

Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*

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Ergebnisse der letzten Woche

Funktionelle Charakterisierung

tir1-Mutanten in Auxin-Antwort beeinträchtigt

tir1-axr1-Doppelmutante additiv/synergistische Interaktion

TIR1 dominant-rezessiv vererbt (redundante Funktion)

Genetische Charakterisierung

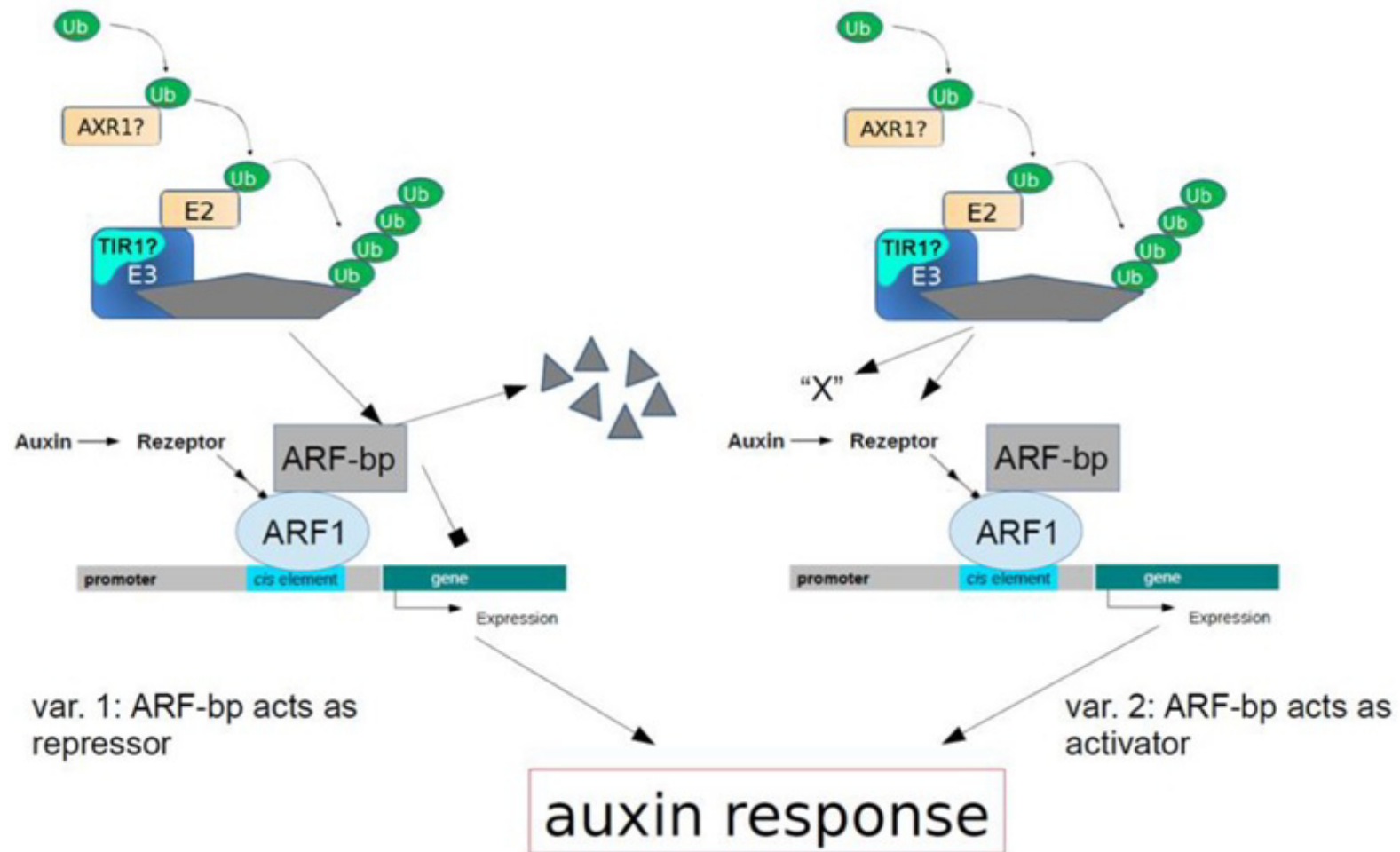
Auf Chromosom 3, Position 128 lokalisiert

F-Box und LRR-Domäne

Mögliche Beteiligung an Ubiquitin-Protein-Ligase-Komplex

Verwandte in Arabidopsis und Reis (LRFs)

Bisheriges Modell



Weitere Erkenntnisse

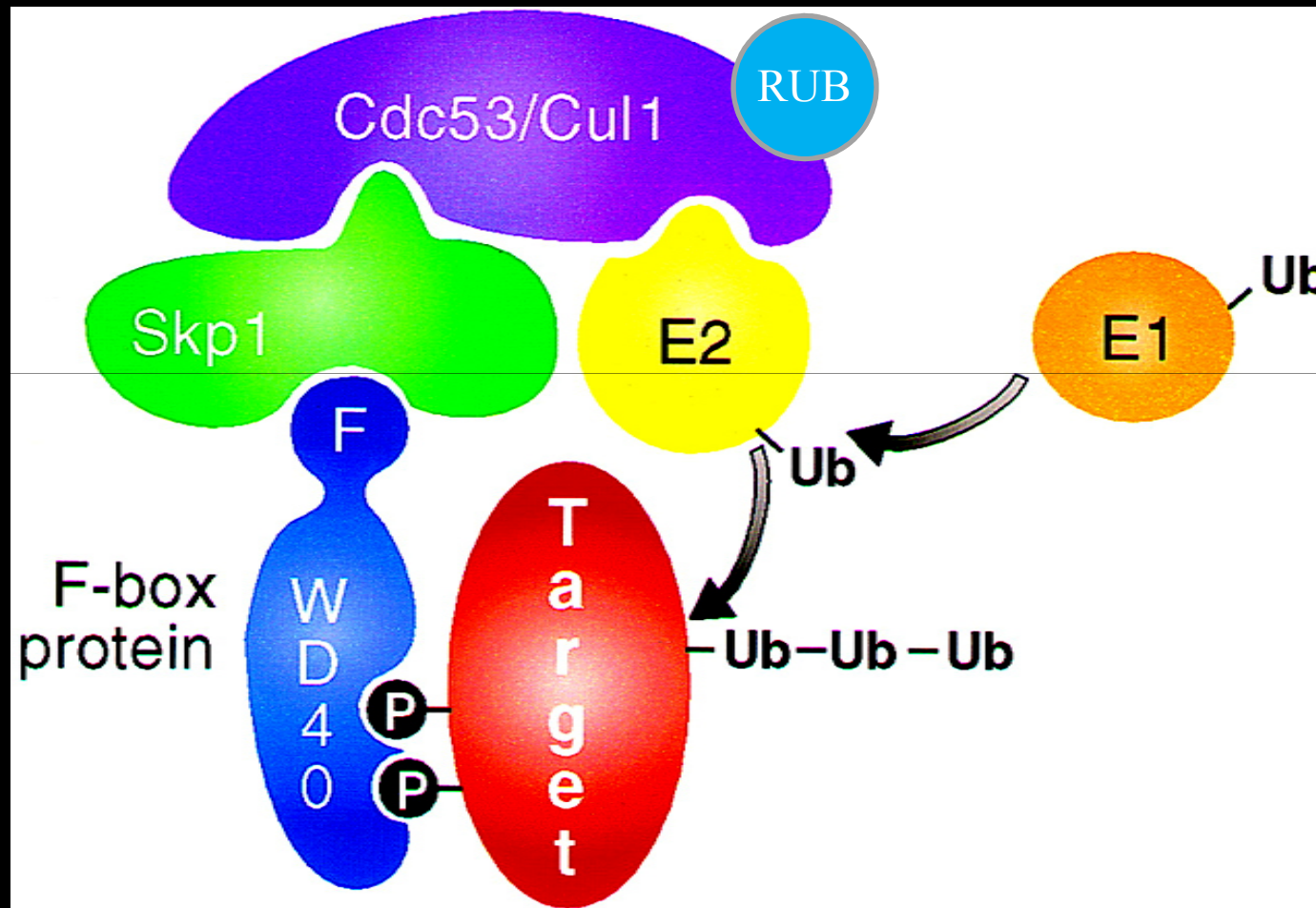
Mutationen in *SAR1* als Supressor von *axr1*

→ selber Pathway
(Cernac et al. 1997)

E-Box Proteine interagieren mit Skp1 und Cdc53

→ Bildung von Ubiquitin-Ligase-Komplexen (SCFs)
(Krek 1998; Patton et al. 1998; Mantis 1999)

Ubiquitin-Ligase-Komplex (SCF)



(Mantis 1999)

The diagram illustrates the ubiquitin-proteasome pathway. Ubiquitin (Ub) is activated by E1 (ubiquitin-activating enzyme) using ATP, forming a Ub-E1 complex. This complex is transferred to E2 (ubiquitin-conjugating enzyme), forming a Ub-E2 complex. E2 then interacts with E3 (ubiquitin-ligase) and a substrate protein. E3 facilitates the transfer of Ub from E2 to the substrate, forming a Ub-substrate complex. The Ub-substrate complex is then targeted to the 26S proteasome, which degrades the substrate into protein fragments. Ubiquitin is released from the proteasome and recycled back to E1.

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Molecular Cancer Therapeutics Reviews

ACR

Ubiquitin-Ligase-Komplex (SCF)

AXR1 interagiert mit ECR1

- Aktivierung Ubiquitin-related Protein (RUB)
(del Pozo et al. 1998)

Cdc53/Cullin target für RUB/NEDD-8

- Annahme: RUB wirkt auf SCF Komplex ein
(Lammer et al. 1998)

Cdc53-related Protein: At-CUL1

- Substrat für RUB Konjugation
- AXR1-ECR1 E1-like dimer aktiviert RUB
- RUB konjugiert At-CUL1
- ➔ Verzögerung der Bildung oder Modifizierung des SCF Komplexes

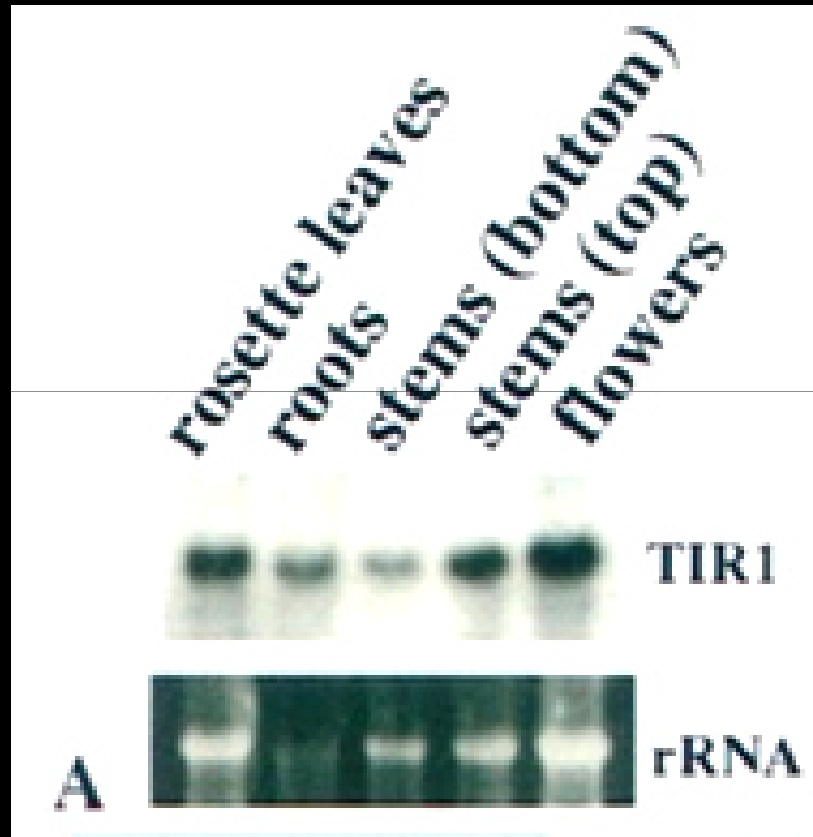
Fragestellung

Wann und in welchen Geweben wird *TIR1* exprimiert?

Bildet TIR1 mit einem oder mehreren Proteinen Komplexe?

Ergebnisse

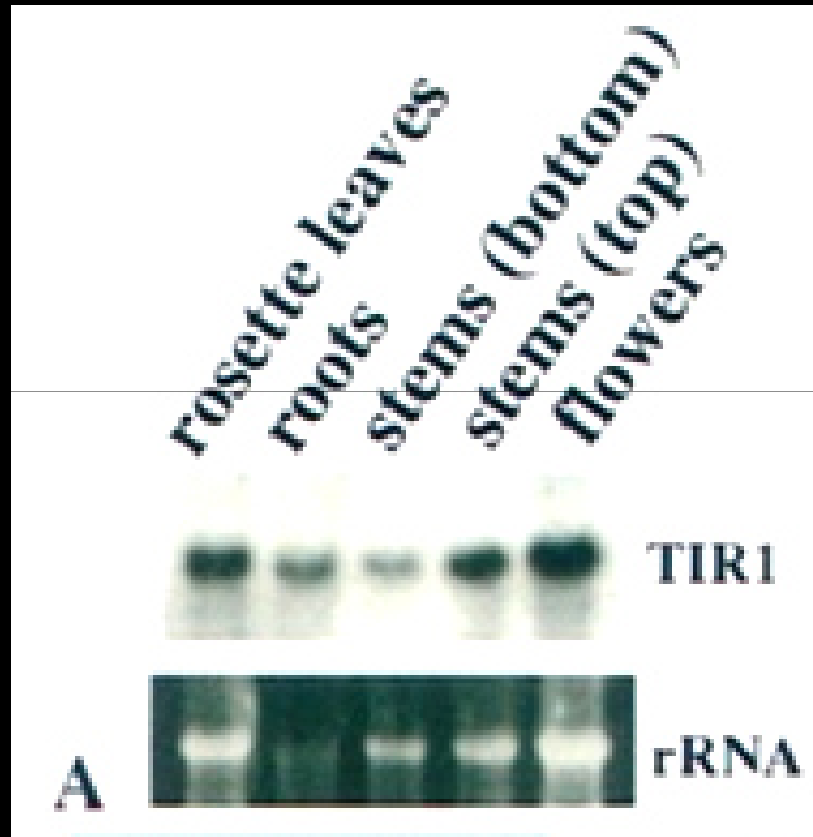
- Expression *TIR1*



A: Northern Hybridisierung
→ Transkriptlevel *TIR1*

Ergebnisse

- Expression *TIR1*



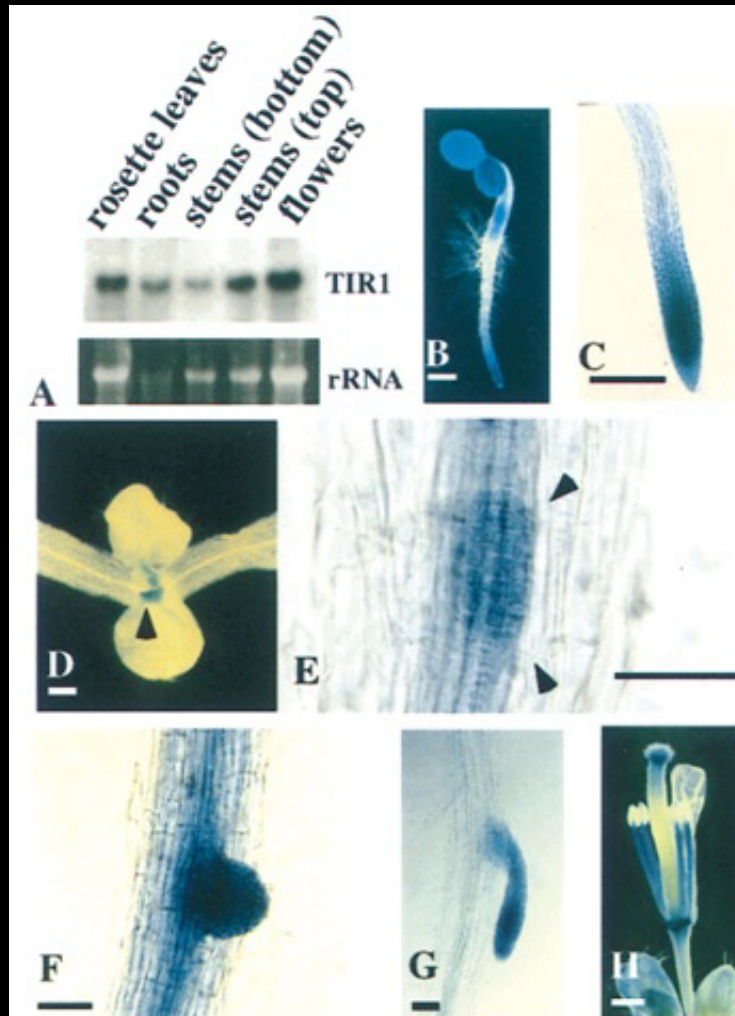
A: Northern Hybridisierung

→ Transkriptlevel *TIR1*

In allen gezeigten Geweben
exprimiert

Ergebnisse

- Expression *TIR1*



B-H: *TIR1*_{PRO-gus}

B: Seedling

C: Apikalmeristem (Wurzel)

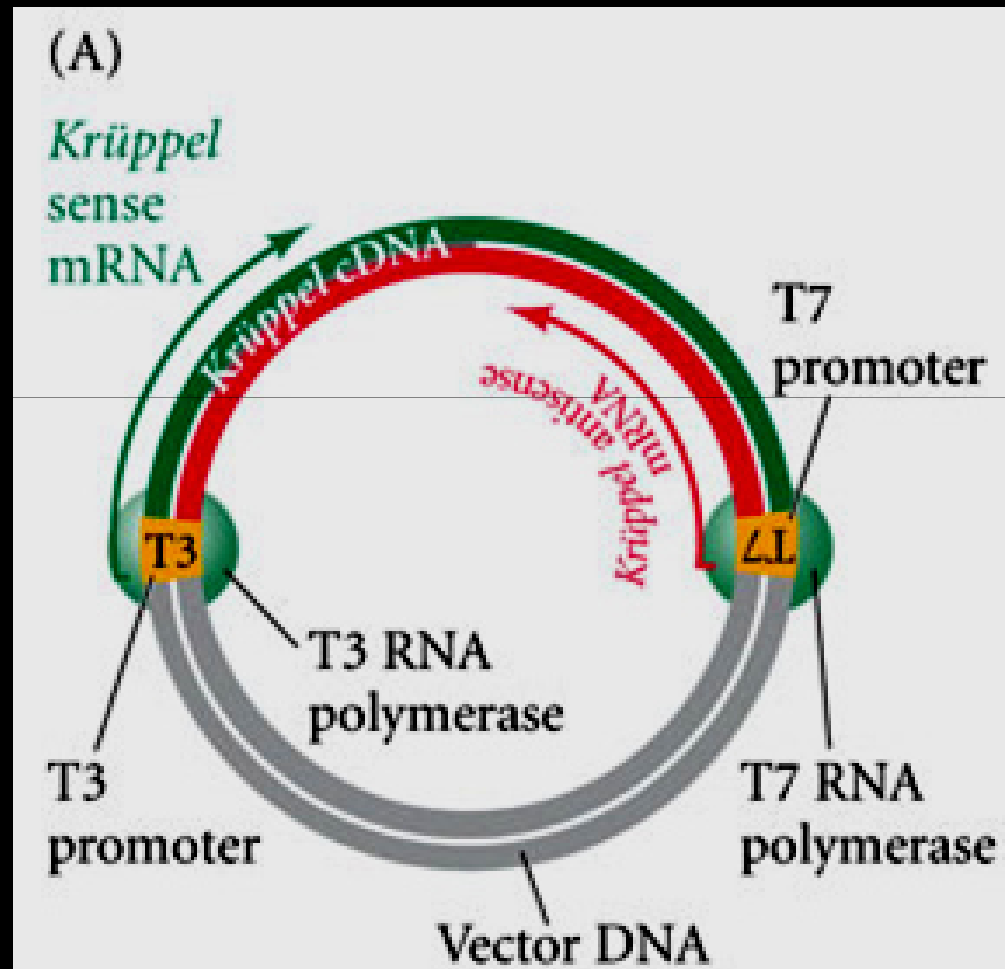
D: Apikalmeristem (Spross)

E-G: lateral Wurzel
Primordium/Meristem

H: floral stigma

Ergebnisse

- Methode: In situ Hybridisierung



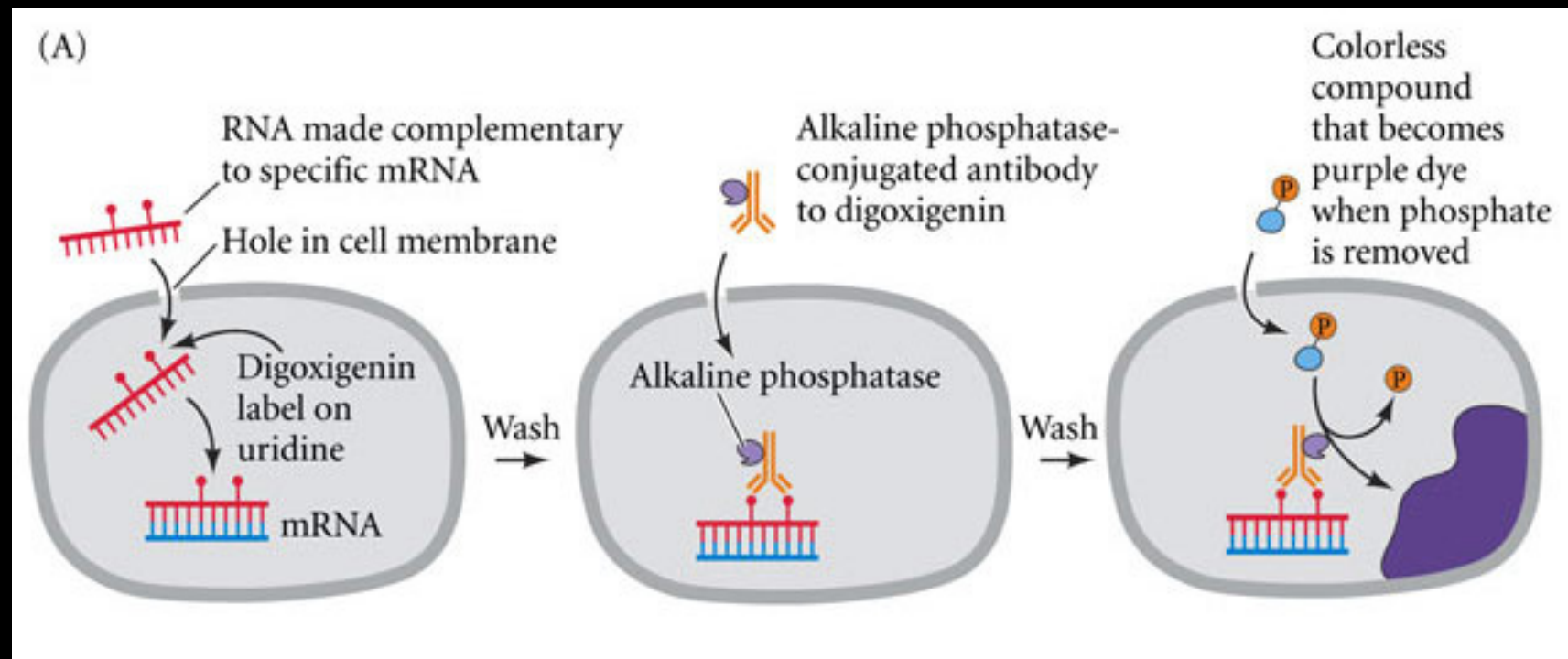
<http://10e.devbio.com/article.php?id=295>

Ergebnisse

- Methode: In situ Hybridisierung

Sonden mit cDNA von *TIR1* erzeugt

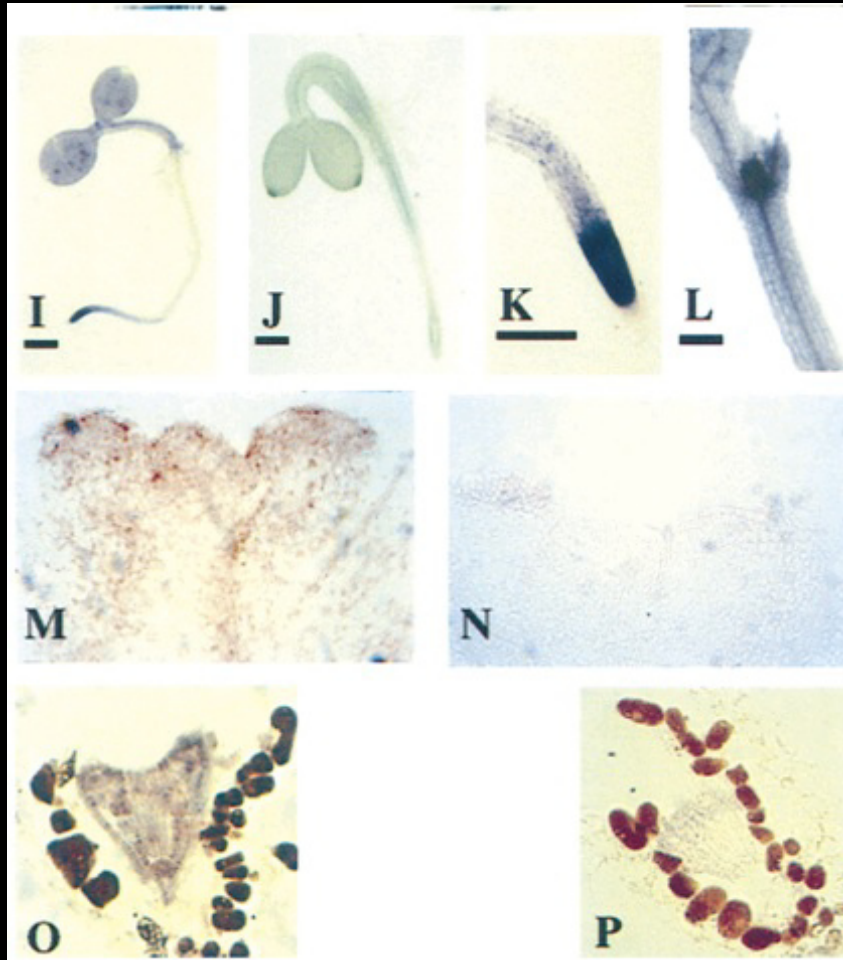
RNA muss fixiert und vor Abbau/Verdau geschützt werden



<http://10e.devbio.com/article.php?id=295>

Ergebnisse

- Expression *TIR1*



B-H: *TIR1-gus*

I,K,L,M: meristematisches
Gewebe

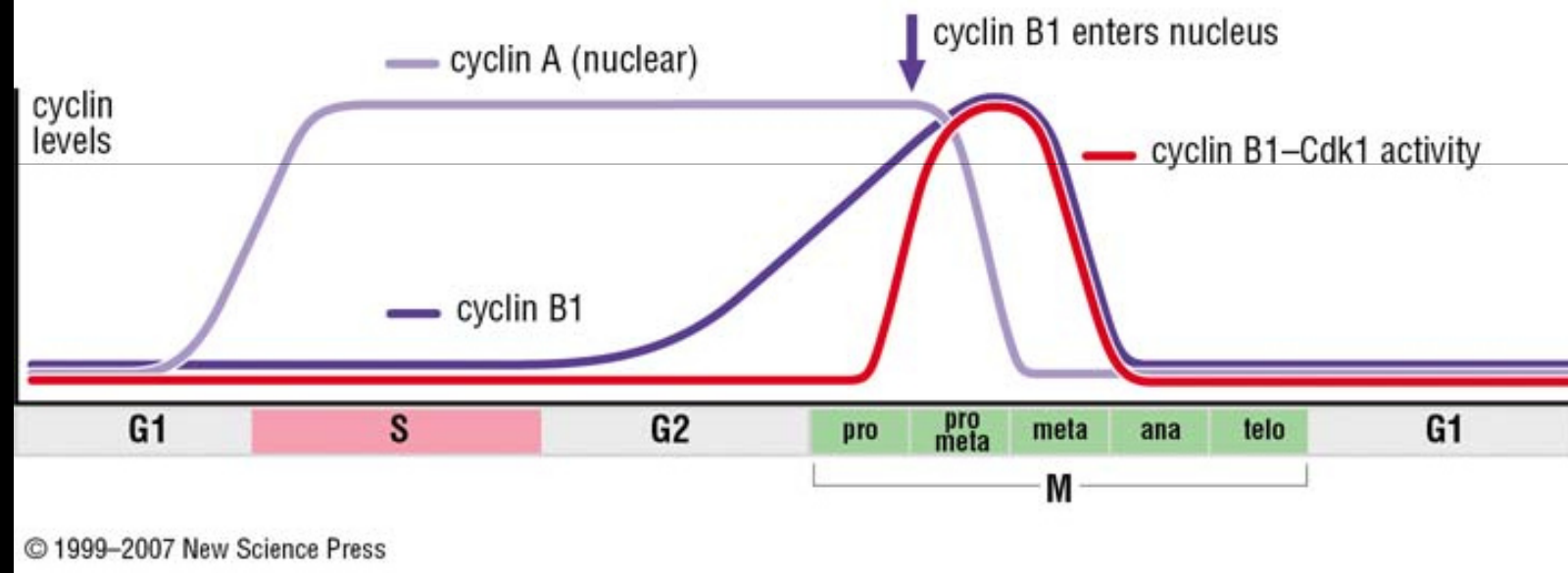
O: Expression in
Embryoentwicklung

J,N,P: sense strand control
hybridization

Ergebnisse

- Methode: Lateral root studies

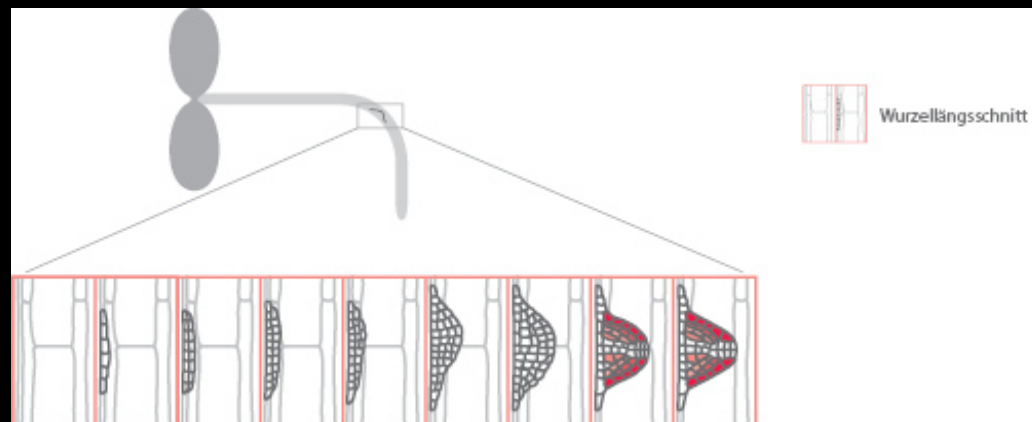
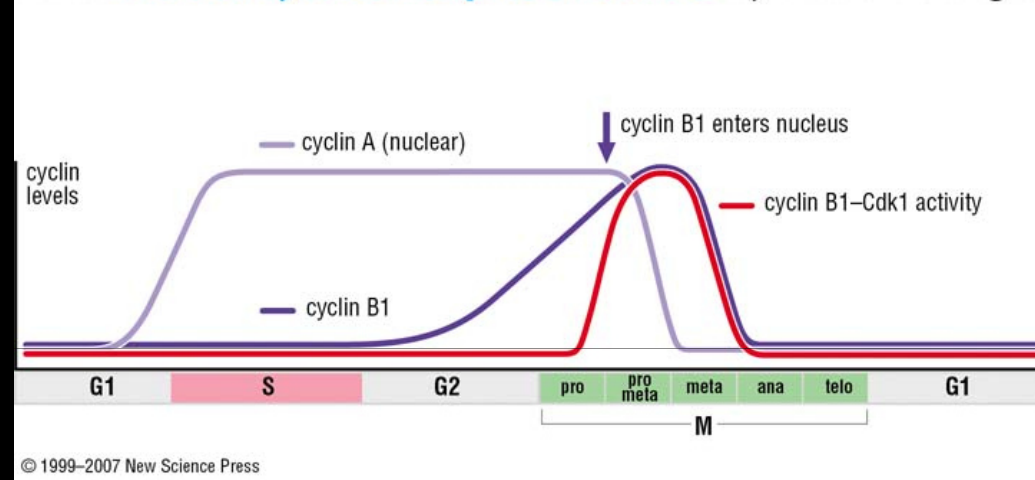
From **The Cell Cycle: Principles of Control** by David O Morgan



Ergebnisse

- Methode: Lateral root studies

From **The Cell Cycle: Principles of Control** by David O Morgan

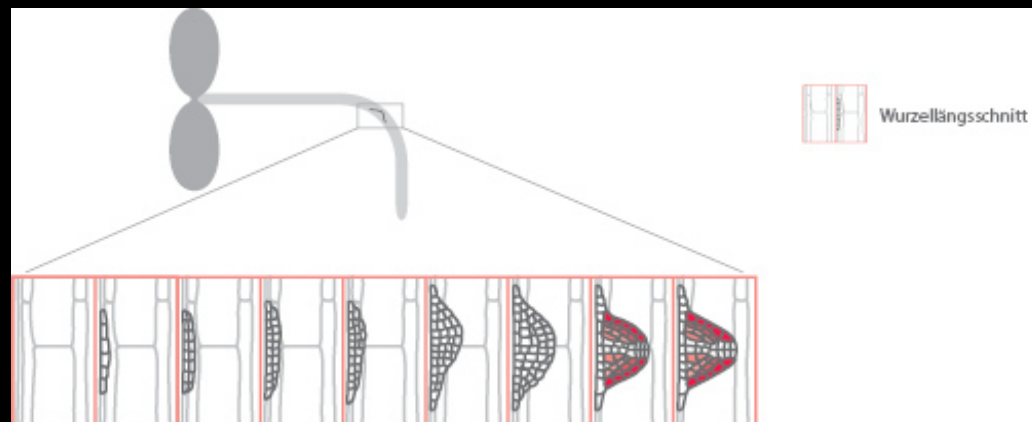
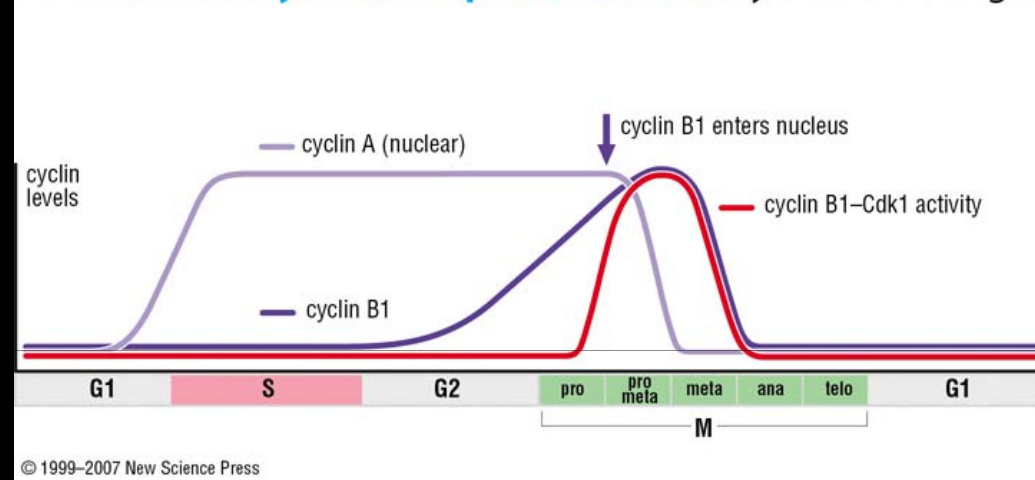


<https://www.helmholtz-muenchen.de>

Ergebnisse

- Methode: Lateral root studies

From **The Cell Cycle: Principles of Control** by David O Morgan



<https://www.helmholtz-muenchen.de>

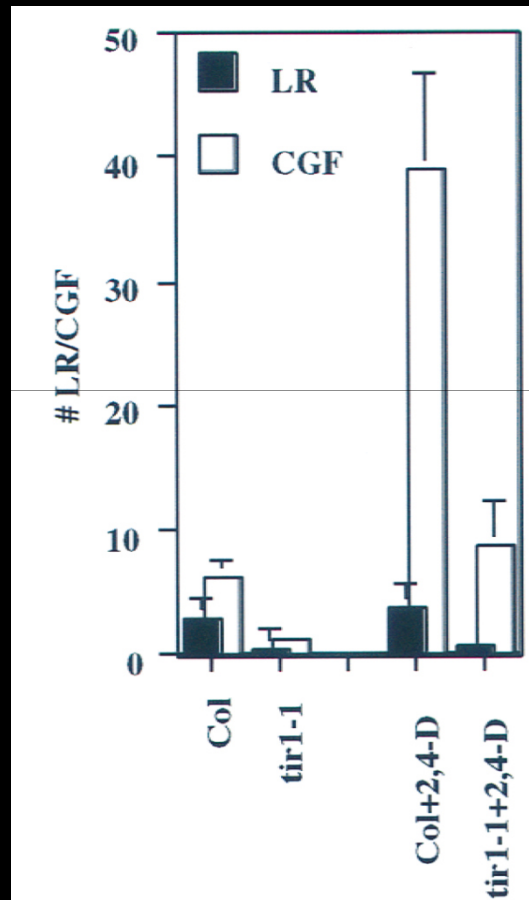
cyclAt-gus

Ausdifferenzierte Zellen
beginnen sich zu teilen

Detektion mit Reporter
in Anfangsstadien

Ergebnisse

- *TIR1*: laterale Wurzelbildung

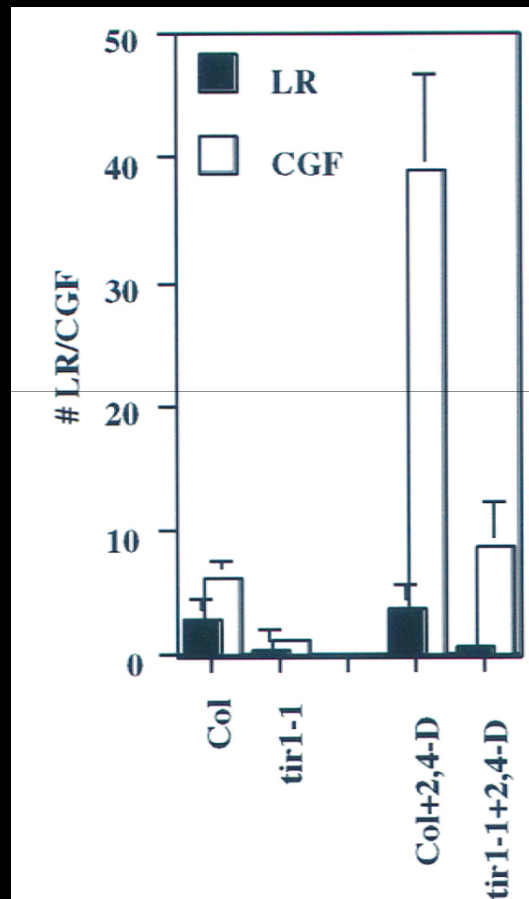


LR: Lateral roots

CGF: *cyc1At-gus* foci

Ergebnisse

- *TIR1*: laterale Wurzelbildung



LR: Lateral roots

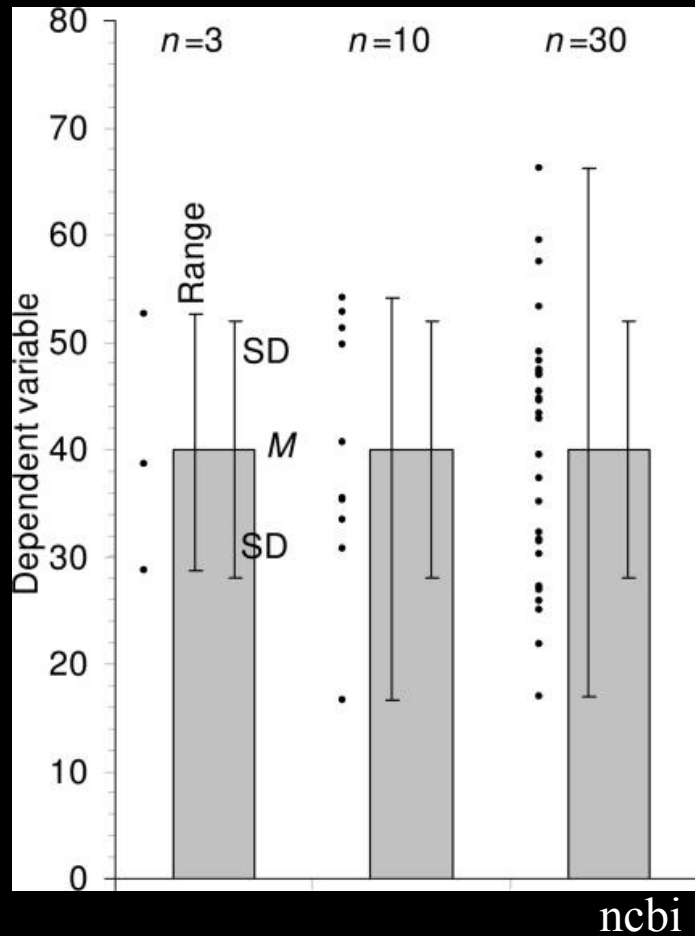
CGF: *cyc1At-gus* foci



David L. Vaux: Know when your numbers are significant

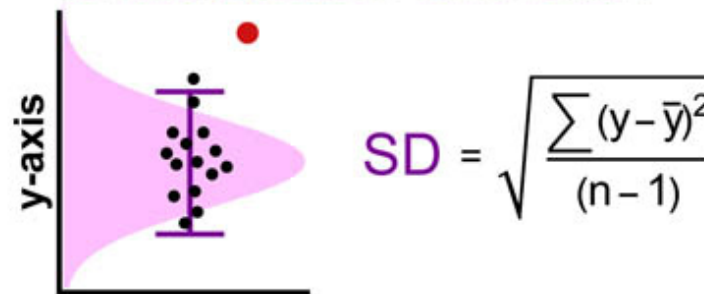
Ergebnisse

- Exkurs Statistik



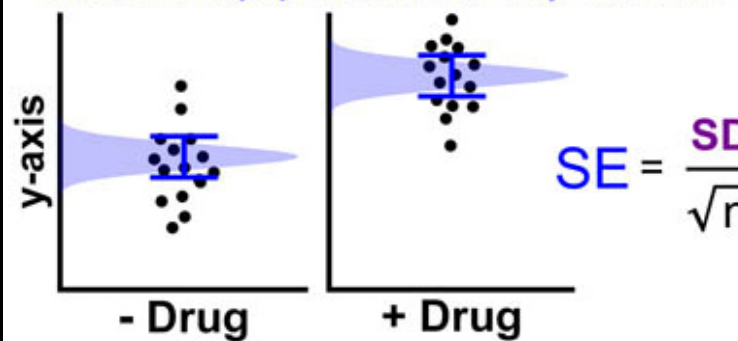
Standard Deviation(SD) (Descriptive)

Q's w/n a population: Is this "normal"?



Standard Error(SE) (Inferential)

Q's between populations: Are they "different"?



<http://www.practicalscience.com>

Ergebnisse

- Exkurs Statistik

STATISTICS GLOSSARY

Some common statistical concepts and their uses in analysing experimental results.

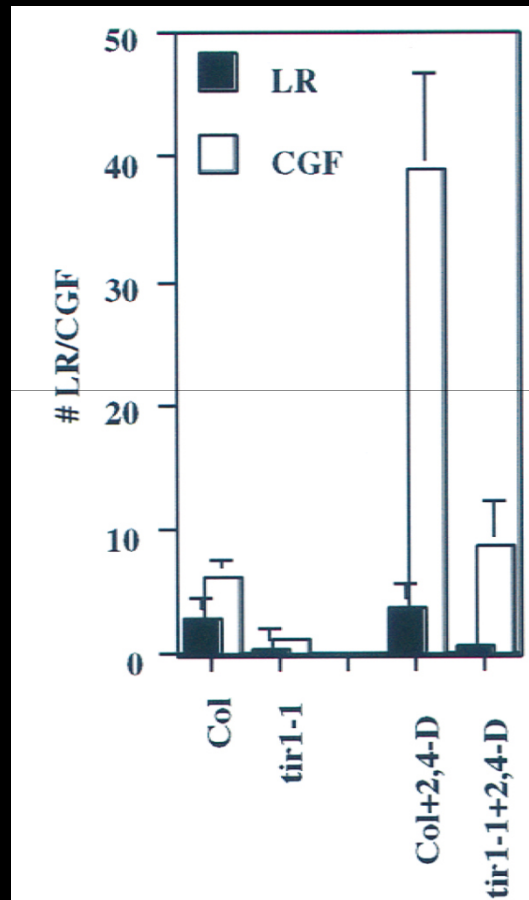
Term	Meaning	Common uses
Standard deviation (s.d.)	The typical difference between each value and the mean value.	Describing how broadly the sample values are distributed. $s.d. = \sqrt{(\sum (x - \text{mean})^2 / (N - 1))}$
Standard error of the mean (s.e.m.)	An estimate of how variable the means will be if the experiment is repeated multiple times.	Inferring where the population mean is likely to lie, or whether sets of samples are likely to come from the same population. $s.e.m. = s.d. / \sqrt{N}$
Confidence interval (CI; 95%)	With 95% confidence, the population mean will lie in this interval.	To infer where the population mean lies, and to compare two populations. $CI = \text{mean} \pm s.e.m. \times t_{(N-1)}$
Independent data	Values from separate experiments of the same type that are not linked.	Testing hypotheses about the population.
Replicate data	Values from experiments where everything is linked as much as possible.	Serves as an internal check on performance of an experiment.
Sampling error	Variation caused by sampling part of a population rather than measuring the whole population.	Can reveal bias in the data (if it is too small) or problems with conduct of the experiment (if it is too big). In binomial distributions (such as live and dead cell counts) the expected s.d. is $\sqrt{N \times p \times (1 - p)}$; in Poisson distributions (for example, cells per field) the expected s.d. is $\sqrt{\text{mean}}$.

N , number of independent samples; t , the t -statistic; p , probability.

David L. Vaux: Know when your numbers are significant

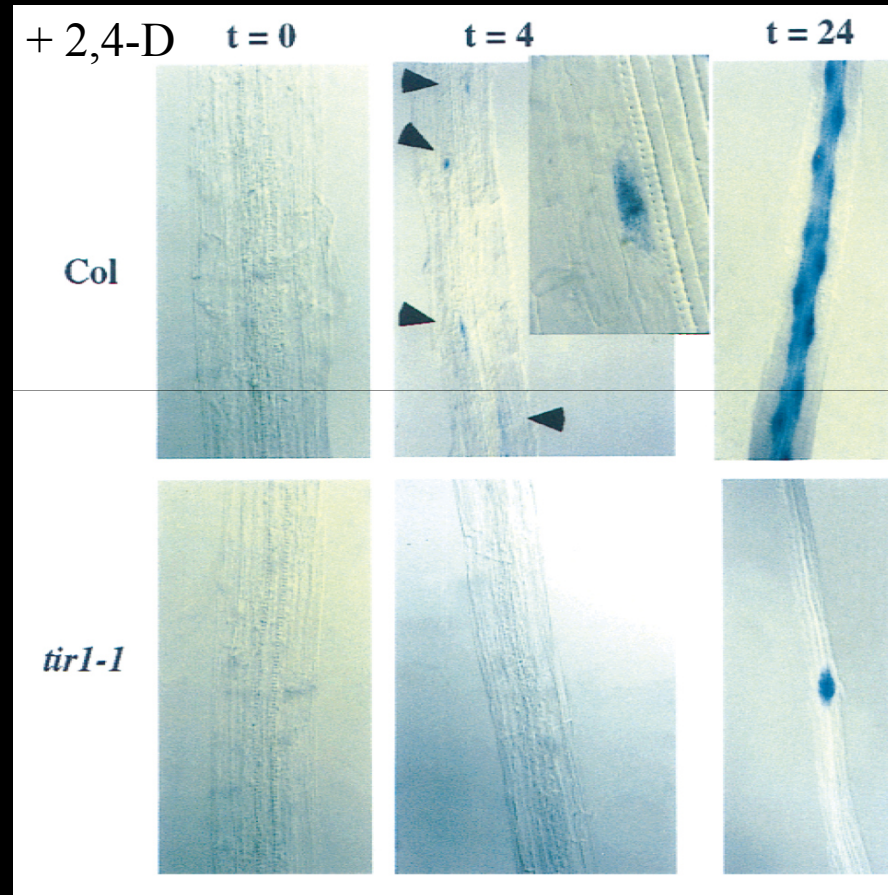
Ergebnisse

- *TIR1* in lateraler Wurzelbildung



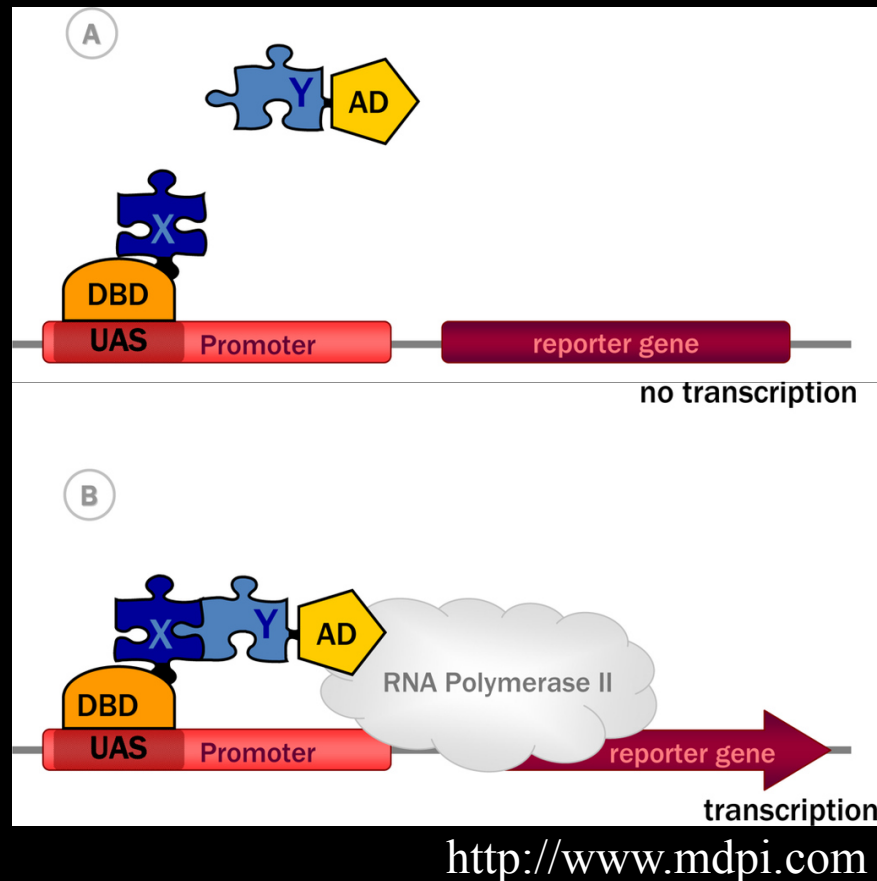
LR: Lateral roots

CGF: *cyc1At-gus* foci



Ergebnisse

- Methode: Yeast two-hybrid



Interaktionspartner TIR1?

Gal4 DNA-binding domain-TIR1

→ bait

Interaktionspartner = Prey

Histidin-Mangelmedium

→ Zwei Klassen (Klone)
identifiziert

→ cDNA sequenziert

→ ASK1 und ASK2

Ergebnisse

- Sequence alignment

ASK2	~~~~~	~~~~~	~~~~~	~~MSTVRKIT	LKSSDGENFE
ASK1	~~~~~	~~~~~	~~~~~	~~MS~ARKIV	LKSSDGESFE
HsSklp1	~~~~~	~~~~~	~~~~~	~~~~~MPSIK	LQSSDGEIFE
ScSklp1	MVTSNVVLVS	GEGERFTVDK	KIAERSLLLK	NYLNDMHDSN	LQNNSDSDSD
ASK2	IDEAVALESQ	TIKHIE...	..DDCTDNGI	PLPNVTSKIL	SKVIEYCKRH
ASK1	VEEAVALESQ	TIHHIVE...	..DDCVDNGV	PLPNVTSKIL	AKVIEYCKRH
HsSklp1	VDVETAKOSV	TIKTHLEDLG	MDDEGDDDEV	PLPNVETAIL	RKVLQWCTEH
ScSklp1	SDSETNERSK	DNNNGDDDE	DDDEIV...M	PVPNVSSVL	QKVIENAKEH
ASK2	VEEAKSETT	ADAPATTTT	TVASGSSDED	LKNDSEFVK	VDQGTFLDLI
ASK1	VEEAKAKAA	VEGAA.....	TSDDD	LKNDADFVK	VDQATLFEI
HsSklp1	KDDPPPPED.		DENKERTDD	IPVNDSEFVK	VDQGTFLFELI
ScSklp1	RDSNFPDED	DDSRKSAP..		VDSNDSEFVK	VDQATLFEI
ASK2	LAANYLNIKG	LLDLTCQTVA	DMIKGKTPEE	IRKTFNIKND	FTPEEEEEVR
ASK1	LAANYLNIKN	LLDLTCQTVA	DMIKGKTPEE	IRKTFNIKND	FTPEEEEEVR
HsSklp1	LAANYLDIKG	LLDVTCQTVA	KMIKGKTPEE	IRKTFNIKND	FTEEEEQVR
ScSklp1	LAANYLNIKP	LLDAGCKVVA	EMIIGKSPEE	IRKTFNIVND	FTPEEEAAR
ASK2	RENQWAFE*				
ASK1	RENQWAFE*				
HsSklp1	RENQWCEK				
ScSklp1	RENWADR				

Homologie

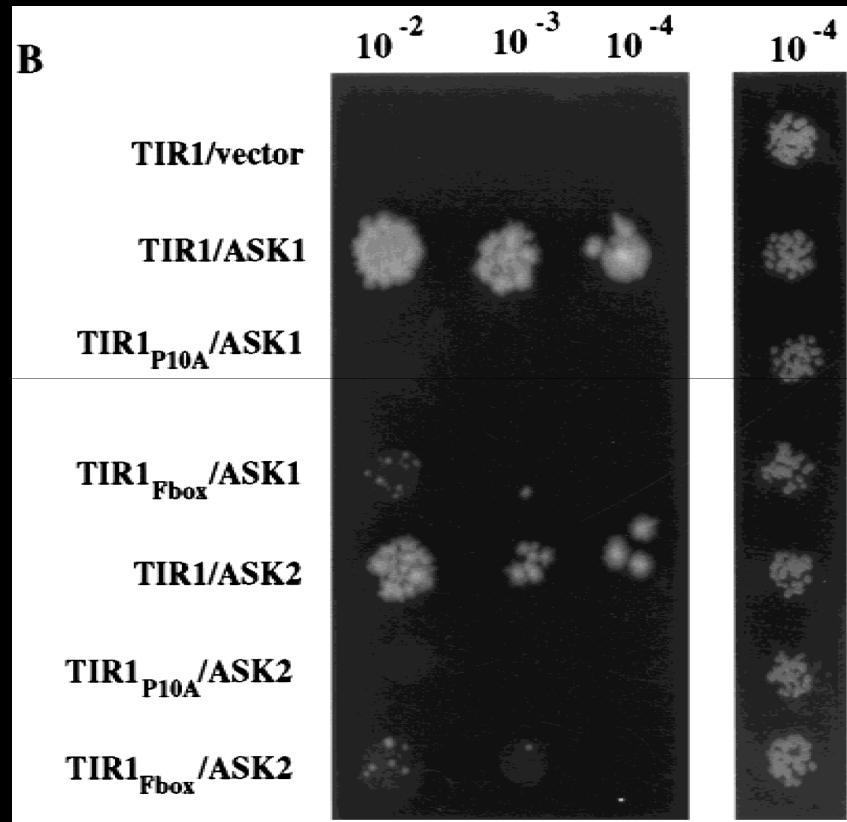
ASK1 und ASK2

HsSklp1

ScSklp1

Ergebnisse

- *TIR1* interagierende Proteine



Hefen mit unterschiedlichen two-hybrid Plasmiden (10d)

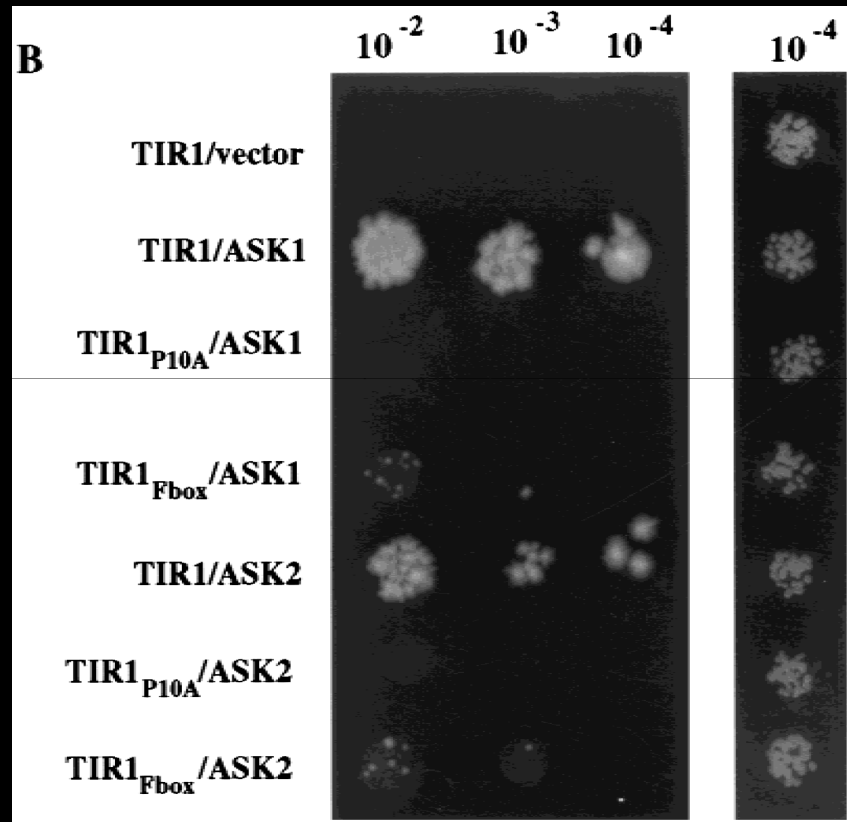
Negativkontrolle auf Vollmedium (3d)

P10A = F-Box Mutiert

Fbox = Nur F-Box

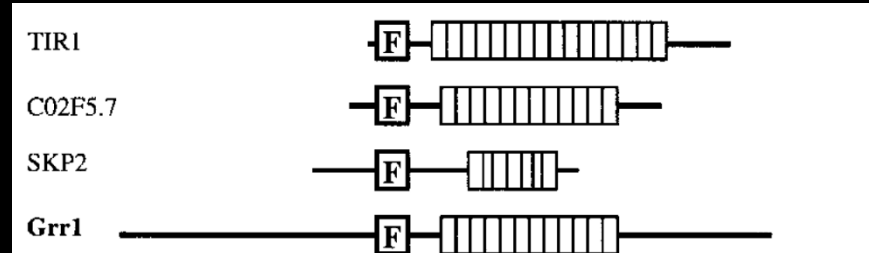
Ergebnisse

- *TIR1* interagierende Proteine



P10A = F-Box Mutiert

Fbox = Nur F-Box

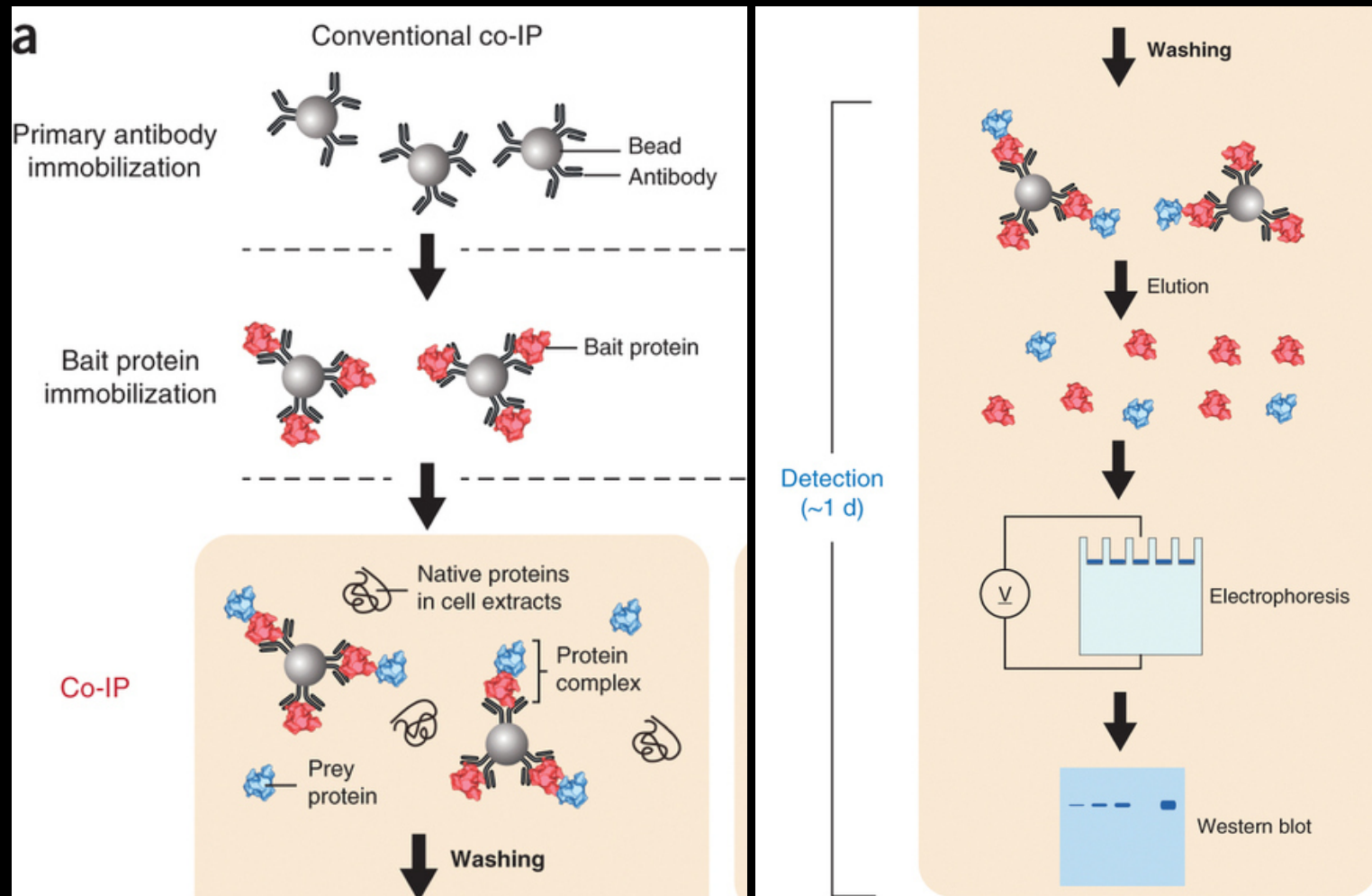


Hefen mit unterschiedlichen two-hybrid Plasmiden (10d)

Negativkontrolle auf Vollmedium (3d)

Ergebnisse

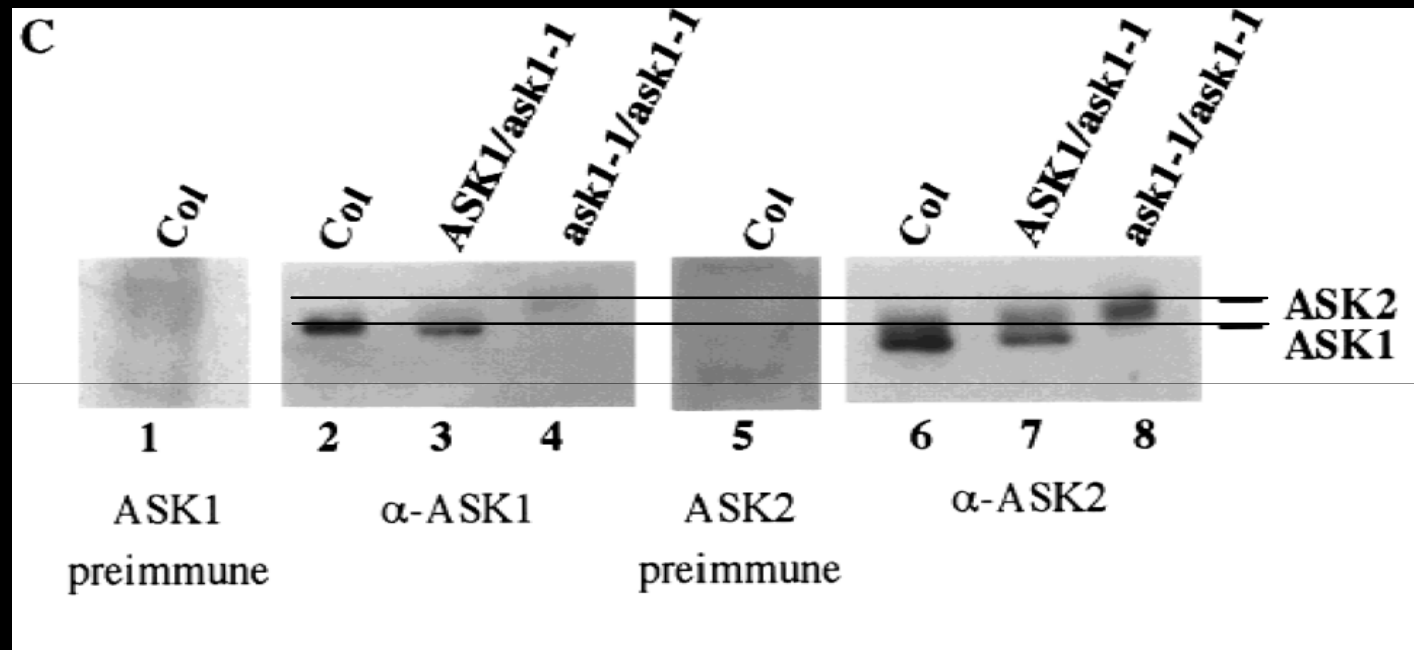
- Methode: Coimmunoprecipitation



<http://www.nature.com/nprot/journal/v8/n10/images/nprot.2013.116-F1.jpg>

Ergebnisse

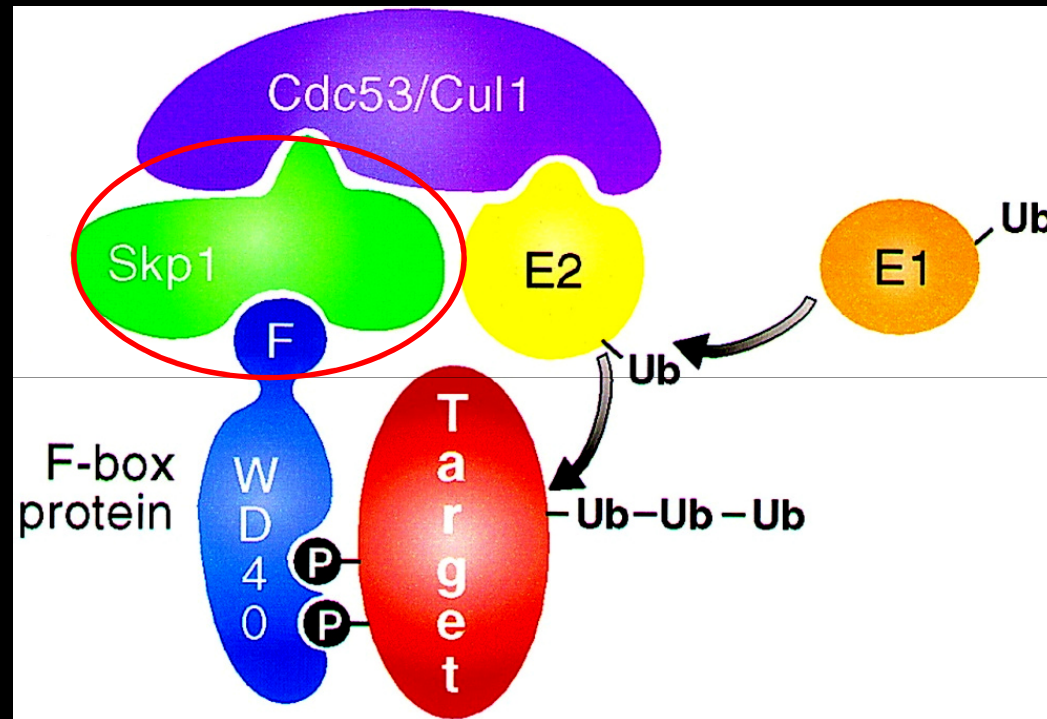
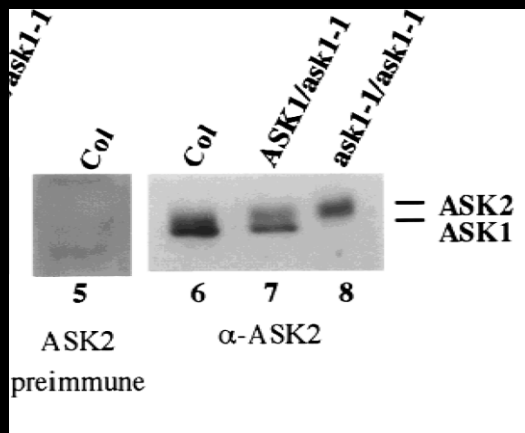
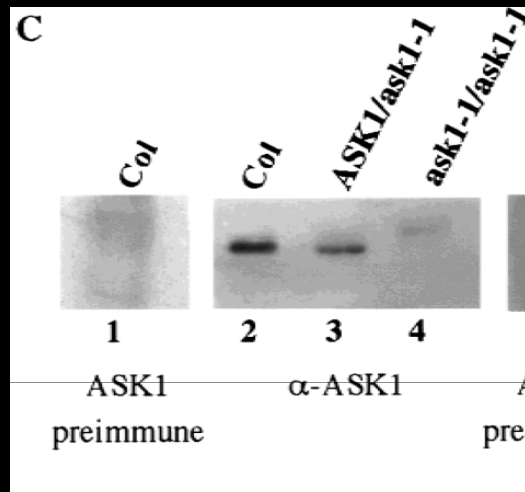
- *TIR1* bildet SCF-like Komplexe



Preimmune?

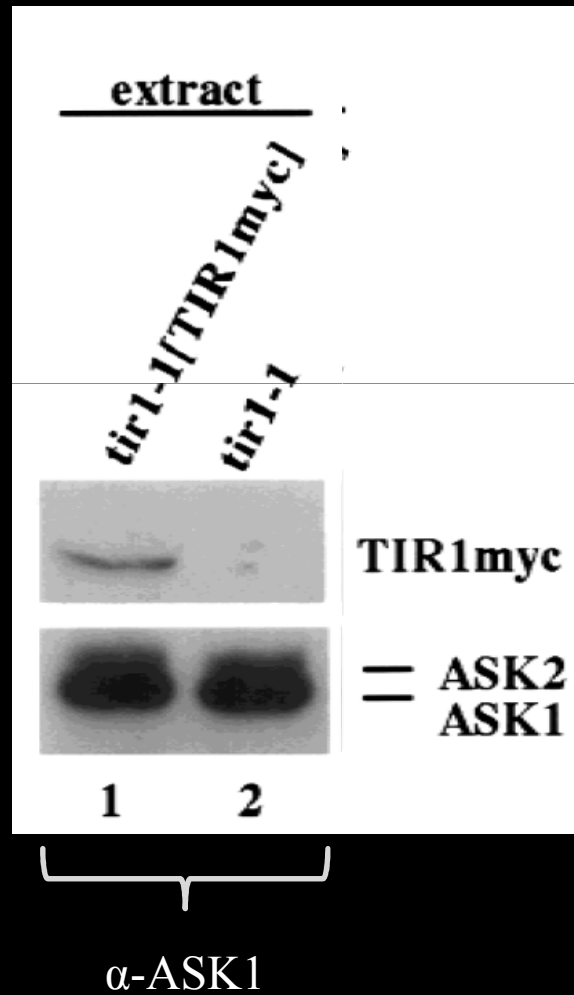
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



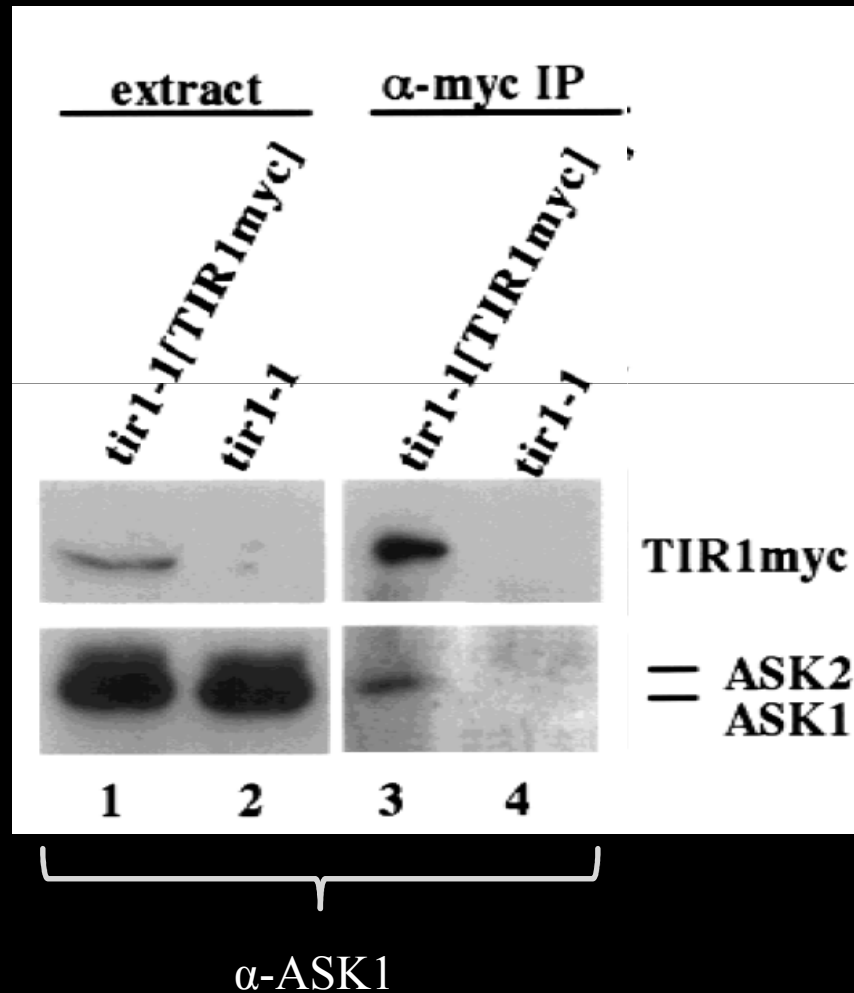
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



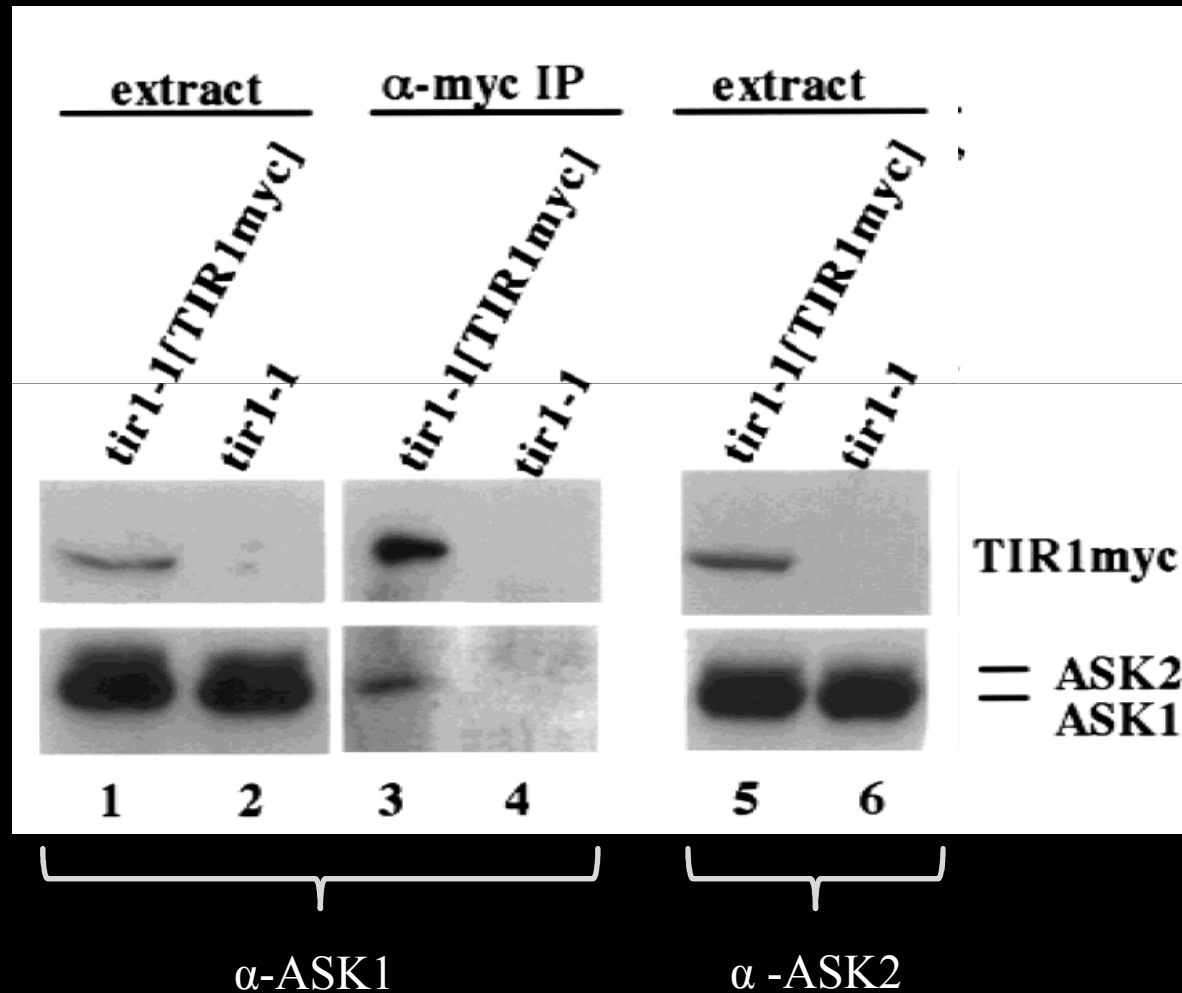
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



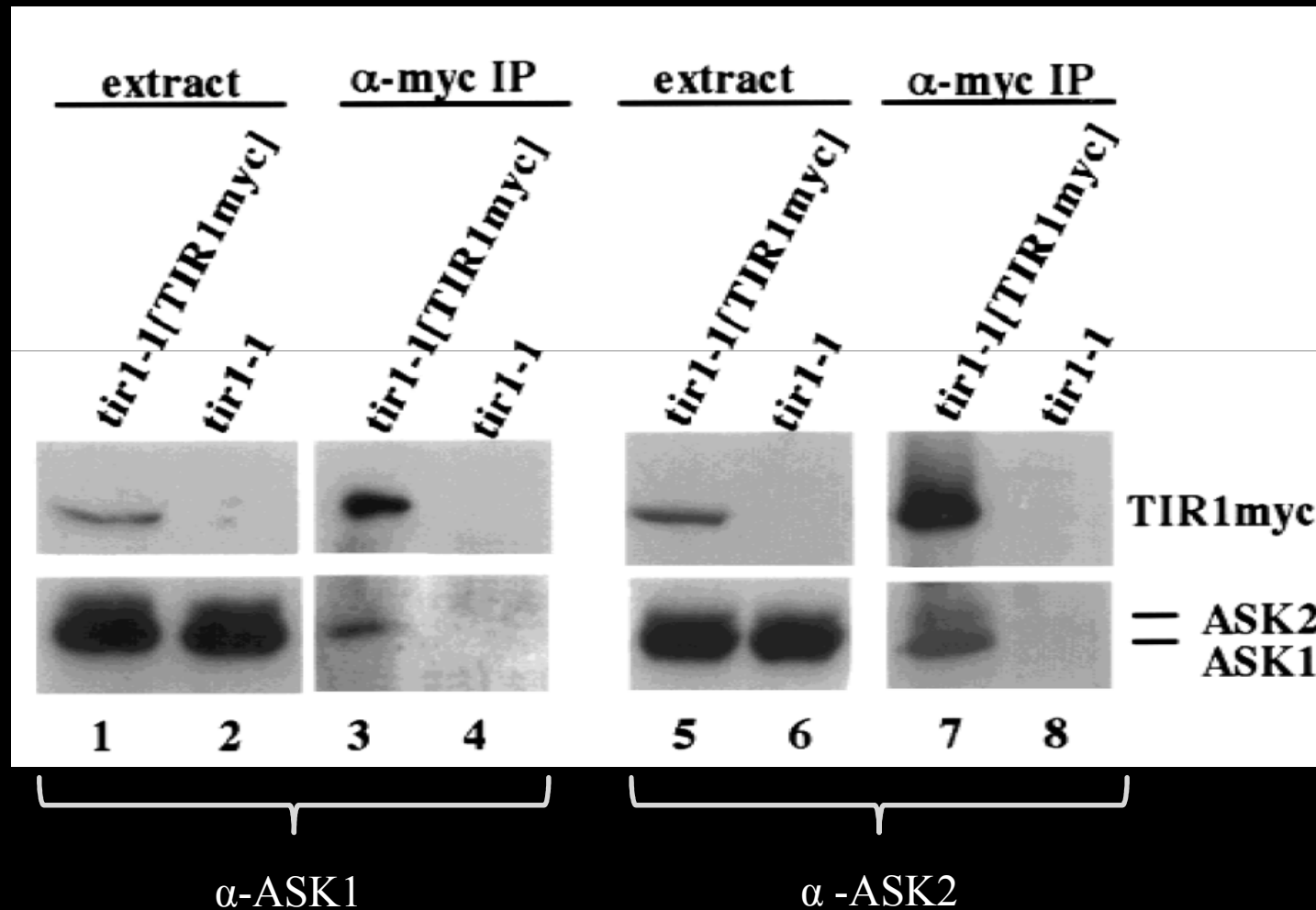
Ergebnisse

- *TIR1* bildet SCF-like Komplexe

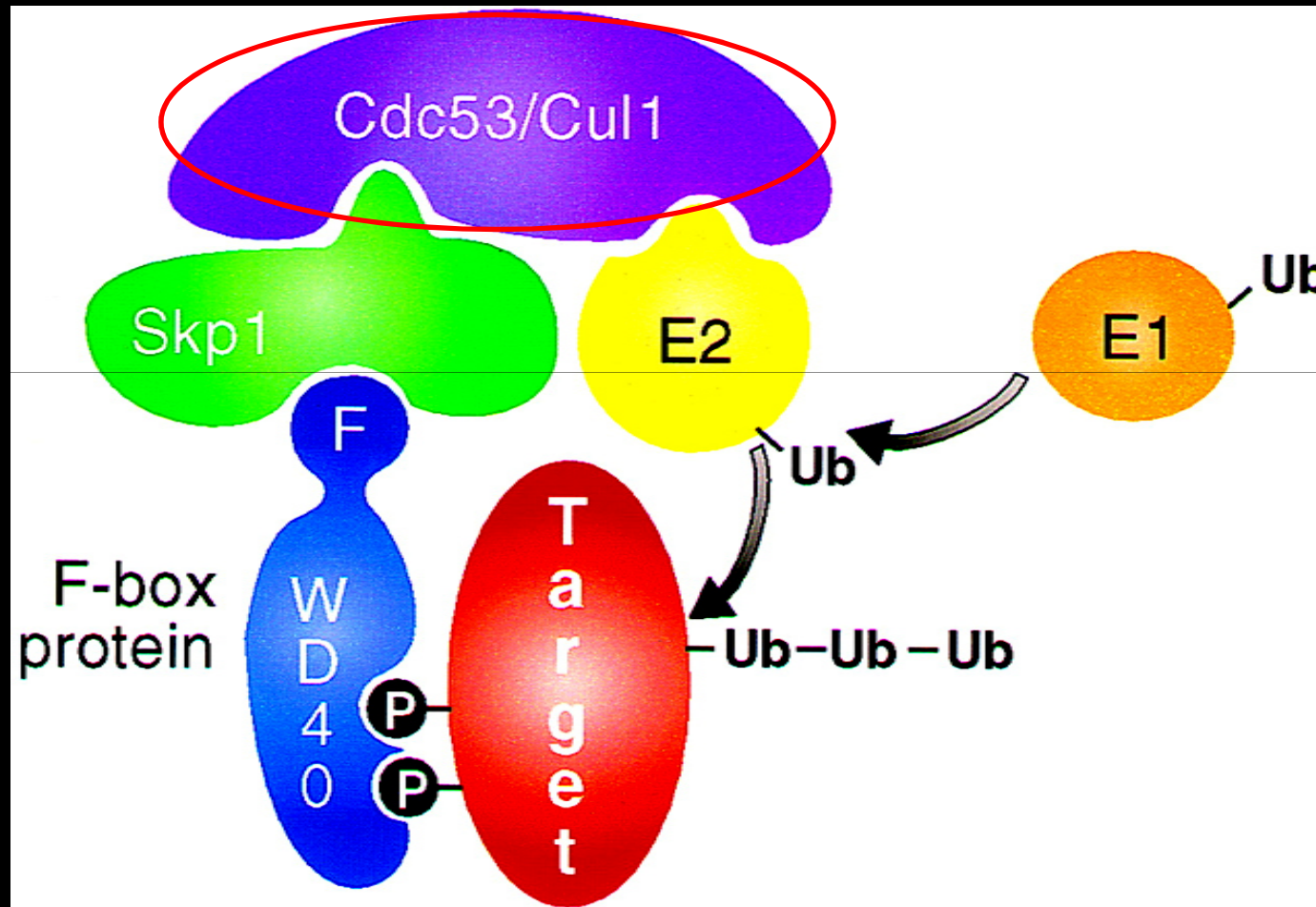


Ergebnisse

- *TIR1* bildet SCF-like Komplexe



Ubiquitin-Ligase-Komplex (SCF)



(Mantis 1999)

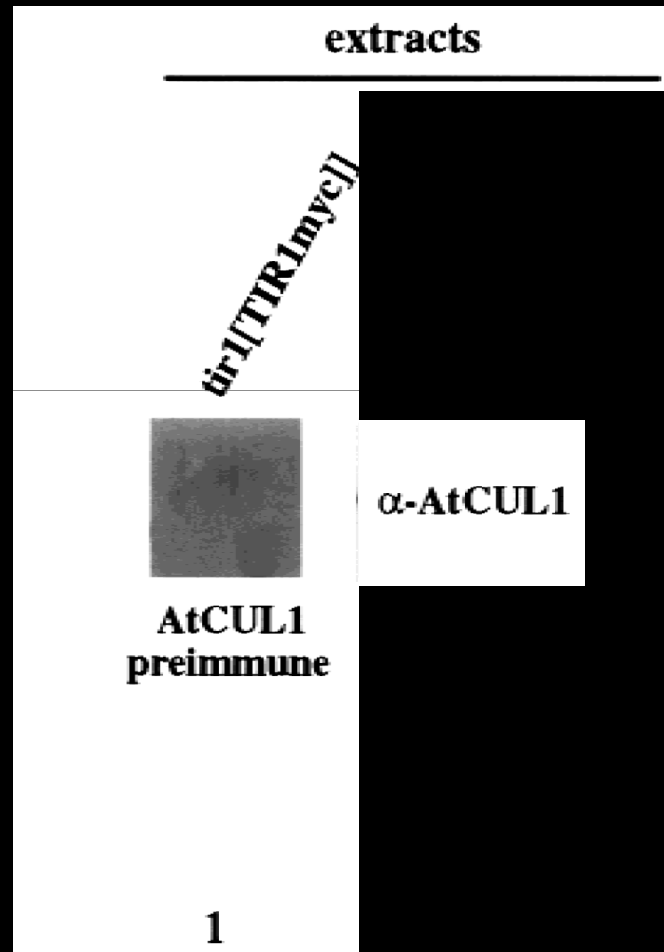
Ergebnisse

- *TIR1* bildet SCF-like Komplexe

A	AtCul1	~~~~~	~~~~~	~~~~~	MERRTIDLEQ	GWDYMQTGIT	RLKRILEGLN
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	MSETLPRSD	LEATWNFIEP	GINQILGNK	NQASTSKRVY	KILSPITWYME	~~~~~
	AtCul1	EPAFDSEQYM	MLYTTIYNMC	EQKPPHDYSQ	QLYDKYREAF	EYINSTVMP	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	VYTAIYNYCV	NKSRSSGHFS	EDSRFGOSTI	LVGSEIYEKL	KNYLKNYIL	~~~~~
	AtCul1	ALREKHDEPM	PRELPKRWGN	HKVMVRNLSR	PEYVLDYVFI	ARPS	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	NFKQSNSETF	FOFYVKEWKR	FTIGALFLEH	ADYMHRYMV	OKERSDGEHE	~~~~~
	AtCul1	LPPLREVGPT	CFRDLVYNEL	HSKVKQAVIA	LVDKEEGEQ	EDRALLKNVL	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	IFDVNTLQEM	TWKEVMFDPS	KDVLINELLD	QVTLGREGQI	EDRSNISTAI	~~~~~
	AtCul1	DIYVEINGMG	QMER	NEEDFESFMI	QDTSSIYSRK	ASSWIQEDSC	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	KSLVALGIDP	QDLKKLNLMV	HIQVFEKPFL	KKQOEYTOY	TMDYLEKESV	~~~~~
	AtCul1	PDYMLKSEEC	LKQERERVAH	YLHSSSEPKL	VEKVOHELIV	VFAEQLEKE	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	PEYLANEVSKR	LEEECDREVIT	YLDHSTOKPL	IACQVKOLIG	ELHTAILOK	~~~~~
	AtCul1	TEYIFEAHSI	IKREBKANTI	YADDERKKPL	SMALRVLTIT	DE....IEKL	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	ENEFVVLLDA	RDIEKITSTY	ALIRROFTLI	PRMASVEEYH	VKKGENEIS	~~~~~
	AtCul1	HSGCRALDRD	DAVDDLSRMY	RYHKKILRGL	EPVANIEKQH	VFAEG.....	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	..GLDELLDE	NRVPDLAQHY	QDFSVVRGCO	QALLQHWSEY	IKVFG.....	~~~~~
	AtCul1	NALVQQAEDT	ATNOVAN...	TASVQ	EOVLIRKVIE	LHDKRMVYVT	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	STLAMEKHNI	MDQENANPKK	LALMTAESLS	PKDYIKLLE	VHDIYSKIFN	~~~~~
	AtCul1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	AtCul1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	AtCul1	ECFQNETLSE	KALKEAFBIE	CH.....	..KIVACSSS	AELLATPCDN	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	ESFPDDIPLA	KALDIACGNE	ININEFALPA	GSPKSAQSKT	SE..LAKYSDI	~~~~~
	AtCul1	ELKKGSEKIL	SDAETDITL	KVVCLLAYIS	DKDQFAFYR	KDLARILLFD	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	ELKKGSEKIL	SDAETDITL	KVVCLLAYIS	DKDQFAFYR	KDLARILLFD	~~~~~
	AtCul1	NSANDDBES	ILKILKQCG	QFTSKQEGM	VTDLTAREN	QNSREDYLS	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	TSASAEDEN	ILKILKQCG	QFTSKQEGM	VTDLTAREN	QNSREDYLS	~~~~~
	AtCul1	NPAANEG..I	DLTVAVLTIG	FWP.SYKSPD	INLPSEHIKC	VEVFKCFYET	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	EPDYSAKYP	DLTPVLAEN	MWP.SYQVEE	FLPQELAVPS	HEKLESYSQ	~~~~~
	AtCul1	KTYCKRLNMI	YSLGTCHING	KFOOKAI...	BLIVSTHQA	VLLLFNTIDK	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	KHSGPKLQW	TILGHAVLEA	PEKECK...	EFQVSLPQIL	VLL..PNECDG	~~~~~
	AtCul1	LSYTELLAQL	NLSHEDLV..	RLI	ESLSCDVKKI	LKKEPNKTY	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	FTLENIQECH	STTIQHIAAA	MVPFIKPLI	QOVPGLDAL	VKPEIQKLS	~~~~~
	AtCul1	SQNDAPFFNS	RFNDRMRIK	H.....	PLPP	VDERKKVVED	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	EDGDRIFNG	EPKHKLFRIK	INQIQ.....	MKET	VEEQVSTTER	~~~~~
	AtCul1	VQDRQYQID	AAIVRINKSR	KVLGHQOLVS	EOVEQLSRMF	KPDIAKPKR	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	ENTERQFTE	ACIVRINKAK	RNLGHTLVN	ECLAQSHQRE	NAKVSMEKRA	~~~~~
	AtCul1	NEGLTARDYL	ERDKNPNMF	RYLA	~~~~~	~~~~~	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	IESLIQDYL	ERDKNPNMF	RYLA	~~~~~	~~~~~	~~~~~
	AtCul1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	AtCul1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	HsCul-4A	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	Cdc53	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~

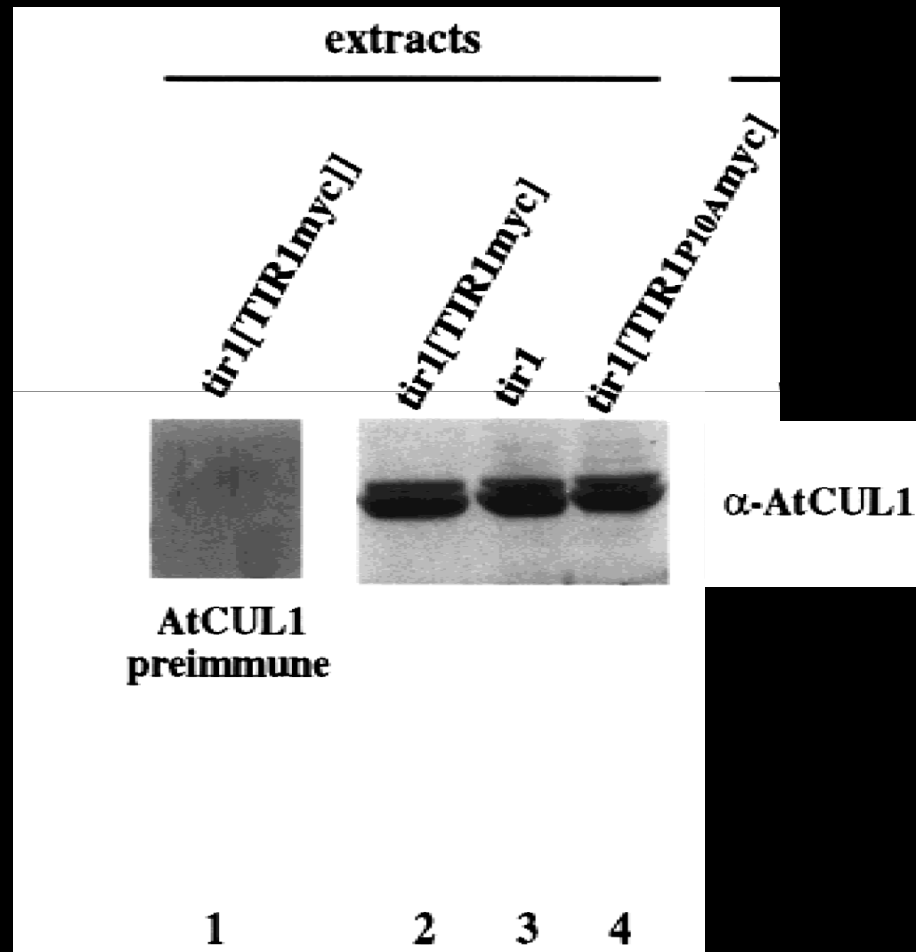
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



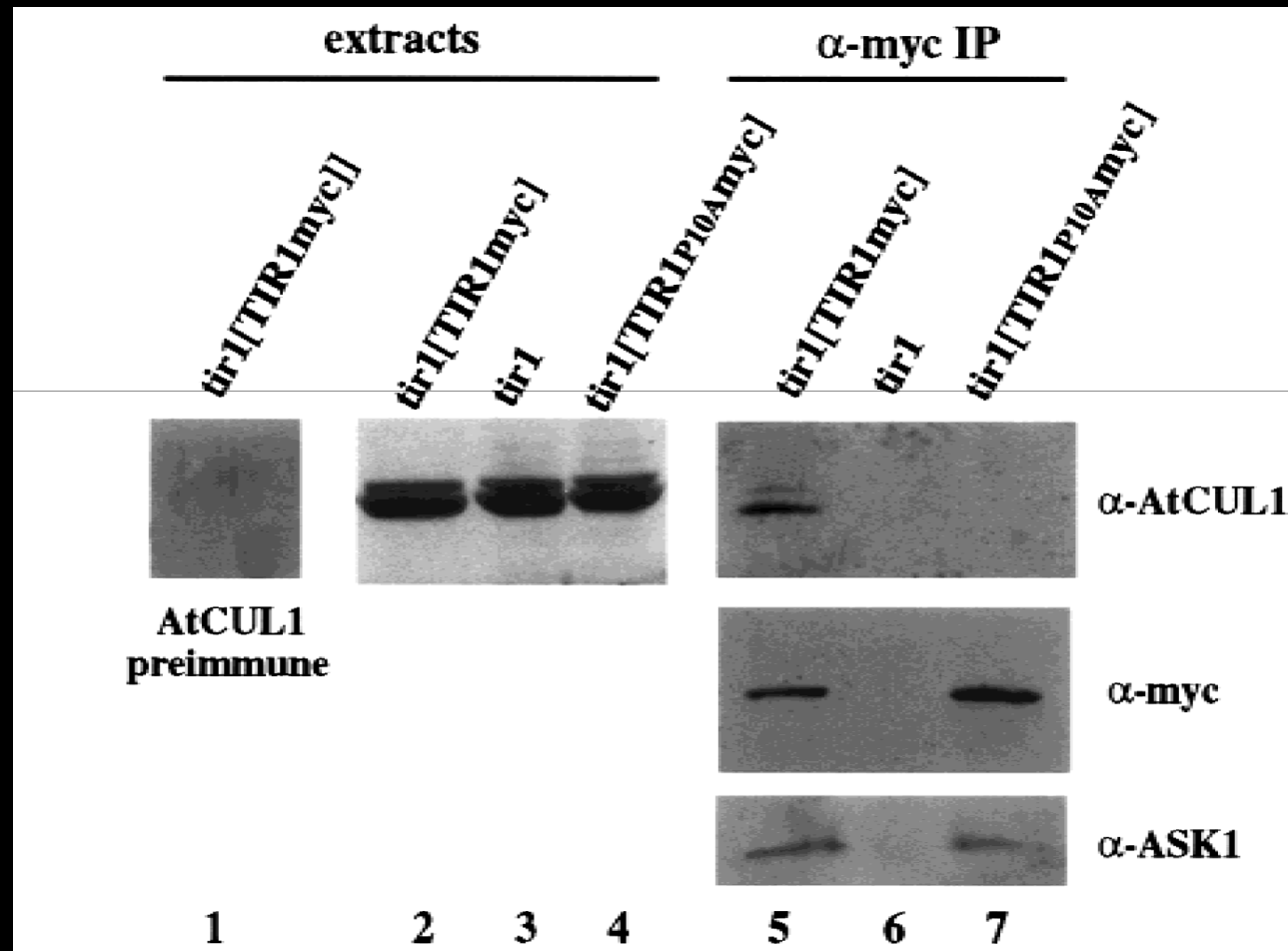
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



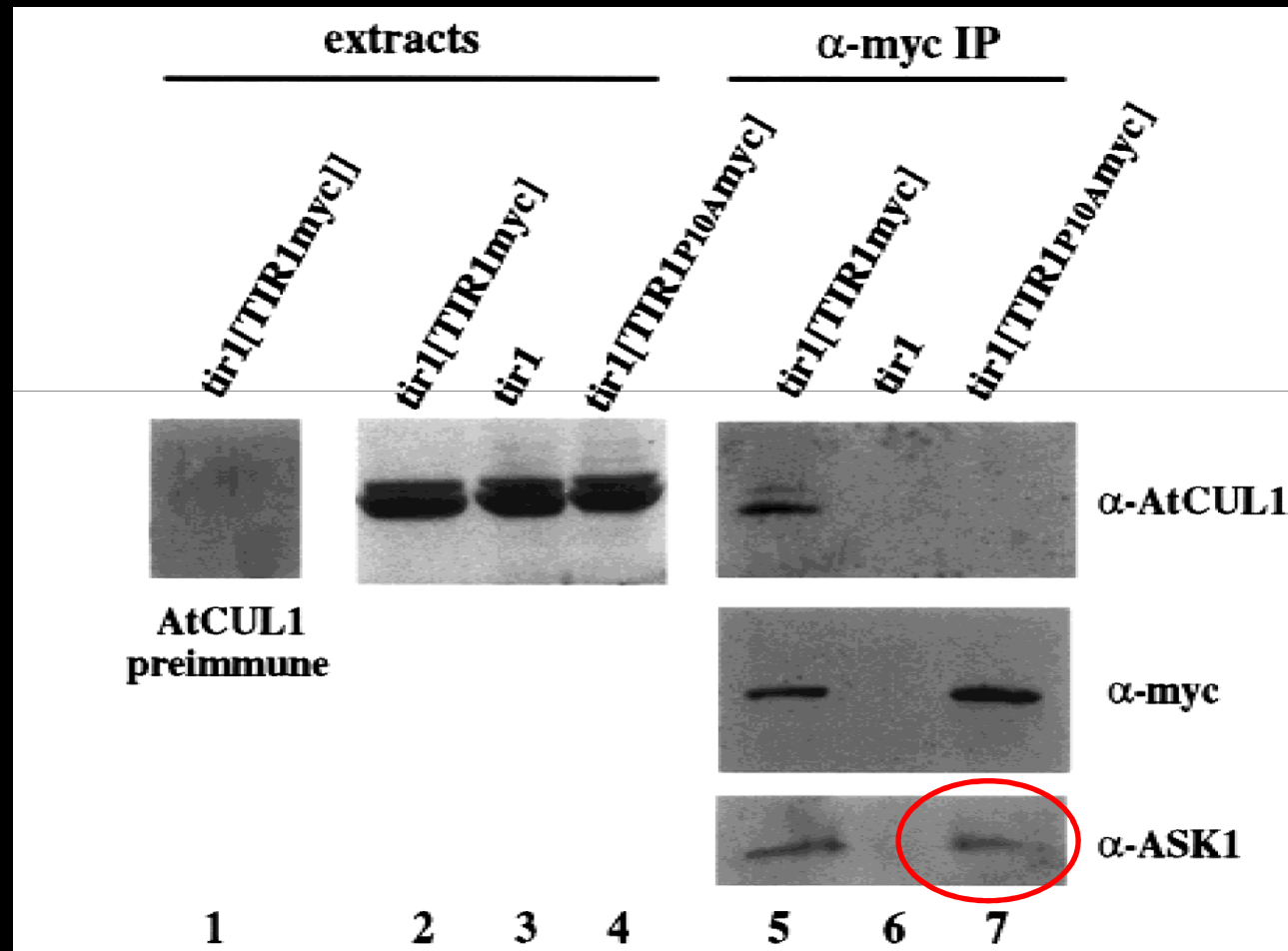
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



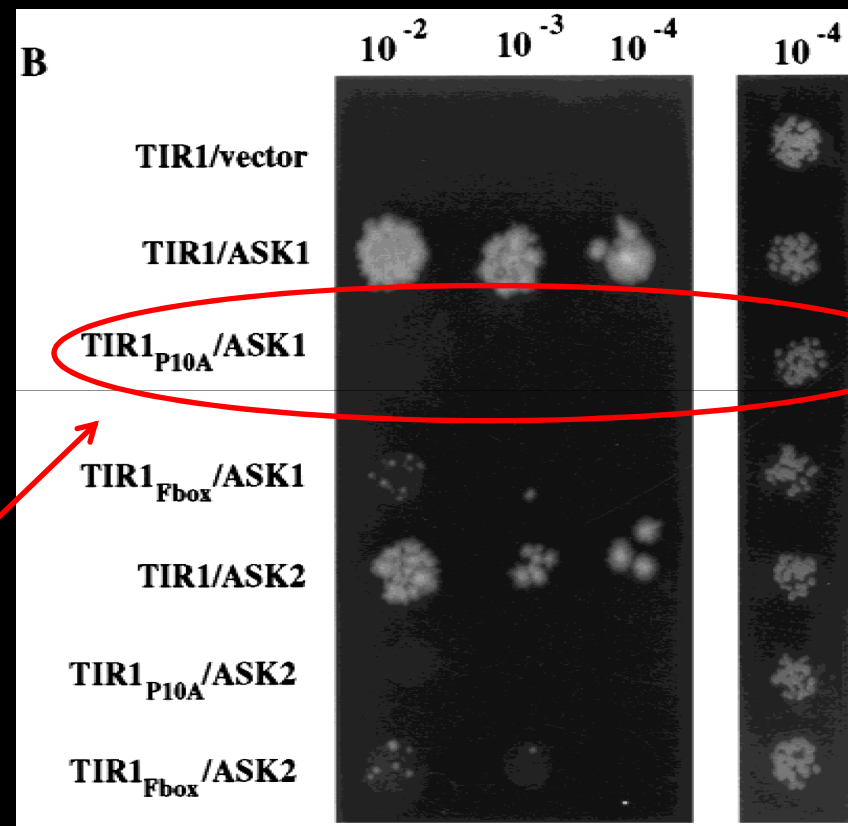
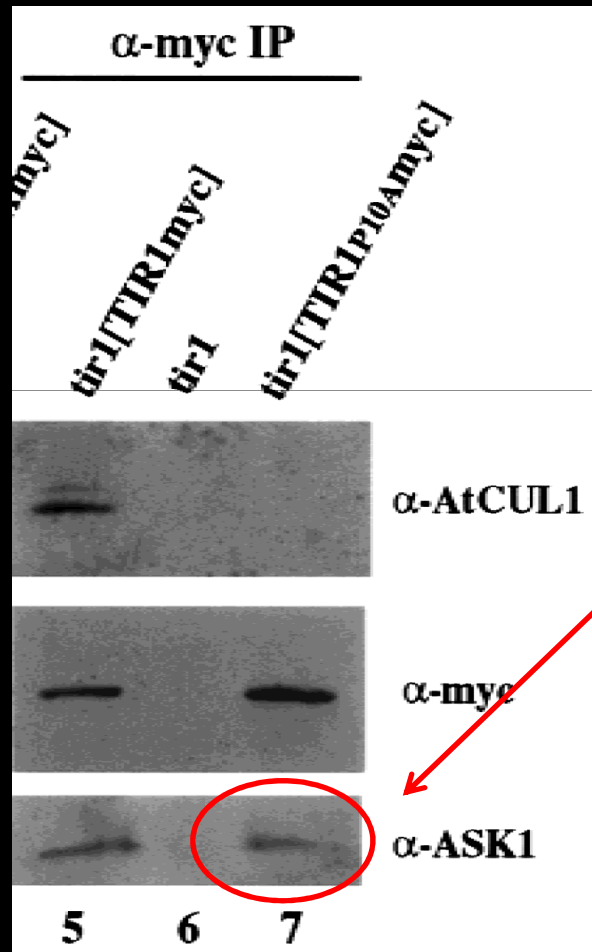
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



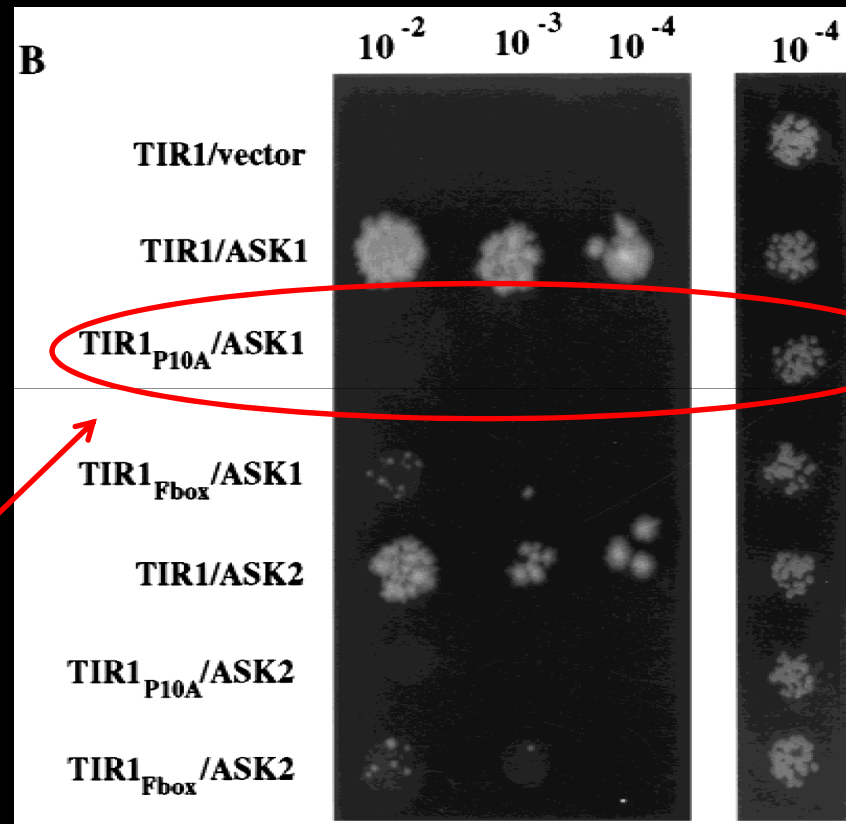
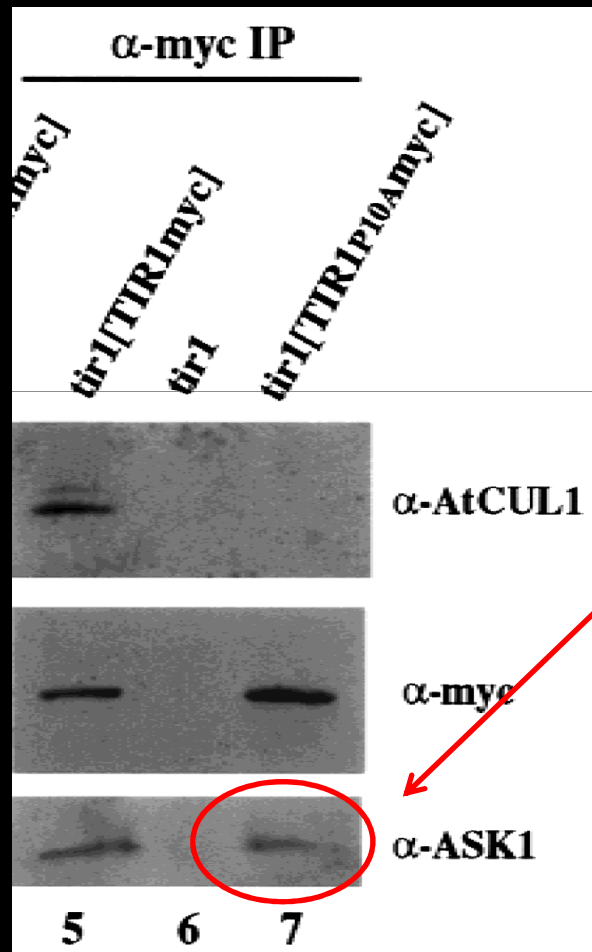
Ergebnisse

- *TIR1* bildet SCF-like Komplexe



Ergebnisse

- *TIR1* bildet SCF-like Komplexe

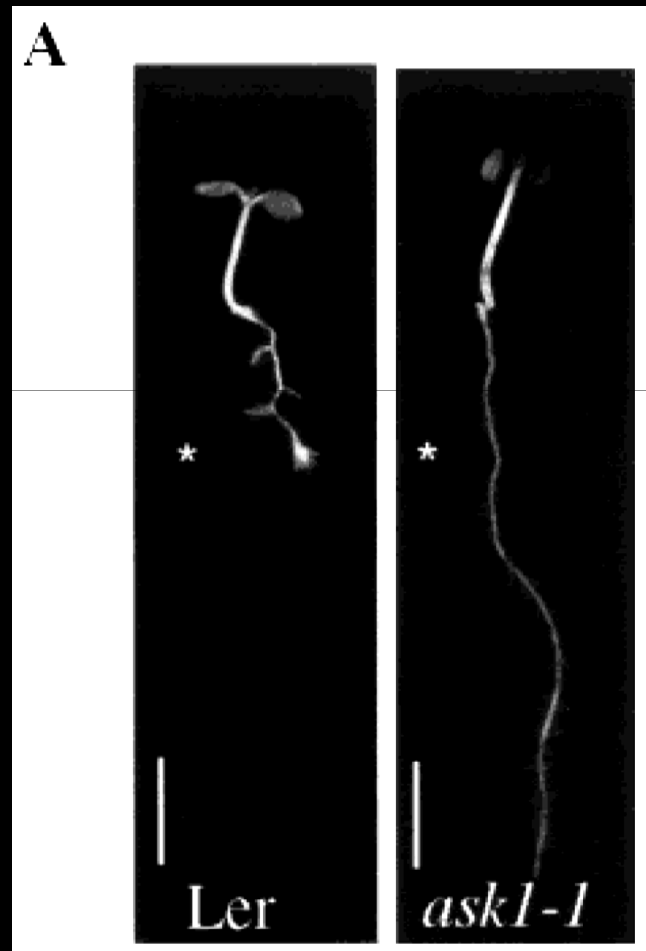


Y2H versus *in planta*

Mögliche Kompensationsreaktionen

Ergebnisse

- *TIR1* bildet SCF-like Komplexe



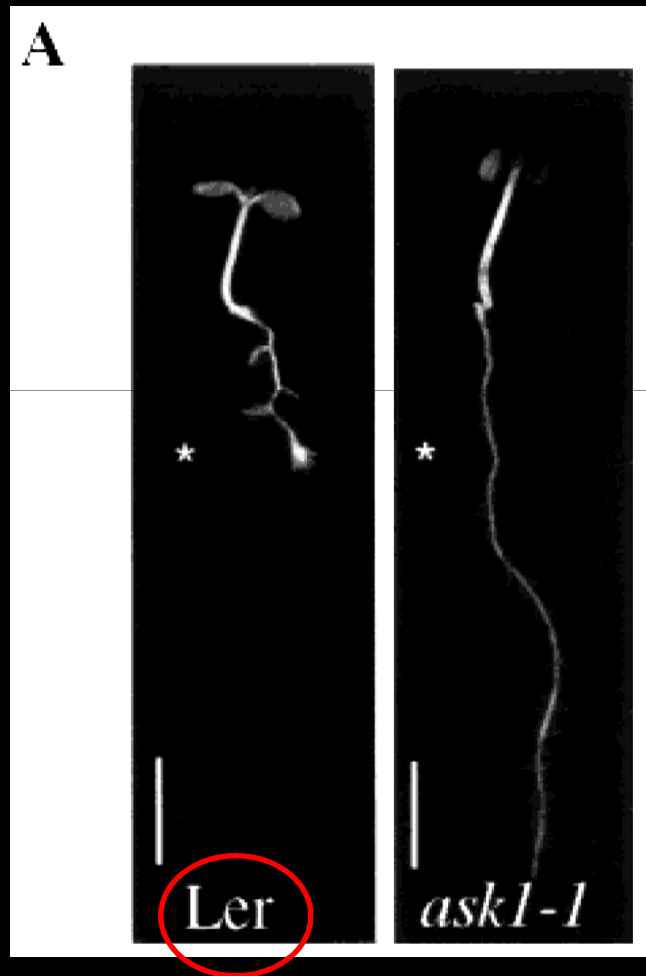
Normalmedium 4d

+ 0,085 μ M 2,4-D

* = Wurzellänge vor Applikation

Ergebnisse

- *TIR1* bildet SCF-like Komplexe



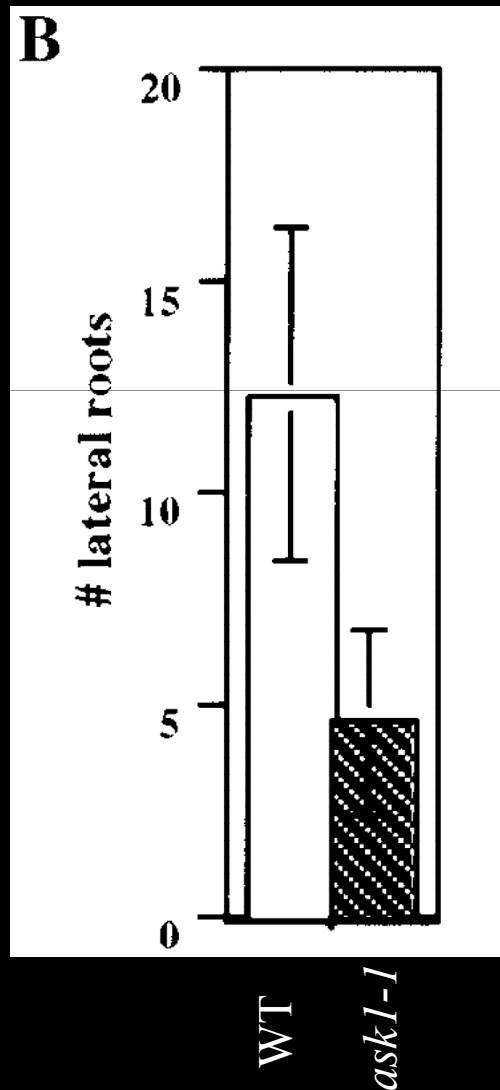
Normalmedium 4d

+ 0,085 μ M 2,4-D

* = Wurzellänge vor Applikation

Ergebnisse

- *TIR1* bildet SCF-like Komplexe

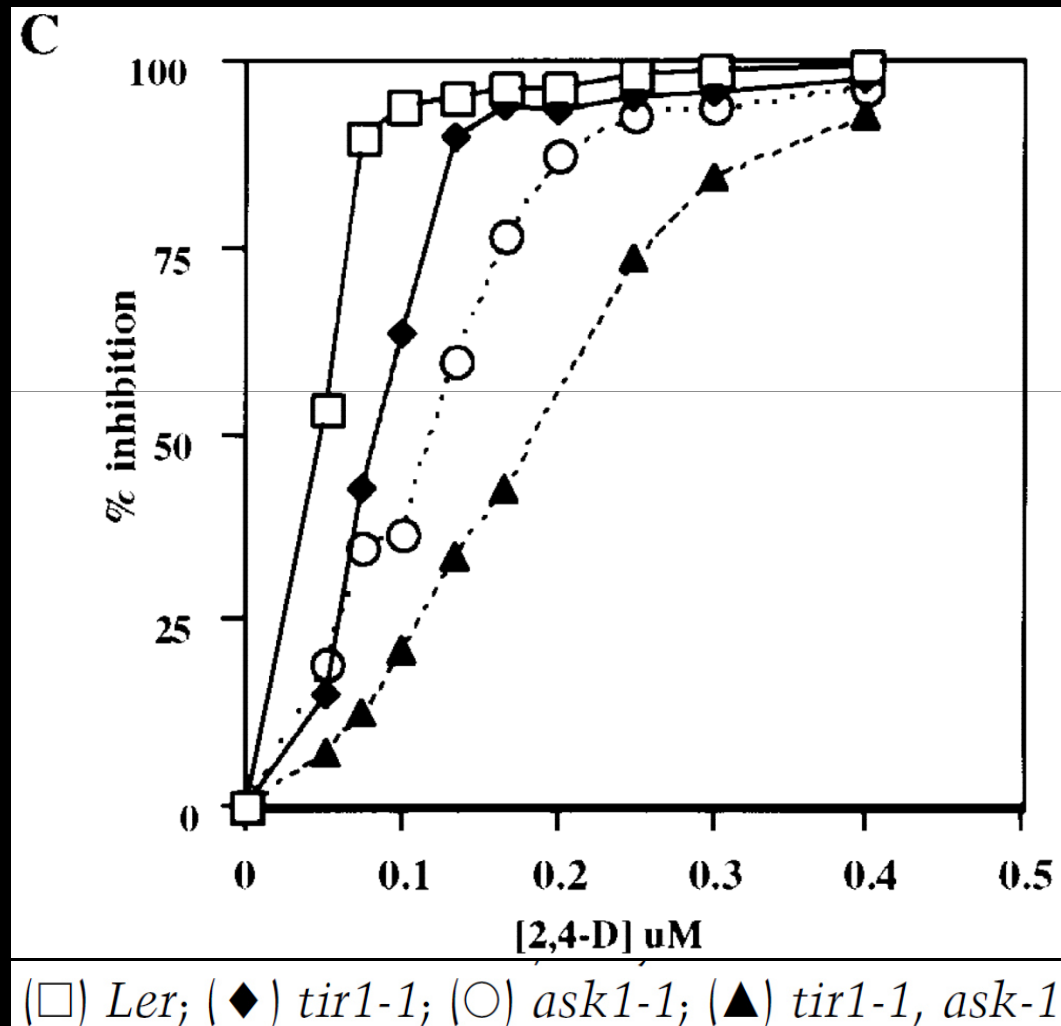


WT und Mutante auf Normalmedium (11d)

n = ?

Ergebnisse

- *TIR1* bildet SCF-like Komplexe



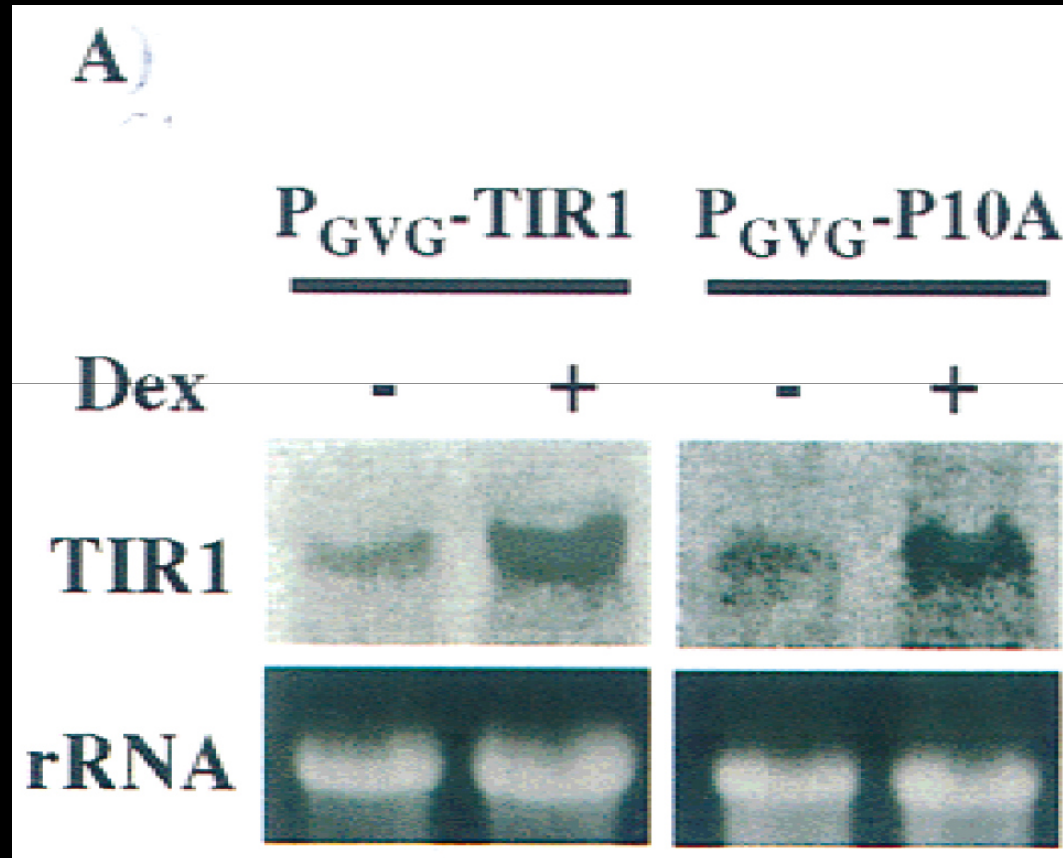
Dosiseffekt

Verminderung des
Wurzelwachstums

Jeder Punkt:
 $n = 10$, S.E $\leq 10\%$

Ergebnisse

- *TIR1* Überexpression

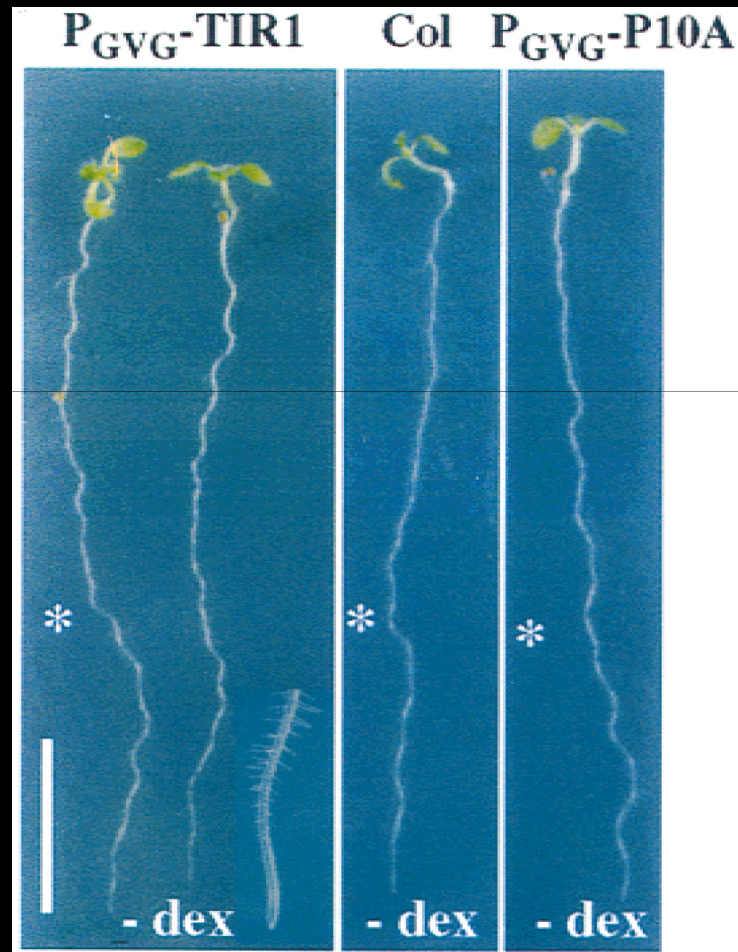


P_{GVG} = Dexamethazone
aktivierbarer
Promotor

GVG = Gal4-VP16-glucocorticoid receptor
Dex = Dexamethazone

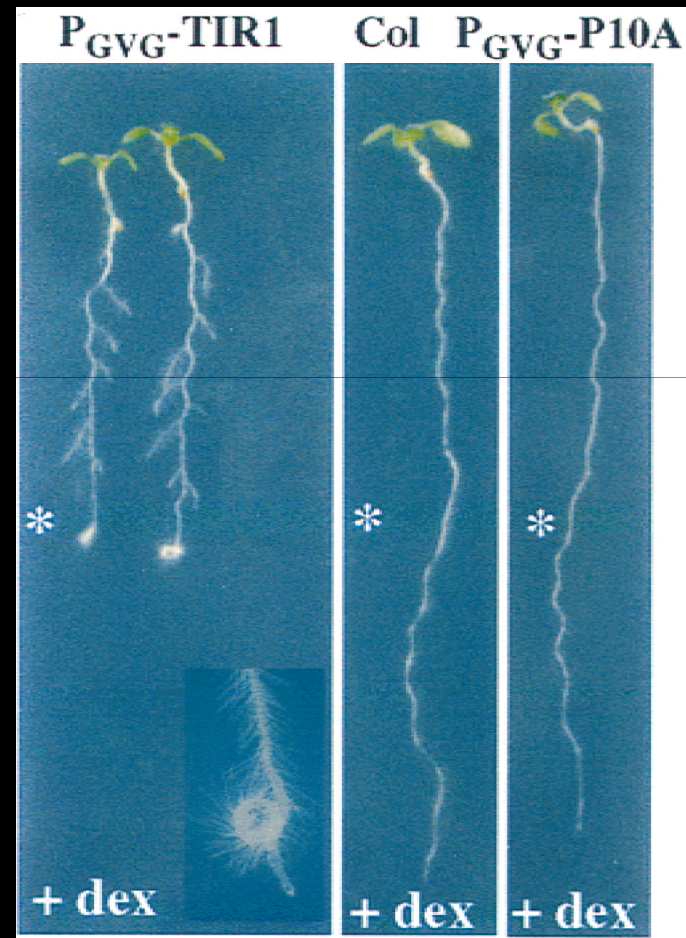
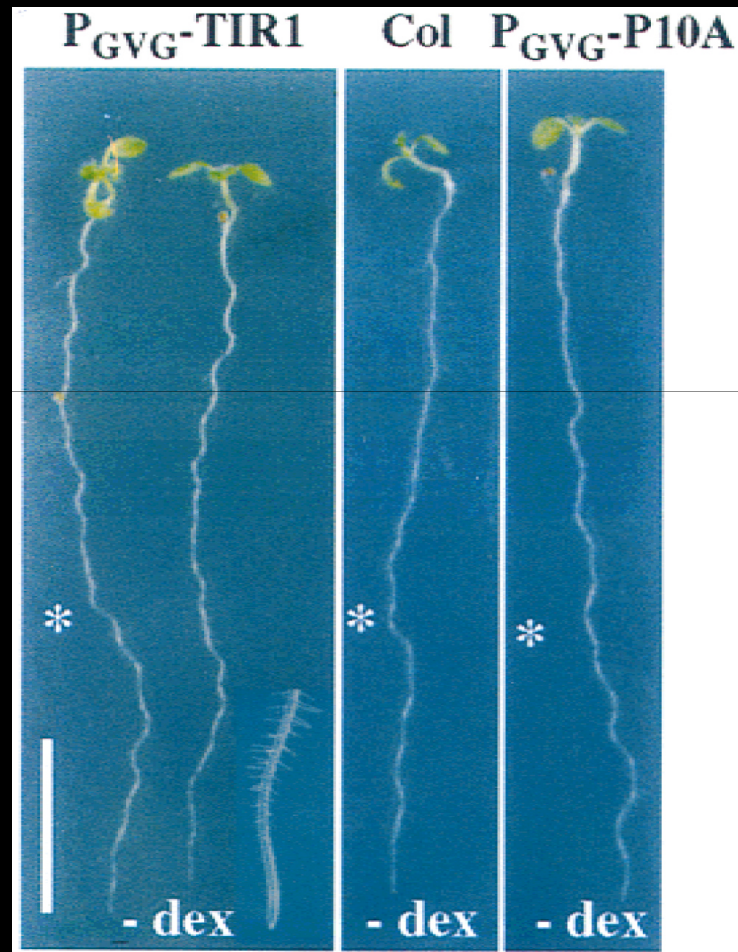
Ergebnisse

- *TIR1* Überexpression



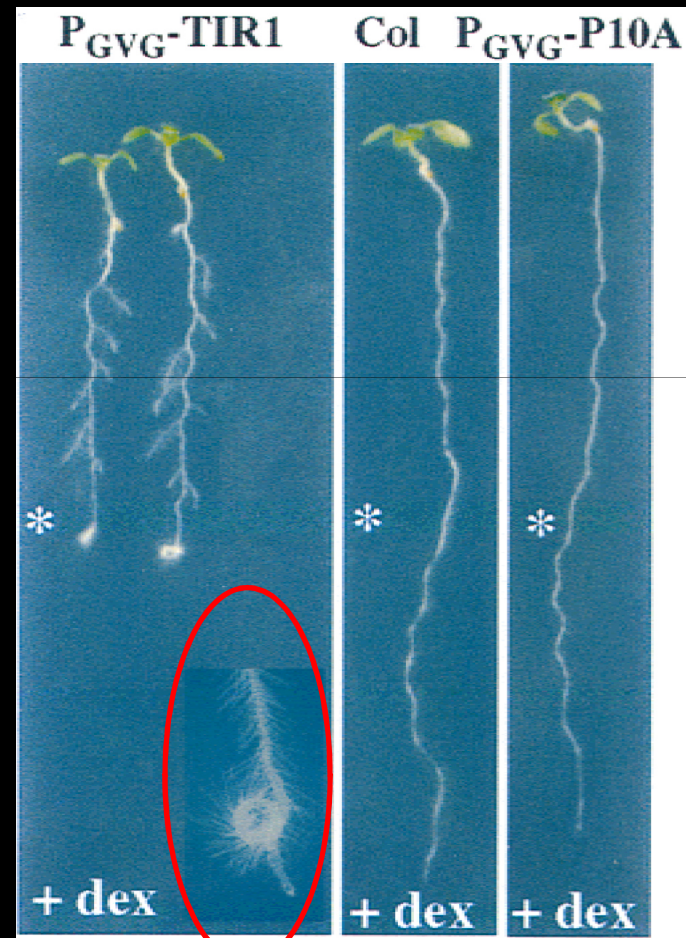
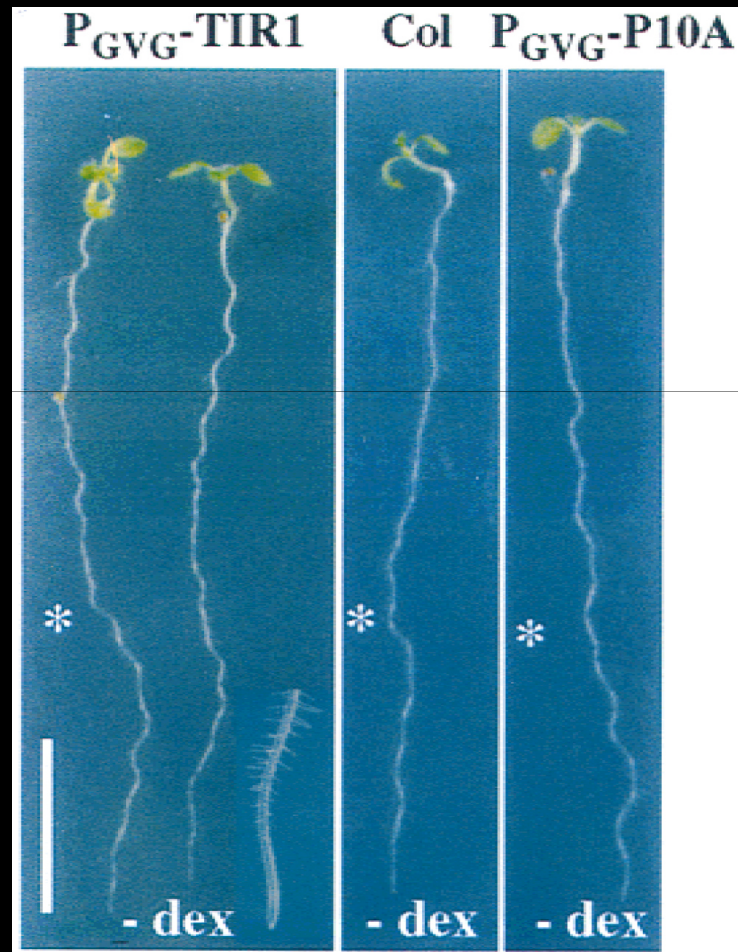
Ergebnisse

- *TIR1* Überexpression



Ergebnisse

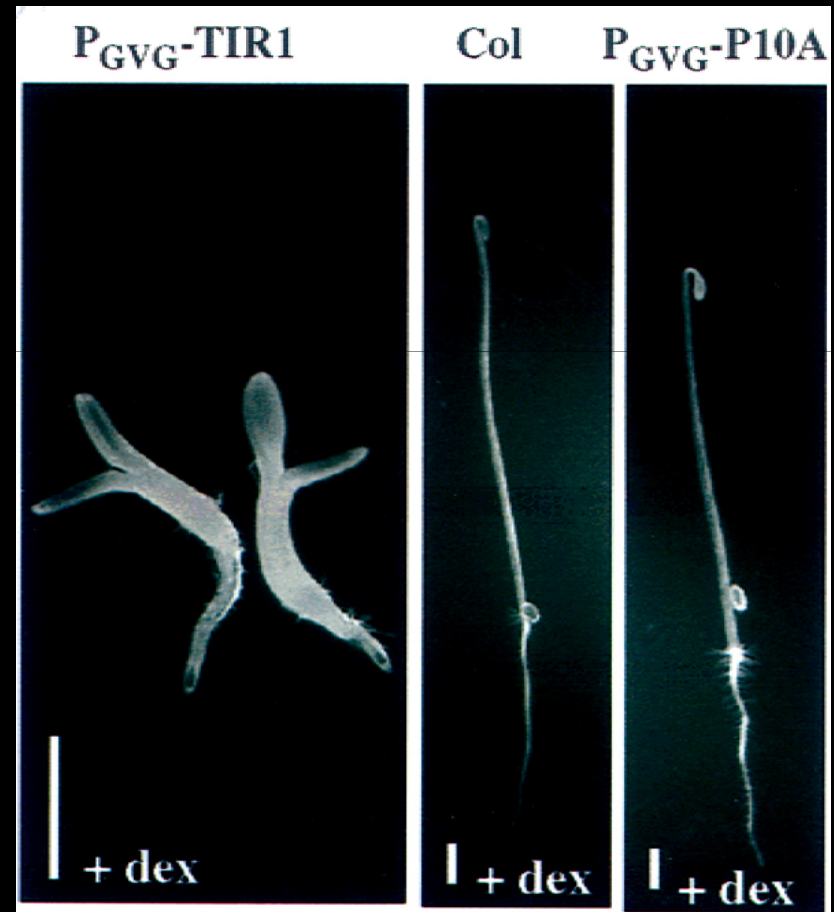
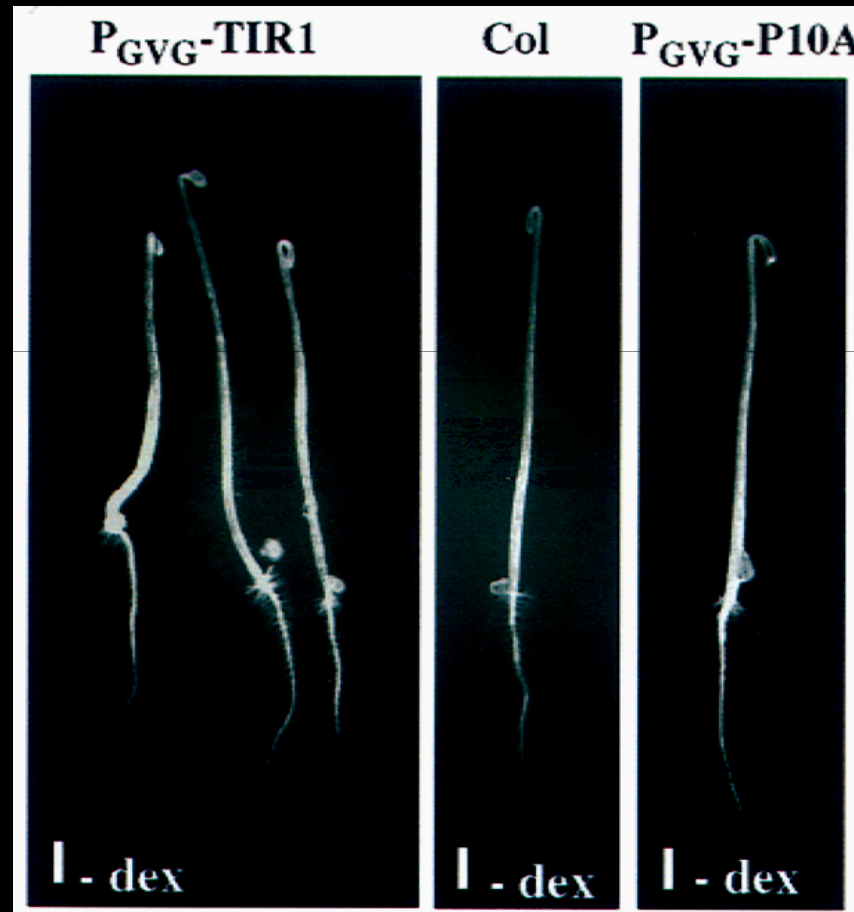
- *TIR1* Überexpression



Bekannter Phänotyp?

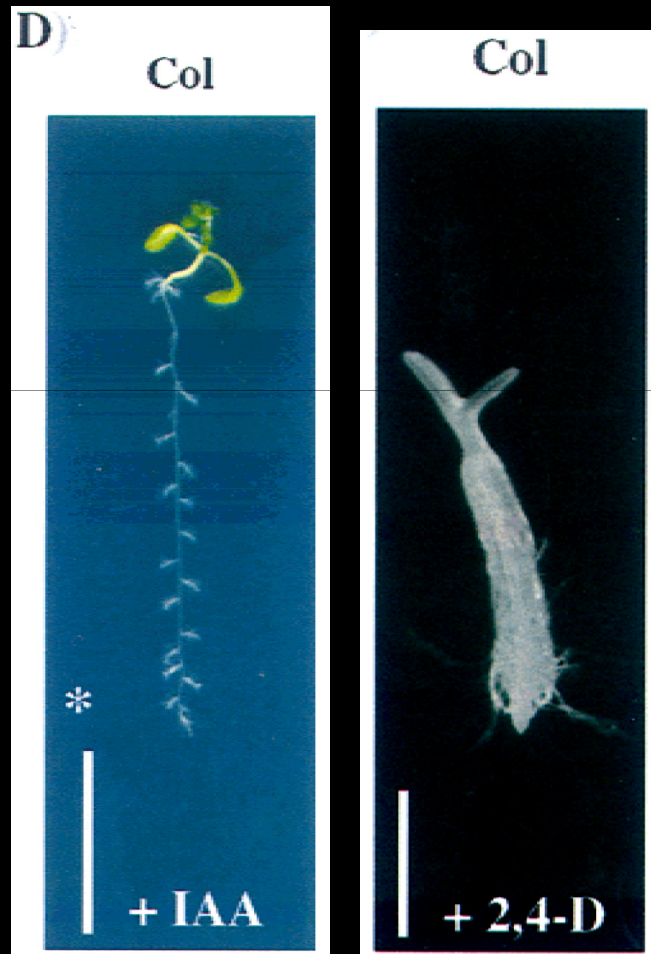
Ergebnisse

- *TIR1* Überexpression



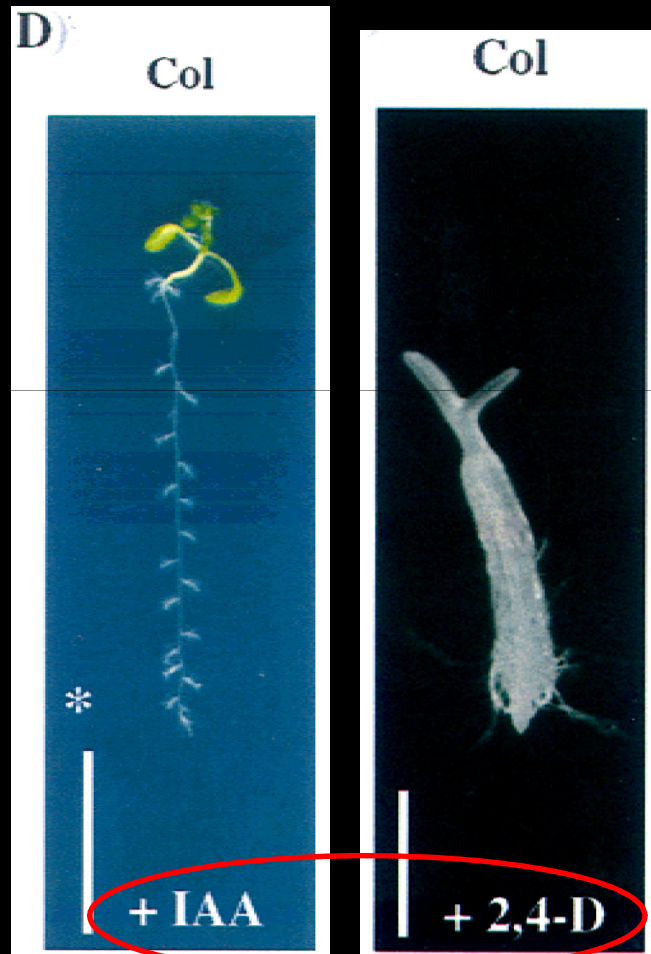
Ergebnisse

- *TIR1* Überexpression



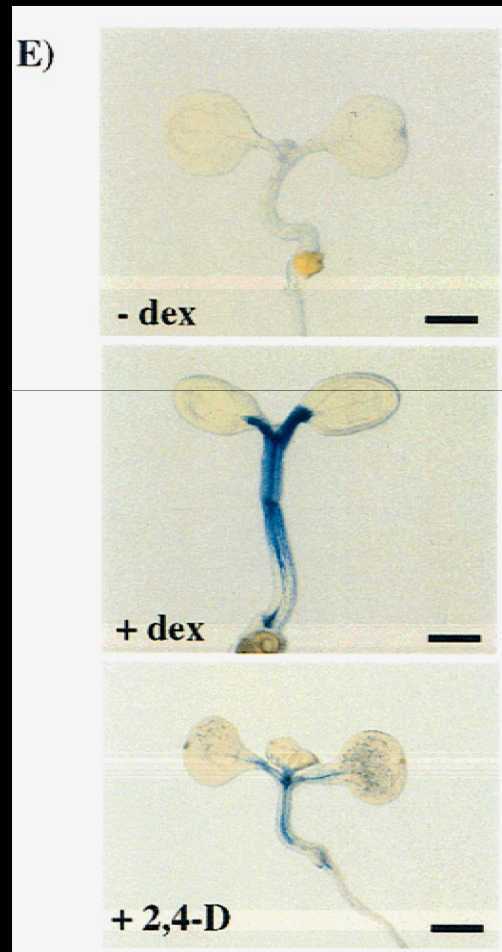
Ergebnisse

- *TIR1* Überexpression



Ergebnisse

- *TIR1* Überexpression



P_{GVG} -TIR1

WT

pIAA4-gus in *PGVG-TIR1*

Wild-type *pIAA4-gus*

pIAA4 = Auxin Reporter

Zusammenfassung

Ruegger et al. 1998

F-Box Protein TIR1 – Auxin response

TIR1 Teil eines SCF-ubiquitin-ligase Komplex?

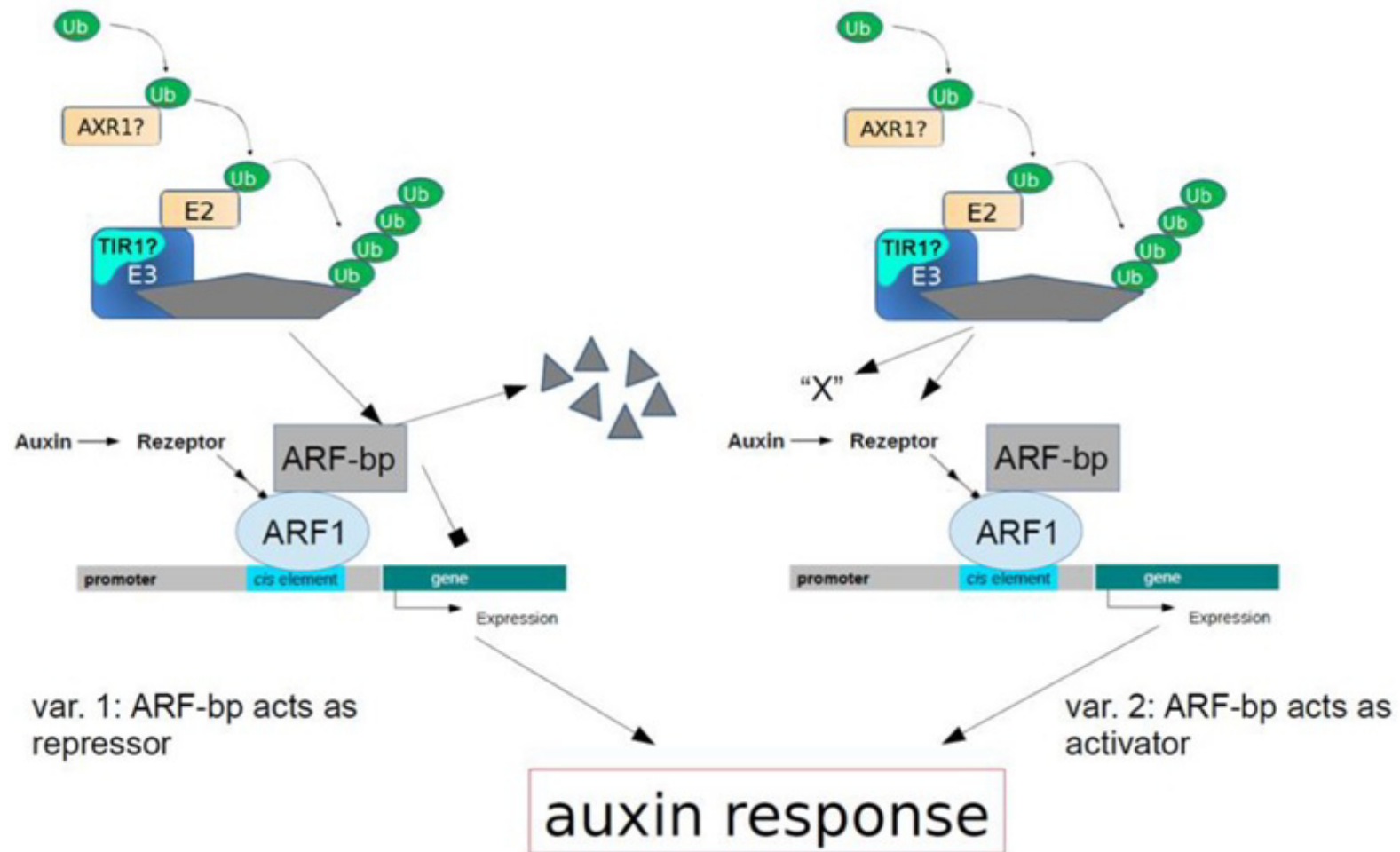
Neue Ergebnisse:

Identifizierung von *A. Skp1p* und Cdc53p-related Protein

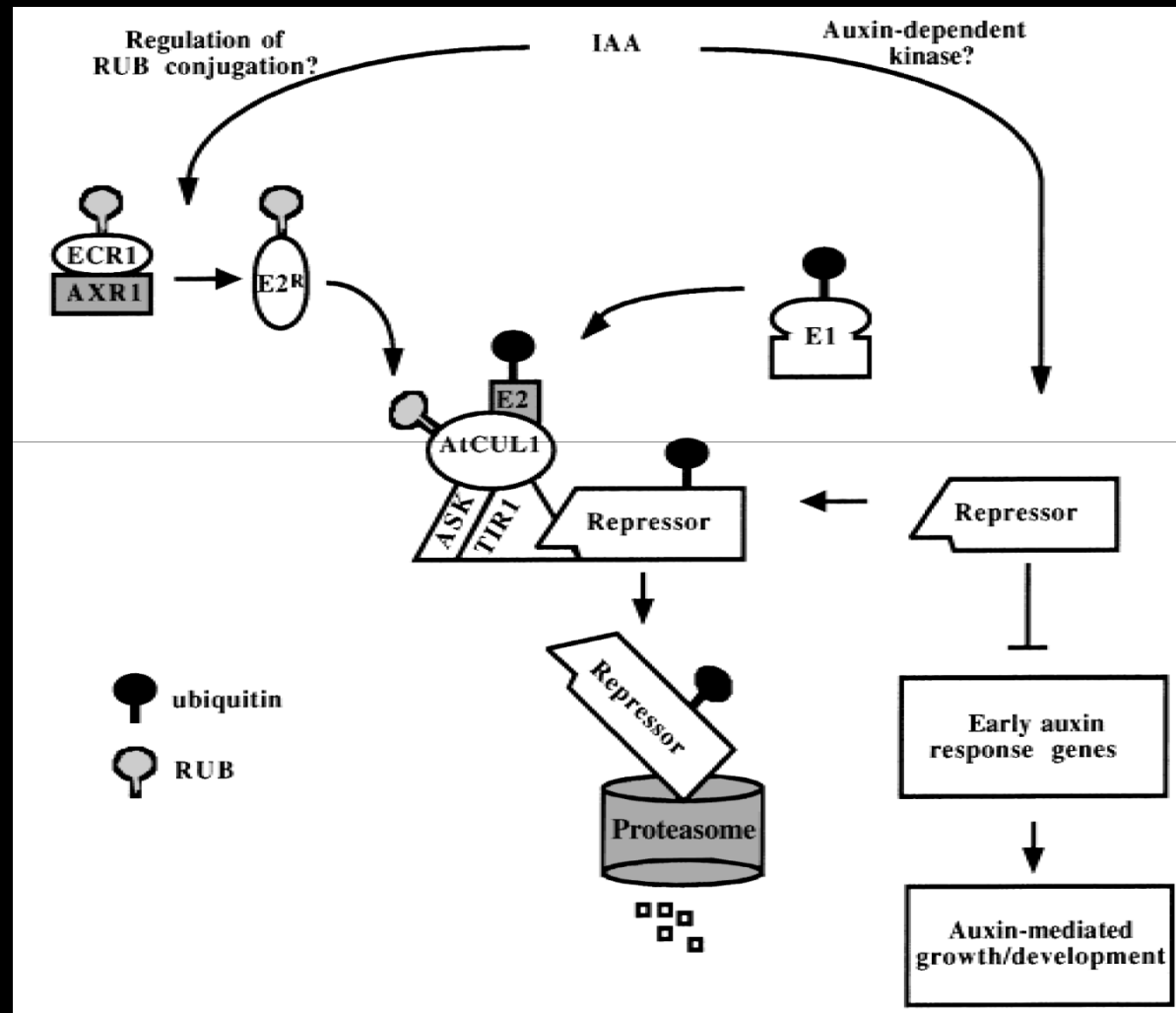
Interaktion mit SCF Komplex = SCF^{TIR1}

SCF^{TIR1} Komplex ist für Auxin-response notwendig

Bisheriges Modell



Aktualisiertes Modell



Nächste Stunde:

Auxin regulates SCF^{TR1}-dependent degradation of AUX/IAA proteins

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