CG920 Genomics

Lesson 7

Protein Interactions in Gene Regulations

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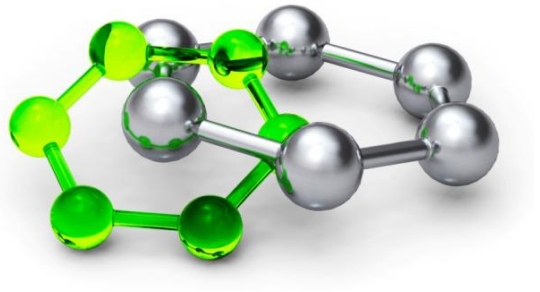

Literature

- Literature sources for Chapter 06:

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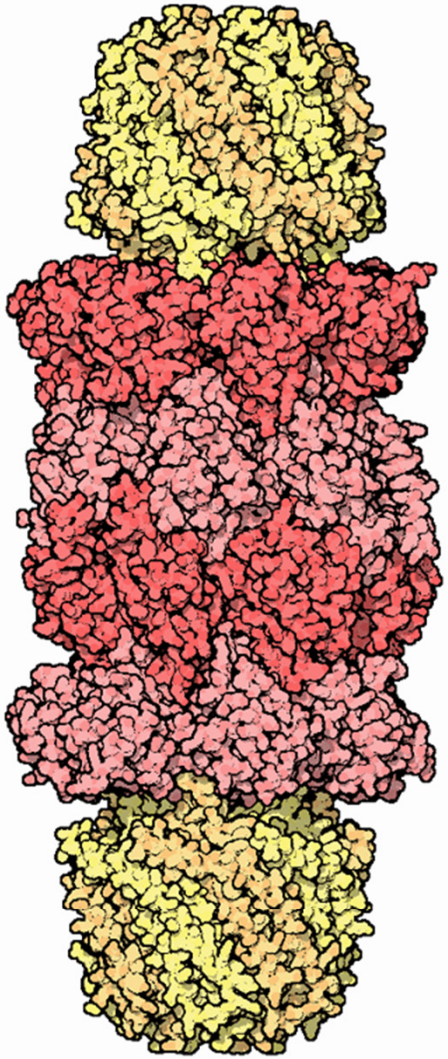
Outline

- 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

Importance of Protein Interactions

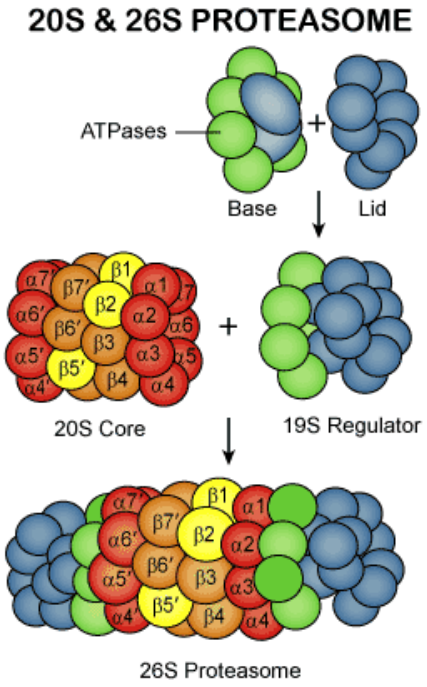
- **Functional importance of specific protein interactions**
  - Most of the proteins in the cell exist in the form of complexes which may further interact with each other
    - Proteasome
      - protein complex responsible for the degradation of obsolete proteins in the cell

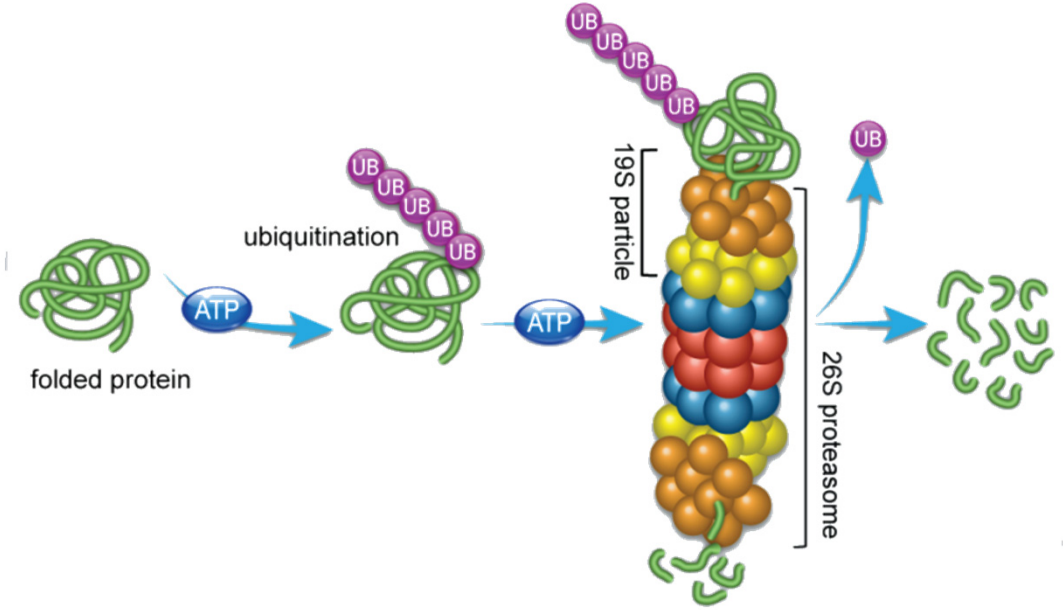


The importance of protein interactions

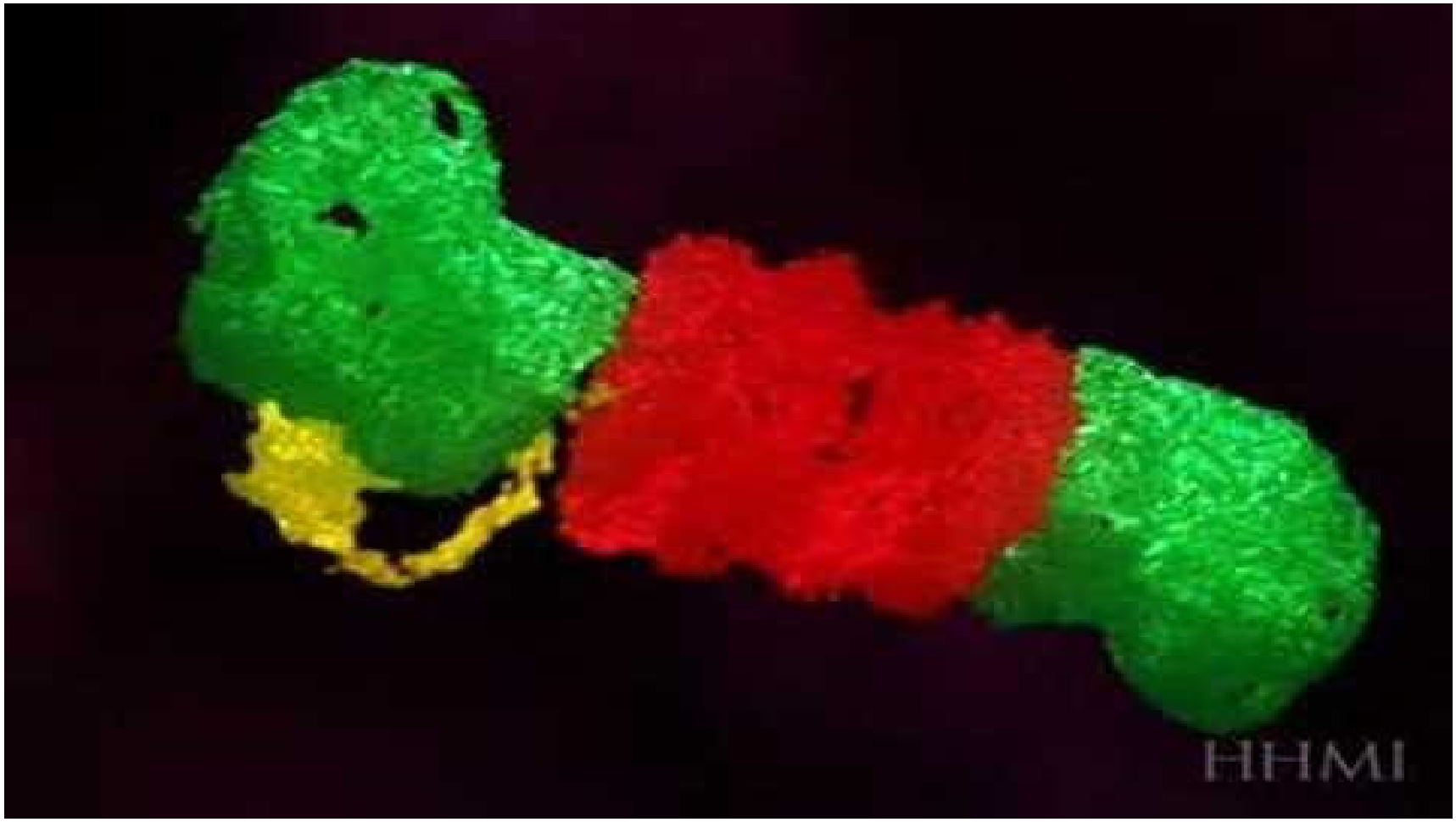
Proteasome

- Consisting of a **core**, also being designated as **20S** and **regulatory portions** (19 or 11S)
- Allows **targeted degradation** of proteins labelled by a specific marker - small polyppetide (76 aa) called **ubiquitin**



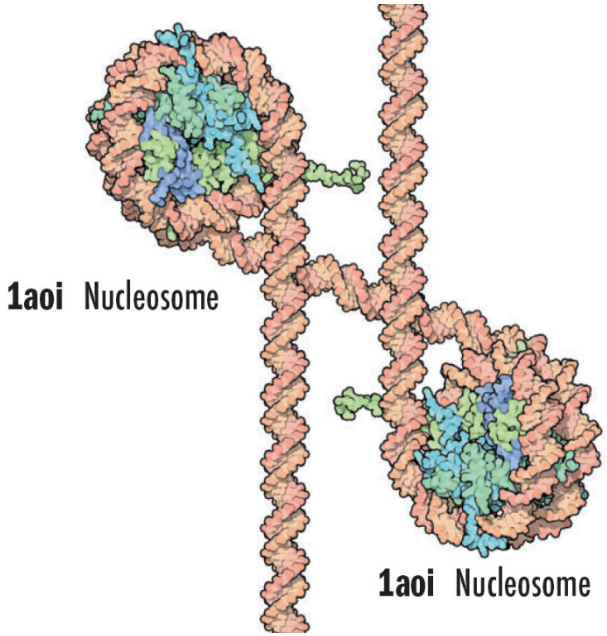


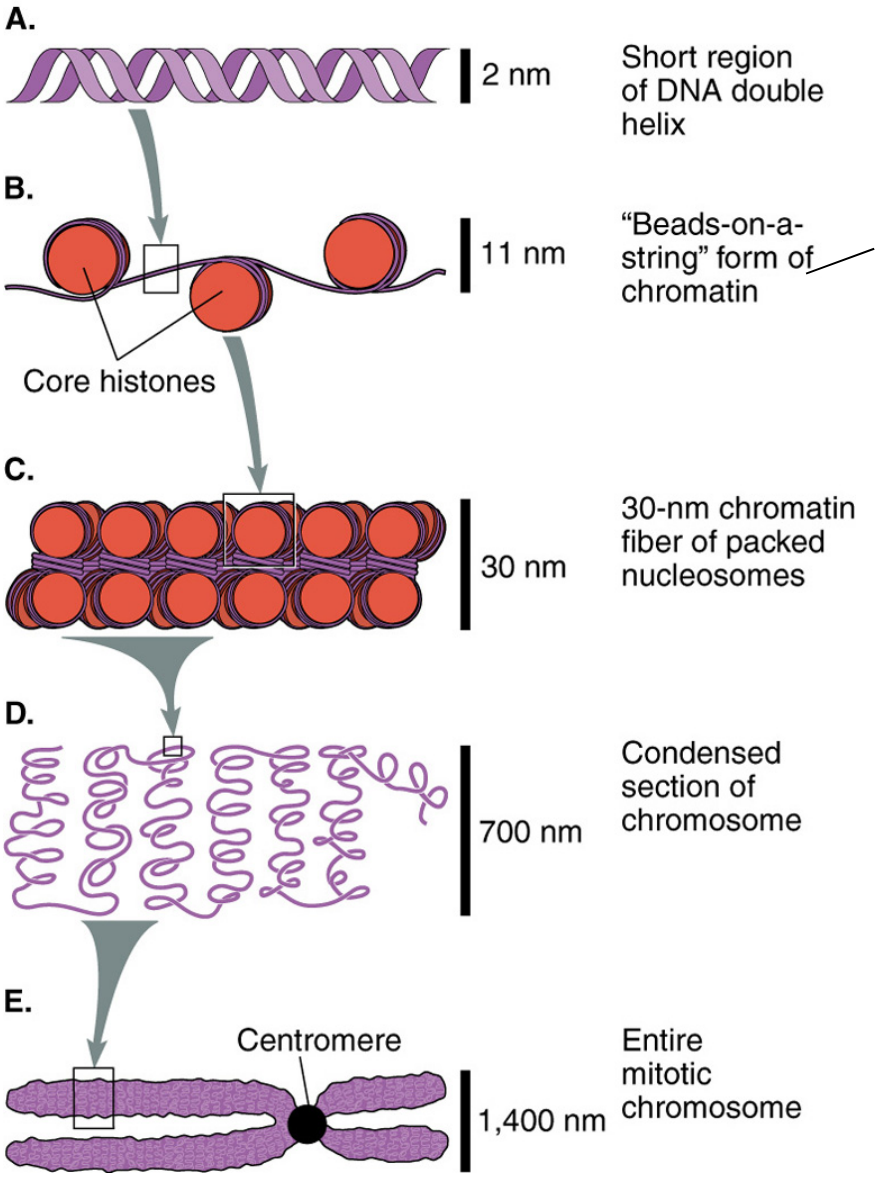
Proteasome –targeted proteolysis



Importance of Protein Interactions

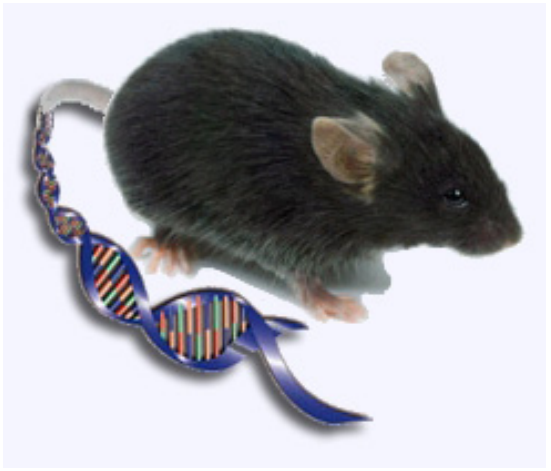
- Functional importance of specific protein interactions
  - Chromatin structure

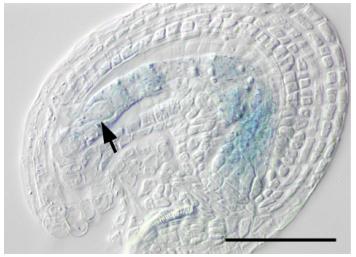




Regulation by histone acetyl transferases or histone deacteylases

DNA methylation in animals vs. in plants





methylation status

CpG

methylation status

CpG or CpNpG

CpNpNp

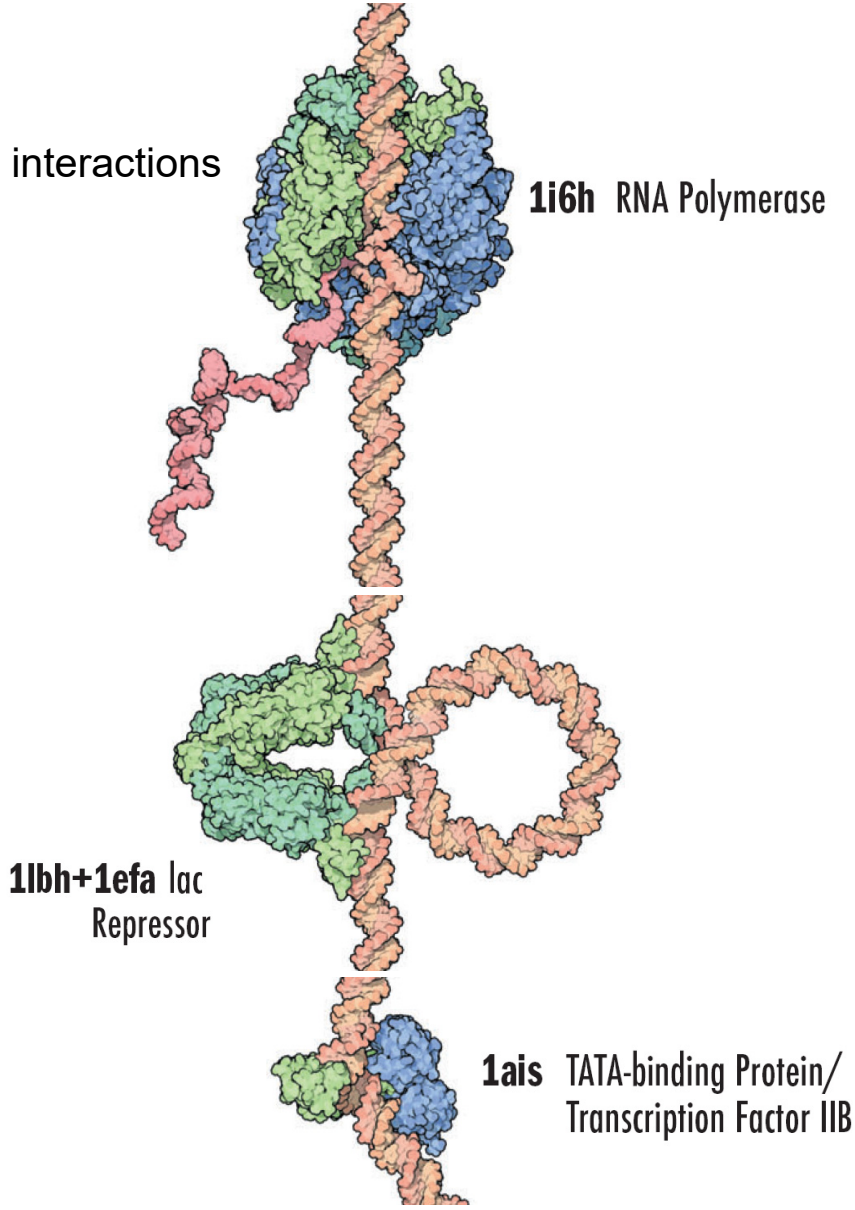
Cell-specific methylation allows maintain of tissue-specific gene expression profiles

↓

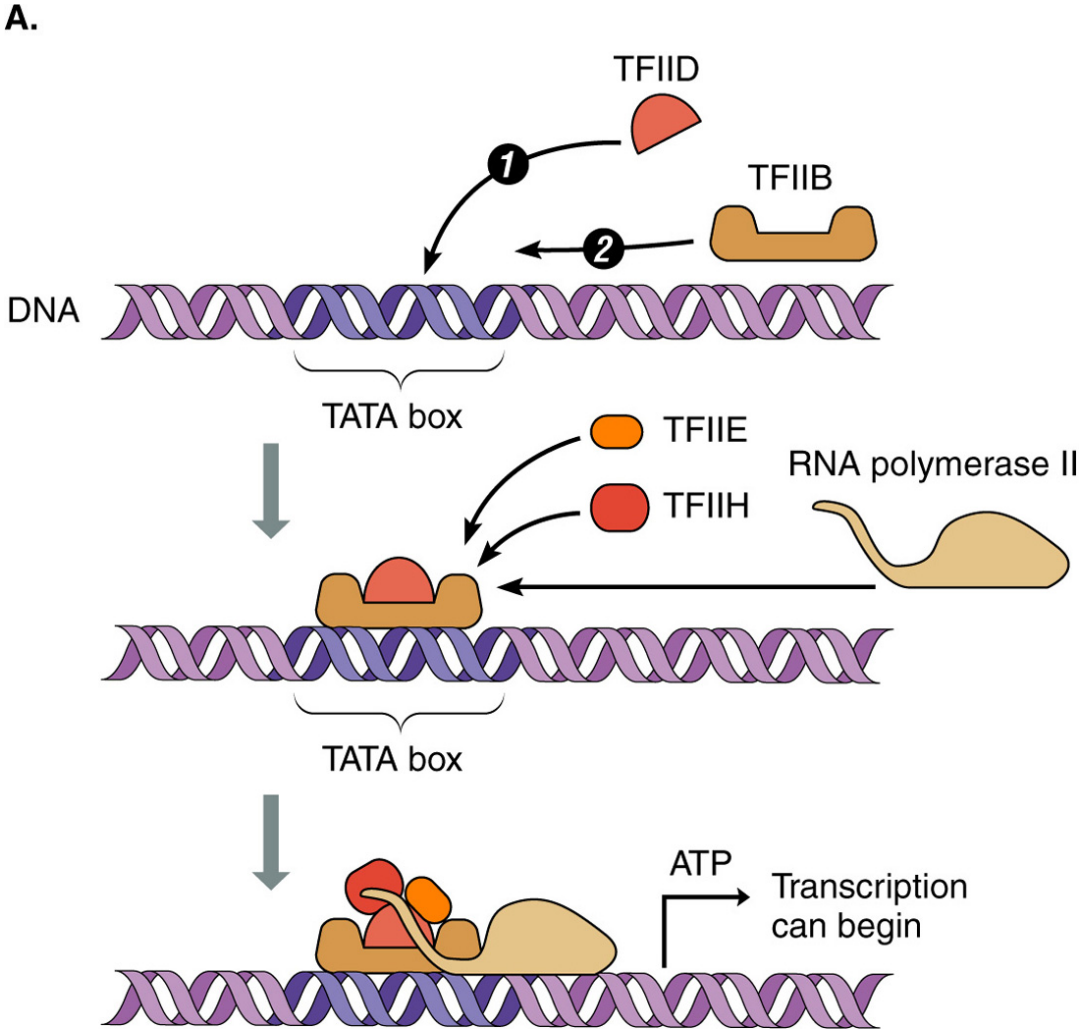
Imprinting and "cell memory" → **Mechanism of transcriptional regulation by DNA methylation mostly unknown**

Importance of Protein Interactions

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



Initiation of Transcription



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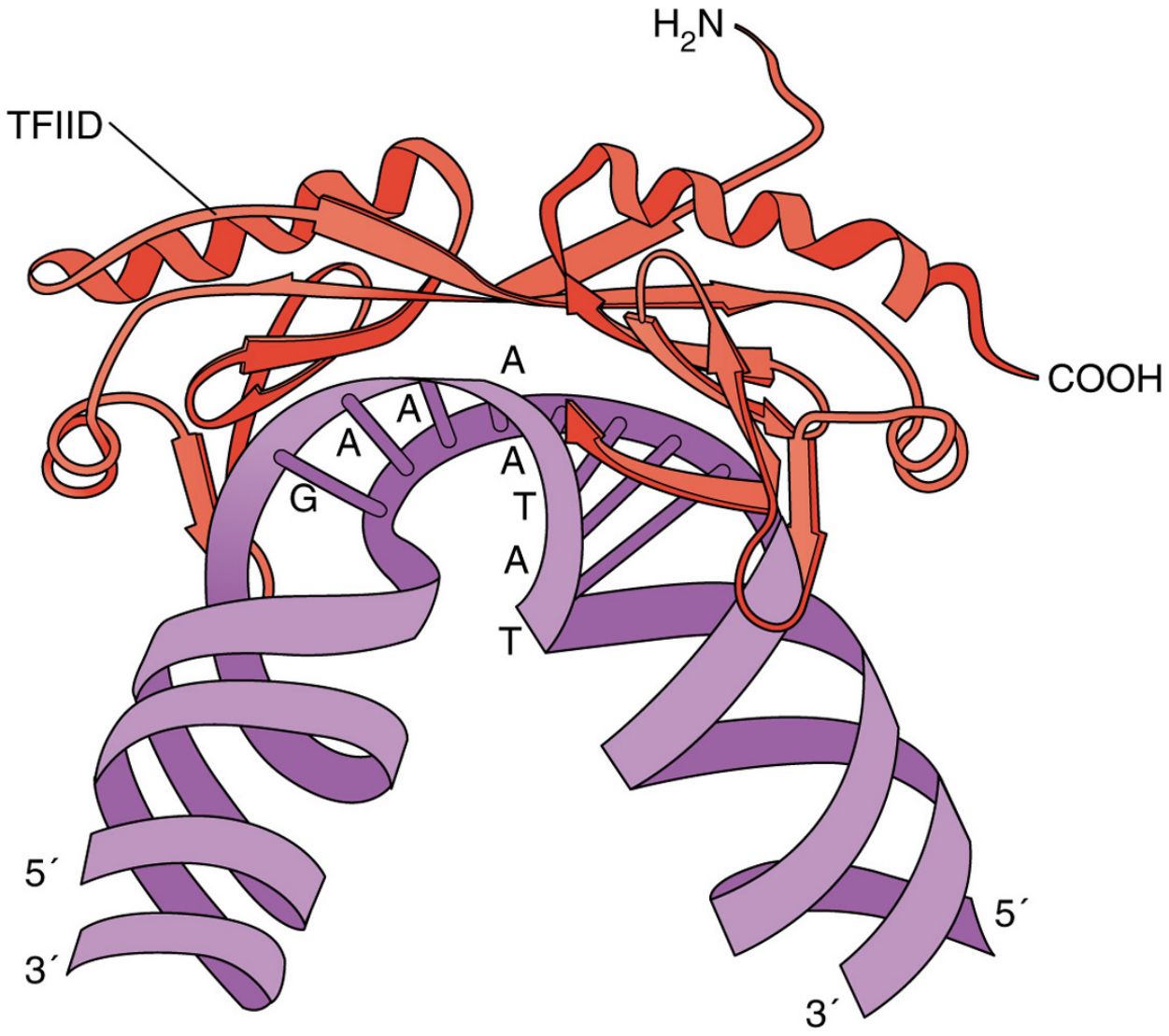

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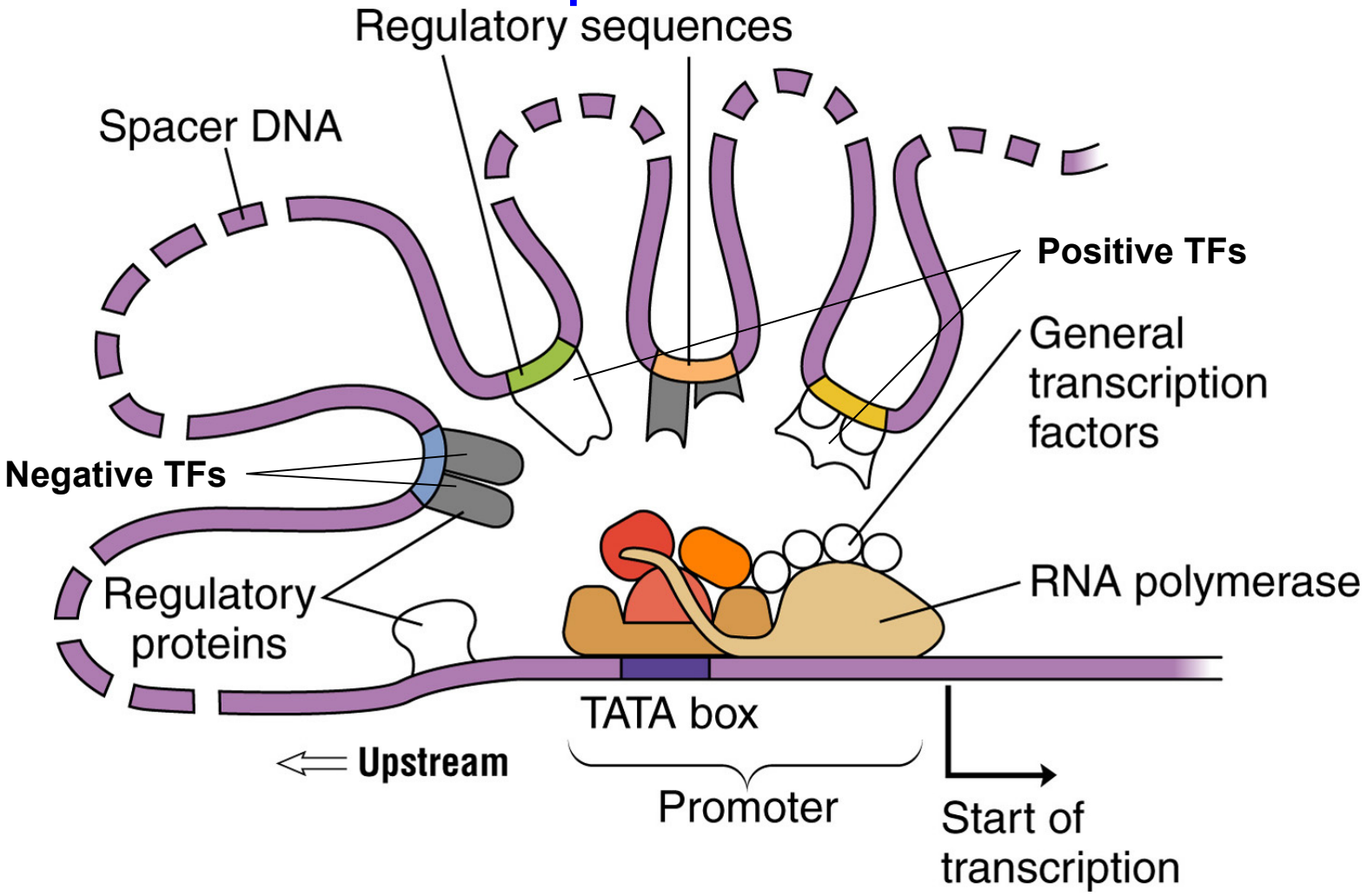

Transcriptional Regulation by TAFs

Signal recognition

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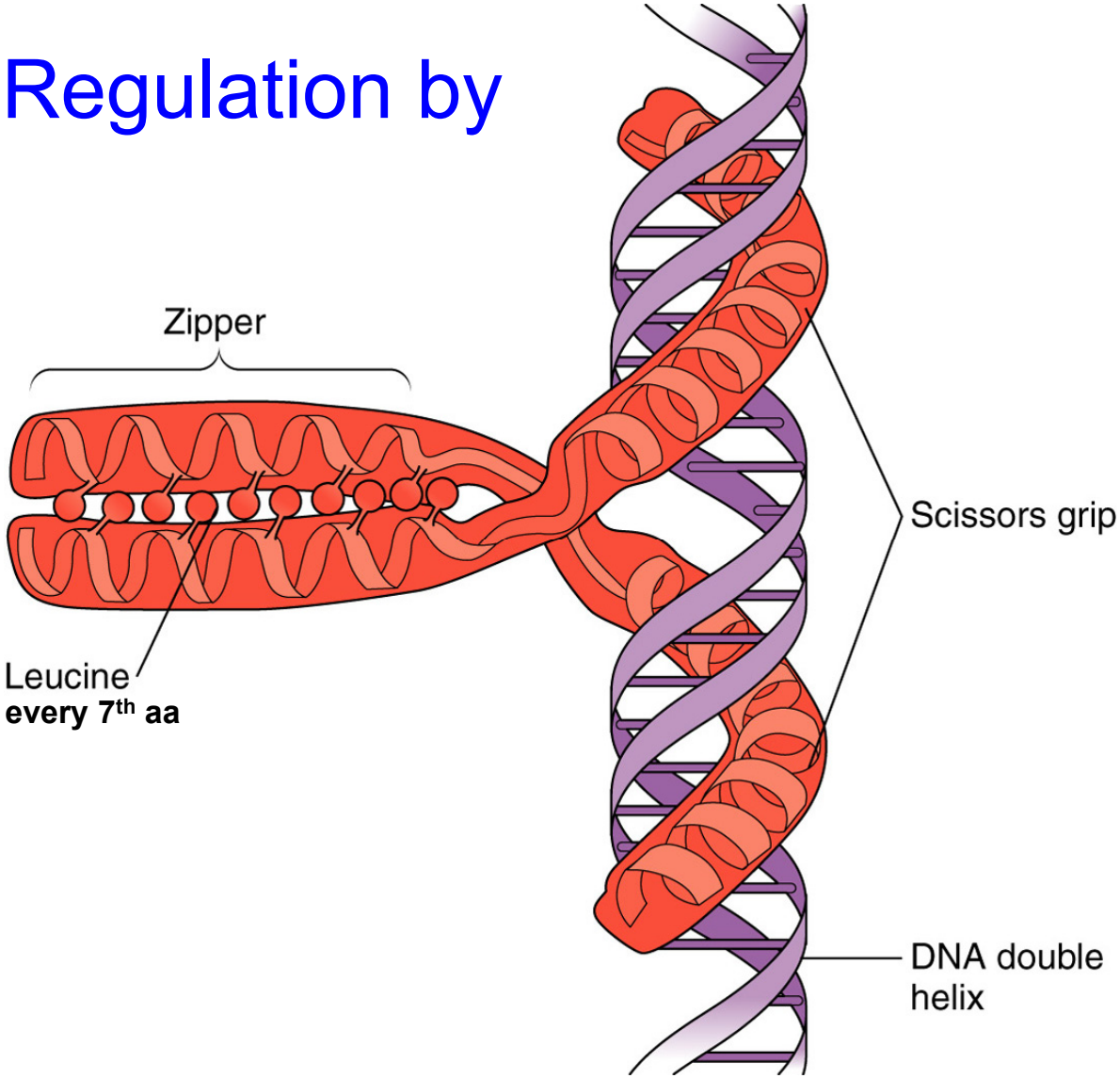
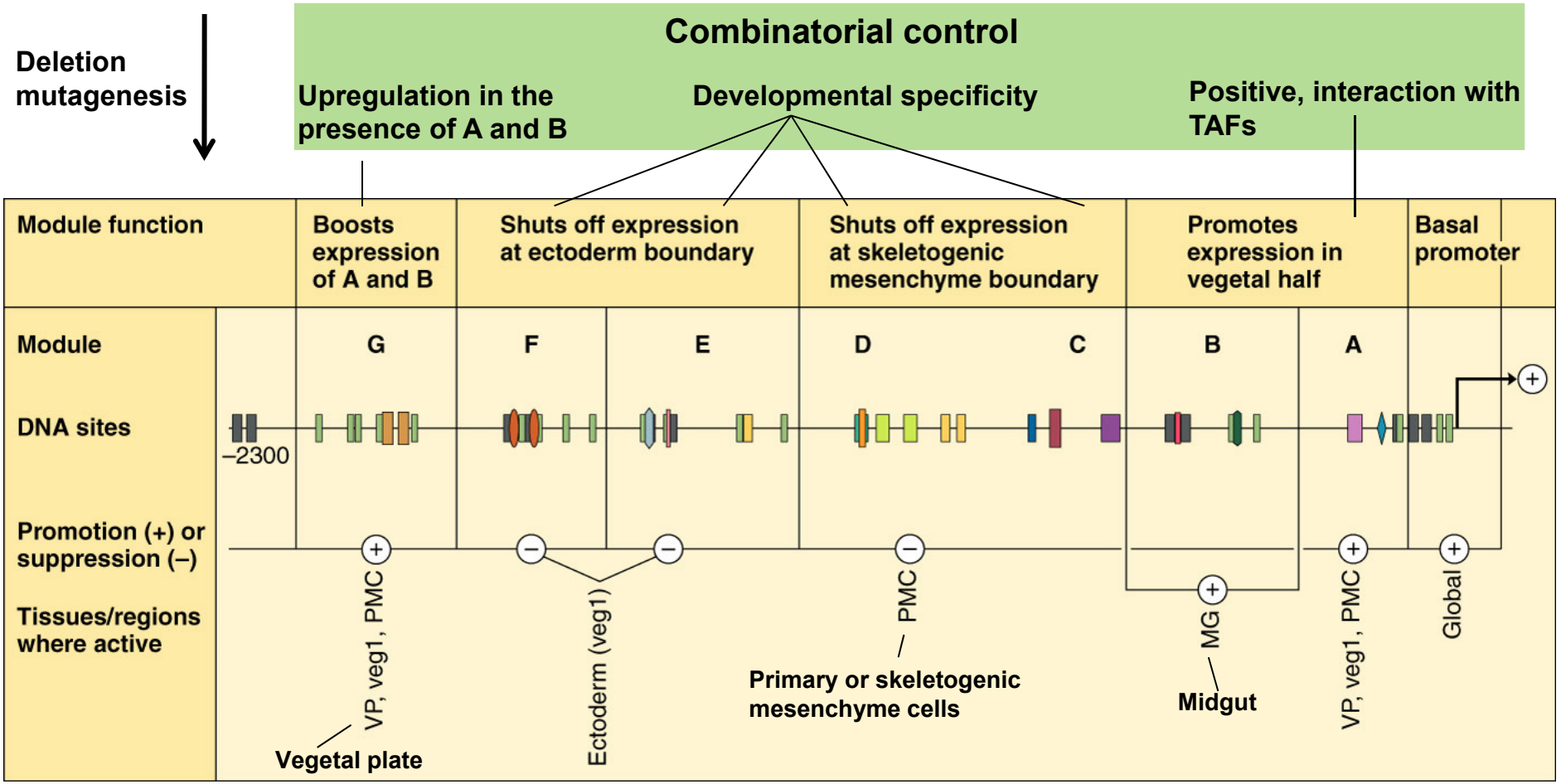
Dimerization →

DNA binding and transcription activation



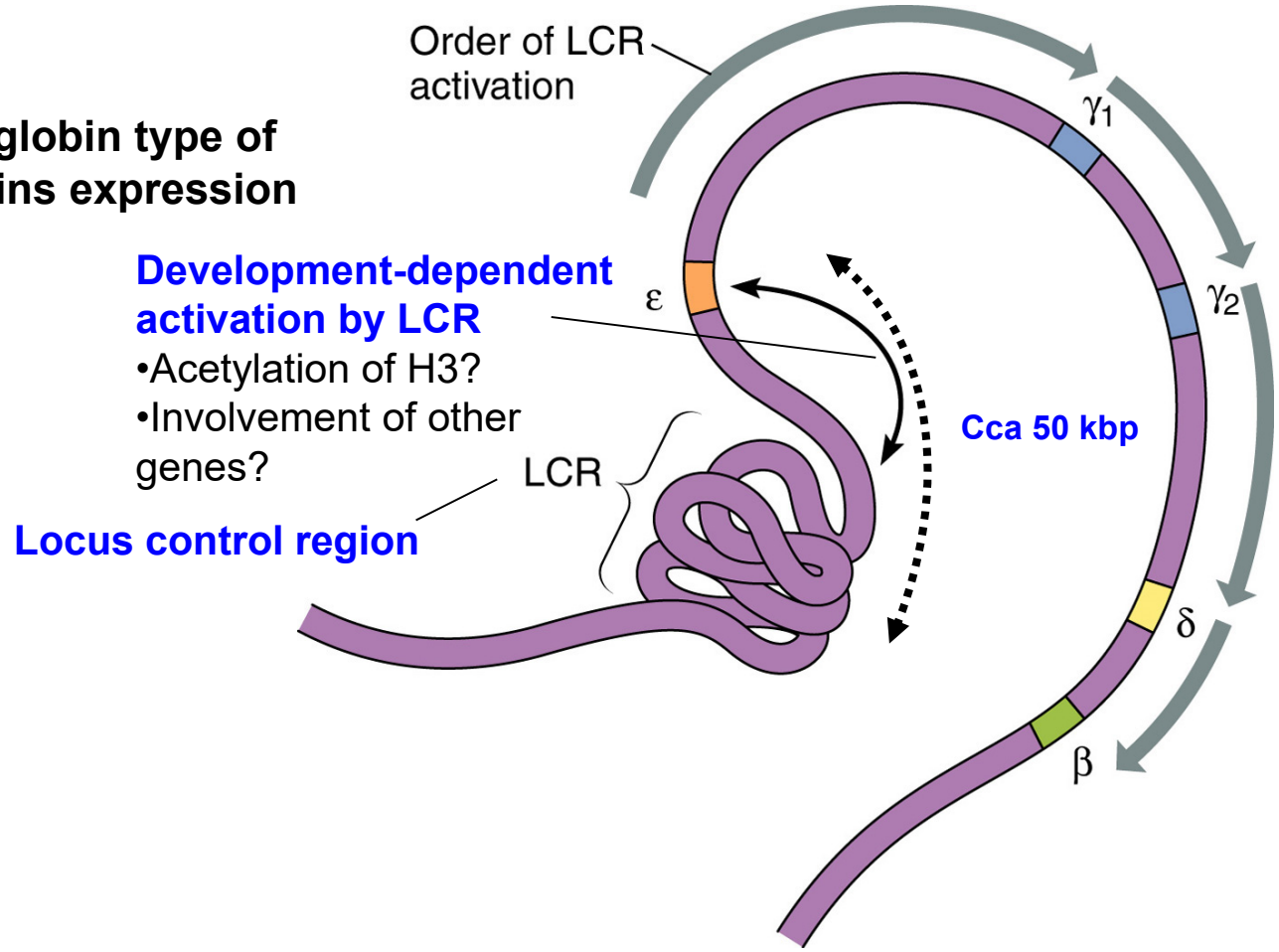
Multifactorial Promoters Control

ProENDO16:REPORTER **(sea urchin)**



Multifactorial Promoters Control

Regulation of β-globin type of hemoglobin chains expression

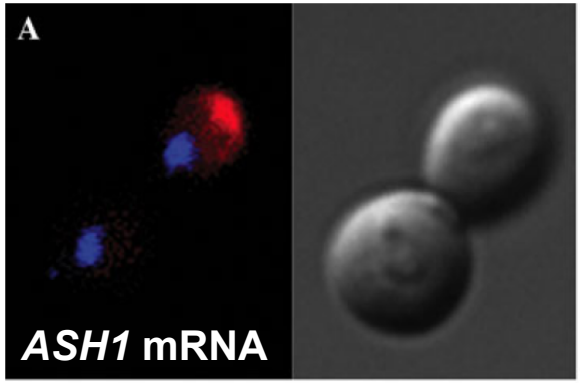


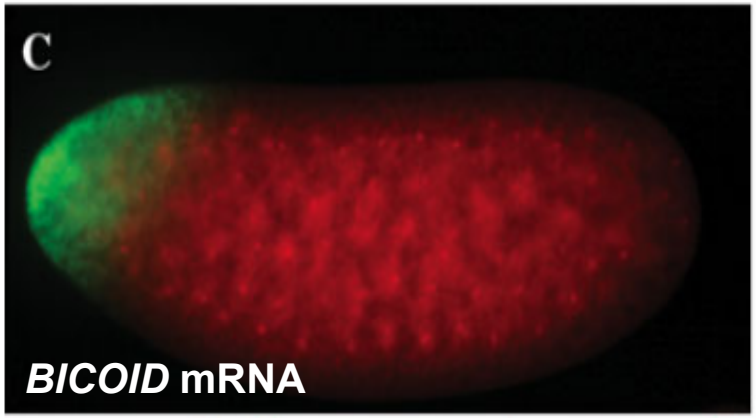
Importance of Protein Interactions

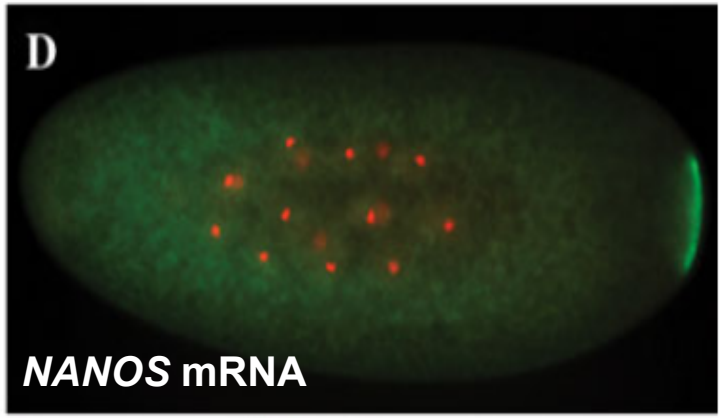
- Functional importance of specific protein interactions
  - Chromatin structure
  - Regulation of transcription
  - mRNA localization

mRNA localization

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



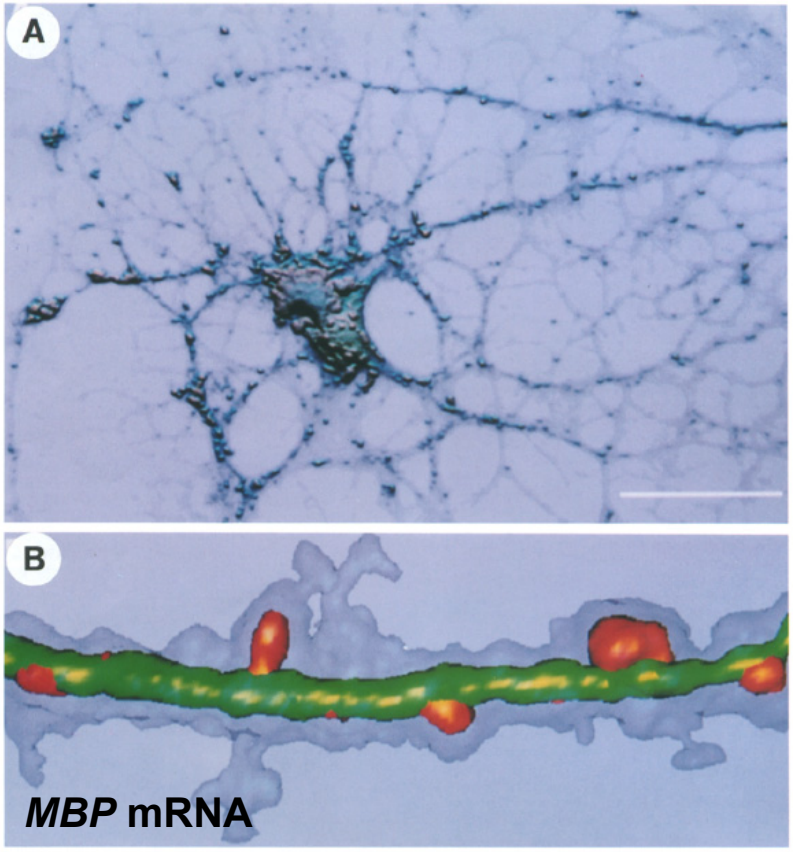




Shahbabian and Chartrand, 2012

mRNA localization

- **Role of mRNA localization**
  - Attenuating the expression of potentially toxic proteins
    - Localization of expression of MYELIN BASIC PROTEIN (MBP) into myelination regions of nerve cells

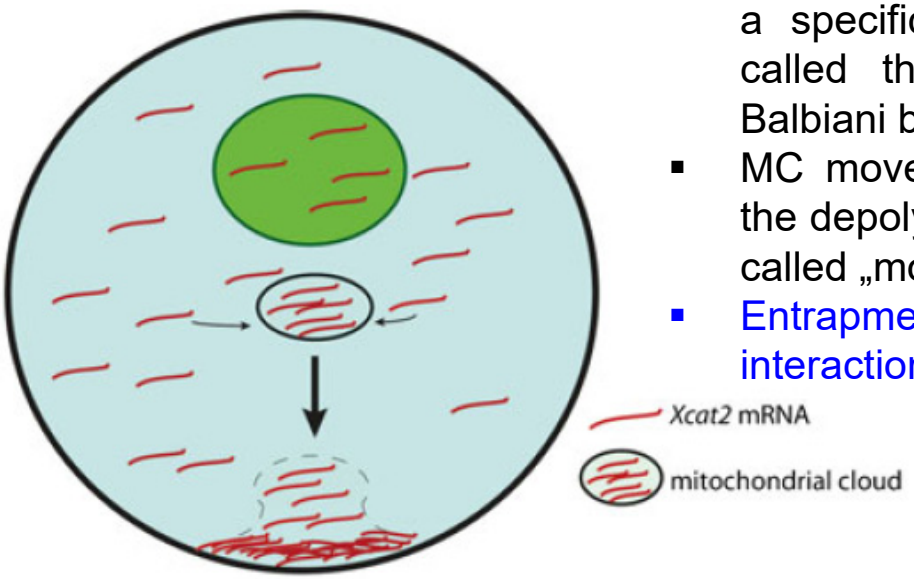


Ainger et al., 1993

mRNA localization

Mechanisms

- **Diffusion and entrapment of mRNA**



Shahbabian and Chartrand, 2012

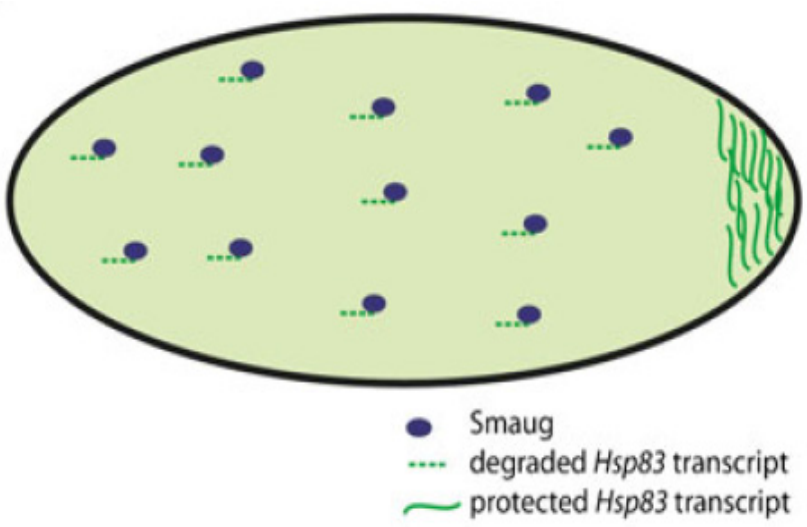
- During the early stages of *Xenopus* oogenesis, *Xcat-2* mRNA is restricted to a specific structure in the cytoplasm called the mitochondrial cloud (MC, Balbiani body)
- MC movement is partly dependent on the depolymerization of microtubuls (so-called „molecular motor“)
- Entrapment on the vegetal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

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

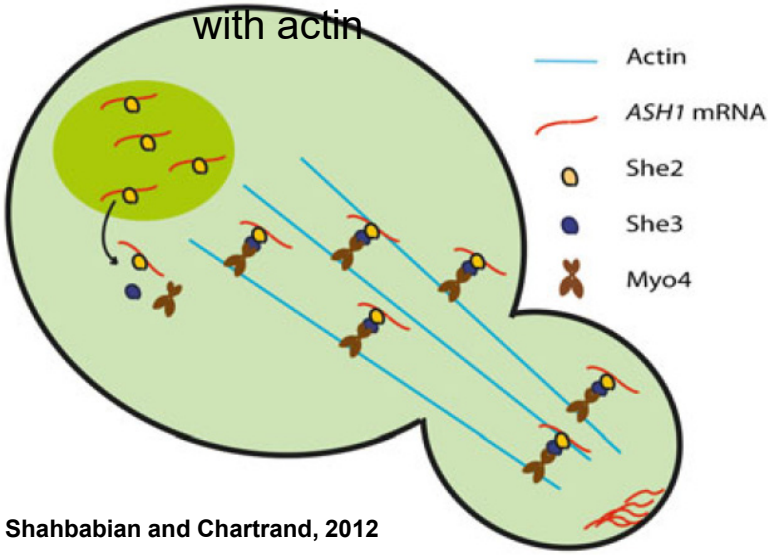


- HIE elements are recognized by SMAUG protein, which mediates binding of degradation complex CCR4/POP2/NOT
- In the posterior pole the *Hsp83* mRNA is protected from the effects of SMAUG by the so-called HPE element in 3'UTR; mechanism of this protection is still unknown

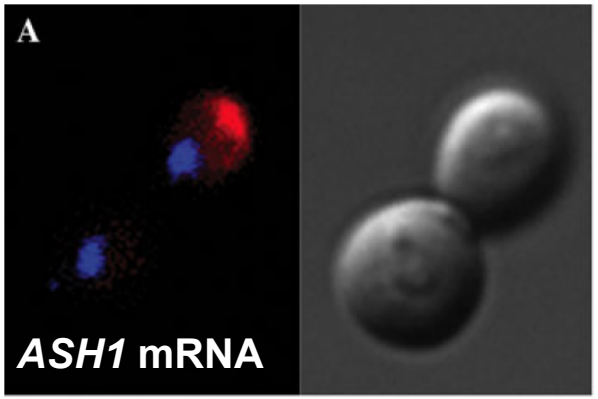
mRNA localization

Mechanisms

- **Active transport of mRNA**
  - Asymmetric Synthesis of HO1 (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

Importance of Protein Interactions

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

Importance of Protein Interactions

- Functional importance of specific protein interactions
  - Chromatin structure
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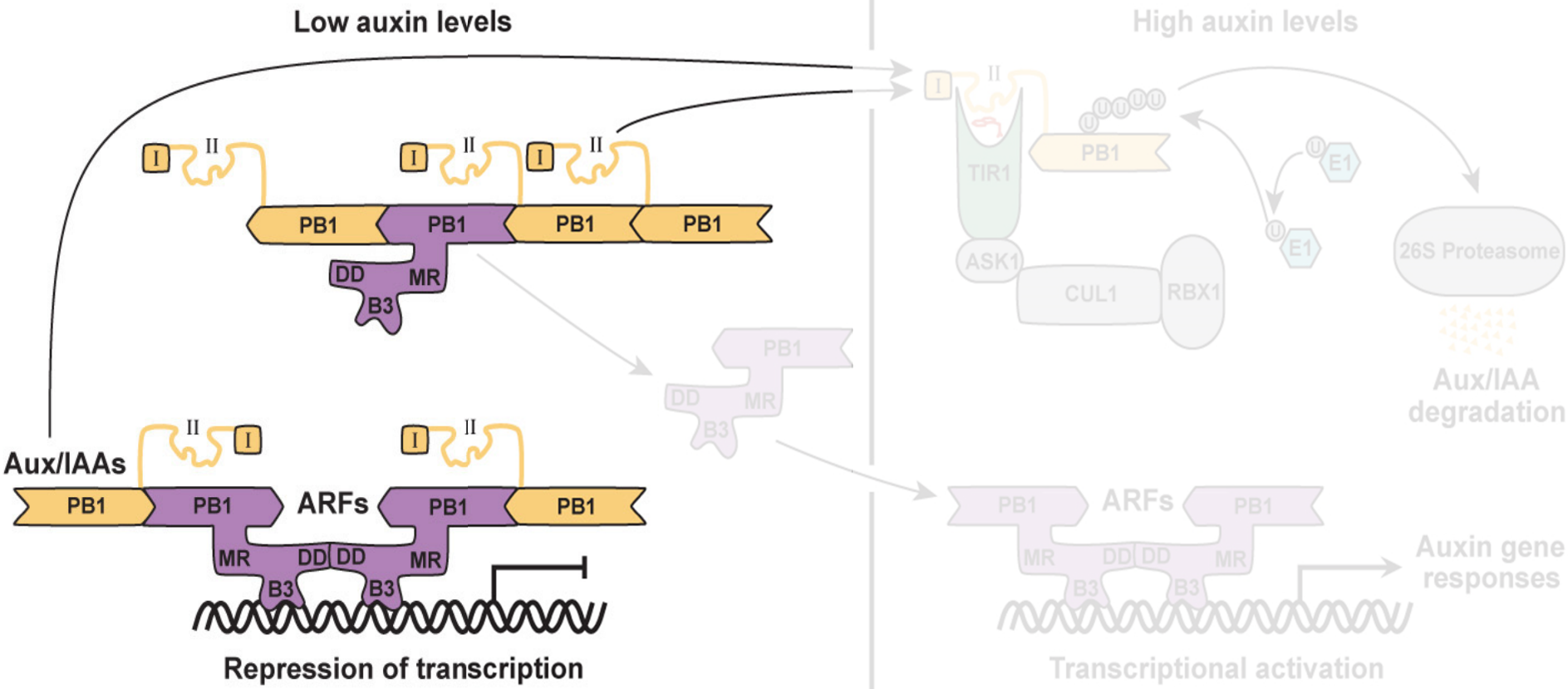
  - hnRNA splicing
  - Protein stability

Auxin Signalling



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

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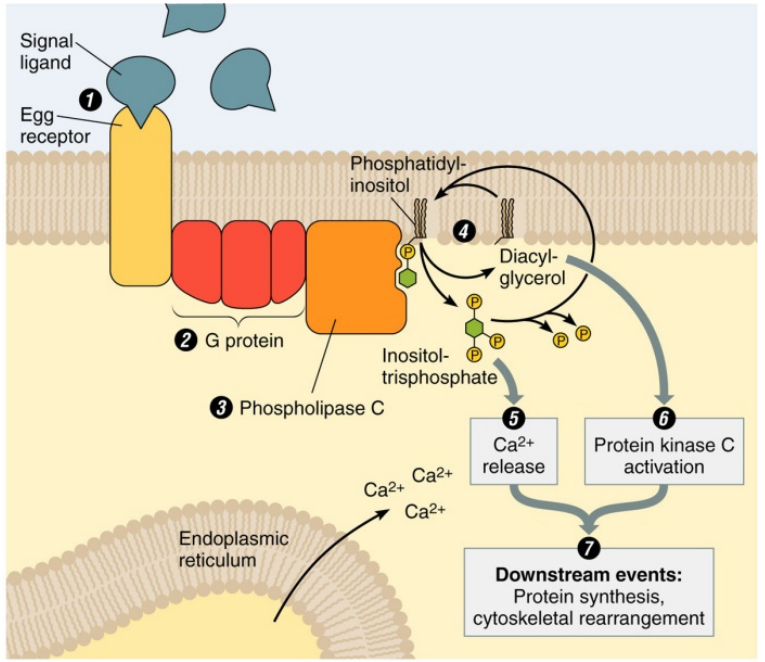
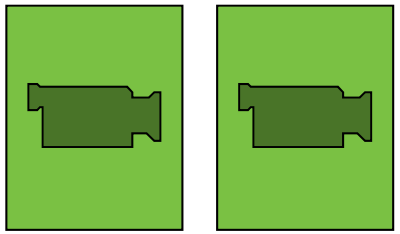
Importance of Protein Interactions

- Functional importance of specific protein interactions
  - Chromatin structure
  - Regulation of transcription
  - mRNA localization
  - hnRNA splicing
  - Protein stability
  - Signal transduction

Signal transduction

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





Outline

- Functional importance of the specific interactions of proteins in the regulation of gene expression
  - Chromatin structure
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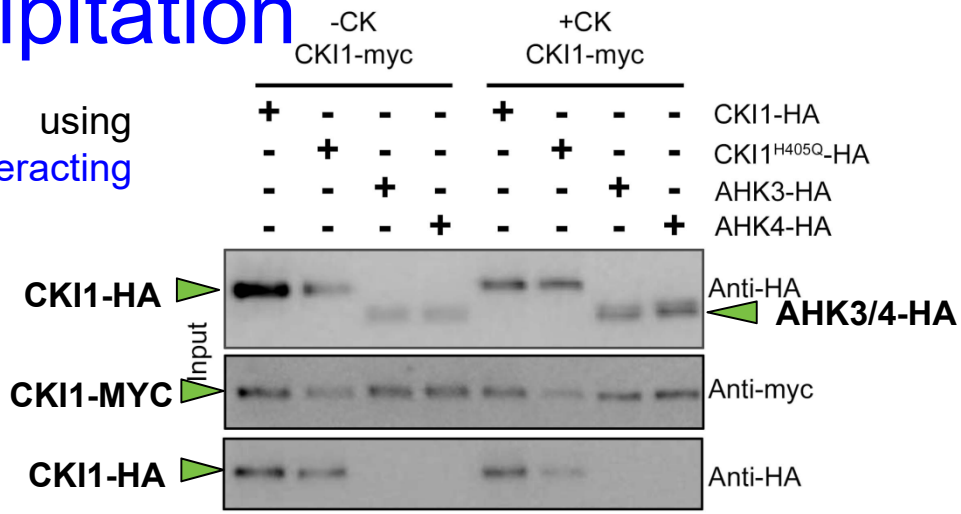
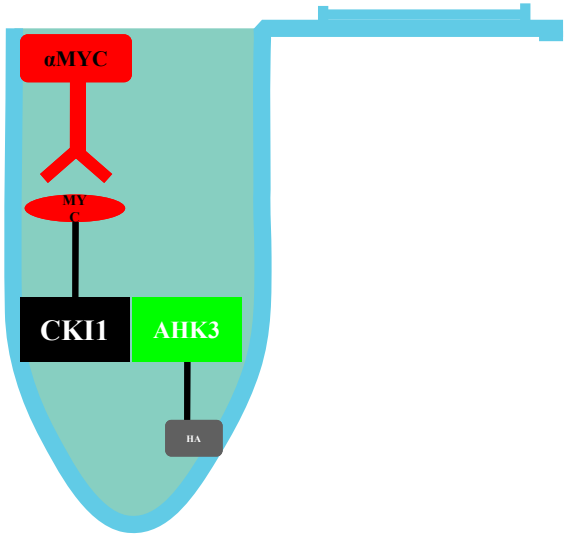
- Methods of analysis of protein interactions *in vivo*
  - Co-immunoprecipitation

PI *in vivo*

Co-immunoprecipitation

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





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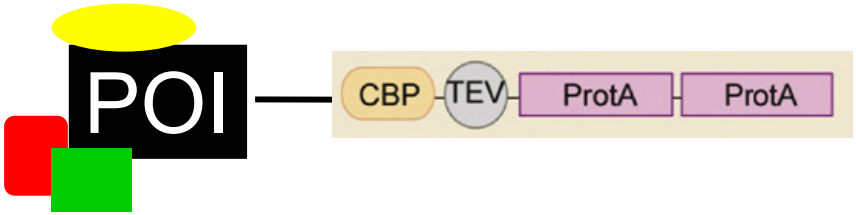
PI *in vivo*

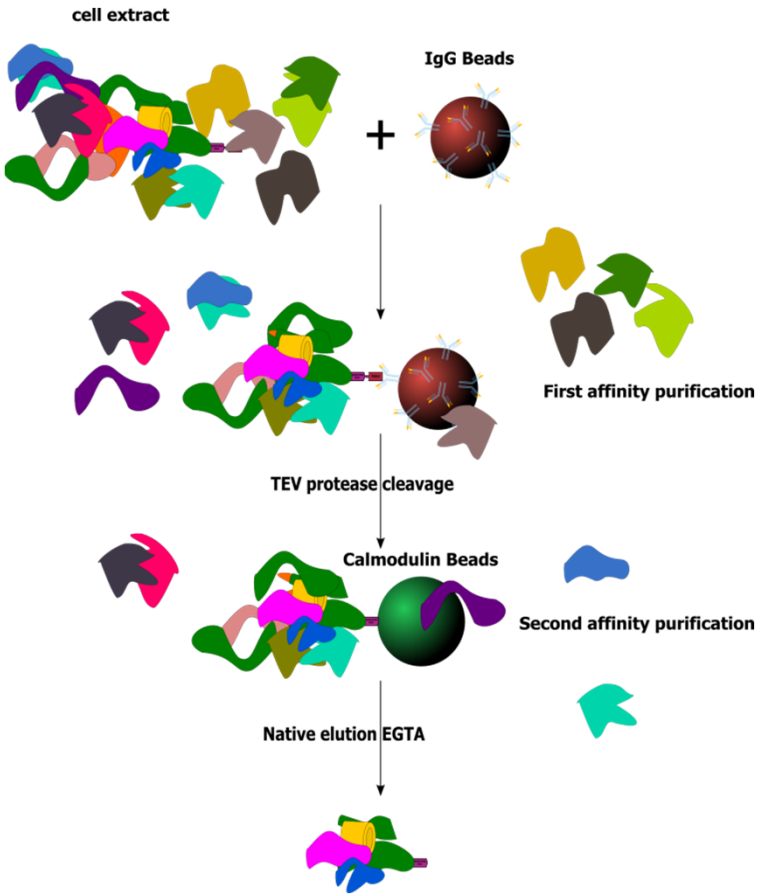
Tandem affinity purification (TAP-tag)

- 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 specifity



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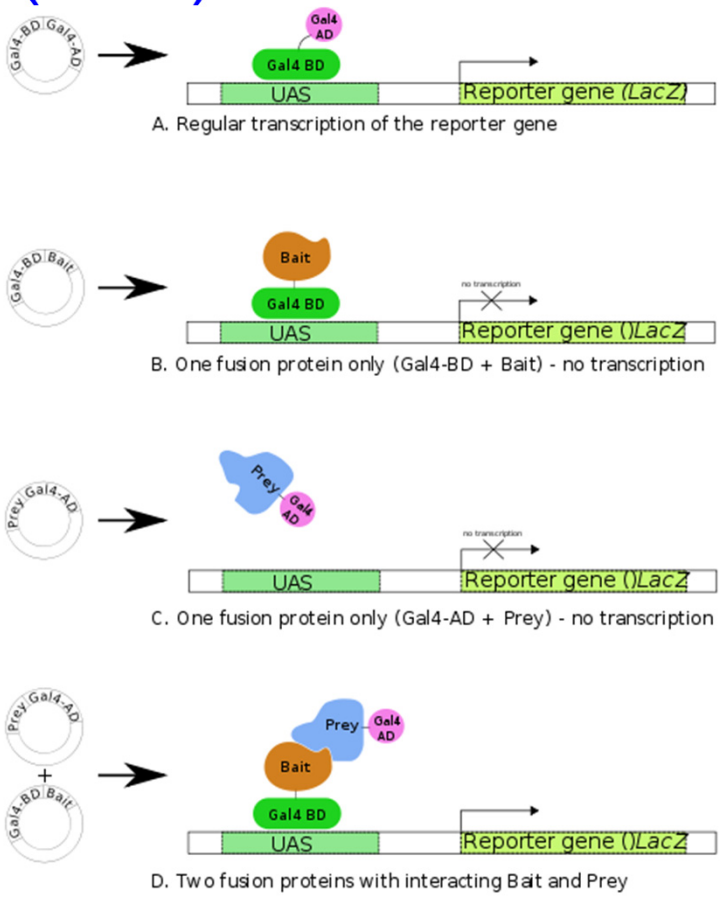
PI *in vivo*

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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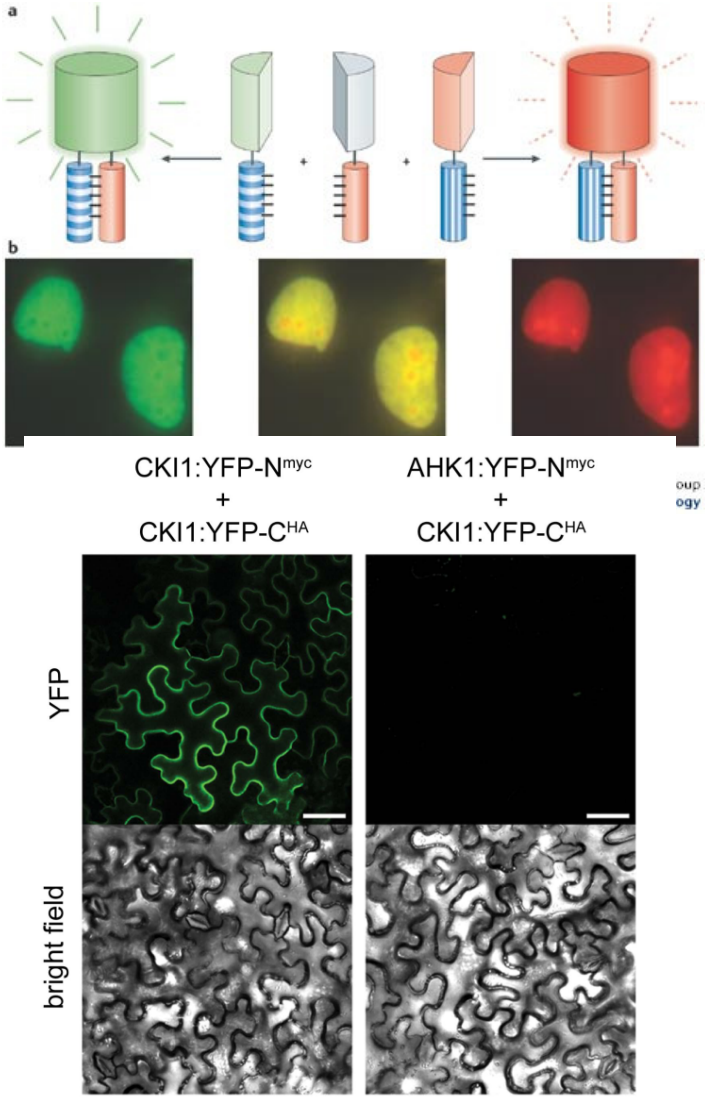
  - Bimolecular fluorescence complementation (BiFC)

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 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*

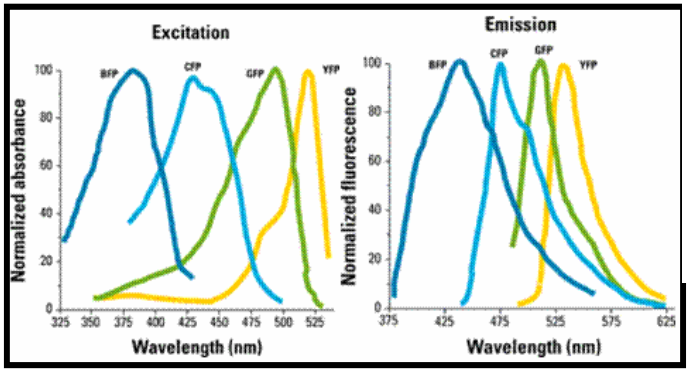
Membrane Recruitment Assay (MeRA)

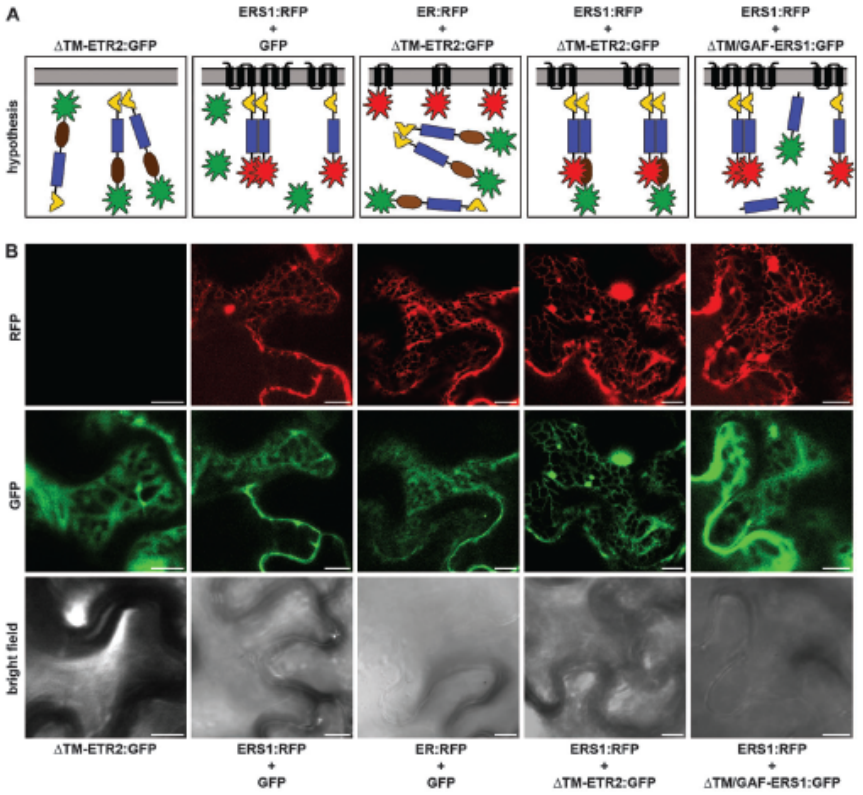
- Method for identification of interactions of cytoplasmic proteins with the membrane proteins

Membrane protein is fused with a 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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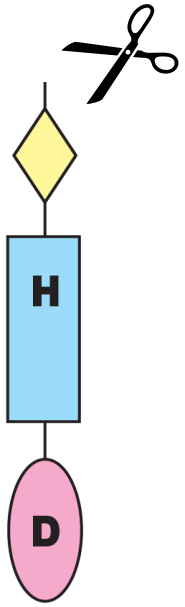
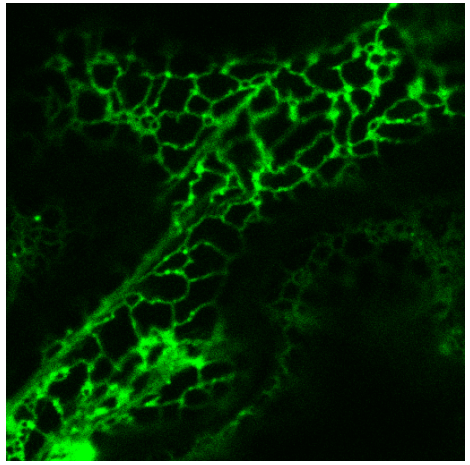
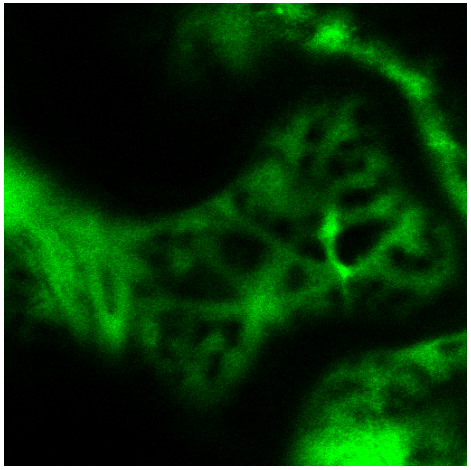
PI *in vivo*

Membrane Recruitment Assay (MeRA)

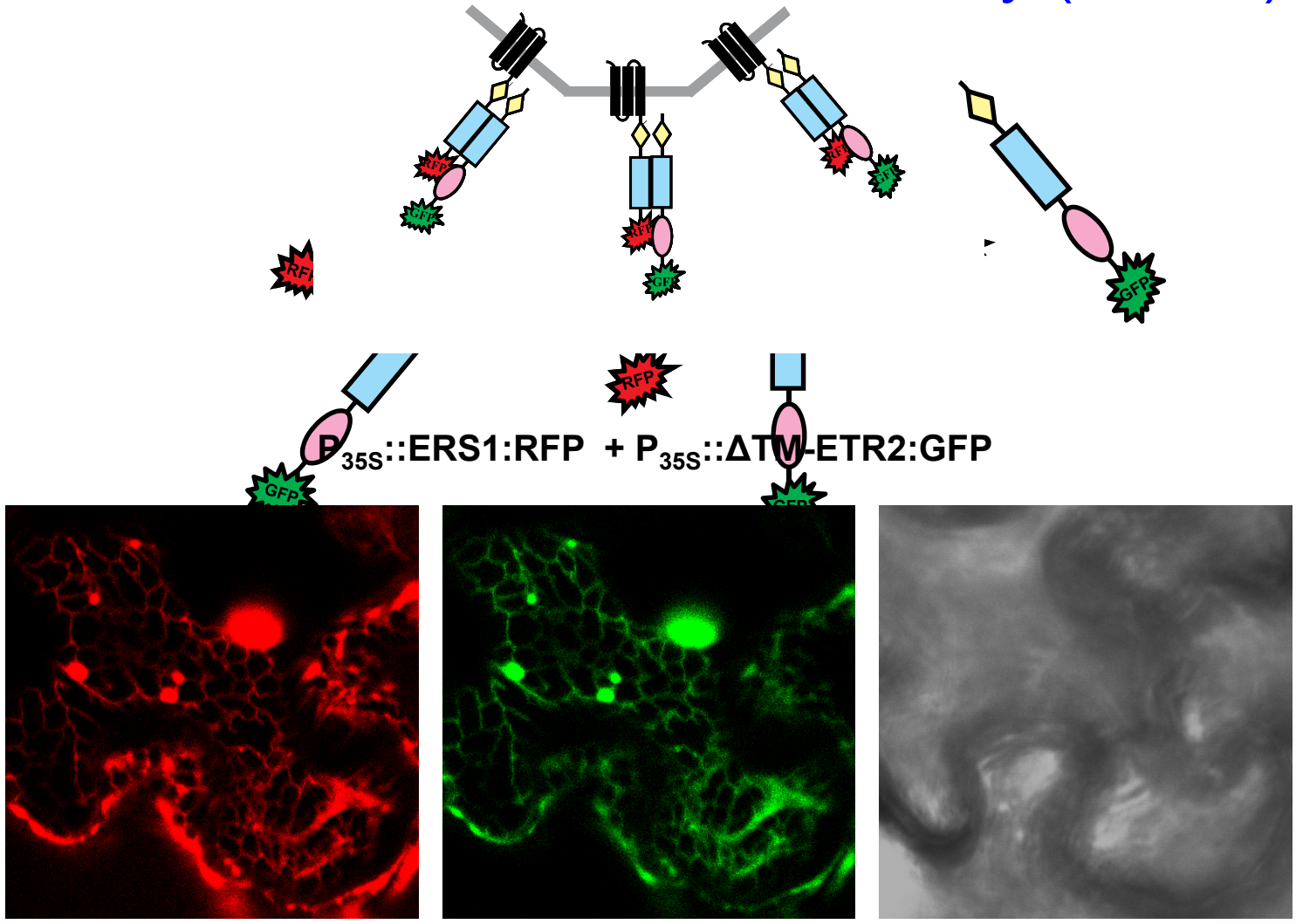






PI *in vivo*

Membrane Recruitment Assay (MeRA)

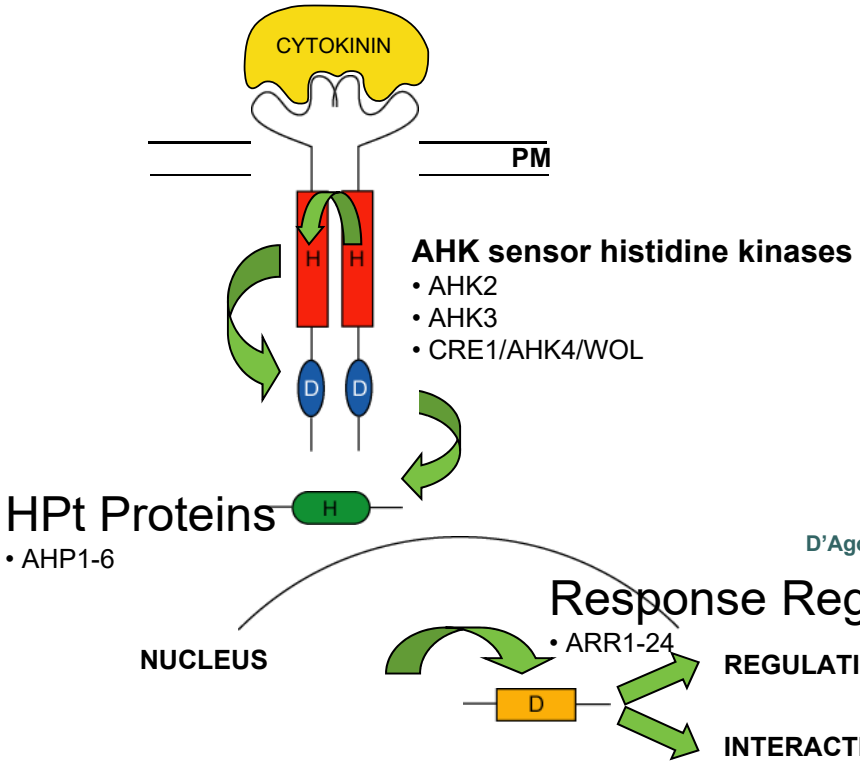


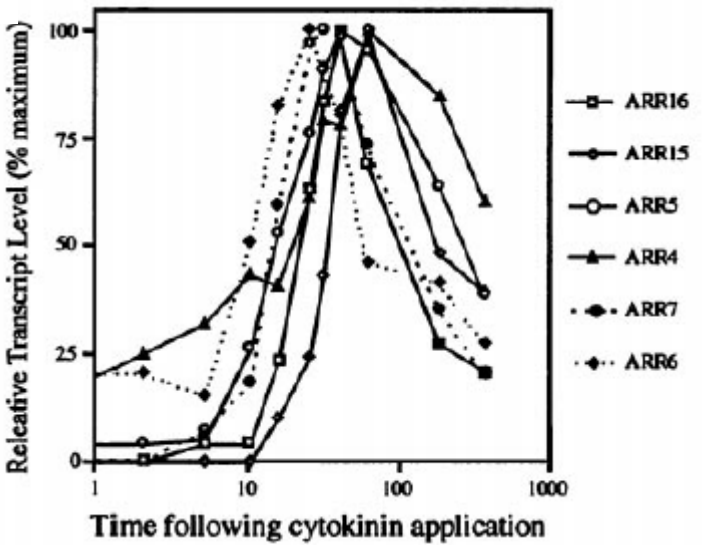
Outline

- Functional importance of the specificic 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

Signal Transduction via MSP

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

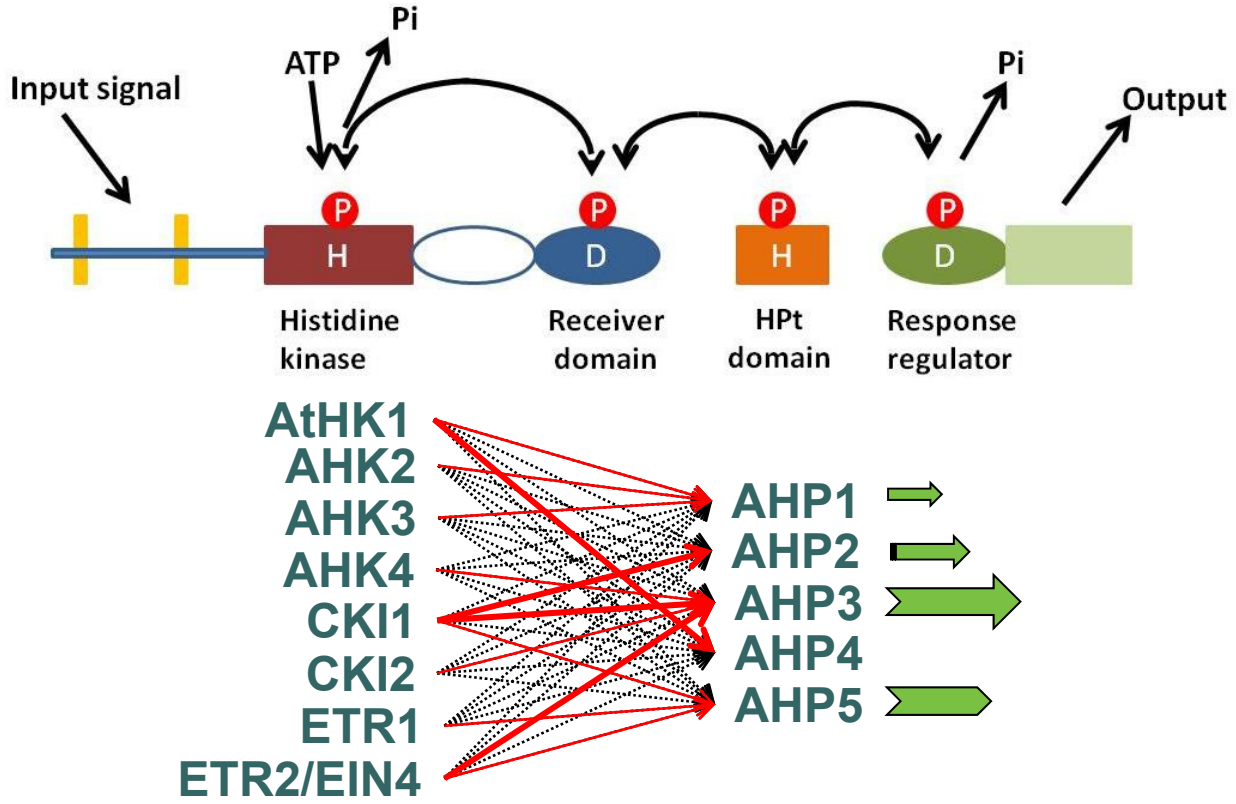




D'Agostino et al., Plant Phys, 2000

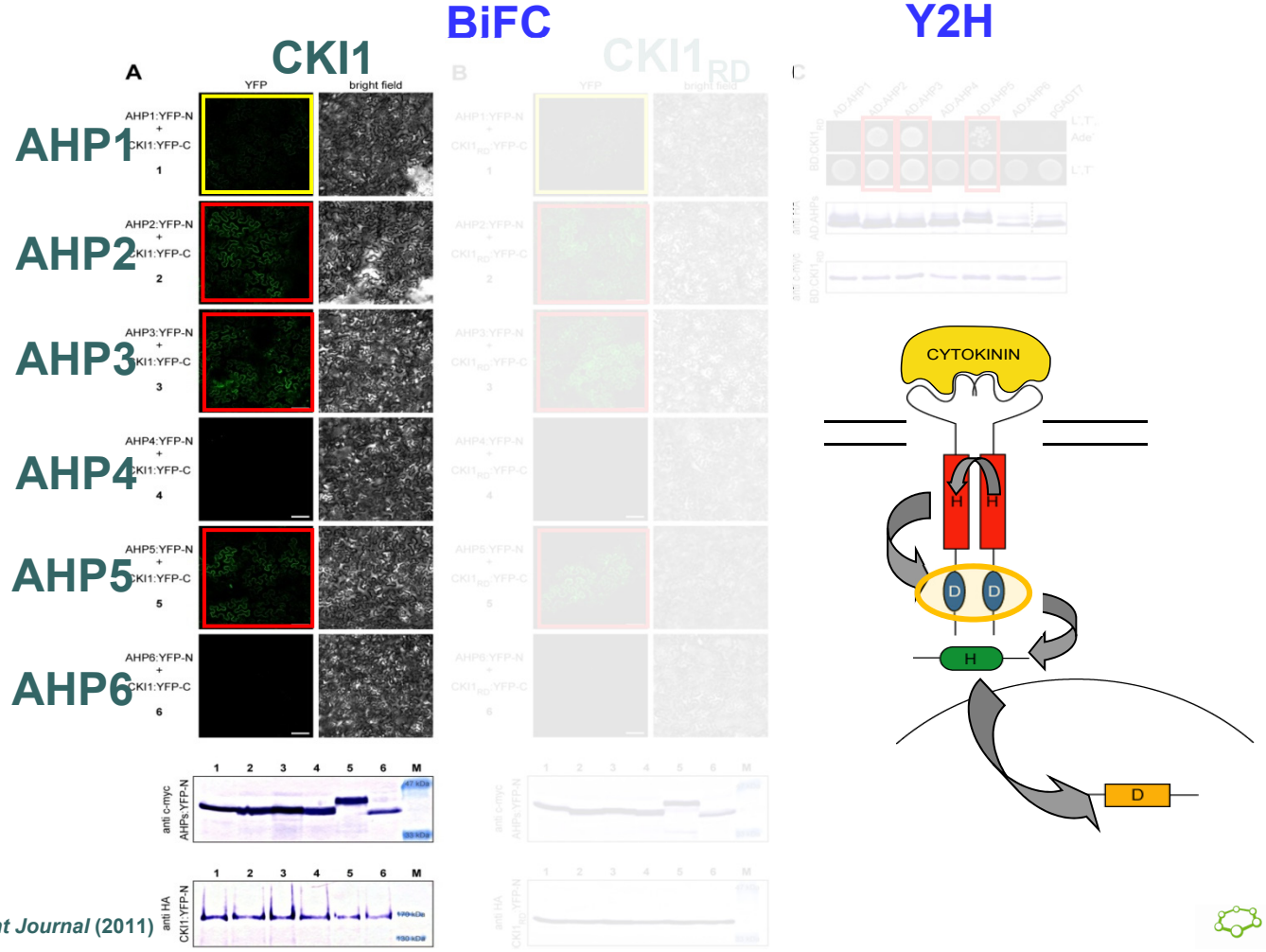
Is there any specificity in plant MSP?

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



Specificity of CKI1 signalling

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

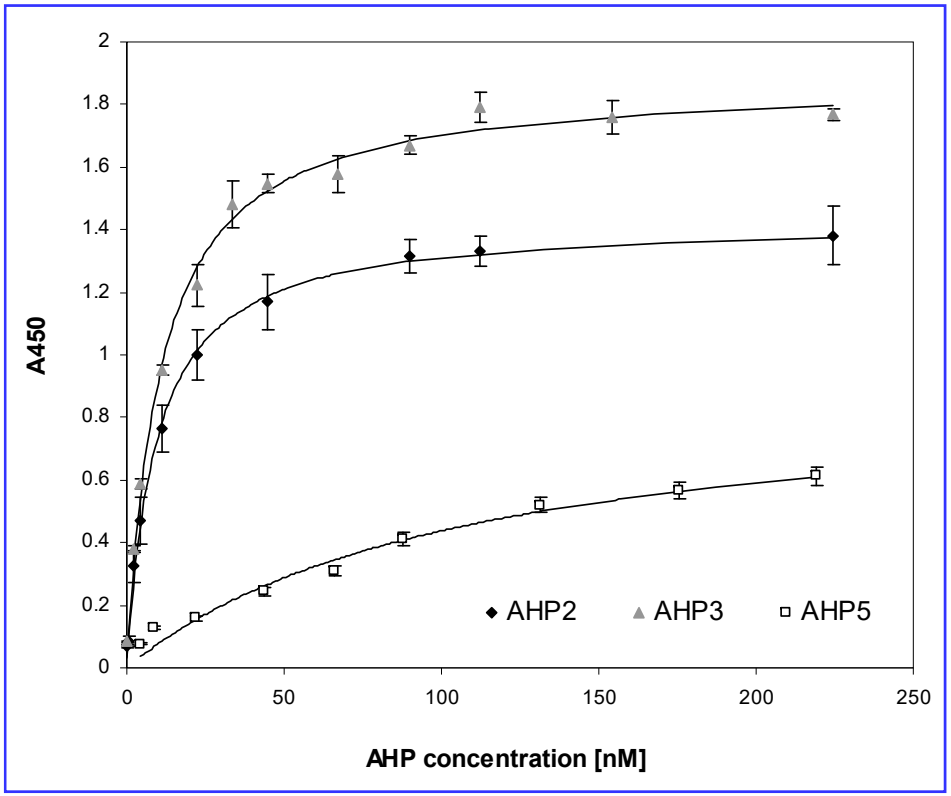


Pekárová et al., *Plant Journal* (2011)

CEITEC

Specificity of CKI1 Signalling

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



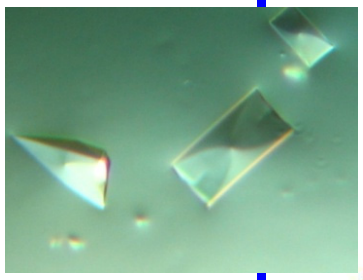
AHP3: K_d ~ 10,5 nM

AHP2: K_d ~ 9,17 nM

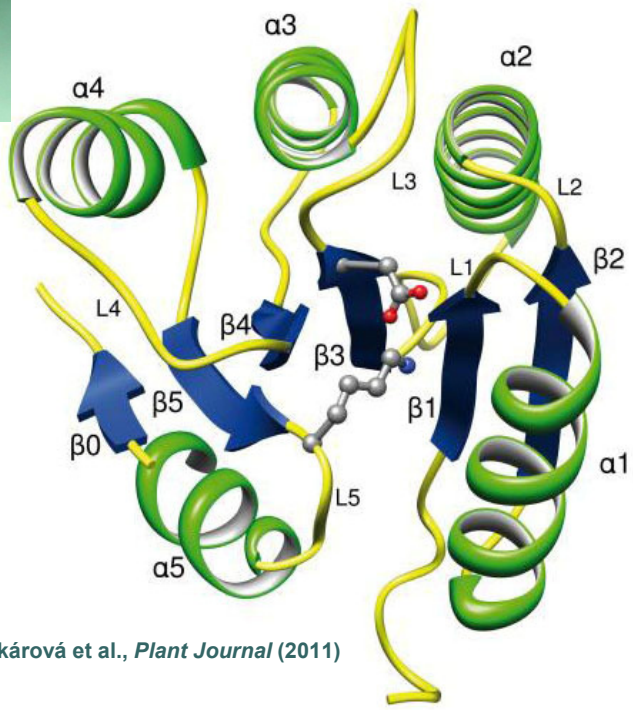
AHP5: K_d ~ 108 nM

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

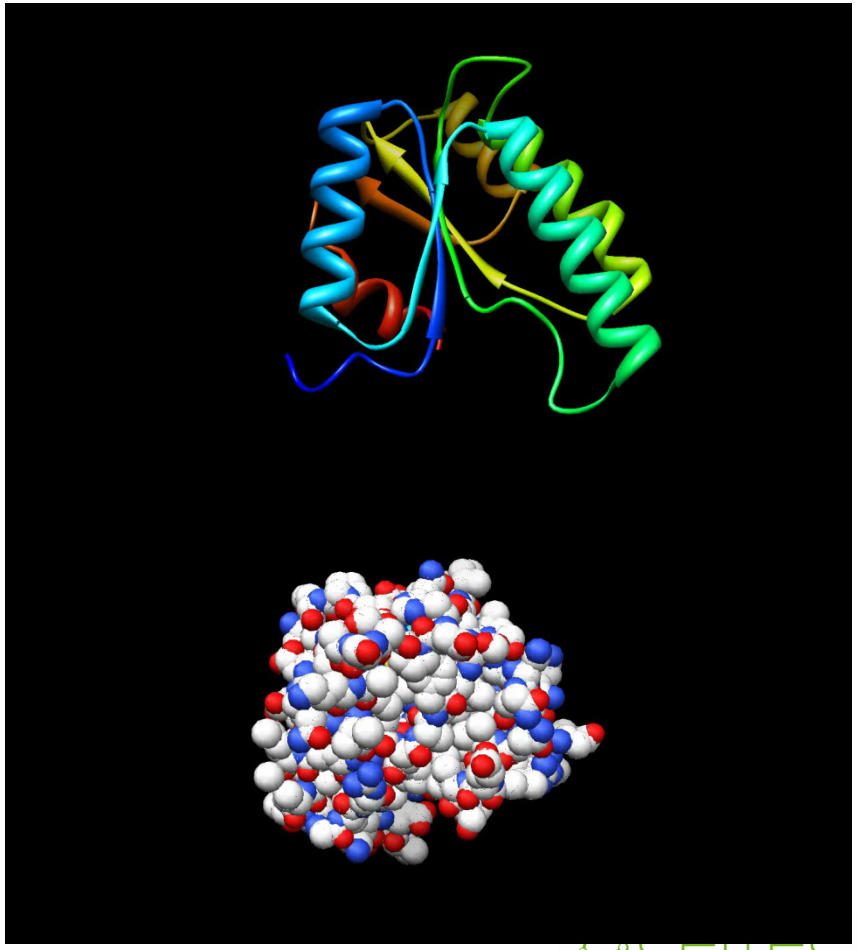
Structure of $CKI1_{RD}$



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

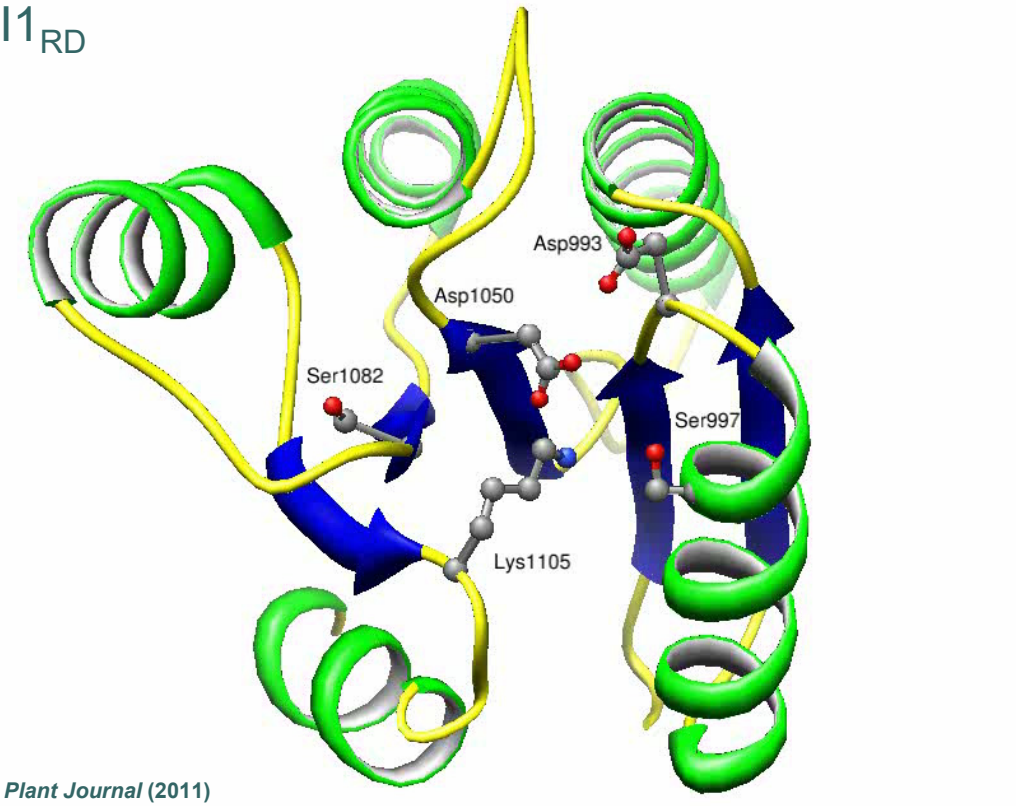


Pekárová et al., *Plant Journal* (2011)



Dynamics of $CKI1_{RD}$

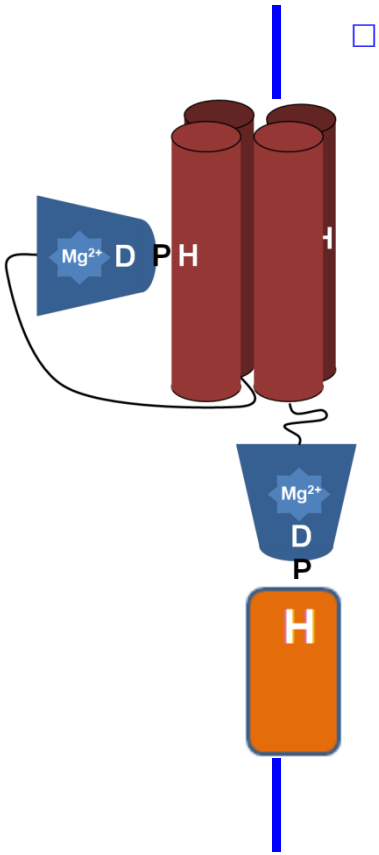
- ***Mg^{2+}binding*** leads to ***remodelling of active centre*** of $CKI1_{RD}$



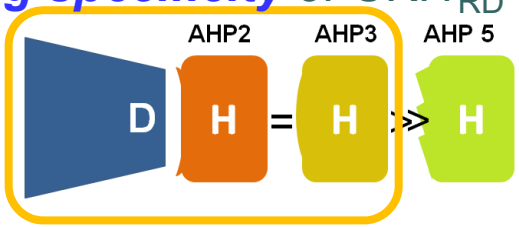
Pekárová et al., *Plant Journal* (2011)

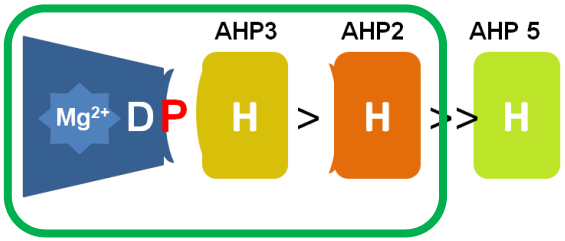
$CKI1_{RD}$ structural changes are associated with its binding specificity

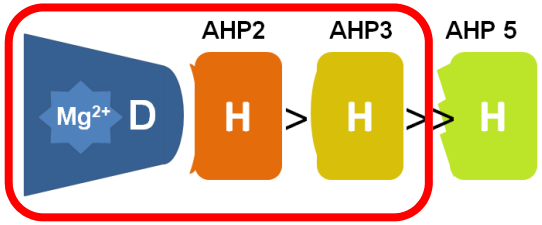
- ***Mg^{2+}***- and ***BeF_3^-***-induced ***structural changes fine-tune binding specificity*** *of* $CKI1_{RD}$



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



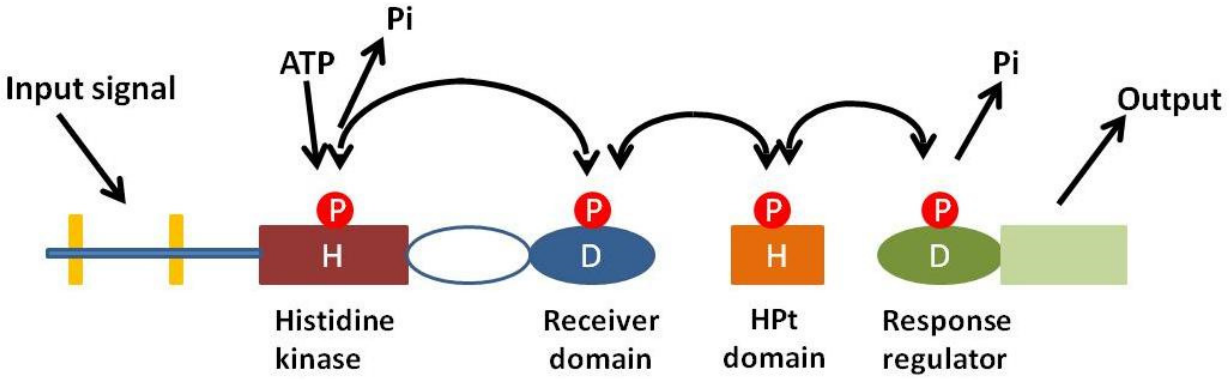


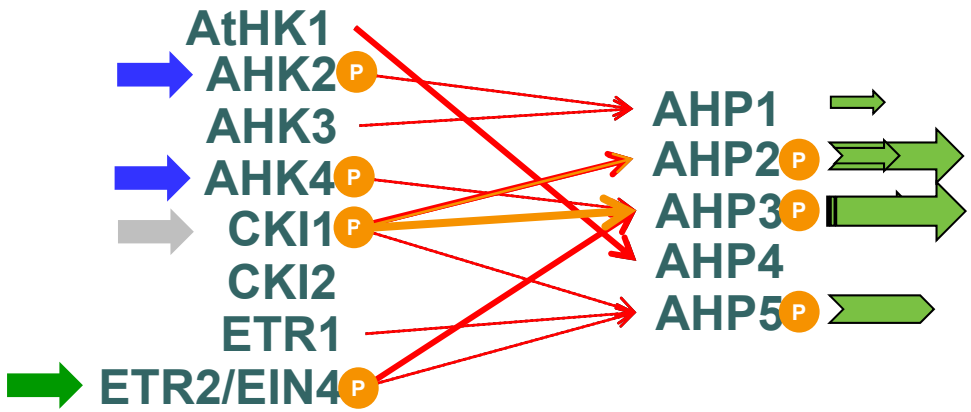


Ligand	0	Mg^{2+}	BeF_3^-
AHP2	9.17 ± 0.49	6.2 ± 0.98	11.6 ± 2.0
AHP3	10.5 ± 0.73	12.9 ± 0.72	8.0 ± 0.42
AHP5	108 ± 18	152 ± 26	119 ± 32

Model Suggestion

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





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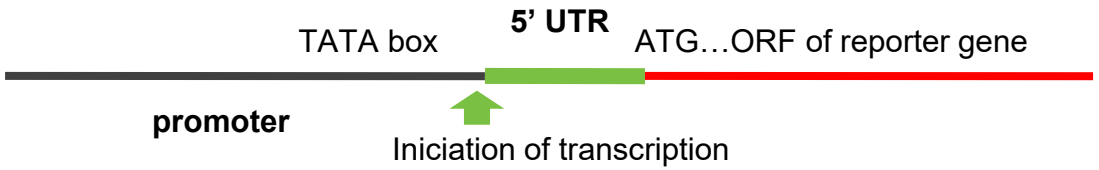
Discussion

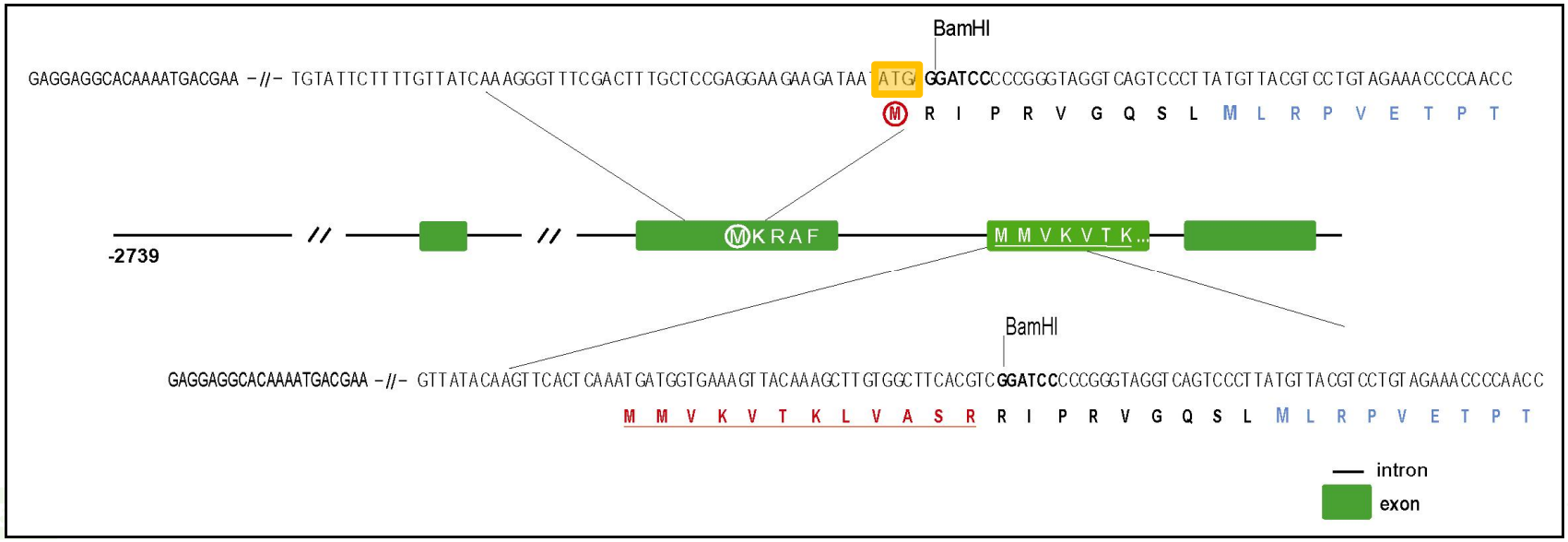
Outline

- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene

Transcriptional Fusion

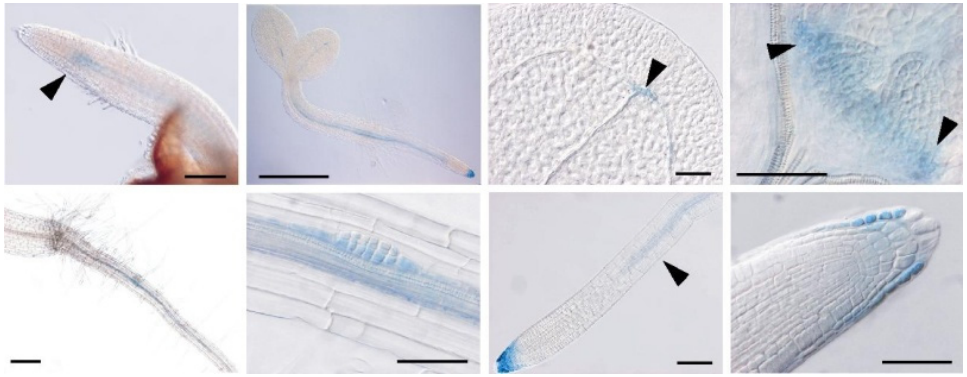
- Identification and cloning of the promoter region of the gene
- Preparation of recombinant DNA carrying the promoter and the reporter gene (uidA, GFP)

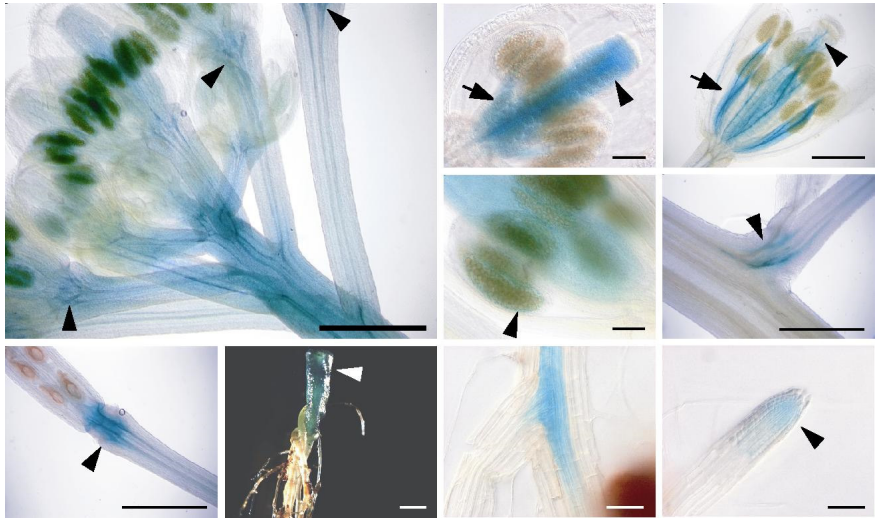




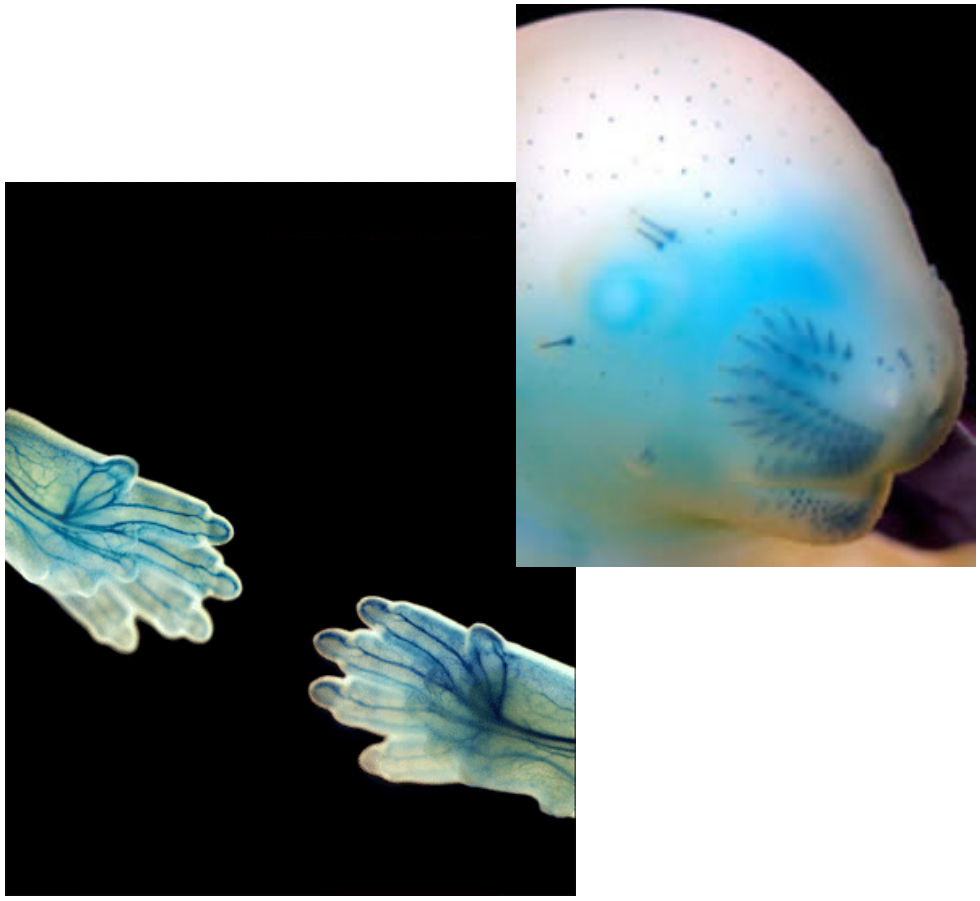
Transcriptional Fusion

- Identification and cloning of the promoter region of the gene
- Preparation of recombinant DNA carrying the promoter and the reporter gene (uidA, GFP)
- Preparation of transgenic organisms carrying this recombinant DNA and their histological analysis





GUS Reporter in Mouse Embryos



Outline

- Methods of gene expression analysis
  - Qualitative analysis of gene expression
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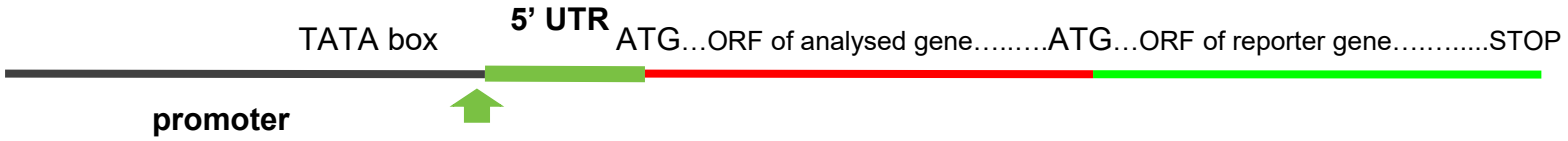
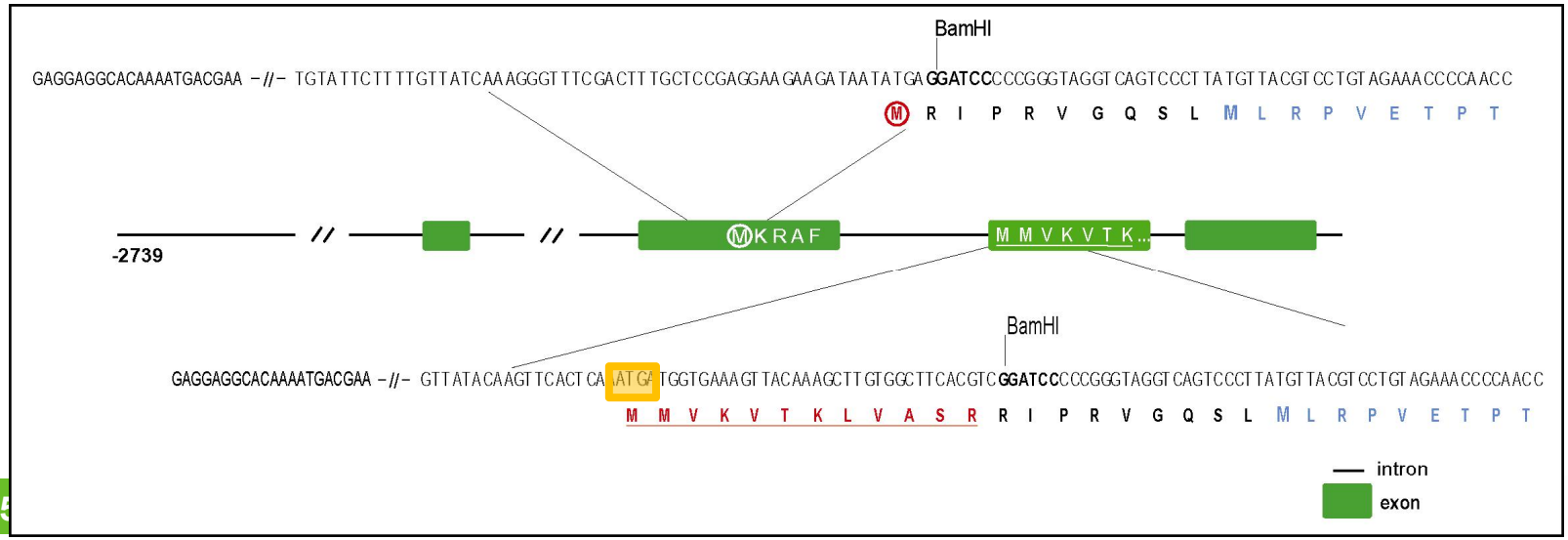
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene

Translational Fusion

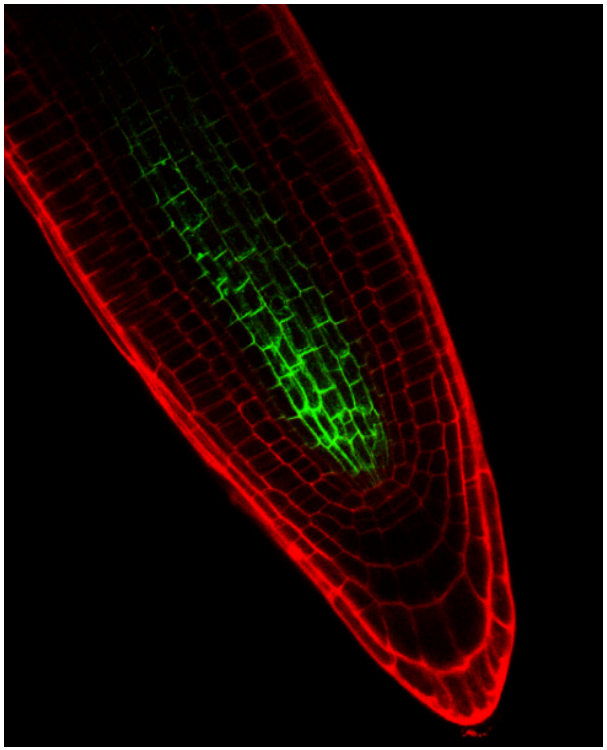
- Identification and cloning of the promoter and coding region of the analyzed gene
- Preparation of a recombinant DNA carrying the promoter and the coding sequence of the studied gene in a fusion with the reporter gene (uidA, GFP)



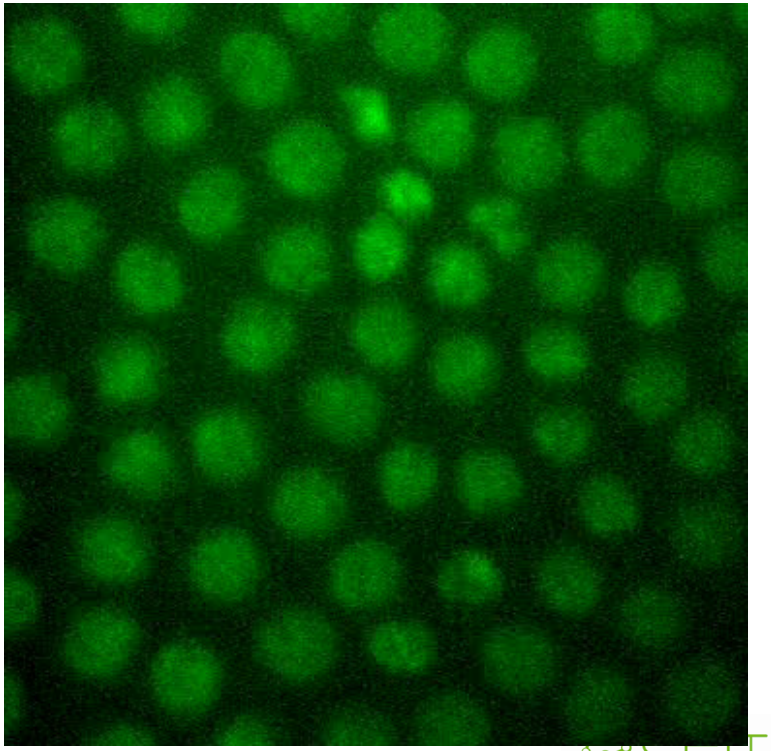


Translational Fusion

- Preparation of transgenic organisms carrying the recombinant DNA and their histological analysis
- Compared to transcriptional fusion, translation fusion allows analysis of intercellular localization of gene product (protein) or its dynamics

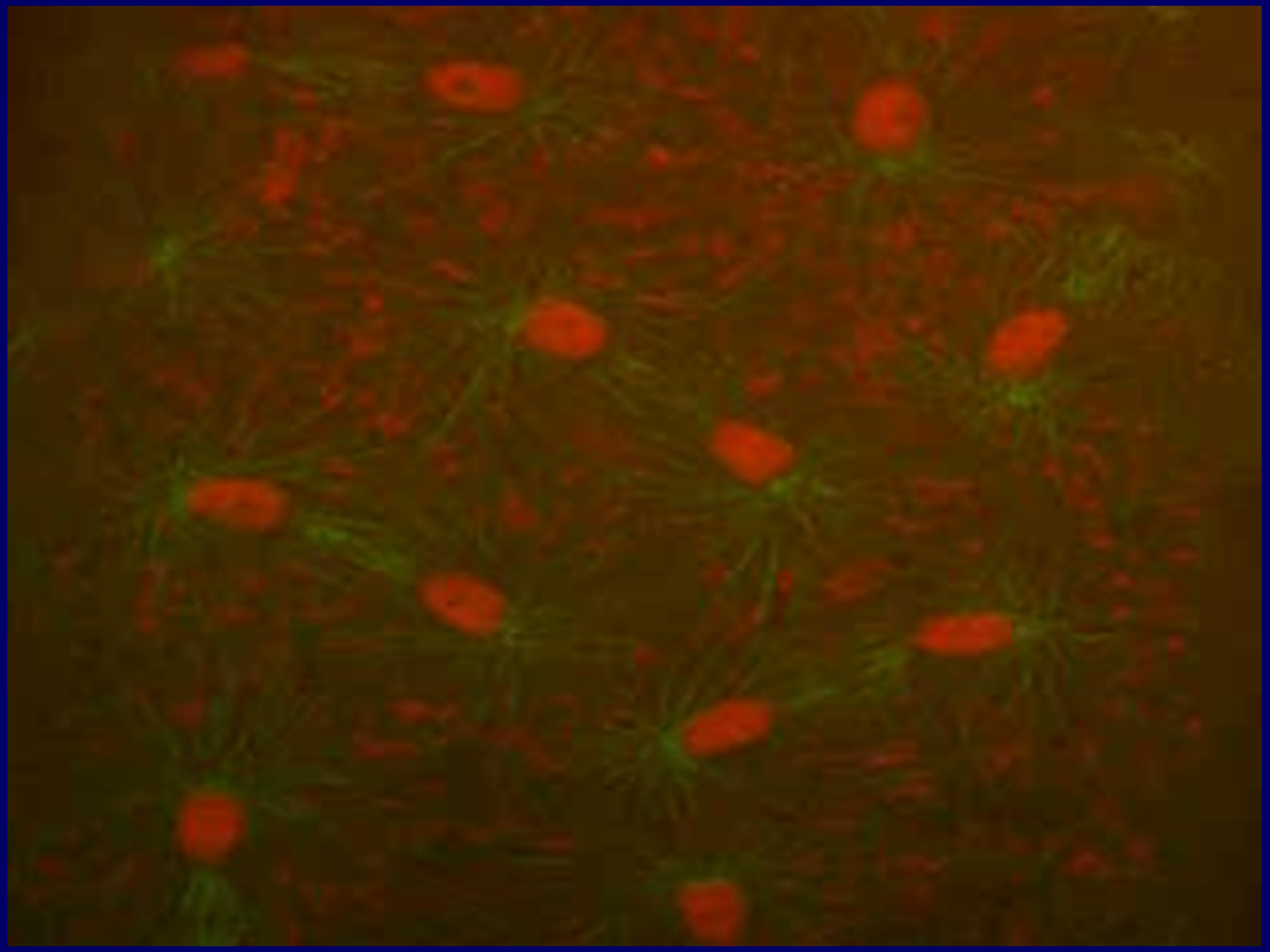


PIN1-GFP in *Arabidopsis*



Histone 2A-GFP in *Drosophila* embryo by PAM

Translational Fusion



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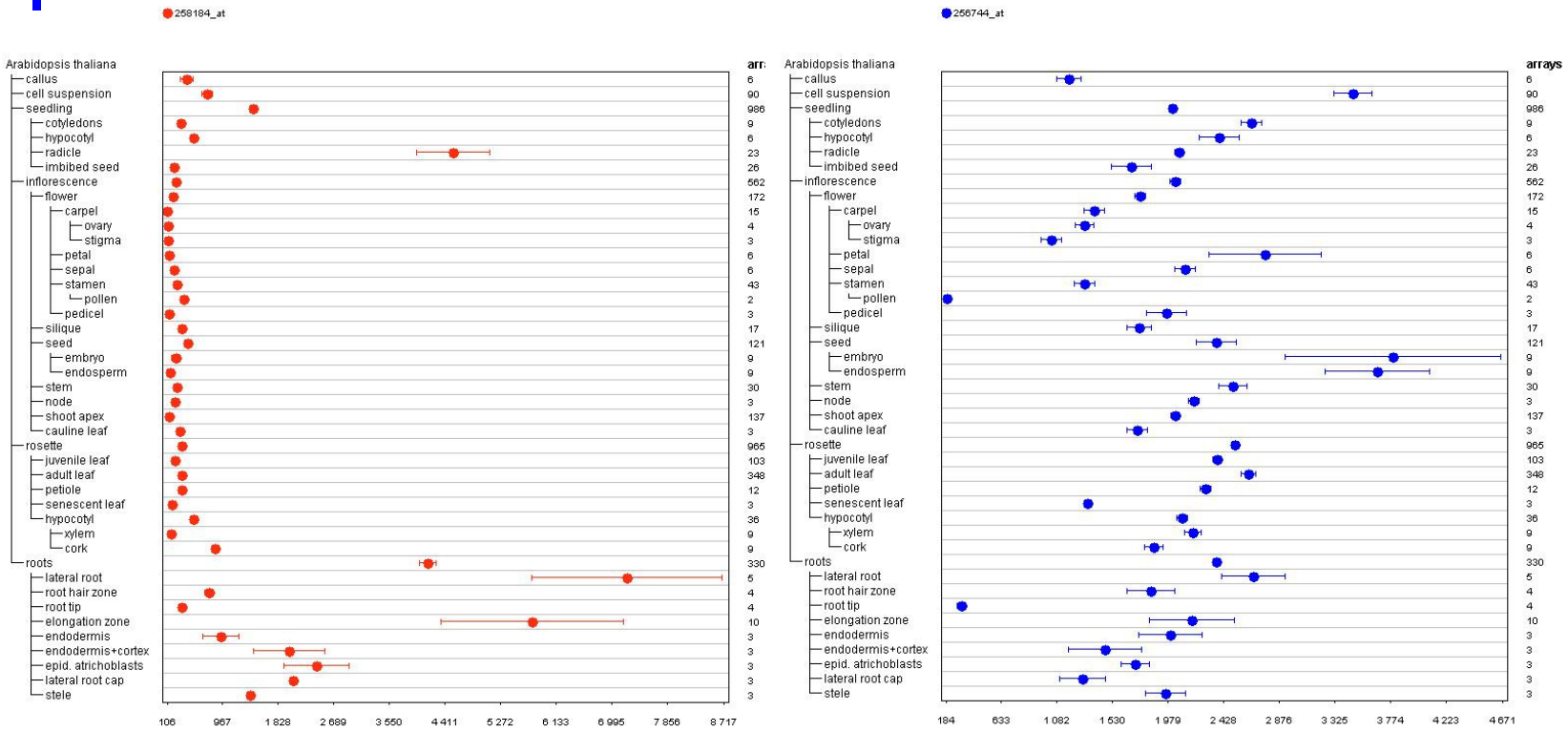
Databases

- **Analysis of expression using Genevestigator** (***AHP1*** and ***AHP2***, *Arabidopsis*, Affymetrix ATH 22K Array)



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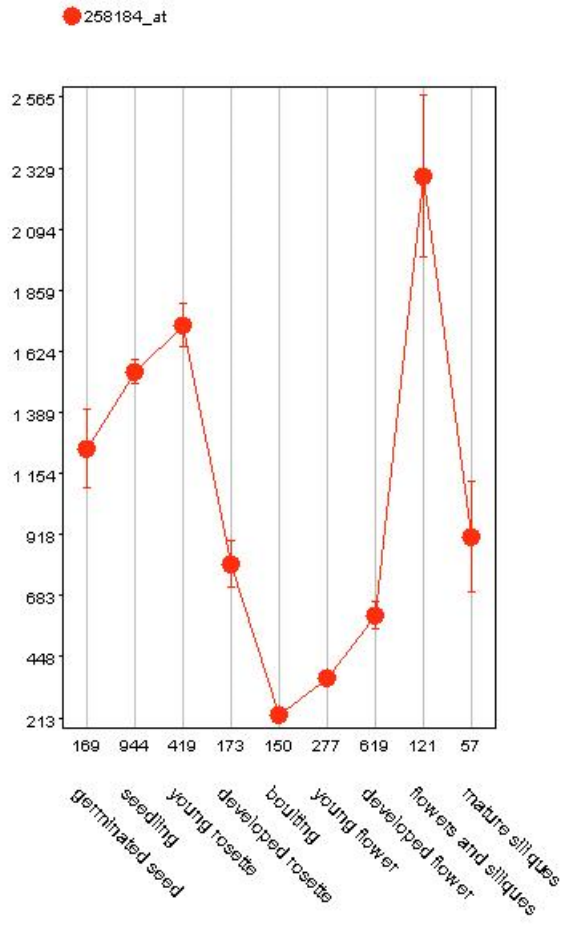

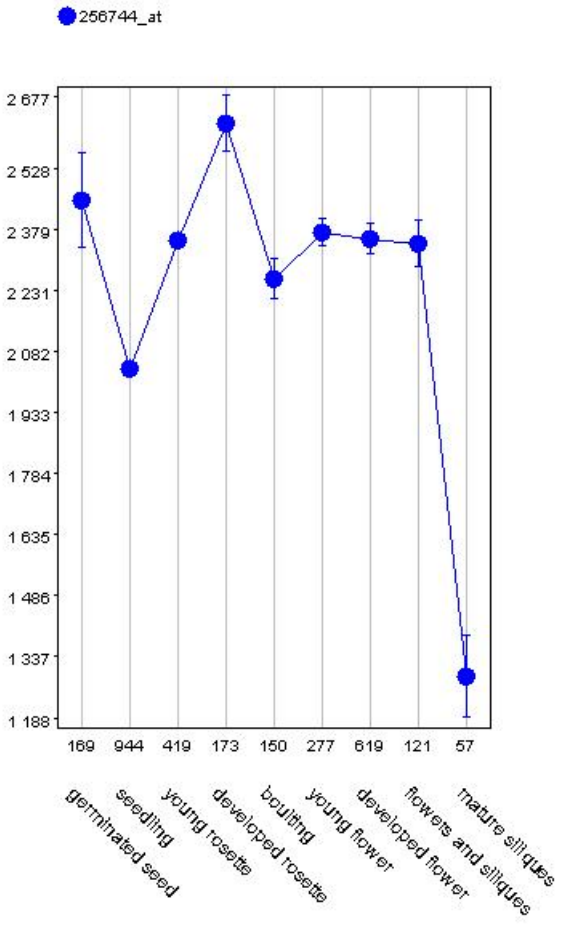

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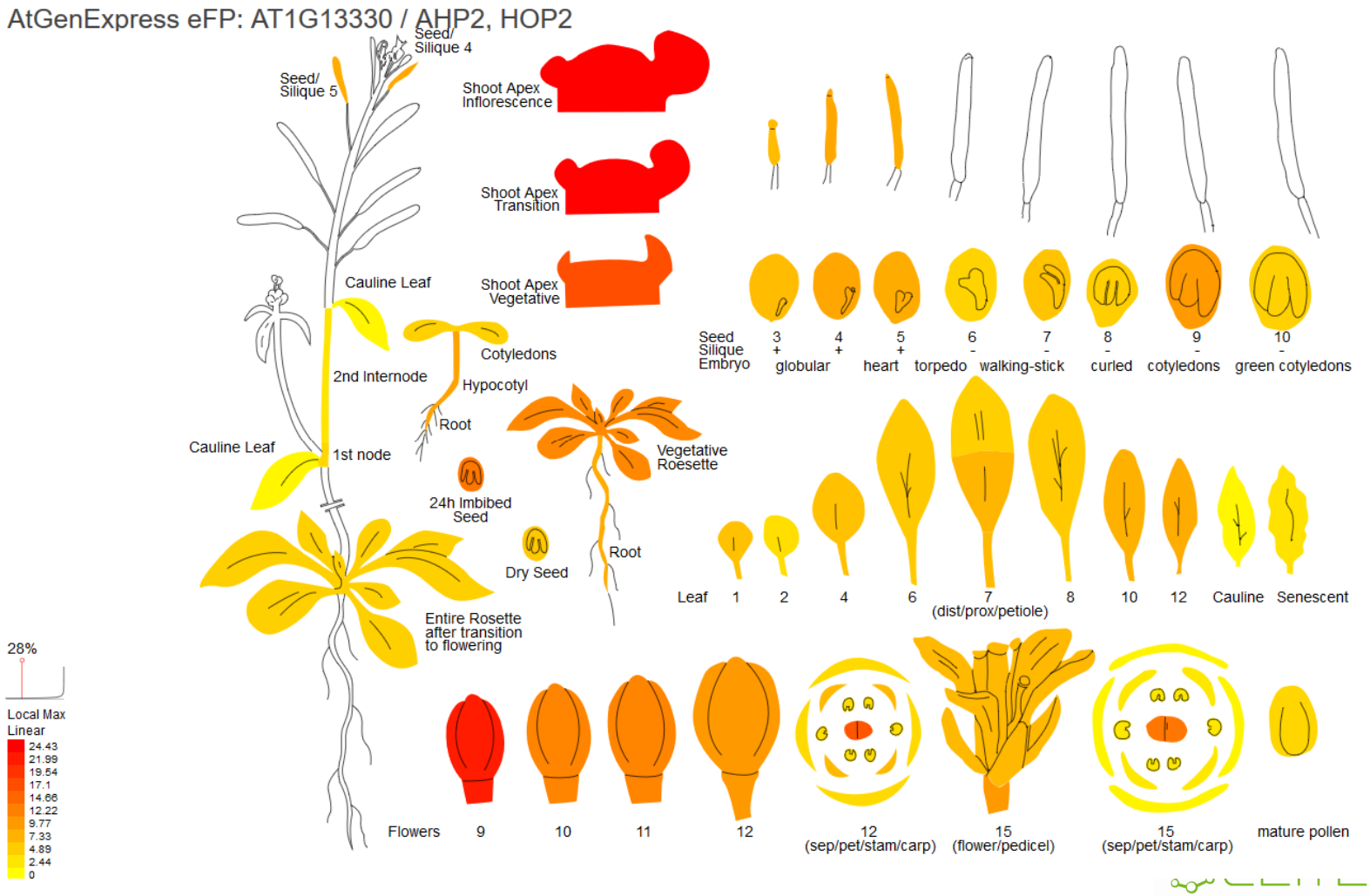
Databases

- **Analysis of expression using ePlant**



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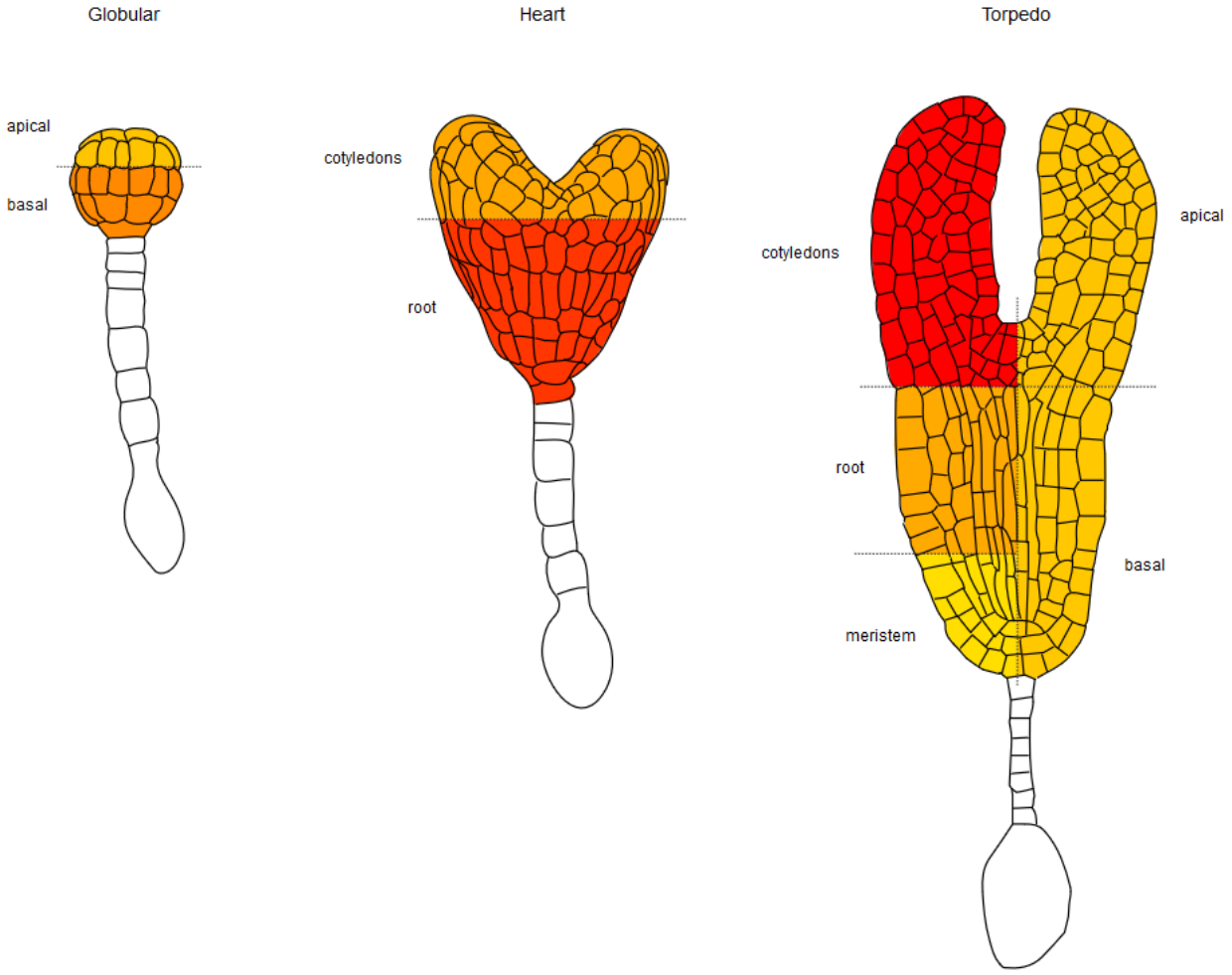

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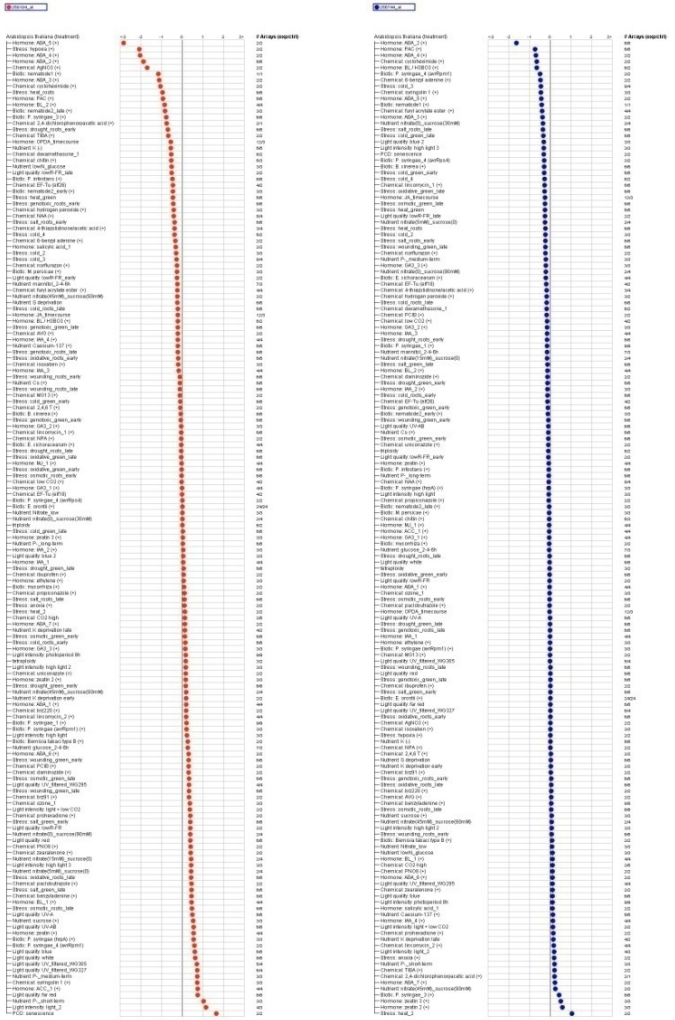

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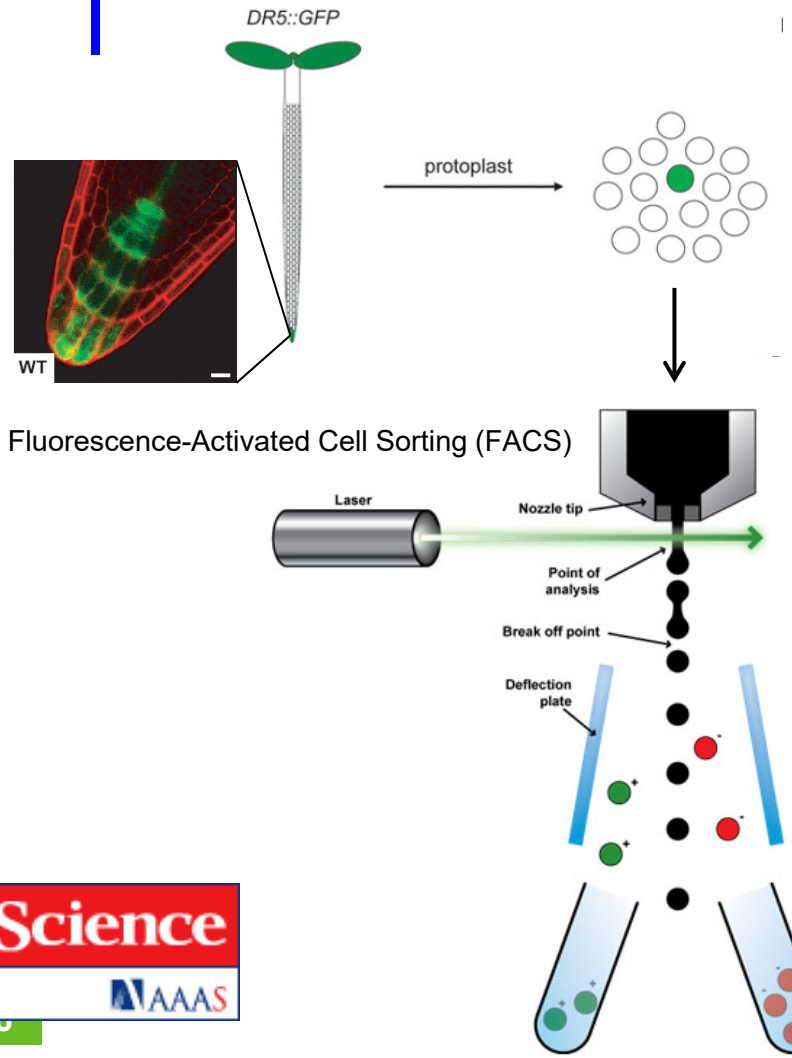
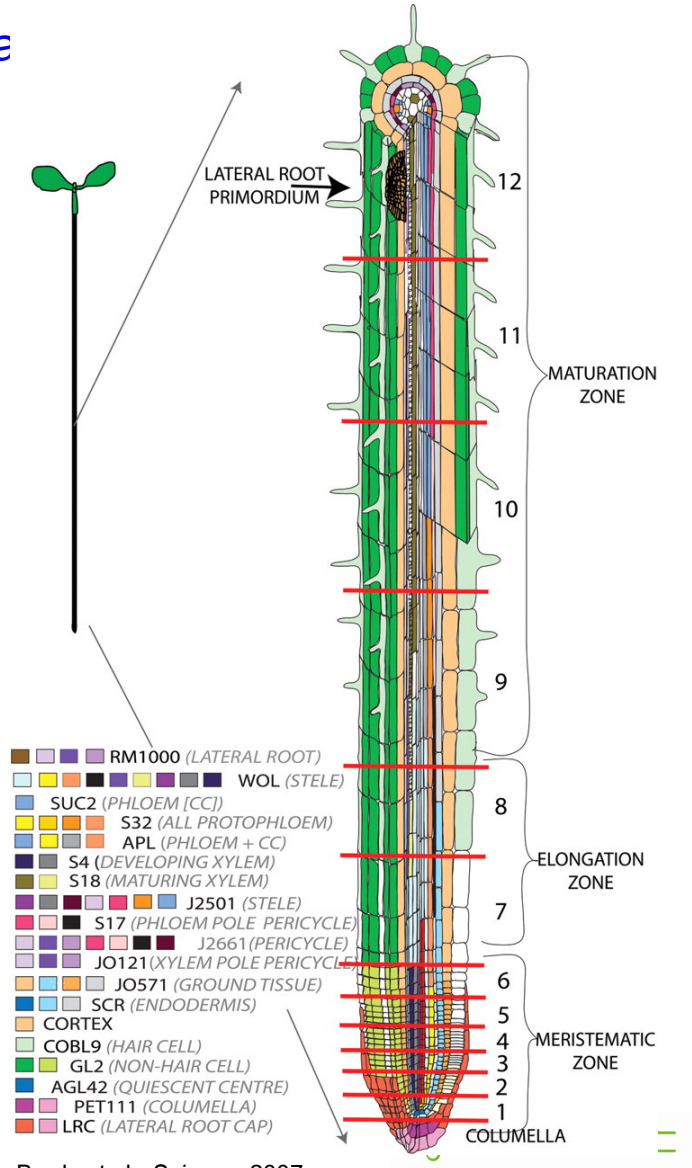
    - Tissue- and cell-specific gene expression analysis

Expression Maps - RNA

- High-Resolution Expression Map in Ara

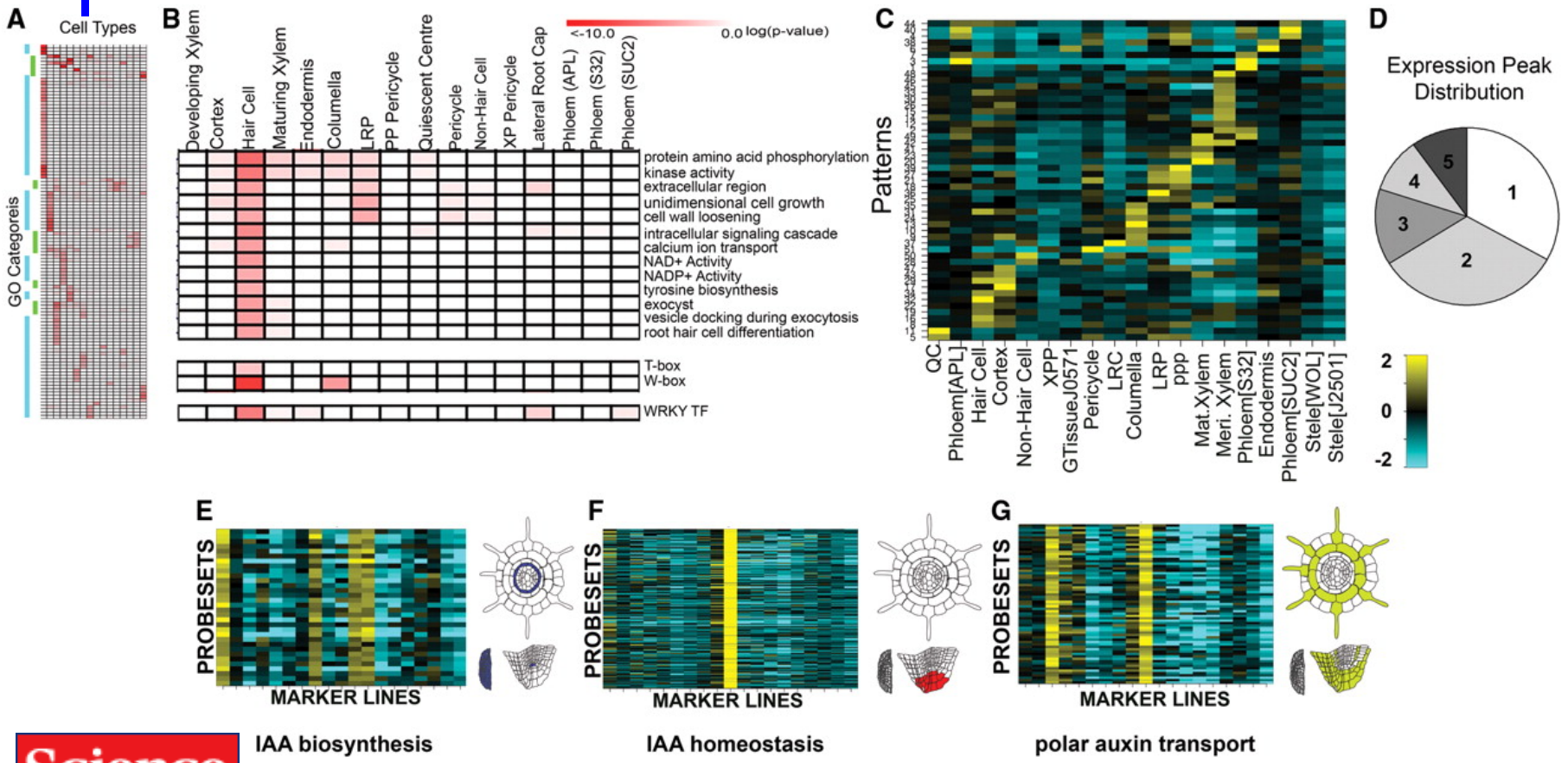




Brady et al., *Science*, 2007

Expression Maps - RNA

- High-Resolution Expression Map in Arabidopsis Root



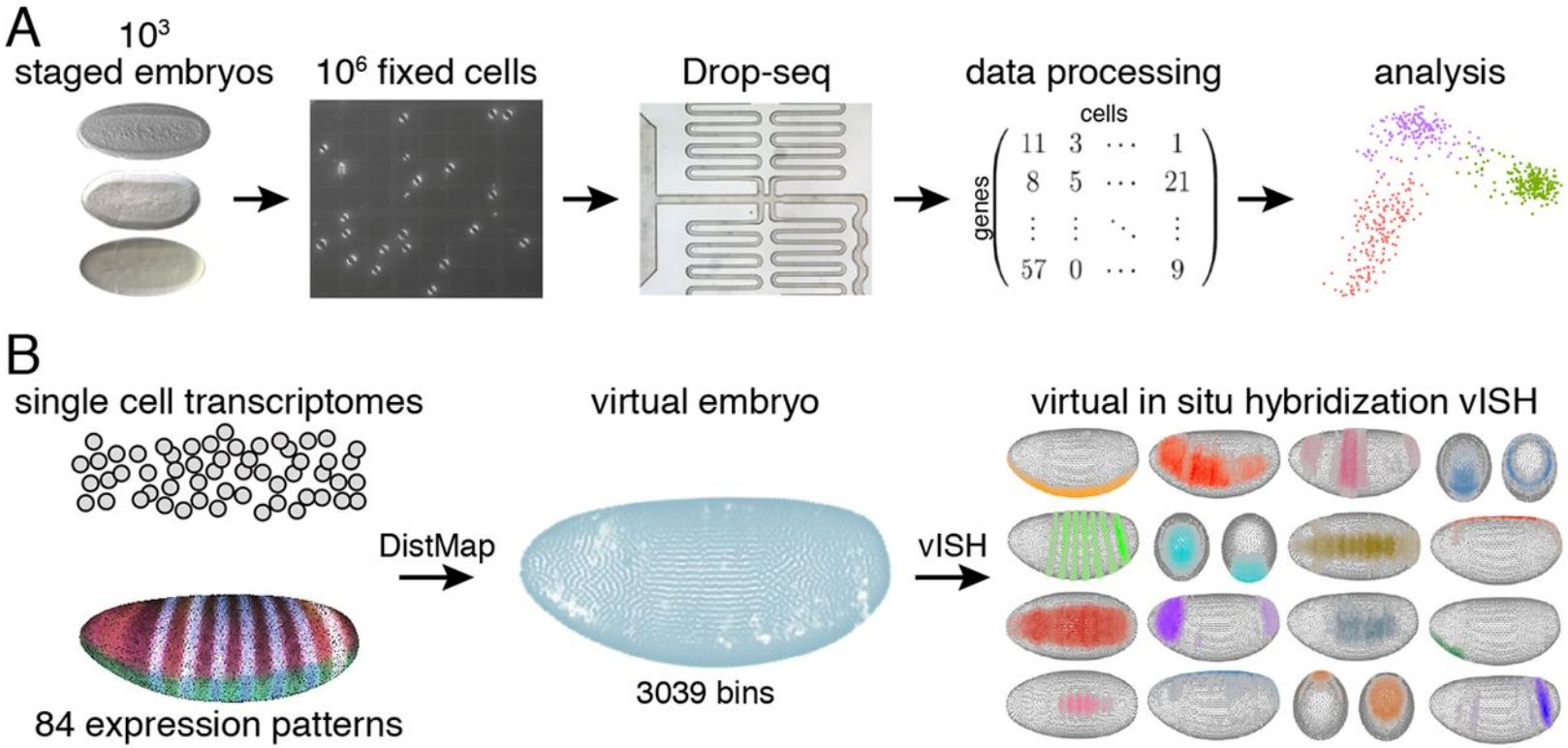
Science
AAAS

Brady et al., *Science*, 2007

CEITEC

Expression Maps - RNA

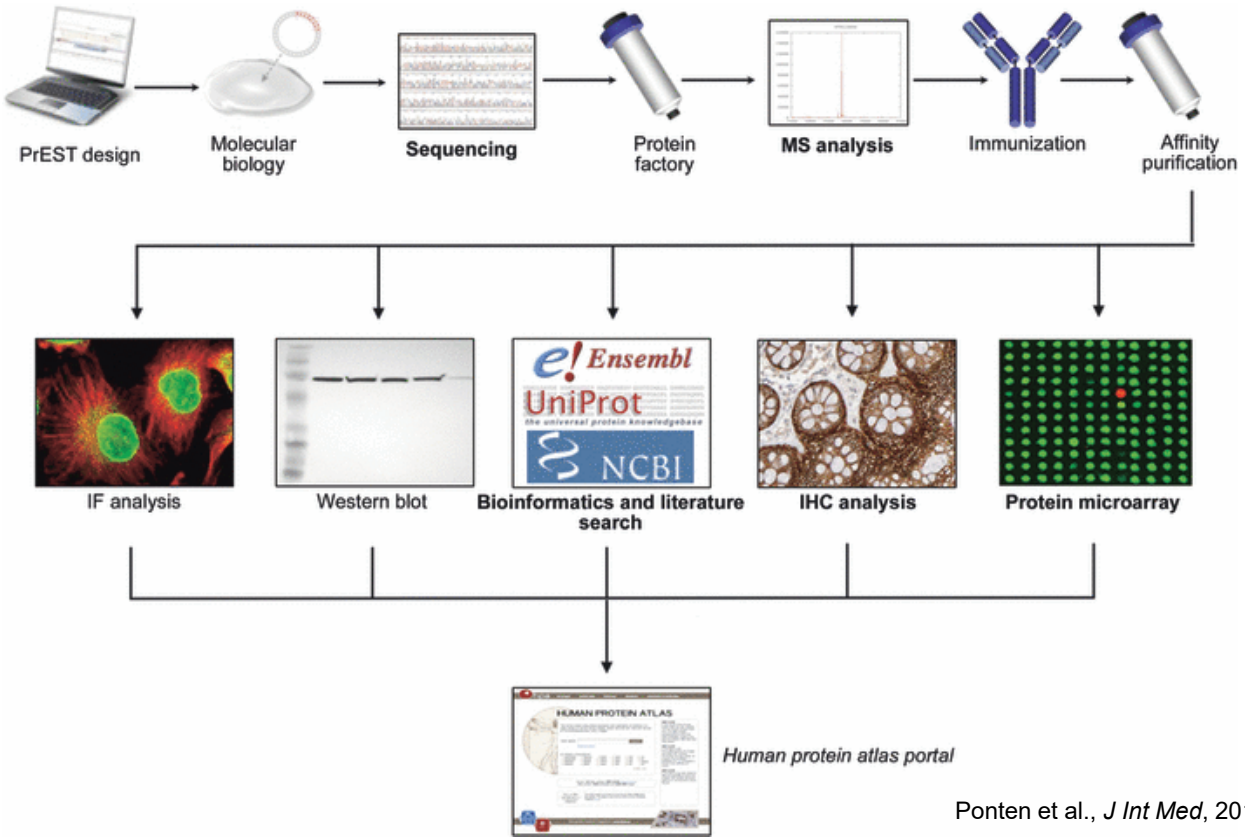
- High-Resolution Expression Map in Drosophilla



Nikos Karaiskos et al. Science 2017;science.aan3235

Expression Maps - Proteins

- Human Protein Atlas



Ponten et al., *J Int Med*, 2011

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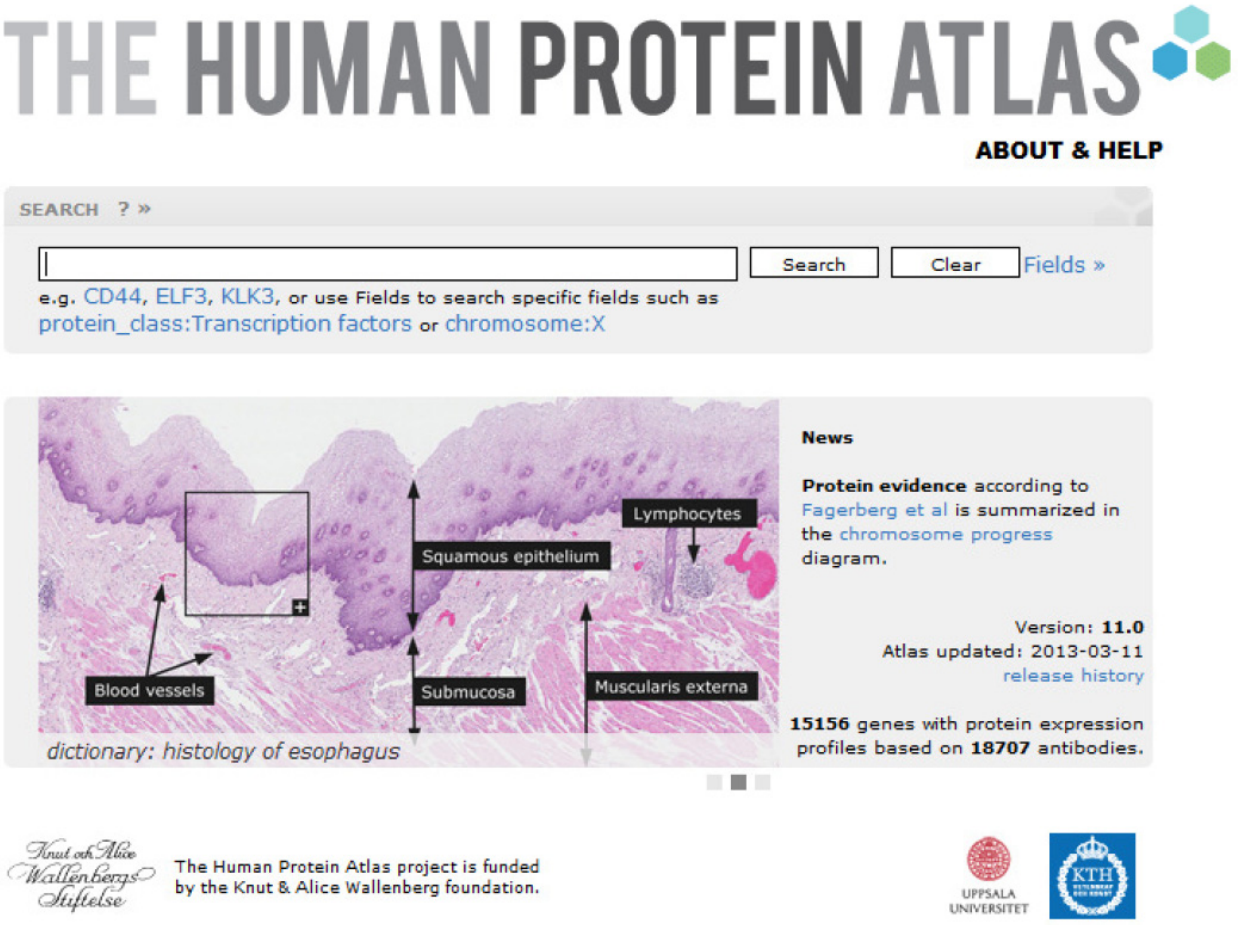

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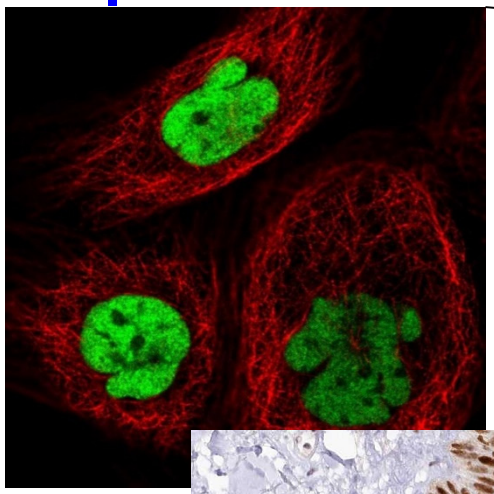
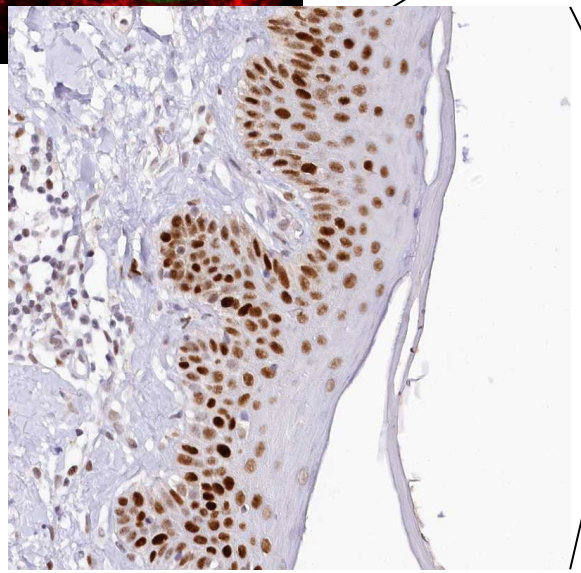
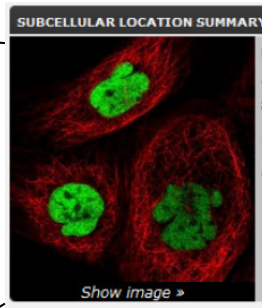






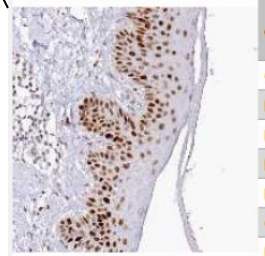
SUBCELLULAR LOCATION SUMMARY

Main location(s)	Nucleus but not nucleoli
Additional location(s)	
Staining summary	Localized to the nucleus but excluded from the nucleoli.
Reliability (APE)	High
Antibodies in assay	CAB039238, CAB039239

MORE SUBCELL DATA

NORMAL TISSUE & ORGAN SUMMARY

Expression summary	Fractions of cells showed weak nuclear and/or cytoplasmic expression.
Tissue specificity	Expressed in 11 out of 82 cell types
Reliability (APE)	High
Antibodies in assay	CAB002973, CAB039238, CAB039239



Organ	No of cell types	Protein expression
CNS (brain)	11	
Hematopoietic (blood)	8	
Liver and pancreas	5	
Digestive (GI-tract)	13	
Respiratory (lung)	4	
Cardiovascular	1	
Female tissues	13	
Placenta	2	
Male tissues	5	
Urinary tract (kidney)	3	
Skin and soft tissues	14	
Endocrine tissues	3	

MORE TISSUE DATA

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  - Quantitative analysis of gene expression
    - DNA and protein chips

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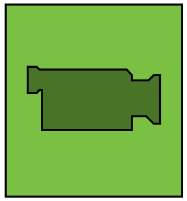
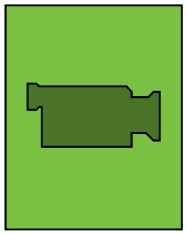
DNA Chips

- Method, which provides quick comparison of a large number of genes/proteins between the test sample and control
- Oligo DNA chips are used the most



- There are commercialy available kits for the whole genome
  - company Operon (Qiagen), 29.110 of 70-mer oligonucleotides representing 26.173 genes coding proteins, 28.964 transcripts and 87 microRNA genes of *Arabidopsis thaliana*
  - Possibility of use for the preparation of photolithography chips – facilitation of oligonucletide synthesis e.g. for the whole human genome (about 3,1 x 10^9 bp) jit is possible to prepare 25-mers in only 100 steps, by this technique



- Chips not only for the analysis of gene expression, but also for e.g. Genotyping (SNPs, sequencing with chips, …)

Affymetrix ATH1 *Arabidopsis* genome array

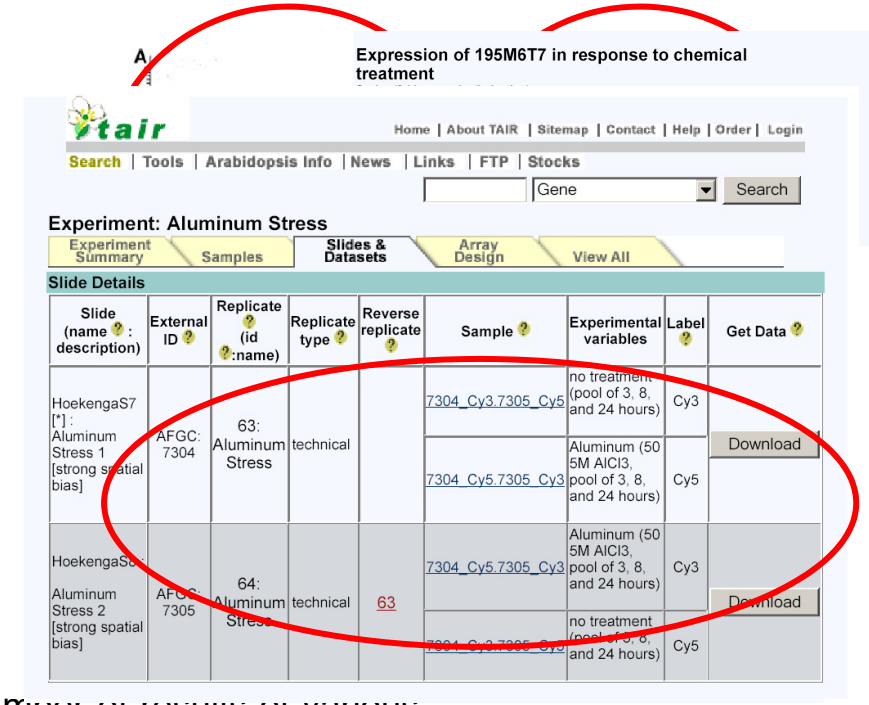
Critical Specifications	
Number of arrays	One
Number of sequence represented	>24,000 gene sequences
Feature size	18 µm
Oligonucleotide probe length	25-mer
Probe pairs/sequence	11
Control sequences	*E. coli* genes *bioB, bioC, bioD.* *B. subtilis* gene *lysA.* Phage P1 *cre* gene. Arabidopsis maintenance genes GAPDH, Ubiquitin, and Actin
Detection sensitivity	1:100,000*

*As measured by detection in comparative analysis between a complex target containing spiked control transcriptions and a complex target with no spikes.

DNA Chips

- For the correct interpretation of the results, good knowledge of advanced statistical methods is required
- It is necessary to include a sufficient number of controls and repeats

  - Control of accuracy of the measurement (repeated measurements on several chips with the same sample, comparing the same samples analysed on different chips with each other)
  - Control of reproducibility of measurements (repeated measurements with different samples isolated under the same conditions on the same chip – comparing with each other)
  - Identification of reliable measurement treshold
  - Finally comparing the experiment with the control or comparing different conditions with each other -> the result



- Currently there's been a great number of results of various experiments in publicly accessible databases

Che et al., 2002

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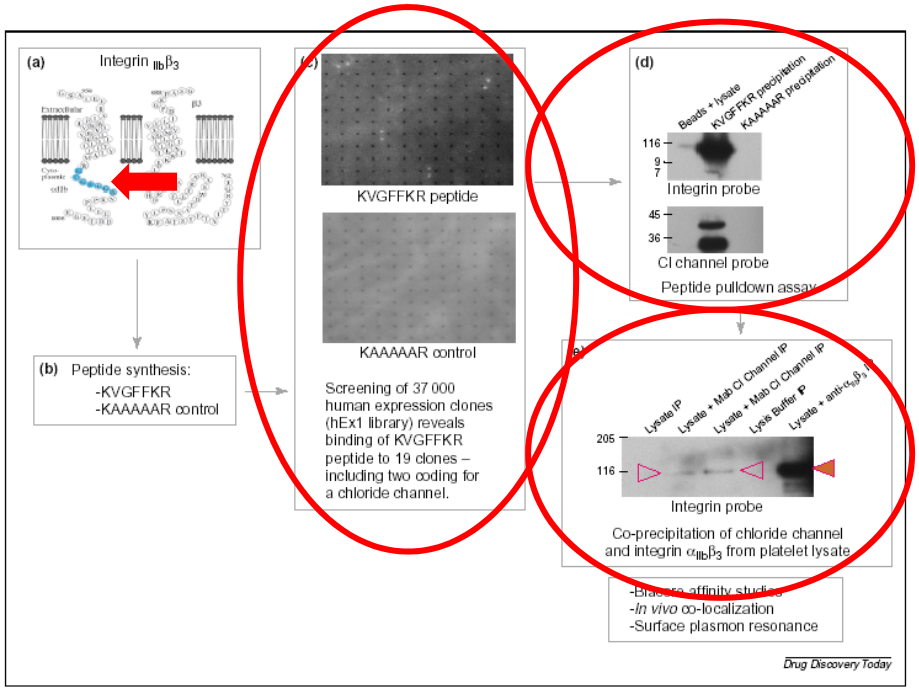
Protein Chips

- Protein chips
  - Chips with high density containing 10^4 proteins
  - Analysis of protein-protein interactions, kinase substrates and interactions with small molecules
  - Possibility of using antibodies – more stable than proteins

Protein Chips

- Identification of proteins interacting with integrin $\alpha_{IIb}\beta_3$ cytoplasmic domain of platelets
  - Expression of cytoplasmic part as a fusion peptide biotin-KVGFFKR
  - Analysis of binding to the protein chip containing 37.000 clones of *E. coli* expressing human recombinant proteins
  - Confirmation of interaction by pull-down analysis of peptides and by coprecipitation of whole proteins as well (e.g. chloride channel Icln)
  - Other use: e.g. in the identification of kinase substrates, when substrates are bound to the chip and exposed to kinases in the presense of radiolabeled ATP (786 purified proteins of barely, of which 21 were identified as CK2α kinase substrates; Kramer et al., 2004)



Lueking et al., 2005

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Outline

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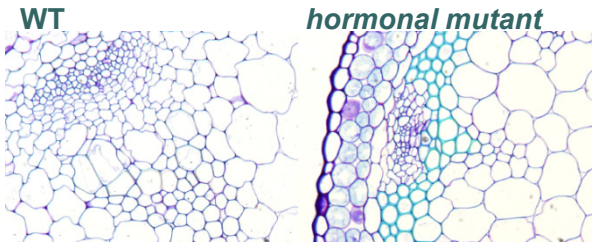
Next Gen Transcriptional Profiling

- ***Transcriptional profiling*** via ***RNA sequencing***



mRNA mRNA

cDNA cDNA

Sequencing by Illumina and **number of transcripts** determination

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Results of –omics Studies vs Biologically Relevant Conclusions

- Transcriptional profiling yielded more then **7K differentially regulated genes**…

Ddii et al., *unpublished*

gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_change)	test_stat	p_value	q_value	significant
AT1G07795	1:2414285-2414967	WT	MT	OK	0	1,1804	1.79769e+308	1.79769e+308	6.88885e-05	0,000391801	yes
HRS1	1:4556891-4558708	WT	MT	OK	0	0,696583	1.79769e+308	1.79769e+308	6.61994e-06	4.67708e-05	yes
ATMLO14	1:9227472-9232296	WT	MT	OK	0	0,514609	1.79769e+308	1.79769e+308	9.74219e-05	0,000535055	yes
NRT1.6	1:9400663-9403789	WT	MT	OK	0	0,877865	1.79769e+308	1.79769e+308	3.2692e-08	3.50131e-07	yes
AT1G27570	1:9575425-9582376	WT	MT	OK	0	2,0829	1.79769e+308	1.79769e+308	9.76039e-06	6.647e-05	yes
AT1G60095	1:22159735-22162419	WT	MT	OK	0	0,688588	1.79769e+308	1.79769e+308	9.95901e-08	9.84992e-07	yes
AT1G03020	1:698206-698515	WT	MT	OK	0	1,78859	1.79769e+308	1.79769e+308	0,00913915	0,0277958	yes
AT1G13609	1:4662720-4663471	WT	MT	OK	0	3,55814	1.79769e+308	1.79769e+308	0,00021683	0,00108079	yes
AT1G21550	1:7553100-7553876	WT	MT	OK	0	0,562868	1.79769e+308	1.79769e+308	0,00115582	0,00471497	yes
AT1G22120	1:7806308-7809632	WT	MT	OK	0	0,617354	1.79769e+308	1.79769e+308	2.48392e-06	1.91089e-05	yes
AT1G31370	1:11238297-11239363	WT	MT	OK	0	1,46254	1.79769e+308	1.79769e+308	4.83523e-05	0,000285143	yes
APUM10	1:13253397-13255570	WT	MT	OK	0	0,581031	1.79769e+308	1.79769e+308	7.87855e-06	5.46603e-05	yes
AT1G48700	1:18010728-18012871	WT	MT	OK	0	0,556525	1.79769e+308	1.79769e+308	6.53917e-05	0,000374736	yes
AT1G59077	1:21746209-21833195	WT	MT	OK	0	138,886	1.79769e+308	1.79769e+308	0,00122789	0,00496816	yes
AT1G60050	1:22121549-22123702	WT	MT	OK	0	0,370087	1.79769e+308	1.79769e+308	0,00117953	0,0048001	yes
AT4G15242	4:8705786-8706997	WT	MT	OK	0,00930712	17,9056	10,9098	-4,40523	1.05673e-05	7.13983e-05	yes
AT5G33251	5:12499071-12500433	WT	MT	OK	0,0498375	52,2837	10,0349	-9,8119	0	0	yes
AT4G12520	4:7421055-7421738	WT	MT	OK	0,0195111	15,8516	9,66612	-3,90043	9.60217e-05	0,000528904	yes
AT1G60020	1:22100651-22105276	WT	MT	OK	0,0118377	7,18823	9,24611	-7,50382	6.19504e-14	1.4988e-12	yes
AT5G15360	5:4987235-4989182	WT	MT	OK	0,0988273	56,4834	9,1587	-10,4392	0	0	yes

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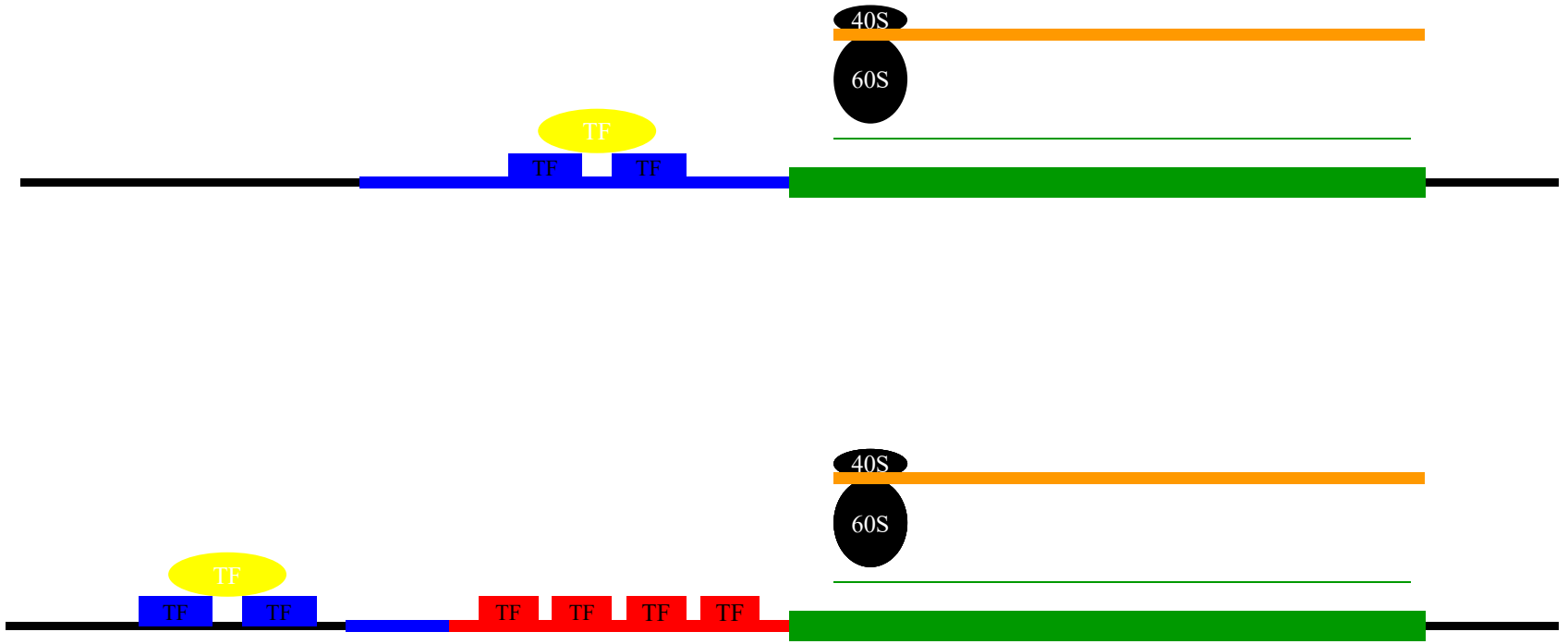
  - T-DNA activation mutagenesis

Gain-of-Function Approaches

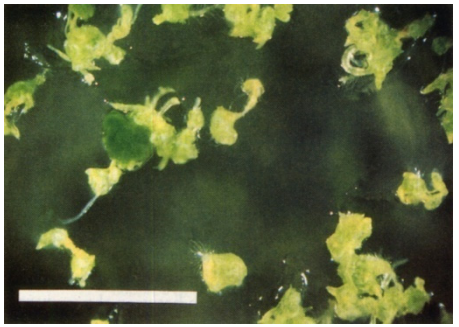
- Methods for identification of gene function using gain-of-function approaches
  - T-DNA activation mutagenesis
    - Method enabling isolation of dominant mutants by random insertion of constitutive promoter, resulting in overexpression of the gene and therefore in corresponding phenotypic changes
    - First step: preparation of mutant library prepared by tansformation of a strong constitutive promoter or enhancer
    - Next step: search of interesting phenotypes
    - Identification of the affected gene, e.g. by plasmid-rescue

Activation Mutagenesis



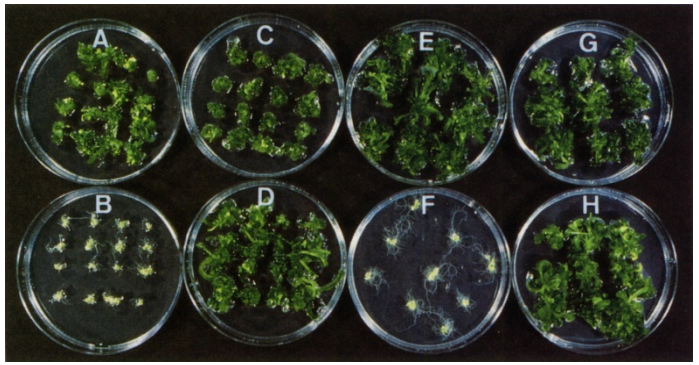
Isolation of *CKI1* Gene

- Tatsuo Kakimoto, *Science* 274 (1996), 982-985 *
- Isolation of the gene using activation mutagenesis



*

- Mutant phenotype is a phenocopy of exogenous application of cytokinins (*CKI1*, ***C***YTO***K***ININ ***I***NDEPENDENT *1*)



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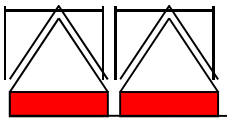
Regulated Expression Systems

activator
x

activator x reporter

reporter



pOP

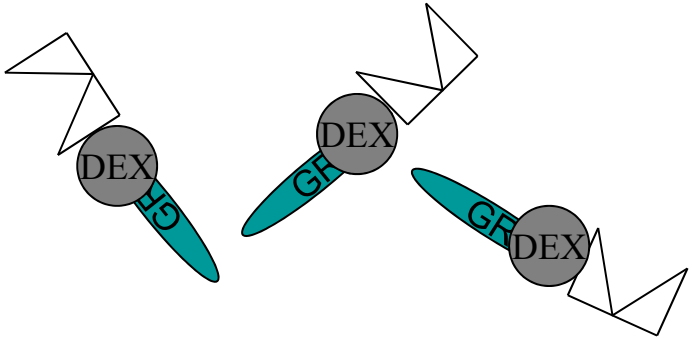
Regulated Expression Systems

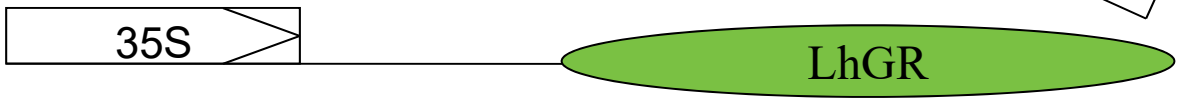
activator
x

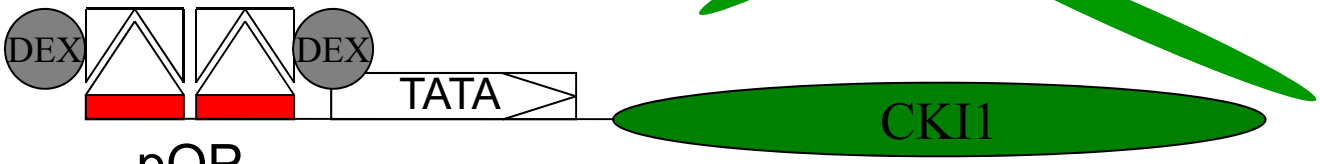
reporter

activator x reporter

+DEX







pOP

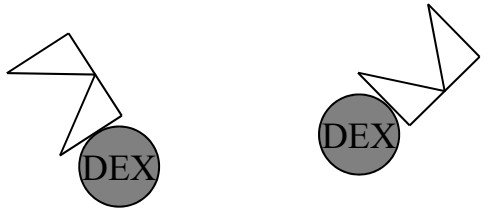
Regulated Expression Systems

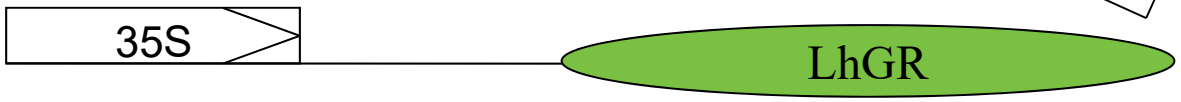
activator
X

reporter

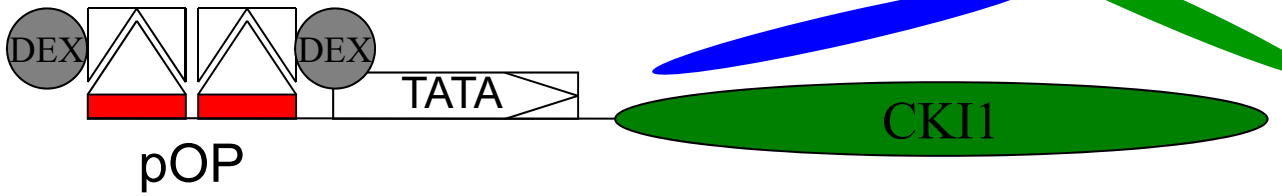
activator x reporter

+DEX



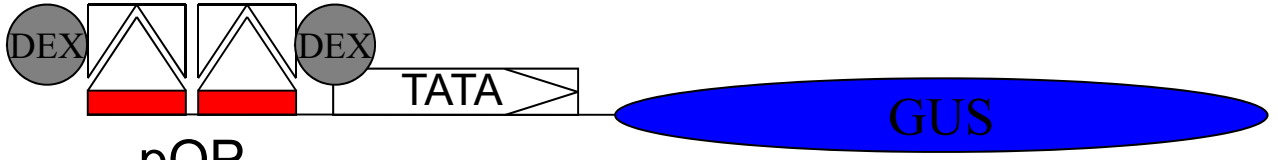


wt Col-0



pOP

4C



pOP

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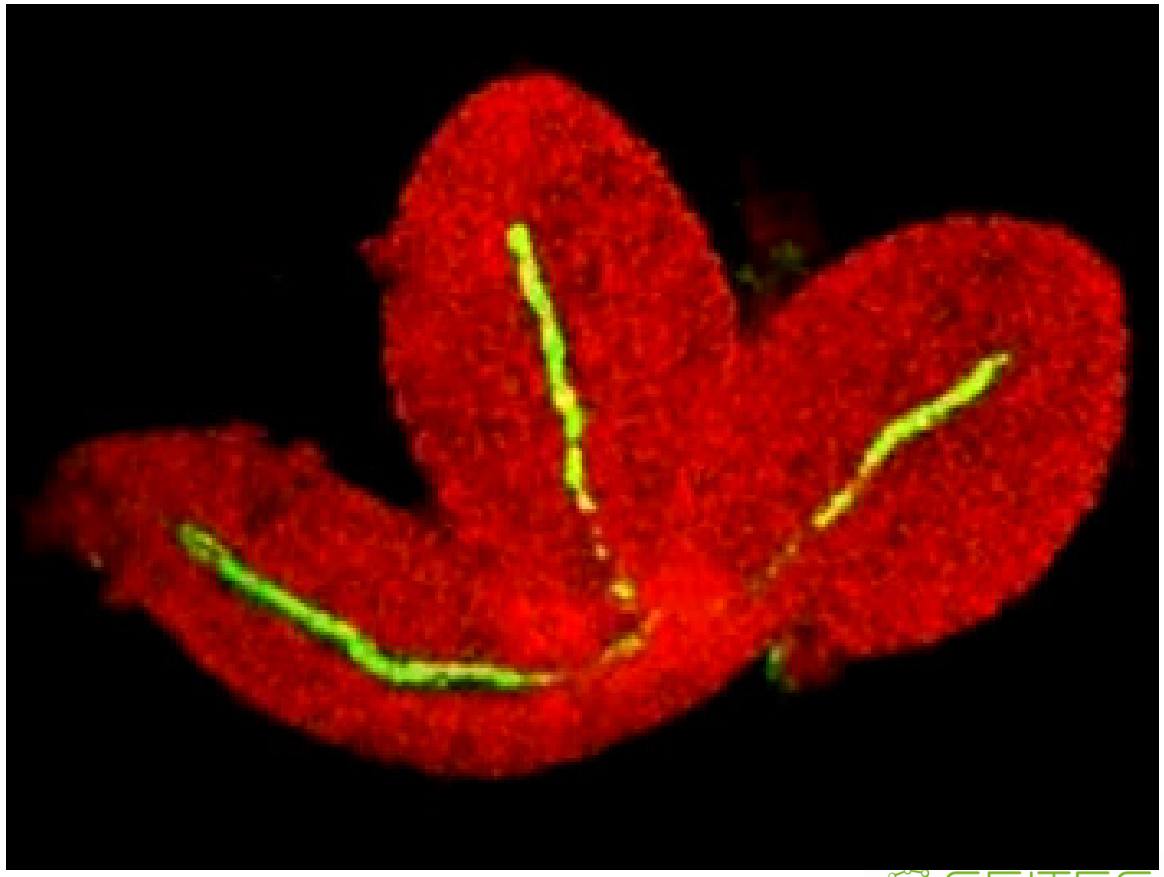
Regulated Expression Systems

- Regulatable gene expression systems
  - Time- or site-specific regulation of gene expression, leading to a change in phenotype and thereby identification of the natural function of the gene
    - pOP system
    - UAS system



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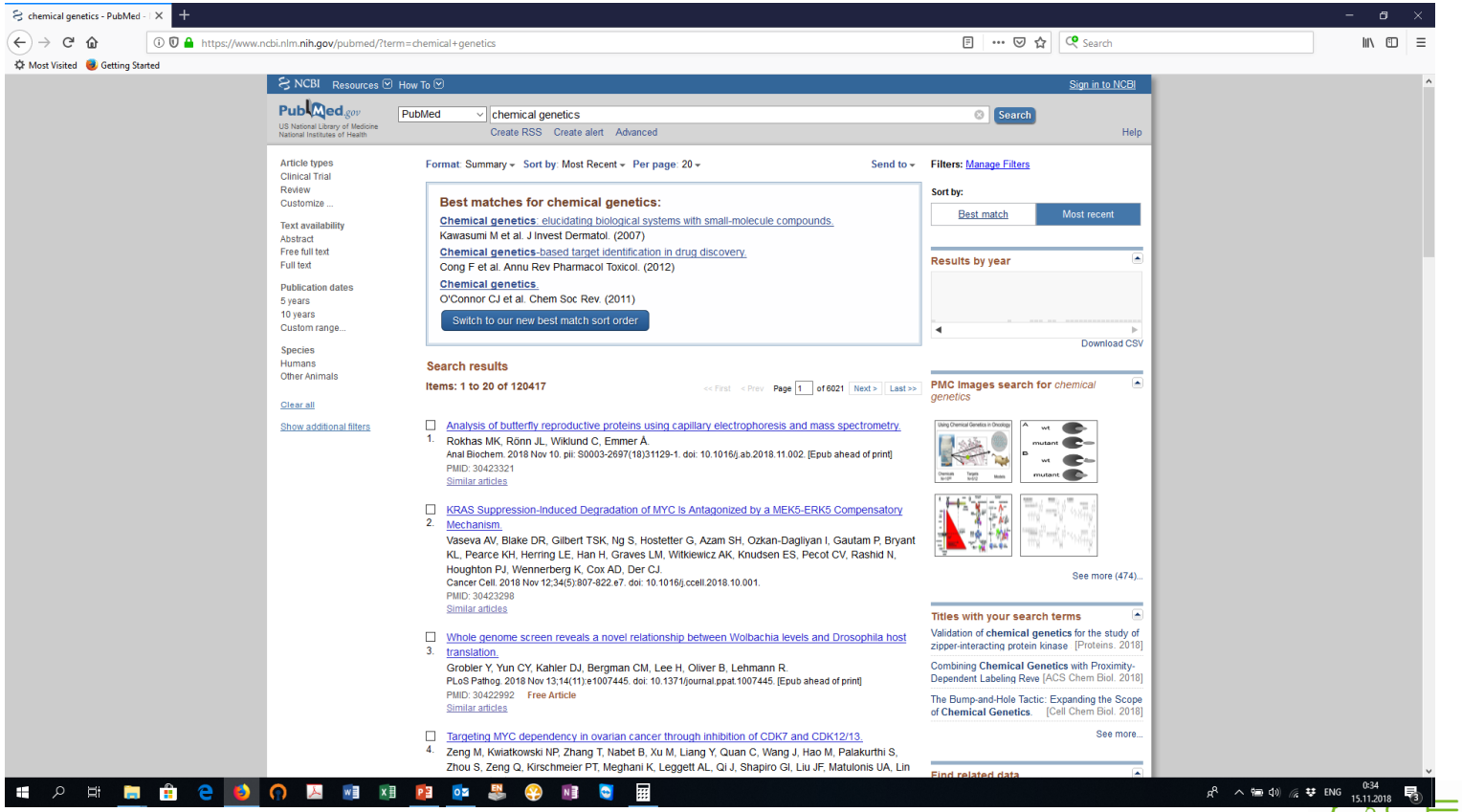
Chemical Genetics

- New trends
  - **„chemical genetics“** – more than **50.000/120.417** records in PubMed database (16.10. **2008**/15.11. **2018**, **an increase of >240 %**)

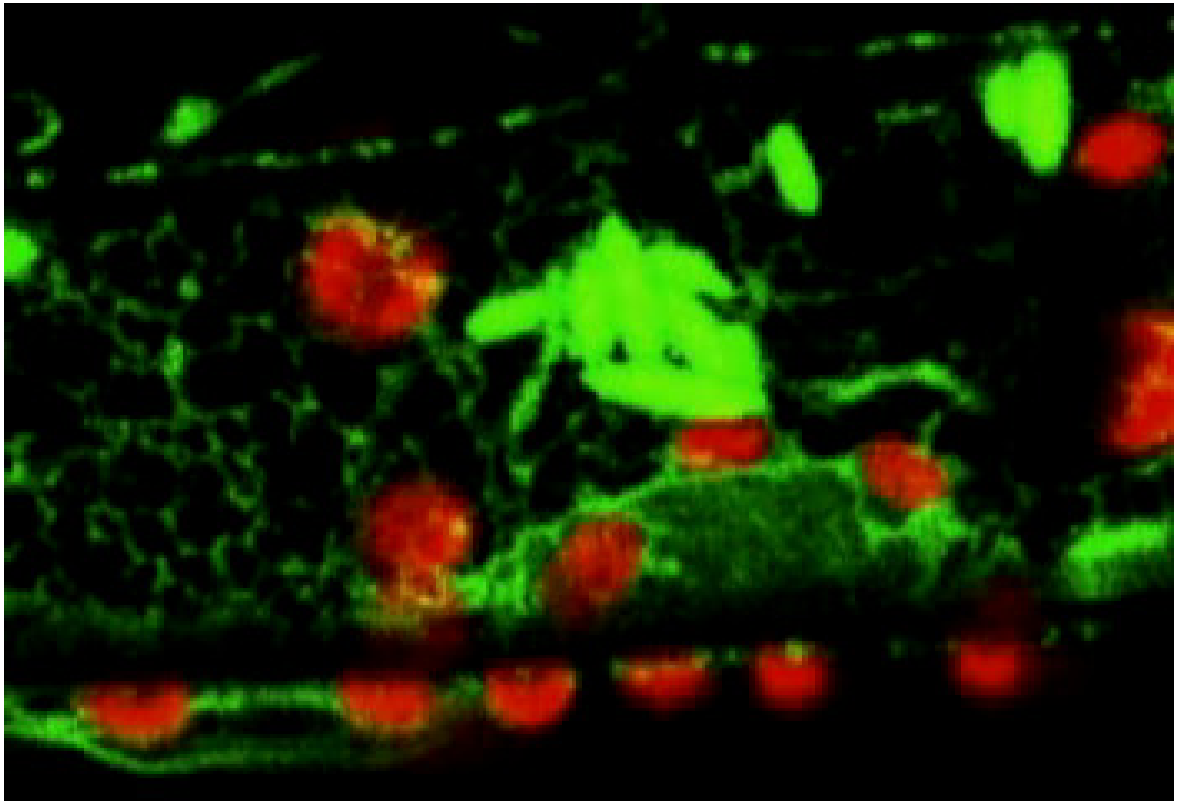


Chemical Genetics

- New trends
  - **„chemical genetics“** – more than **50.000/130.437** records in PubMed database (16.10. **2008/**24.10. **2019**, **an increase of >260 %**)
    - Like in the case of genetics, there are also „**forward**“ and „**reverse**“ genetics approaches
    - Unlike in „classical“ genetics approaches, **the subject of study** is not a gene, but a **protein**
    - Chemical genetics tries to identify either the **target protein** after a chemical treatment and after following phenotypic changes („forward“ chemical genetics) or **chemicals able to interact with protein of interest** („reverse“ chemical genetics)
    - For that purpose there are carried out **searches in the libraries** of various **chemicals** (thousands of entries, comercially available)
    - example: **analysis of endomembrane transport** in plants

Chemical Genetics

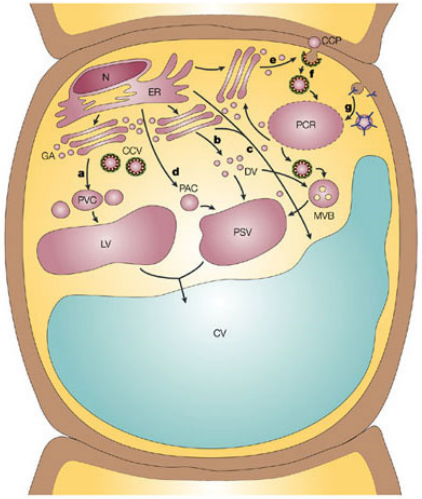
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
  - In plants cells there occurr very dynamic processes mediated mainly by endomembrane transport

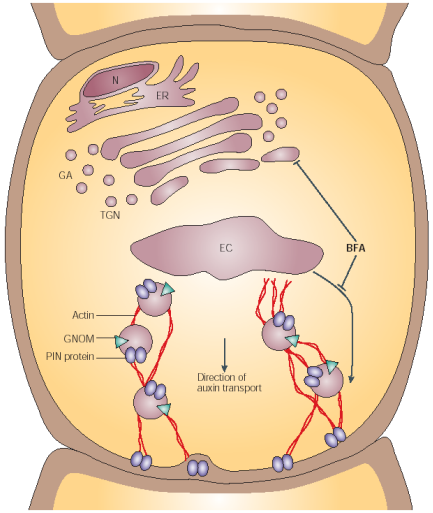


GFP targeted to the ER

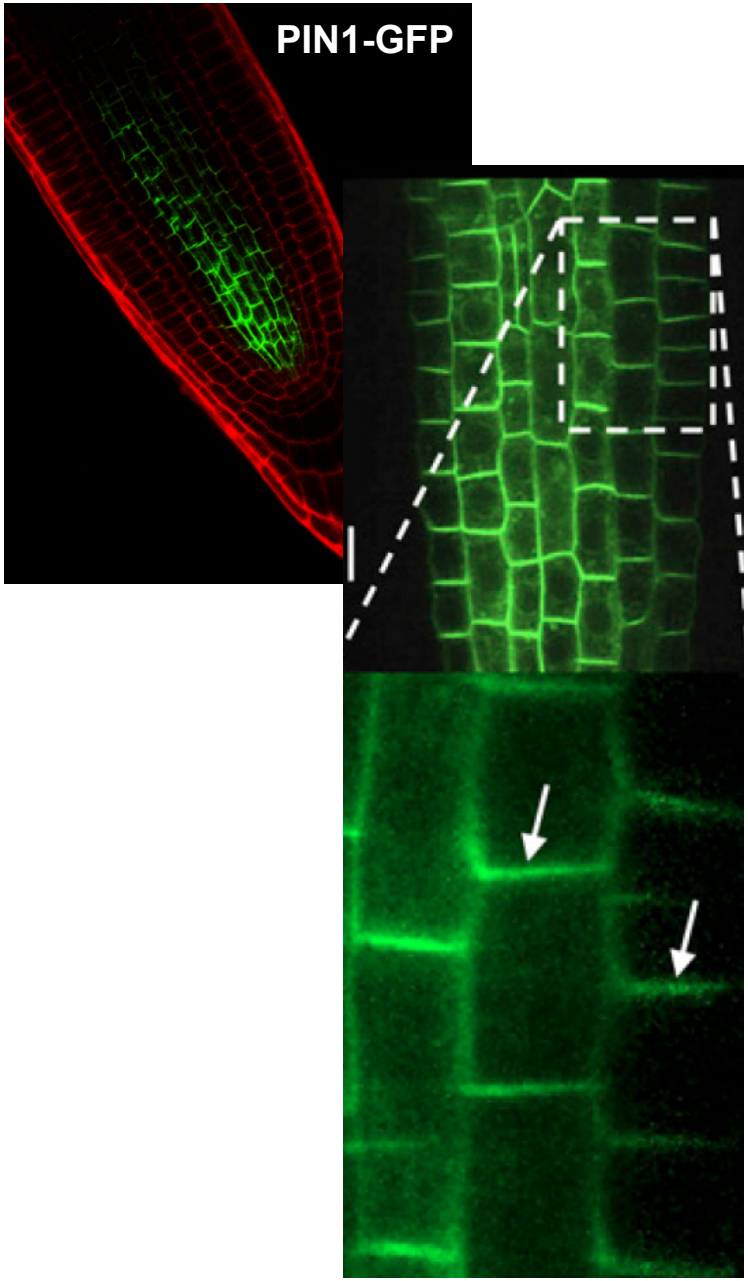
Chemical Genetics

- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
  - In plants cells there occurr very dynamic processes mediated mainly by endomembrane transport (see film, GFP targeting to the ER)
  - Endomembrane transport is an important regulatory mechanism in signal transduction and regulation of cellular processes

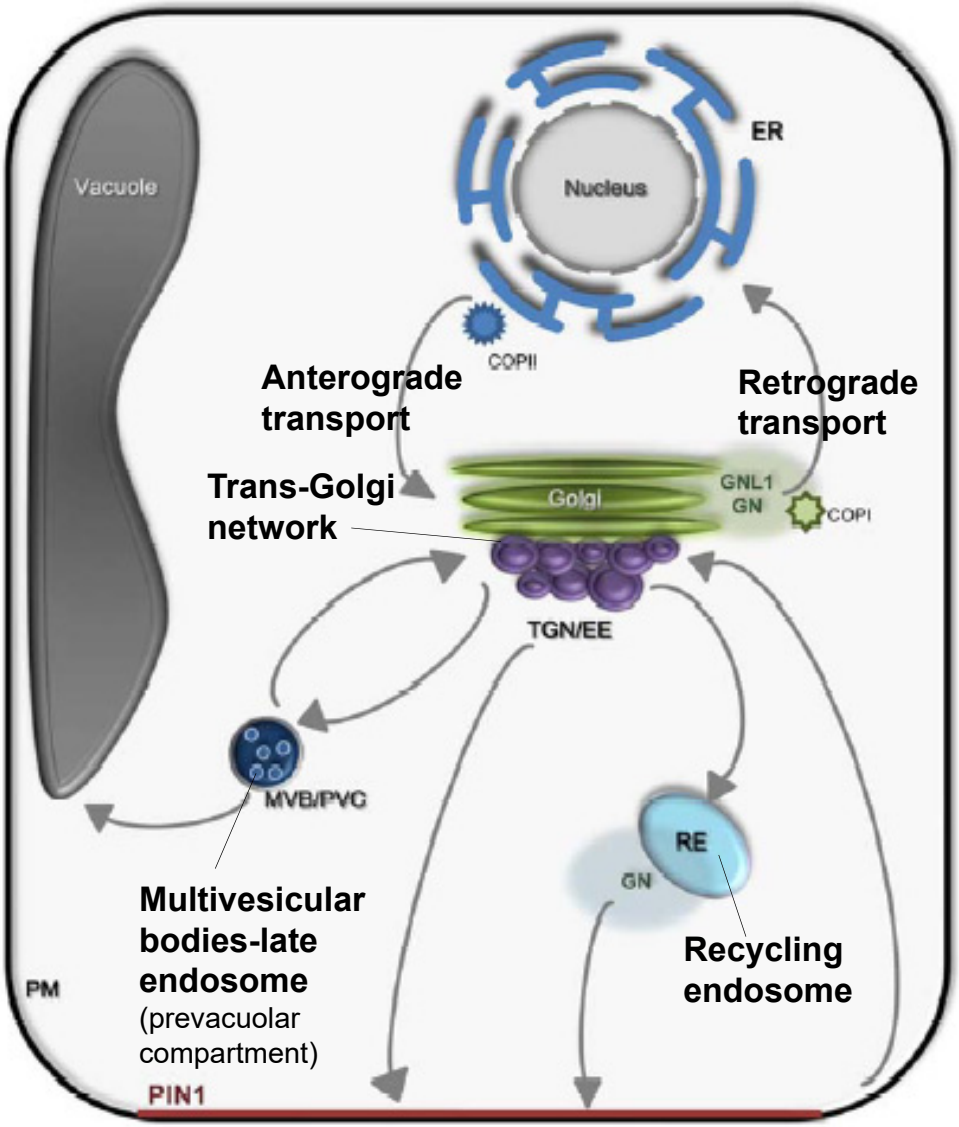




CEITEC

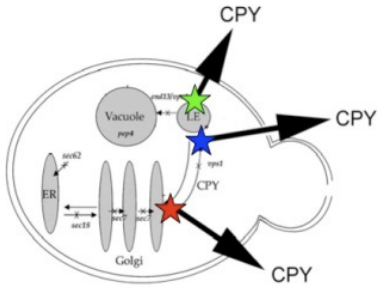


Huang et al., 2010

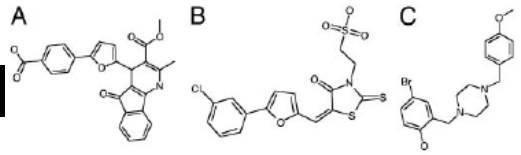


Chemical Genetics

- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
  - By searching in the „library“ of chemicals there were identified those, that lead to the secretion of enzyme (carboxypeptidase Y) in yeast (*S. cerevisiae)* – this enzyme is normally transported to the vacuole via the endomembrane transport
    - Analysis of changes in secretion using dot-blot and immunodetection of carboxypeptidase Y in the culture medium with monoclonal antibodies

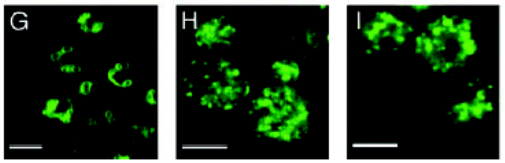


Chemical structure of sortins



Immunodetection of carboxypeptidase

Detection of vacuole phenotype (tonoplast shape) of yeast by staining with a specific color (MDY-64)



Zouhar et al., 2004

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Chemical Genetics

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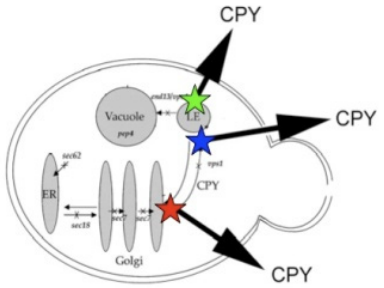
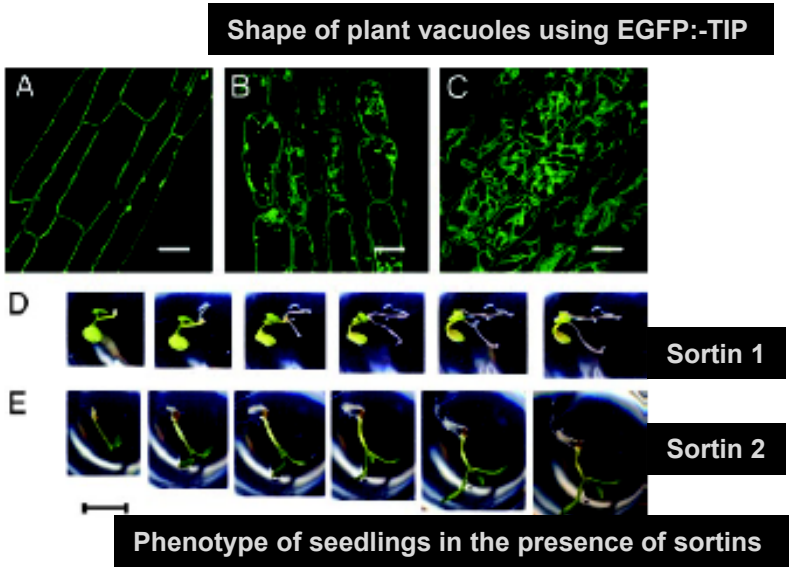
    - Analysis of changes in secretion using dot-blot and immunodetection of carboxypeptidase Y in the culture medium with monoclonal antibodies
  - Identified compounds („sortins“) were able to induce similar changes in *Arabidopsis* as well – transport mechanisms are conserved in yeast and in plants
  - For detailed identification of the molecular proces affected by one of the identified „sortins“, the analysis of its influence on a secretion of a marker protein (AtCPY) was performed – sortin 1 specifically inhibits only this secretory pathway
  - Identifcation of mutants with altered sensitivity to sortin 1 (hyper- or hypo-sensitive mutants) by EMS mutagenesis





Zouhar et al., 2004

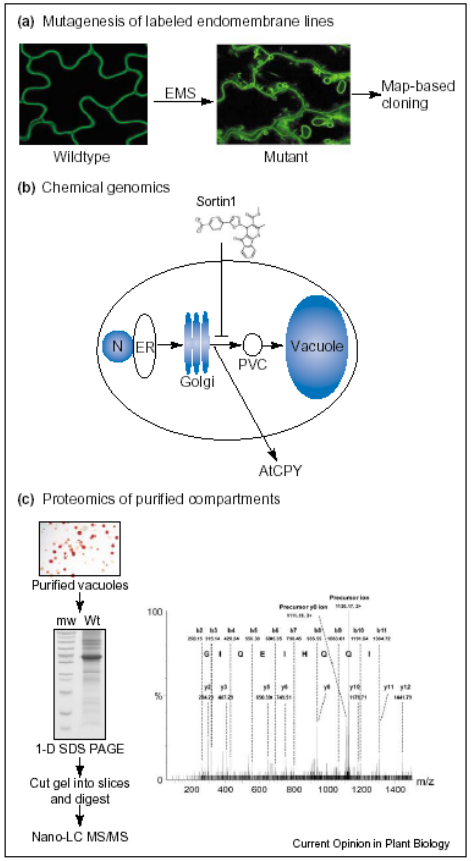
CEITEC

- Analysis of mechanisms of endomembrane transport by chemical genetics approaches – summary

  - GFP::d-TIP vacuole membrane (tonoplast) labelling and identification of mutations leading to altered tonoplast morphology

  - Chemical genetics in combination with classical genetics – identification of proteins participating in regulation of endomembrane transport

  - Proteomics approaches – identification and analysis of vacuole proteome



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Discussion