Bi8940 Developmental Biology
Lesson 9

Morphogenesis

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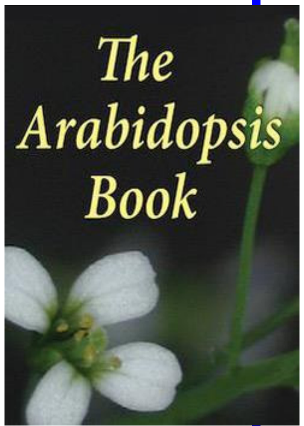
Literature



- **Fred H. Wilt and Sarah Hake, Principles of Developmental Biology** (W.W. Norton & Company, New York, London, 2004)
- **Capron A, Chatfield S, Provart N, Berleth T 2009**. Embryogenesis: Pattern Formation from a Single Cell. *The Arabidopsis Book*. Rockville, MD: American Society of Plant Biologists, doi: 10.1199/tab.0126, http://www.aspb.org/publications/arabidopsis/.
- Selected original papers in scientific journals

Outline of Lesson 9

Morphogenesis

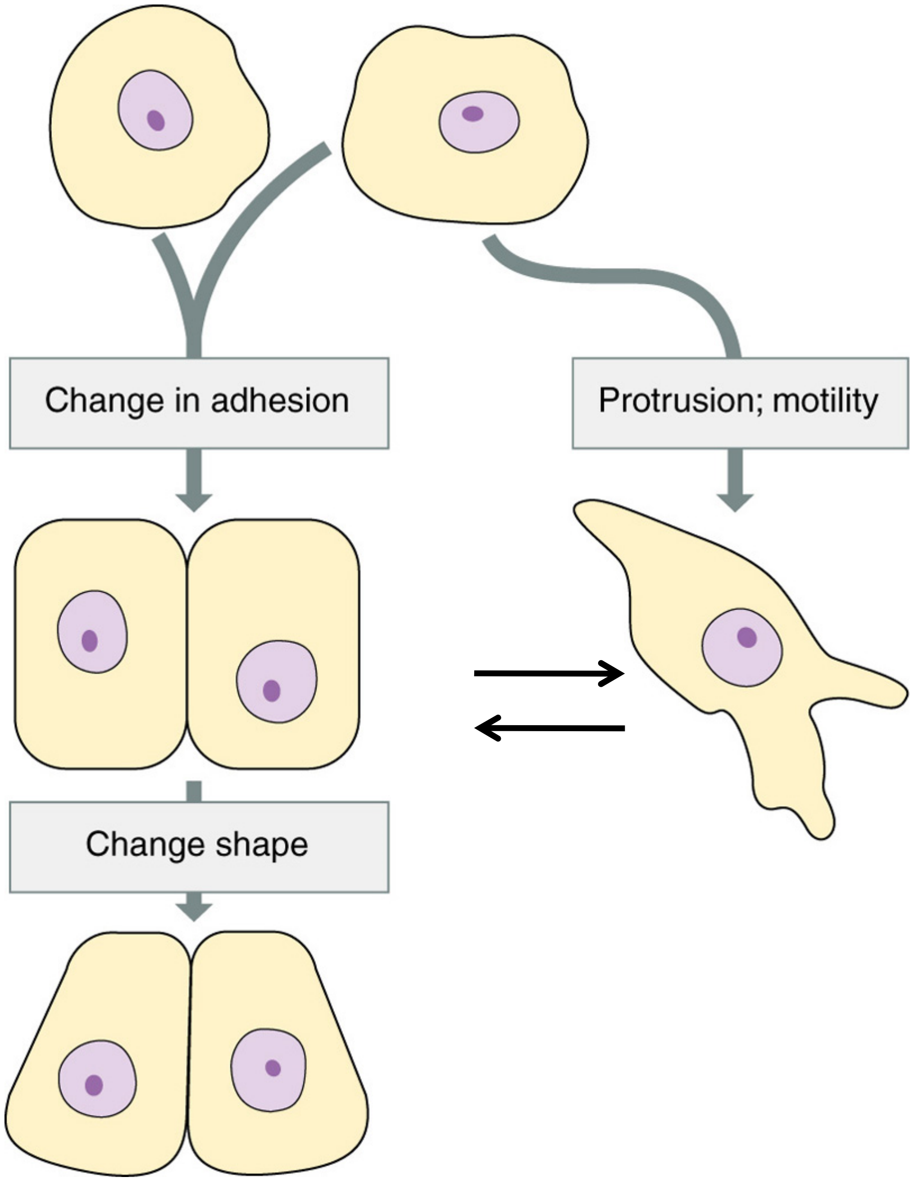
- **Morphogenesis in animals**
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants
  - Morphogenic manoeuvres
  - Changes in the cell motility and tissue interactions during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

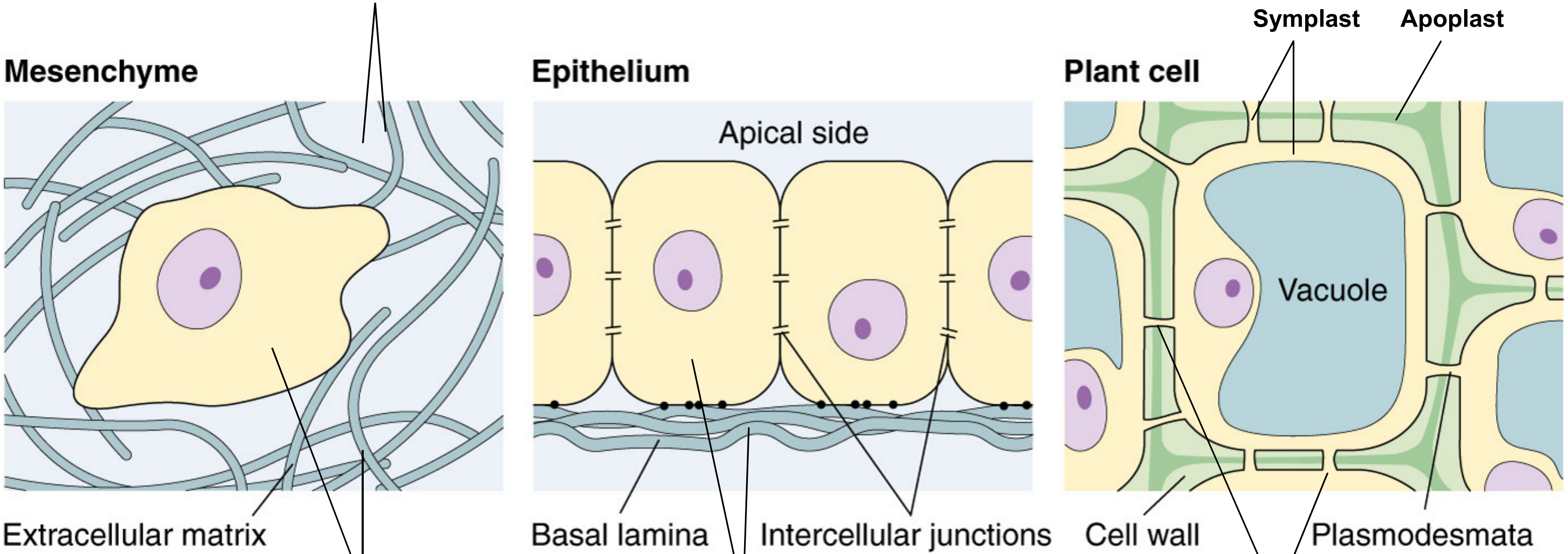
Outline of Lesson 9

Morphogenesis

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


Change in adhesion
Protrusion; motility
Change shape

Extracellular matrix
- •Colllagen
- •Proteoglycans
- •Glycoproteins
- •Signalling molecules



Interactions with extracellular matrix

Cell-to-cell communication and interactions with basal lamina

Cell-to-cell communication and interactions with extracellular space

Outline of Lesson 9

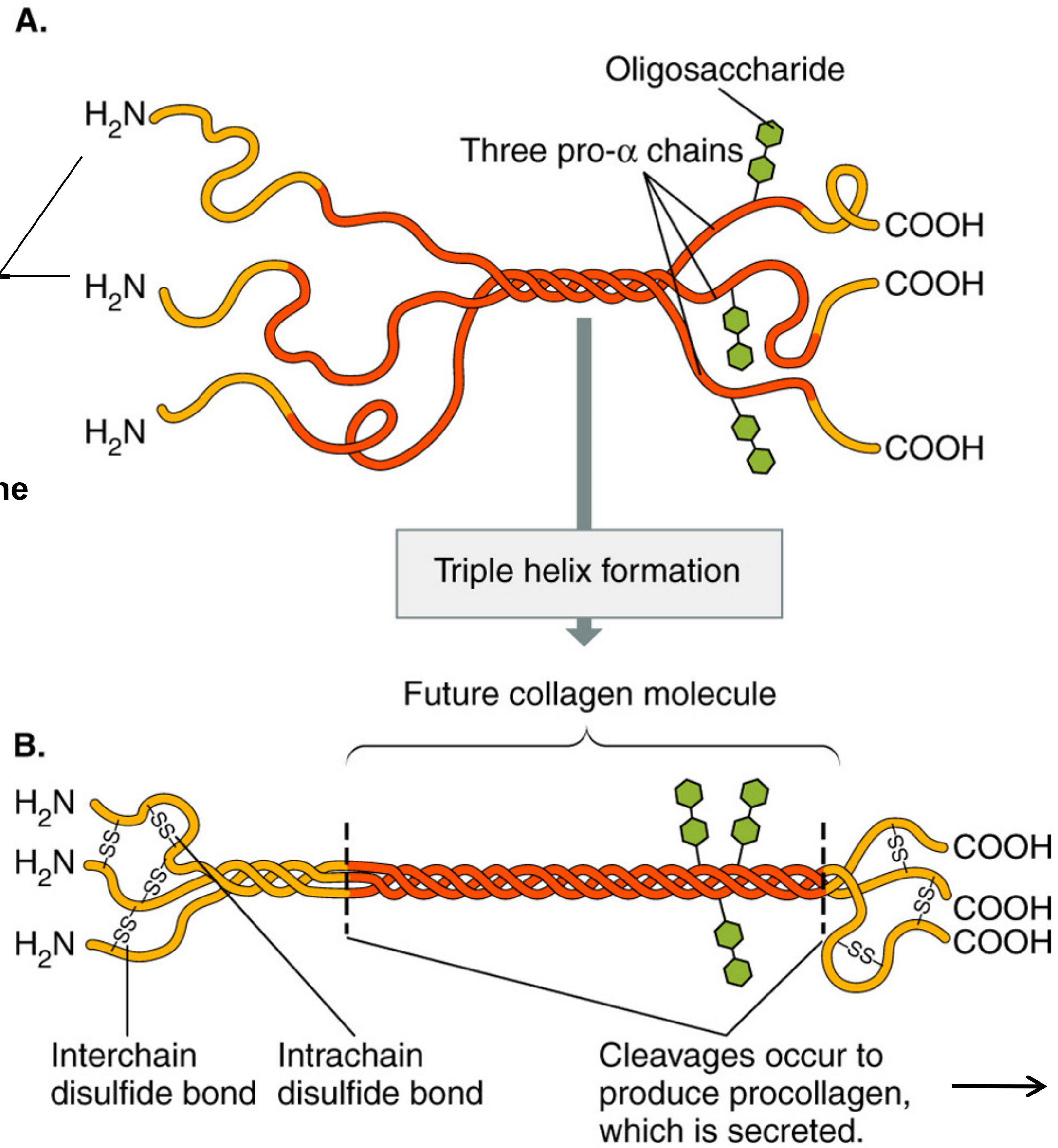
Morphogenesis

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

Collagen

Prolin- and hydroxyprolin-rich aa chains (Gly-X-Y)n

Proline of hydroxyproline



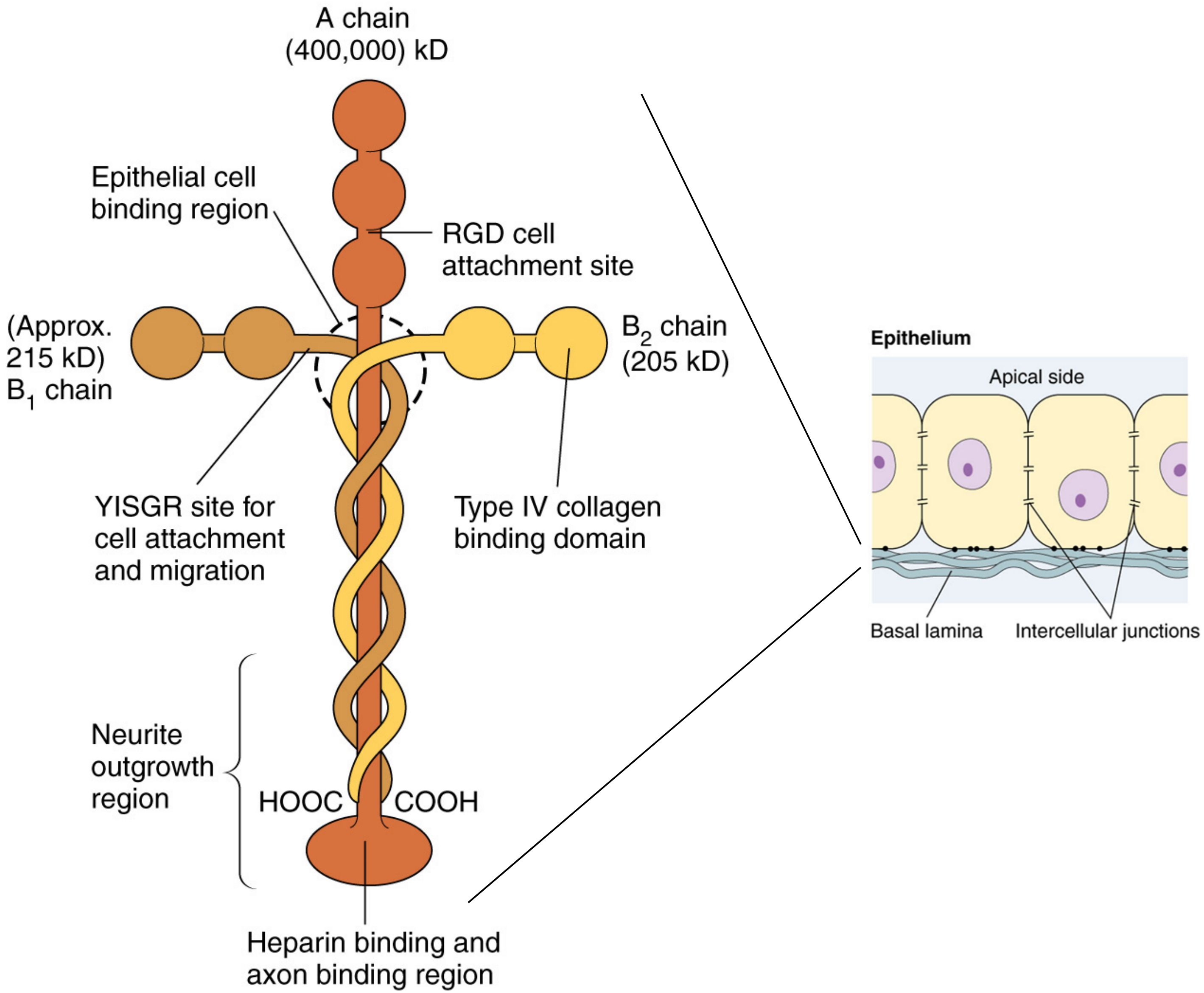
Cell-environment-dependent regulation

Final assembly in the extracellular space

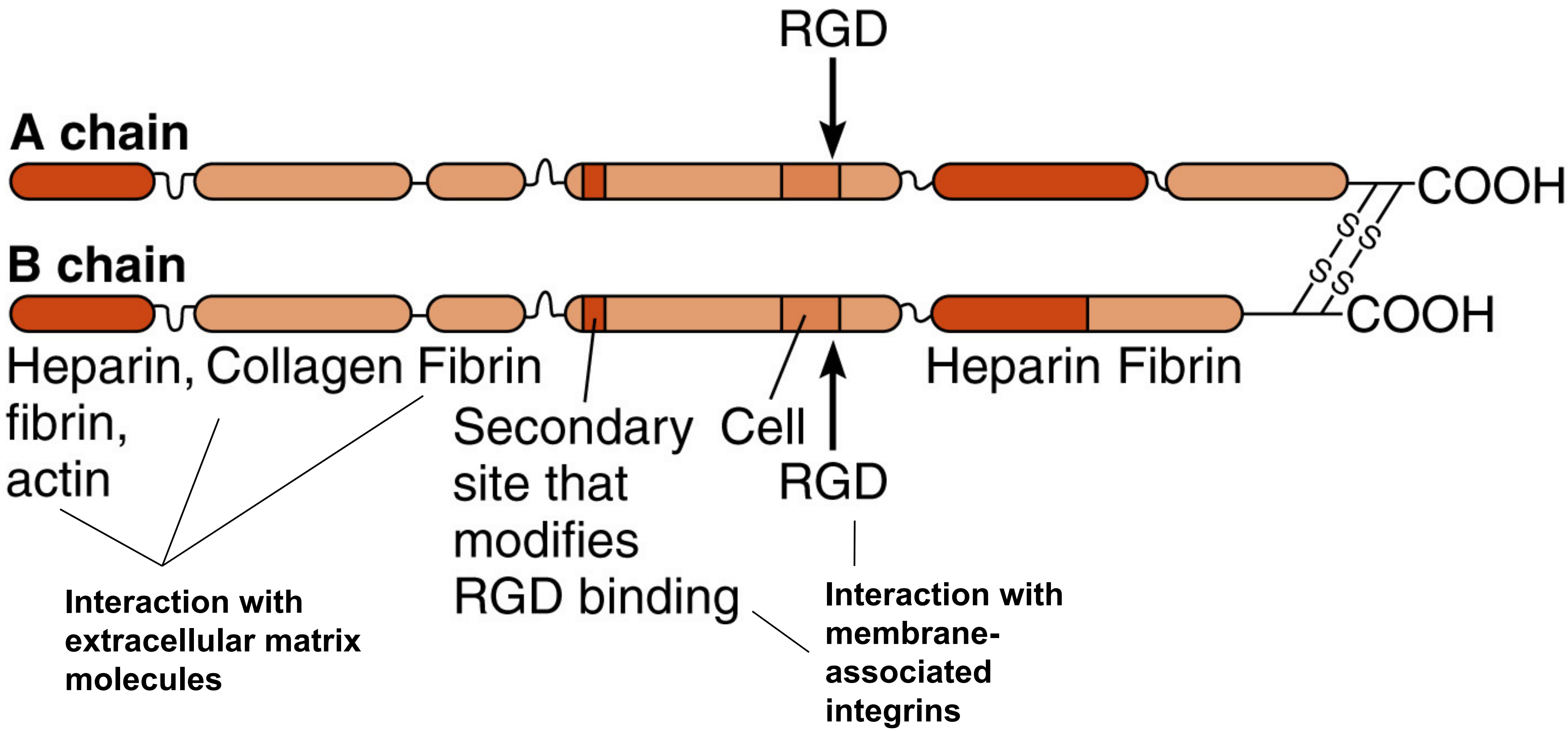
Table 12.1 The Major Types of Collagen

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

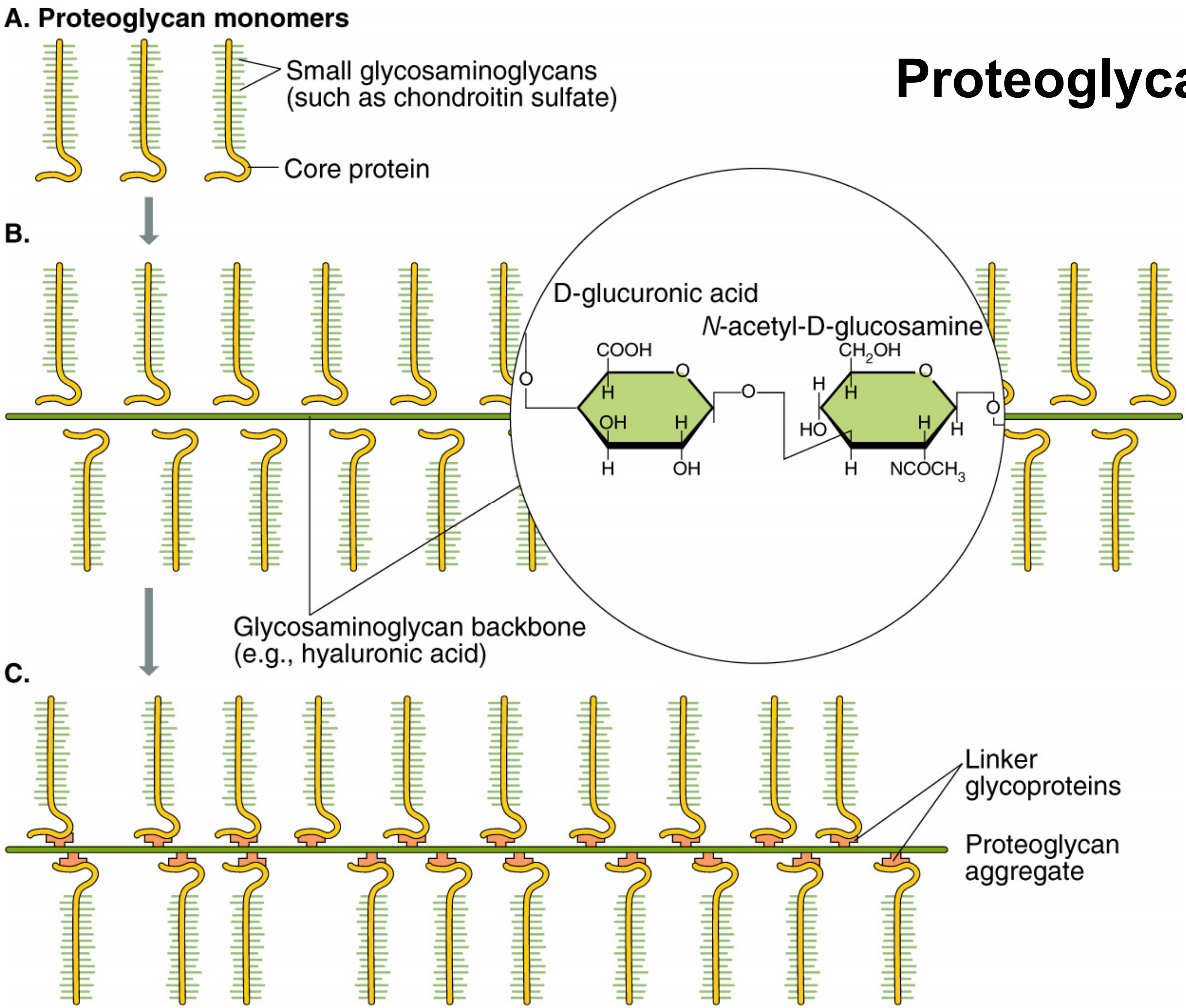
Laminin

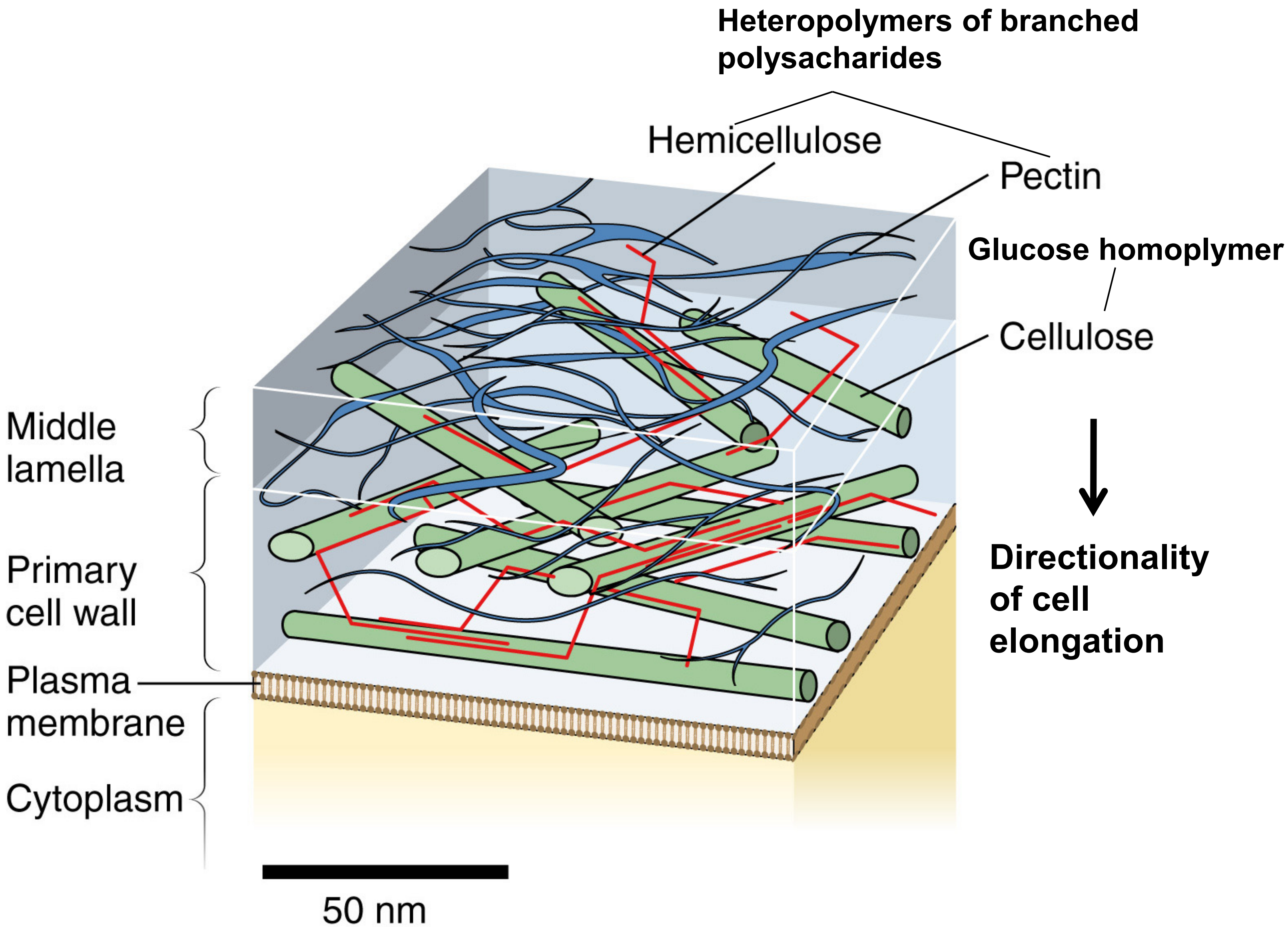

A chain
(400,000) kD
Epithelial cell
binding region
RGD cell
attachment site
(Approx.
215 kD)
B_1 chain
B_2 chain
(205 kD)
YISGR site for
cell attachment
and migration
Type IV collagen
binding domain
Neurite
outgrowth
region
HOOC
COOH
Heparin binding and
axon binding region
Epithelium
Apical side
Basal lamina
Intercellular junctions

Fibronectin



Proteoglycans



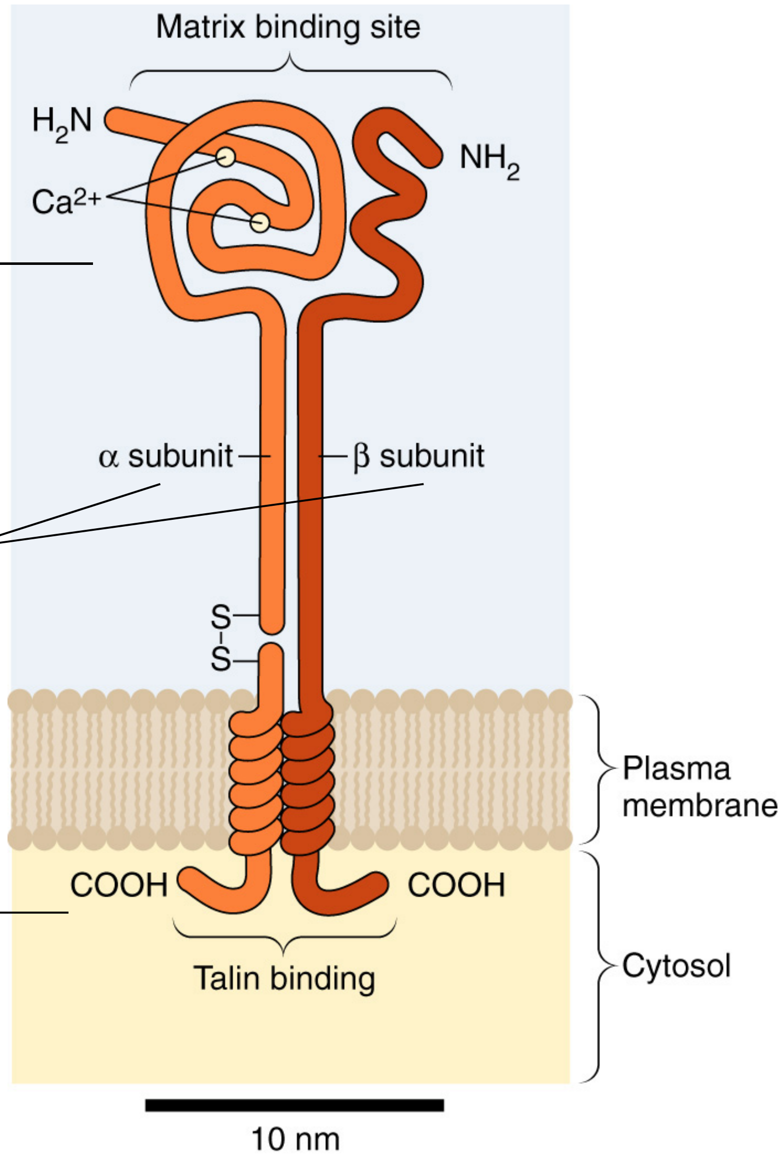

Heteropolymers of branched polysacharides
Hemicellulose
Pectin
Glucose homoplymer
Cellulose
Middle lamella
Primary cell wall
Plasma membrane
Cytoplasm
50 nm
Directionality of cell elongation

Integrins

Interaction with extracellular matrix molecules

Encoded by gene families

Interaction with internal proteins, e.g. cytoskeleton



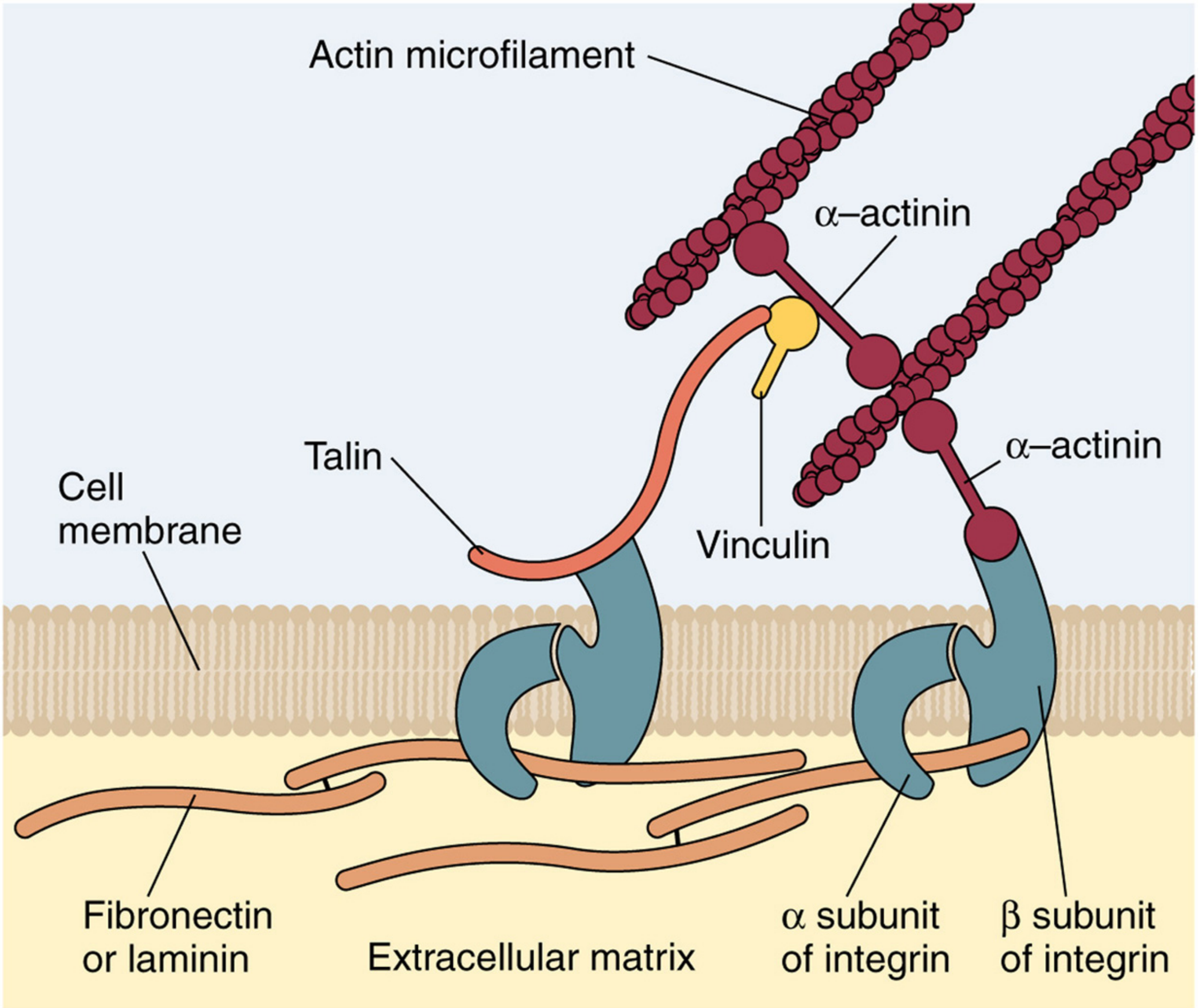
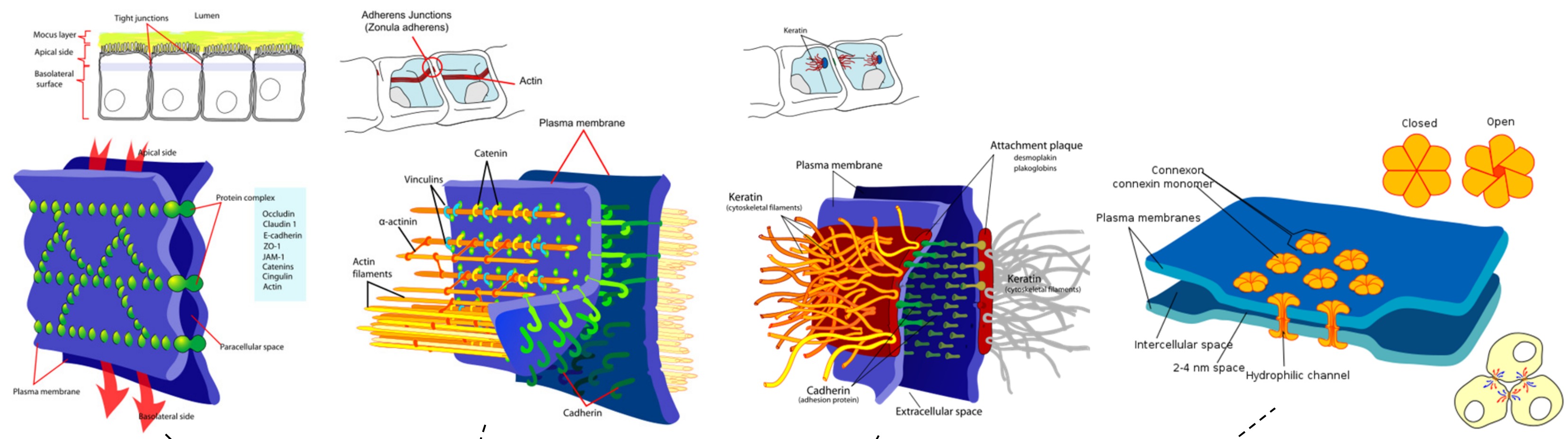
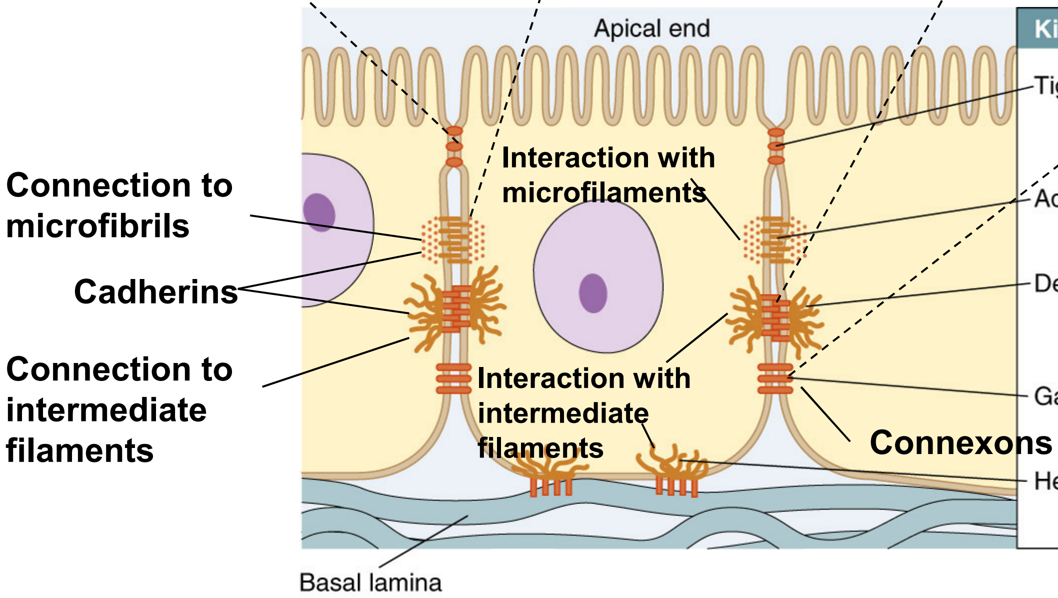

Actin microfilament
α–actinin
α–actinin
Talin
Vinculin
Cell
membrane
Fibronectin
or laminin
Extracellular matrix
α subunit
of integrin
β subunit
of integrin

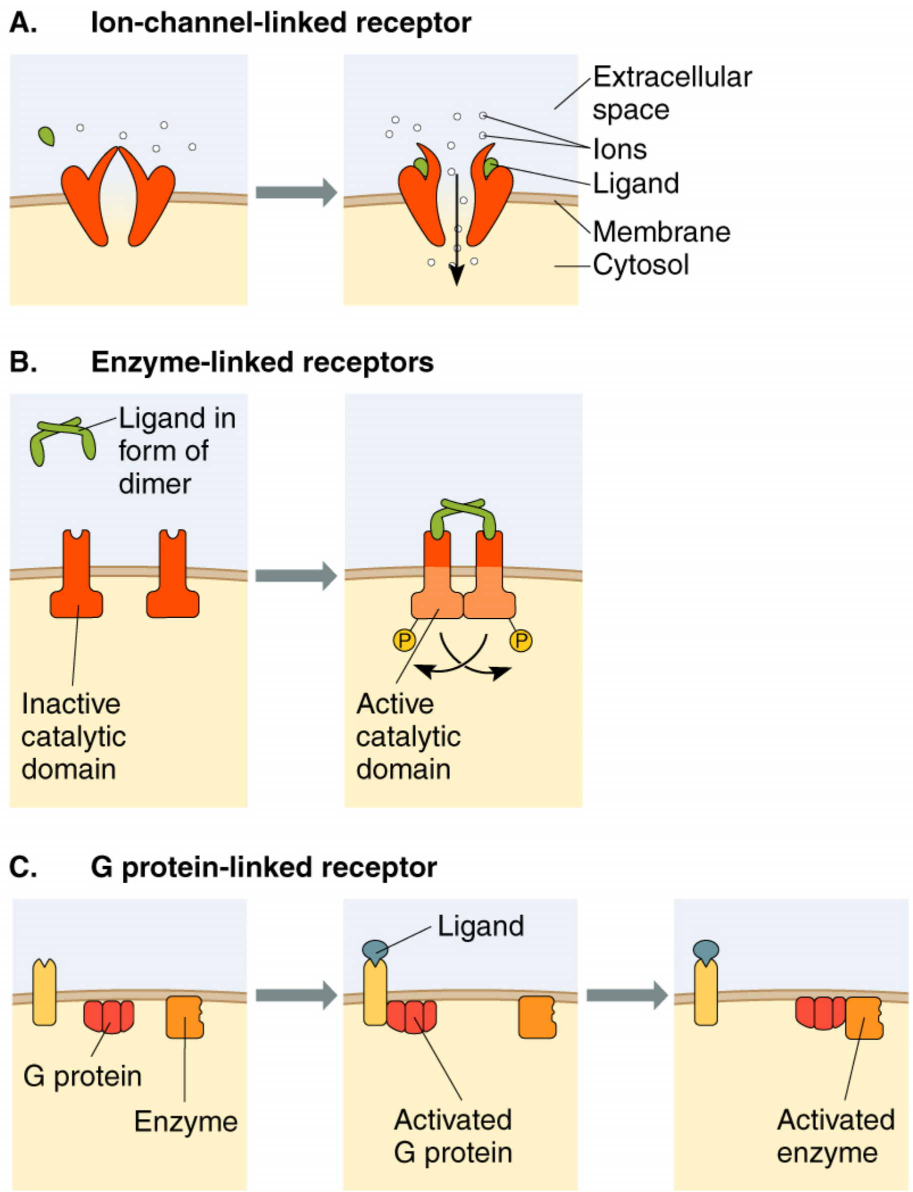
TABLE 12.2 SOME LIGANDS OF INTEGRIN DIMERS

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

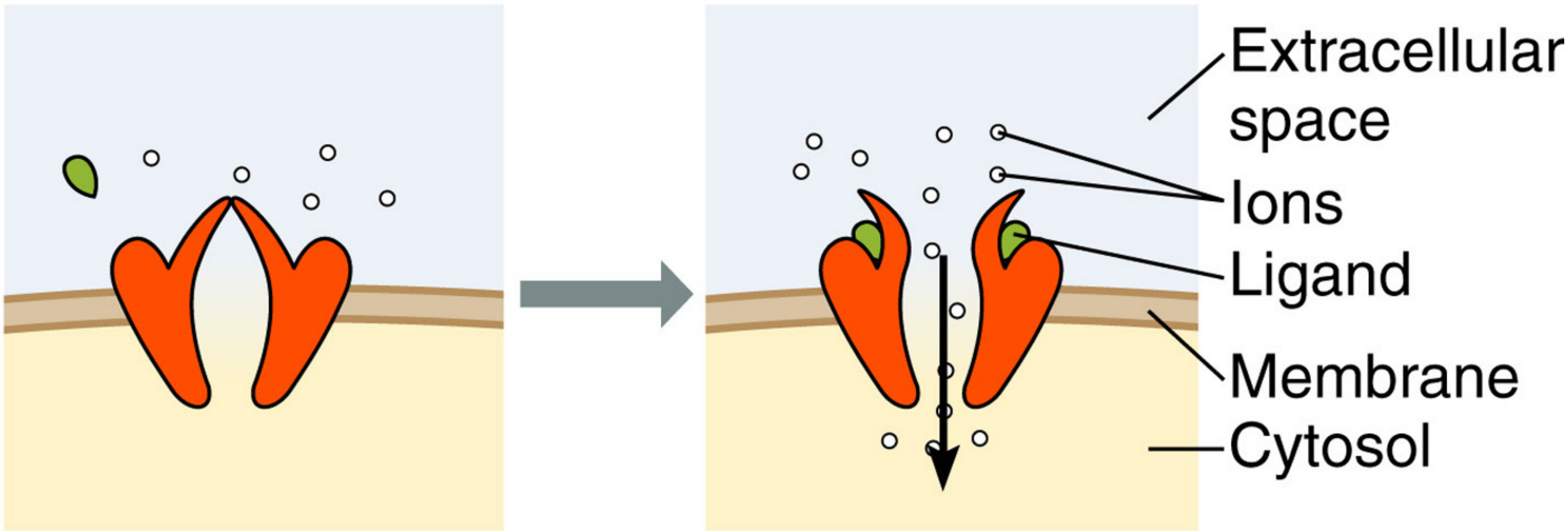




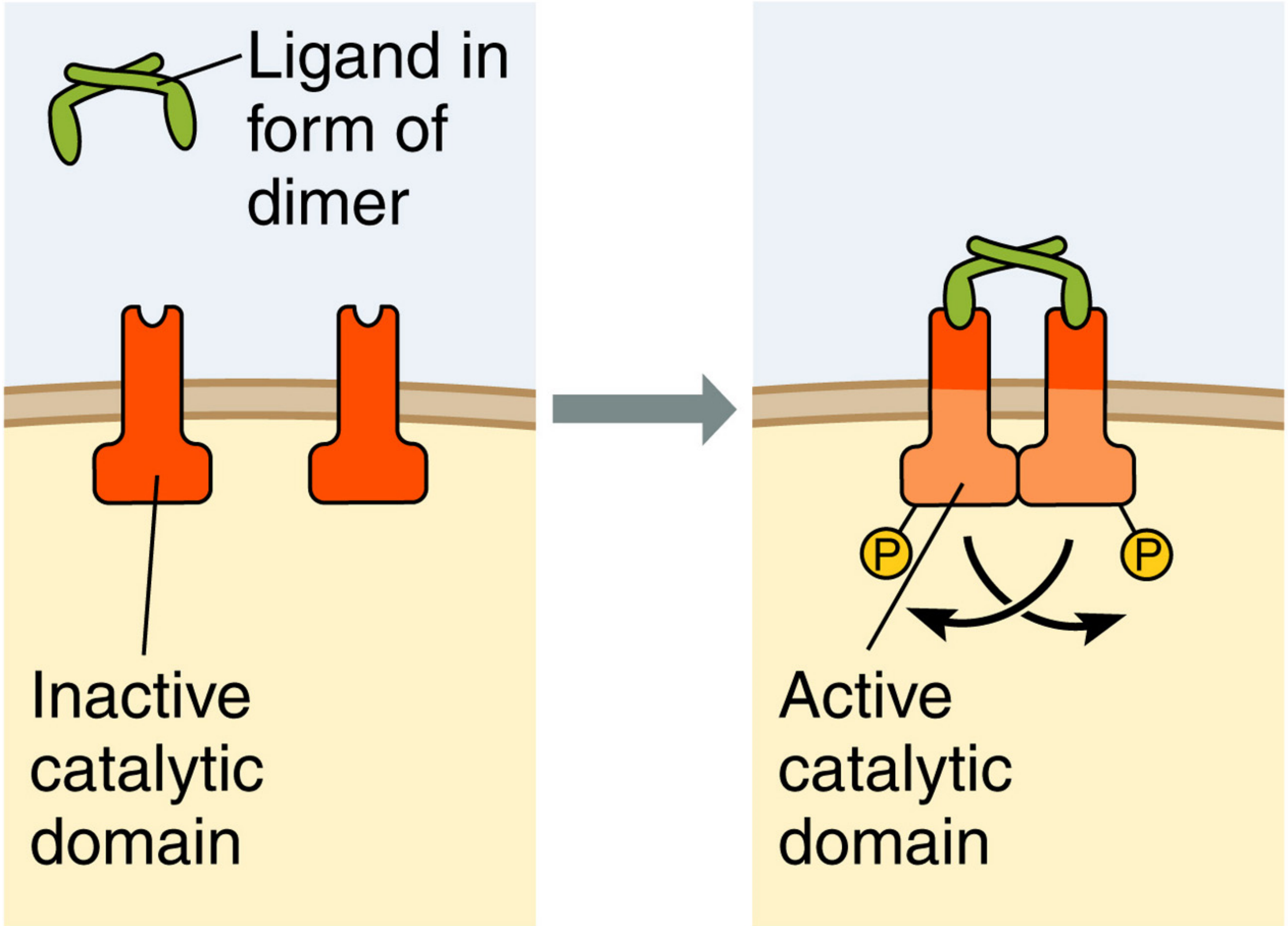
Kind of junction	Function
Tight	Seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them
Adherens	Joins an actin bundle in one cell to a similar bundle in a neighboring cell
Desmosome	Anchors the tough intermediate filaments in one cell to those in a neighboring cell
Gap	Allows passage of small water-soluble ions and molecules between cells
Hemidesmosome	Anchors intermediate filaments in a cell to the basal lamina

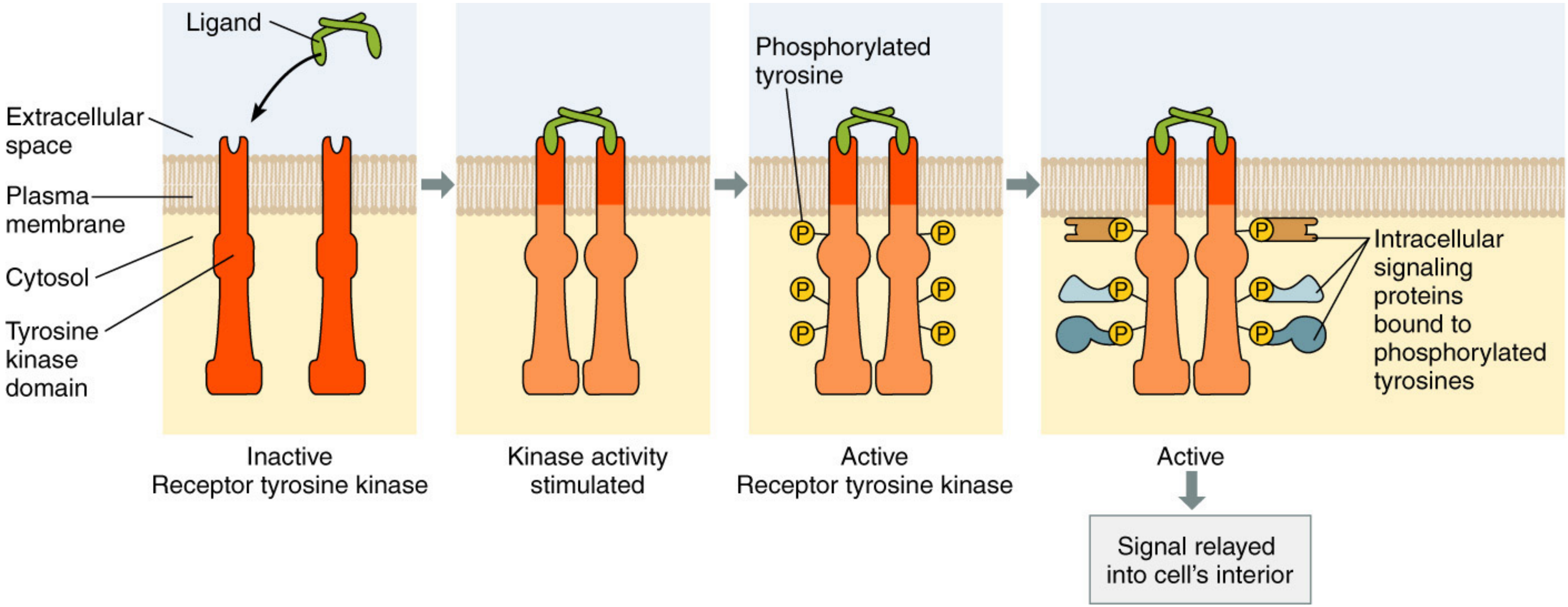

A. Ion-channel-linked receptor
Extracellular space
Ions
Ligand
Membrane
Cytosol
B. Enzyme-linked receptors
Ligand in form of dimer
Inactive catalytic domain
P
P
Active catalytic domain
C. G protein-linked receptor
G protein
Enzyme
Ligand
Activated G protein
Activated enzyme

A. Ion-channel-linked receptor

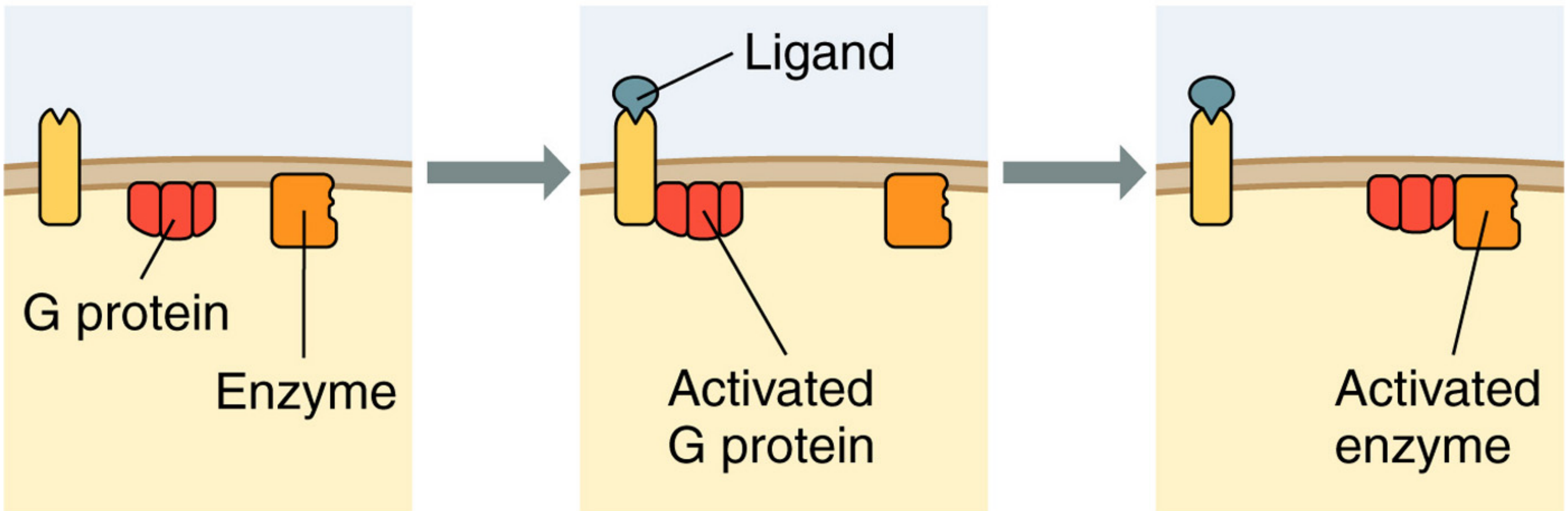


B. Enzyme-linked receptors




Ligand
Extracellular space
Plasma membrane
Cytosol
Tyrosine kinase domain
Inactive
Receptor tyrosine kinase
Kinase activity stimulated
Phosphorylated tyrosine
Active
Receptor tyrosine kinase
Intracellular signaling proteins bound to phosphorylated tyrosines
Active
Signal relayed into cell's interior

C. G protein-linked receptor



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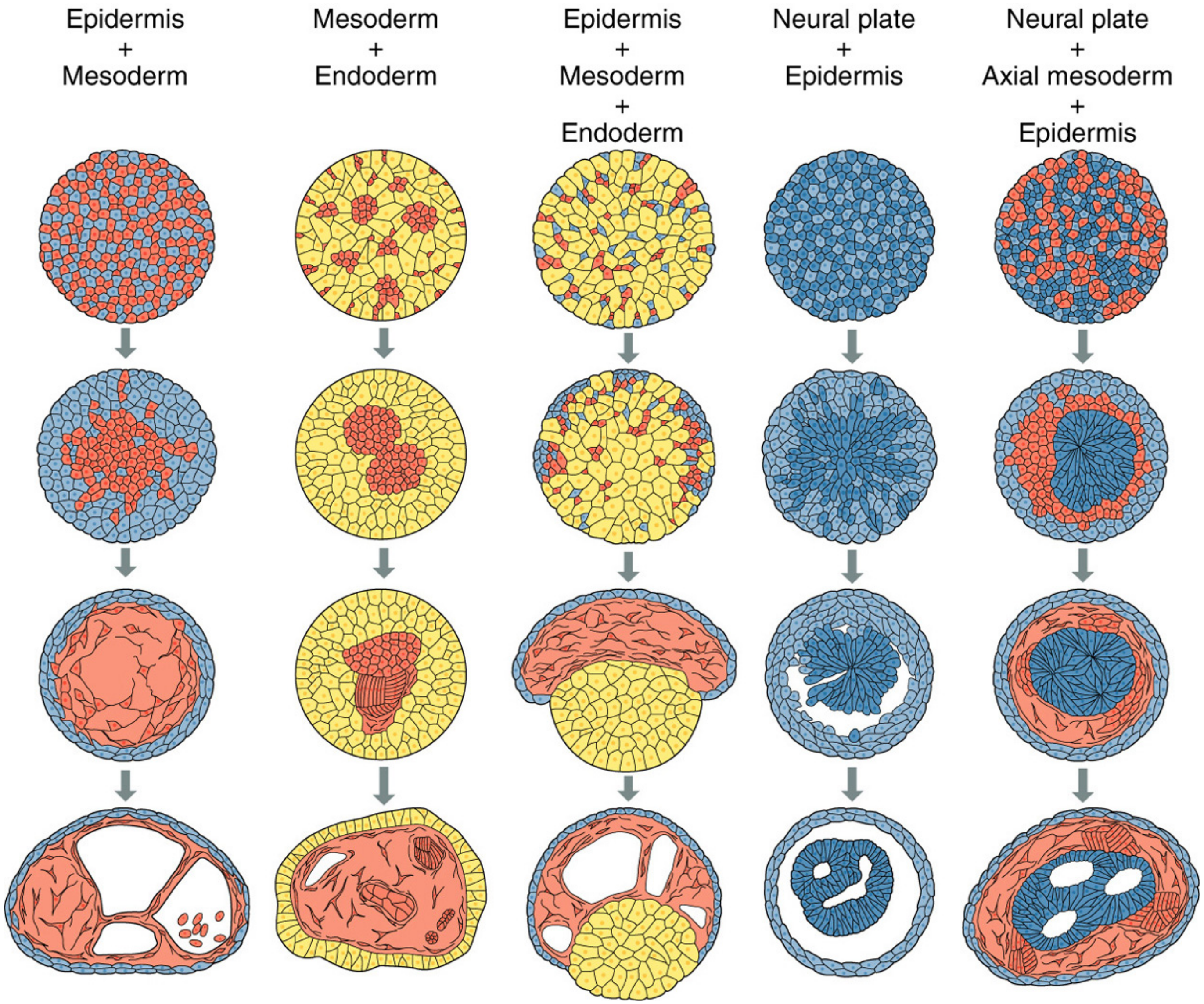
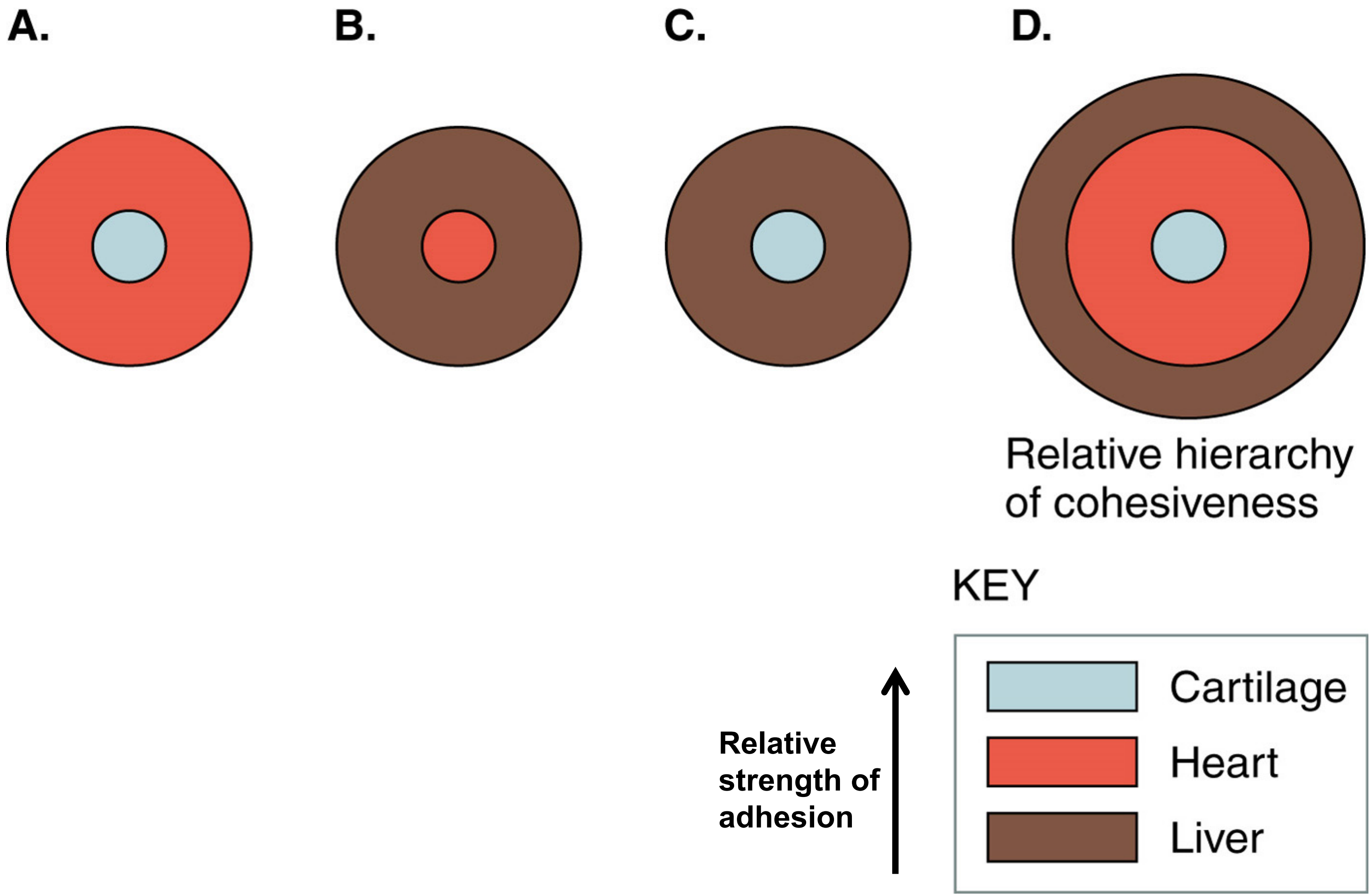
- Morphogenesis in animals
  - Changes in the cell adhesion, protrusion and motility
  - Extracellular matrix regulators of morphogenesis
  - Specificity of cell aggregations and its molecular determinants


Epidermis + Mesoderm
Mesoderm + Endoderm
Epidermis + Mesoderm + Endoderm
Neural plate + Epidermis
Neural plate + Axial mesoderm + Epidermis

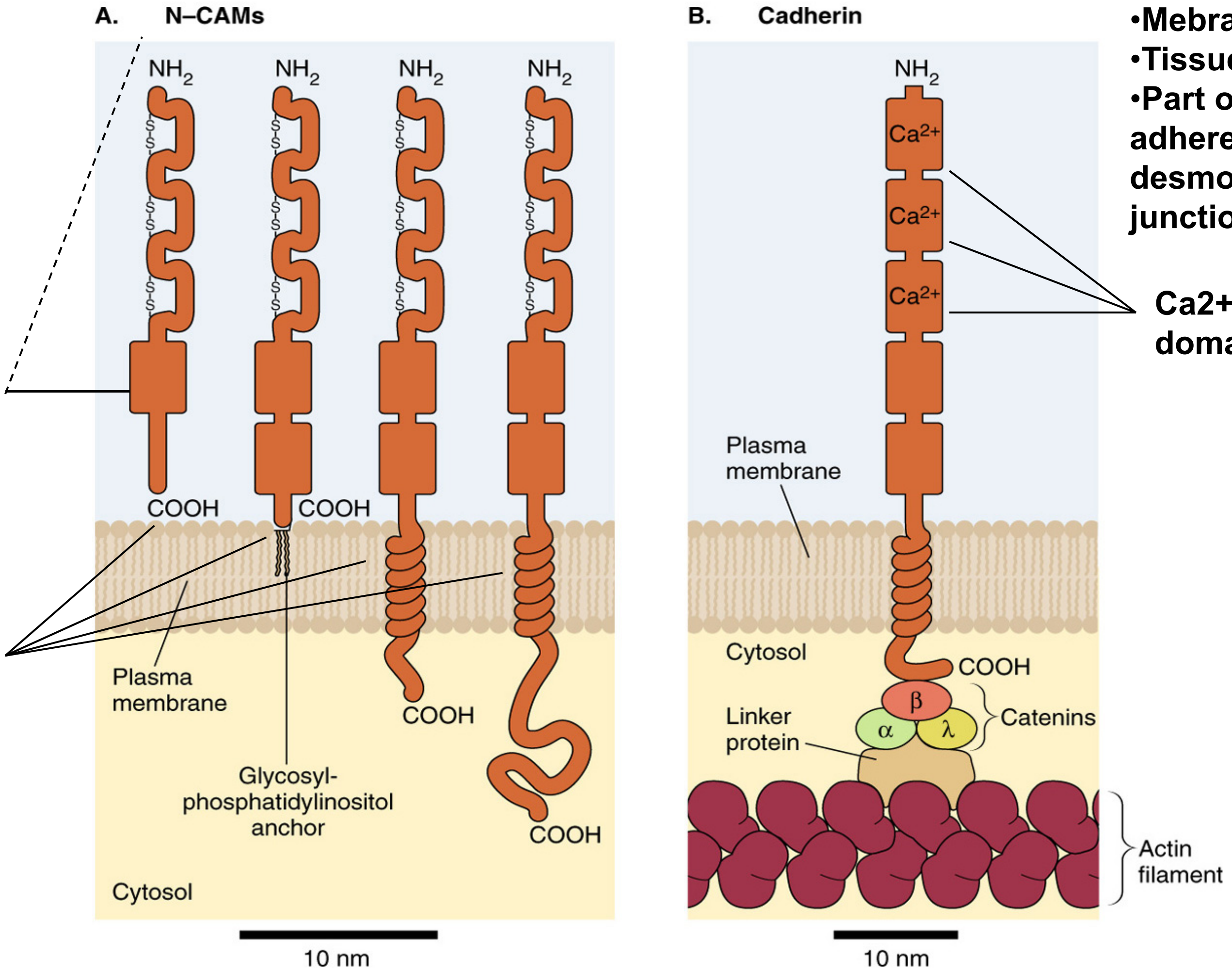

A.
B.
C.
D.
Relative hierarchy of cohesiveness
KEY
Relative strength of adhesion
Cartilage
Heart
Liver

Cell Adhesion Molecules (CAMs) Cadherins

•Homophilic heterophilic
•With or without Ca2+
•Membrane-bound or free

5 looped extracellular domains in total (Ig-like]

Different subtypes of CAMs

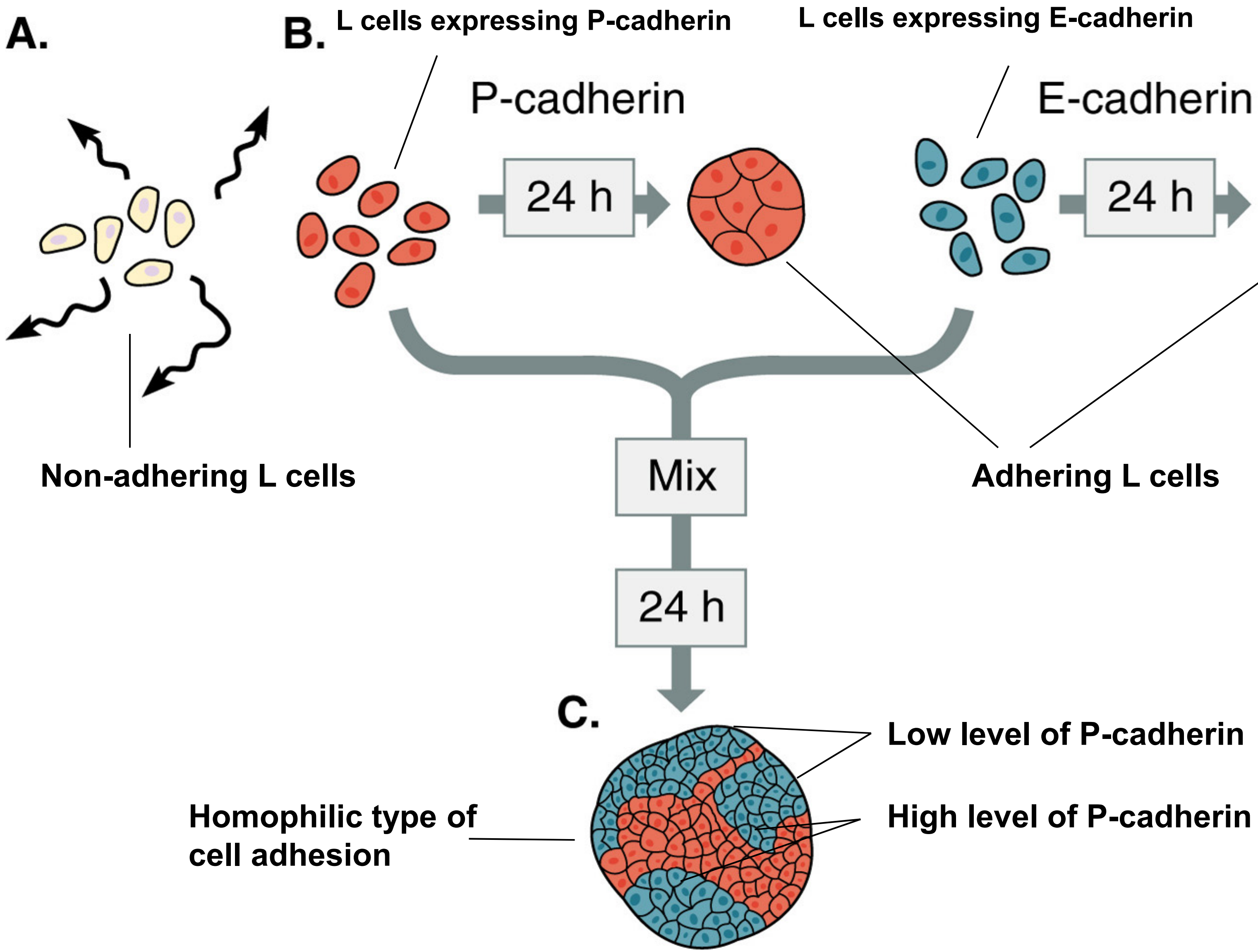


•Homophilic
•Ca2+-dependent
•Mebrane-bound
•Tissue specific
•Part of the adherens and desmosome junctions

Ca2+ 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^{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


A.
B.
L cells expressing P-cadherin
L cells expressing E-cadherin
P-cadherin
E-cadherin
24 h
24 h
Non-adhering L cells
Mix
Adhering L cells
24 h
C.
Low level of P-cadherin
High level of P-cadherin
Homophilic type of
cell adhesion

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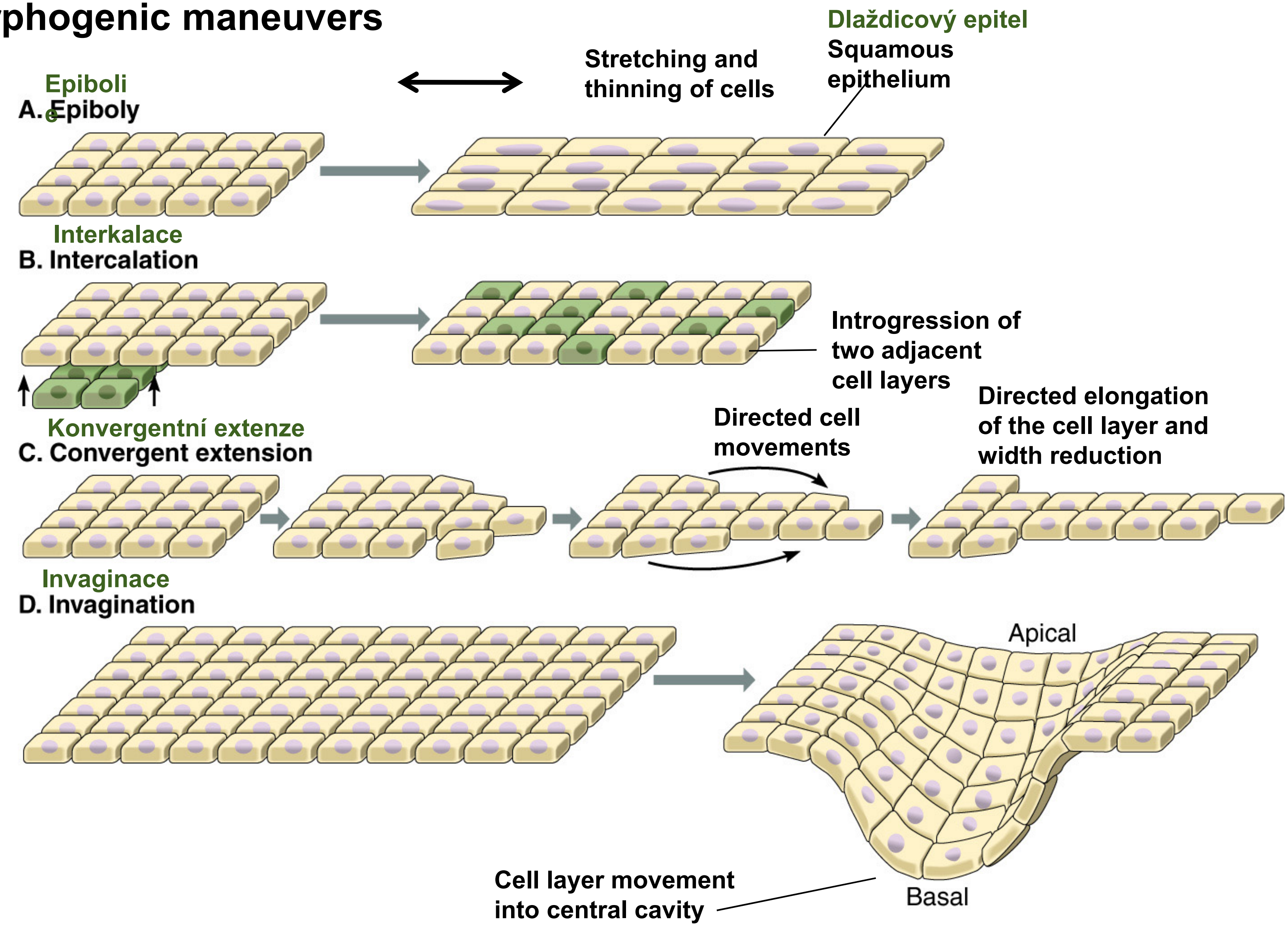
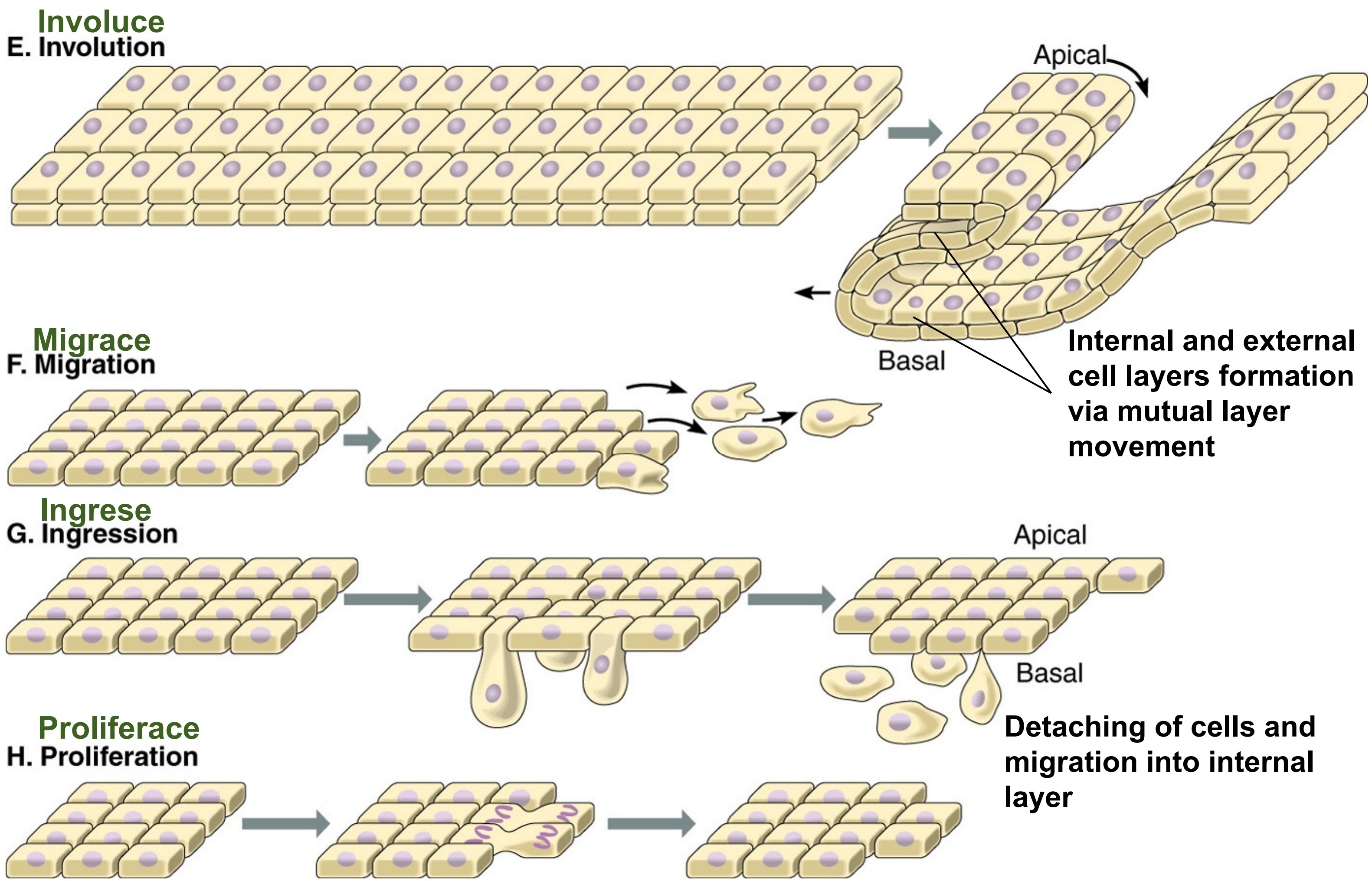
  - Morphogenic manoeuvres

Morphogenic maneuvers



Morphogenic maneuvers



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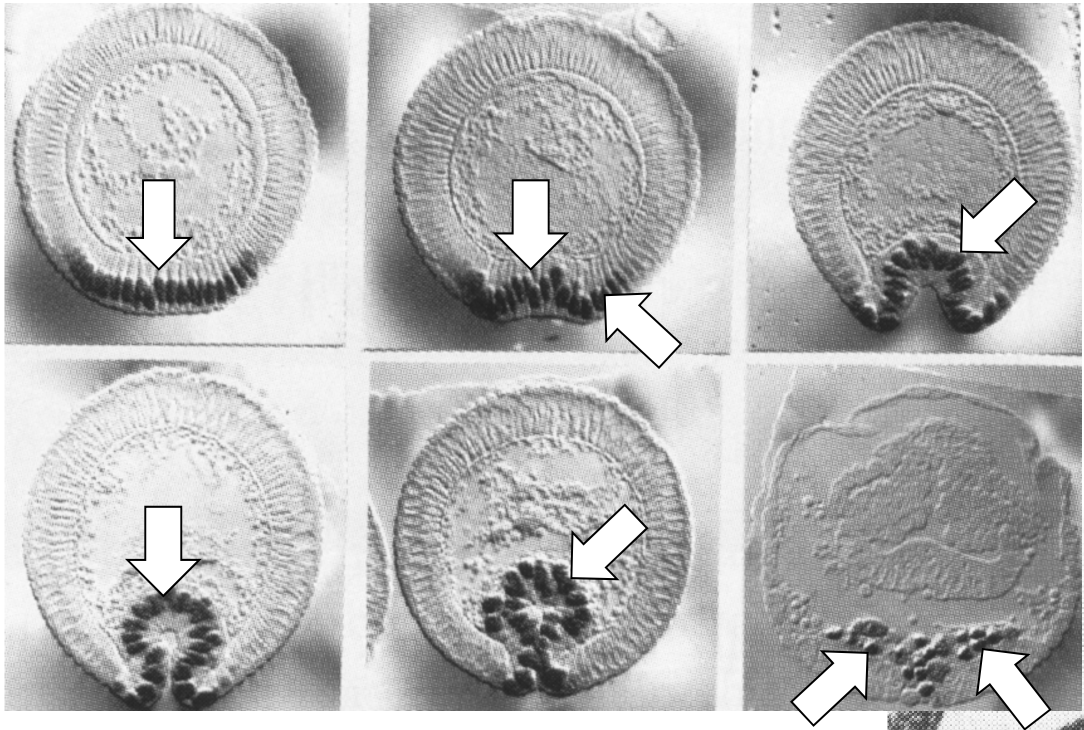
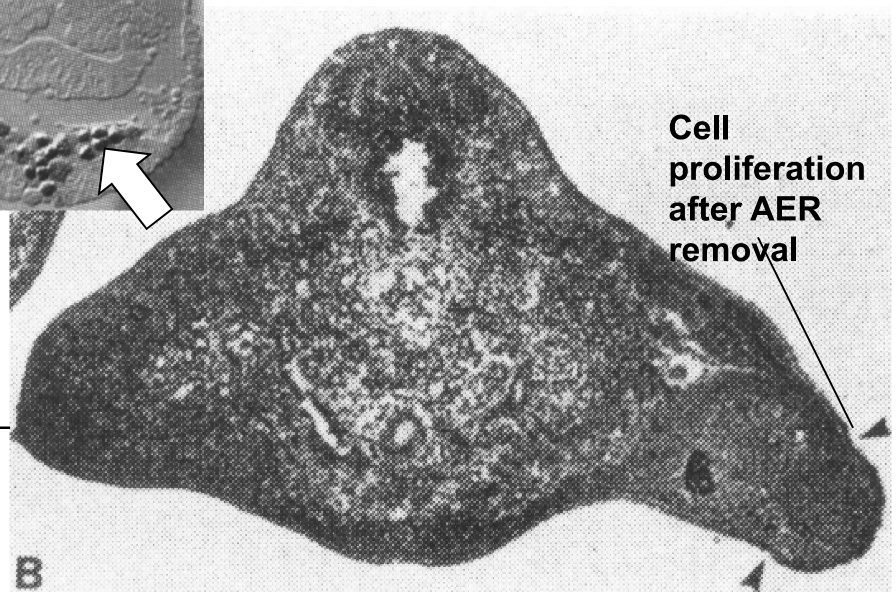
TWIST localization during neural furrow formation in *Drosophila*



Regulation of cell proliferation by AER in mouse



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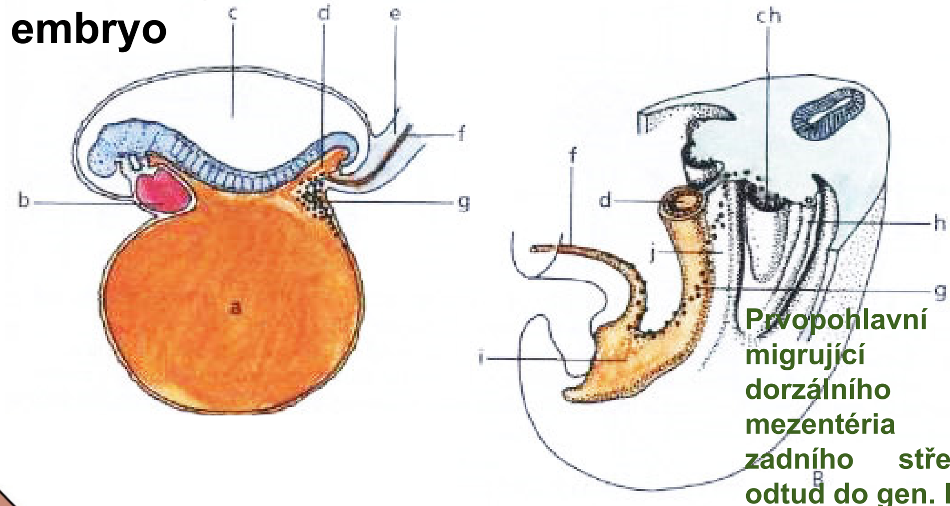
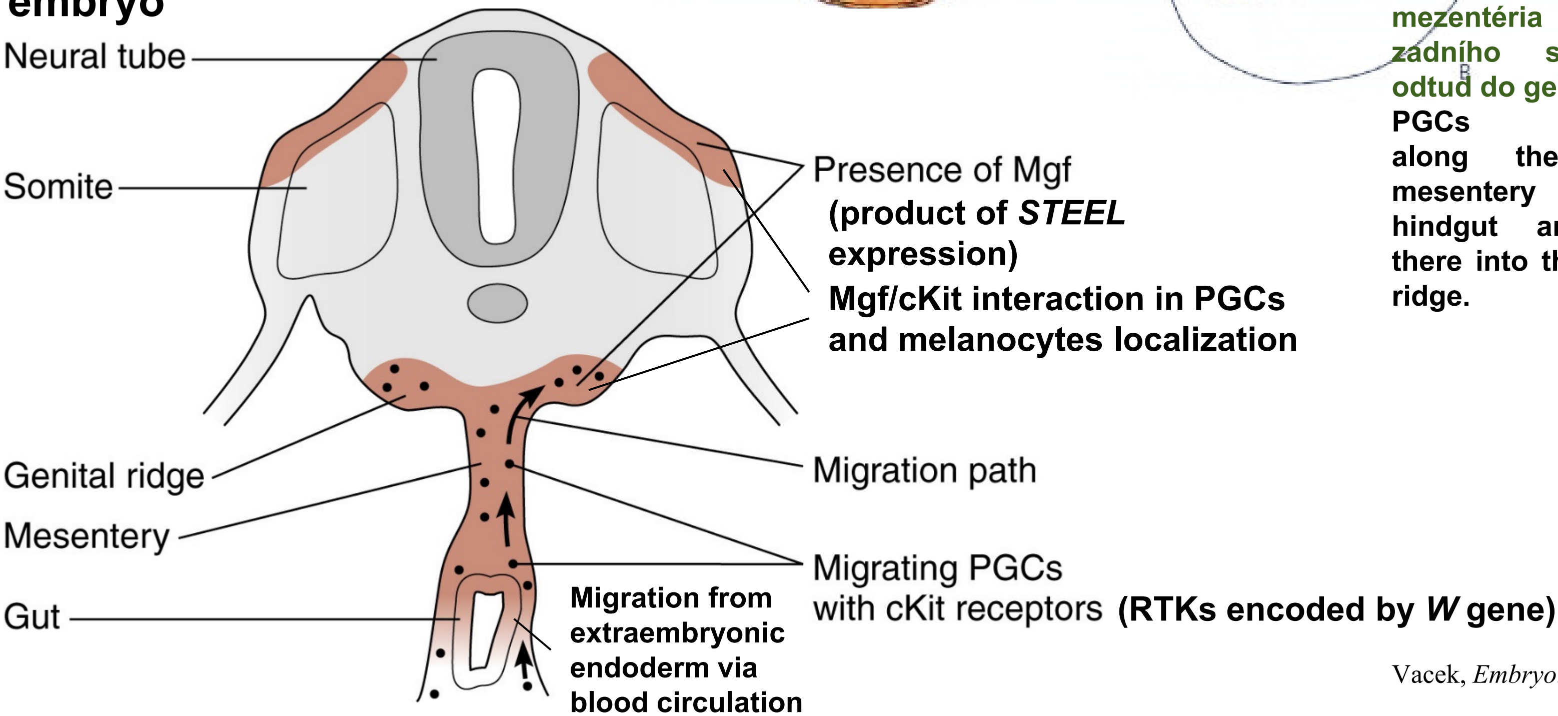
PGCs migration in human embryo



Prvopohlavní buňky migrující podél dorzálního mezentéria do zadního střeva a odtud do gen. Lišty

PGCs migrating along the dorzal mesentery into the hindgut and from there into the genital ridge.

PGCs migration in chicken embryo



Vacek, *Embryologie* (2006)

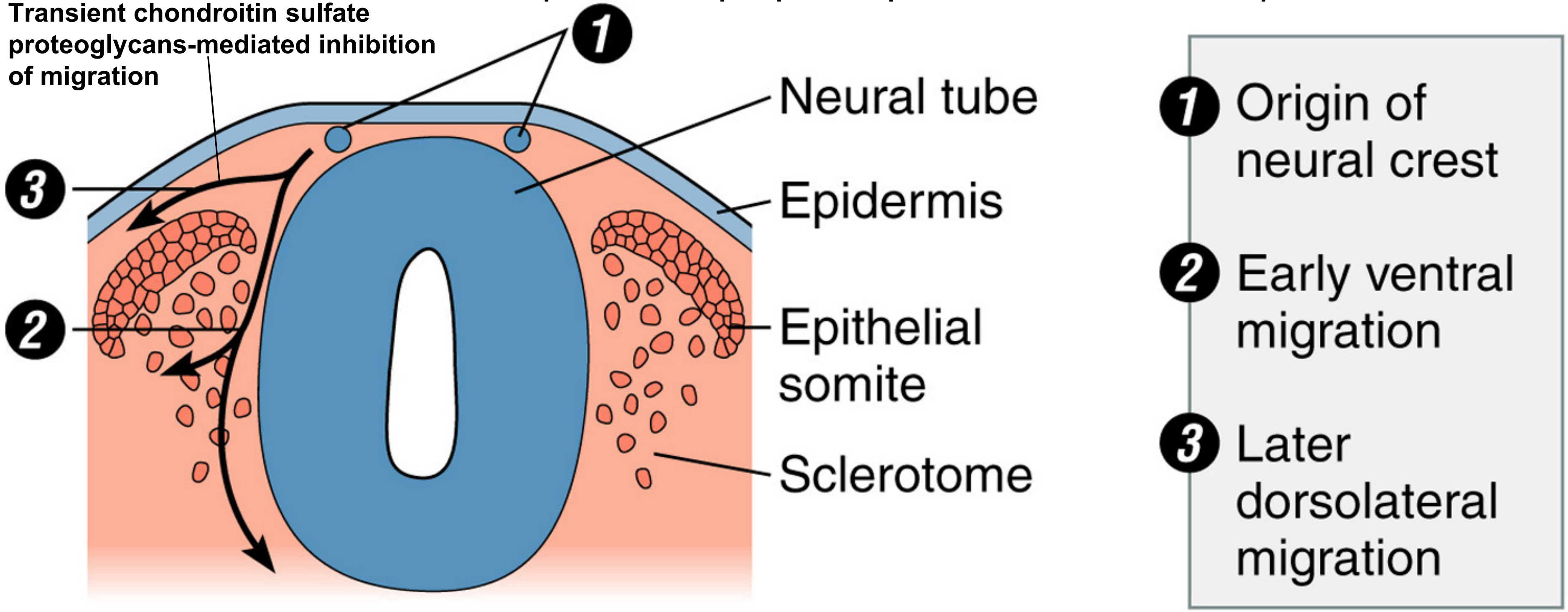
Neural crest cells migration

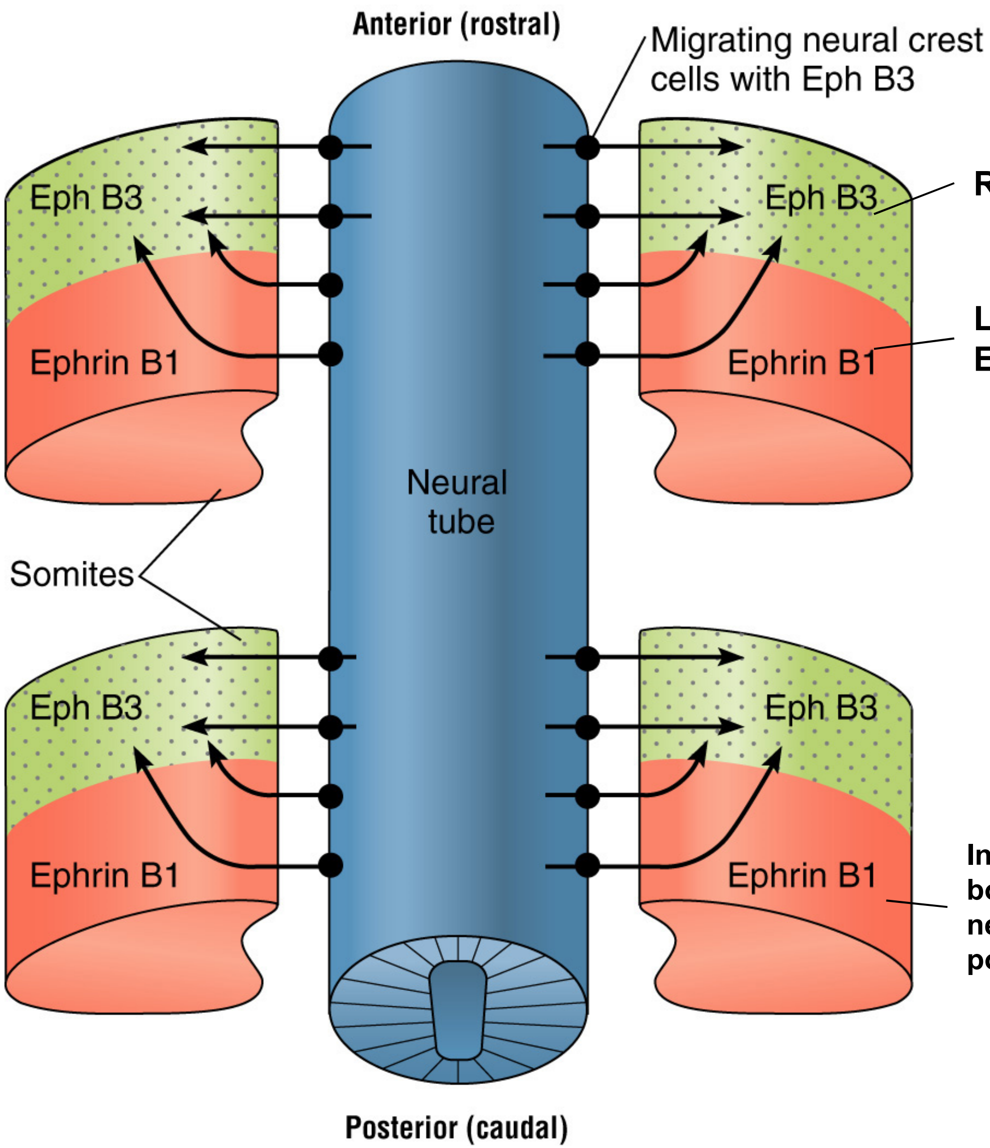
↓ adhesivity and ↑ migration

↑

Stimulation of neural crest formation via BMP4 production in prospective epidermis ⟶ Reduction of N- and E-cadherins and N-CAM production

Transient chondroitin sulfate proteoglycans-mediated inhibition of migration



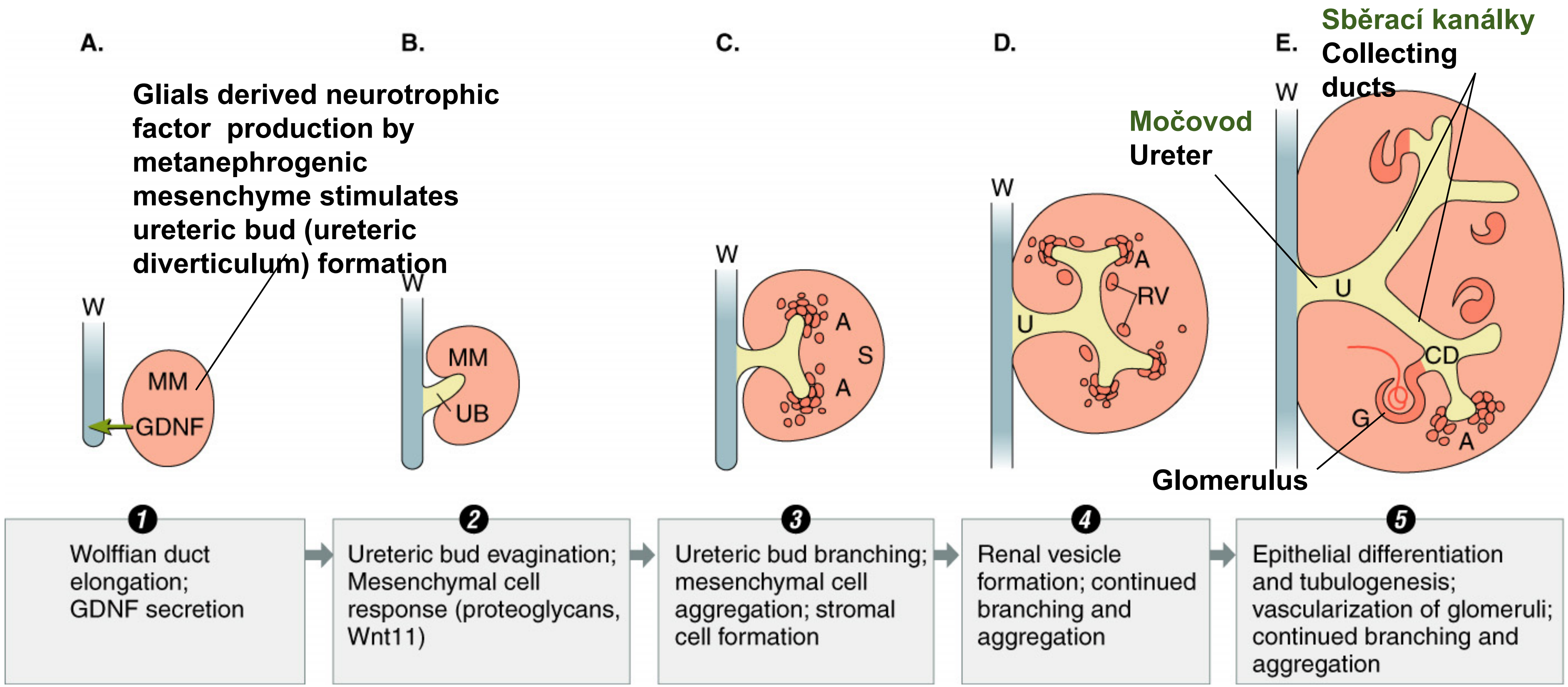


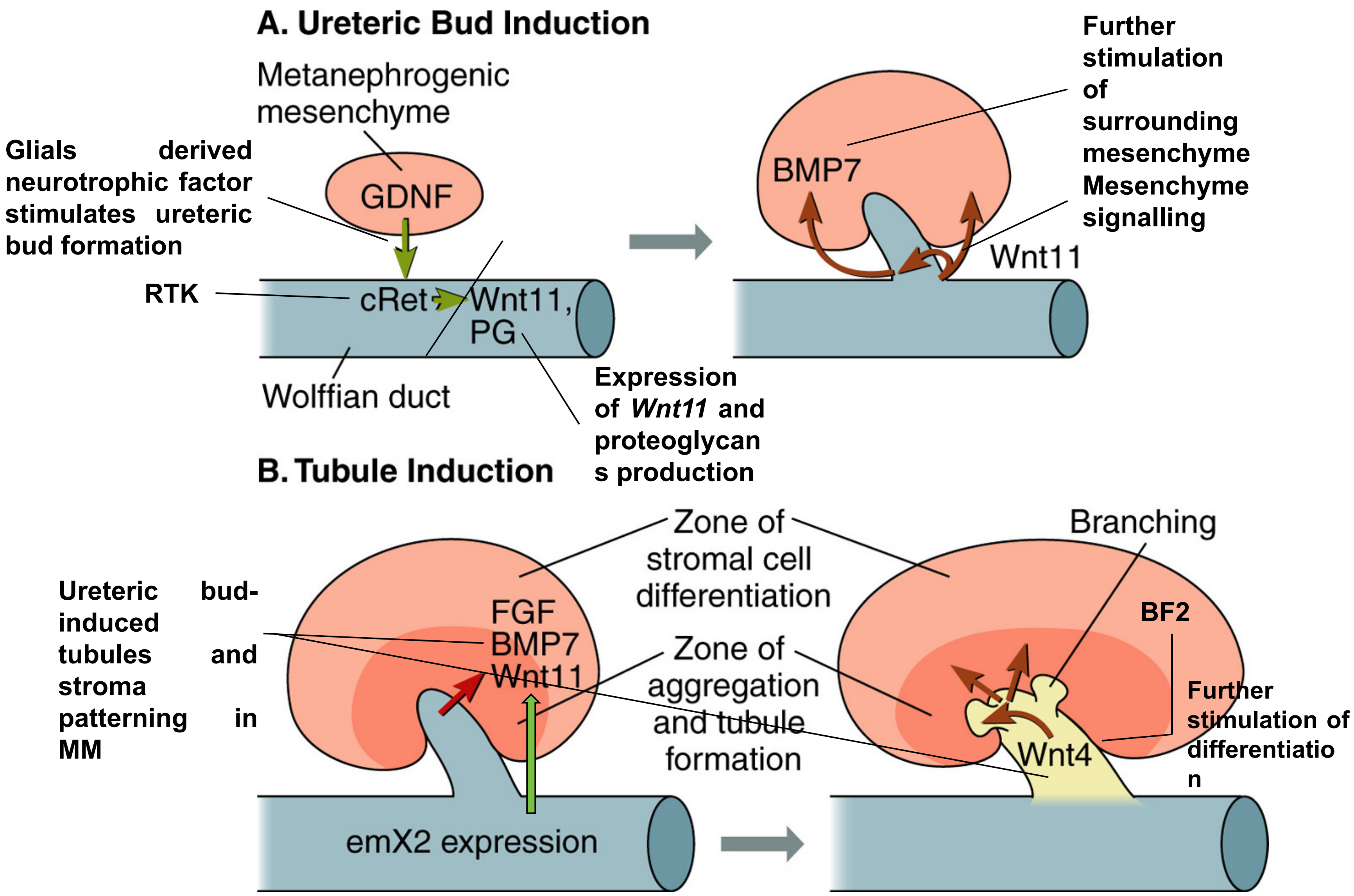
RTK

Ligand of EphB3

Interaction with substrate-bound Ephrin B1 traps the neural crest cells in the posterior somite portion

Kidney development




A. Ureteric Bud Induction
Metanephrogenic mesenchyme
GDNF
Glials derived neurotrophic factor stimulates ureteric bud formation
RTK
cRet
Wnt11, PG
Wolffian duct
Expression of *Wnt11* and proteoglycans production
BMP7
Wnt11
Further stimulation of surrounding mesenchyme Mesenchyme signalling
B. Tubule Induction
Ureteric bud-induced tubules and stroma patterning in MM
FGF
BMP7
Wnt11
emX2 expression
Zone of stromal cell differentiation
Zone of aggregation and tubule formation
Branching
BF2
Wnt4
Further stimulation of differentiation

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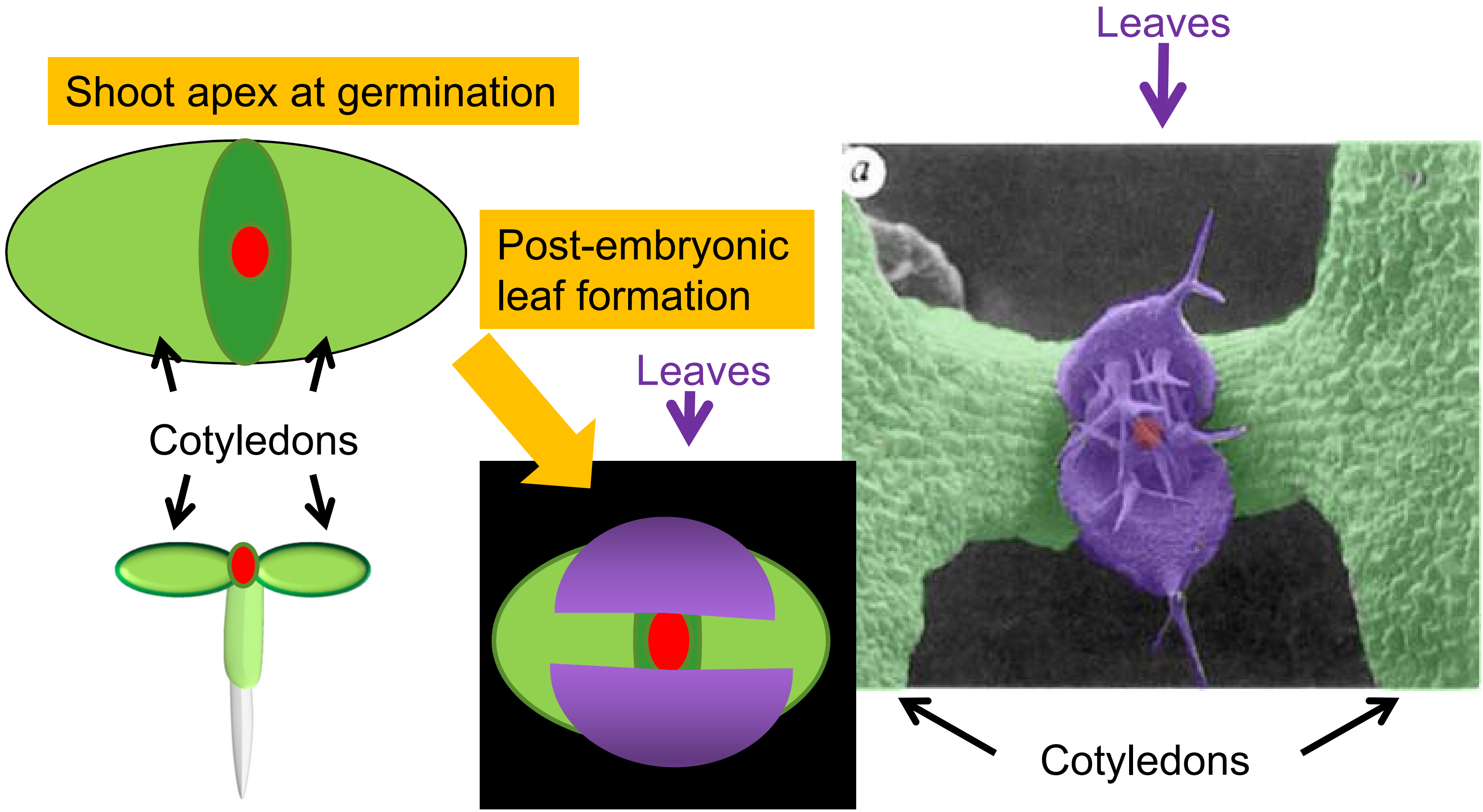
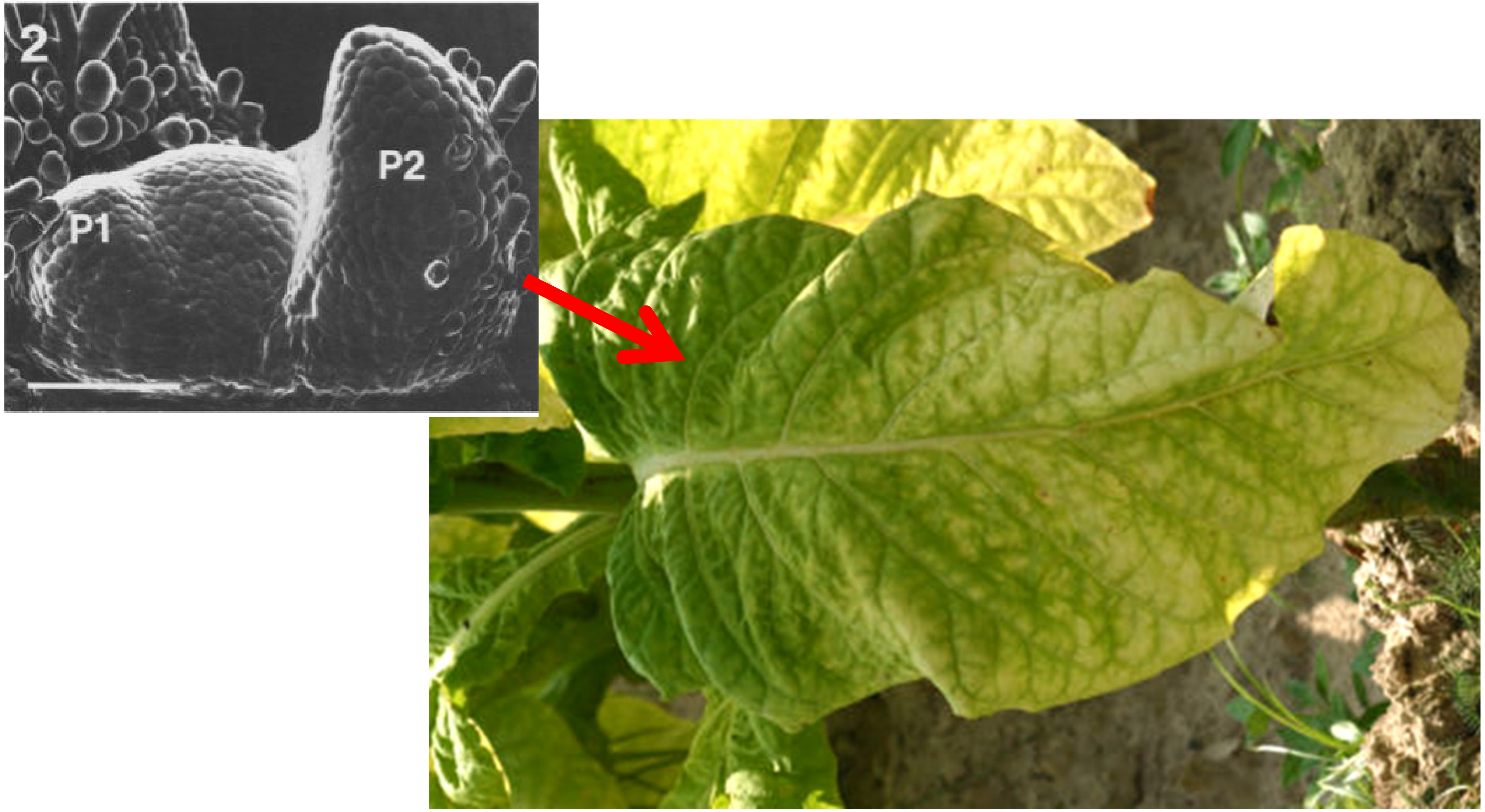
Origin of leaves

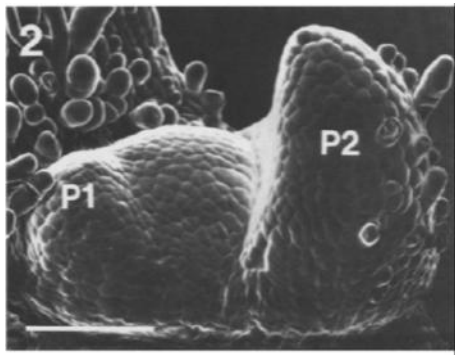


Reprinted by permission from Macmillan Publishers, Ltd: NATURE. Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K. (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. Nature 379: 66-69.

How does a leaf primordium become a leaf?



How does a leaf primordium become a leaf?



0.2 mm

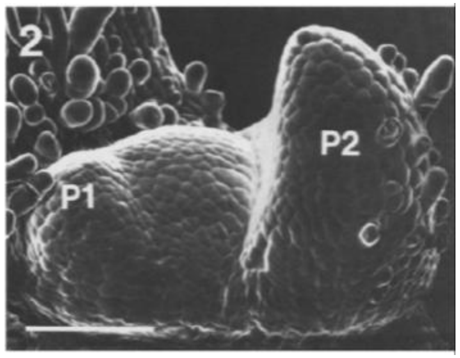
GROWTH

500 mm

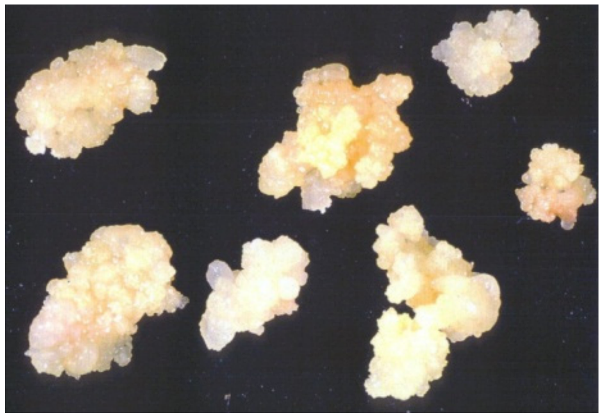
The leaf primordium increases in length ~ 2500 fold by cell division and cell expansion.

CEITEC

How does a leaf primordium become a leaf?



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



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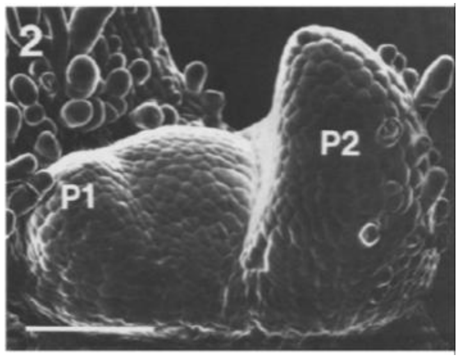
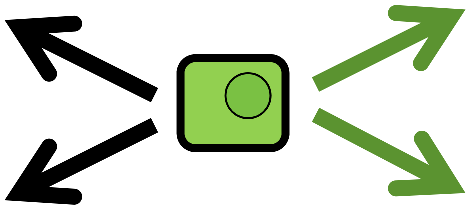


To make a leaf, each cell in the primordium must divide, grow and differentiate in a controlled way.



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

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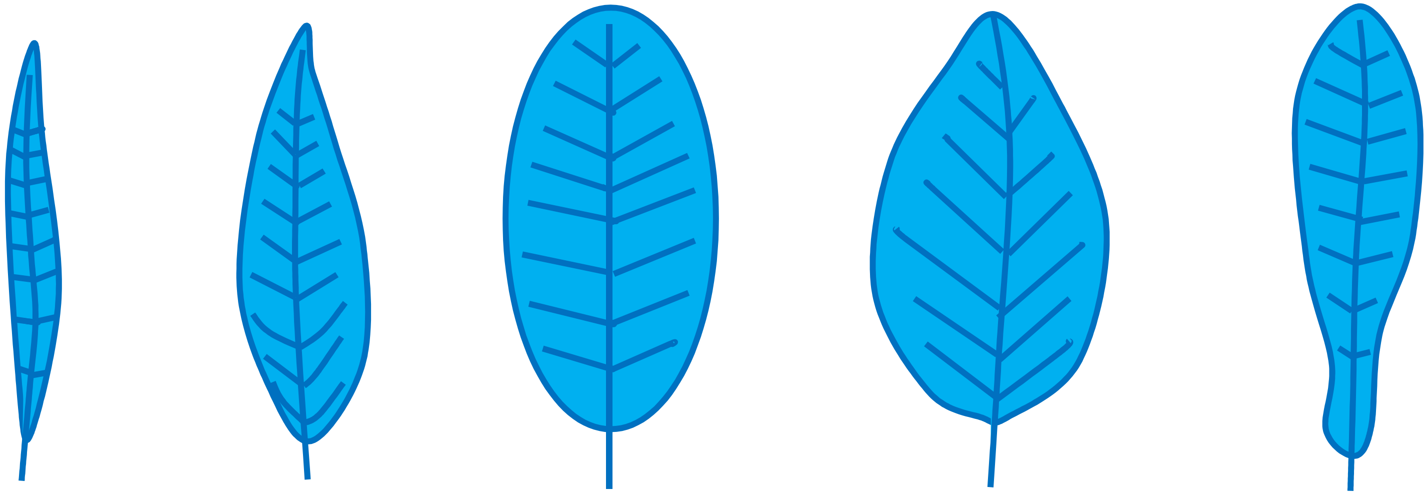
Leaf forms



Podlouhlý
Linear

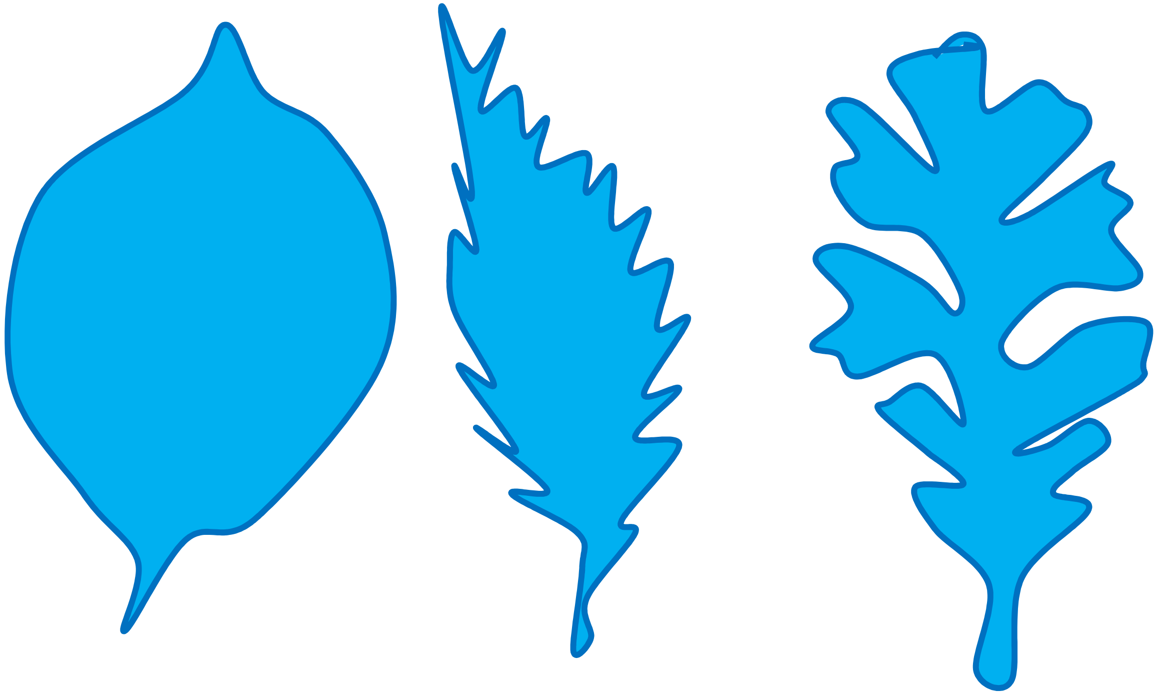
Kopinatý
Lanceolate

Eliptický
Elliptical

Vejčitý
Ovate

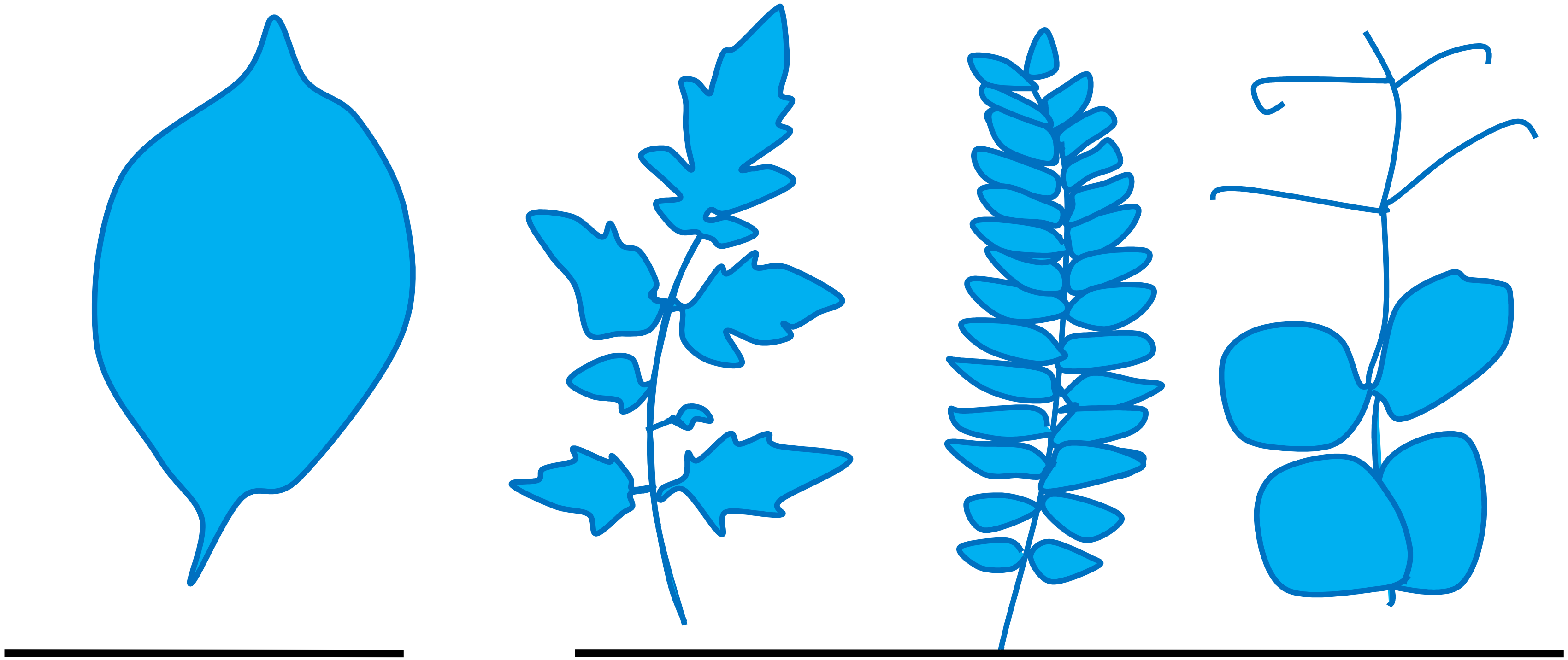
Kopistovitý
Spatulate

Leaf forms



Hladký Laločnatý
Dílný/Sečný
Smooth Serrated Lobed

Leaf forms



Jednoduchý
Simple

Složený
Compound

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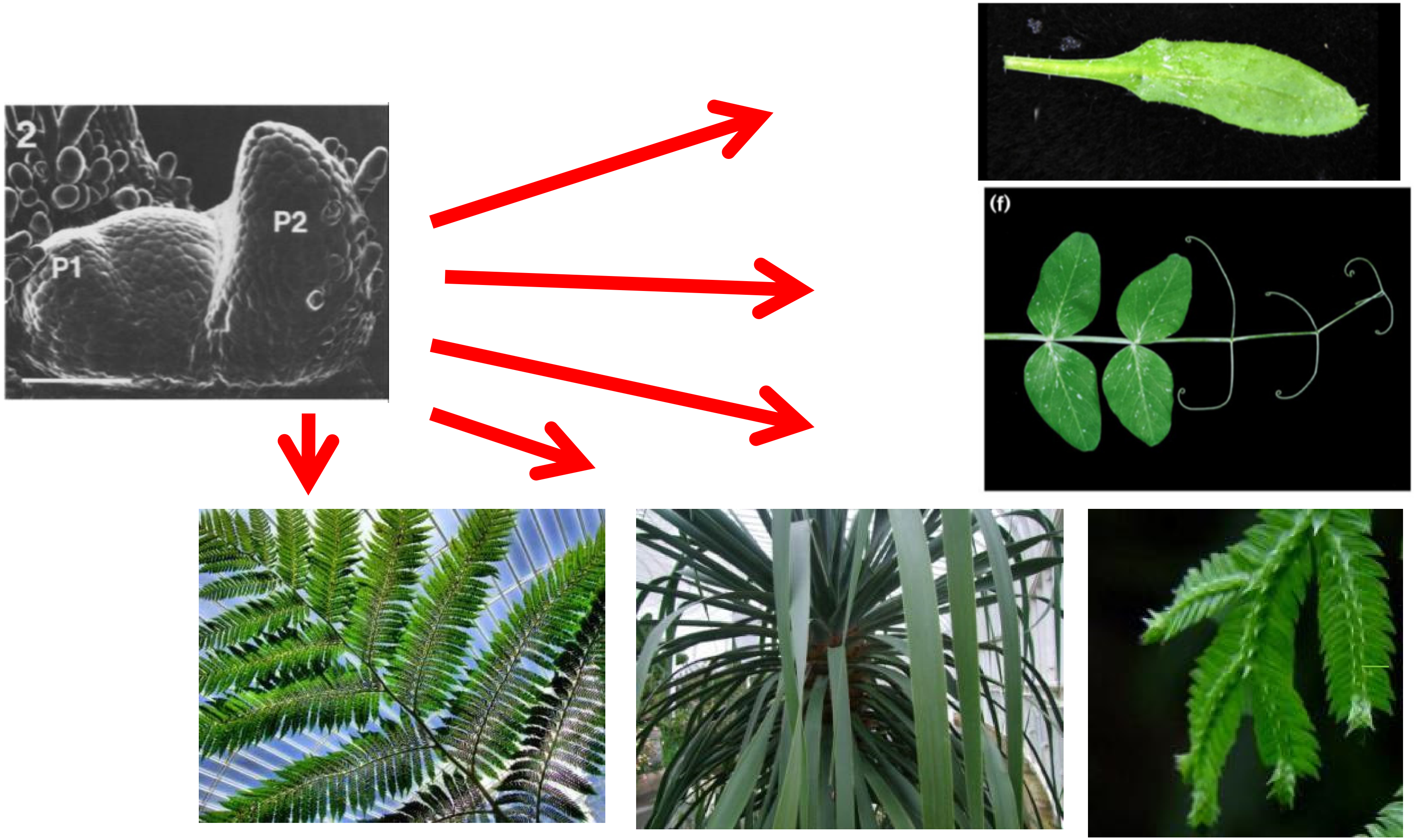
  - The role of oriented cell division and its relative distribution

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.

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

↓

CYCD3

↓

Cell division

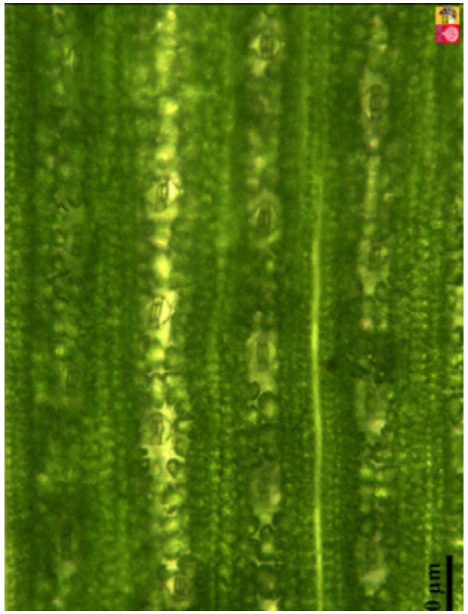
ant-1 WT ANT-OX

→

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

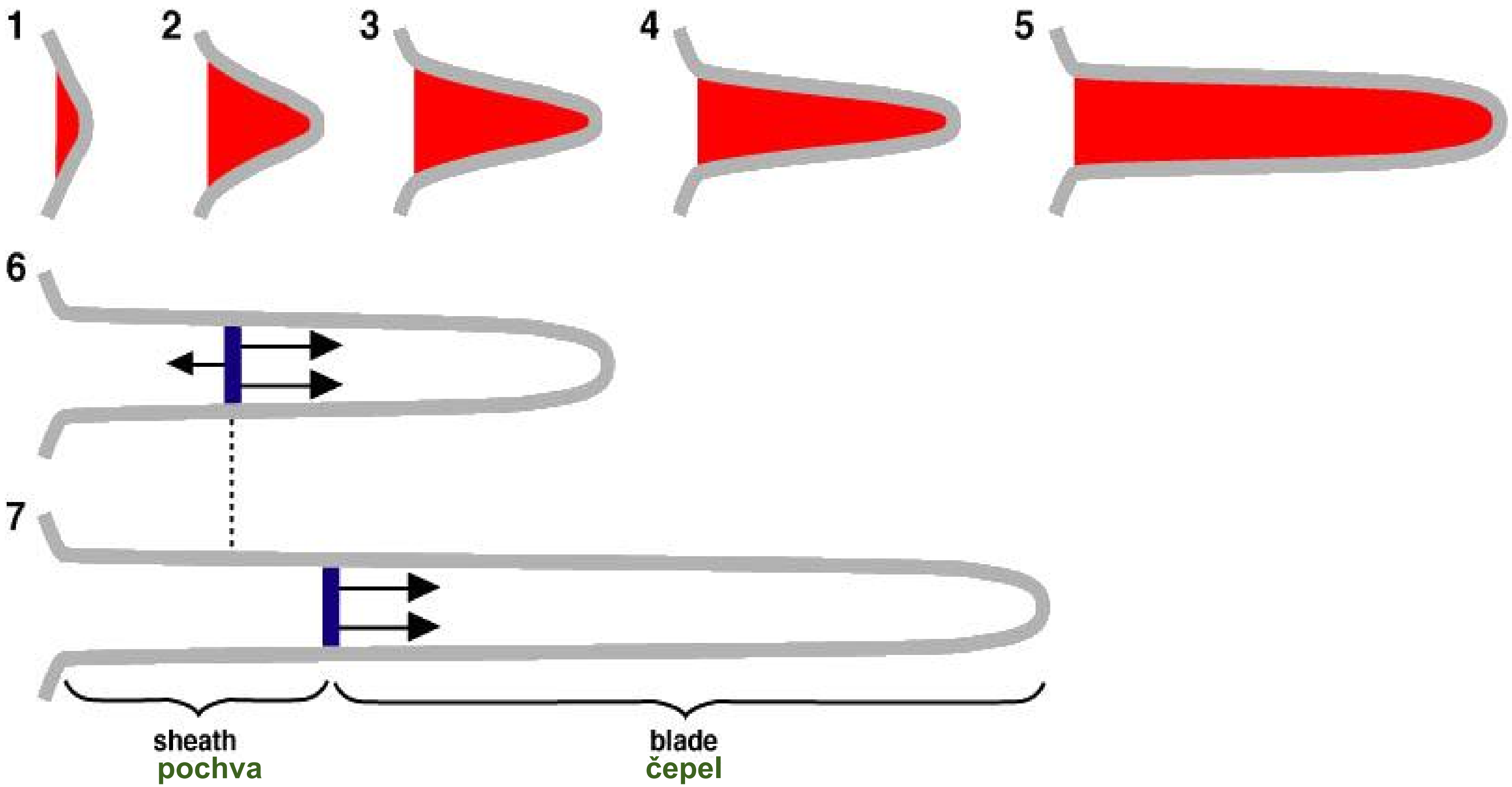
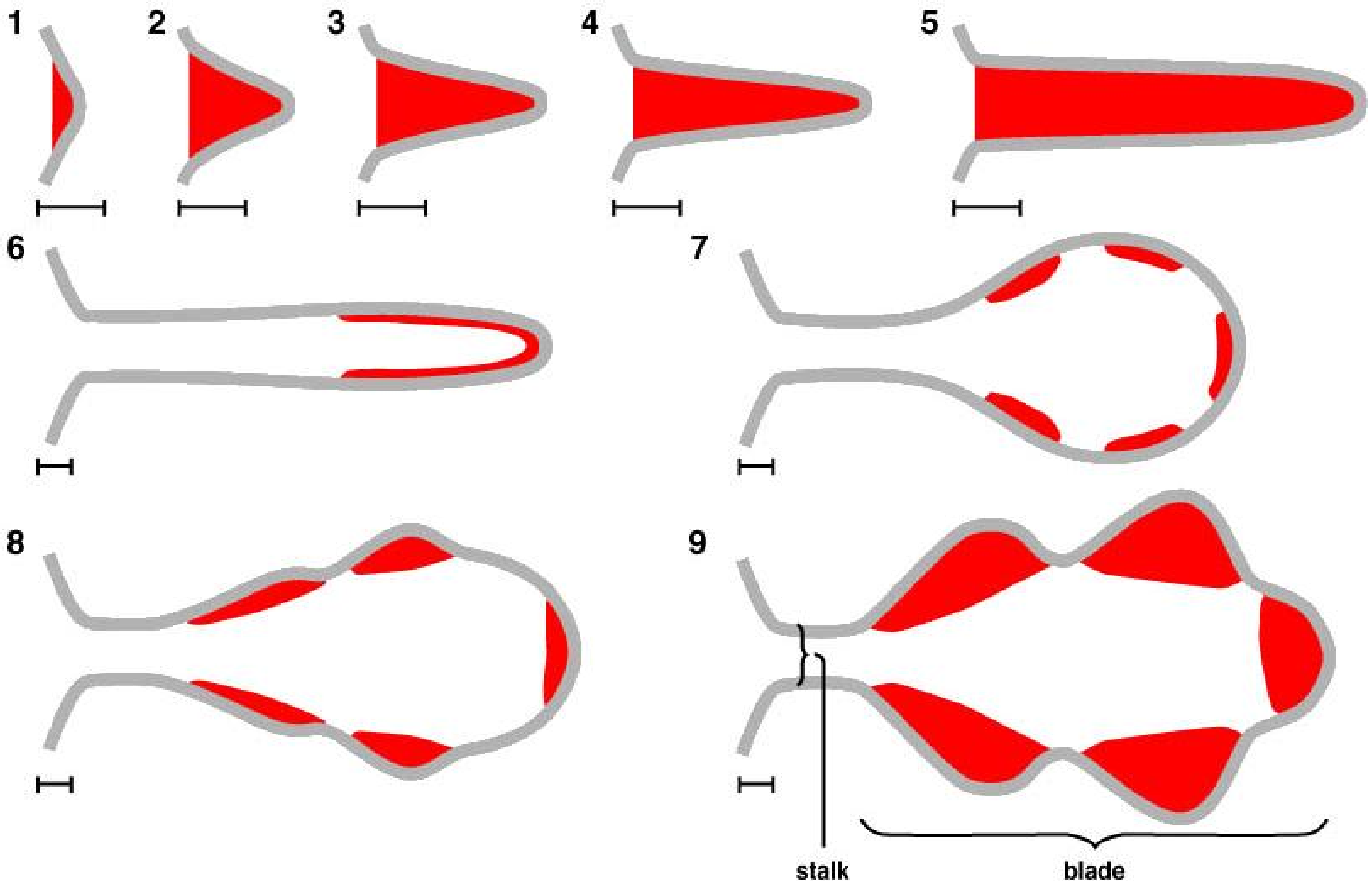


Image courtesy of J. Derksen, J. Hiddink and E.S. Pierson Copyright Radboud University Nijmegen

Leaf growth in dicots

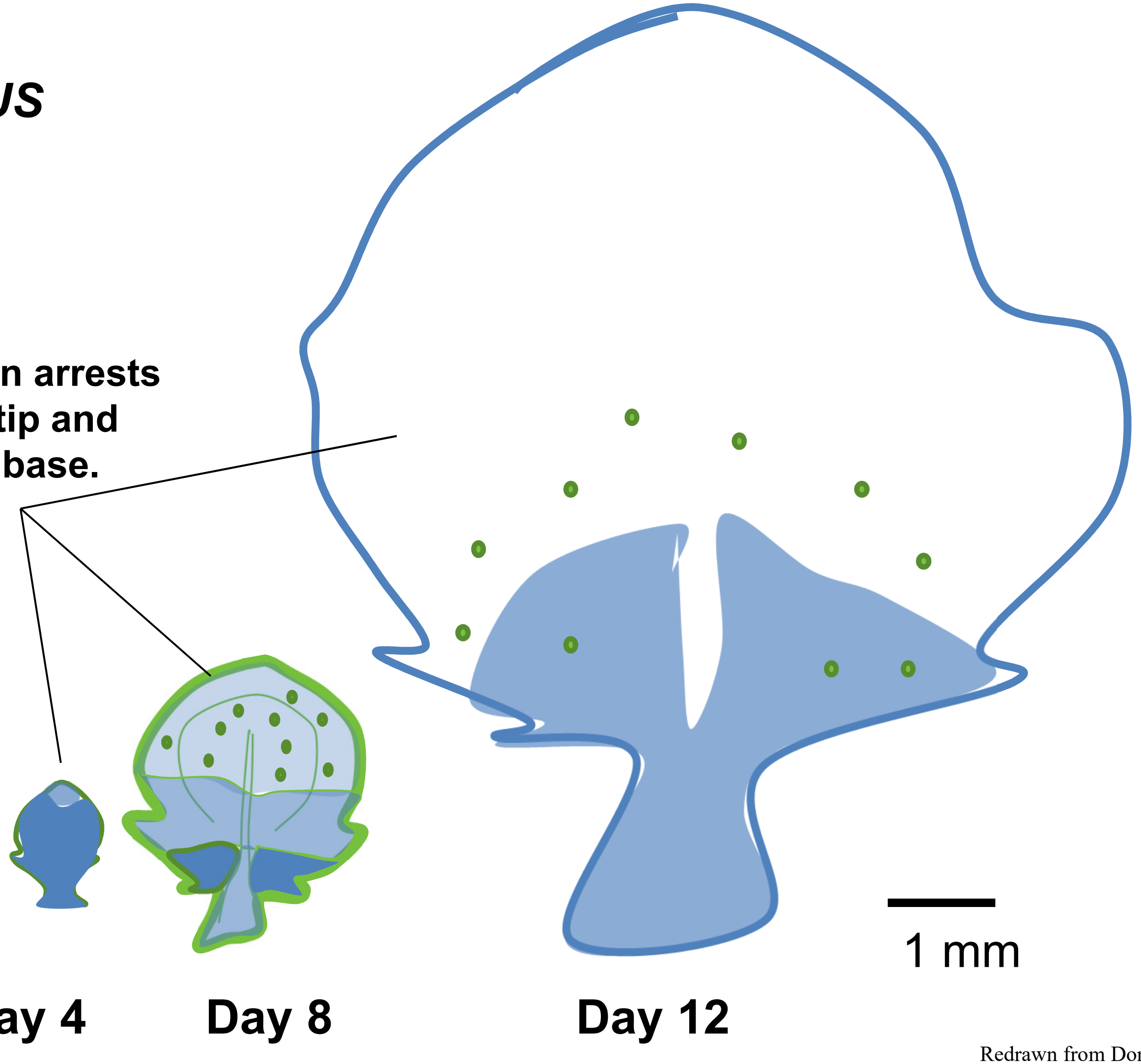


[Image](#) courtesy of J. Derksen, J. Hiddink and E.S. Pierson Copyright Radboud University Nijmegen

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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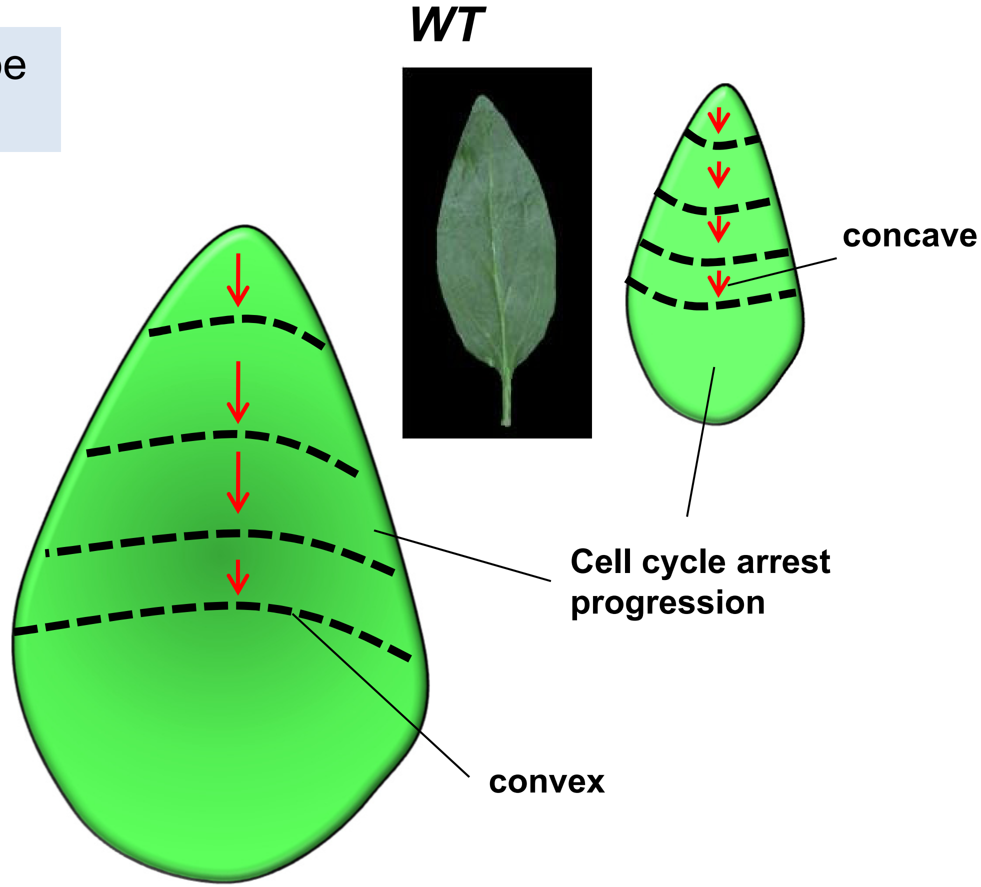
Altering the pattern of cell divisions alters leaf shape

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

cincinnata (cin)



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.

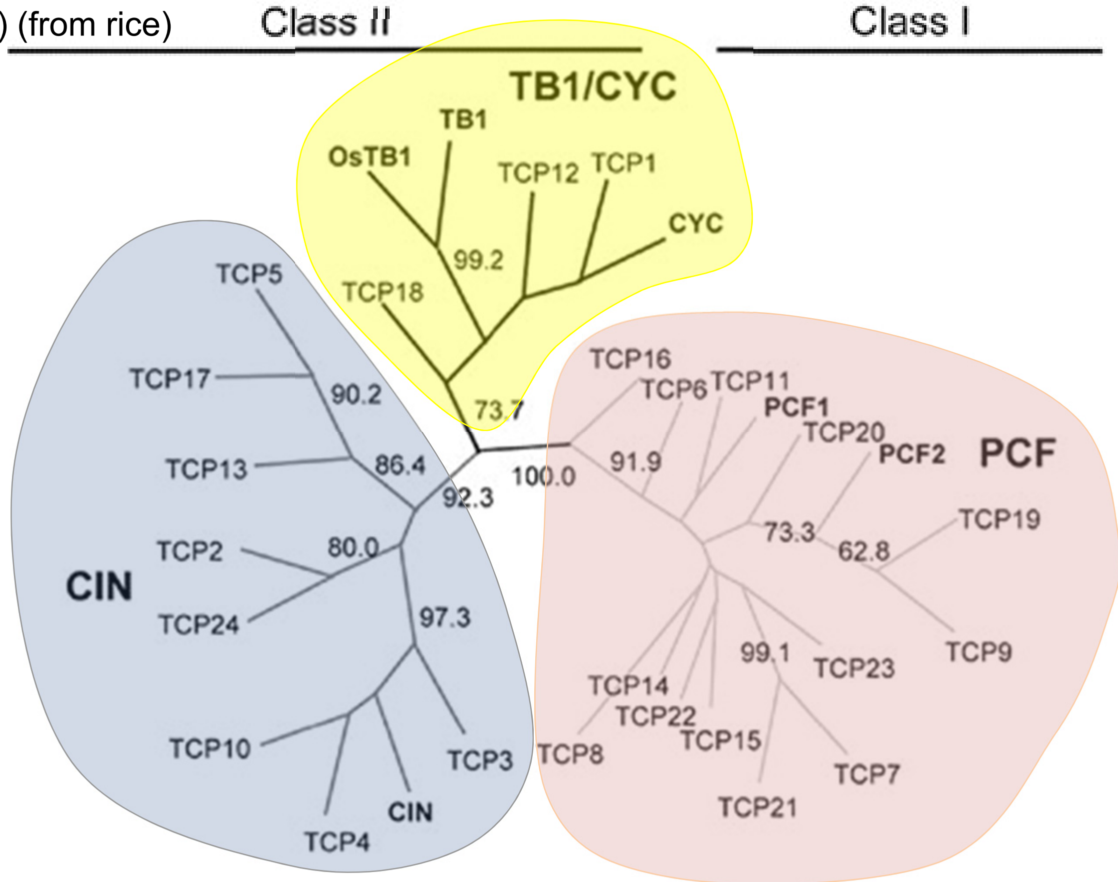
TCP genes

- ***T**EOSINTE BRANCHED1* (*TB1*) (from maize),
- ***C**YCLOIDEA* (*CYC*) (from *Antirrhinum*), and
- ***P**ROLIFERATING CELL FACTOR* (*PCF*) (from rice)

Basic-helix-loop-helix TFs

↓

Cell division



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

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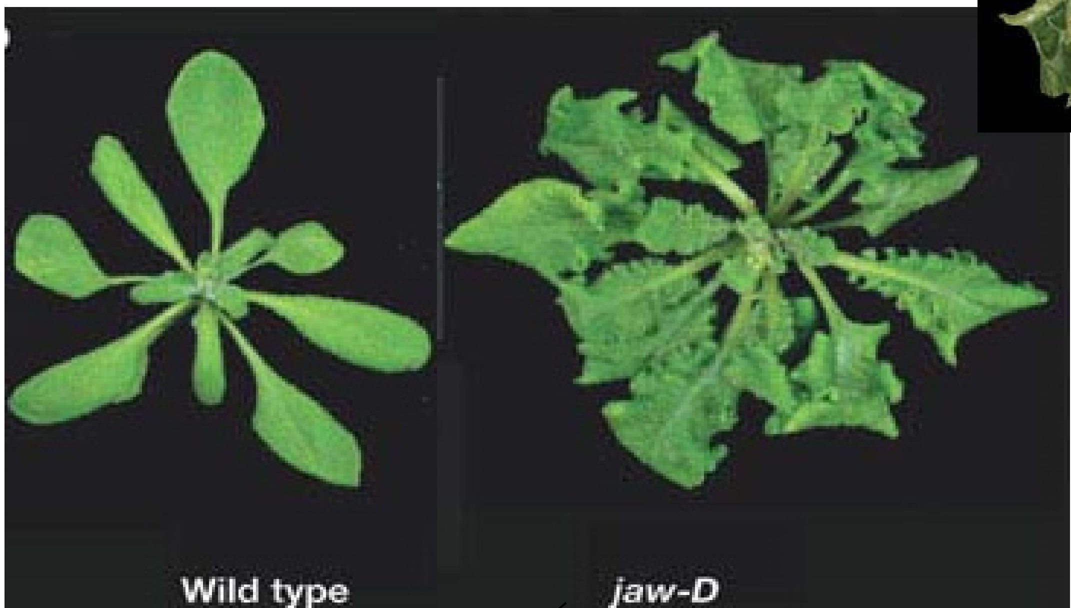
Over-accumulation of a miRNA (miR-JAW, in the *jaw-D* mutant) causes a similar phenotype

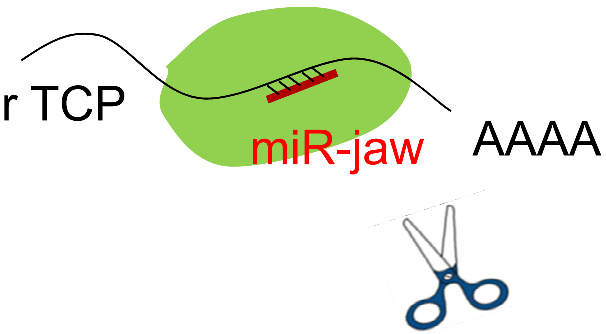
cin

WT ***Pro35S:miR-JAW***

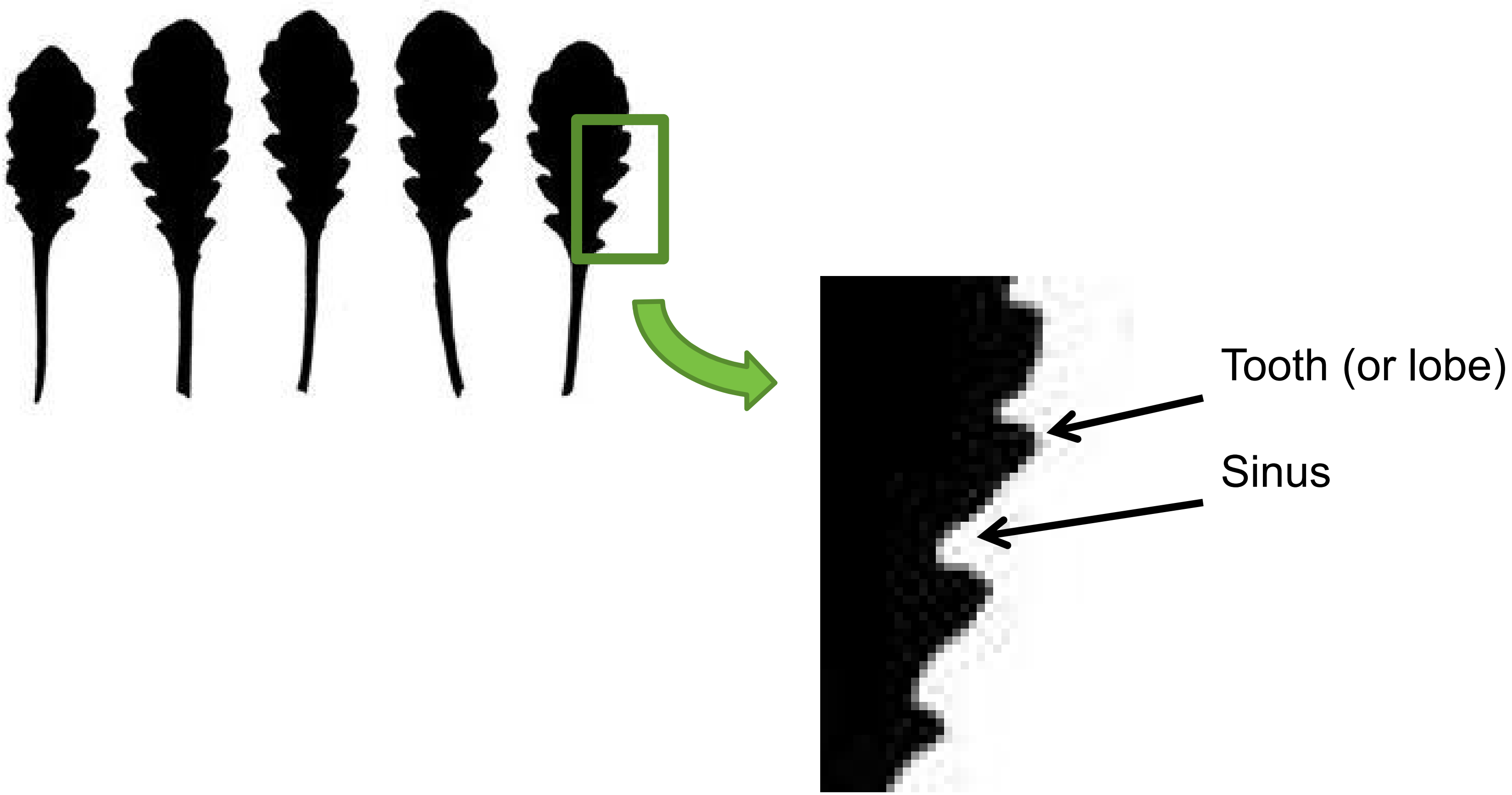


Phenocopy of *cincinnata*

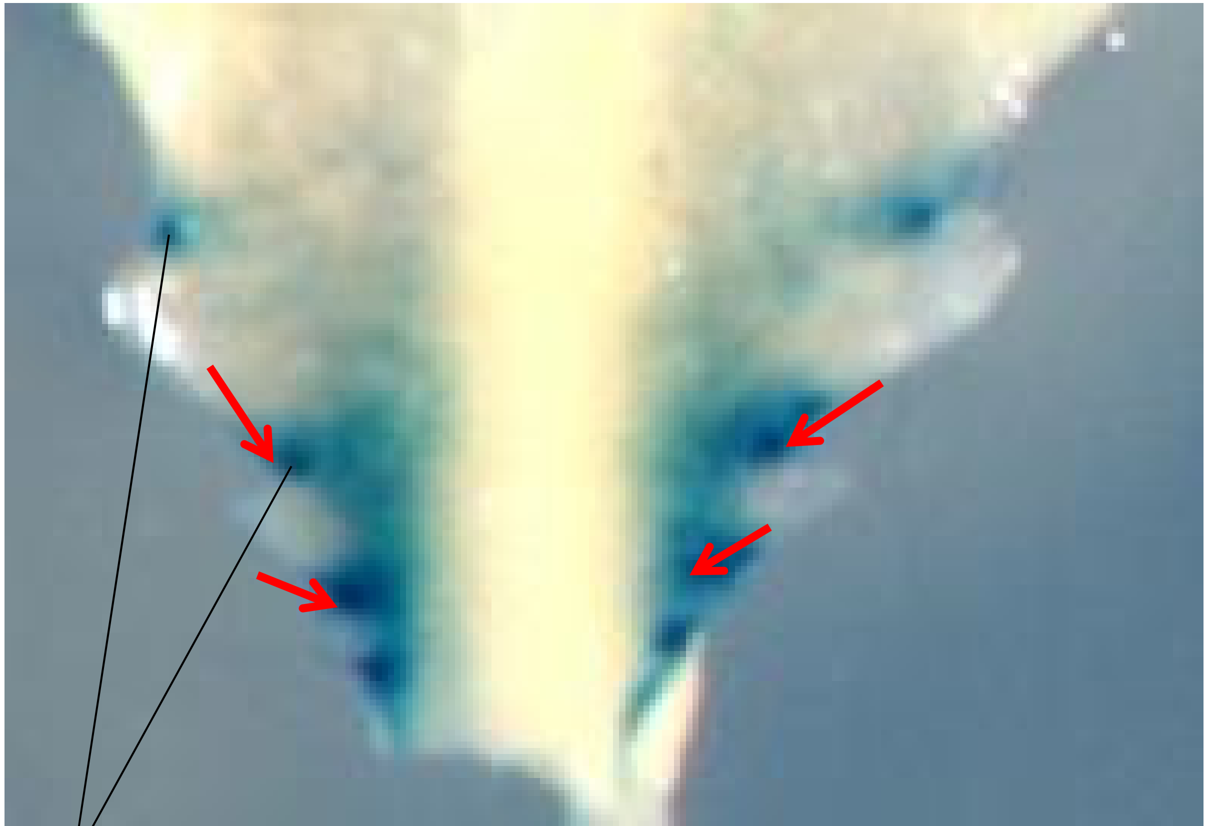
miR-jaw targets RNA from *TCP* genes (like *CIN*) for degradation



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.

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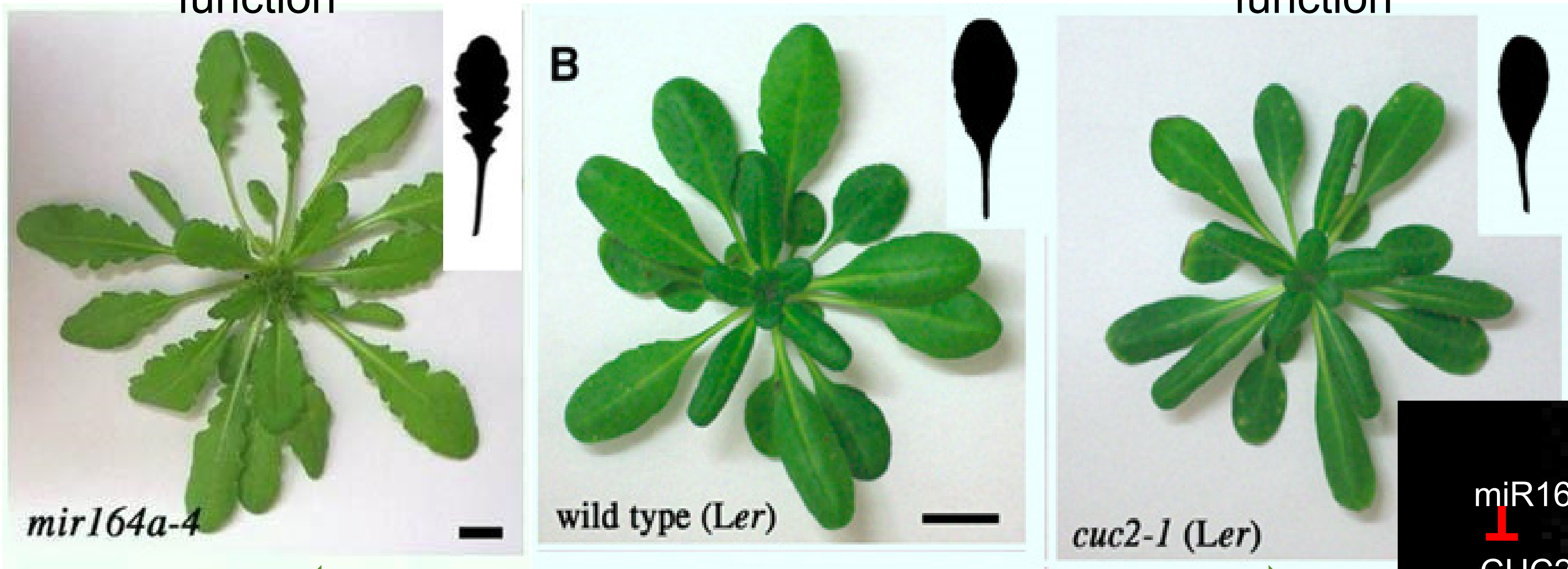
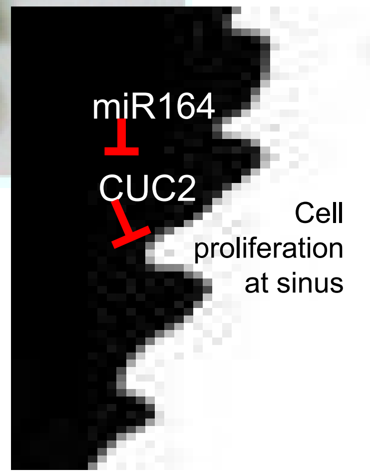
CUC2 expression is controlled by *miR164*

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.

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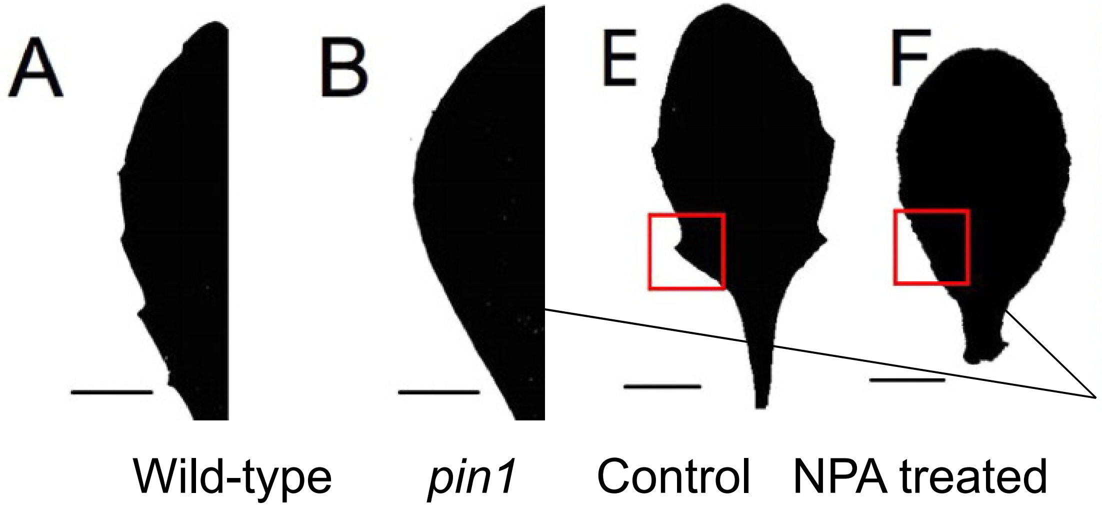
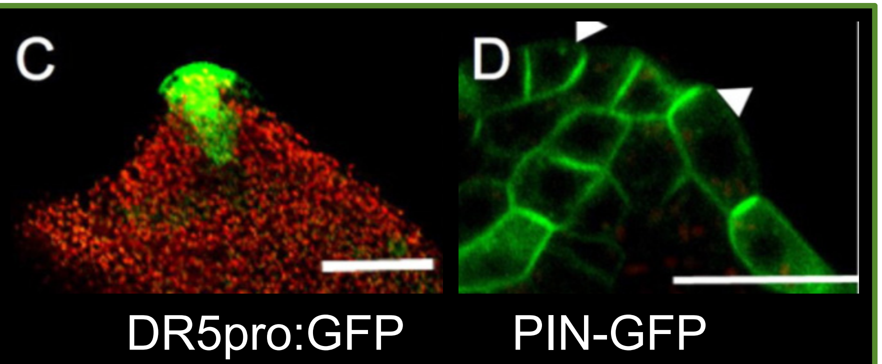
    - Auxin-regulated positional information for cell division

A local auxin maximum specifies the outgrowths of the leaf margin



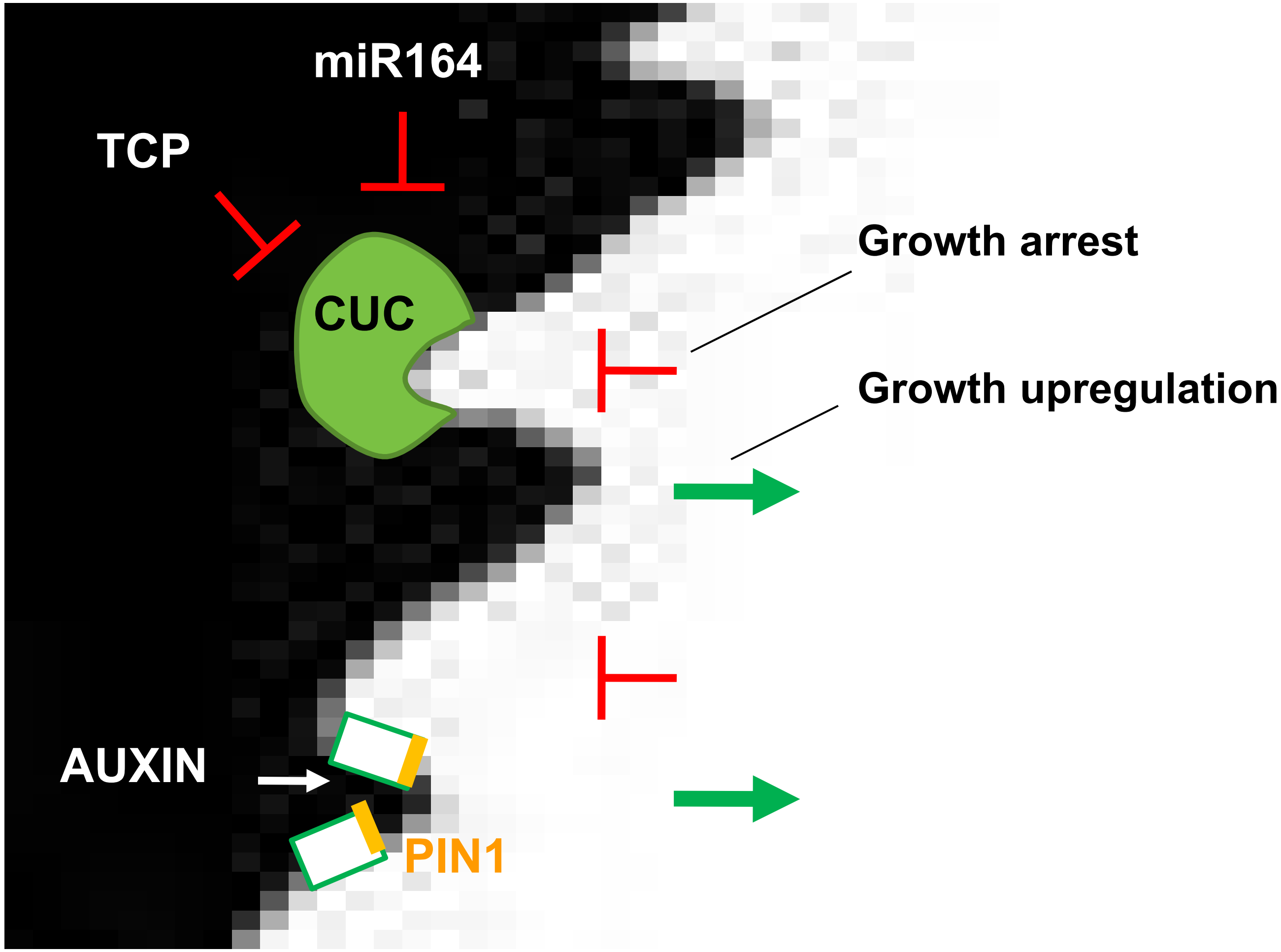
Interfering with auxin transport results in smooth-margined leaves

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.

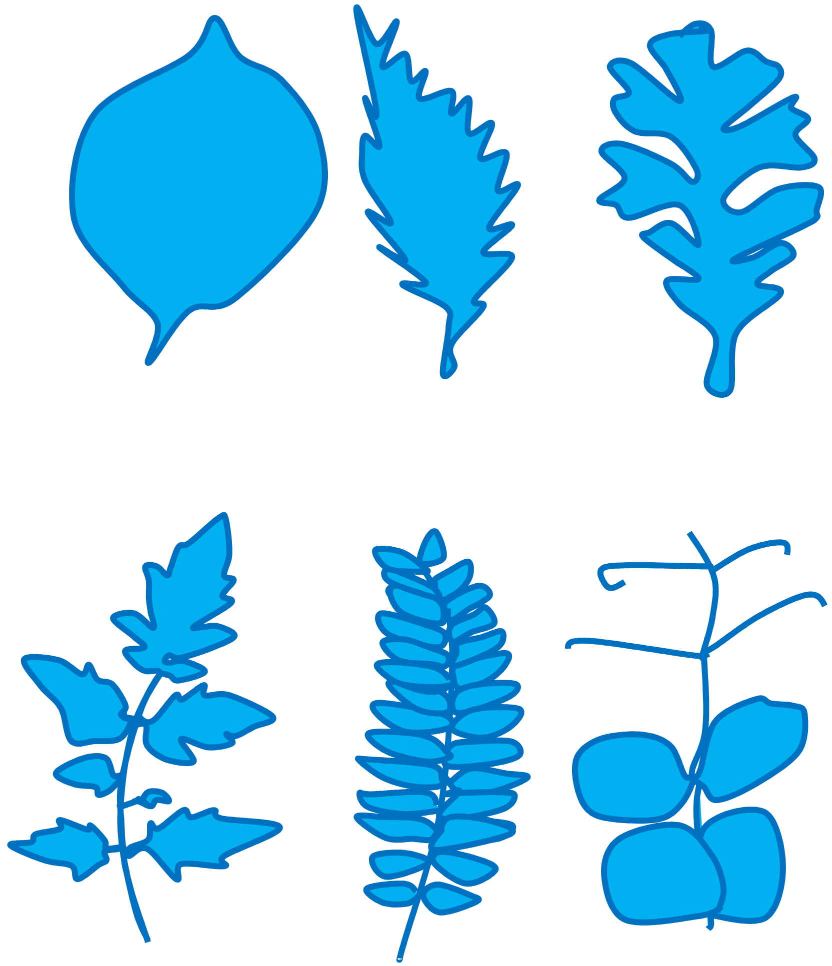
Summary - Control of leaf margin shape



What determines if a leaf is simple or compound?

Simple

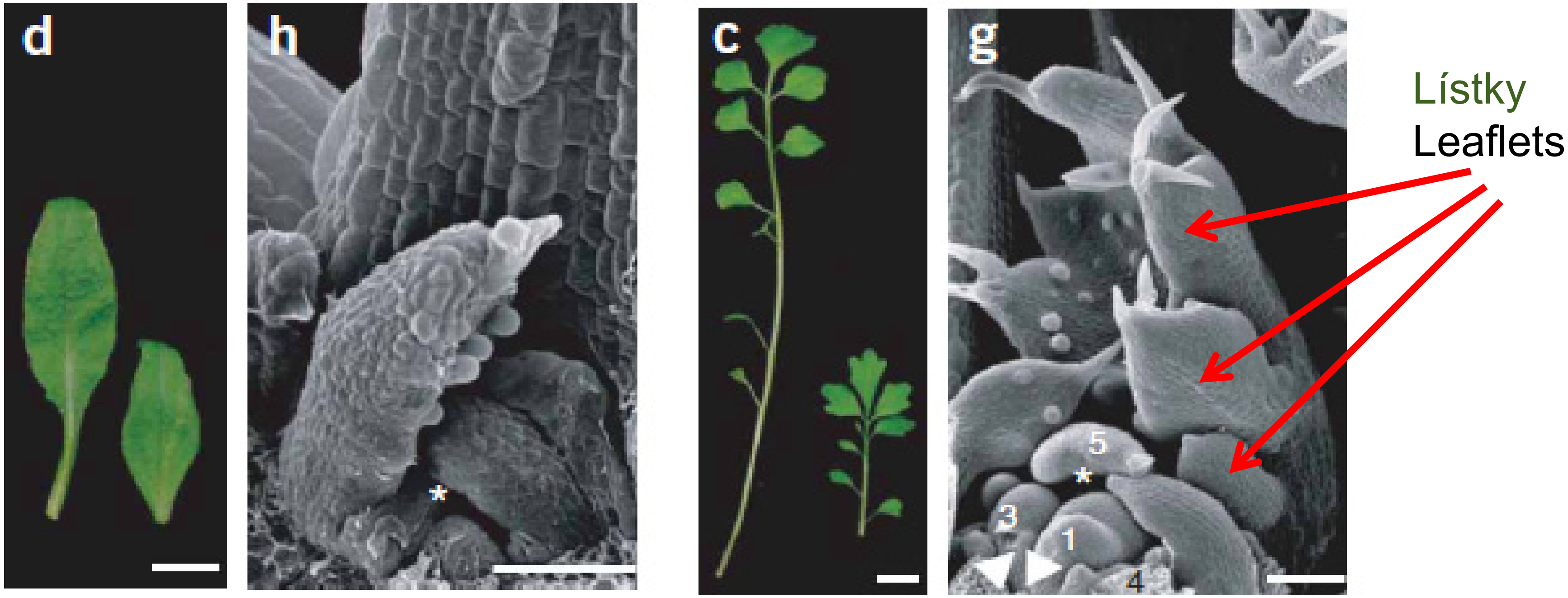
Compound



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

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

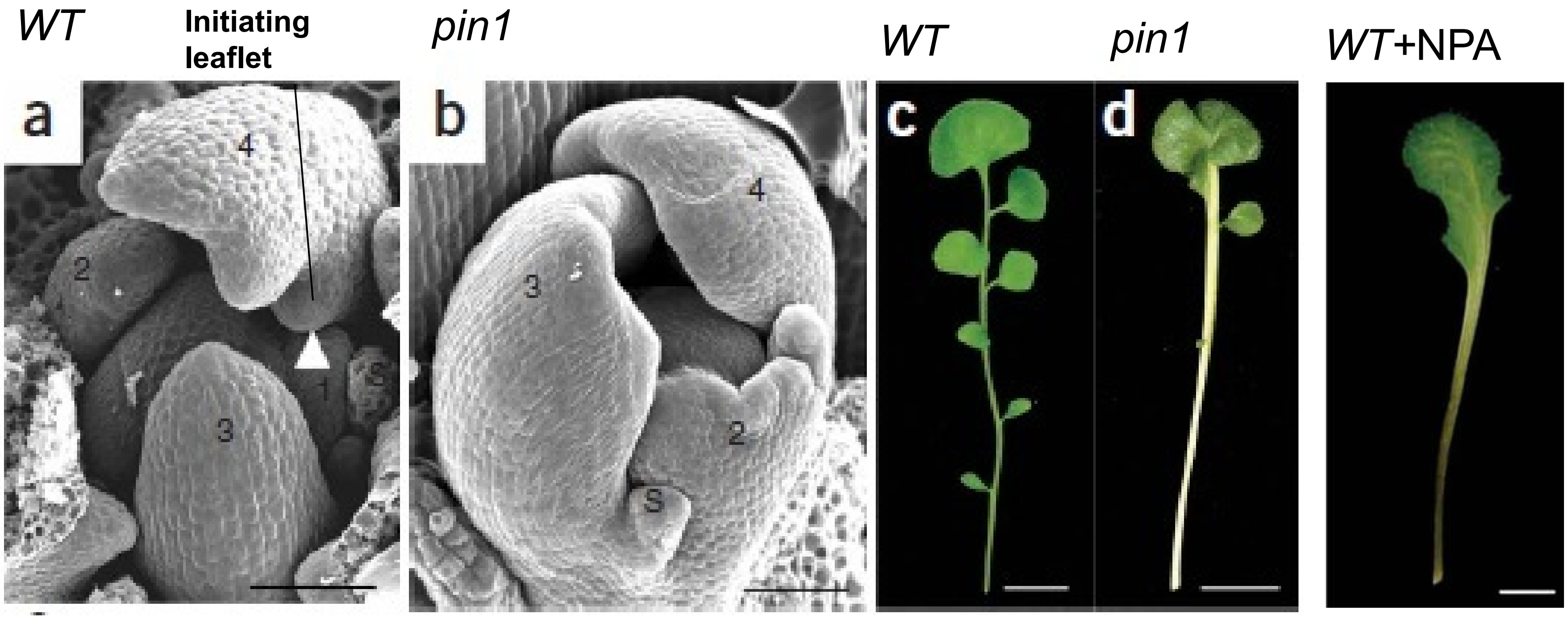
Arabidopsis thaliana *Cardamine hirsuta* řeřišnice srstnatá



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

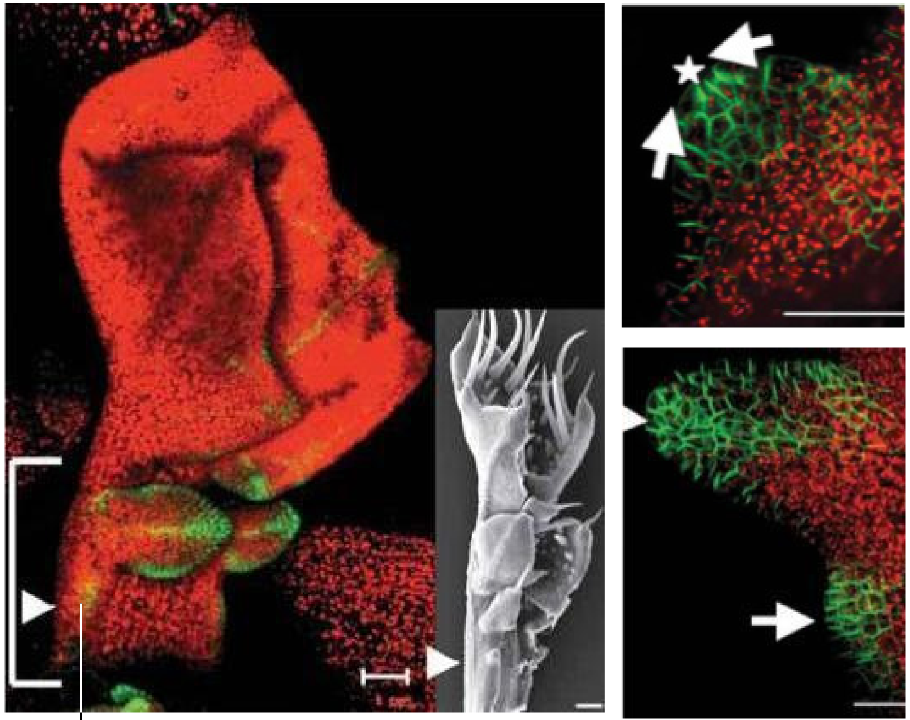
Cardamine hirsuta



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)

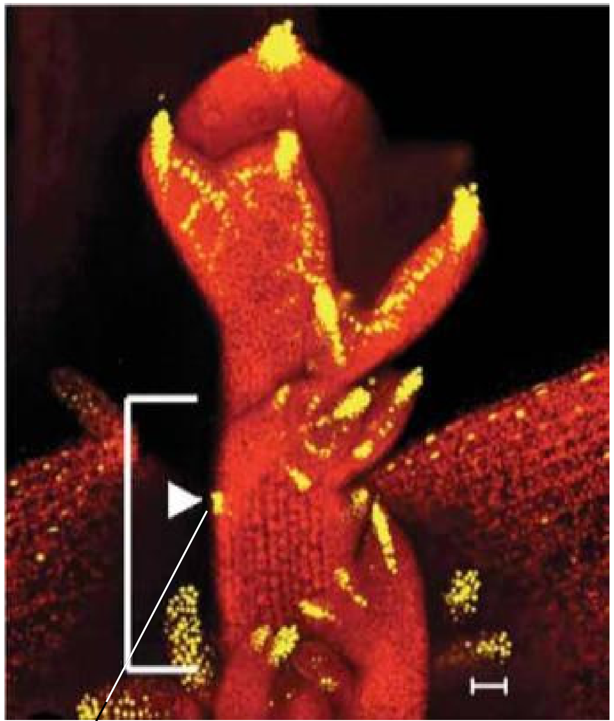
A PIN1-generated auxin maximum precedes leaflet formation

ProPIN1:PIN1-GFP



PIN1 **expression in the prospective leaflet position**

DR5:YFP



Auxin accumulation in the prospective leaflet position

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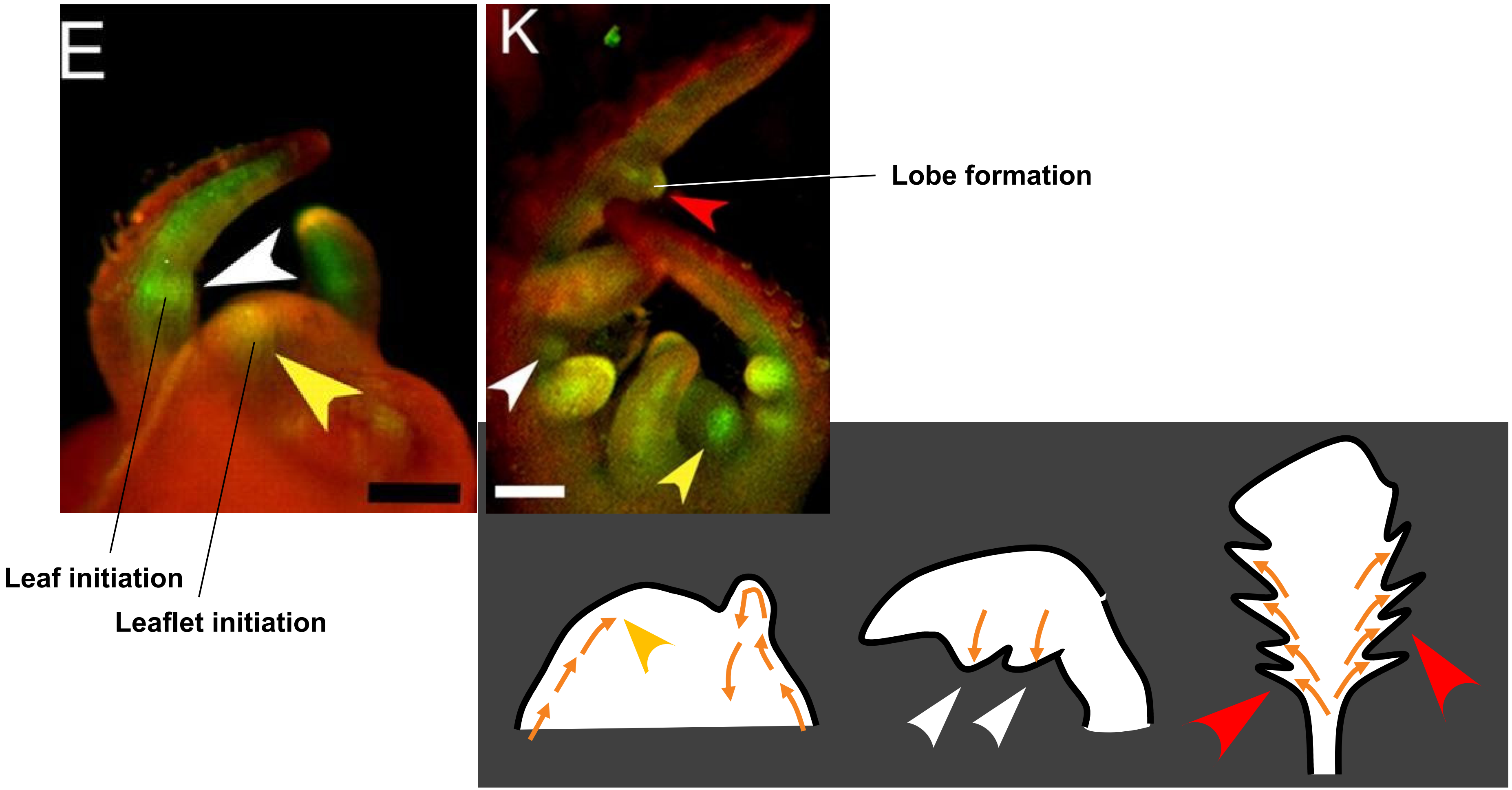
Auxin has a recurring role in leaf development



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

CEITEC

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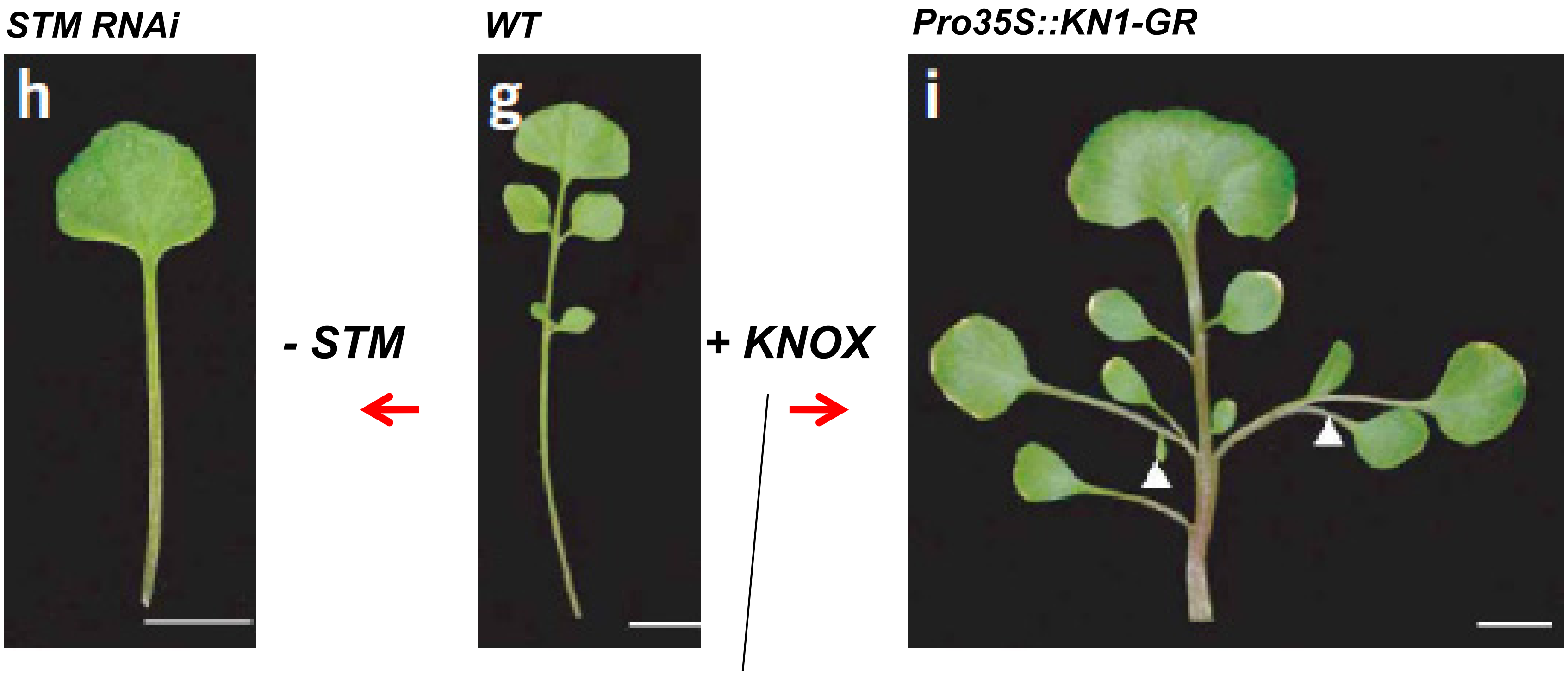
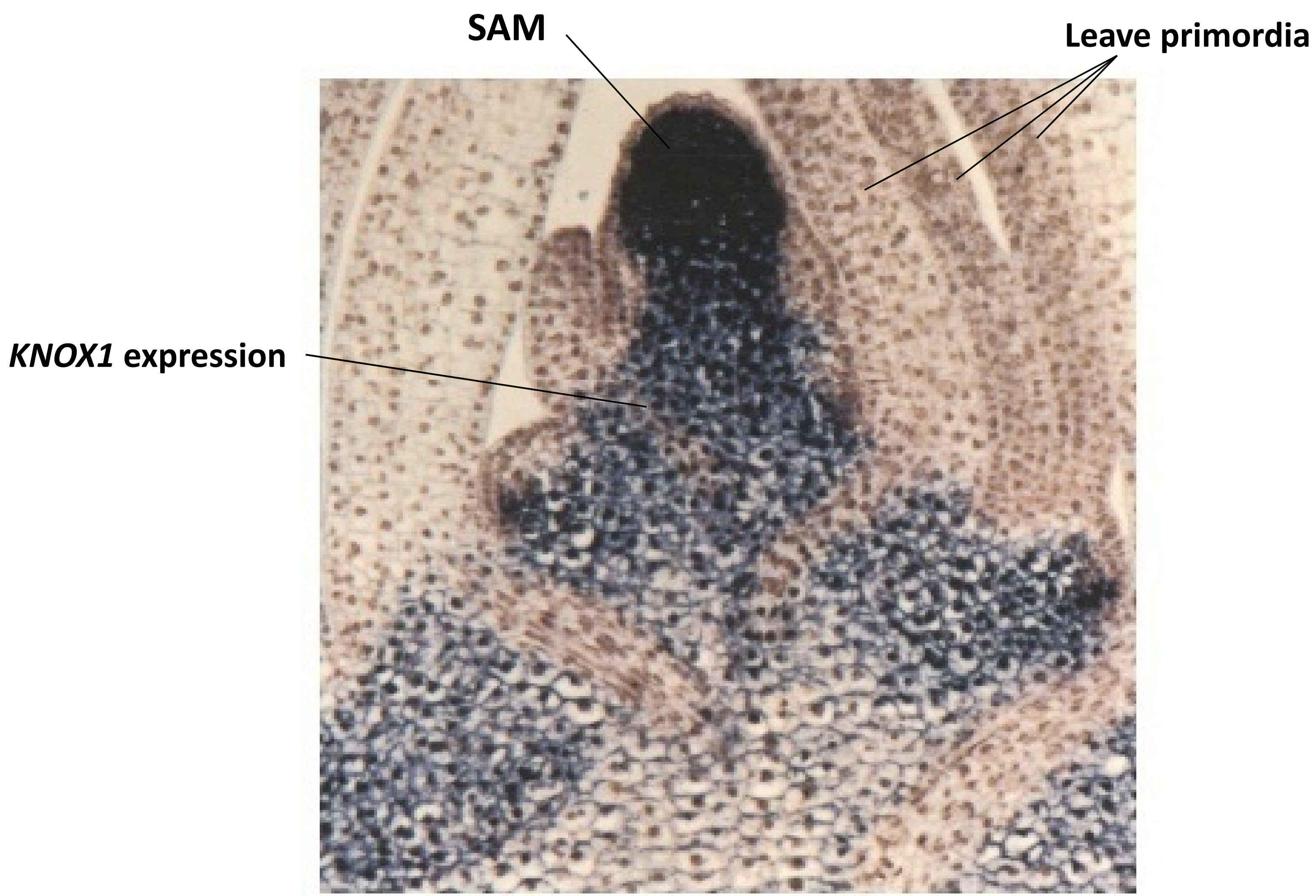
    - *KNOX* and boundary genes in the leaf complexity

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

Cardamine hirsuta

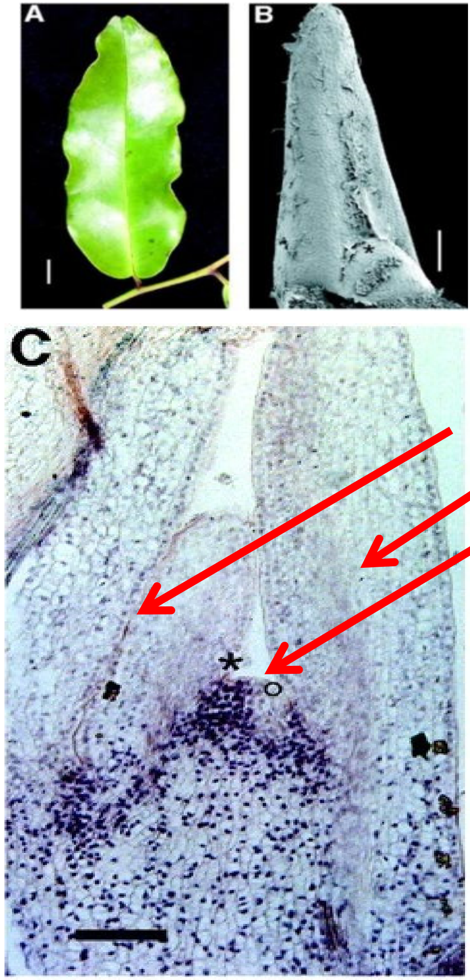


WT **KNOTTED-like homeobox TFs**



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

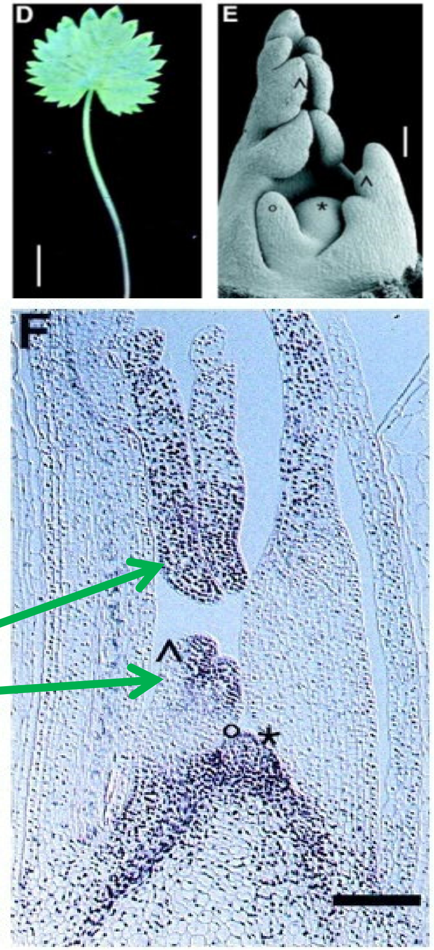


Amborella trichopoda

In plants with <u>compound</u> leaves, *KNOX1* expression turns back on in primordia

***KNOX1* OFFin leaves**

Compound leaf

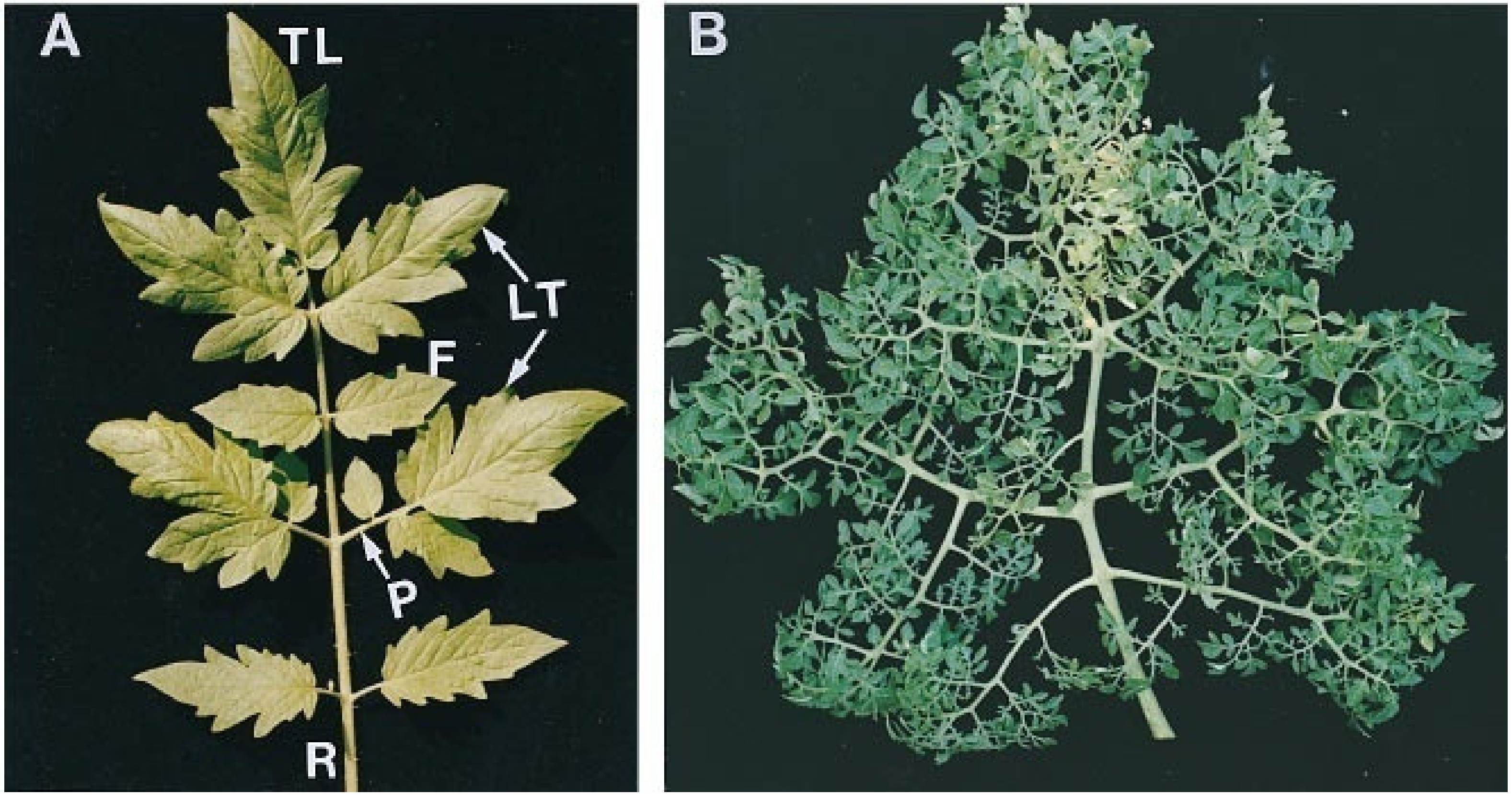


***KNOX1* ON in leaves**

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 *KNOXI* gene expression during development. Science 296: 1858-1860. Reprinted with permission from AAAS.

Tomato

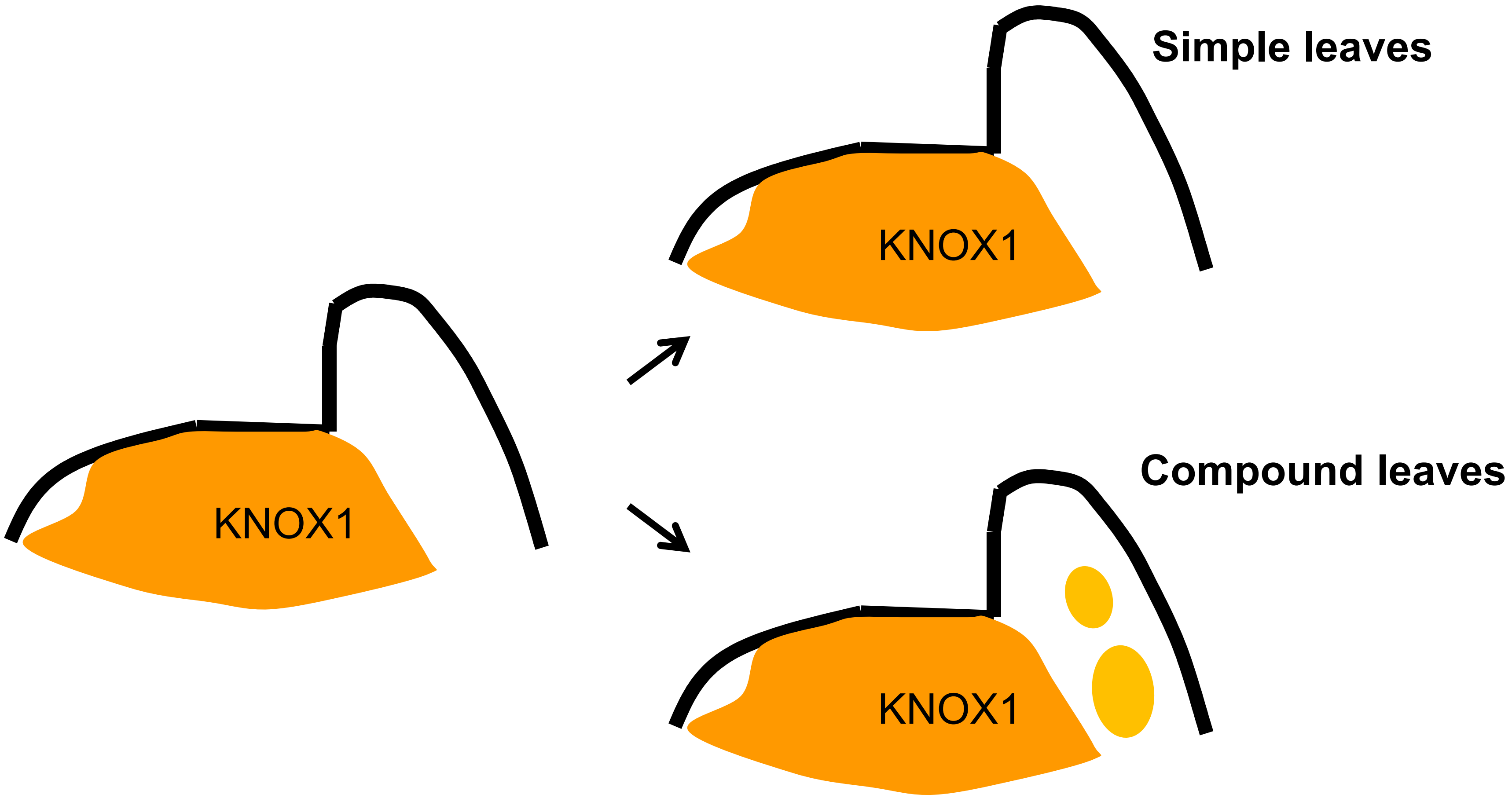


WT

Pro35S:KNOTTED1

Homeobox TF gene from maize

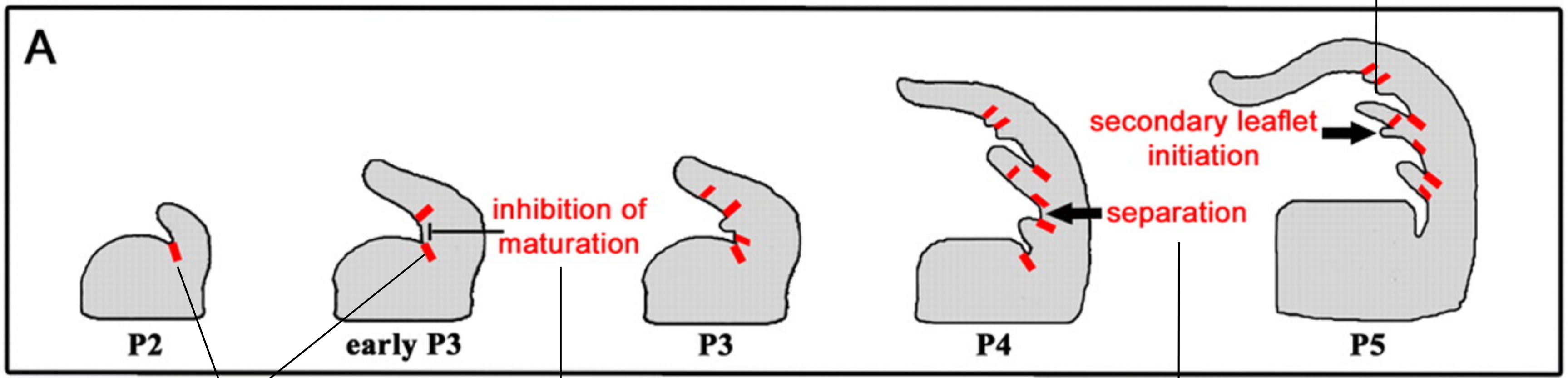
KNOX1 genes have a recurring role in leaf development



Boundary genes have a recurring role in leaf development

Secondary leaflet initiation and separation

Compound leave formation in tomato



GOBLET expression

Allows novel leaflet formation

Boundary formation and Ireaflet separation

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**

Discussion