

CG920 Genomics

Lesson 6

Protein Interactions in Gene Regulations

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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

Literature

- Literature sources for Chapter 06:

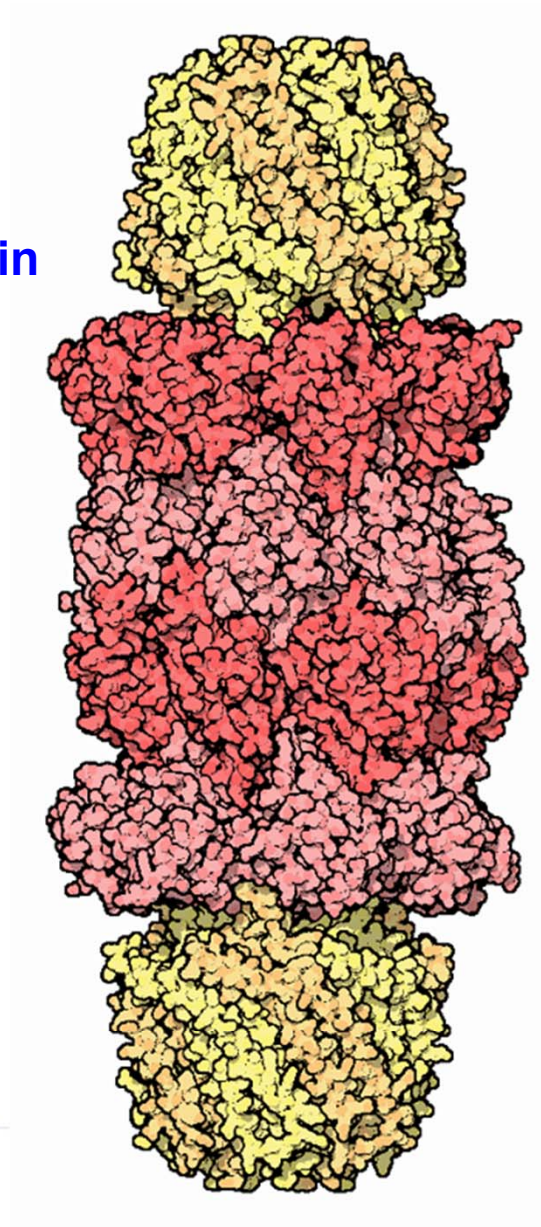
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- Hu, C.D., and Kerppola, T.K. (2003). Simultaneous visualization of multiple protein interactions in living cells using multicolor fluorescence complementation analysis. *Nat. Biotechnol.* 21, 539-545.
- Shahbadian, K., and Chartrand, P. (2012). Control of cytoplasmic mRNA localization. *Cellular and molecular life sciences : CMLS* 69, 535-552.
- Van Leene, J., Witters, E., Inze, D., and De Jaeger, G. (2008). Boosting tandem affinity purification of plant protein complexes. *Trends Plant Sci* 13, 517-520.
- Walter, M., Chaban, C., Schutze, K., Batistic, O., Weckermann, K., Nake, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., Harter, K., and Kudla, J. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J* 40, 428-438.

Outline

- Functional importance of the specific 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

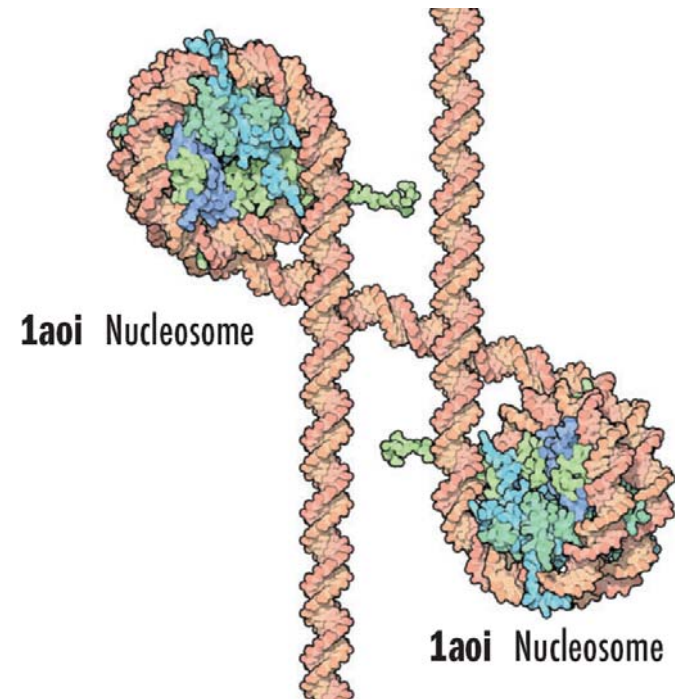
Importance of Protein Interactions

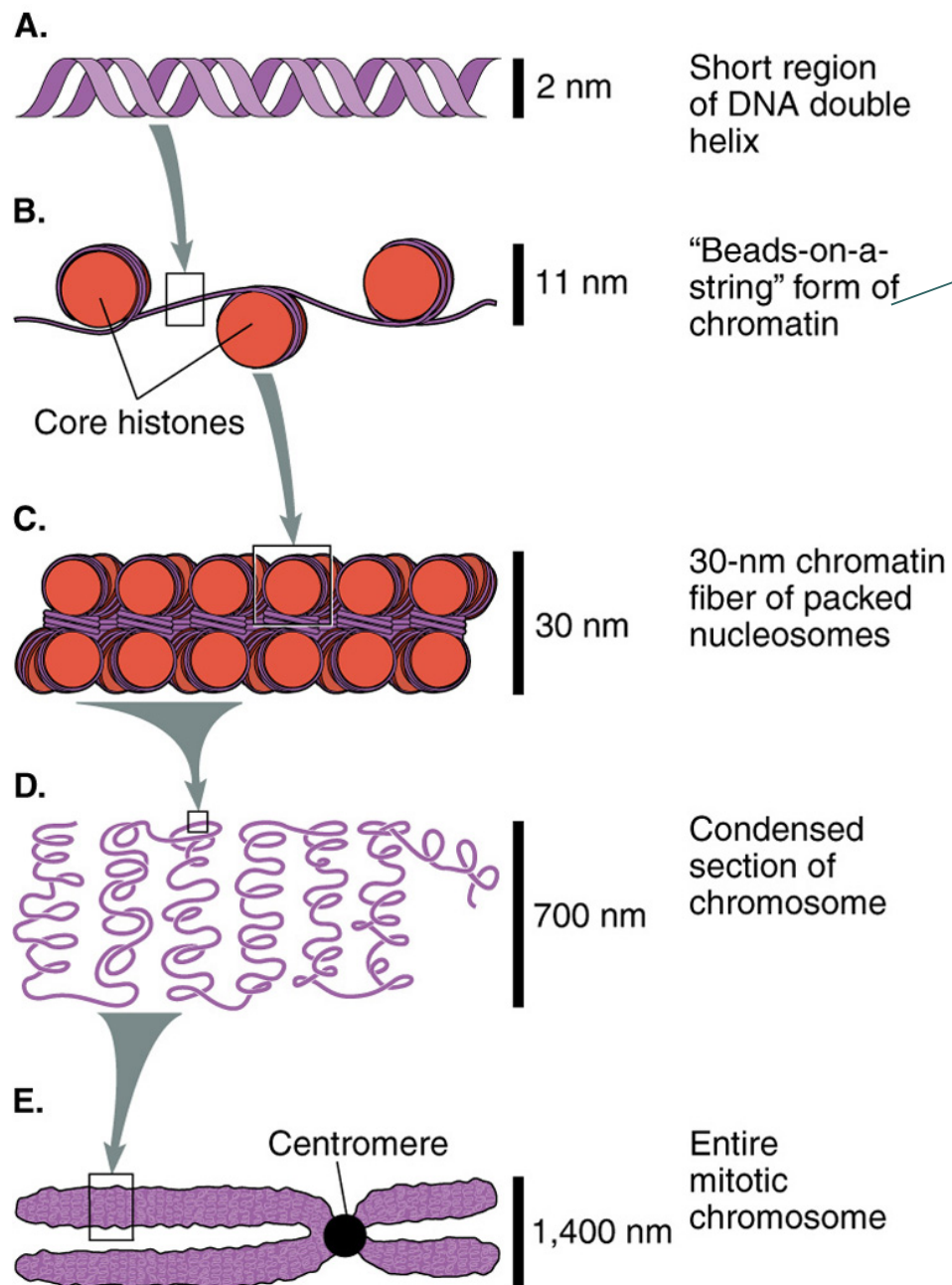
- **Functional importance of specific protein interactions**
 - Most of the proteins in the cell exist in the form of complexes which may further interact with each other
 - **Proteasome**
 - protein complex responsible for the degradation of obsolete proteins in the cell



Importance of Protein Interactions

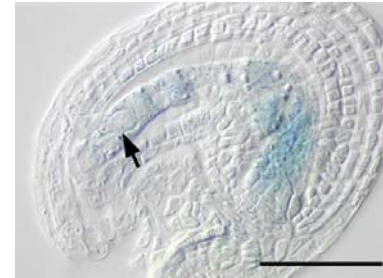
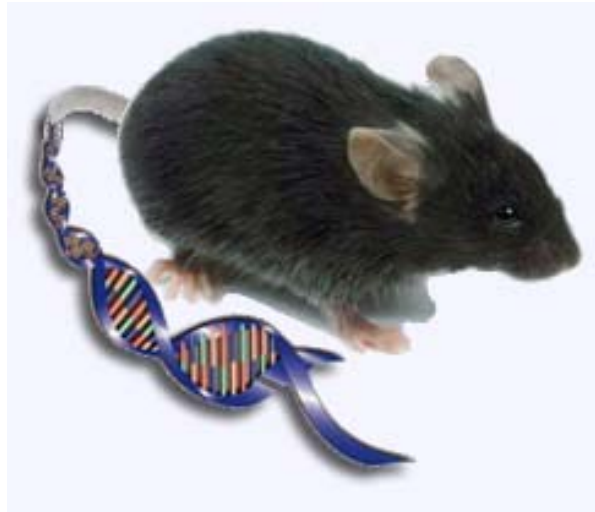
- Functional importance of specific protein interactions
 - Chromatin structure





Regulation by **histone acetyl transferases** or **histone deacteylases**

DNA methylation in animals vs. in plants



methylation status

CpG

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



Imprinting and “cell memory”



Mechanism of transcriptional regulation by DNA methylation mostly unknown

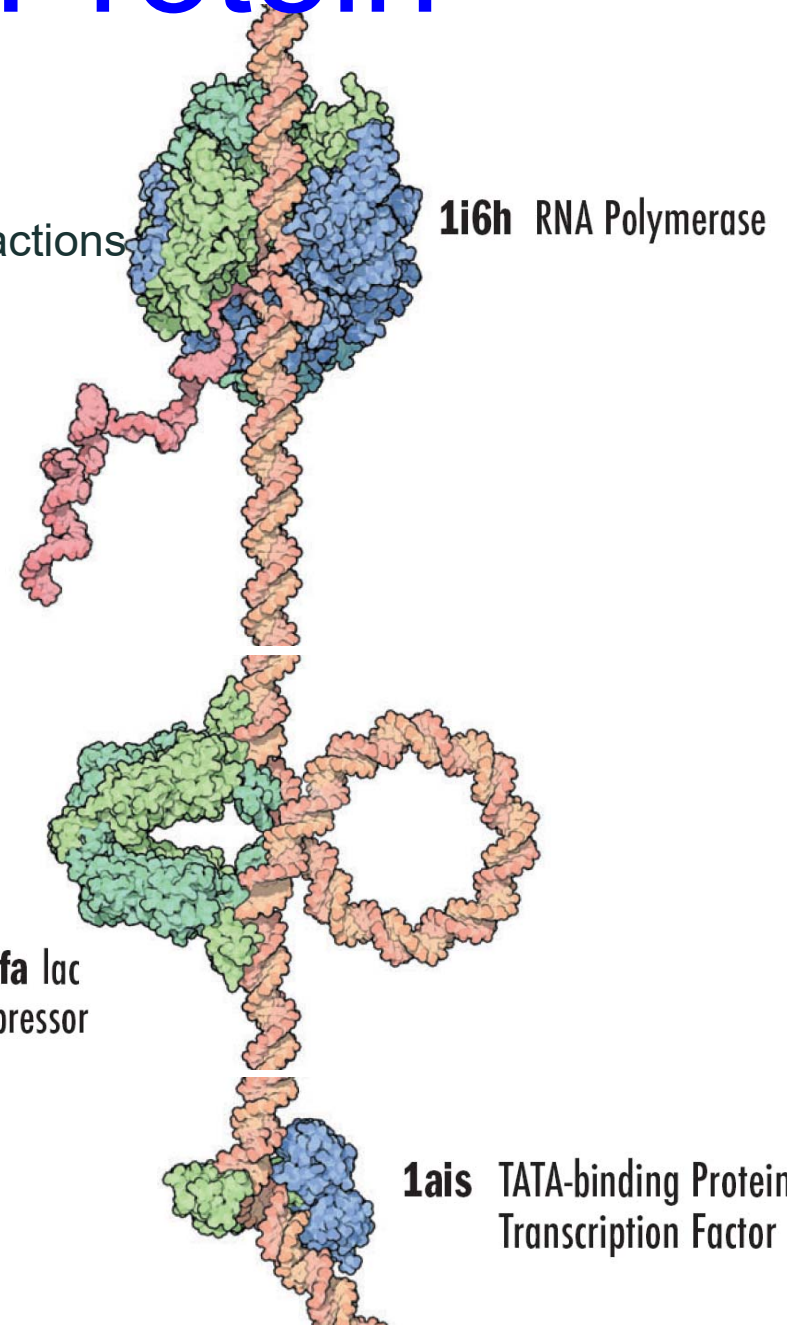
methylation status

CpG or CpNpG

CpNpNp

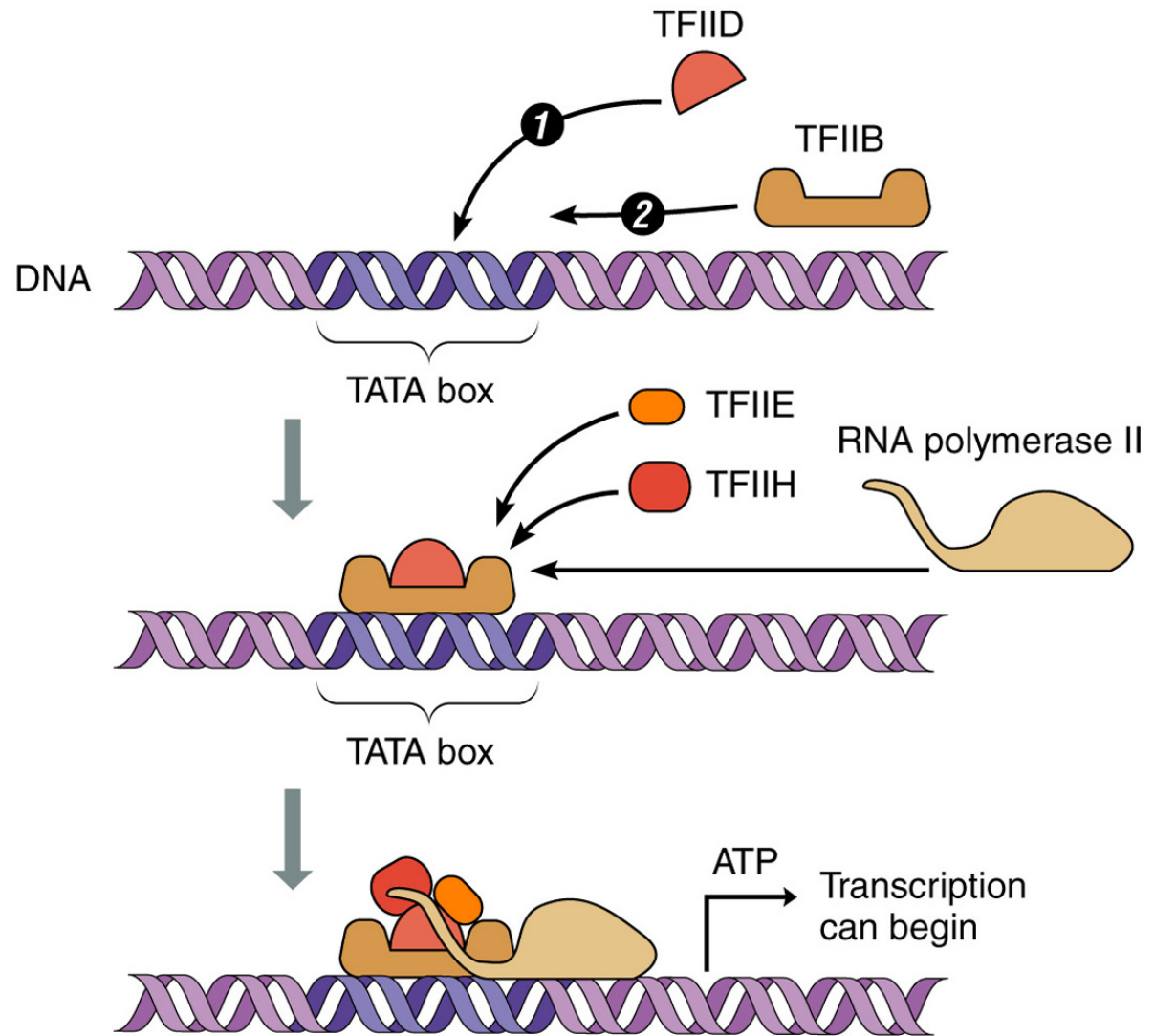
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- Functional importance of specific protein interactions
 - Chromatin structure
 - Regulation of transcription

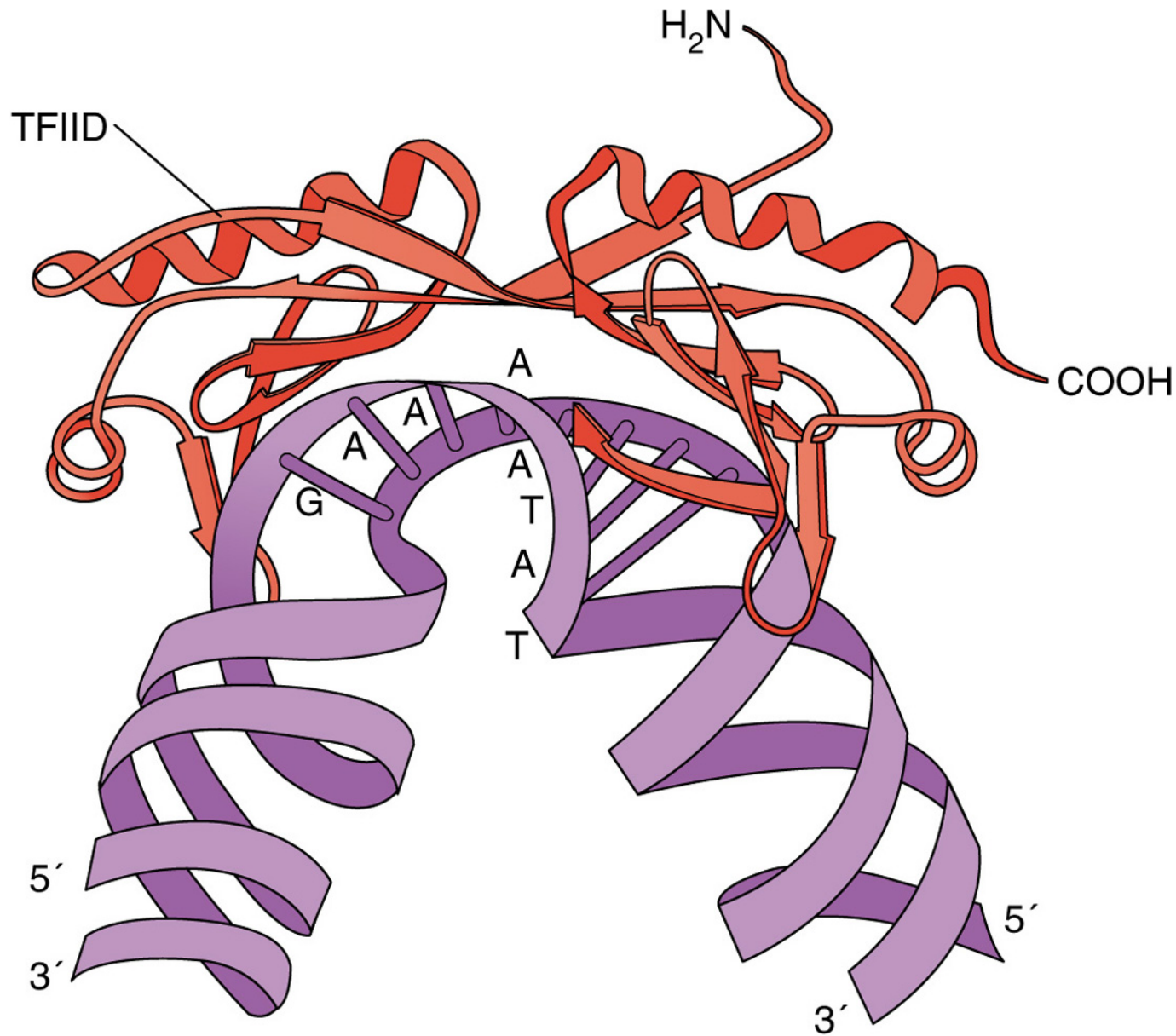


Initiation of Transcription

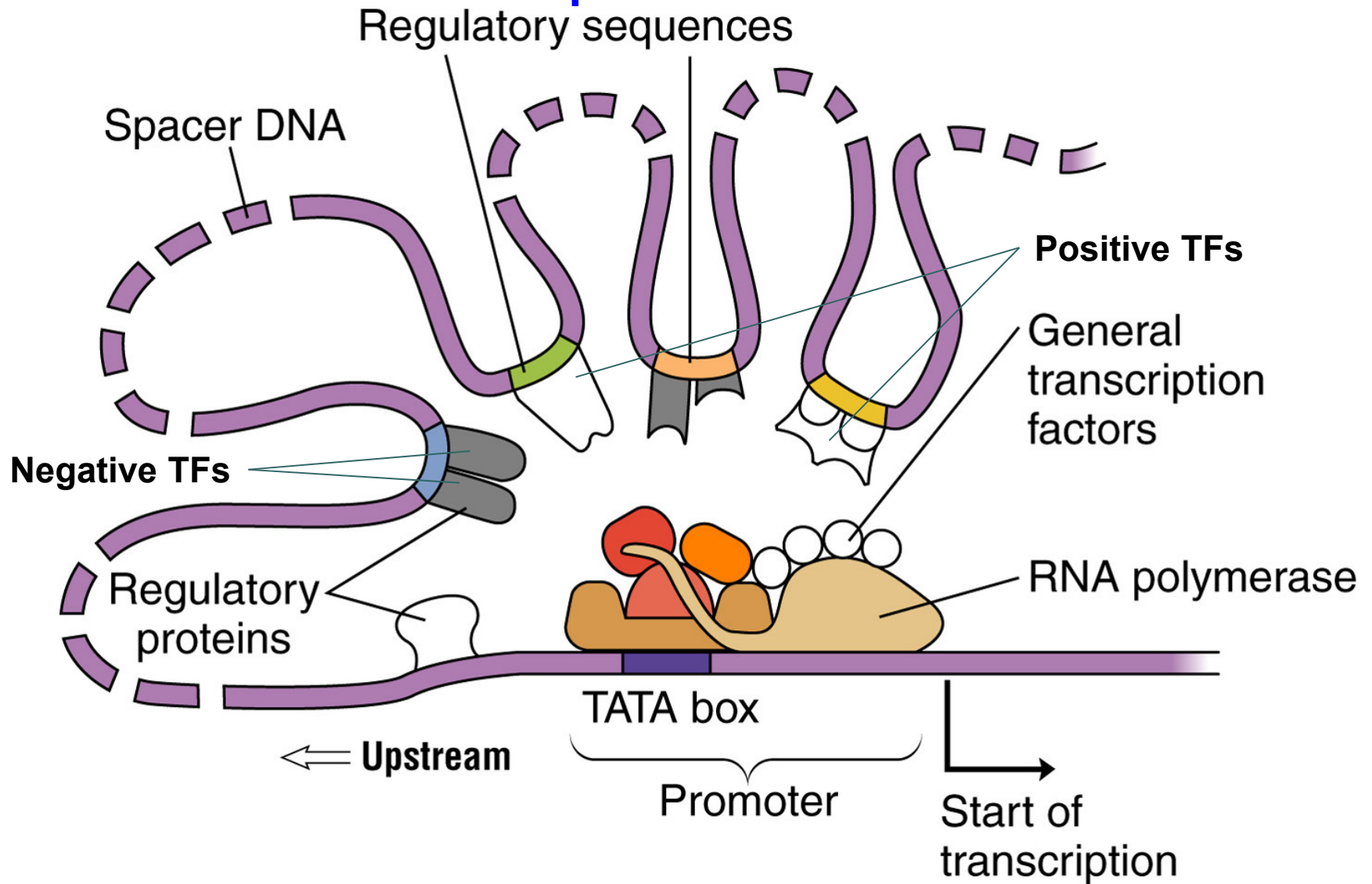
A.



B.



Initiation of Transcription



Transcriptional Regulation by TAFs

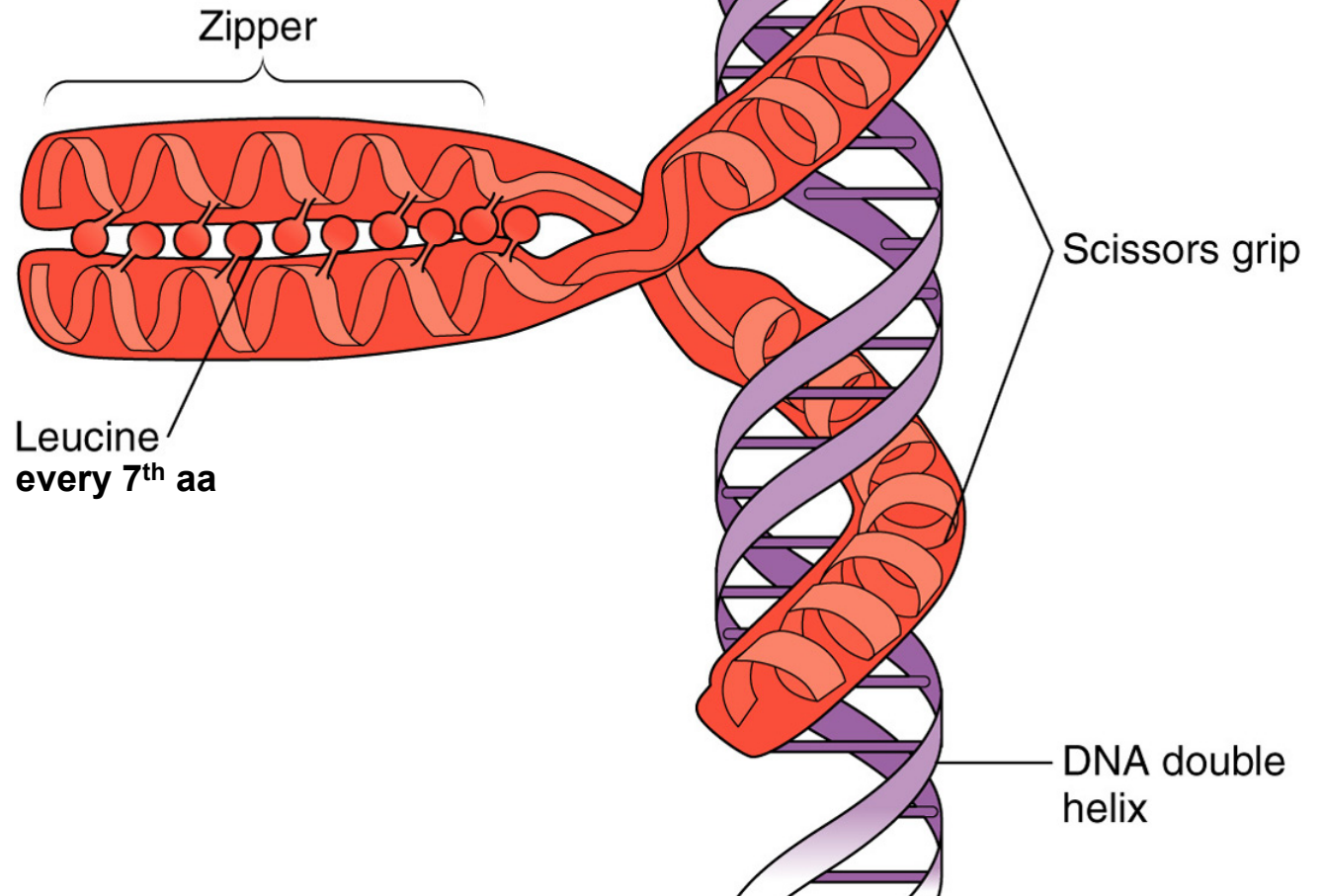
Signal recognition



Dimerization

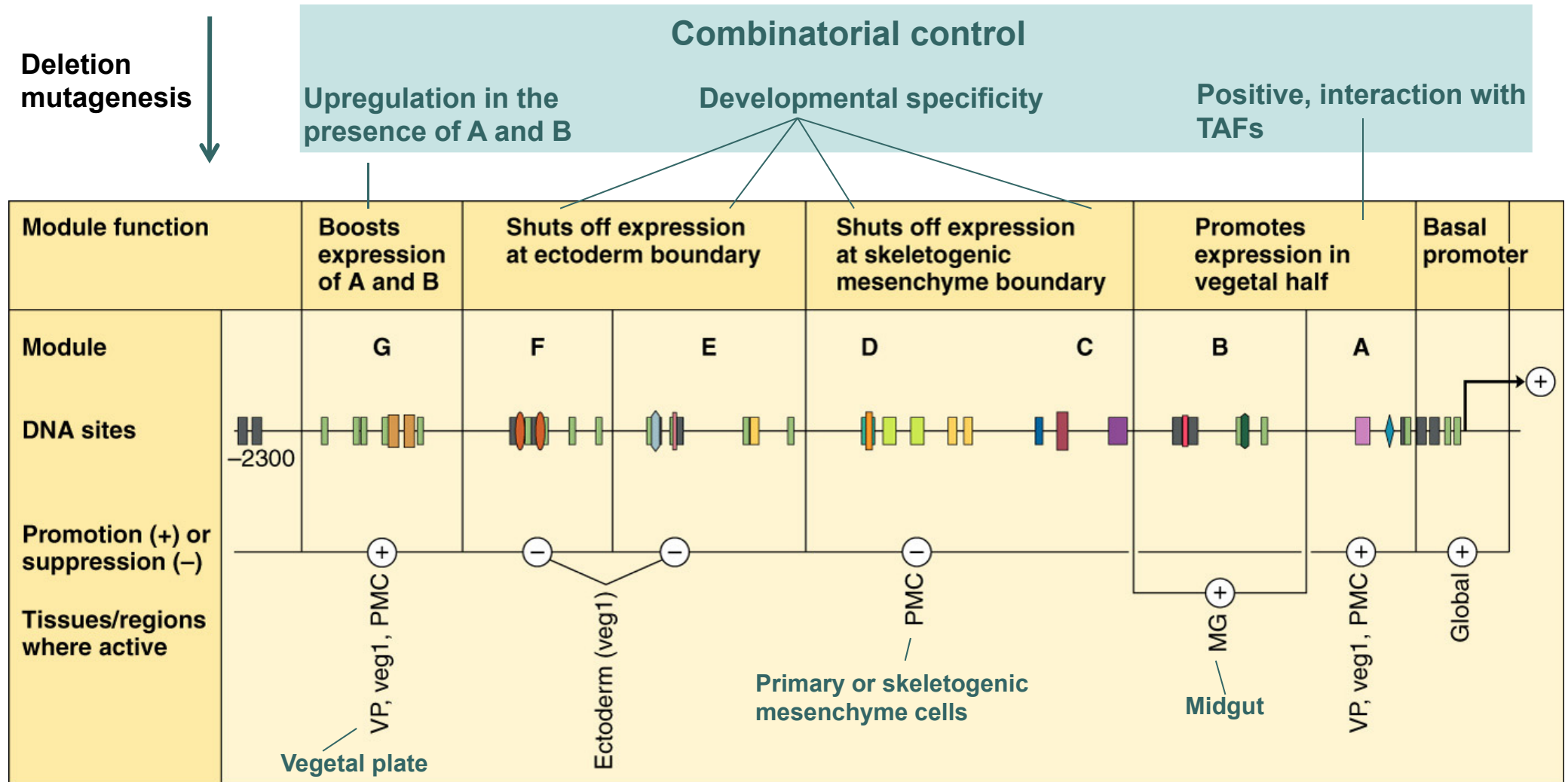


DNA binding and transcription activation



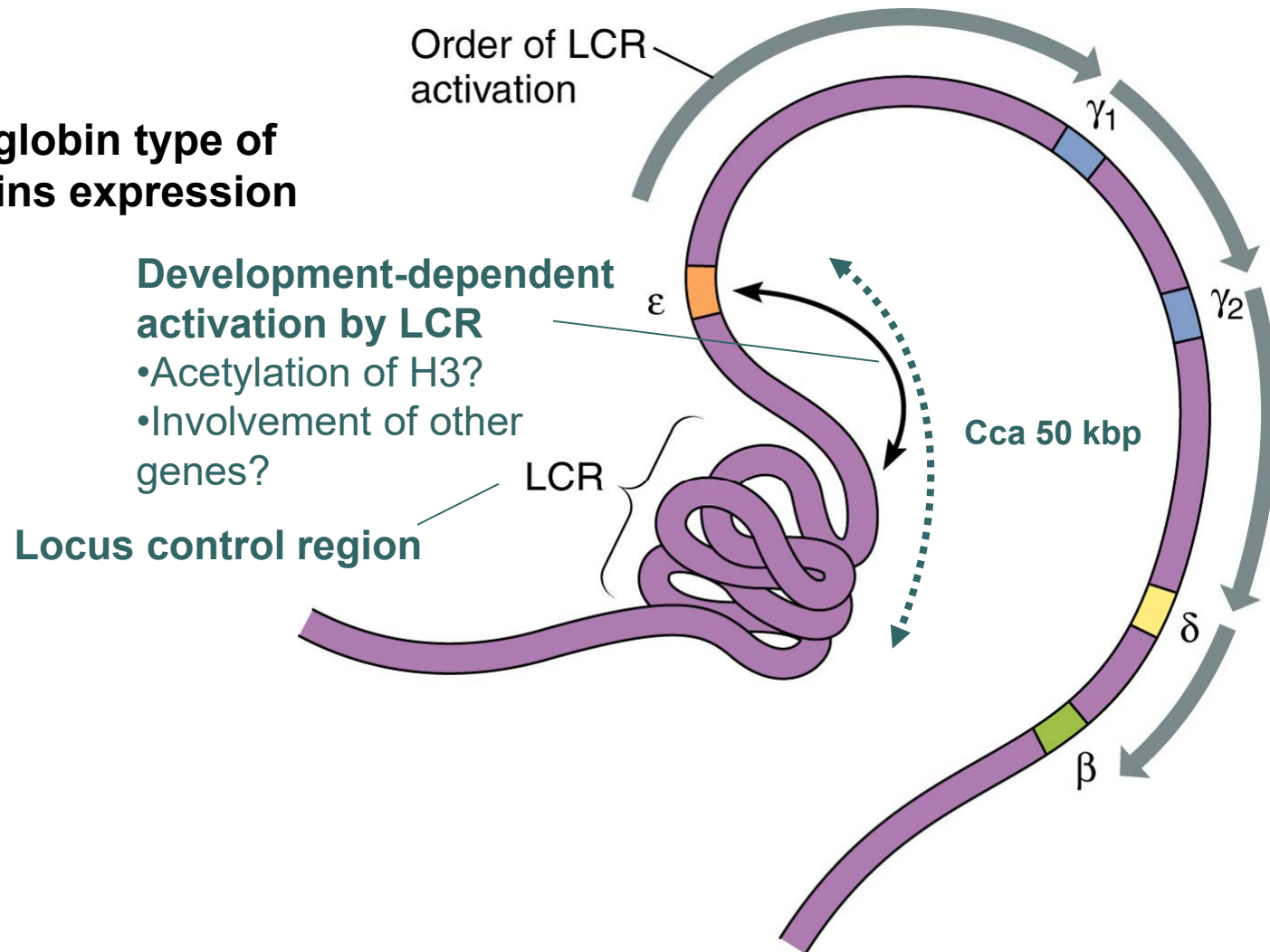
Multifactorial Promoters Control

ProENDO16:REPORTER (sea urchin)



Multifactorial Promoters Control

Regulation of β -globin type of hemoglobin chains expression

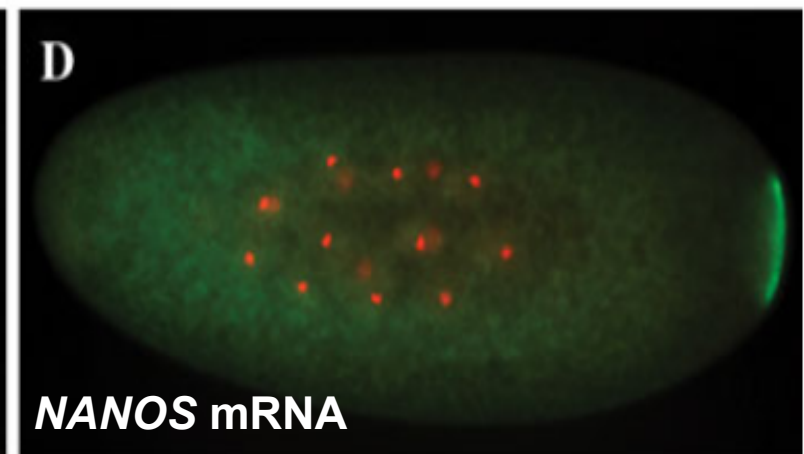
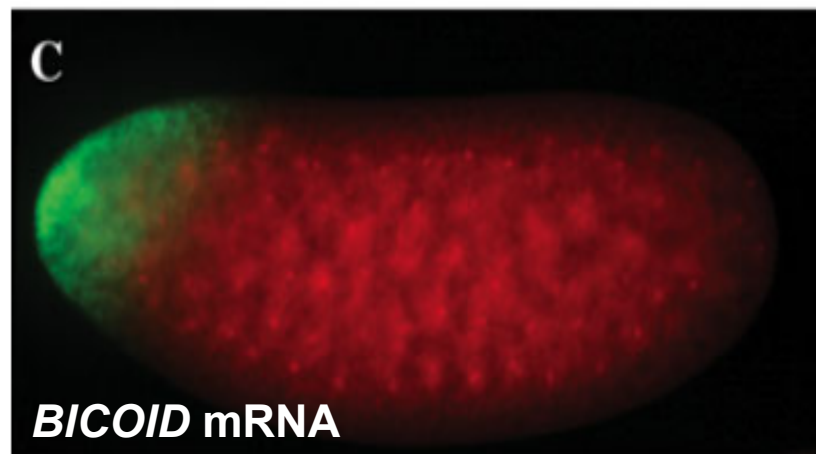
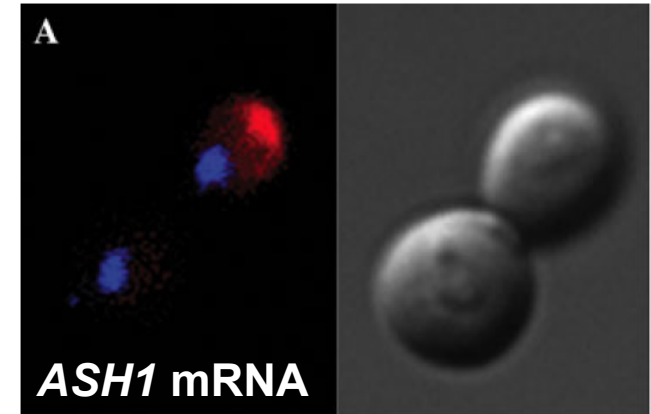


Importance of Protein Interactions

- Functional importance of specific protein interactions
 - Chromatin structure
 - Regulation of transcription
 - mRNA localization

mRNA localization

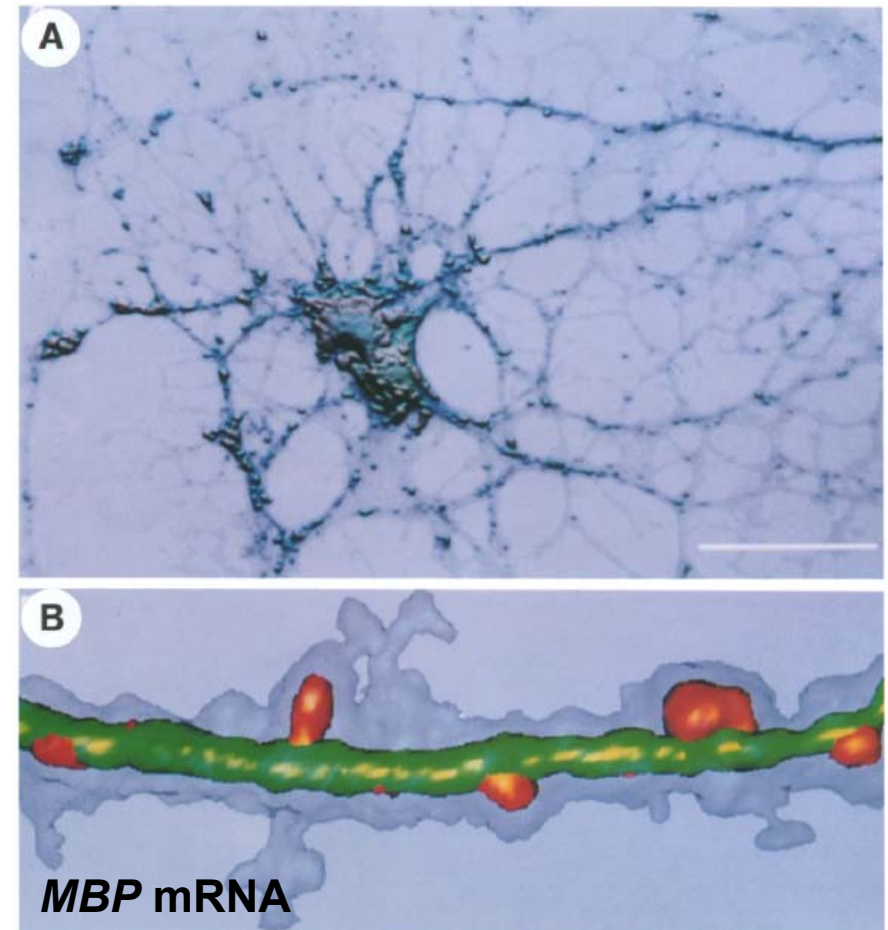
- Importance of mRNA localization
 - Control over spatiotemporal localization of gene product (protein)
 - Asymmetric cell division during development
 - Embryo polarization



Shahbadian and Chartrand, 2012

mRNA localization

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



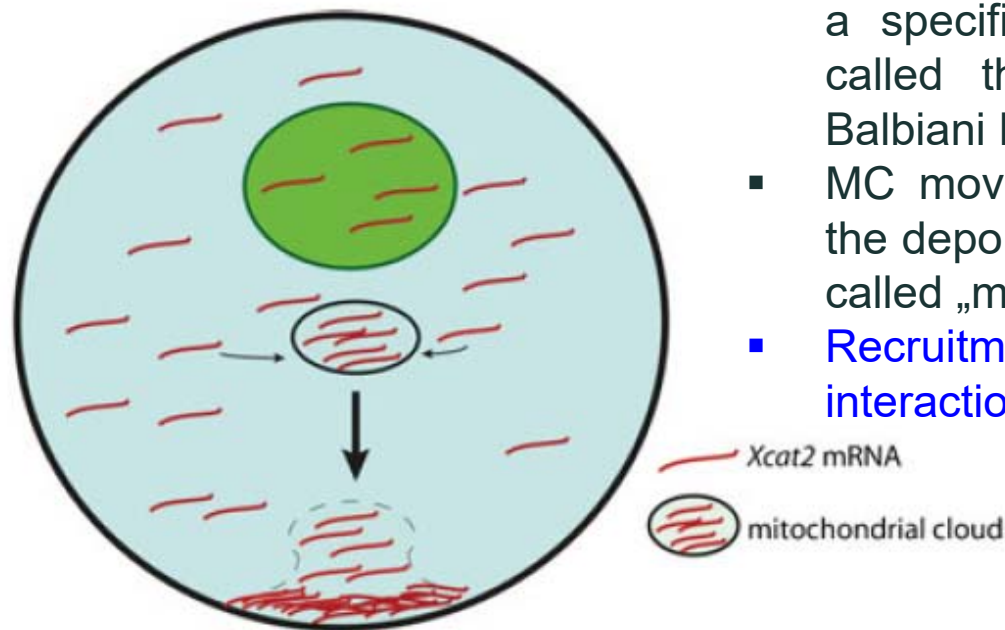
Ainger et al., 1993

mRNA localization

Mechanisms

- **Diffusion and recruitment of mRNA**

- 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“)
- **Recruitment** on the **vegetal pole** via interaction of MC and ER



Shahbadian and Chartrand, 2012

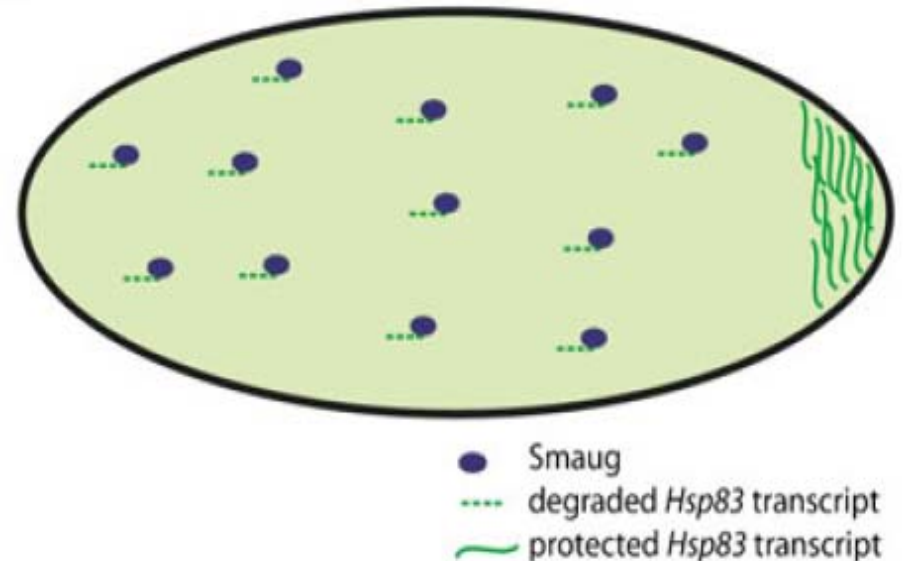
mRNA localization

Mechanisms

Shahbadian and Chartrand, 2012

Localized mRNA degradation

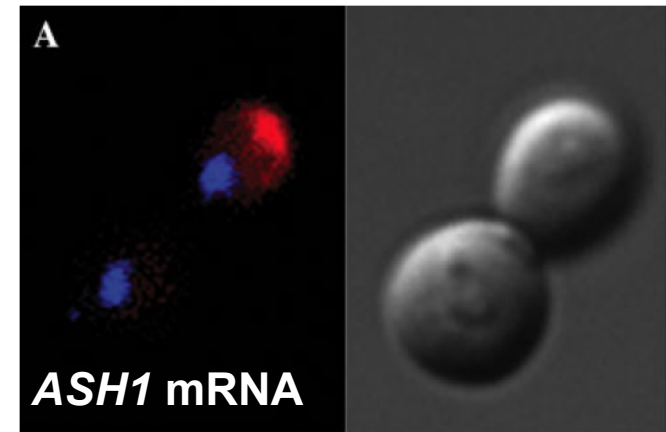
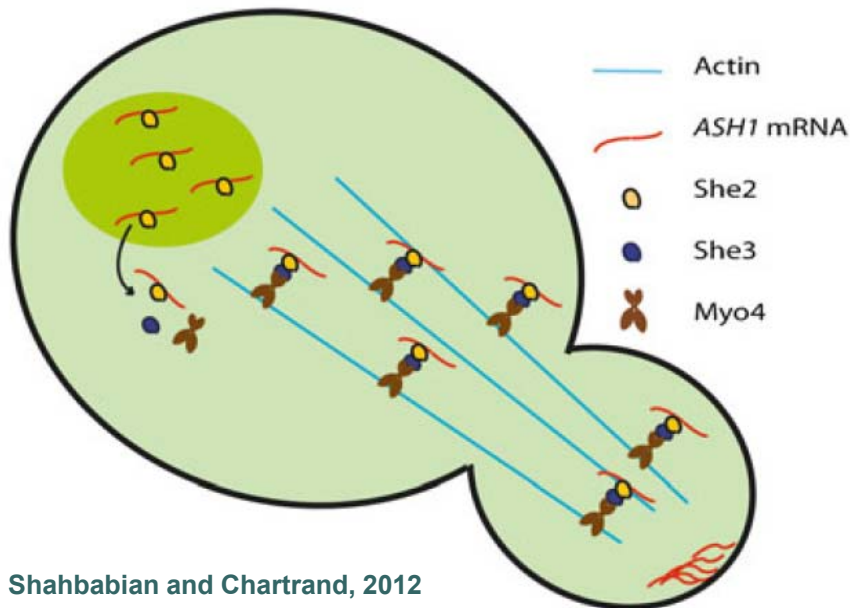
- During embryogenesis in *Drosophila m.* *Hsp83* mRNA is localized at the posterior pole of embryo, similarly to *NANOS* mRNA
- *Hsp83* mRNA is localized in the whole embryo, however, it is destabilized by cis elements both in 3'UTR (HDE) and in coding region (HIE).
- HIE elements are recognized by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



mRNA localization

Mechanisms

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



Shahbadian 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

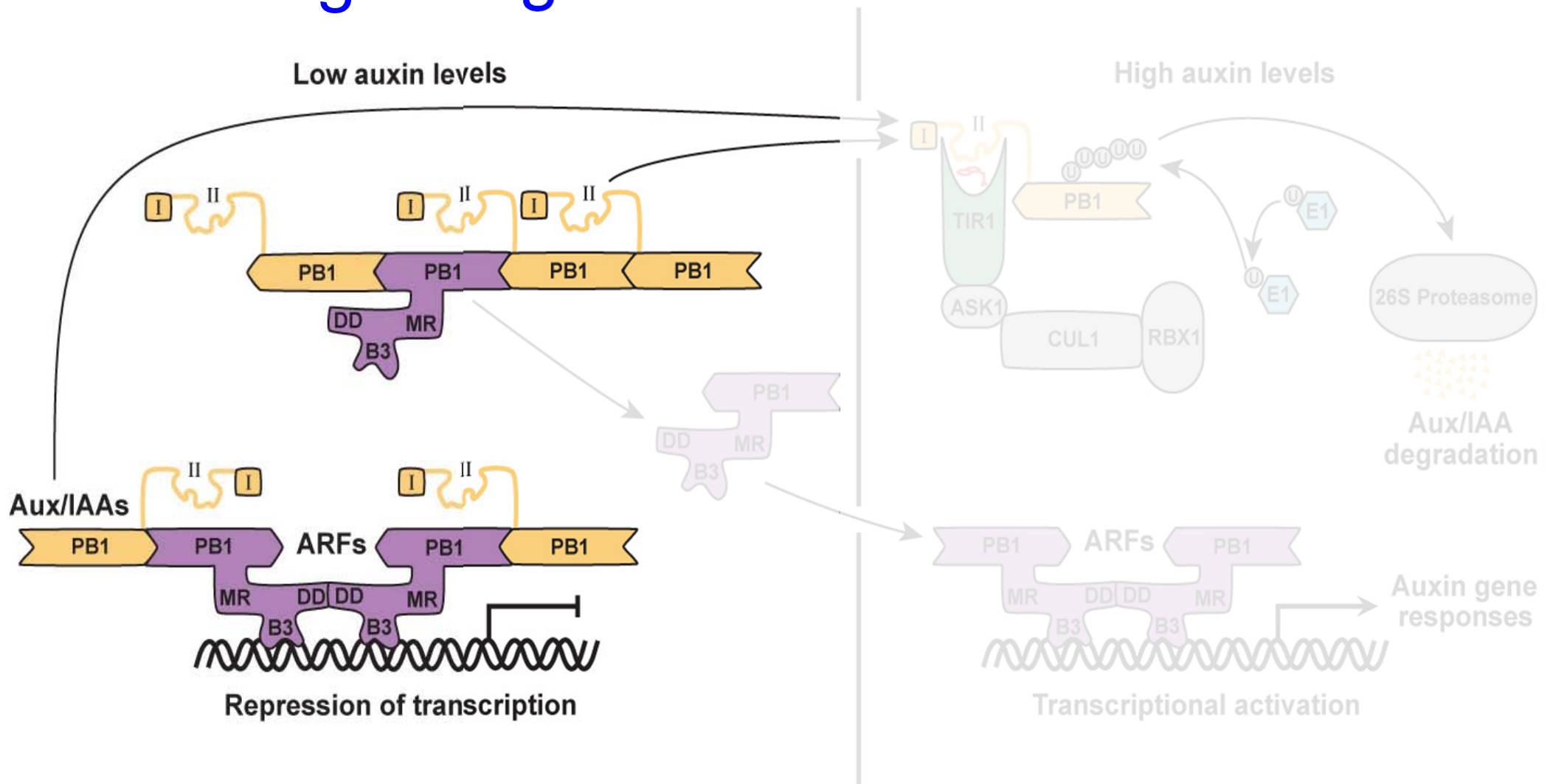
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Importance of Protein Interactions

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 - Protein stability

Auxin Signalling



Jing and Strader, *Plant Structural Biology, Hormonal Regulations* (2018)

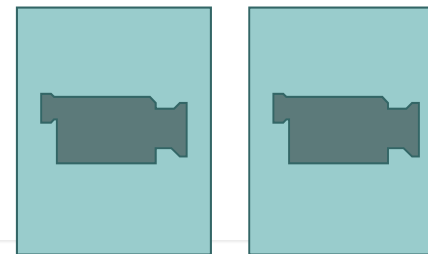
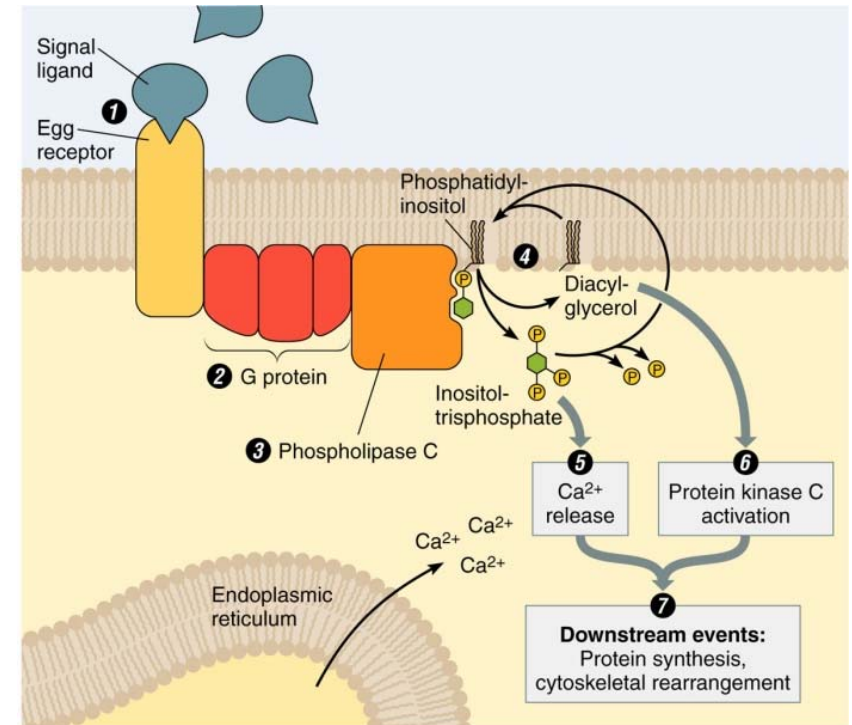
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 - Signal transduction

Signal transduction

- PI and signal transduction

- through G protein and phospholipase C
- Signalling cascades using cAMP



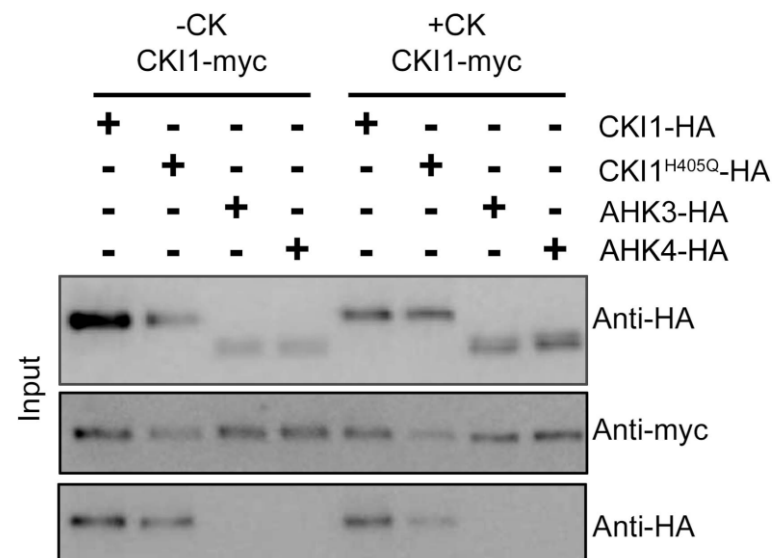
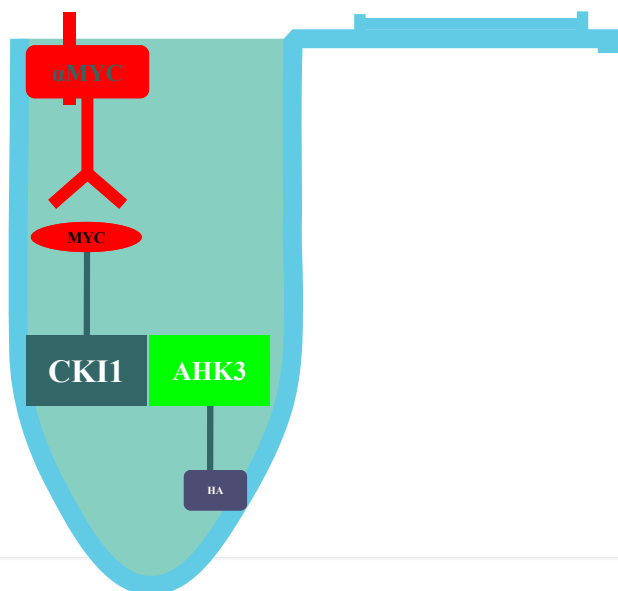
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PI *in vivo*

Co-immunoprecipitation

- Isolation of protein complexes using antibodies recognizing one of the interacting proteins



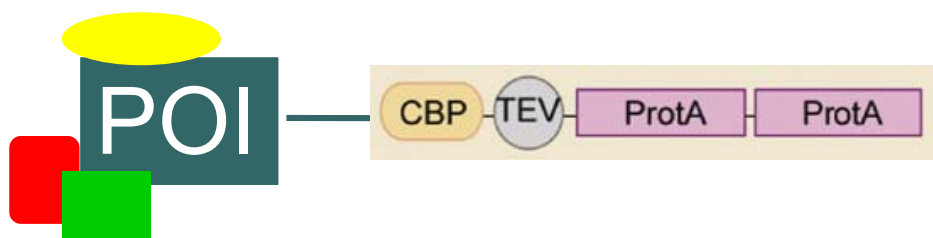
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PI *in vivo*

Tandem affinity purification (TAP-tag)

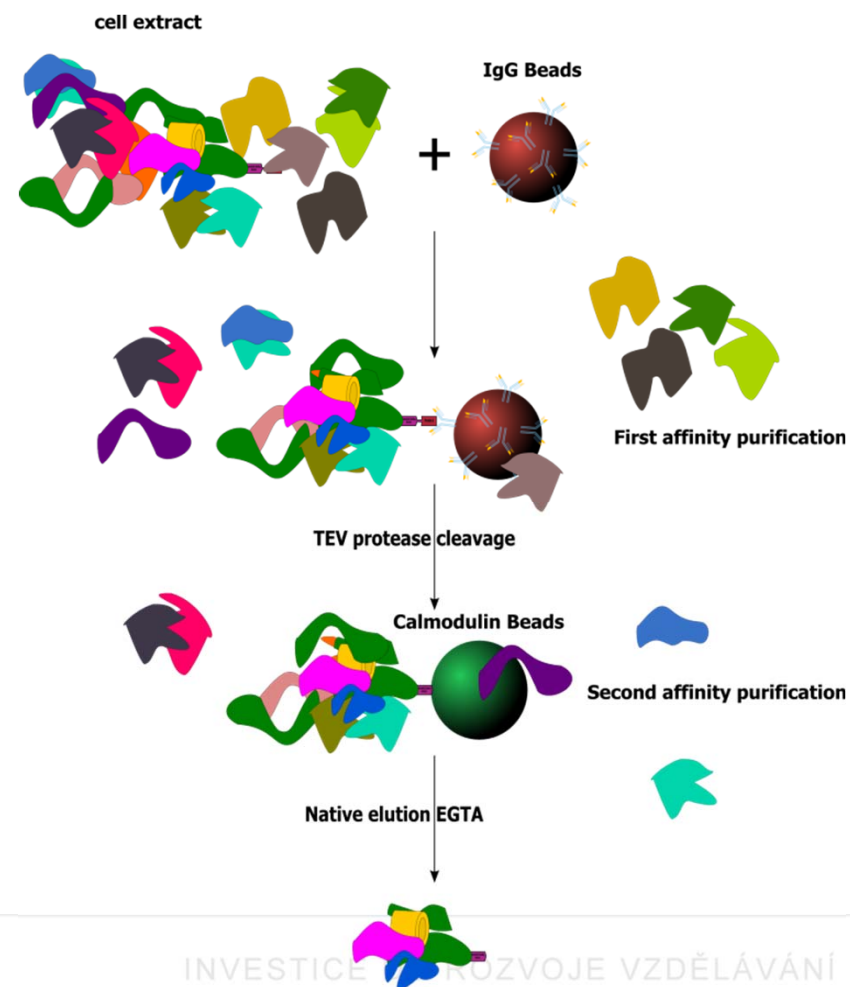
- Isolation of protein complexes using recombinant proteins fused with two different binding domains - tags



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

- Isolated protein complexes are separated using 1D ELFO and then identified by MS

- Advantage:** using two independent protein domains for affinity purification -> therefore **high specificity**



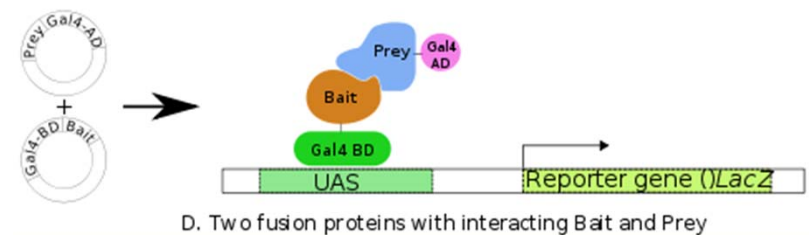
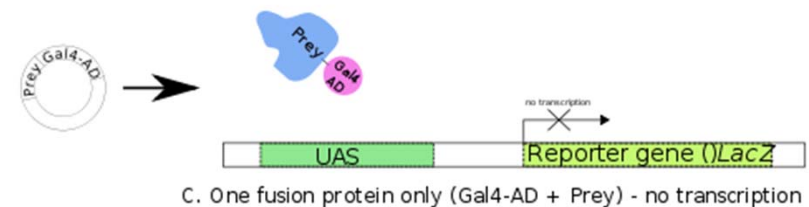
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PI *in vivo*

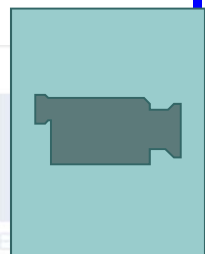
Yeast two-hybrid assay (Y2H)

- 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 for interaction partners in expression libraries of individual organisms



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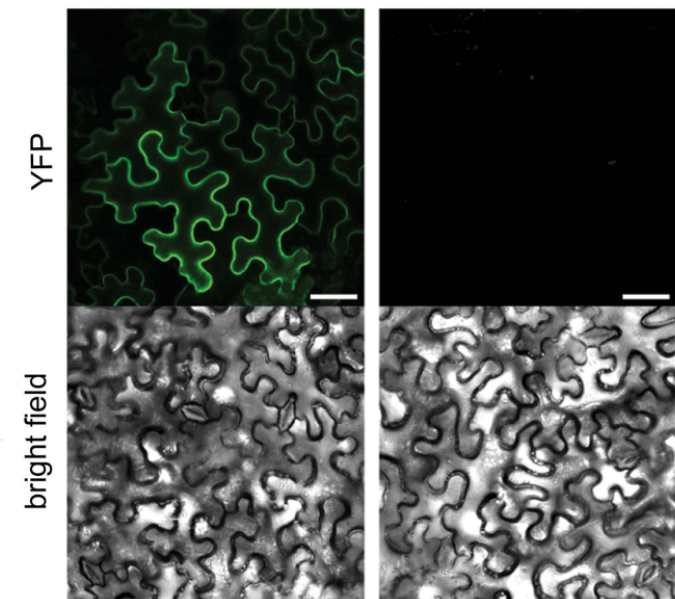
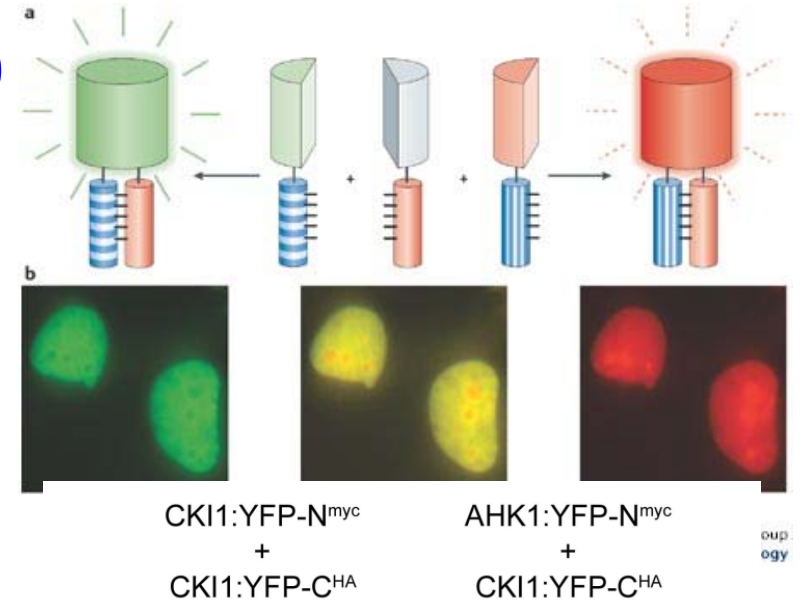
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PI *in vivo*

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 appears
- Apart from identification of the interaction, this method allows you to localize the interaction within the cell



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PI *in vivo*

Membrane Recruitment Assay (MeRA)

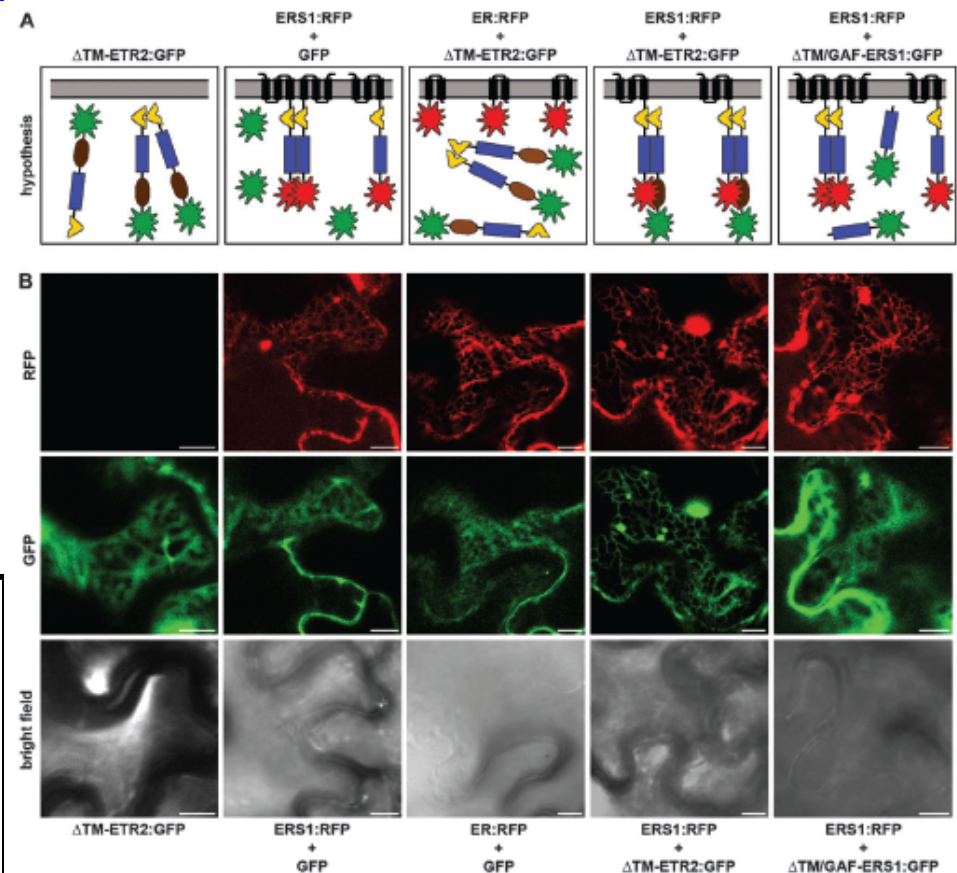
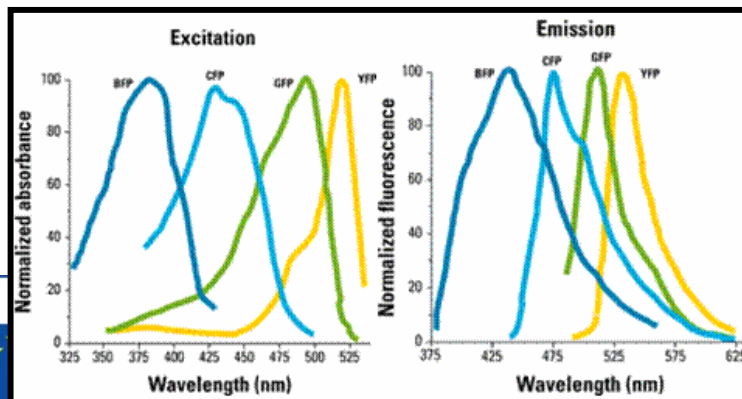
- Method for identification of interactions of cytoplasmic proteins with the membrane proteins



Membrane protein is fused with a fluorescent 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

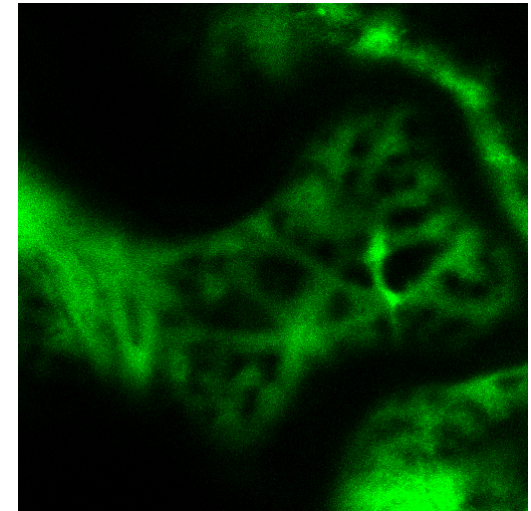
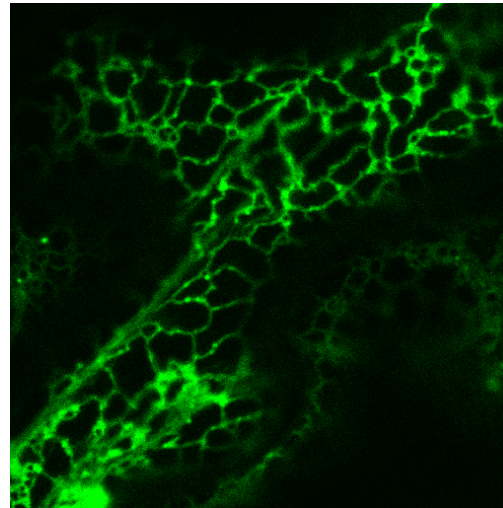
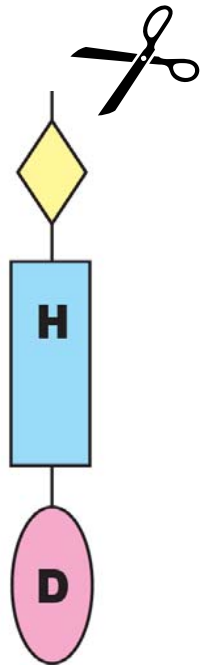


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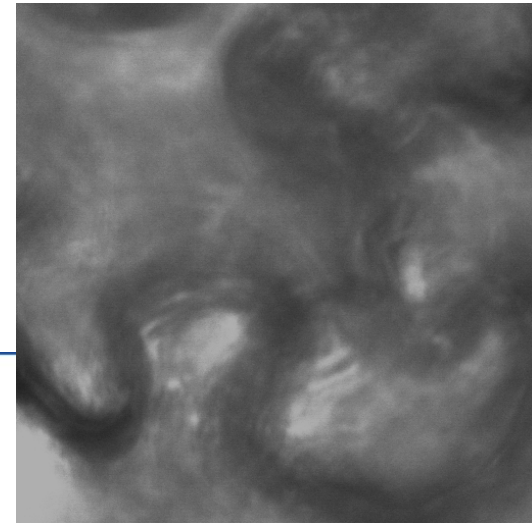
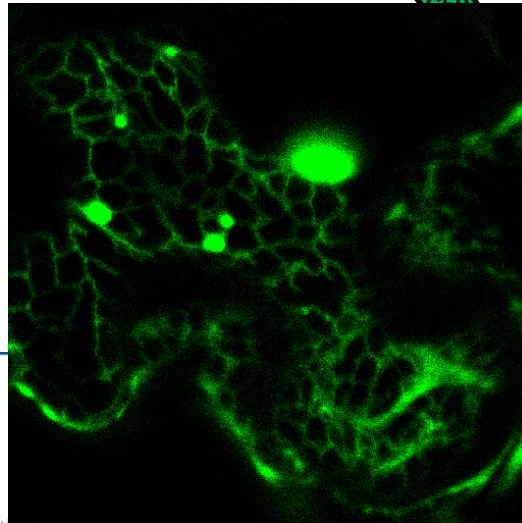
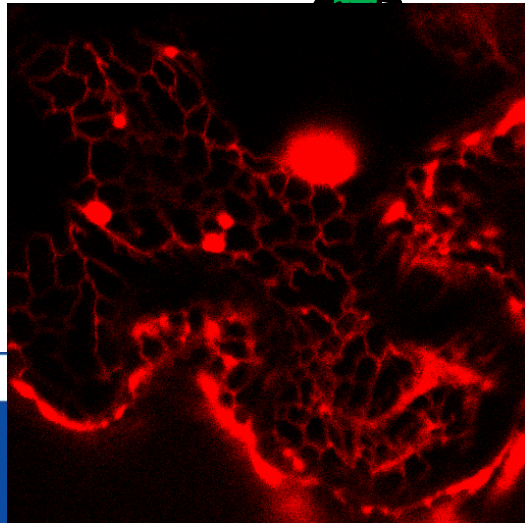
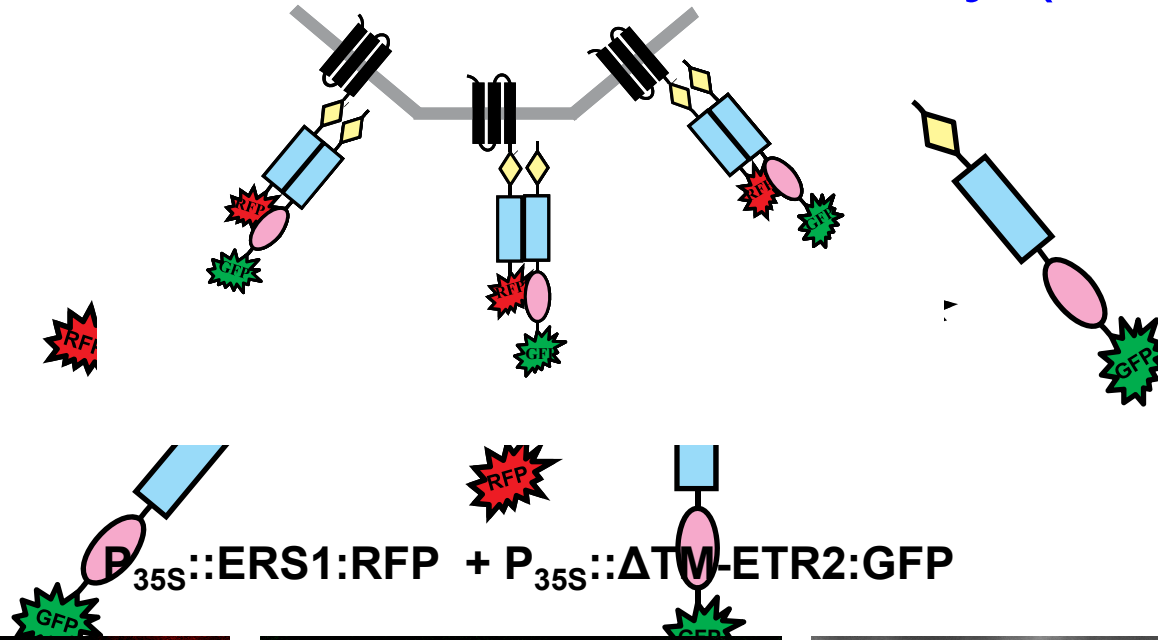
PI *in vivo*

Membrane Recruitment Assay (MeRA)



PI *in vivo*

Membrane Recruitment Assay (MeRA)

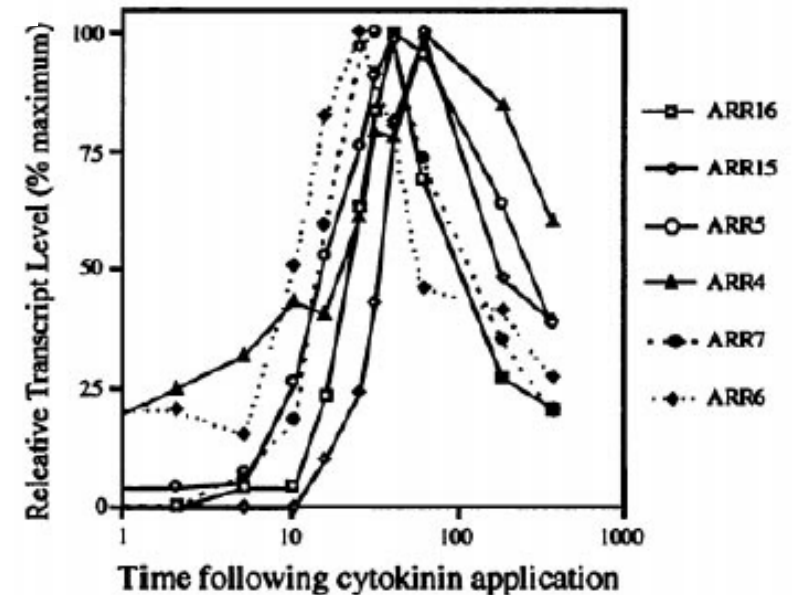
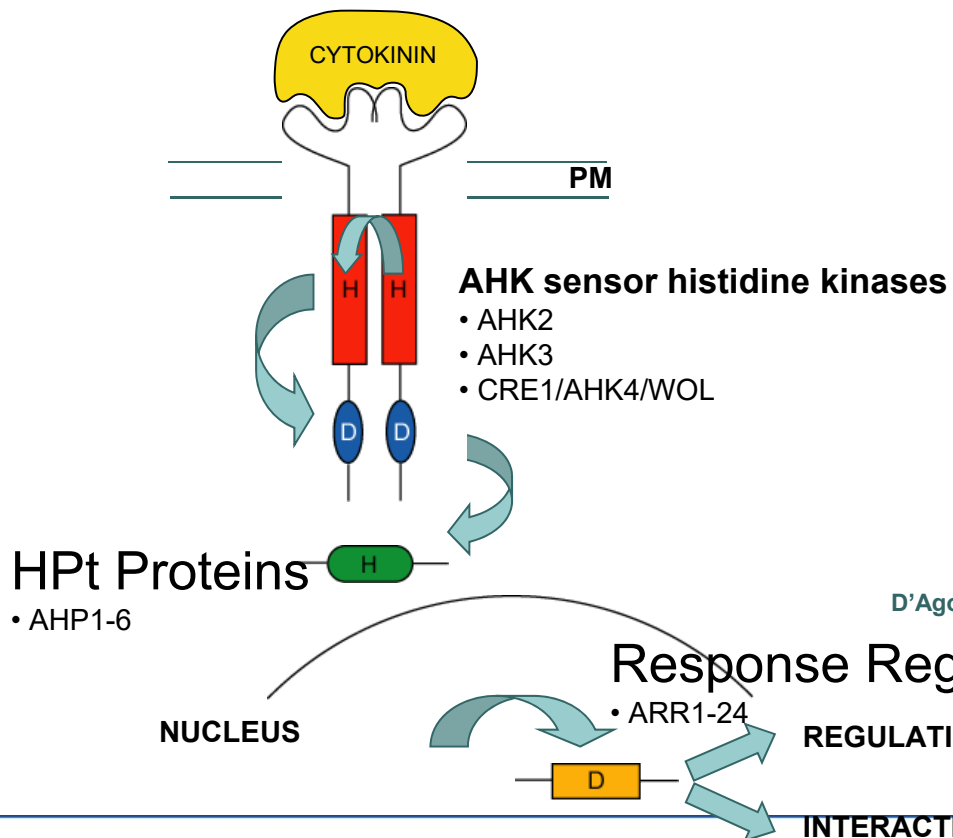


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Signal Transduction via MSP

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

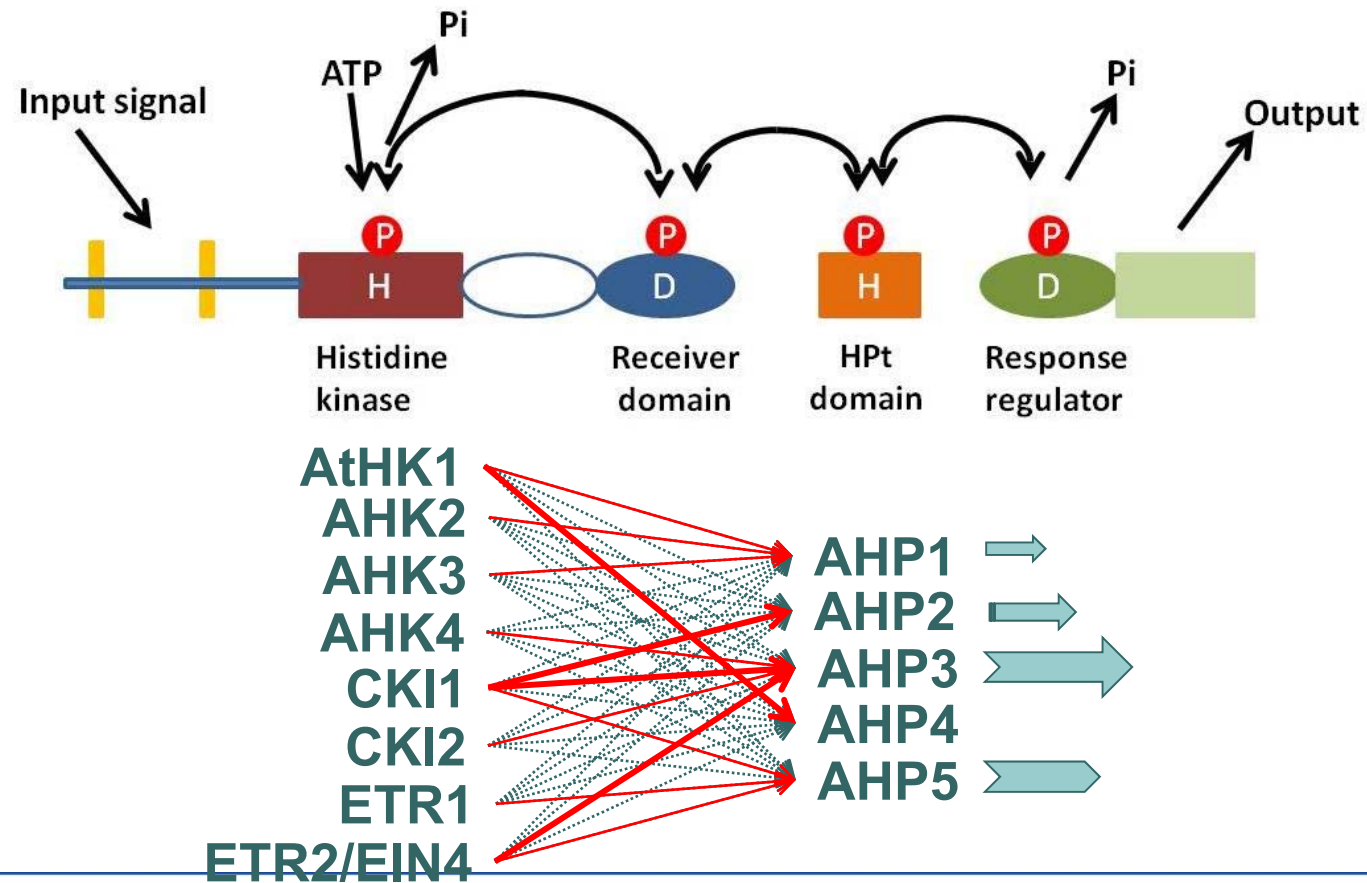


D'Agostino et al., Plant Phys, 2000

CK primary response genes
- Type-A ARR expression

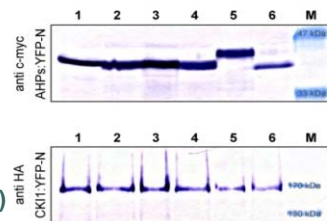
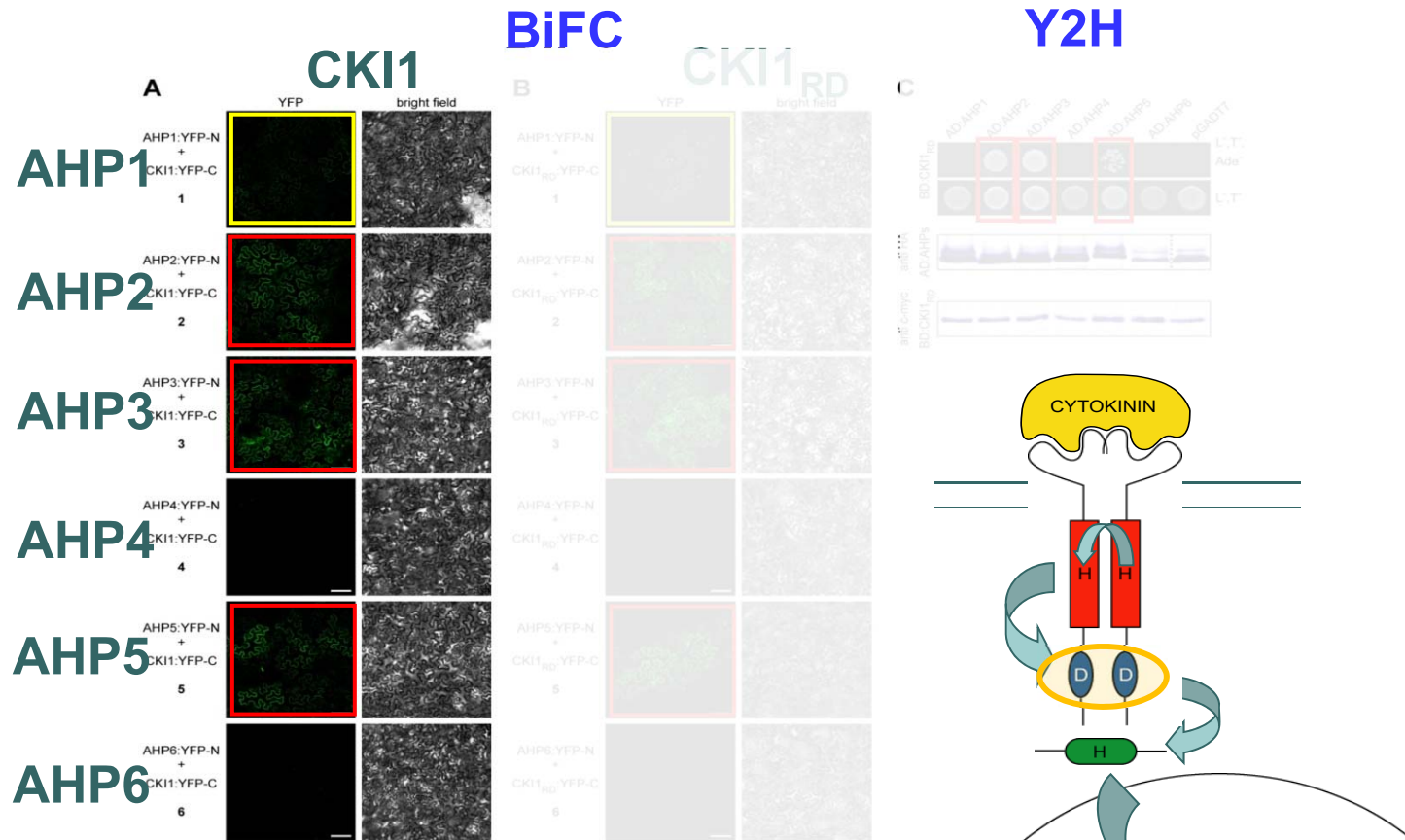
Is there any specificity in plant MSP?

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



Specificity of CKI1 signalling

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



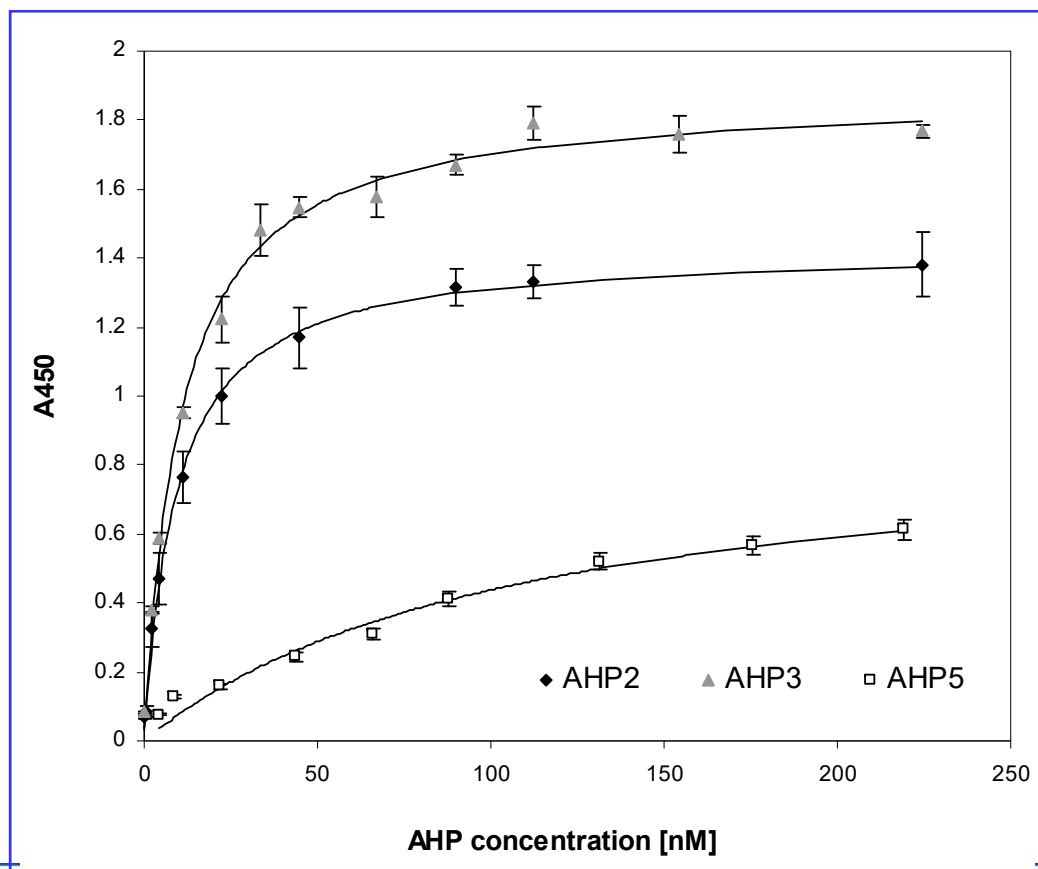
VZDĚLÁVÁNÍ

spolufinancována
sociálním fondem

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Specificity of CKI1 Signalling

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



AHP3: $K_d \sim 10,5$ nM

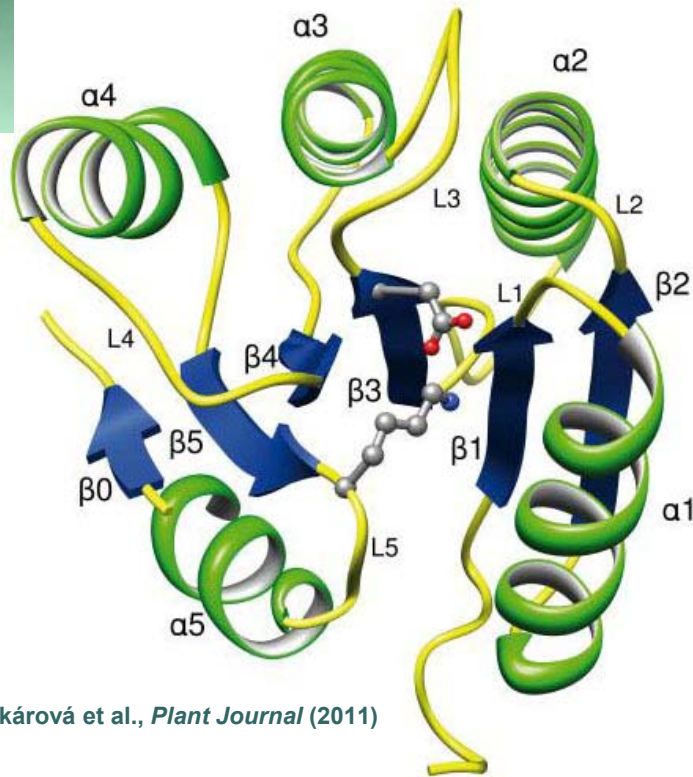
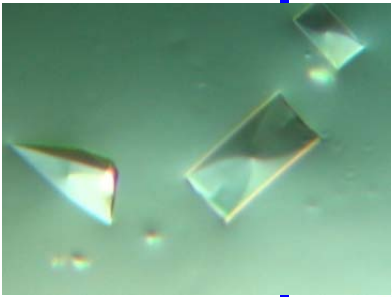
AHP2: $K_d \sim 9,17$ nM

AHP5: $K_d \sim 108$ nM

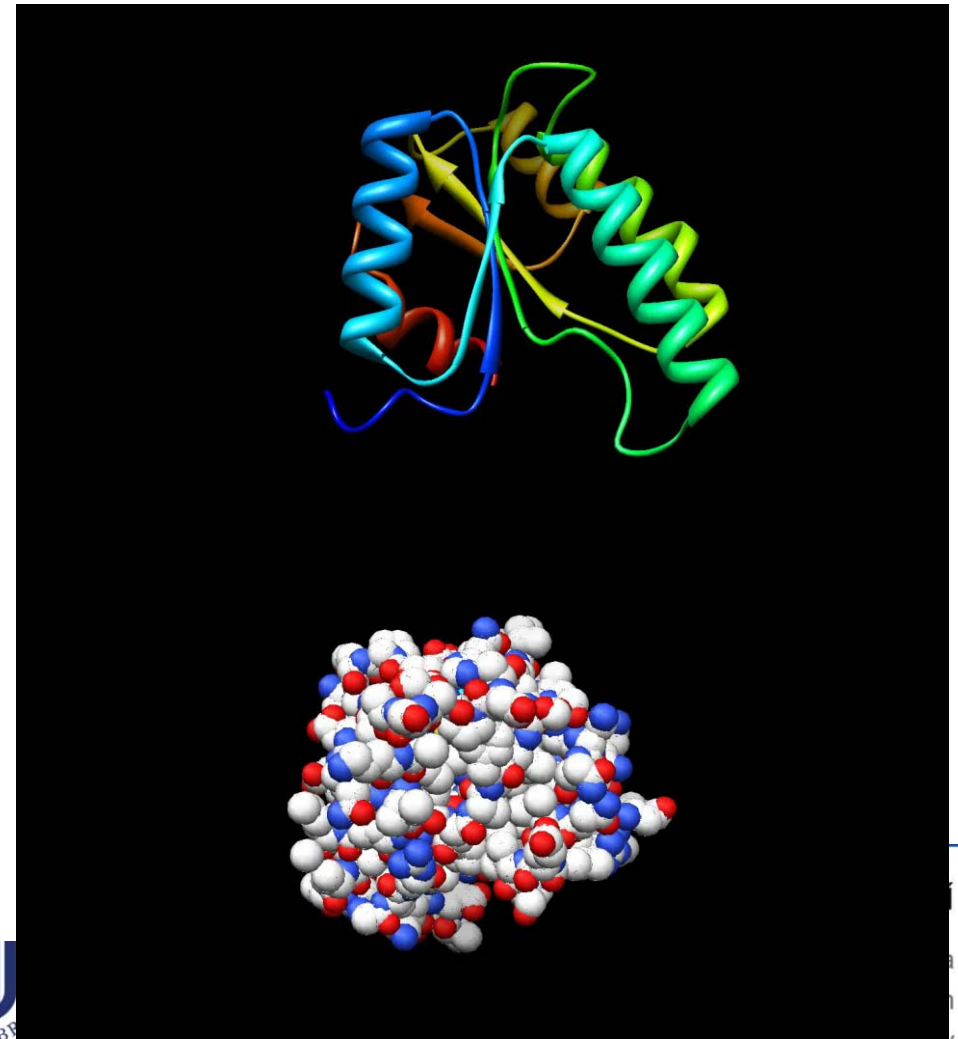
Pekárová et al., *Plant Journal* (2011)

Structure of CKI1_{RD}

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

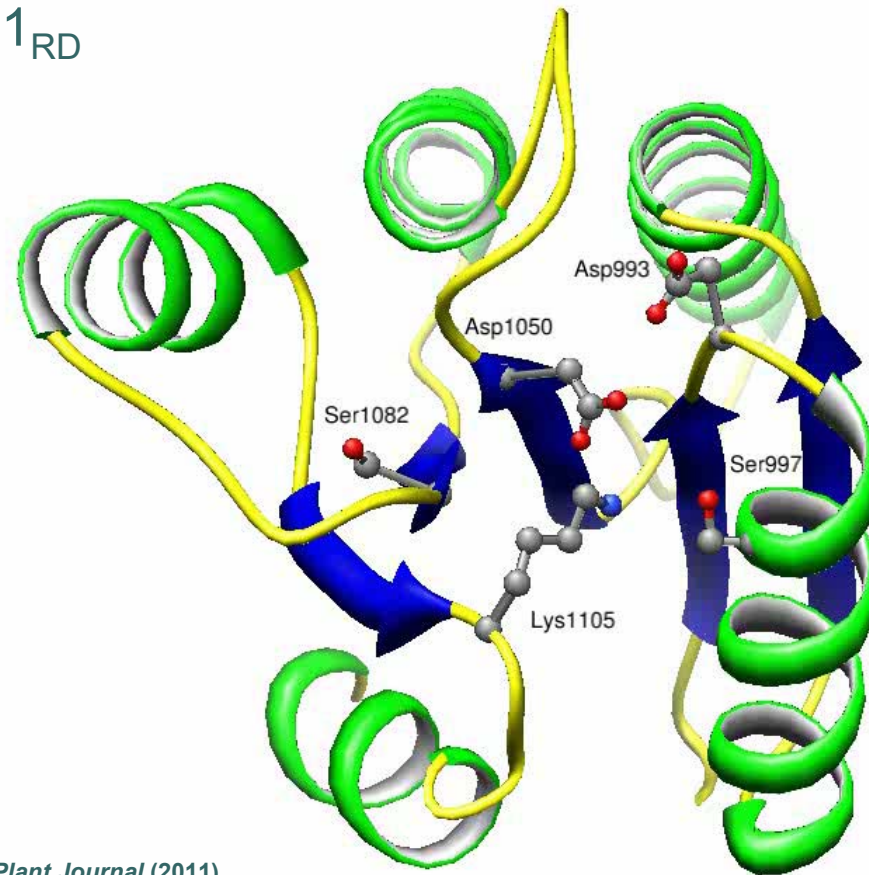


Pekárová et al., *Plant Journal* (2011)



Dynamics of CKI1_{RD}

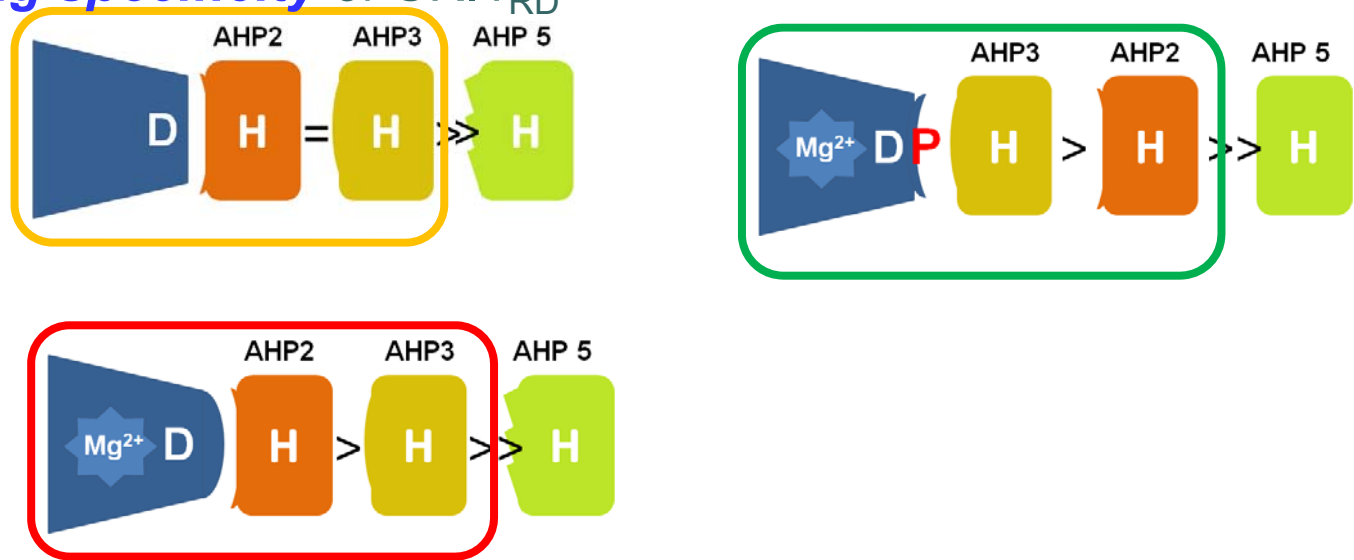
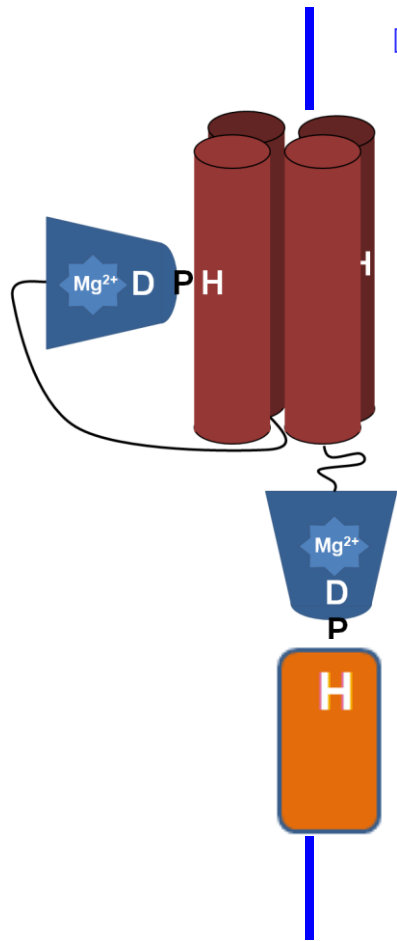
- *Mg²⁺ binding* leads to *remodelling of active centre* of CKI1_{RD}



Pokárová et al., *Plant Journal* (2011)

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

- *Mg²⁺*- and *BeF₃⁻*-induced *structural changes fine-tune binding specificity of CKI1_{RD}*



Ligand

0

Mg²⁺

BeF₃⁻

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

Pekárová et al., *Plant Journal* (2011)

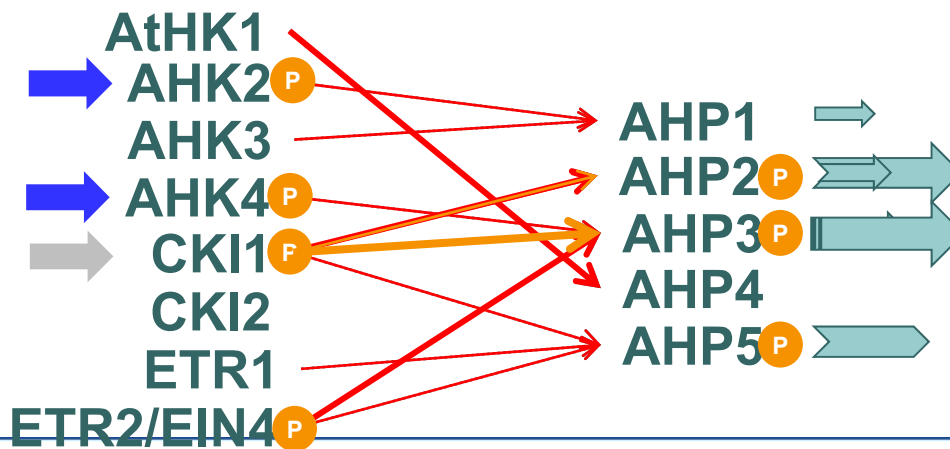
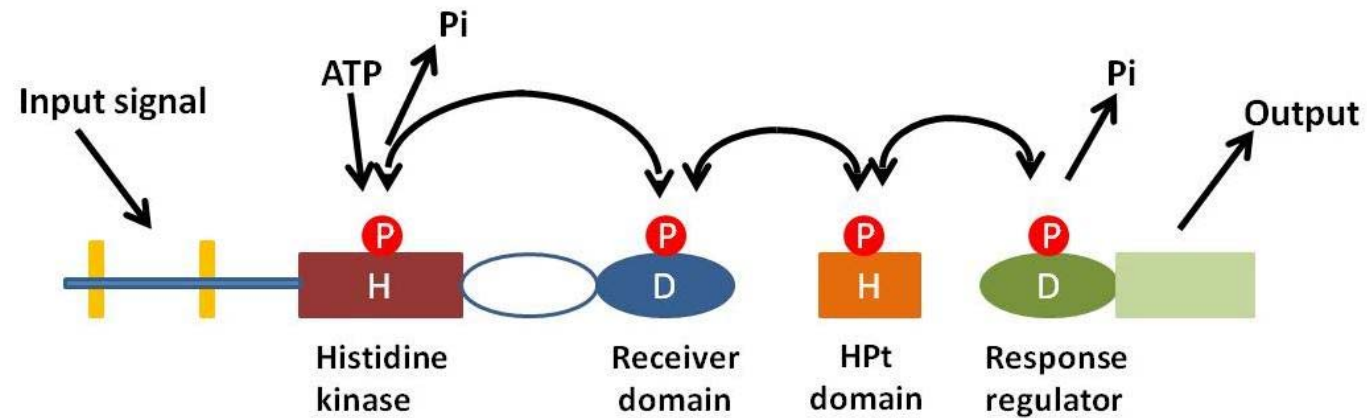


ÁVÁNÍ

ncována
n fondem
republiky

Model Suggestion

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



Summary

- Functional importance of the specific interactions of proteins in the regulation of gene expression
 - Chromatin structure
 - Regulation of transcription
 - mRNA localization
 - mRNA stability
 - 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

Discussion



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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