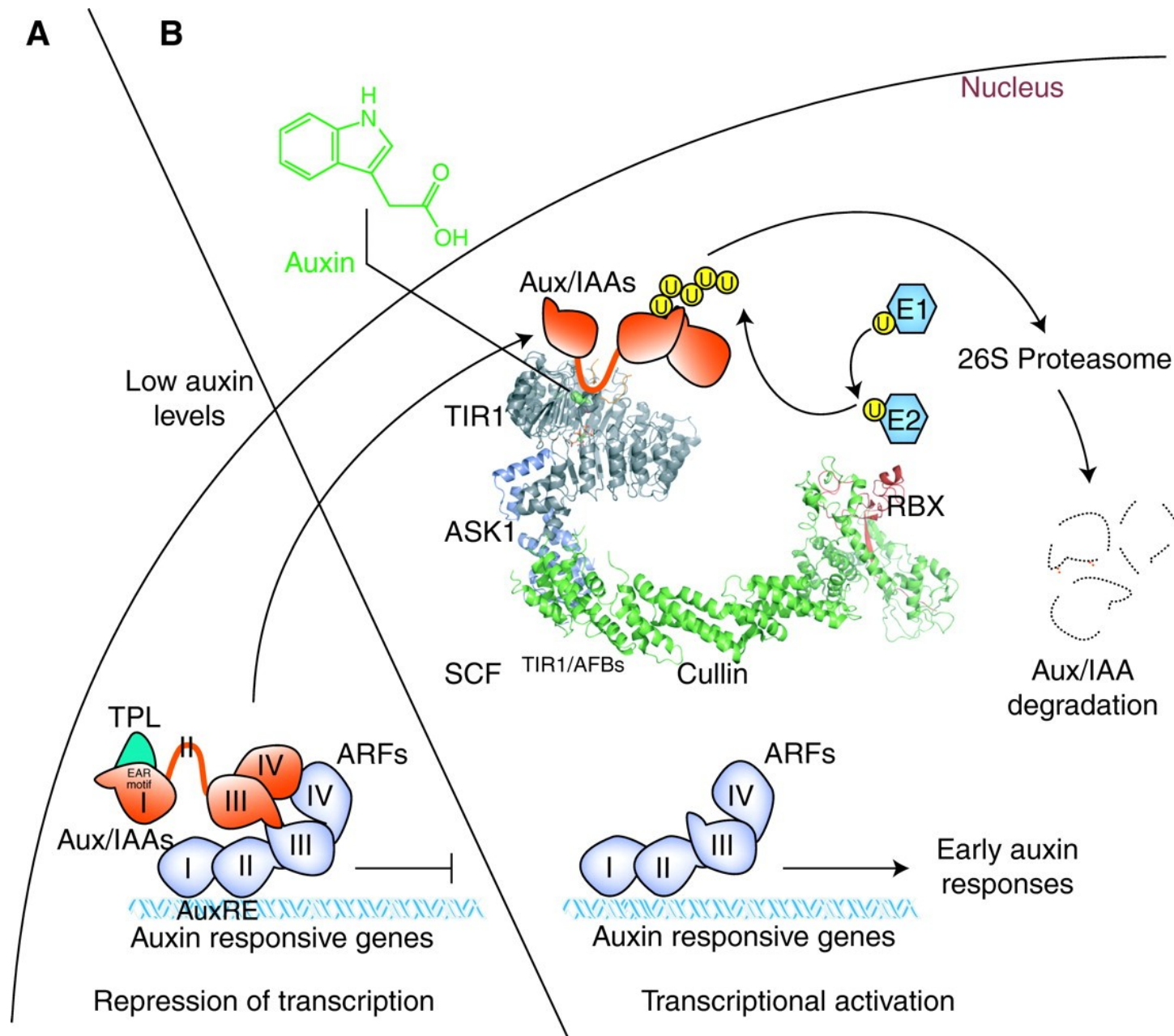


Molekulare Mechanismen der Signaltransduktion



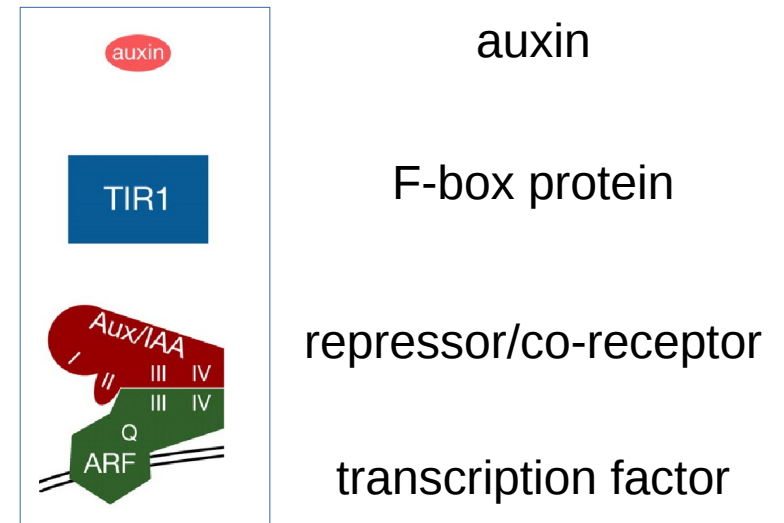
13 – experimental approaches
and current research topics in auxin biology
<http://tinyurl.com/Modul-MMS>

auxin signal transduction model



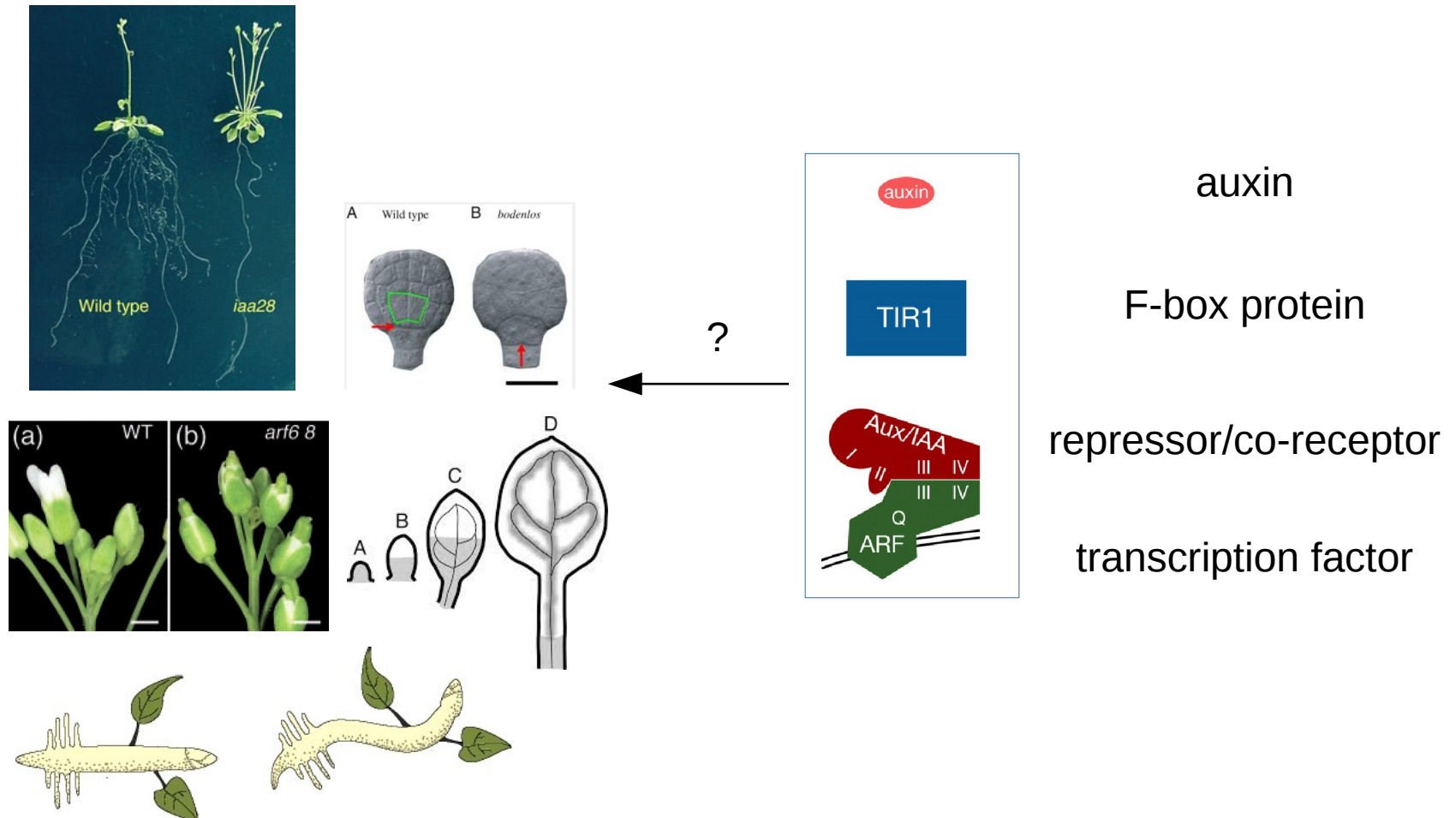
open questions to be answered

simple and short
signal transduction



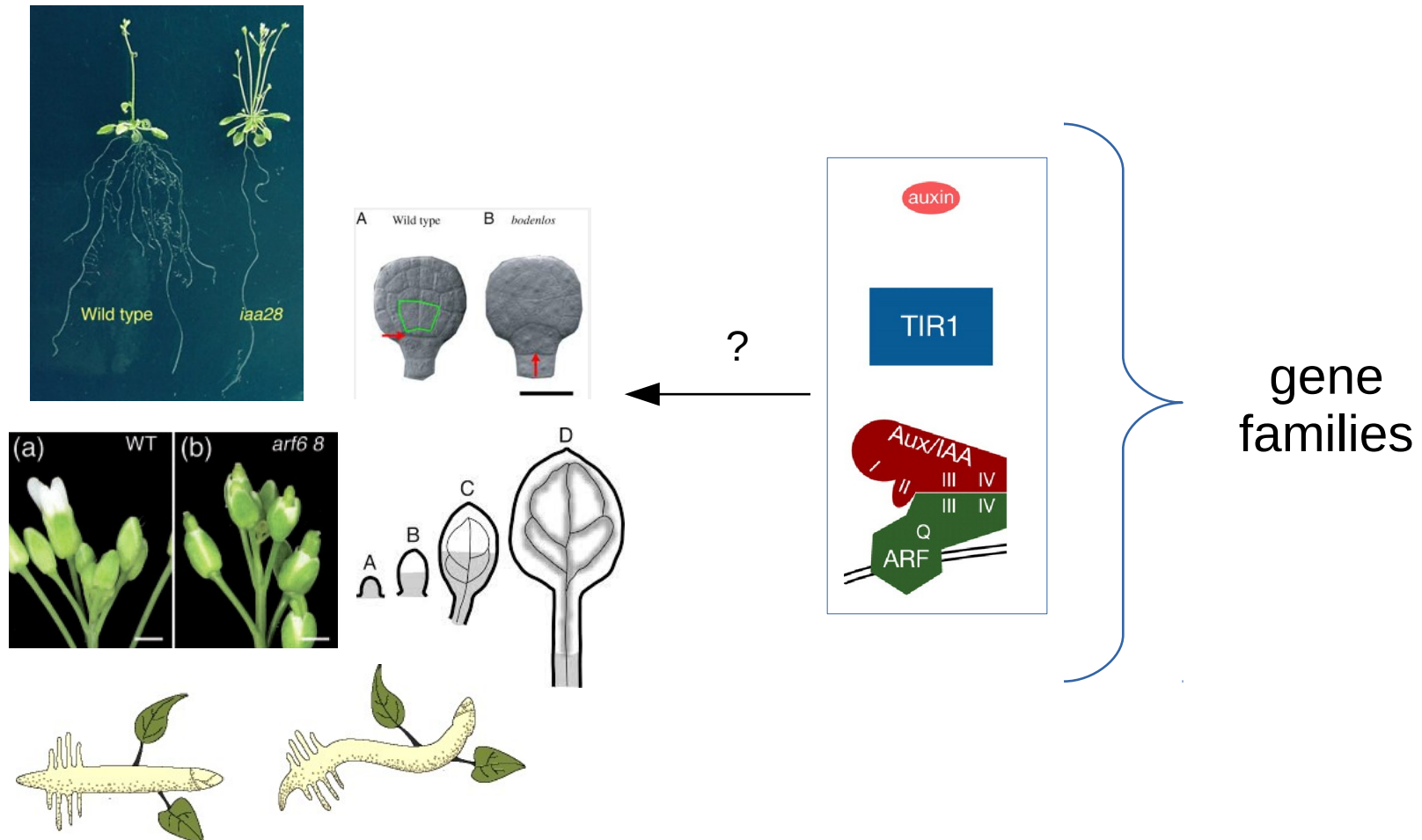
open questions to be answered

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



open questions to be answered

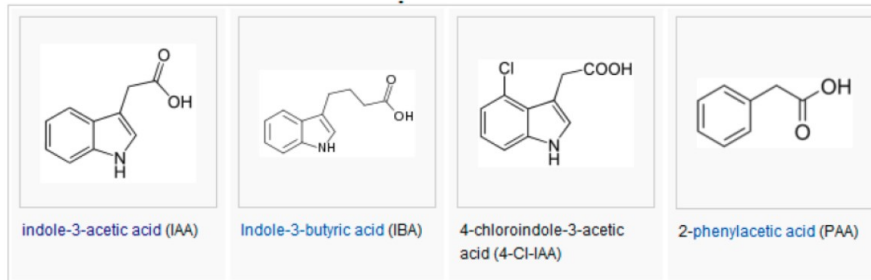
1. how does this simple signal transduction pathway allow the differential activation of diverse set of responses?



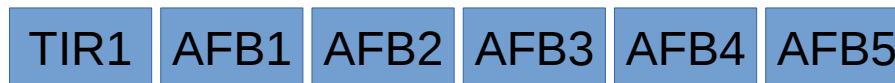
open questions to be answered

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

4 - ? x



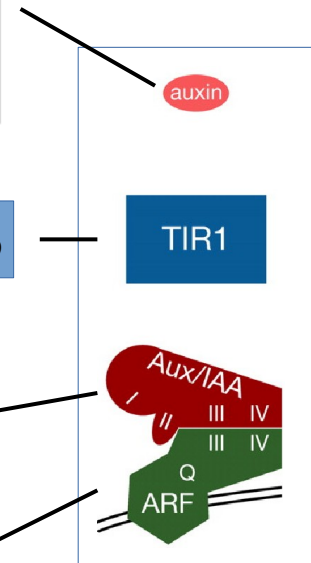
6 x



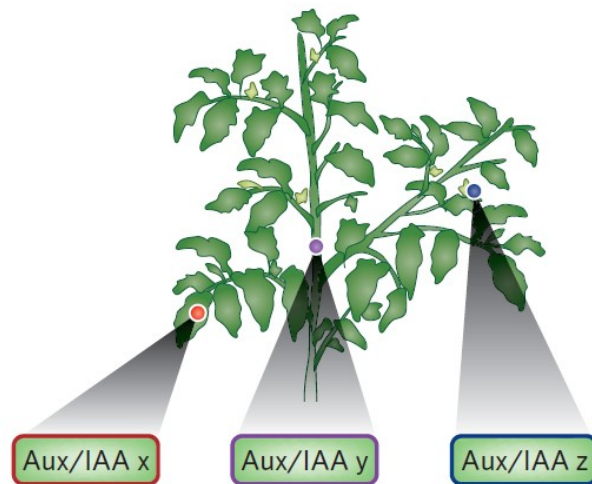
29 x



23 x



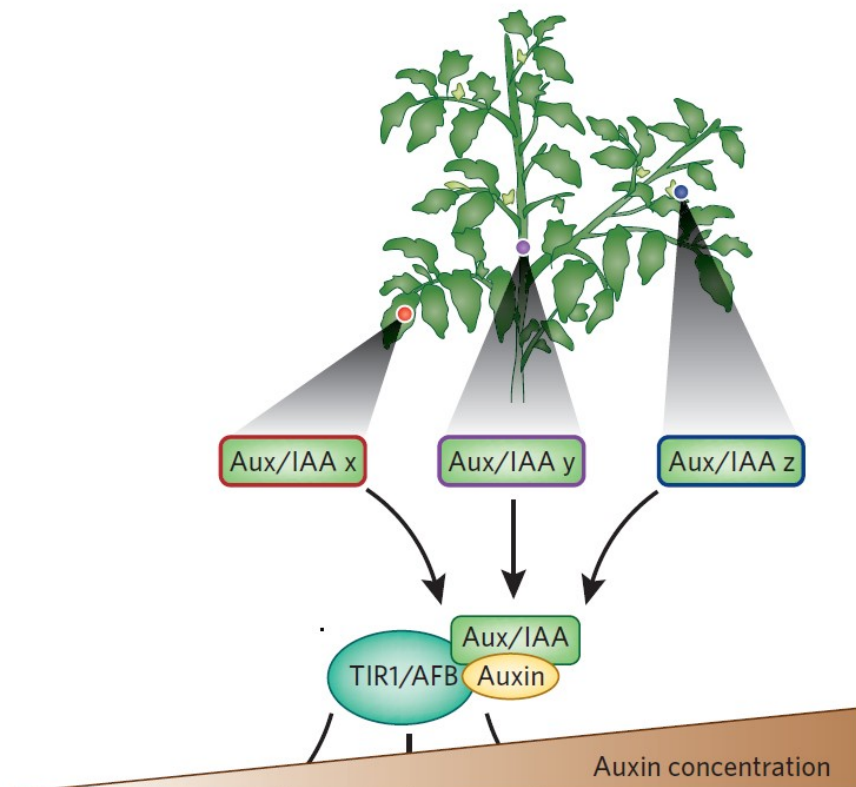
putative mechanism of differential auxin responses



Hypothesis:

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

putative mechanism of differential auxin responses

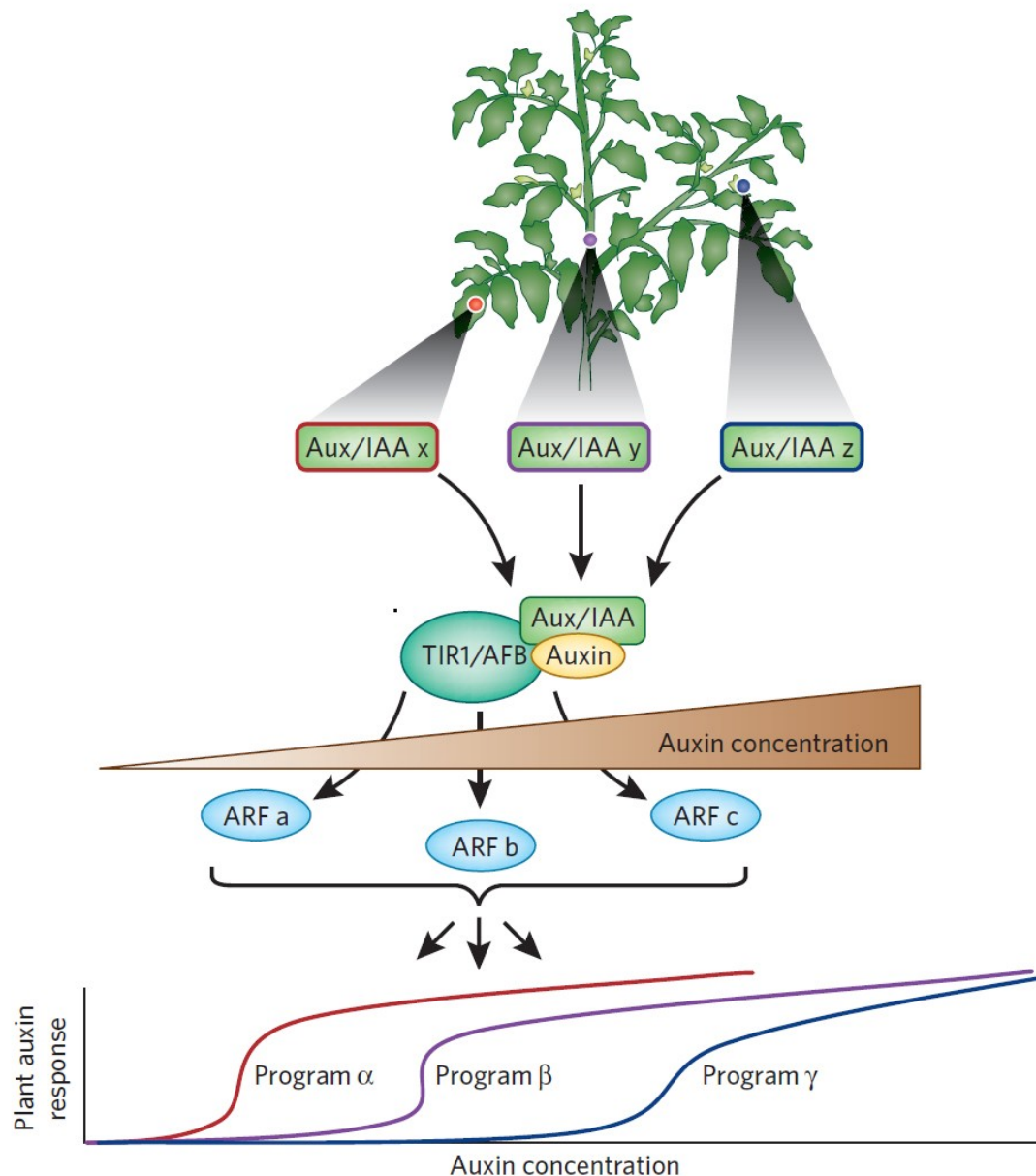


Hypothesis:

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

different combinations of TIR1/AFBs and Aux/IAAs have differential auxin-sensing capacities

putative mechanism of differential auxin responses



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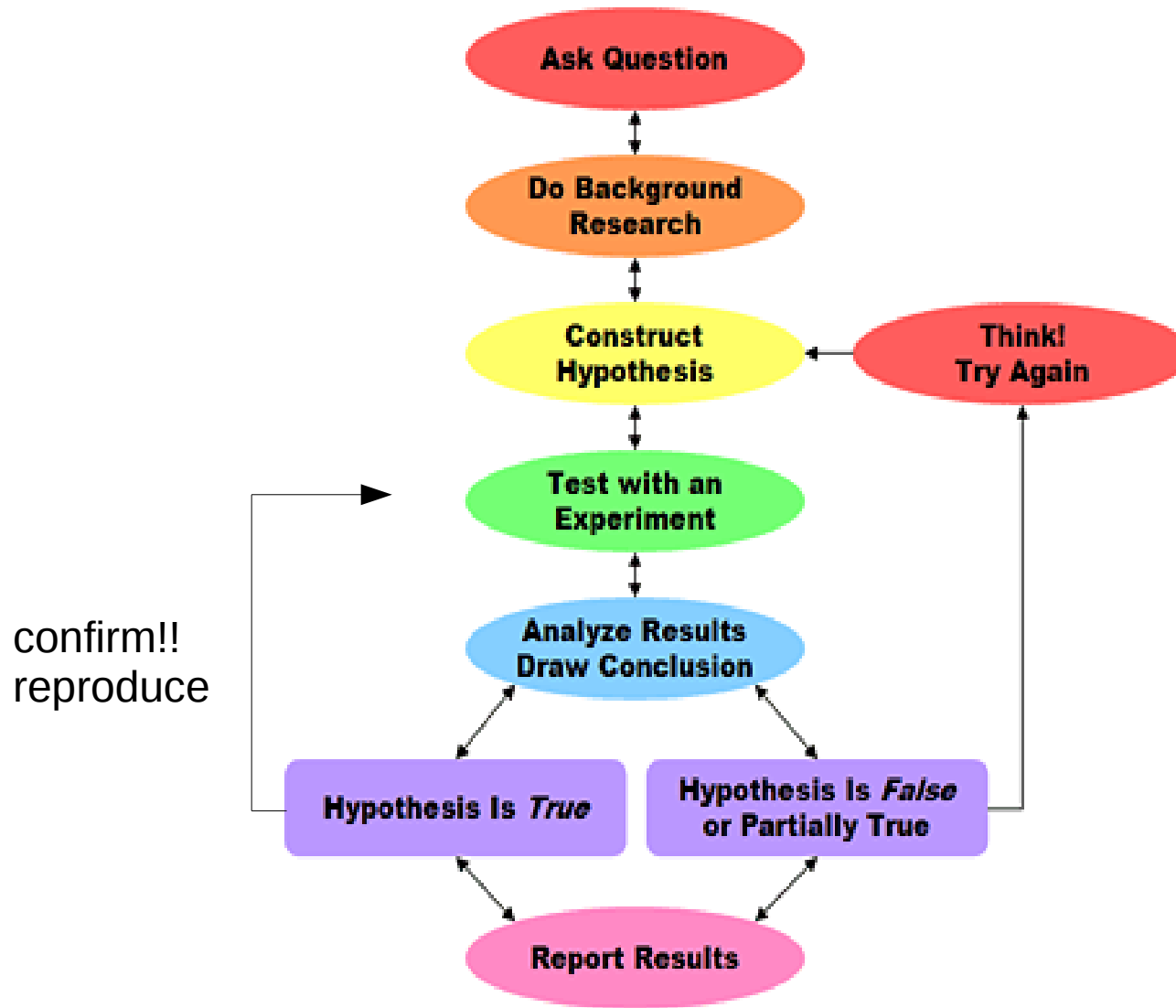
different combinations of TIR1/AFBs and Aux/IAAs have differential auxin-sensing capacities

differential presence of ARFs trigger specific downstream responses

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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 - identification of targets
 - identification of putative ligands

general concepts of experimental design



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

general concepts of experimental design

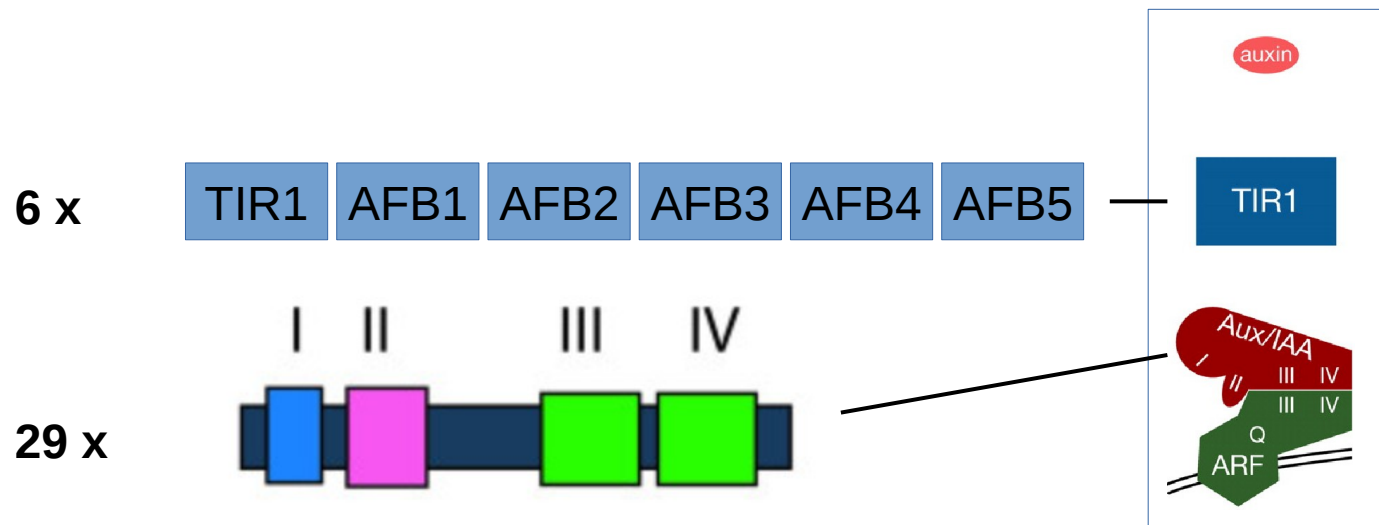
- think about variables that can affect the experiment
 - factors that can be controlled
 - factors that cannot be controlled (but measured?)
 - unknowns
 - choose appropriate statistics to estimate impact on variability!
- consider objectivity – if doubtful: double-blind your samples!
 - extremely important 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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open questions to be answered

- **redundant /specific co-receptor properties of TIR1/ARF – AUX/IAA pairs**



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
-

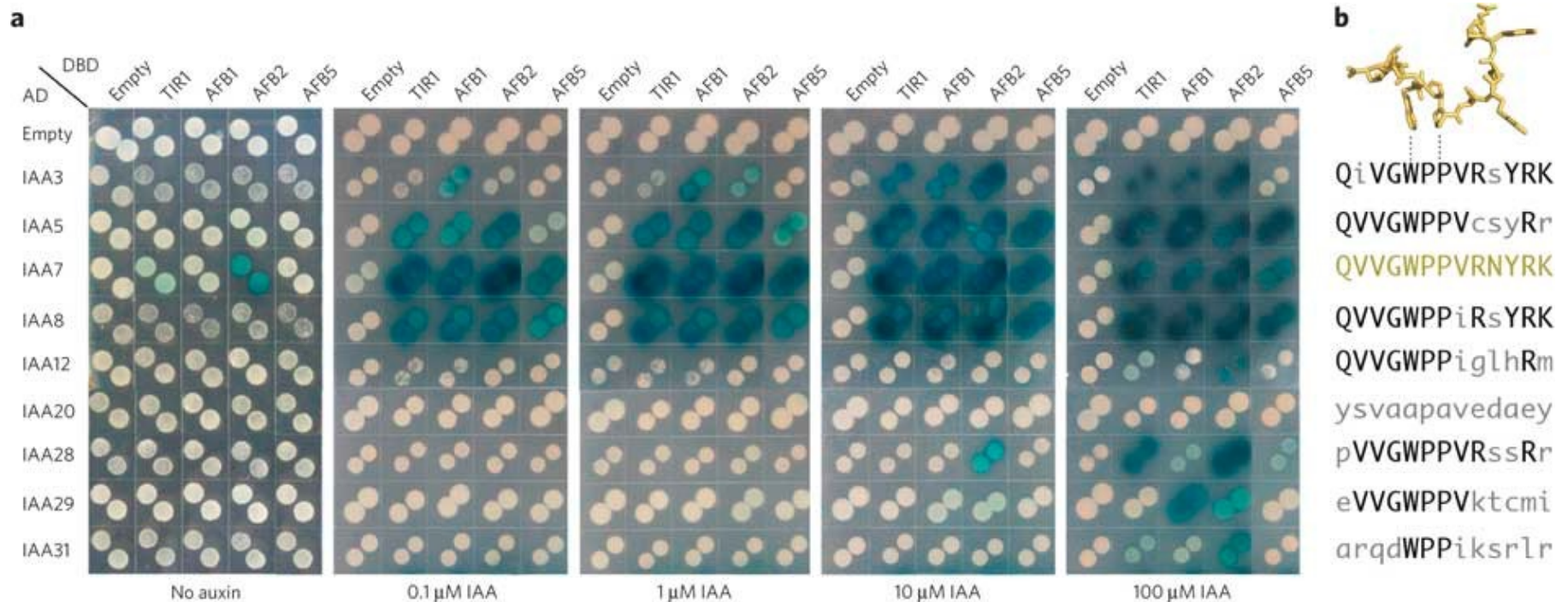
What are the necessary factors to consider + controls to test?

Q1: Redundancy / specificity of co-receptors

approaches:

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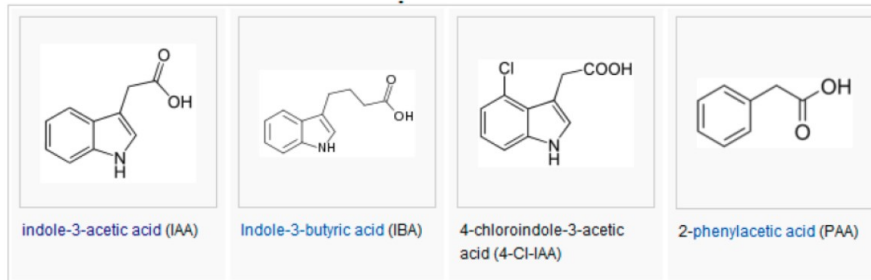
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open questions to be answered

- **redundant /specific co-receptor properties of TIR1/ARF – AUX/IAA pairs**

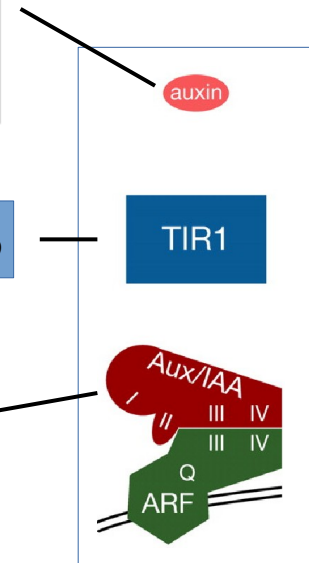
4 - ? x



6 x

TIR1 AFB1 AFB2 AFB3 AFB4 AFB5

29 x



Q1: Redundancy / specificity of co-receptors

approaches:

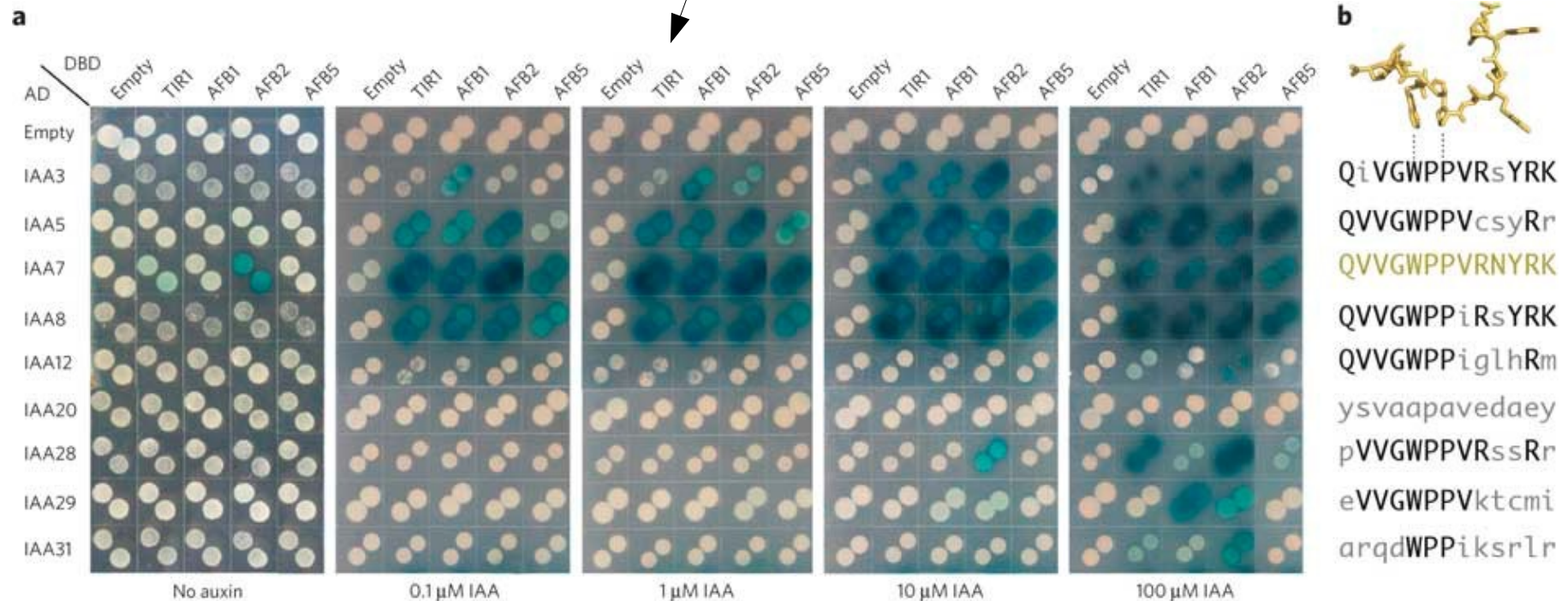
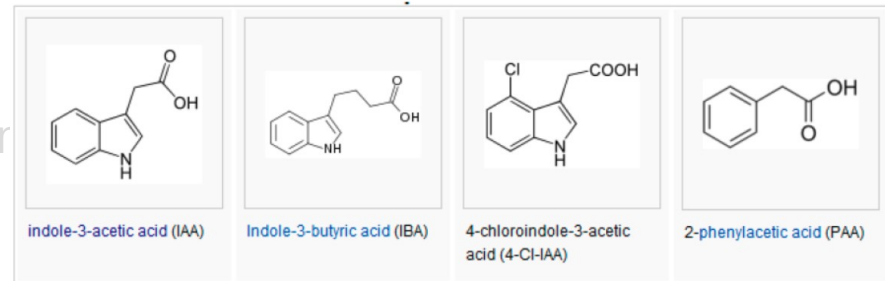
1. Y2H with all possible combinations

2. pull down assays

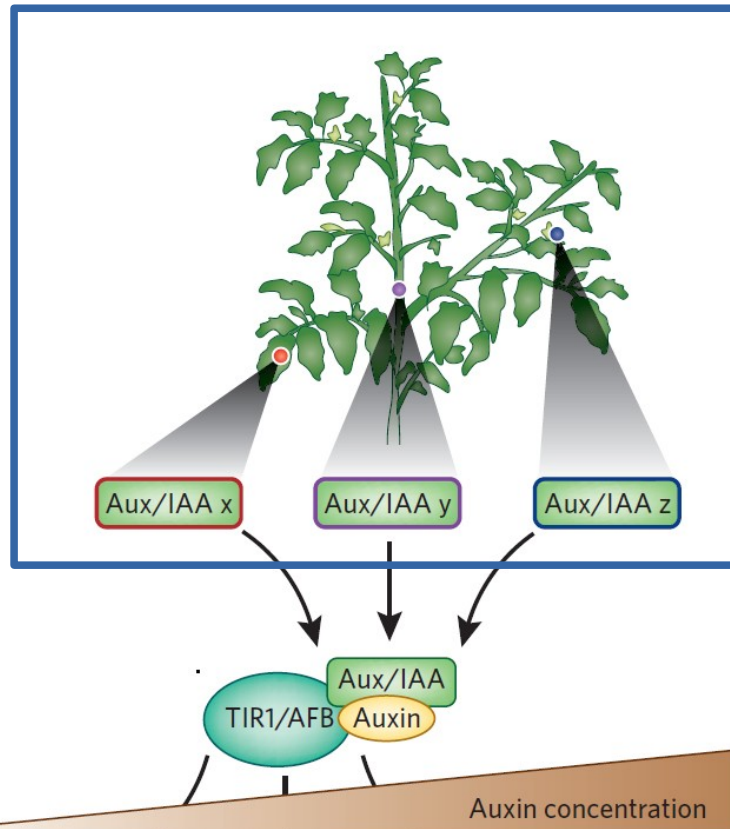
3. genetic interactions – combination of gain

differential sensing?

What are the necessary factors to



putative mechanism of differential auxin responses



Hypothesis:

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

different combinations of TIR1/AFBs and Aux/IAAs have differential auxin-sensing capacities



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

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

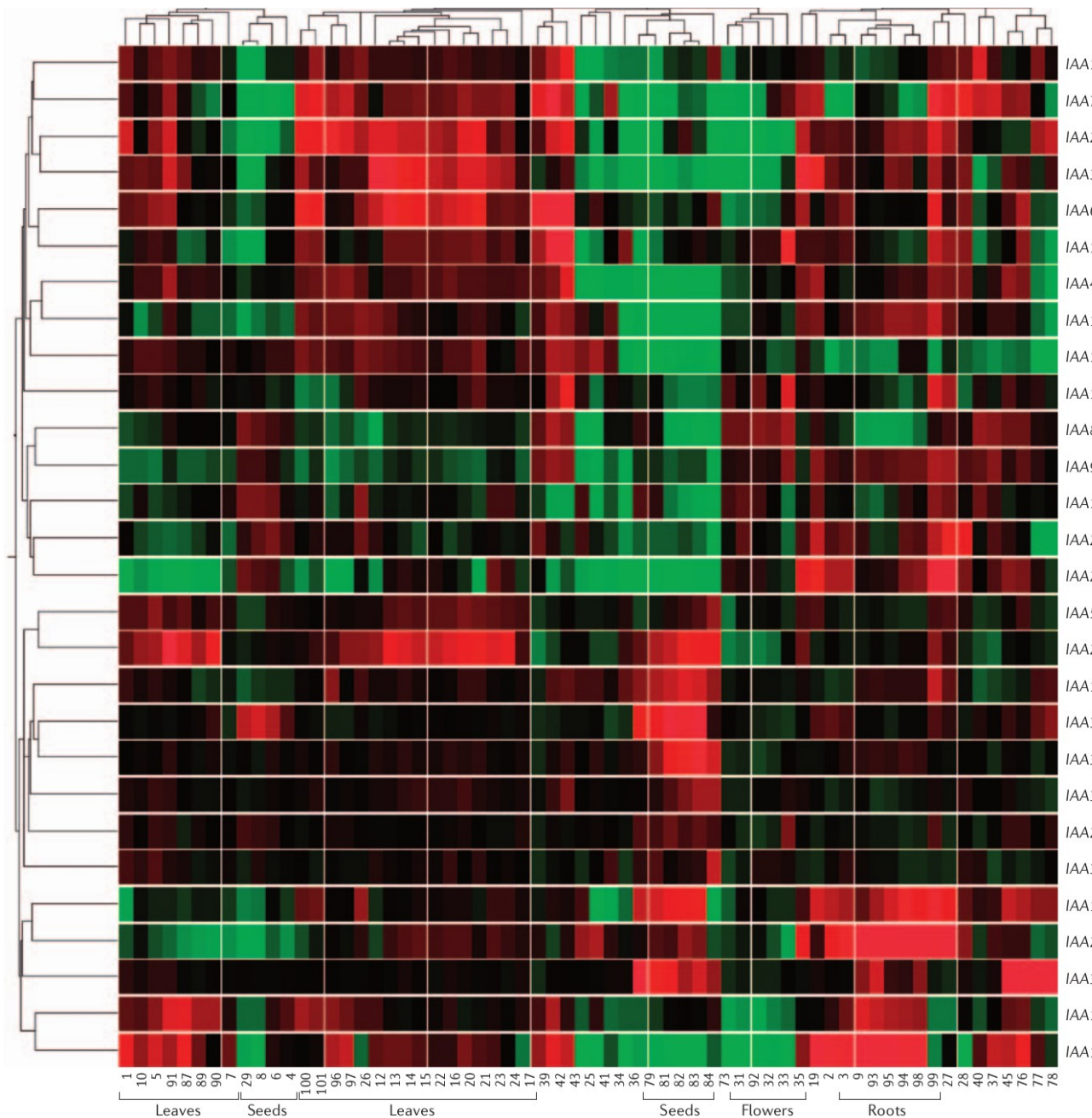
mutant/OEx
phenotypes



- 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

microarray expression
data from different
experiments were
analyzed together

identification of
potentially
redundant
Aux/IAAs

red: high expression
green low expression

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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

AtIAA6	:	-----IAAK-----EGIALEITELRLGLPGDNYS-----EISVC	:	28
AtIAA7	:	-----IIG-----QIMILKATELGLGLPGAE-----AVESPA	:	28
AtIAA8	:	MSYRLLSVDKDELVTSPCLKERNYLGSDCSVDSSSTIPNVVGKSNLNFKAATELRLGLPESQSPERETDFGLLSPRTPDEKLLFPLLPSKDNGSATTTGHK	:	100
FaIAA1	:	-----IAFQ-----AEDLLEATELRLGLPG-----TKETE	:	26
FaIAA2	:	-----MTSTVAMGGGVMEEDRKYSLLNEEETELRLGLPGALK-----DGDQGV	:	43
MdIAA16	:	-----VGSVIHE-----QQNQKYSLLNEEETELRLGLPGILS-----TAGKDGE	:	40
SlIAA17	:	-----MSS-----NKLDEEETELRLGLPGARK-----NVYGDND	:	30
LcIAA1	:	-----MSLENAN-----QSPDSVDLSLNEEETELRLGLPG-----	:	29
Domain I				
AtIAA6	:	##GSSKGGGRVLSDMITSSALDEN-----ENSIVSSVED-----ESLIVVMSIAVGVPPVCSYRRKGNNEEASK-----	:	91
AtIAA7	:	KSAVGSIRGESETVDLMNLQSN-----KEGSVDKKNVSAVPKEKTLTKDPSKPPAKAQQVVGWPPVRYRKKMTQQKITSSGAEEASSEKAGNF	:	117
AtIAA8	:	NVVSGNKRGEADTWDEFSGVGKSVRPGGGINMMLSPKVKDYSKSYQEERSHAKGGLNNNAKKAQQVVGWPPPIRSYRKNMTASSTSKNTDEV	:	192
FaIAA1	:	QQTNNKRALP--VEHESNASSA-----PKPPPSAAQLVGWPPVRSYRKKCVQAKKTE-----	:	77
FaIAA2	:	KSCSNKRGFSETVDLKNFSSN-----DDVSRSGRDQGVETKKEKDASAAP-----APAPRAKAAQVVGWPPVRSFRKMTVAGQKKS--TDQDQAEKS--	:	130
MdIAA16	:	ASKNTGKRGEAETVDLKNLSSE-----DQSAGDEDQVVDKKEKDVAQVPRSNPTKPHAAKAAQVVGWPPVRSFR--MTAVQKDV--SDE--GEK----	:	126
SlIAA17	:	TCNVNGRGE--VDLKNLSSD-----INNPKNST-----HKTAAKAAQVVGWPPVRSFRKMTTSQKLD--REN-----	:	91
LcIAA1	:	---GVKRGES--VDNLGDSR-----RGGGGGDKRCSGENDEMSS-----DKPASKAAQVVGWPPVRLSRKNVKS-----	:	92
Domain II				
AtIAA6	:	-----AIGVVKVSMGAPYMRKIDLGSSNSYINLVTDENLEGCLGIC-----FAKEGKKCEYIIITYEDKDRDMLVGDVPWMEFVES	:	169
AtIAA7	:	GGGAAGAGIVVKVSMGAPYLRRKVDLNYKSYQDLSDAIKMFSSFTMCNYGAQGM--IDEMNESKLIDLLNSSEYVPSYEDKGDWMLVGDVPWMEFVES	:	215
AtIAA8	:	GKPGGLGVIVVKVSMGAPYLRRKVDLRYTYSYQDLSSALERMESCFTLCQGLHGAQGRERSSIDKQDLHGSEFVLTYEDKGDWMLVGDVPWMEFVES	:	292
FaIAA1	:	--GESGGMVVKVSMGAPYLRRKMDLKYKGYPEILKALEEMEK--KVE-----EYCEKEGYNRSEFVPTTYEDKGDWMLVGDVPWMEFVES	:	162
FaIAA2	:	GSTSTSAAVVKVSMGAPYLRRKVDLKYQSYQELSTALGKMESFTIINCQSQGM--KDEMNESKLIDLLNGSEYVPSYEDKGDWMLVGDVPWMEFVES	:	228
MdIAA16	:	--TNSSAAVVKVSMGAPYLRRKVDLKYKSYQELSTALGKMESFTIICSYGSQGM--KDEMNESKLIDLLNGSEYVPTTYEDKGDWMLVGDVPWMEFVES	:	222
SlIAA17	:	-----DNILVVKVSMGAPYLRRKVDLNYKSYQELDALIKMENSFTI--VQGM--KDEMHGKIMDLNLSYVPTTYEDKGDWMLVGDVPWMEFVES	:	180
LcIAA1	:	-----CKVVKVAVDGAAPYLRRKVDLEMYTSYQQLTALAEEMFSCFTIR-----NYLNERKIMDQVNGVYVPTTYEDKGDWMLVGDVPWMEFVES	:	176
Domain III				
AtIAA6	:	#####CKRLRIVKRSDITGFGLOQD-----	:	189
AtIAA7	:	CKRLRIMKSEAVGLAPRAVEKCKNYS--	:	243
AtIAA8	:	CKLKLIMKGSDSLGLAPGAVEK--SKNTERV	:	321
FaIAA1	:	CKRLRIMKSEAKGLGCAV-----	:	181
FaIAA2	:	CKRLRIMKSEAIGLAPRAVEK--FKNYS--	:	255
MdIAA16	:	CKRLRIMKSEAIGLAPRAVEK--CKNYS--	:	249
SlIAA17	:	CKRLRIMKSEAIGLAPRAVEK--CKNNG--	:	208
LcIAA1	:	CKRLRIMKSEAIGLAPRTTPPNSSST---	:	203
Domain IV				

→ site-directed mutagenesis, sequence swaps, etc

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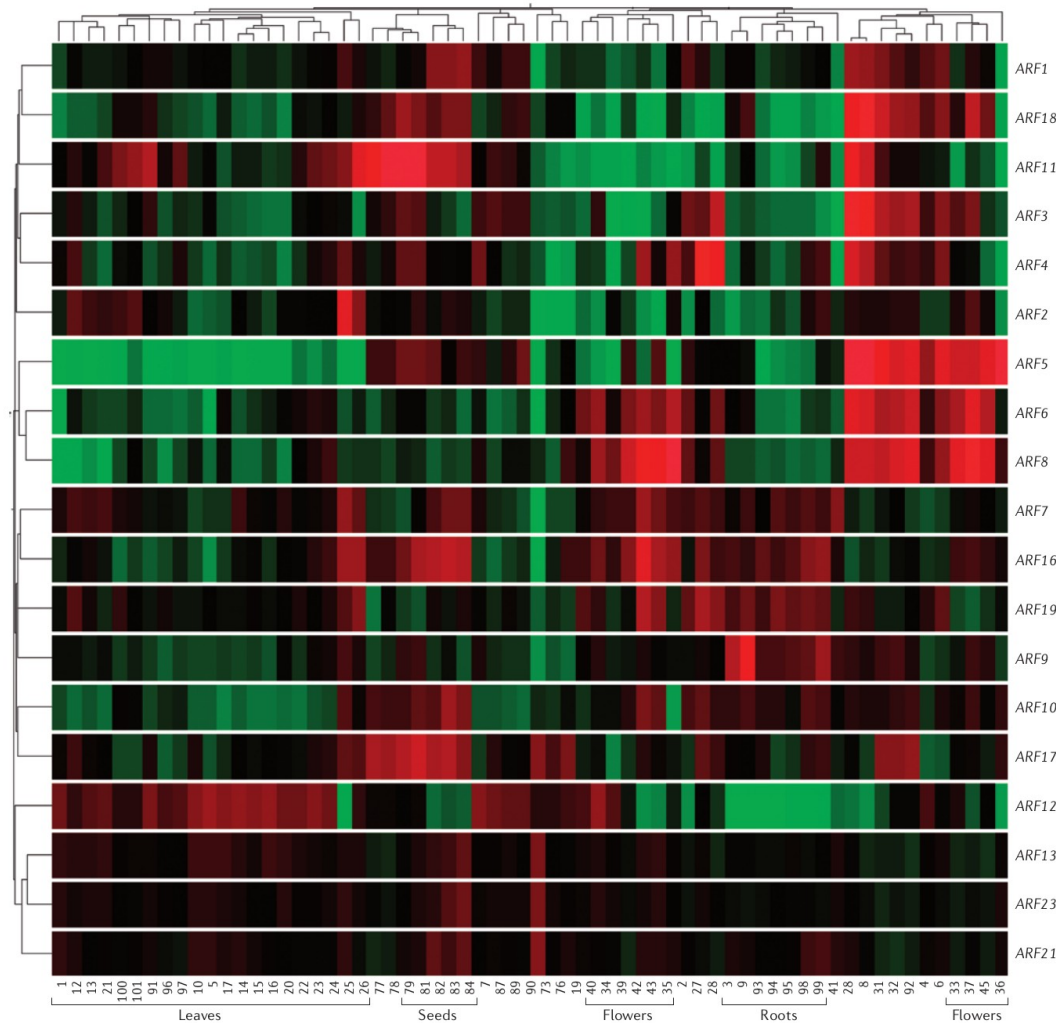
redundancy / specificity of ARFs



approaches:

1. partial swaps of domains → identification of variable sequence stretches 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 / specificity 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