CG920 Genomics

Lesson 6

Protein Interactions in Gene Regulations

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Literature

- Literature sources for Chapter 06:

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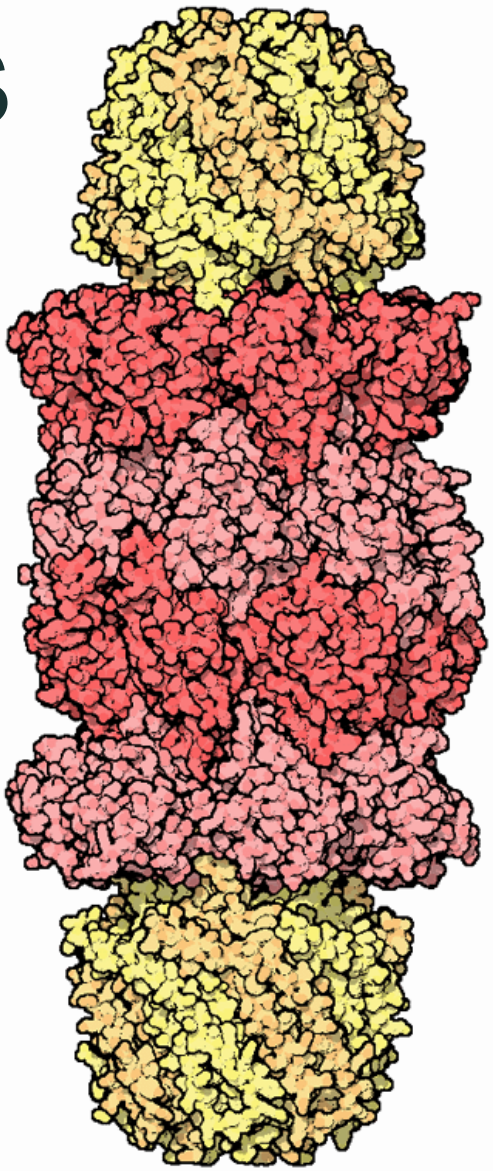
Outline

- Functional importance of the specificic interactions of proteins in the regulation of gene expression
  - Chromatin structure
  - Regulation of transcription
  - mRNA localization
  - Protein stability
  - Signal transduction
- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation
  - The tandem affinity purification (TAP-tag)
  - Yeast two-hybrid assay (Y2H)
  - Bimolecular fluorescence complementation (BiFC)
  - Membrane Recruitment Assay (MeRA)
- Practical use of methods for *in vivo* studies of protein interactions

The importance of protein interactions

- **Functional importance of specific protein interactions**
  - Most of the proteins in the cell exist in the form of complexes which may further interact with each other
    - Proteasome
      - protein complex responsible for the degradation of obsolete proteins in the cell



Once obsolete proteins are tagged with at least four ubiquitin molecules, they are destroyed by proteasomes. Proteasomes are voracious protein shredders, but the destructive machinery is carefully protected so that it can't attack all of the normal proteins in the cell. The proteasome, shown here from PDB entry 1fnt, is shaped like a cylinder, with its active sites sheltered inside the tube. The caps on the ends regulate entry into the destructive chamber, where the protein is chopped into pieces 3 to 23 amino acids long.

Most of the non-lysosomal proteolysis that occurs in eukaryotic cells is performed by a nonspecific and abundant barrel-shaped complex called the 20S proteasome. Substrates access the active sites, which are sequestered in an internal chamber, by traversing a narrow opening (alpha-annulus) that is blocked in the unliganded 20S proteasome by amino-terminal sequences of alpha-subunits. Peptide products probably exit the 20S proteasome through the same opening. 11S regulators (also called PA26 (ref. 4), PA28 (ref. 5) and REG) are heptamers that stimulate 20S proteasome peptidase activity in vitro and may facilitate product release in vivo. Here we report the co-crystal structure of yeast 20S proteasome with the 11S regulator from Trypanosoma brucei (PA26). PA26 carboxy-terminal tails provide binding affinity by inserting into pockets on the 20S proteasome, and PA26 activation loops induce conformational changes in alpha-subunits that open the gate separating the proteasome interior from the intracellular environment. The reduction in processivity expected for an open conformation of the exit gate may explain the role of 11S regulators in the production of ligands for major histocompatibility complex class I molecules. (PDB, Whitby et al., (2000) Nature **408:** 115-120, http://www.rcsb.org/pdb/explore/explore.do?structureId=1fnt).

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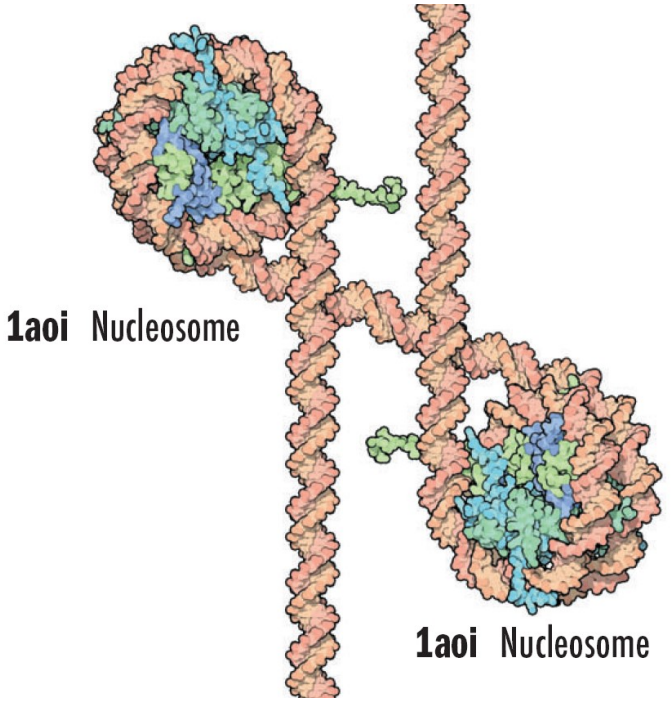
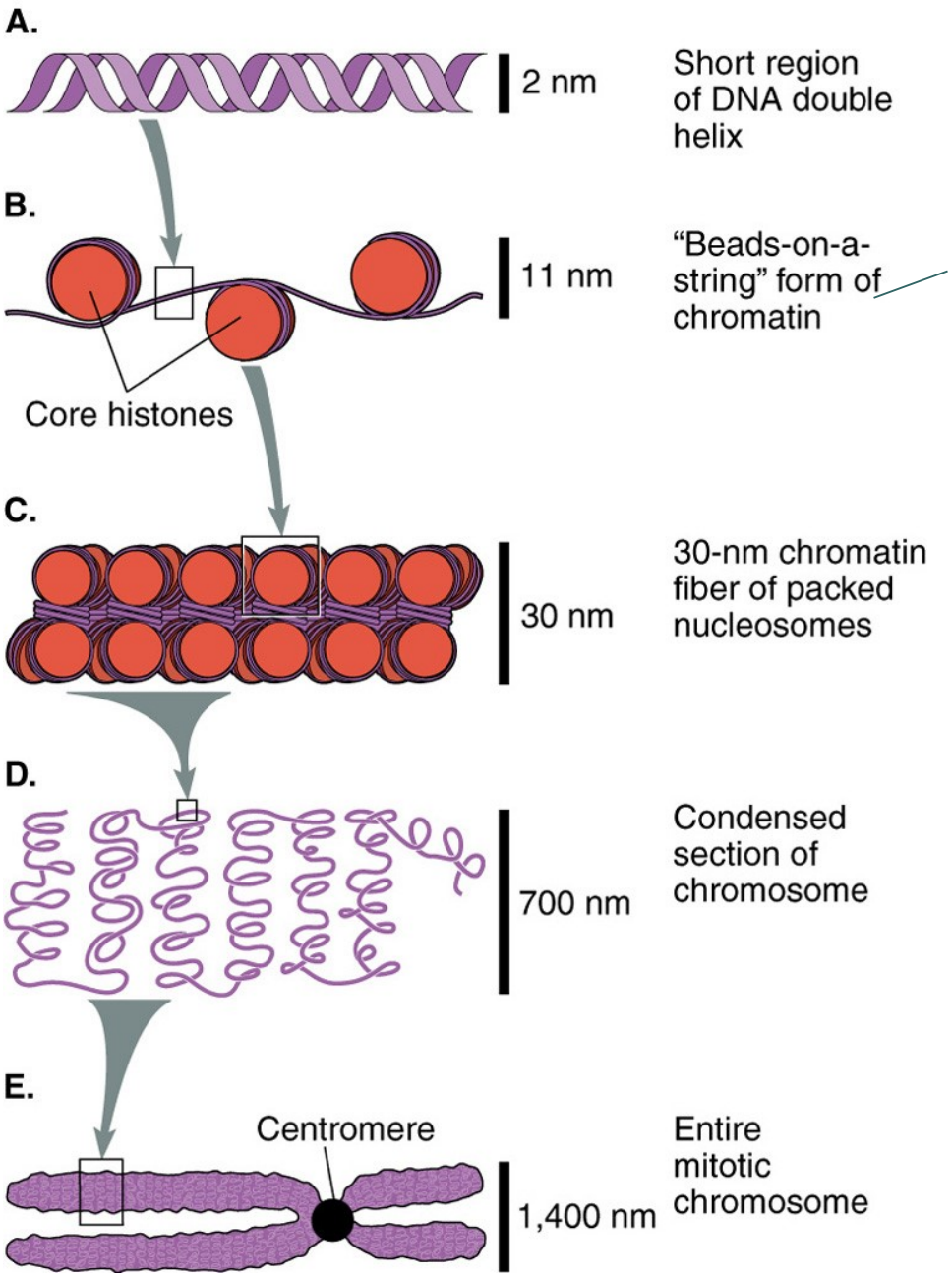
The importance of protein interactions

- Functional importance of specific protein interactions
  - Chromatin structure





Regulation by histone acetyl transferases or histone deacteylases

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Regulation of the chromatin structure represents one of the very basal gene expression regulatory levels. Chromatin is a substrate for DNA-dependent RNA polymerases that transcript the DNA encoded information into the “words and sentences” of RNA.

Regulation of chromatin structure and its accessibility to DNA-dependent RNA polymerases depends on many factors, one of the most important is the regulation of chromatin binding to nucleosomes and chromatin methylation.

Regulation of chromatin interaction with histones, the positively charged proteins forming the core of nucleosomes, is performed via modification of acetylation status of the N-terminal portion of histones, especially histones H3 and H4. This occurs via action of histone acetyl transferases or histone deacteylases.

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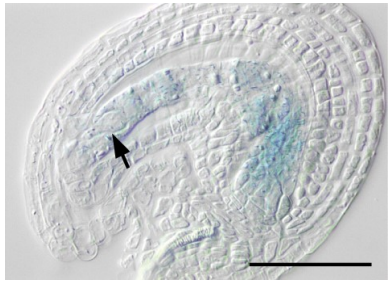
DNA methylation in animals vs. in plants



methylation status

CpG

methylation status

CpG or CpNpG

CpNpNp

Cell-specific methylation allows maintain of tissue-specific gene expression profiles

↓

Imprinting and “cell memory” → **Mechanism of transcriptional regulation by DNA methylation mostly unknown**

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Modification of the chromatin methylation is performed via DNA methyltransferases.
Interestingly, there is difference in the methylation in animals and in plants.
In animals, the methylation takes place mostly on the cytosine that occurs next to guanosine (the sequence is denoted as CpG). In mammals, 60-90% of all CpGs are methylated.
In plants, cytosines are methylated both symmetrically (CpG or CpNpG) and asymmetrically (CpNpNp), where N is any nucleotide.
Methylation status is usually “reset” in the zygote and is reconstituted during development again. E.g. the methylation is very low in the mouse embryo at the blastula stage, however, DNA derived from later stages when organogenesis is initiated is substantially more modified by methylation.
DNA methylation also stably alters the gene expression pattern in cells such that cells can "remember where they have been"; in other words, cells programmed to be pancreatic islets during embryonic development remain pancreatic islets throughout the life of the organism without continuing signals telling them that they need to remain islets.
DNA methylation is involved in the genomic imprinting, i.e. the genes originating from both parents are often diversely methylated, which results into differential expression of parental genomes (for the importance of the imprinting in the parental conflict and epigenetics, see the lecture “Bi0580 Developmental genetics” by prof. Vyskot).
Up to know it is not clear how methylation regulates transcription. Possibly, methylation status affects chromatin configuration or binding general repressor factors.

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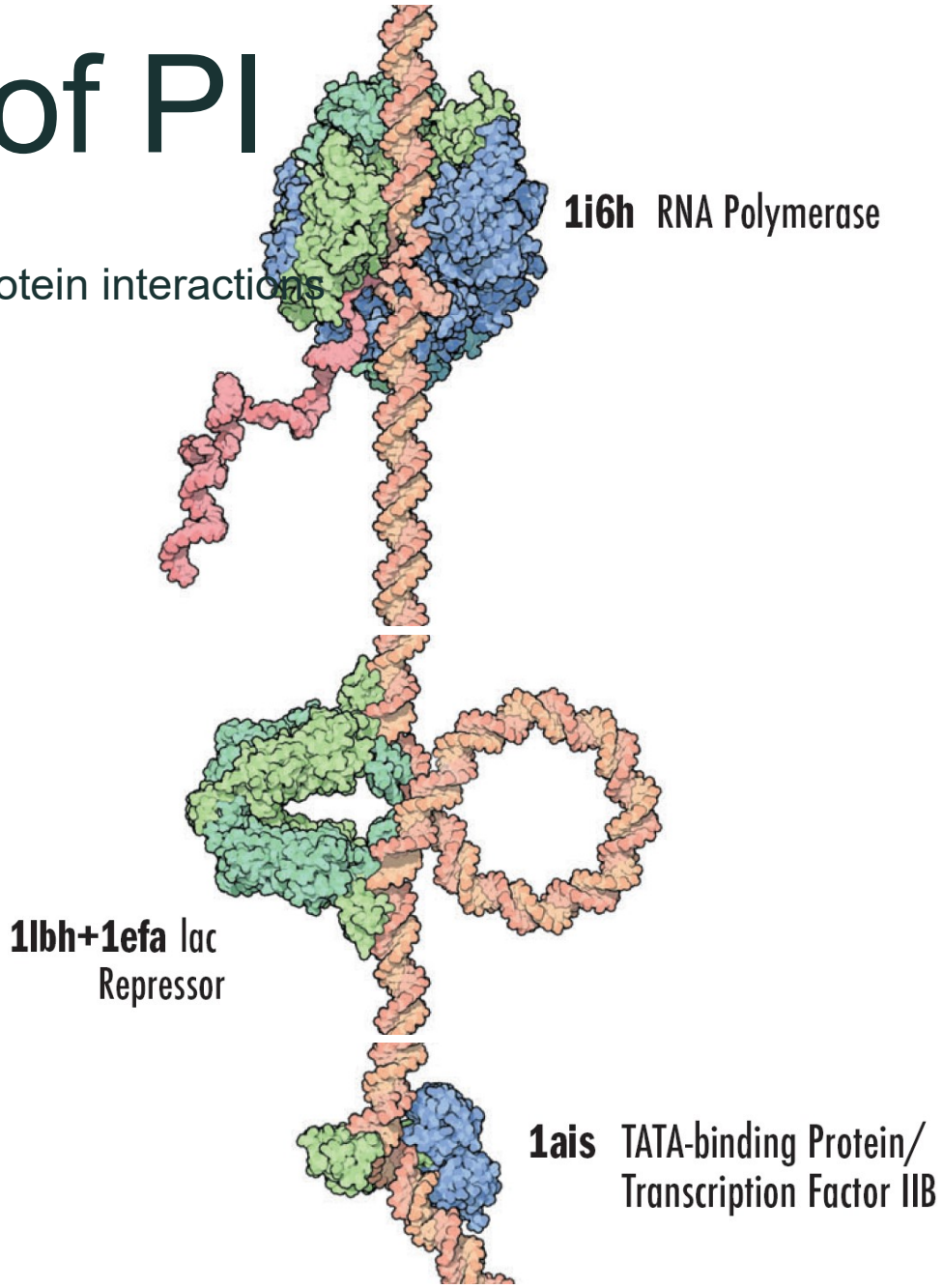
Importance of PI

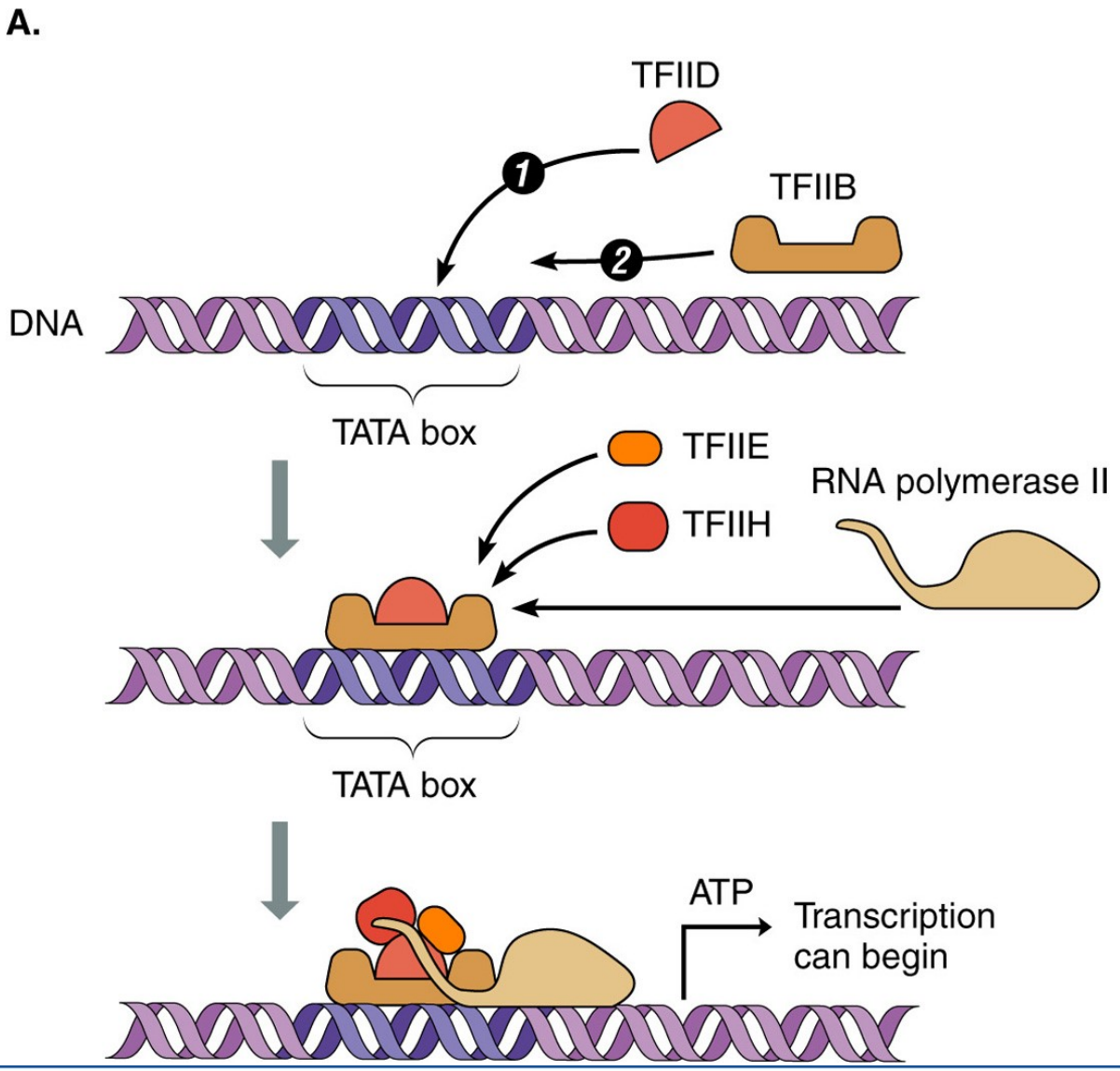
- Functional importance of specific protein interactions
  - Regulation of transcription



Formation of transcription initiation complex

Regulation of transcription occurs via specific interaction of both general and tissue specific transcription factors (TFs) with promoter and/or enhancer sequences.

The scheme above shows simplified subsequent formation of the complex of TFs involved in the regulation of transcription. Interaction of general TFIID with the TATA box induces distortion of the DNA structure (see the next slide).



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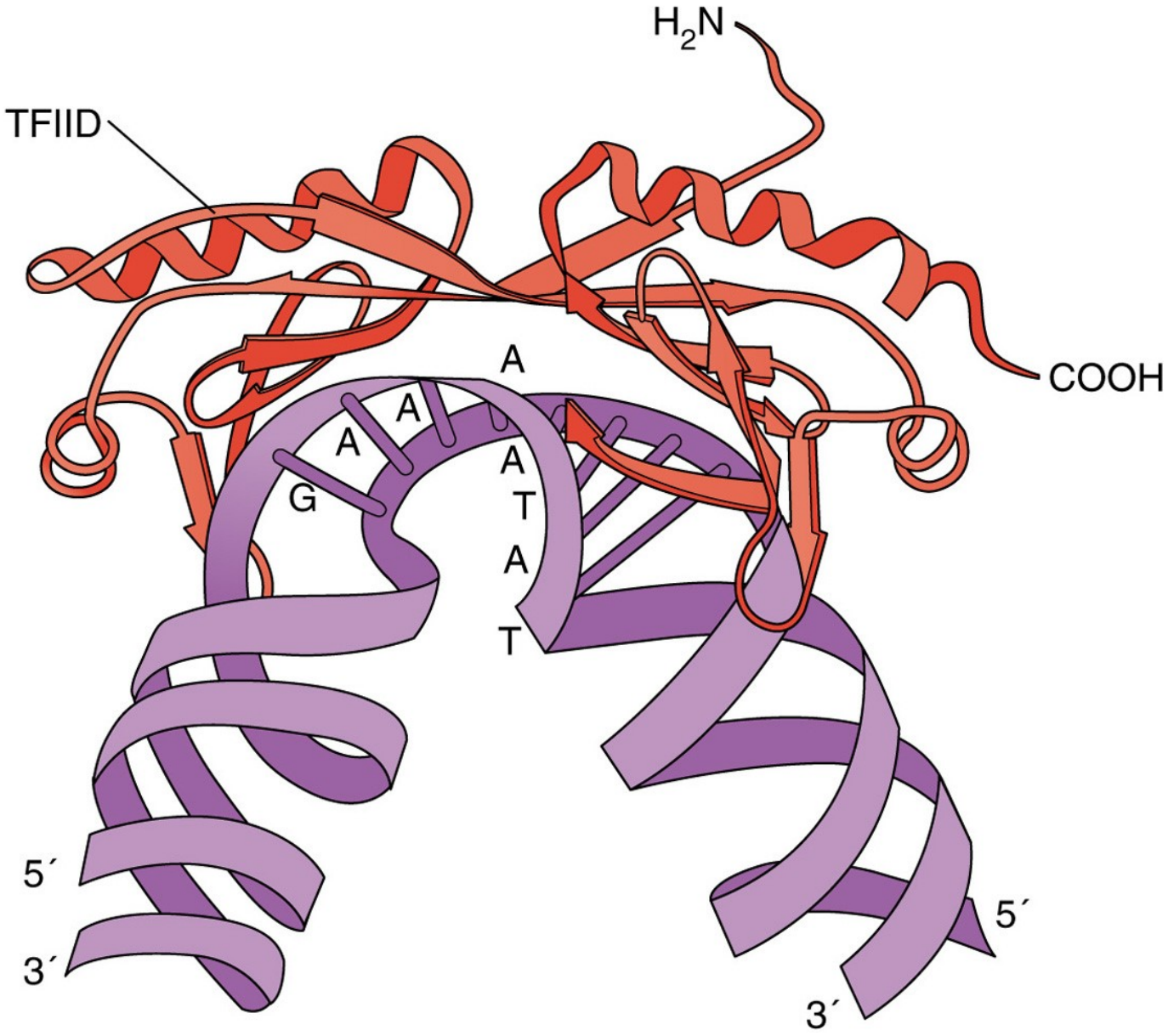
B.

Induction of structural changes upon interaction of TFIID with DNA. This may be important for the assembly of other TFs involved in the formation of transcription initiation complex.

This change of confirmation provides a kind of “signature” that is recognized by other proteins and NA polymerase to recognize the proper binding site. However, there are also TATA box-less promoter, where probably other types of “signatures” occur.



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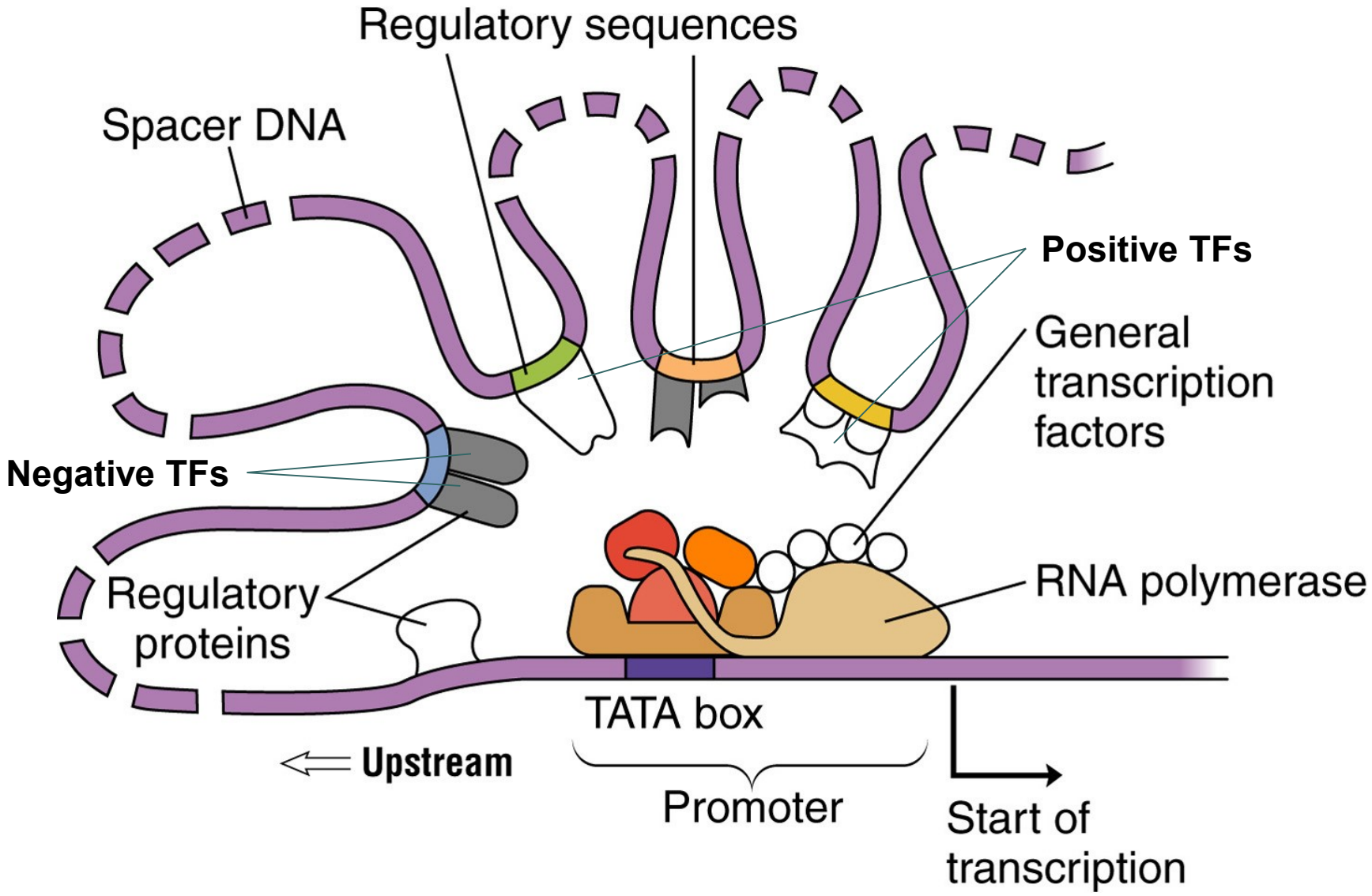
Formation of transcription initiation complex



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The scheme showing the formation of the transcription initiation complex and the interaction of both positive (open symbols) and negative (solid symbols) factors.

These proteins bind to the regulatory sequences that might be hundreds or even thousands of base pairs away from the promoter. These protein interact with each other and with the RNA polymerase, integrating thus many signals into a “yes” or “no” response of the basal promoter, i.e. the region adjacent to the TATA box and recognized by the RNA polymerase.

The individual positive or negative factors are complex and their activity might be regulated by their phosphorylation status or via their interaction with other proteins (i.e. momomeric or dimeric) etc..

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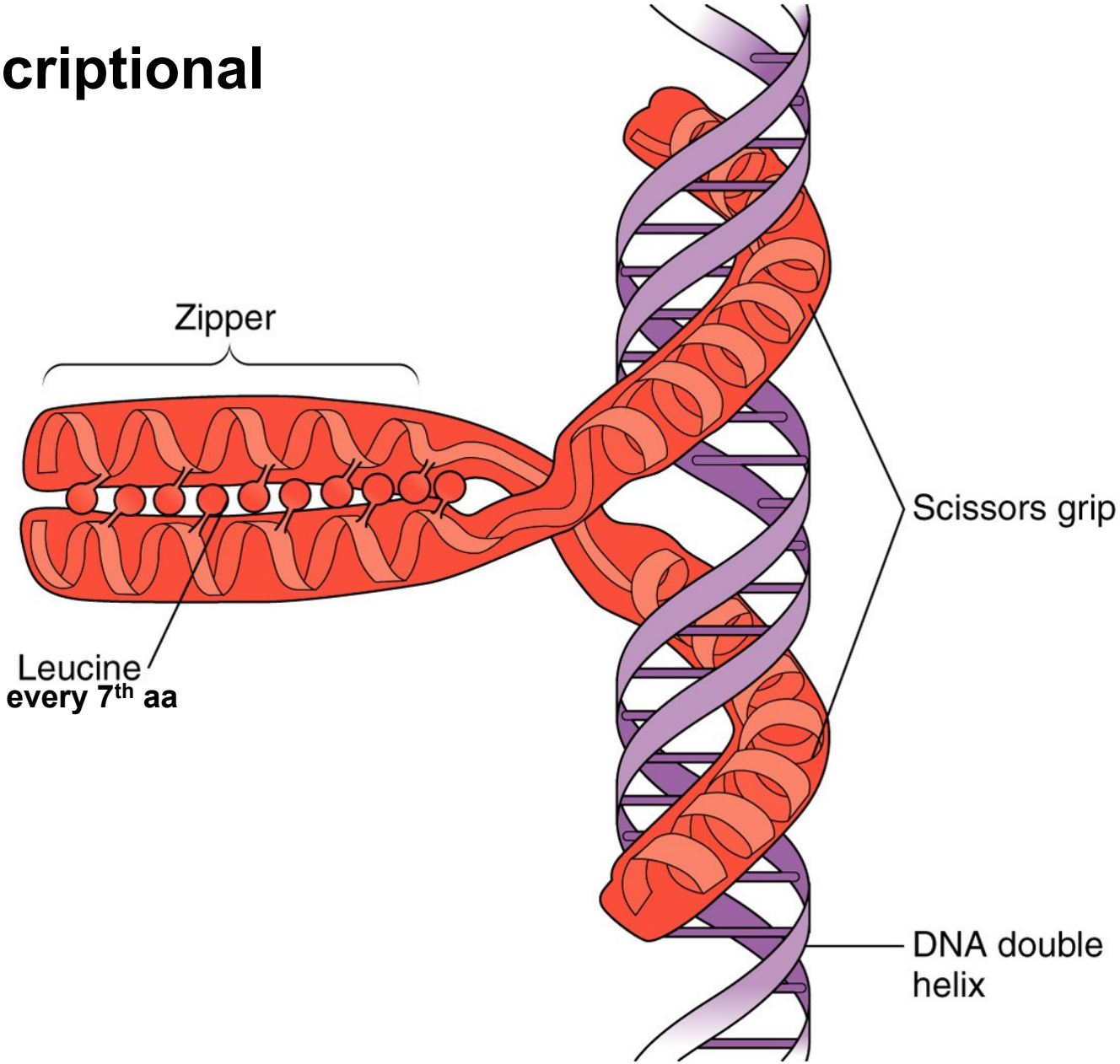
Mechanism of transcriptional regulation by TAFs

Signal recognition

↓

Dimerization →

DNA binding and transcription activation



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There is a whole family of transcription activating factors (TAFs) that interact with signalling molecules, e.g. steroid hormones, thyroid hormones or retinoic acid and in a response to the signal transfer to the nucleus where they regulate transcription.

One of the type of TAF are leucine zipper or bZIP type TAFs. These TAFs are dimeric, with leucine-rich hydrophobic face formed by the Leu that occurs every 7th aa.

That allows the factor to take the proper configuration, which provides the dimer with the ability to bind DNA via charged aa.

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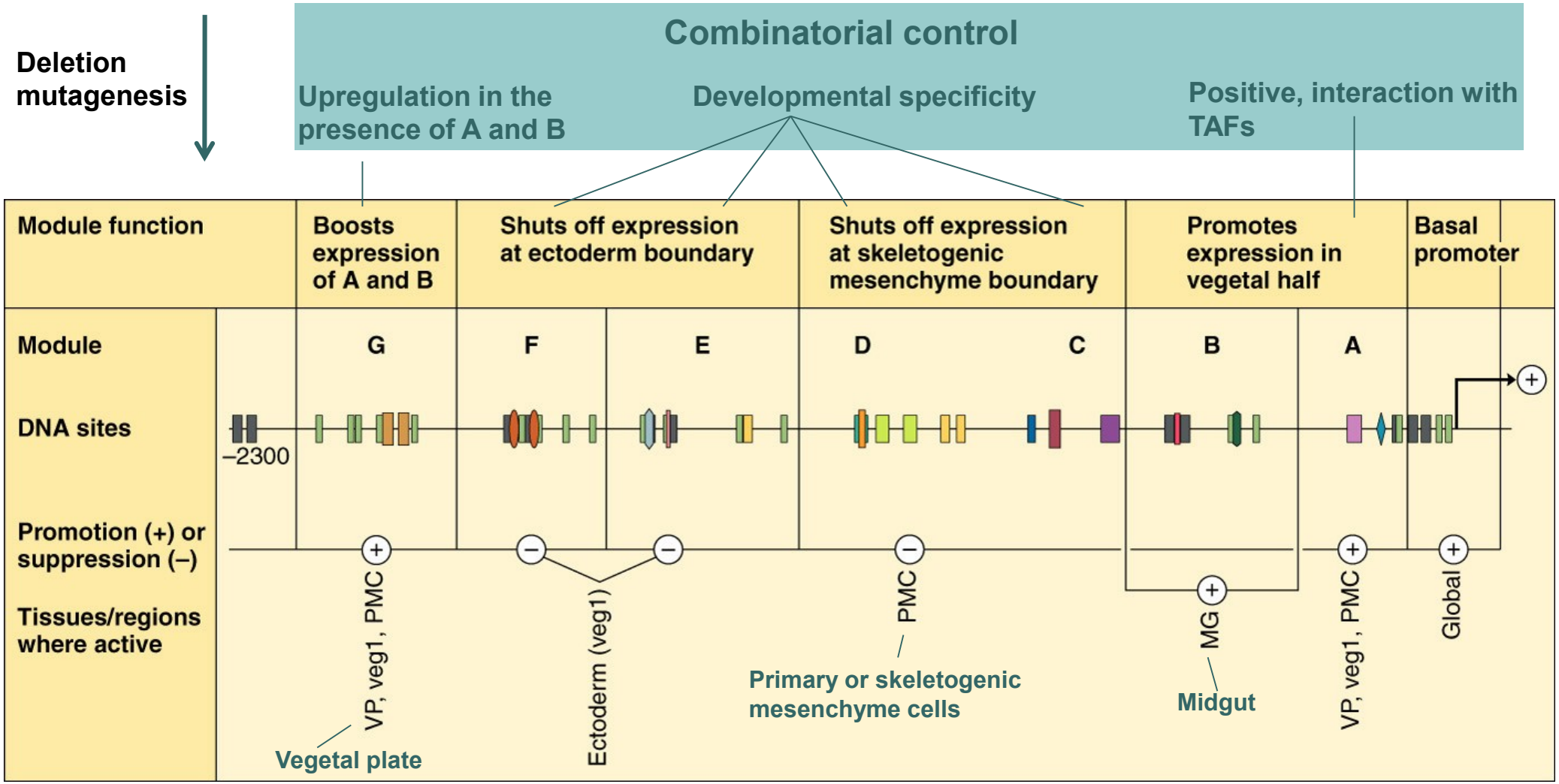
“Microprocessor-like” acting promoters

ProENDO16:REPORTER **(sea urchin)**



An example of the “microprocessor”-like acting promoter is a promoter of the *endo16* gene from the sea urchin.
There have been identified several gene regulatory modules in the *endo16* gene that have positive or negative regulatory role. These modules were identified via formation of deletion mutants of the transcriptional fusions with reporter gene.
The analysis has revealed that the module A has a positive function and must interact with its cognate TAFs for transcription to occur.
Module G enhances the expression when the A and B are active.
C, D, E and F are responsible for the specificity of the expression of endo16 during sea urchin development.
Each of the modules has several protein interaction sites, some of them general, other unique. Site for the protein SpGCF1 is present in many modules and is probably responsible for looping of chromatin, allowing thus bringing of distal regulatory modules close to the basal promoter.
This type of regulation, i.e. based on the different activities of diverse regulatory sequences is sometimes called *combinatorial* and is common for development of many living creatures.
In the combinatorial type of regulations, some modules may act synergistically, some of them antagonistically, some may have both positive and negative roles (e.g. the module B, see the figure). This variability allows very precise and responsive regulations towards changing environmental conditions.

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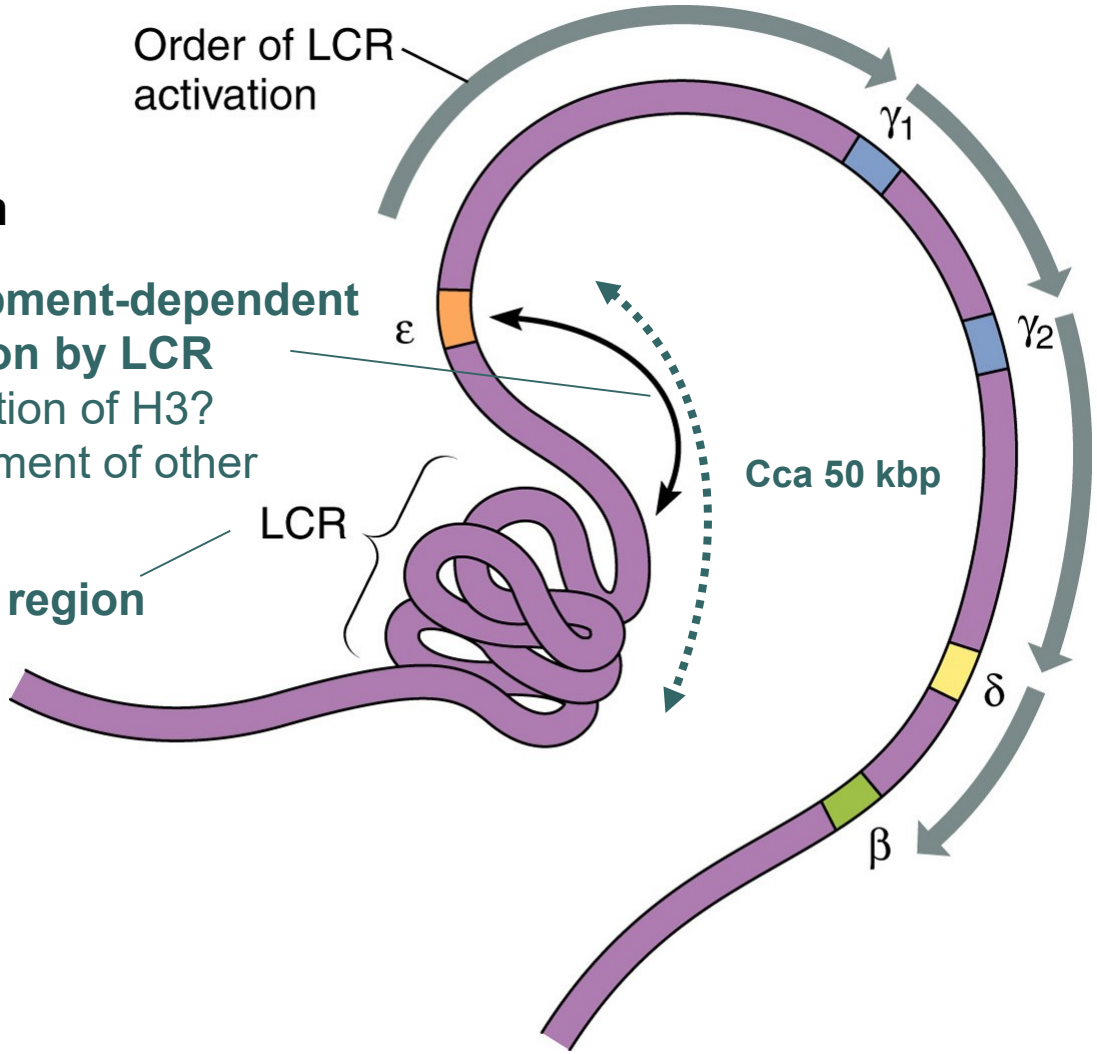
“Microprocessor-like” acting promoters

Regulation of β-globin type of hemoglobin chains expression



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An example of the combinatorial gene regulation is the regulation of β-globin type of hemoglobin chains of humans.

As discussed in the Lesson 5 (course Bi8940 Developmental biology), the type of hemoglobin produced by the fetus changes during development. The hemoglobin present in the liver-produced hemoglobin is composed of two α- and two β-type chains. The β-type hemoglobin chains are of several developmental types, produced by ε, γ1, γ2 and β (in this order). In addition, there is minor adult type of β-type hemoglobin, called δ globin.

The genes for the β-type chains are aligned on the chromosome in the order, in which they are expressed during development (see the figure).

For the expression of individual cell types is distinctive an upstream regulatory sequence called locus control region (LCR). LCR is located about 50 kbp away from the most proximal ε gene.

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The LCR structure is different in erytrocyte precursor cells in comparison to other cells that could be demonstrated by the changes in the sensitivity to low concentrations of DNase, suggesting low amount of nucleosomes bound.

For the expression of the particular genes, the interaction of their regulatory sequences with LCRs is necessary. Because of LCR can interact only with one regulatory sequence at a time, only one type of genes for the particular β-type chain is activated (the first interaction of LCR with ε gene, which is later in development replaced by the other one, is shown by the double-headed arrow).

The underlying molecular mechanisms of the specific pattern of the LCR movement from the most proximal towards the most distal gene cannot be satisfactory explained.

Probably, acetylation of H3 histones might play a role and possibly, other genes outside of the β-type chain family are involved in the regulation of LCR activity. That seems to be confirmed by the identification of other human genes with similar structure, suggesting common regulatory mechanisms via LCRs.

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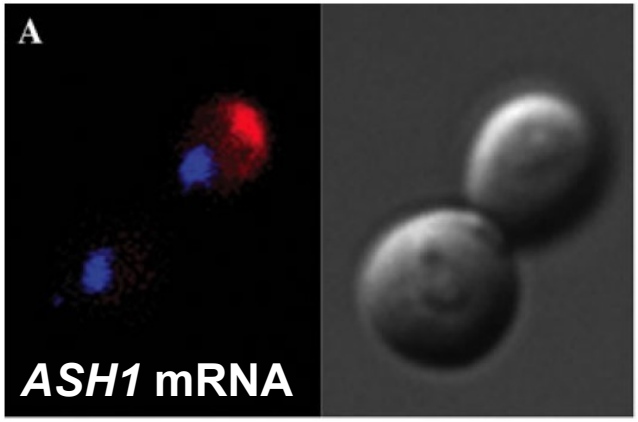
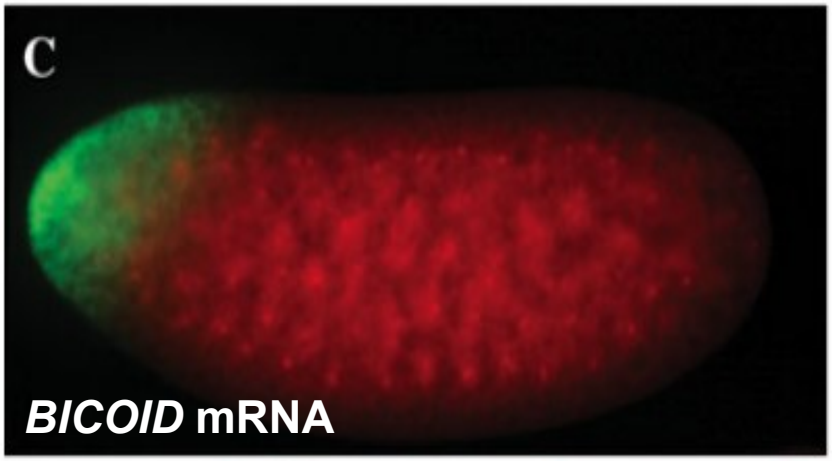
Importance of PI

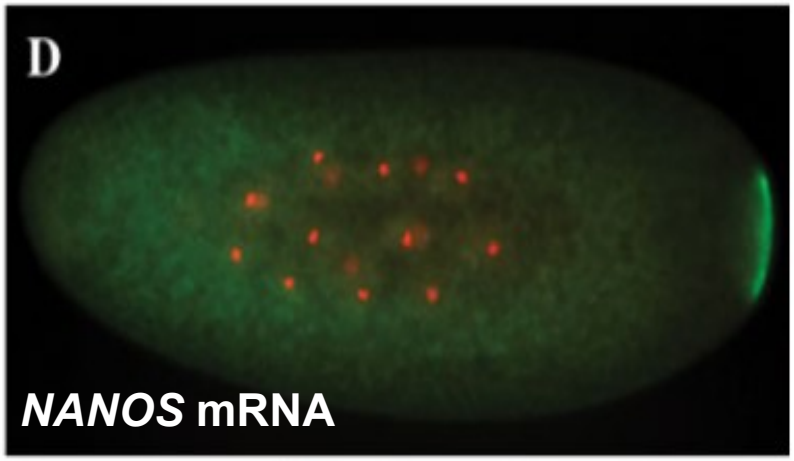
- Functional importance of specific protein interactions
  - mRNA localization

mRNA localization

- **Importance of mRNA localization**
  - Spatiotemporal localization of gene product (protein)
    - Asymmetric cell division during development
    - Polarization of embryo







Shahbabian and Chartrand, 2012

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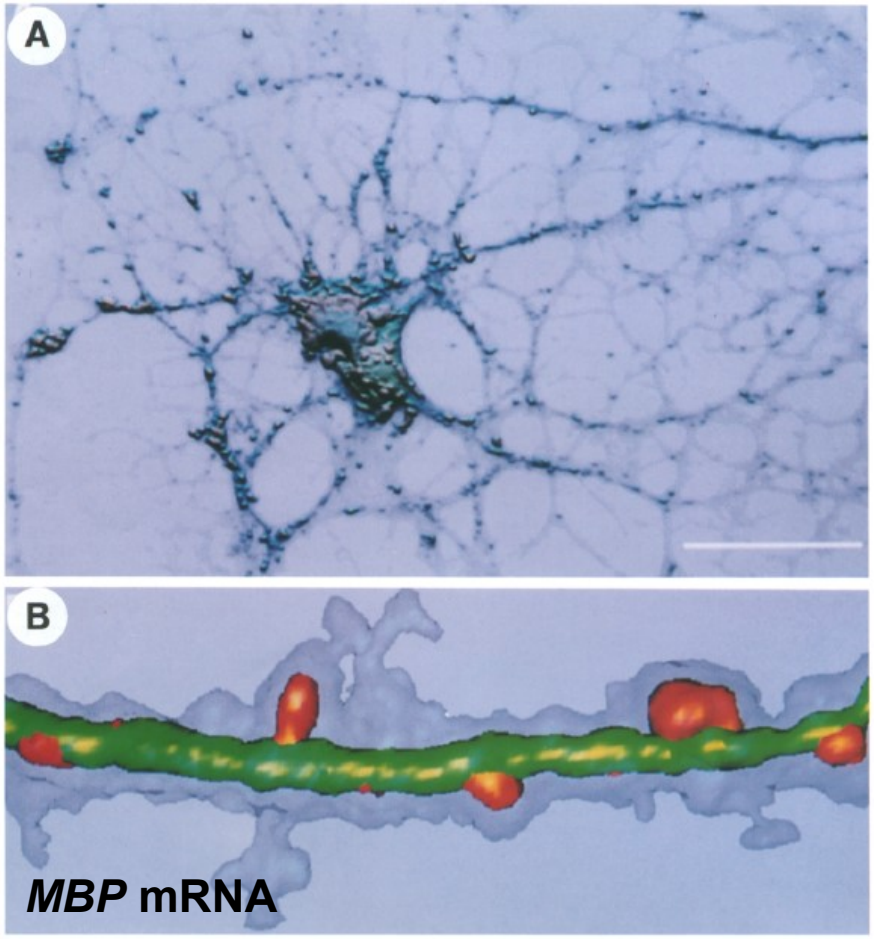
mRNA localization

- **Role of mRNA localization**
  - Downregulation of expression of potentially toxic proteins
    - Localization of expression of MBP into myelination regions of nerve cells



Ainger et al., 1993

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Myelin basic protein (MBP) is a protein believed to be important in the process of myelination of nerves in the nervous system.

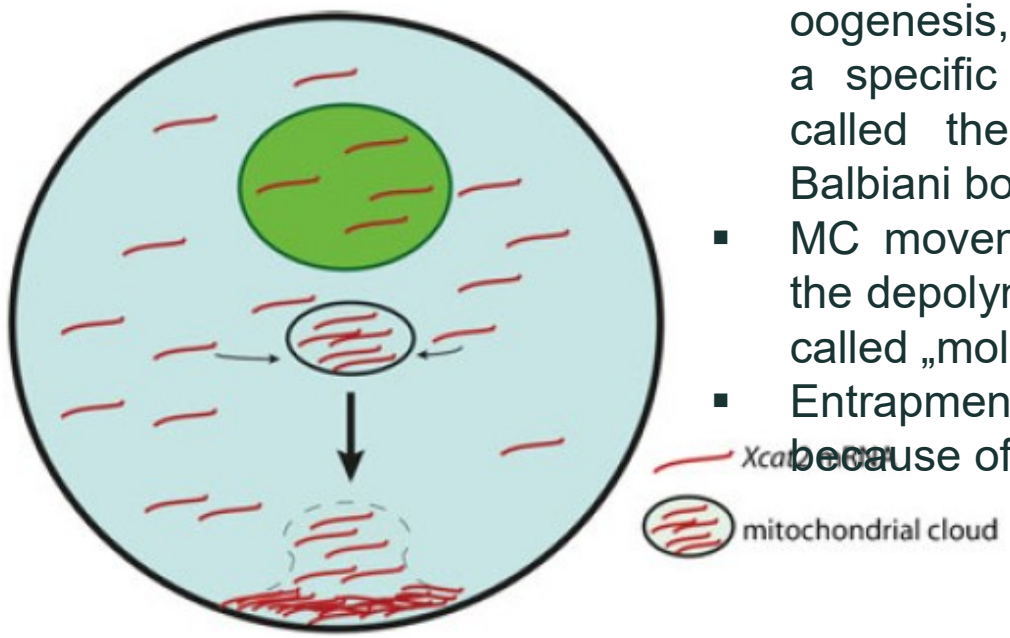
The images show localization of mRNA for MBP. Digoxigenin-labeled MBP RNA was microinjected into mouse oligodendrocytes growing in primary culture. The injected RNA appeared as small granules which were present throughout the cytoplasm and processes, and was also found dispersed in the peripheral membranes of the cell.

To analyze the three dimensional distribution of microinjected labeled MBP mRNA throughout the cell, consecutive optical sections through a single oligodendrocyte were collected, reconstructed, and visualized using volume rendering (Fig. A) or isosurface rendering (Fig. B) techniques. An oligodendrocyte microinjected with MBP mRNA, visualized by volume rendering is shown in Fig. A. RNA granules were observed throughout the perikaryon and in some, but not all, processes. The granules in the perikaryon and in the processes appeared to be equivalent in size. In some regions the granules in the processes were aligned in tracks. Although not apparent from this image, the nucleus was devoid of granules.

mRNA localization

Mechanisms

- **Diffusion-entrapment mechanism of mRNA**



Shahbabian and Chartrand, 2012

- During the early stages of *Xenopus* oogenesis, *Xcat-2* mRNA is restricted to a specific structure in the cytoplasm called the mitochondrial cloud (MC, Balbiani body)
- MC movement is partly dependent on the depolymerization of microtubuls (so-called „molecular motor“)
- Entrapment on the vegetal pole occurrs because of interaction of MC and ER

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Another well studied example of the diffusion-entrapment mechanism is the Xenopus Xcat-2 mRNA, which encodes a Nos related zinc-finger RNA-binding protein.
During the early stages of Xenopus oogenesis, Xcat-2 mRNA is restricted to a specific structure in the cytoplasm called the mitochondrial cloud (MC). The mitochondrial cloud, also called Balbiani body, consists mostly of mitochondria and small vesicles, and is the source of germinal granule material [32]. The movement of the MC in the cytoplasm results in the localization of the Xcat-2 mRNA at the vegetal cortex (Shahbabian and Chartrand, 2012).

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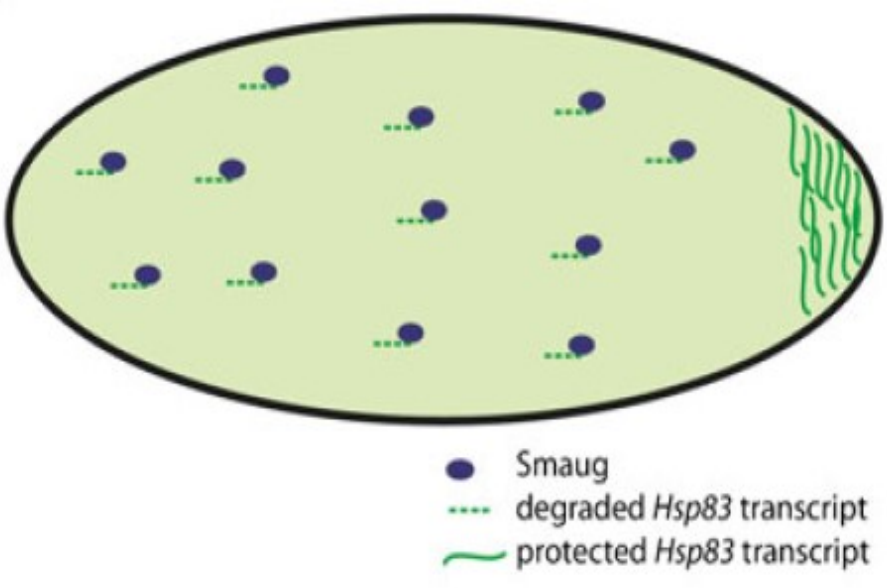
mRNA localization

Mechanisms

- **Localized mRNA degradation**
  - During embryogenesis in *Drosophila m.* *Hsp83* mRNA is localized at the posterior pole of embryo, similarly to *NANOS* mRNA
  - *Hsp83* mRNA is localized in the whole embryo, however, it is destabilized by cis elements both in 3'UTR (HDE) and in coding region (HIE).

Shahbabian and Chartrand, 2012



- HIE elements are recognized by SMAUG protein, which mediates binding of degradation complex CCR4/POP2/NOT
- In the posterior pole the *Hsp83* mRNA is protected from the effects of SMAUG by the so-called HPE element in 3'UTR; mechanism of this protection has been still unknown

Localized stabilization of a transcript is another mechanism by which an mRNA can be subcellularly targeted. In this case, an mRNA is rapidly degraded in most parts of the cell, but it is protected from degradation at a specific location. The hsp83 mRNA, which encodes a heat shock protein in Drosophila, is a well-characterized example of this kind of localization (Fig. 2b). This transcript is localized at the posterior pole of the early Drosophila embryo by the selective stabilization of the mRNA at the posterior pole and degradation of the transcript elsewhere in the cytoplasm.

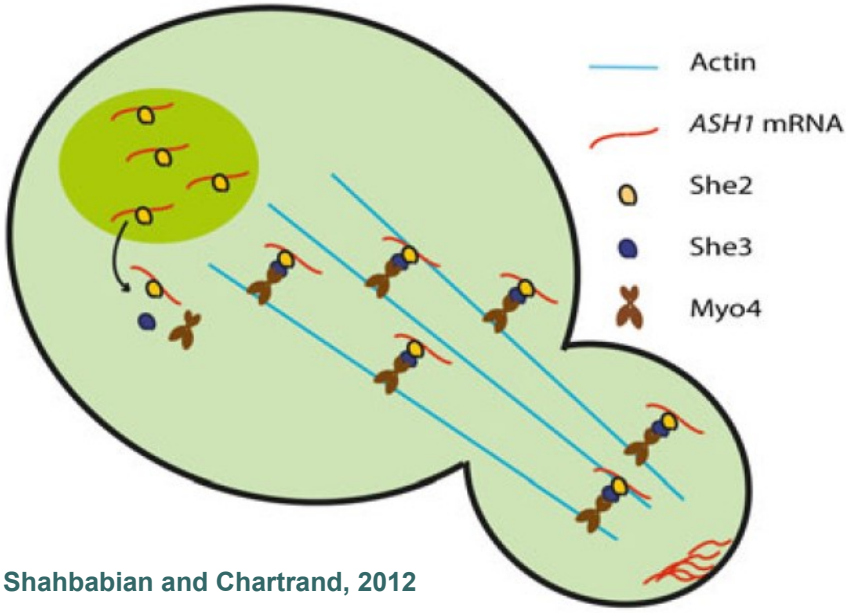
The level of hsp83 mRNA, which is a maternally encoded transcript, decreases more rapidly in embryos than in unfertilized eggs, which suggests that two separate mechanisms control the stability of this transcript [38]. These two independent pathways, which are called ‘‘maternal’’ and ‘‘zygotic’’ pathways, use maternally and embryonic encoded proteins, respectively, to degrade the hsp83 transcript [38]. By analyzing the 3’UTR of hsp83 mRNA, a region from nucleotides 253–349 was identified as the Hsp83 degradation element (HDE), which directs the destabilization of this mRNA in unfertilized eggs. However, this region has no effect in the zygotic degradation pathway, and transcripts without the HDE domain are subject to degradation by the embryonic degradation machinery [38].

The hsp83 ORF has also been shown to affect the stability of the transcript. A region at the 3' end of the ORF, which comprises 615 nucleotides, has been found to be responsible for this destabilization, and was consequently called Hsp38 instability element (HIE) [39]. This region, which has the major effect in the destabilization of the transcript, functions together with the HDE for complete degradation. The HIE domain contains six stem-loop structures that are recognized by the maternally encoded RNA-binding protein Smaug [39, 40]. It was shown that in Smaug mutants, degradation and thus localization of hsp83 mRNA are impaired. Smaug recruits the CCR4/POP2/NOT deadenylase complex, triggering deadenylation and thus degradation of the hsp83 transcript [40]. Although Smaug is present throughout the pole plasm, the hsp83 mRNA is protected from Smaug action at the posterior pole. This protection is related to a 57 nt region in the 3'UTR (nucleotides 351–407) downstream of HDE, which is called HPE (Hsp83 protection element). HPE is sufficient to confer stability to an unstable transcript at the pole plasm [40]. The mechanism by which this domain functions is not clear, and may include interaction of trans-acting factors that block the availability of the transcript to Smaug (Shahbabian and Chartrand, 2012).

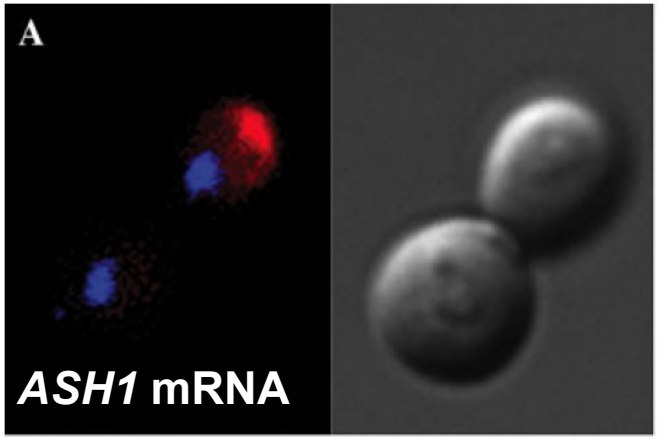
mRNA localization

Mechanisms

- **Active transport of mRNA**
  - ASH1 is represor of the *HO* endonuclease in *S. cereviseae;* inhibition of HO results in inhibition of mating-type switching in daughter cells
  - *ASH1* mRNA is actively transported by „molecular motors“ associated with actin



Shahbabian and Chartrand, 2012



Shahbabian and Chartrand, 2012

- *ASH1* mRNA contains 4 *cis* elements (3 in the coding sequence and 1 in the 3’UTR), which are recognized by RNA-binding protein SHE2
- SHE2 interacts with SHE3, an adaptor protein, which links SHE2 to the molecular motor MYO4, which then binds to actin and allows transport of *ASH1* mRNA into the daughter cell

Localization of ASH1 mRNA is essential for the asymmetric distribution of Ash1, which acts as a transcriptional repressor of the HO endonuclease and results in inhibition of mating-type switching in daughter cells [88, 89]. The ASH1 mRNA contains four localization elements, three in the coding sequence (E1, E2A, and E2B) and one overlapping the end of the coding sequence and the 3'UTR (E3) [25, 90]. While the presence of these four elements leads to an optimal localization, deletion analysis revealed that each element is sufficient for localization of a reporter mRNA to the bud.

When each of these elements was inserted in multiple copies in the 30UTR, the new constructs showed nearly normal localization. However, for these mRNAs, the asymmetric distribution of Ash1 was impaired, suggesting that the position of these elements is important for Ash1 sorting but not for ASH1 mRNA localization [91]. Although the primary sequences of the four ASH1 localization elements are different, they all fold into a stem-loop structure that contains a few conserved nucleotides [92, 93]. All four elements interact with the same RNA binding protein called She2, which is involved in the localization of bud-localized mRNAs in S. cerevisiae.

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She2 forms a tetramer under physiological conditions, and mutations that disrupt this tetrameric state abolish its RNA-binding capacity and impair She2-dependent localization to the bud tip [94]. She2 interacts directly with the C-terminal domain of She3, an adaptor protein that links the She2–mRNA complex to the molecular motor Myo4 (Fig. 2c) [55, 95, 96]. Recent evidence also suggests that She3, besides its role in connecting the She2–RNA complex to Myo4, is itself able to bind RNA and acts synergistically with She2 to increase the affinity and specificity of RNA binding [97].

Recent studies on Myo4 helped to explain why multiple localization elements are required for proper ASH1 mRNA localization. Myo4 is a class V myosin whose main function is the transport of mRNAs to the bud tip using actin filaments [98–100]. Myo4, unlike other type V myosins, is a nonprocessive monomer in vivo, but it becomes processive when present in the form of oligomers [101, 102]. Purification of the localization complex associated with a single localization element revealed that multiple copies of Myo4 are associated with this RNA [103]. Moreover, increasing the number of Myo4 attached to the ASH1 mRNA increased the efficiency of localization of this transcript. These results suggest that each localization element interacts with higher order protein complexes in which a She2 tetramer may recruit multiple copies of Myo4, thus ensuring a continuous and processive movement of the mRNP complex into the bud. Moreover, it is possible that a She2 tetramer binds simultaneously to the localization elements of a single transcript or, alternatively, to those of different mRNAs. This would bring multiple mRNAs together within a single complex in which several Myo4 molecules modulate their transport to the bud tip (Shahbabian and Chartrand, 2012).

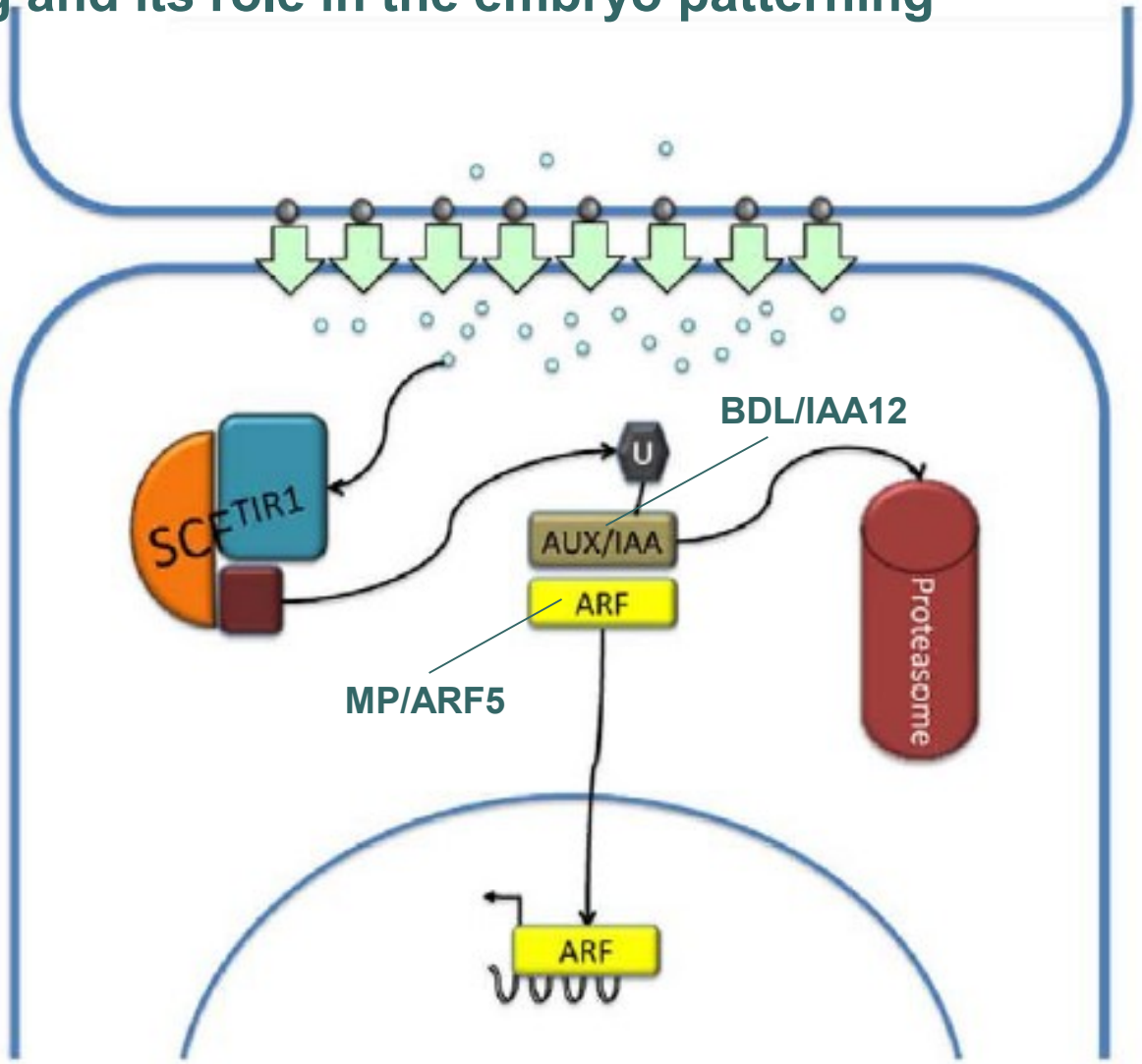
Importance of PI

- Functional importance of specific protein interactions
  - hnRNA splicing

Importance of PI

- Functional importance of specific protein interactions
  - Protein stability

Auxin signalling and its role in the embryo patterning



Capron et al., *Arabidopsis Book* (2009)

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Scheme of the auxin signaling pathway as an example of the role of protein stabilization leading to regulation of gene expression.
Under low intracellulr auxin concentrations, the transcription activators of auxin-regulated genes, which are called auxin responsive factors (ARFs), are in a complex with negative regulators of transcription, so called AUX/|IAA proteins. In the complex, Arfs can not activate transcription.
After auxin is imported into the cell, it binds to the TIR1 protein, that allows interaction with AUX/IAA-ARF complex and targets AUX/IAA protein for the degradation via proteosome.
That allows ARFs to enter nucleus and activate trancription of auxin-induced genes.

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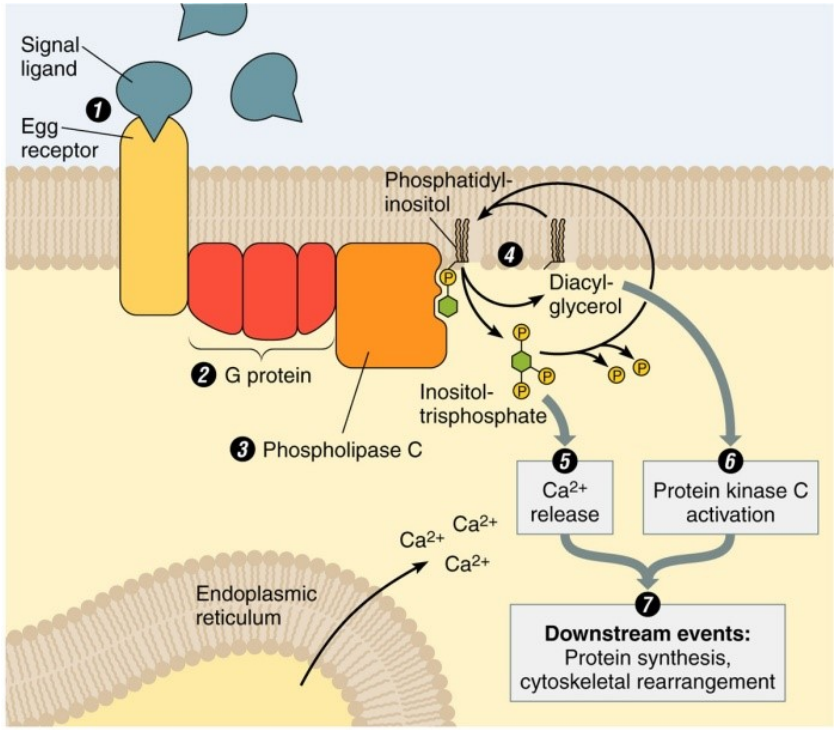
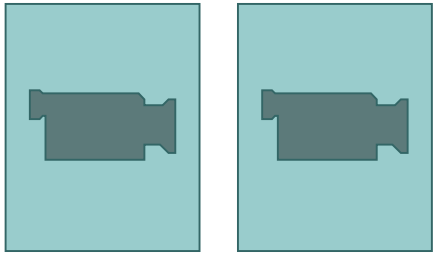
Importance of PI

- Functional importance of specific protein interactions
  - Signal transduction

PI and signal transduction

- **PI and signal transduction**
  - through G protein and phospholipase C
  - Signalling cascades using cAMP





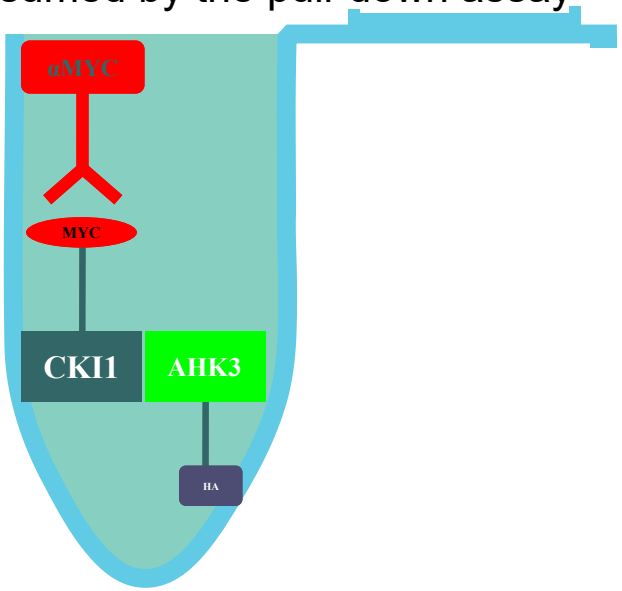
Outline

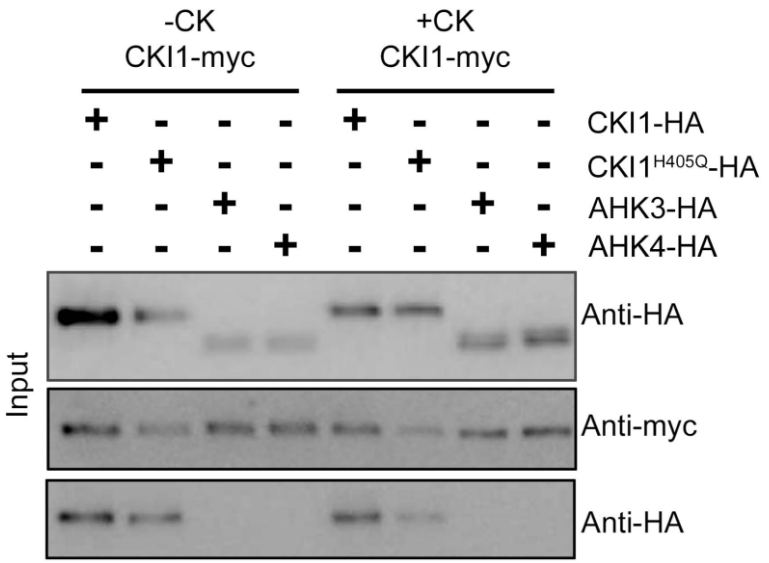
- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation

PI *in vivo*

Co-immunoprecipitation

- Based on the isolation of protein complexes using antibodies recognizing one of the interacting proteins
- The principle of co-immunoprecipitation is used in a method, which confirms interactions of proteins, which is already assumed by the pull-down assay





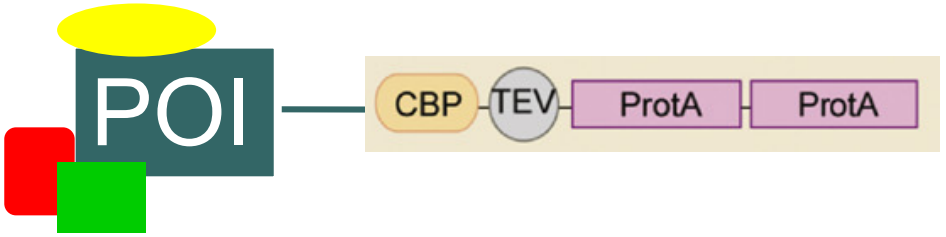
Outline

- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation
  - The tandem affinity purification (TAP-tag)

PI *in vivo*

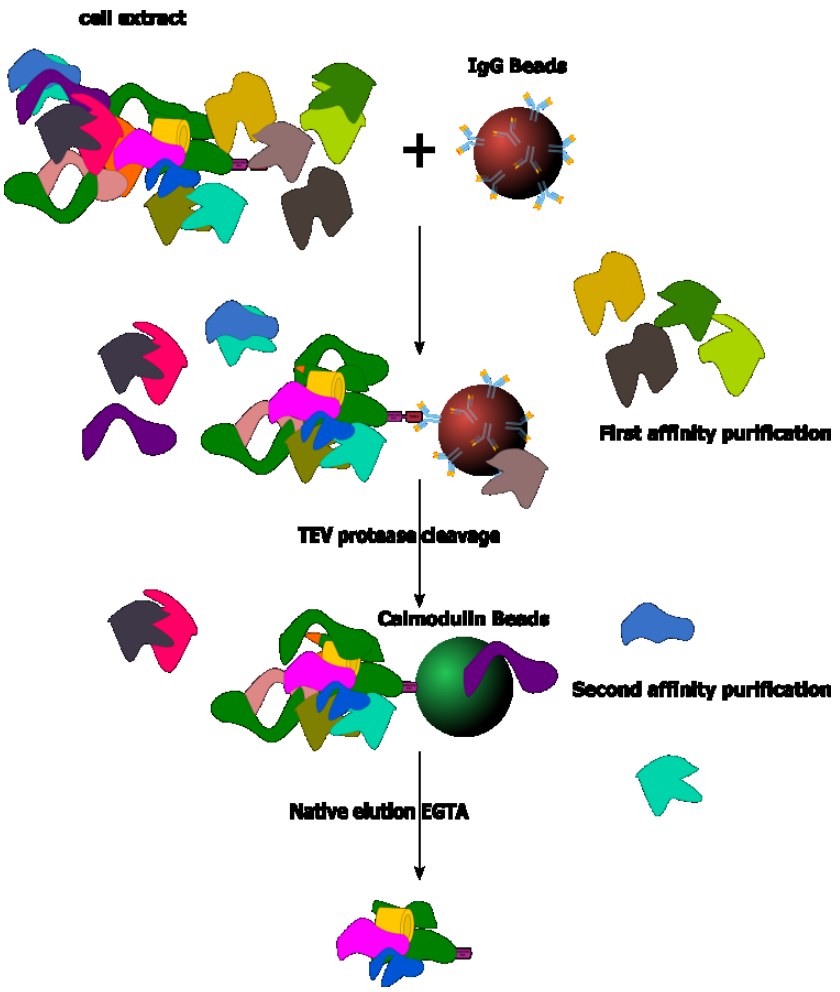
Tandem affinity purification (TAP-tag)

- Isolation of protein complexes using recombinant proteins fused with two different binding domains



  - calmodulin-binding protein (CBP)
  - IgG binding domains of protein A (ProtA)
  - TEV (tobacco etch virus) protease recognition site

- Isolated protein complexes are divided using 1D ELFO and then identified by MS
- Advantage: using two independent protein domains for affinity purification -> therefore high specifity



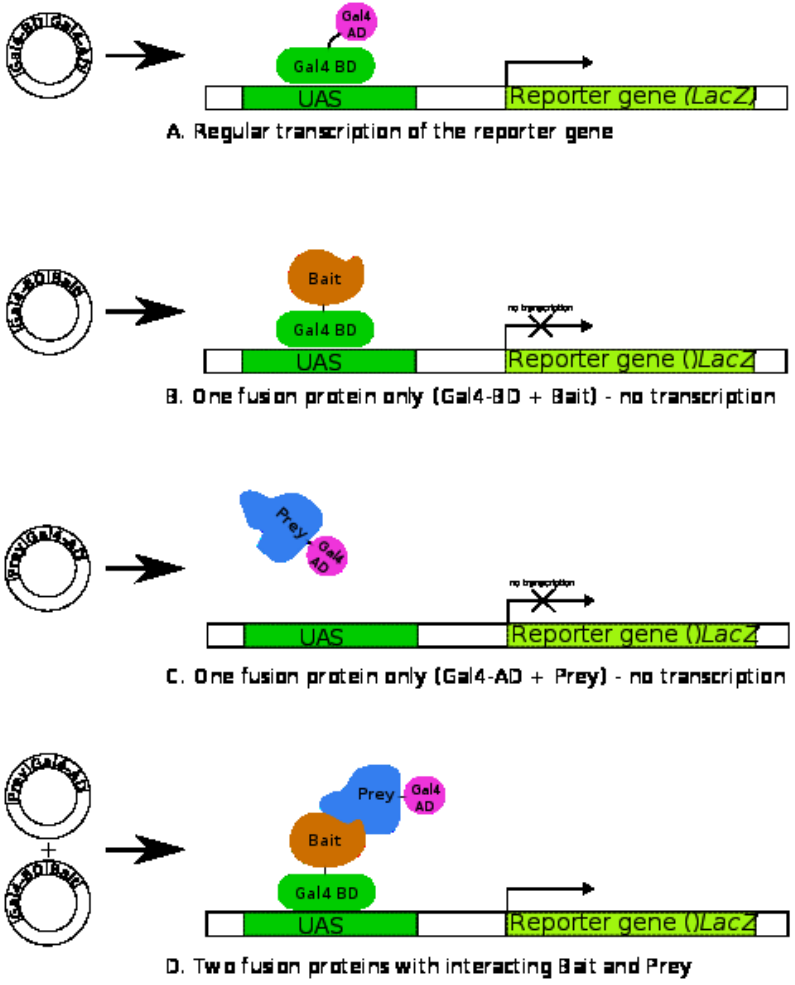
Outline

- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation
  - The tandem affinity purification (TAP-tag)
  - Yeast two-hybrid assay (Y2H)

PI *in vivo*

Yeast two-hybrid assay (Y2H)

- Isolation of protein complexes using recombinant proteins, each fused to a part of Gal4 transcription factor
  - One of the proteins (bait) fused to DNA-binding domain of Gal4 (Gal4-BD)
  - The other protein (prey) fused to activation domain of Gal4 (Gal4-AD)
- Protein interactions enable reconstitution of binding domains with activation domain and triggers the expression of a reporter gene
  - Visual detection (blue color, LacZ)
  - Auxotrophic selection (growth on medium lacking histidine, His)
- Method used for searching interaction partners in expression libraries of individual organisms



Outline

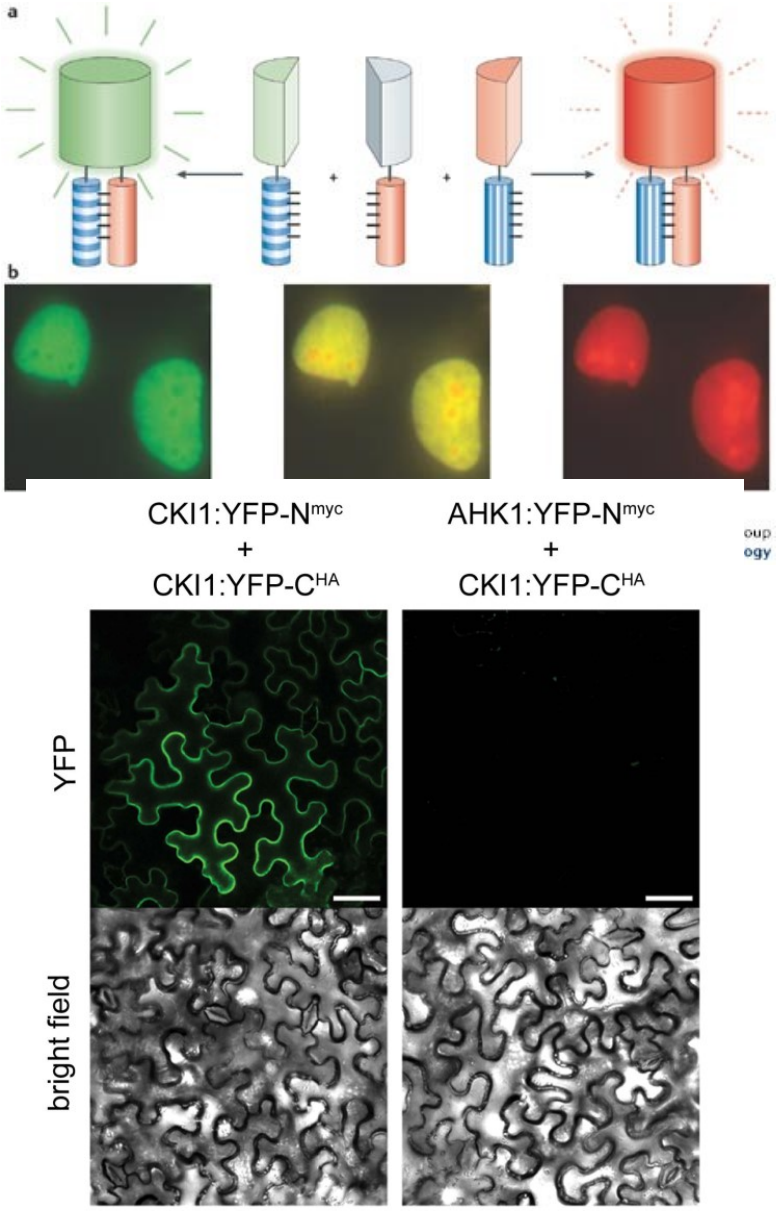
- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation
  - The tandem affinity purification (TAP-tag)
  - Yeast two-hybrid assay (Y2H)
  - Bimolecular fluorescence complementation (BiFC)

PI *in vivo*

Bimolecular fluorescence complementation (BiFC)

- Protein interaction is detected by reassociation of the fluorescent protein
  - Each of the potential interaction partners is fused to one of the subunits of the fluorescent protein, e.g. YFP
  - In case of interaction, the fluorescence reappears

- Apart from identification of the interaction, this method allows you to locate the interaction in the cell



Outline

- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation
  - The tandem affinity purification (TAP-tag)
  - Yeast two-hybrid assay (Y2H)
  - Bimolecular fluorescence complementation (BiFC)
  - Membrane Recruitment Assay (MeRA)

PI *in vivo*

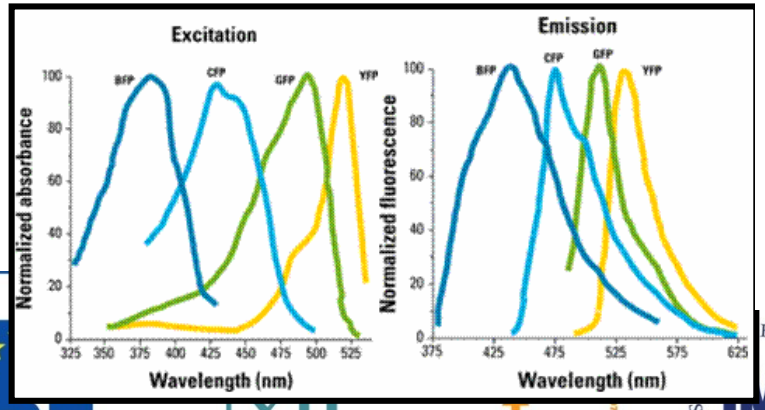
Membrane Recruitment Assay (MeRA)

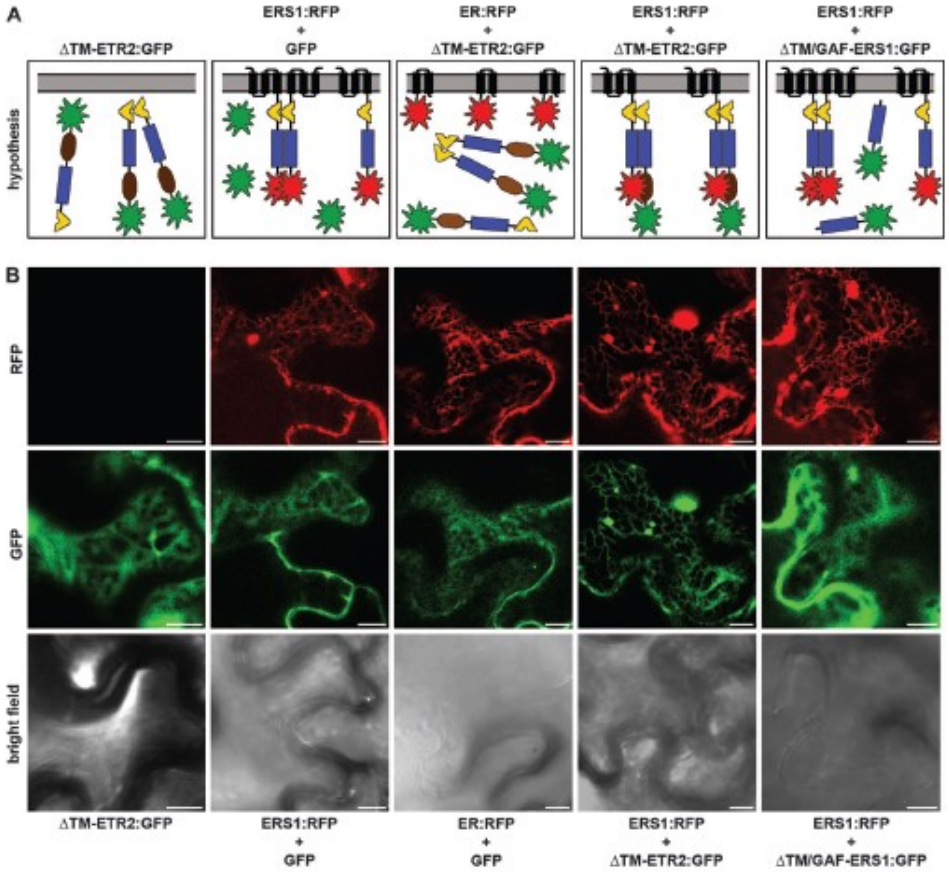
- Method for identification of interactions of cytoplasmic proteins with the membrane proteins

Membrane protein is fused with a fluorescecnt protein

Potential interaction partner is fused with another fluorescent protein with different emission spectra

- In case of interaction the localization of the cytoplasmic protein is changed – it is colocalized on the membrane with the membrane protein





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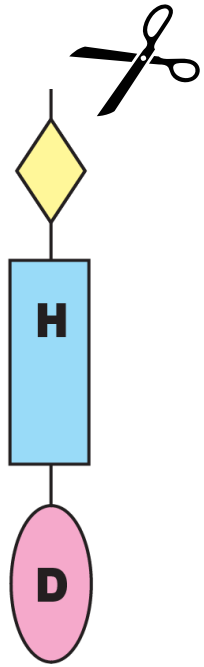
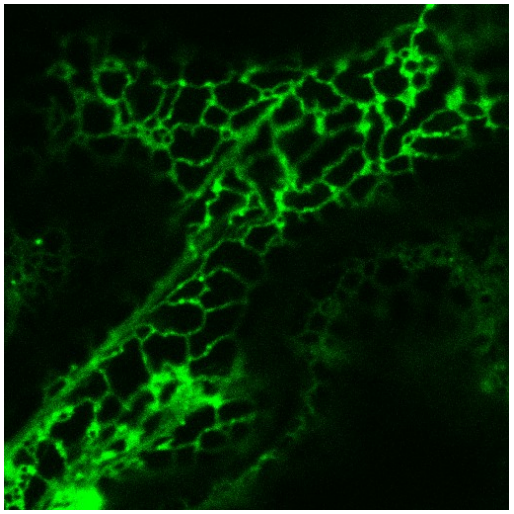
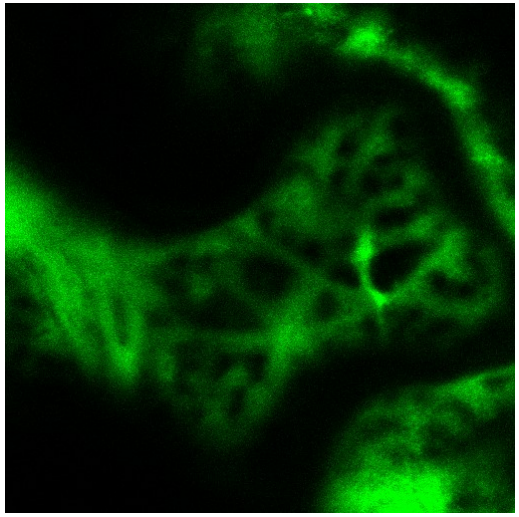
PI *in vivo*

Membrane Recruitment Assay (MeRA)







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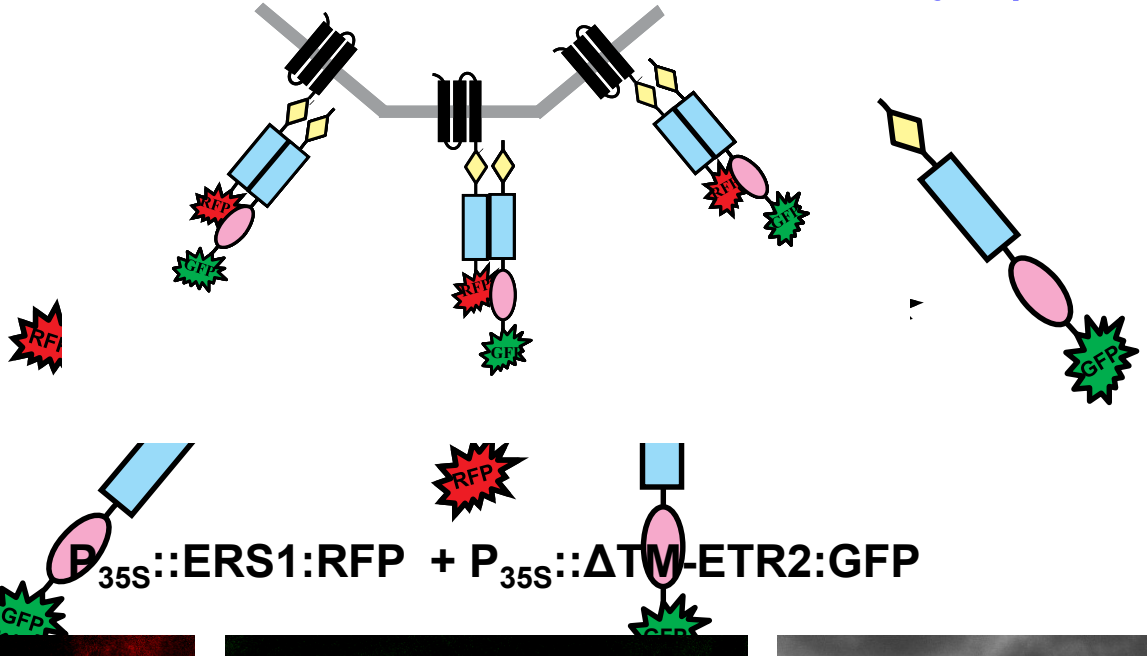
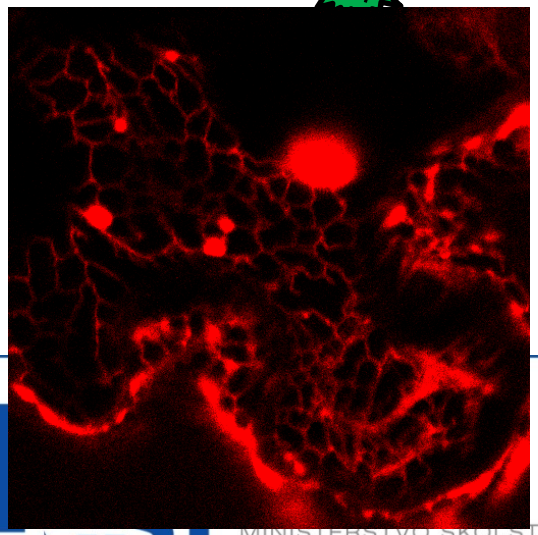
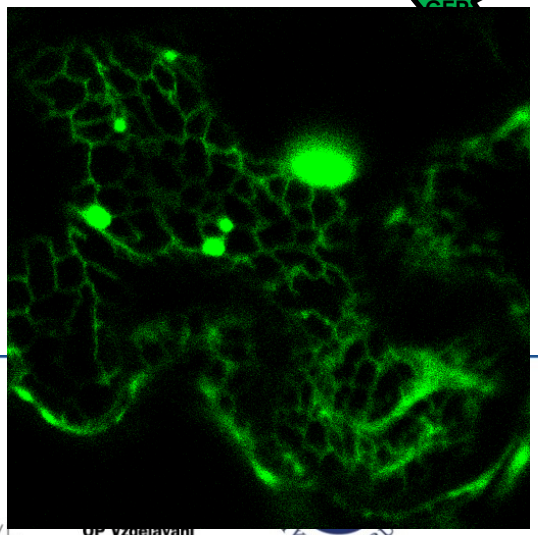
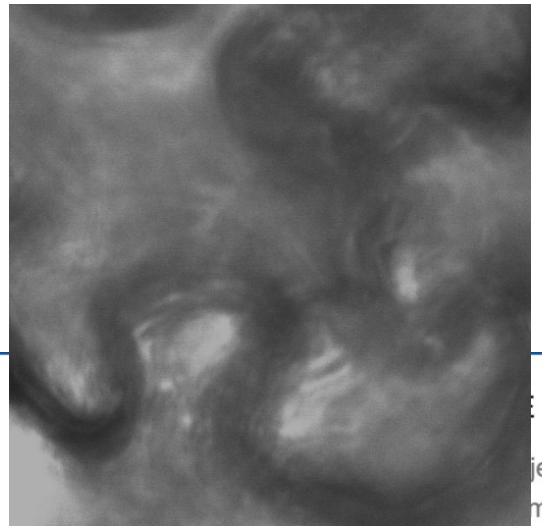
PI *in vivo*

Membrane Recruitment Assay (MeRA)







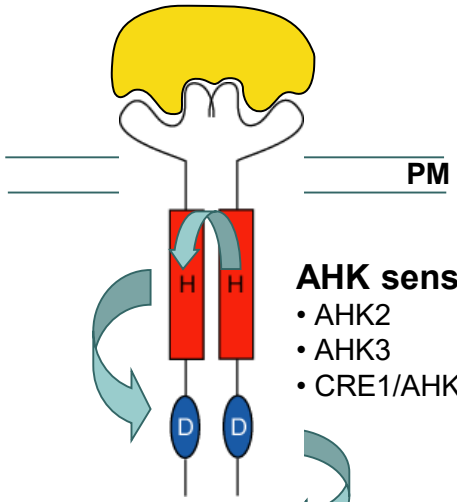


Outline

- Practical use of methods for *in vivo* studies of protein interactions

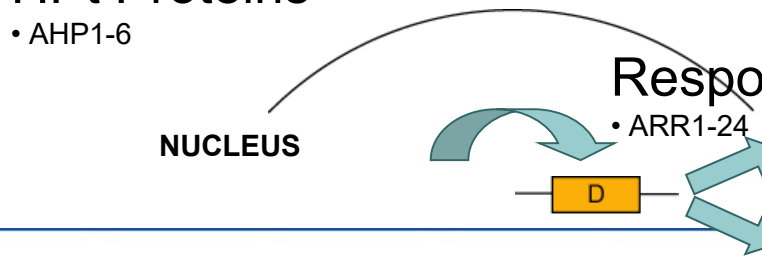
Signal Transduction via MSP

Recent Model of the CK Signaling via Multistep Phosphorelay (MSP) Pathway



AHK sensor histidine kinases
- AHK2
- AHK3
- CRE1/AHK4/WOL

HPt Proteins
- AHP1-6



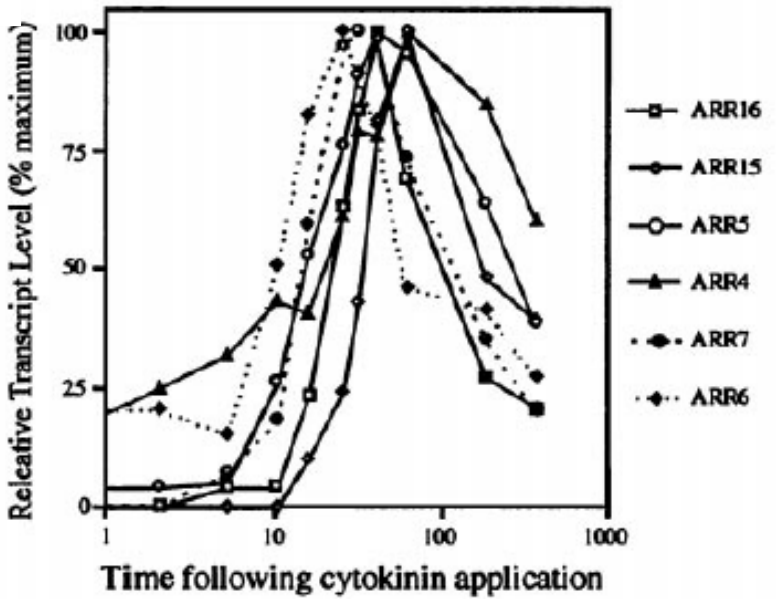
Response Regulators
- ARR1-24

NUCLEUS

REGULATION OF TRANSCRIPTION

CK primary response genes
- Type-A *ARRs* expression

INTERACTION WITH EFFECTOR PROTEINS



D'Agostino et al., Plant Phys, 2000

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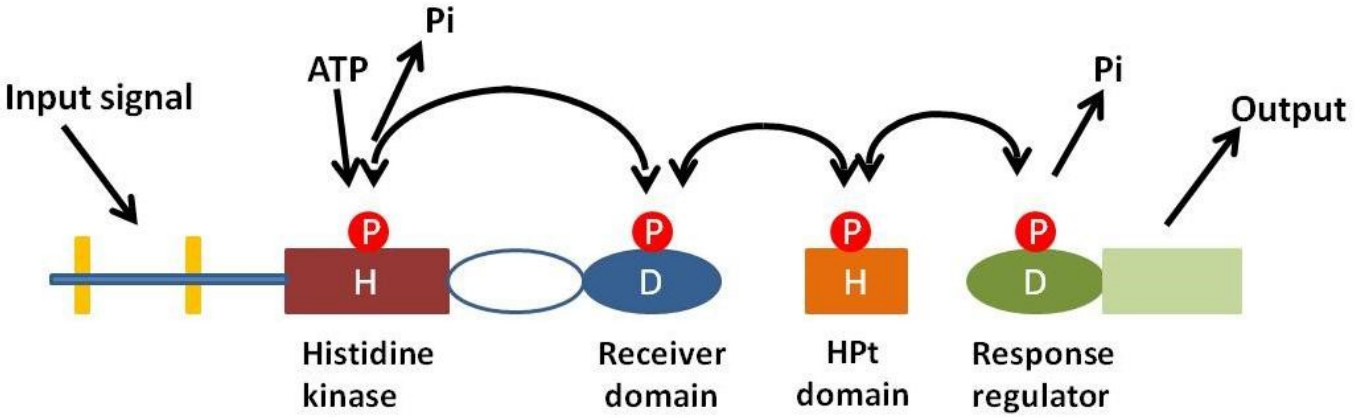
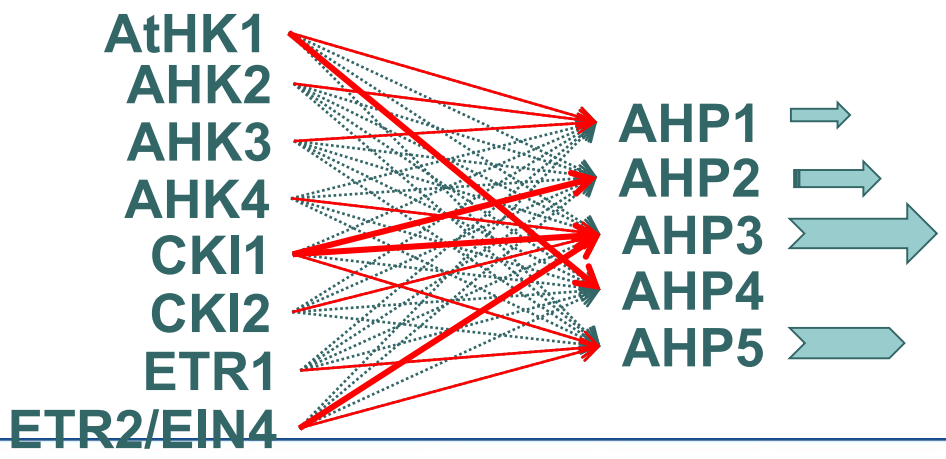
Is there any specificity in plant MSP?

- Is there ***a signalling specificity of MSP*** in plants?





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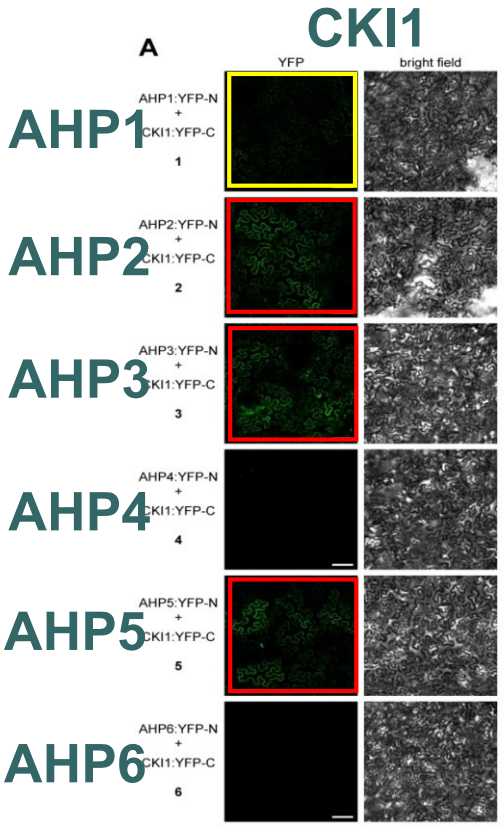
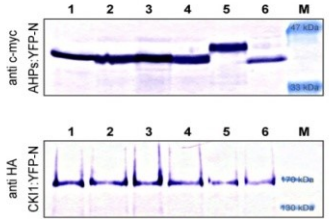
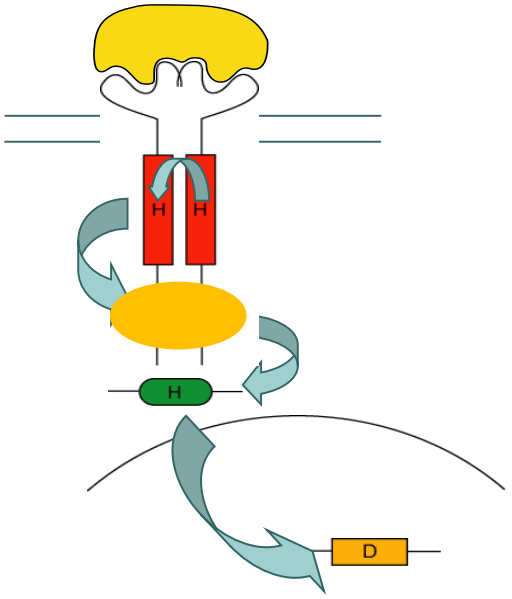
Specificity of CKI1 signalling

- ***CKI1 interacts*** *in vivo* with only ***subset of AHPs***

BiFC **Y2H**







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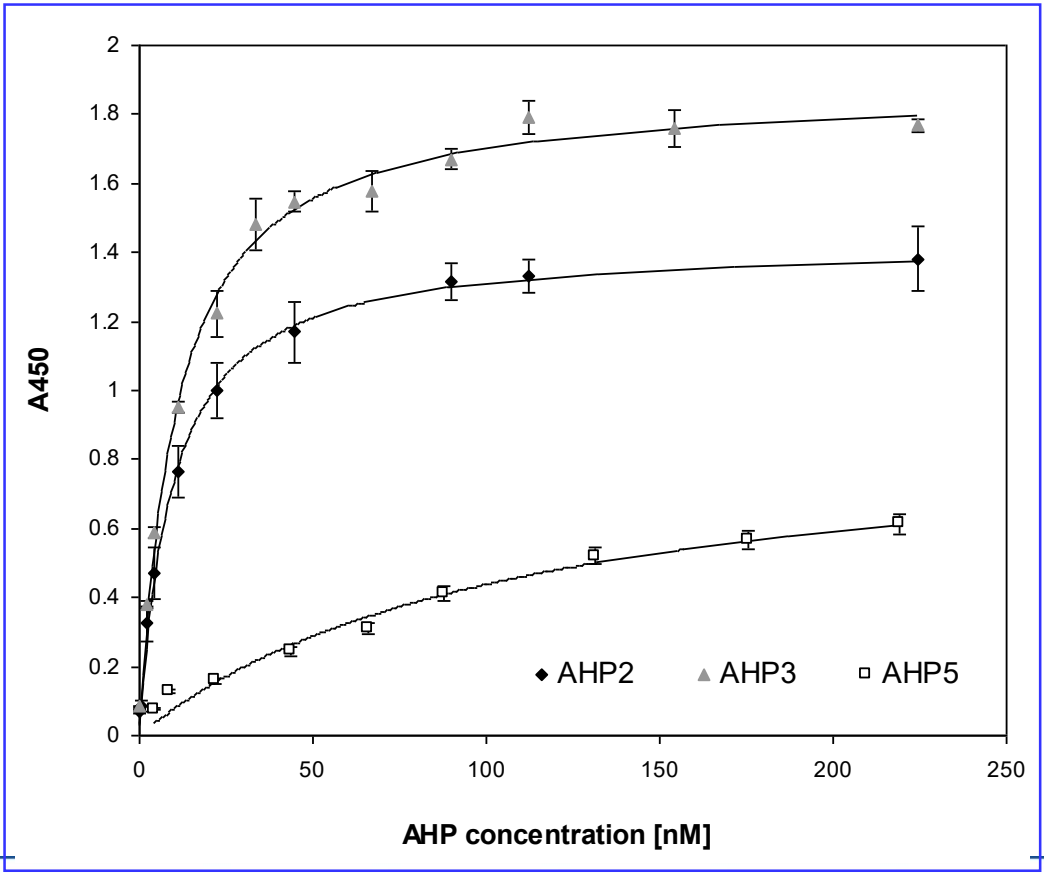
Specificity of CKI1 Signalling

- ***Specificity of CKI1 interaction*** was confirmed ***in vitro***



AHP3: K_d ~ 10,5 nM

AHP2: K_d ~ 9,17 nM

AHP5: K_d ~ 108 nM

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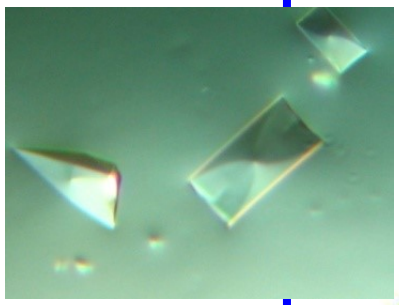
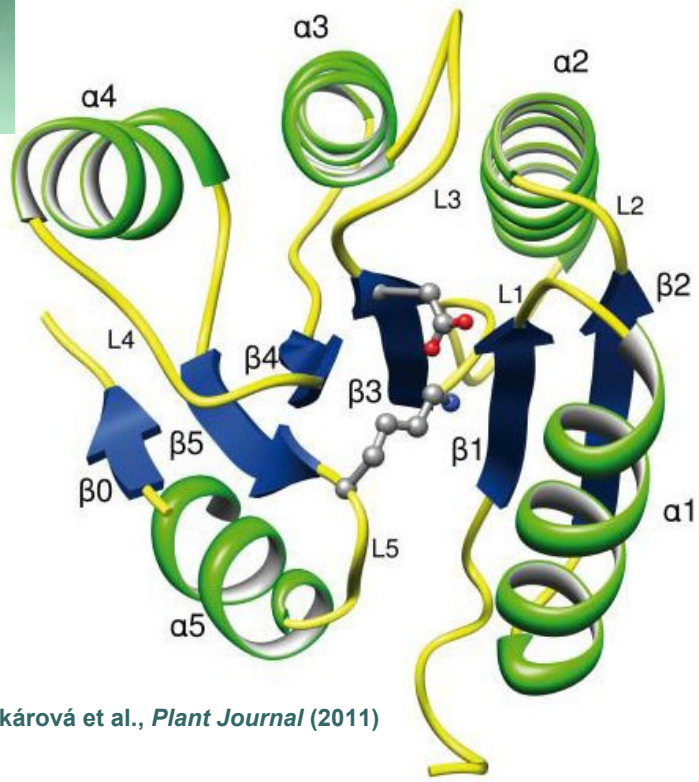
Structure of $CKI1_{RD}$

- X-ray crystallography revealed conserved ***$(\alpha/\beta)_5$ structural fold*** of $CKI1_{RD}$





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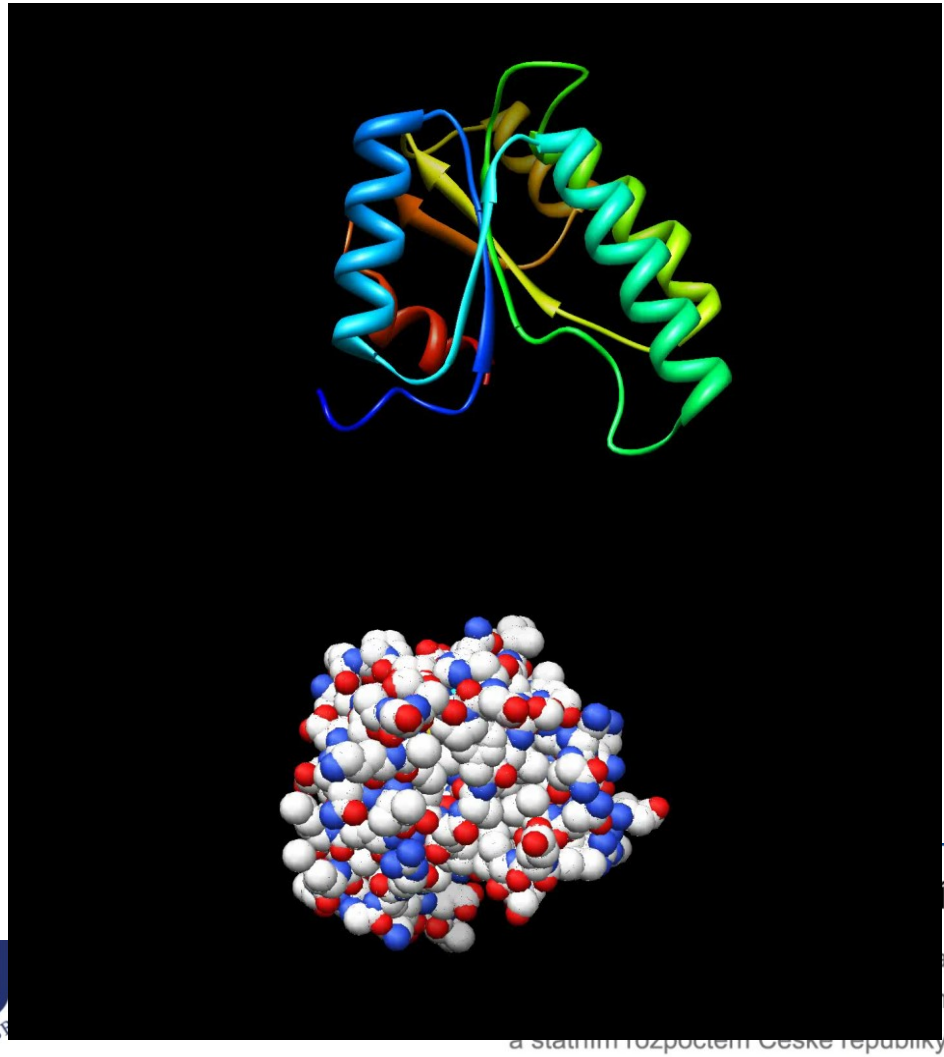

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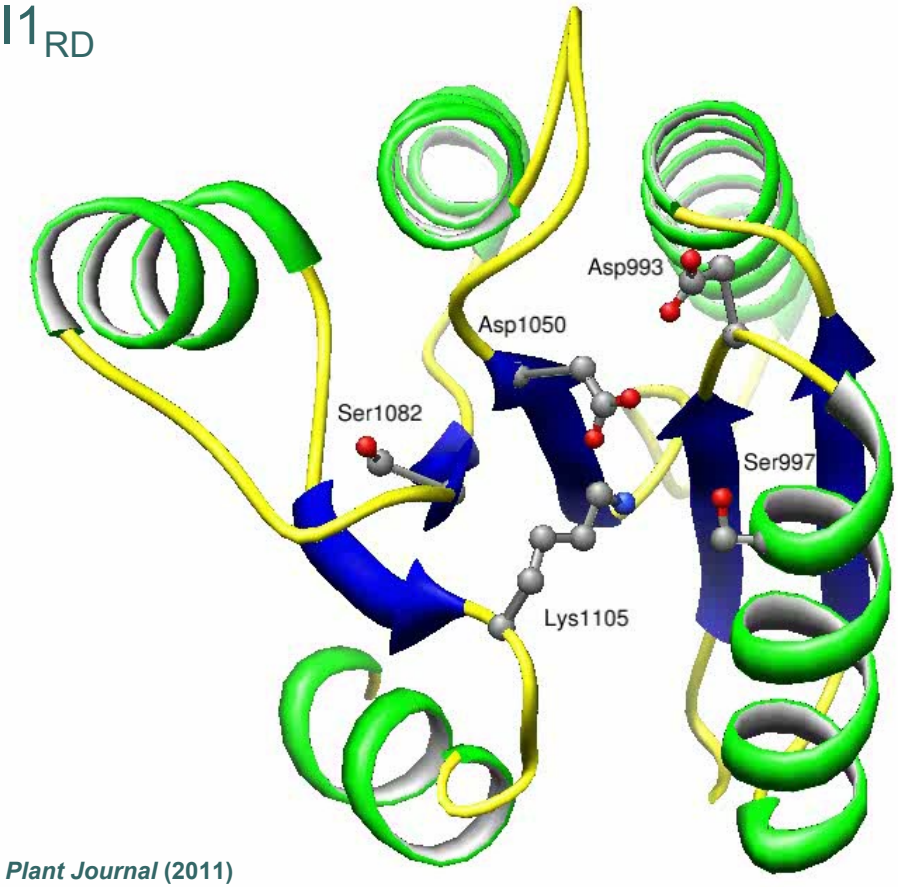
Dynamics of $CKI1_{RD}$

- ***Mg^{2+}binding*** leads to ***remodelling of active centre*** of $CKI1_{RD}$



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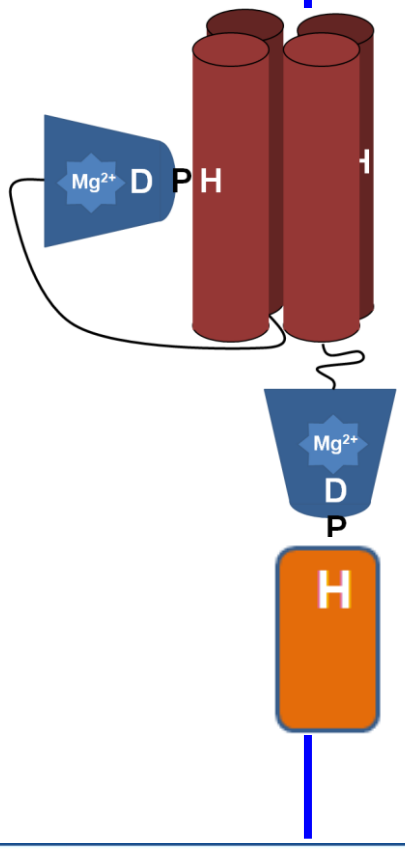
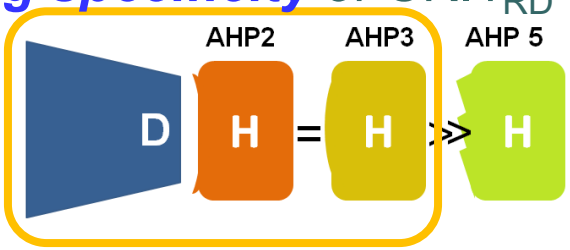
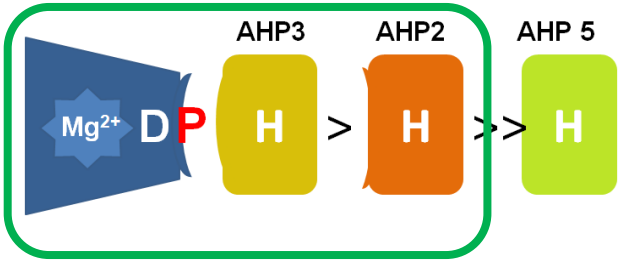
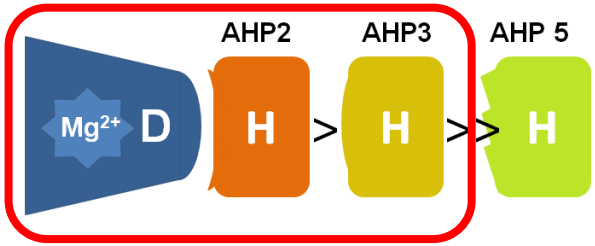
$CKI1_{RD}$ structural changes are associated with its binding specificity

- ***Mg^{2+}***- and ***BeF_3^-***-induced ***structural changes fine-tune binding specificity*** *of* $CKI1_{RD}$









Ligand	0	Mg^{2+}	BeF_3^-
AHP2	9.17 ± 0.49	6.2 ± 0.98	11.6 ± 2.0
AHP3	10.5 ± 0.73	12.9 ± 0.72	8.0 ± 0.42
AHP5	108 ± 18	152 ± 26	119 ± 32

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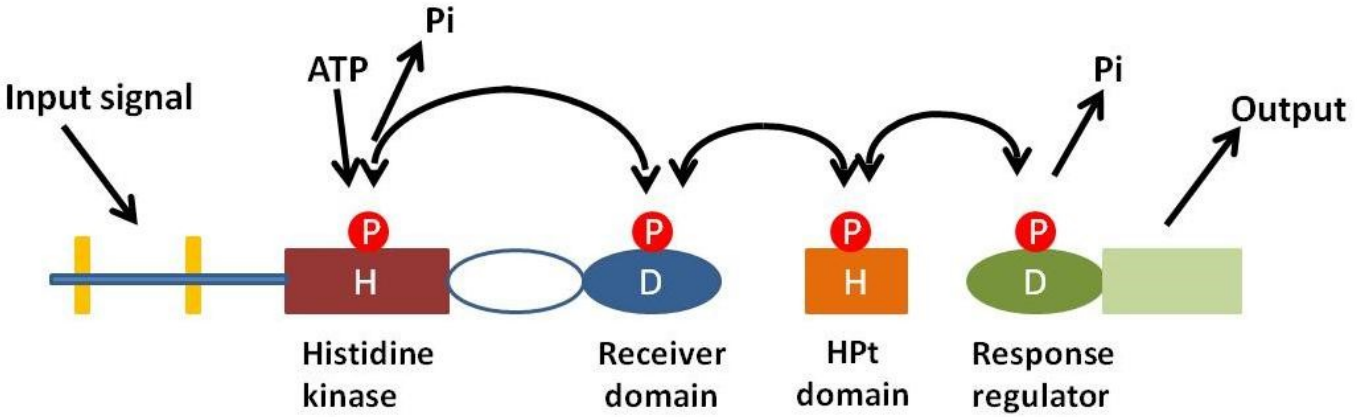
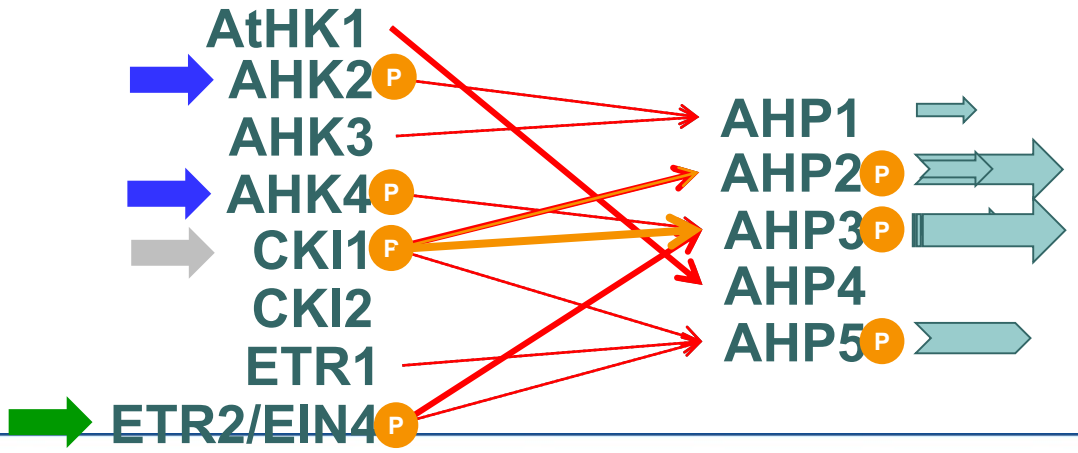
Model Suggestion

- ***YES***, there is ***signalling specificity of MSP*** in plants.





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