SFRZE

2022

2) Gene expression and signal transduction

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

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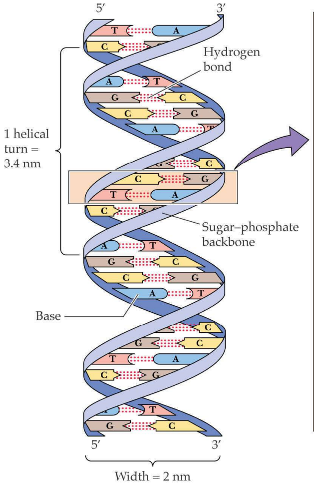
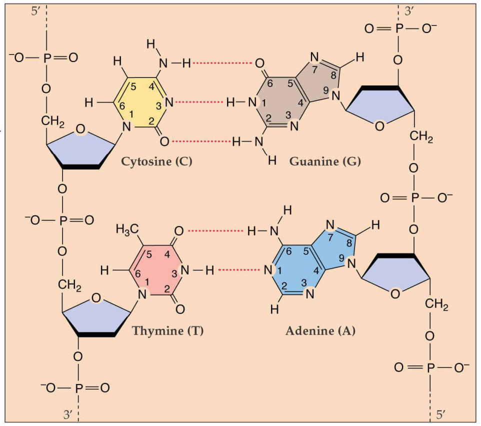
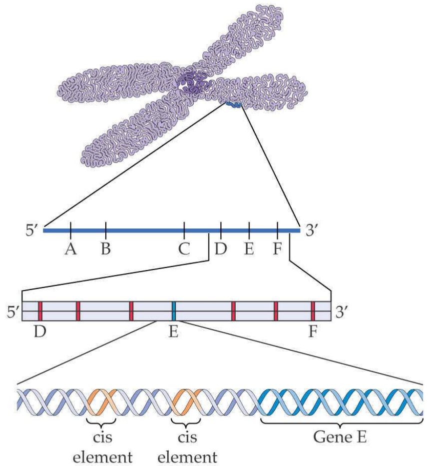
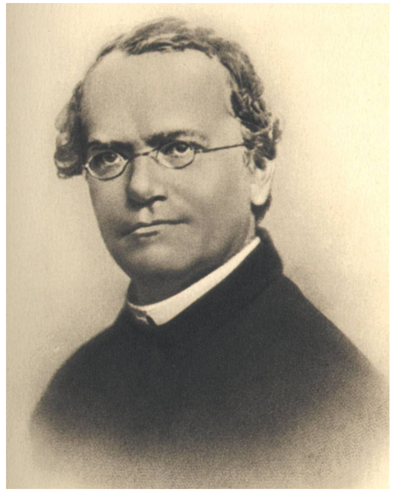
Living cell contains the instructions for building of the whole organisms = GENES

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





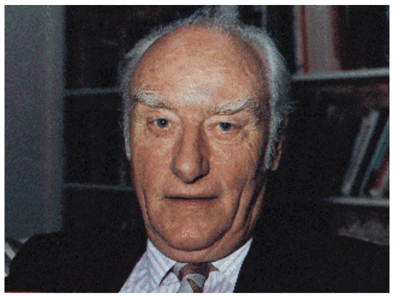




Prof. Gregor Johann Mendel

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

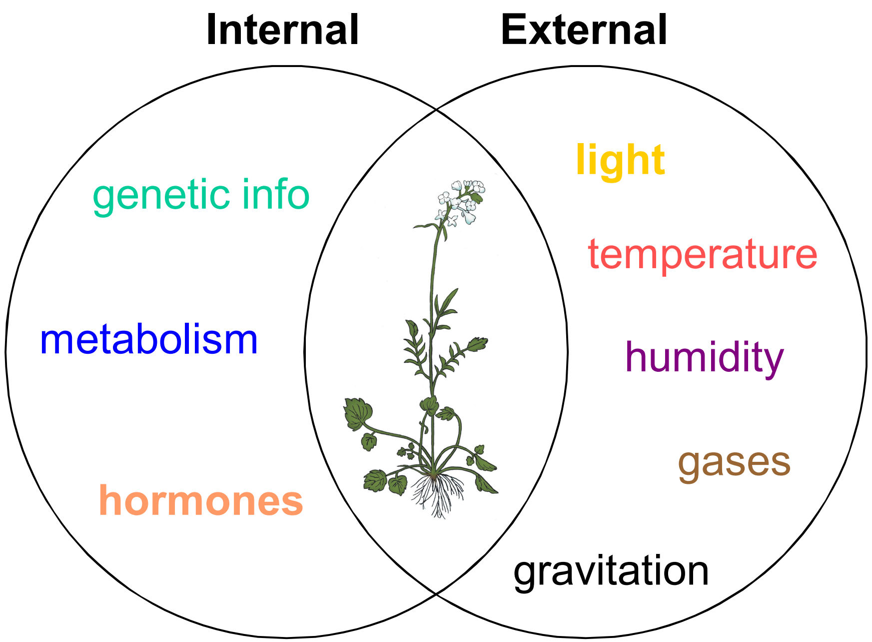
Prof. James Watson (USA)

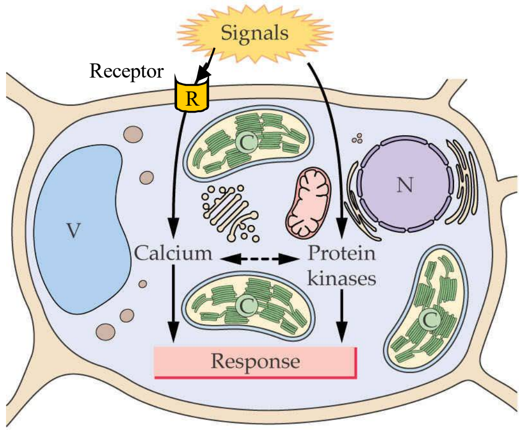


Prof. Francis Crick (UK) († 29.7. 2004)

Development of molecular biology

Development of an organism is regulated by signals (factors)





Signal

Receptor

Amplifier (secondary messenger)

Response
(e.g. gene expression)

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 x 10^6 bp

Drosophila = 2 x 10^8 bp

Human = 3 x 10^9 bp

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

GENOME = DNA coding proteins + DNA noncoding proteins *

(human ~ 5-10%)

Repetitive DNA   Spacer DNA

Size of plant genome is very variable:

Arabidopsis = 1,46 x 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

1st lecture
PMP

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)

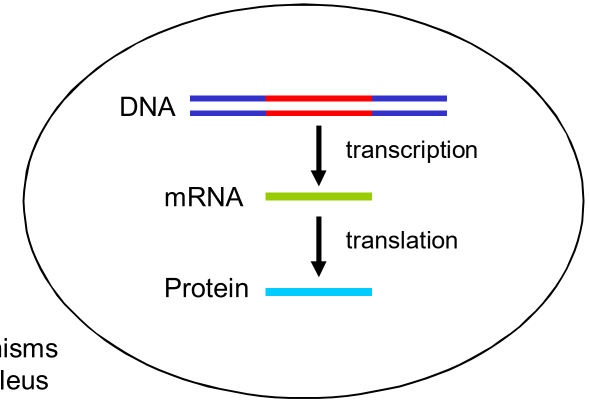
Plants: *UBQ* (for protein ubiquitin), *ACT* (for protein actin)

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.

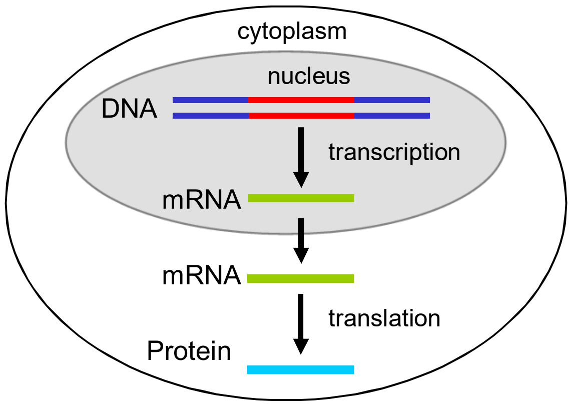
b) Gene expression in plants

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



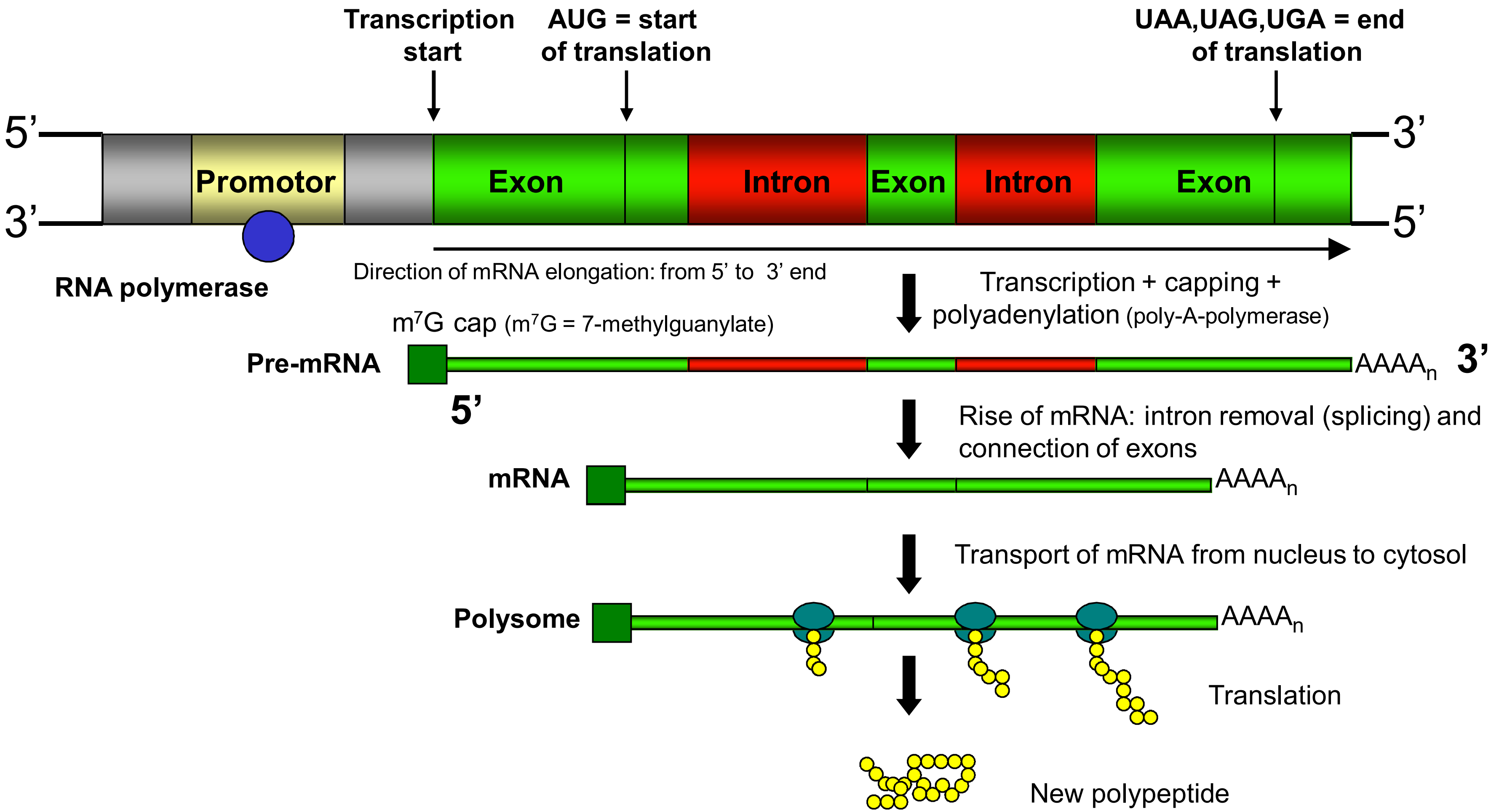
Prokaryotic organisms do not have nucleus

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

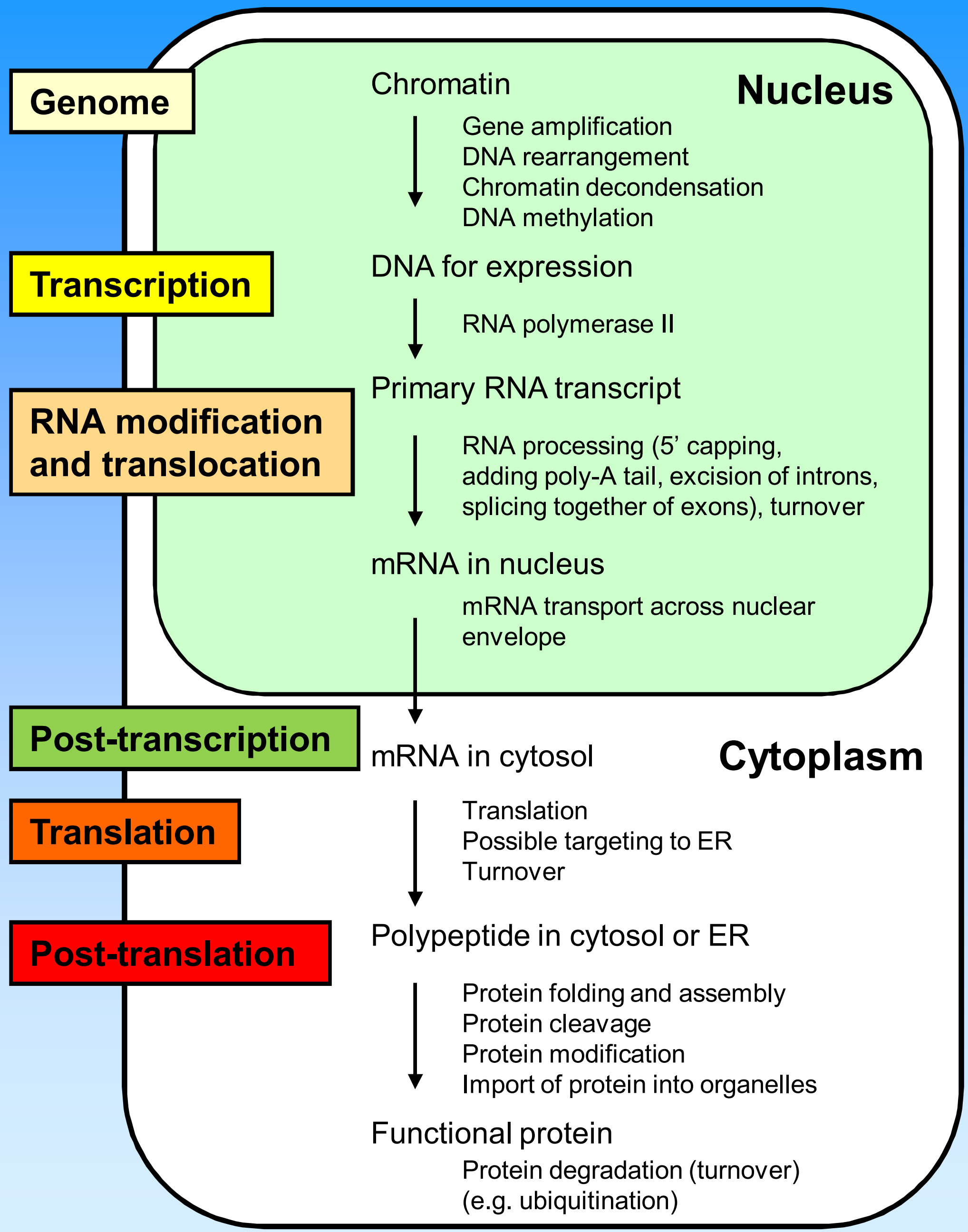


Requirement of additional control and regulatory mechanisms

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

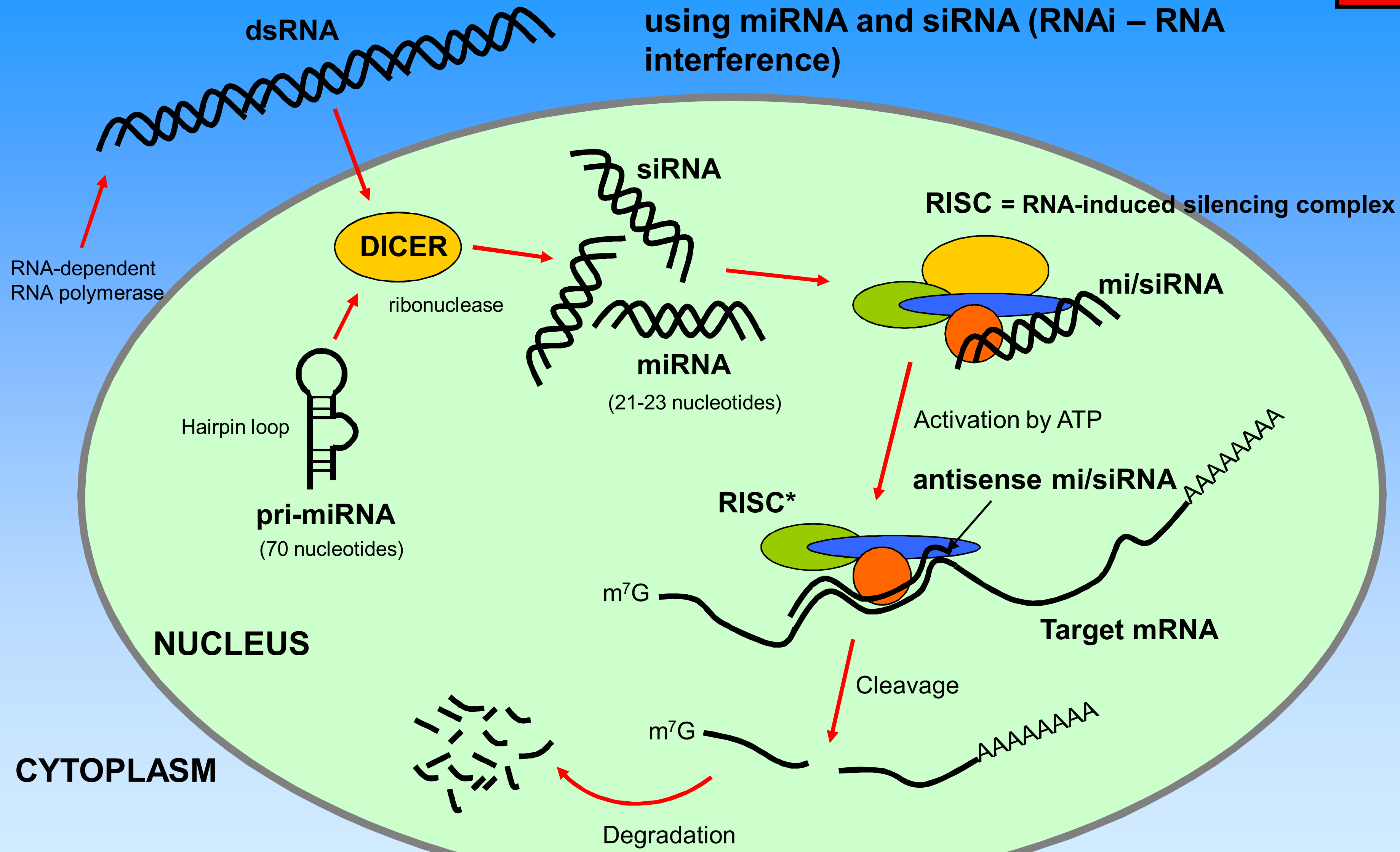
1) Turnover rate of mRNA – dependent on plant tissue and physiological conditions

2) Translatability of the mRNA molecule = ability of mRNA to by 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)



miRNA - microRNA

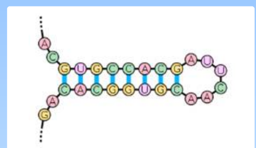
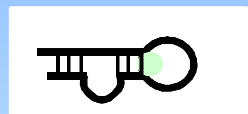
siRNA - short interfering RNA

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

Update 2015

Kamthan A et al. (2015) Frontiers in Plant Science 6, Article 208: 1 – 17

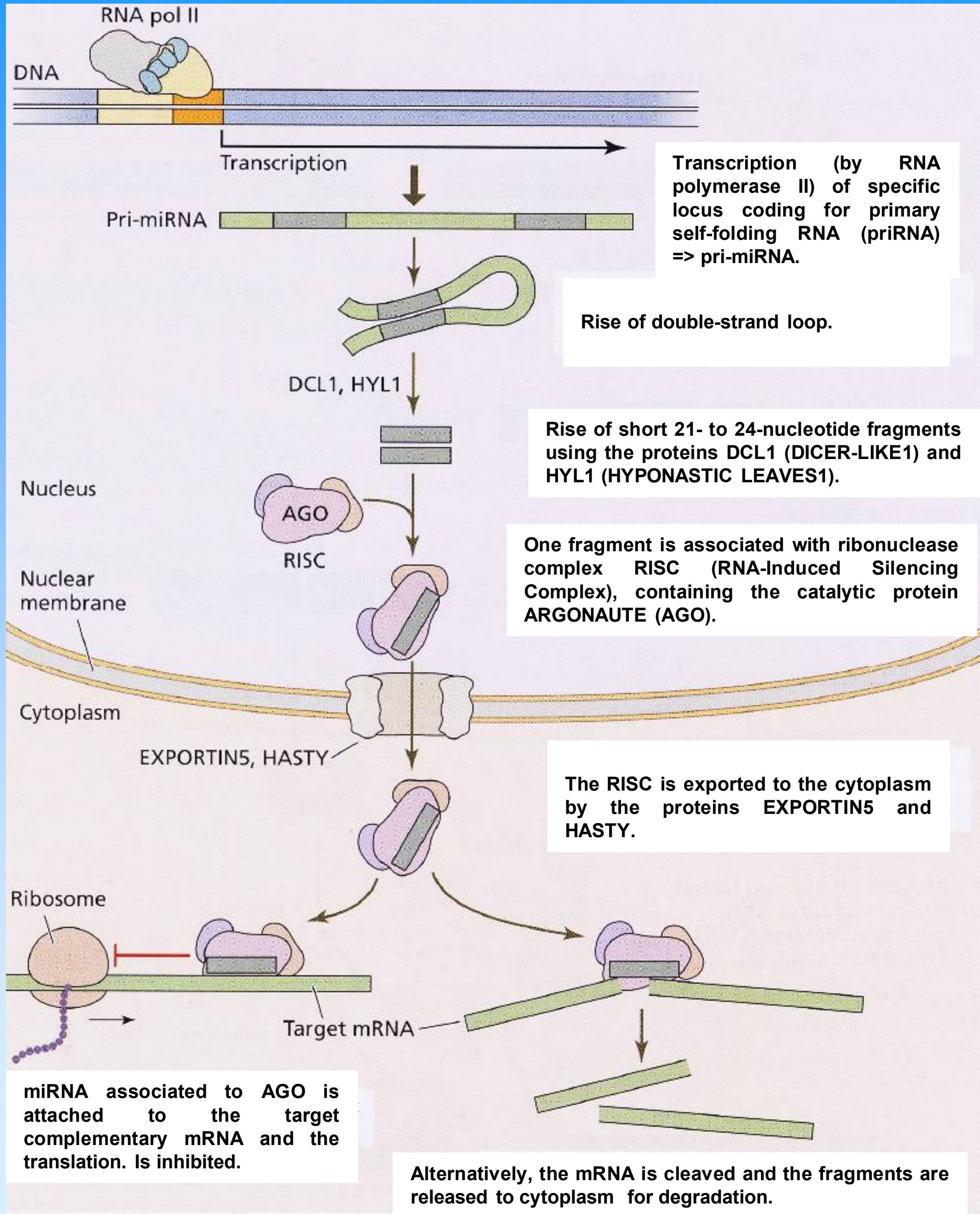
Comparison of miRNA and siRNA

	miRNA	siRNA
Origin	Specific loci in the genome Encoded by own genes	Encoded by transposon, viruses and by heterochromatin
Prekurzor (biogenesis)	One RNA molecule containing sec. stem-loop structure	Long double-stranded RNA molecules or extended hairpins
		
Evolutionary conservation	Almost always conserved in related organisms	Rarely preserved in related organisms
Aim of regulation	Regulate expression of various genes	Mediate the switching off of the genes from which they originate (or very similar genes)

Update 2021

Gao Z et al. (2021) Plant Growth Regulation 93: 1 – 12

Regulation of expression by microRNA (miRNA)



Transcription (by RNA polymerase II) of specific locus coding for primary self-folding RNA (priRNA) => pri-miRNA.

Rise of double-strand loop.

Rise of short 21- to 24-nucleotide fragments using the proteins DCL1 (DICER-LIKE1) and HYL1 (HYPONASTIC LEAVES1).

One fragment is associated with ribonuclease complex RISC (RNA-Induced Silencing Complex), containing the catalytic protein ARGONAUTE (AGO).

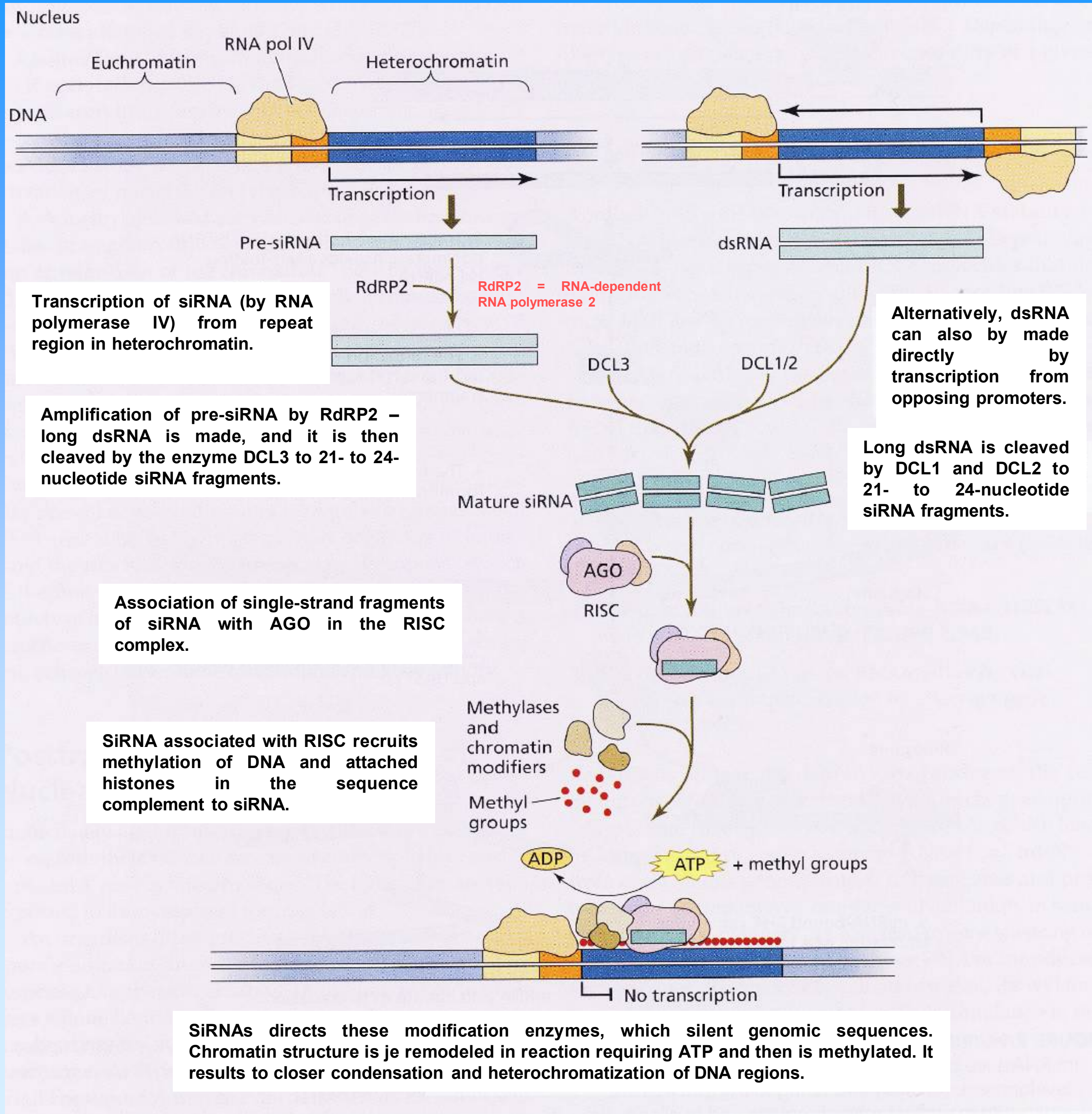
The RISC is exported to the cytoplasm by the proteins EXPORTIN5 and HASTY.

miRNA associated to AGO is attached to the target complementary mRNA and the translation. Is inhibited.

Alternatively, the mRNA is cleaved and the fragments are released to cytoplasm for degradation.

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

Regulation of expression by short interfering RNA (siRNA)



Transcription of siRNA (by RNA polymerase IV) from repeat region in heterochromatin.

Amplification of pre-siRNA by RdRP2 – long dsRNA is made, and it is then cleaved by the enzyme DCL3 to 21- to 24-nucleotide siRNA fragments.

Alternatively, dsRNA can also by made directly by transcription from opposing promoters.

Long dsRNA is cleaved by DCL1 and DCL2 to 21- to 24-nucleotide siRNA fragments.

Association of single-strand fragments of siRNA with AGO in the RISC complex.

SiRNA associated with RISC recruits methylation of DNA and attached histones in the sequence complement to siRNA.

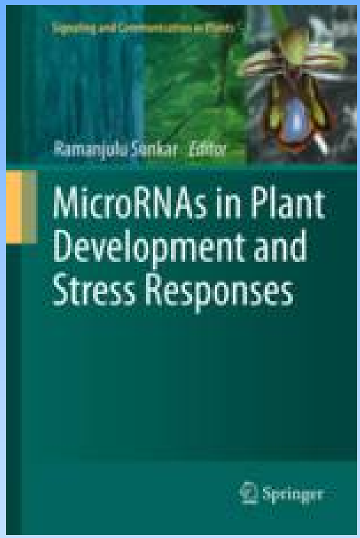
SiRNAs directs these modification enzymes, which silent genomic sequences. Chromatin structure is je remodeled in reaction requiring ATP and then is methylated. It results to closer condensation and heterochromatization of DNA regions.

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

miRNA and siRNA regulate gene expression

Important role in growth and development signaling

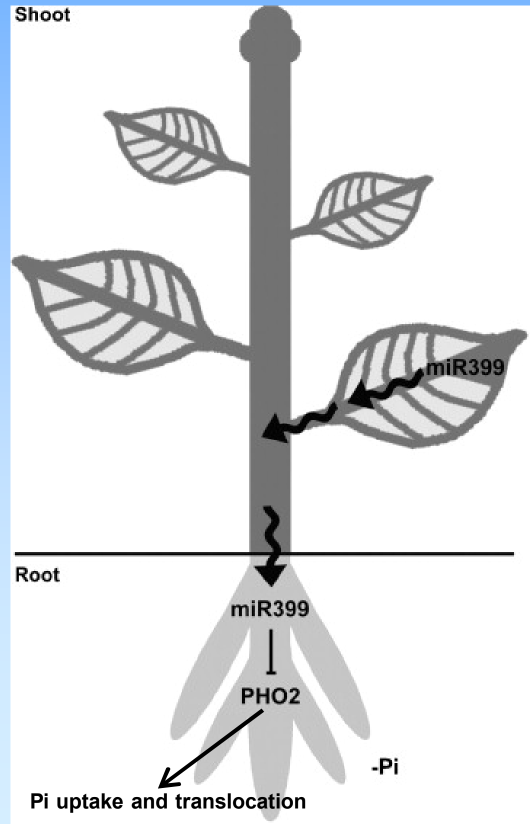
Update 2012



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

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

miRNA are transported for long distances



Update 2019

Fang X et al. (2019) Developmental Cell 48: 371-382

The work shows the existence of a signaling mechanism between chloroplasts and the nucleus, which is involved in miRNA biogenesis under abiotic stresses. Tocopherols (vitamin E), synthesized from tyrosine in chloroplasts, positively regulate miRNA production.

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**

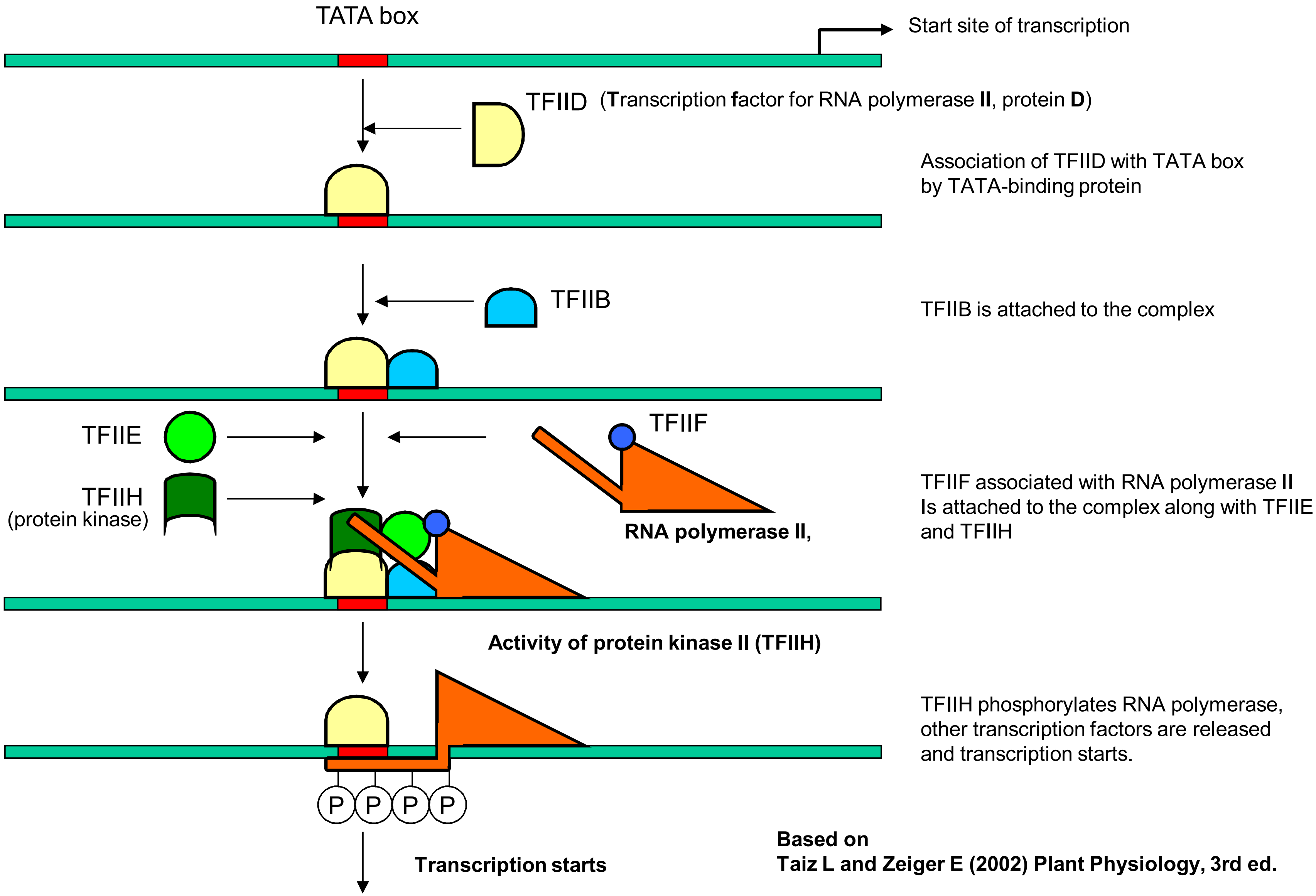
- **I** – located in nucleolus, role in synthesis of ribosomal RNA
- **II** – located in nucleoplasma, role in pre-mRNA synthesis
- **III** - located in nukleoplasma, role in synthesis of tRNA or 5S RNA

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



Based on
Taiz L and Zeiger E (2002) Plant Physiology, 3rd ed.

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

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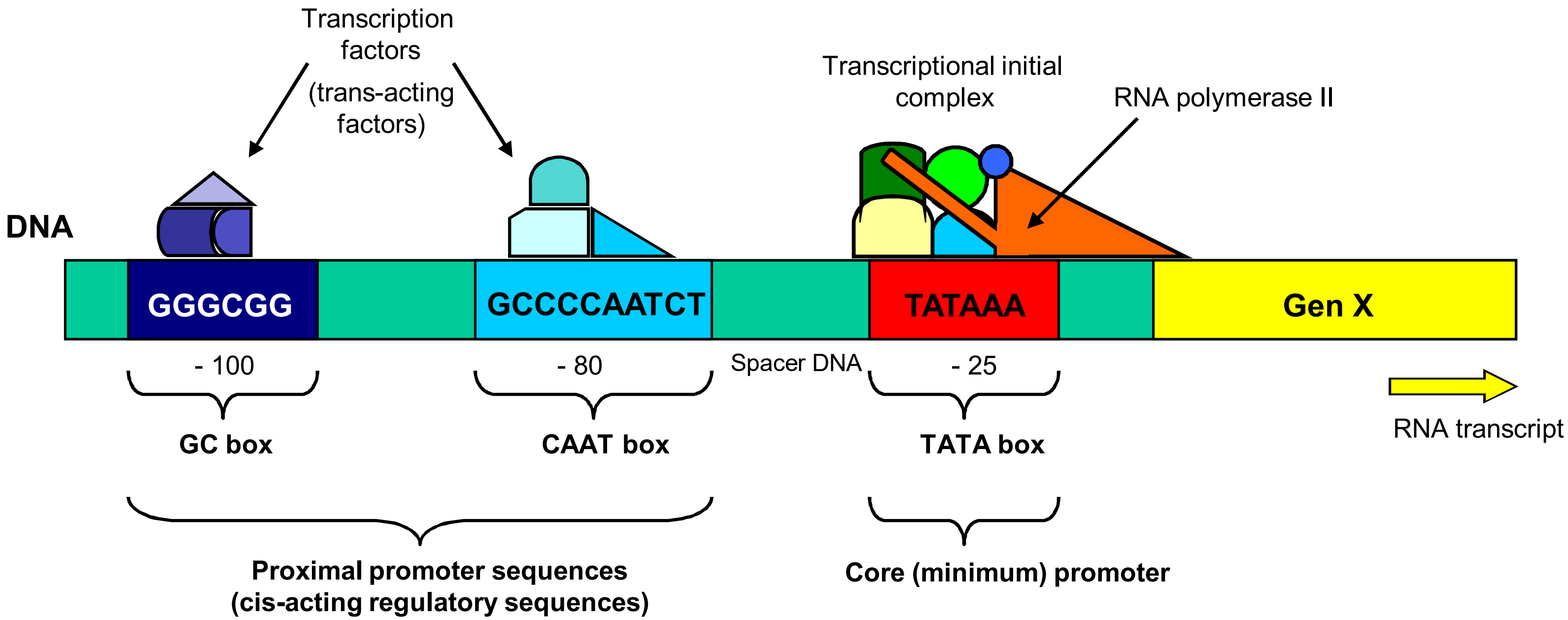
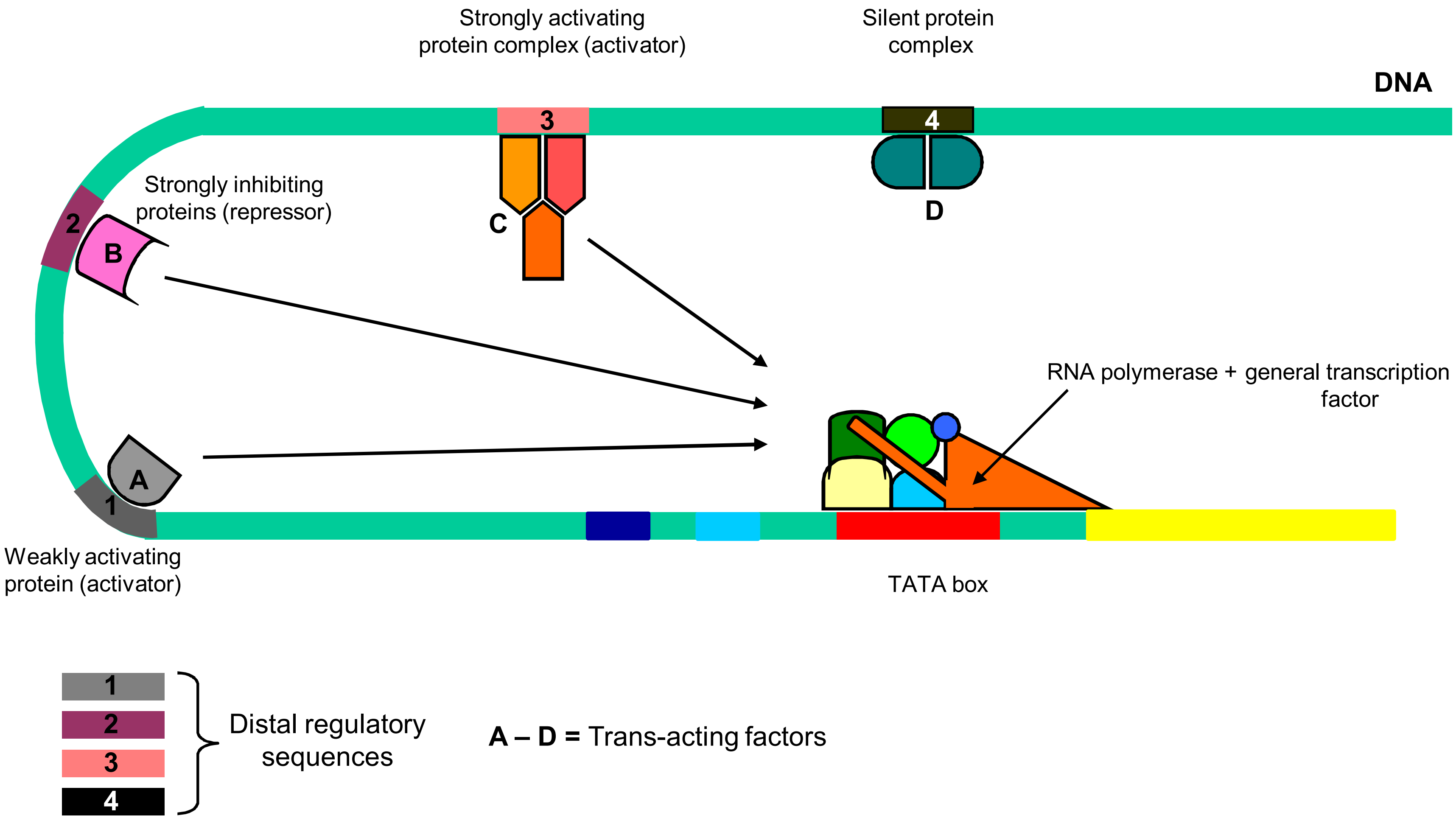
Organization and regulation of typical eukaryotic minimal promoter (core promoter) for RNA polymerase II



Regulation of transcription by distal regulatory sequences

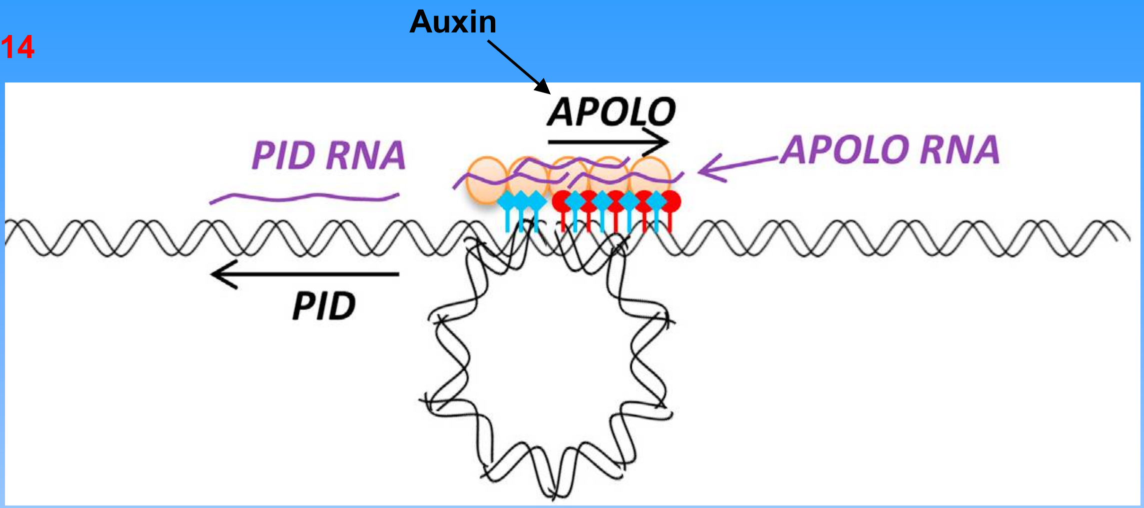


A – D = Trans-acting factors

UPDATE 2014 lincRNA – long intergenic noncoding RNA

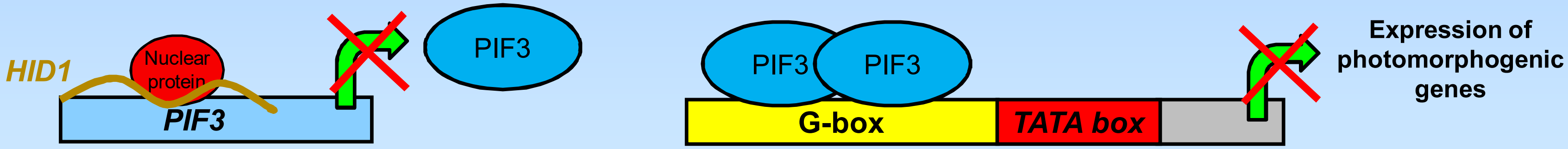
Ariel F et al. (2014) Molecular Cell 55: 1-14

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.

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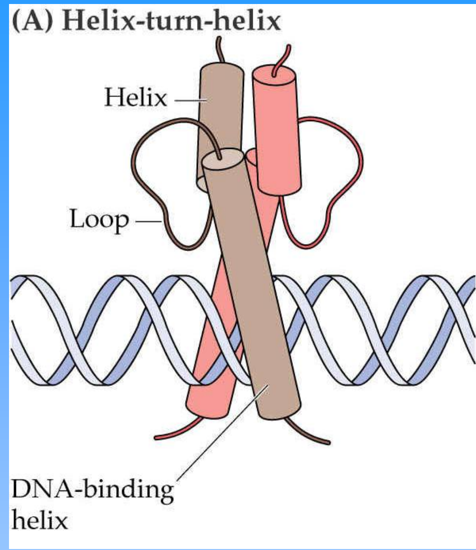
DNA-binding motive

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

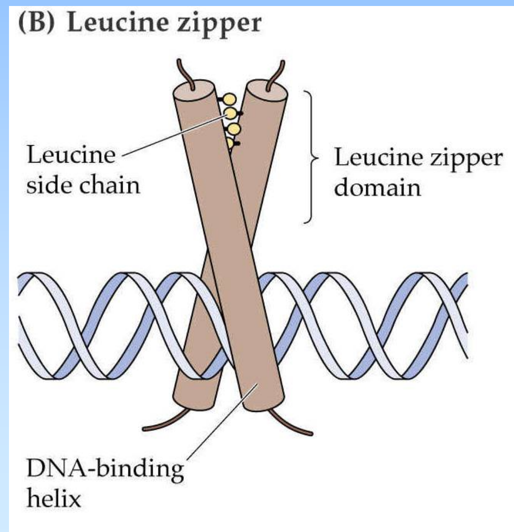
Review about methods for study of transcription factors.

DNA-binding motives

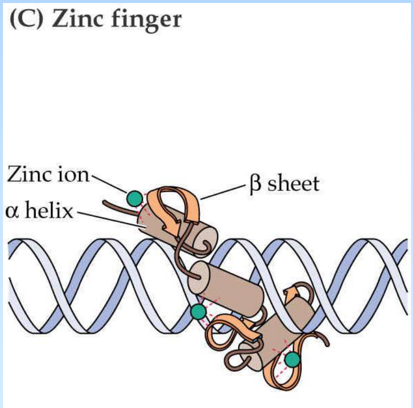
Helix-turn-helix 2 α helixes separated by loop of polypeptide chains; It functions as a dimer; often coded by homeotic genes



Leucine zipper α 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)

Gene expression noise

Genetically identical cells grown in identical conditions can show significantly different levels of gene expression = NOISE

Unicellular organisms - allows certain parts of the unicellular population to prepare for environmental stress.

Multicellular organisms - role of noise unclear; while plasticity allows the plant to respond dynamically to environmental changes and epigenetic inheritance allows plants to acquire a certain memory of previous stresses, the noise of gene expression can allow the plant to bet on protection against an unknown environment.

Update 2020

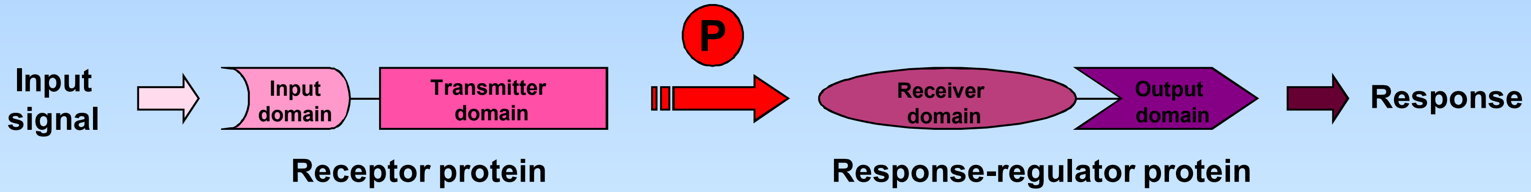
Cortijo S and Locke JCW (2020) Trends in Plant Science, May 25;
DOI:https://doi.org/10.1016/j.tplants.2020.04.017

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

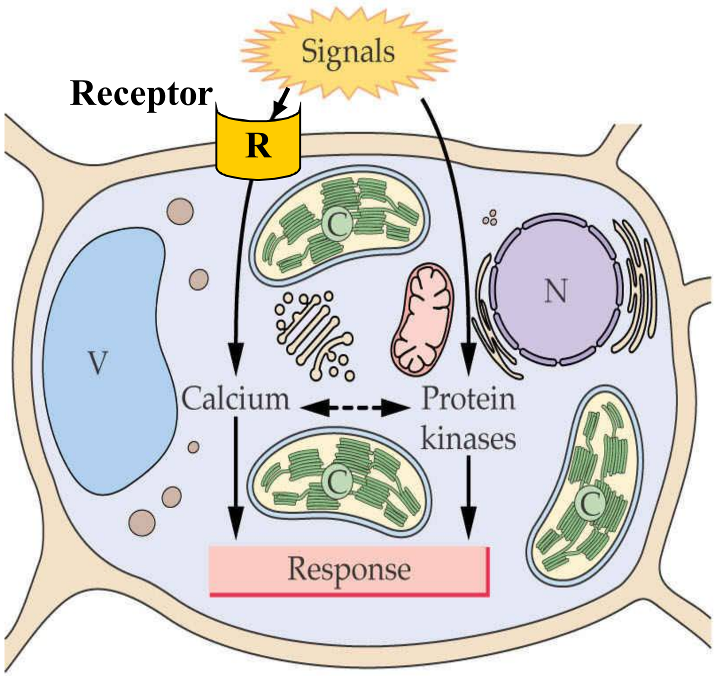
Need of new signaling mechanisms = chemical messengers – secondary messengers

Understanding of essential mechanisms of signaling pathways in animals

Parallel in plants revealed (e.g. brassinosteroids)

Similar signaling mechanisms in plants understood.

Scheme of signal transduction in plants



Signal

↓

Receptor

↓

Amplifier (secondary messenger)

↓

Response
(e.g. gene expression)

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

Signal →
- **External** (light, temperature, gravity, humidity)
- **Internal** (hormones, metabolites, genetic information)

⇓

Receptor →
- **Intracellular** (for lipophilic hormones => easily transported by plasma membrane)
- **Membrane (surface)** (for hormones soluble in water => unable to cross plasma membrane)

⇓

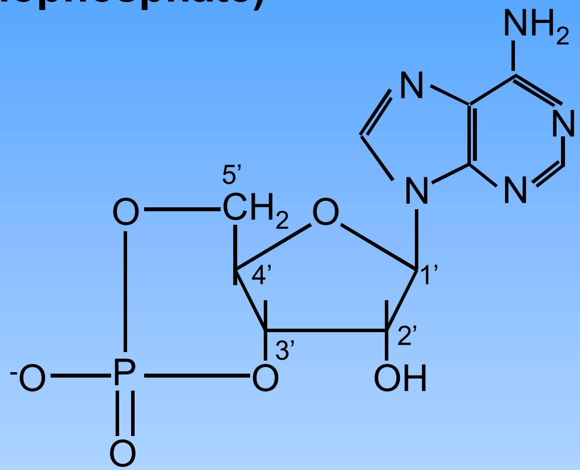
Amplifier (secondary messenger) → Cyclic AMP and GMP, diacylglycerol, inositol-triphosphate, Ca^{2+} ions

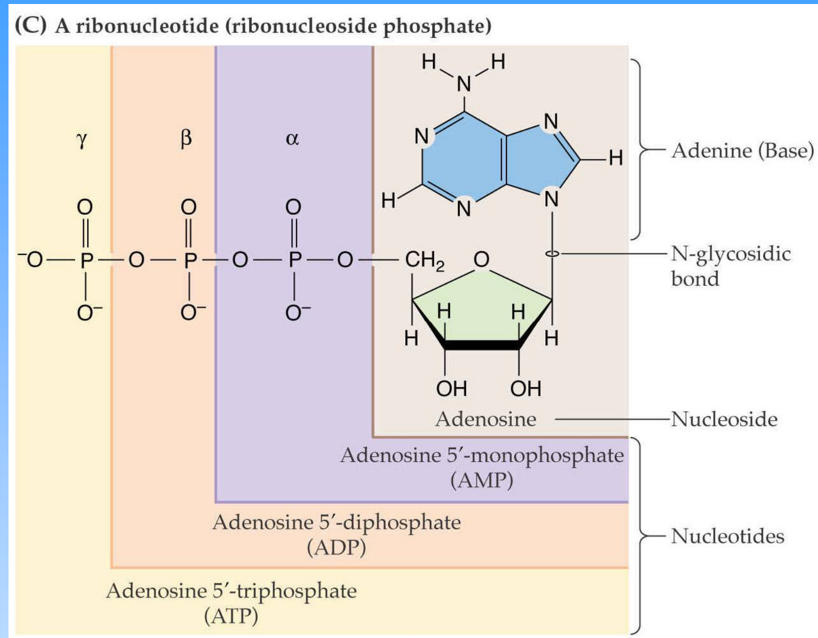
⇓

Response → Gene expression, induction of channel and pump activities

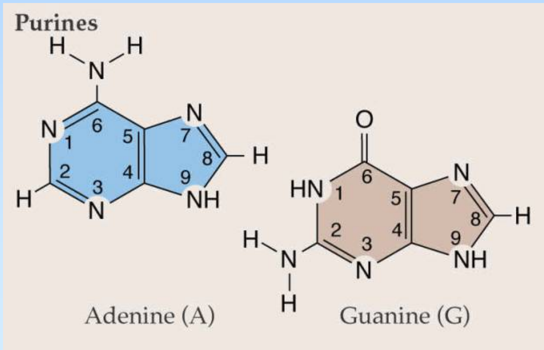
Amplifiers (secondary messengers)

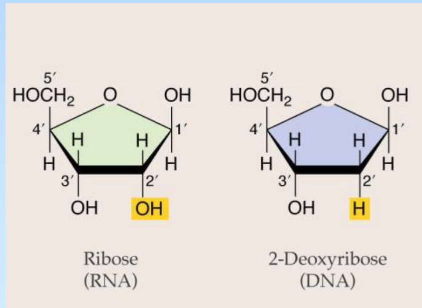
Cyclic AMP (cyclic adenosine monophosphate)





Cyclic GMP (cyclic guanosine monophosphate)





1,2-Diacylglycerol (DAG)

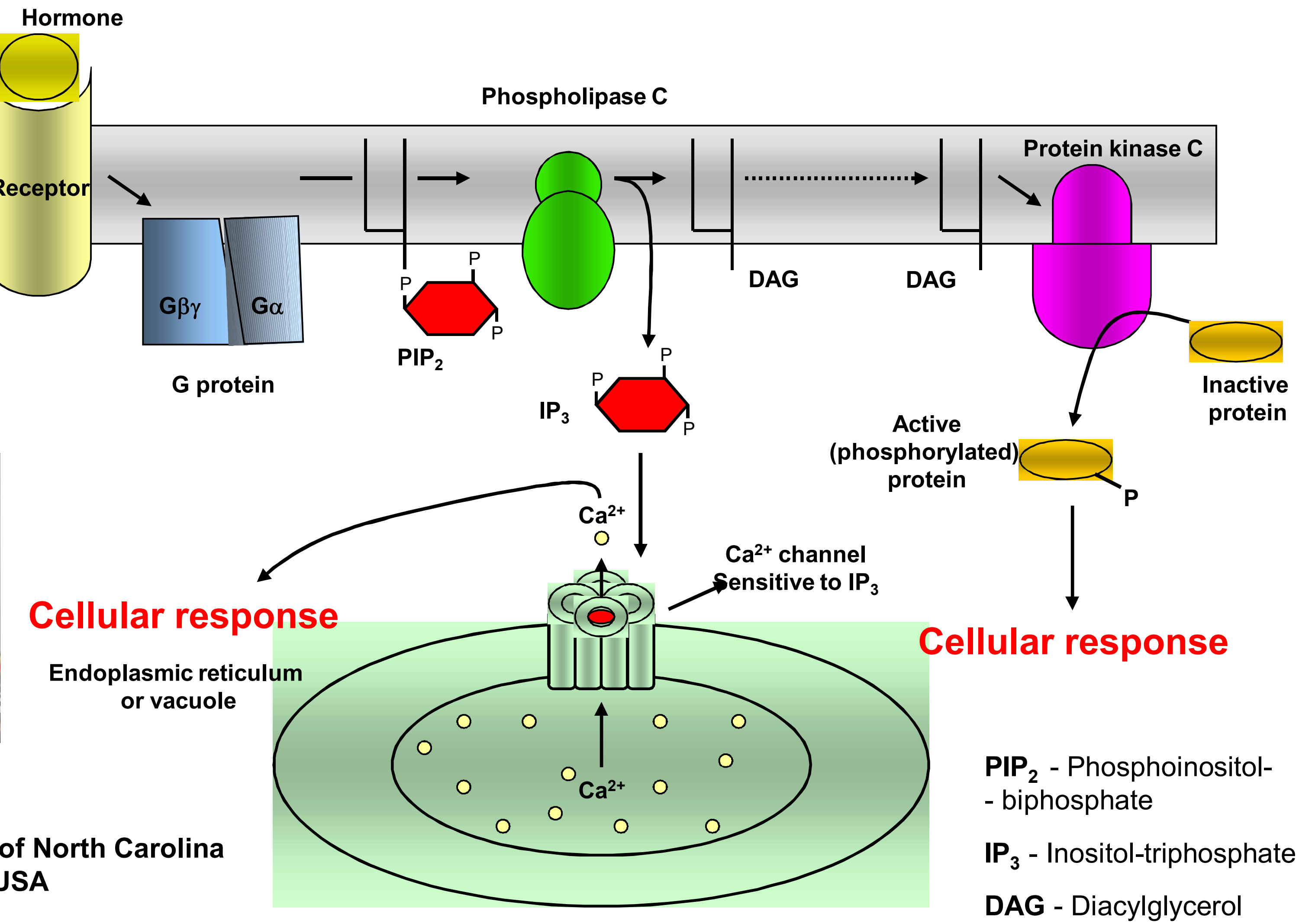
Inositol-1,4,5-triphosphate (IP_3)

Ca^{2+} ions

Nitric oxide N ≡ O

K^+ ions – transport from the cell = switch between metabolic and defense responses during stress

General scheme of inositol-lipid signaling pathway



Alan M Jones

The University of North Carolina at Chapel Hill, USA

PIP_2 - Phosphoinositol-
- biphosphate

IP_3 - Inositol-triphosphate

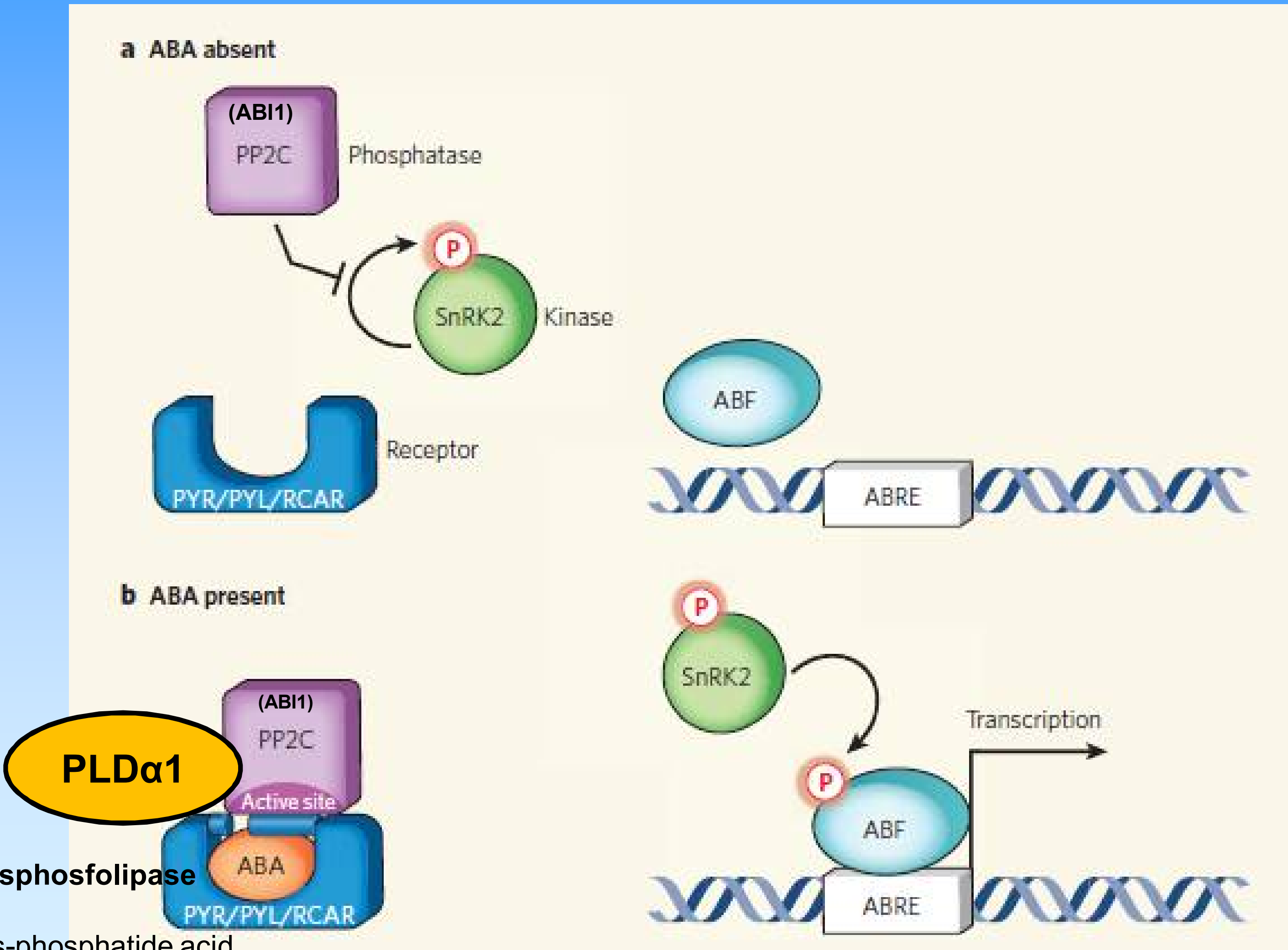
DAG - Diacylglycerol

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

Involvement of lipids in signaling

Update 2020
Hoffmann-Benning S (2020)
Molecular Plant 13: 952-954

ABA signaling during stress (drought, osmotic stress)



PLDα1 - phosphosfolipase

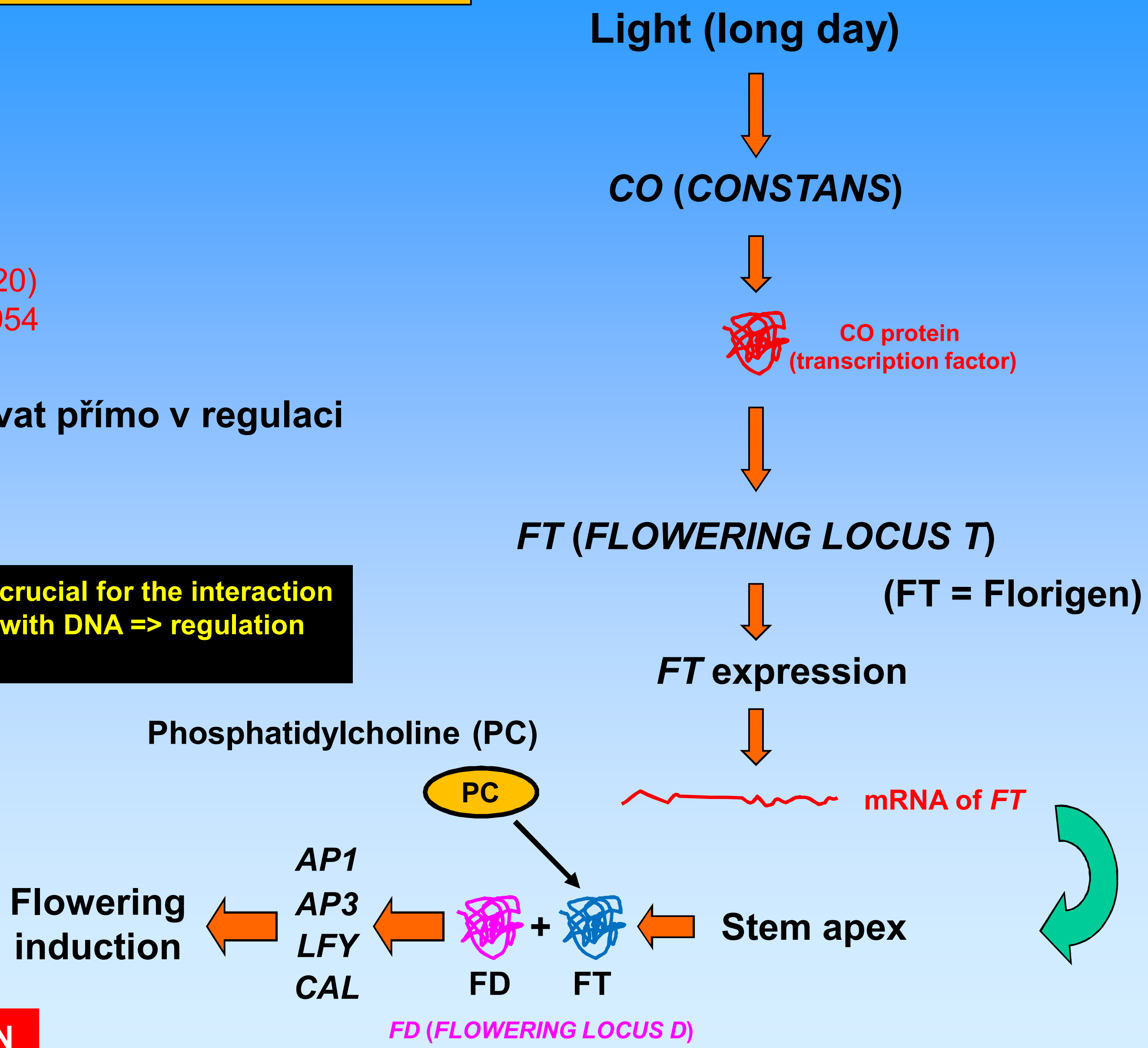
phospholipids-phosphatide acid

Lipid signaling during flowering induction

Update 2020
Hoffmann-Benning S (2020)
Molecular Plant 13: 952-954

Lipidy mohou fungovat přímo v regulaci genové exprese.

The binding of PC to FT is crucial for the interaction of the FT+FD+PC complex with DNA => regulation of transcription



FT protein = FLORIGEN

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:

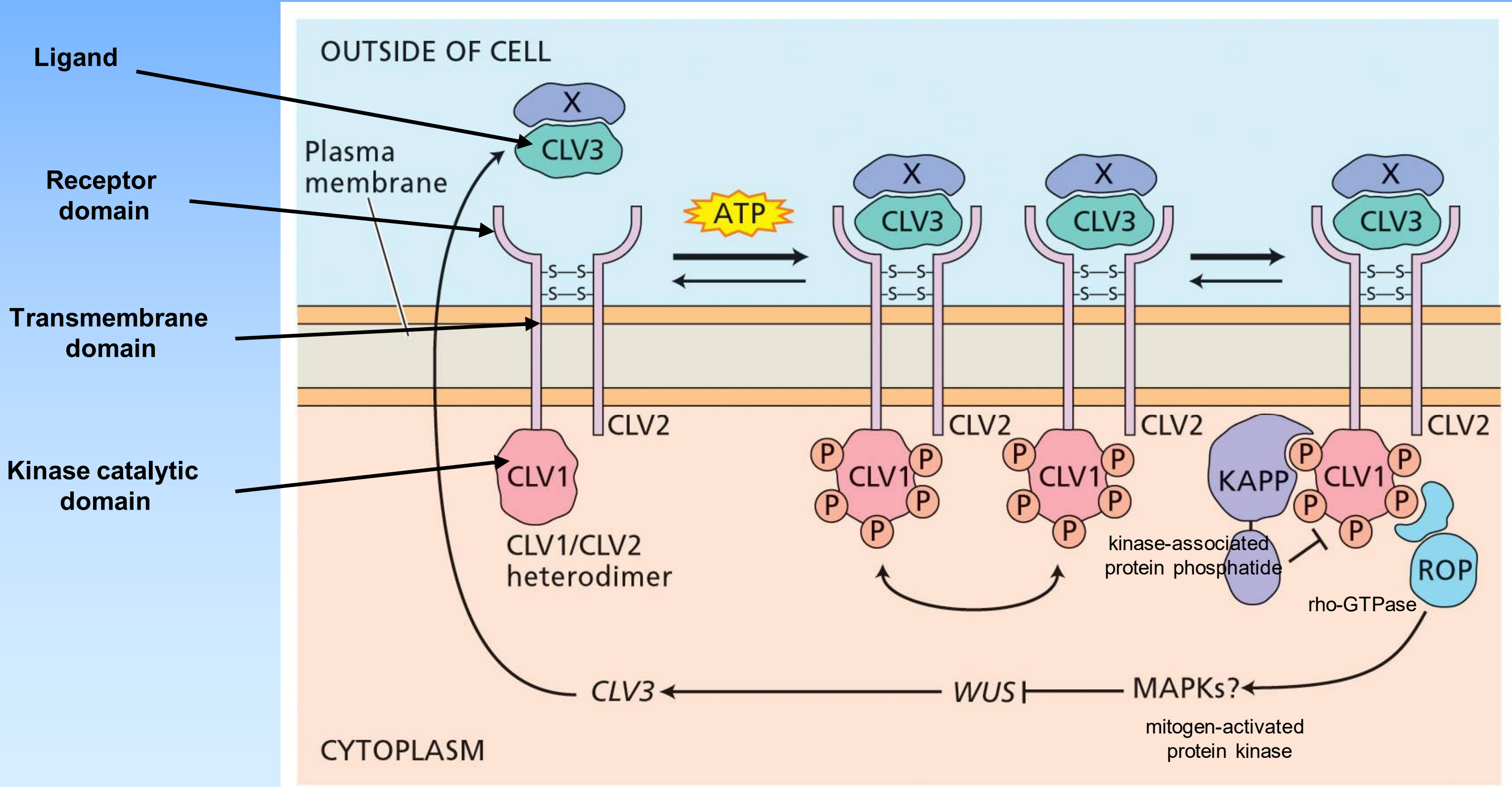
a) **Signal induced by a ligand**
b) **Hormonal signal**
c) **Signal mediated by transfer of mRNA or protein**

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

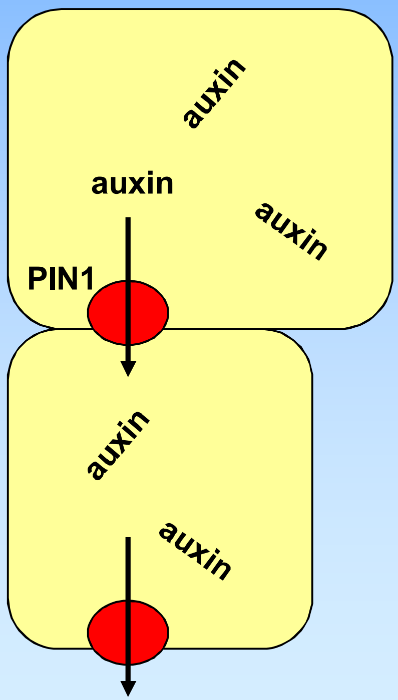


b) Hormonal signal

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.

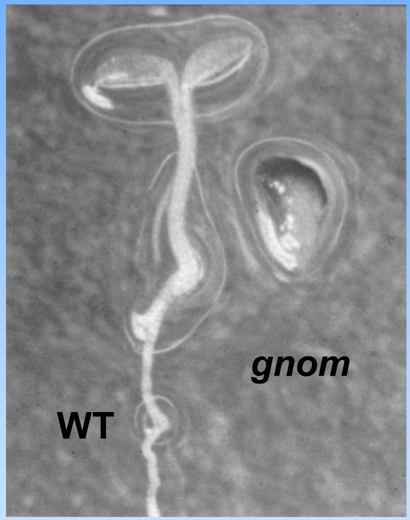
Auxin is distributed to the sites of initiation of lateral organs



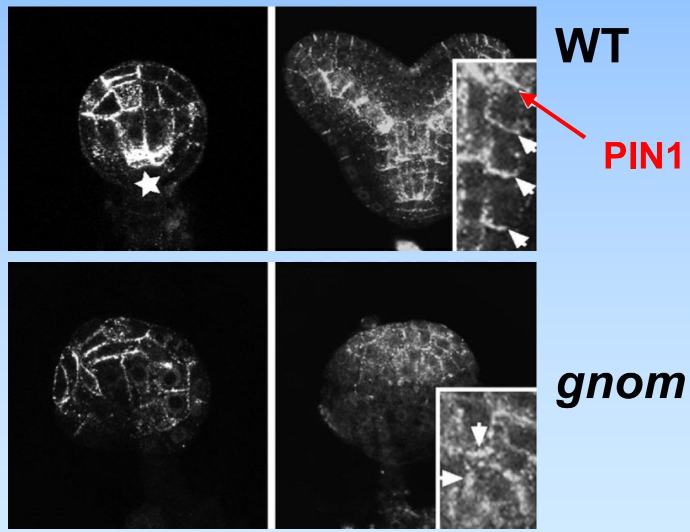
Mutant ***pin1*** – auxin is not distributed to the sites of initiation of lateral organs => it does not form lateral organ primordia

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



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



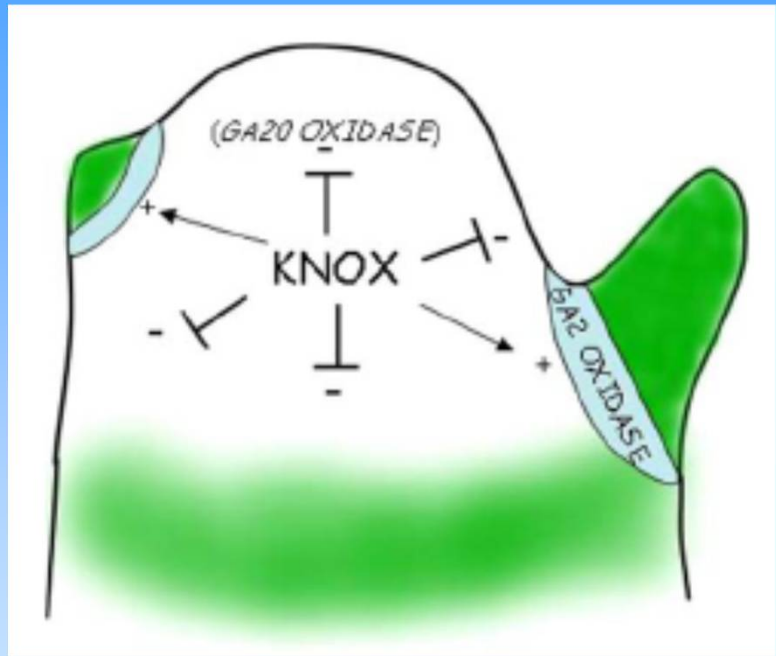
Mutant ***gnom*** is affected in cotyledon and root formation = it lacks axial polarity

GNOM controls apical-basal polarity

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

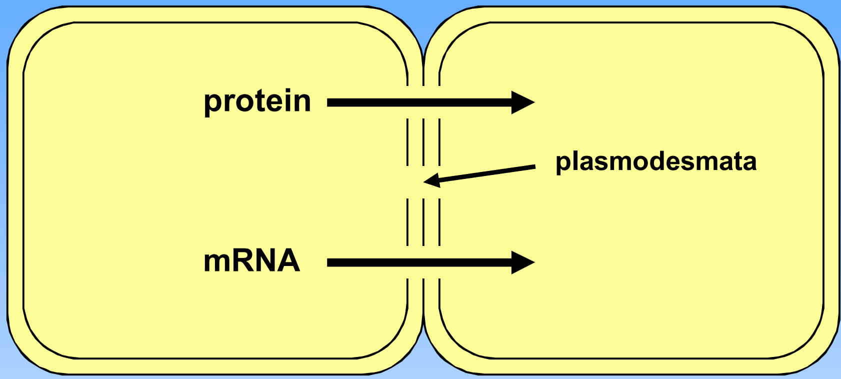
High level of cytokinins – maintenance of undifferentiated meristem

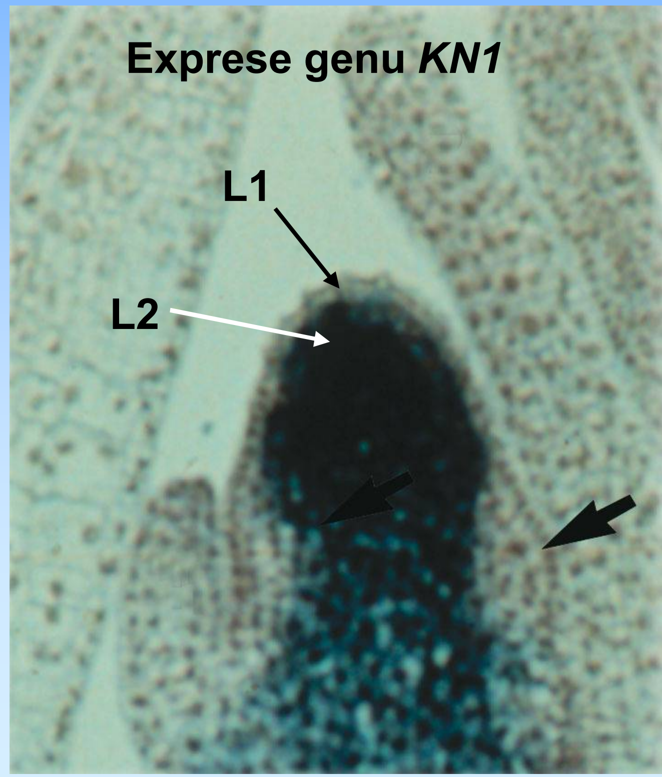
High level of auxins and gibberellins – initiation of lateral organs

c) Signal mediated by transfer of mRNA or protein

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

Oparka K (2005) Plasmodesmata. Blackwell Publishing.





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

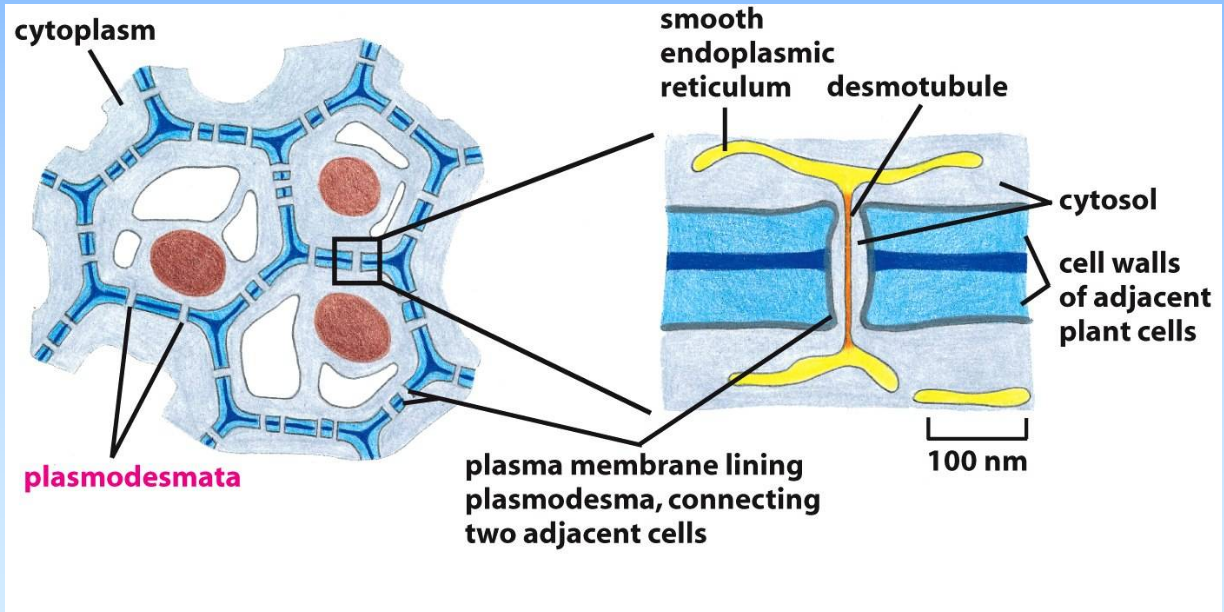
KN1 **mRNA was never detected in L1 zone** ∧ **In L1 zone protein KN1 was revealed.**

KN1 protein was transported into zone L1

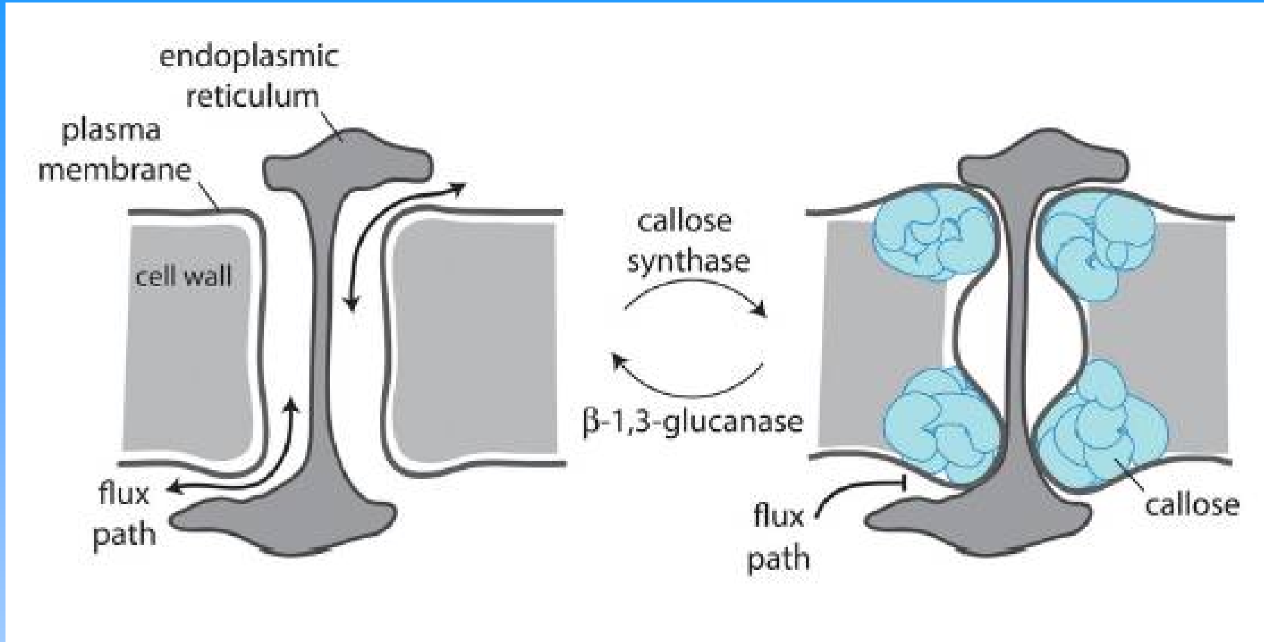
Plasmodesmata – connect cytoplasma of two cells by an aperture in cell wall

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 β-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**

Update 2016

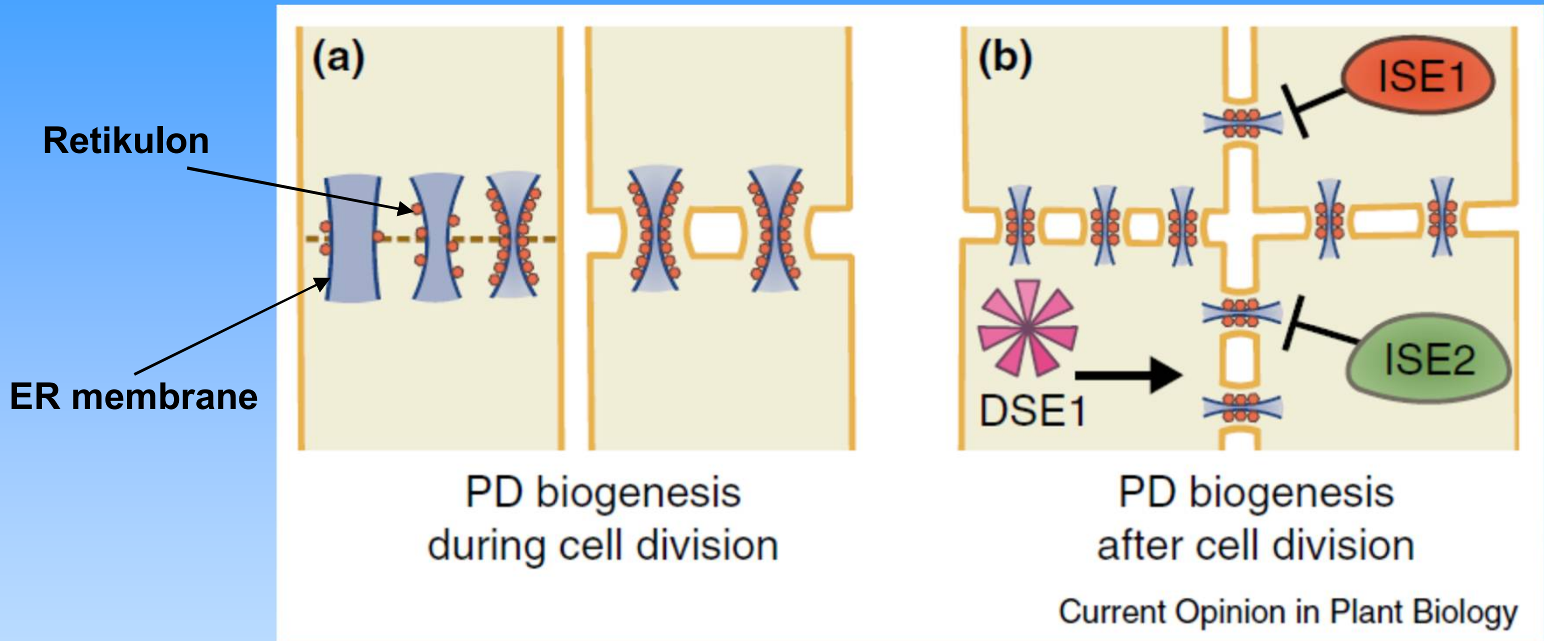
Lim G-H et al. (2016) Cell Host & Microbe 19: 541-549

Viruses encode proteins that manipulate the PD - increase the size of the passageway of the PD => facilitate the movement of viral units from cell to cell.

PD permeability is regulated by PD-localizing proteins (PDLPs): loss of PDLP5 function – increased PD permeability, overexpression of PDLP5 – decreased PD permeability

Update 2017

Brunkard JO and Zambryski PC (2017) Current Opinion in Plant Biology 35: 76-83



Formation of primary plasmodesmata

Reticulons (conservative ER proteins) are involved in the induction of membrane curvature in the ER => formation of the plasmotubule of the primary plasmodesmata

Formation of secondary plasmodesmata

Positive regulation: WD-40-repeat protein DSE1 and choline transporter CHER1 (Choline transporter-like 1)
Negative regulation: signals controlled by the RNA helicases ISE1 (mitochondrial) and ISE2 (chloroplast)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