CG920 Genomics

Lesson 5

Gene Expression and Chemical Genetics

Jan Hejátko

Functional Genomics and Proteomics of Plants,
Mendel Centre for Plant Genomics and Proteomics,
Central European Institute of Technology (CEITEC), Masaryk University, Brno
hejatko@sci.muni.cz, www.ceitec.muni.cz

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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.

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Outline

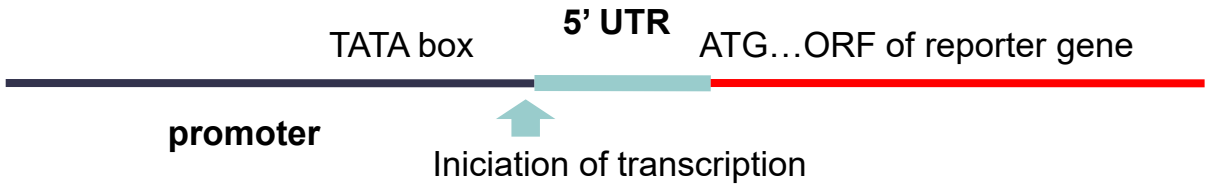
- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene
    - Use of the data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

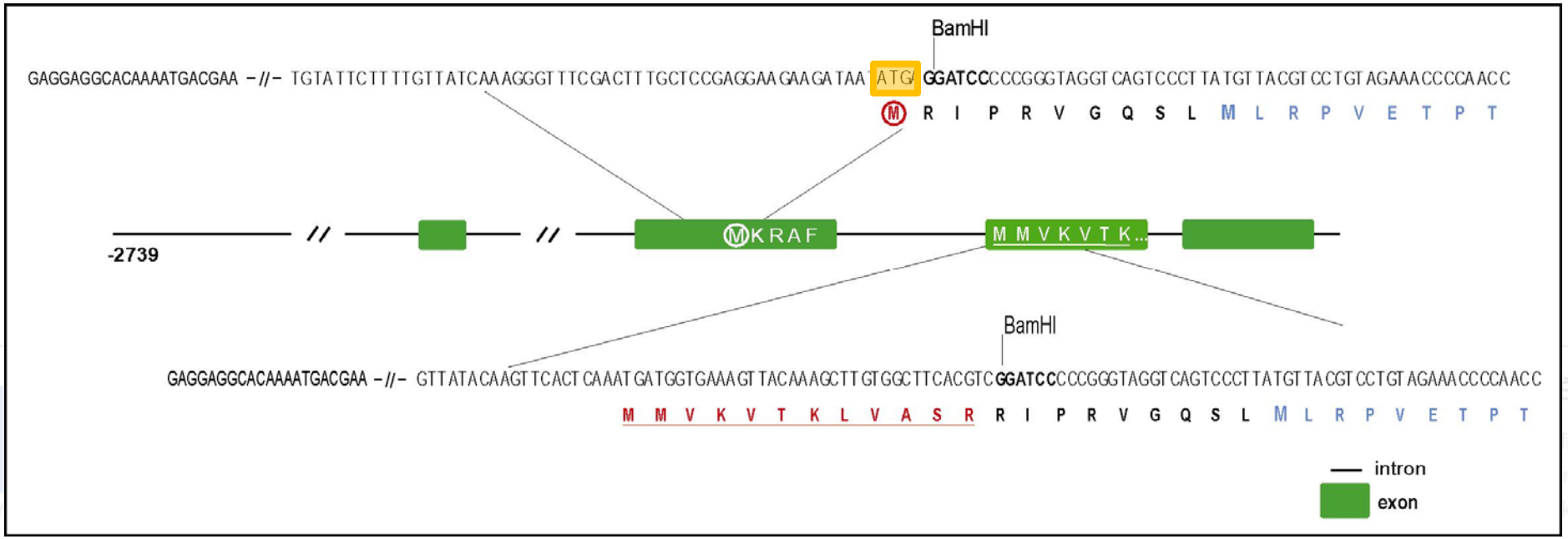
Outline

- 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

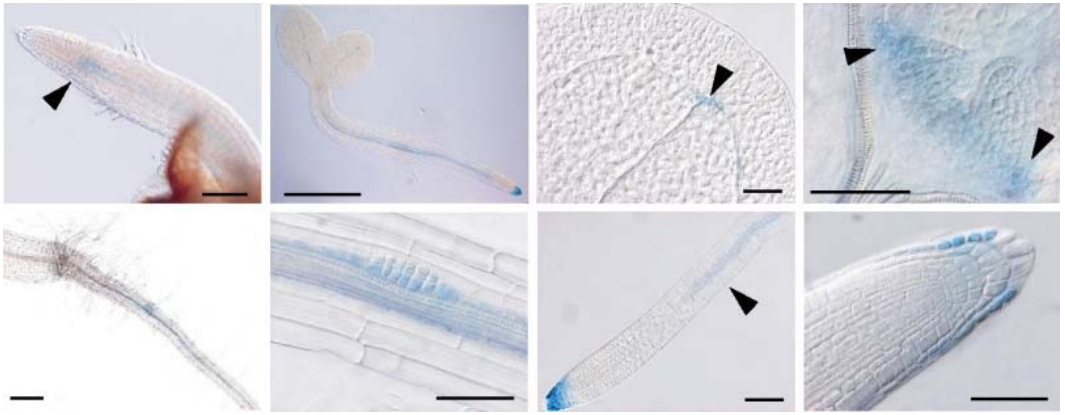
- Identification and cloning of the promoter region of the gene
- Preparation of recombinant DNA carrying the promoter and the reporter gene (uidA, GFP)

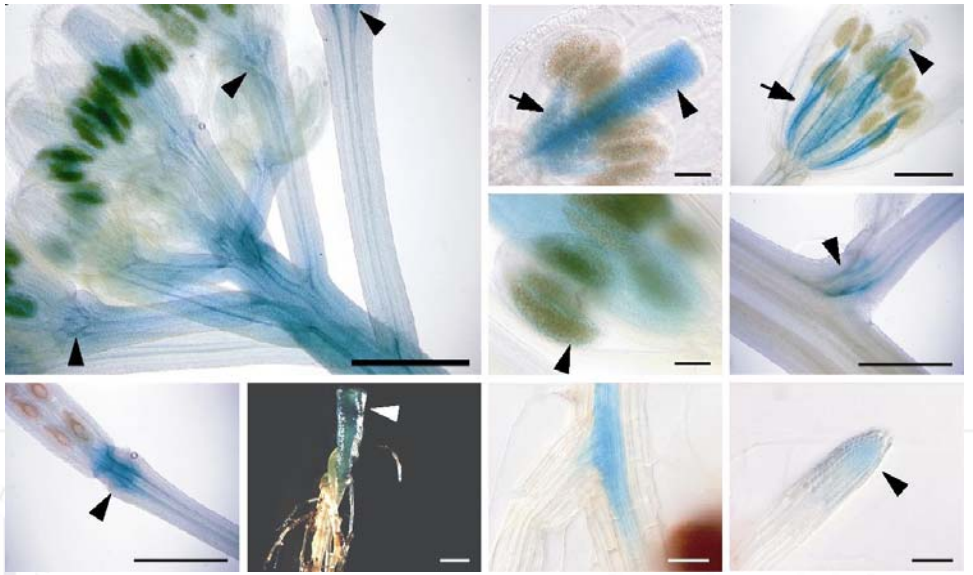




Transcriptional Fusion

- 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





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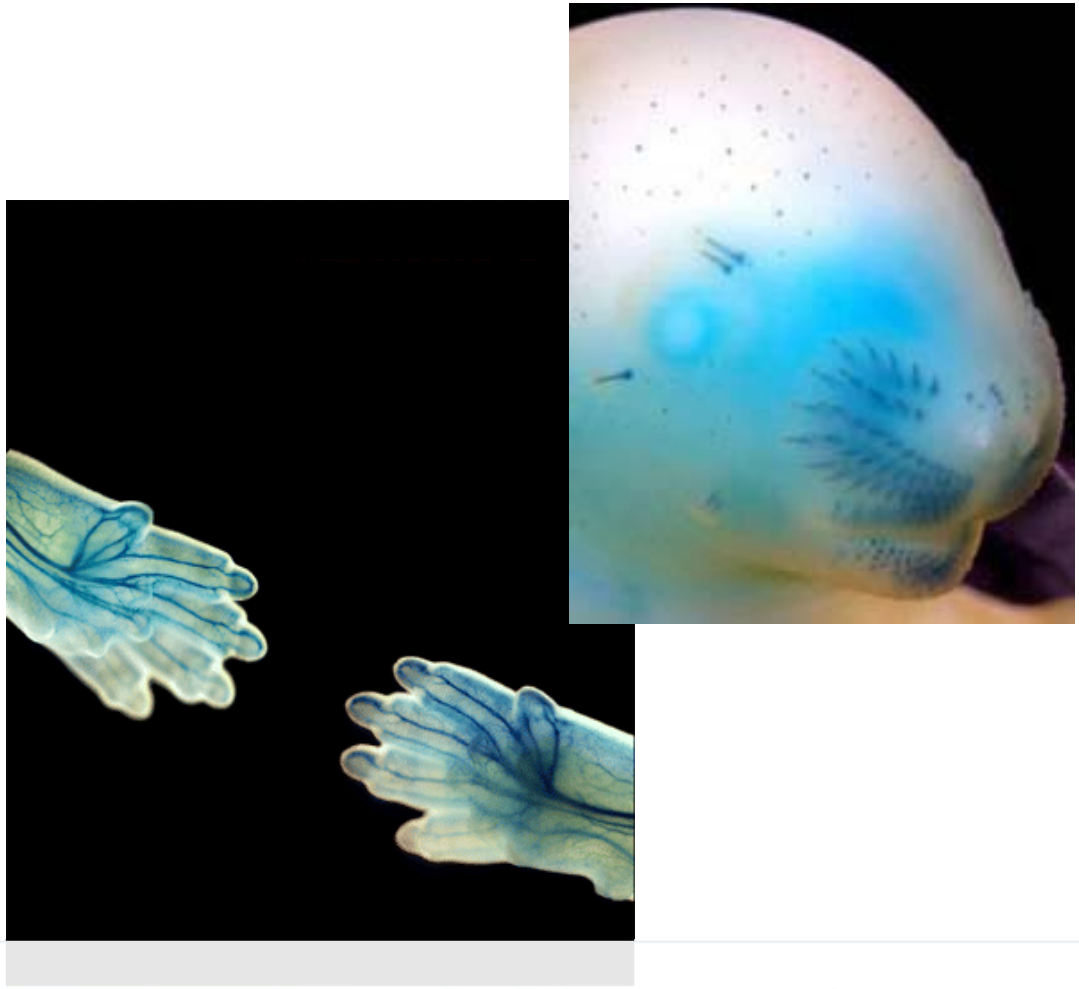
GUS Reporter in Mouse Embryos



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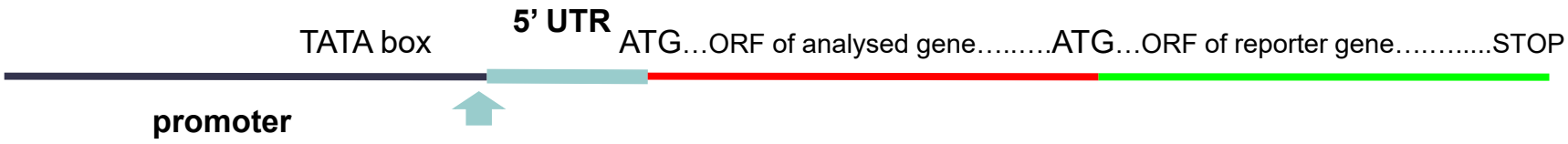
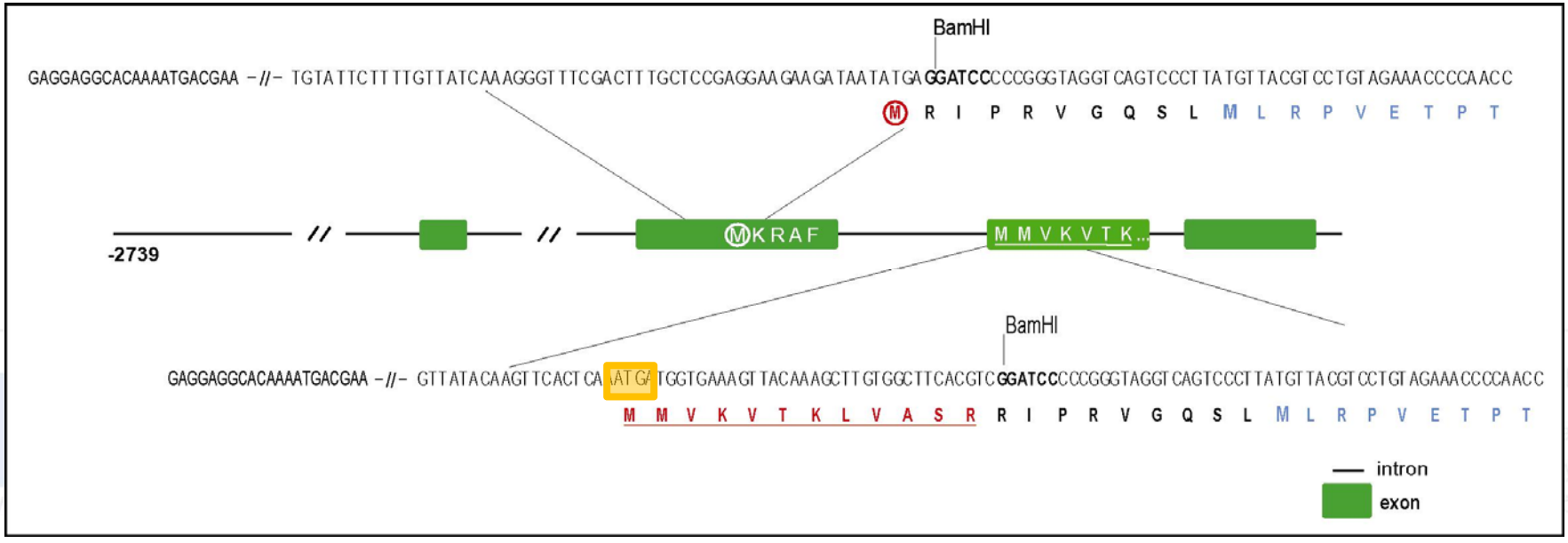
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene

Translational Fusion

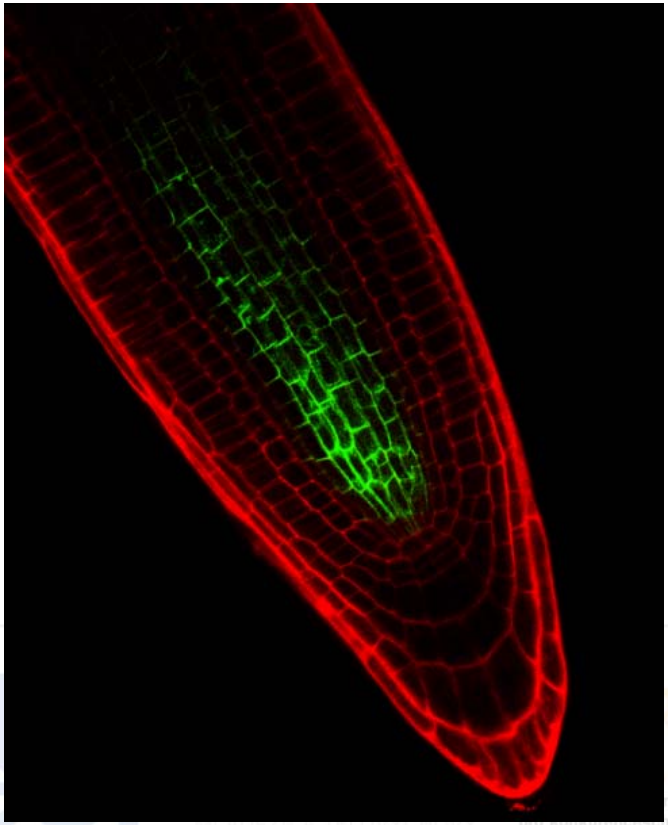
- Identification and cloning of the promoter and coding region of the analyzed gene
- Preparation of a recombinant DNA carrying the promoter and the coding sequence of the studied gene in a fusion with the reporter gene (uidA, GFP)



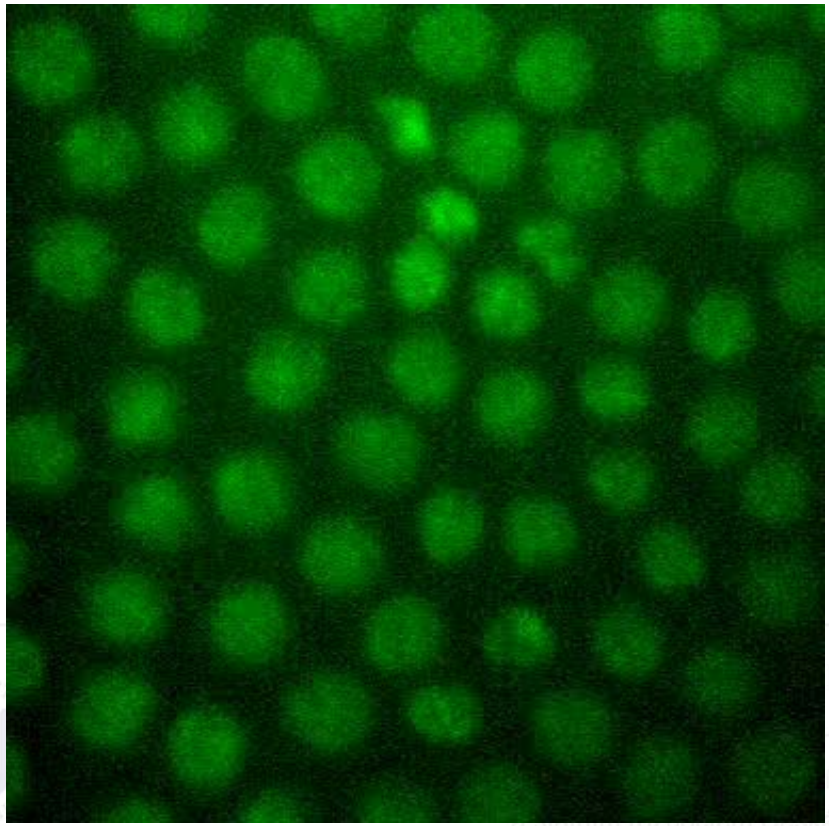


Translational Fusion

- 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

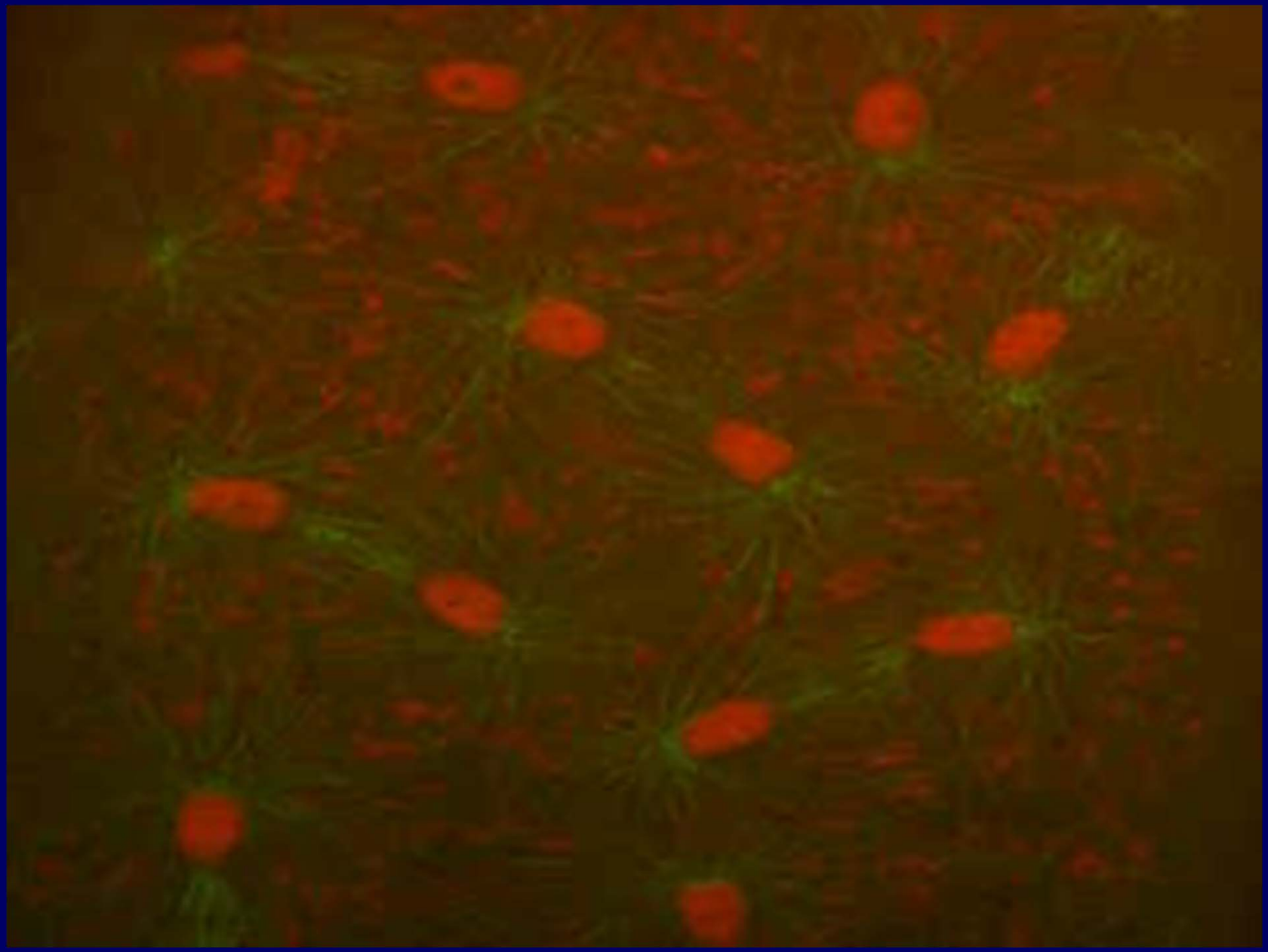


PIN1-GFP in *Arabidopsis*



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

Translational Fusion



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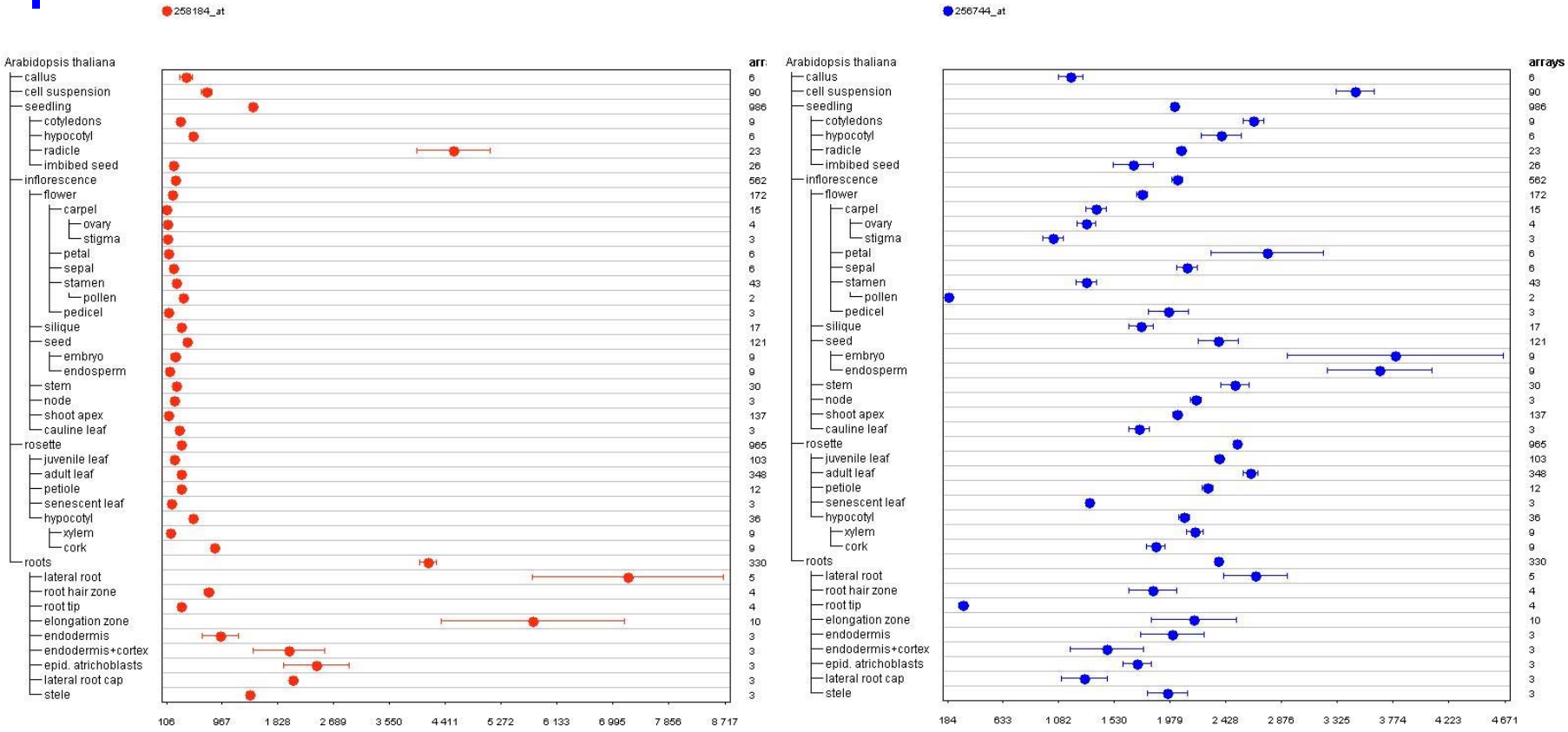
Databases

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



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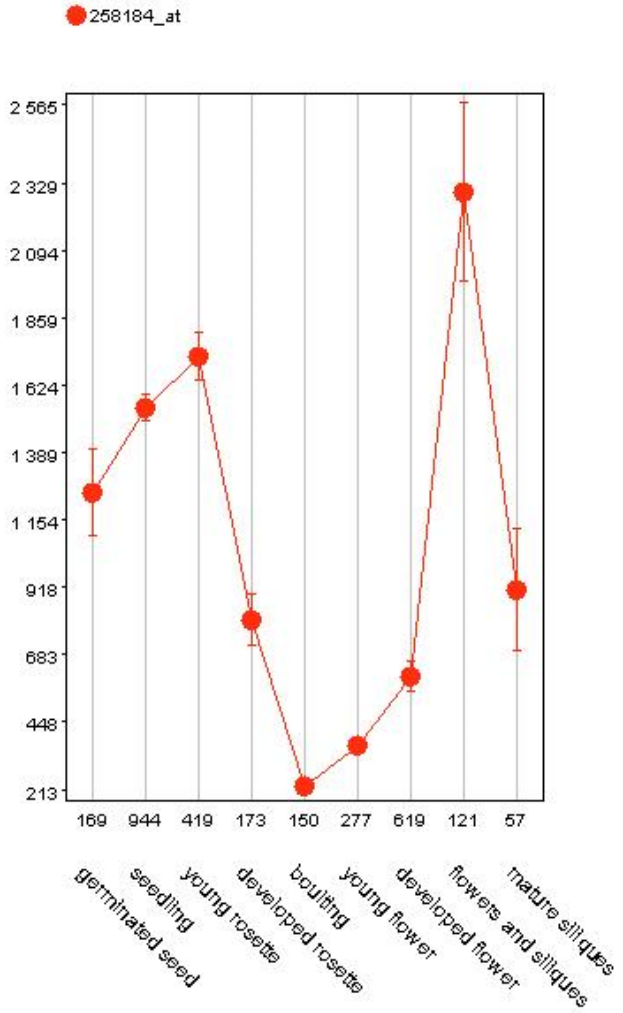

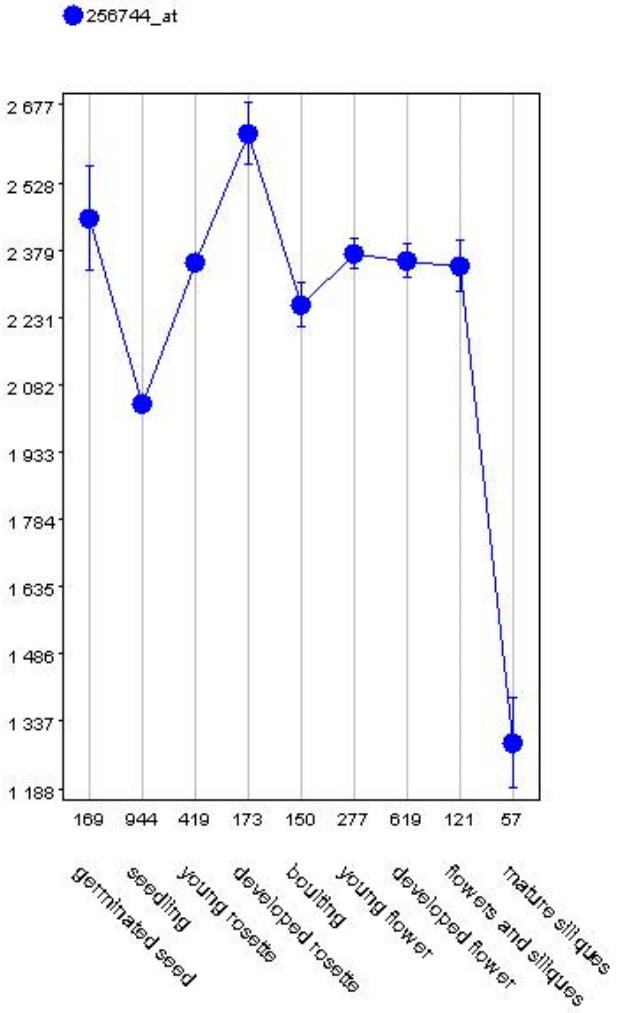

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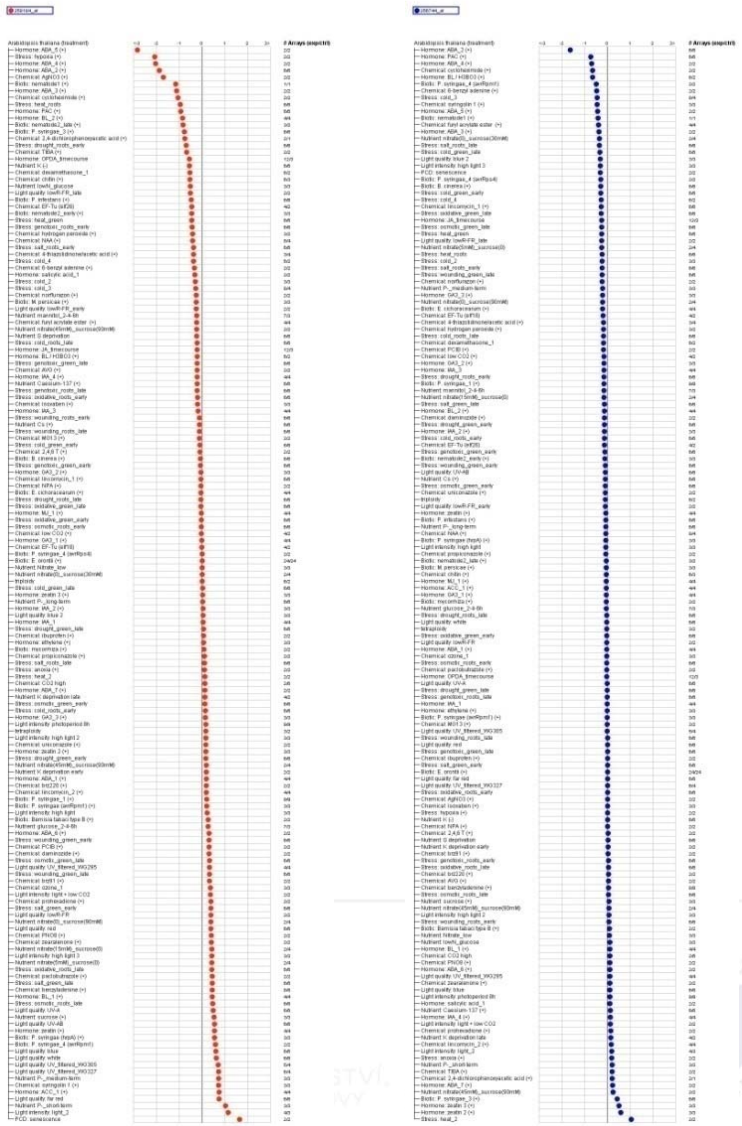

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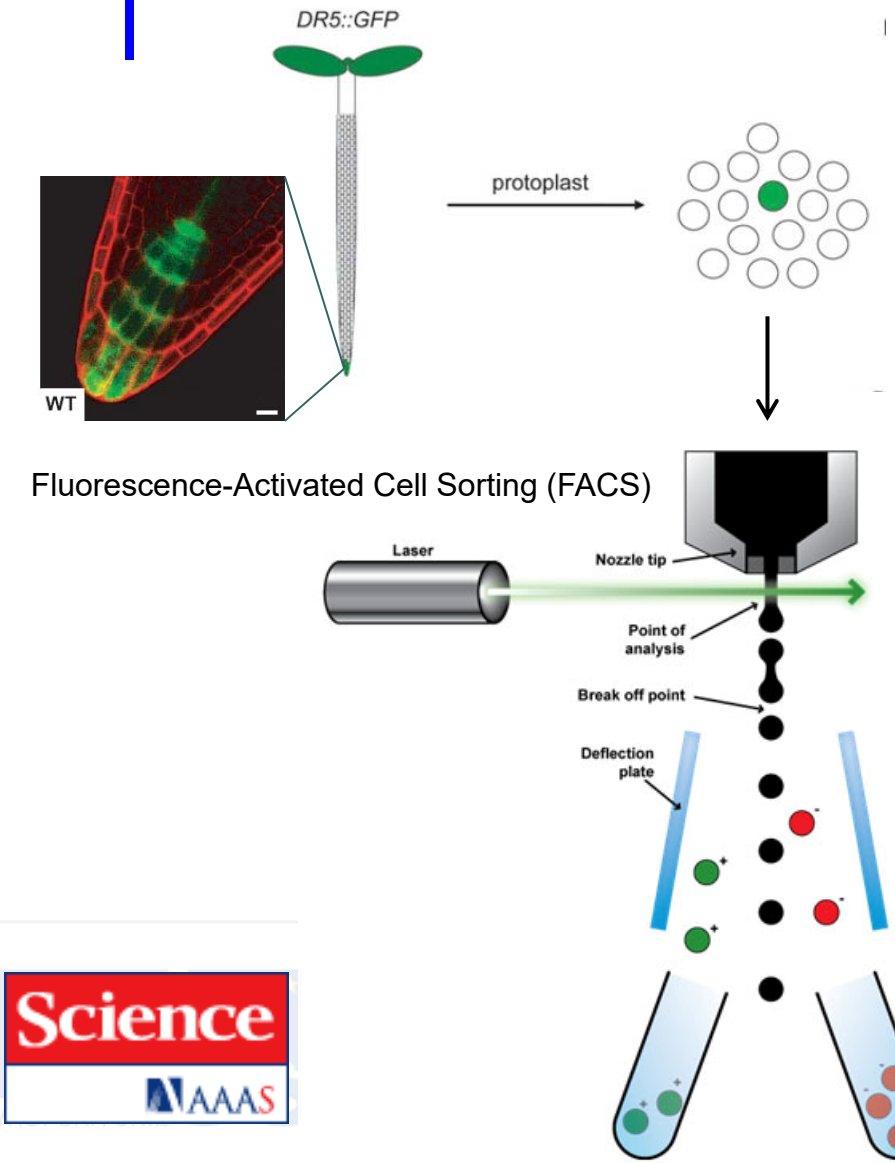
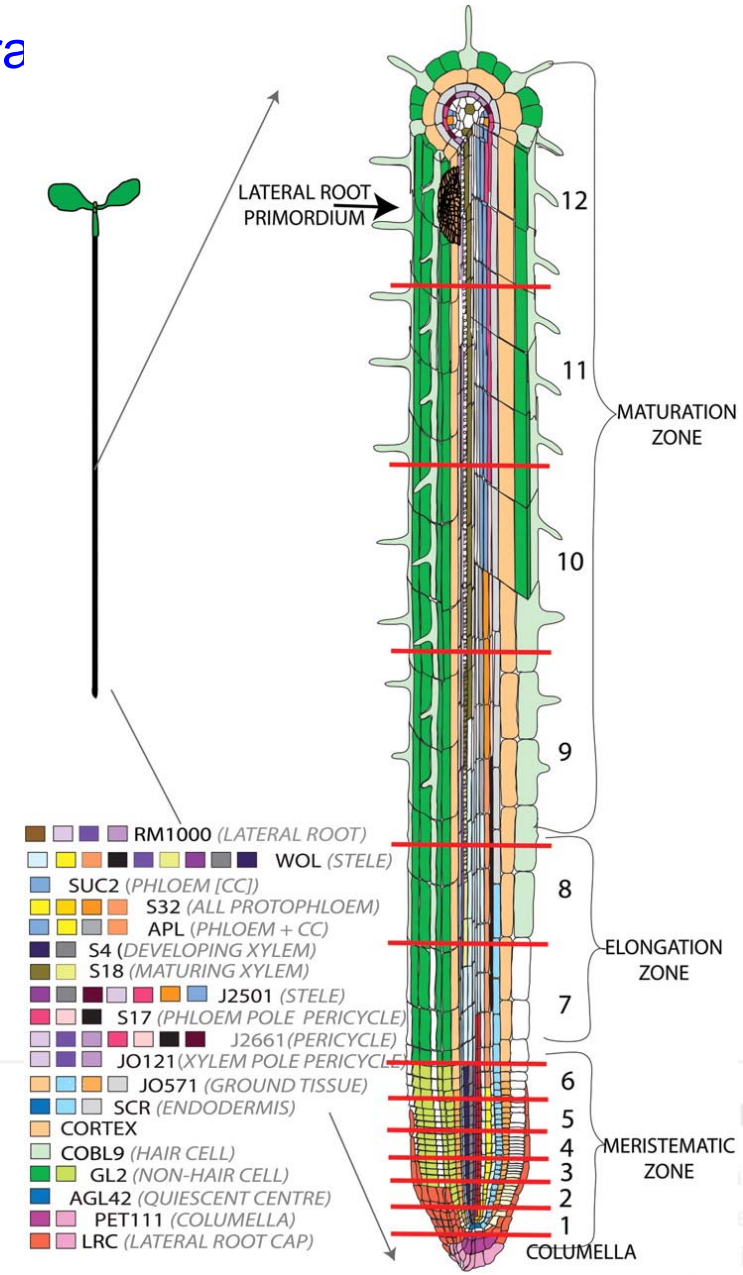
    - Tissue- and cell-specific gene expression analysis

Expression Maps - RNA

- High-Resolution Expression Map in Ara

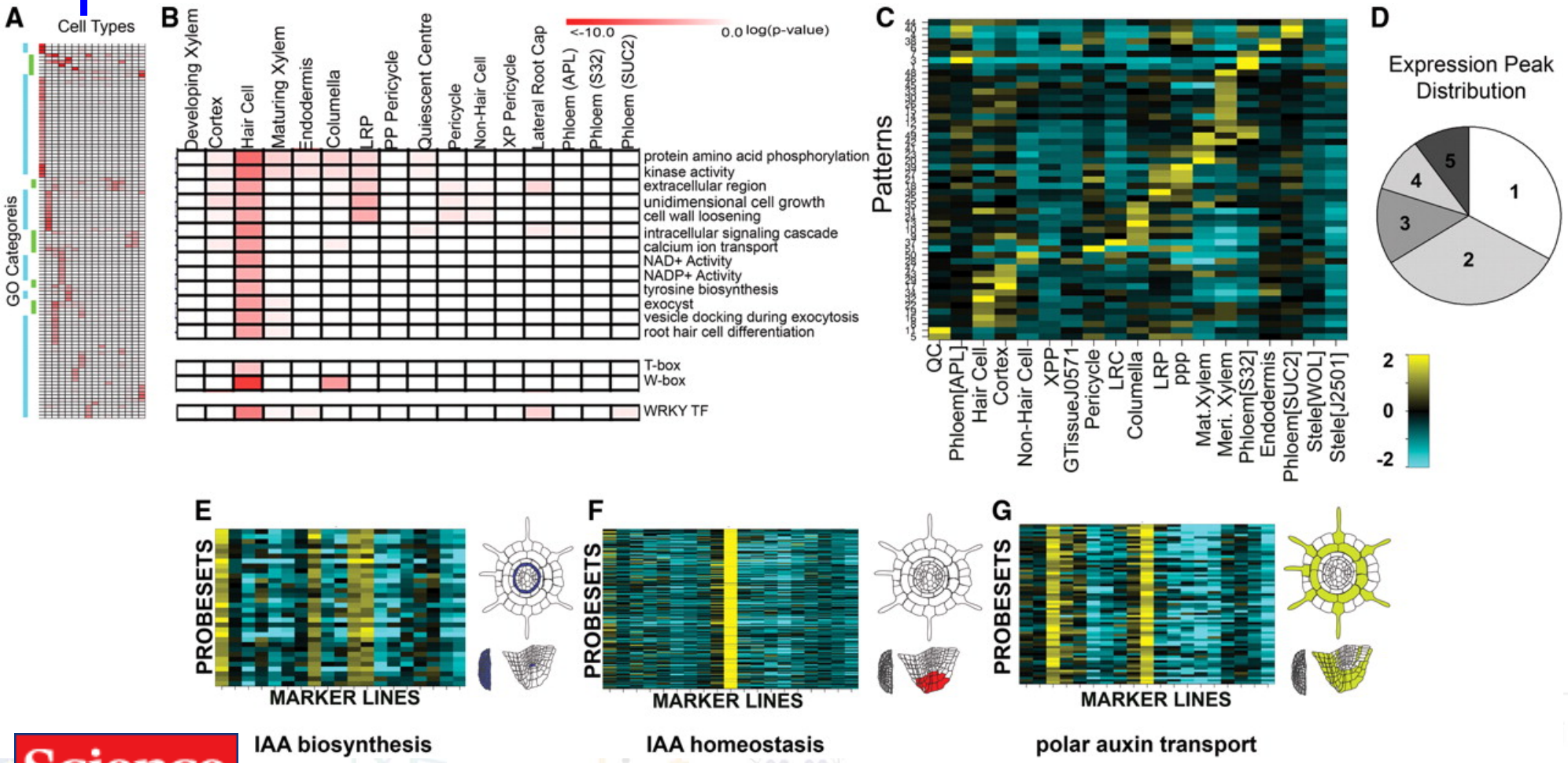




Brady et al., *Science*, 2007

Expression Maps - RNA

- High-Resolution Expression Map in Arabidopsis Root

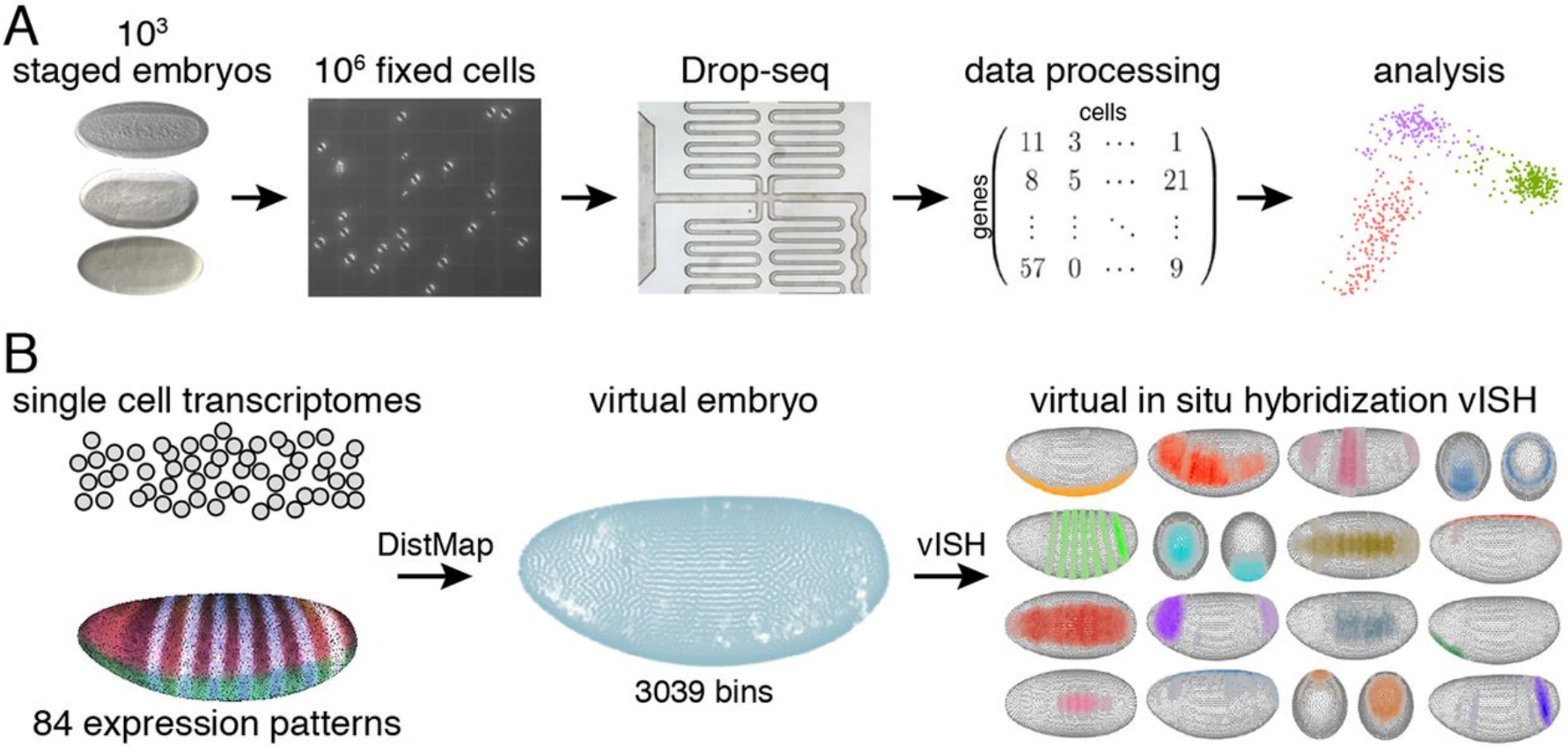


Science
AAAS

Brady et al., *Science*, 2007

Expression Maps - RNA

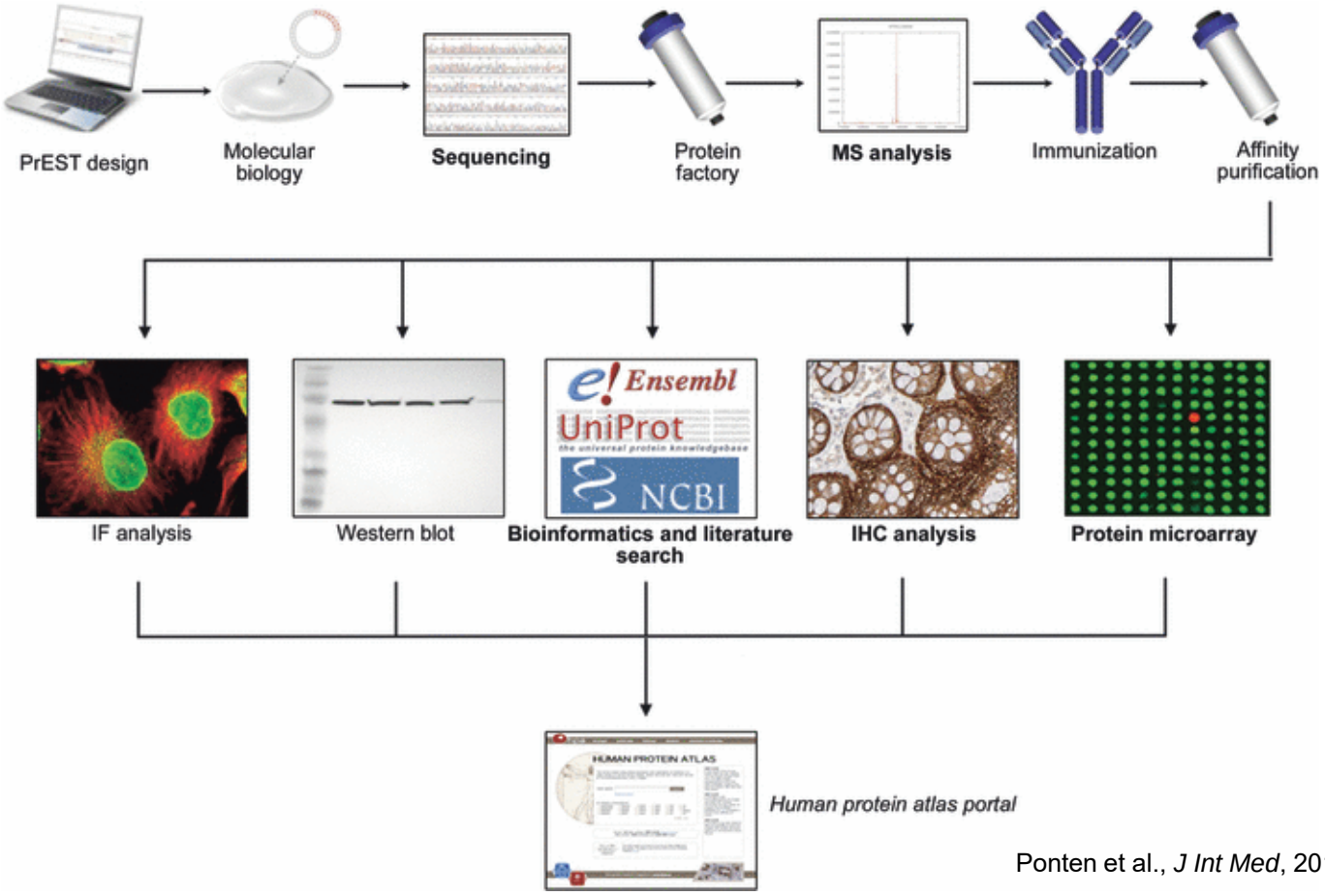
- High-Resolution Expression Map in Drosophilla



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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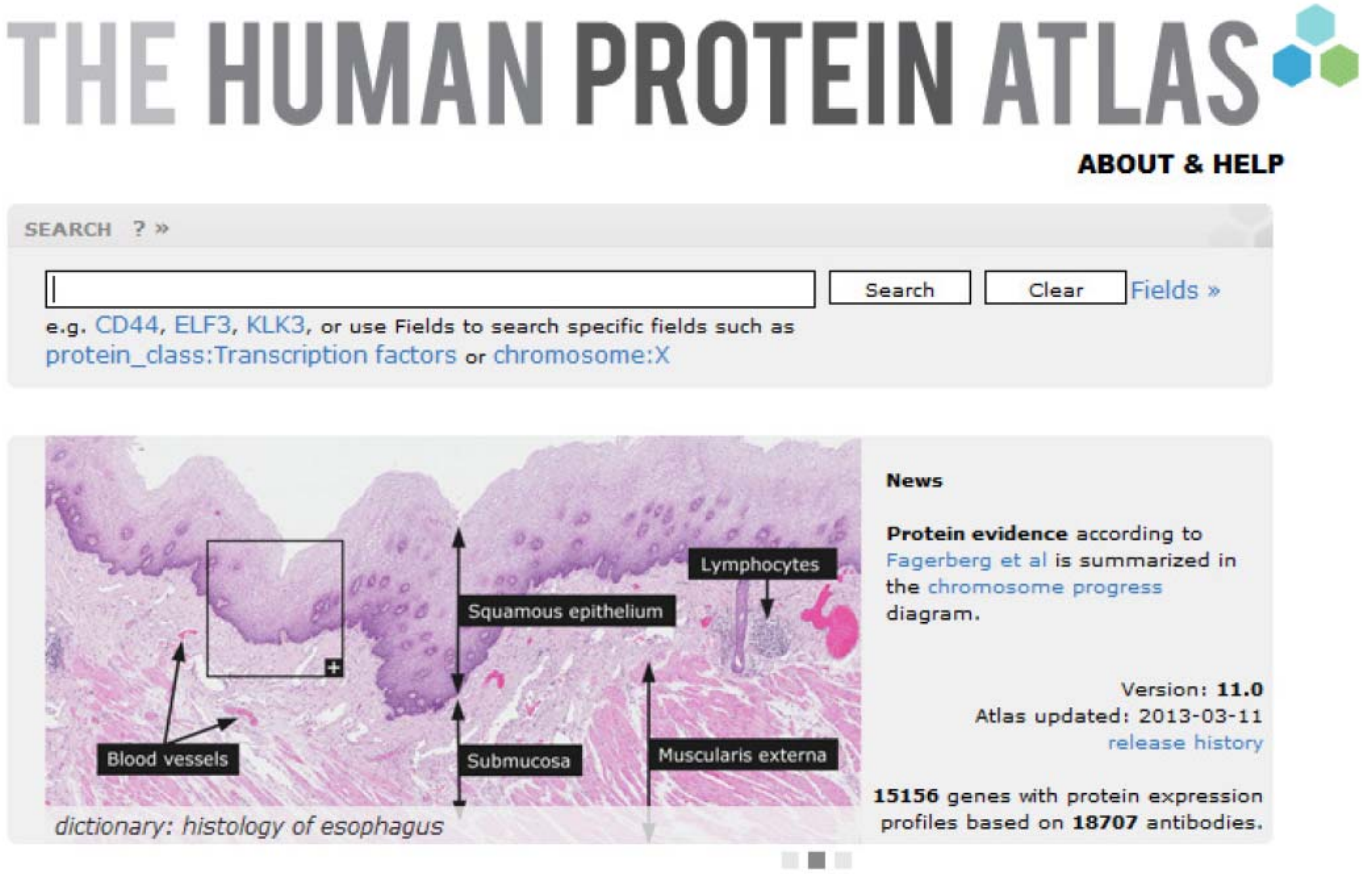
Expression Maps - Proteins

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



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

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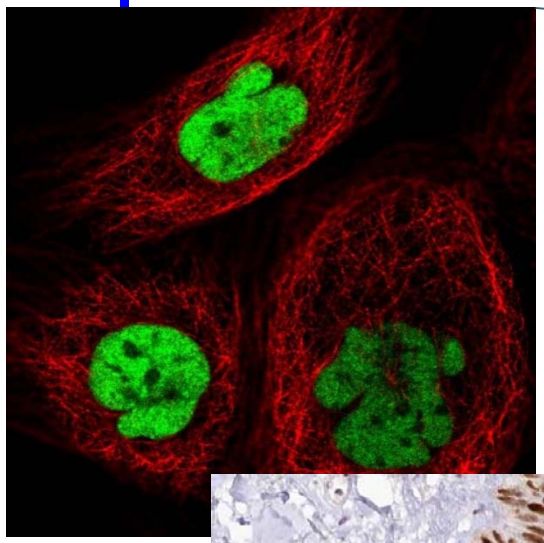
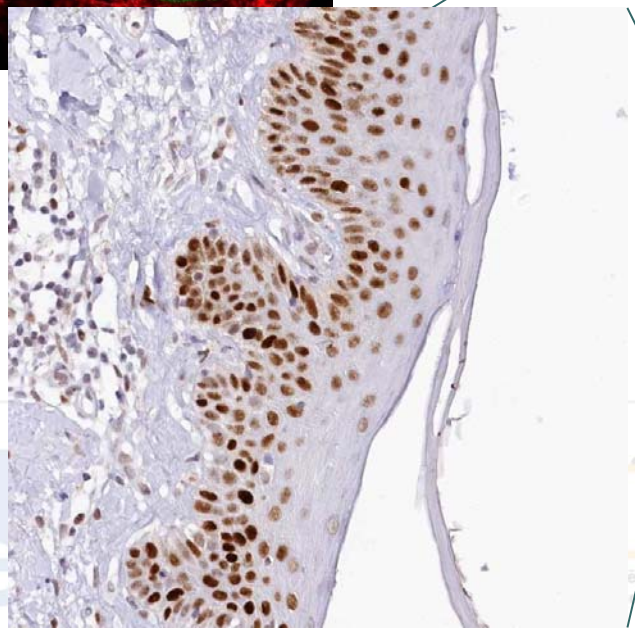
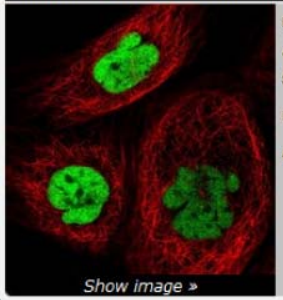




SUBCELLULAR LOCATION SUMMARY

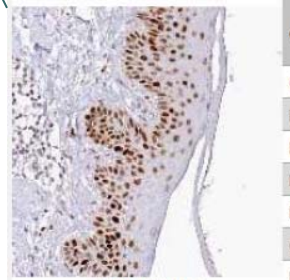


Main location(s)	Nucleus but not nucleoli
Additional location(s)	
Staining summary	Localized to the nucleus but excluded from the nucleoli.
Reliability (APE)	High
Antibodies in assay	CAB039238, CAB039239

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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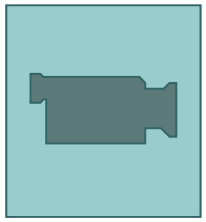
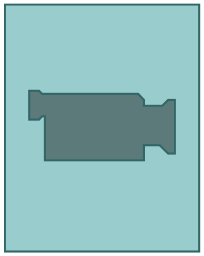
DNA Chips

- 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

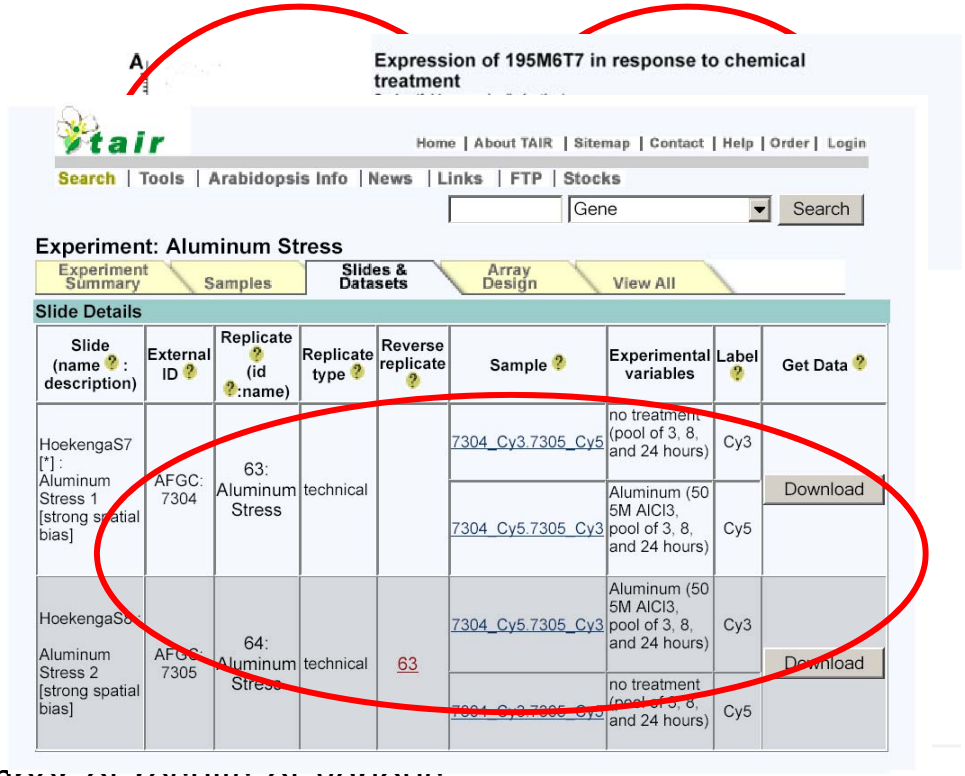
Critical Specifications	
Number of arrays	One
Number of sequence represented	>24,000 gene sequences
Feature size	18 µm
Oligonucleotide probe length	25-mer
Probe pairs/sequence	11
Control sequences	*E. coli* genes *bioB, bioC, bioD*. *B. subtilis* gene *lysA*. Phage P1 *cre* gene. Arabidopsis maintenance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

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

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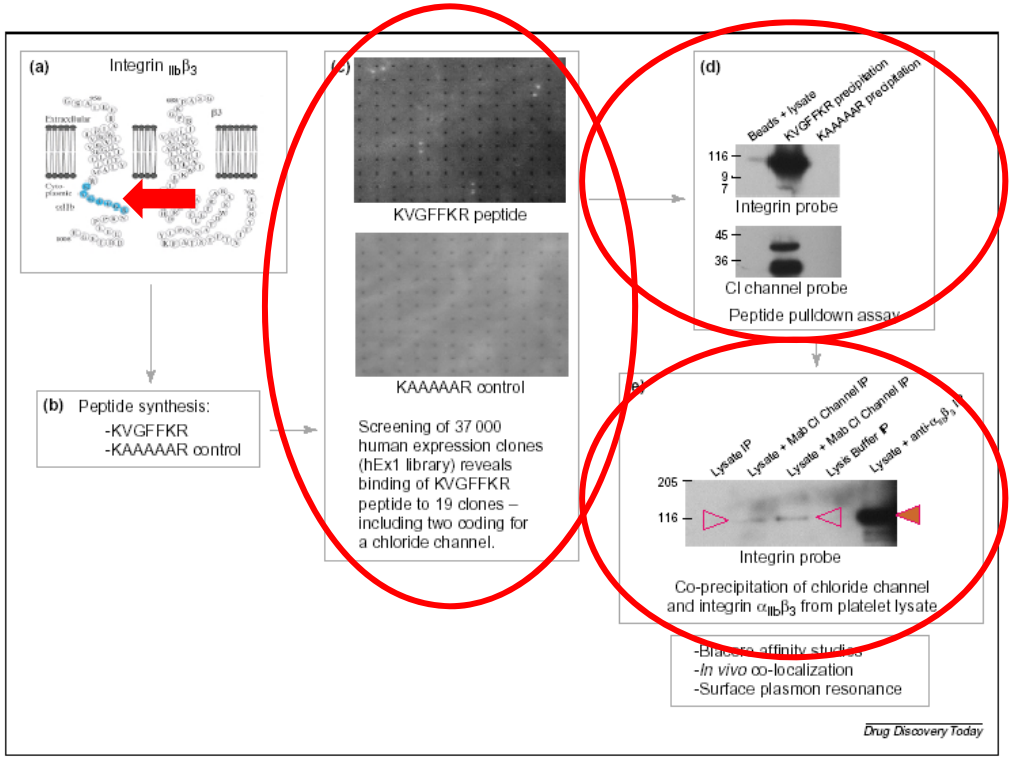
Protein Chips

- 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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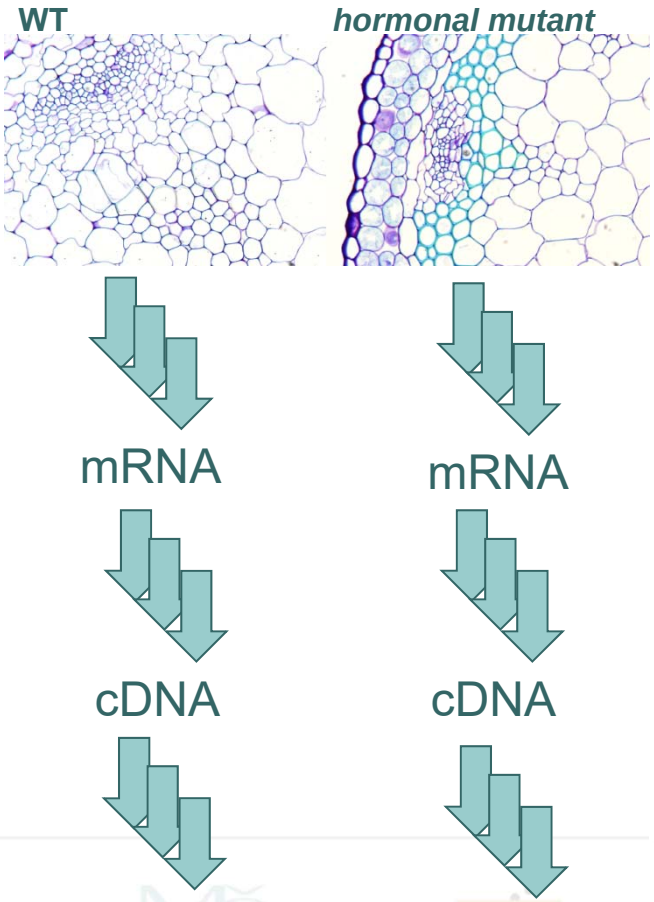
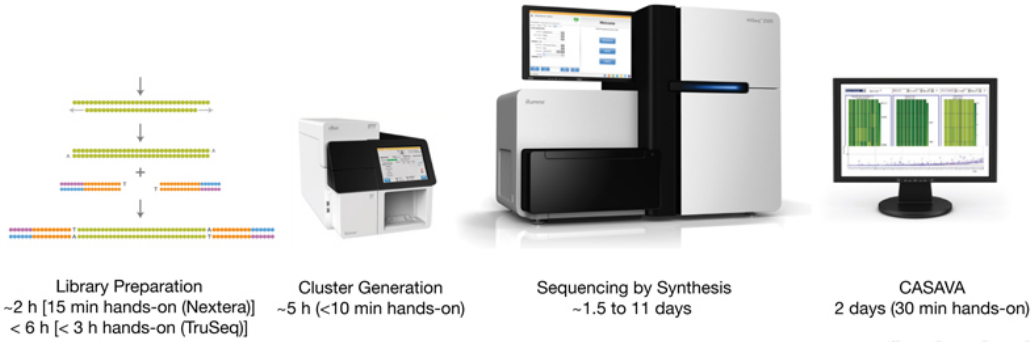
Next Gen Transcriptional Profiling

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





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

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

Outline

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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

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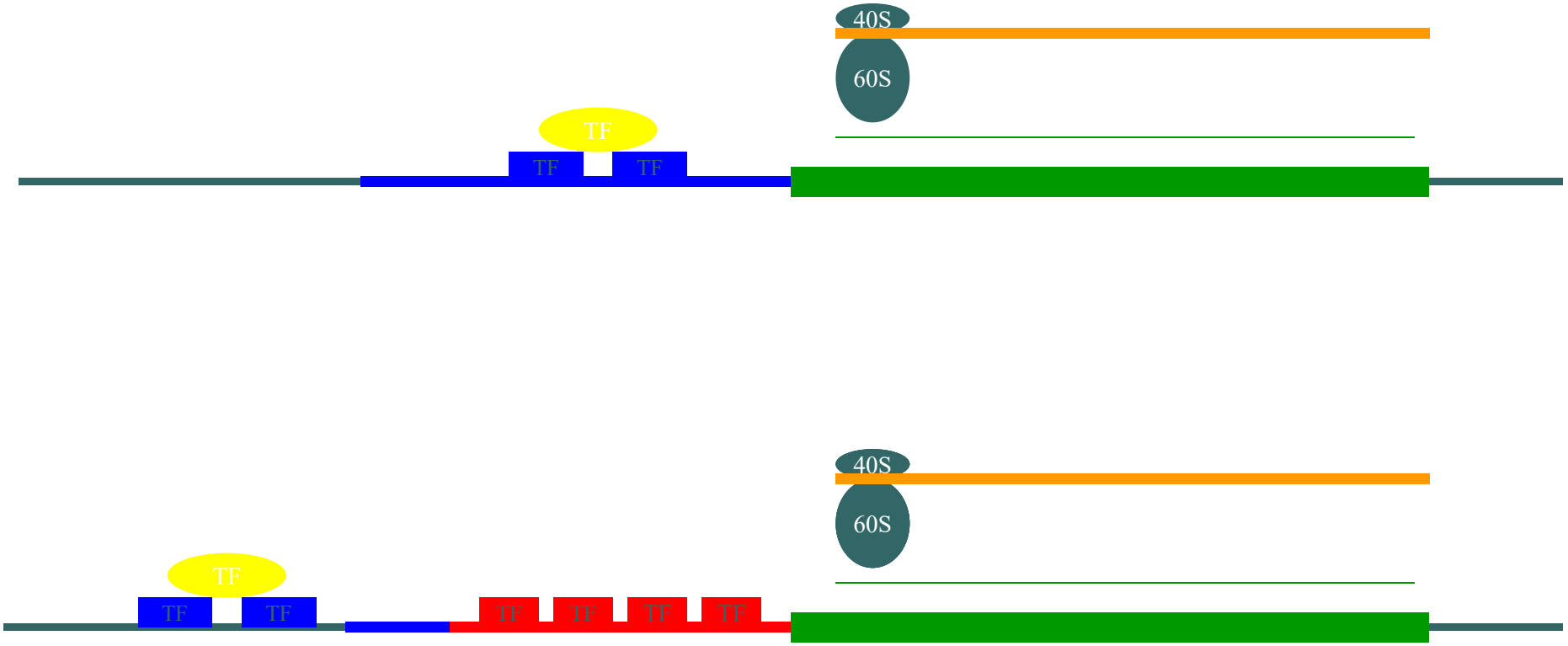
Activation Mutagenesis



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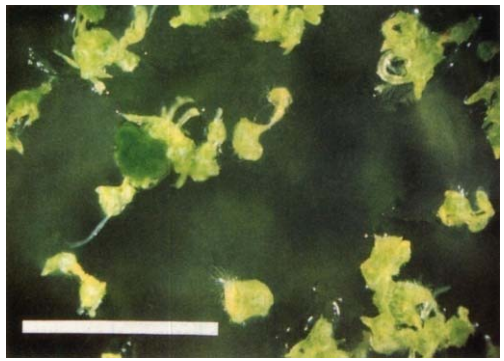
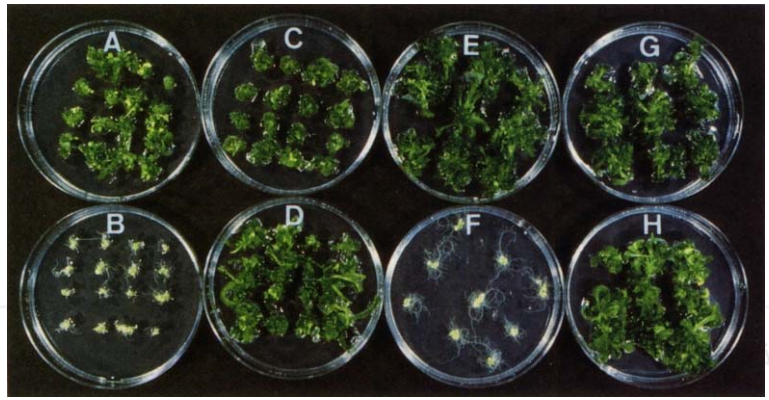
Isolation of *CKI1* Gene

- 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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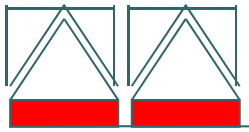
Regulated Expression Systems

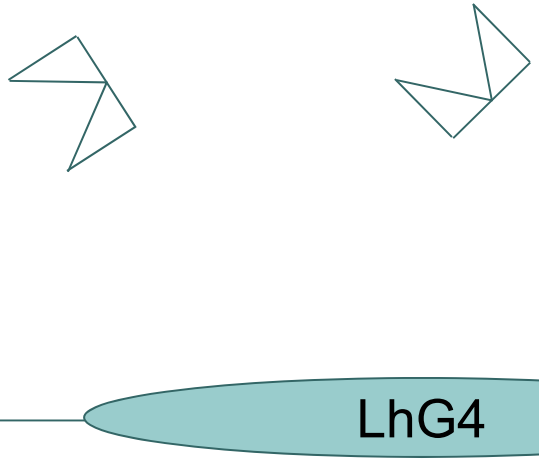
activator
x

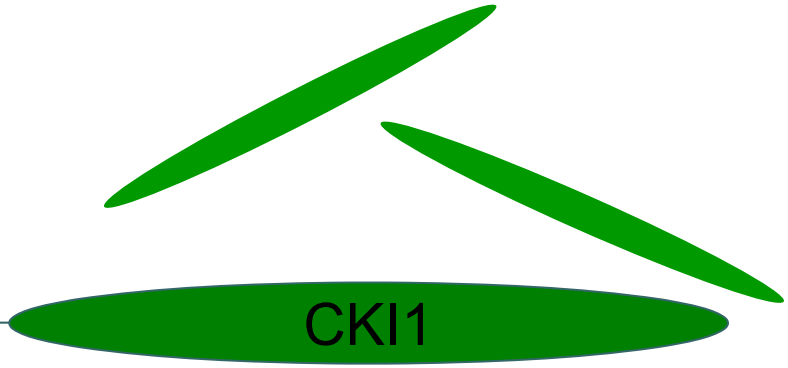
activator x reporter

reporter



pOP





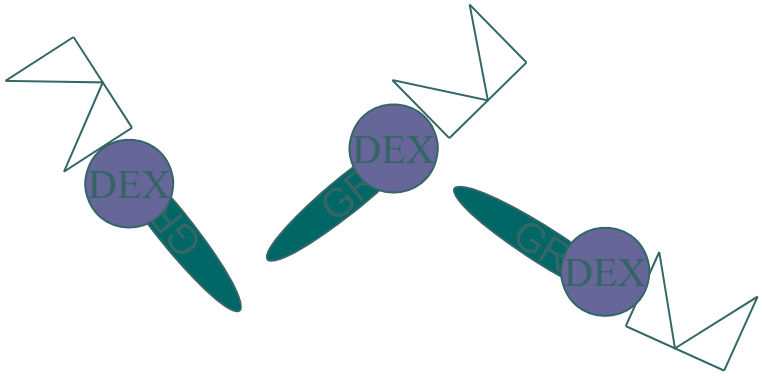
Regulated Expression Systems

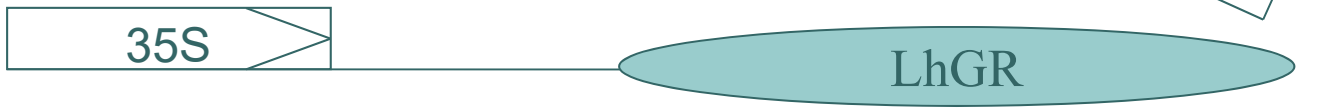
activator
x

reporter

activator x reporter

+DEX





pOP

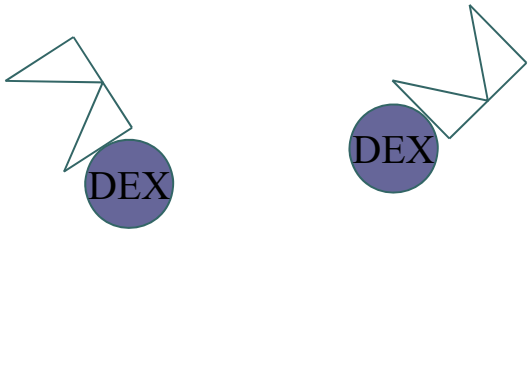
Regulated Expression Systems

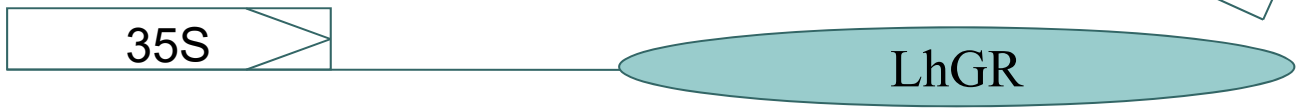
activator
X

reporter

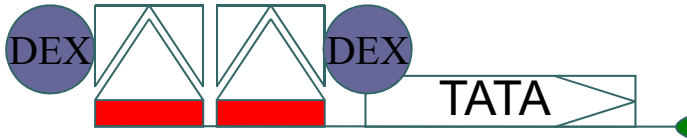
activator x reporter

+DEX

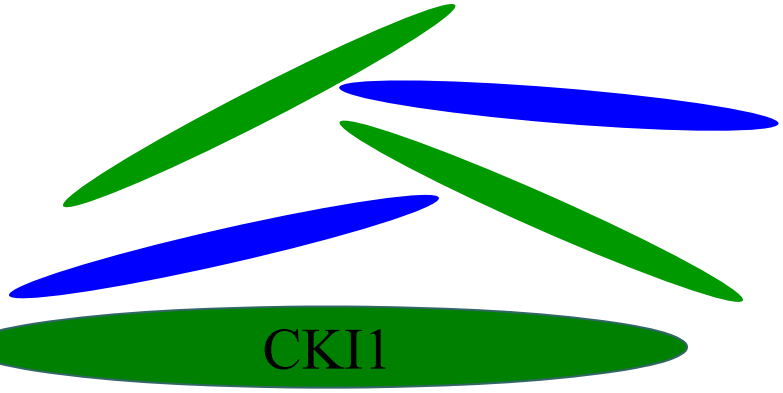




wt Col-0



pOP



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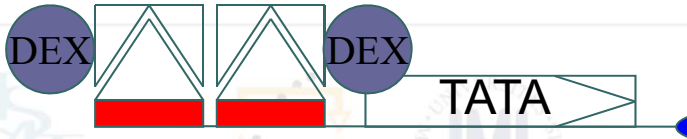
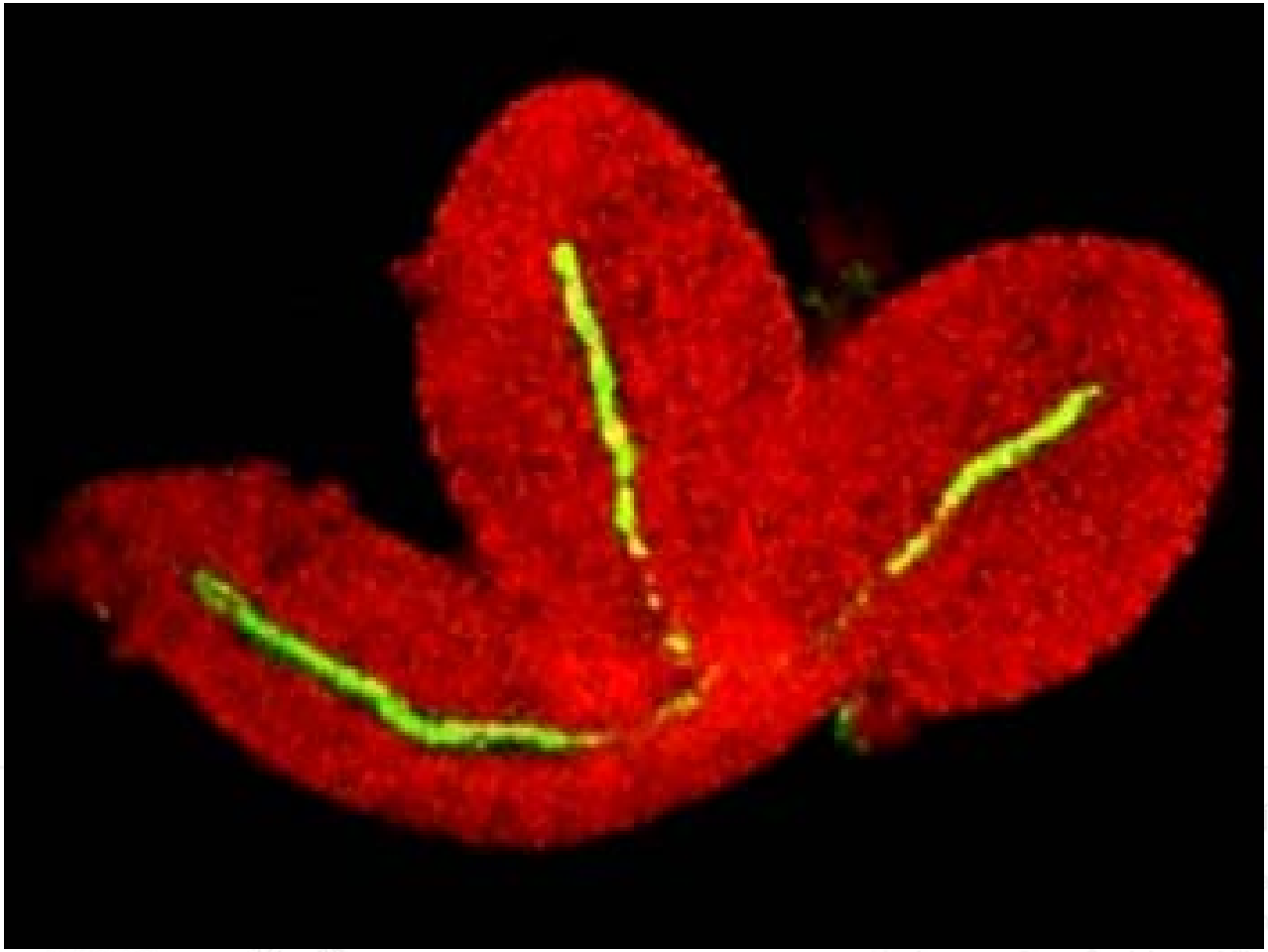


pOP

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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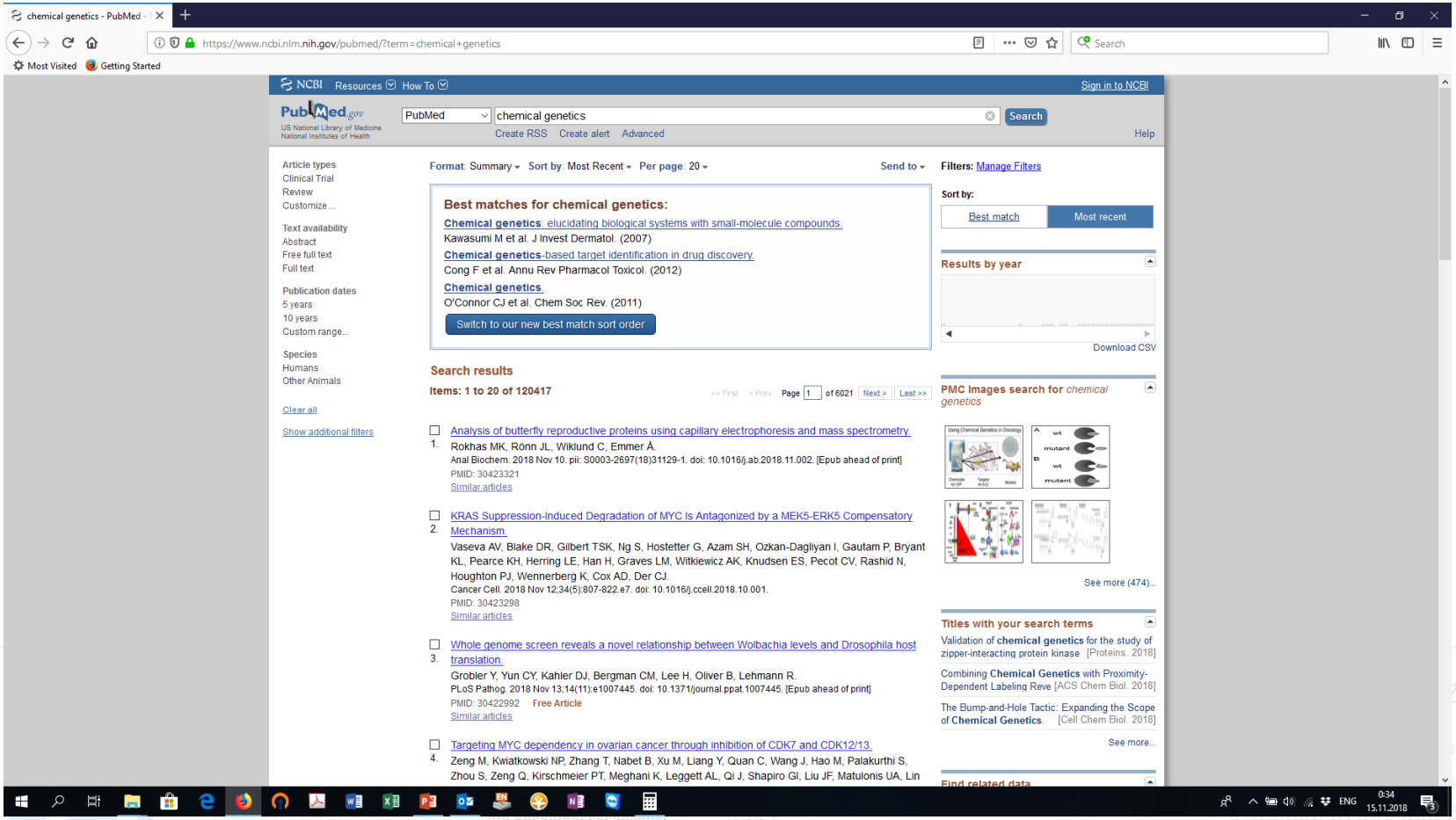
Chemical Genetics

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

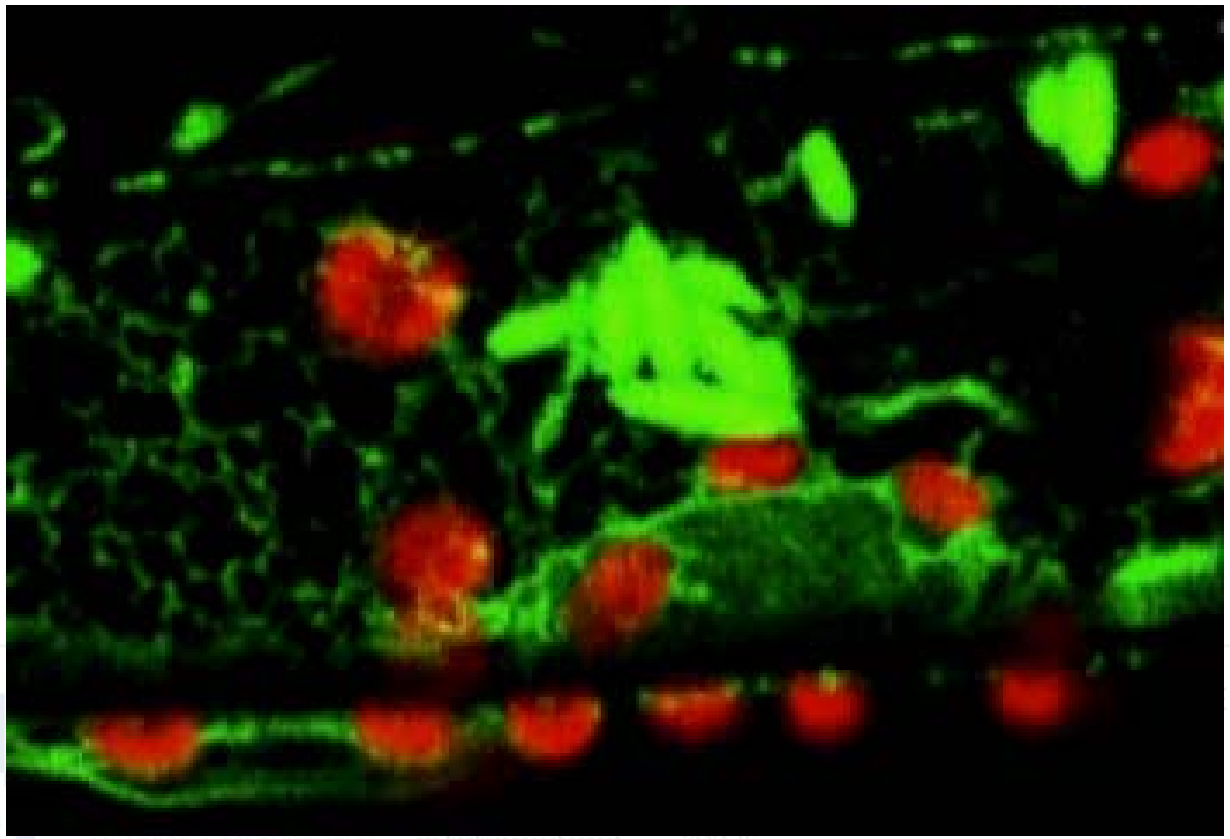


Chemical Genetics

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

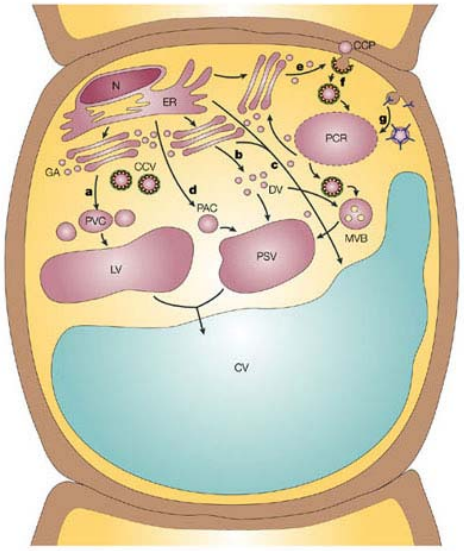
Chemical Genetics

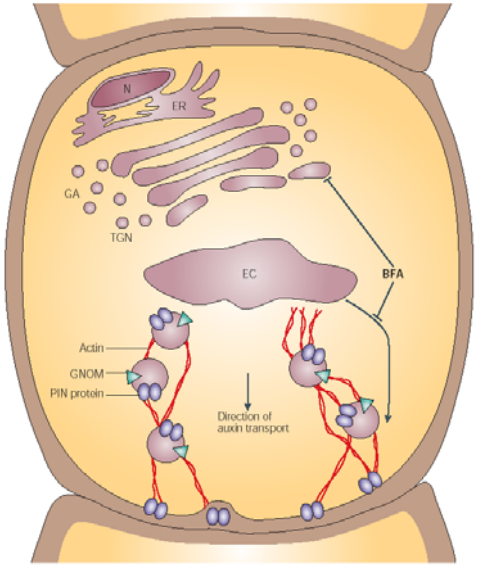
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches
  - In plants cells there occurr very dynamic processes mediated mainly by endomembrane transport (see film, GFP targeting to the ER)

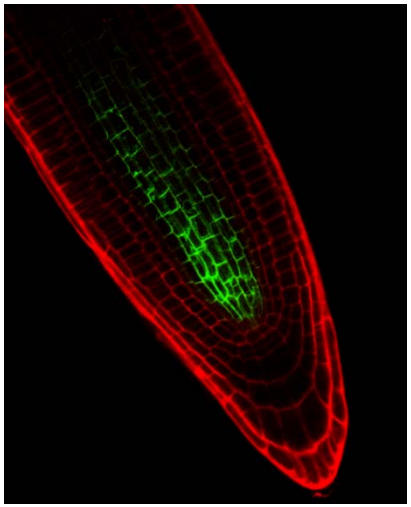


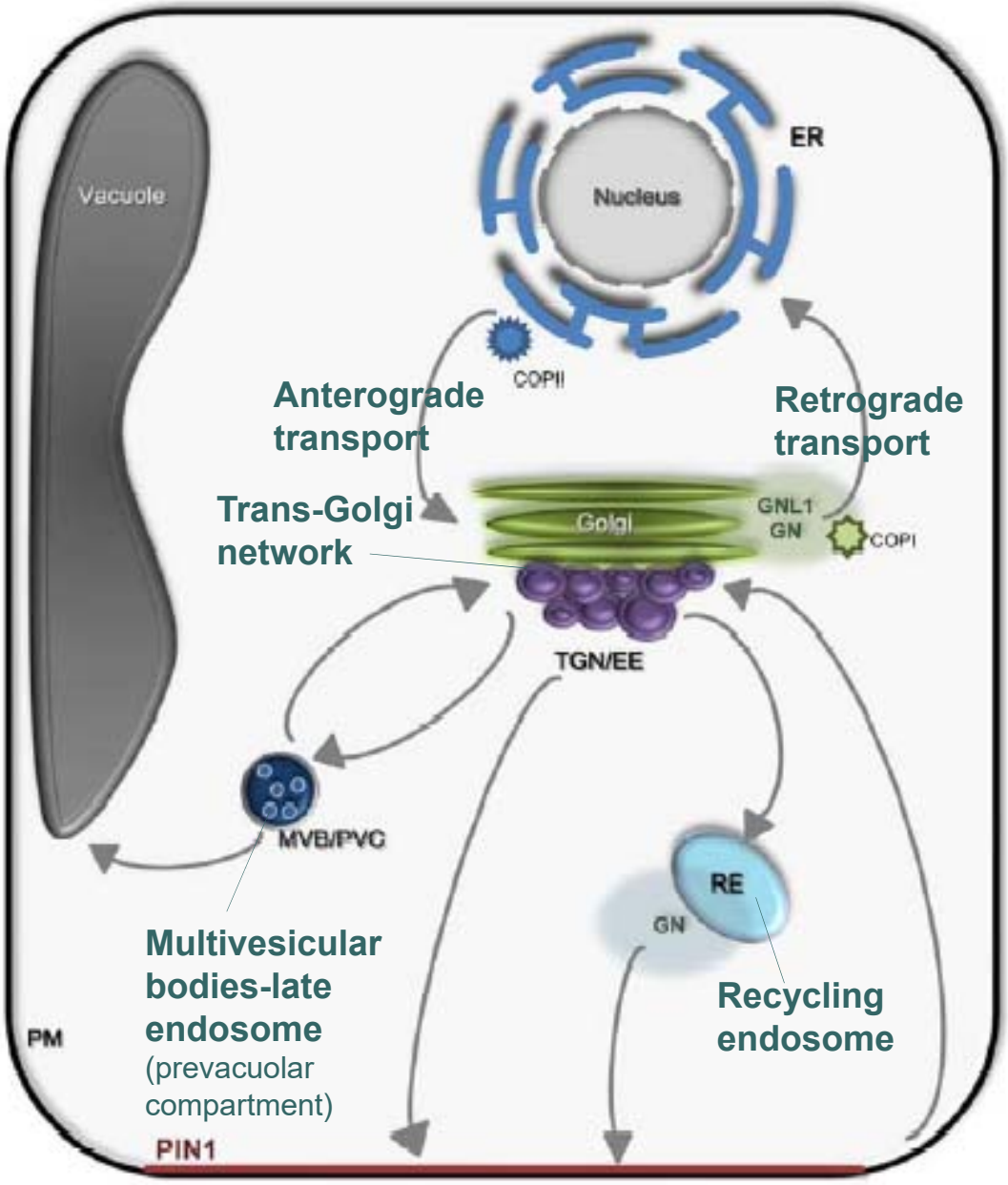
Chemical Genetics

- 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









Richter et al., *E J Cell Biol* (2010)

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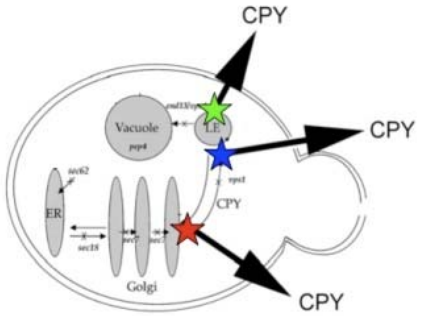
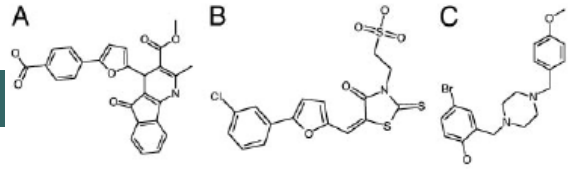
Chemical Genetics

- 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

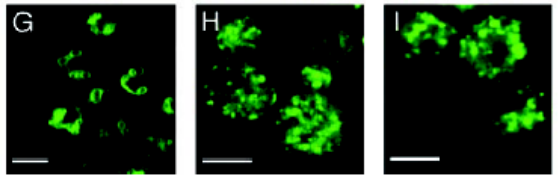


Chemical structure of sortins



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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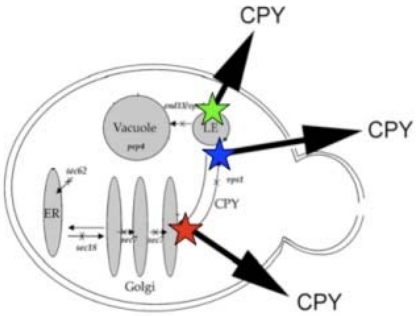
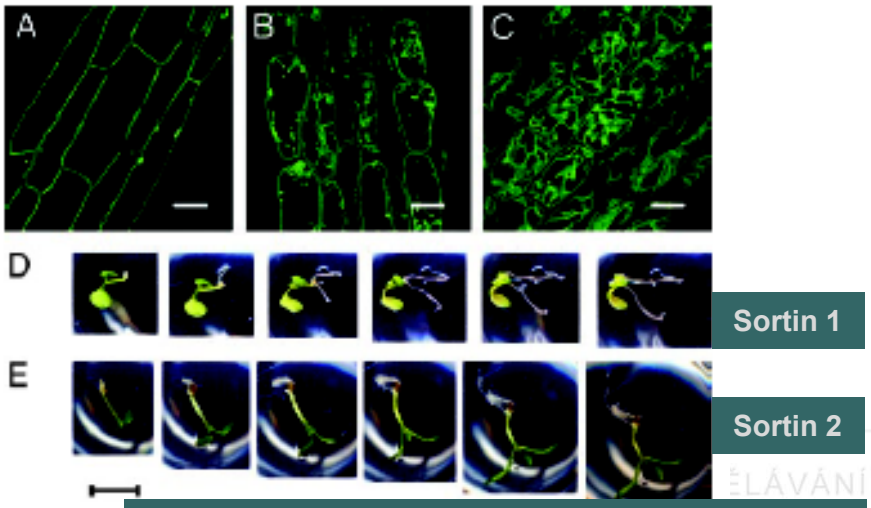
    - Analysis of changes in secretion using dot-blot and immunodetection of carboxypeptidase Y in the culture medium with monoclonal antibodies
  - Identified compounds („sortins“) were able to induce similar changes in *Arabidopsis* as well – transport mechanisms are conserved in yeast and in plants
  - 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



Shape of plant vacuoles using EGFP:-TIP



Phenotype of seedlings in the presence of sortins

Zouhar et al., 2004

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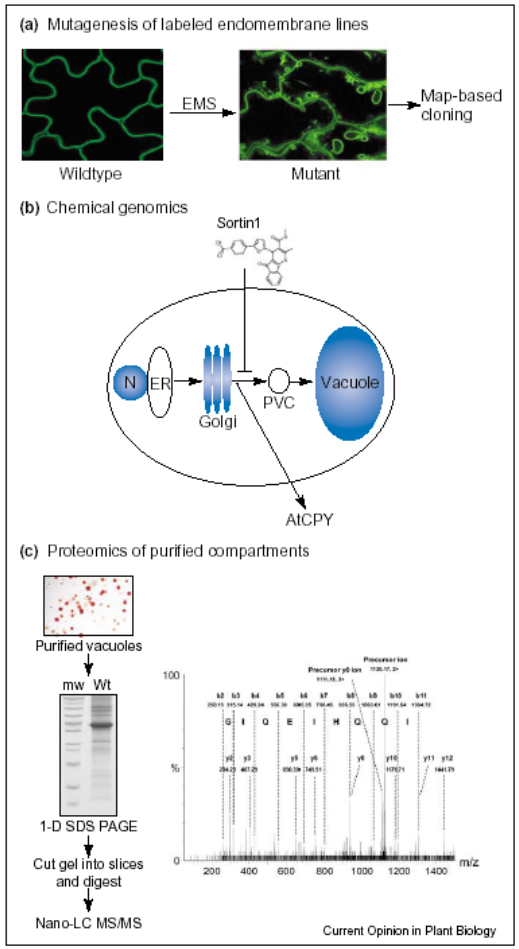
- Analysis of mechanisms of endomembrane transport by chemical genetics approaches – summary

  - 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

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