

CG920 Genomics

Lesson 5

Gene Expression and Chemical Genetics

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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 05:
 - Surpin, M. and Raikhel, N. (2004) Traffic jams affect plant development and signal transduction. *Nature Reviews/Molecular Cell Biology* 5,100-109
 - Zouhar, J., Hicks, G.R. and Raikhel, N.V. (2004) Sorting inhibitors (Sortins): Chemical compounds to study vacuolar sorting in Arabidopsis. *Proceedings of the National Academy of Sciences of the U.S.A.*, 101, 9497–9501
 - Nevo-Dinur, K., Nussbaum-Shochat, A., Ben-Yehuda, S., and Amster-Choder, O. (2011). Translation-independent localization of mRNA in *E. coli*. *Science* 331, 1081-1084.
 - Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* 131, 174-187.
 - Schonberger, J., Hammes, U.Z., and Dresselhaus, T. (2012). In vivo visualization of RNA in plants cells using the lambdaN(22) system and a GATEWAY-compatible vector series for candidate RNAs. *The Plant journal : for cell and molecular biology* 71, 173-181.

Outline

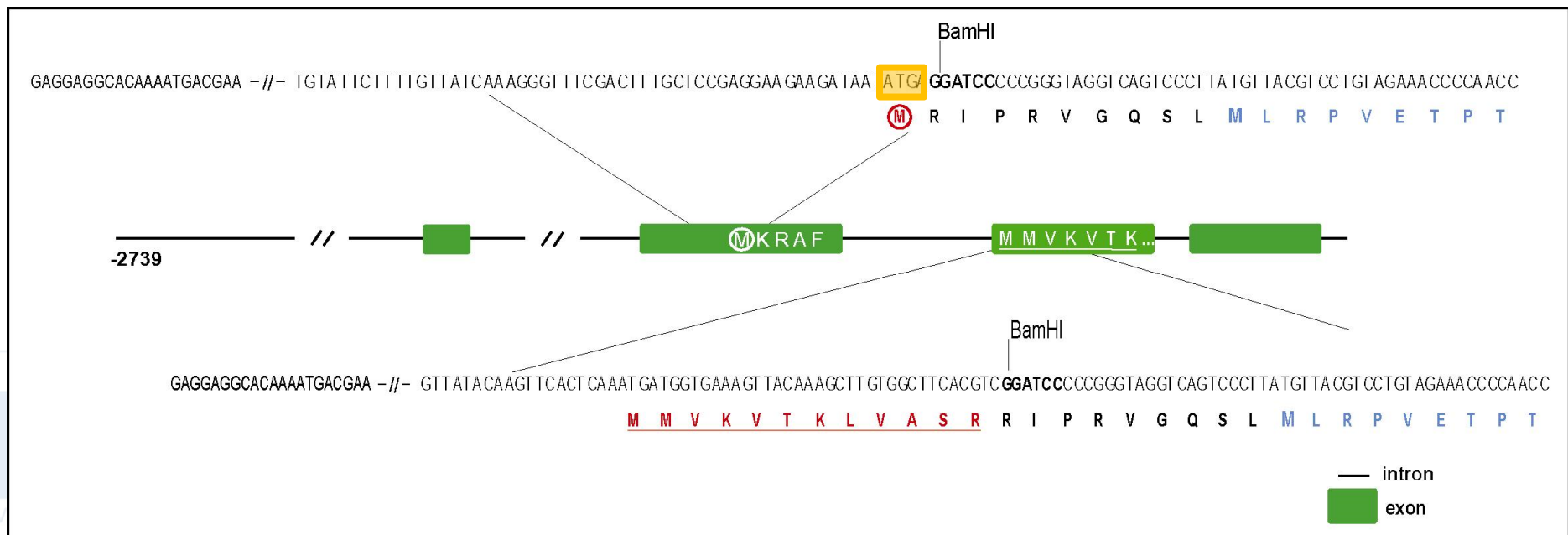
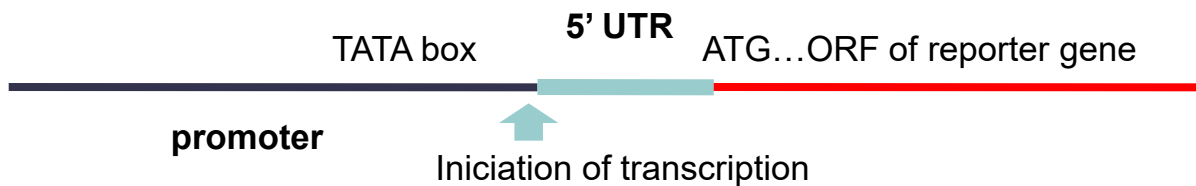
- **Methods of gene expression analysis**
 - **Qualitative analysis of gene expression**
 - Preparation of **transcriptional fusion** of **promoter** of analysed gene with a **reporter gene**
 - Preparation of **translational fusion** of the **coding region** of the analysed gene with **reporter gene**
 - Use of the data available in **public databases**
 - **Tissue-** and **cell-specific** gene expression analysis
 - **Quantitative analysis of gene expression**
 - **DNA and protein chips**
 - **Next generation transcriptional profiling**
- **Regulation of gene expression in the identification of gene function by gain-of-function approaches**
 - **T-DNA activation mutagenesis**
 - **Ectopic expression and regulated gene expression systems**
- **Chemical Genetics**

Outline

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

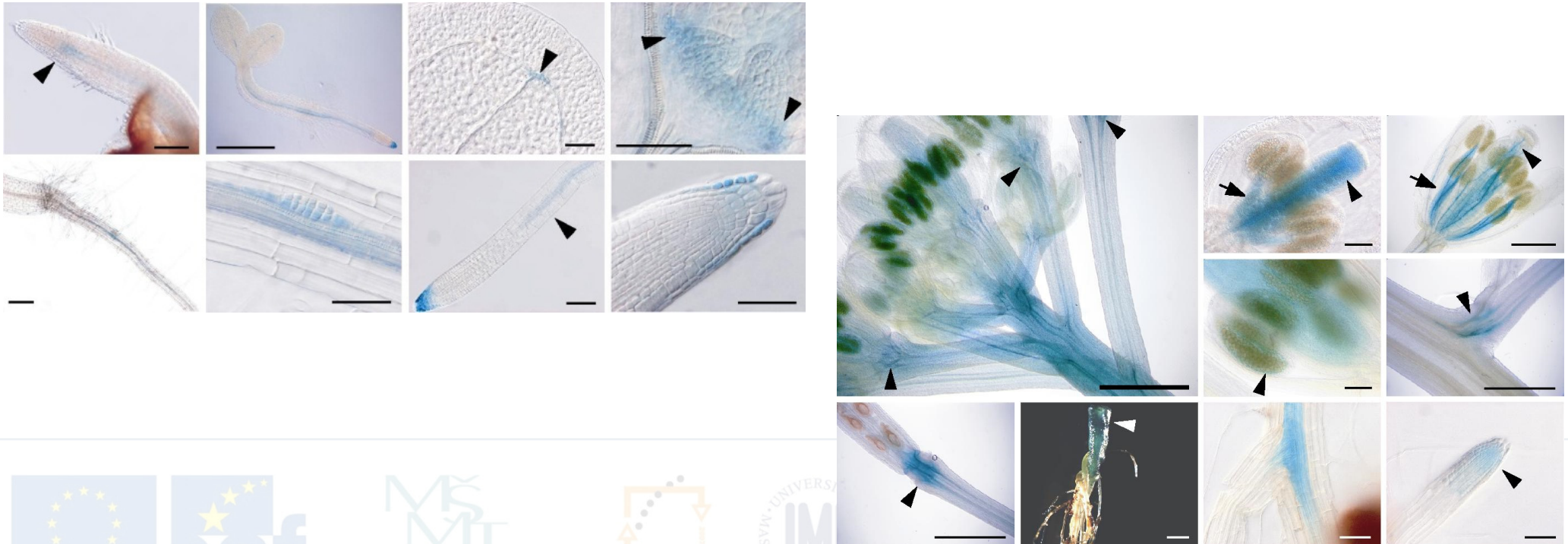
Transcriptional Fusion

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

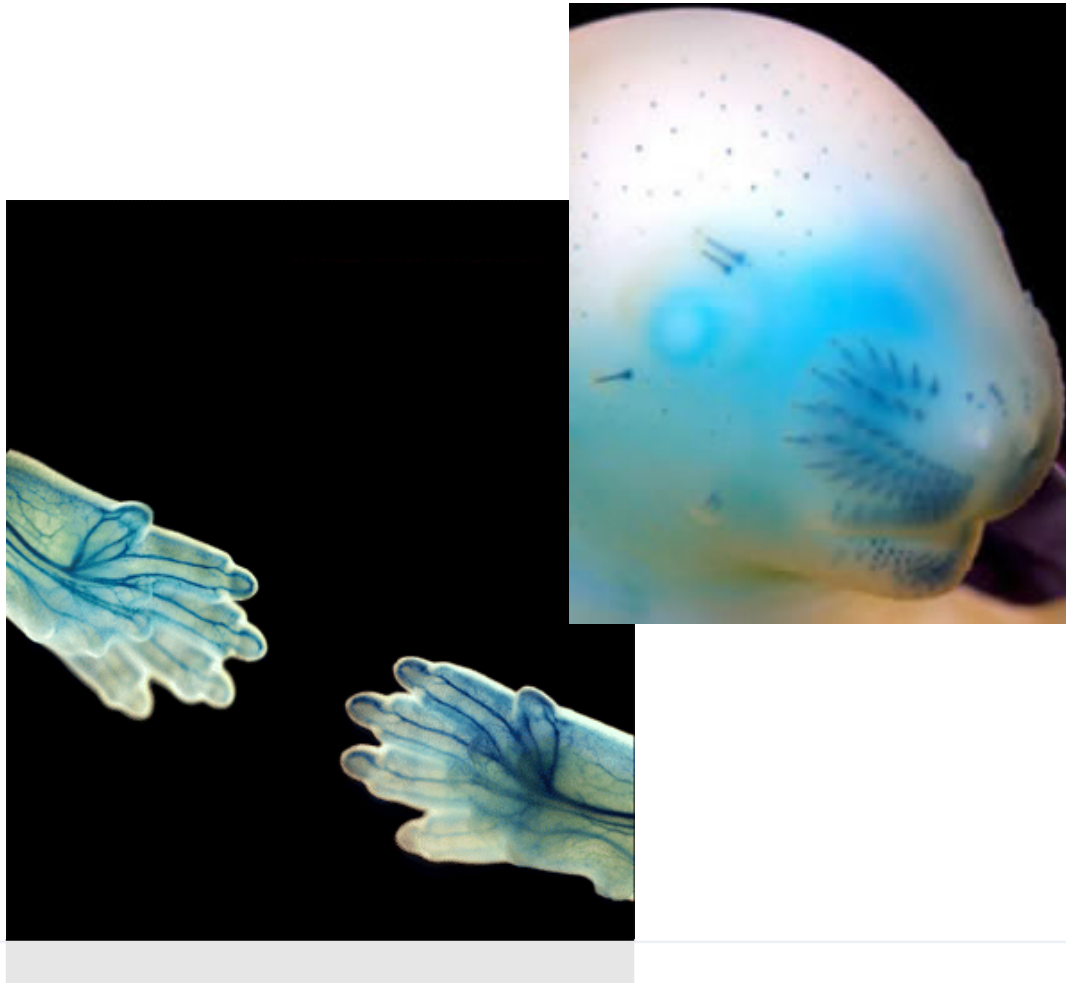


Transcriptional Fusion

- 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

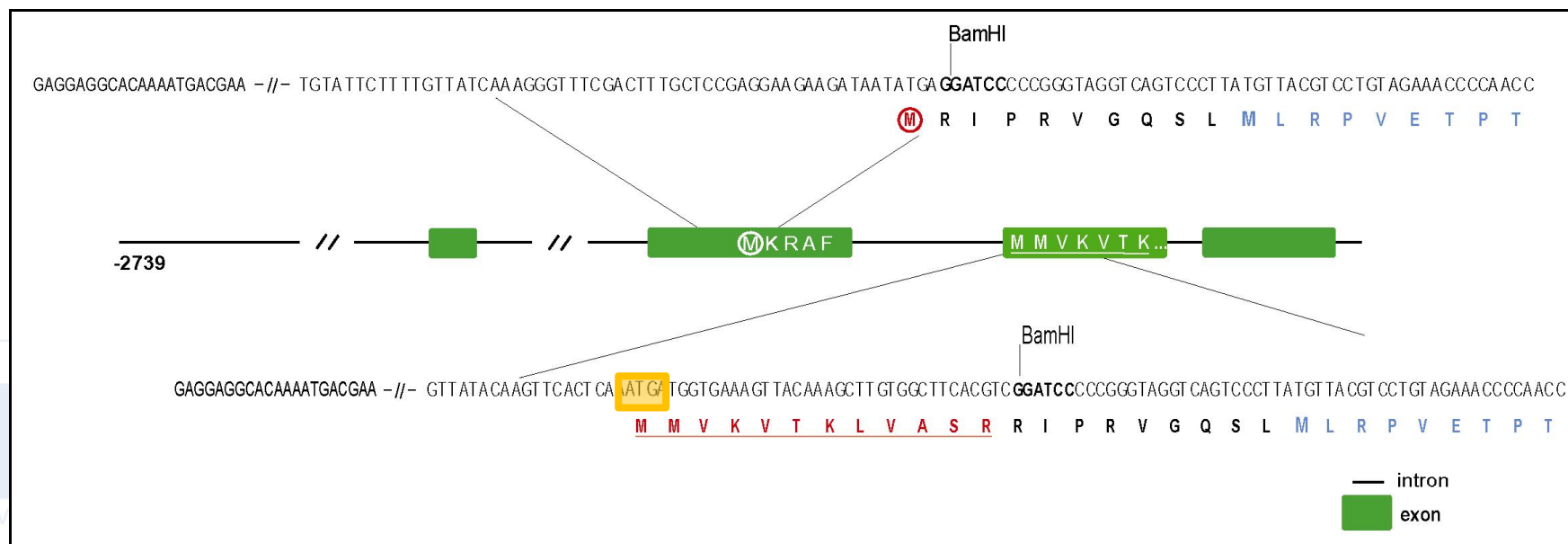
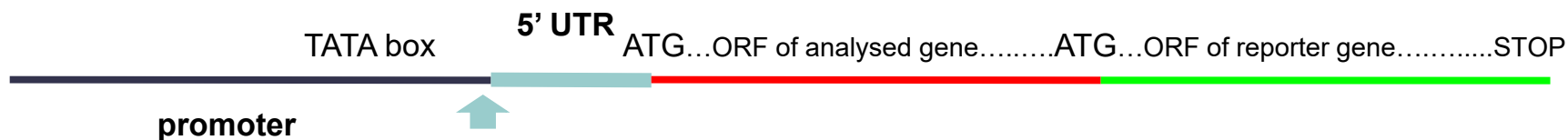


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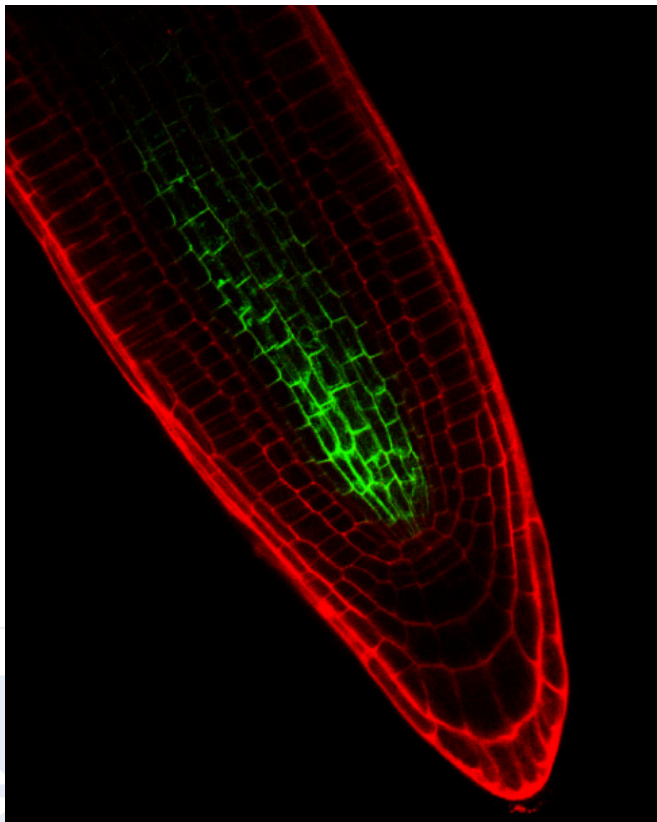
Translational Fusion

- 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)

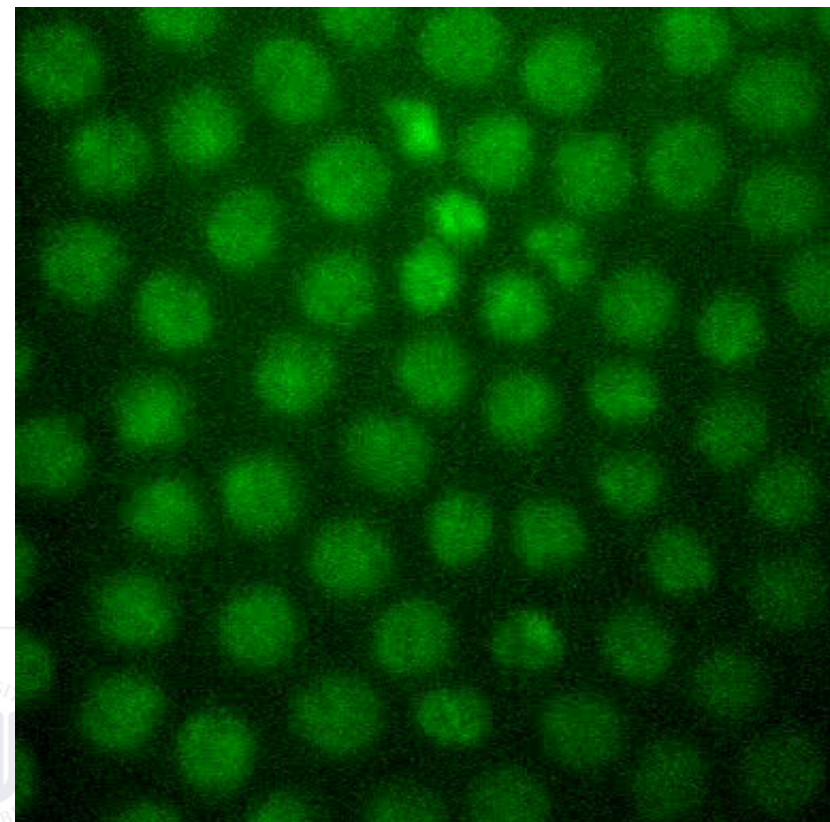


Translational Fusion

- 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

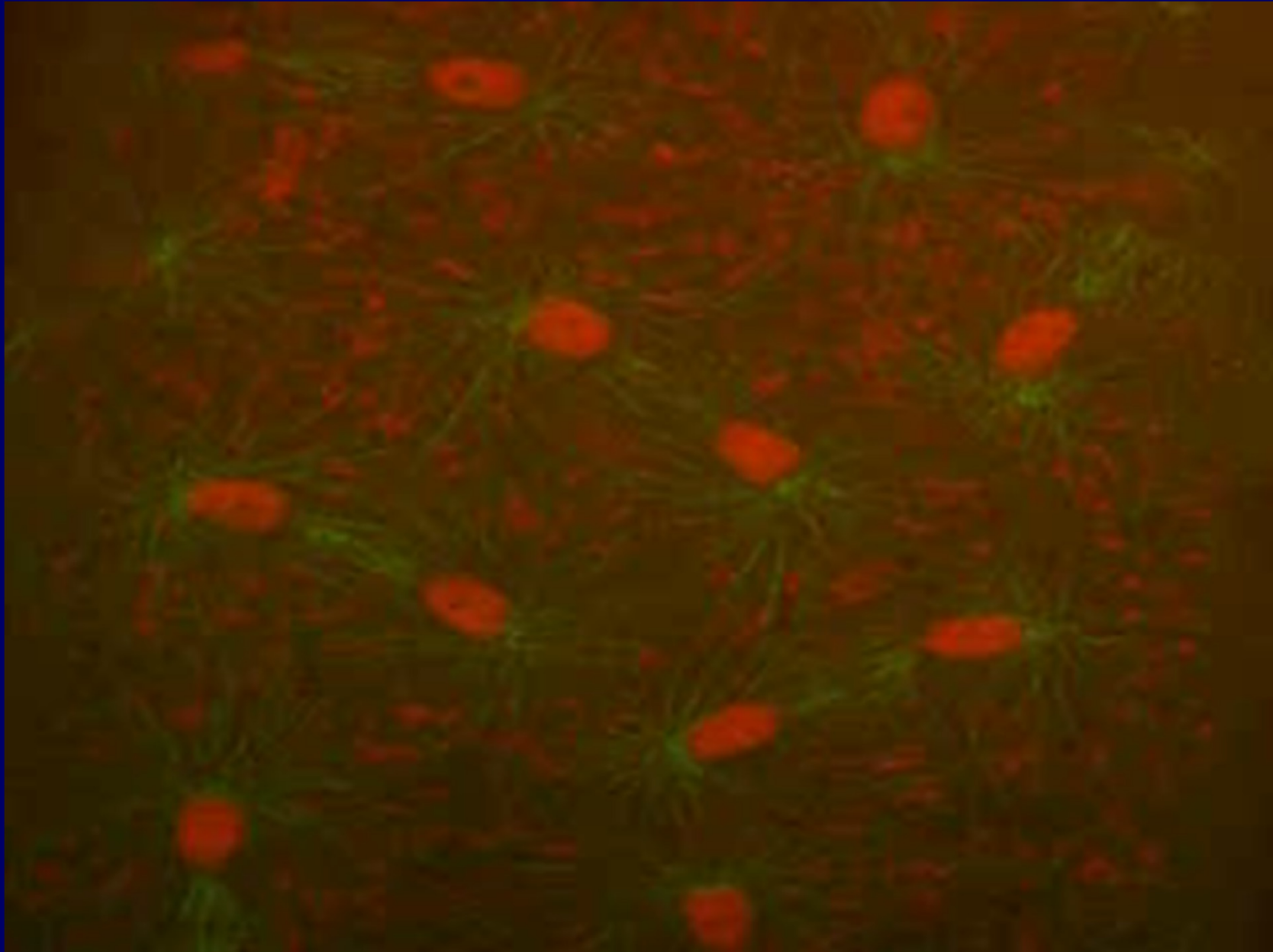


PIN1-GFP in *Arabidopsis*



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

Translational Fusion

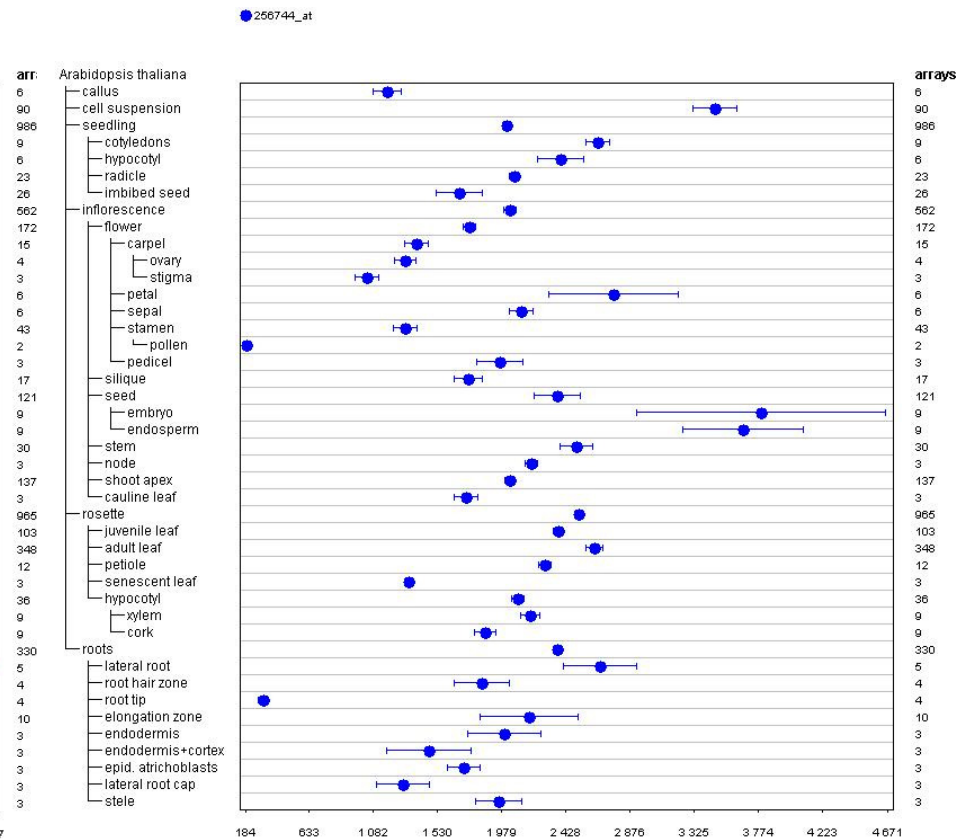
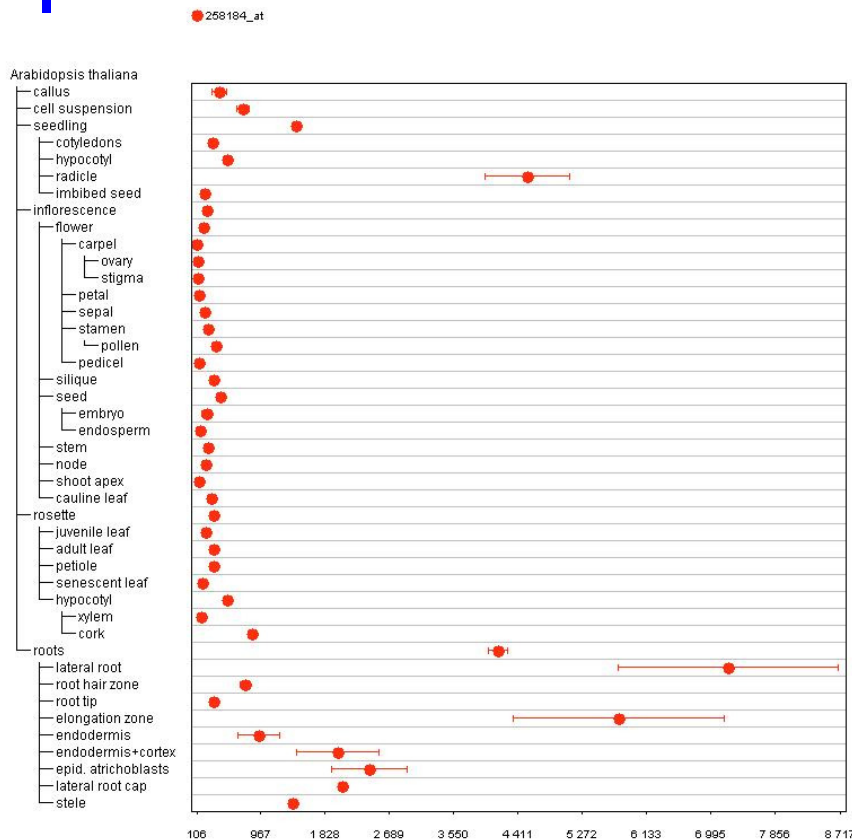


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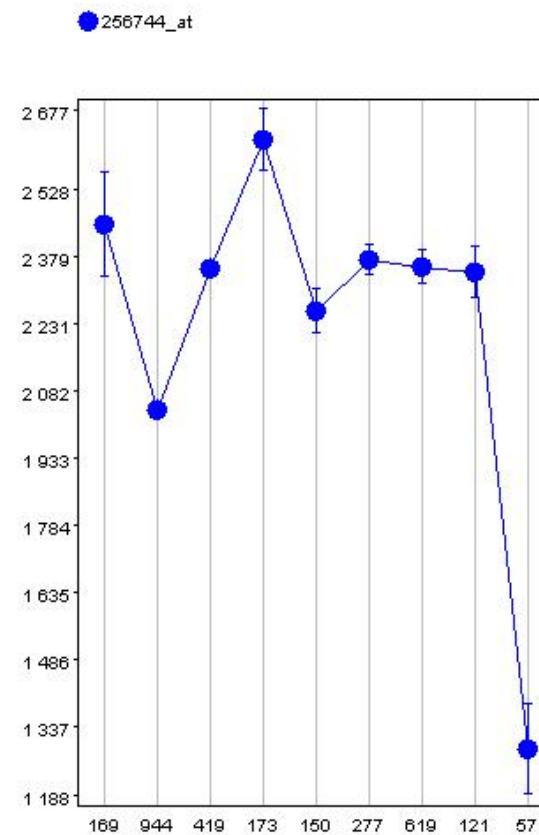
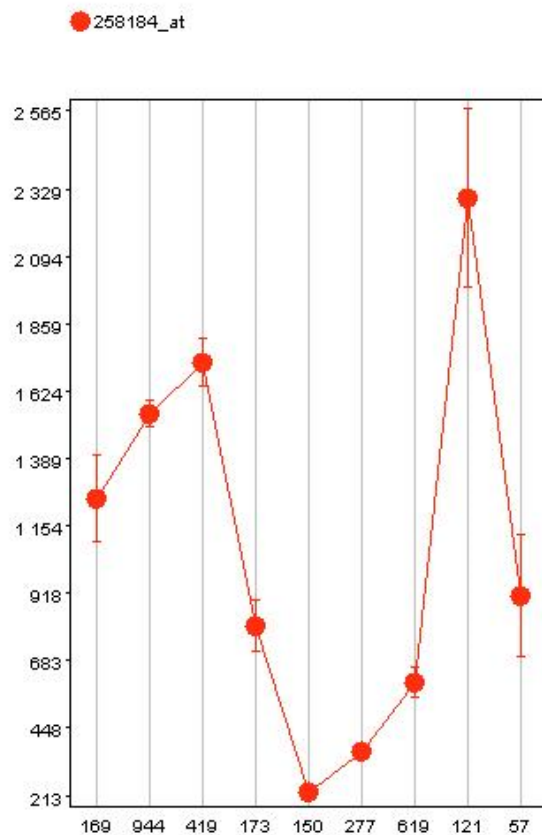
Databases

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



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



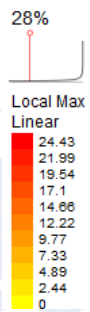
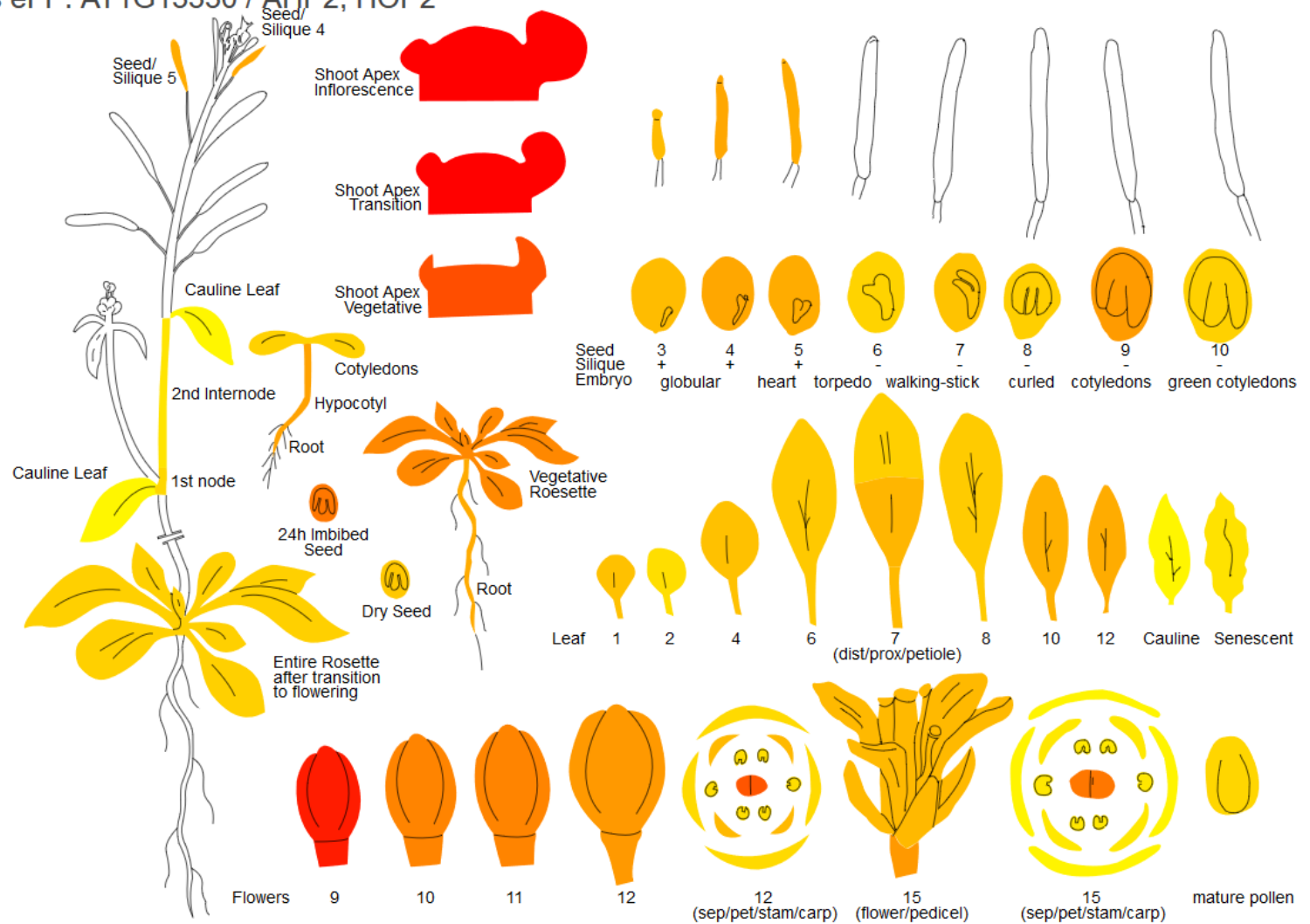
germinated seed
seedling
young rosette
developed rosette
bolting
young flower
developed flower
flowers and siliques
mature siliques

germinated seed
seedling
young rosette
developed rosette
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young flower
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flowers and siliques
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Databases

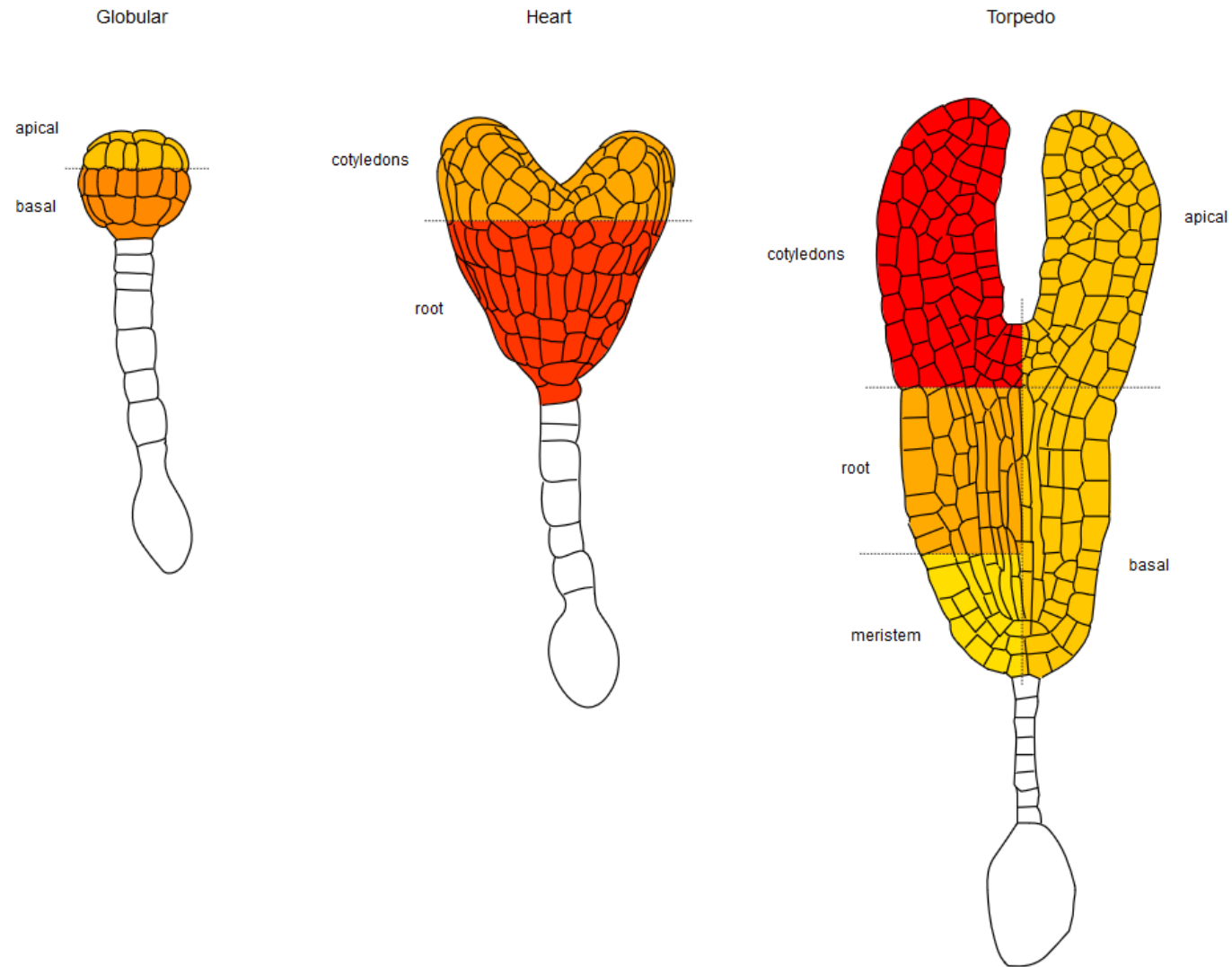
□ Analysis of expression using ePlant

AtGenExpress eFP: AT1G13330 / AHP2, HOP2



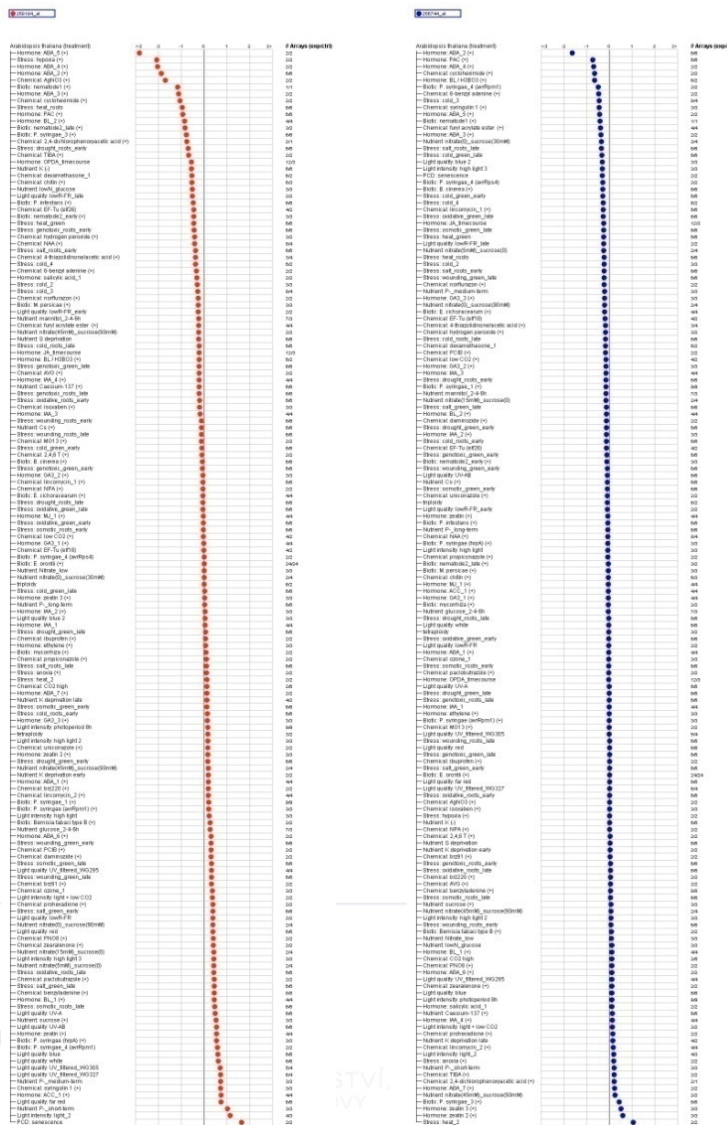
Databases

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Databases

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



STVĚ
VVY

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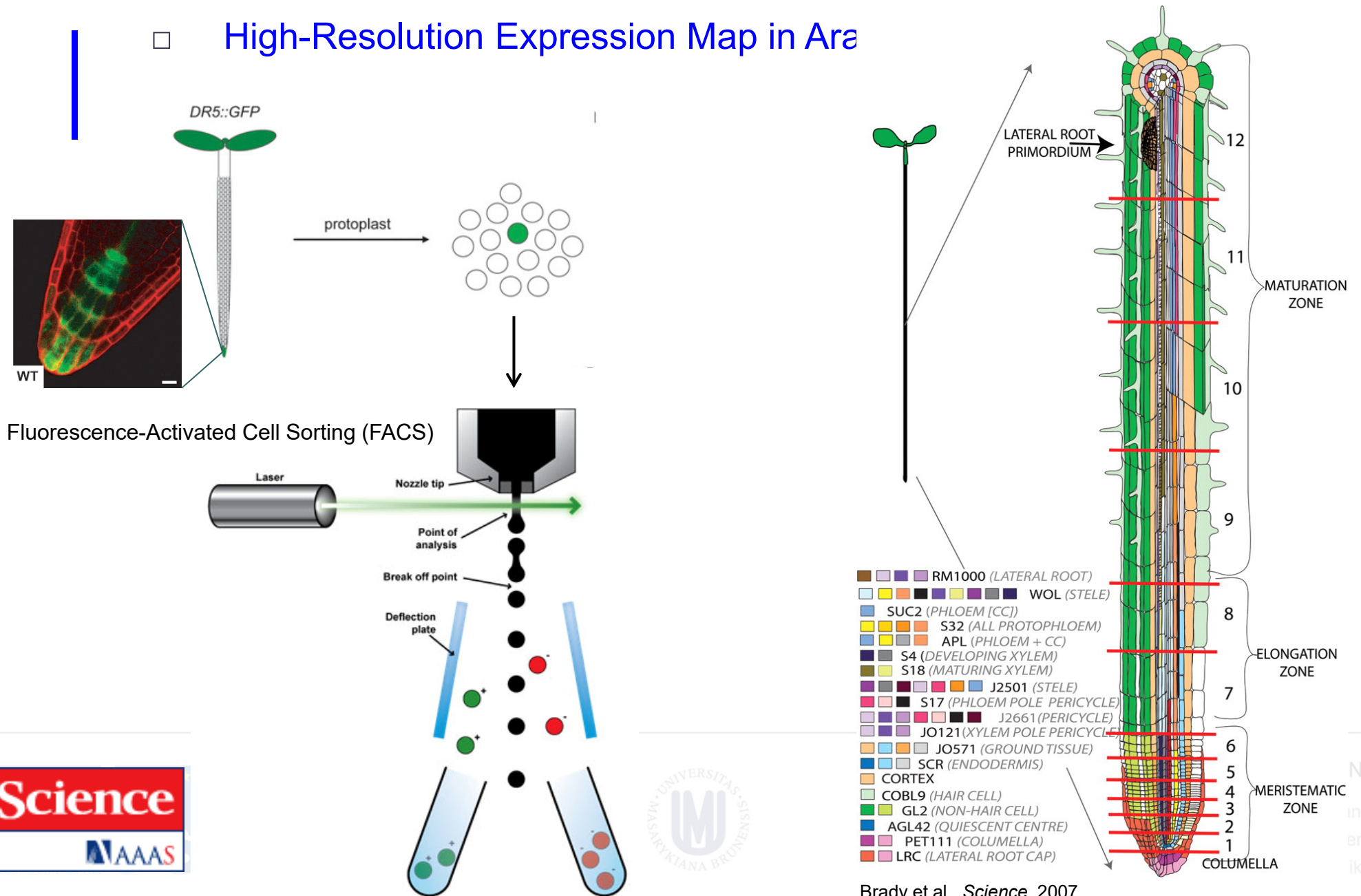


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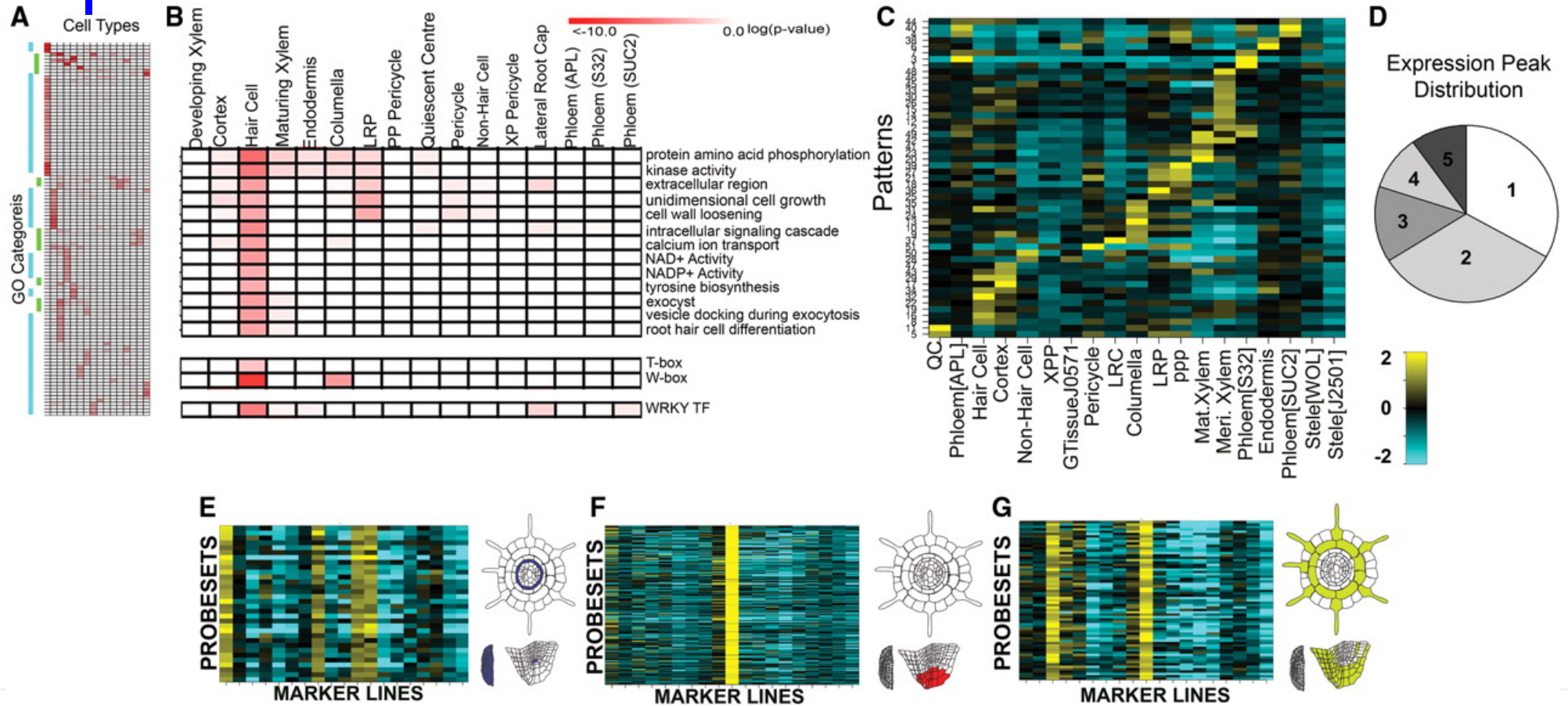
Expression Maps - RNA

High-Resolution Expression Map in Ara



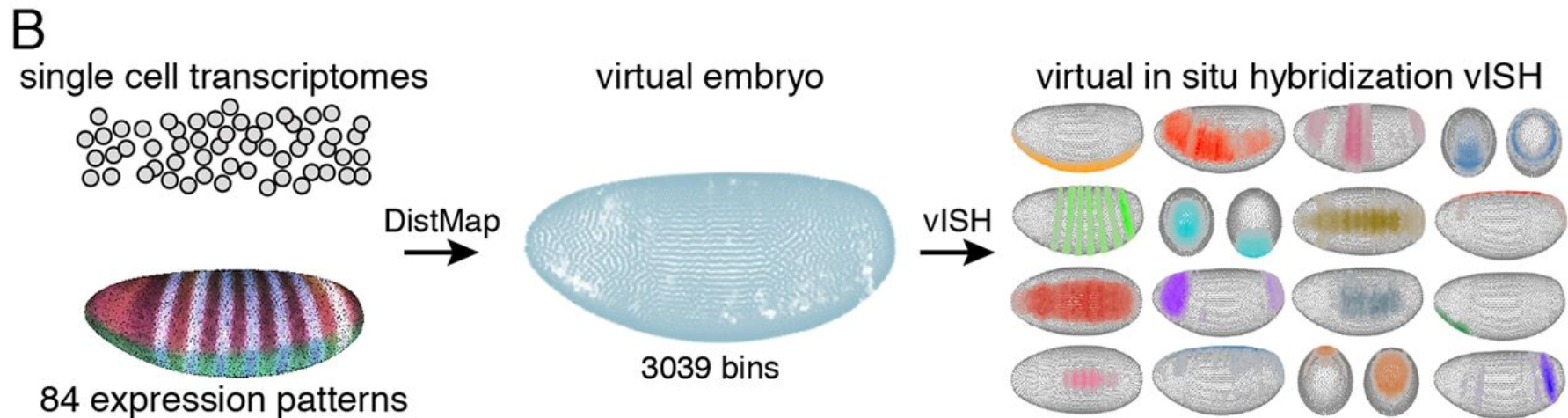
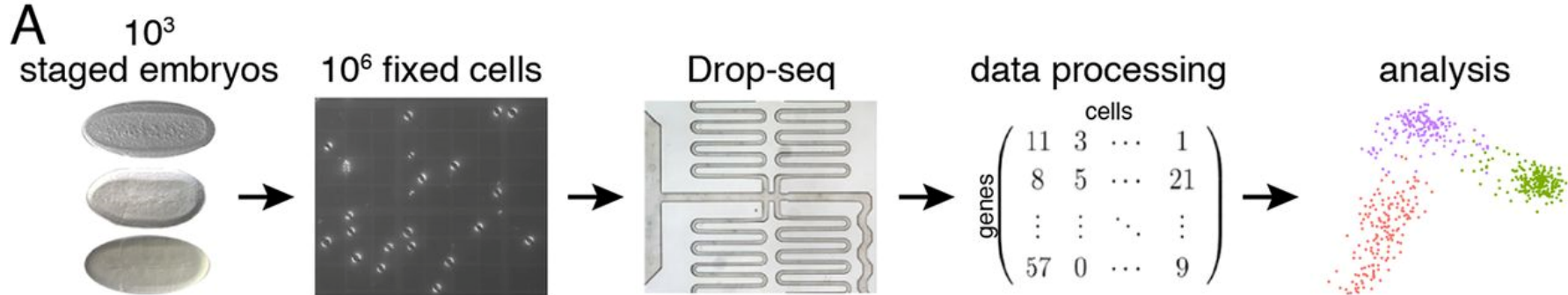
Expression Maps - RNA

High-Resolution Expression Map in Arabidopsis Root



Expression Maps - RNA

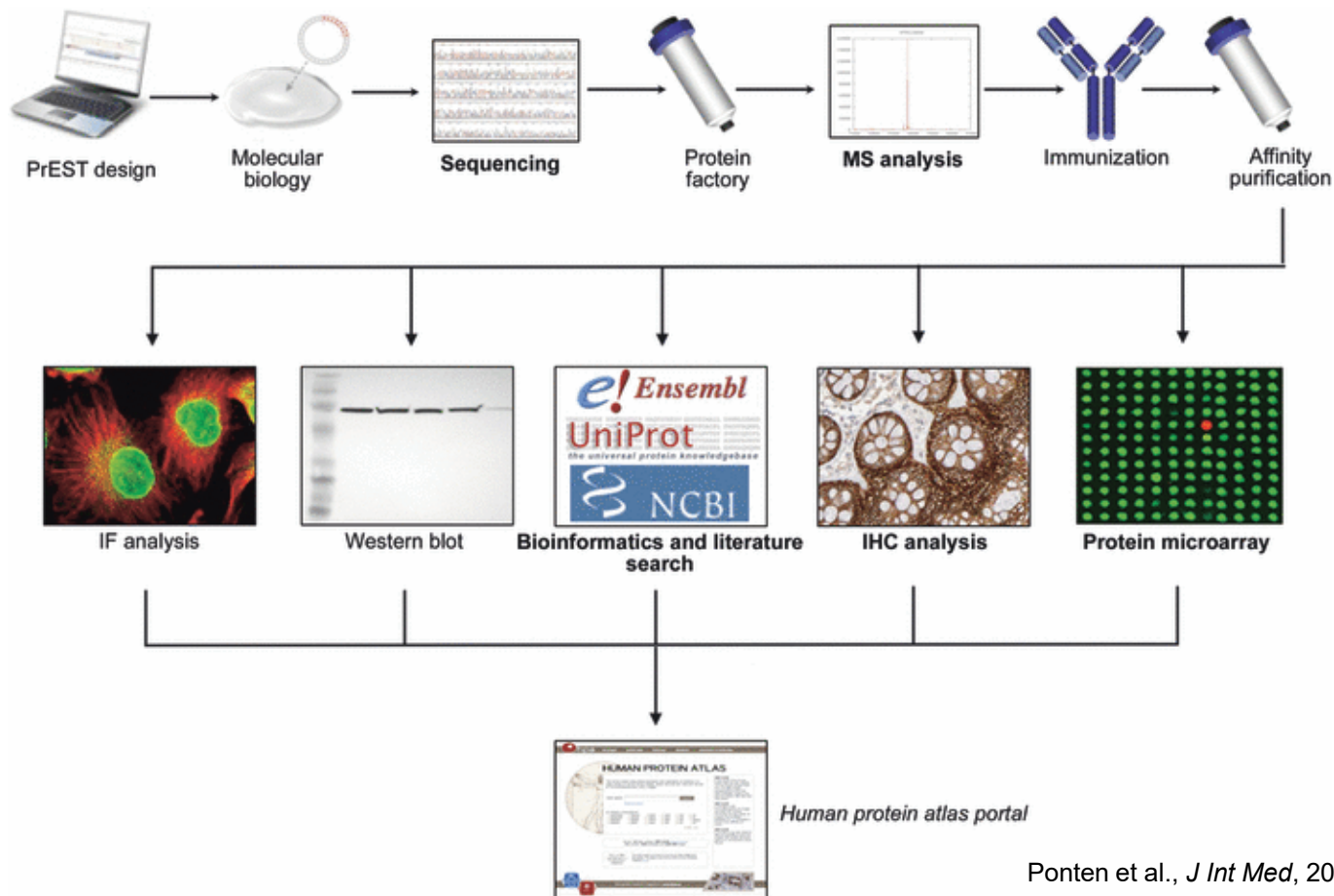
A High-Resolution Expression Map in Drosophilla



Nikos Karaïskos et al. Science 2017;science.aan3235

Expression Maps - Proteins

□ Human Protein Atlas



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

Expression Maps - Proteins

- Human Protein Atlas
(<http://www.proteinatlas.org/>)

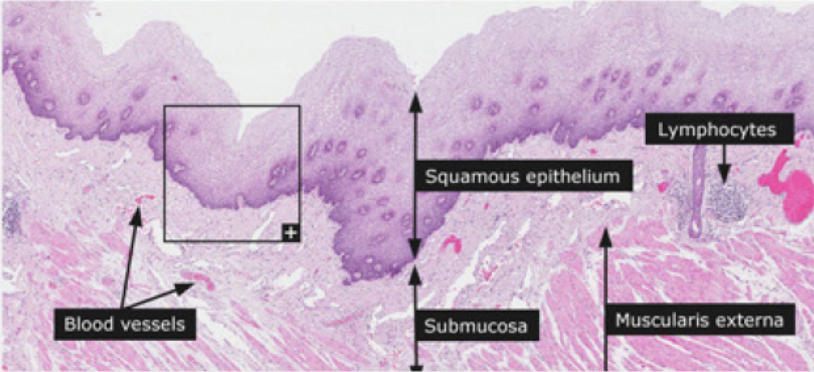
THE HUMAN PROTEIN ATLAS

ABOUT & HELP

SEARCH ? »

Search Clear Fields »

e.g. [CD44](#), [ELF3](#), [KLK3](#), or use Fields to search specific fields such as [protein_class:Transcription factors](#) or [chromosome:X](#)



News

Protein evidence according to [Fagerberg et al](#) is summarized in the [chromosome progress diagram](#).

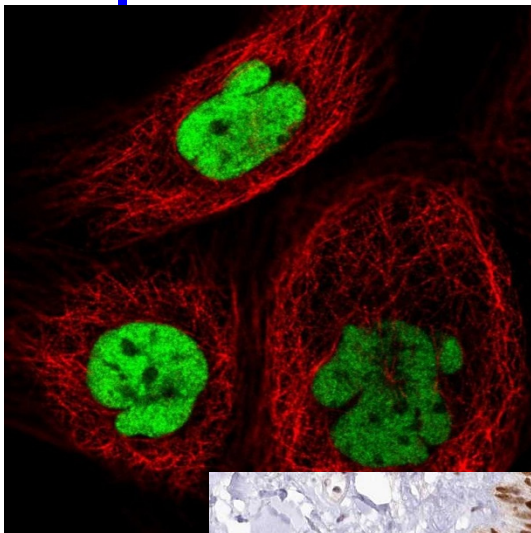
Version: **11.0**
Atlas updated: 2013-03-11
[release history](#)

15156 genes with protein expression profiles based on **18707** antibodies.

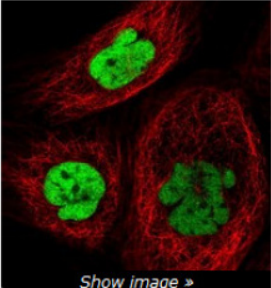
dictionary: histology of esophagus


Expression Maps - Proteins

- Human Protein Atlas (<http://www.proteinatlas.org/>)



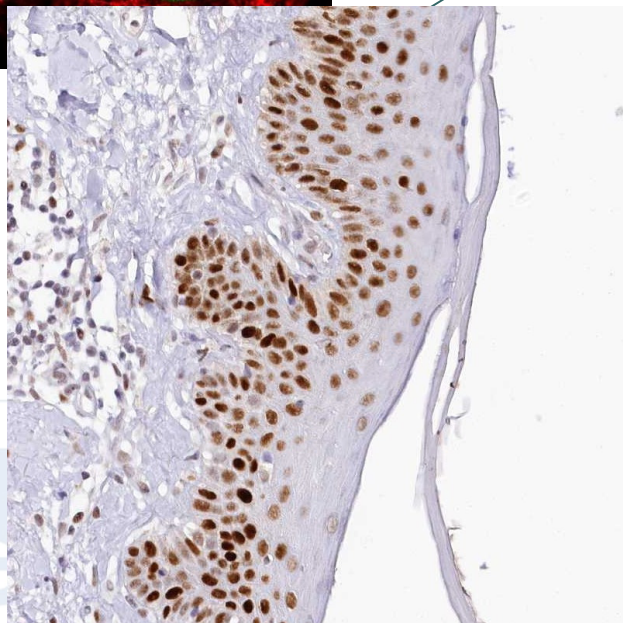
SUBCELLULAR LOCATION SUMMARY ? »



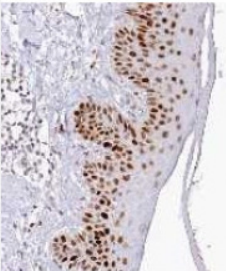
Main location(s) Nucleus but not nucleoli
Additional location(s)
Staining summary Localized to the nucleus but excluded from the nucleoli.
Reliability (APE)  High
Antibodies in assay CAB039238, CAB039239


[Show image »](#)













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	

[Show image »](#)

MORE TISSUE DATA

Outline

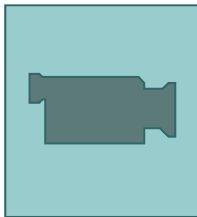
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 - DNA and protein chips

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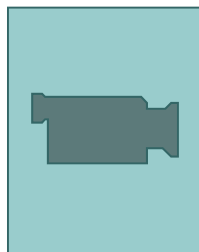
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DNA Chips

- 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 commercially 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 oligonucleotide synthesis e.g. for the whole human genome (about 3,1 x 10⁹ bp) it 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	<i>E. coli</i> genes <i>bioB</i> , <i>bioC</i> , <i>bioD</i> . <i>B. subtilis</i> gene <i>lysA</i> . Phage P1 <i>cre</i> gene. <i>Arabidopsis</i> 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 threshold
- Finally comparing the experiment with the control or comparing different conditions with each other -> the result

Expression of 195M6T7 in response to chemical treatment

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Gene

Experiment: Aluminum Stress

Experiment Summary | Samples | Slides & Datasets | Array Design | View All

Slide Details

Slide (name ? : description)	External ID ?	Replicate (id ? : name)	Replicate type ?	Reverse replicate ?	Sample ?	Experimental variables	Label ?	Get Data ?
HoekengaS7 [*] : Aluminum Stress 1 [strong spatial bias]	AFGC: 7304	63: Aluminum Stress	technical		7304_Cy3.7305_Cy5	no treatment (pool of 3, 8, and 24 hours)	Cy3	Download
					7304_Cy5.7305_Cy3	Aluminum (50 5M AlCl3, pool of 3, 8, and 24 hours)	Cy5	
HoekengaSc Aluminum Stress 2 [strong spatial bias]	AFGC: 7305	64: Aluminum Stress	technical	63	7304_Cy5.7305_Cy3	Aluminum (50 5M AlCl3, pool of 3, 8, and 24 hours)	Cy3	Download
					7304_Cy3.7305_Cy5	no treatment (pool of 3, 8, and 24 hours)	Cy5	

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

Che et al., 2002

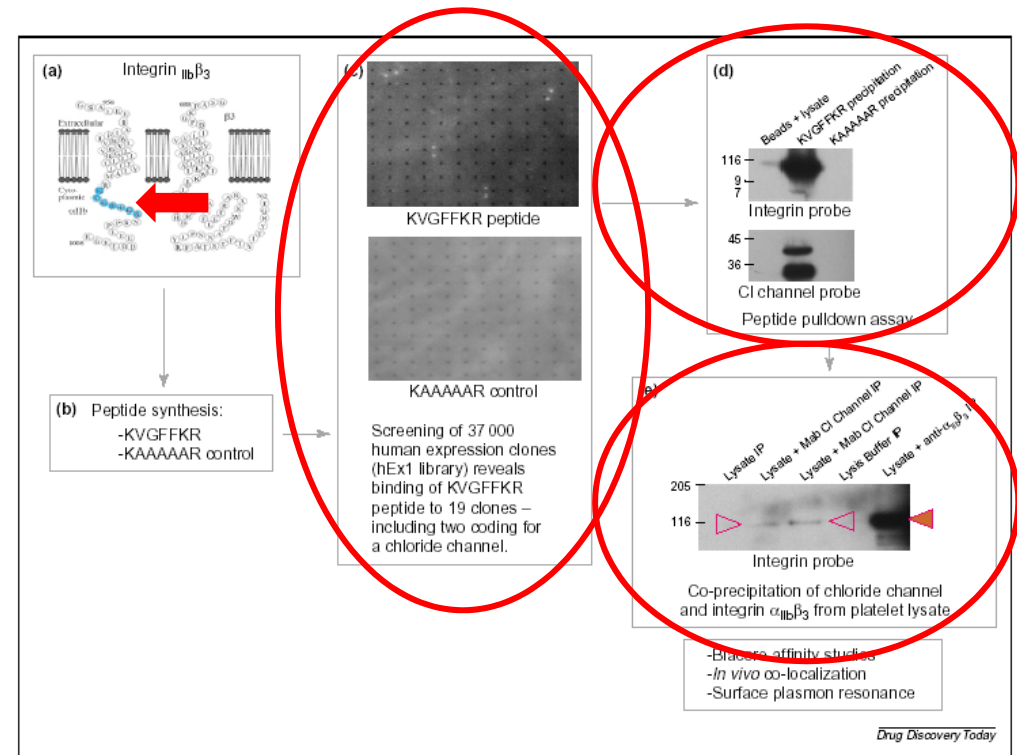
Protein Chips

- 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 Icn)
- Other use: e.g. in the identification of kinase substrates, when substrates are bound to the chip and exposed to kinases in the presence of radiolabeled ATP (786 purified proteins of barely, of which 21 were identified as CK2 α kinase substrates; Kramer et al., 2004)



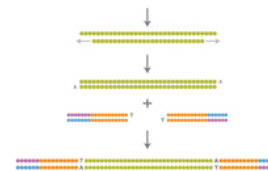
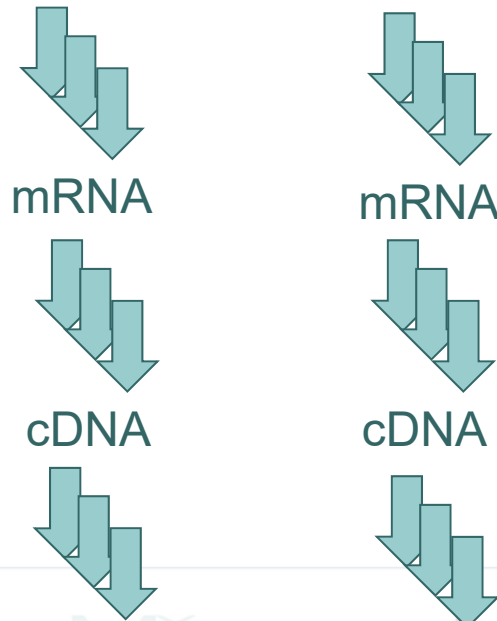
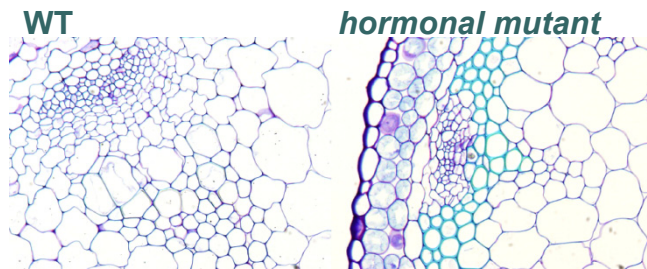
Lueking et al., 2005

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Next Gen Transcriptional Profiling

- *Transcriptional profiling* via *RNA sequencing*



Library Preparation
~2 h [15 min hands-on (Nextera)]
< 6 h [< 3 h hands-on (TruSeq)]



Cluster Generation
~5 h (<10 min hands-on)



Sequencing by Synthesis
~1.5 to 11 days



CASAVA
2 days (30 min hands-on)

Sequencing by Illumina and
number of transcripts determination

Results of –omics Studies vs Biologically Relevant Conclusions

- Transcriptional profiling yielded more than **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	0,00039180	1 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	0,00053505	5 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	0,00028514	3 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	0,00037473	6 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

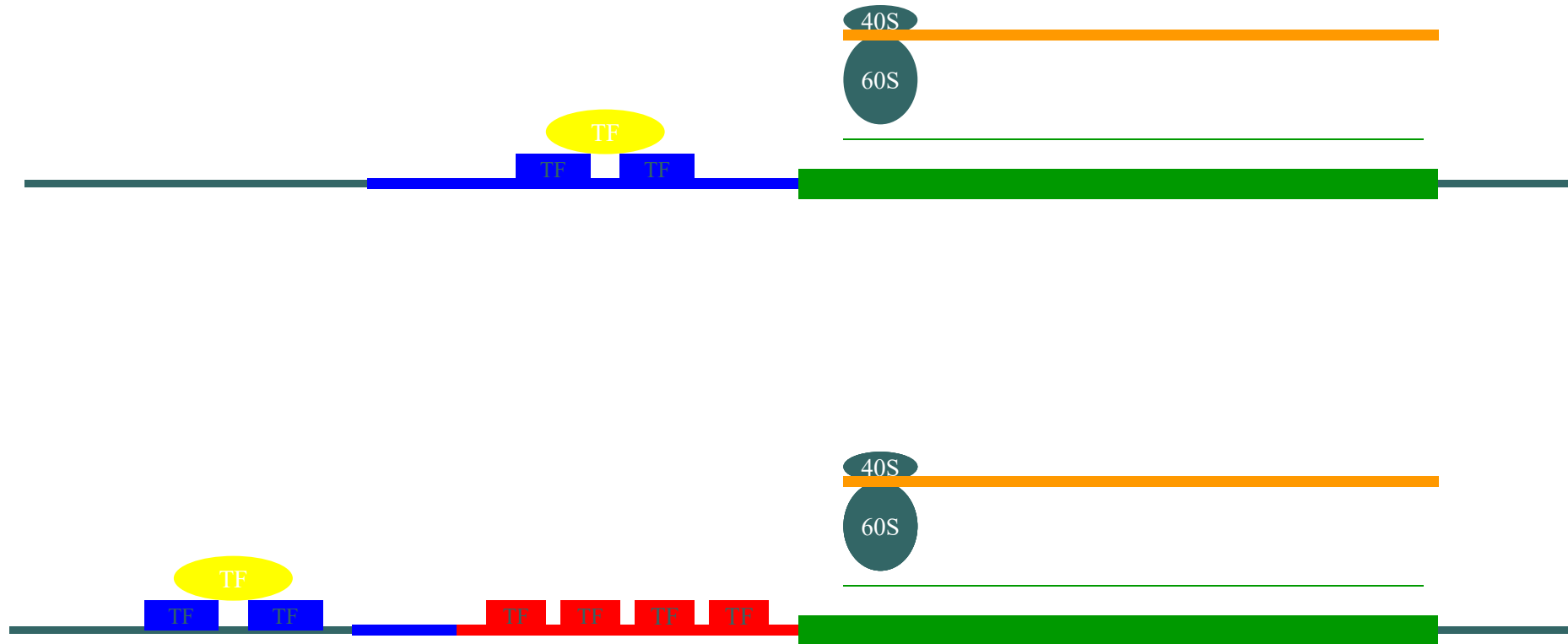
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 - T-DNA activation mutagenesis

Gain-of-Function Approaches

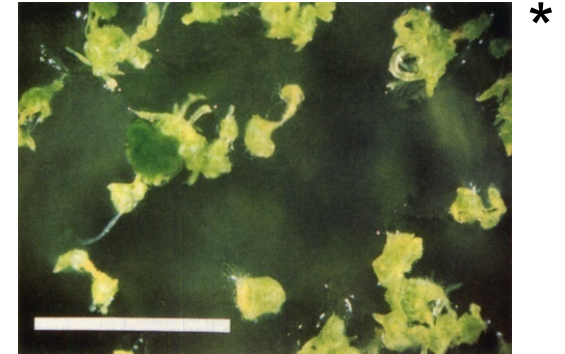
- Methods for identification of gene function using gain-of-function approaches
 - T-DNA activation mutagenesis
 - Method enabling isolation of dominant mutants by random insertion of constitutive promoter, resulting in overexpression of the gene and therefore in corresponding phenotypic changes
 - First step: preparation of mutant library prepared by transformation 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 *CK11* Gene

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

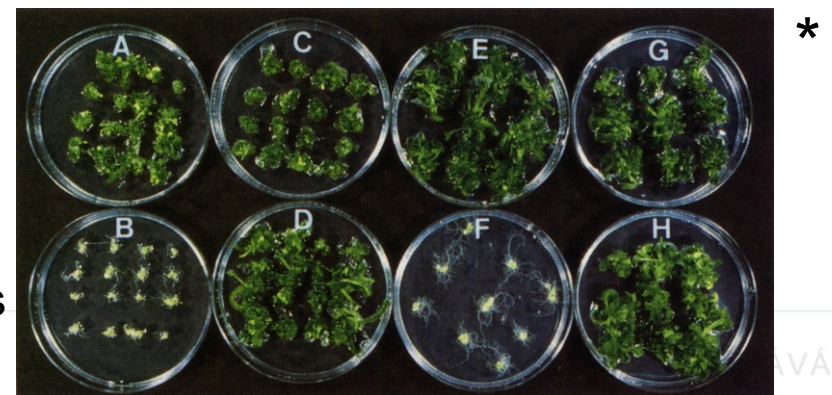


- Mutant phenotype is a phenocopy of exogenous application of cytokinins (*CK11*, *CYTOKININ INDEPENDENT 1*)

K1 plasmid rescue K2 35S::*CK1* cDNA

t-zeatin

no hormones



Outline

- Methods of gene expression analysis
 - Qualitative analysis of gene expression
 - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
 - Preparation of translational fusion of the coding region of the analysed gene with reporter gene
 - Use of the data available in public databases
 - Tissue- and cell-specific gene expression analysis
 - Quantitative analysis of gene expression
 - DNA and protein chips
 - Next generation transcriptional profiling
- Regulation of gene expression in the identification of gene function by gain-of-function approaches
 - T-DNA activation mutagenesis
 - Ectopic expression and regulated gene expression systems

Regulated Expression Systems



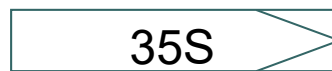
activator
X



activator x reporter



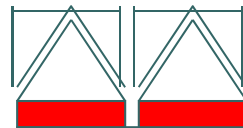
reporter



35S



LhG4



pOP



TATA



CKI1

Regulated Expression Systems



activator
X

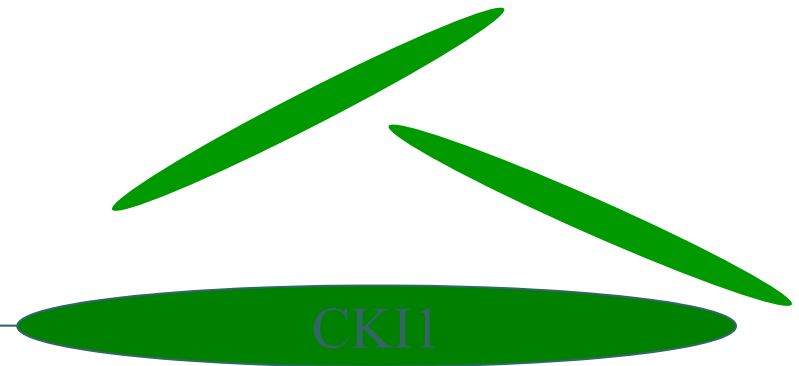
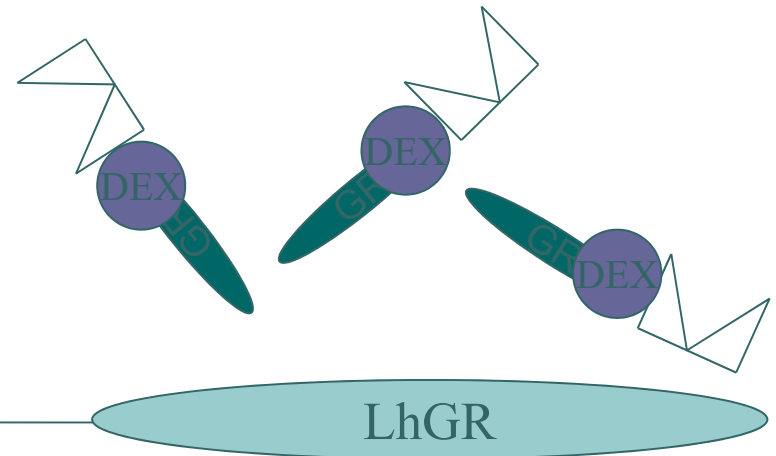


activator x reporter



reporter

+DEX



Regulated Expression Systems



activator
X

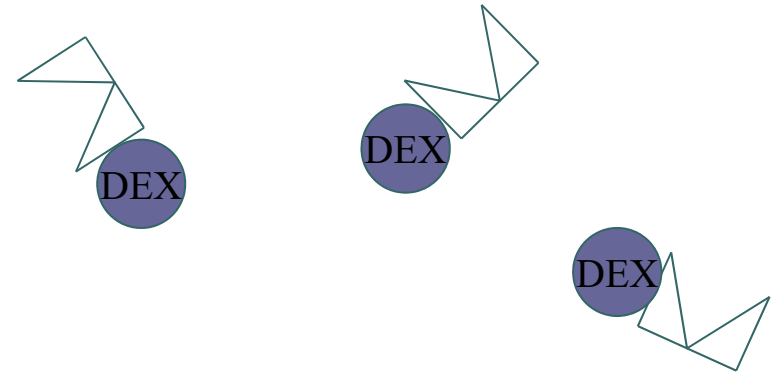
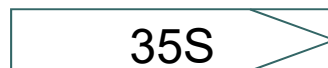


activator x reporter

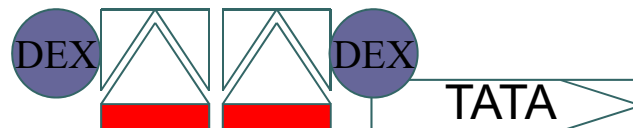


reporter

+DEX

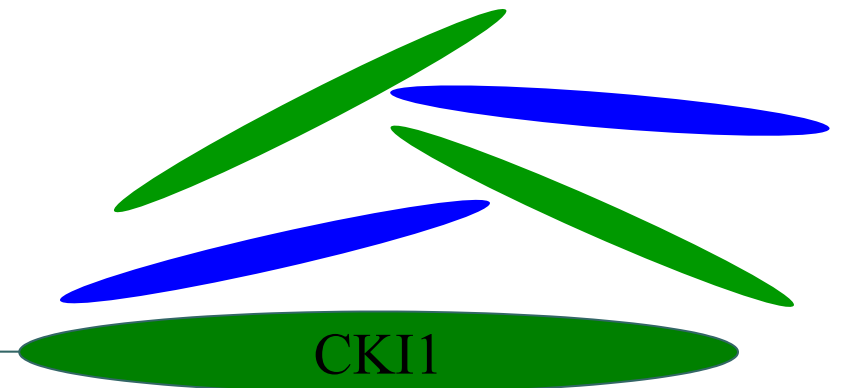


wt Col-0



pOP

TATA



CKI1

4C



pOP

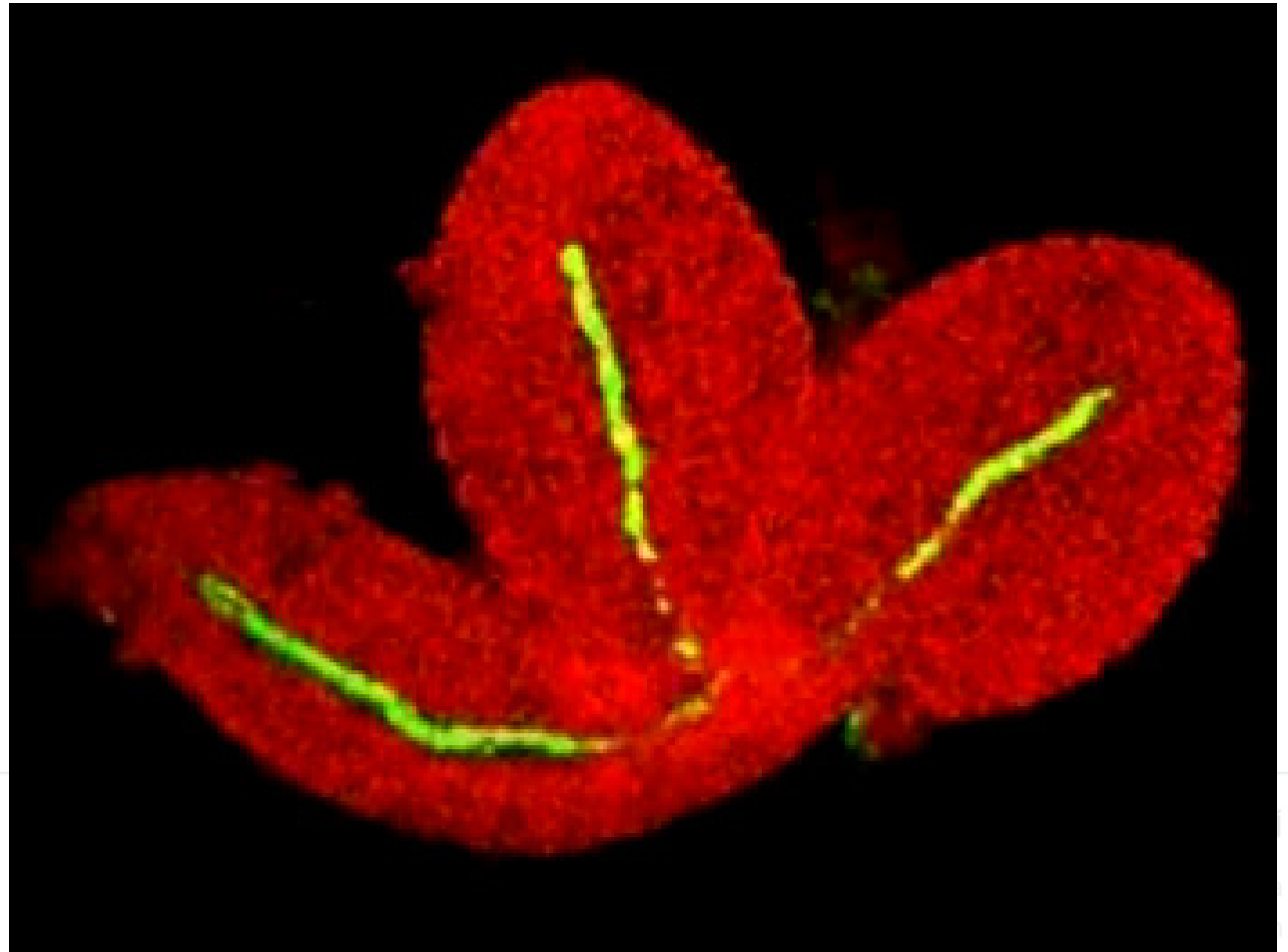
TATA



GUS

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



Outline

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- **Chemical Genetics**

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 %**)

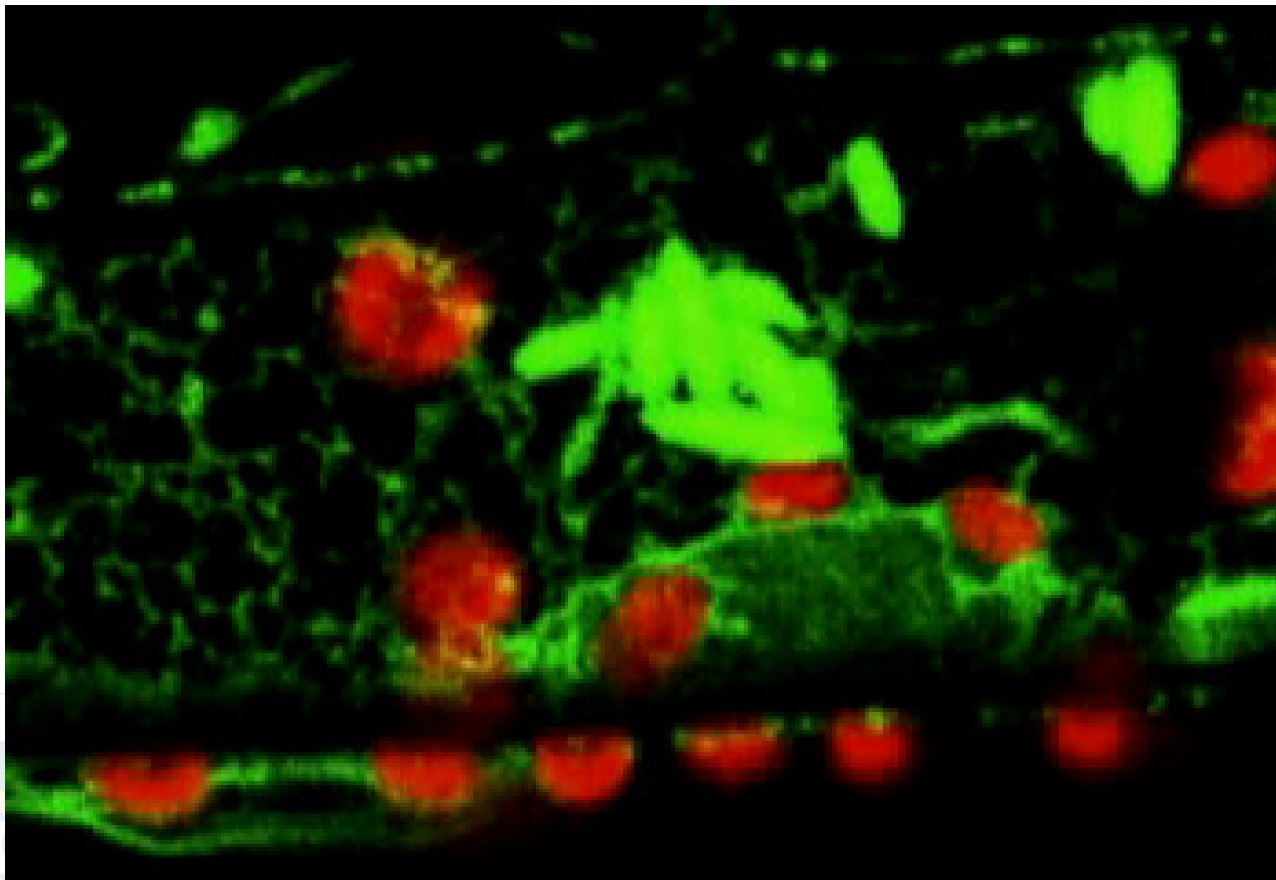
The screenshot shows a web browser window displaying the PubMed search results for the query "chemical genetics". The browser's address bar shows the URL: <https://www.ncbi.nlm.nih.gov/pubmed/?term=chemical+genetics>. The PubMed interface includes a search bar with the query "chemical genetics" and a search button. Below the search bar, there are options for "Format: Summary", "Sort by: Most Recent", and "Per page: 20". A "Best matches for chemical genetics" box highlights several key articles, including "Chemical genetics: elucidating biological systems with small-molecule compounds" by Kawasumi M et al. (2007) and "Chemical genetics-based target identification in drug discovery" by Cong F et al. (2012). The "Search results" section shows a list of items, with the first item being "Analysis of butterfly reproductive proteins using capillary electrophoresis and mass spectrometry" by Rokhas MK, Rönn JL, Wiklund C, Emmer A, published in Anal Biochem in 2018. The second item is "KRAS Suppression-Induced Degradation of MYC Is Antagonized by a MEK5-ERK5 Compensatory Mechanism" by Vaseva AV, Blake DR, Gilbert TSK, Ng S, Hostetter G, Azam SH, Ozkan-Dagliyan I, Gautam P, Bryant KL, Pearce KH, Herring LE, Han H, Graves LM, Witkiewicz AK, Knudsen ES, Pecot CV, Rashid N, Houghton PJ, Wennerberg K, Cox AD, Der CJ, published in Cancer Cell in 2018. The third item is "Whole genome screen reveals a novel relationship between Wolbachia levels and Drosophila host translocation" by Grobler Y, Yun CY, Kahler DJ, Bergman CM, Lee H, Oliver B, Lehmann R, published in PLoS Pathog in 2018. The fourth item is "Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13" by Zeng M, Kwiatkowski NP, Zhang T, Nabet B, Xu M, Liang Y, Quan C, Wang J, Hao M, Palakurthi S, Zhou S, Zeng Q, Kirschmeier PT, Meghani K, Leggett AL, Qi J, Shapiro GI, Liu JF, Matulonis UA, Lin. The right sidebar contains sections for "Sort by:" (Best match, Most recent), "Results by year", "PMC Images search for chemical genetics" (showing a diagram of a protein structure), and "Titles with your search terms" (listing related articles like "Validation of chemical genetics for the study of zipper-interacting protein kinase"). The bottom of the image shows a Windows taskbar with various application icons and a system tray with the date 15.11.2018 and time 9:34.

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, commercially available)
 - example: **analysis of endomembrane transport** in plants

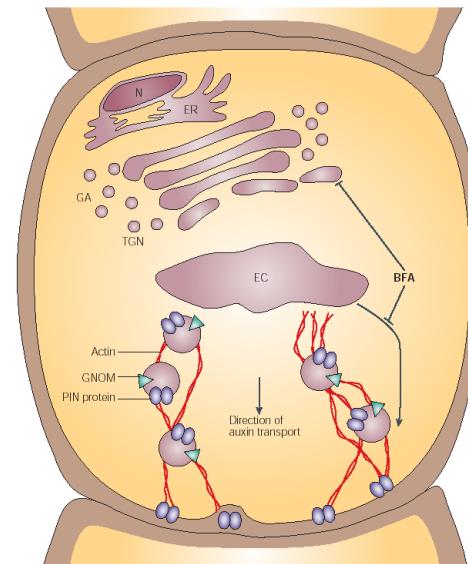
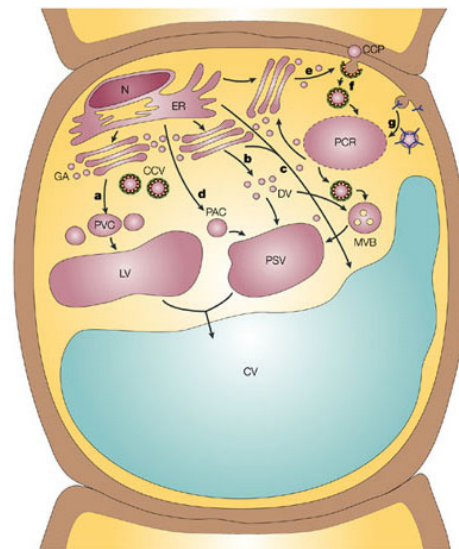
Chemical Genetics

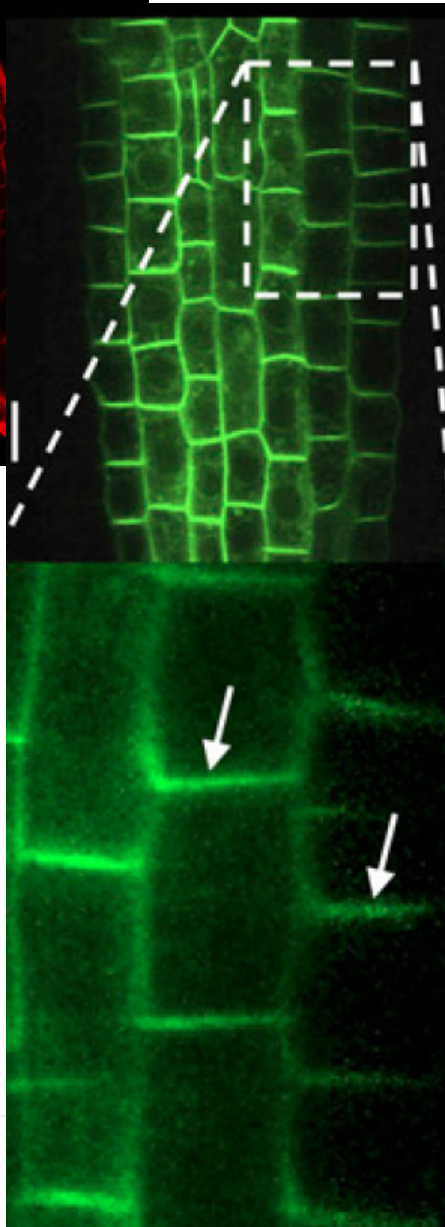
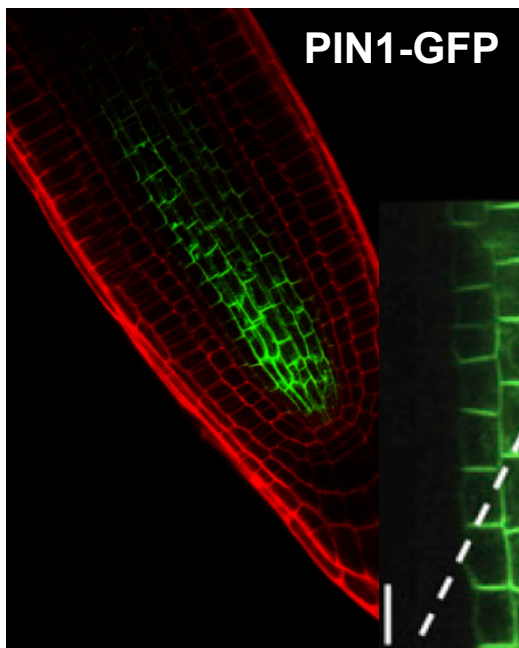
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
 - In plants cells there occur very dynamic processes mediated mainly by endomembrane transport



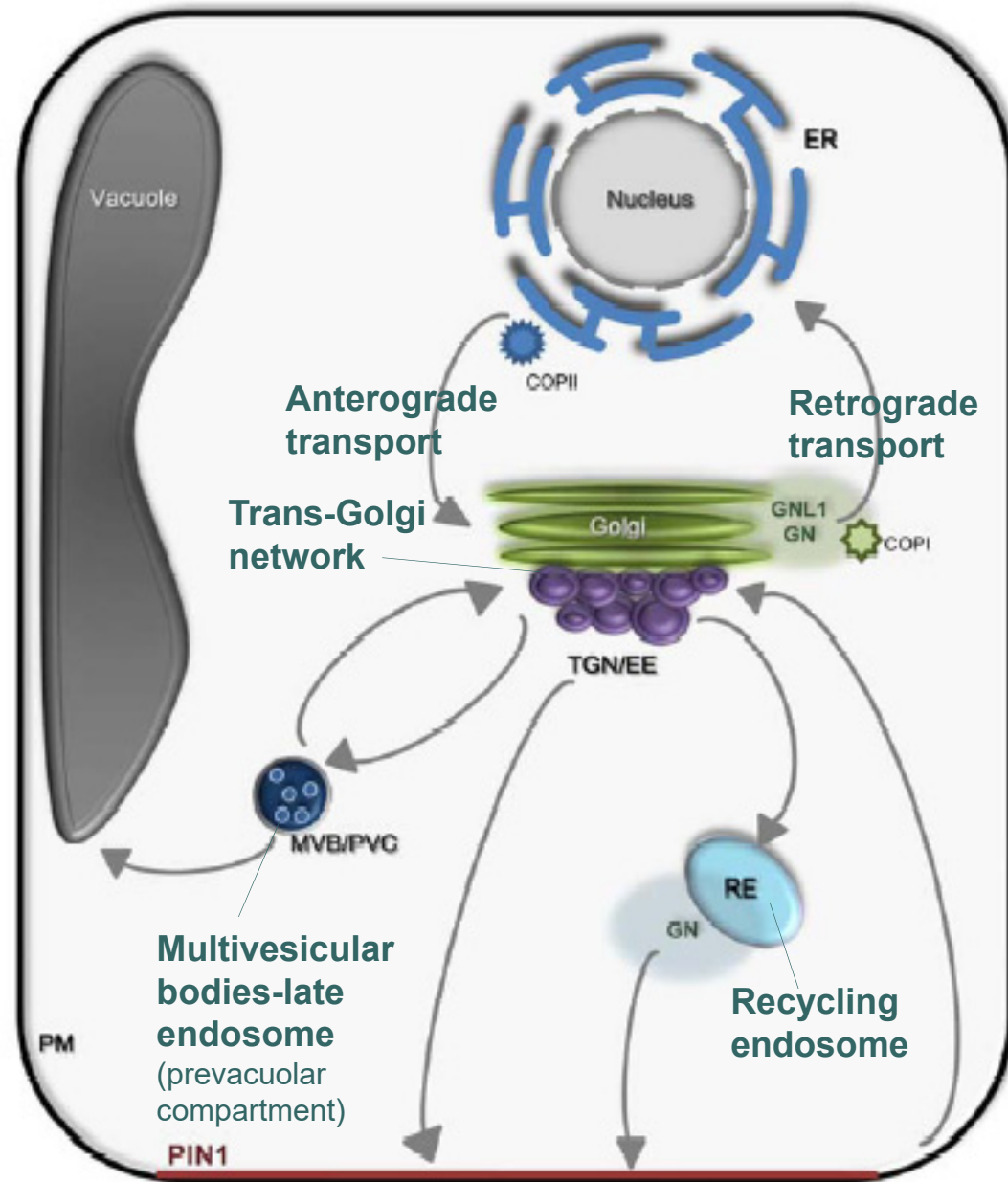
Chemical Genetics

- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
 - In plants cells there occur 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





Huang et al., 2010

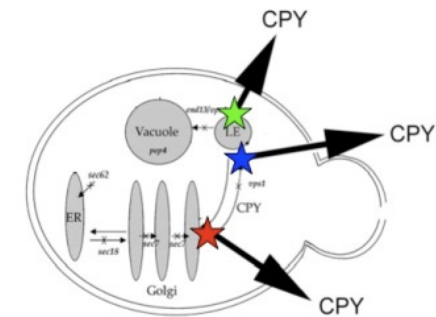


Chemical Genetics

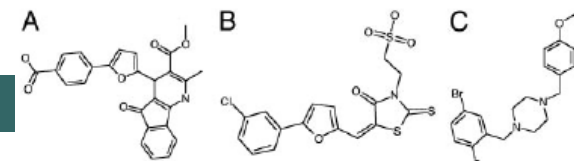
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches

- 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

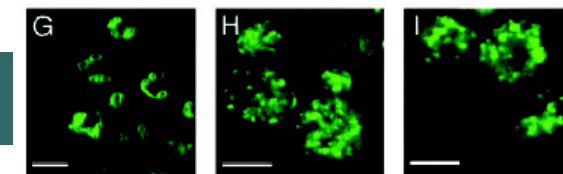


0 2.5 5 10 25 50 100 [mg/L]

Immunodetection of carboxypeptidase



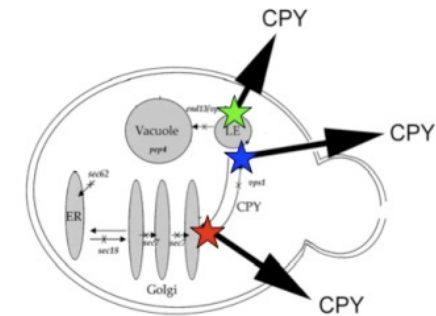
Detection of vacuole phenotype (tonoplast shape) of yeast by staining with a specific color (MDY-64)



Chemical Genetics

- Analysis of mechanisms of endomembrane transport by chemical genetics approaches

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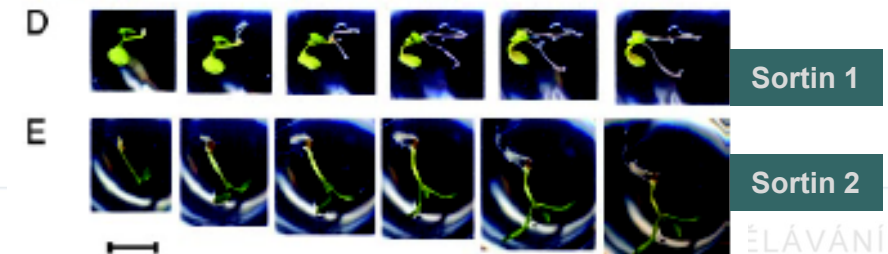
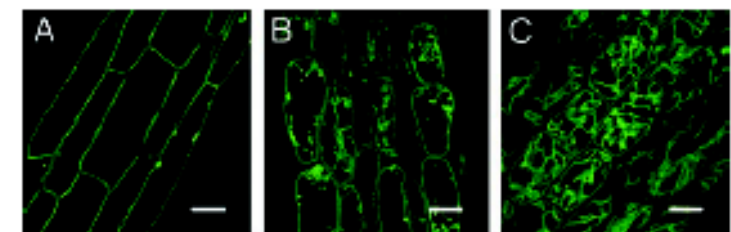


- Analysis of changes in secretion using dot-blot and immunodetection of carboxypeptidase Y in the culture medium with monoclonal antibodies

- 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 process 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

Shape of plant vacuoles using EGFP:-TIP



Phenotype of seedlings in the presence of sortins

- Identification of mutants with altered sensitivity to sortin 1 (hyper- or hypo-sensitive mutants) by EMS mutagenesis

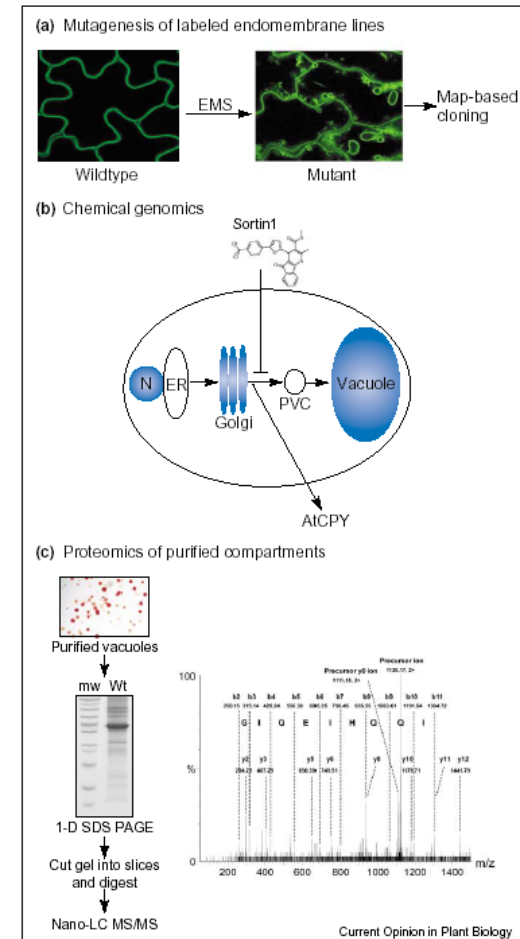
Zouhar et al., 2004
 financným fondem
 a státním rozpočtem České republiky

- 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



Summary

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Discussion



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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