

# Bi8940 Developmental biology

## Lesson 9

### Morphogenesis

Jan Hejátko

**Laboratory of Molecular Plant Physiology,**  
Department of Functional Genomics and Proteomics,  
Masaryk University,  
Brno, Czech Republic

[hejatko@sci.muni.cz](mailto:hejatko@sci.muni.cz), [www.sci.muni.cz/FGP/](http://www.sci.muni.cz/FGP/)



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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



# Outline of Lesson 9

## Morphogenesis

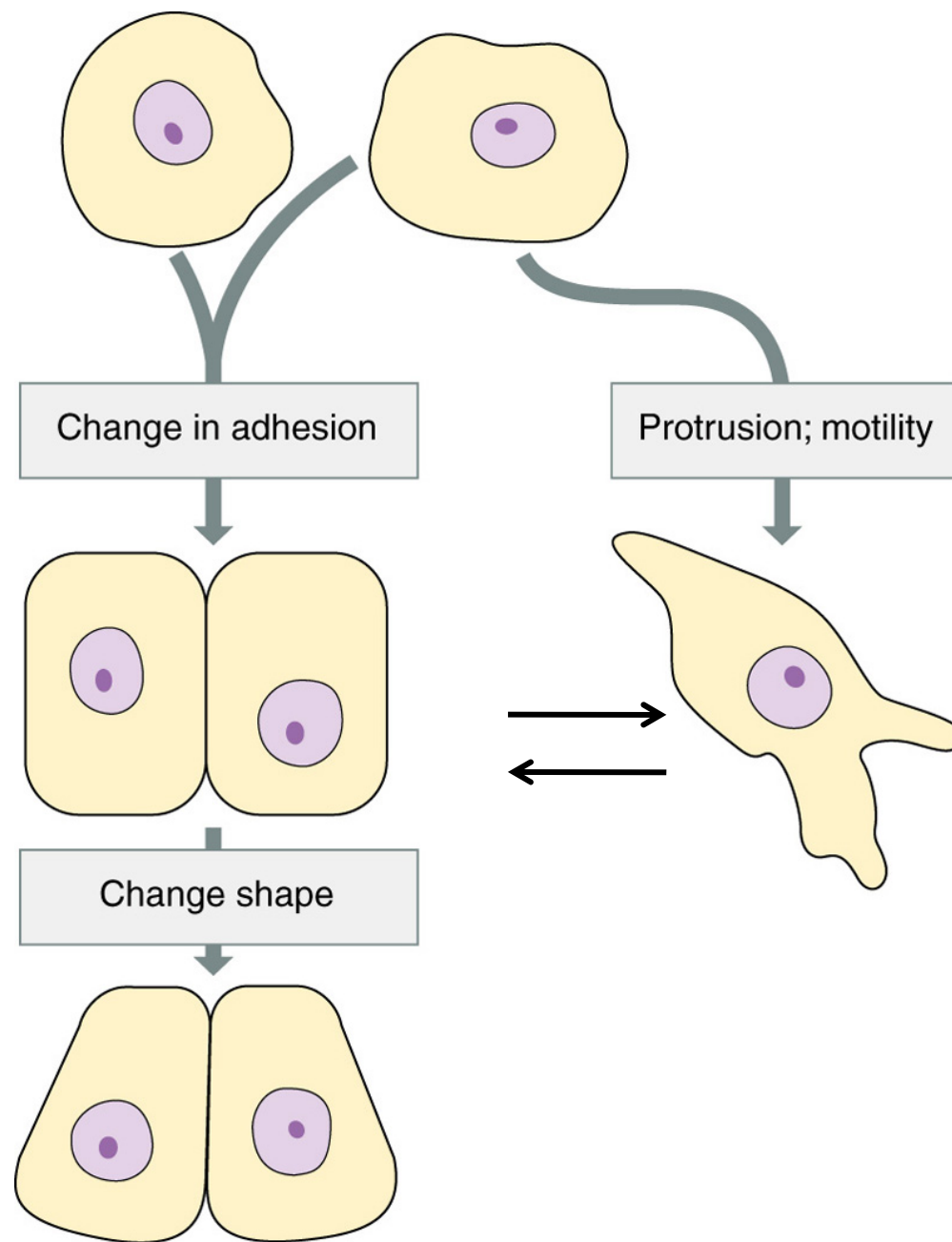
- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres
  - Changes in the cell motility and tissue interactions during organogenesis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



# Outline of Lesson 9

## Morphogenesis

- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility

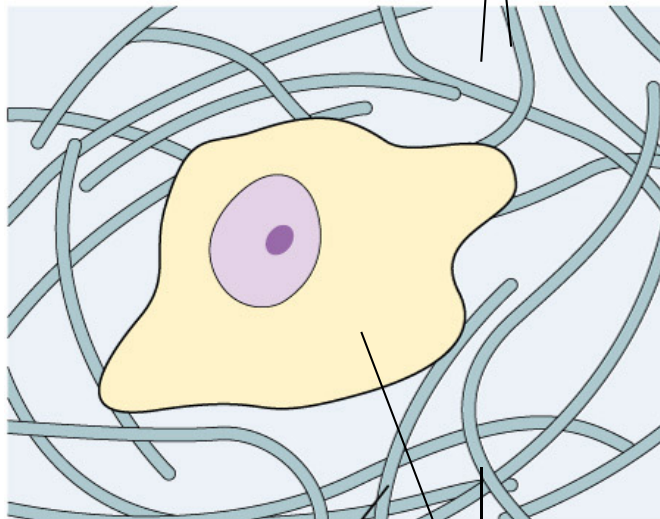




## Extracellular matrix

- Collagen
- Proteoglycans
- Glycoproteins
- Signalling molecules

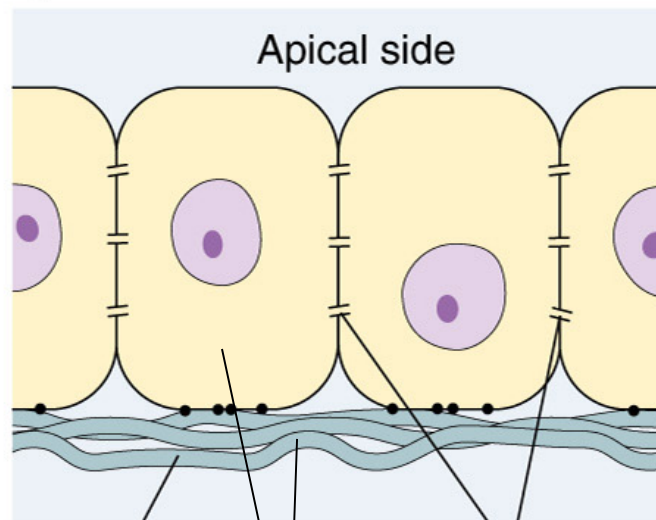
### Mesenchyme



Extracellular matrix

Interactions with  
extracellular matrix

### Epithelium

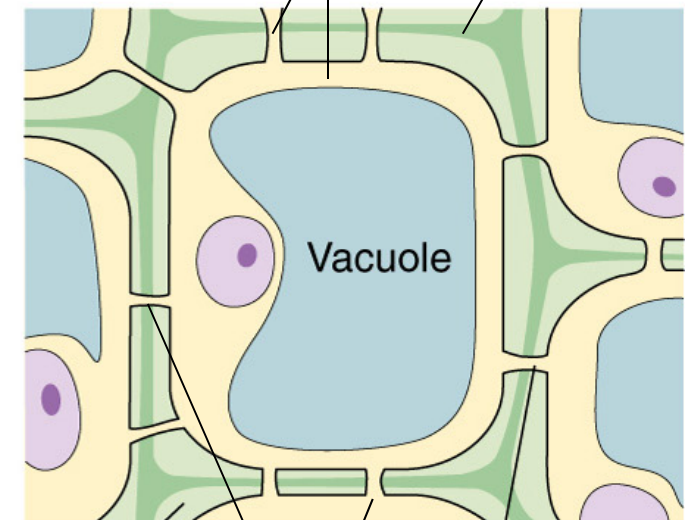


Basal lamina

Intercellular junctions

Cell-to-cell communication  
and interactions with basal  
lamina

### Plant cell



Cell wall

Plasmodesmata

Cell-to-cell communication  
and interactions with  
extracellular space



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## Morphogenesis

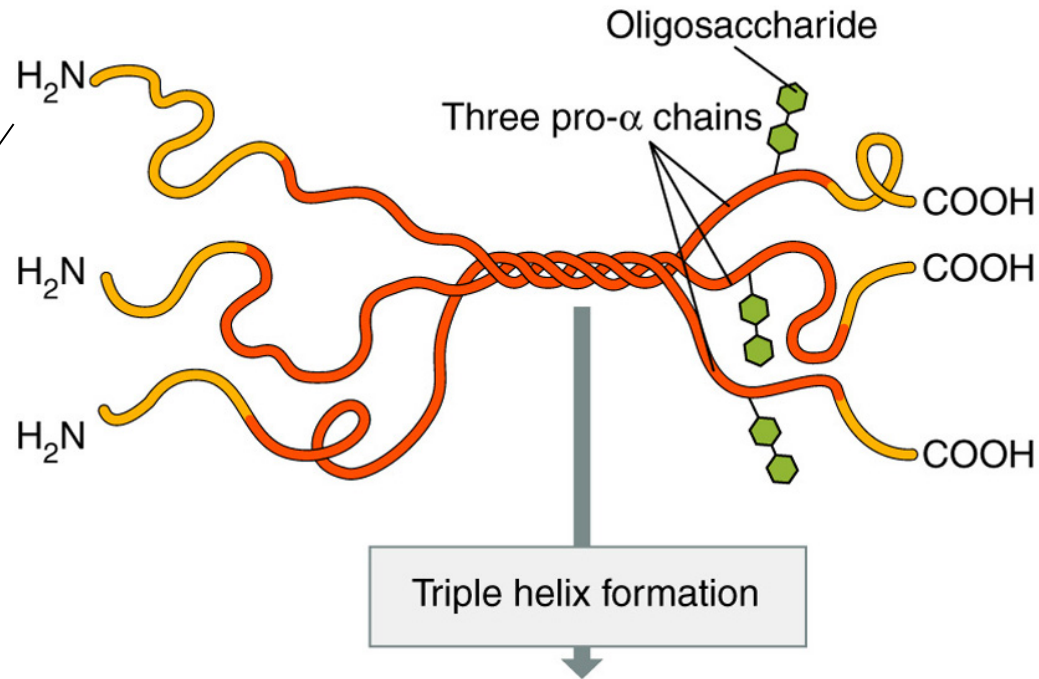
- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis

# Collagen

A.

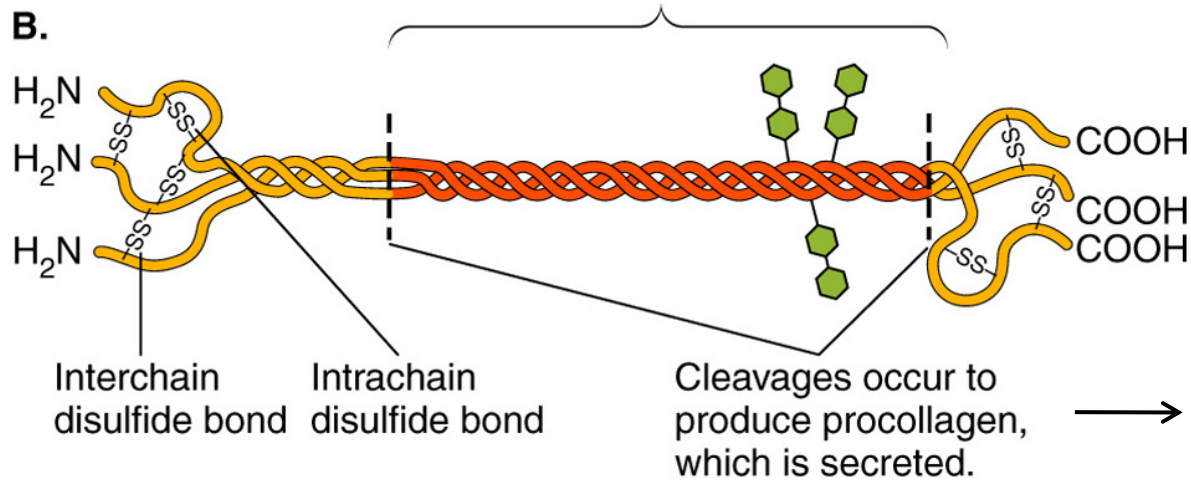
Prolin- and  
hydroxyprolin  
rich aa chains  
(Gly-X-Y)<sub>n</sub>

Proline of  
hydroxyproline



Future collagen molecule

B.



Cell-environment-  
dependent  
regulation

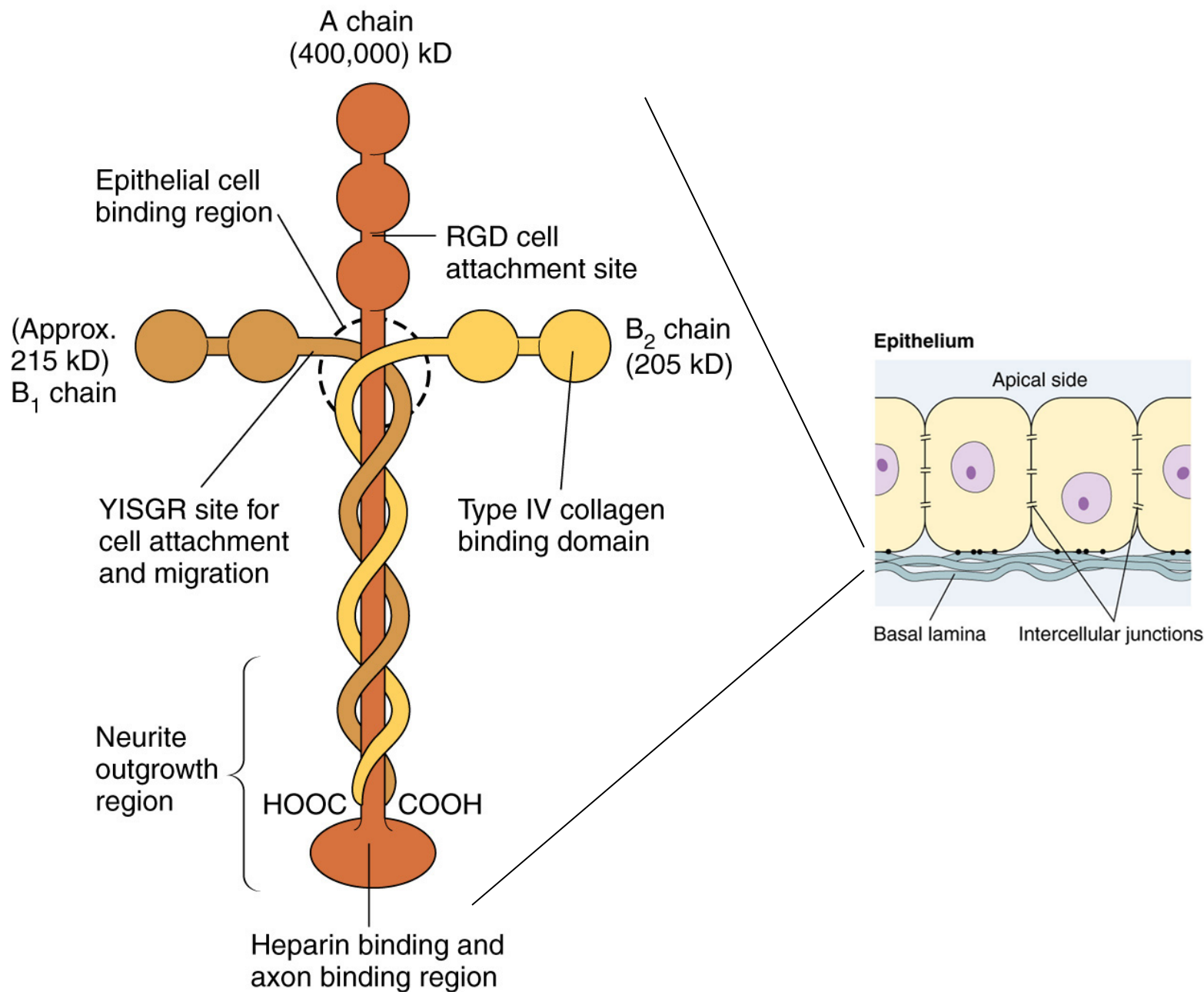
Final assembly in  
the extracellular  
space



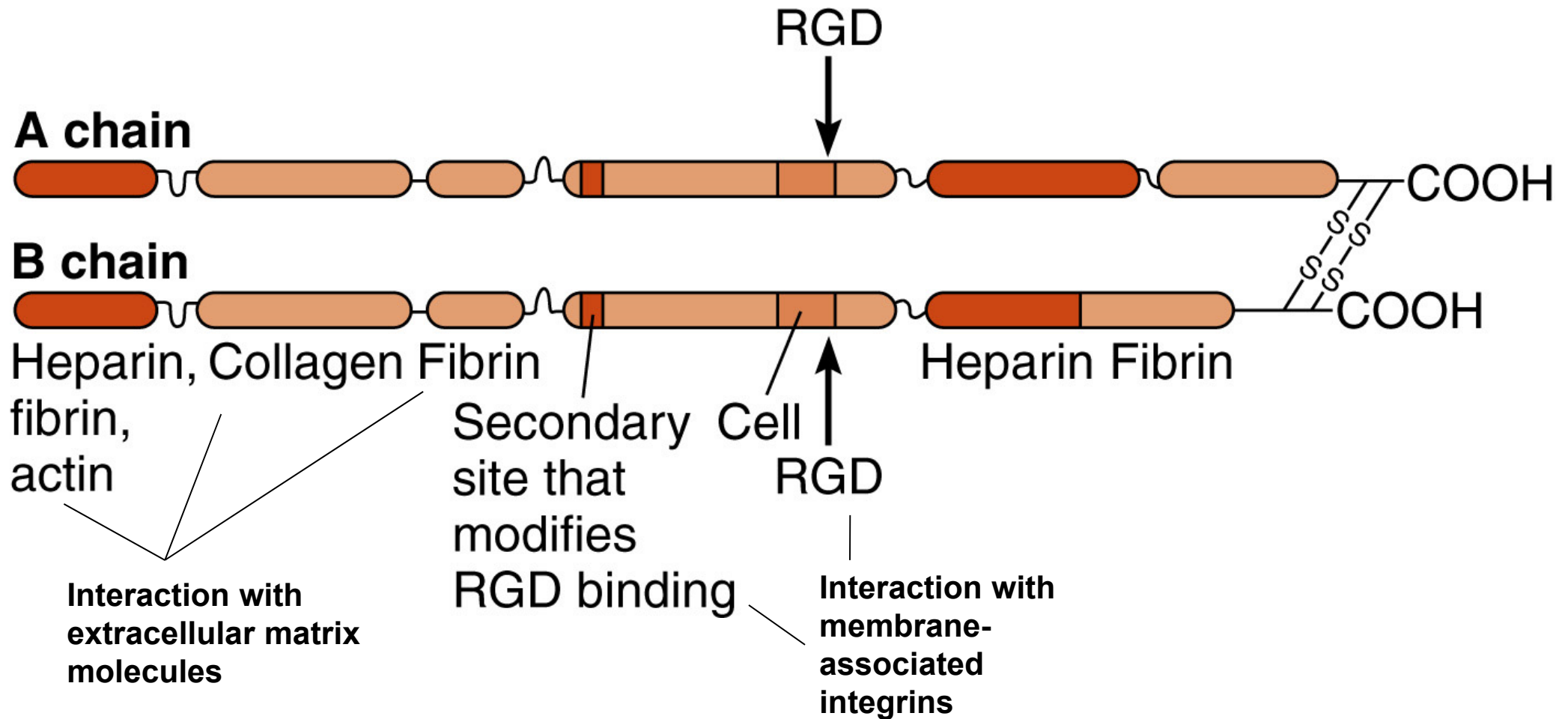
**TABLE 12.1 THE MAJOR TYPES OF COLLAGEN**

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

# Laminin

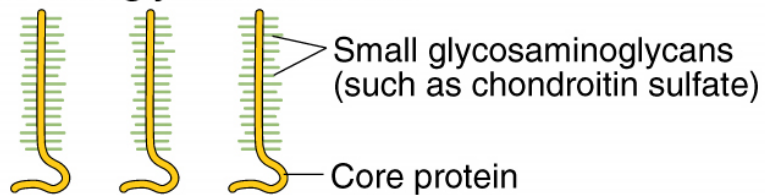


# Fibronectin



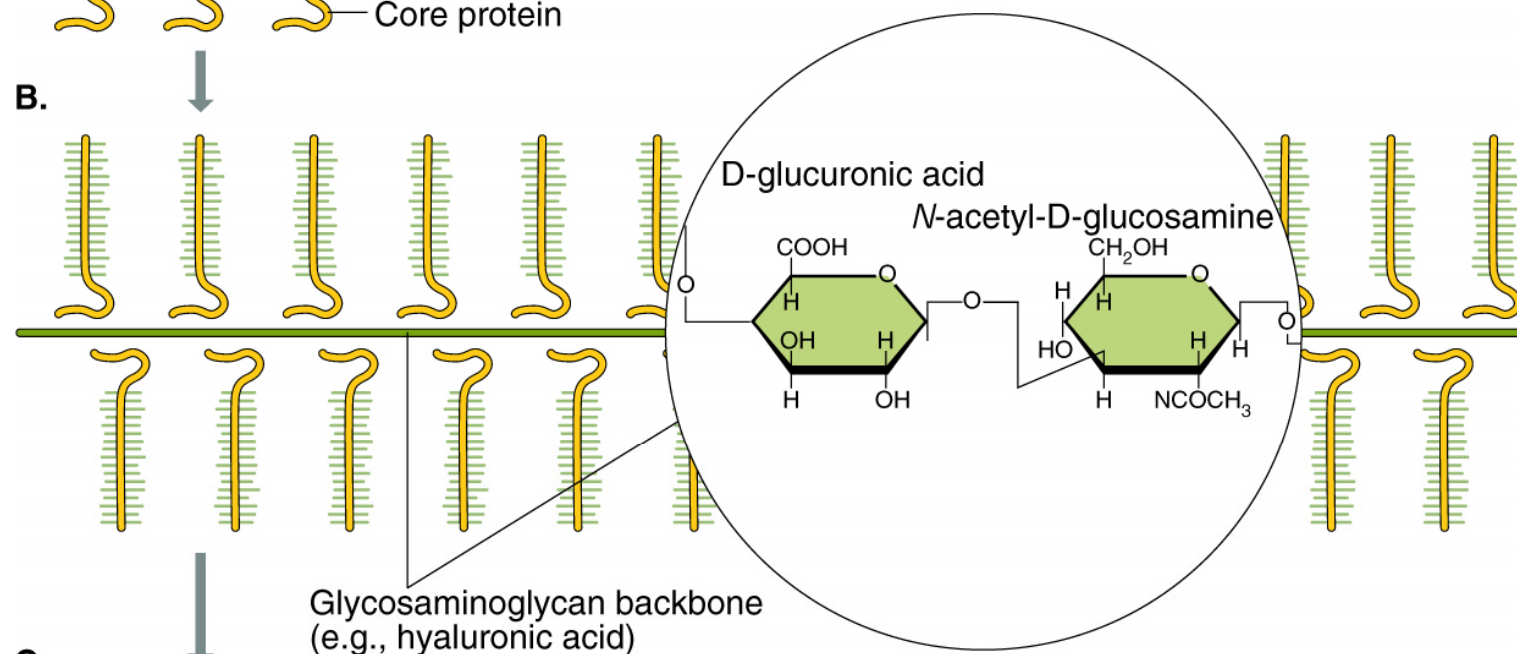


## A. Proteoglycan monomers

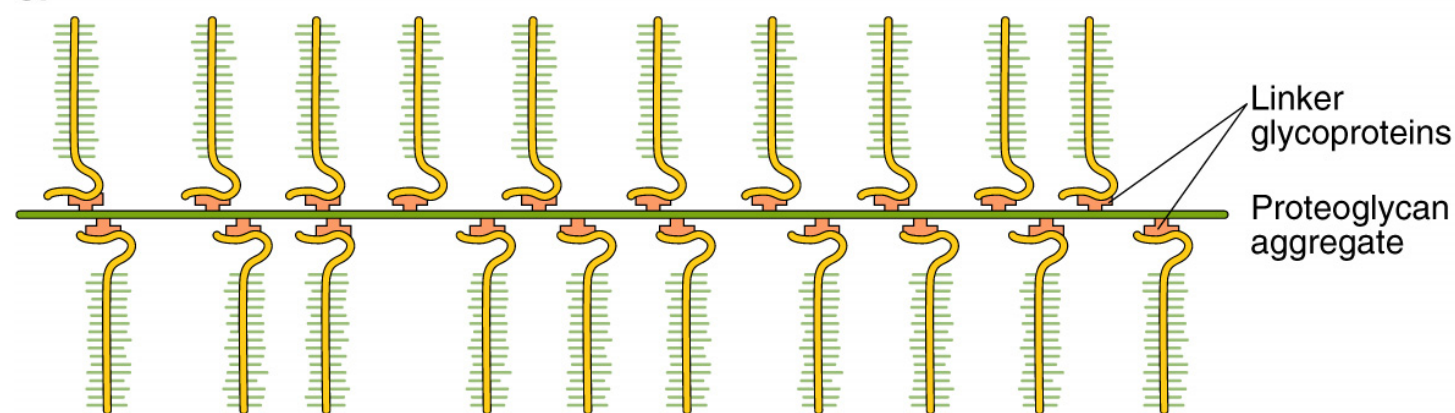


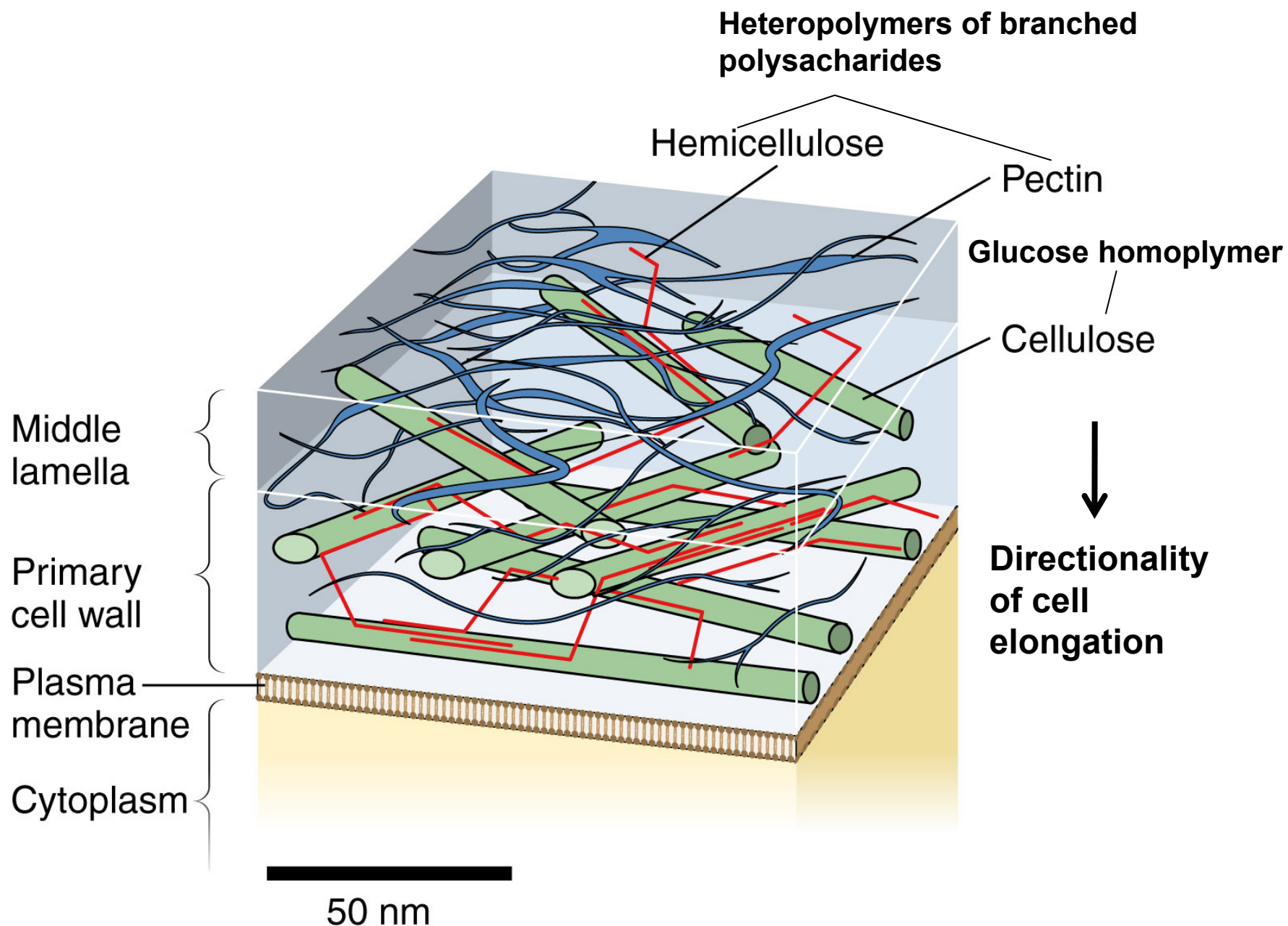
# Proteoglycans

B.



C.





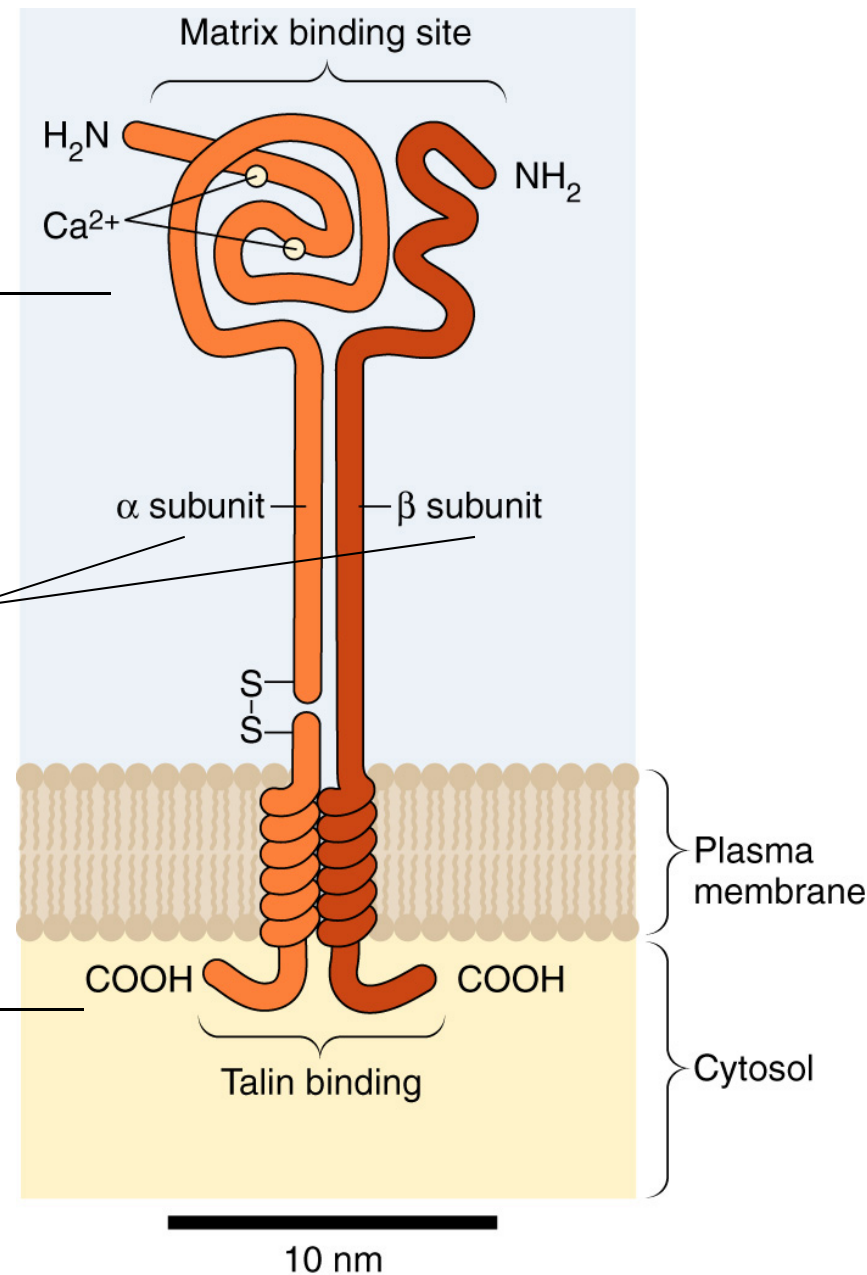


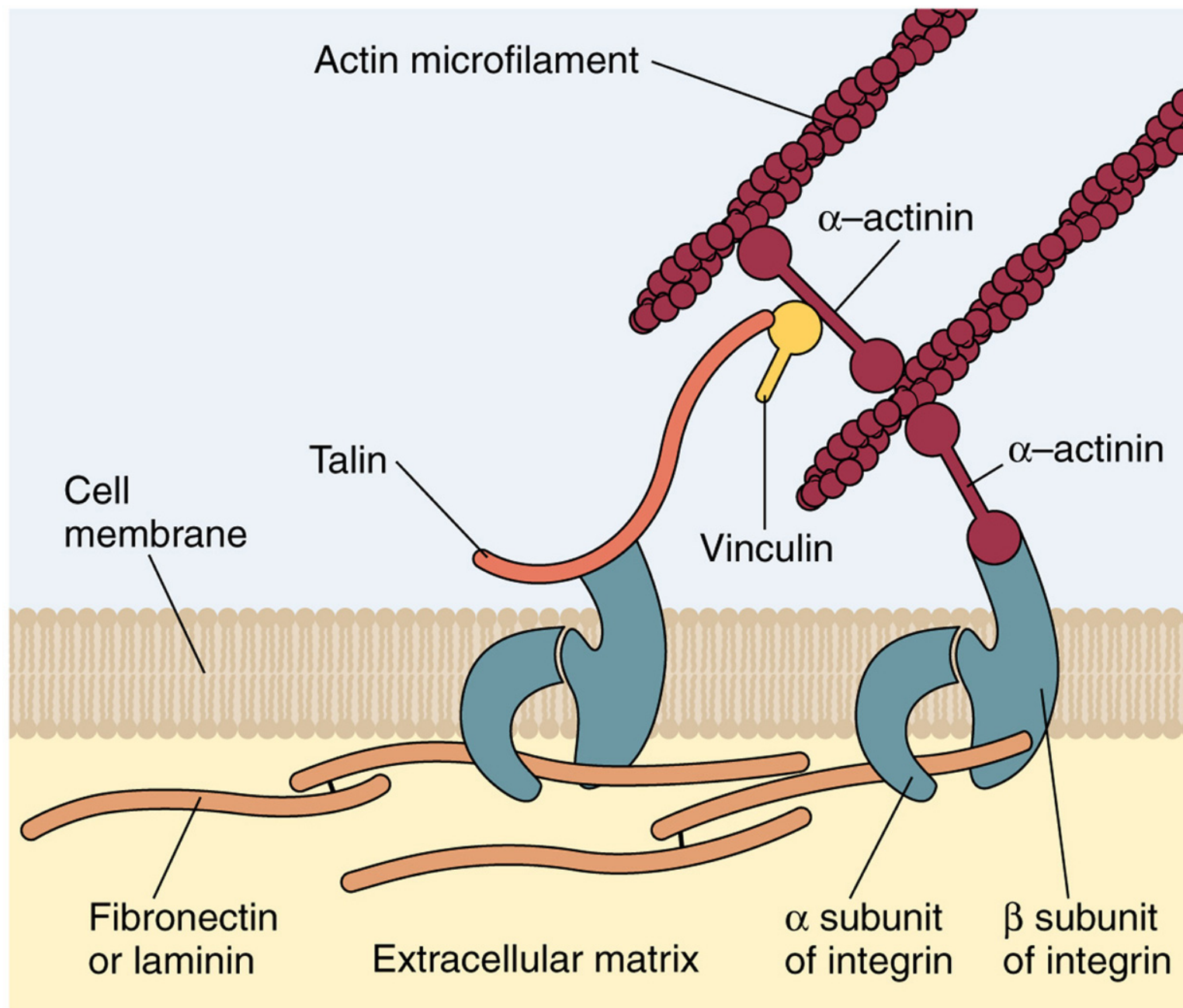
# Integrins

Interaction with  
extracellular  
matrix molecules

Encoded by  
gene  
families

Interaction with  
internal proteins,  
e.g. cytoskeleton



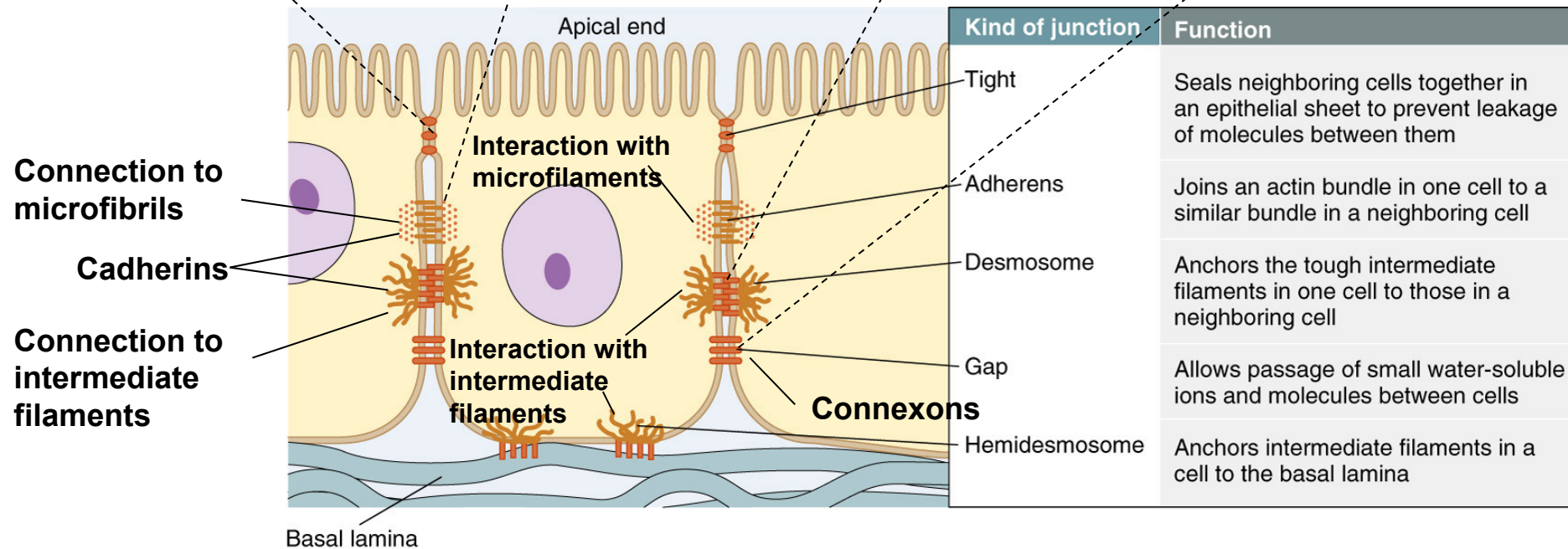
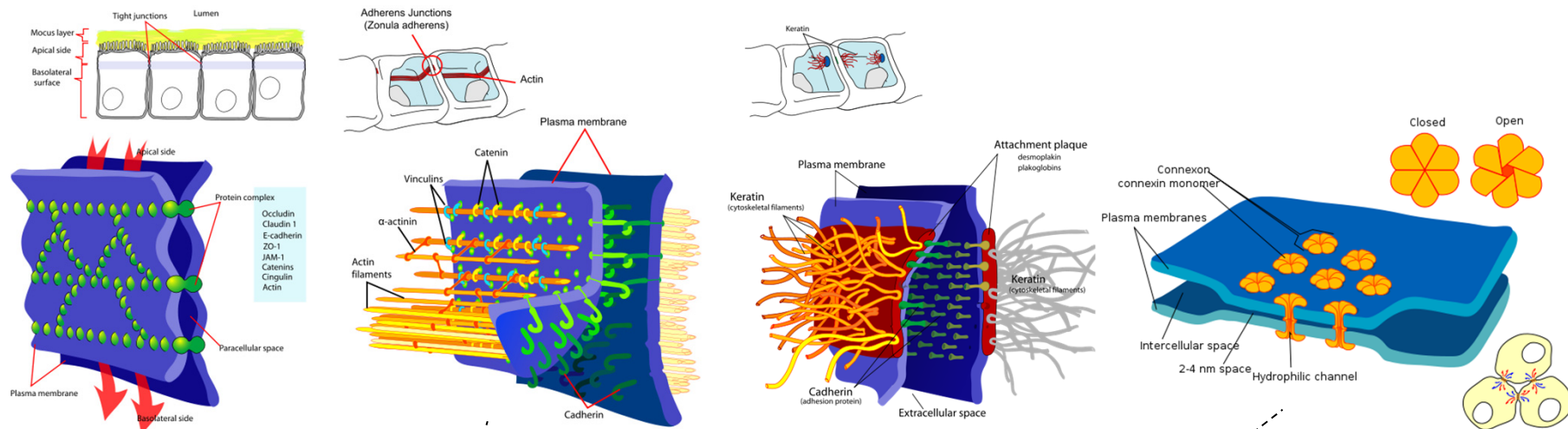




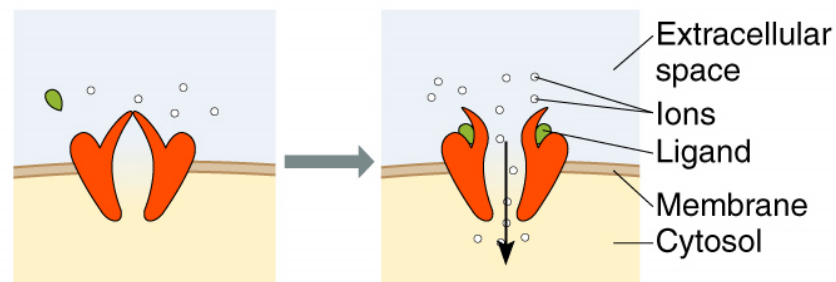
# TABLE 12.2 SOME LIGANDS OF INTEGRIN DIMERS

Major $\beta$ Subunit	Ligand of Integrin Dimer Subunits	Types of $\alpha$ Subunits
$\beta_1$	Collagen Laminin Fibronectin	1, 2, 3 1, 2, 3, 6 3, 4, 5 V
$\beta_2$	I-CAM Fibrinogen	2L, 2M 2M
$\beta_3$	Fibrinogen Fibronectin	V, 2b V, 2b
$\beta_4$	Basal lamina	6

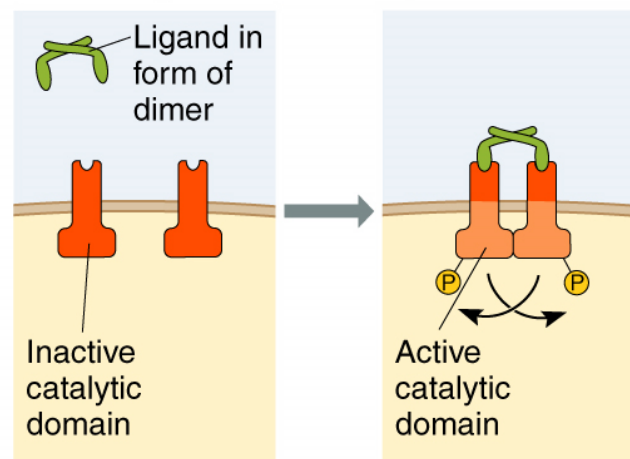




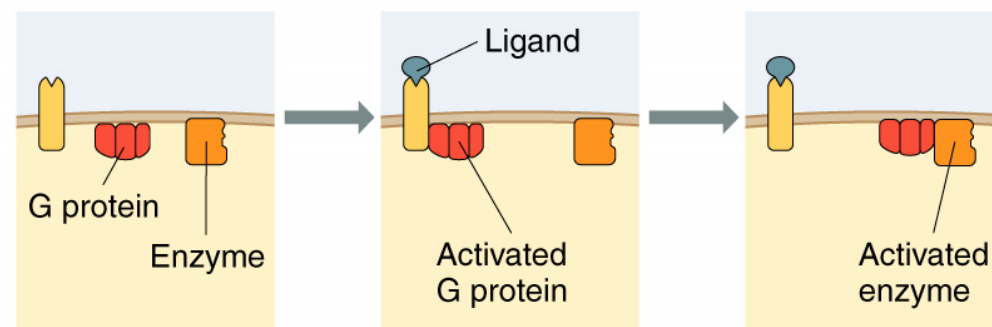
### A. Ion-channel-linked receptor



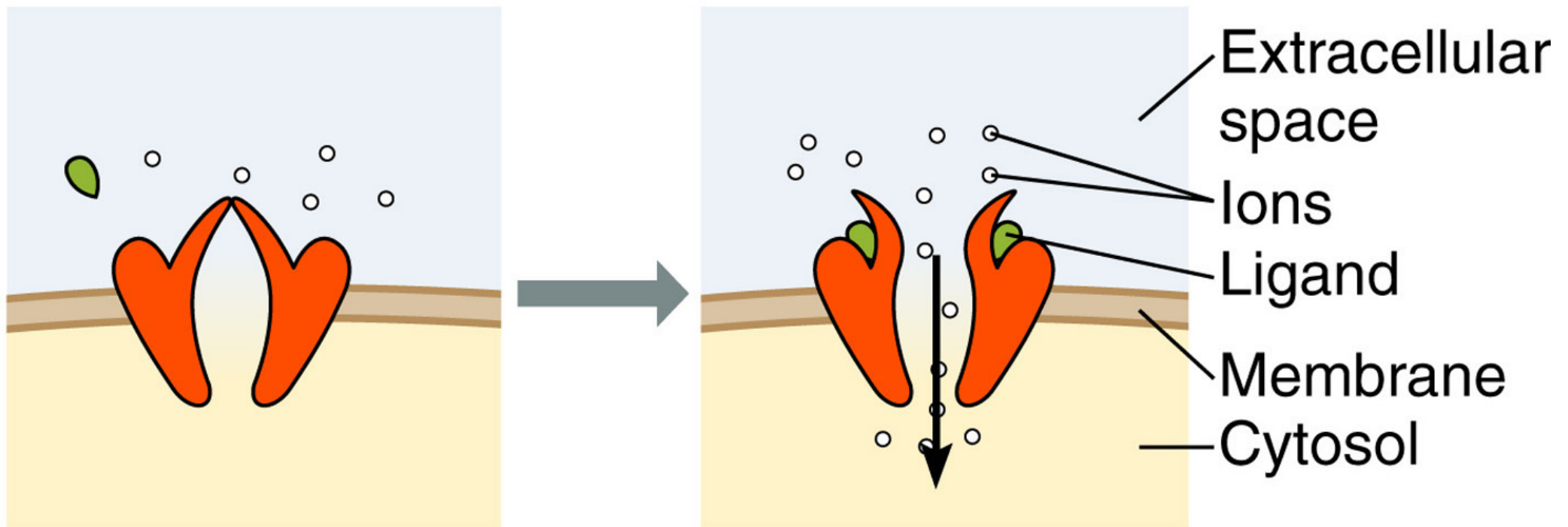
### B. Enzyme-linked receptors



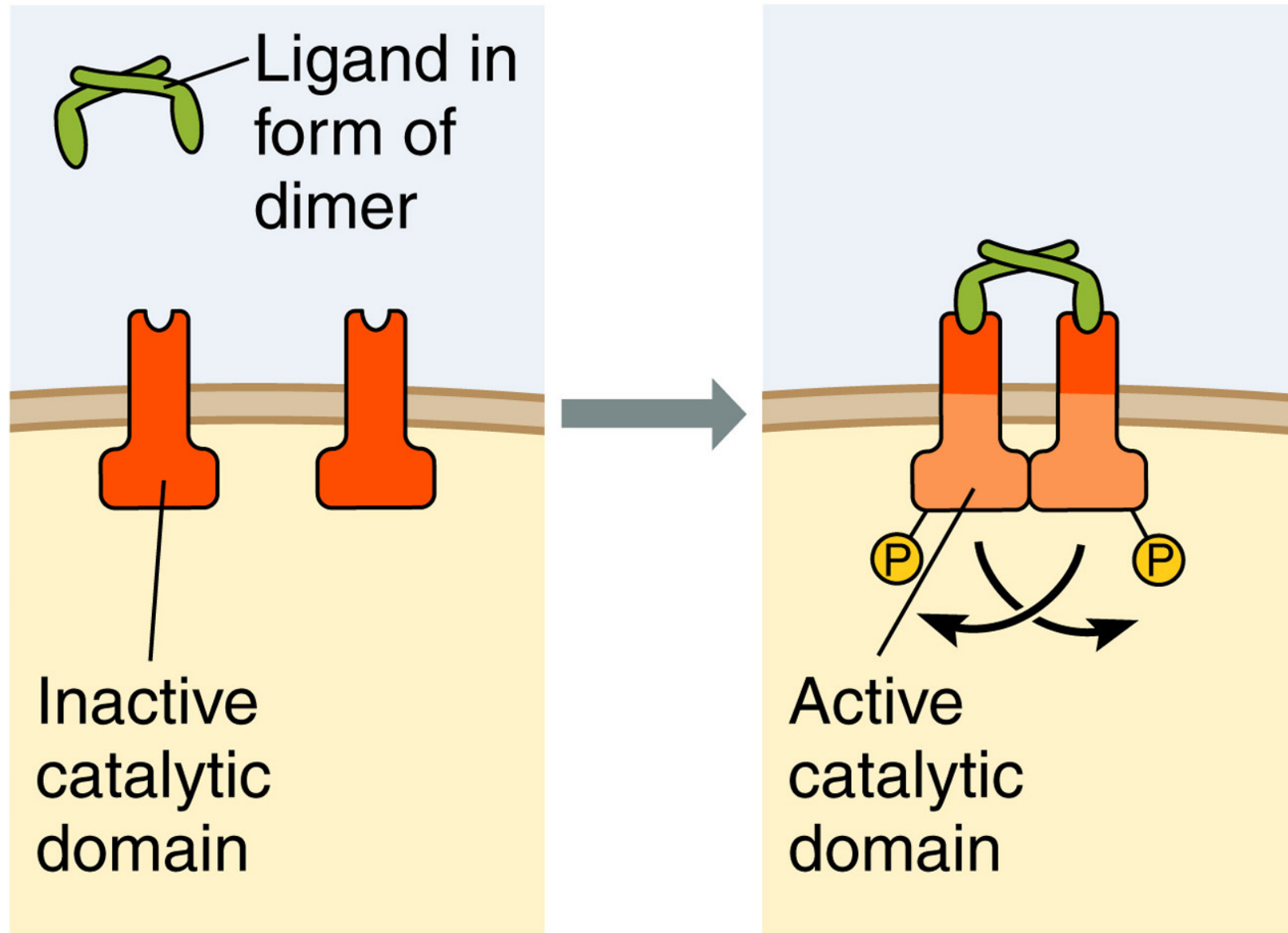
### C. G protein-linked receptor



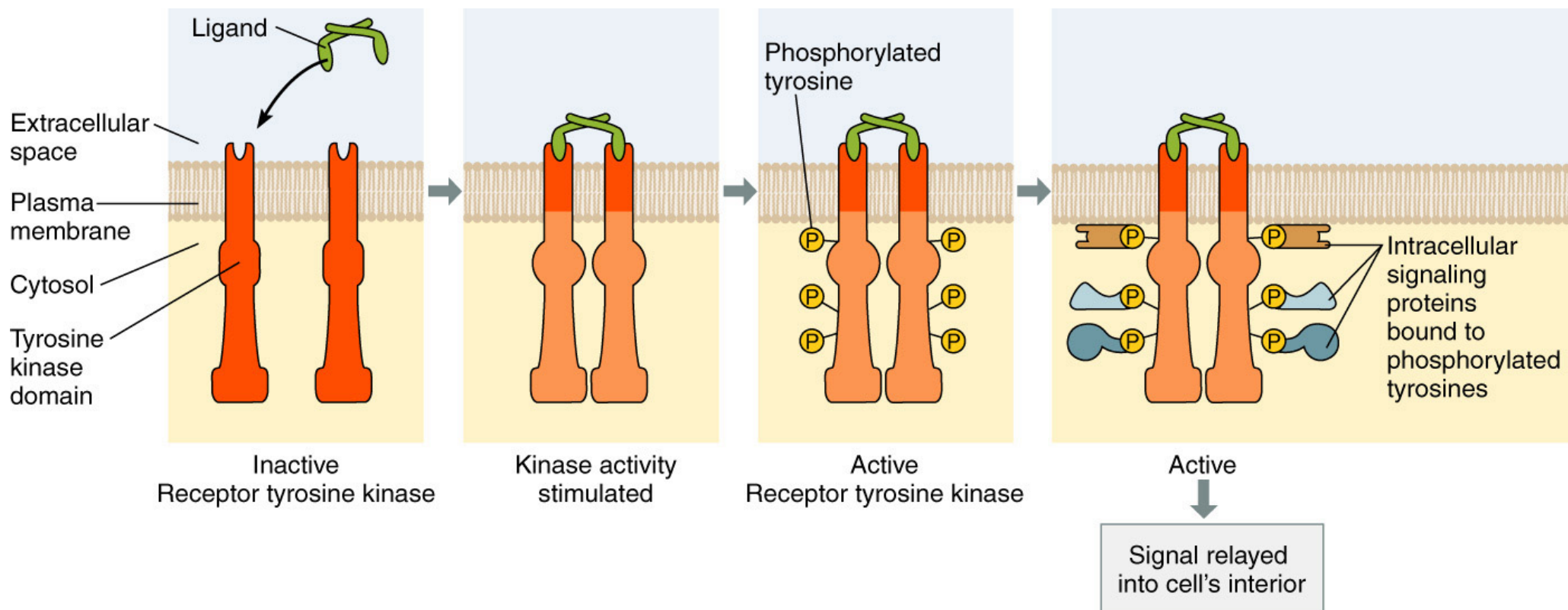
## A. Ion-channel-linked receptor



## B. Enzyme-linked receptors

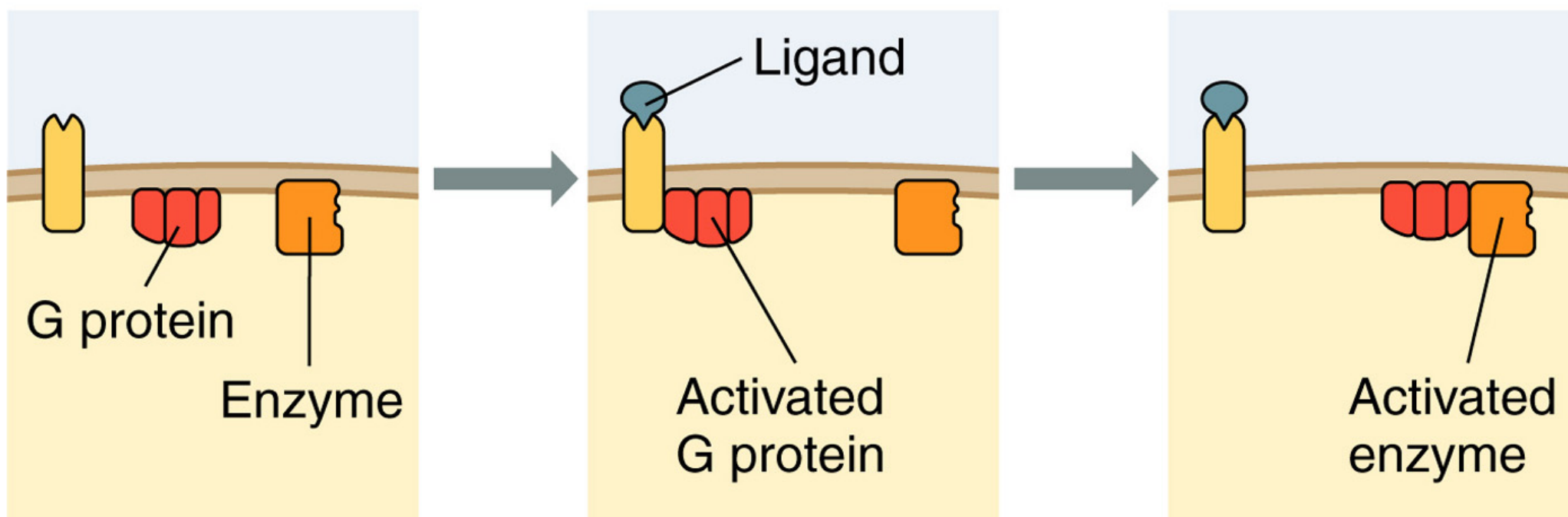








## C. G protein-linked receptor

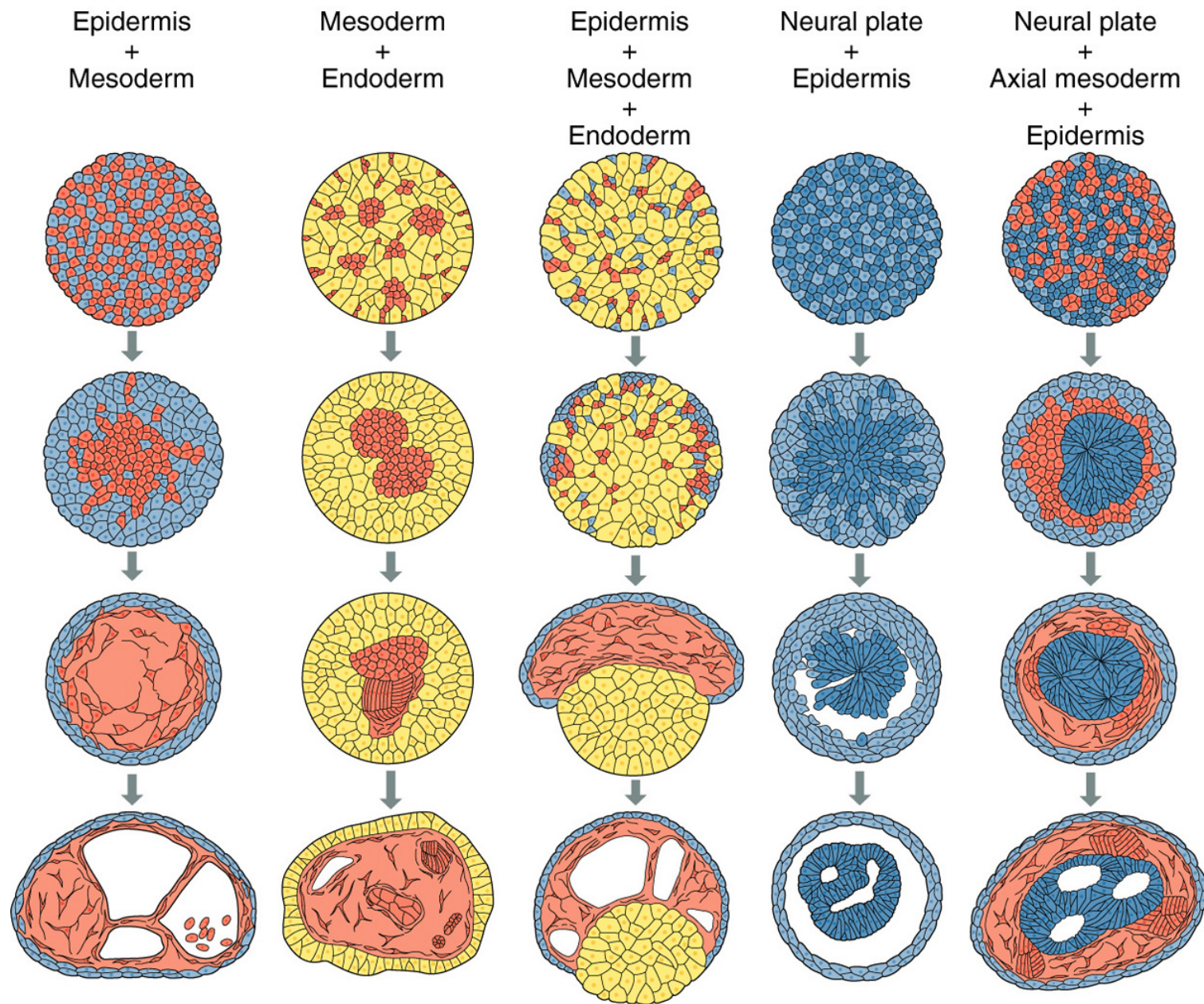




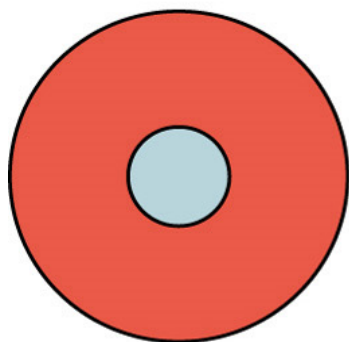
# Outline of Lesson 9

## Morphogenesis

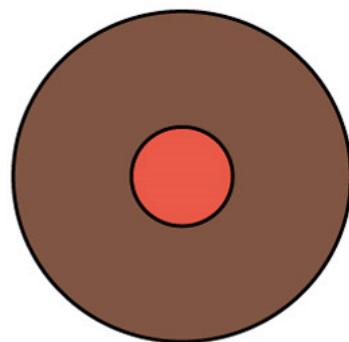
- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants



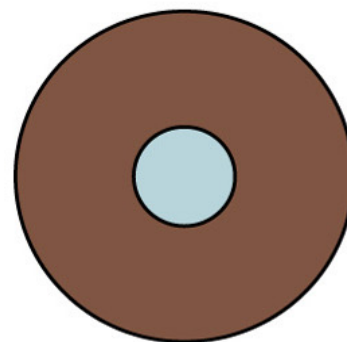
A.



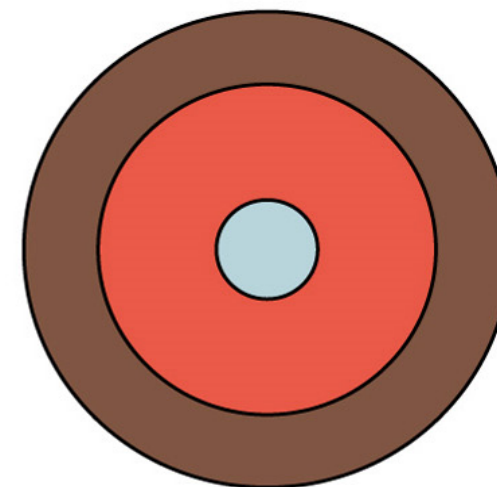
B.



C.



D.



Relative hierarchy  
of cohesiveness

KEY



Relative  
strength of  
adhesion



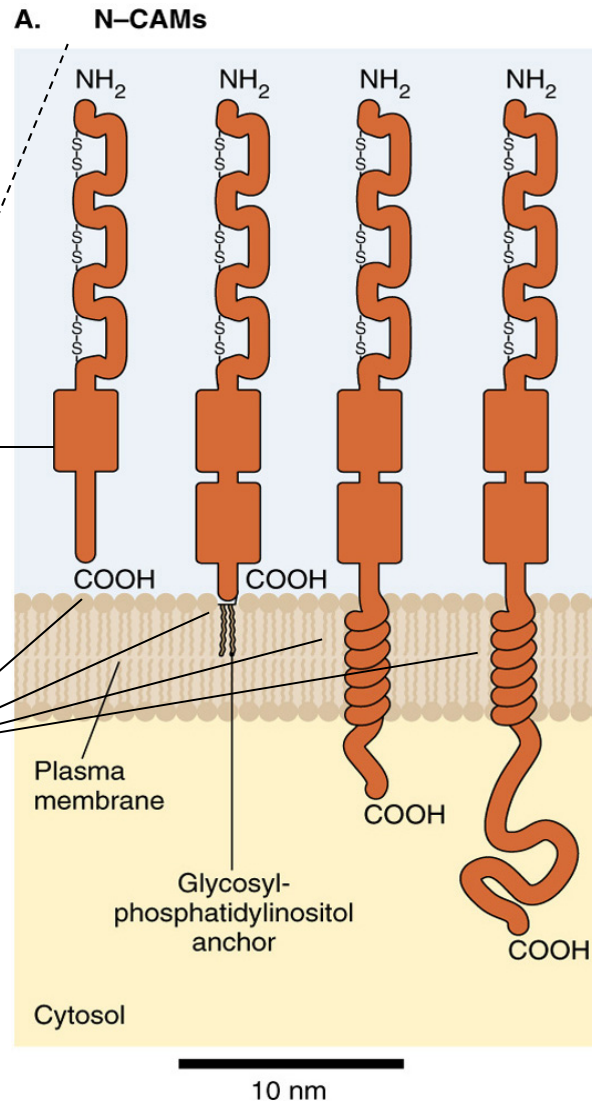


# Cell Adhesion Molecules (CAMs)

- Homophilic
- heterophilic
- With or without  $\text{Ca}^{2+}$
- Membrane-bound or free

5 looped extracellular domains in total (Ig-like)

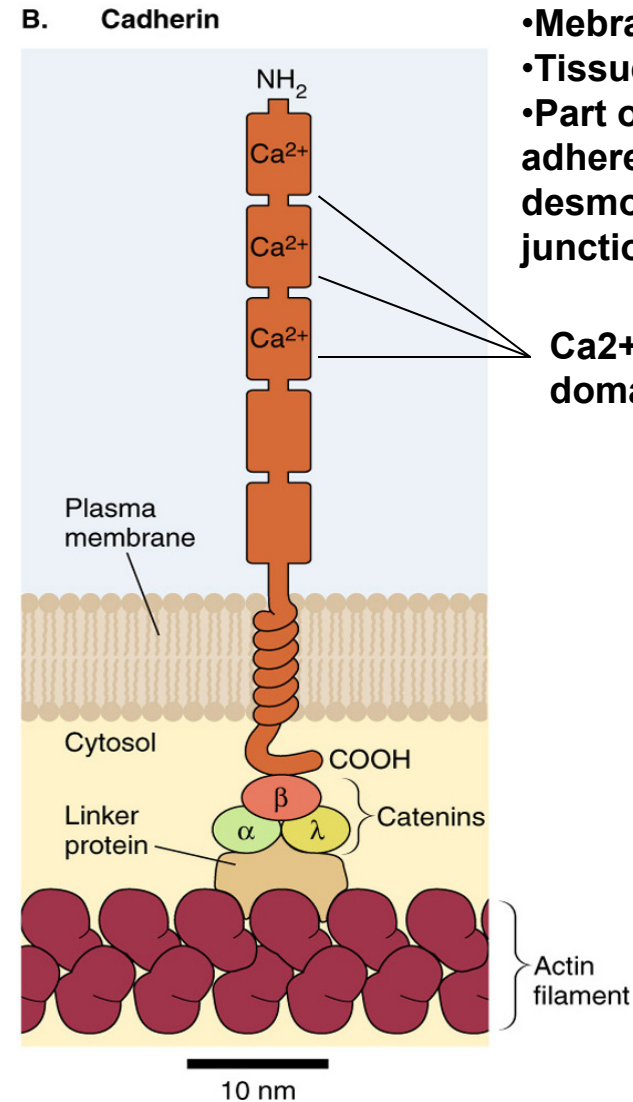
Different subtypes of CAMs



# Cadherins

- Homophilic
- $\text{Ca}^{2+}$ -dependent
- Membrane-bound
- Tissue specific
- Part of the adherens and desmosome junctions

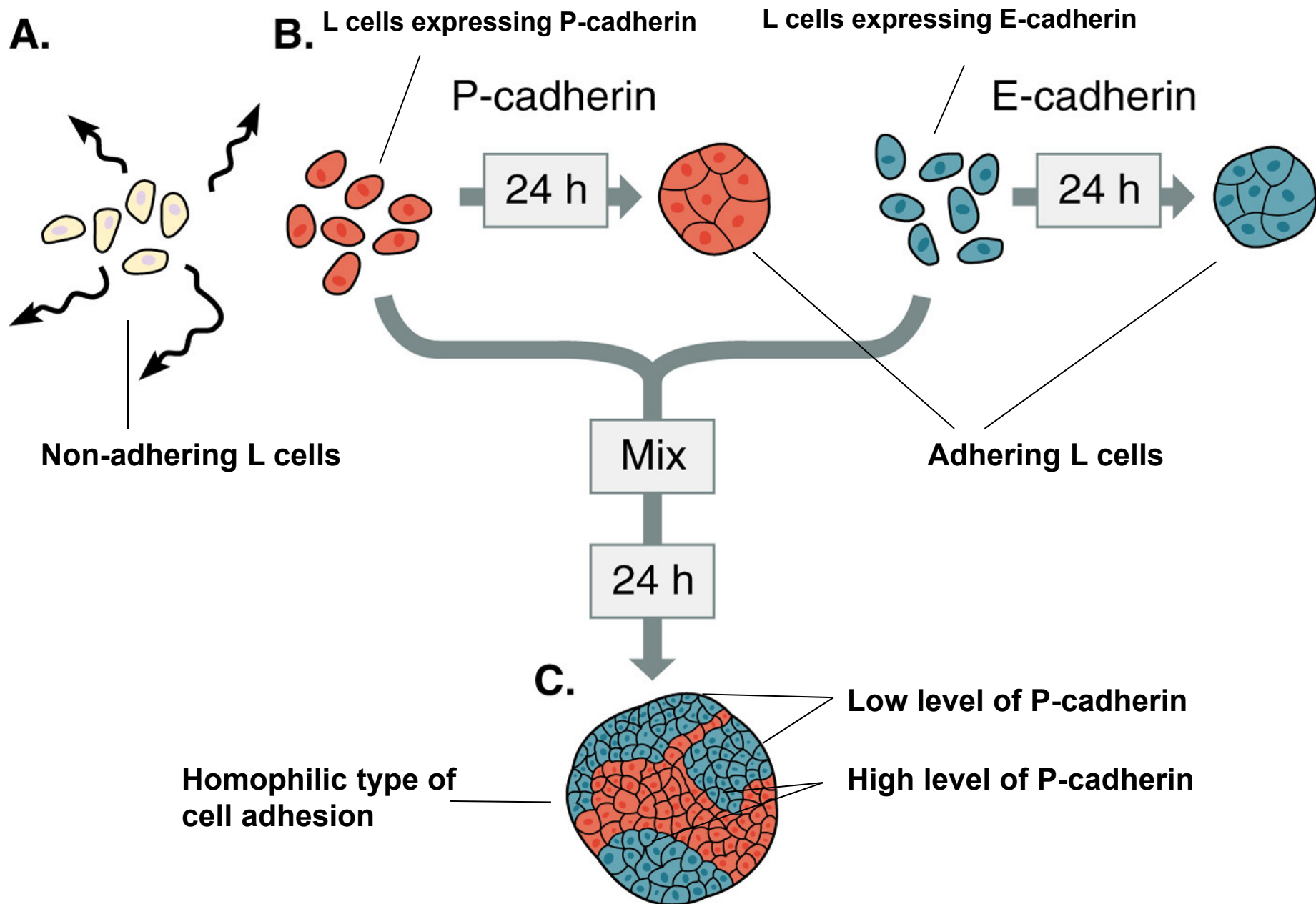
$\text{Ca}^{2+}$  binding domains





## 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 <sup>2+</sup>	Blastomeres
A-CAM	Homophilic	Ca <sup>2+</sup>	Mesoderm, lens, muscle
P-cadherin	Homophilic	Ca <sup>2+</sup>	Endoderm, placenta
N-cadherin	Homophilic	Ca <sup>2+</sup>	Central nervous system
EP-cadherin (C-cadherin)	Homophilic	Ca <sup>2+</sup>	Cleavage stage blastomeres
Integrins	Heterophilic	Varies	Extracellular matrix







# Outline of Lesson 9

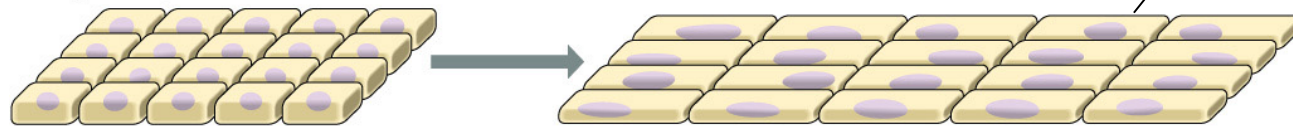
## Morphogenesis

- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
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  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres

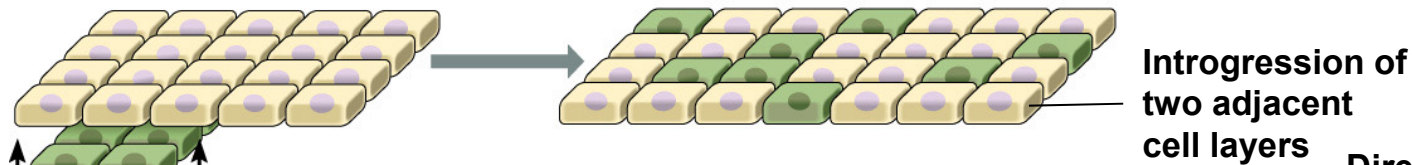


# Morphogenic maneuvers

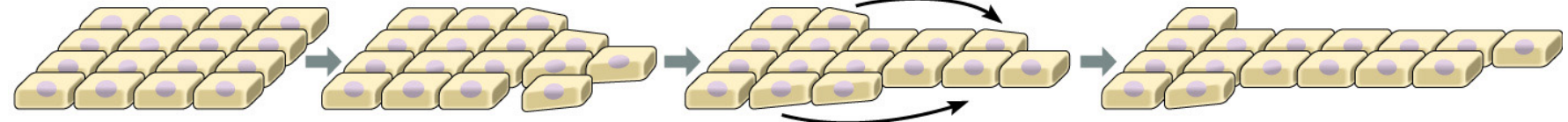
## Epibolie A. Epiboly



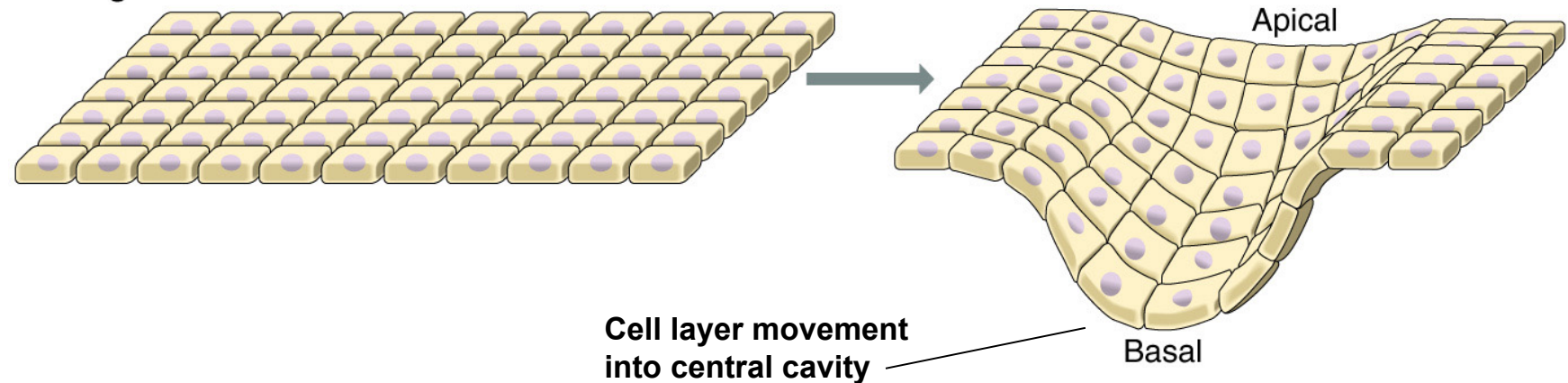
## Interkalace B. Intercalation



## Konvergentní extenze C. Convergent extension

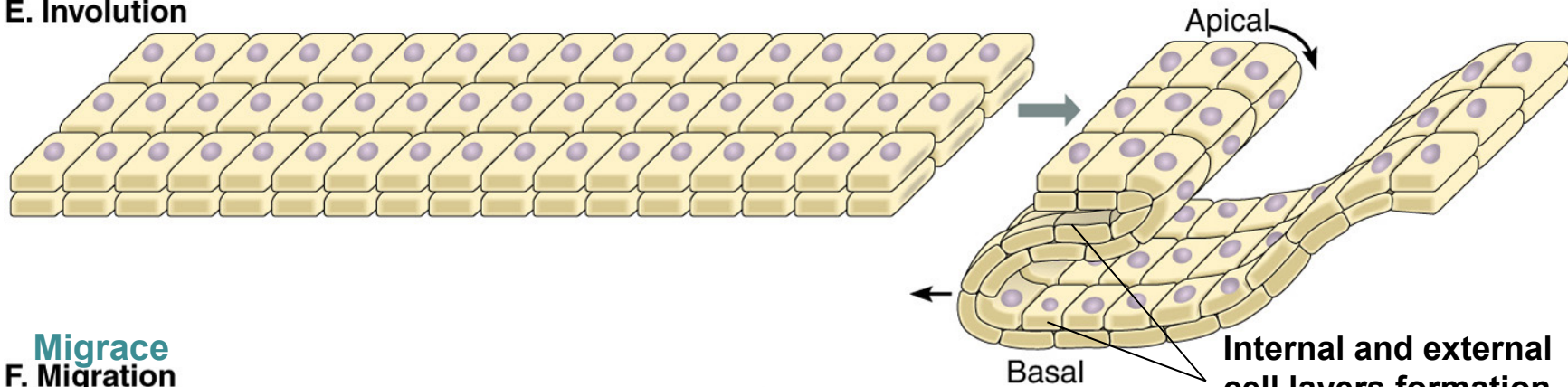


## Invaginace D. Invagination

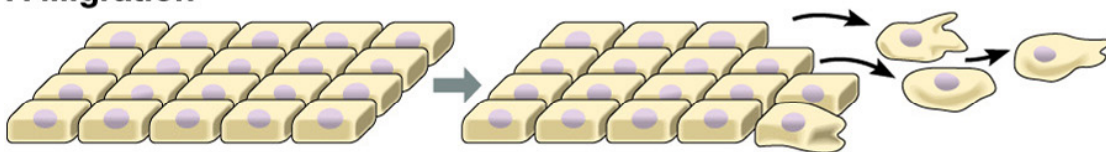


# Morphogenic maneuvers

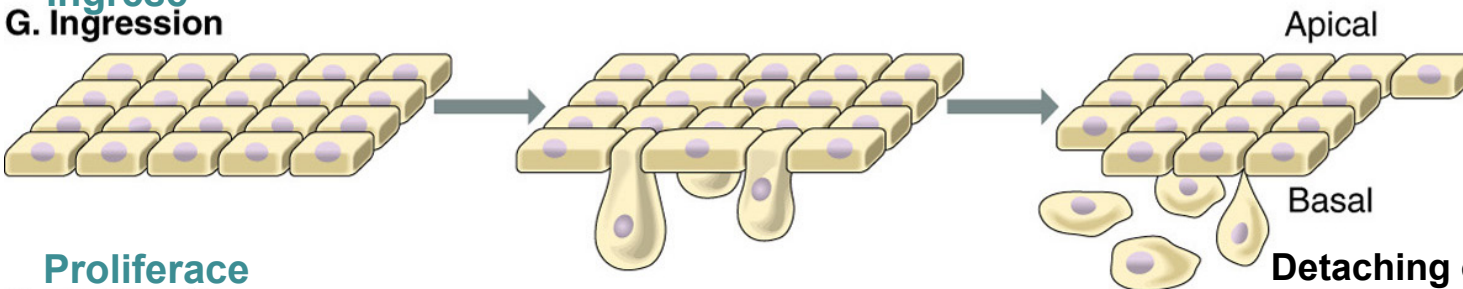
## Involuce E. Involution



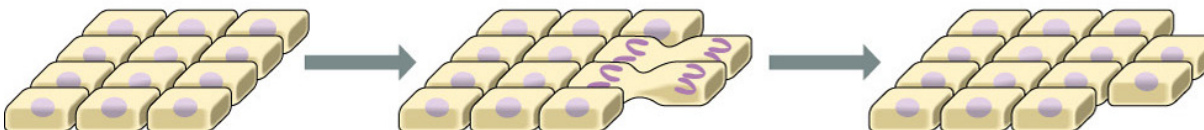
## Migrace F. Migration



## Ingrese G. Ingression

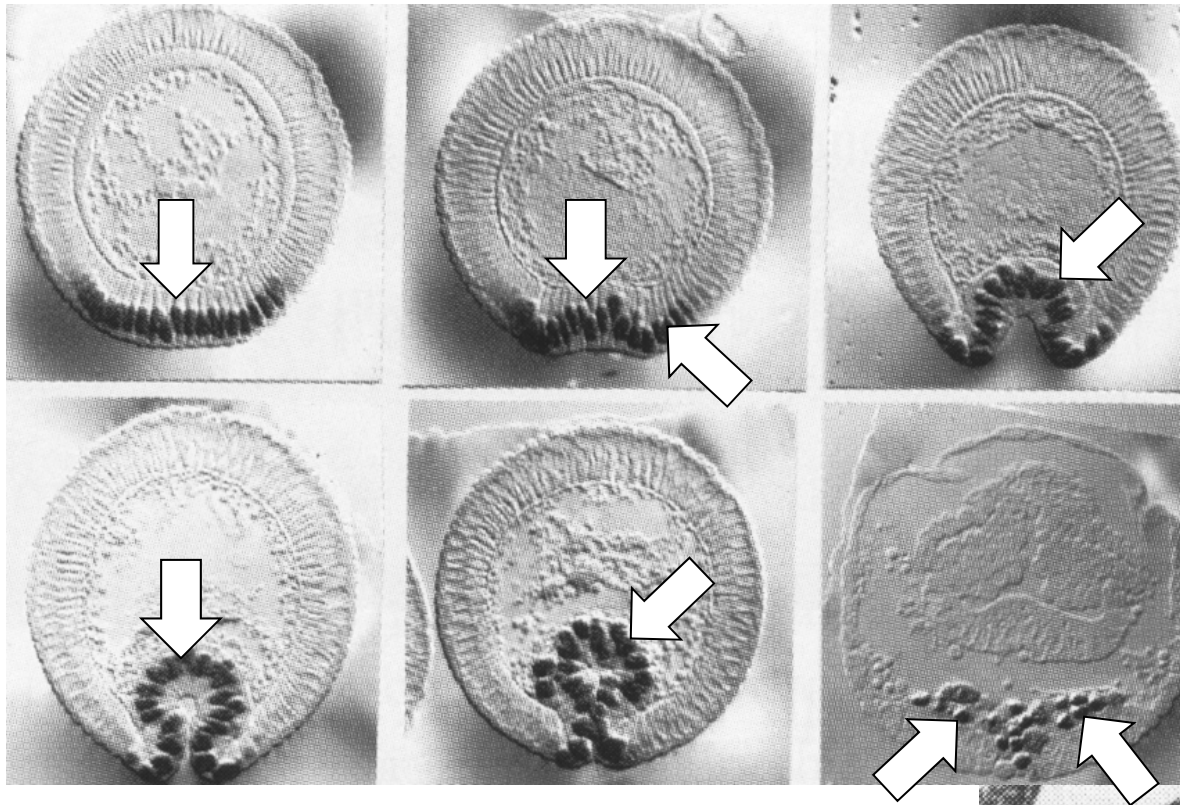


## Proliferace H. Proliferation

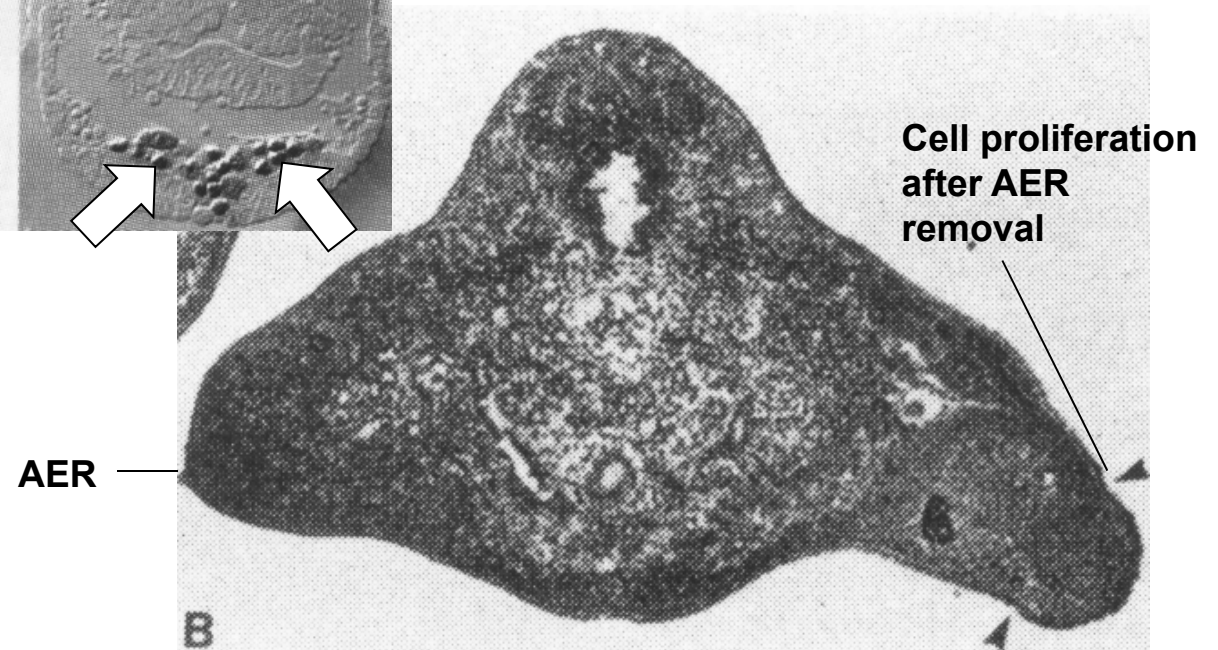




# TWIST localization during neural furrow formation in *Drosophila*



## Regulation of cell proliferation by AER in mouse



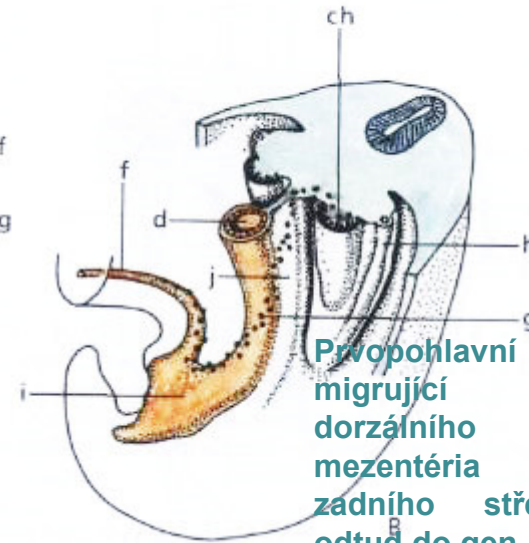
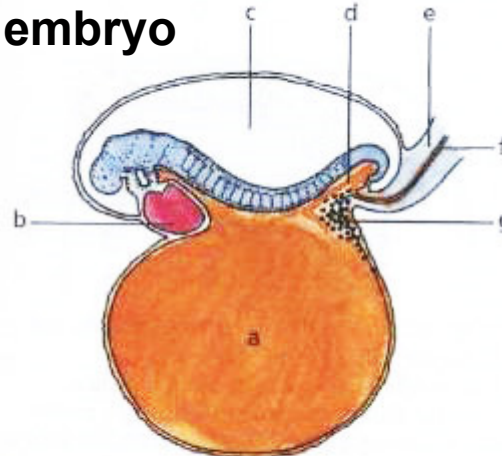


# Outline of Lesson 9

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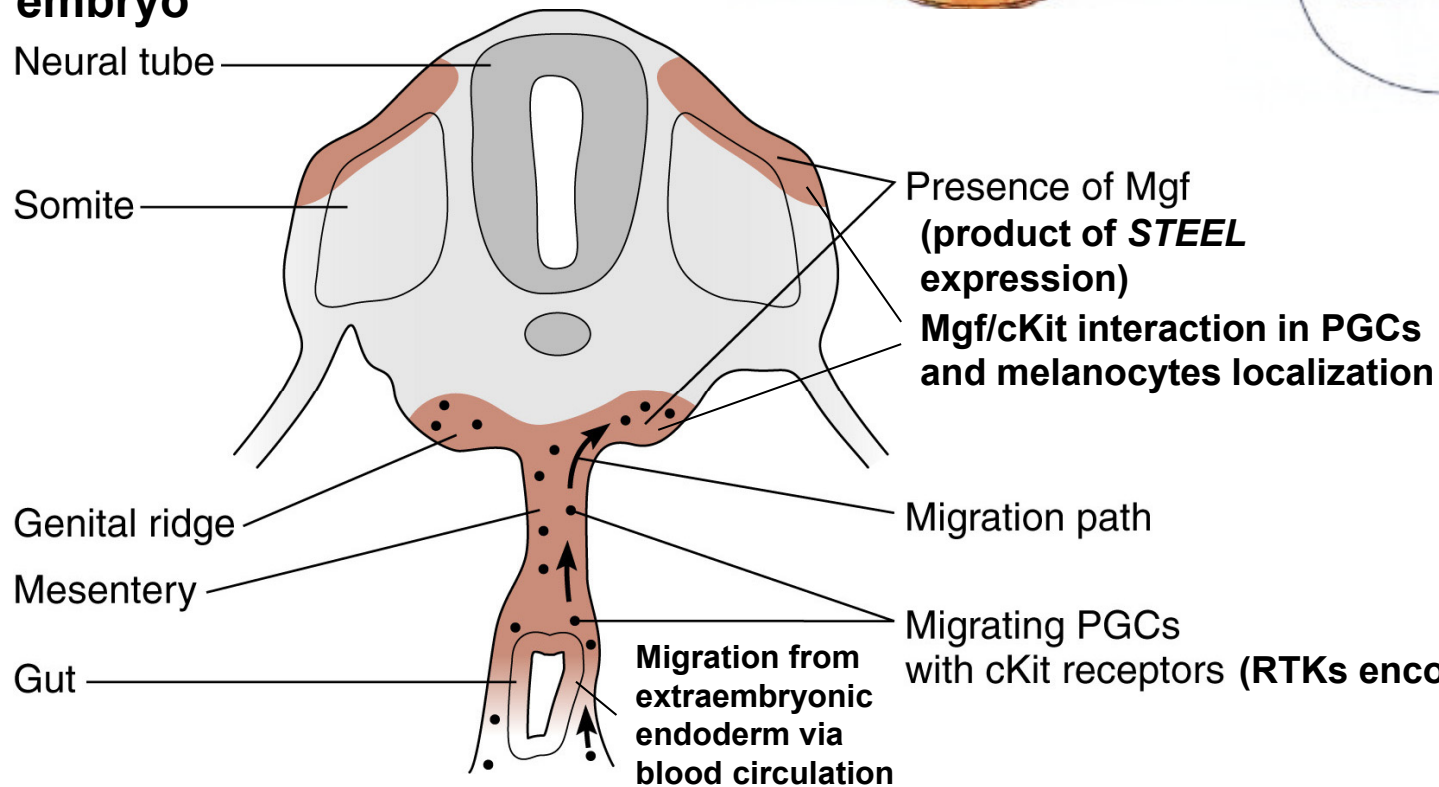
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## PGCs migration in human embryo



Prvopohlavní buňky migrují podél dorzálního mezentéria do zadního střeva a odtud do gen. Lišty  
PGCs migrating along the dorsal mesentery into the hindgut and from there into the genital ridge.

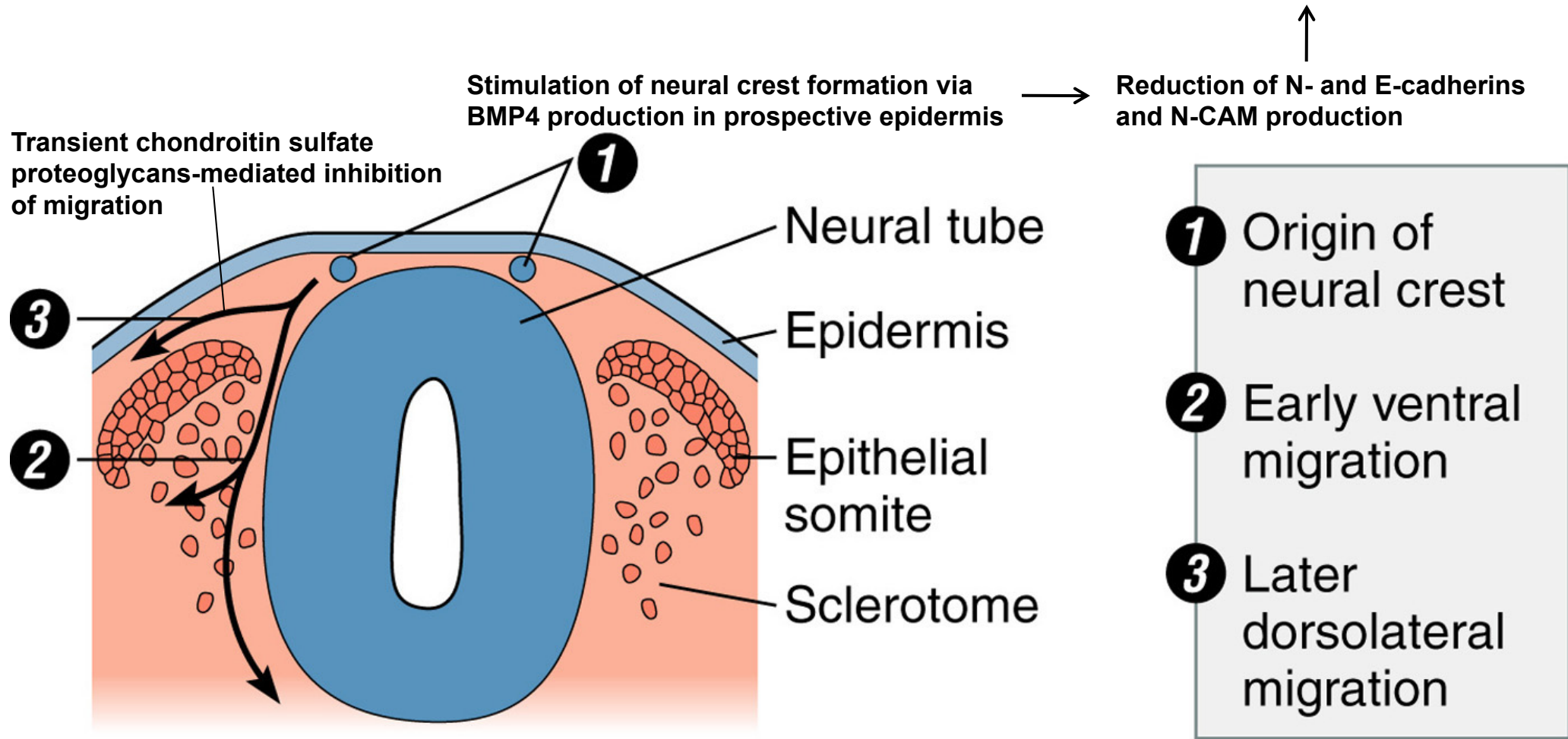
## PGCs migration in chicken embryo

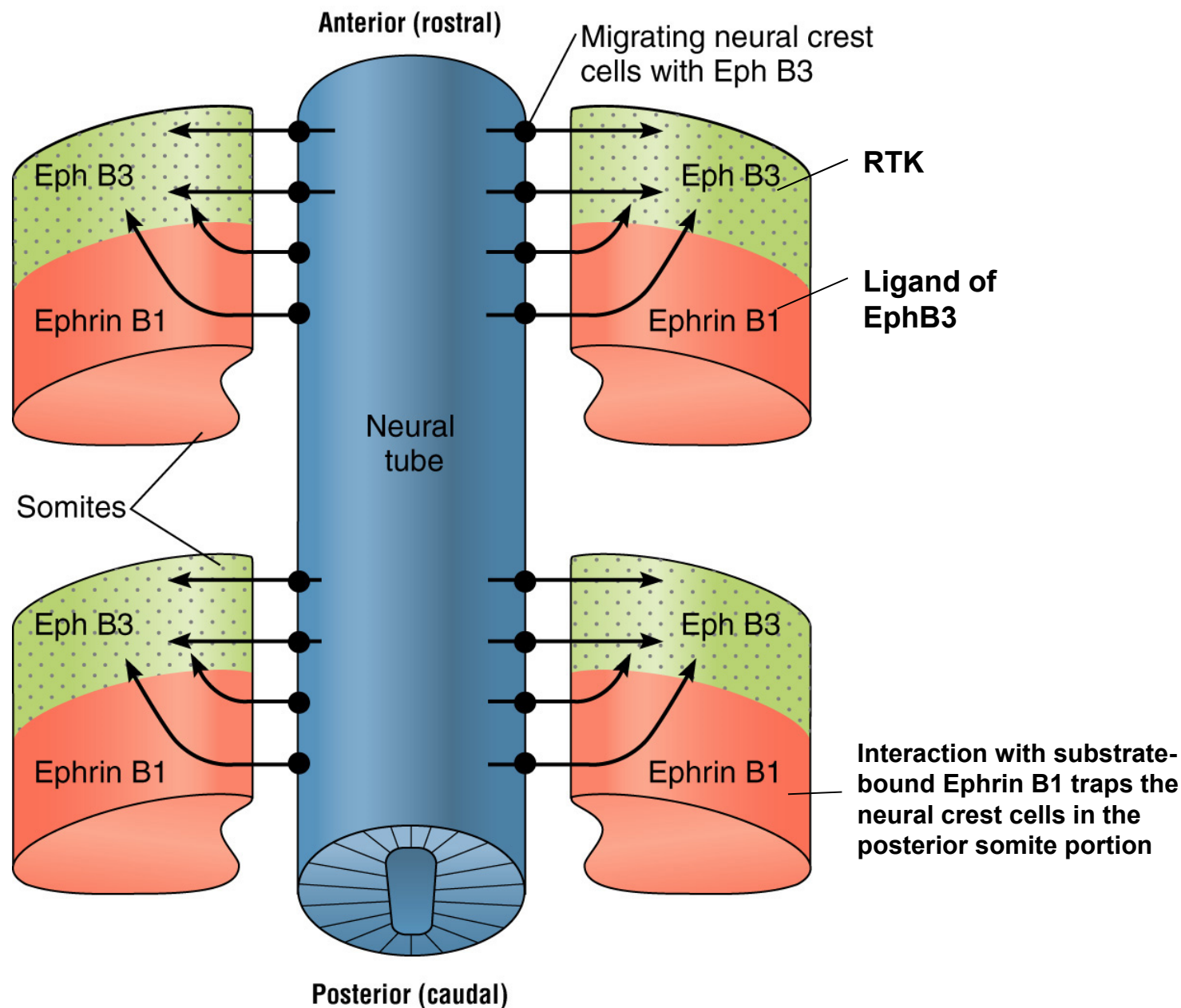


Vacek, *Embryologie* (2006)

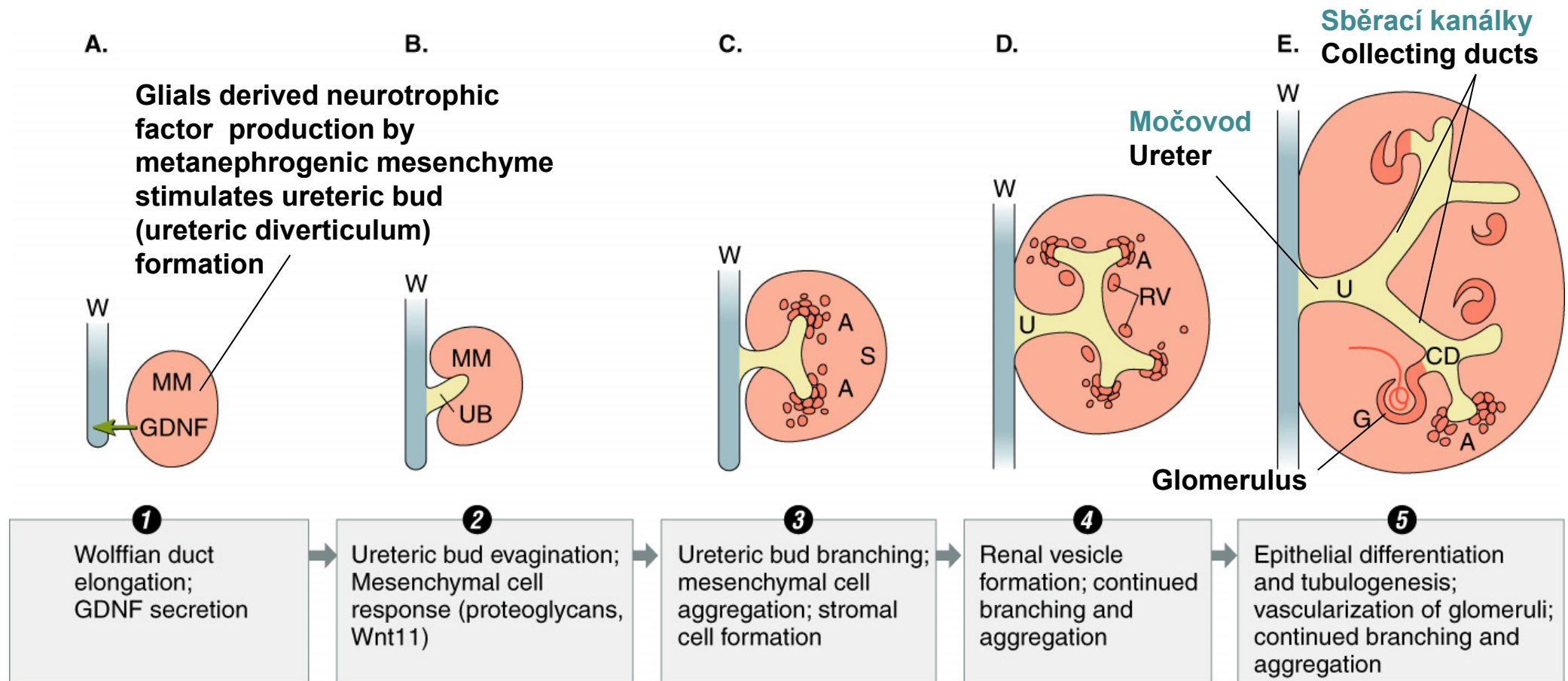


# Neural crest cells migration



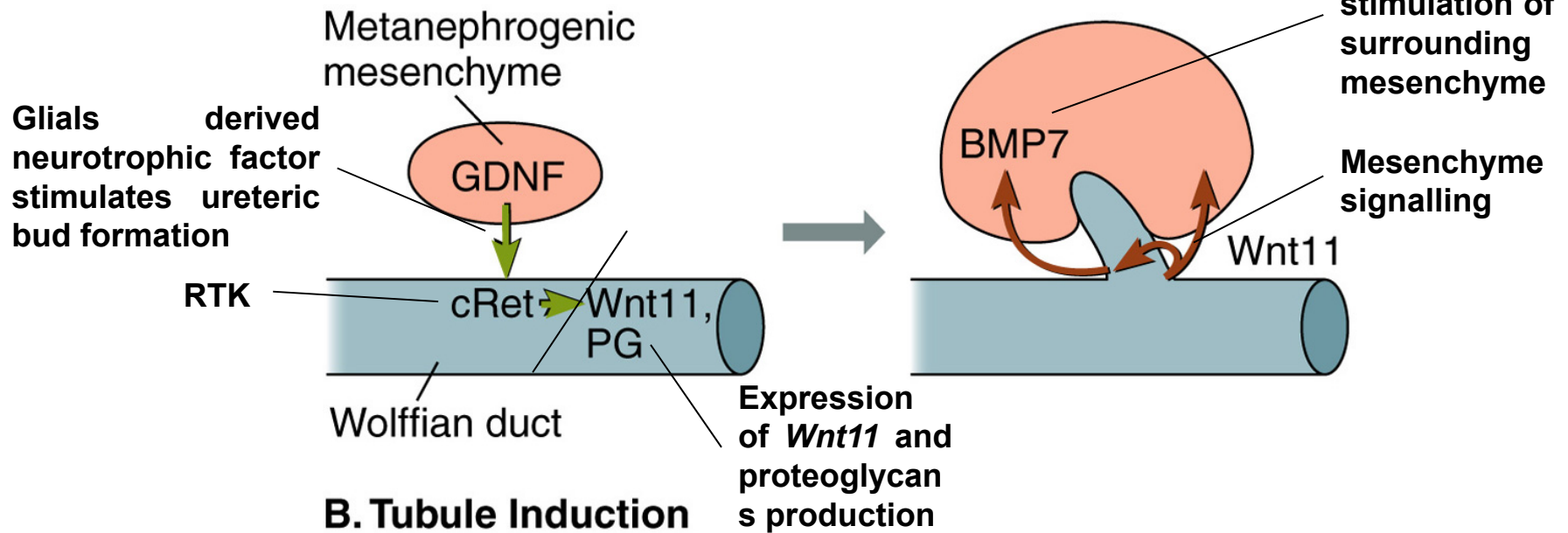


# Kidney development

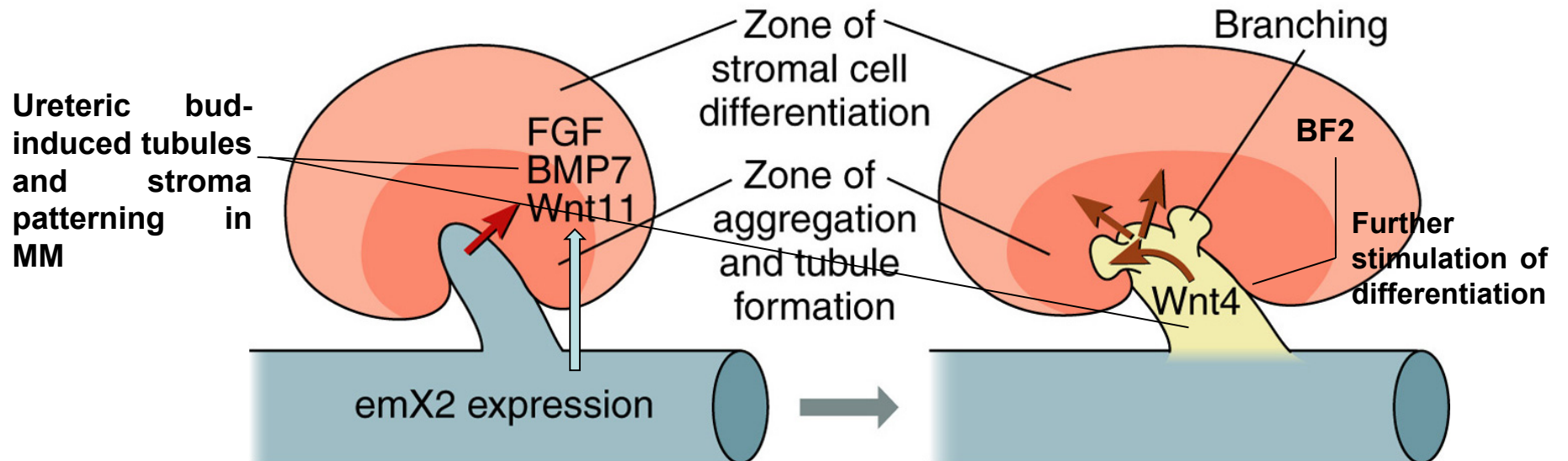




## A. Ureteric Bud Induction



## B. Tubule Induction



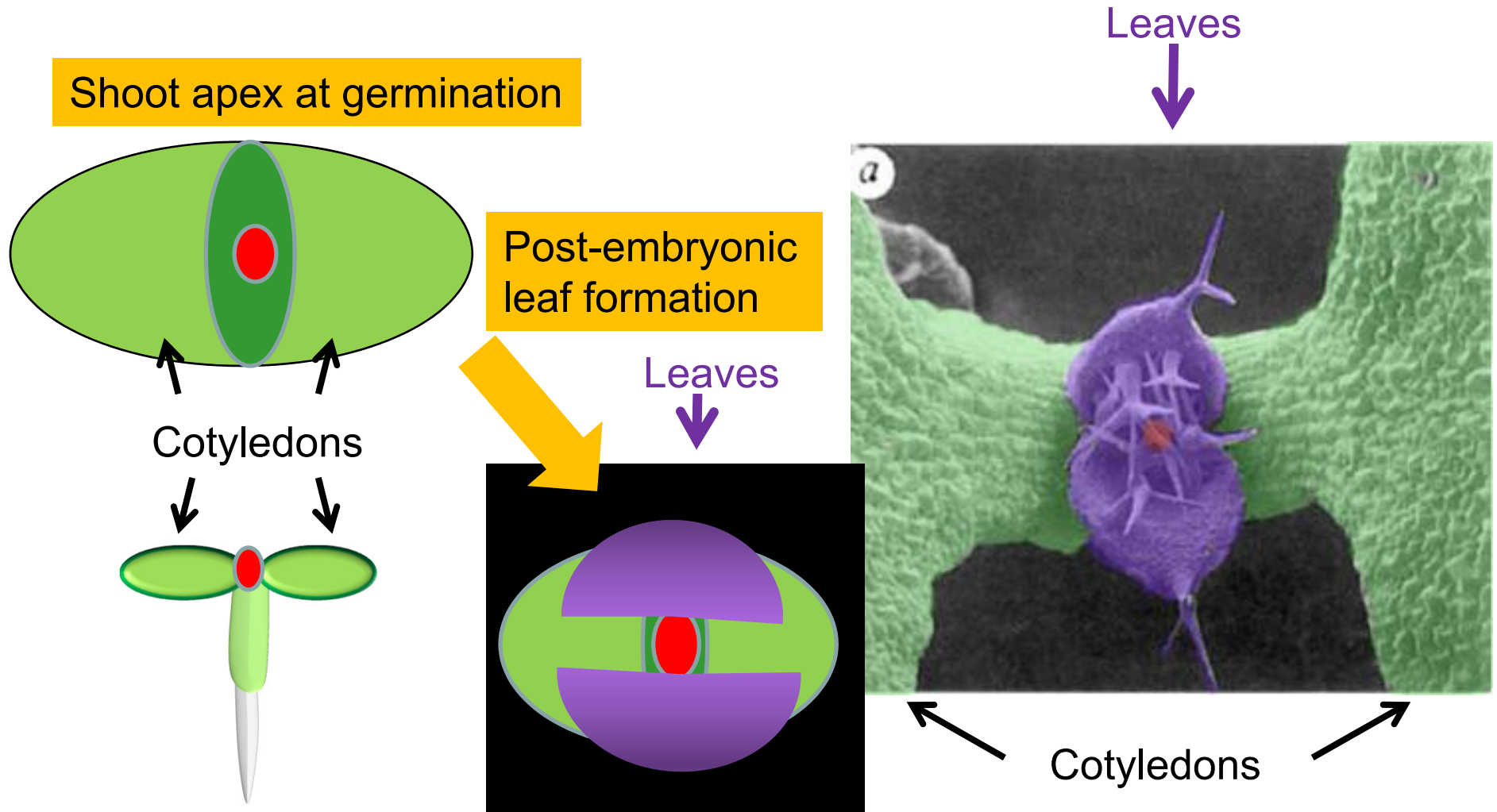


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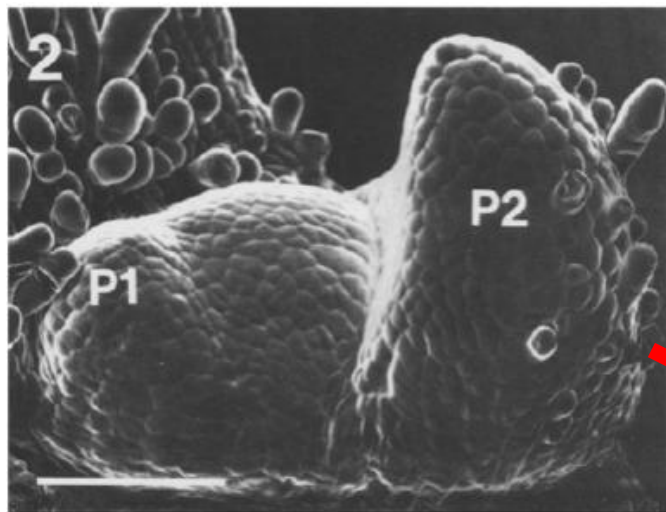
# Origin of leaves



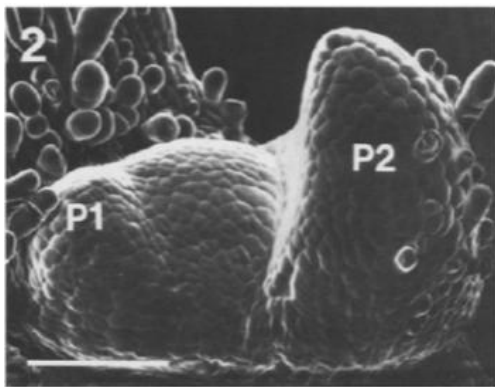
Reprinted by permission from Macmillan Publishers, Ltd: [NATURE](#). Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K. (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* 379: [66-69](#).



# How does a leaf primordium become a leaf?



# How does a leaf primordium become a leaf?



0.2 mm

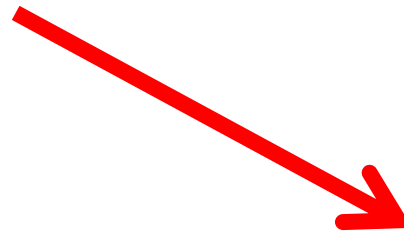
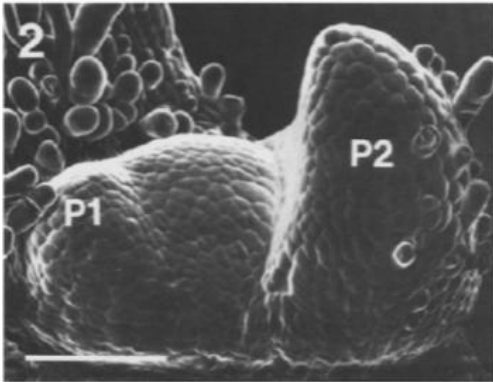
GROWTH



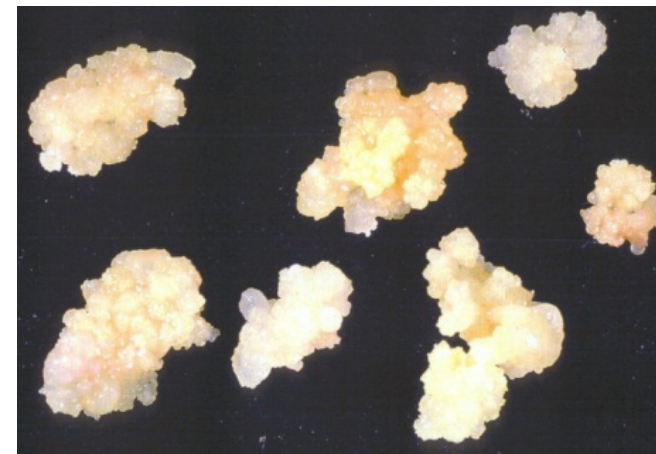
500 mm

The leaf primordium increases in length ~ 2500 fold by cell division and cell expansion.

# How does a leaf primordium become a leaf?

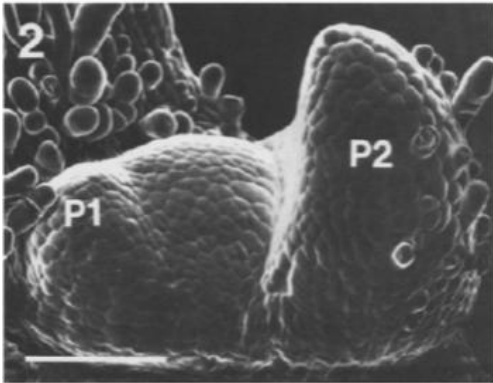


But unregulated growth doesn't make a leaf, it makes an undifferentiated tissue called callus

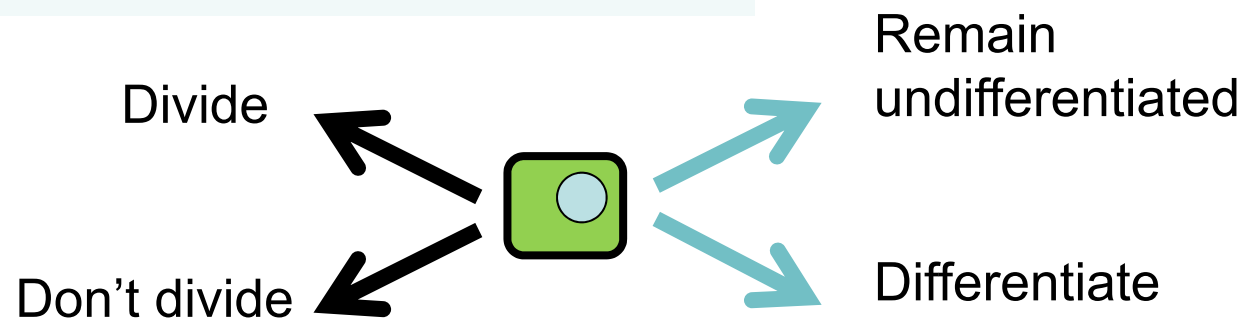




# How does a leaf primordium become a leaf?



To make a leaf, each cell in the primordium must divide, grow and differentiate in a controlled way.



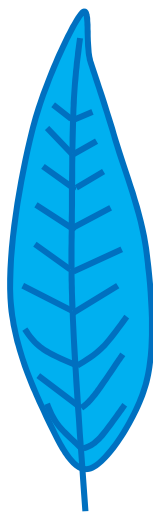
- Leaf diversity
- What determines leaf size and shape?
- What determines if a leaf is simple or compound?
- What controls cell differentiation?



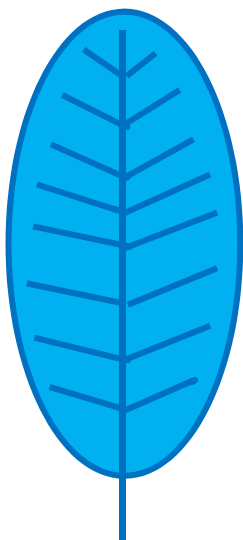
# Leaf forms



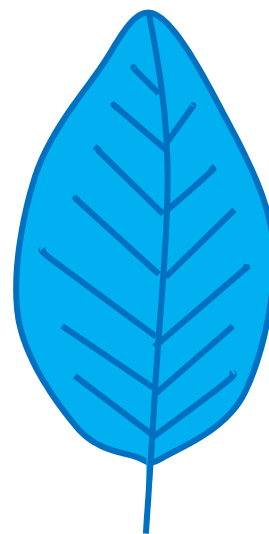
Podlouhlý  
Linear



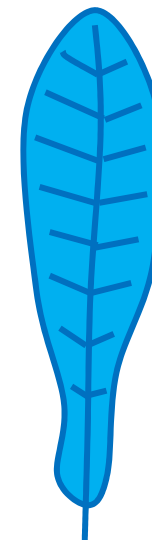
Kopinatý  
Lanceolate



Eliptický  
Elliptical



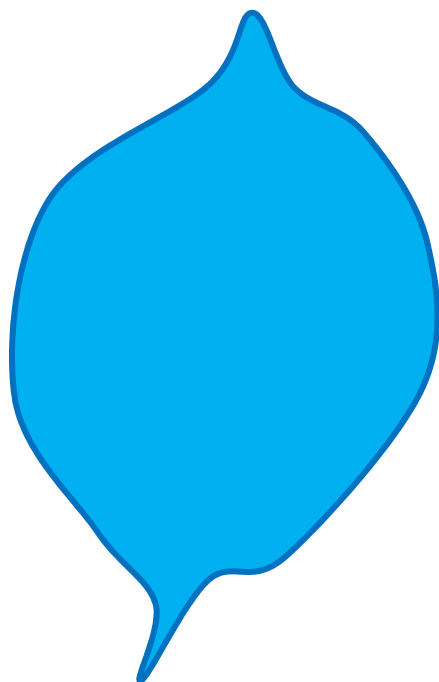
Vejčitý  
Ovate



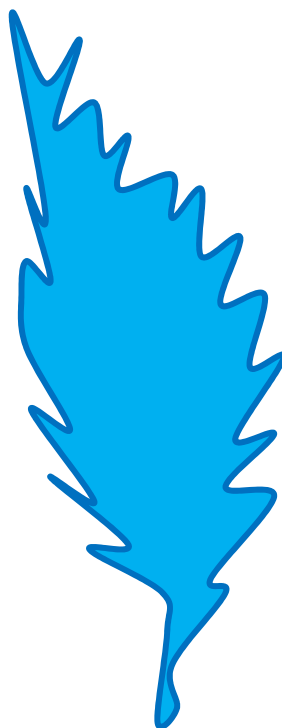
Kopistovitý  
Spatulate



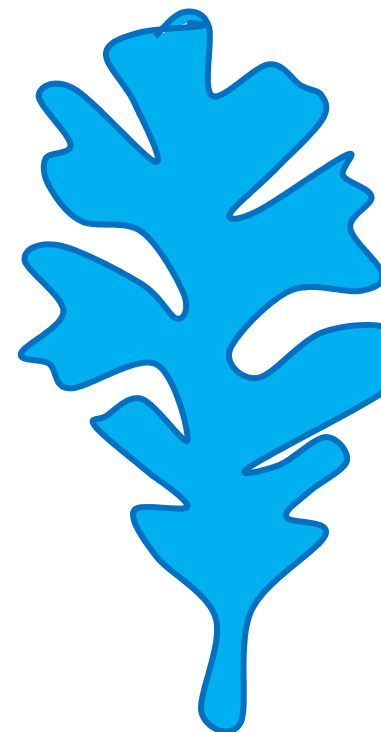
# Leaf forms



Hladký  
Smooth

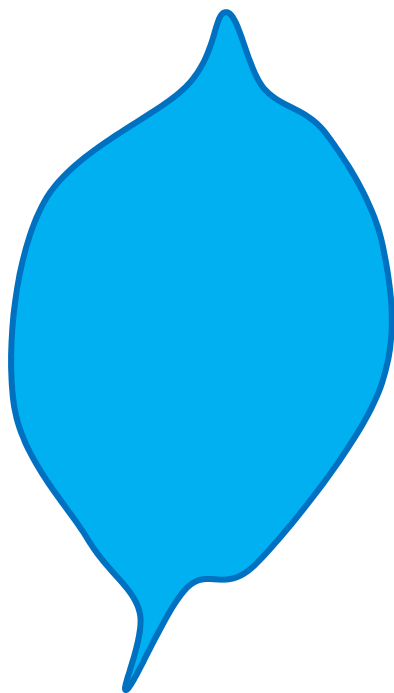


Laločnatý  
Serrated



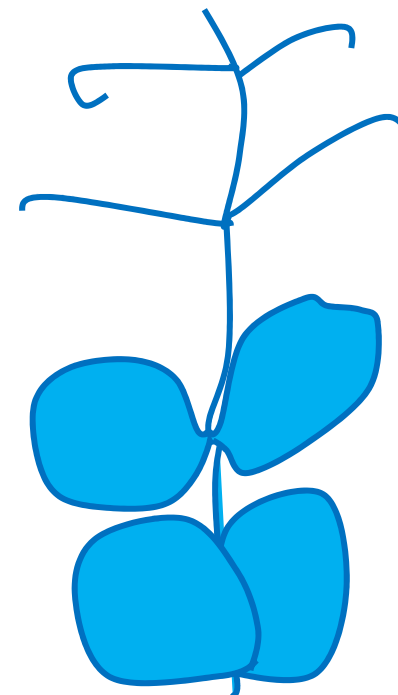
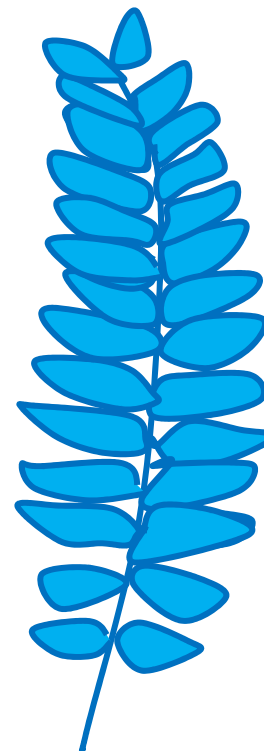
Dílný/Sečný  
Lobed

# Leaf forms



---

Jednoduchý  
Simple



---

Složený  
Compound



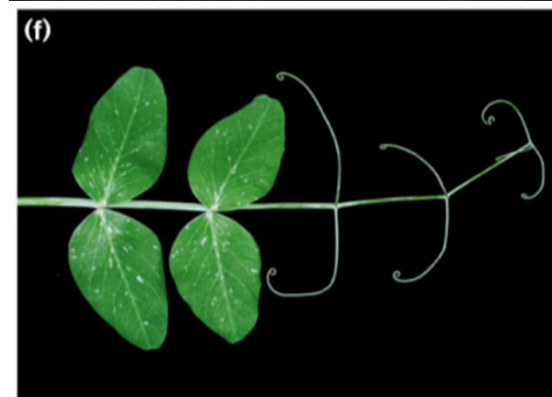
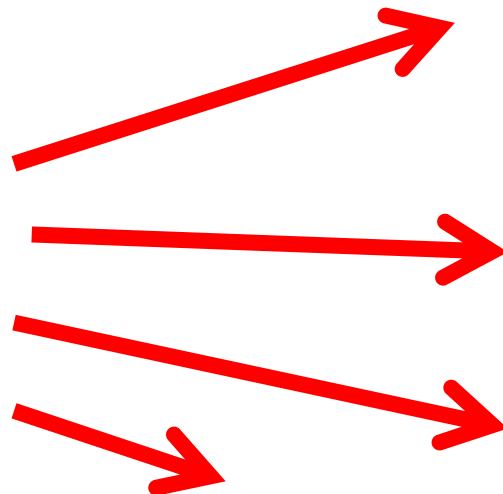
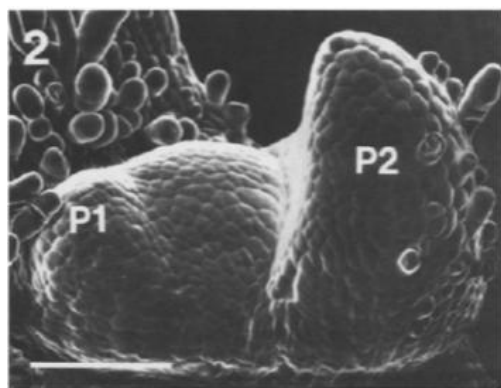
# Outline of Lesson 9

## 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



# What determines the size and shape of a leaf?



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



Uniform  
growth



Differential  
growth



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

# 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



Reprinted by permission from Macmillan Publishers, Ltd: [NATURE](#) 425: 257-263. Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D. Control of leaf morphogenesis by microRNAs. Copyright (2003).



# Increasing the number of cell divisions increases leaf size



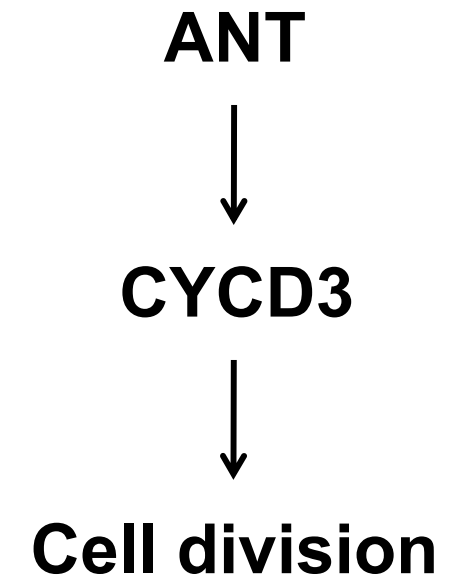
*ant-1*

WT

ANT-OX

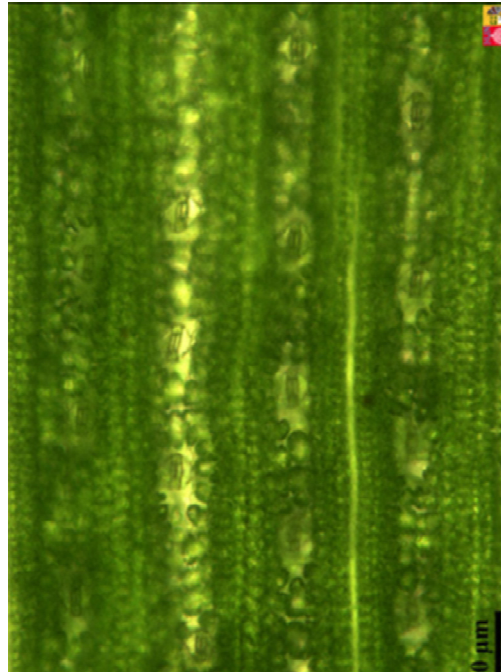


Increasing number of cell cycles in developing leaf



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.

# Patterns of cell divisions (and expansion) determine leaf shape



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

# Monocot leaves grow linearly

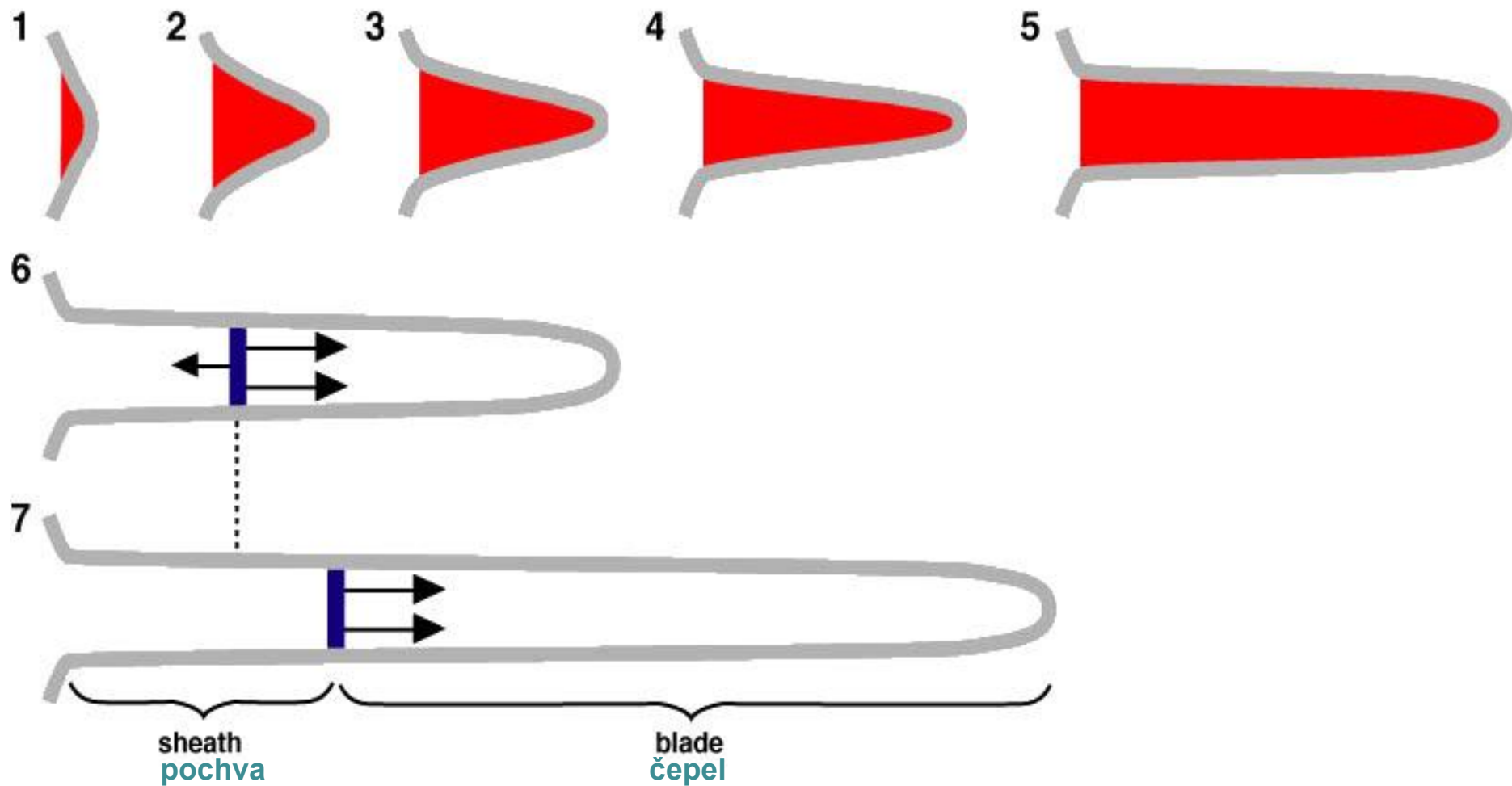


Image courtesy of J. Derksen, J. Hiddink and E.S. Pierson Copyright Radboud University Nijmegen



# Leaf growth in dicots

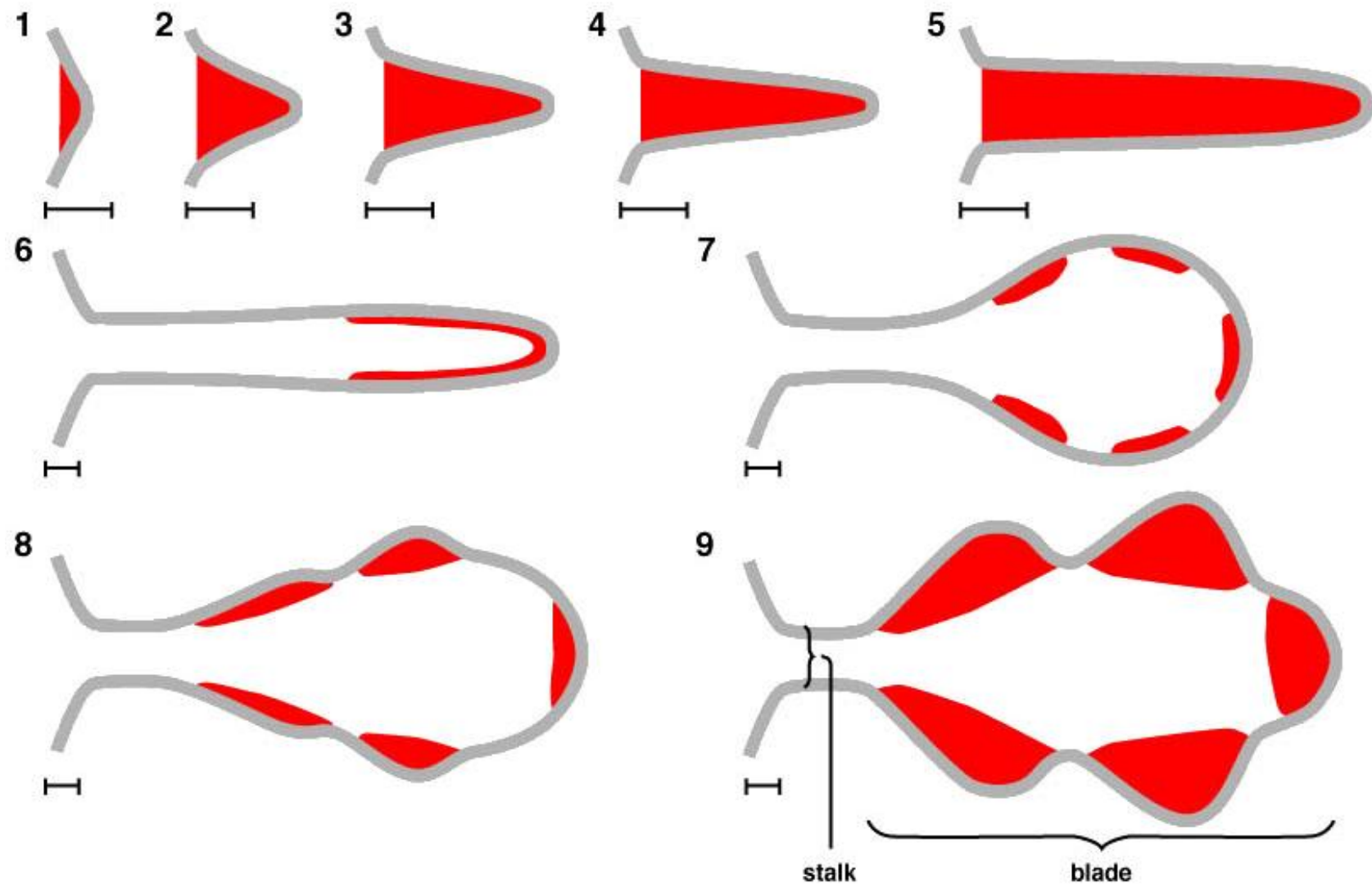


Image courtesy of J. Derksen, J. Hiddink and E.S. Pierson Copyright Radboud University Nijmegen

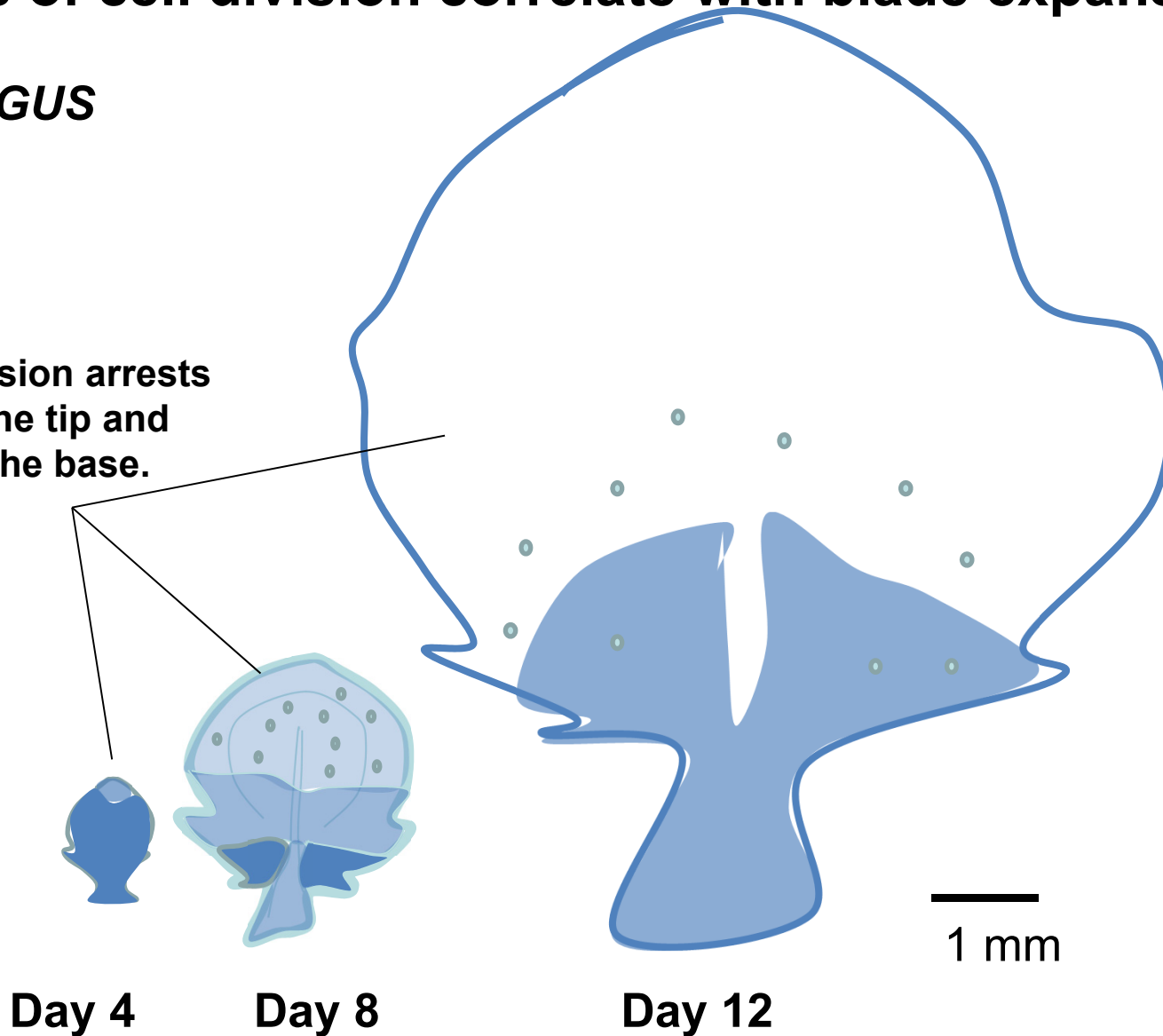




# Patterns of cell division correlate with blade expansion

*ProCYC:GUS*

Cell division arrests  
first at the tip and  
later at the base.



Redrawn from Donnelly et al., (1999) Dev Biol 215: 407-419.



# Outline of Lesson 9

## Morphogenesis

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- Morphogenesis in plants
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  - The role of oriented cell division and its relative distribution
    - Regulation of cell division by TCP and boundary genes

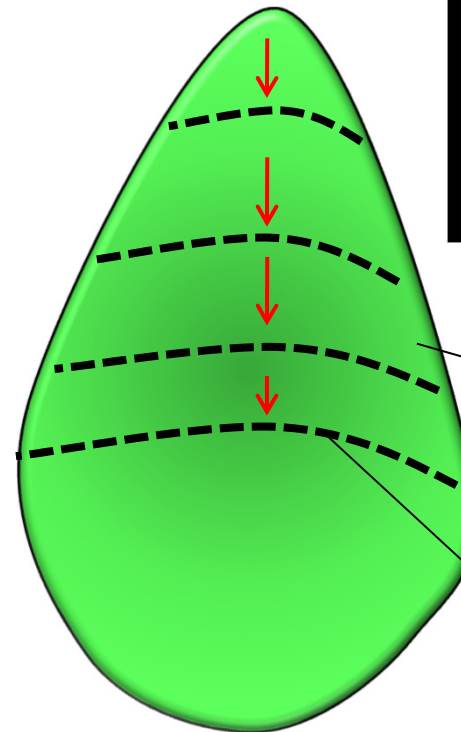
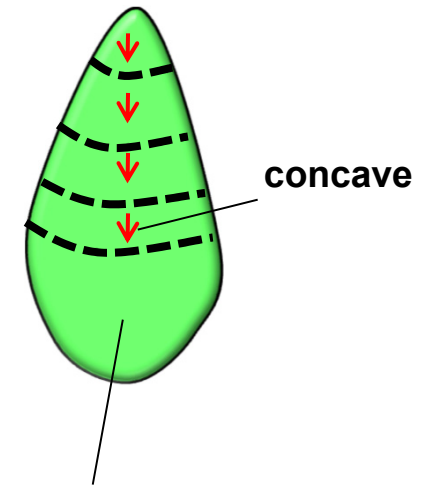
# Altering the pattern of cell divisions alters leaf shape

*CINCINNATA* (*CIN*) encodes a TCP-type transcription factor



***cincinnata (cin)***

**WT**



Cell cycle arrest progression

convex

Crawford, B.C.W., Nath, U., Carpenter, R., and Coen, E.S. (2004) *CINCINNATA* controls both cell differentiation and growth in petal lobes and leaves of *Antirrhinum*. *Plant Physiol.* 135: [244253](#).

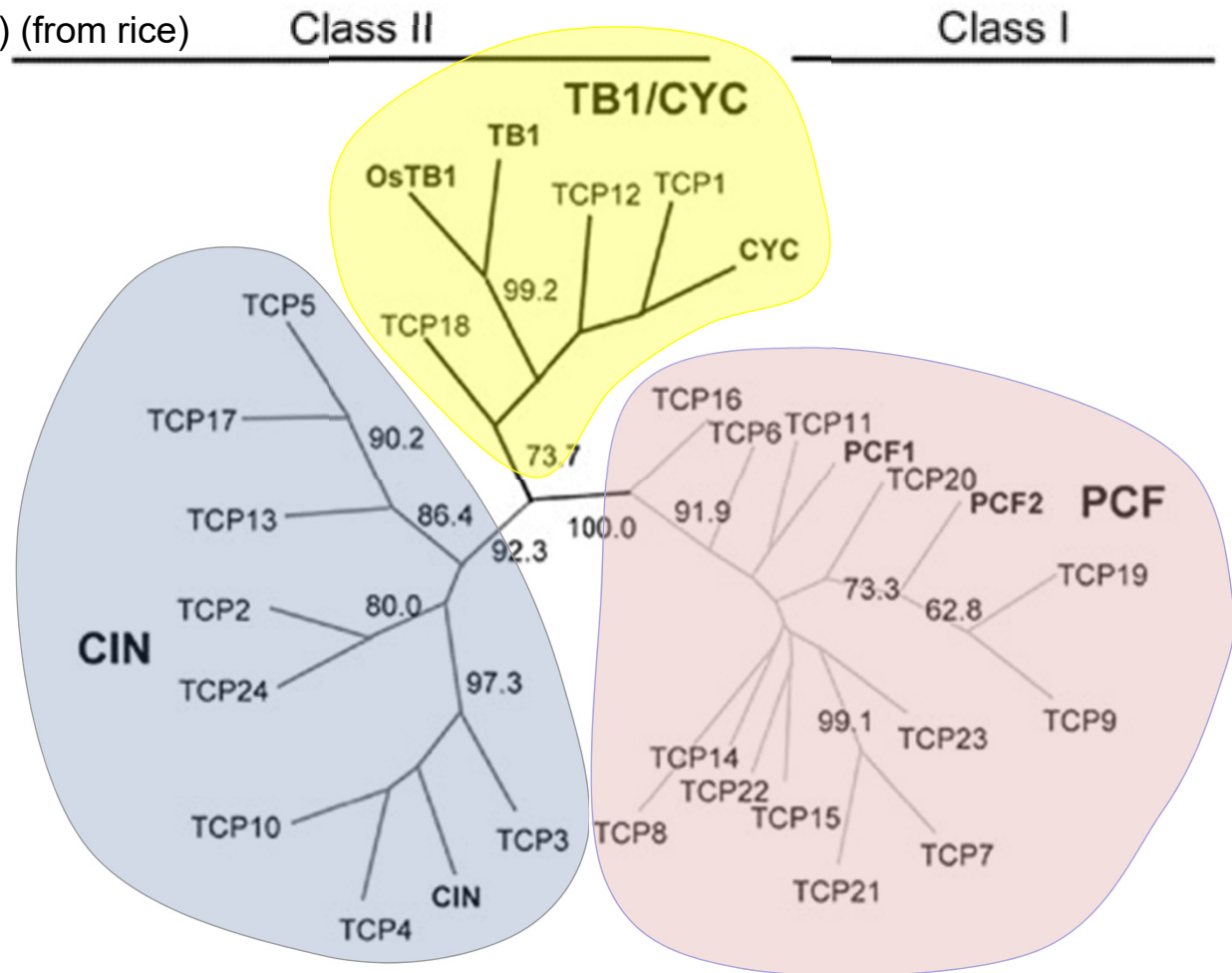
# TCP genes

- *TEOSINTE BRANCHED1* (*TB1*) (from maize),
- *CYCLOIDEA* (*CYC*) (from *Antirrhinum*), and
- *PROLIFERATING CELL FACTOR* (*PCF*) (from rice)

Basic-helix-loop-helix TFs



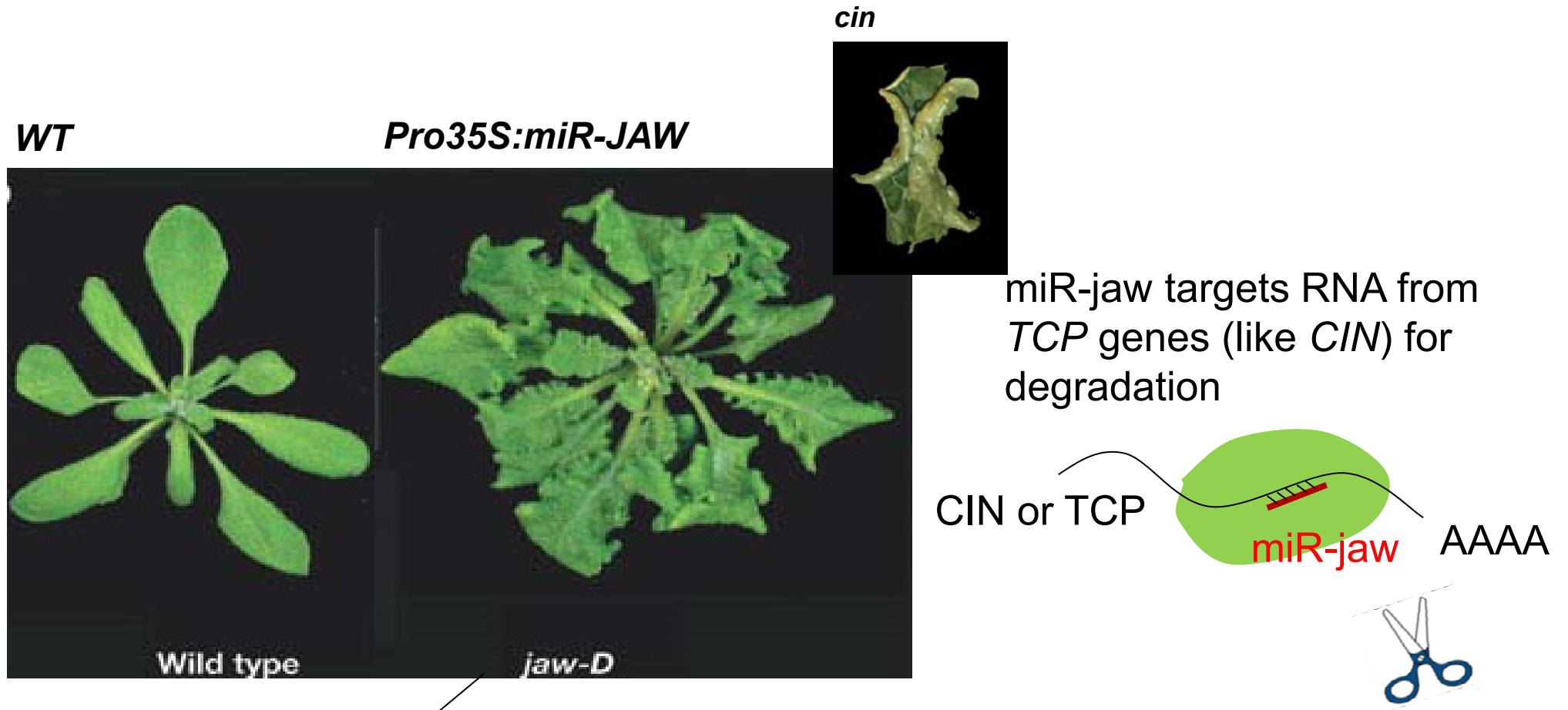
Cell division



Aguilar-Martínez, J.A., Poza-Carrón, C., and Cubas, P. (2007) *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds. *Plant Cell* 19:458-472.



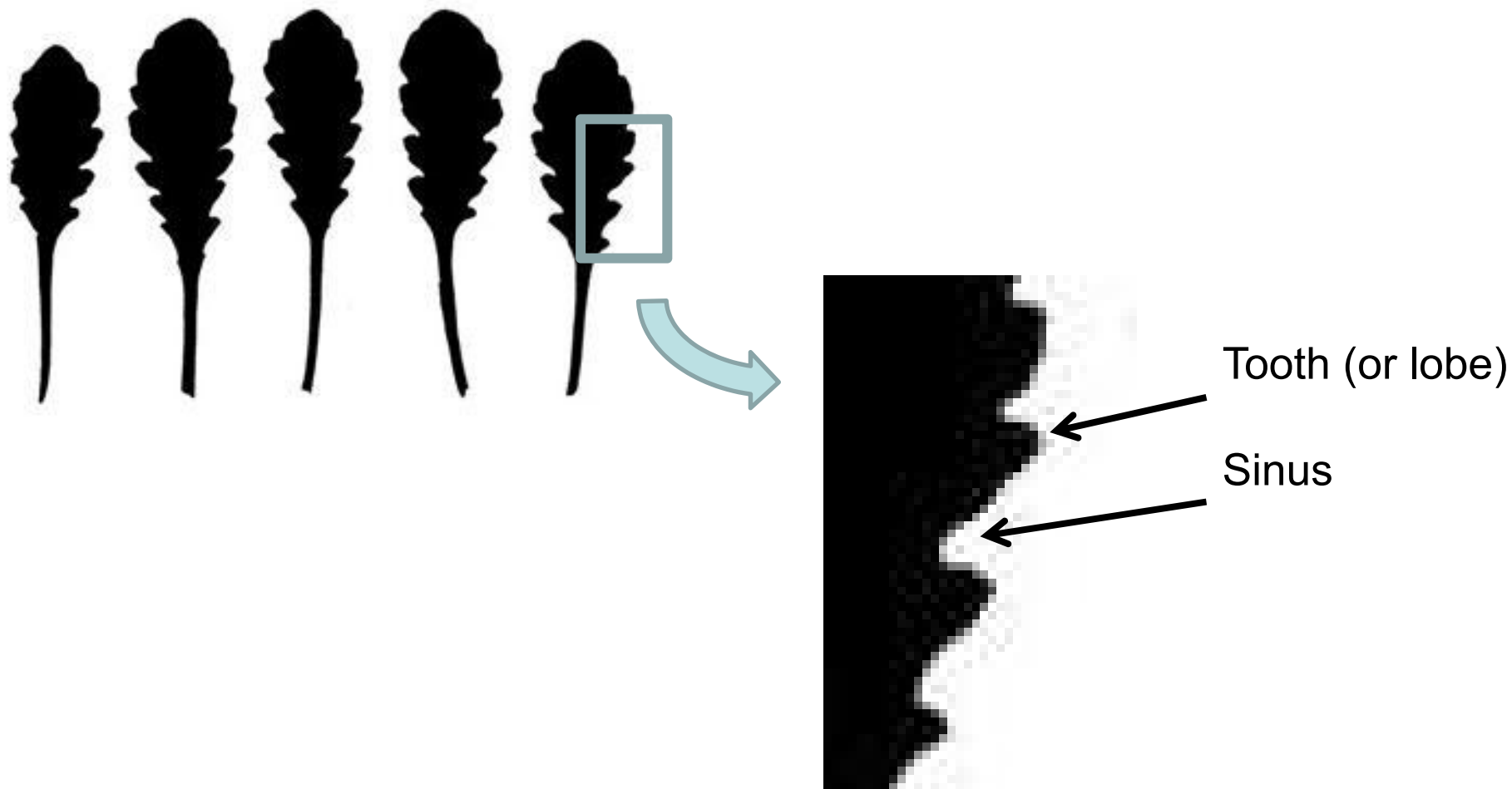
# Over-accumulation of a miRNA (miR-JAW, in the *jaw-D* mutant) causes a similar phenotype



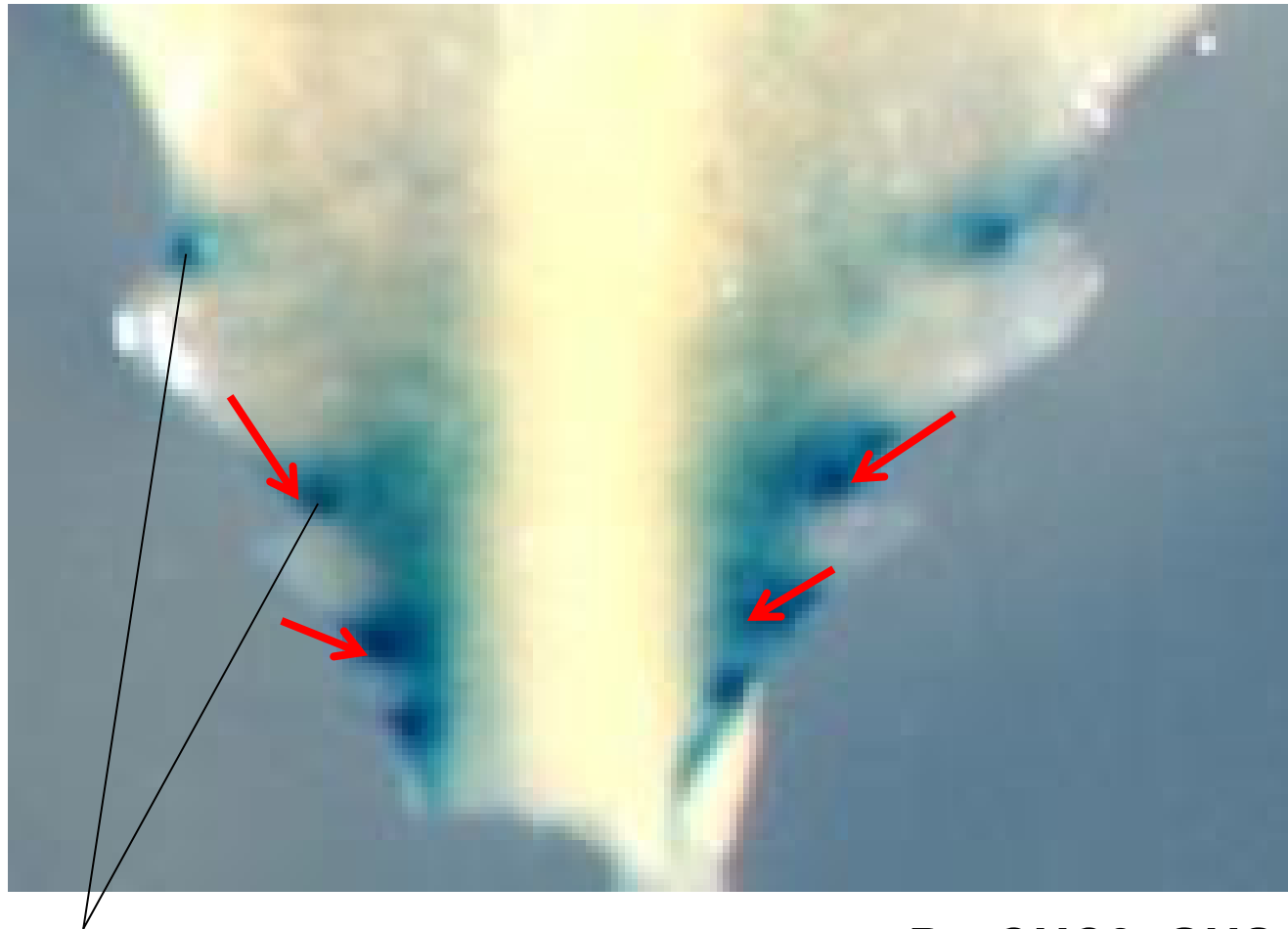
**Phenocopy of *cinninnata***

Reprinted by permission from Macmillan Publishers, Ltd: [NATURE](#) 425: 257-263. Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D. Control of leaf morphogenesis by microRNAs. Copyright (2003).

# Control of cell divisions underlies growth of leaf margins



# Control of cell divisions underlies growth of leaf margins



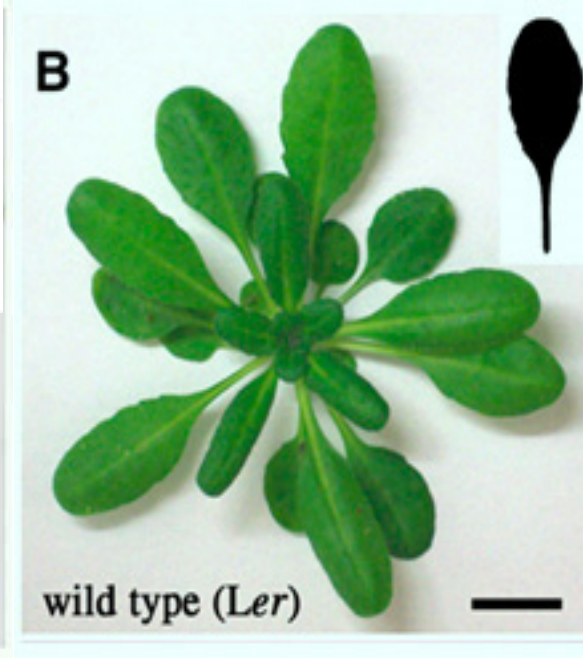
***CUC2* contributes to the formation of serrations**

***ProCUC2 :GUS***

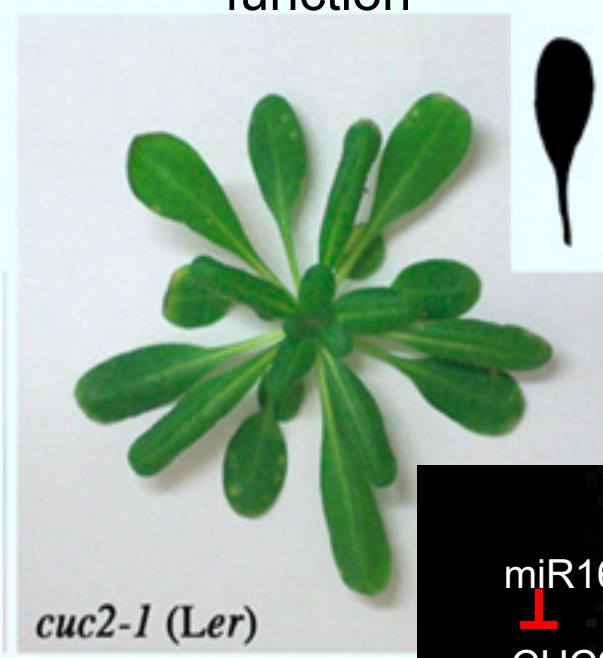
Nikovics, K., Blein, T., Peaucelle, A., Isida, T., Morin, H., Aida, M., and Laufs, P. (2006) The balance between the *MIR164A* and *CUC2* genes controls leaf margin serration in Arabidopsis. Plant Cell 18: [2929-2945](#).

# *CUC2* expression is controlled by *miR164*

Loss of *miR164*  
function

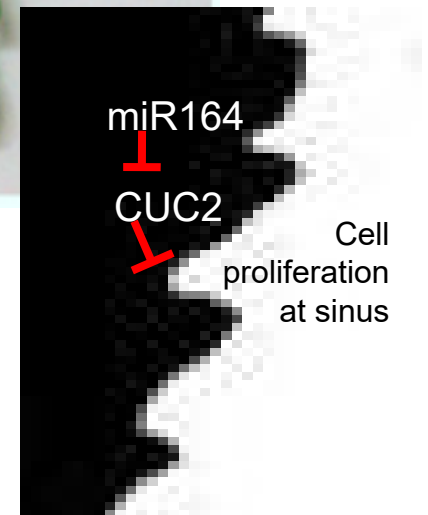


Loss of *CUC2*  
function



More serrated

Less serrated



Nikovics, K., Blein, T., Peaucelle, A., Isida, T., Morin, H., Aida, M., and Laufs, P. (2006) The balance between the *MIR164A* and *CUC2* genes controls leaf margin serration in Arabidopsis. Plant Cell 18: [2929-2945](#).



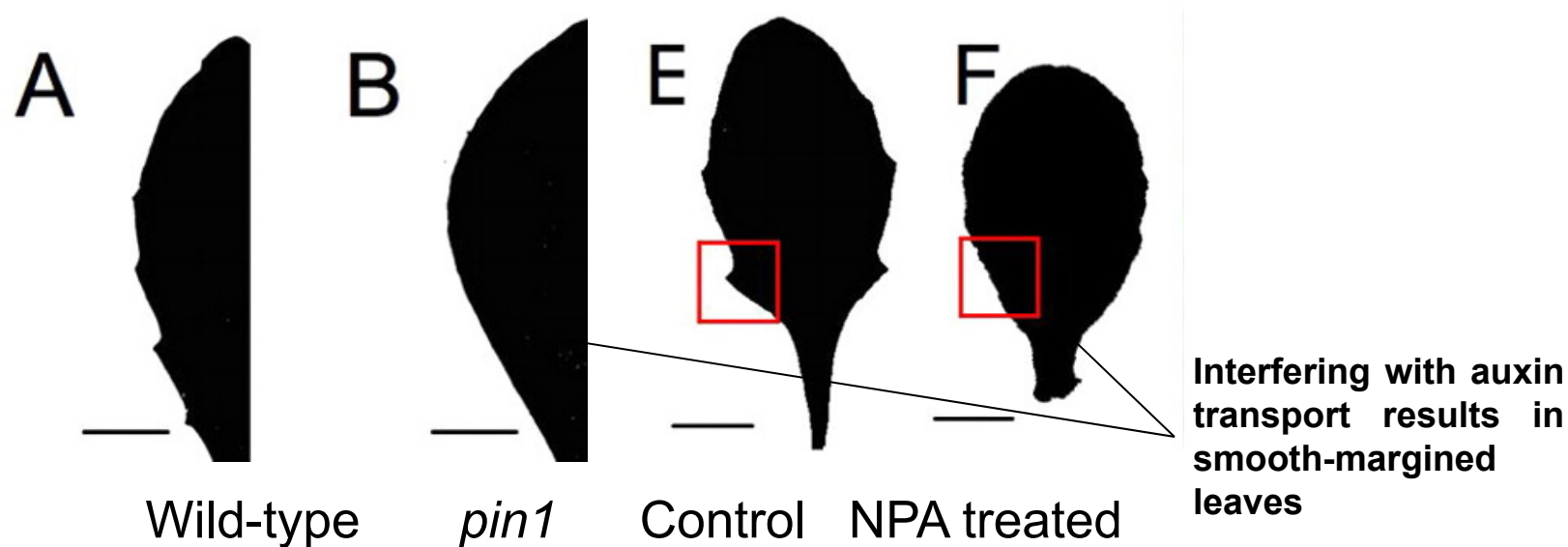


# Outline of Lesson 9

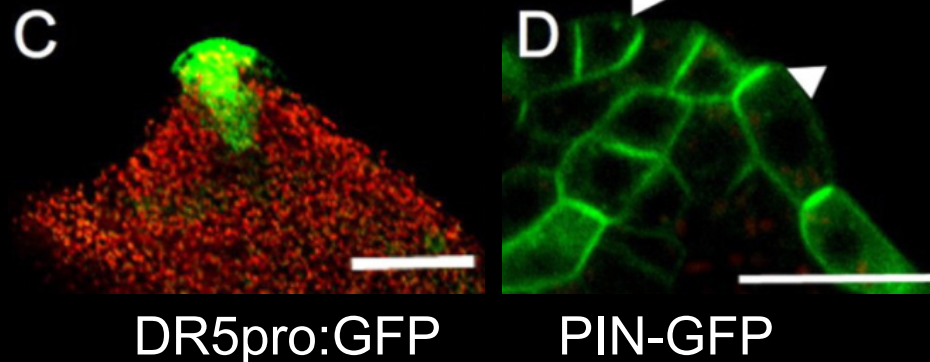
## Morphogenesis

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    - Regulation of cell division by TCP and boundary genes
    - Auxin-regulated positional information for cell division

# A local auxin maximum specifies the outgrowths of the leaf margin

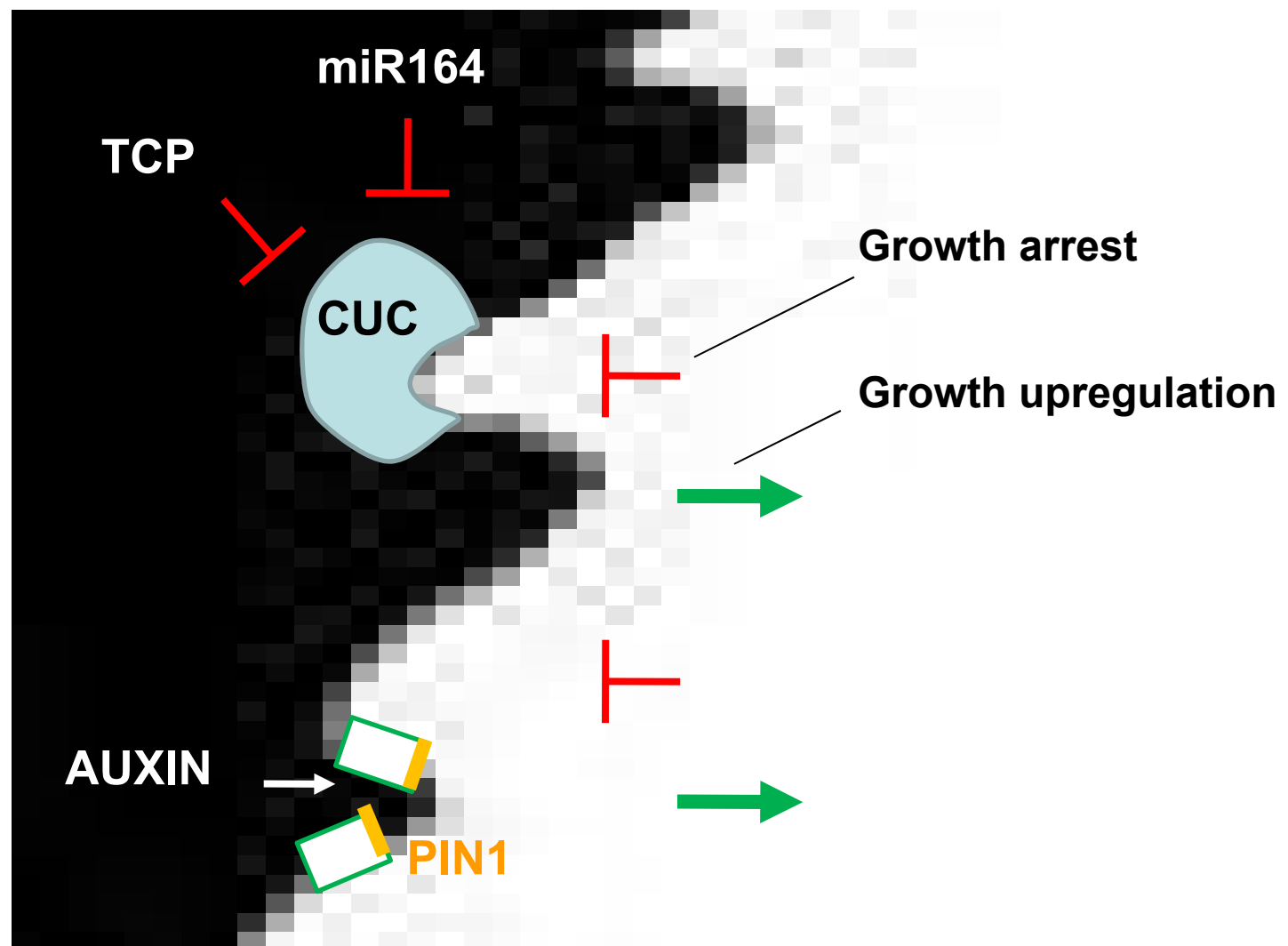


PIN1 orientation produces an auxin maximum at the serration tip



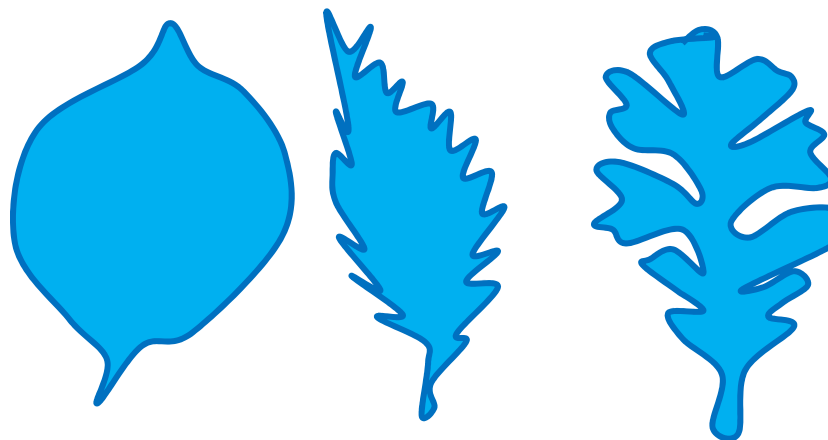
Reproduced with permission Hay, A., Barkoulas, M., and Tsiantis, M. (2006) ASYMMETRIC LEAVES1 and auxin activities converge to repress *BREVIPEDICELLUS* expression and promote leaf development in *Arabidopsis*. [Development](#) 133, 3955-3961.

# Summary - Control of leaf margin shape

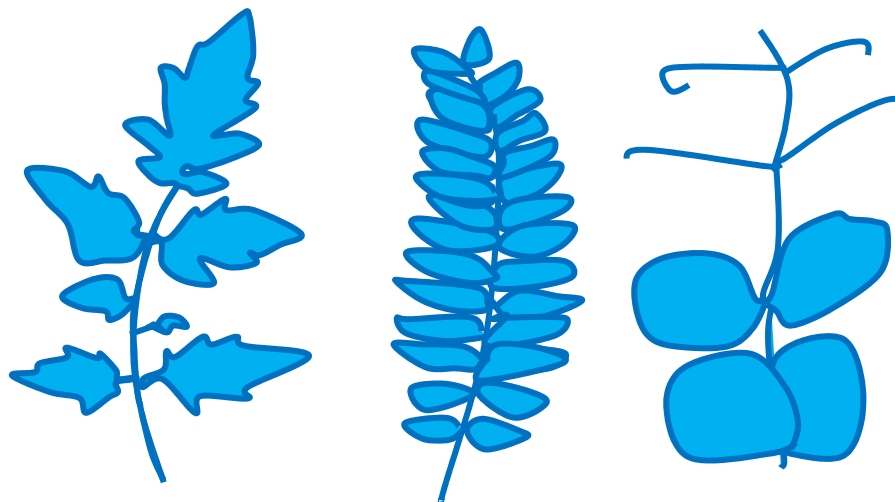


# What determines if a leaf is simple or compound?

**Simple**



**Compound**

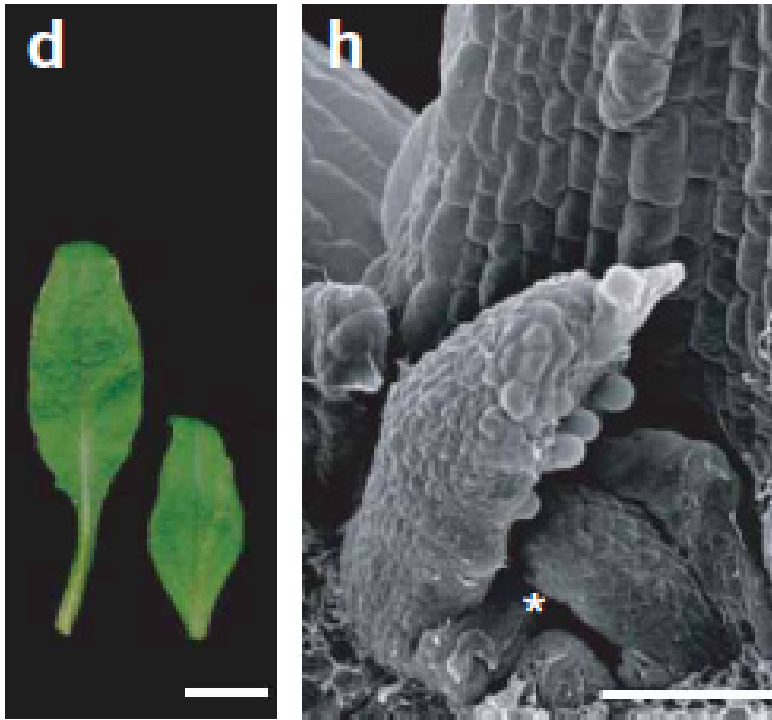


Redrawn from Champagne, C., and Sinha, N. (2004). Development 131:4401-4412

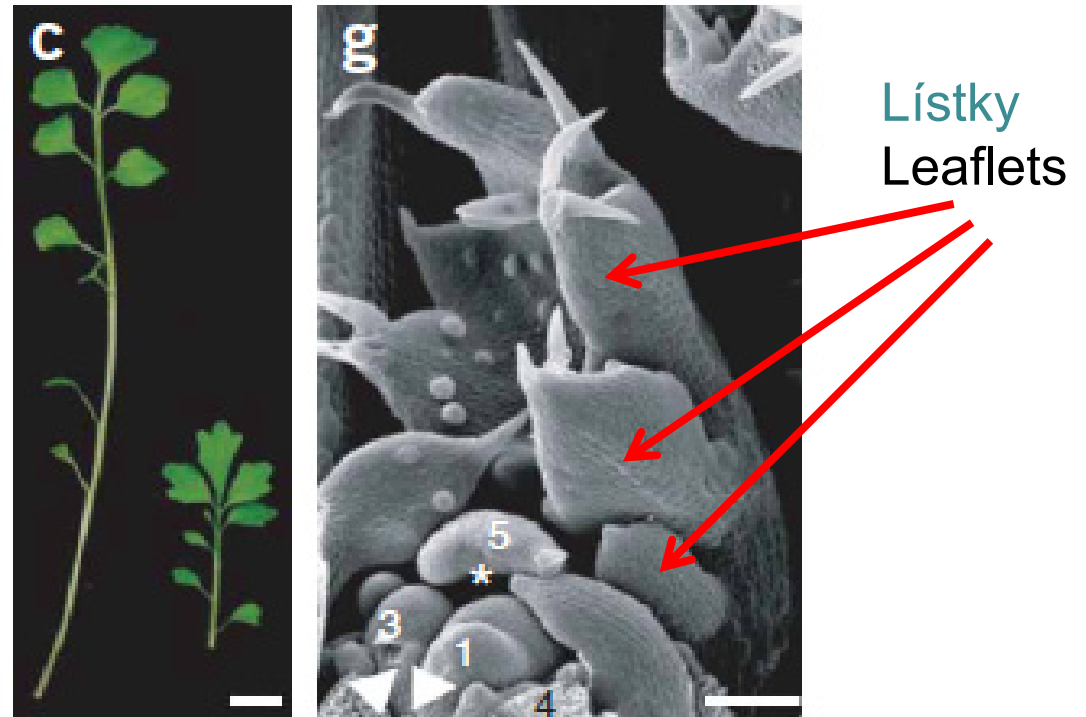


# *Cardamine hirsuta* is closely related to *Arabidopsis thaliana* but has compound leaves

*Arabidopsis thaliana*



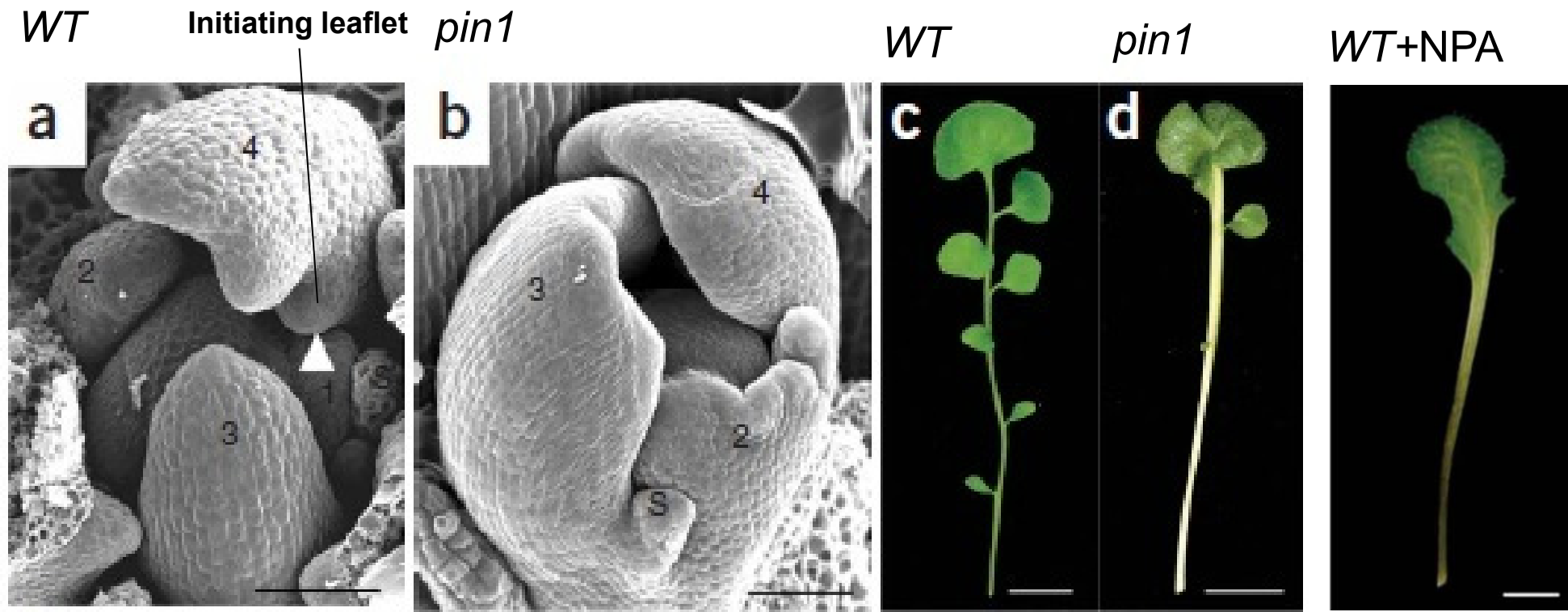
*Cardamine hirsuta* řeřišnice srstnatá



Reprinted by permission from Macmillan Publishers, Ltd: [NATURE GENETICS](#) 38: 942-947. Hay, A., and Tsiantis, M. The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. Copyright (2006).

# Polar auxin transport is necessary for compound leaf formation

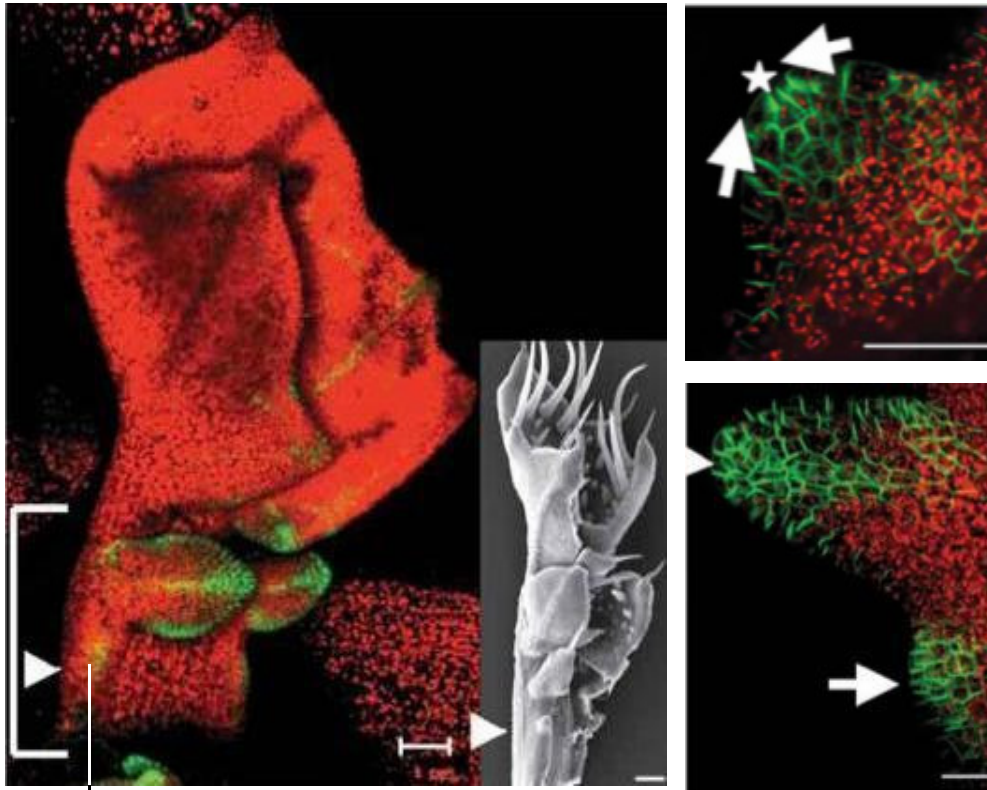
*Cardamine hirsuta*



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)

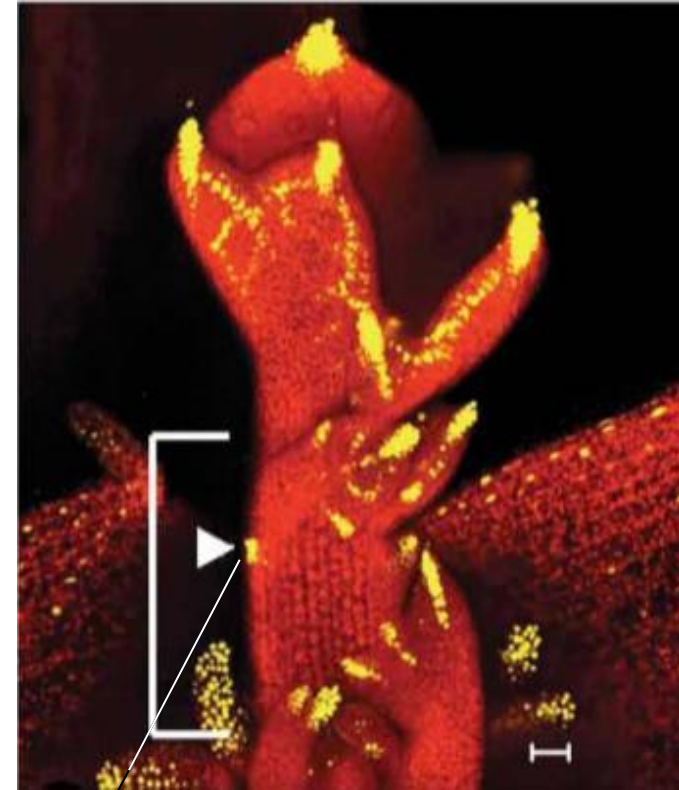
# A PIN1-generated auxin maximum precedes leaflet formation

*ProPIN1:PIN1-GFP*



**PIN1 expression in the prospective leaflet position**

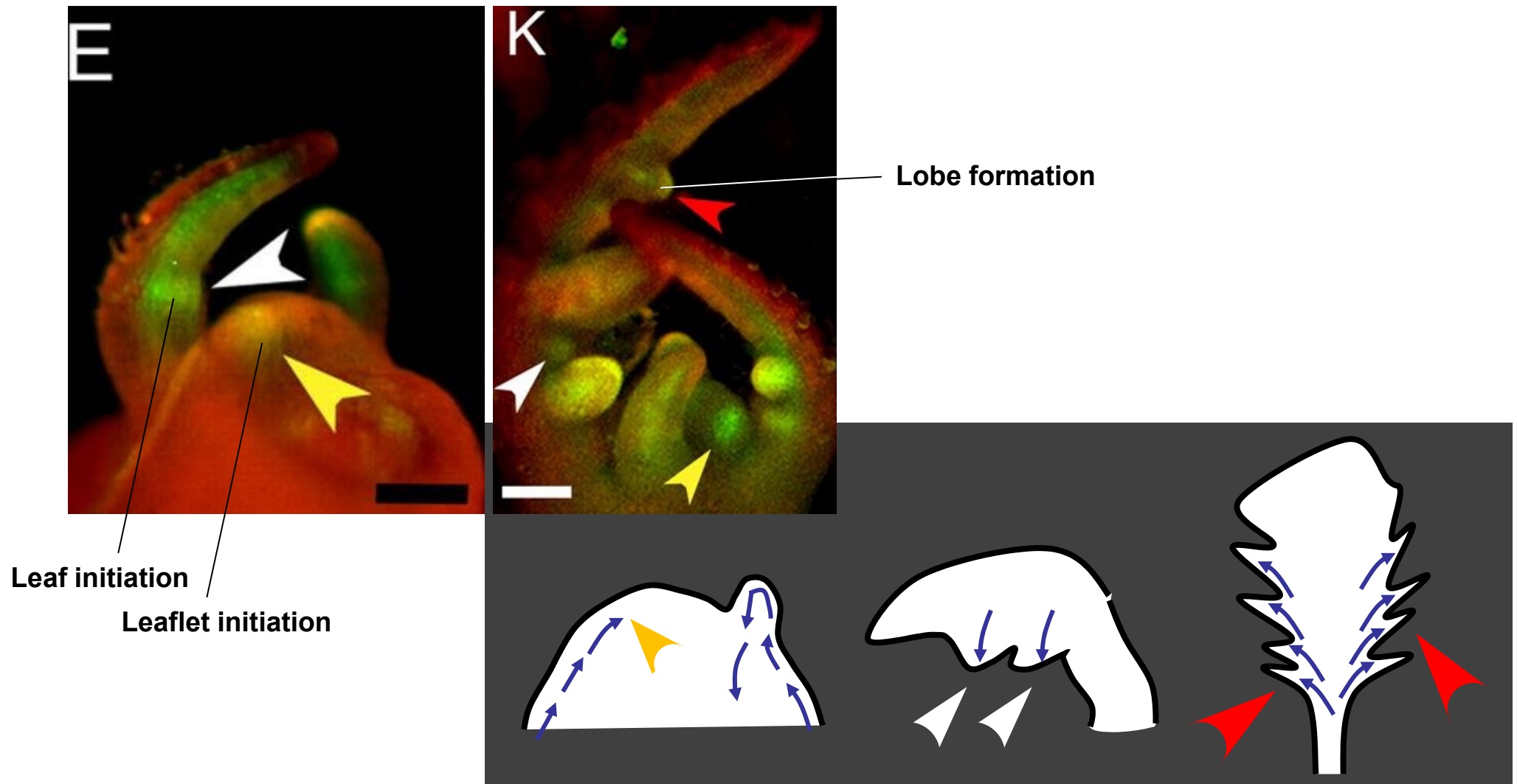
*DR5:YFP*



**Auxin accumulation in the prospective leaflet position**

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

# Auxin has a recurring role in leaf development



Koenig, D., Bayer, E., Kang, J., Kuhlemeier, C., and Sinha, N. (2009) Auxin patterns *Solanum lycopersicum* leaf morphogenesis. *Development* **136**: [2997-3006](#).





# Outline of Lesson 9

## Morphogenesis

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  - 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

# Expression of *KNOX1* transcription factor gene correlates with leaf complexity

*Cardamine hirsuta*

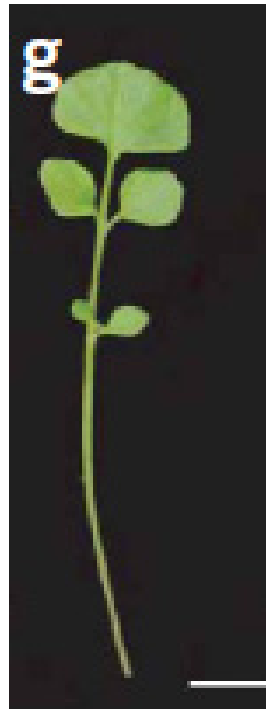
*STM RNAi*



- *STM*



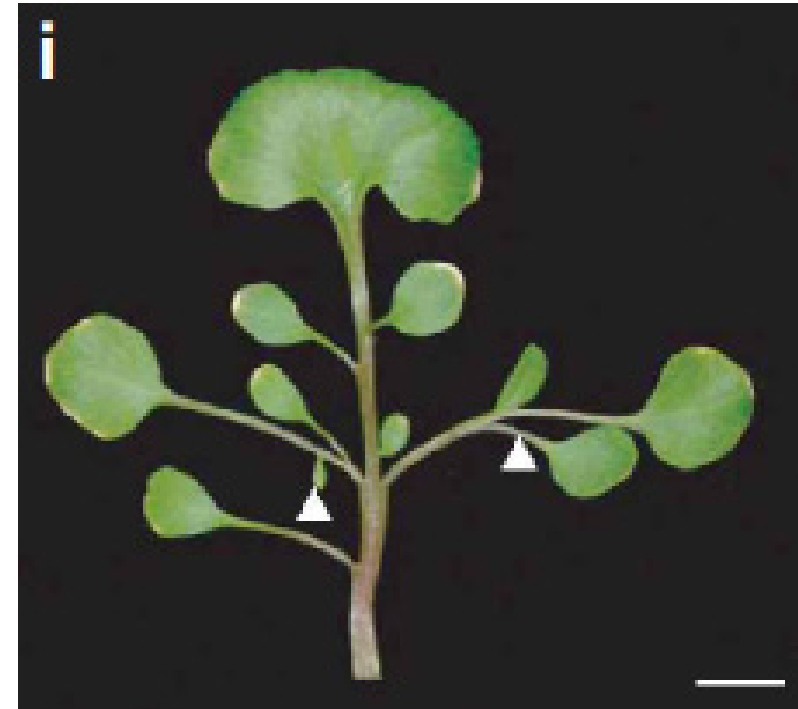
*WT*



+ *KNOX*



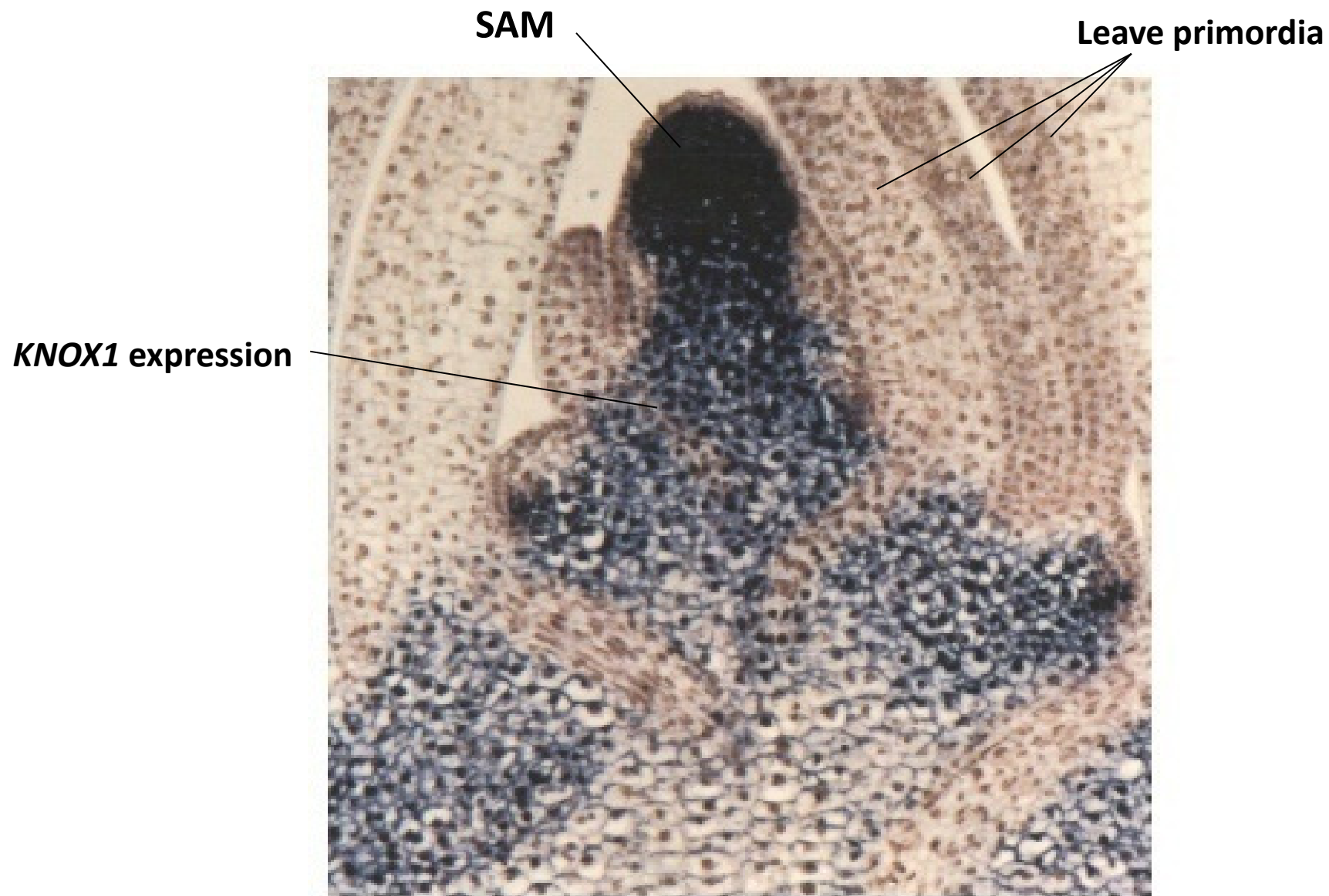
*Pro35S::KN1-GR*



*WT*

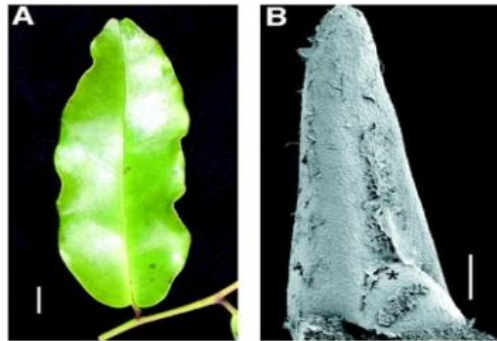
KNOTTED-like homeobox TFs

Reprinted by permission from Macmillan Publishers, Ltd: [NATURE GENETICS](#) 38: 942-947. Hay, A., and Tsiantis, M. The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. Copyright (2006).



Jackson, D., Veit, B., and Hake, S. (1994) Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. [Development](#) 120: 405–413. Reproduced with permission.

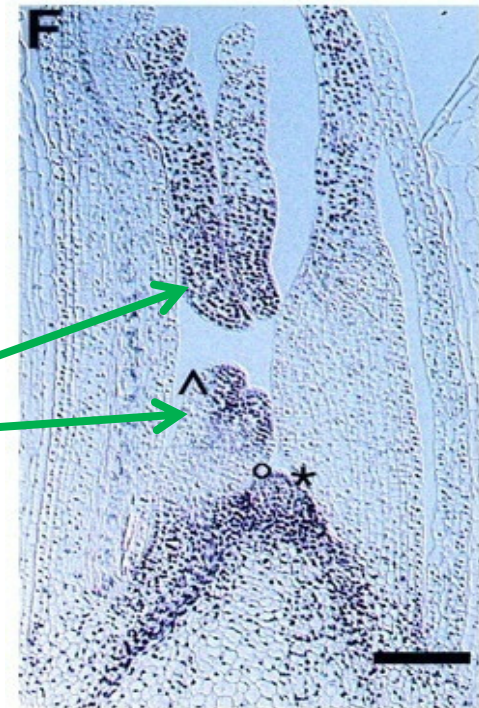
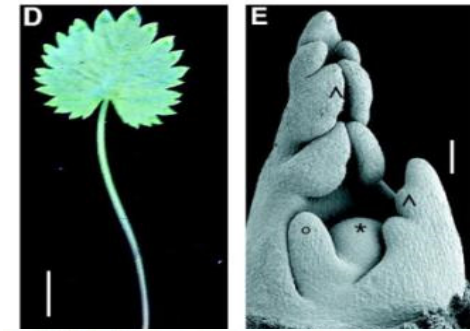
## Simple leaf



*Amborella trichopoda*

In plants with  
compound leaves,  
***KNOX1*** expression  
turns back on in  
primordia

## Compound leaf



*Pimpinella anisum* anýz vonný

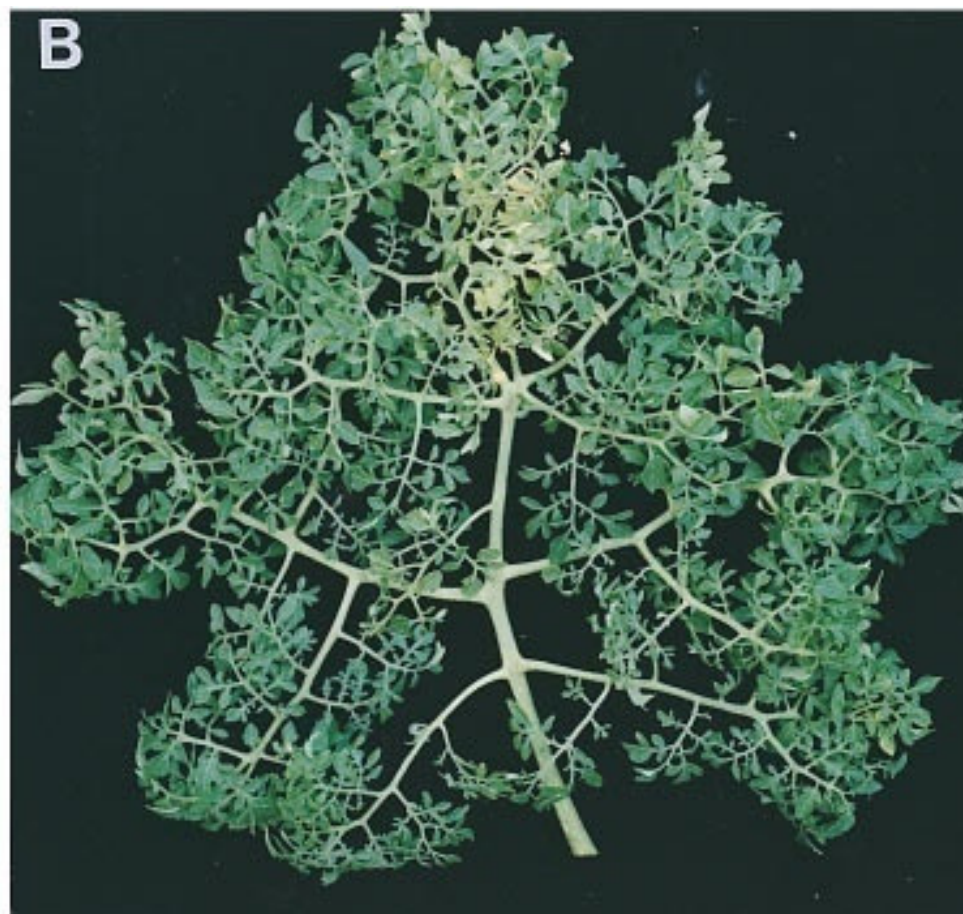
From Bharathan, G., Goliber, T.E., Moore, C., Kessler, S., Pham T., and Sinha, N.R. (2002) Homologies in leaf form inferred from *KNOX1* gene expression during development. Science 296: [1858-1860](#). Reprinted with permission from AAAS.



# Tomato



**WT**

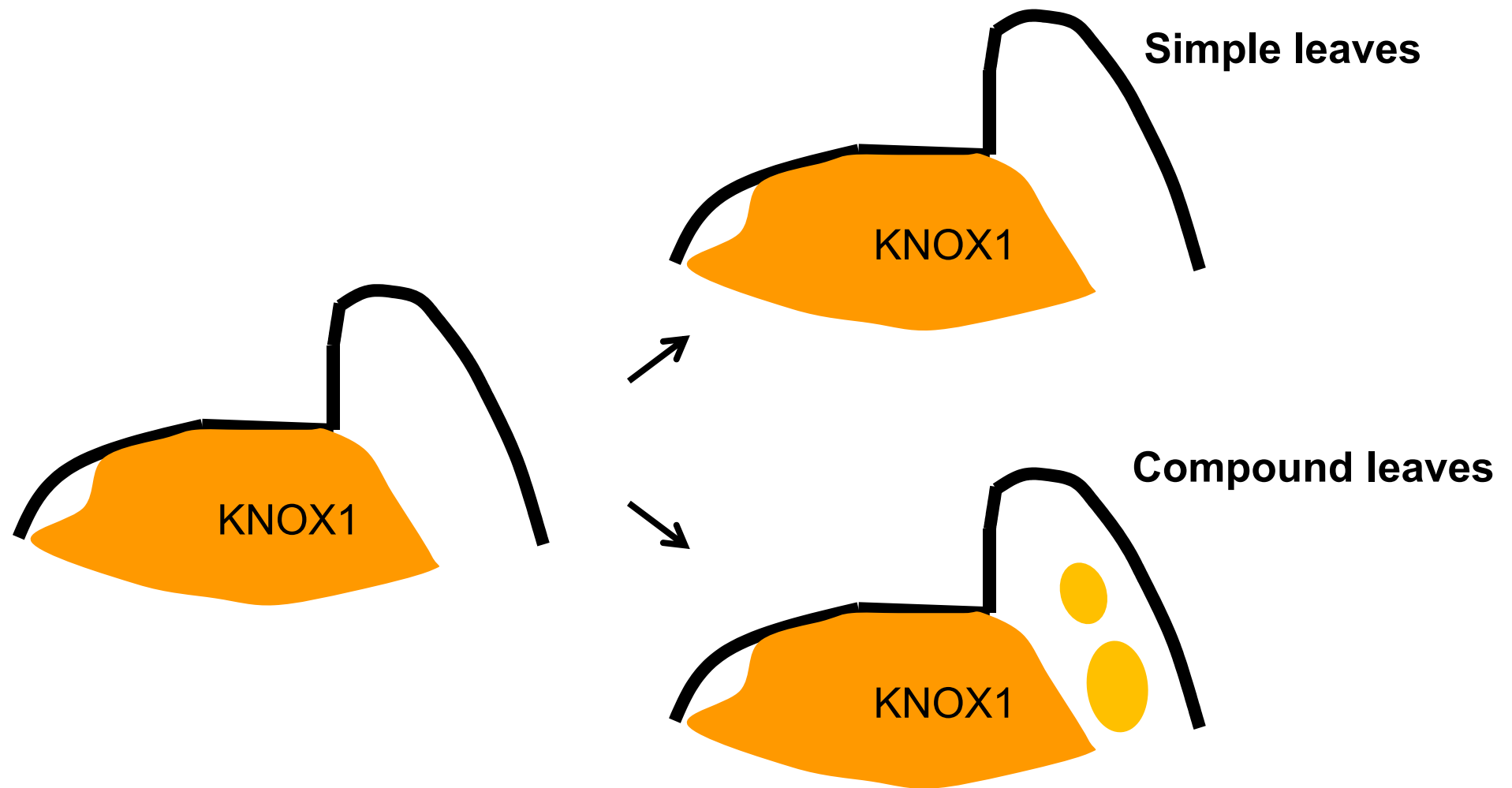


***Pro35S:KNOTTED1***

Homeobox TF gene from  
maize

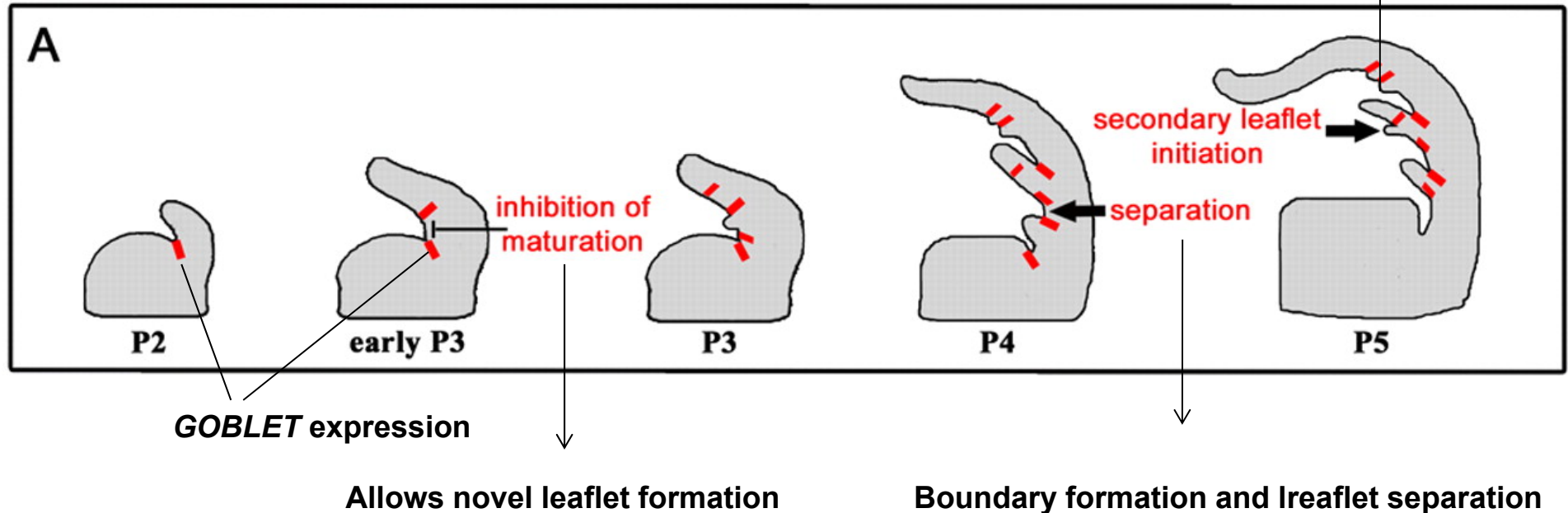
Reprinted from [Cell](#), 84 (5). Hareven, D., Gutfinger, T., Parnis, A., Eshed, Y., Lifschitz, E. The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. [735–744](#). Copyright Cell Press (1996), with permission from Elsevier.

# ***KNOX1* genes have a recurring role in leaf development**



# Boundary genes have a recurring role in leaf development

## Compound leaf formation in tomato



Berger, Y., Harpaz-Saad, S., Brand, A., Melnik, H., Sirding, N., Alvarez, J.P., Zinder, M., Samach, A., Eshed, Y., and Ori, N. (2009) The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves *Development* 136, [823-832](#). Reproduced with permission.



# 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**