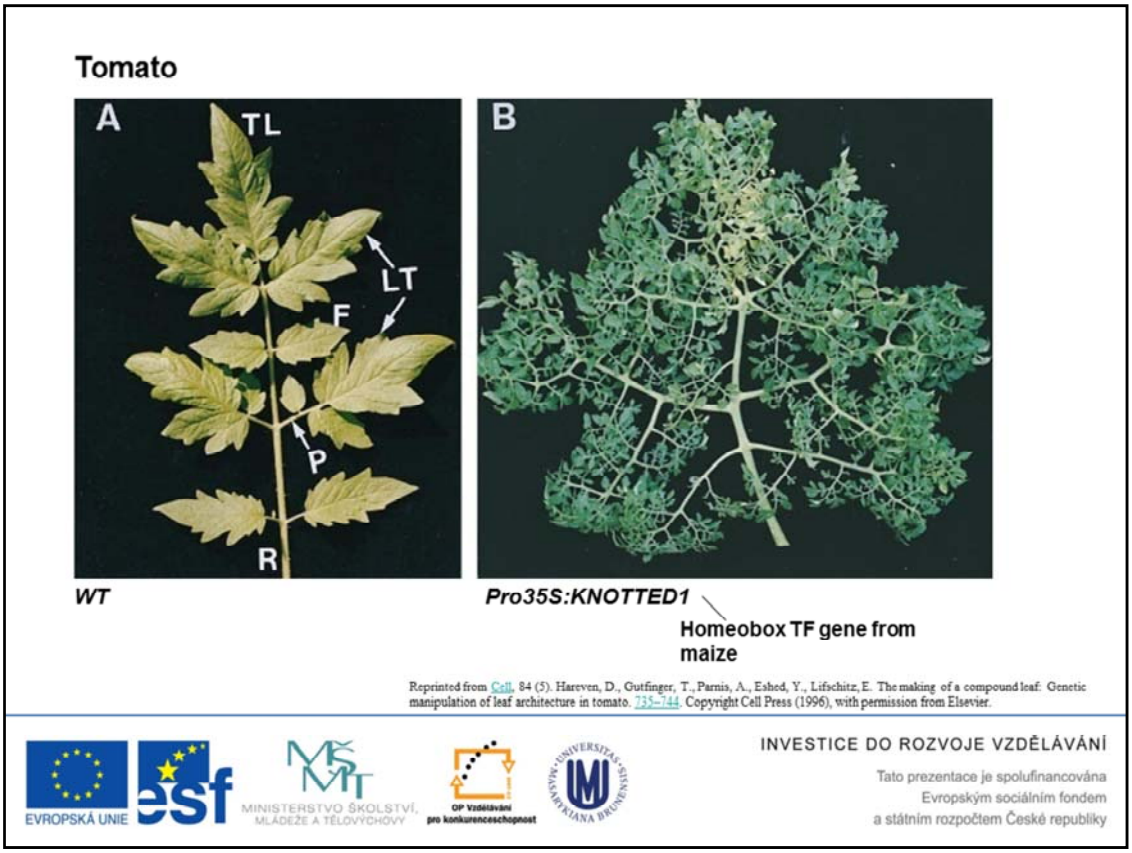


In most of the plants with simple leaves, the *KNOX* genes are expressed in shoot apical meristem (SAM) but not in incipient leaf primordia.

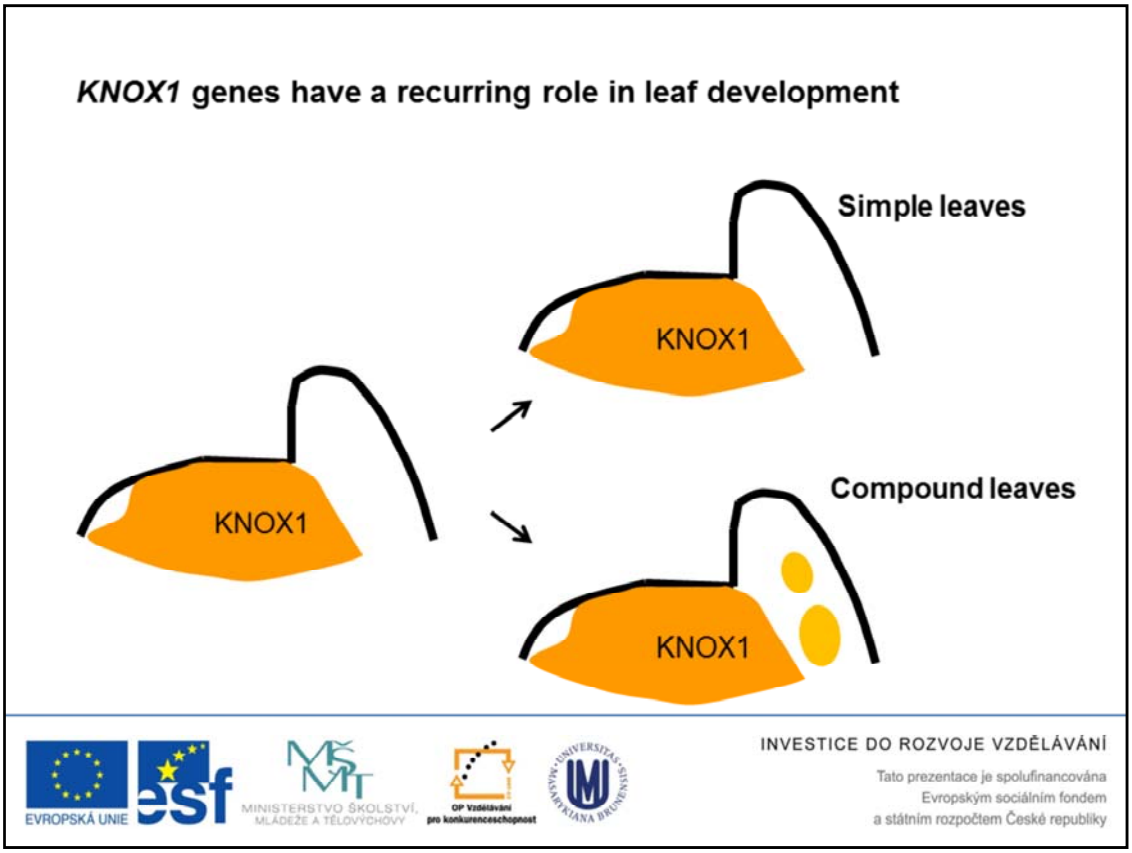


However, in plants with compound leaves, the *KNOX1* is usually induced in the leaf primordia, leading to the leaflets formation.

Some plants with *KNOX* expression in leaves make simple leaves due to secondary morphogenesis – see Bharathan et al., (2002).



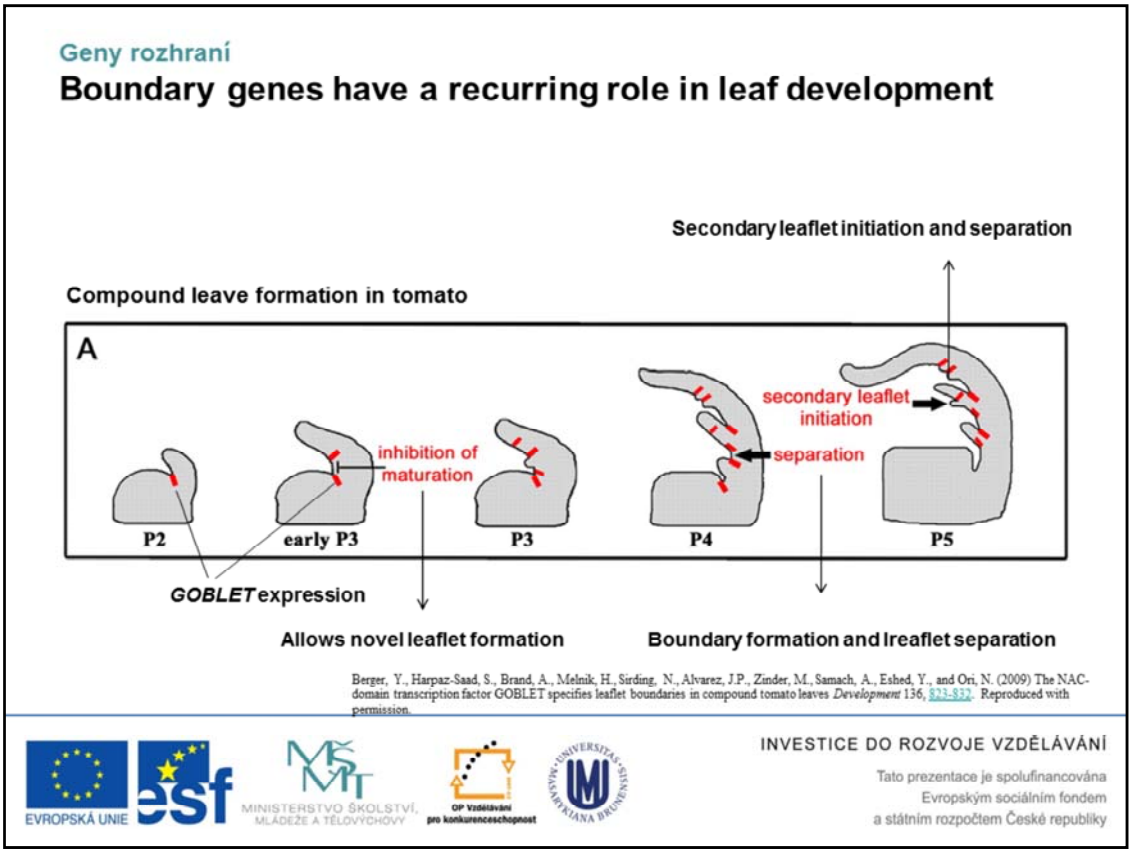
In plants with compound leaves, over-expression of *KNOX1* genes can make leaves ultra-compound.



KNOX1 genes are repressed at the site of leaf primordium initiation.

Simple leaves (e.g. Arabidopsis) *KNOX1* genes stay off .

Compound leaves (e.g. tomato) *KNOX1* genes turn on again in developing leaf primordia, conferring prolonged organogenic activity on the leaf edges.



Boundary genes coordinate auxin gradients and gene expression patterns during leaf and leaflet initiation and leaf serrations.

The tomato *GOBLET (GOB)* gene is related to *CUC2* and sets up boundaries throughout tomato leaf development. *GOBLET* expression is shown in red.

In early P3 primordia, *GOB* is expressed at the leaf margin prior to leaflet initiation and inhibits maturation in the adjacent area, enabling future leaflet initiation.

During primary leaflet formation, restricted *GOB* expression in space and time allows proper leaflet separation.

In the terminal leaflet, *GOB* expression enables the development of lobes and serrations. In plastochron 5 (P5), stripes of GOB expression in the primary leaflet flanks enable initiation and separation of secondary leaflets.

Key Concepts

Morphogenesis

- In animals, **regulated cell motility** and **adhesion** is necessary for **proper morphogenesis**
- The **interaction between cells** and **surrounding environment** is critical for the changes in **adhesion** and/or **cell motility**
- Presence of specific **interacting molecules** and **their quantity** allows **formation of self-organizing system** based on the **selective cellular adhesiveness**
- **Cellular interactions** and **signalling** are critical for **proper organogenesis**
- **Morphogenesis in plants** is regulated by **direction** and **localization** of **cell division** and **cell elongation**
- Auxin-provided **positional information** and **spatial-specific** regulated **gene expression** are involved in the **modulation** of **cell division** and **organ** (leaf) **patterning**

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