

## **2) Gene expression and signal transduction**

- a) Size and organization of plant genome**
- b) Gene expression in plants**
- c) Signal transduction in plants**

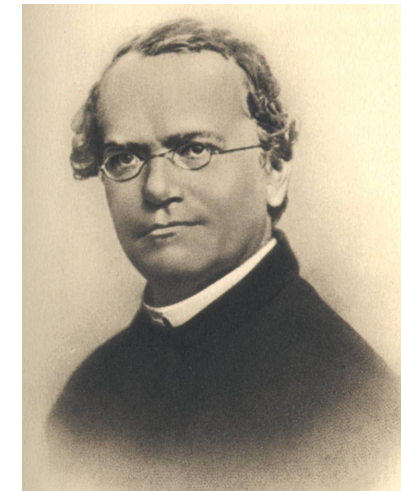
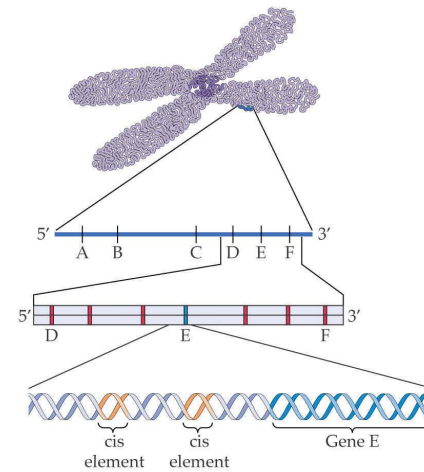
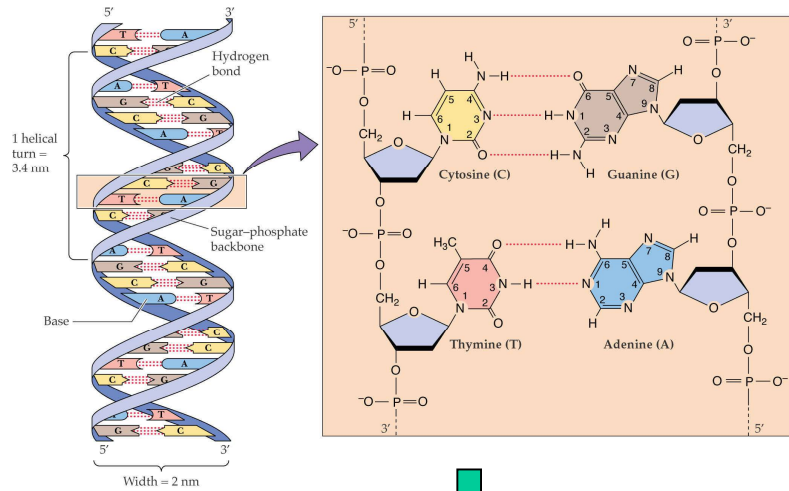
**Martin Fellner**

**Laboratory of Growth Regulators**

**Faculty of Science, Palacky University in Olomouc  
and Institute of Experimental Botany  
Czech Academy of Science**

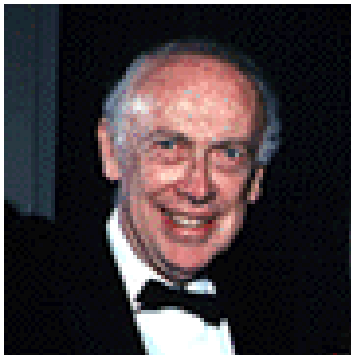
Living cell contains the instructions for building of the whole organisms = GENES

Linearly ordered GENES create the chromosome (J. G. Mendel 1865)

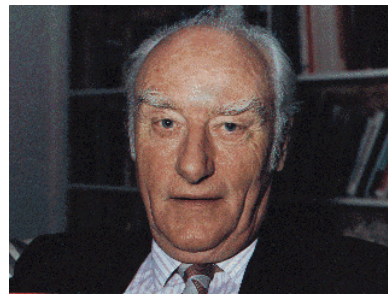


Prof. Gregor Johann Mendel

1953 – structure of DNA (Nobel Price in Medicine 1962)



Prof. James Watson (USA)

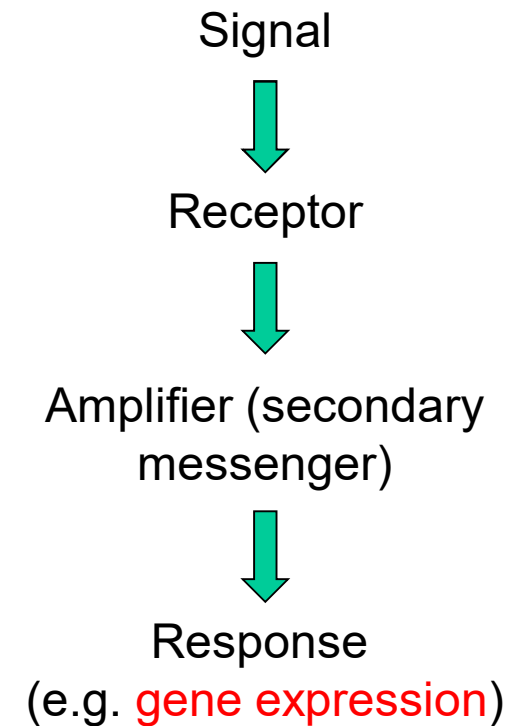
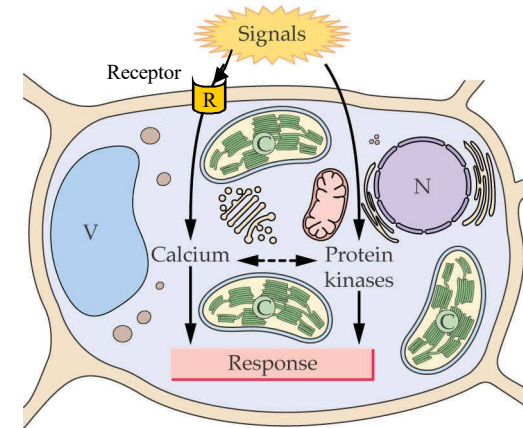
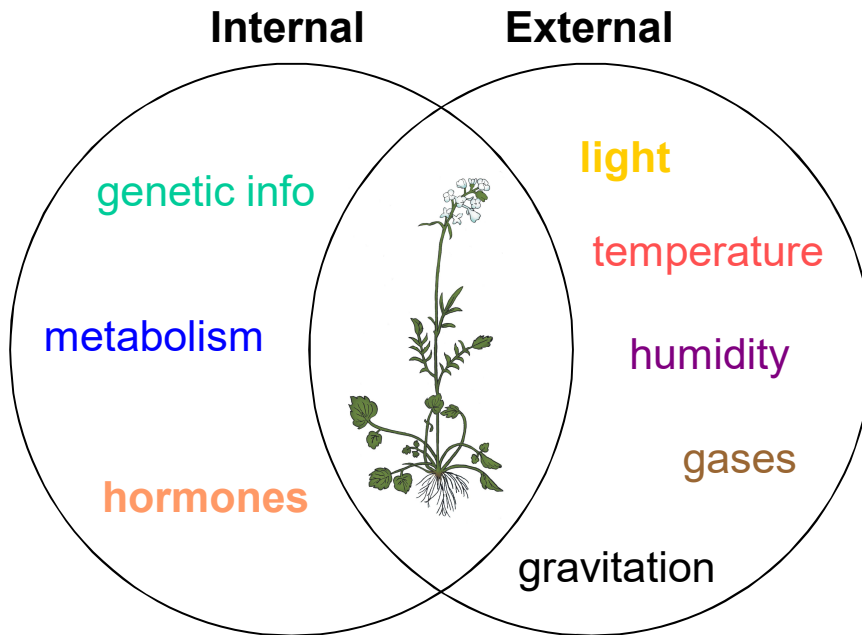


Prof. Francis Crick (UK) († 29.7. 2004)



Development of molecular biology

Development of an organism is regulated by signals (factors)



## a) Size and organization of plant genome

**GENE** = sequence of DNA, which codes RNA molecule directly involved in the production of the enzyme or structural protein of a cell (the term gene was used first in 1909 By W. L. Johannsen)

Genes on the chromosome form binding groups = they are inherited together

**GENOME** = total amount of DNA (i.e. genetic information) in cell (i.e. in nucleus + organelles)

Growth, development and response of an organism to environment is programmed turning on and switching off the genes (i.e. programmed expression)



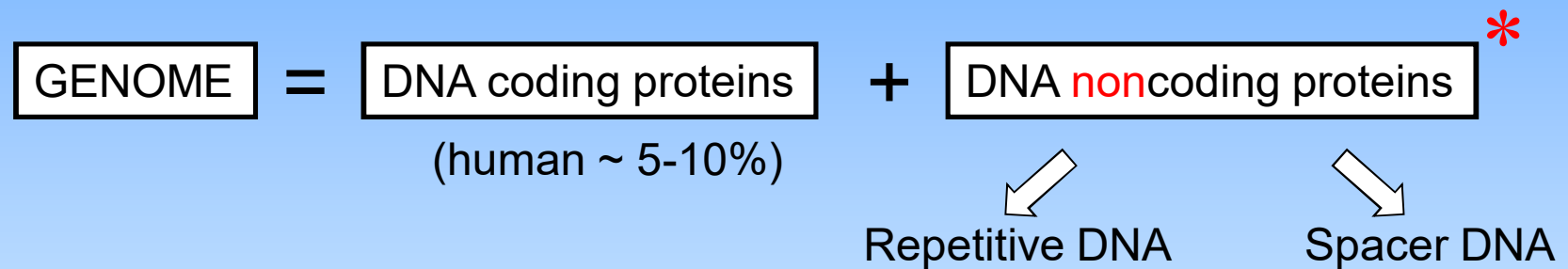
Changes in assembly of enzymes and structural proteins



Growth, development, response of organism

Haploid configuration: *E. coli* =  $4,7 \times 10^6$  bp  
*Drosophila* =  $2 \times 10^8$  bp  
*Human* =  $3 \times 10^9$  bp

Size of eukaryotic genome does not determine the complexity of the organism, since not all DNA code for genes:



Size of plant genome is very variable:

*Arabidopsis* =  $1,46 \times 10^8$  bp

*Trillium* =  $10^{11}$  bp

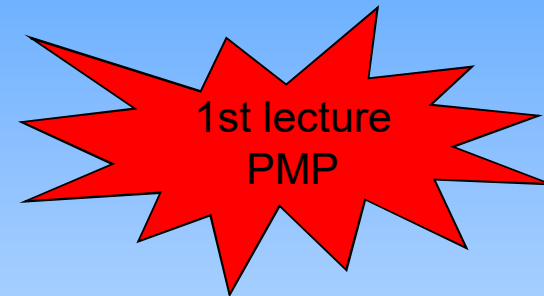
Species of genus *Vicia* differ each to other even 20-times in their genome size.



*Trillium grandiflorum*

Differences in genome sizes is determined by differential amounts of repetitive and spacer DNA.

*Arabidopsis* = the smallest genome among all plant species, as it contains only 10% of repetitive DNA => *Arabidopsis* - model plant



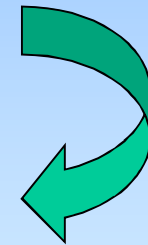
Genome sequencing projects => known genomes of many organisms

**Bacteria = 500 genes – 8 thousands genes**

**Yeast = 6 thousands genes**

***Drosophila* = 12 thousands genes**

***Arabidopsis* = 26 thousands genes (1 gen = ~ 5 kb)**



The most of haploid genomes in plants contains 20–30 thousands genes on average.  
Today's conception - 12 thousands genes sufficient to form an eukaryotic organism.



**Housekeeping genes** = constitutive expression of a gene (genes coding proteins, which play important role in many types of cells)

Plants: *UBQ* (for protein ubiquitin), *ACT* (for protein actin)

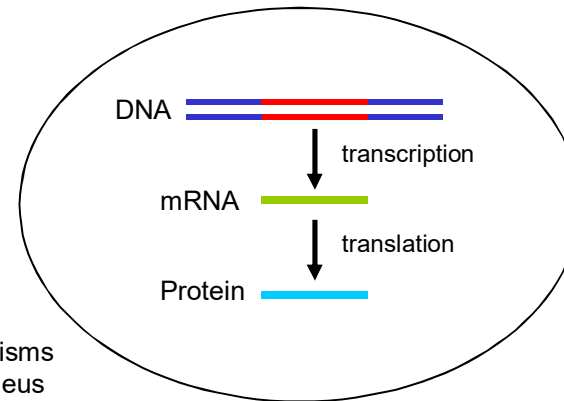
Human: *UBQ*, *EMC7* (ER membrane protein complex subunit 7)



**Regulated genes** = genes are turning on or switching off based on the needs of the organism or based on the response of the organism to specific stimuli.

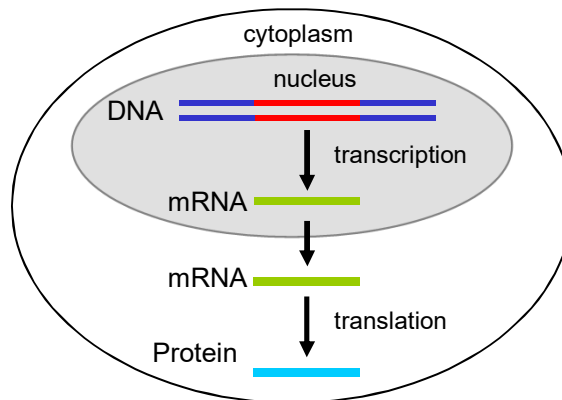
## b) Gene expression in plants

**Prokaryotic organisms:** transcription and translation are temporally and spatially connected = protein synthesis starts before completing the synthesis of mRNA.



Prokaryotic organisms  
do not have nucleus

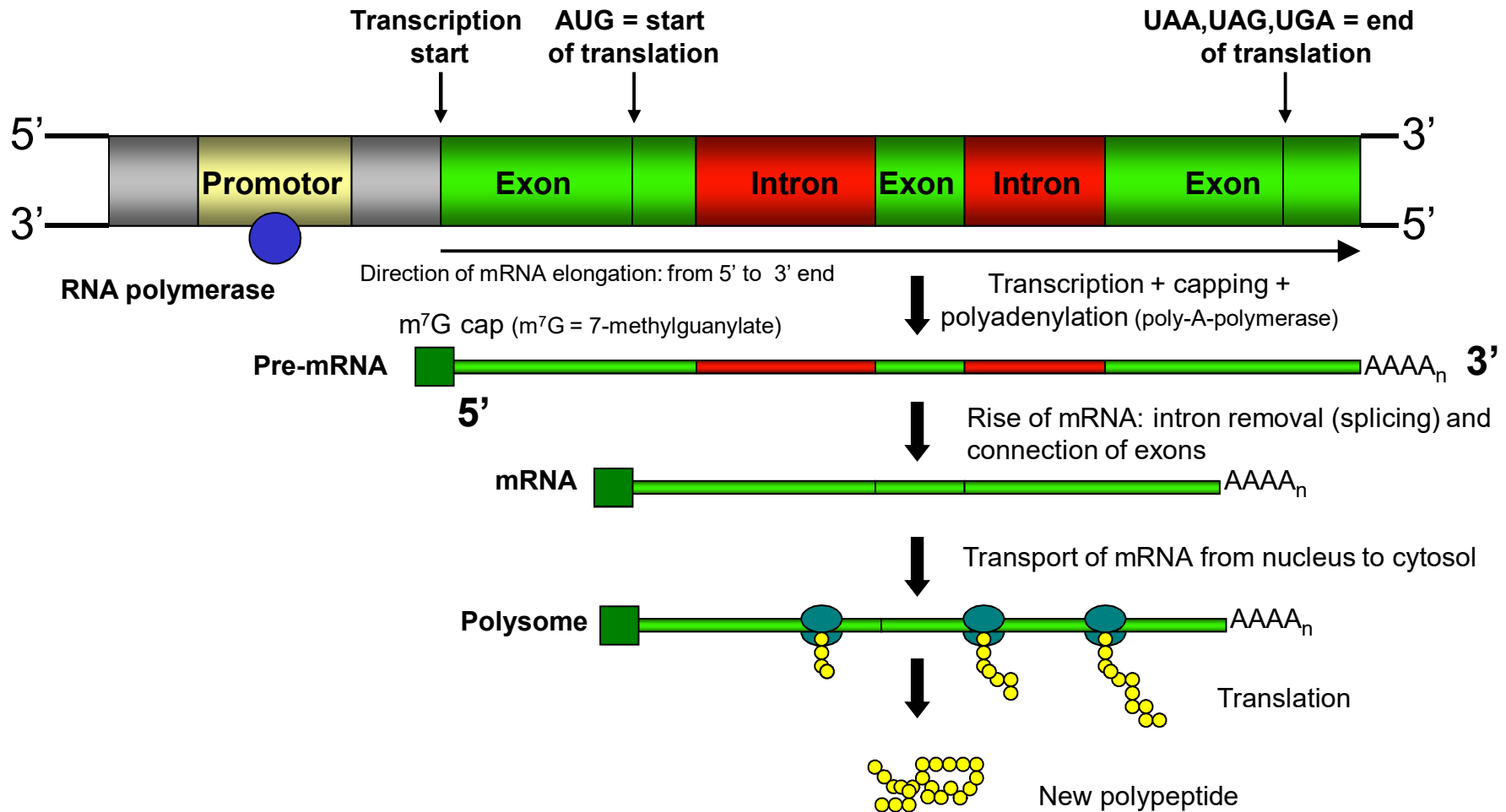
**Eukaryotic organisms:** transcription and translation are temporally and spatially separated = mRNA is transported into cytosol, where protein synthesis starts.



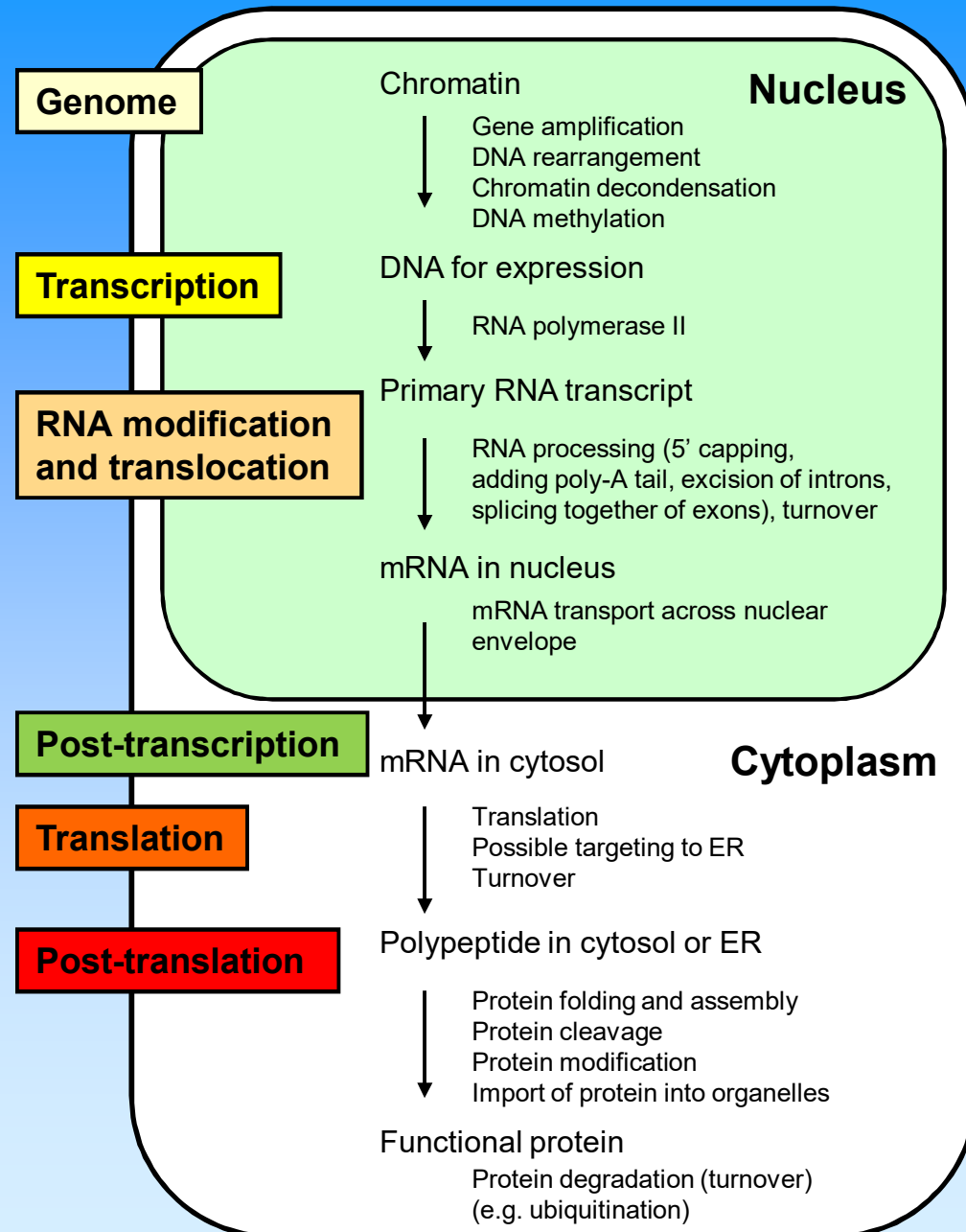
Requirement of additional control  
and regulatory mechanisms

## Organization of genome in eukaryotic organisms:

- one gene codes for one polypeptide
- nuclear genome does not contain operons
- exons function instead of operons and noncoding DNA regions are called introns



## Various levels of regulation of the expression of eukaryotic gene



## Post-transcriptional regulatory mechanisms

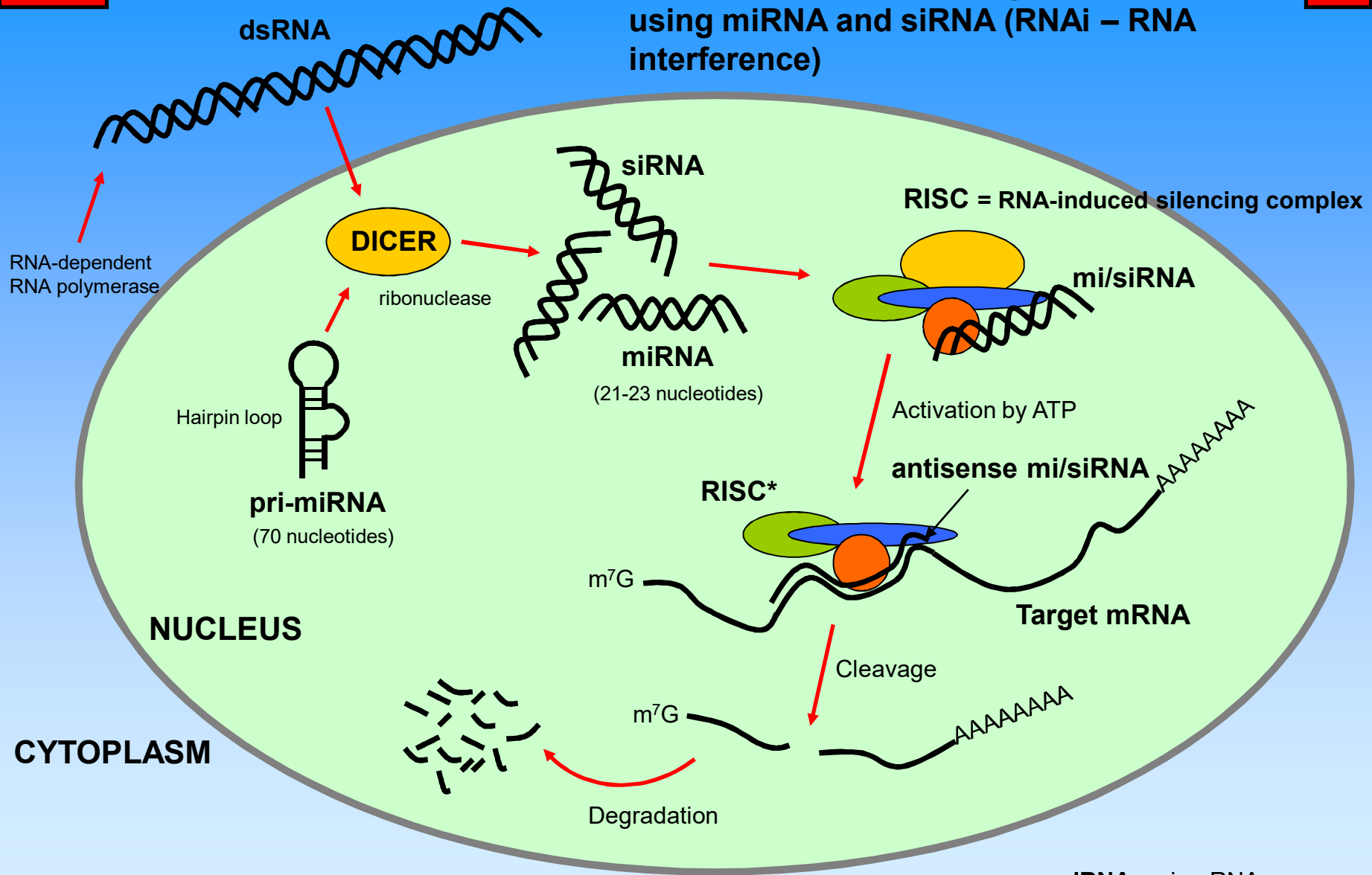
1) Turnover rate of mRNA – dependent on plant tissue and physiological conditions

2) Translatability of the mRNA molecule = ability of mRNA to be translated into molecule of the protein

Factors affecting translatability:

- Secondary and tertiary structure of RNA – accessibility of AUG to ribosome
- Codon bias – amount of rare codons
- Cellular localization of translation – free polysomes or polysomes bound to ER

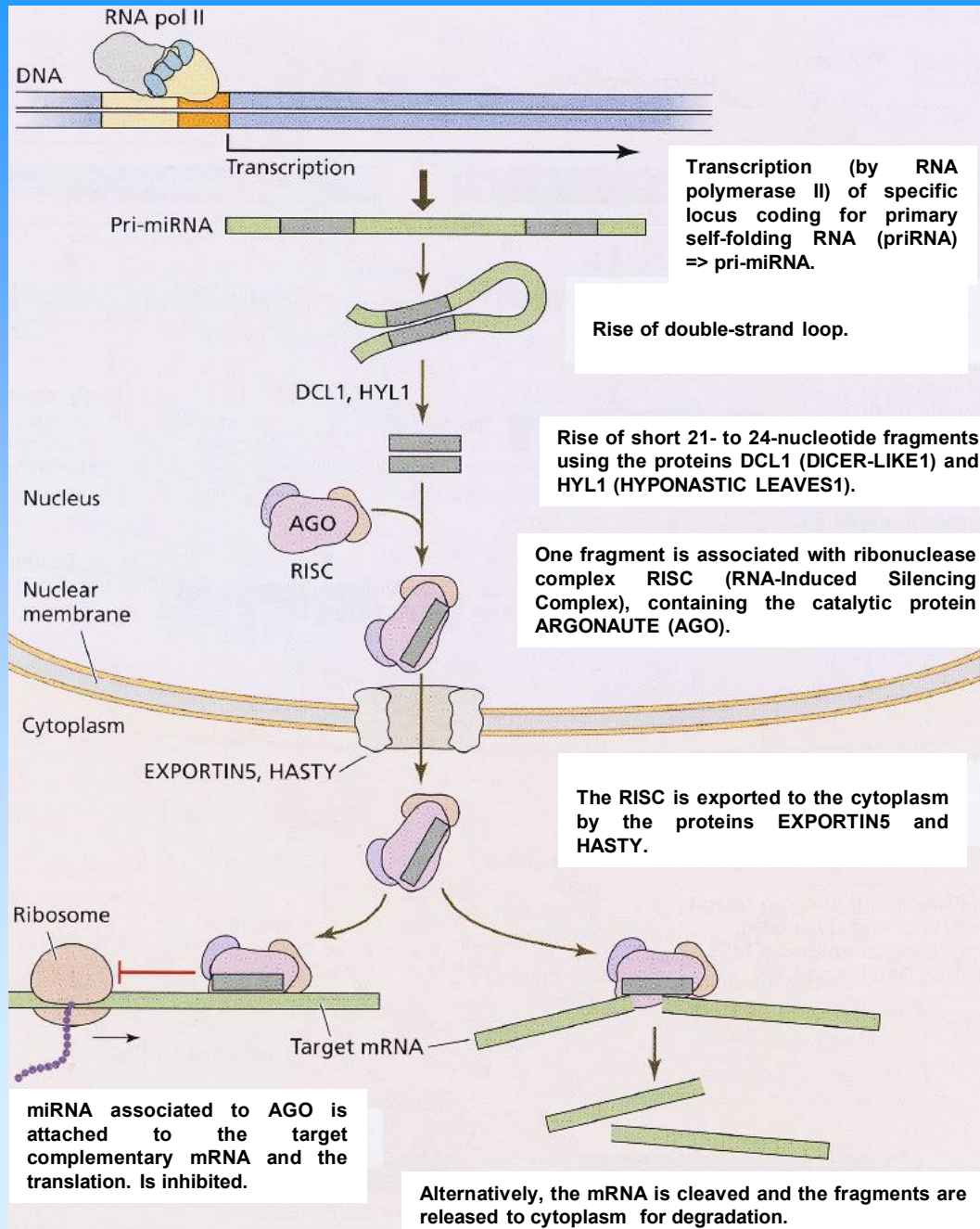
### Model of suppression of gene expression using miRNA and siRNA (RNAi – RNA interference)



Based on Taiz L and Zeiger E (2006) Plant Physiology, 4th ed.

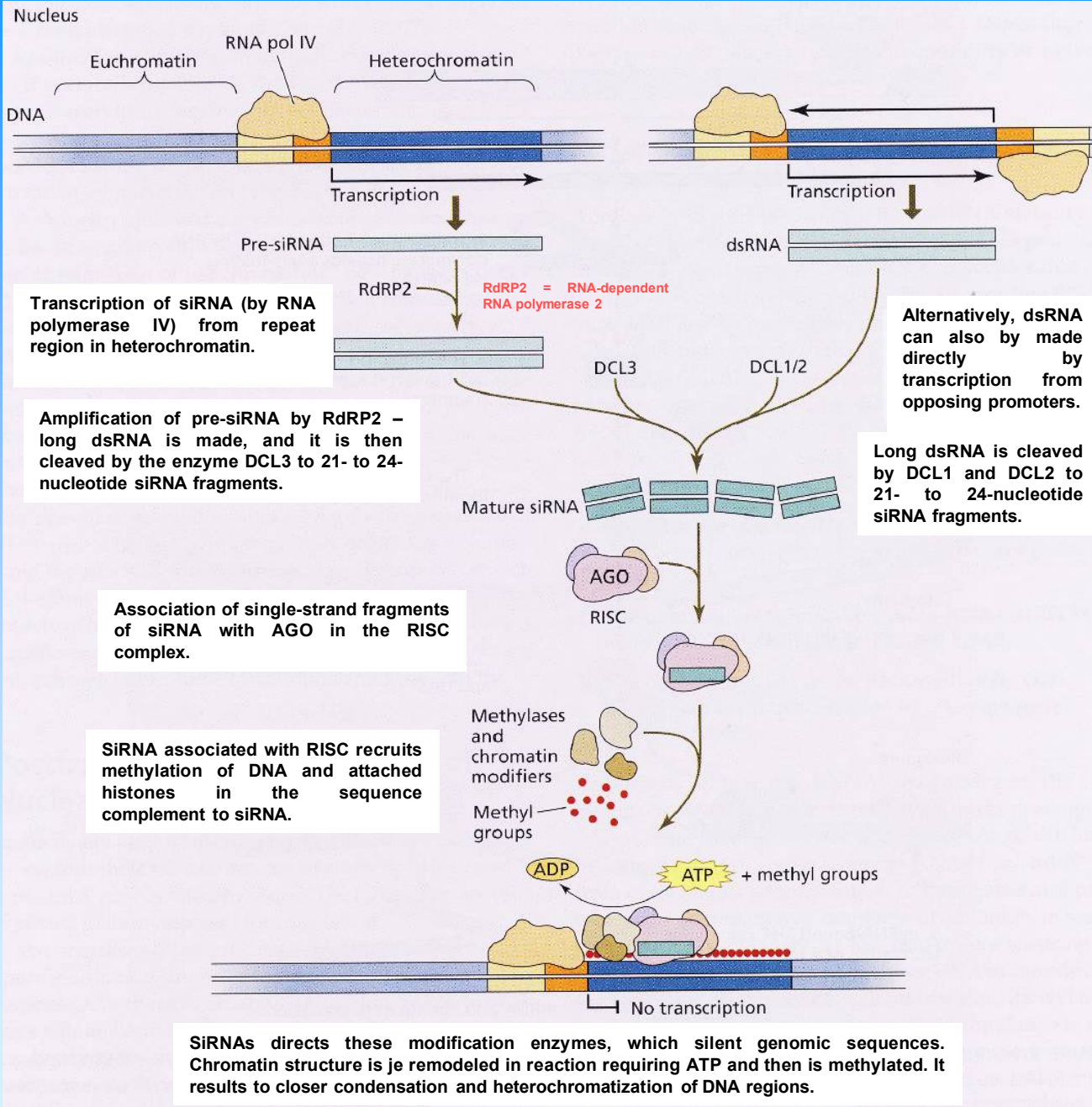
**miRNA** - microRNA  
**siRNA** - short interfering RNA

# Regulation of expression by microRNA (miRNA)



Based on  
 Taiz L and Zeiger E (2010)  
 Plant Physiology, 5th ed.

# Regulation of expression by short interfering RNA (siRNA)



Podle  
 Taiz L and Zeiger E (2010)  
 Plant Physiology, 5th ed.

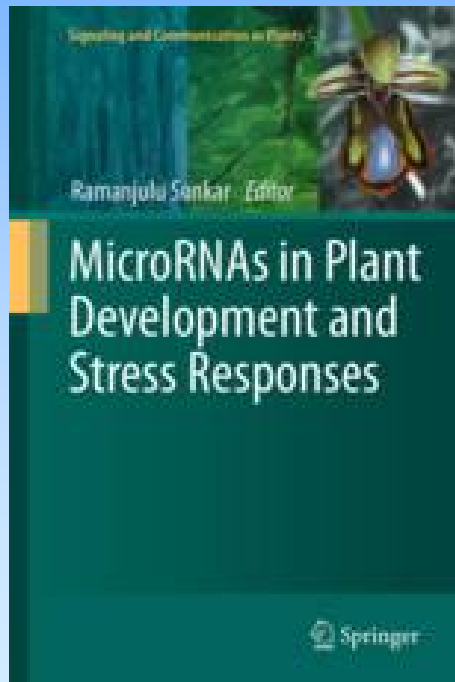
miRNA and siRNA regulate gene expression

Important role in growth and development signaling

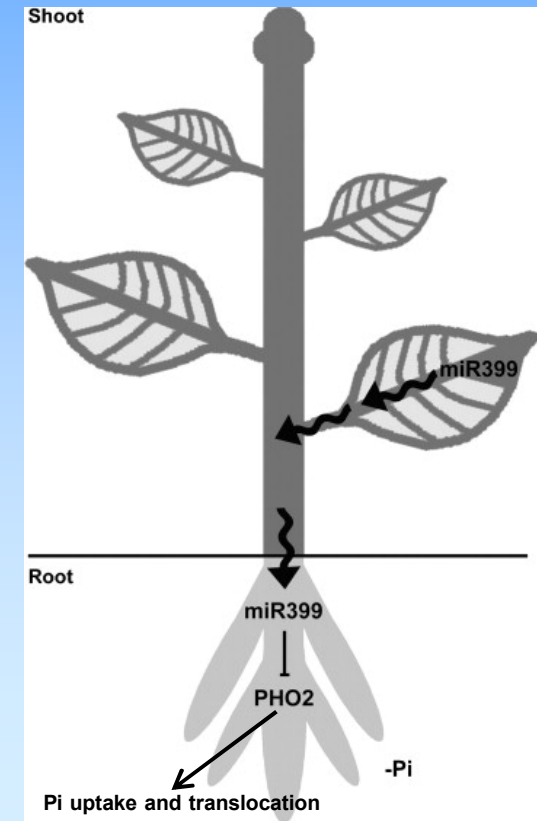
**Marín-González E et al. (2012) Plant Science 196: 18-30**

miRNA are transported for long distances

**Update 2012**



**Sunkar R (2012) MicroRNAs in Plant Development and Stress Responses. Springer**



## Regulation of gene expression on transcriptional level

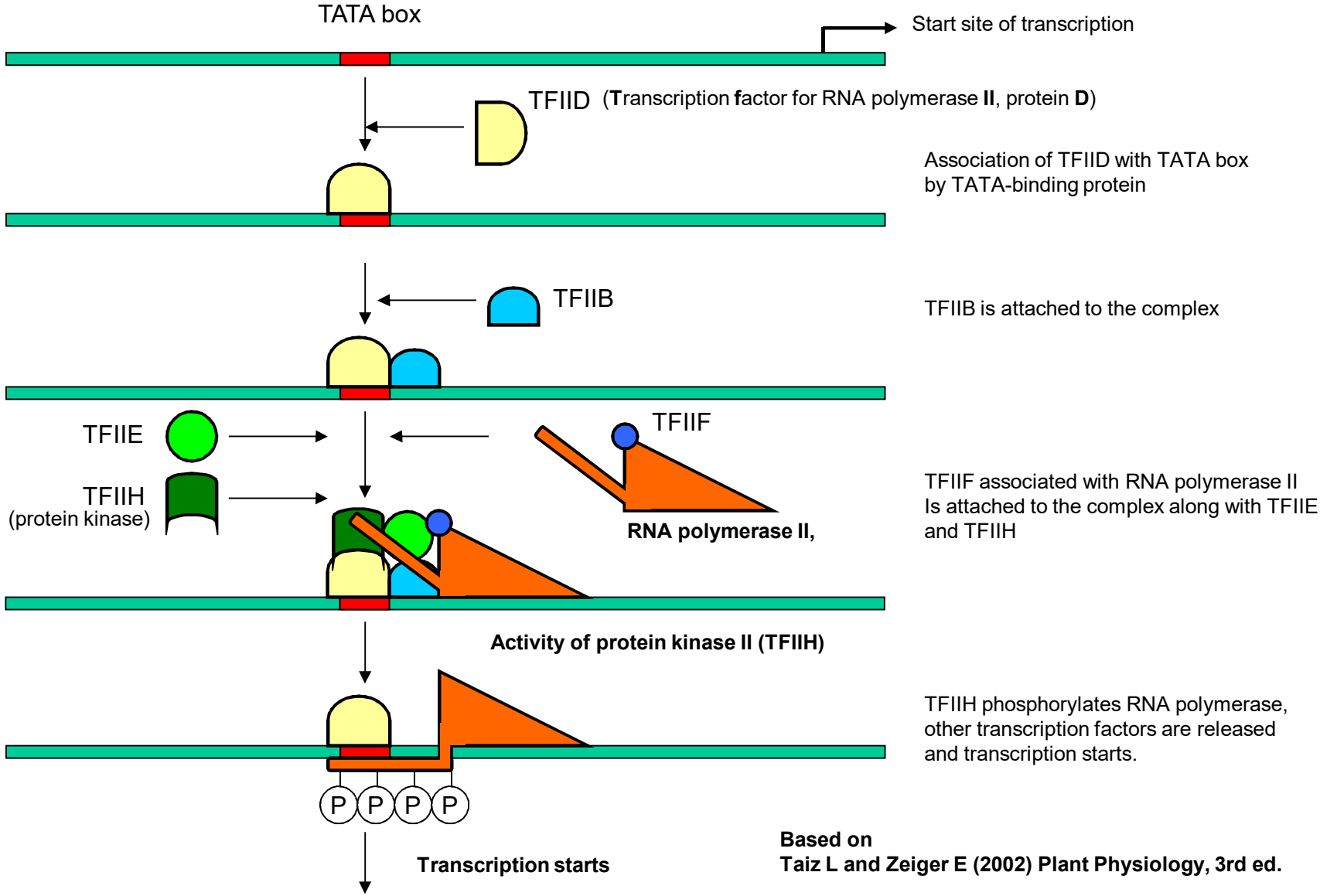
Transcription in eukaryotes is much complex compared to procaryota. Three basic differences in transcription between eu- and prokaryota:

- 1) **Eukaryotes use at least 3 different RNA polymerases: I, II a III**
  - I – located in nucleolus, role in synthesis of ribosomal RNA
  - II – located in nucleoplasma, role in **pre-mRNA** synthesis
  - III - located in nukleoplasma, role in synthesis of tRNA or 5S RNA
  
- 2) **Eukaryotic RNA polymerase requires other proteins – i.e. general transcription factors** – providing correct position of RNA polymerase at the correct start site; transcription factors form large subunit complexes.

TF and miRNA share general regulatory strategy:

- a) TF and miRNA are defined for individual types of cells
- b) TF and miRNA controls tens or hundreds of target genes
- c) Most of genes is regulated by combination of TFs or miRNAs

# Complex of transcription factors regulating transcription



3) **Eukaryotic promoters (= sequence upstream of initiation site) are complicated**

**Structure of eukaryotic promoter:**

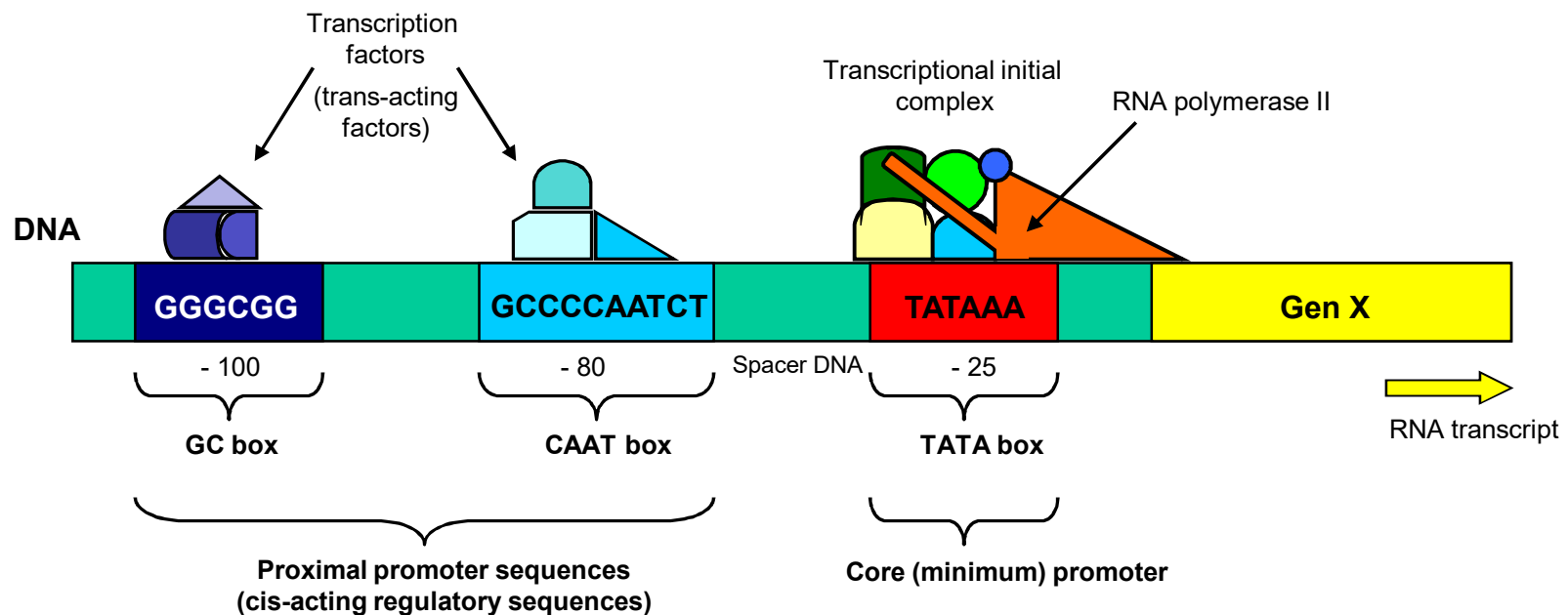
- core (minimal promoter) = minimal sequence required for expression
- regulatory sequence = sequence controlling the activity of minimal promoter

**Each polymerase I, II and III targets different type of promoter.**

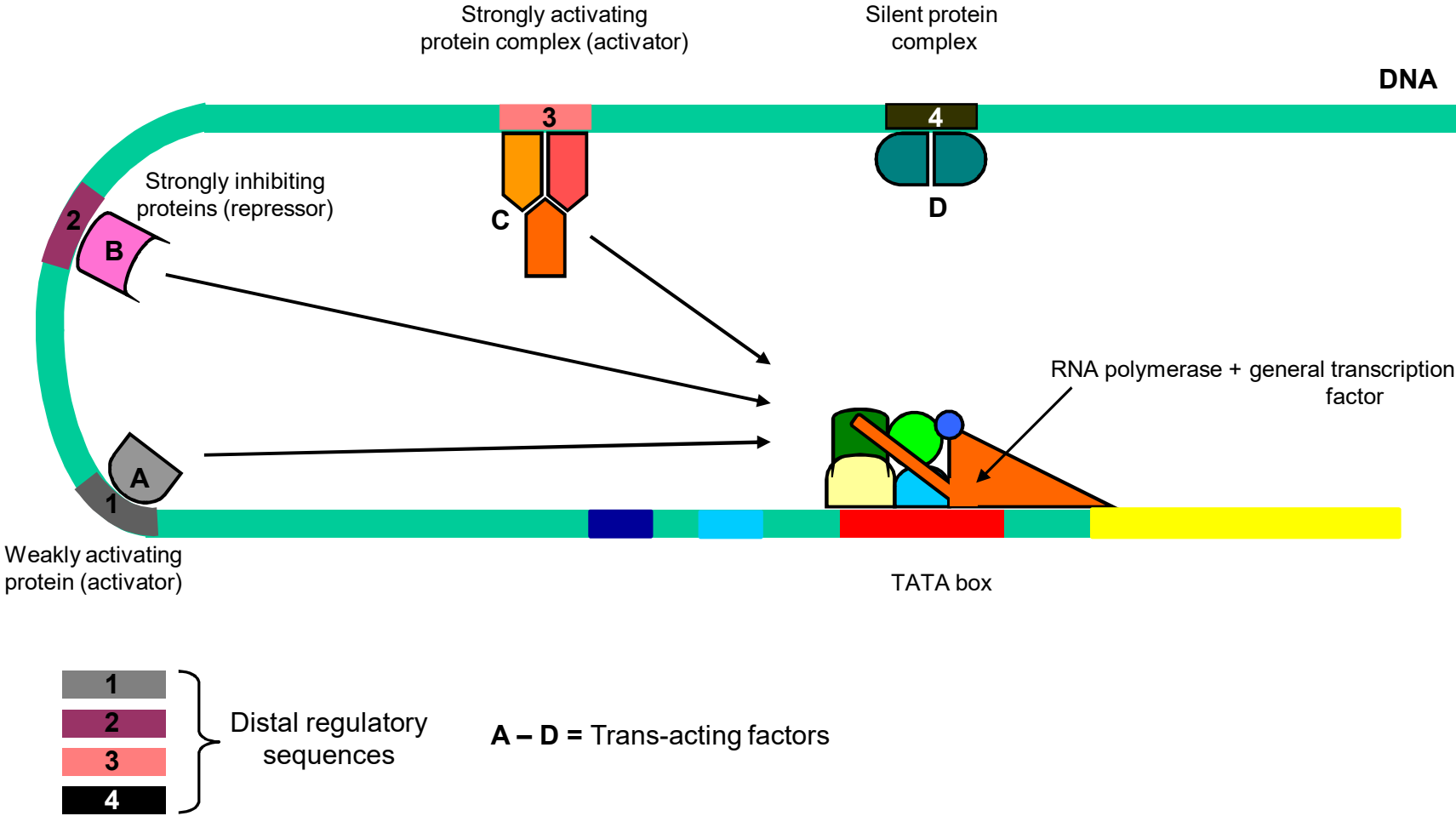
**Typical promoter for RNA polymerase II**



## Organization and regulation of typical eukaryotic minimal promoter (core promoter) for **RNA polymerase II**



# Regulation of transcription by distal regulatory sequences

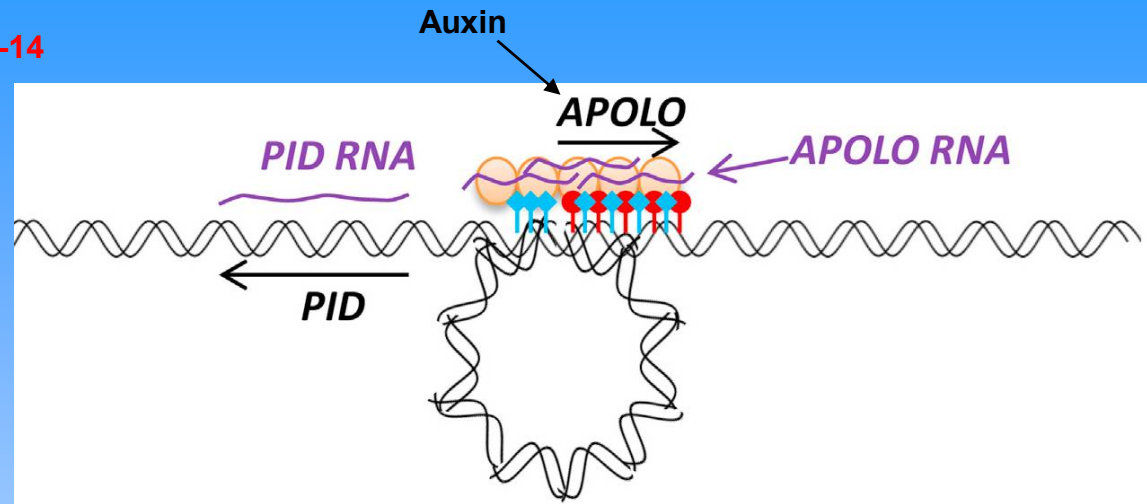


UPDATE 2014

lincRNA – long intergenic noncoding RNA

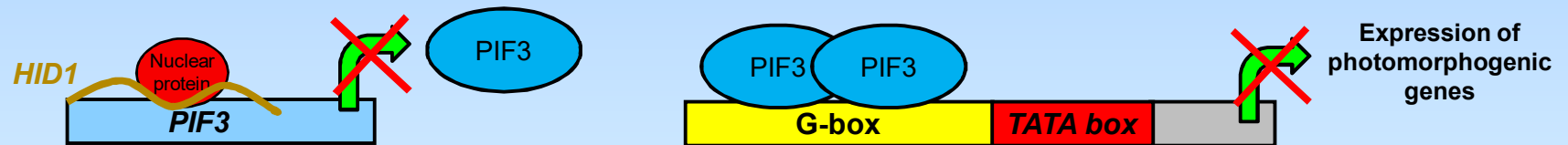
Ariel F et al. (2014) *Molecular Cell* 55: 1-14

Noncoding RNA *APOLO*



Wang Y et al. (2014) *PNAS* 111: 10359-10364

Noncoding RNA *HID1*



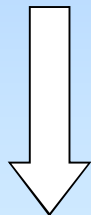
Noncoding RNA (DNA) plays a role in mechanisms of plant adaptation to variable external factors.

## Structural motives of transcription factors

**Transcription factors consist of 3 structural parts:**

- **DNA-binding domain**
- **Transcription-activating domain**
- **Ligand-binding domain**

**In order to bind to DNA, the DNA-binding domain of the transcription factor must heavily interact with double helix DNA by means of hydrogen bonds, ion and hydrophobic bonds.**



**DNA-binding motive**

**Wehner N et al. (2011) *Frontiers in Plant Science* 2(68): 1-7**

Review about methods for study of transcription factors.

## DNA-binding motives

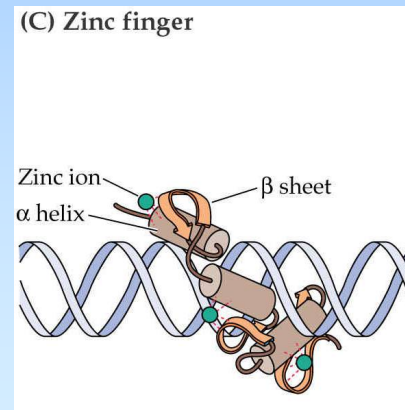
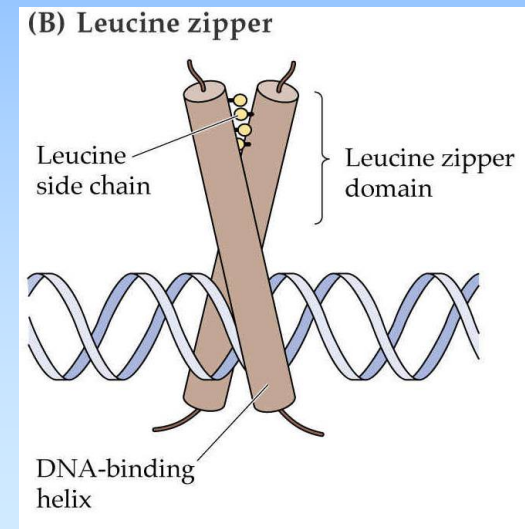
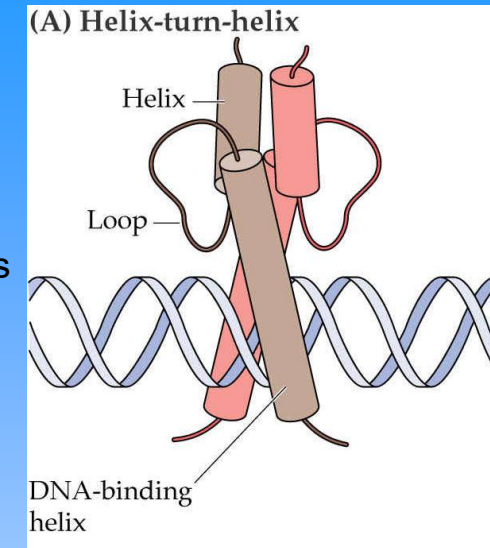
**Helix-turn-helix** 2  $\alpha$  helices separated by loop of polypeptide chains; It functions as a dimer; often coded by homeotic genes

**Leucine zipper**  $\alpha$  helix about 30 amino acids, each seventh amino acid is leucine; it functions as dimer

**Zinc finger** Various structures, where Zn plays important role; to DNA is bound as monomer or dimer; *COP1*-plays a role in photomorphogenesis

**Basic zipper (bZip)** (e.g. bound to ABA-response element)

**Helix-loop-helix** (e.g. expression of genes regulated by phytochromes)



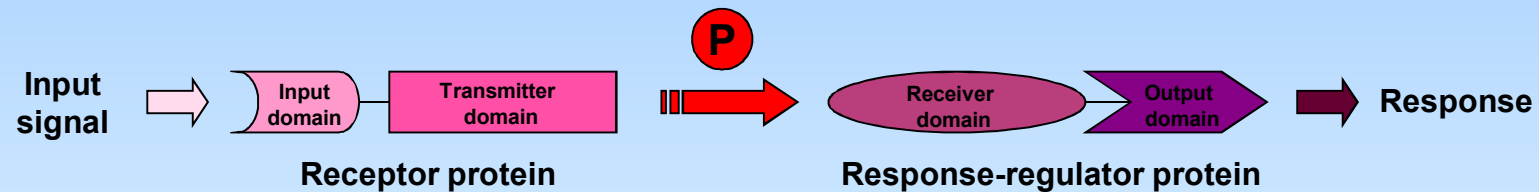
## c) Signal transduction in plants

Signaling pathways coordinate gene expression with environmental conditions surrounding plants or with changes in their development.

Ability of plant to respond to all stimuli around



Two-component system of signal transfer and regulation of expression:



Based on  
Taiz L and Zeiger E (2006) Plant Physiology, 4th ed.

Multicellular organisms → Necessity to coordinate developmental responses and responses to external stimuli

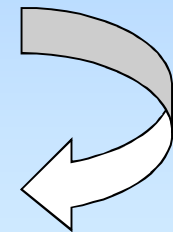


Requirement of new signaling mechanisms more complex in comparison with two-component system

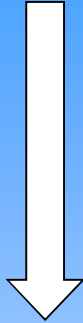
Plants are sessile organisms

→ No nervous system

Need of new signaling mechanisms = chemical messengers – secondary messengers



## Understanding of essential mechanisms of signaling pathways in animals

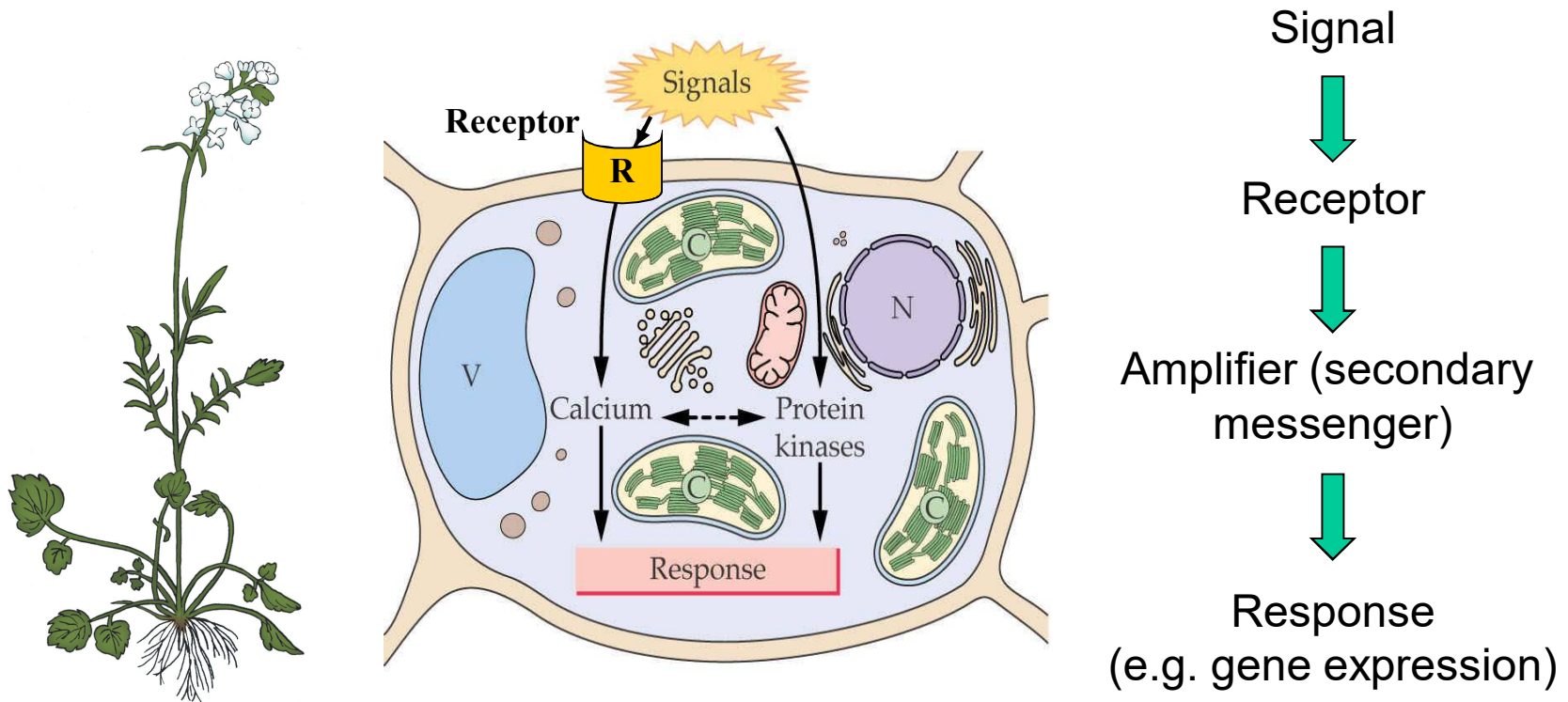


Parallel in plants revealed (e.g. brassino**steroids**)



Similar signaling mechanisms in plants understood.

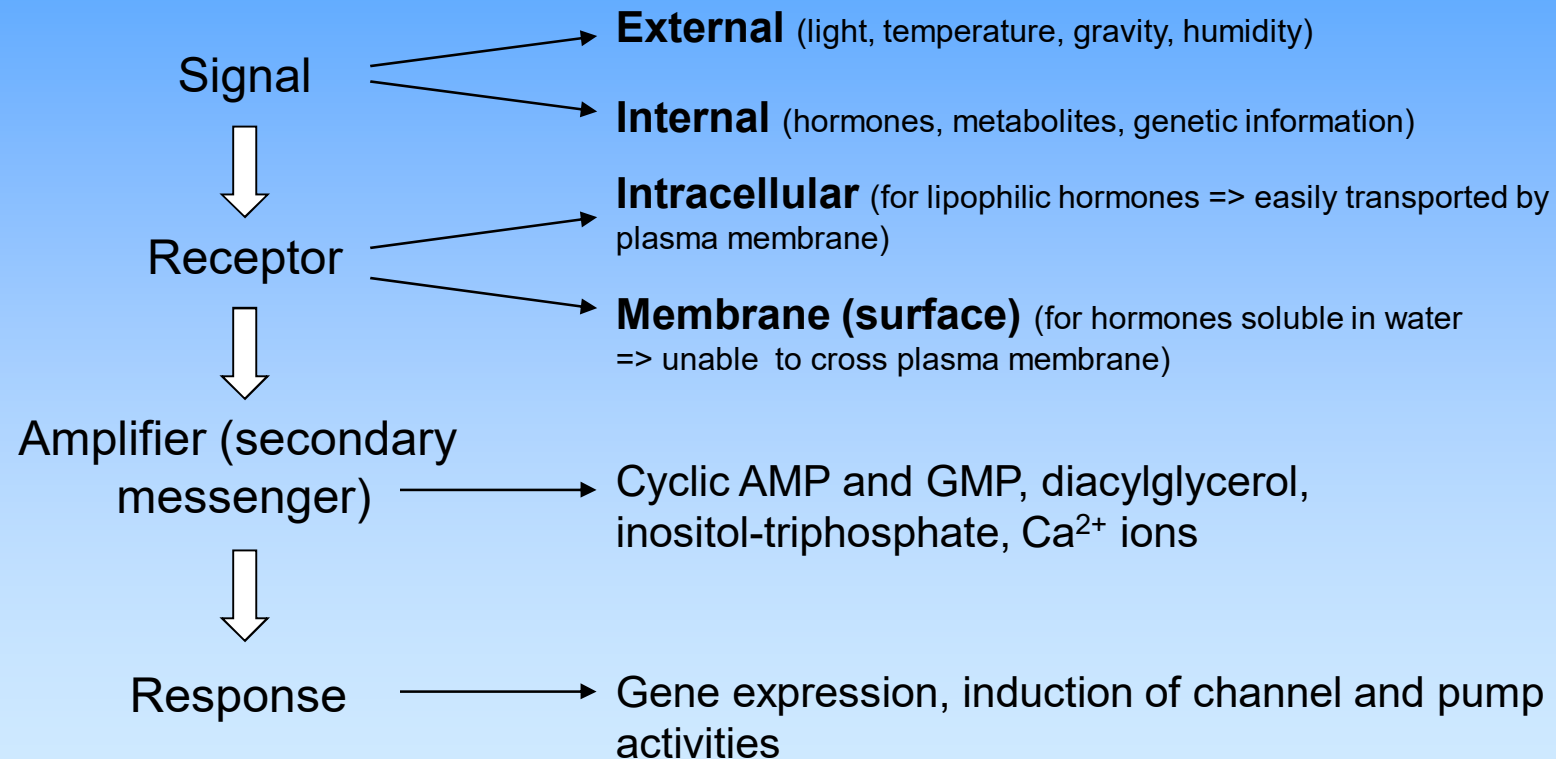
## Scheme of signal transduction in plants



López-Bucio et al. (2006) *Current Opinion in Plant Biology* 9: 523 - 529

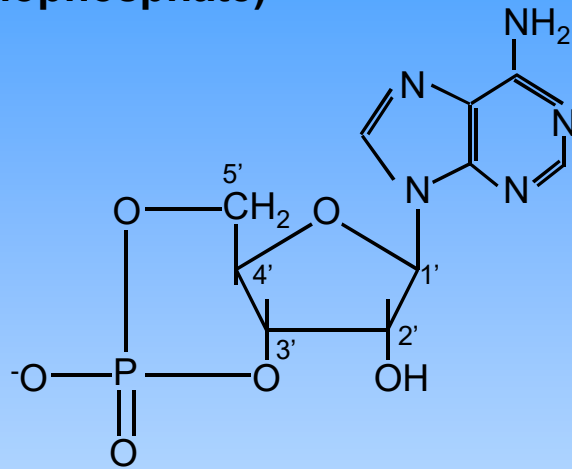
New types of signal molecules important for plant and growth development.

## Scheme of signal transduction in plants



# Amplifiers (secondary messengers)

**Cyclic AMP (cyclic adenosine monophosphate)**



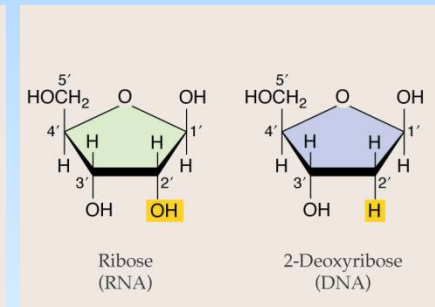
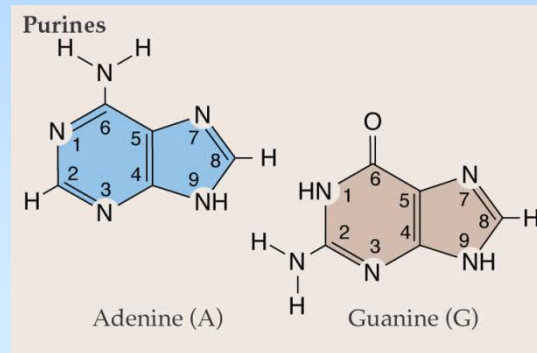
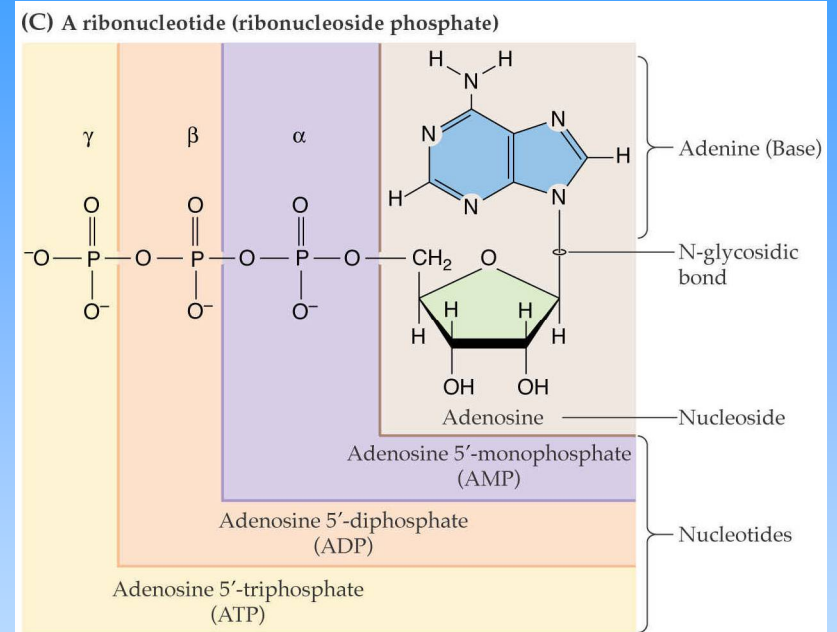
**Cyclic GMP (cyclic guanosine monophosphate)**

**1,2-Diacylglycerol (DAG)**

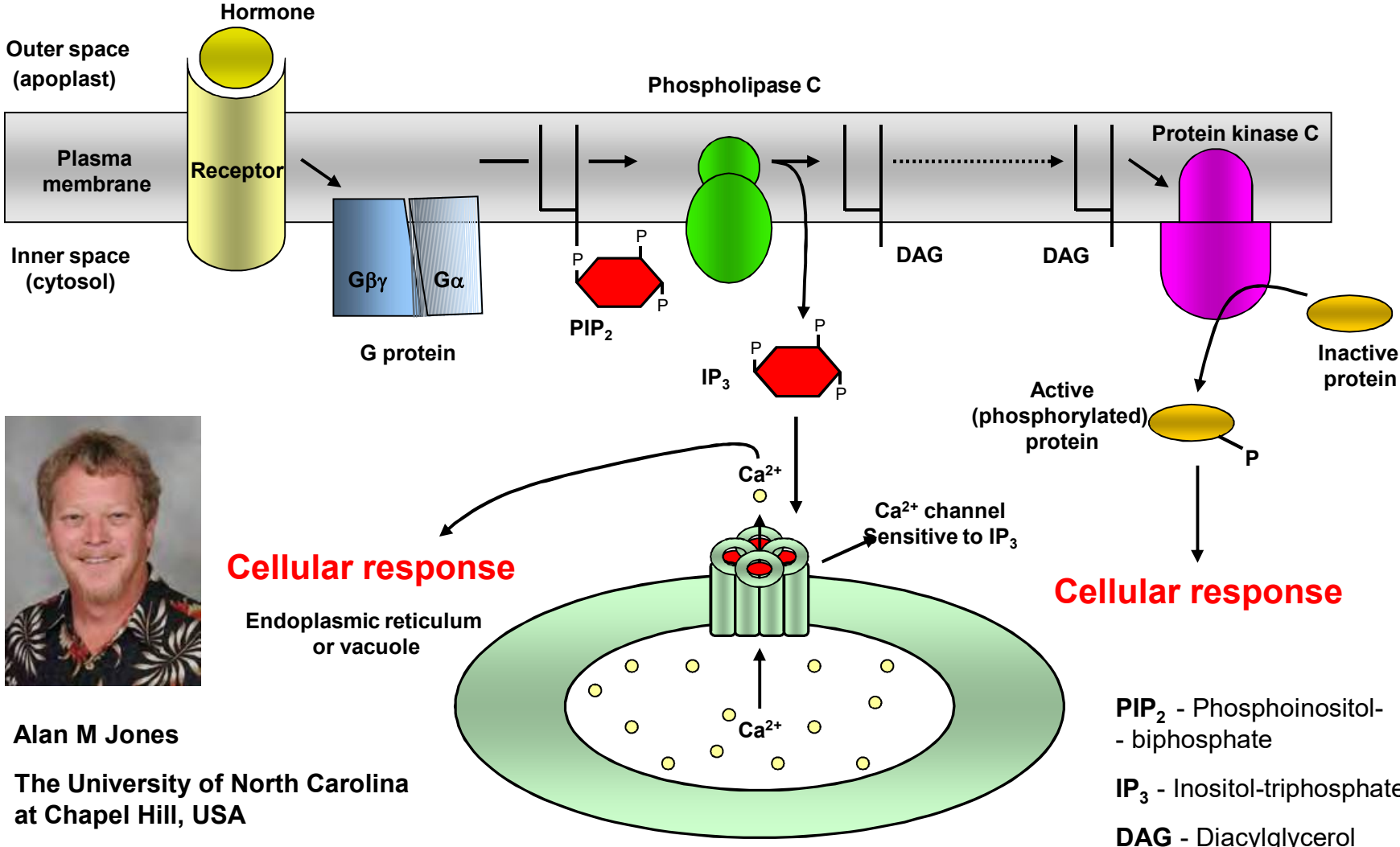
**Inositol-1,4,5-triphosphate (IP<sub>3</sub>)**

**Ca<sup>2+</sup> ions**

**Nitric oxide N ≡ O**



# General scheme of inositol-lipid signaling pathway



**Alan M Jones**  
 The University of North Carolina  
 at Chapel Hill, USA

**Cellular response**

Endoplasmic reticulum  
 or vacuole

**Cellular response**

- PIP<sub>2</sub> - Phosphoinositol-bisphosphate
- IP<sub>3</sub> - Inositol-triphosphate
- DAG - Diacylglycerol

Based on Taiz L and Zeiger E (2006) Plant Physiology, 4th ed.

## **Signal transduction from cell to cell regulates growth and development**

Fate of a cell is determined by its position in plant (location in the organ).

Cell monitors its own position by communication with neighboring cells.

### **Mechanisms of communication:**

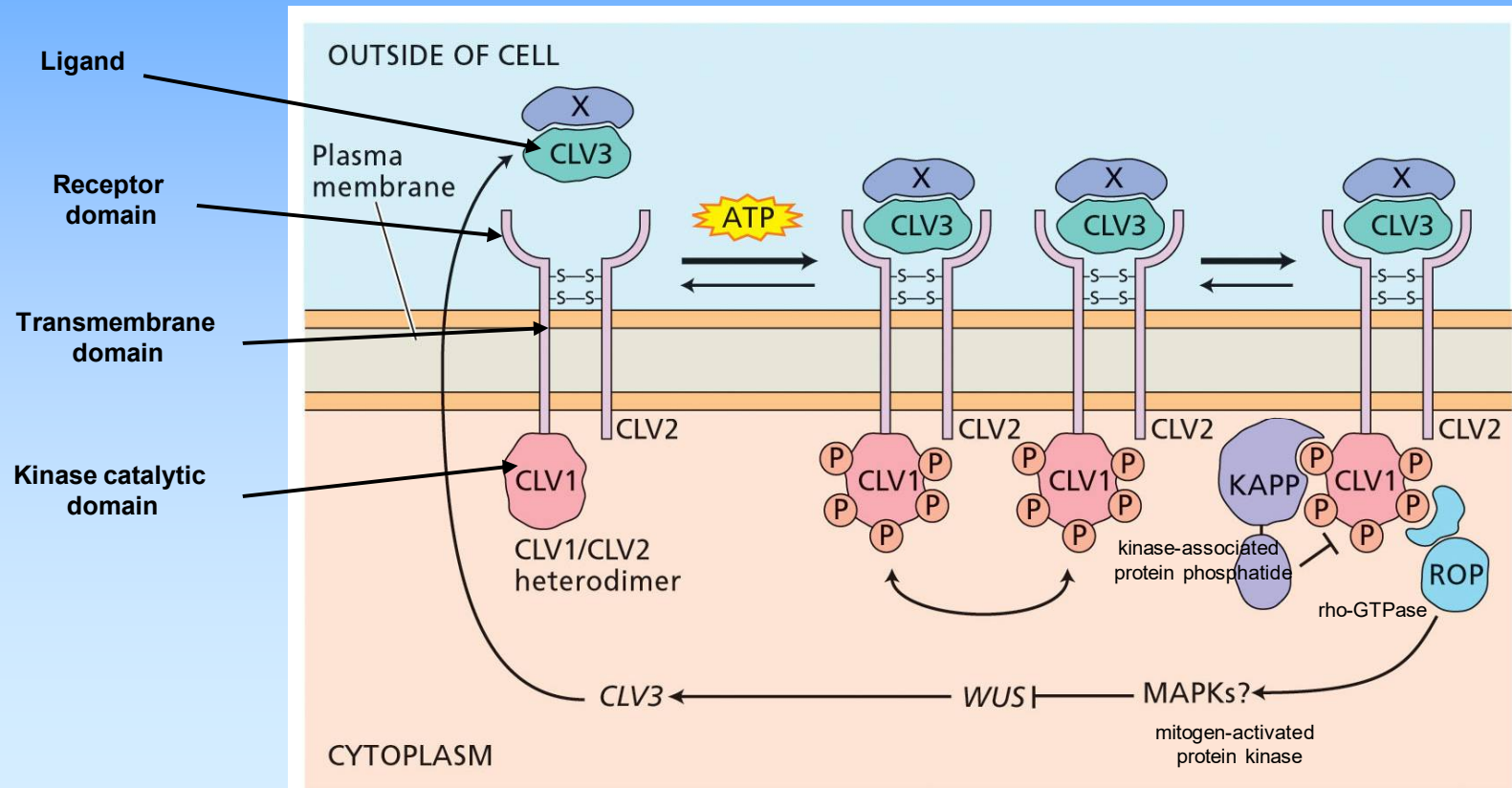
- a) Signal induced by a ligand**
- b) Hormonal signal**
- c) Signal mediated by transfer of mRNA or protein**

## a) Signal induced by a ligand

Model of the CLV1/CLV2 receptor-kinase signaling pathway regulating development of shoot apical meristem.

CLV1/CLV2 - receptor protein kinase = integral membrane protein

Clark SE (2001) Nature Reviews Molecular Cell Biology 2:276-284

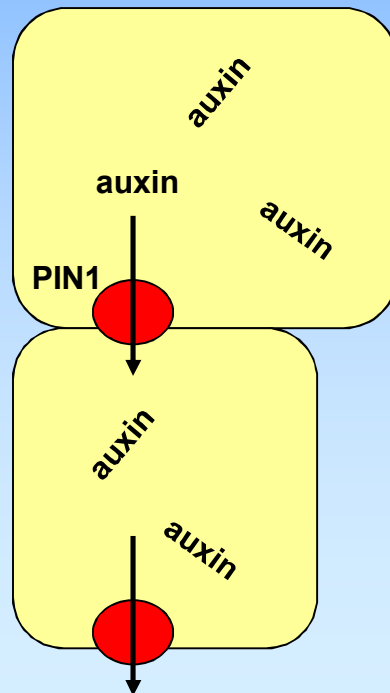


## b) Hormonal signal

### Auxin – important plant hormone

**PIN1** protein – located at the basal sites of the cell; it transports auxin from one cell to another cell – auxin distribution.

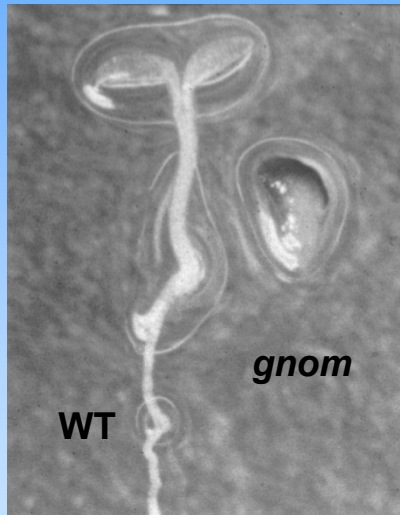
Auxin is distributed to the sites of initiation of lateral organs



Mutant *pin1* – auxin is not distributed to the sites of initiation of lateral organs => it does not form lateral organ primordia

Auxin plays a role in axial polarity of plants and in the development of vascular system. Gene ***GNOM*** plays a role in auxin signaling.

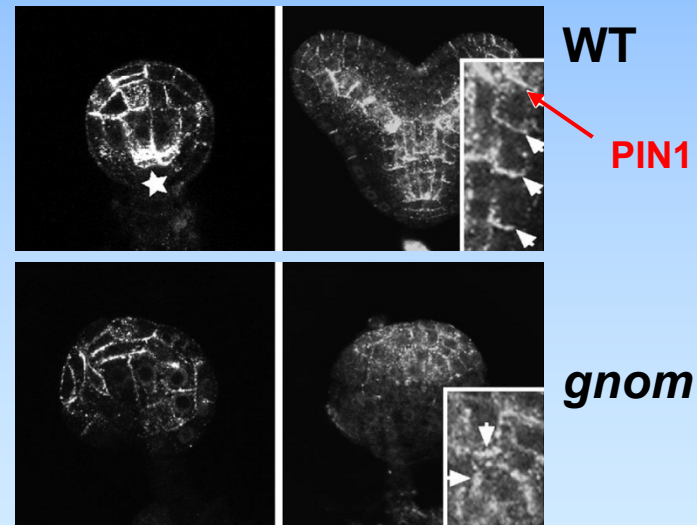
### ***GNOM***



Mutant ***gnom*** is affected in cotyledon and root formation = it lacks axial polarity

***GNOM*** controls apical-basal polarity

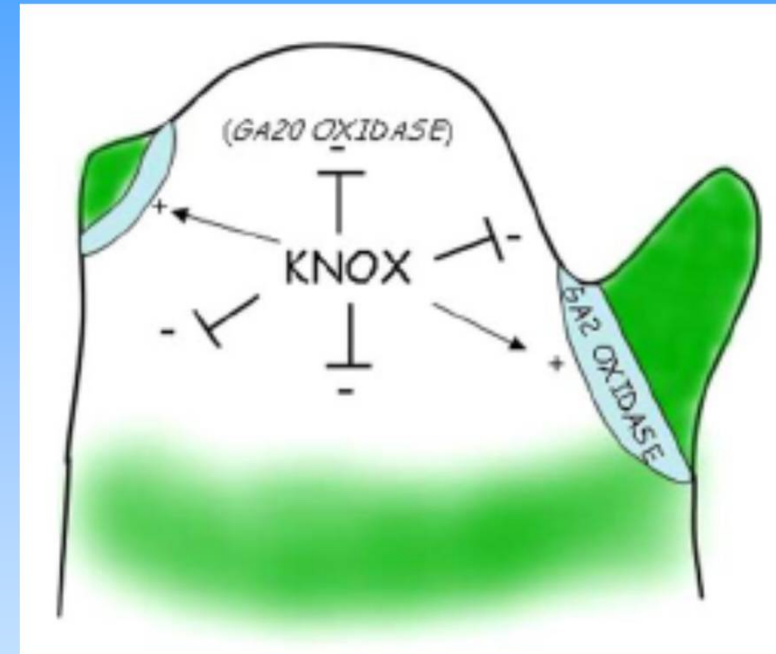
***GNOM*** is essential for correct **PIN1** localization at the basal sites of the cell.



## Expression of KNOX directs synthesis and metabolism of gibberellins

Expression of KNOX => biosynthesis of GAs (GA20 oxidase) in the central part of the meristem is blocked

Expression of KNOX => stimulation of the conversion of inactive GAs to active GAs (GA2 oxidase) in the sites of leaf initiation



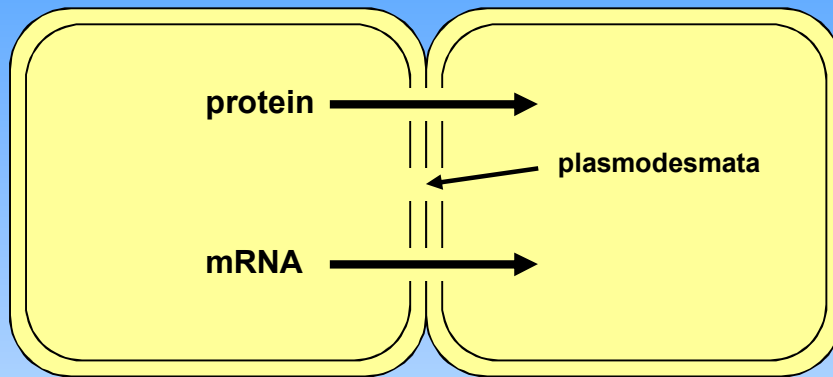
Veit B (2009) Plant Mol Biol 69: 397-408

High level of **cytokinins** – maintenance of undifferentiated meristem

High level of **auxins** and **gibberellins** – initiation of lateral organs

c) Signal mediated by transfer of mRNA or protein

Symplastic communication between cells = by means of **plasmodesmata**



*KN1* is expressed only in L2 zone of apical meristem.

*KN1* mRNA was never detected in L1 zone

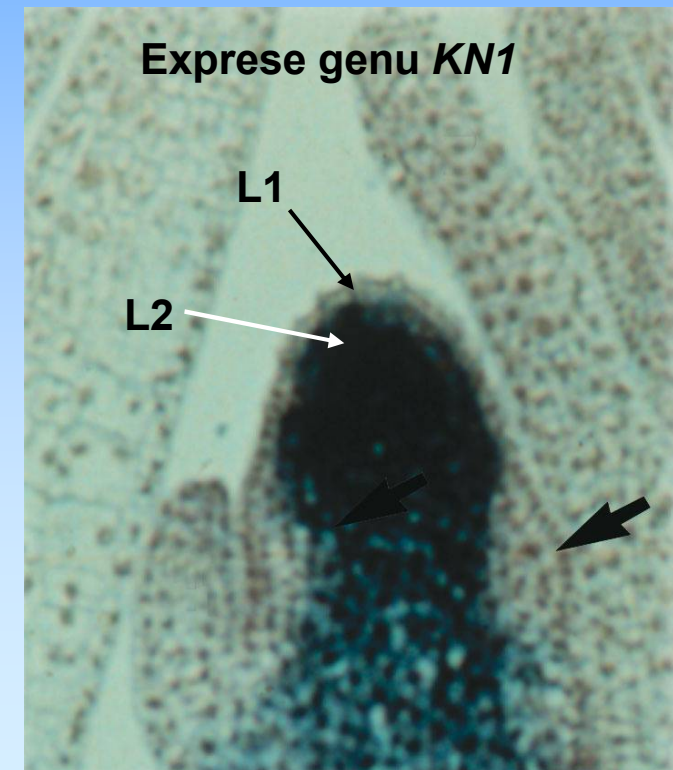
In L1 zone protein *KN1* was revealed.



*KN1* protein was transported into zone L1



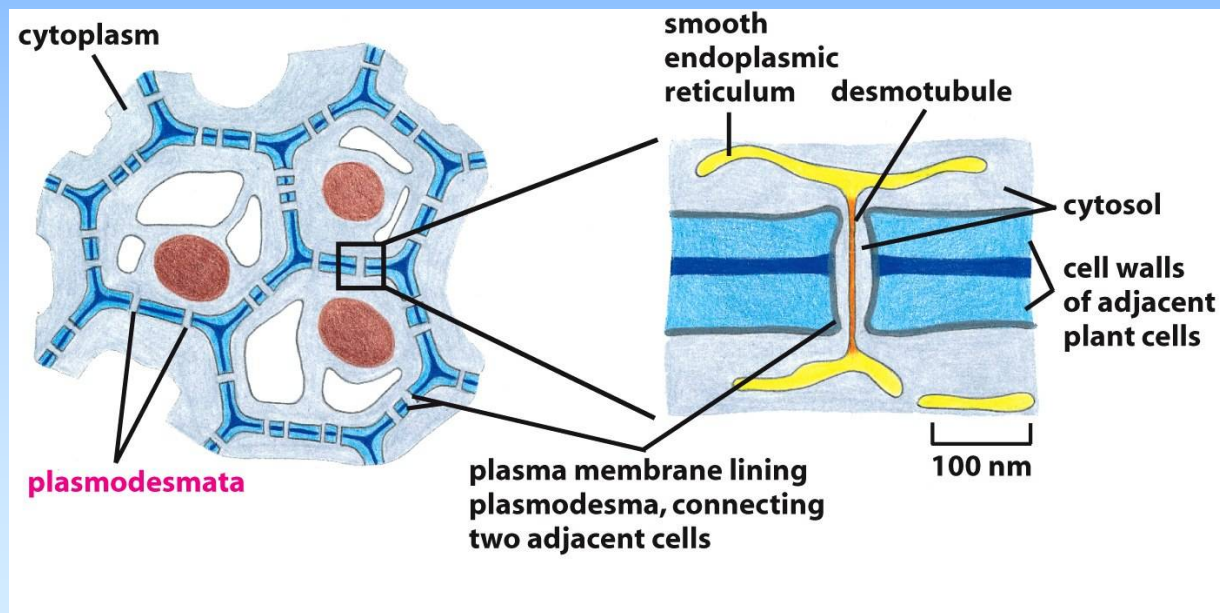
Oparka K (2005) *Plasmodesmata*. Blackwell Publishing.



**Plasmodesmata** – connect cytoplasm of two cells by an aperture in cell wall

**Big molecules (proteins, e.g. KN1; viral proteins)** – active transport through desmotubule from ER to ER; ability to actively spread the aperture

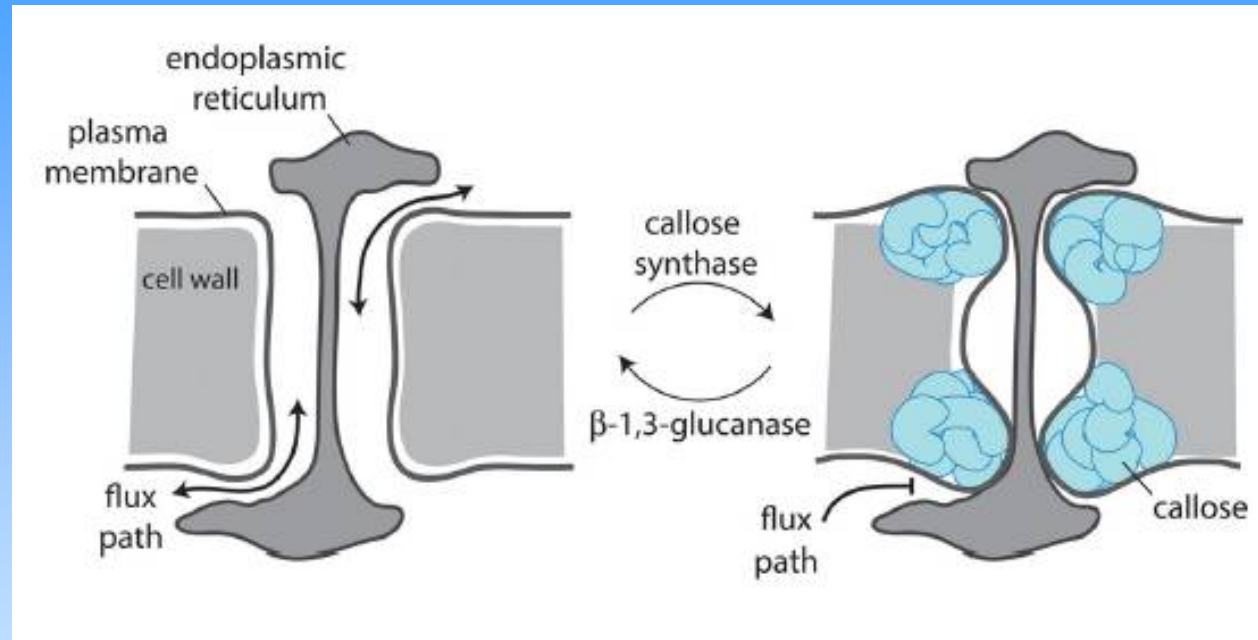
**Smaller molecules (RNA, small proteins)** – passive transport through plasmodesmata around desmotubule



Alberts B et al. (2008) Molecular Biology of The Cell. Garland Science, str. 1158.

## UPDATE 2012

Maule AJ et al. (2012) *Frontiers in Plant Science* 3: 1-5



Synthesis of callose – enzyme glykosyl synthase  
(callose synthase)

Degradation of callose – enzyme  $\beta$ -1-3-glucanase

Signals triggering expression of genes involved in deposition or degradation of callose:

- stress (viruses)
- ROS (reactive oxygen species) – influence local redox status or cell status