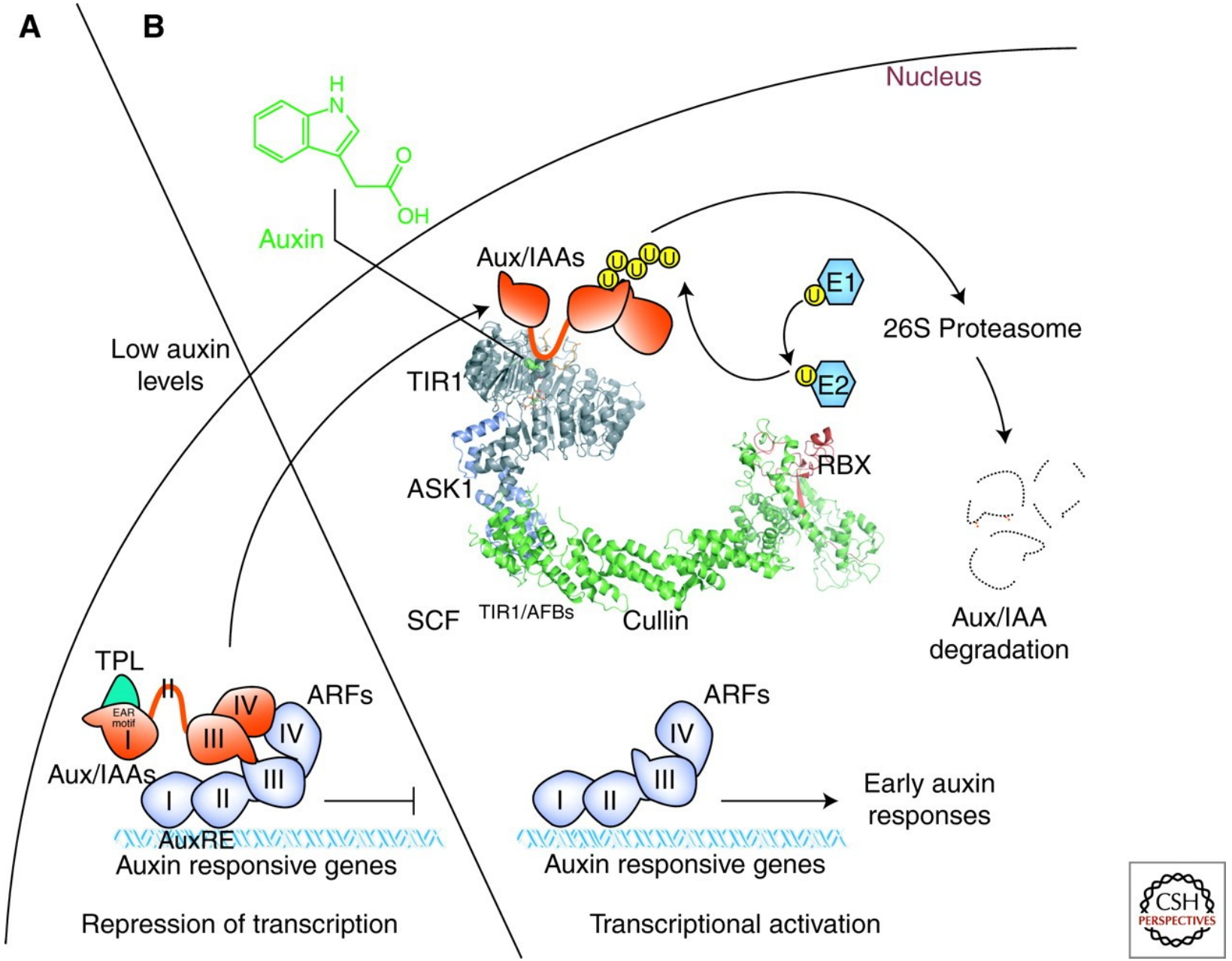
Molekulare Mechanismen der Signaltransduktion

13 – experimental approaches
and current research topics in auxin biology
http://tinyurl.com/Modul-MMS

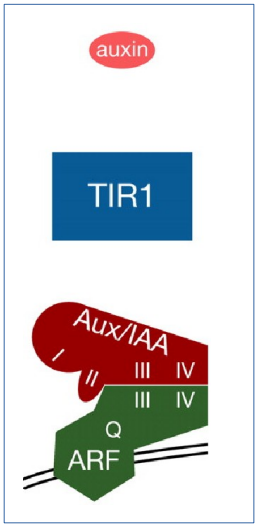
http://1.bp.blogspot.com/-9F1l8KcH52s/TdAOKUhlqSI/AAAAAAAADDA/gIycVE2Qgf8/s400/The-secret-of-experimental-.gif

auxin signal transduction model



open questions to be answered

simple and short
signal transduction



auxin

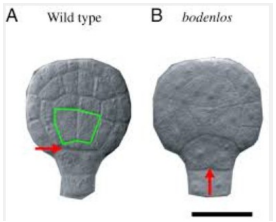
F-box protein

repressor/co-receptor

transcription factor

open questions to be answered

1. how does this simple signal transduction pathway allow the differential activation of diverse set of responses?



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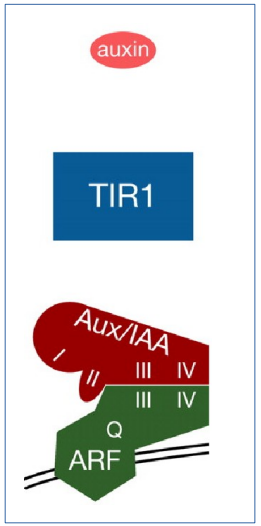

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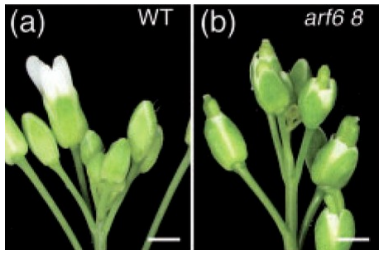

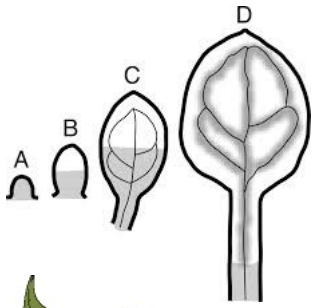

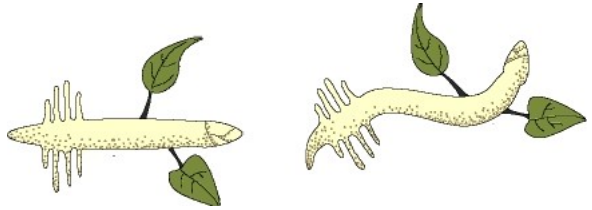

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http://d2qiws50qrj9uc.cloudfront.net/content/develop/133/23/4609/F1.large.jpg

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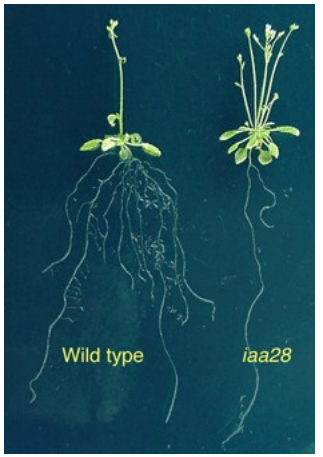

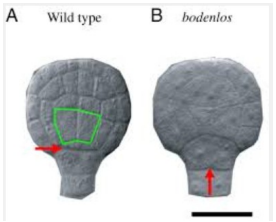

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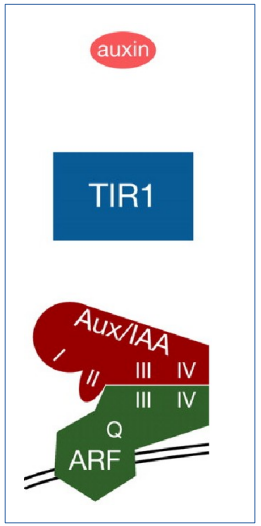
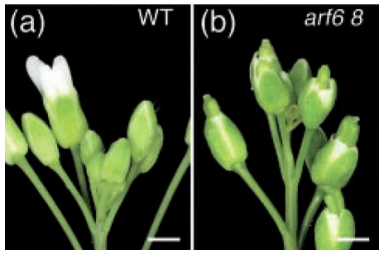
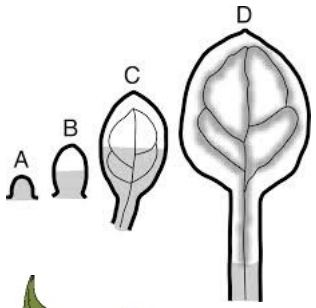
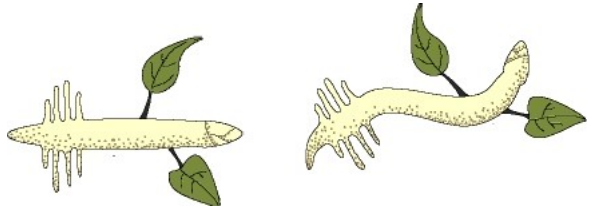


gene families







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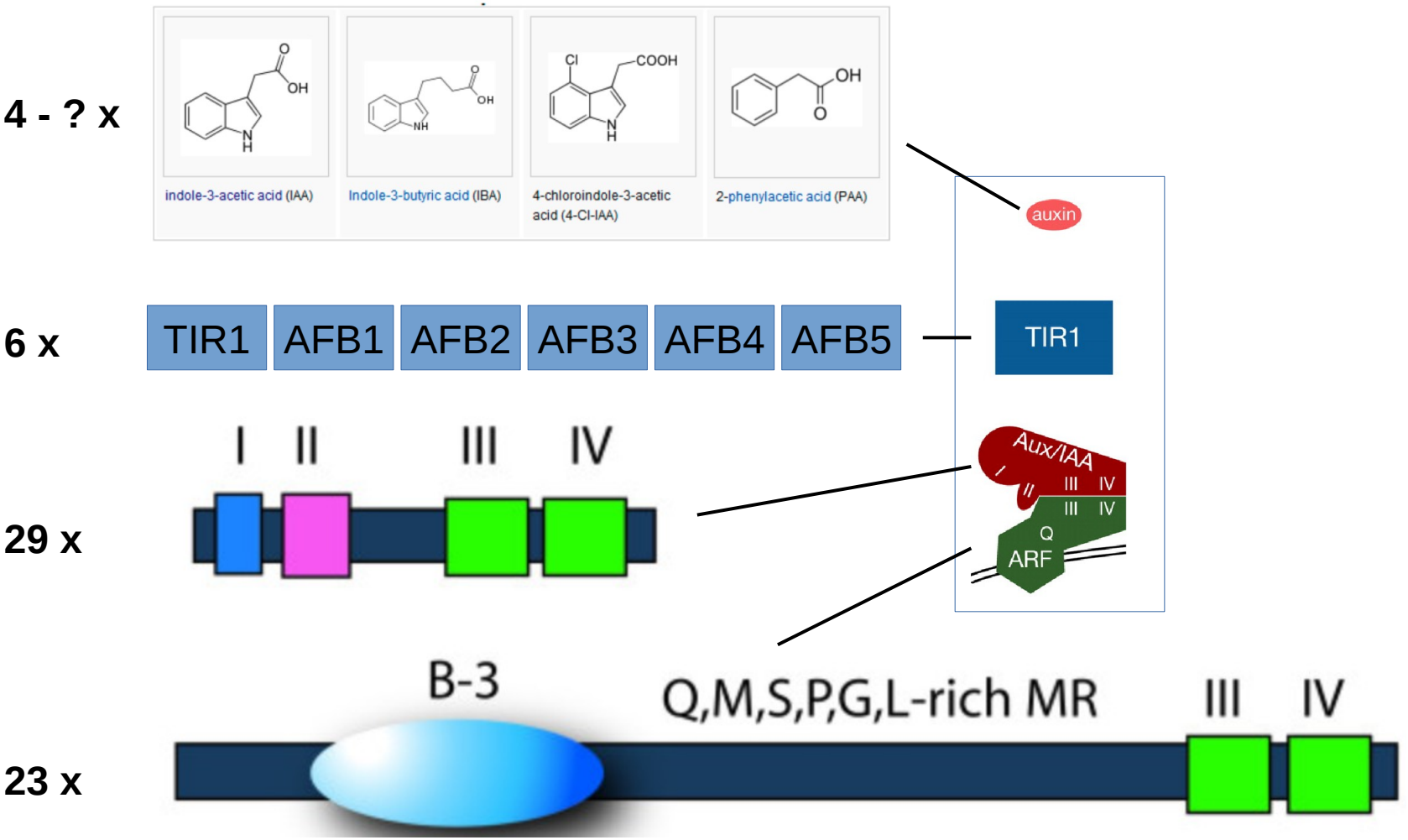

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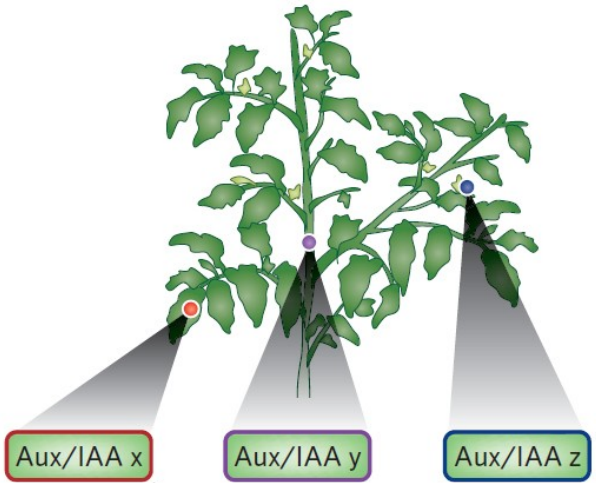
putative mechanism of differential auxin responses



Hypothesis:

differential expression of *Aux/IAA* genes (spatio-temporal)

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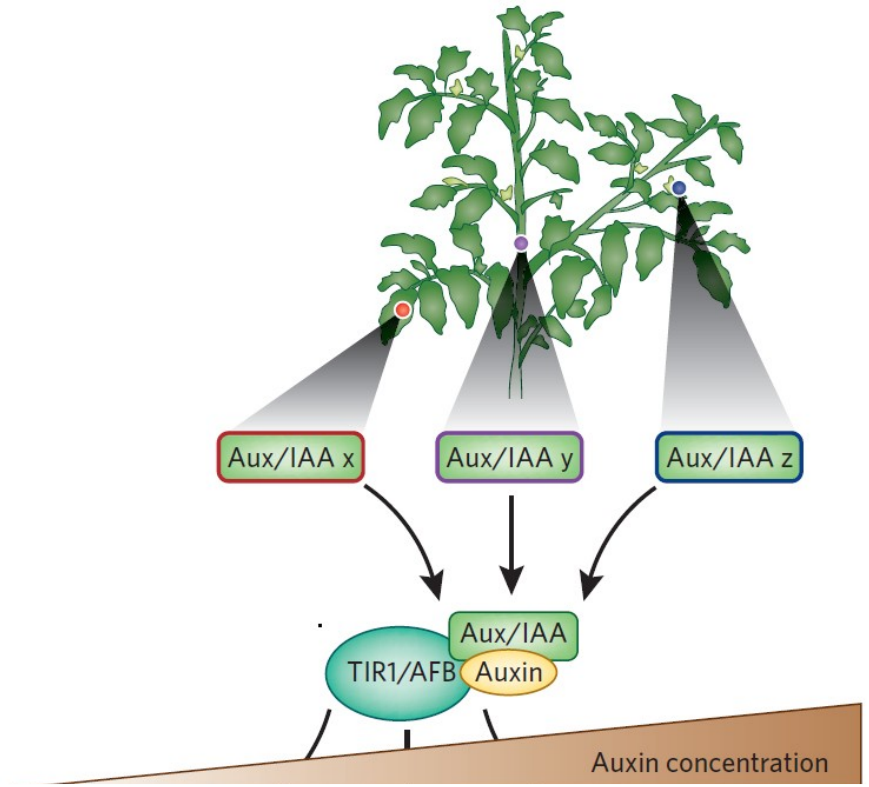

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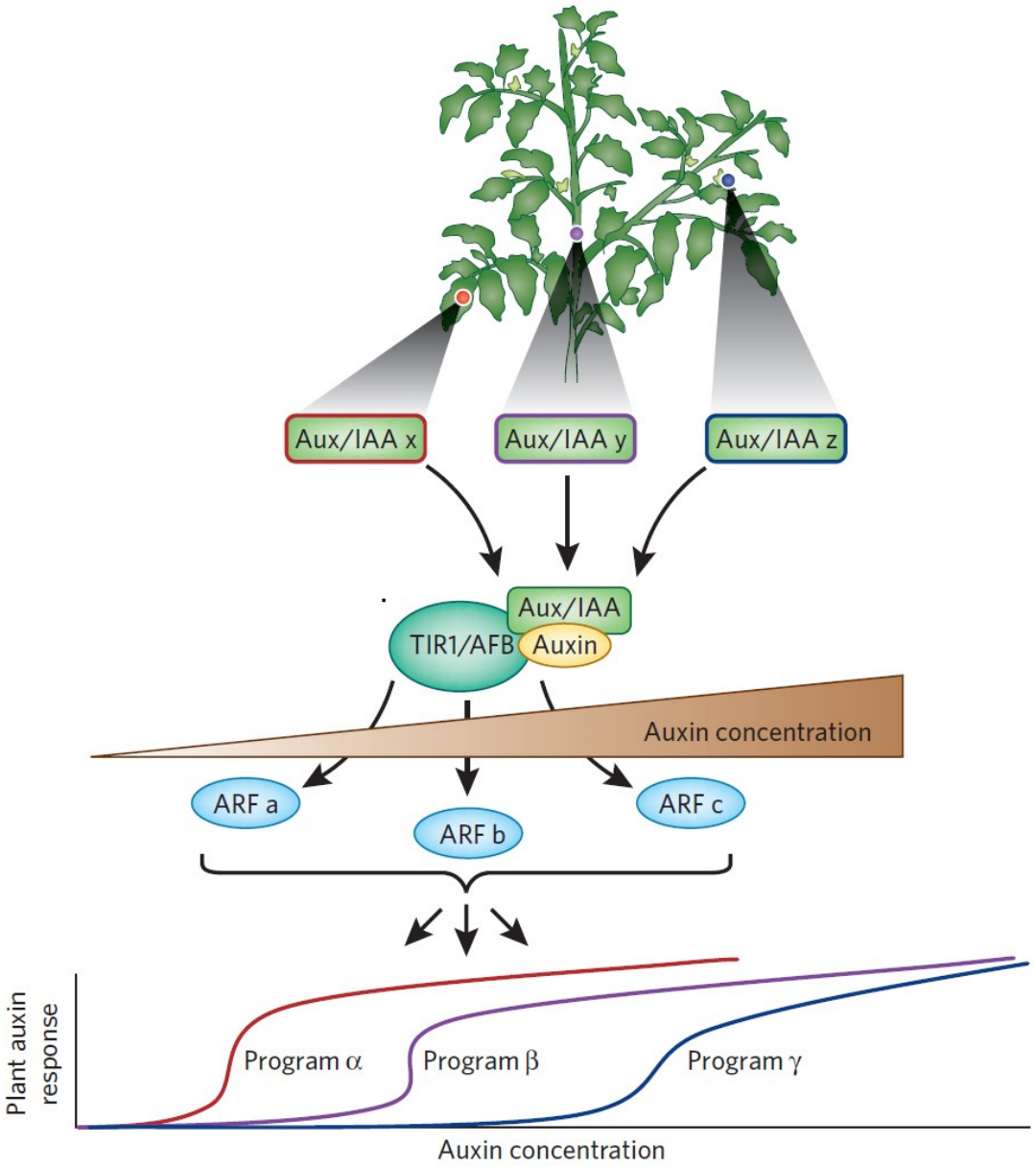

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differential presence of ARFs trigger specific downstream responses

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examples of current research topics

- TIR1 / AFBs
  - auxin binding properties
  - redundant /specific co-receptor properties of TIR1/ARF – AUX/IAA pairs
- structural properties of AUX/IAAs
  - domain structures
  - functions of domain I and linker sequences
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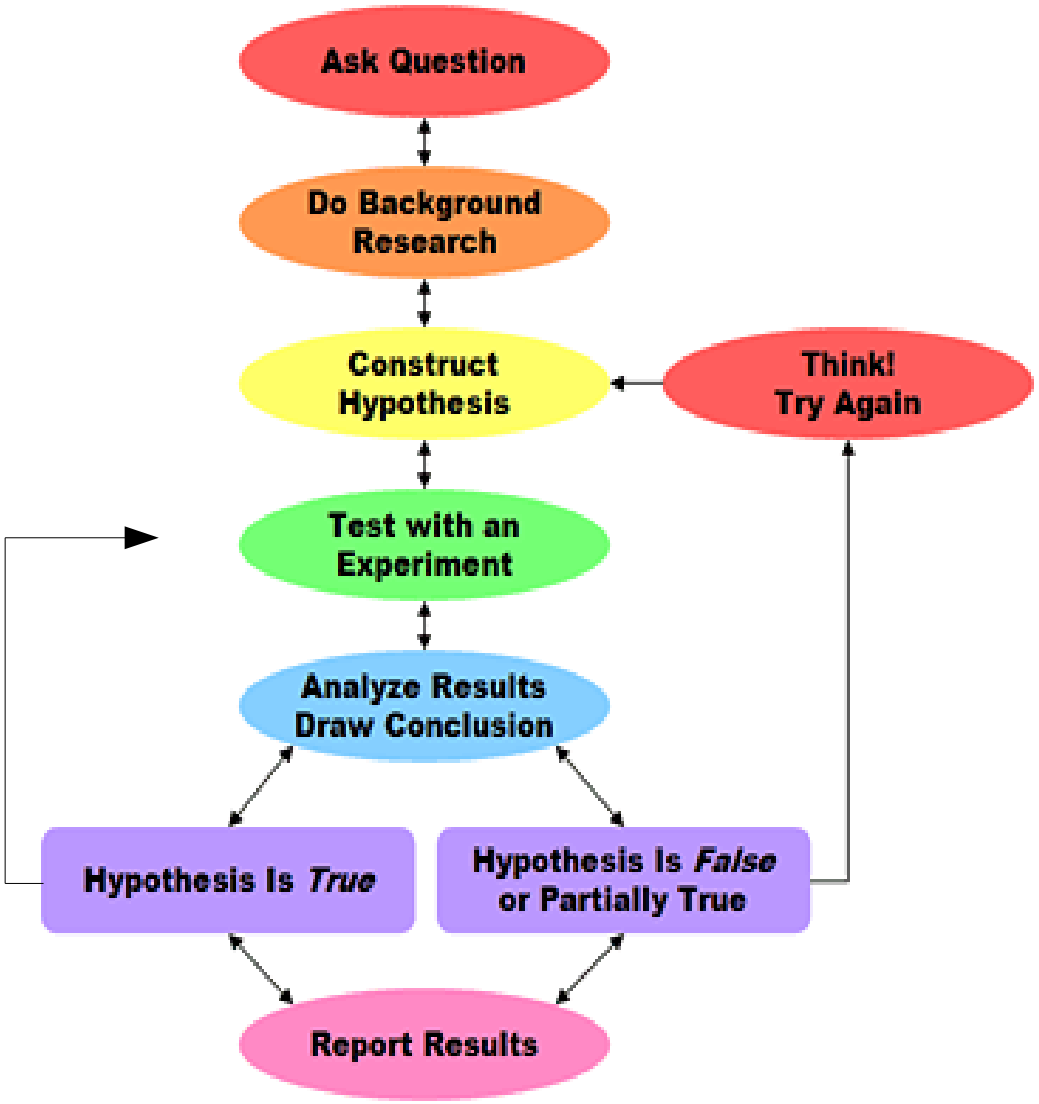
general concepts of experimental design



confirm!!
reproduce

also: proper documentation!!!! notes need to enable reproduction of experiment later

general concepts of experimental design

- think about variables that can affect the experiment
  - factors that can be controlled
  - factors that cannot be controlled (but measured?)
  - unknowns

    → choose appropriate statistics to estimate impact on variability!

- consider objectivity – if doubtful: double-blind your samples!

    → extremely important for qualitative data

- randomization of samples to counteract growth effects
- technical factors (chamber effects?) - reciprocal set-up of experiments
- controls!

    → more than one might be needed to account for different steps in the experiment

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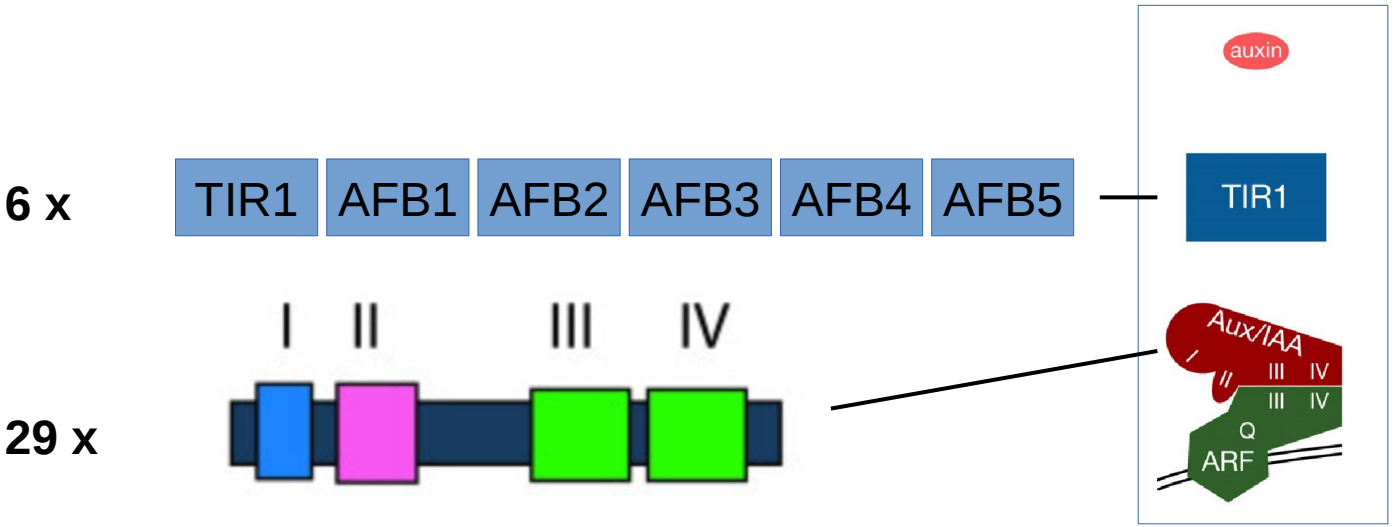

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Q1: Redundancy / specificity of co-receptors

approaches:

1. Y2H with all possible combinations

2. pull down assays

3. split-YFP/BIFC/yellow cameleon

4. genetic interactions – combination of gain- and loss-of-function mutants

others:

- expression analysis of tir1, afb1, afb2… mutants – in response to auxin, different tissues…
- radioactive ligand binding + competition experiments → determine binding specificities
- ……

What are the necessary factors to consider + controls to test?

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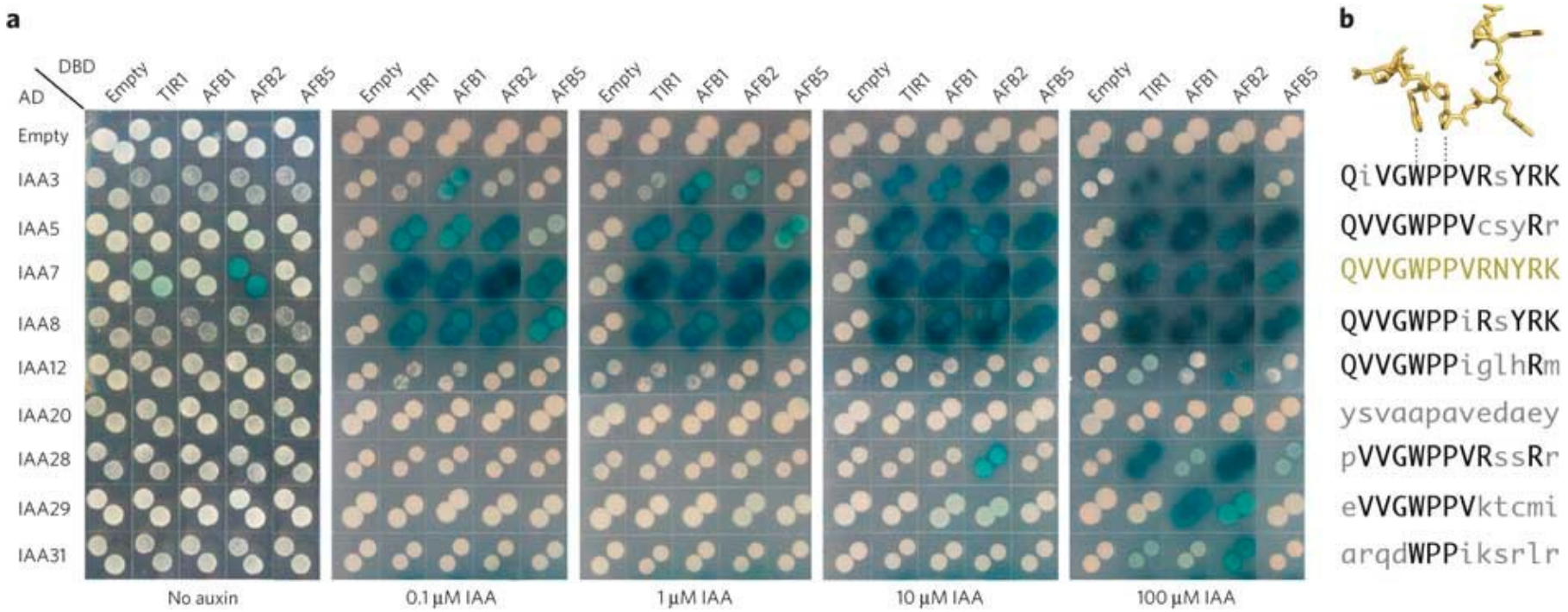

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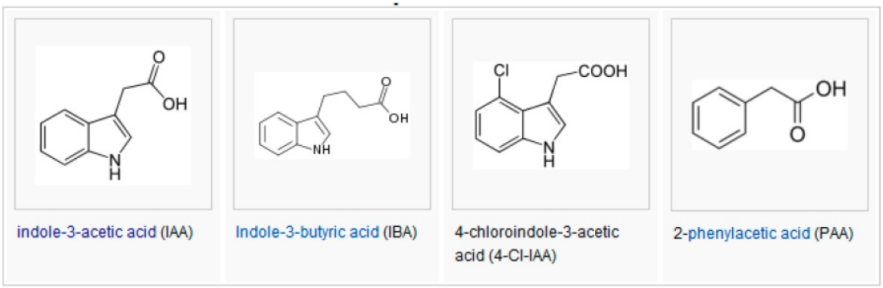
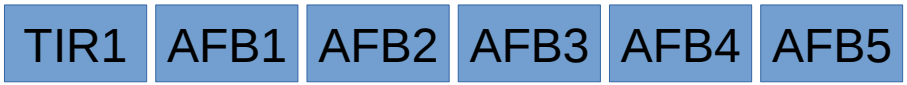
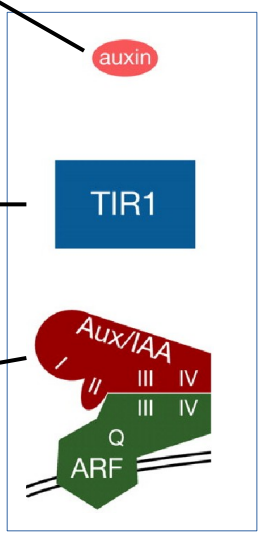
4 - ? x



6 x



29 x



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differential sensing?

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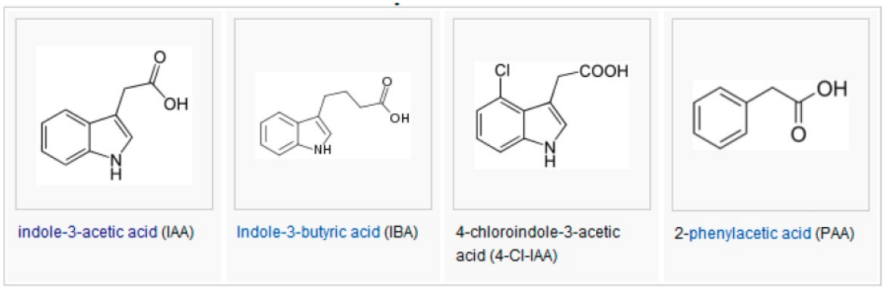

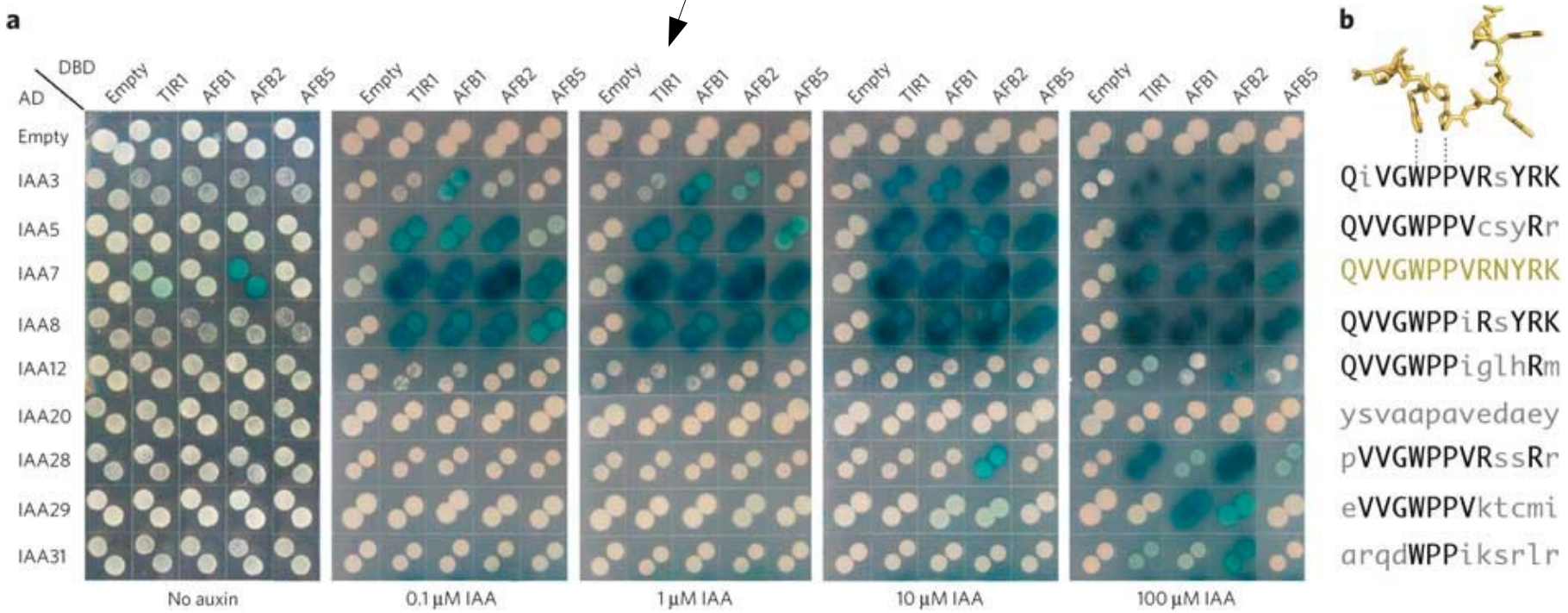

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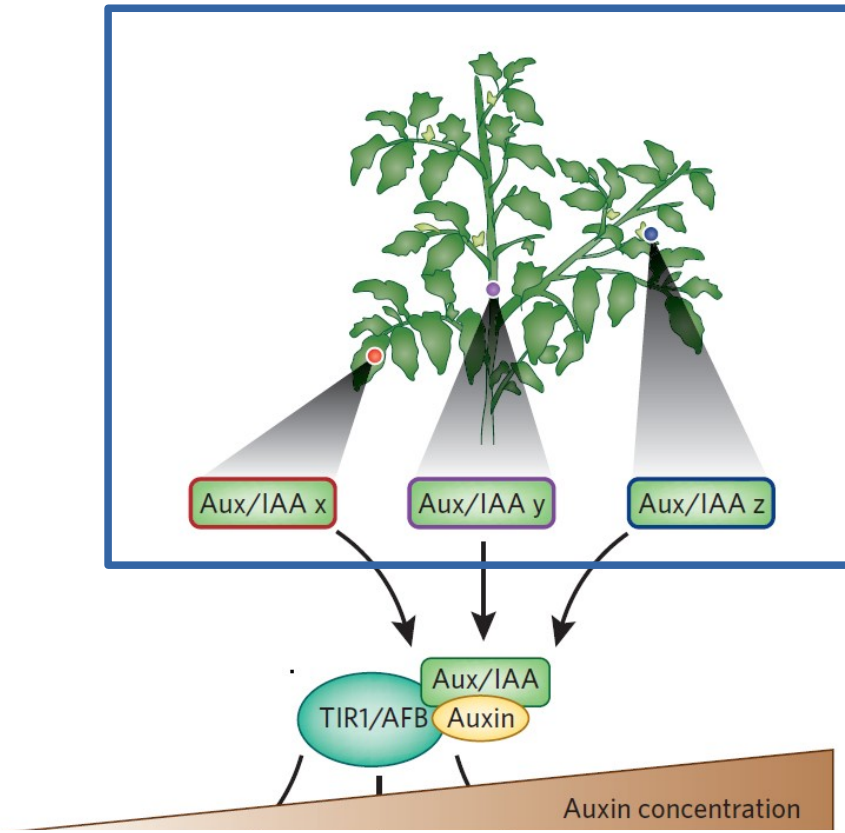

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putative mechanism of differential auxin responses

gene
expression profiles

- qRT-PCR of individual genes
- analysis of whole genome data (microarray, RNAseq)

protein
presence/levels

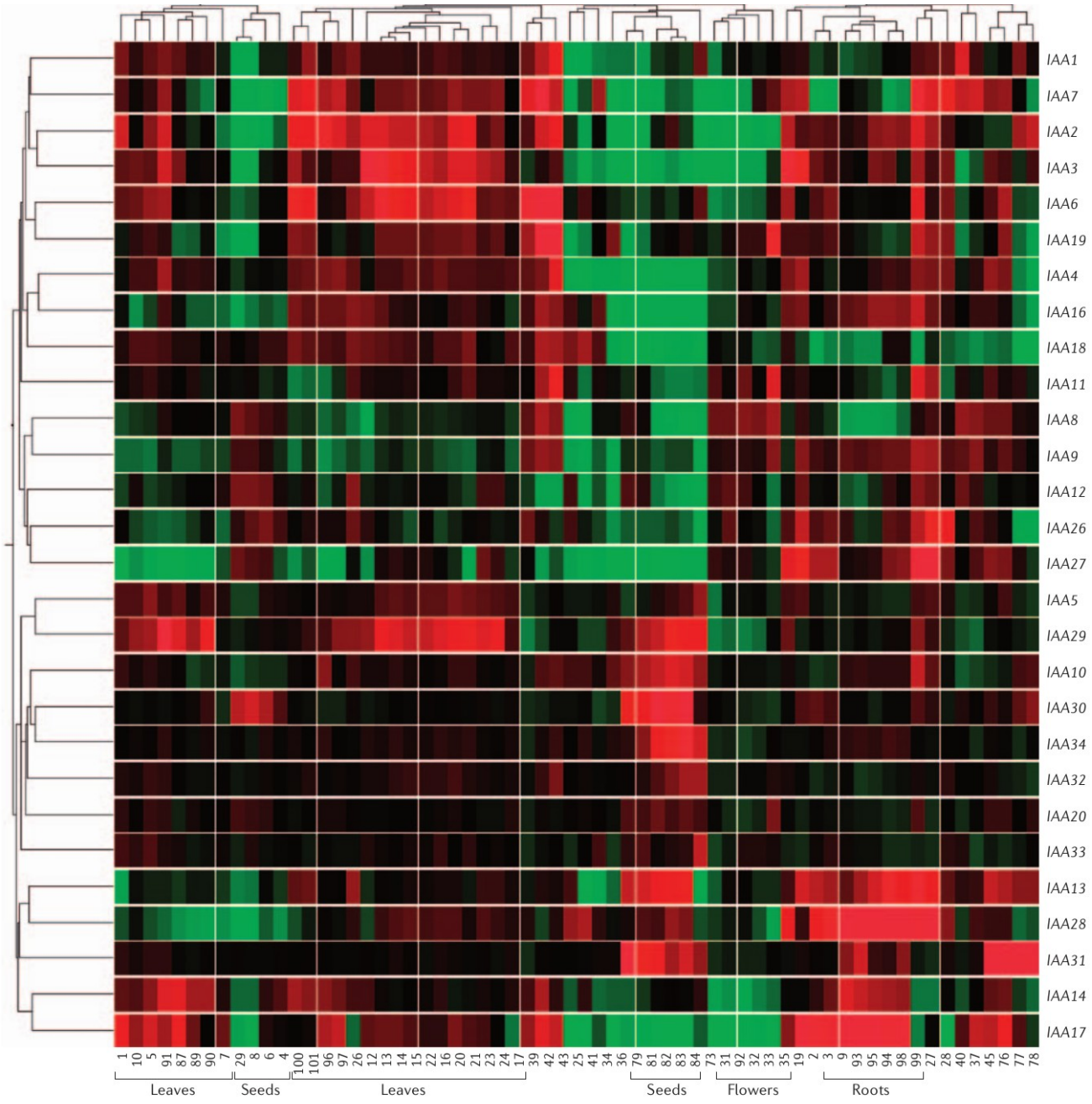
- Western blot of tissue extracts
- antibodies?
- tagged constructs

mutant/OEx
phenotypes

- phenotypic analysis of loss- or gain-of-function lines

how to determine spatio-temporal specificities of AUX/IAAs?

redundancy / specificity of AUX/IAAs



→ cluster analysis

microarry expression data from different experiments were analyzed together

identification of potentially redundant Aux/IAAs

red: high expression
green low expression

Teale et al. 2006

examples of current research topics

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AUX/IAA domains

Question: which domains/linkers are relevant for AUX/IAA specificity in co-receptor function?

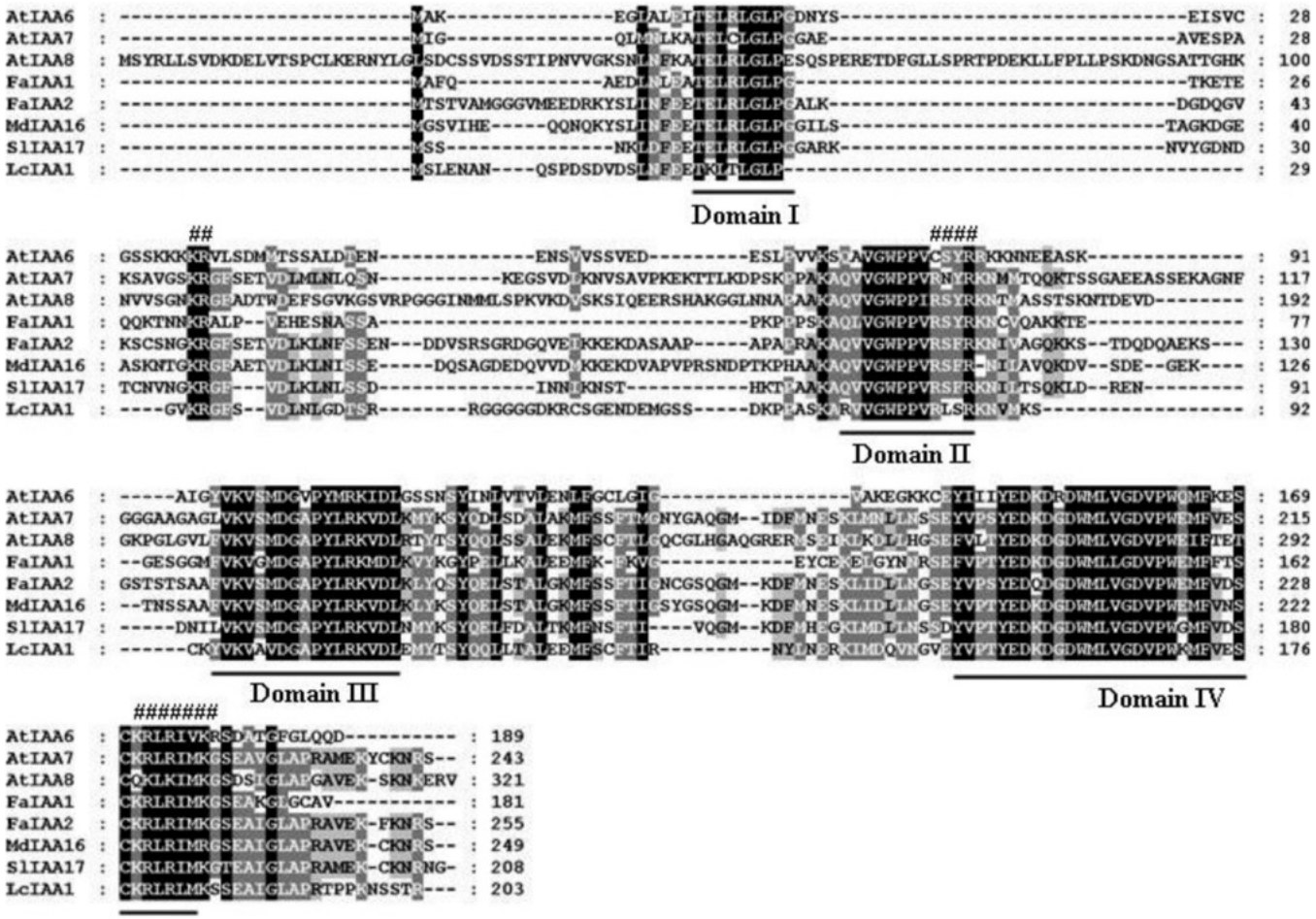
AUX/IAA domains

Question: which other domains contribute to AUX/IAA stability and which sequences determine specificity of co-receptor activity?

- truncate proteins
- site-directed mutagenesis
  sequence alignments → determine “interesting” amino acids
- domain/sequence swaps → chimeric proteins

crystallization and structure elucidation

identification of conserved and variable sequences



→ site-directed mutagenesis, sequence swaps, etc

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redundancy / specificitiy of ARFs

approaches:

1. partial swaps of domains → identification of variable sequence streches via alignment

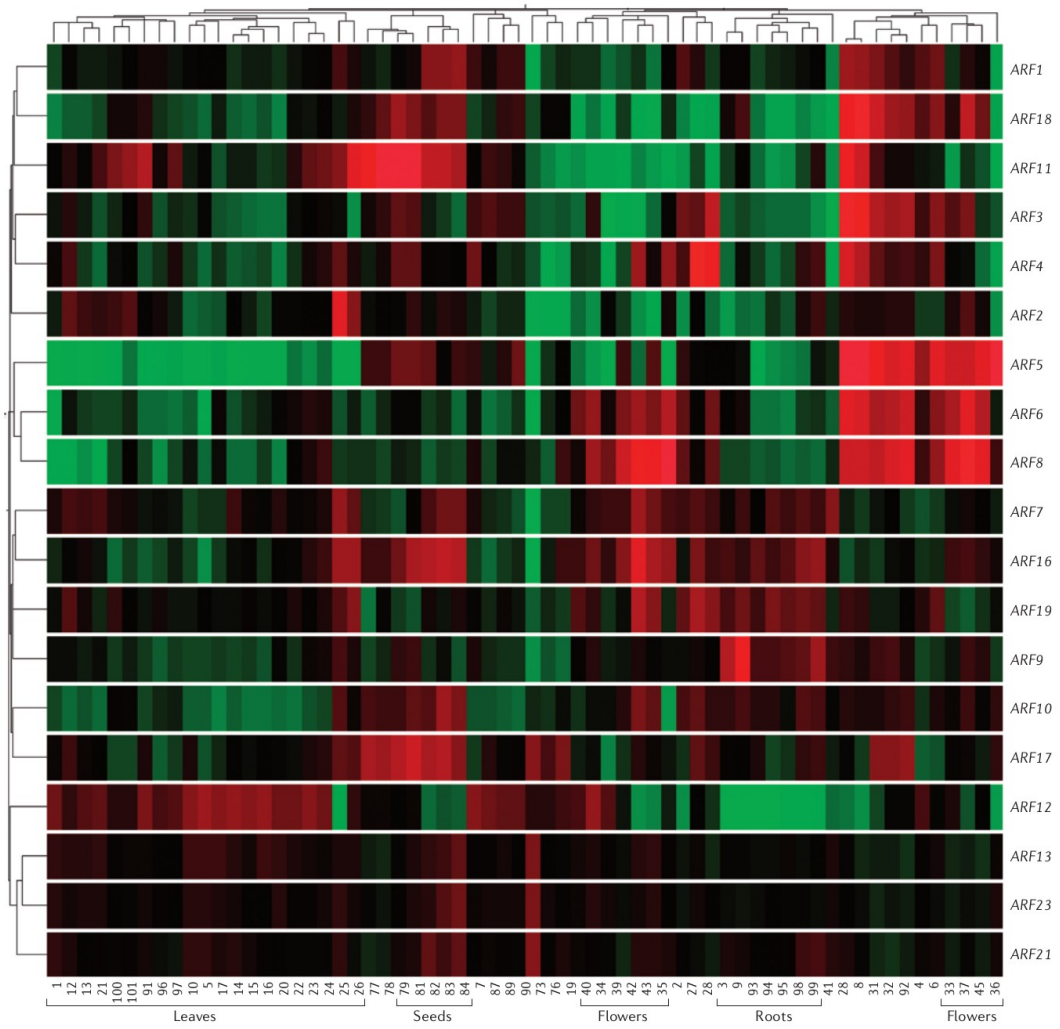
2. K.O mutants / OX lines → gene expression profiles (RNAseq, microarrays) + phenotypic characterization of single or higher order mutants

3. CHIP-Seq analysis → identification of direct target genes

4. interaction studies with AUX/IAAs

5. expression analysis of ARF gene family

redundancy / specificitiy of ARFs



partially redundant / partially specific → different ARF combinations in different tissues (and/or timepoints)

→ identification of pairs/groups for generation of higher order mutants