

# CG920 Genomics

## Lesson 6

### Gene Expression and Chemical Genetics

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And

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**M U N I**  
**S C I**



# 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

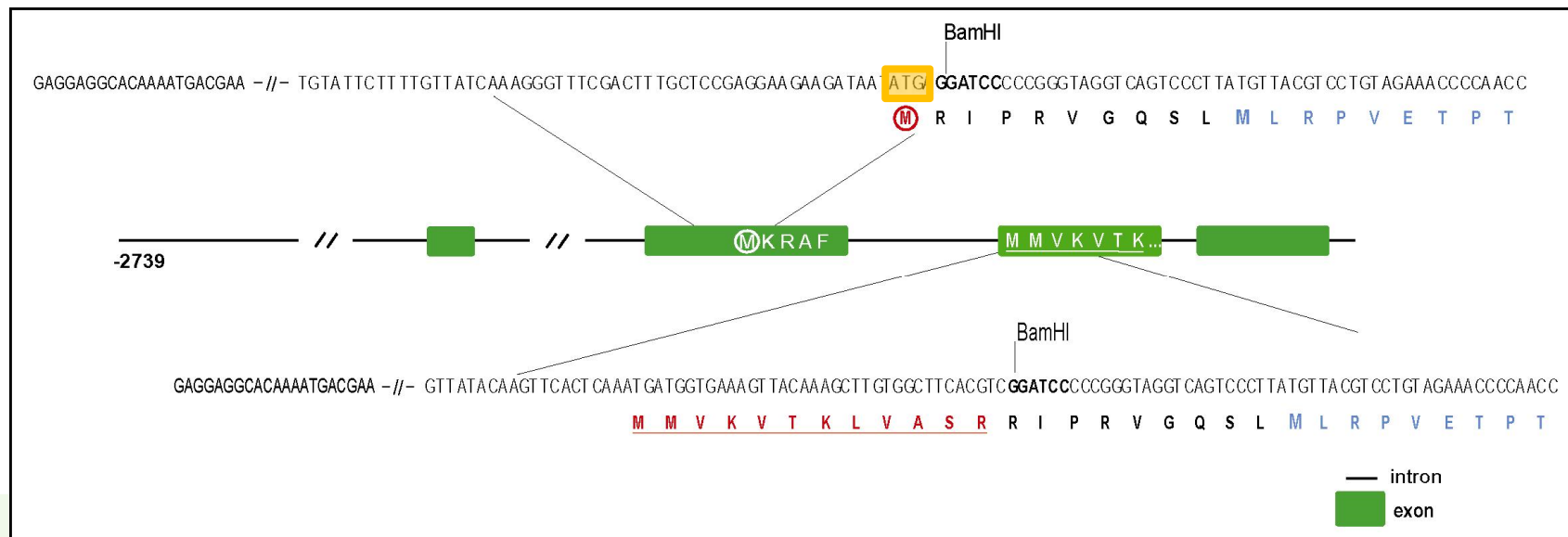
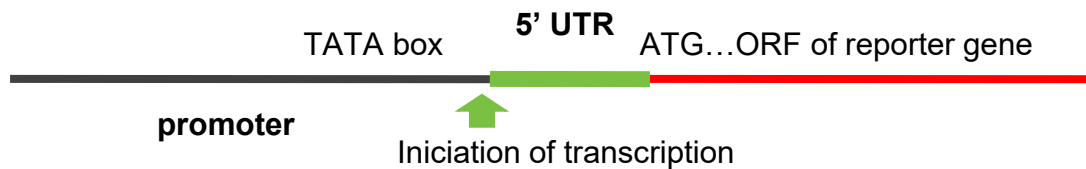
- **Methods of gene expression analysis**
  - **Qualitative analysis of gene expression**
    - Preparation of **transcriptional fusion** of **promoter** of analysed gene with a **reporter gene**
    - Preparation of **translational fusion** of the **coding region** of the analysed gene with **reporter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

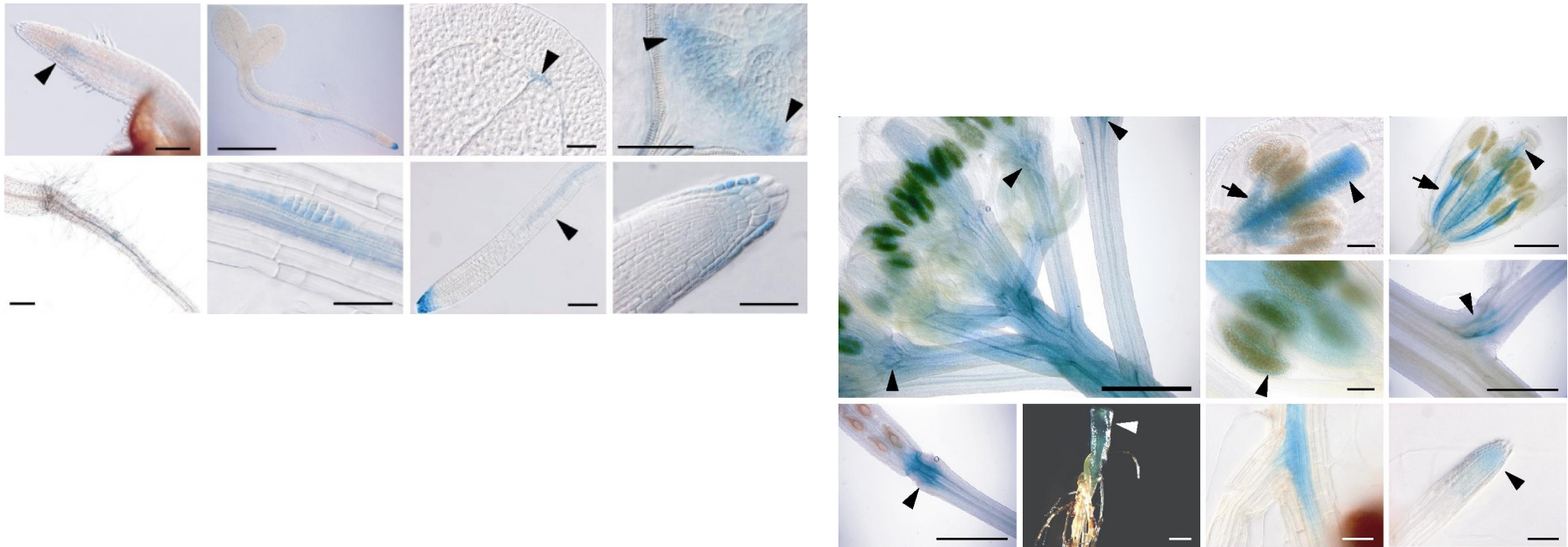
# Transcriptional Fusion

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

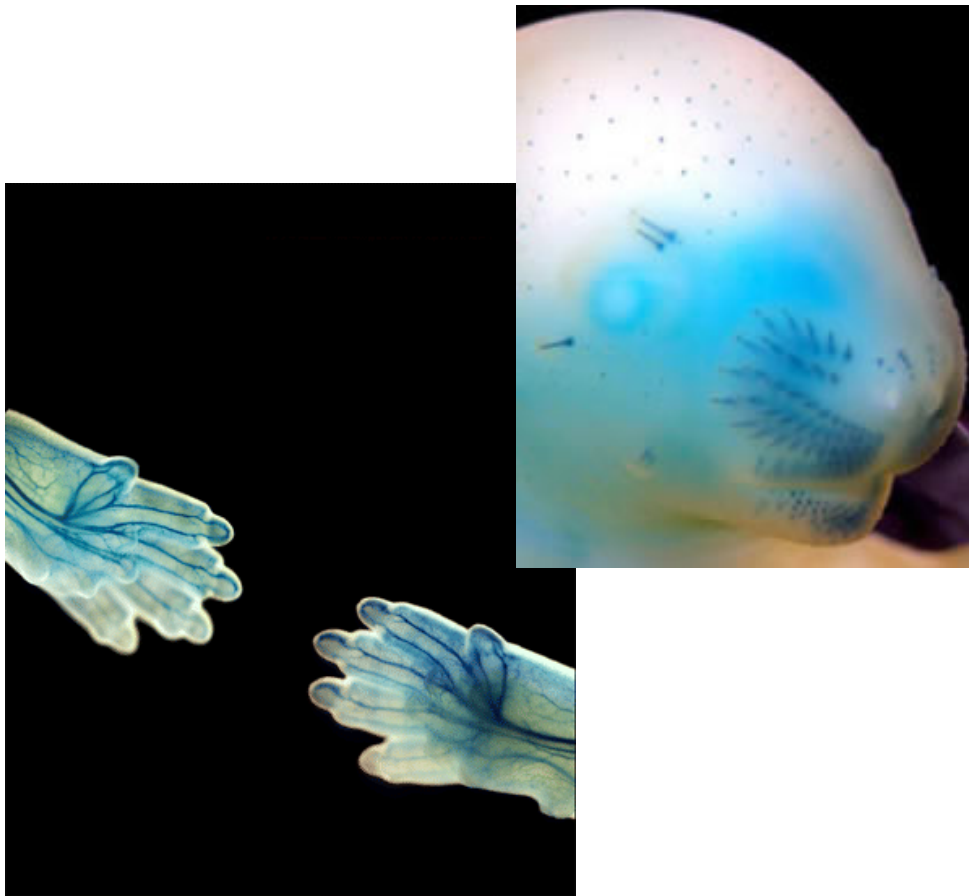


# Transcriptional Fusion

- 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



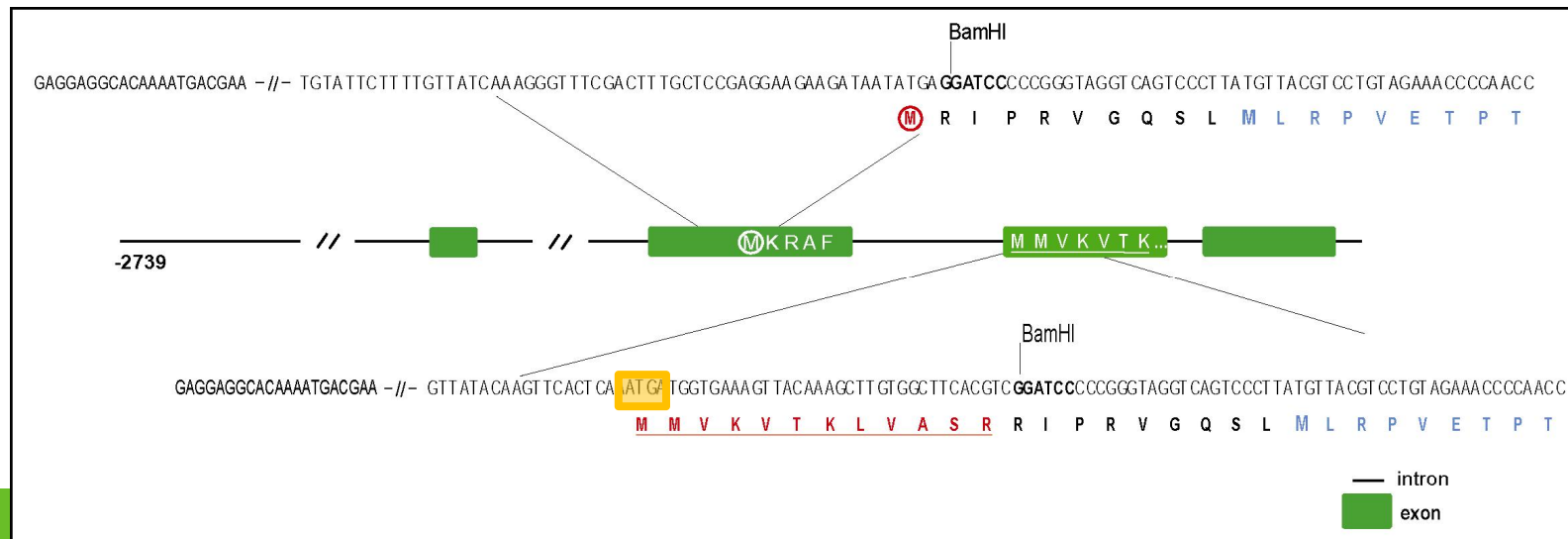
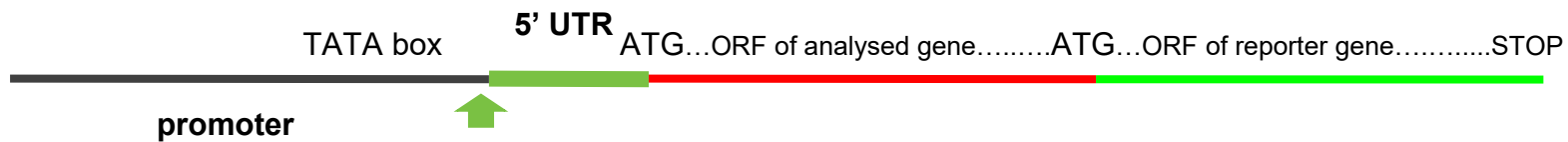
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- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
    - Preparation of translational fusion of the coding region of the analysed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



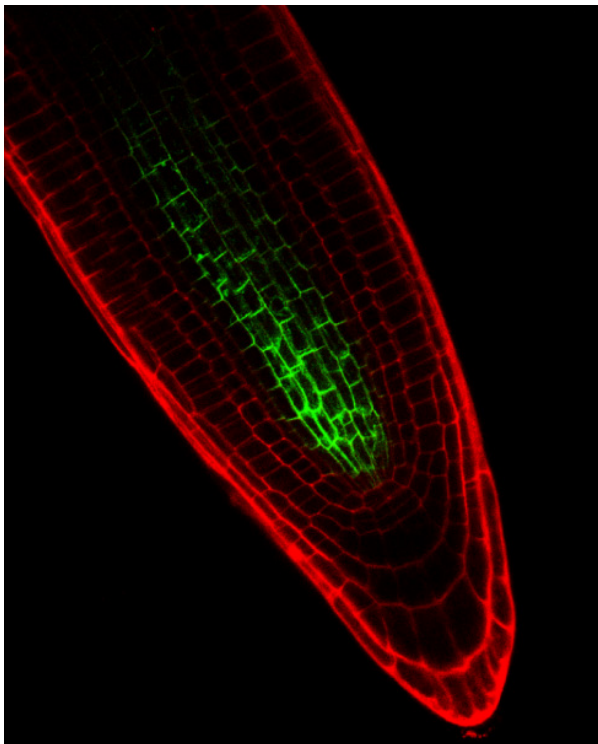
# Translational Fusion

- 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)

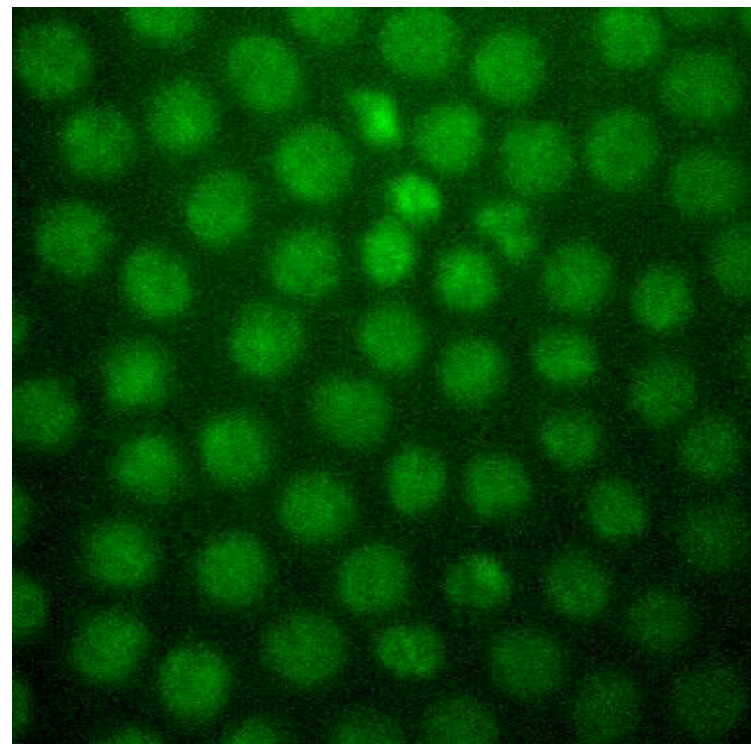


# Translational Fusion

- 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

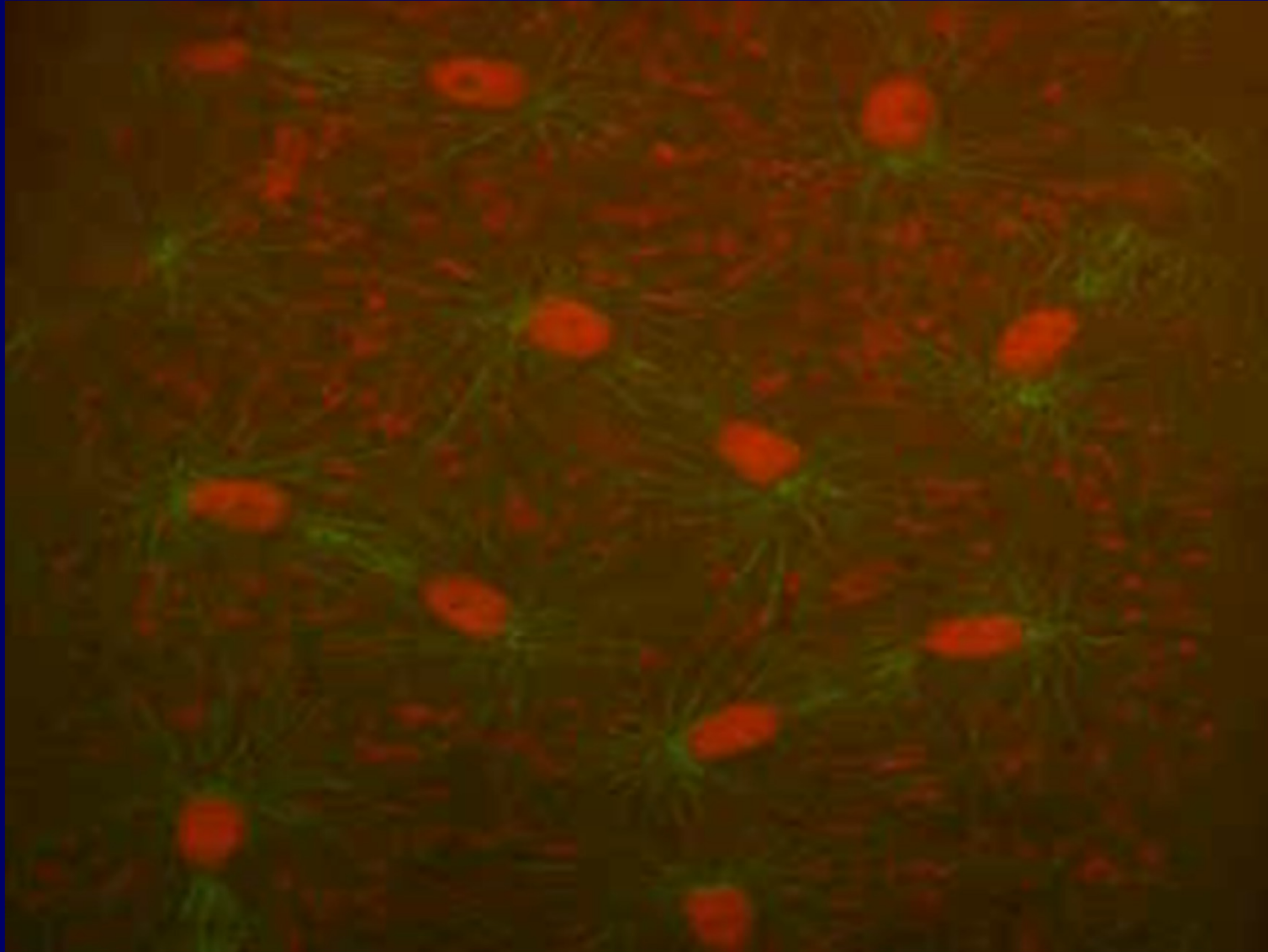


PIN1-GFP in *Arabidopsis*



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

# Translational Fusion

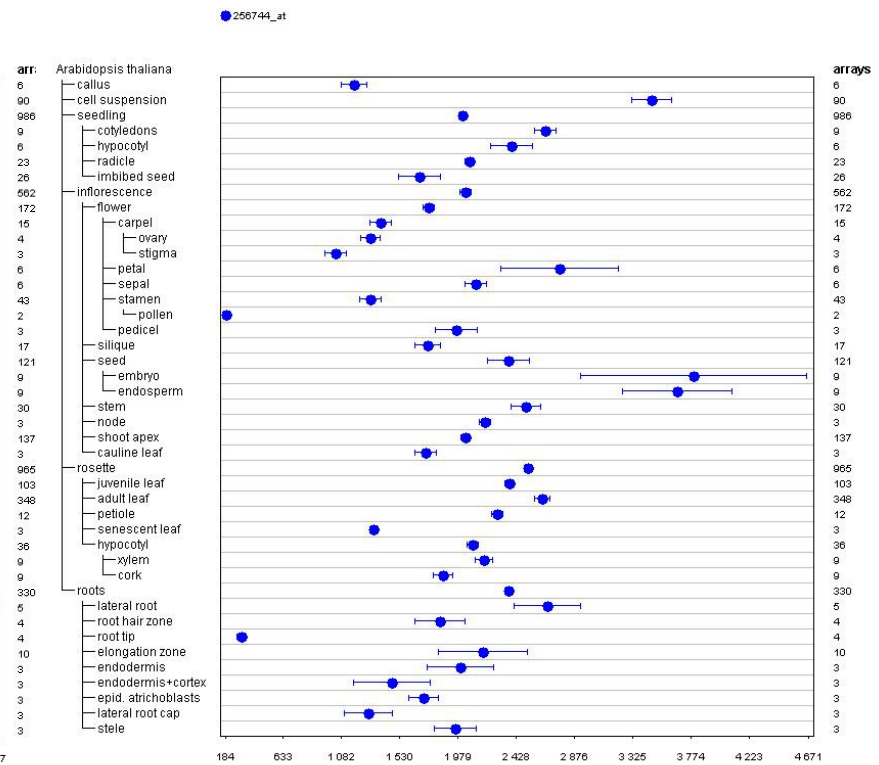
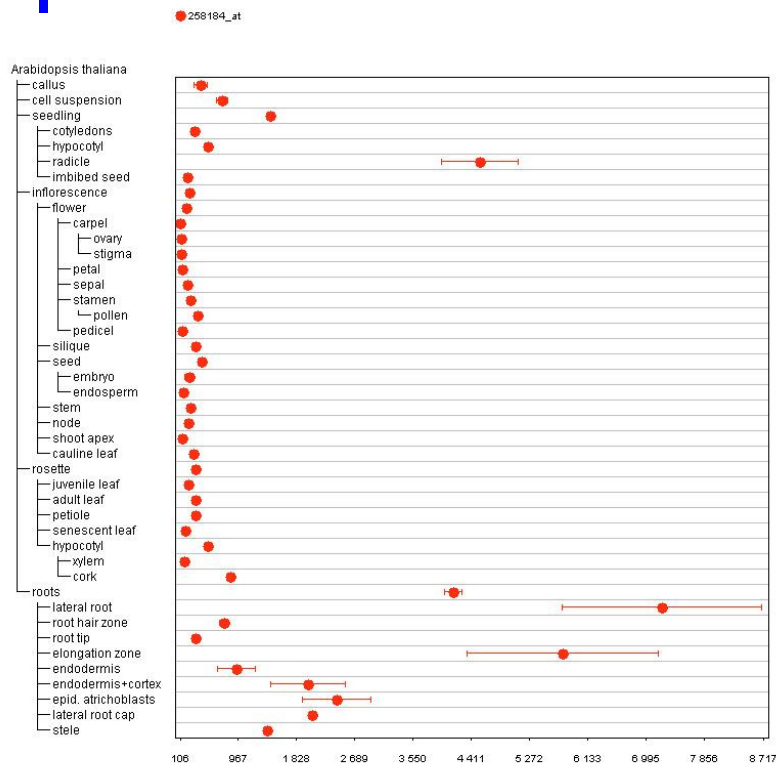


# Outline

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    - 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

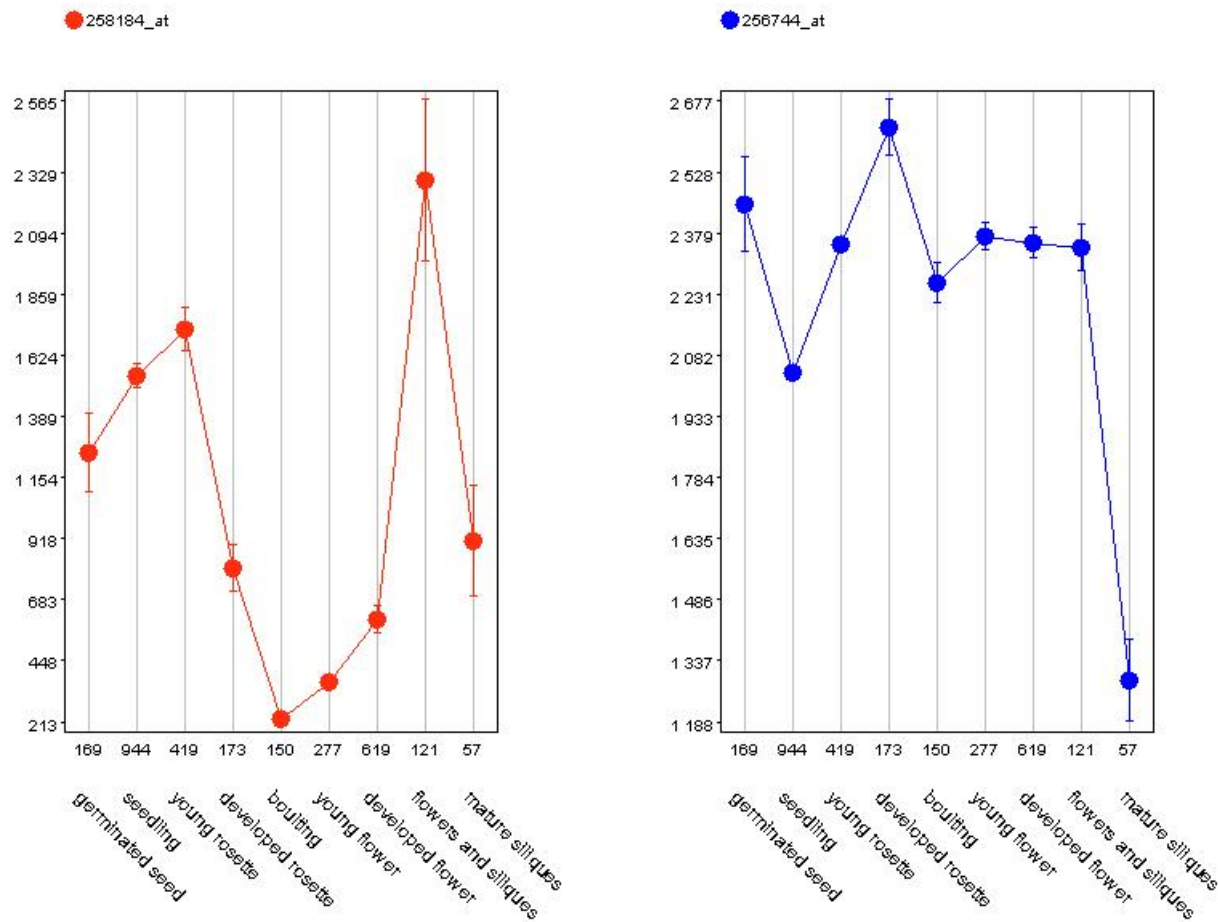
# Databases

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



# Databases

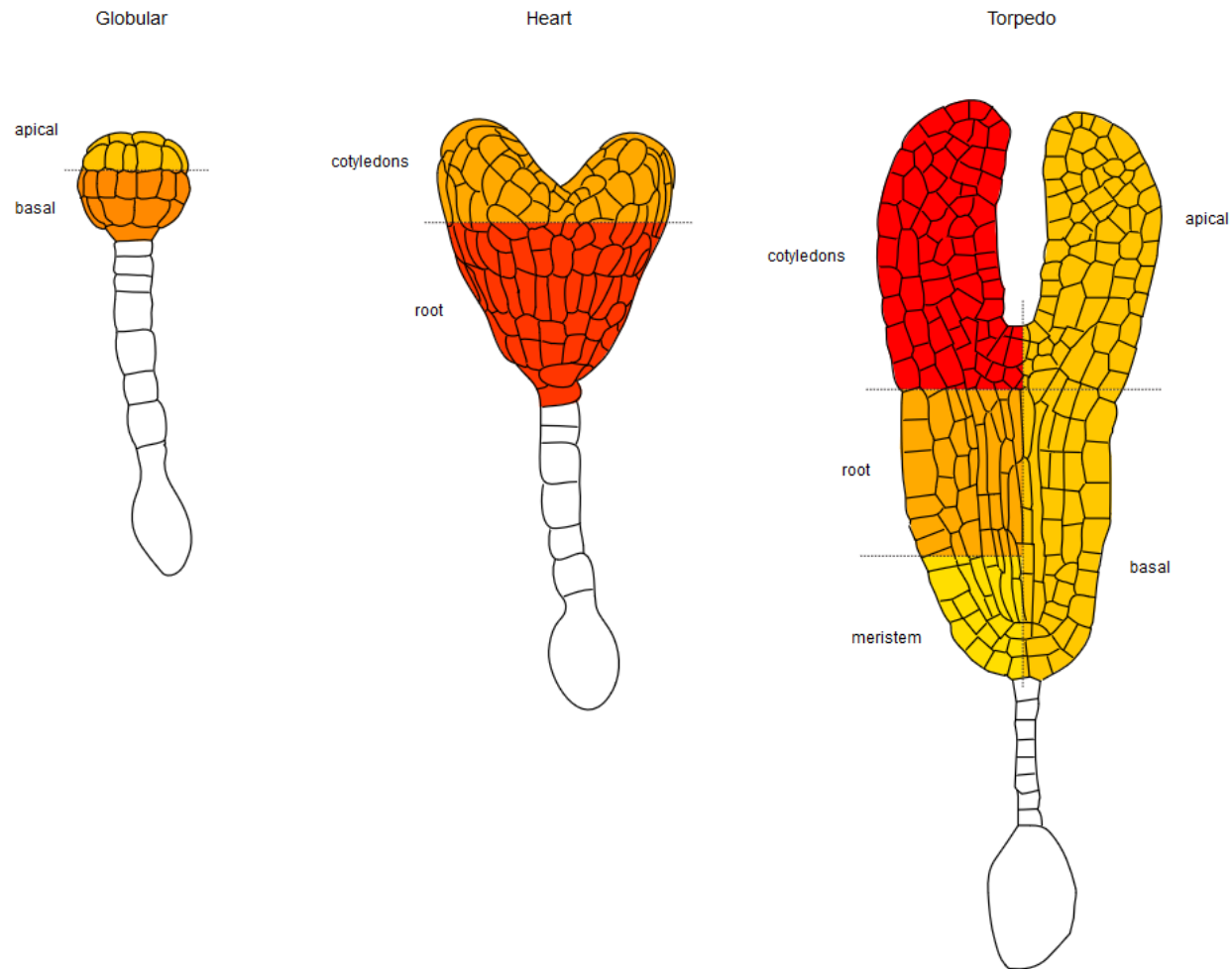
- Analysis of expression using Genevestigator (*AHP1* and *AHP2*, *Arabidopsis*, Affymetrix ATH 22K Array)





# Databases

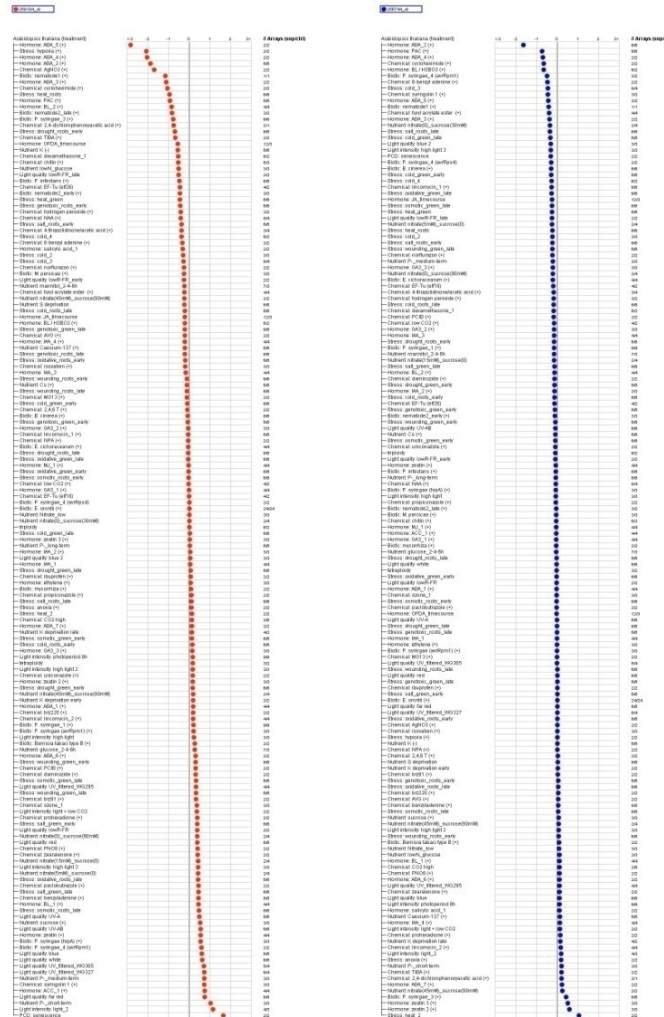
- Analysis of expression using ePlant





# Databases

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

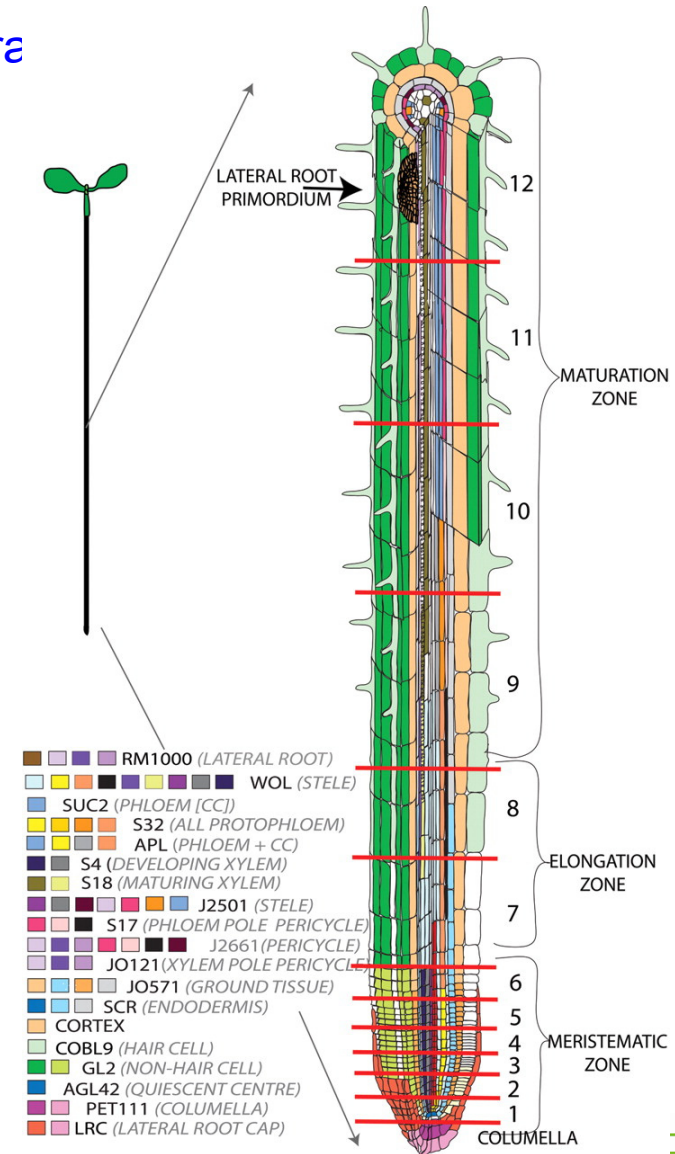
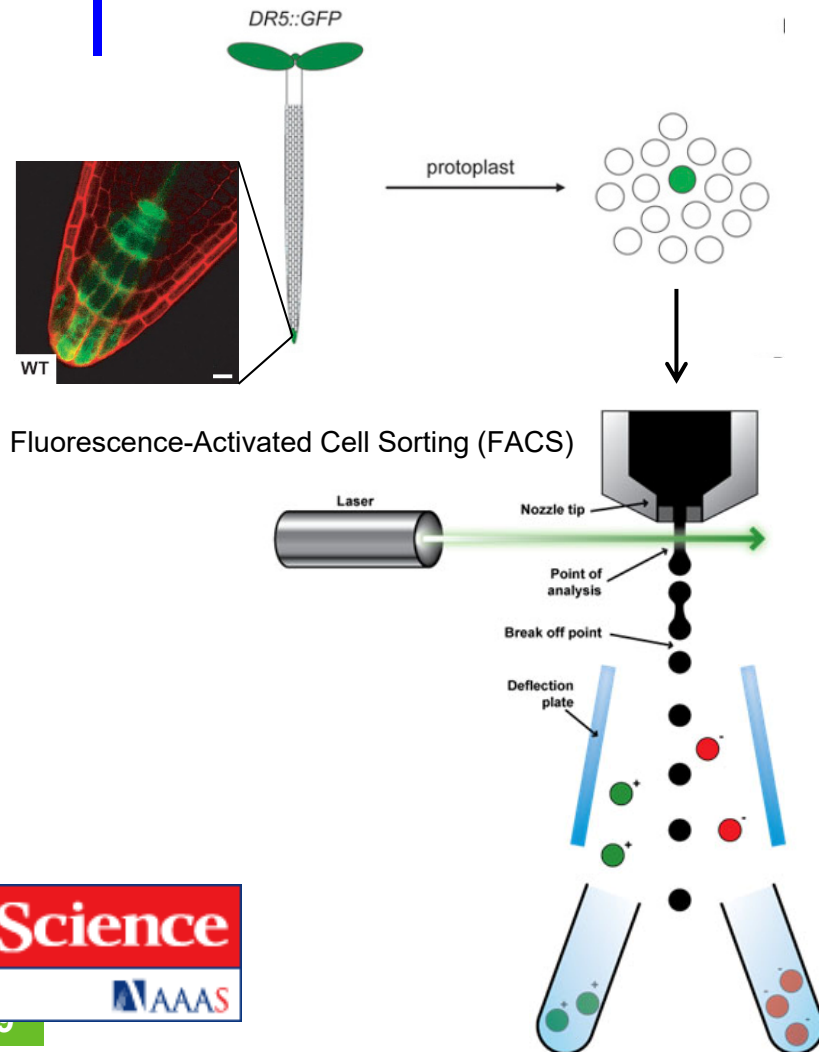


# Outline

- Methods of gene expression analysis
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    - 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

# Expression Maps - RNA

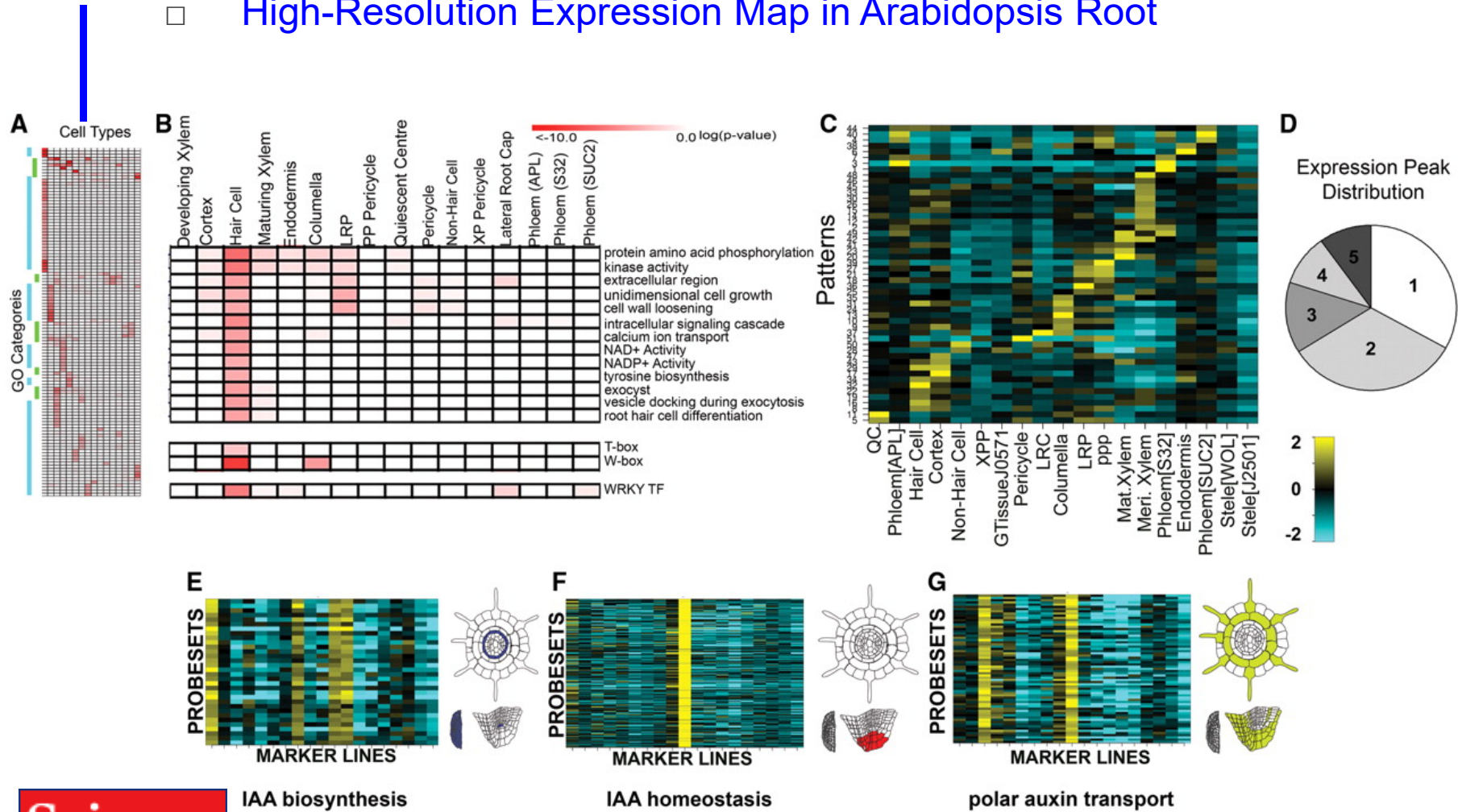
## High-Resolution Expression Map in Arabidopsis



Brady et al., *Science*, 2007

# Expression Maps - RNA

## High-Resolution Expression Map in Arabidopsis Root



IAA biosynthesis

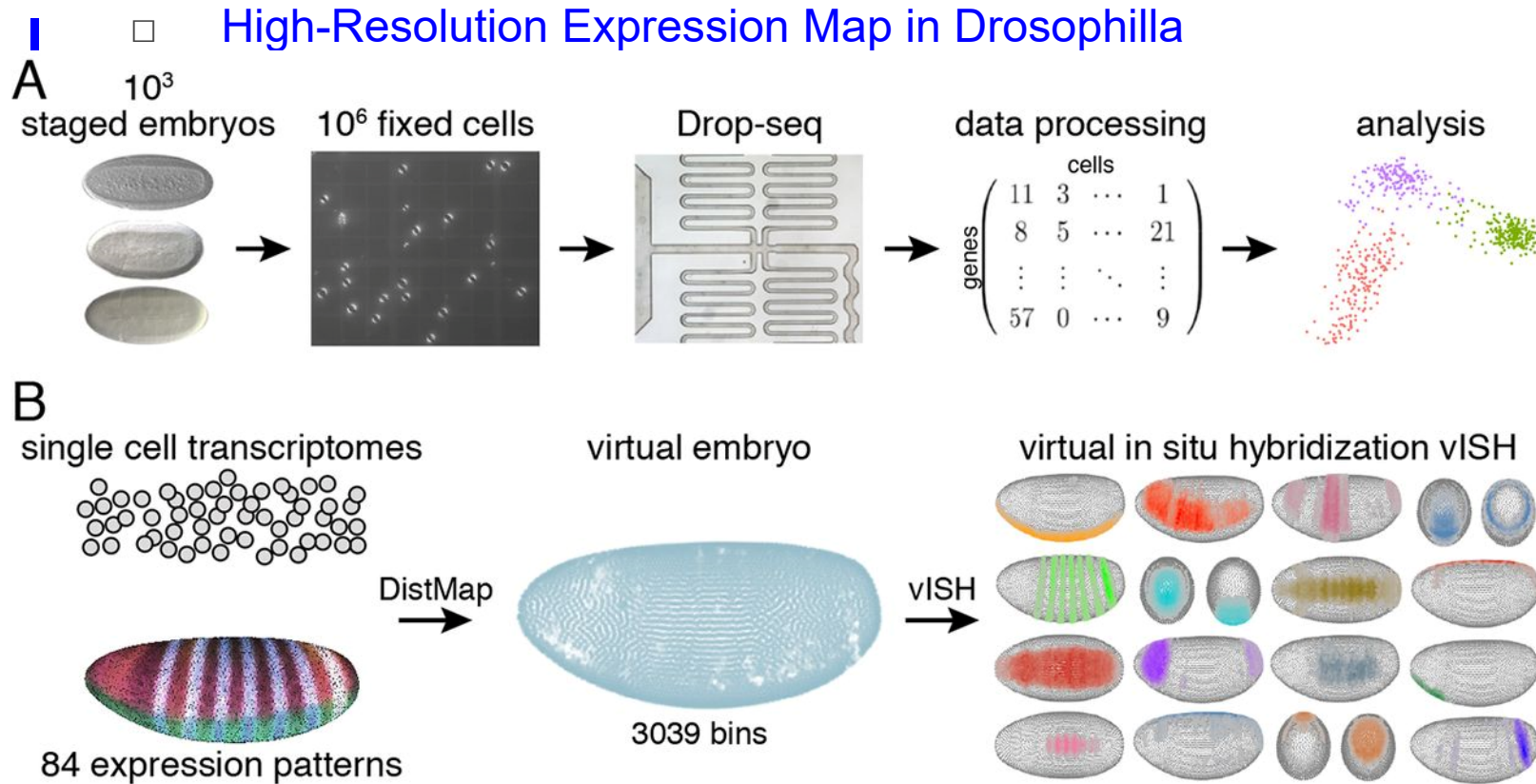
IAA homeostasis

polar auxin transport

Brady et al., *Science*, 2007



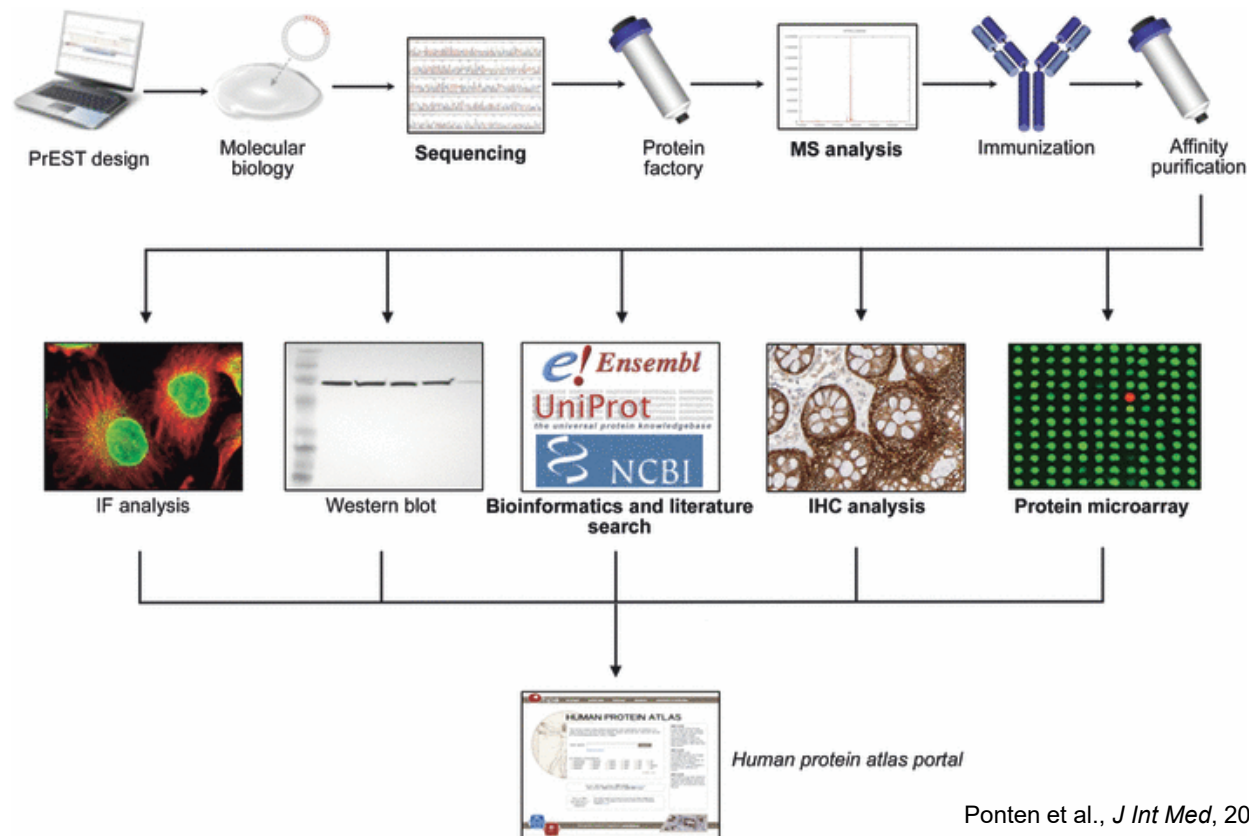
# Expression Maps - RNA



Nikos Karaiskos 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

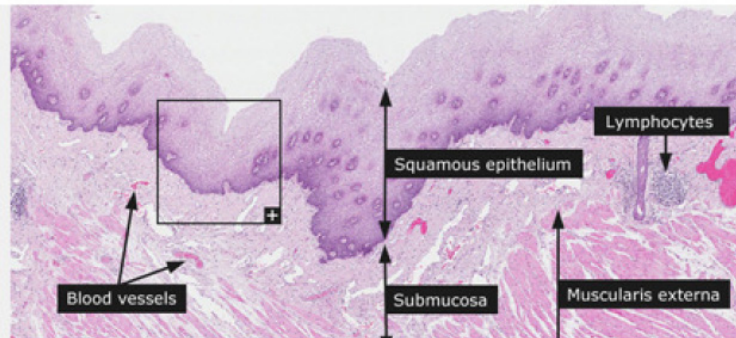
SEARCH ? »

e.g. CD44, ELF3, KLK3, or use Fields to search specific fields such as  
protein\_class:Transcription factors or chromosome:X

Search

Clear

Fields »



dictionary: histology of esophagus

### 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.

*Knut och Alice  
Wallenbergs  
Stiftelse*

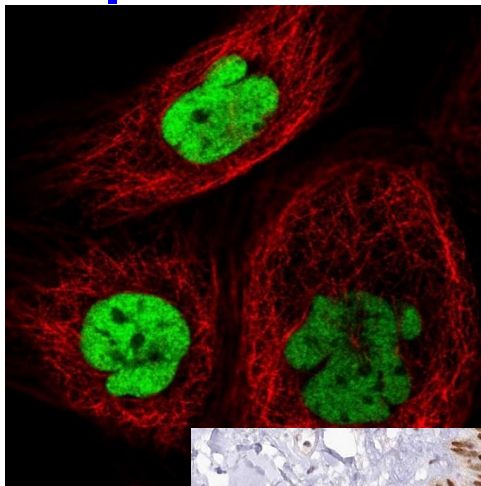
The Human Protein Atlas project is funded by the Knut & Alice Wallenberg foundation.

UPPSALA  
UNIVERSITET

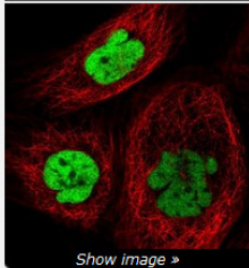


# Expression Maps - Proteins

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



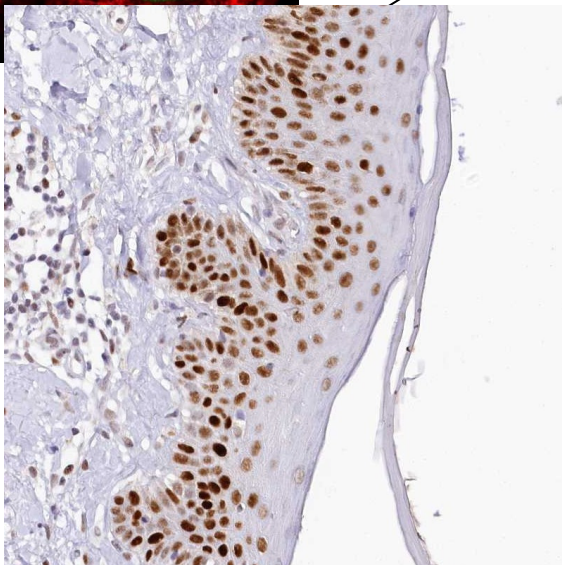
**SUBCELLULAR LOCATION SUMMARY** ? >



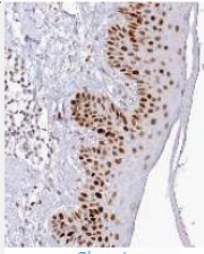
**Main location(s)** Nucleus but not nucleoli  
**Additional location(s)**  
**Staining summary** Localized to the nucleus but excluded from the nucleoli.  
**Reliability (APE)** High  
**Antibodies in assay** CAB039238, CAB039239

[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	<input type="text"/>
Hematopoietic (blood)	8	<input type="text"/>
Liver and pancreas	5	<input type="text"/>
Digestive (GI-tract)	13	<input type="text"/>
Respiratory (lung)	4	<input type="text"/>
Cardiovascular	1	<input type="text"/>
Female tissues	13	<input type="text"/>
Placenta	2	<input type="text"/>
Male tissues	5	<input type="text"/>
Urinary tract (kidney)	3	<input type="text"/>
Skin and soft tissues	14	<input type="text"/>
Endocrine tissues	3	<input type="text"/>

[Show image >](#)

**MORE TISSUE DATA**

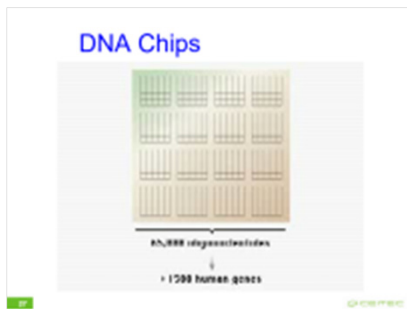


# Outline

- 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

# 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  $3,1 \times 10^9$  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, ...)

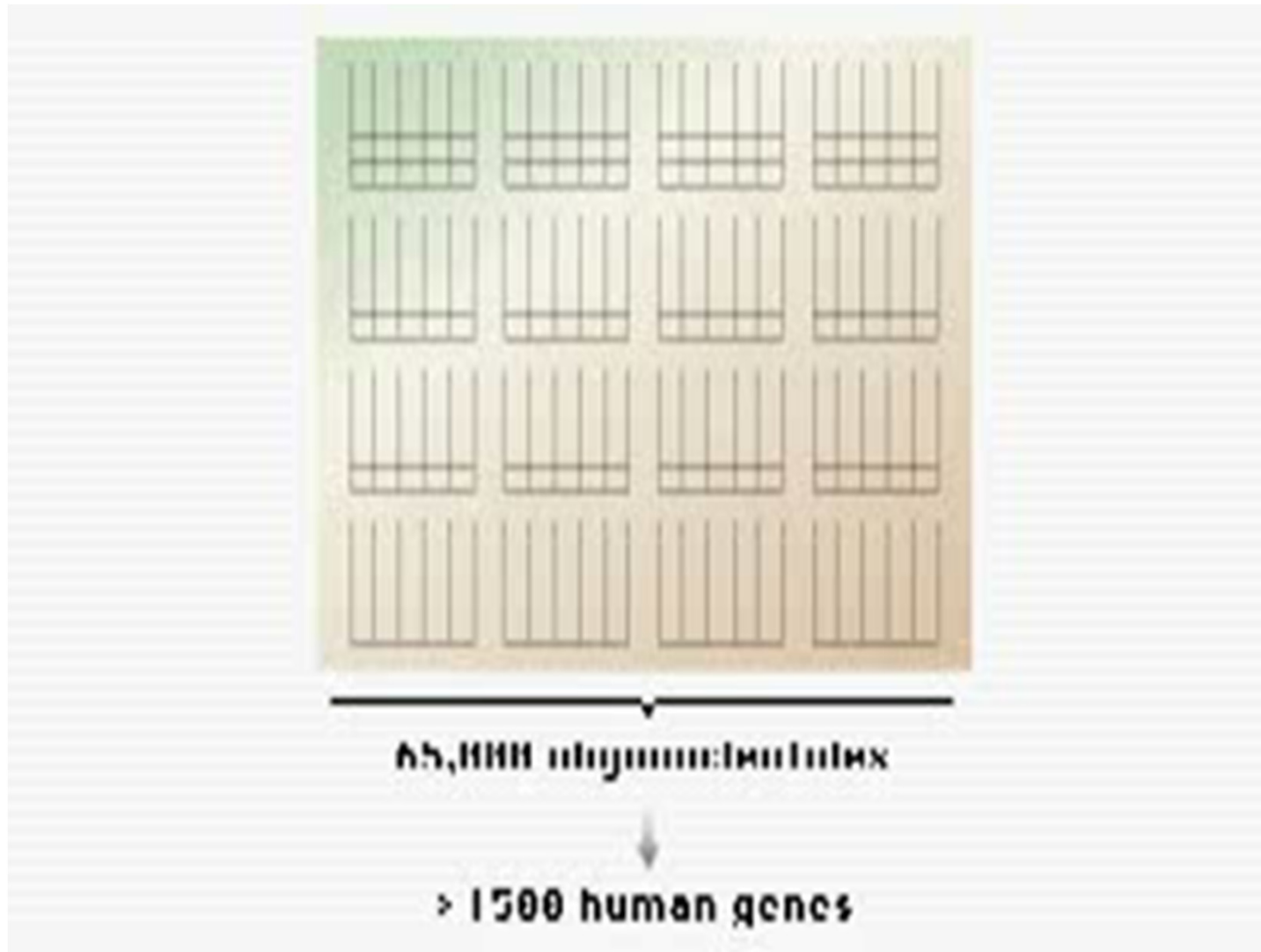


Affymetrix ATH1 *Arabidopsis* genome array

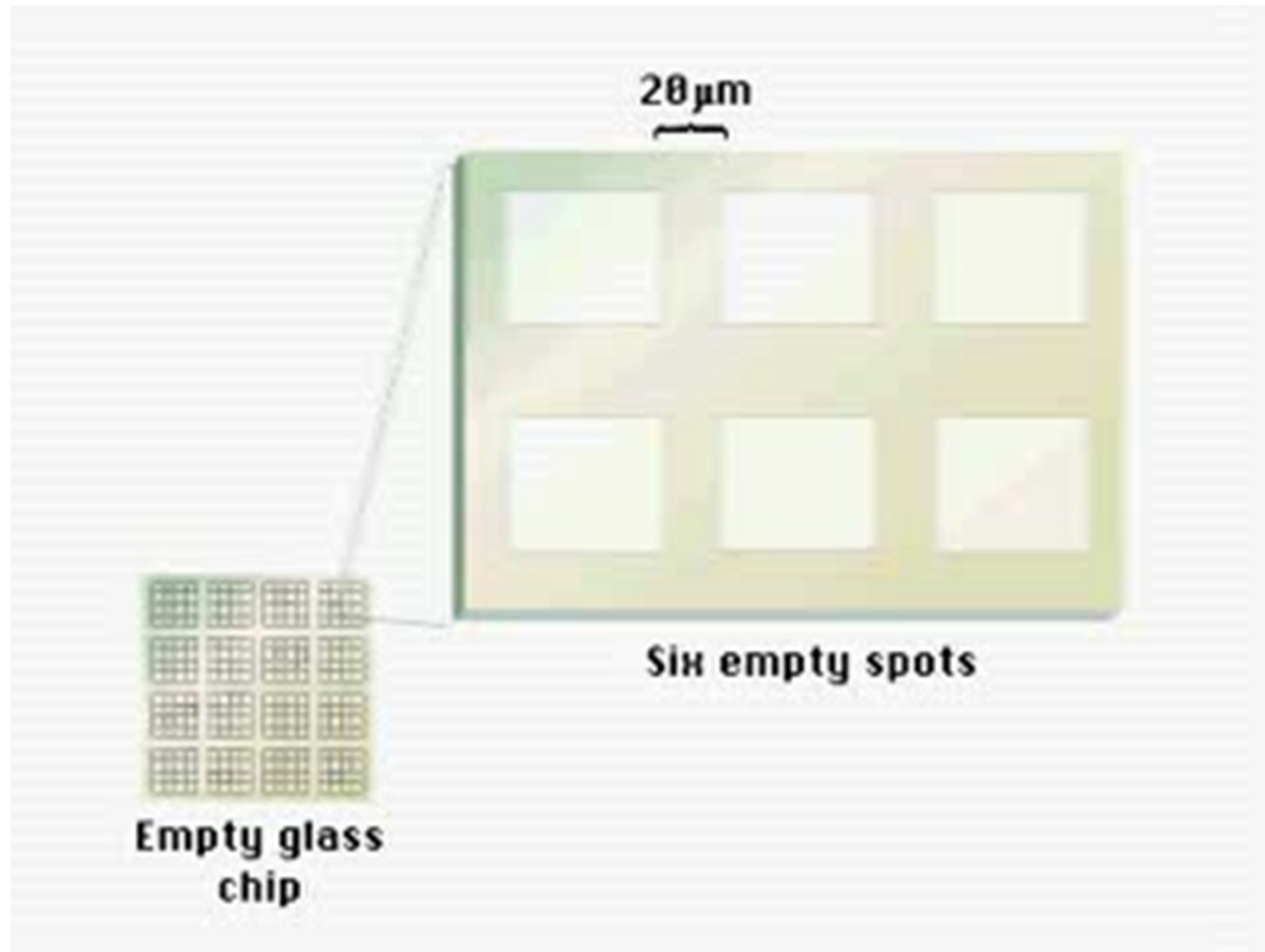
Critical Specifications	
Number of arrays	One
Number of sequence represented	>24,000 gene sequences
Feature size	18 µm
Oligonucleotide probe length	25-mer
Probe pairs/sequence	11
Control sequences	<i>E. coli</i> genes <i>bioB</i> , <i>bioC</i> , <i>bioD</i> . <i>B. subtilis</i> gene <i>lysA</i> . Phage 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



# Photolithography



# 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	
HoekengaS8 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

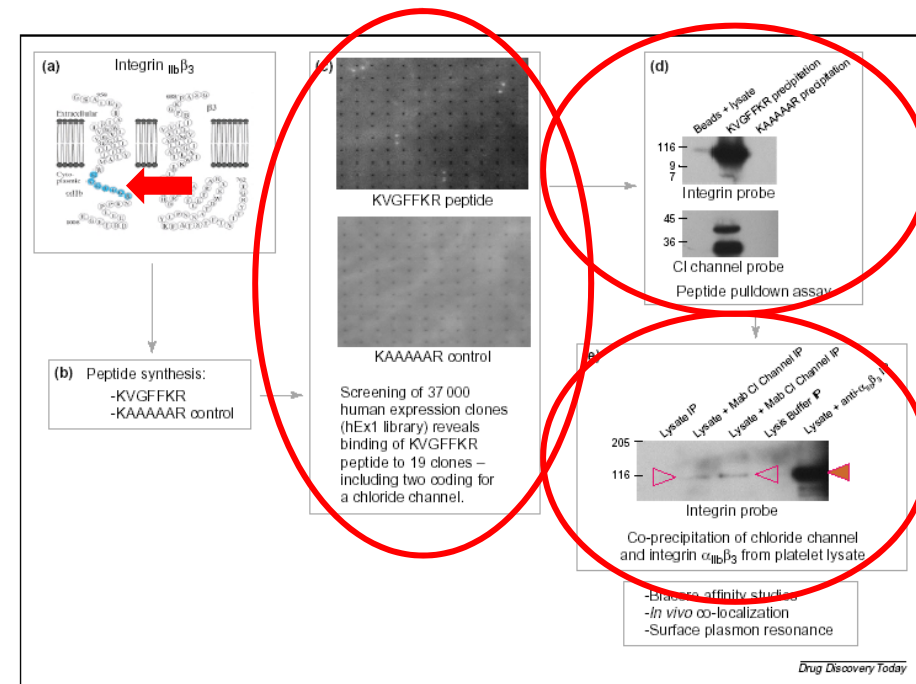
# Protein Chips

- 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 $\alpha$  kinase substrates; Kramer et al., 2004)



Lueking et al., 2005

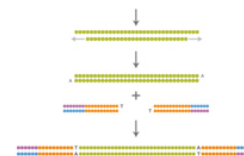
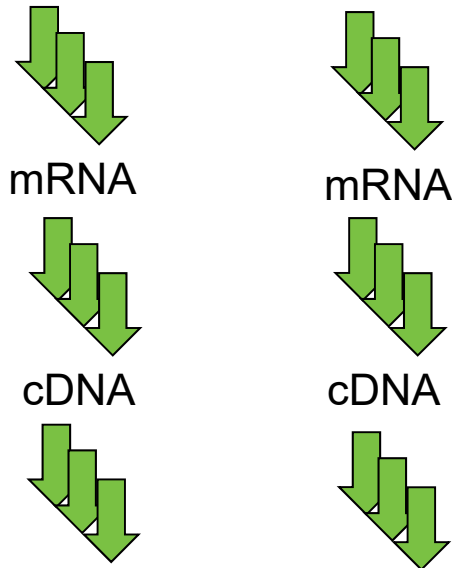
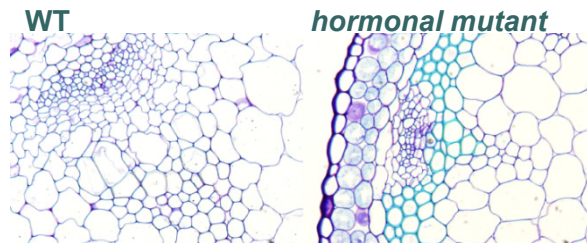
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    - Use of the data available in **public databases**
    - **Tissue- and cell-specific** gene expression analysis
  - **Quantitative analysis of gene expression**
    - **DNA and protein chips**
    - **Next generation transcriptional profiling**



# 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	6.8885e-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

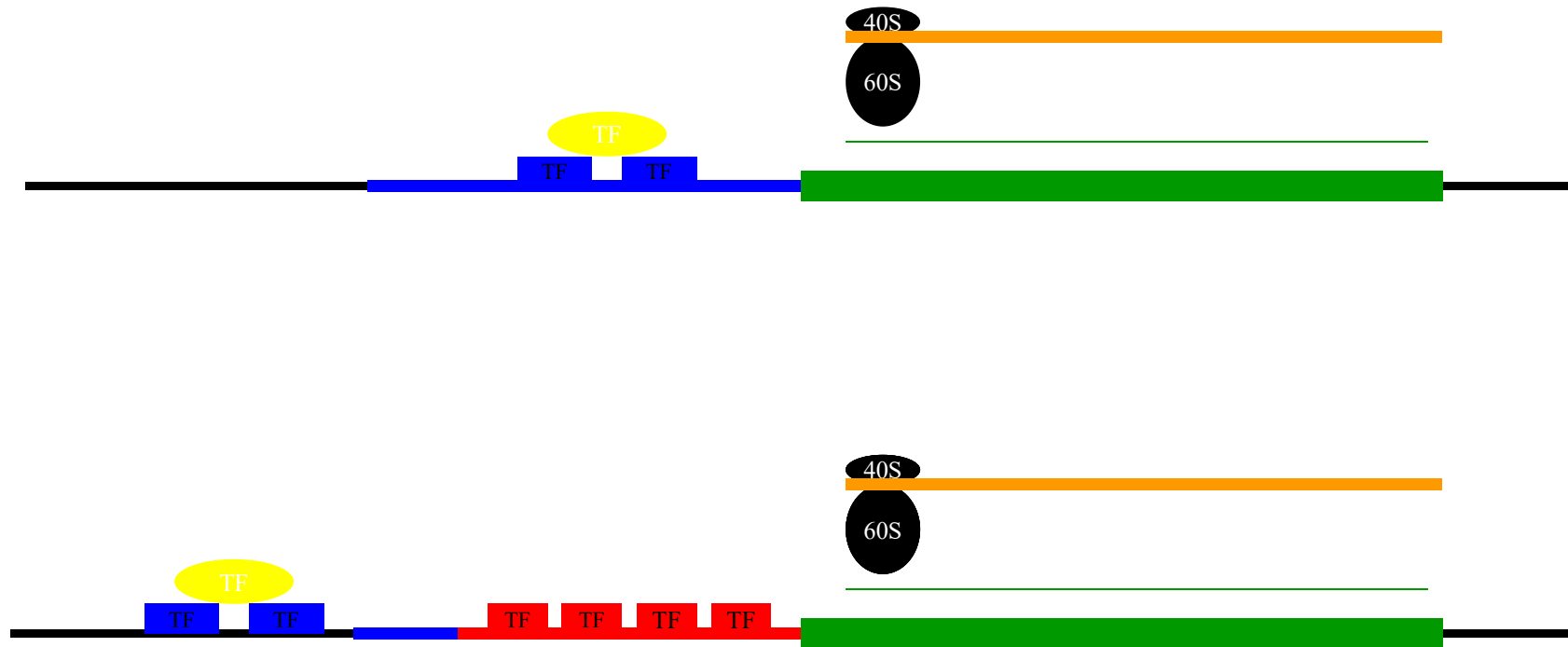
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  - Quantitative analysis of gene expression
    - DNA and protein chips
    - Next generation transcriptional profiling
- **Regulation of gene expression in the identification of gene function by gain-of-function approaches**
  - T-DNA activation mutagenesis

# Gain-of-Function Approaches

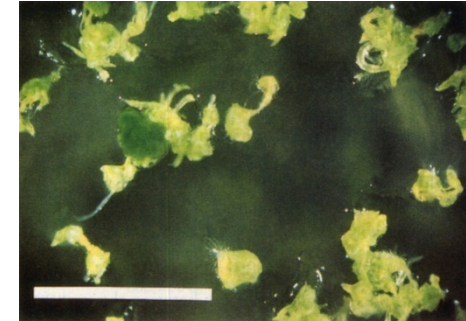
- Methods for identification of gene function using gain-of-function approaches
  - T-DNA activation mutagenesis
    - Method enabling isolation of dominant mutants by random insertion of constitutive promoter, resulting in overexpression of the gene and therefore in corresponding phenotypic changes
    - 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

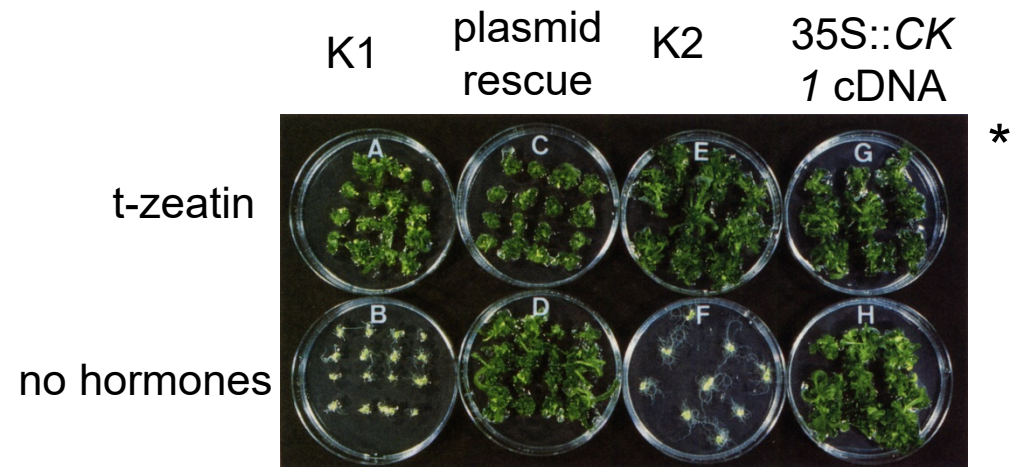


# Isolation of *CKI1* Gene

- 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*, *CYTOKININ INDEPENDENT 1*)



# 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



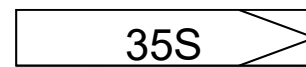
activator  
X



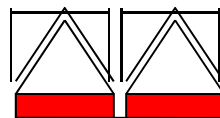
activator x reporter



reporter



LhG4



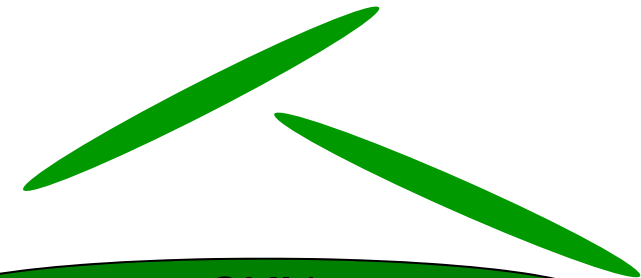
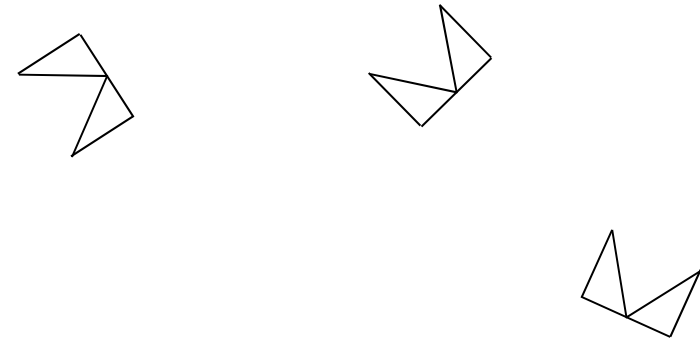
pOP



TATA



CKI1





# Regulated Expression Systems



activator X

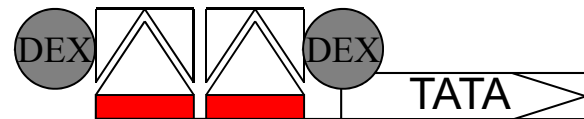
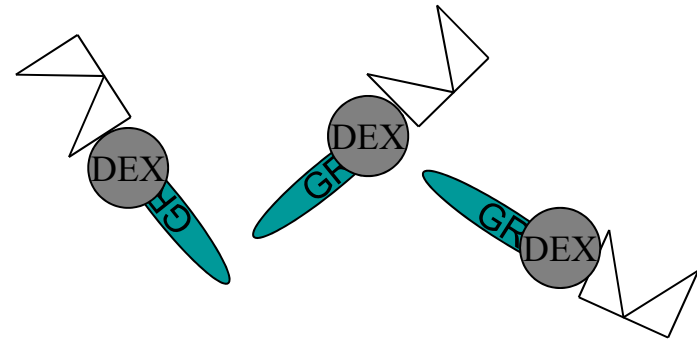
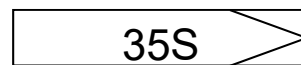


activator x reporter

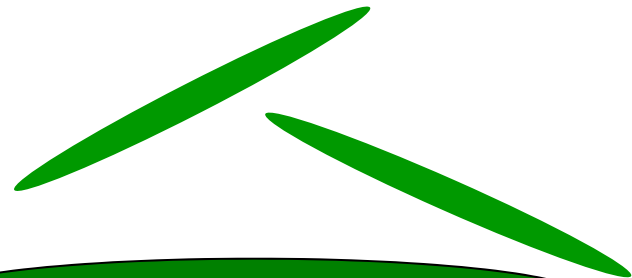


reporter

+DEX



pOP



# Regulated Expression Systems



activator X

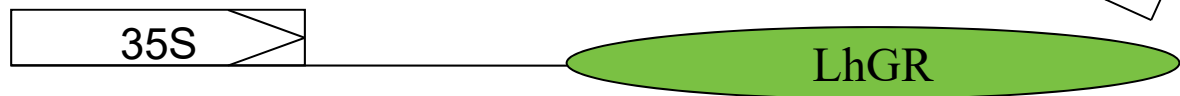


activator x reporter

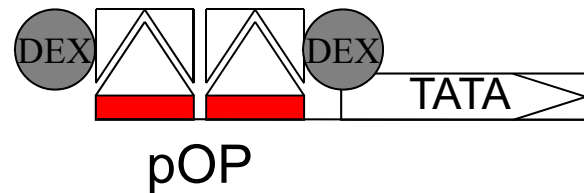


reporter

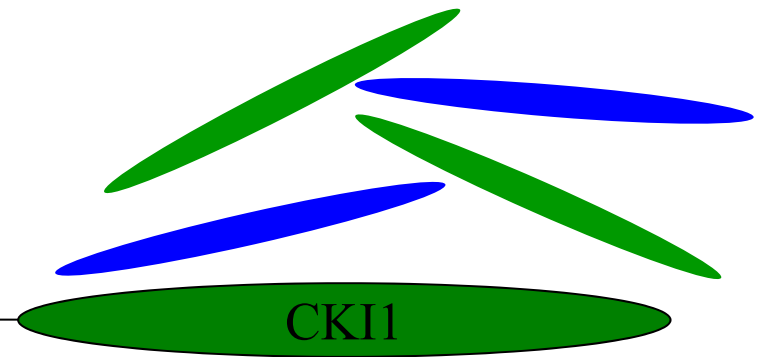
+DEX



wt Col-0

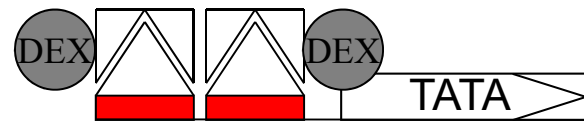


pOP



CKI1

4C



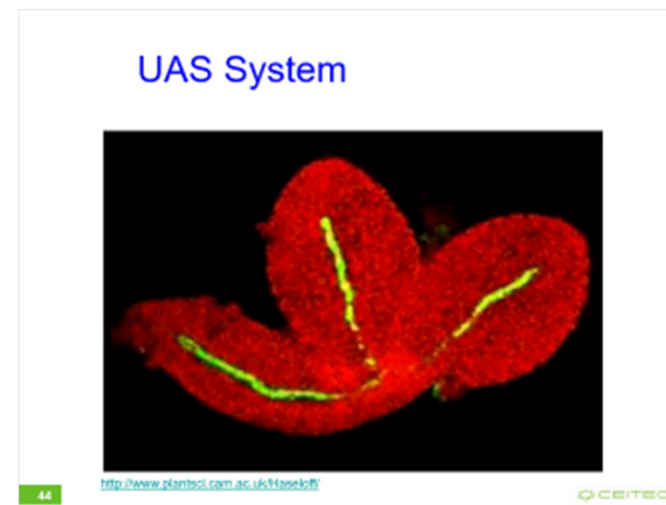
pOP



GUS

# Regulated Expression Systems

- Regulated transgene expression systems
  - Allow **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



# UAS System



<http://www.plantsci.cam.ac.uk/Haseloff/>

# 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**
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    - **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**

# 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 displays a web browser window with the URL <https://www.ncbi.nlm.nih.gov/pubmed/?term=chemical+genetics>. The page is the NCBI PubMed search results page for the query "chemical genetics".

**Search Results Summary:**

- Format: Summary | Sort by: Most Recent | Per page: 20
- Filters: Manage Filters
- Sort by: Best match | Most recent
- Results by year: [Graph]
- PMC images search for chemical genetics: [Image thumbnails]
- Titles with your search terms: [List of titles]
- Find related data: [Link]

**Best matches for chemical genetics:**

- [Chemical genetics: elucidating biological systems with small-molecule compounds.](#)  
Kawasumi M et al. *J Invest Dermatol.* (2007)
- [Chemical genetics-based target identification in drug discovery.](#)  
Cong F et al. *Annu Rev Pharmacol Toxicol.* (2012)
- [Chemical genetics.](#)  
O'Connor CJ et al. *Chem Soc Rev.* (2011)

**Search results**

Items: 1 to 20 of 120417

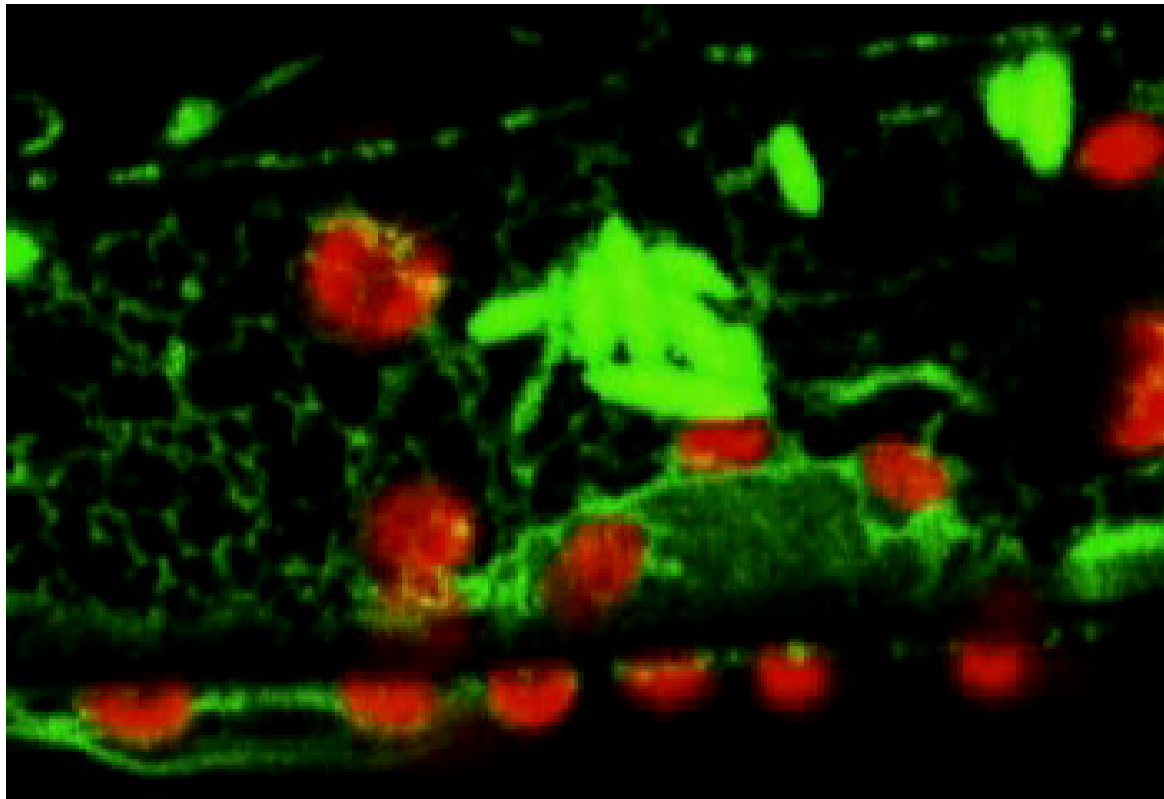
- [Analysis of butterfly reproductive proteins using capillary electrophoresis and mass spectrometry.](#)  
Rokhas MK, Rönn JL, Wiklund C, Emmer A. *Anal Biochem.* 2018 Nov 10; pii: S0003-2697(18)31129-1. doi: 10.1016/j.ab.2018.11.002. [Epub ahead of print]  
PMID: 30423321  
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- [Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13.](#)  
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, Matulis UA, Lin

# 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

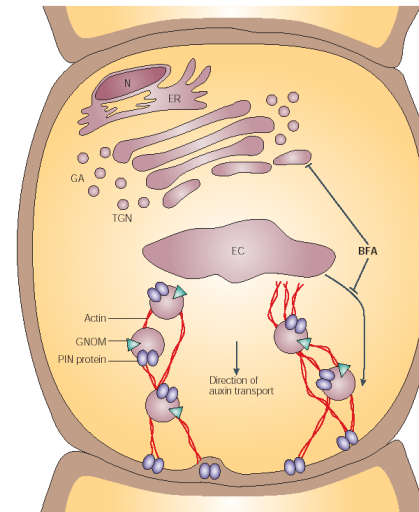
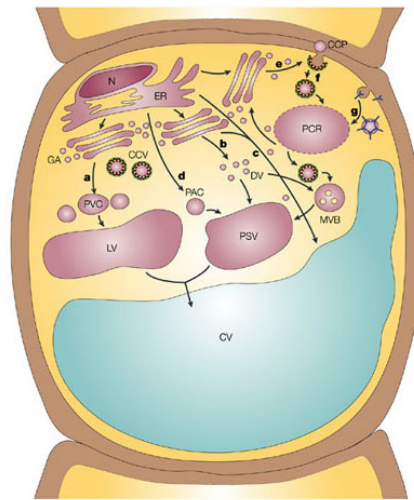


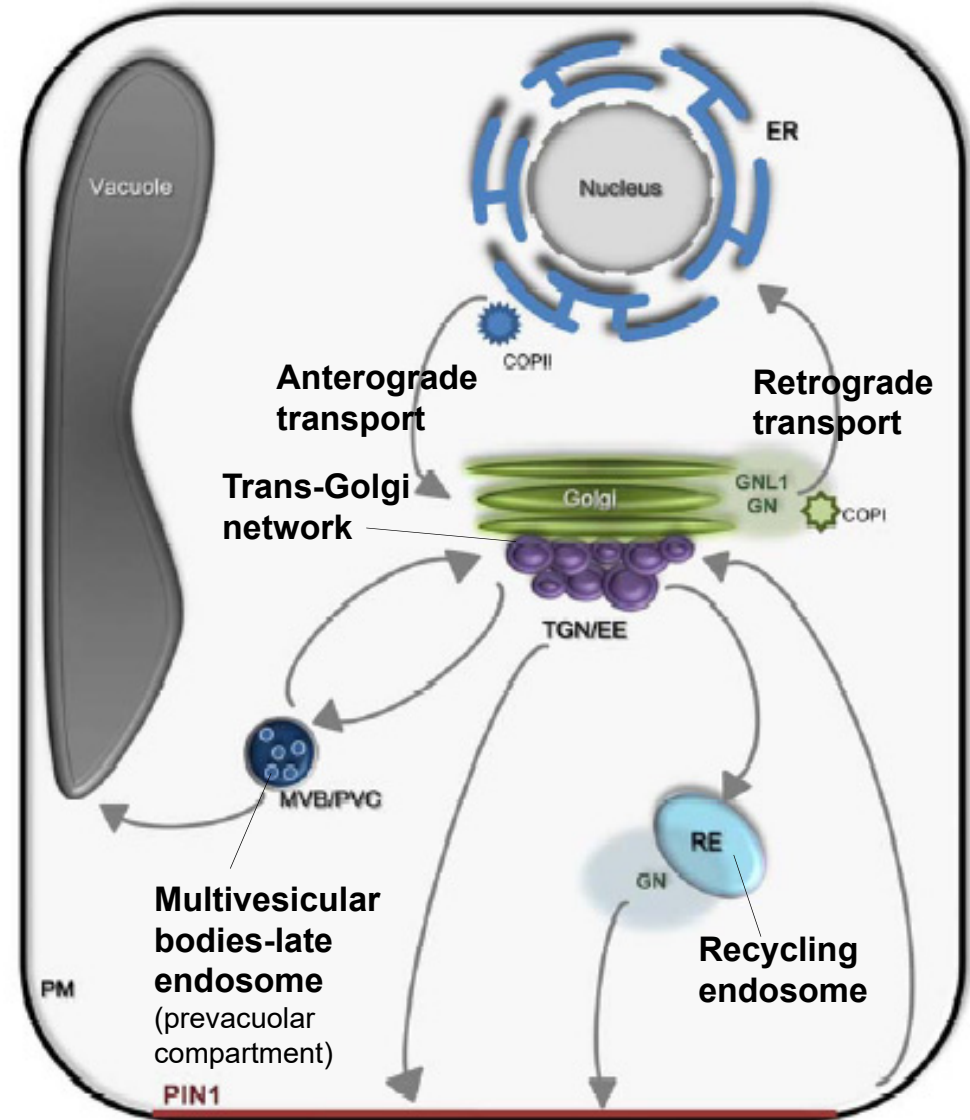
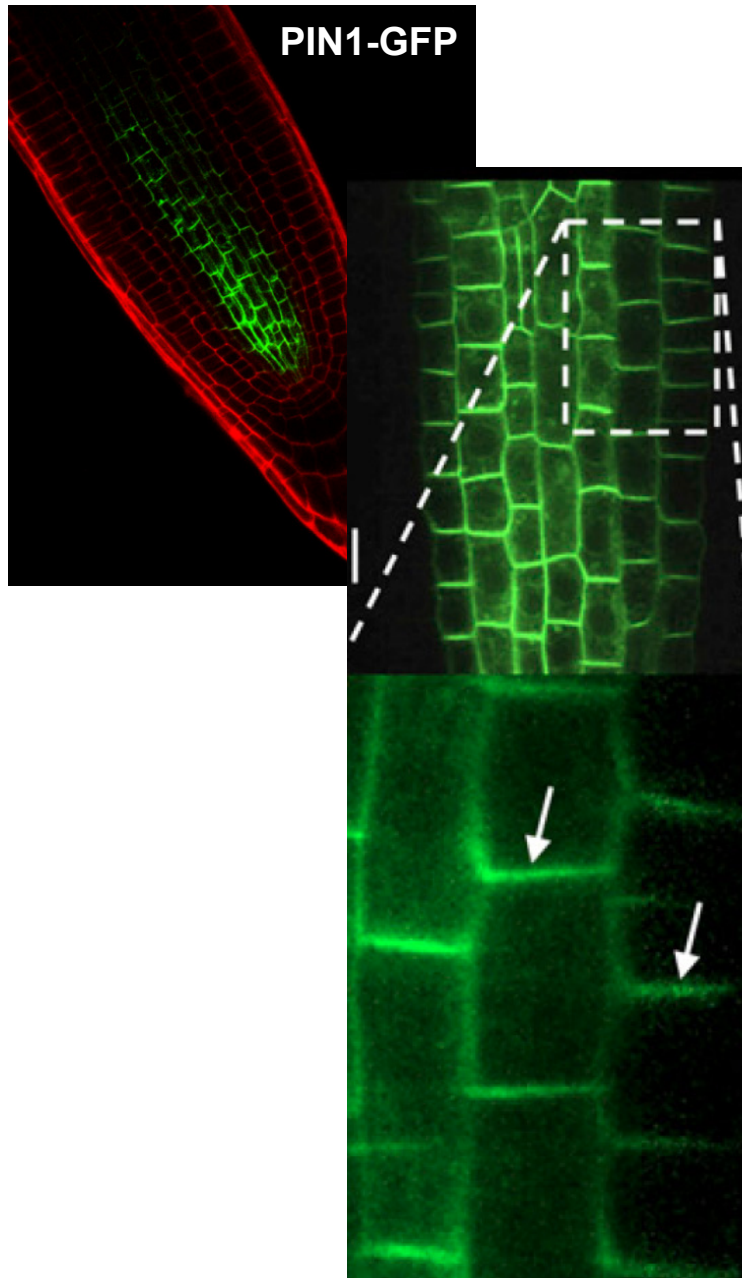
GFP targeted to the ER



# 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



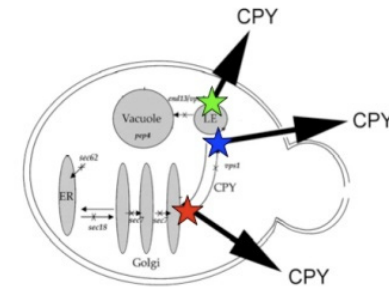


Huang et al., 2010

# Chemical Genetics

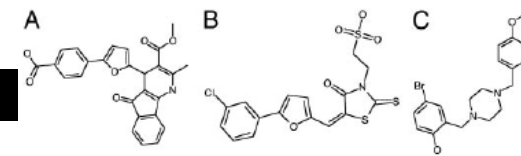
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches

- 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

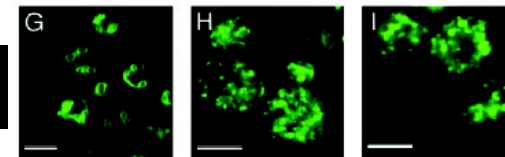


0 2.5 5 10 25 50 100 [mg/L]

Immunodetection of carboxypeptidase



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

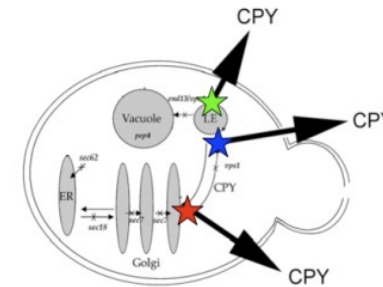


Zouhar et al., 2004

# Chemical Genetics

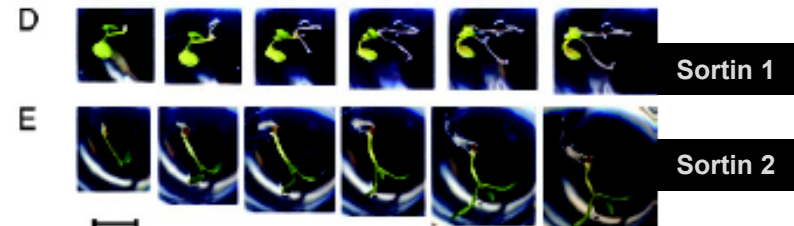
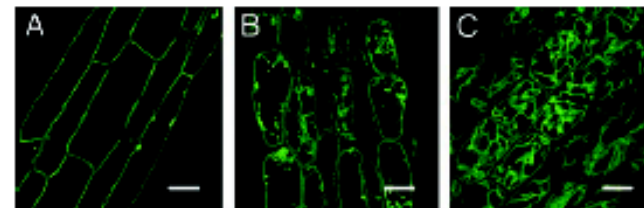
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- Identified compounds („sortins“) were able to induce similar changes in *Arabidopsis* as well – transport mechanisms are conserved in yeast and in plants
- For detailed identification of the molecular process affected by one of the identified „sortins“,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

Shape of plant vacuoles using EGFP:-TIP

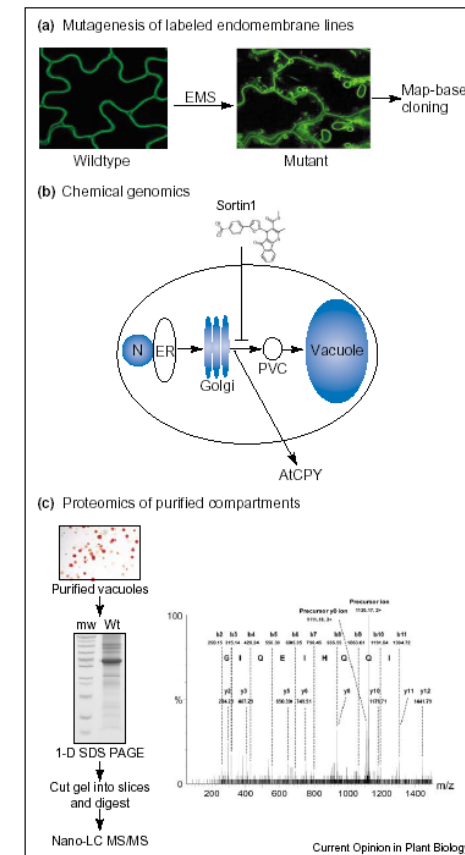


Phenotype of seedlings in the presence of sortins

Zouhar et al., 2004

- 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