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

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Received April 28, 1999; revised version accepted May 21, 1999.

Ergebnisse der letzten Woche

Funktionelle Charakterisierung

tir1-Mutanten in Auxin-Antwort beeinträchtigt

tir1-axr1-Doppelmutante additiv/synergistische Interaktion

TIR1 dominant-rezessiv vererbt (rendundante Funktion)

Genetische Charakterisierung

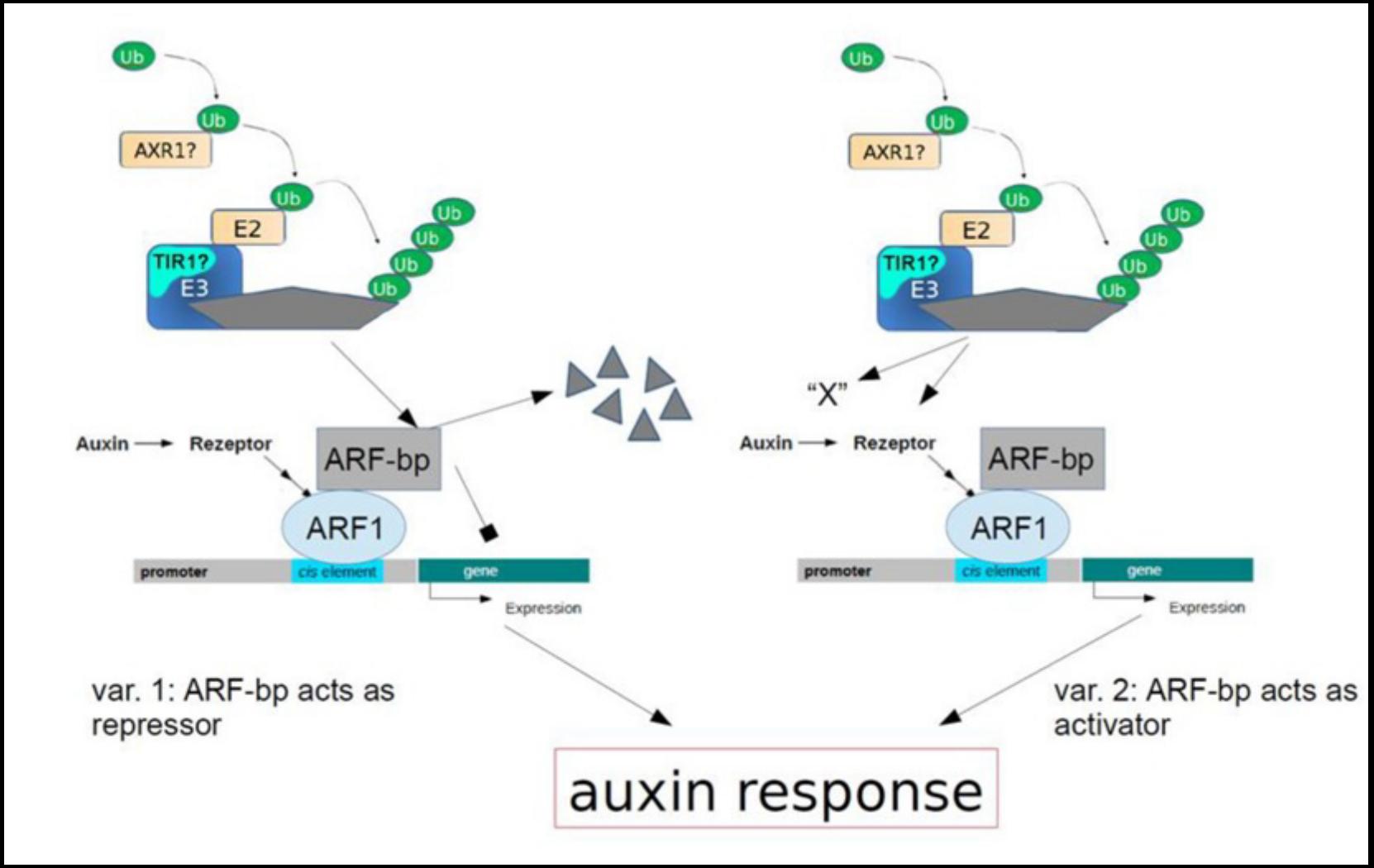
Auf Chromosom 3, Position 128 lokalisiert

F-Box und LRR-Domäne

Mögliche Beteiligung an Ubiquitin-Protein-Ligase-Komplex

Verwandte in Arabidopsis und Reis (LRFs)

Bisheriges Modell



Weitere Erkenntnisse

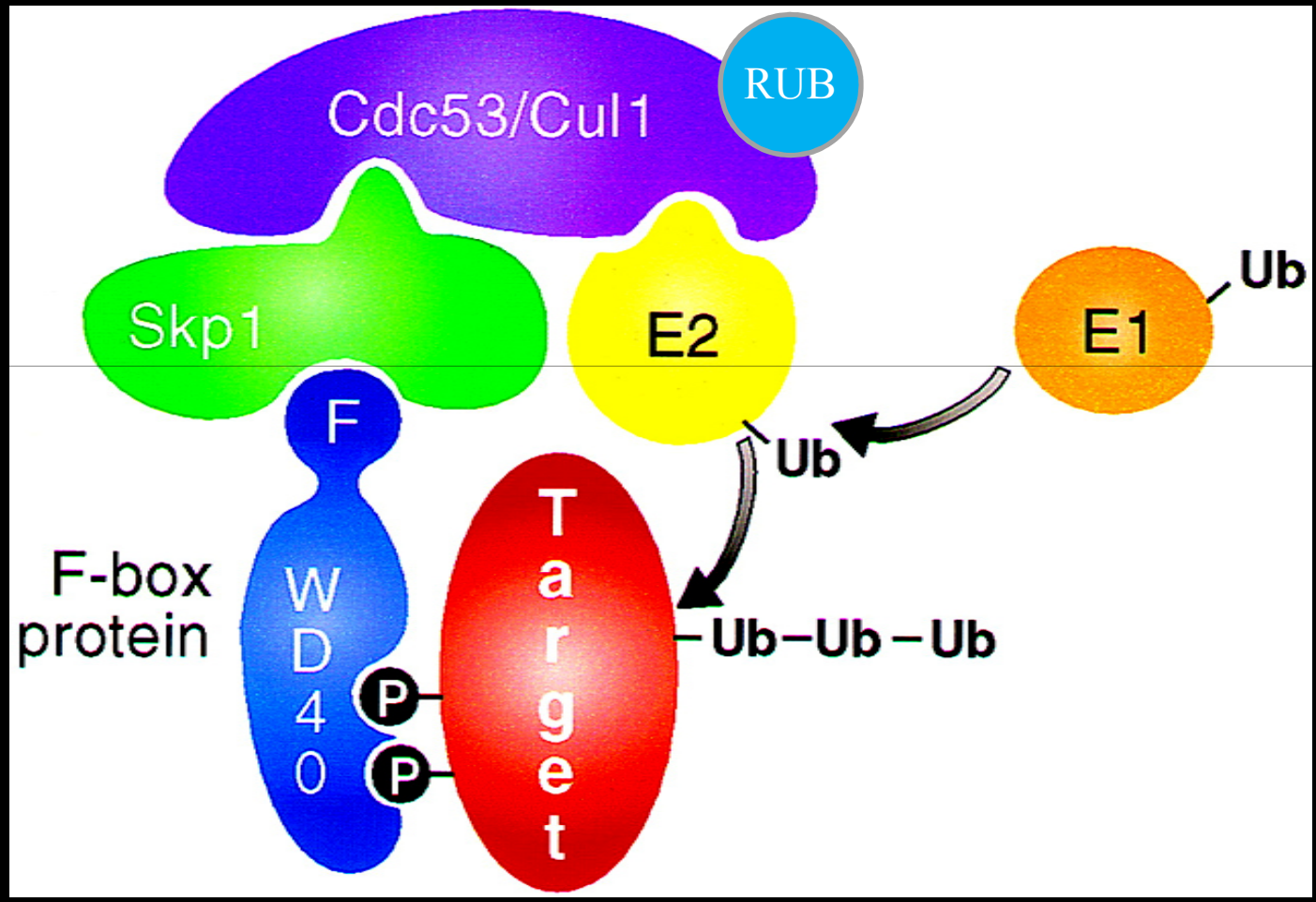
Mutationen in *SAR1* als Supressor von *axr1*

→ selber Pathway
(Cernac et al. 1997)

F-Box Proteine interagieren mit Skp1 und Cdc53

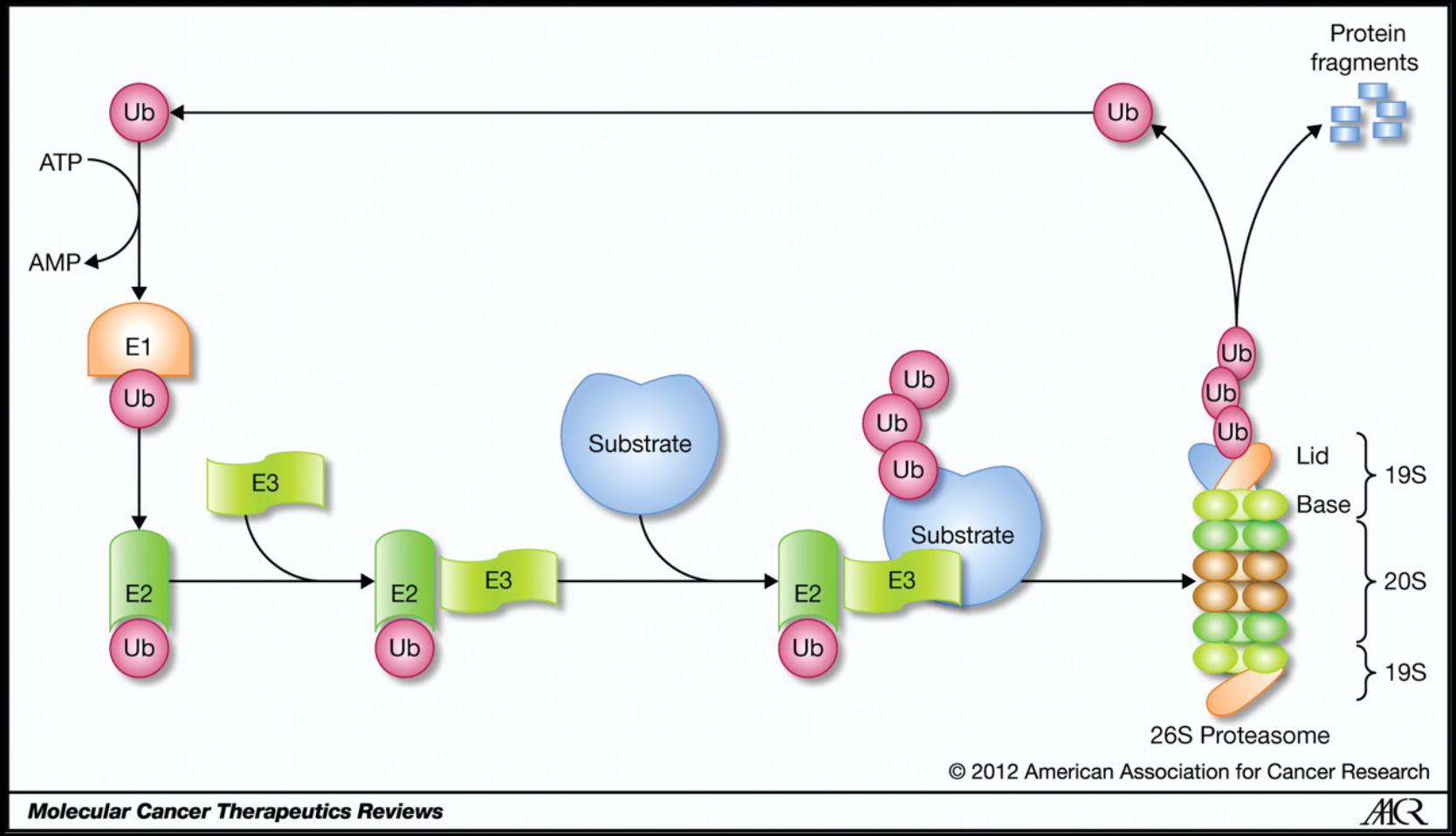
→ Bildung von Ubiquitin-Ligase-Komplexen (SCFs)
(Krek 1998; Patton et al. 1998: Mantis 1999)

Ubiquitin-Ligase-Komplex (SCF)



(Mantis 1999)

Ubiquitin-Proteasom-System



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

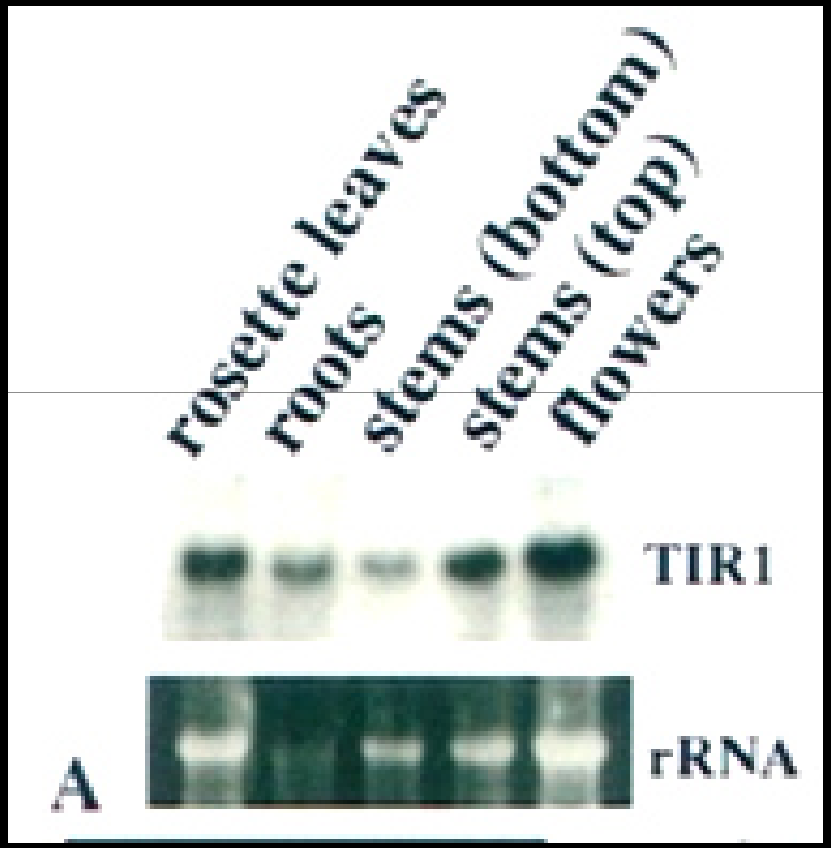
→ Substrat für RUB Konjugation
→ AXR1-ECR1 E1-like dimer aktiviert RUB
→ RUB konjugiert At-CUL1
➔ Verzögerung der Bildung oder Modifizierung des SCF Komplexes

Fragestellung

Wann und in welchen Geweben wird *TIR1* exprimiert?

Bildet TIR1 mit einem oder mehreren Proteinen Komplexe?

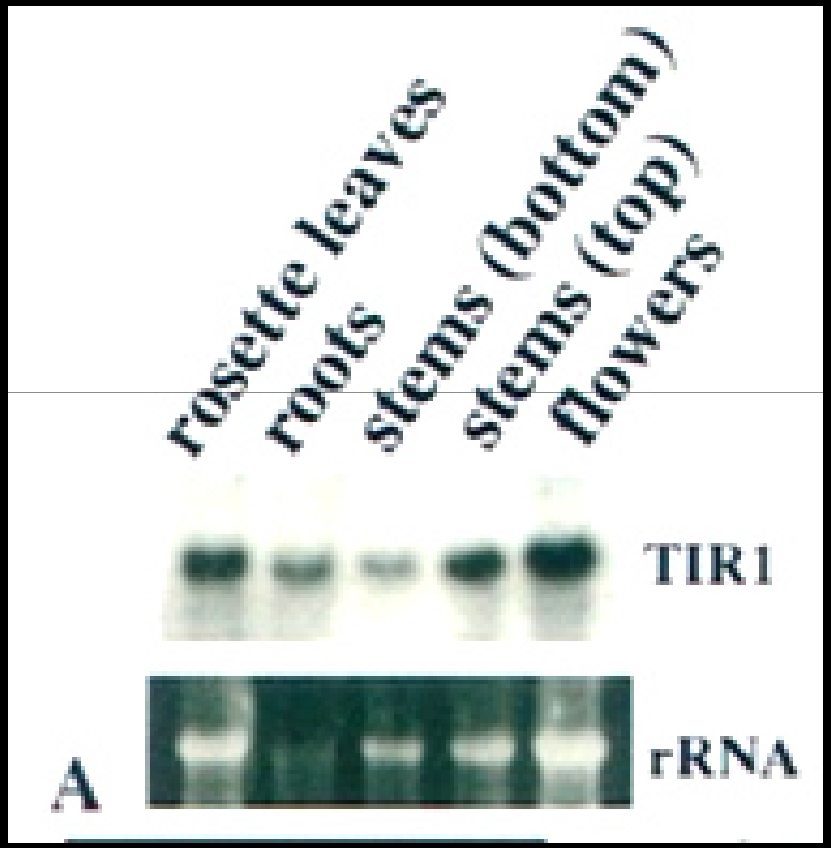
Ergebnisse
- Expression *TIR1*



A: Northern Hybridiserung

→ Transkriptlevel *TIR1*

Ergebnisse
- Expression *TIR1*

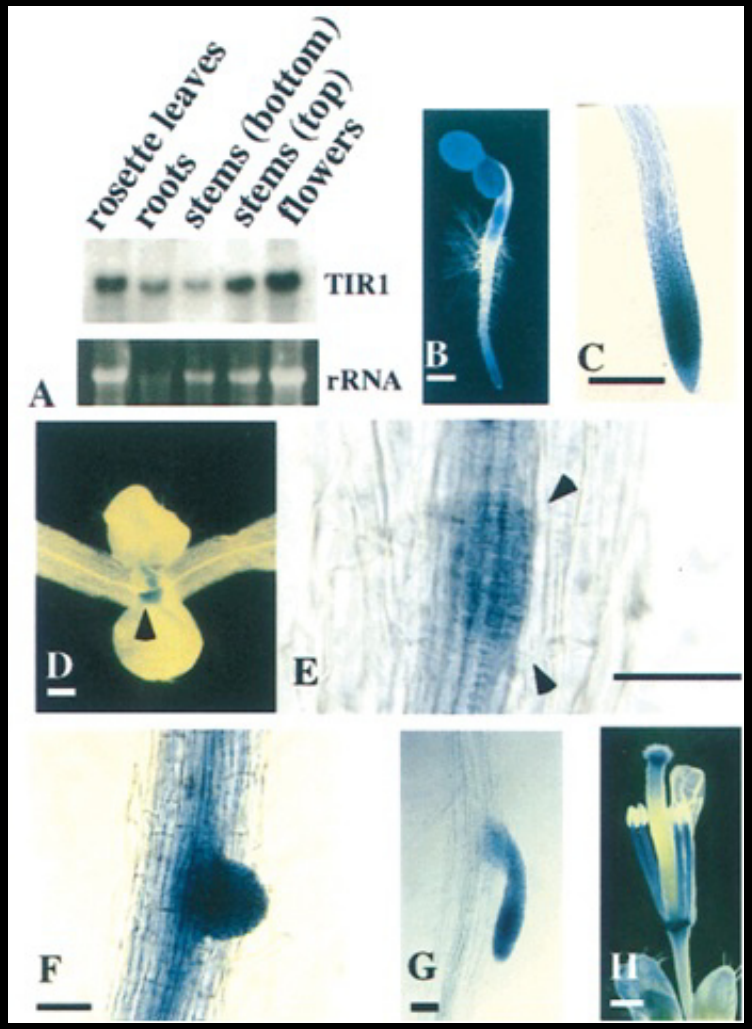


A: Northern Hybridiserung

→ Transkriptlevel *TIR1*

In allen gezeigten Geweben exprimiert

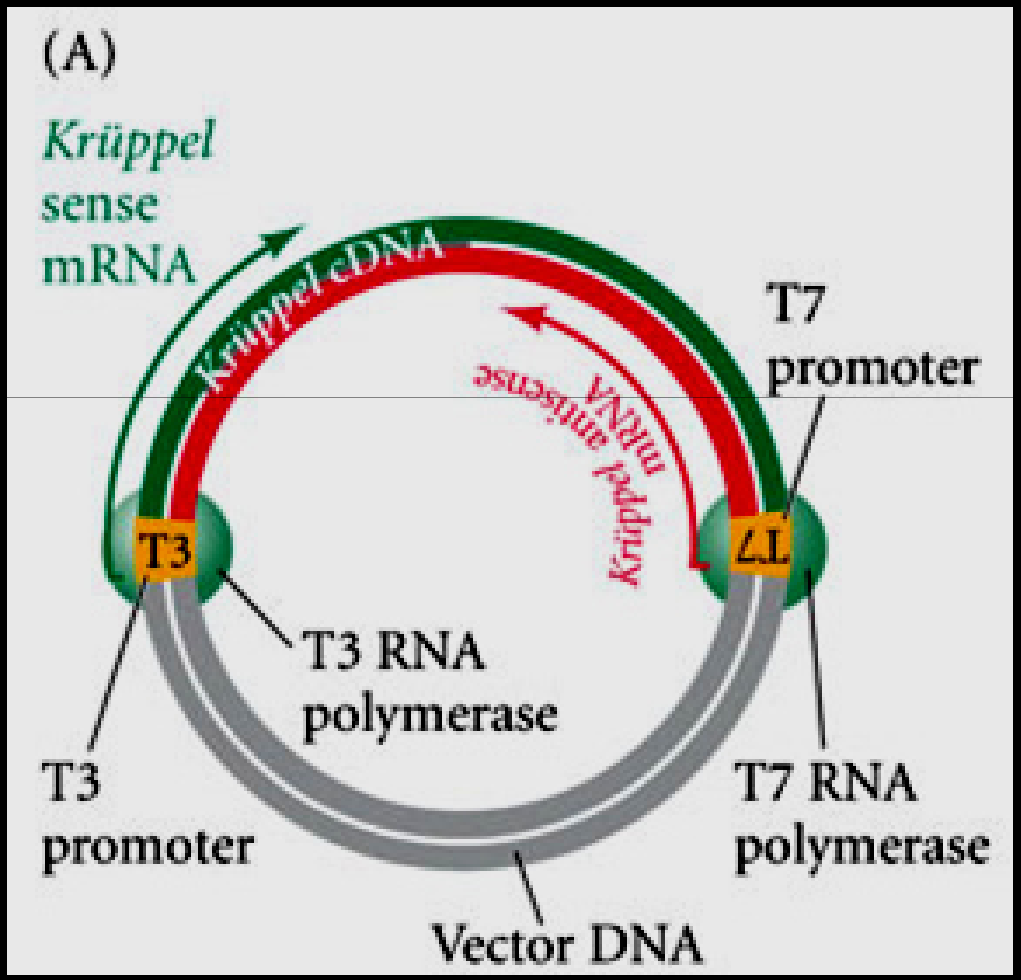
Ergebnisse
- Expression *TIR1*



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

- B: Seedling
- C: Apikalmeristem (Wurzel)
- D: Apikalmeristem (Spross)
- E-G: lateral Wurzel Primordium/Meristem
- H: floral stigma

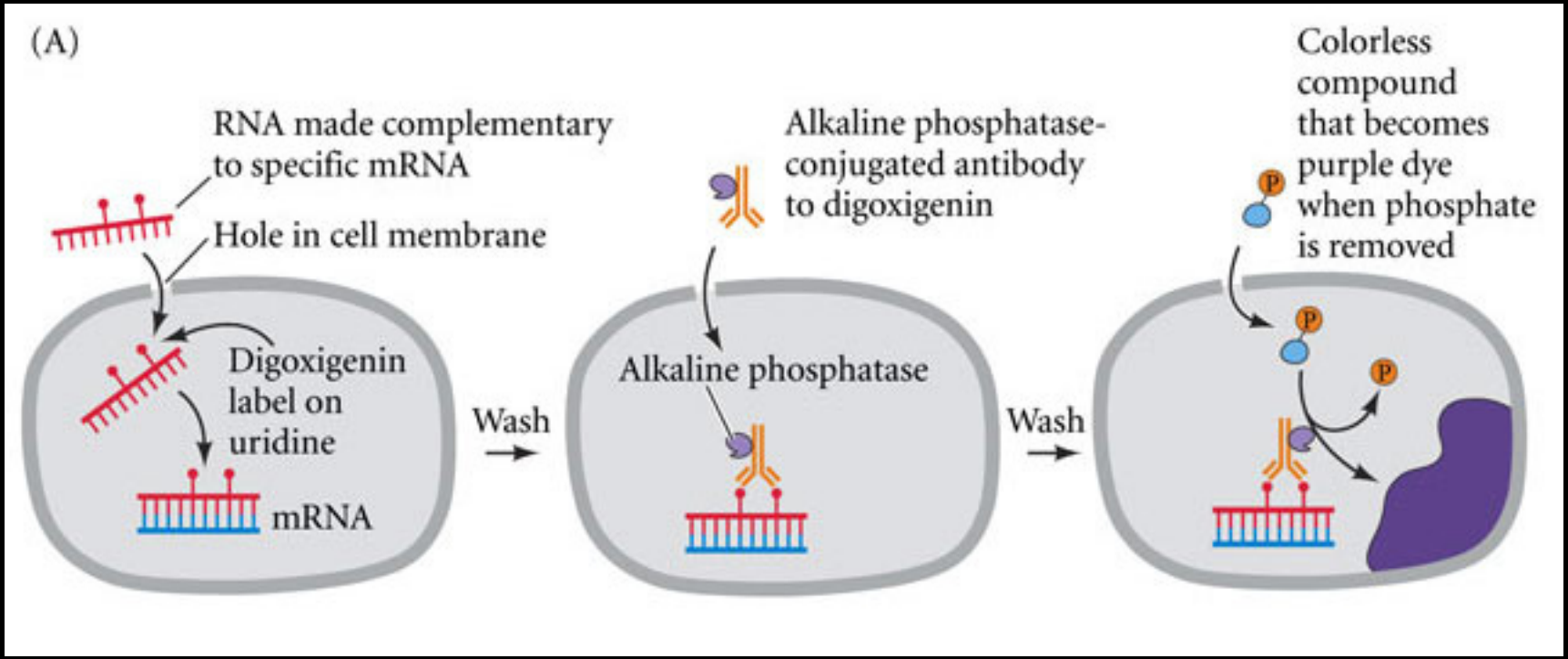
Ergebnisse
- Methode: In situ Hybridisierung



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



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Ergebnisse
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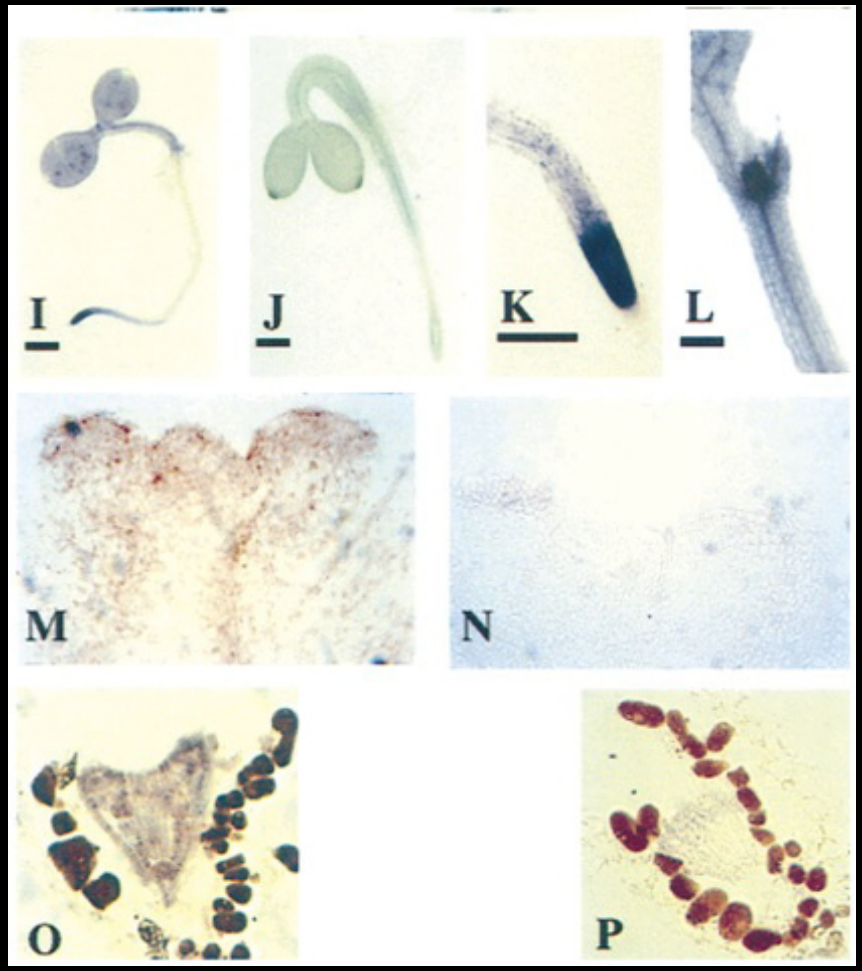


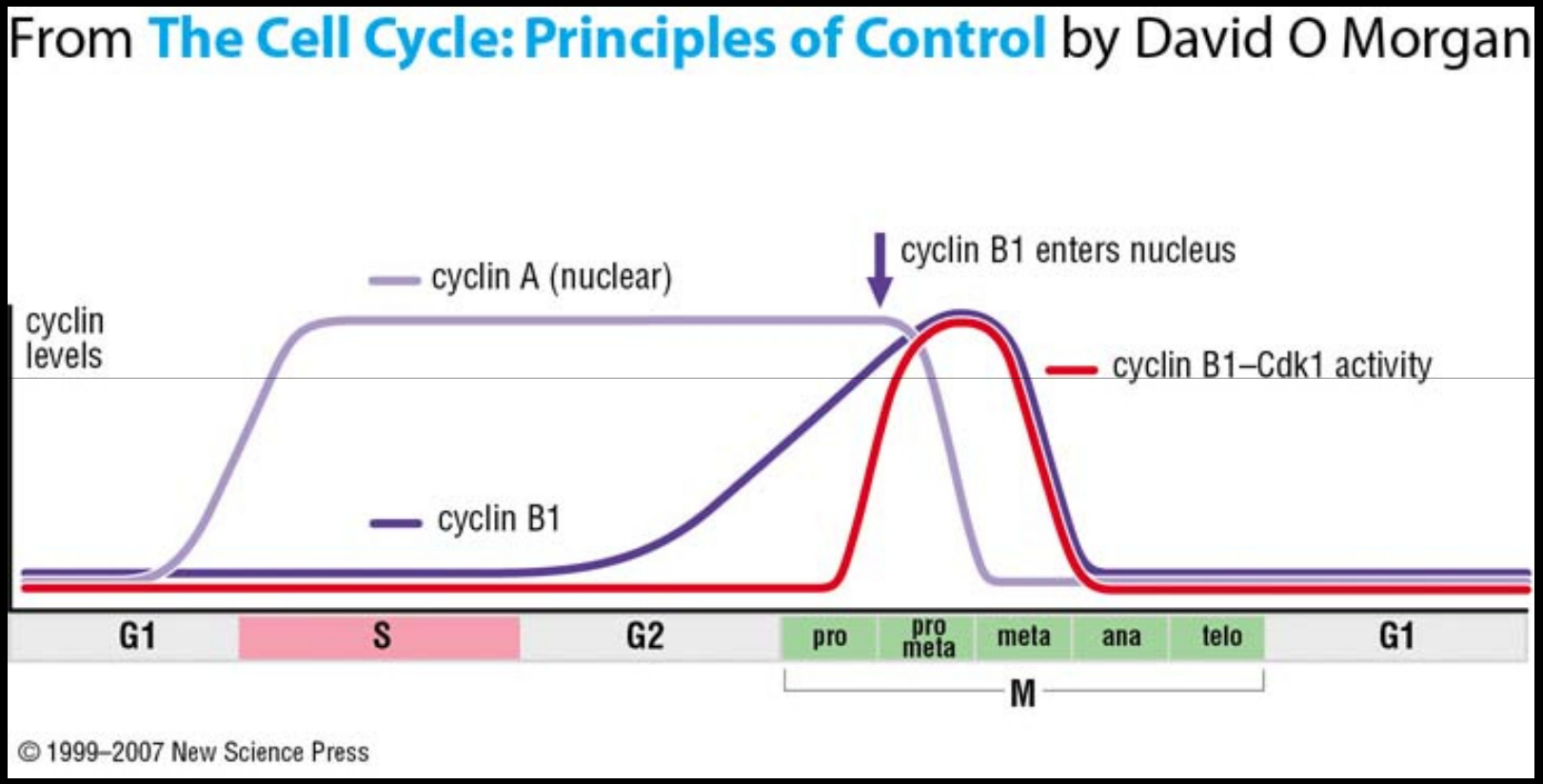
B-H: *TIR1-gus*

I,K,L,M: meristematisches Gewebe

O: Expression in Embryoentwicklung

J,N,P: sense strand control hybridzation

Ergebnisse
- Methode: Lateral root studies



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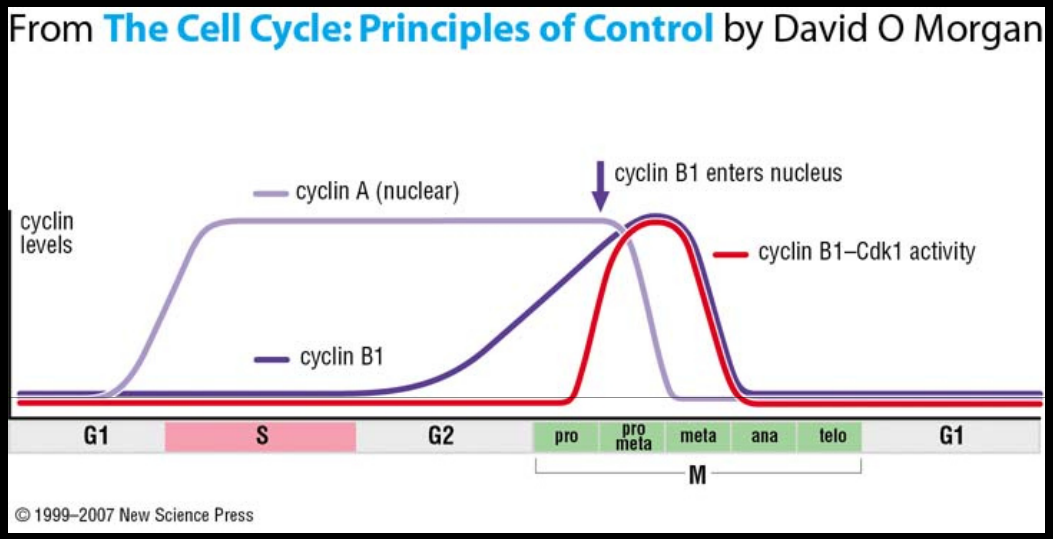

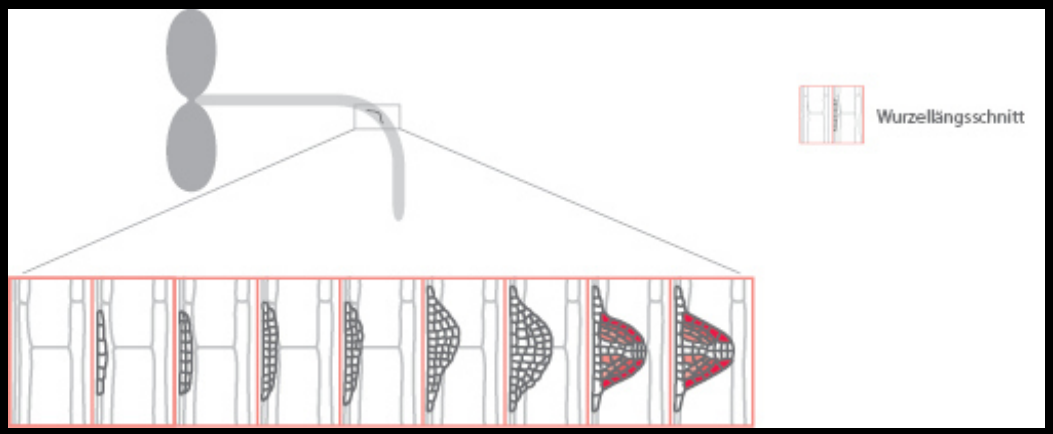

https://www.helmholtz-muenchen.de

Ergebnisse
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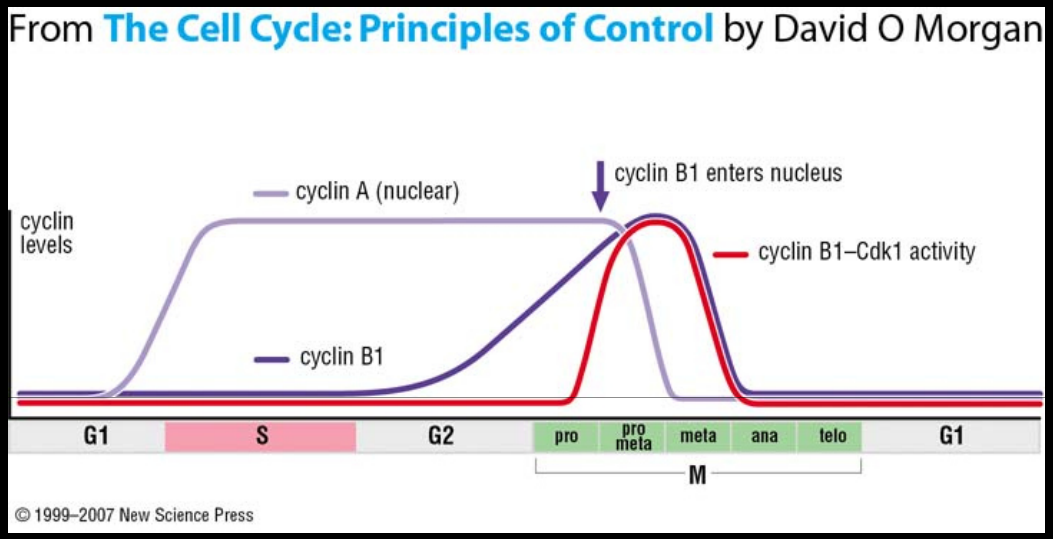

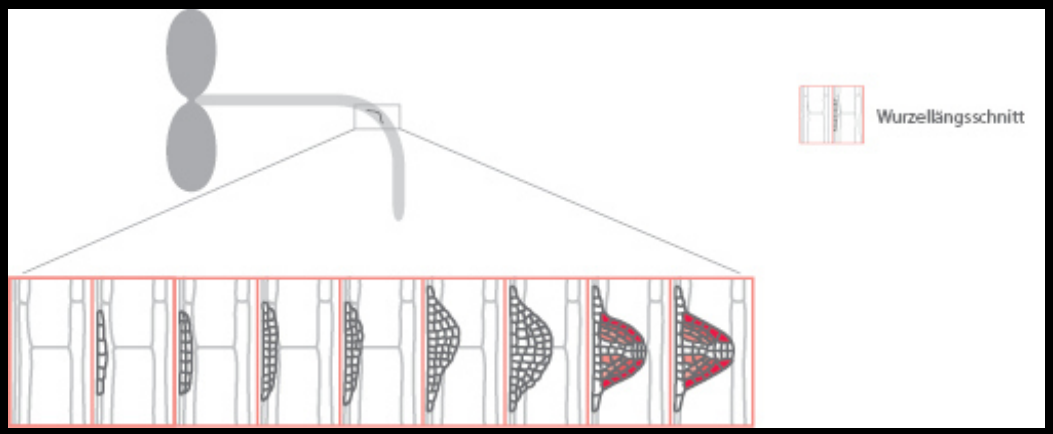

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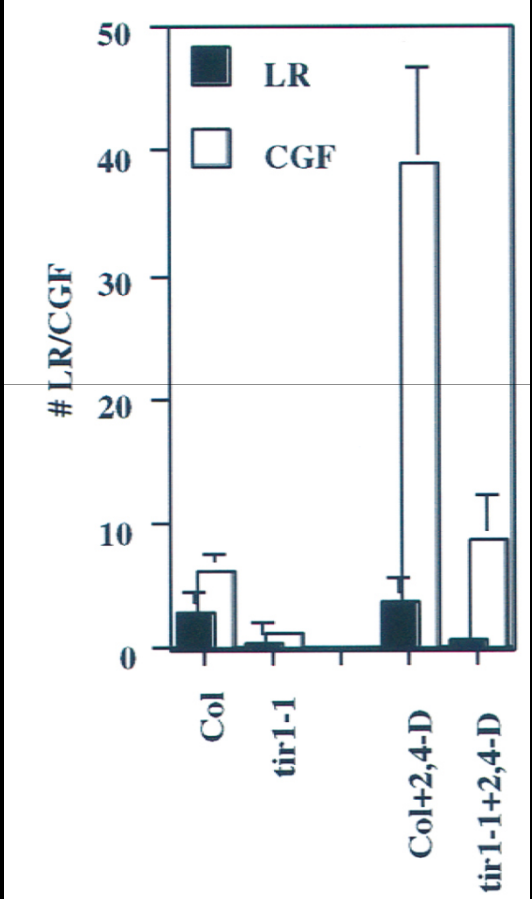
cyc1At-gus

Ausdifferenzierte Zellen beginnen sich zu teilen

Detektion mit Reporter in Anfangsstadien

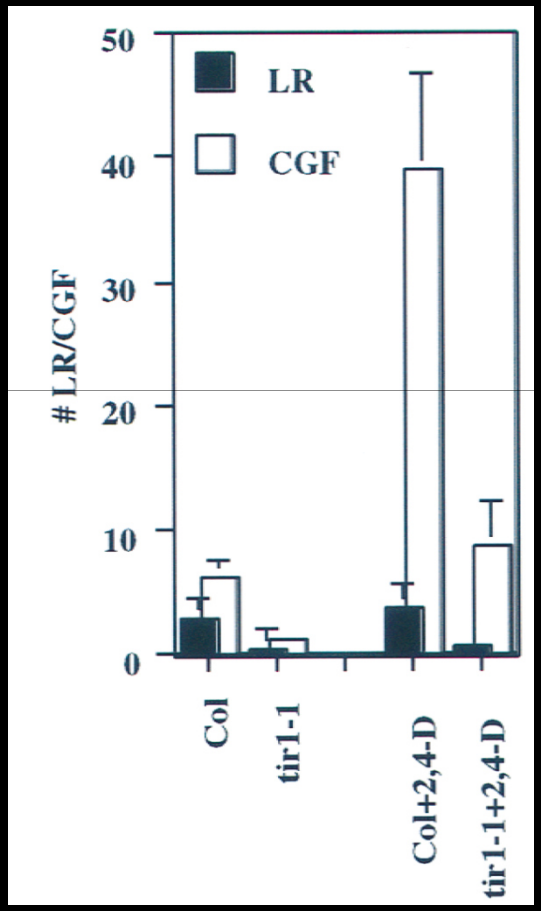
Ergebnisse
- *TIR1:* laterale Wurzelbildung



LR: Lateral roots

CGF: *cyc1At-gus* foci

Ergebnisse
- *TIR1:* laterale Wurzelbildung

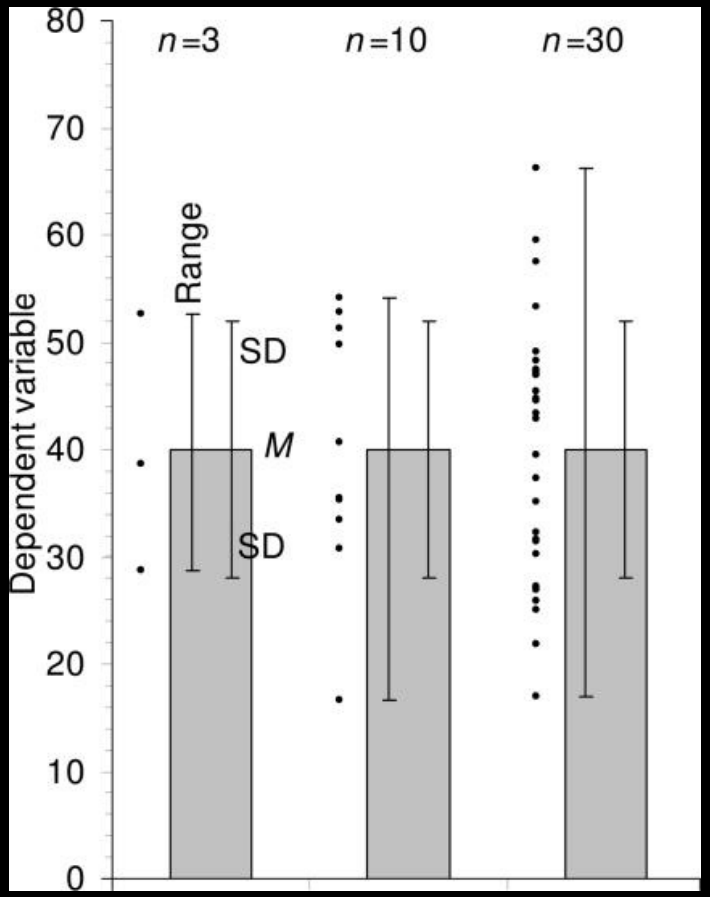


LR: Lateral roots

CGF: *cyc1At-gus* foci

David L. Vaux: Know when your numbers are significant

Ergebnisse
- Exkurs Statistik



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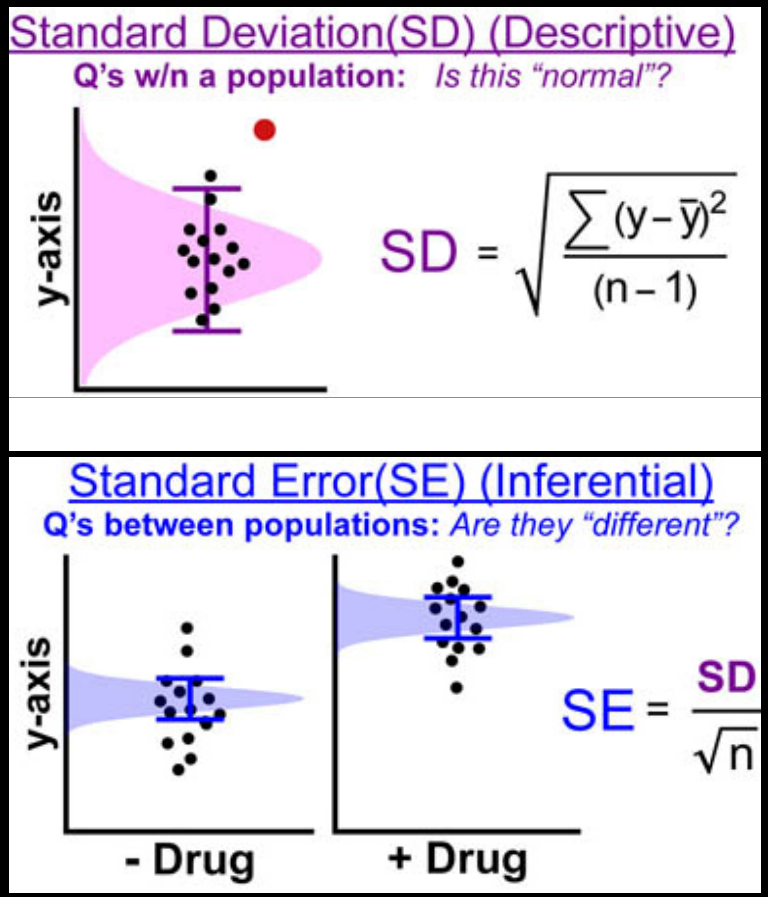


http://www.practicallyscience.com

Ergebnisse
- Exkurs Statistik

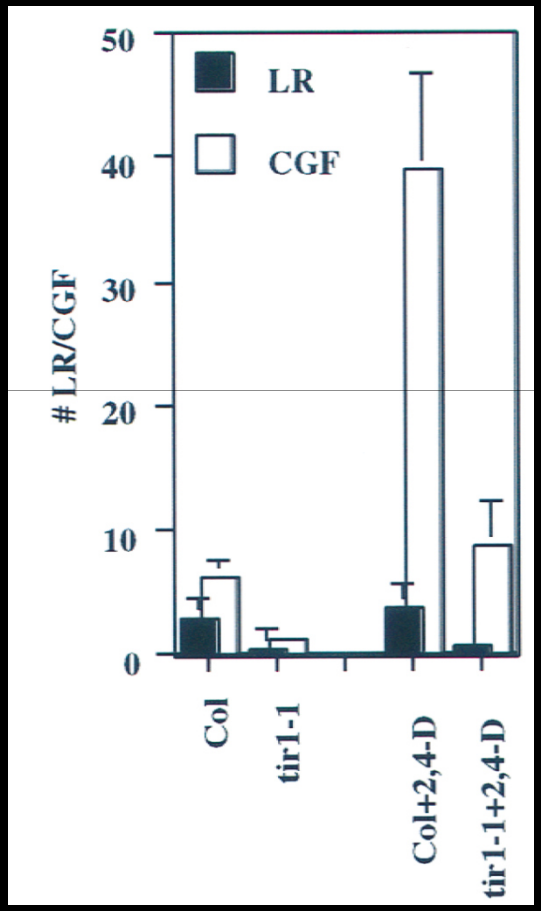
STATISTICS GLOSSARY
Some common statistical concepts and their uses in analysing experimental results.

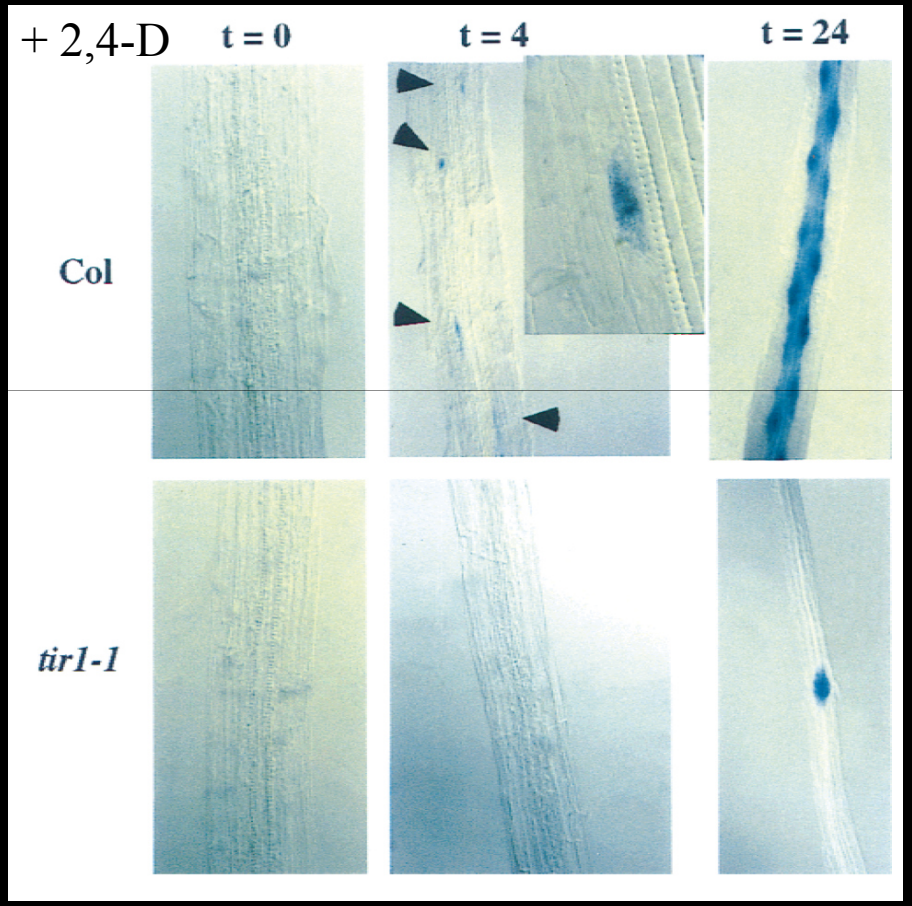
Term	Meaning	Common uses
Standard deviation (s.d.)	The typical difference between each value and the mean value.	Describing how broadly the sample values are distributed. s.d. $= \sqrt{(\sum (x - \text{mean})^2/(N-1))}$
Standard error of the mean (s.e.m.)	An estimate of how variable the means will be 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. $= \text{s.d.}/\sqrt{N}$
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 $= \text{mean} \pm \text{s.e.m.} \times t_{(N-1)}$
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

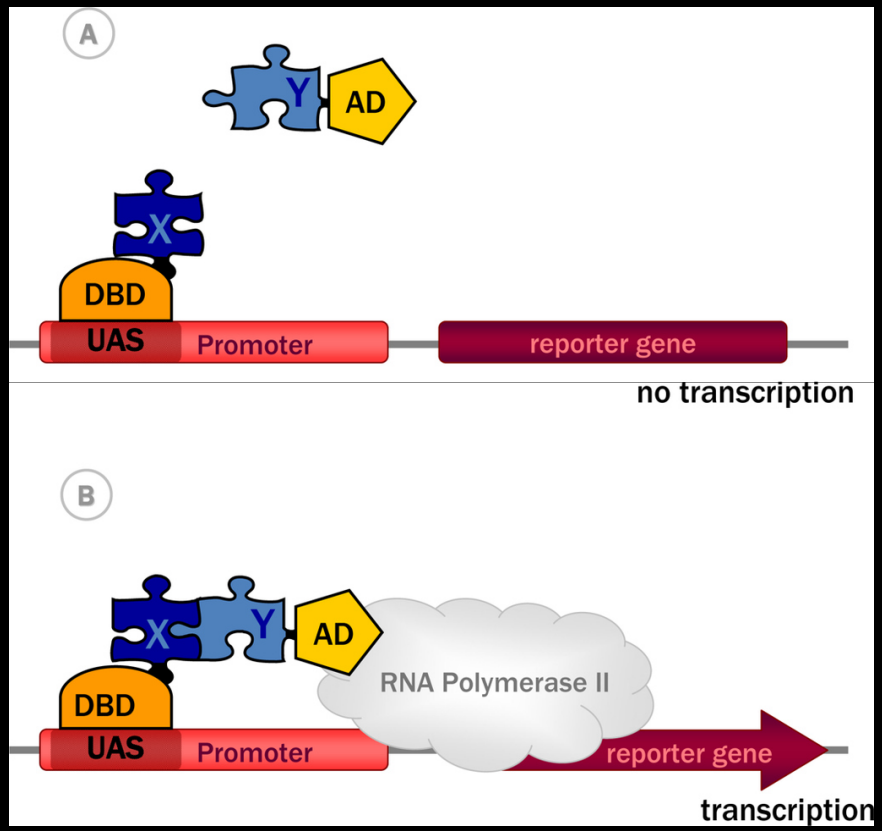




LR: Lateral roots

CGF: *cyc1At-gus* foci

Ergebnisse
- Methode: Yeast two-hybrid



http://www.mdpi.com

Interaktionspartner TIR1?

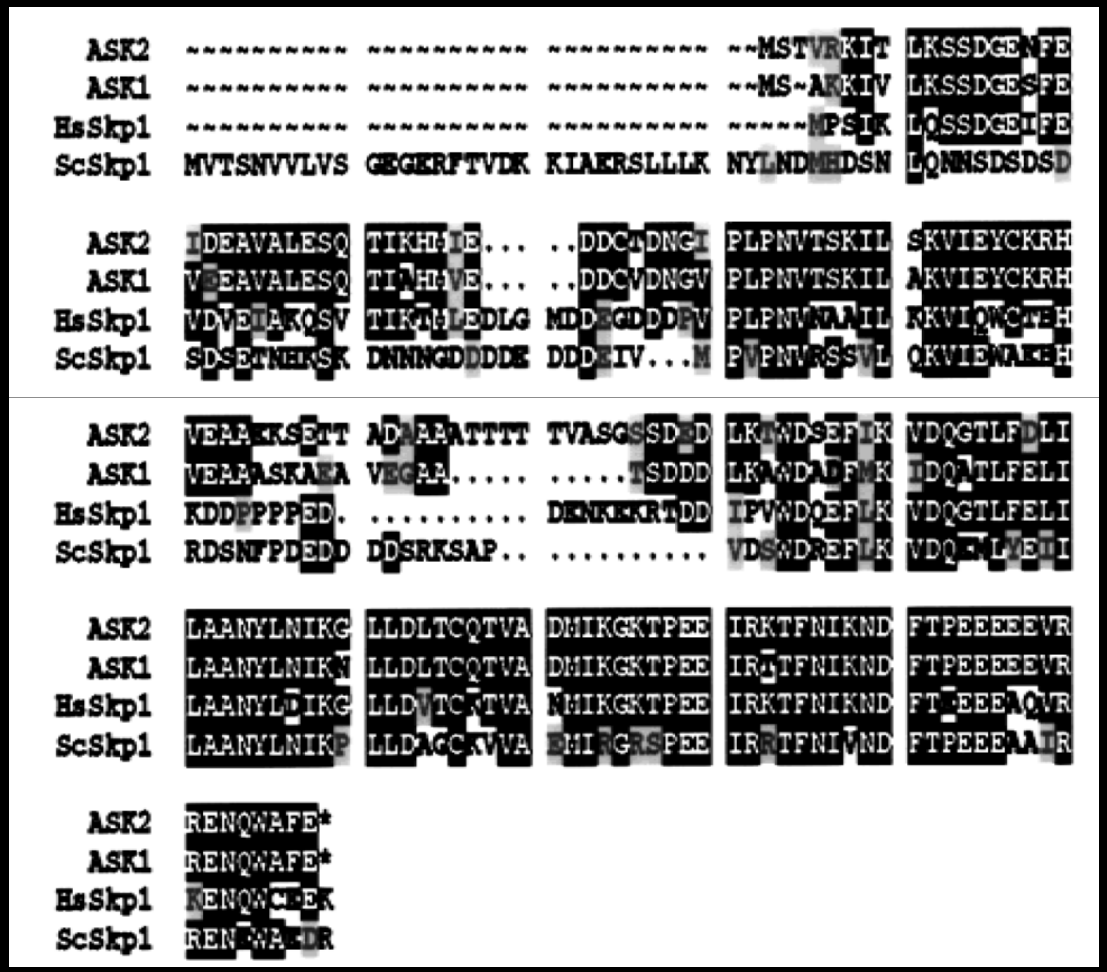
Gal4 DNA-binding domain-TIR1
→ bait
Interaktionspartner = Prey

Histidin-Mangelmedium

→ Zwei Klassen (Klone) identifiziert
→ cDNA sequenziert

→ASK1 und ASK2

Ergebnisse
- Sequence alignment



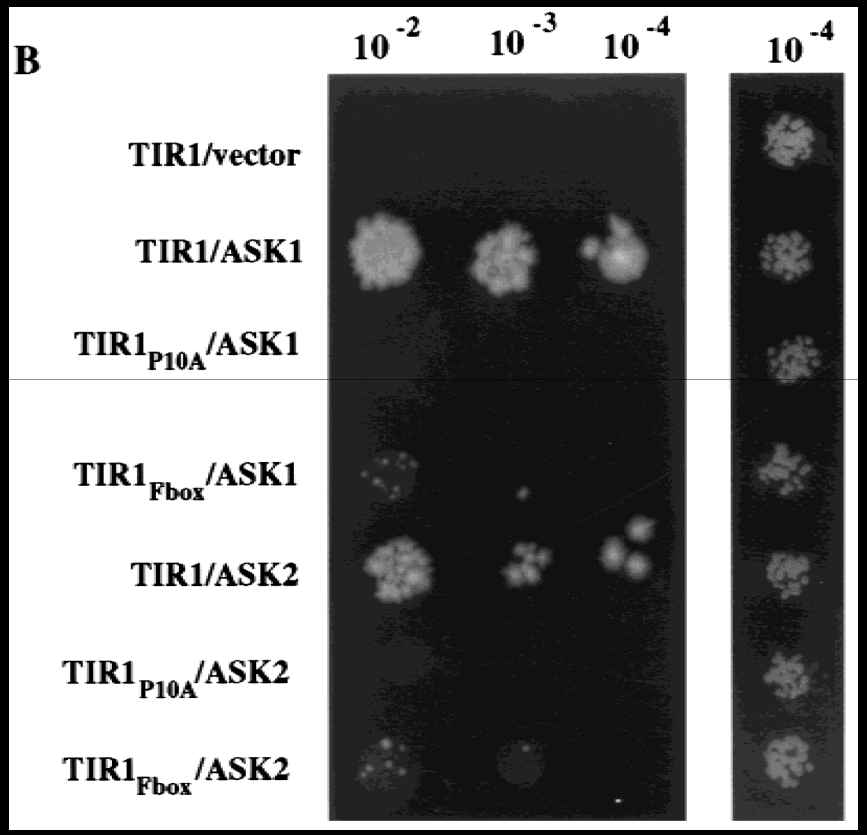
Homologie

ASK1 und *ASK2*

HsSkp1

ScSkp1

Ergebnisse
- *TIR1* interagierende Proteine

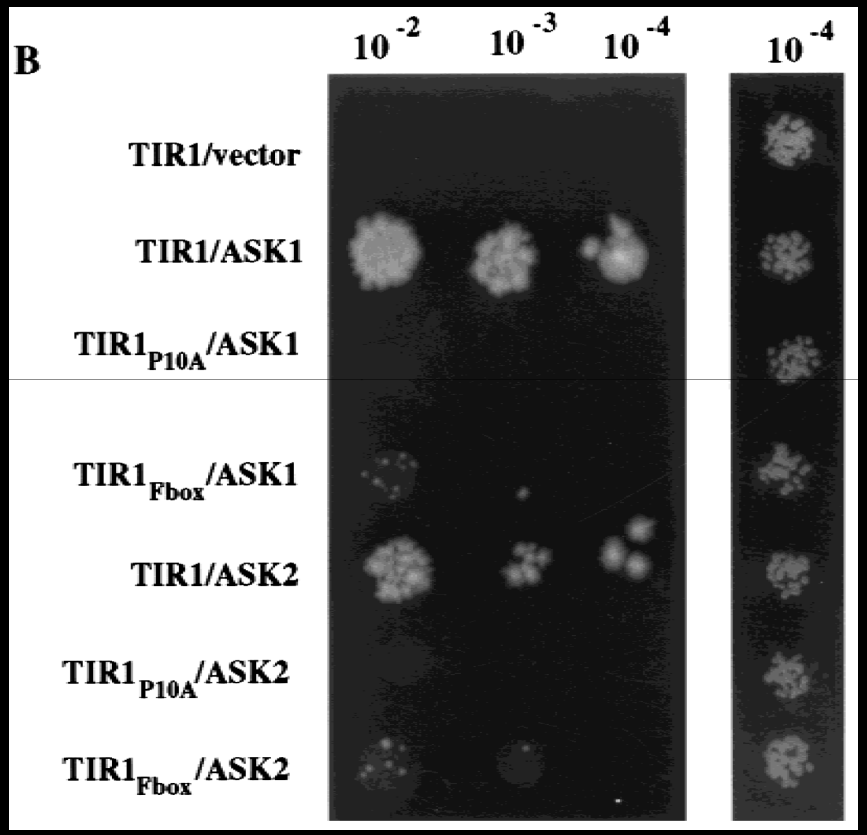


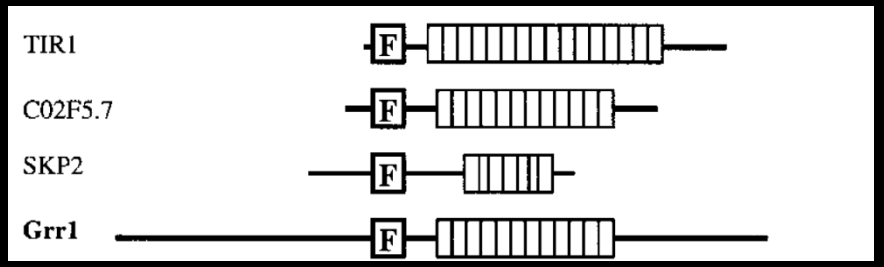
Hefen mit unterschiedlichen two-hybrid Plasmiden (10d)

Negativkontrolle auf Vollmedium (3d)

P10A = F-Box Mutiert
Fbox = Nur F-Box

Ergebnisse
- *TIR1* interagierende Proteine





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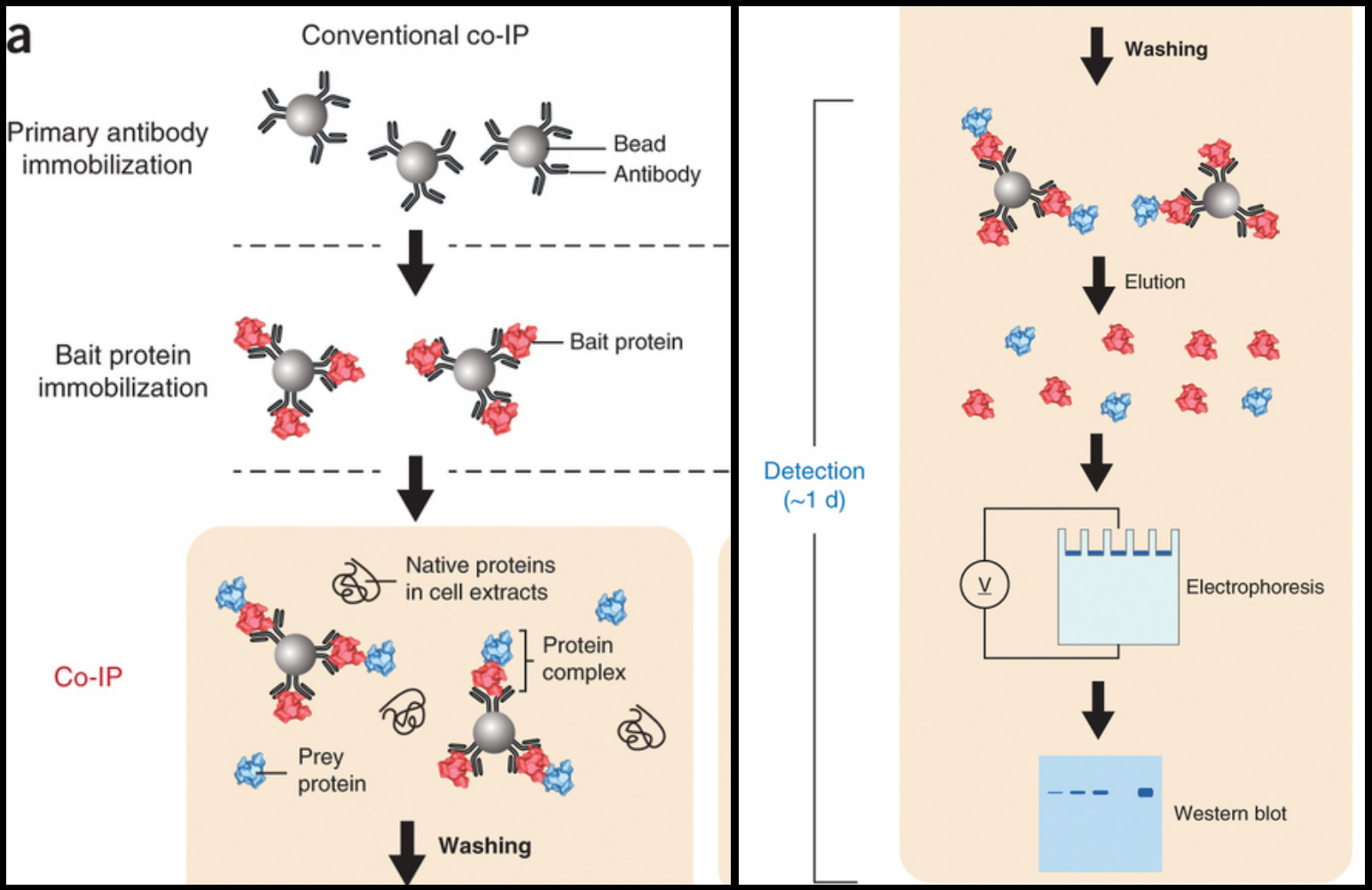
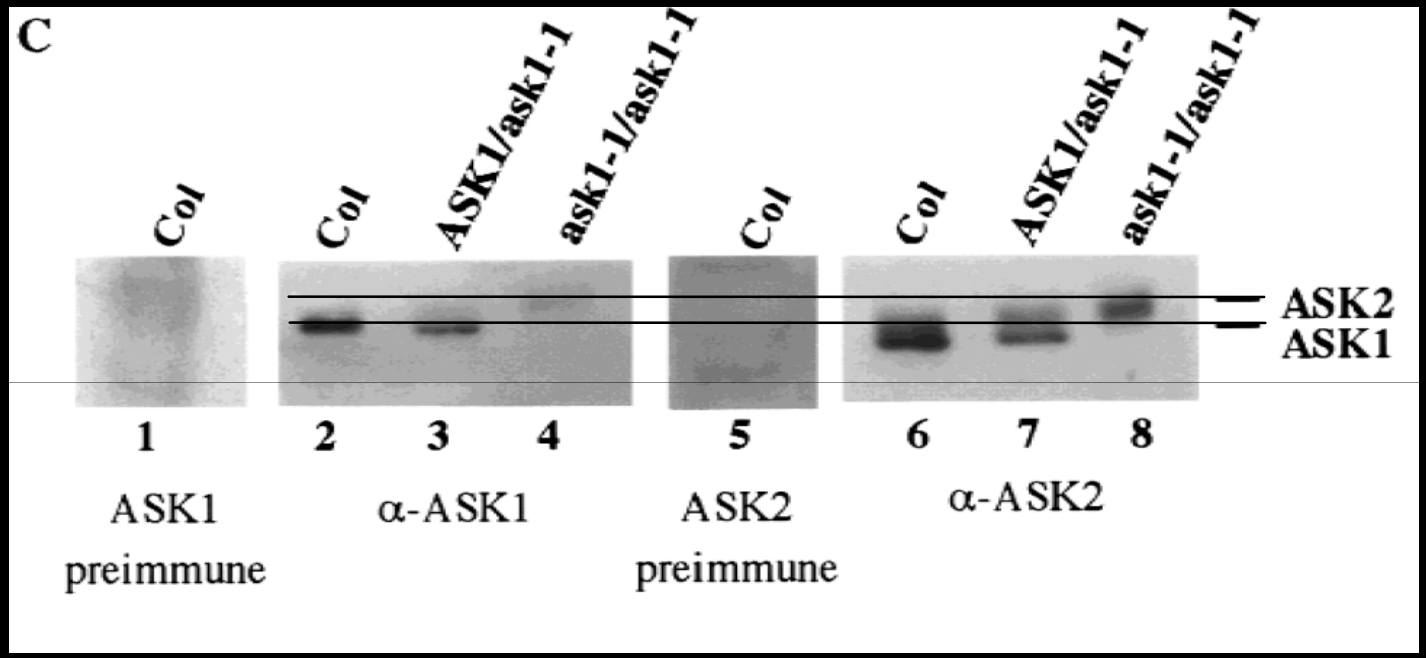
Ergebnisse
- Methode: Coimmunoprecipitation



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

Ergebnisse
- *TIR1* bildet SCF-like Komplexe



Preimmune?

Ergebnisse
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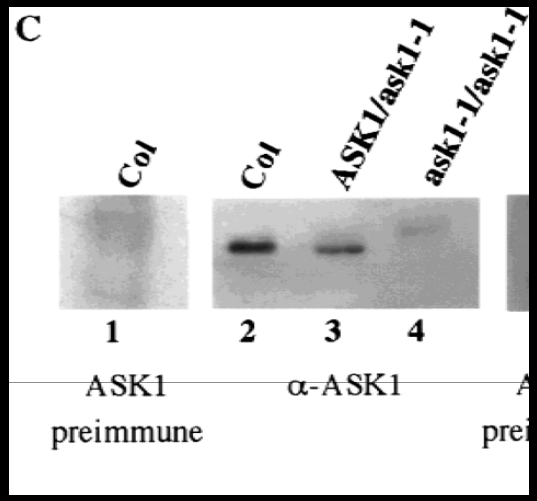

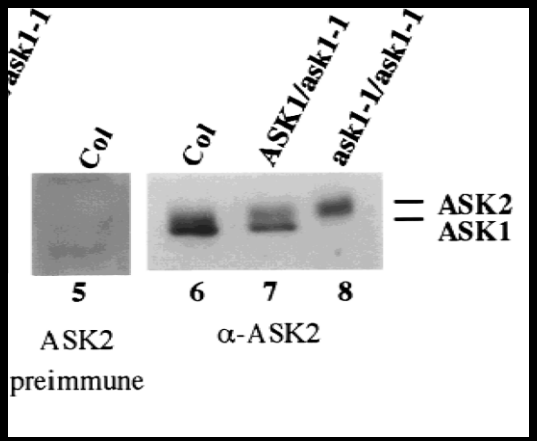

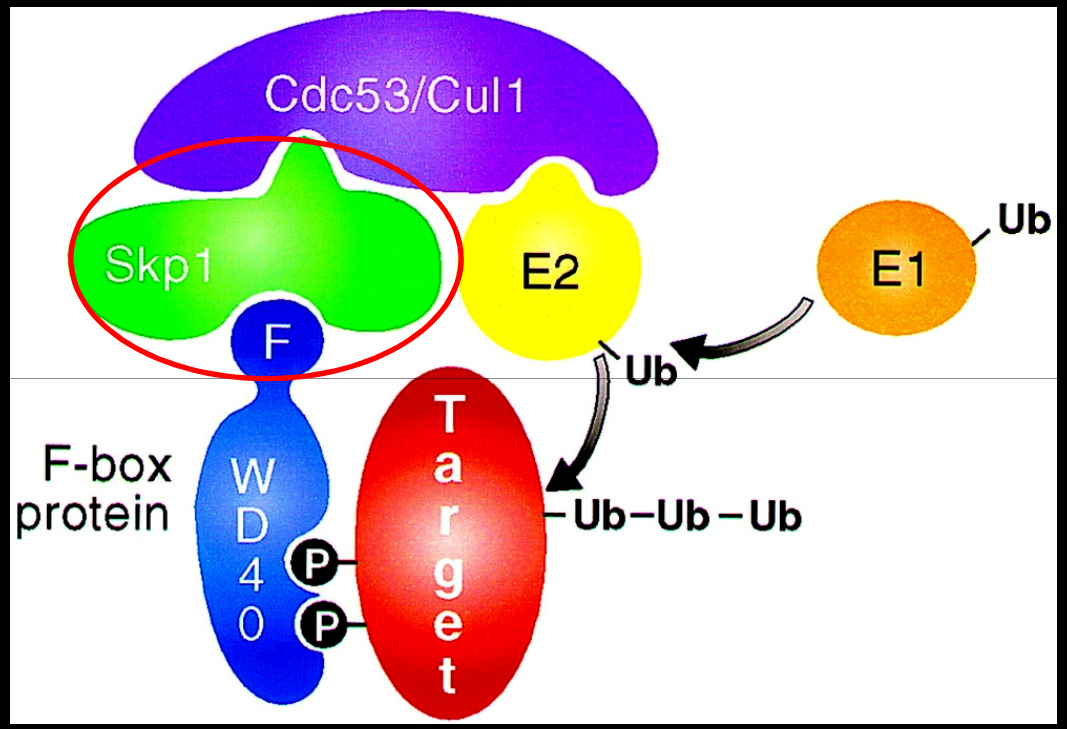

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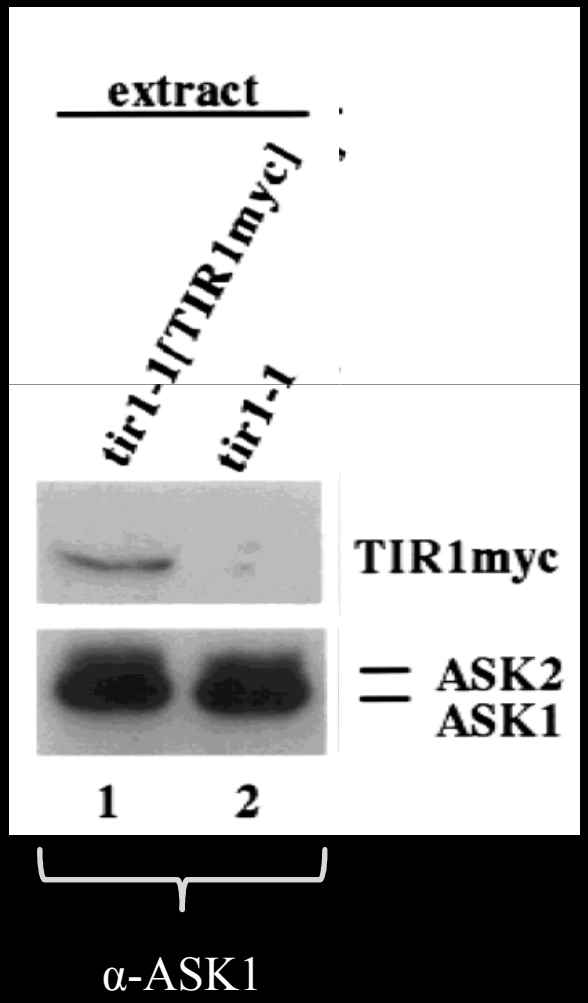

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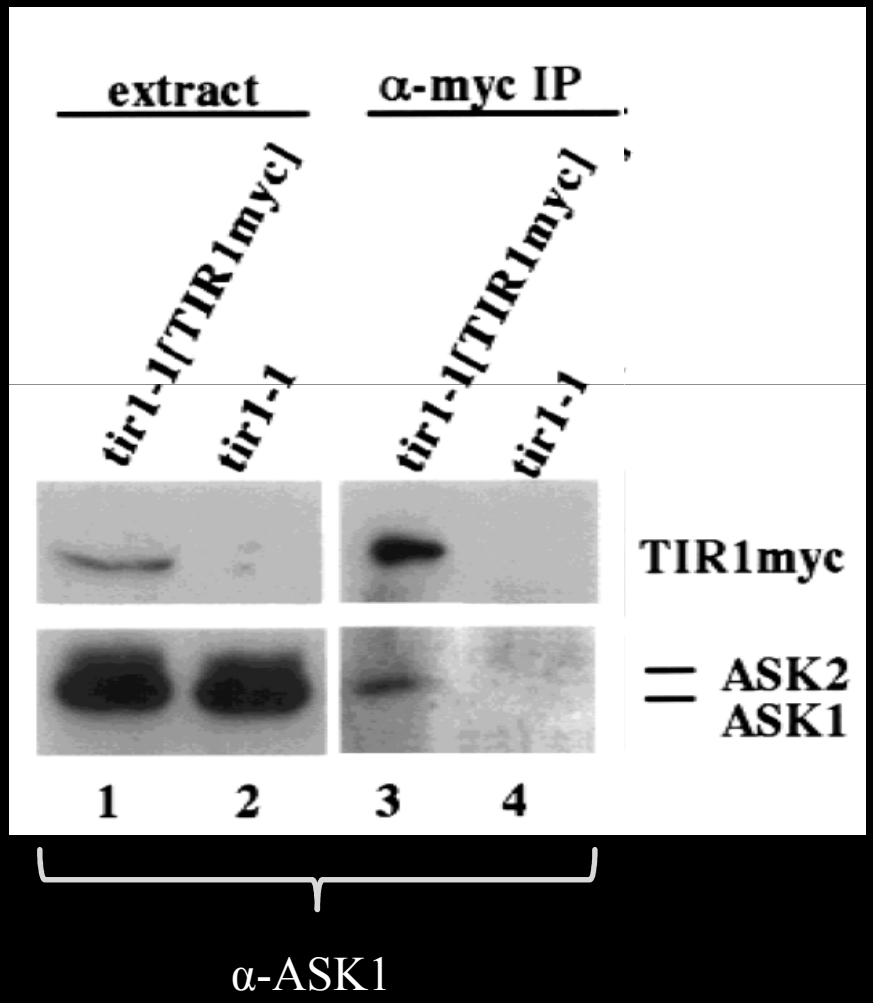

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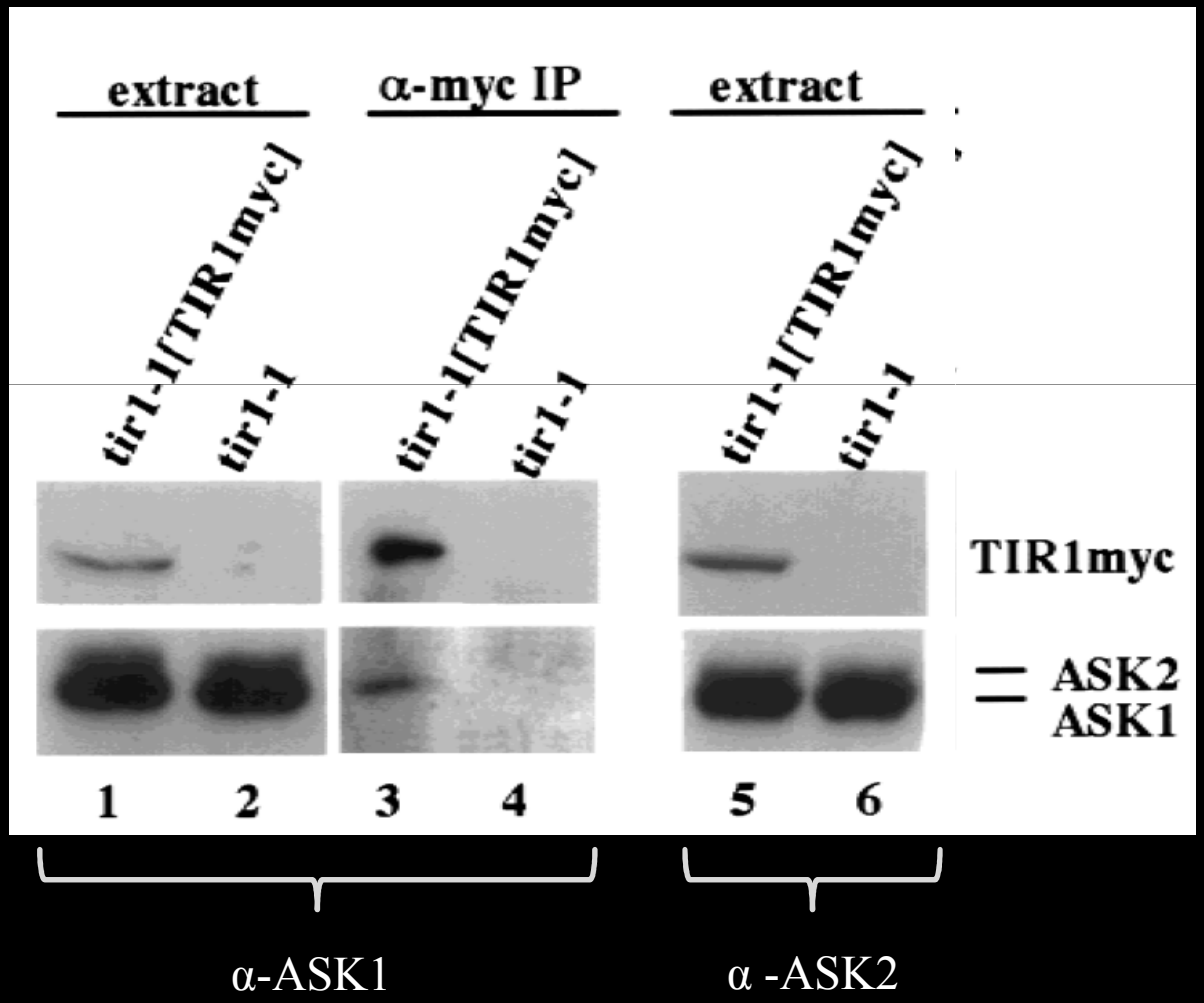

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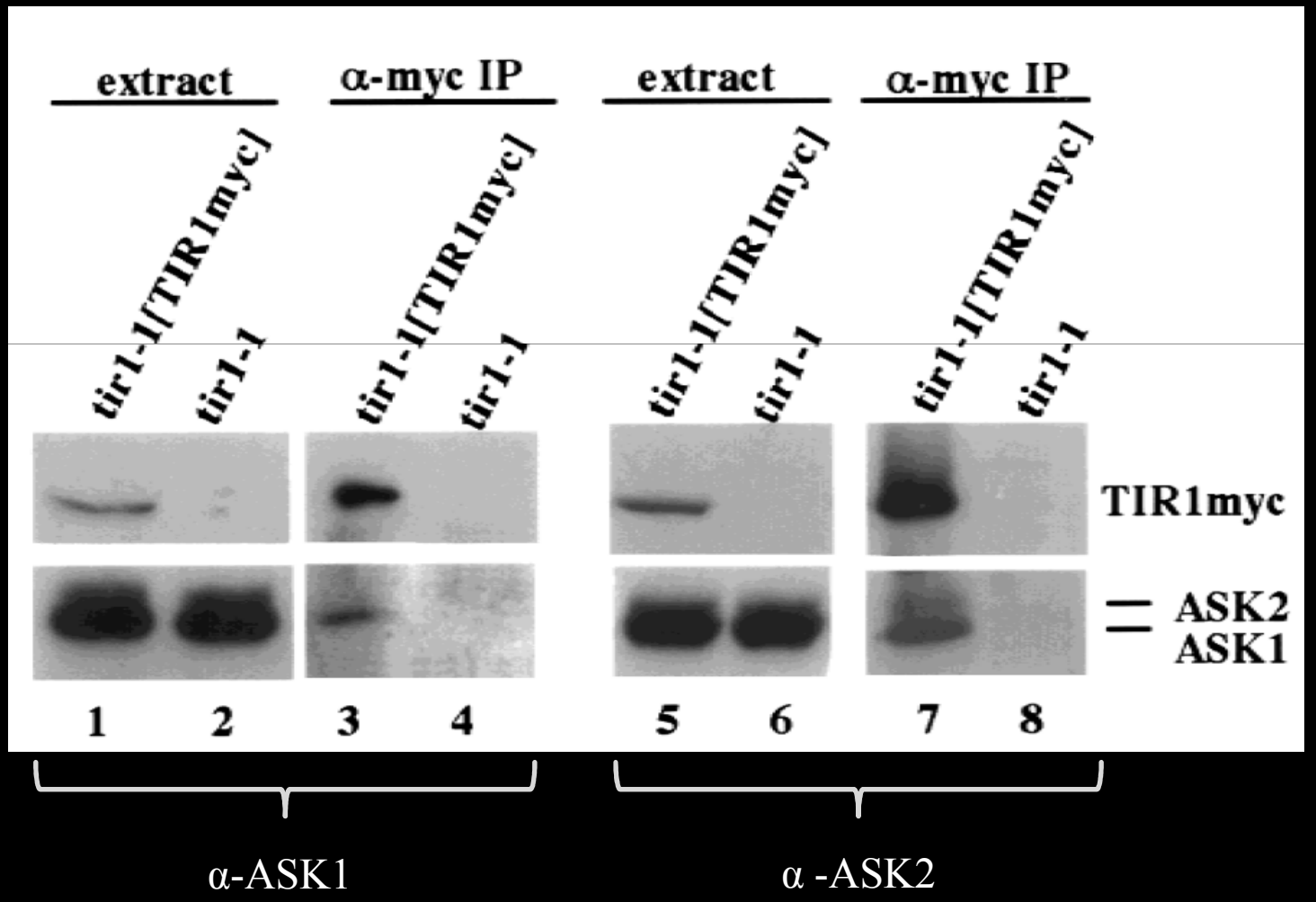

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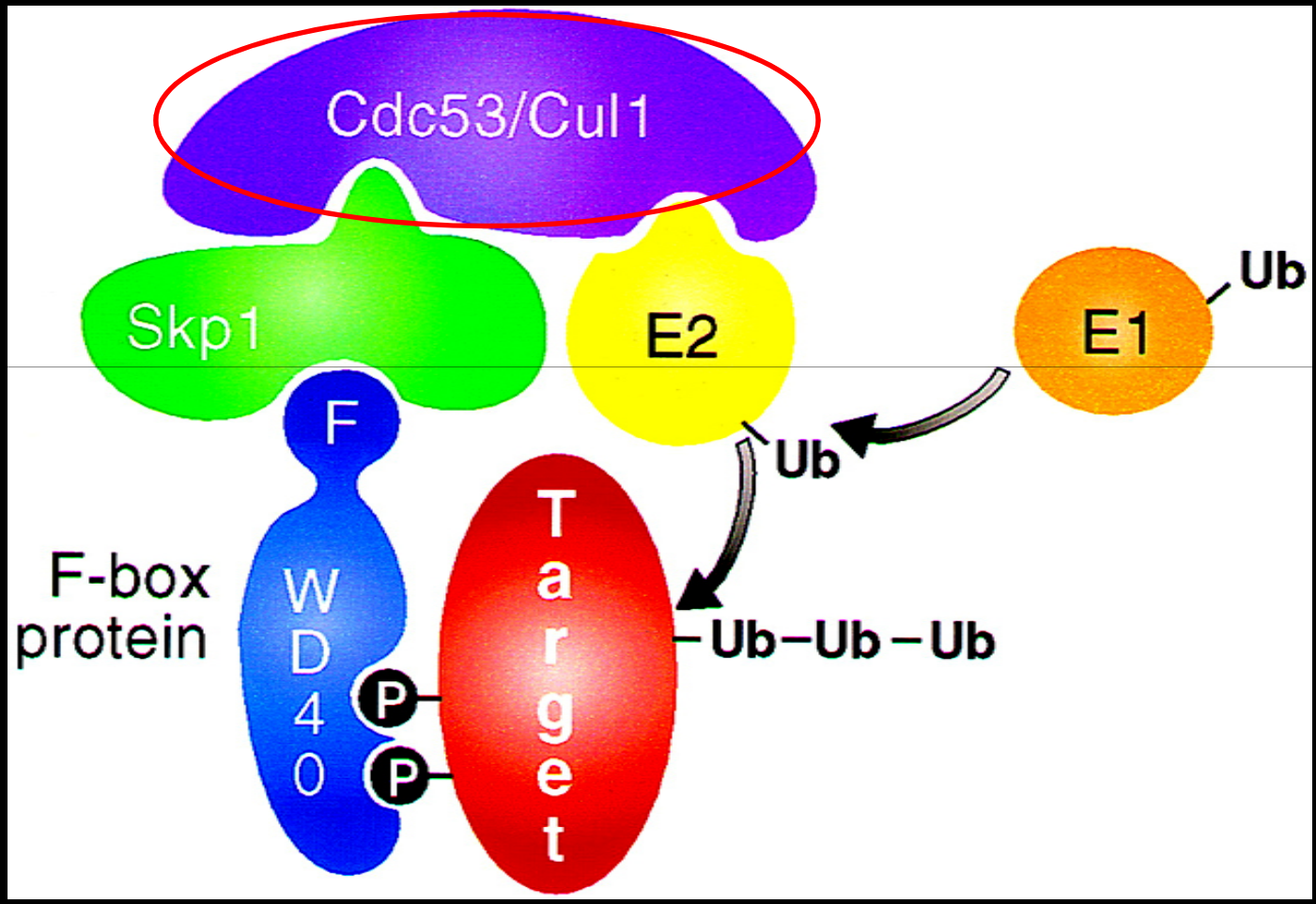

(Mantis 1999)

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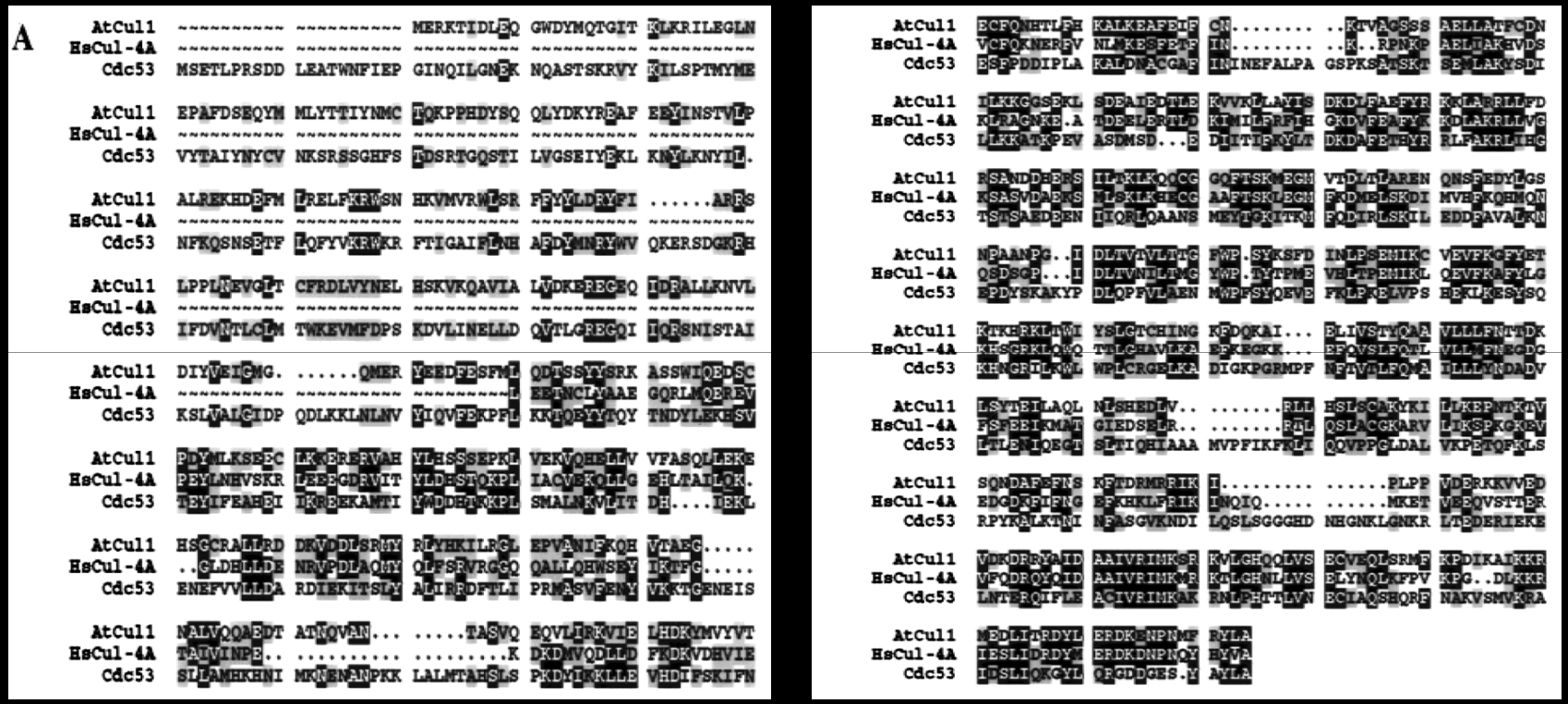

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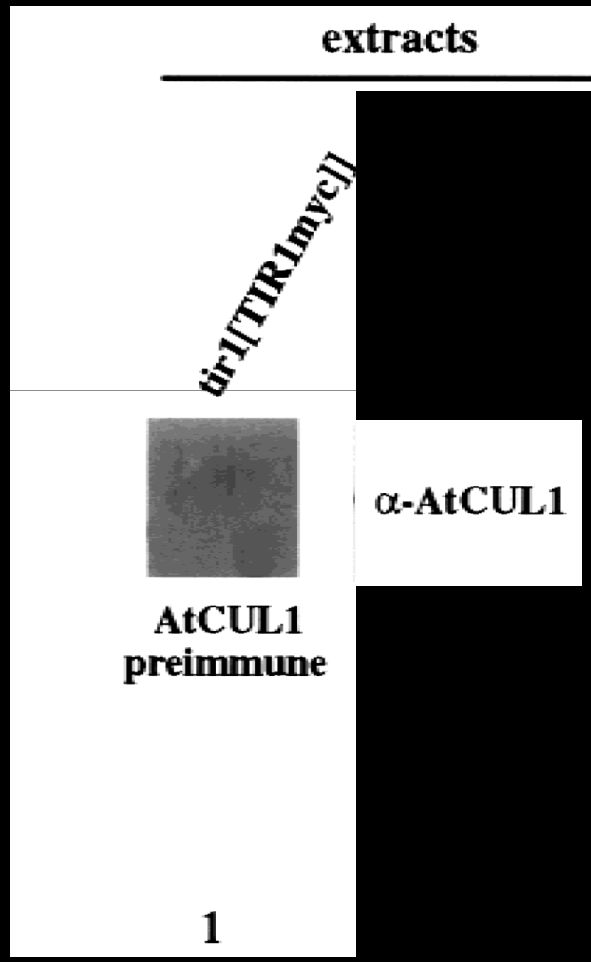

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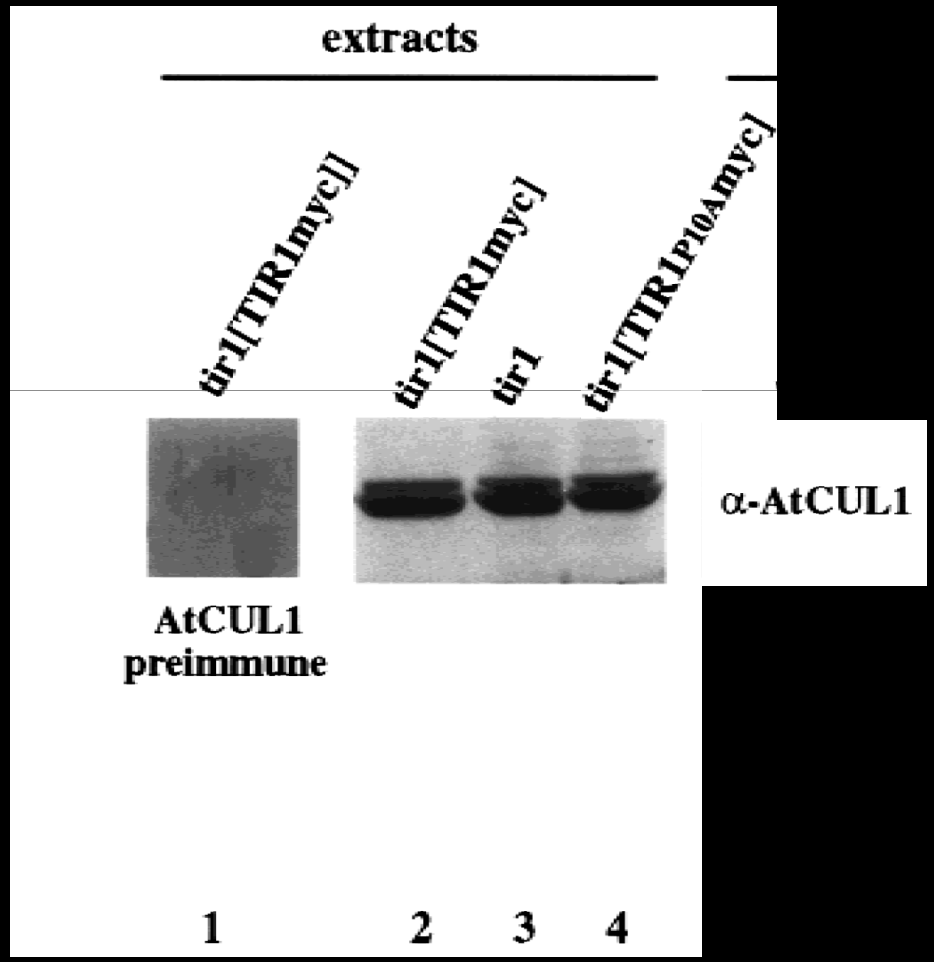

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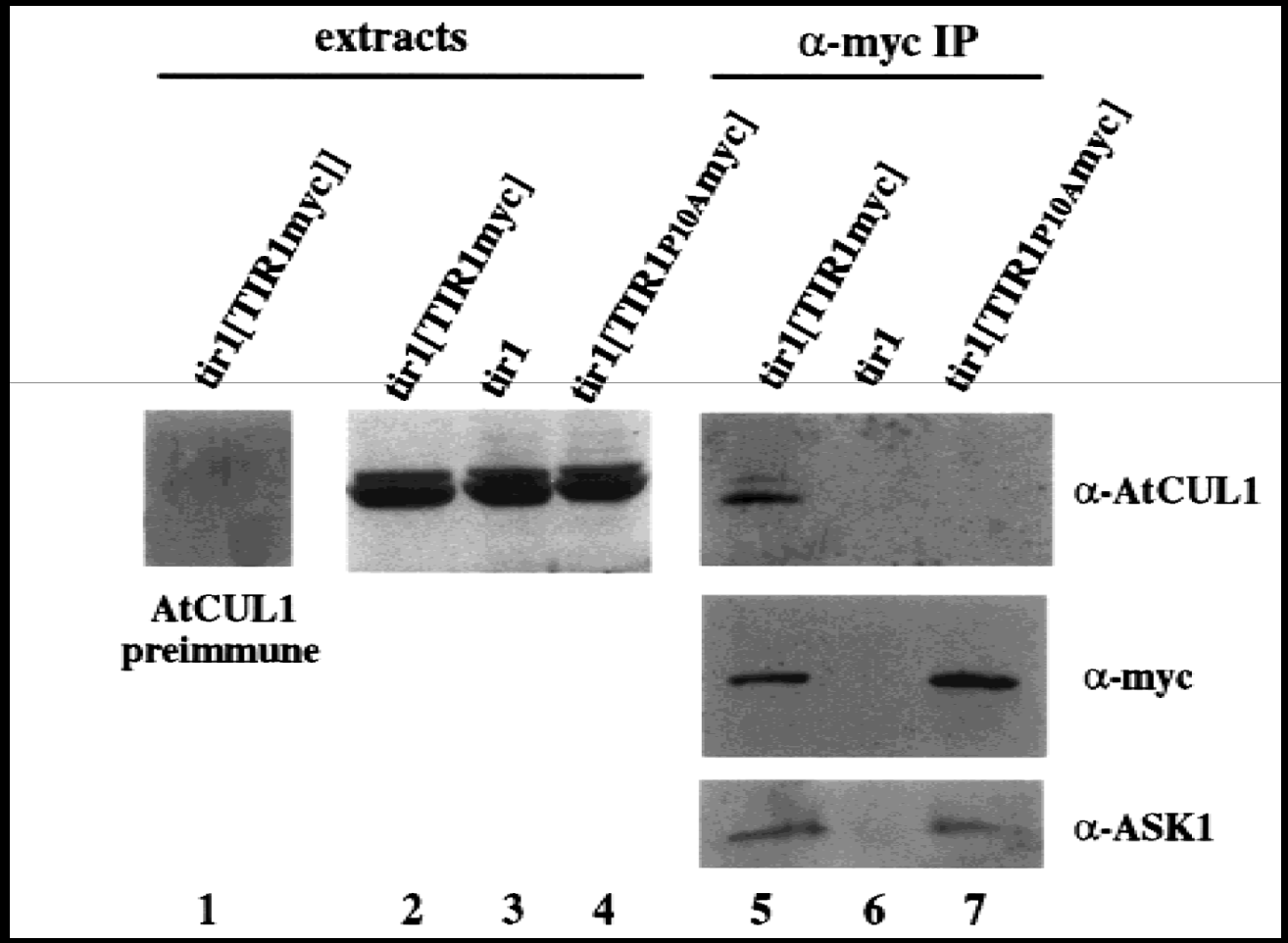

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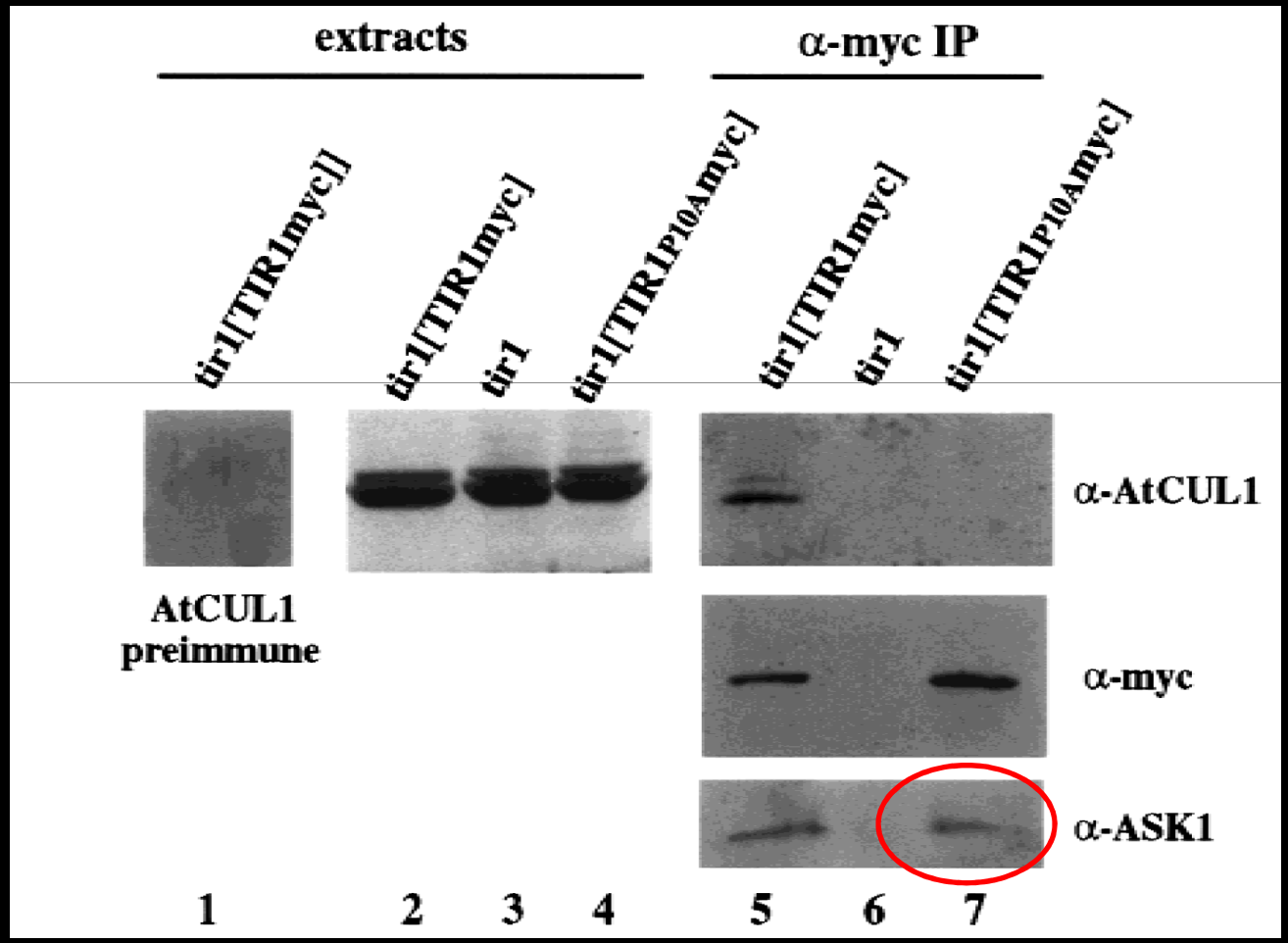

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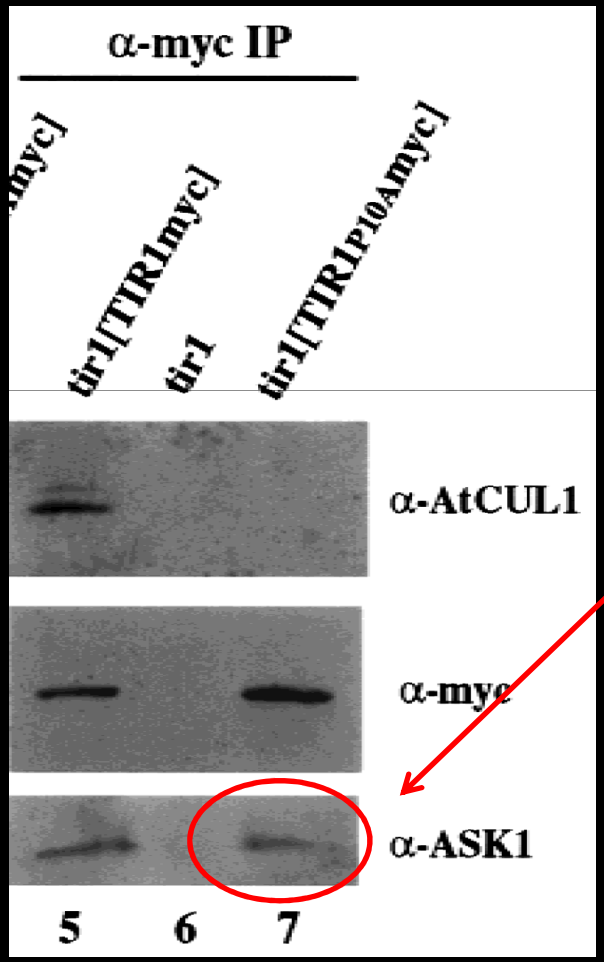

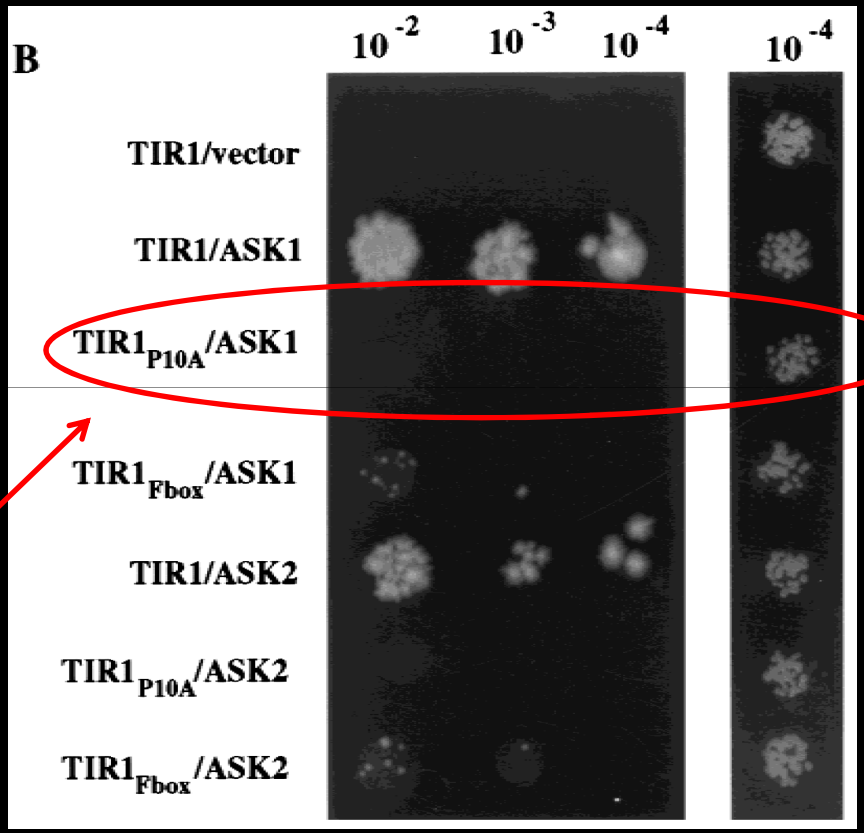

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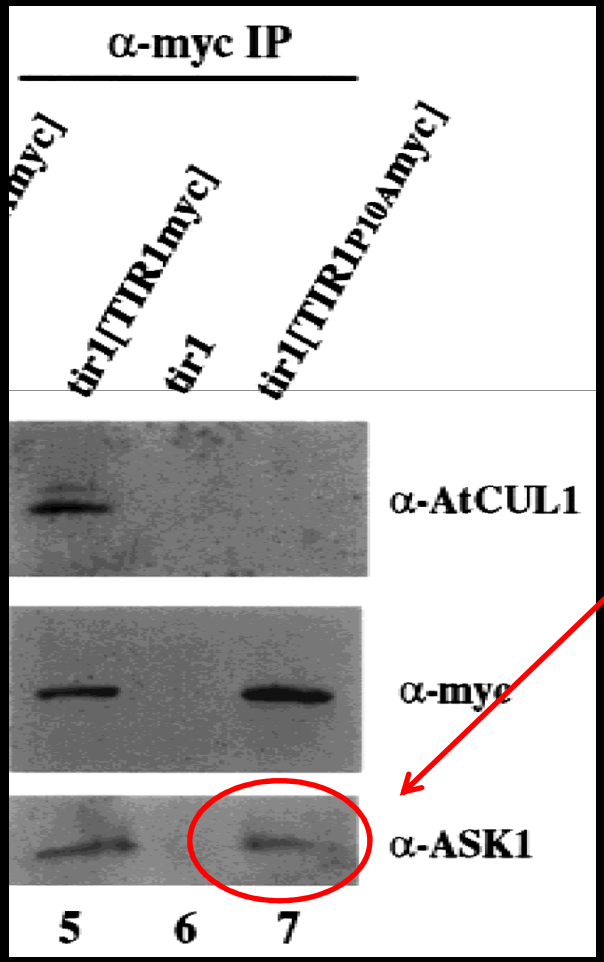
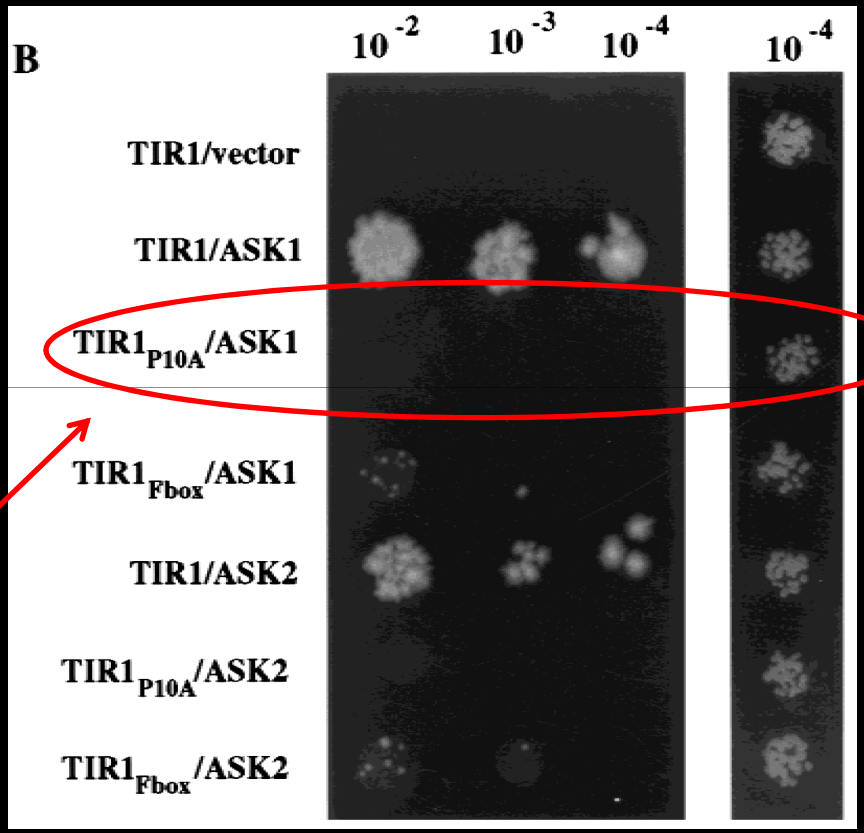
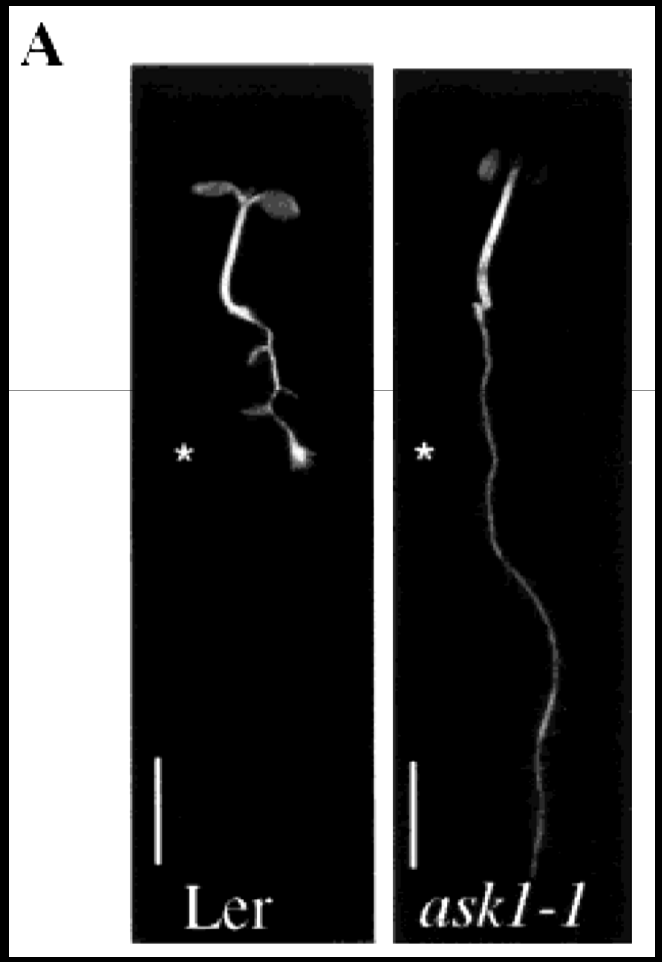
- *TIR1* bildet SCF-like Komplexe





Y2H versus *in planta*
Mögliche Kompensationsreaktionen

Ergebnisse
- *TIR1* bildet SCF-like Komplexe

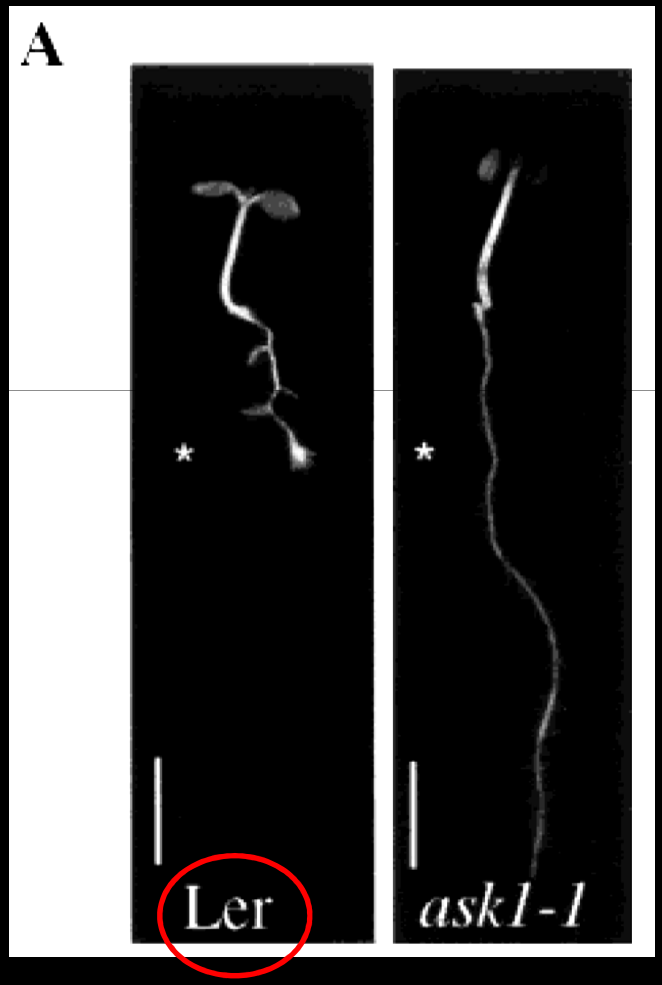


Normalmedium 4d

+ 0,085 µM 2,4-D

* = Wurzellänge vor Applikation

Ergebnisse
- *TIR1* bildet SCF-like Komplexe

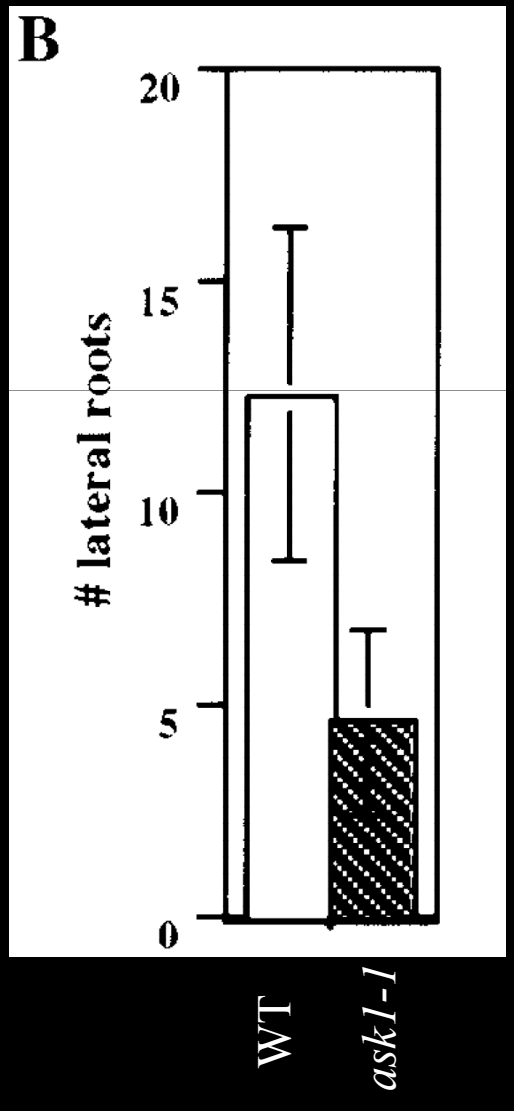


Normalmedium 4d

+ 0,085 µM 2,4-D

* = Wurzellänge vor Applikation

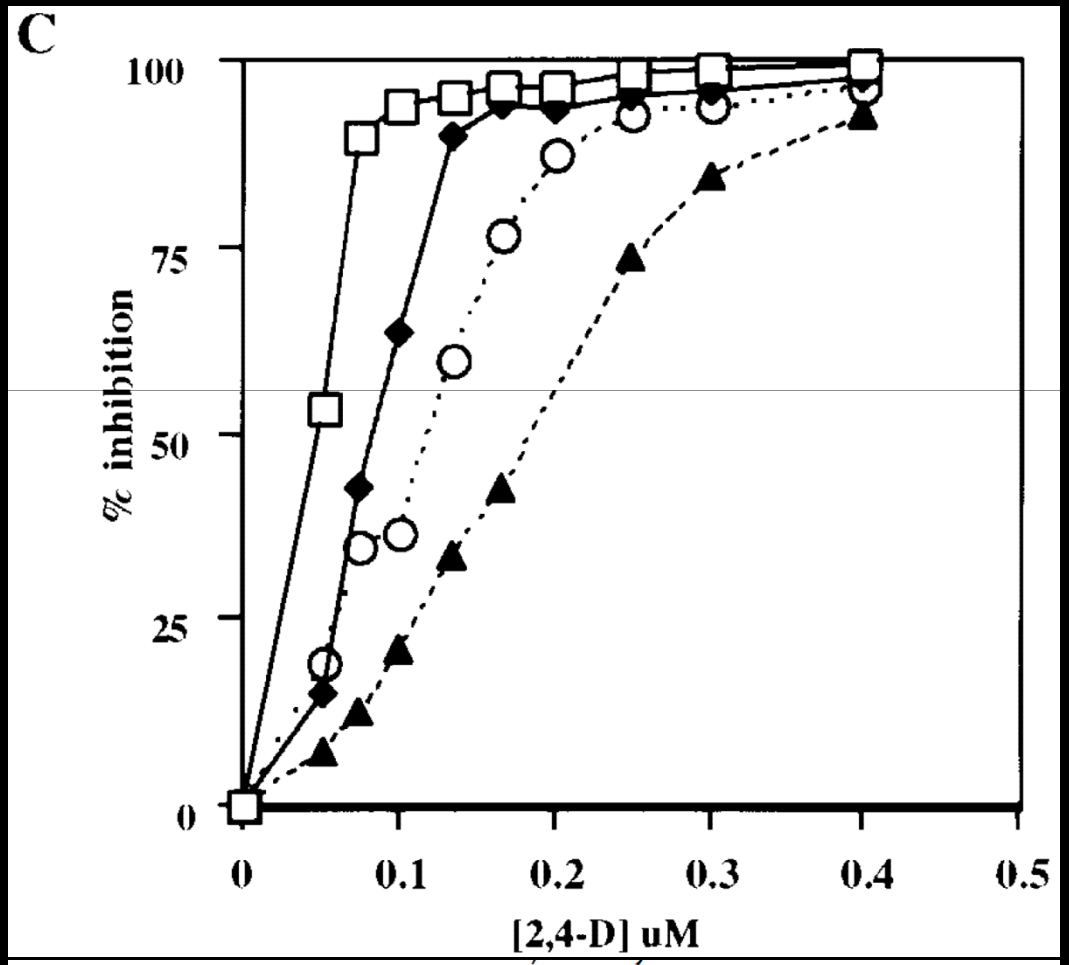
Ergebnisse
- *TIR1* bildet SCF-like Komplexe



WT und Mutante auf Normalmedium (11d)

n = ?

Ergebnisse
- *TIR1* bildet SCF-like Komplexe



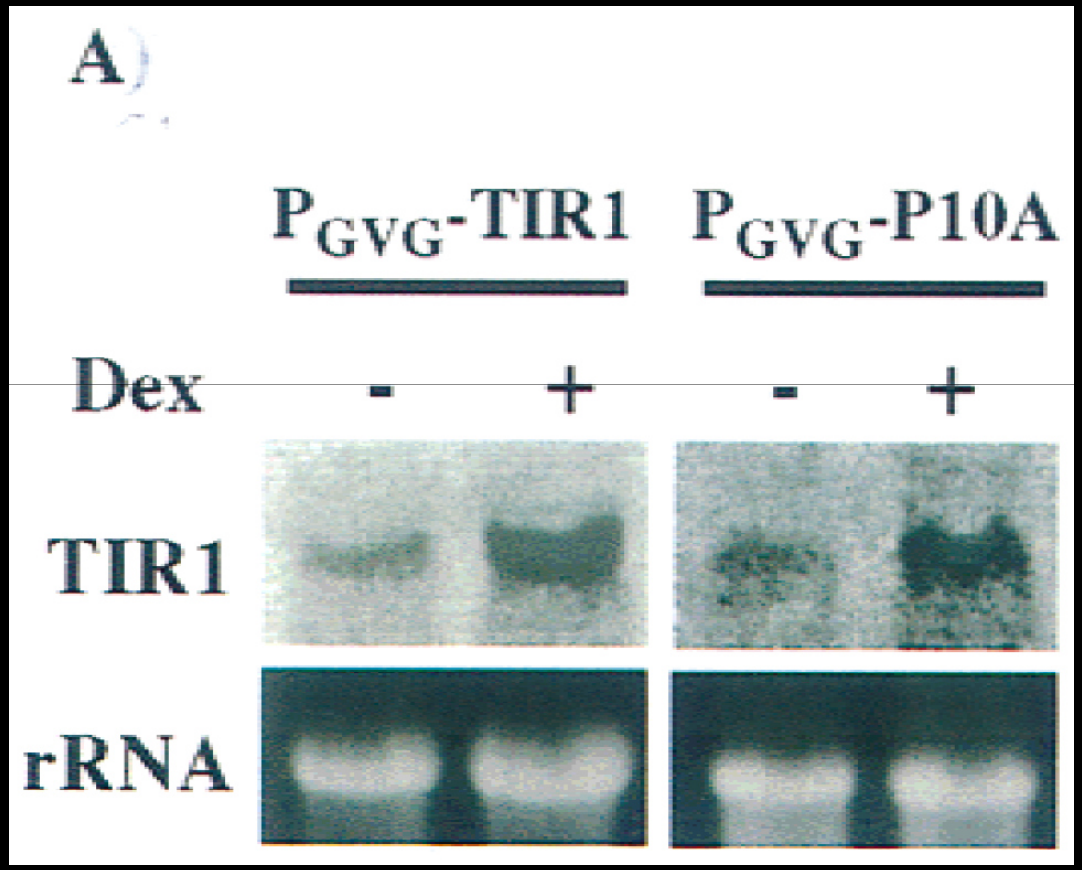
(□) *Ler*; (◆) *tir1-1*; (○) *ask1-1*; (▲) *tir1-1, ask-1*

Dosiseffekt

Verminderung des Wurzelwachstums

Jeder Punkt:
n = 10, S.E ≤ 10%

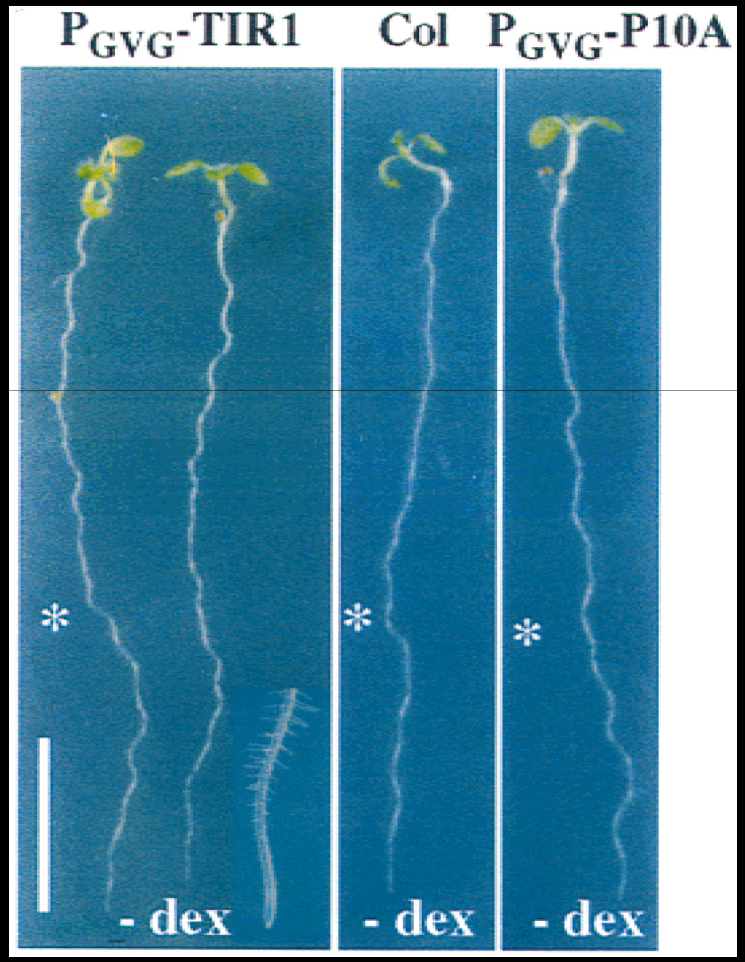
Ergebnisse
- *TIR1 Üb*erexpression



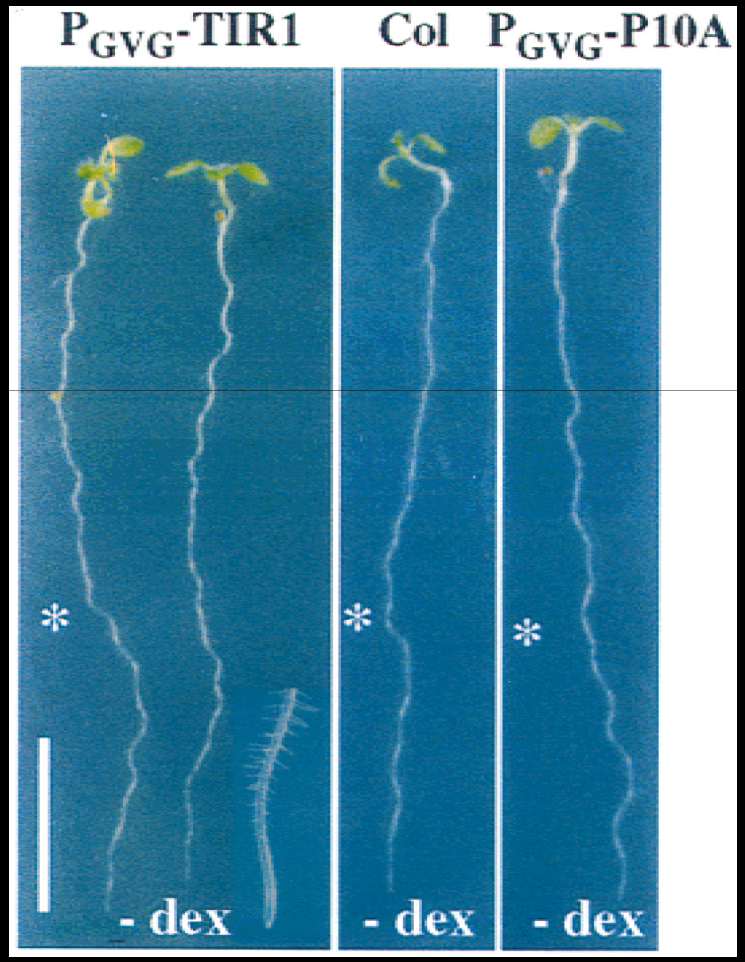
P_{GVG} = Dexamethazone aktivierbarer Promotor

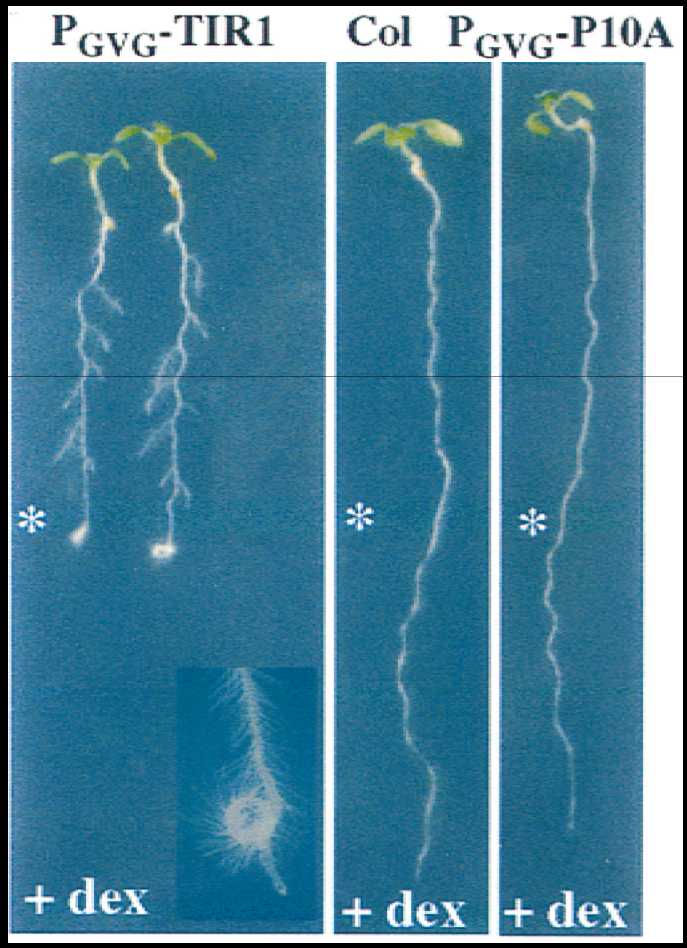
GVG = Gal4–VP16-glucocorticoid receptor
Dex = Dexamethazone

Ergebnisse
- *TIR1 Üb*erexpression

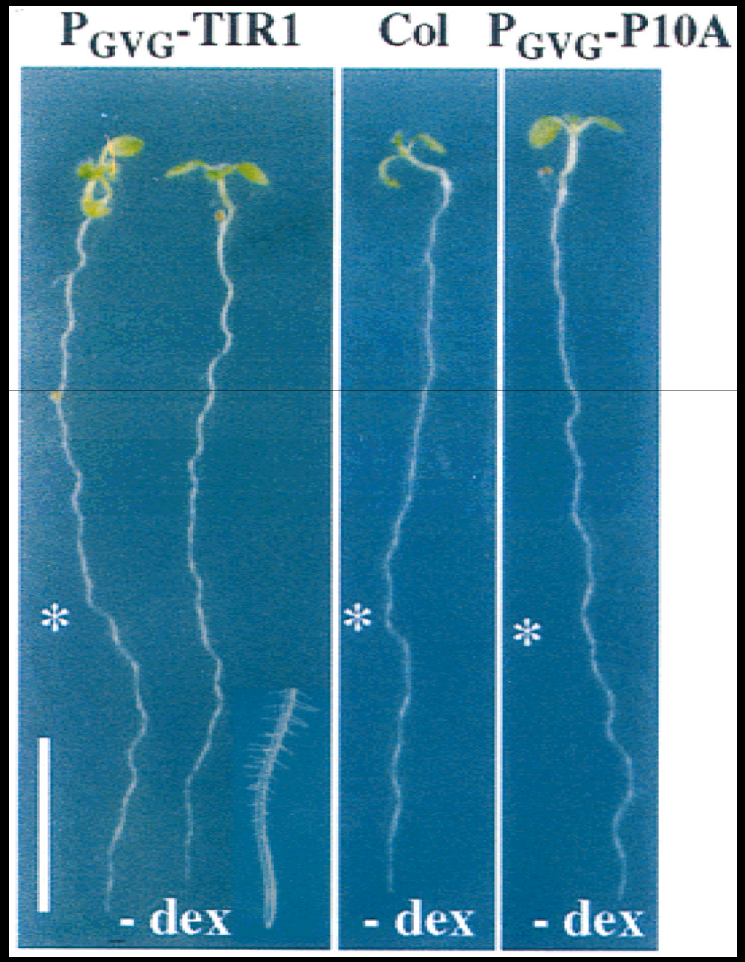


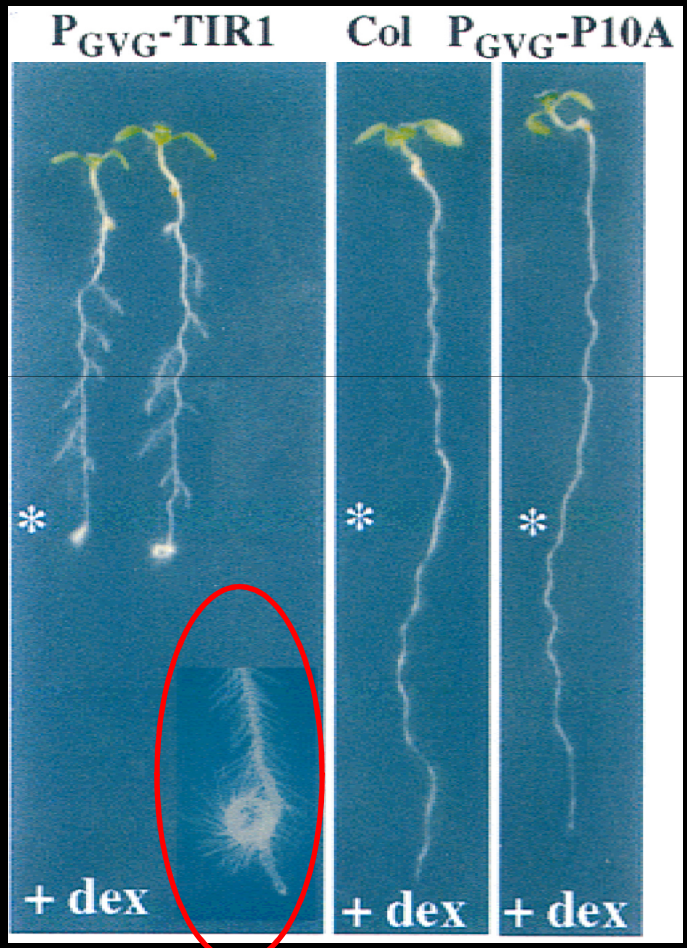
Ergebnisse
- *TIR1 Üb*erexpression





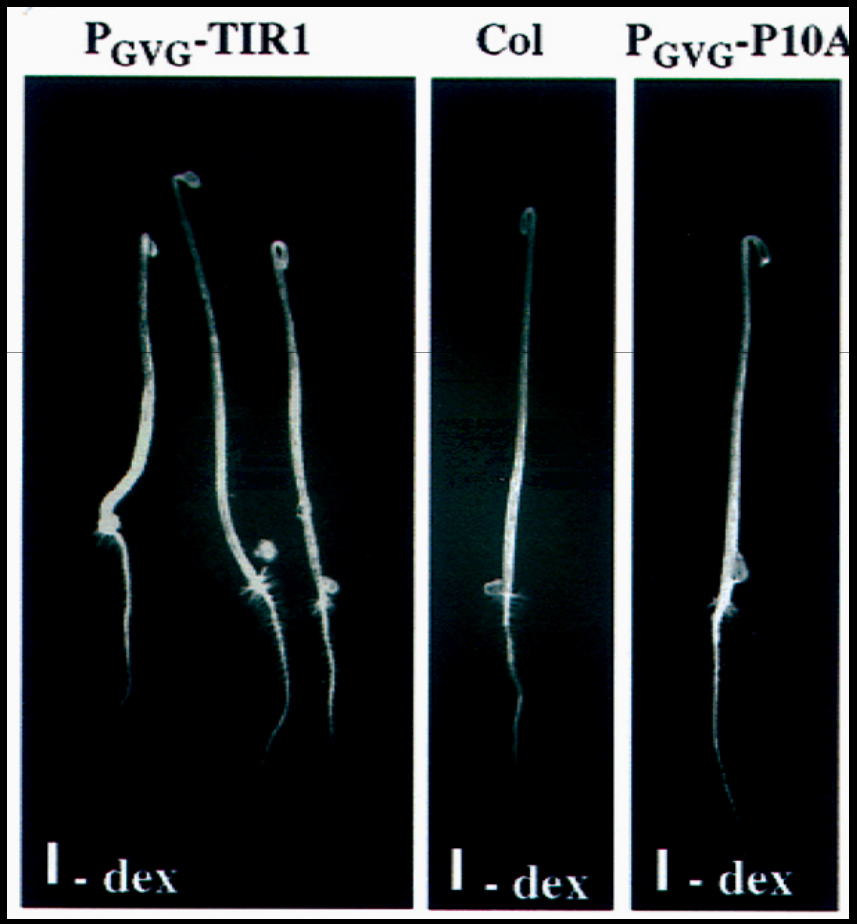
Ergebnisse
- *TIR1 Üb*erexpression

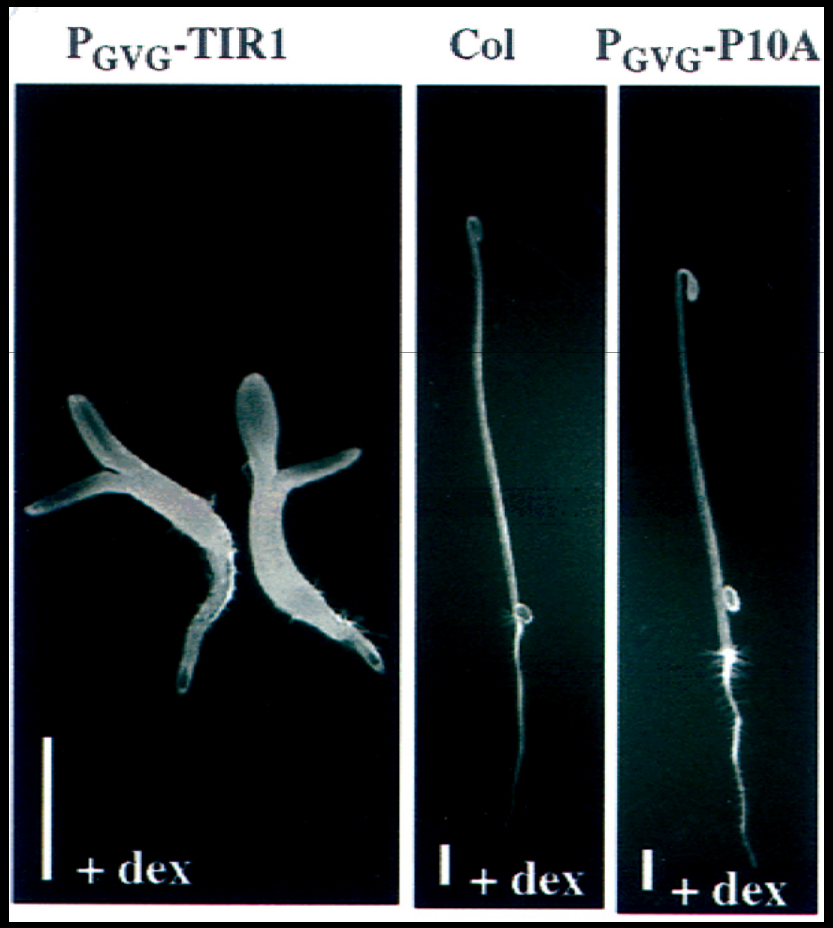




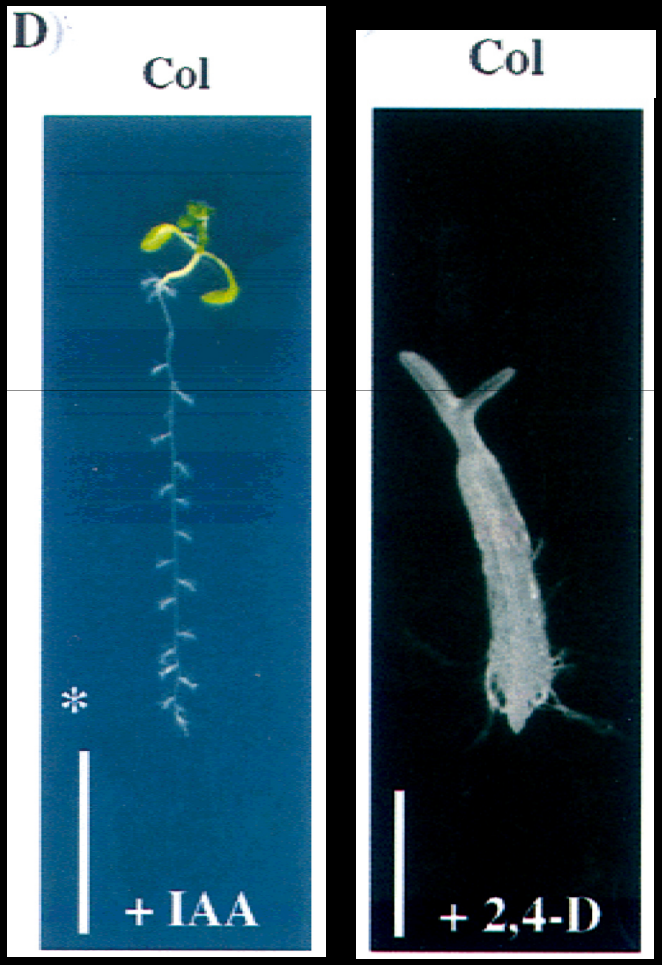
Bekannter Phänotyp?

Ergebnisse
- *TIR1 Üb*erexpression

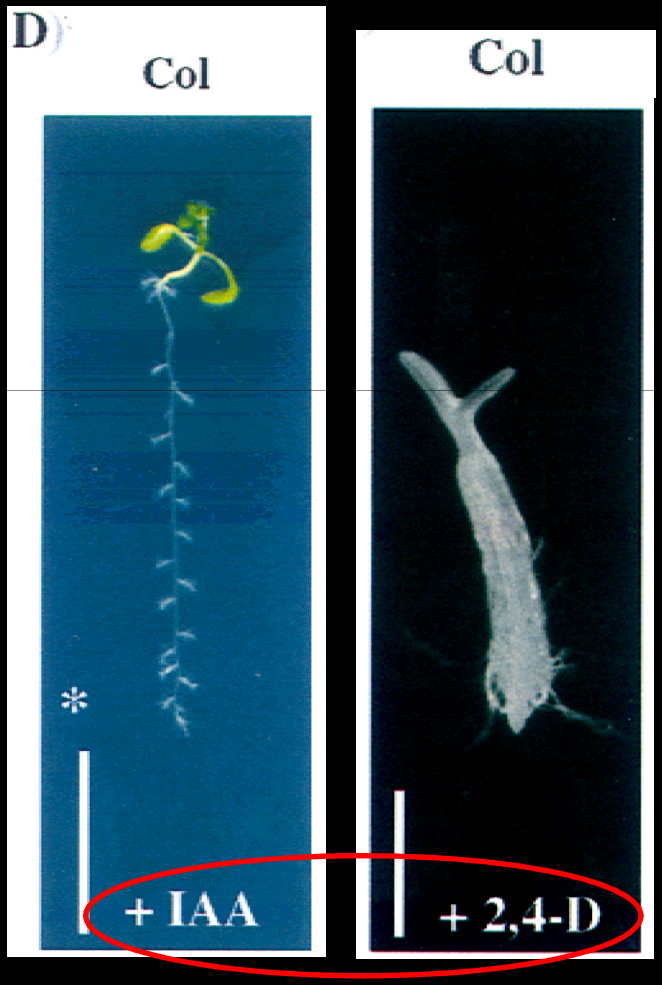




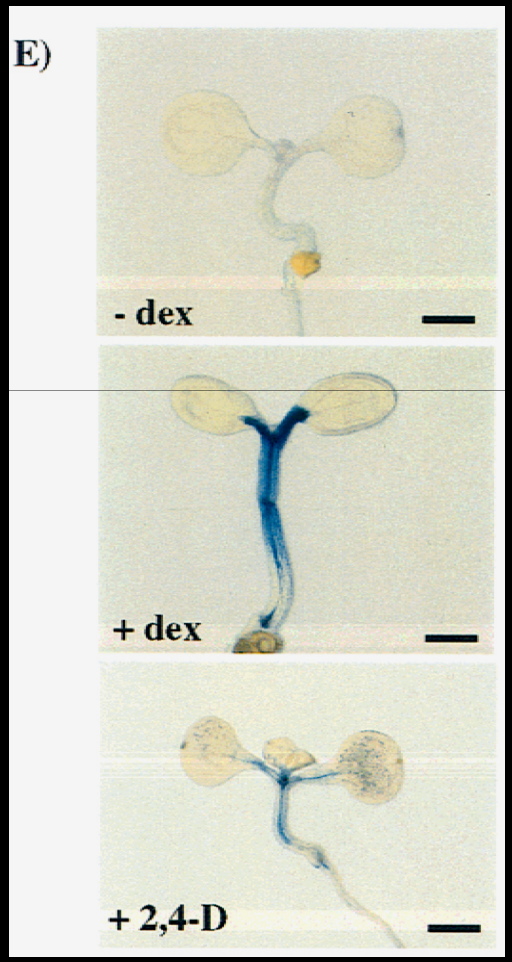
Ergebnisse
- *TIR1 Üb*erexpression



Ergebnisse
- *TIR1 Üb*erexpression



Ergebnisse
- *TIR1 Überexpression*



P_{GVG}-TIR1

WT

pIAA4–gus in *PGVG–TIR1*

Wild-type *pIAA4–gus*

pIAA4 = Auxin Reporter

Zusammenfassung

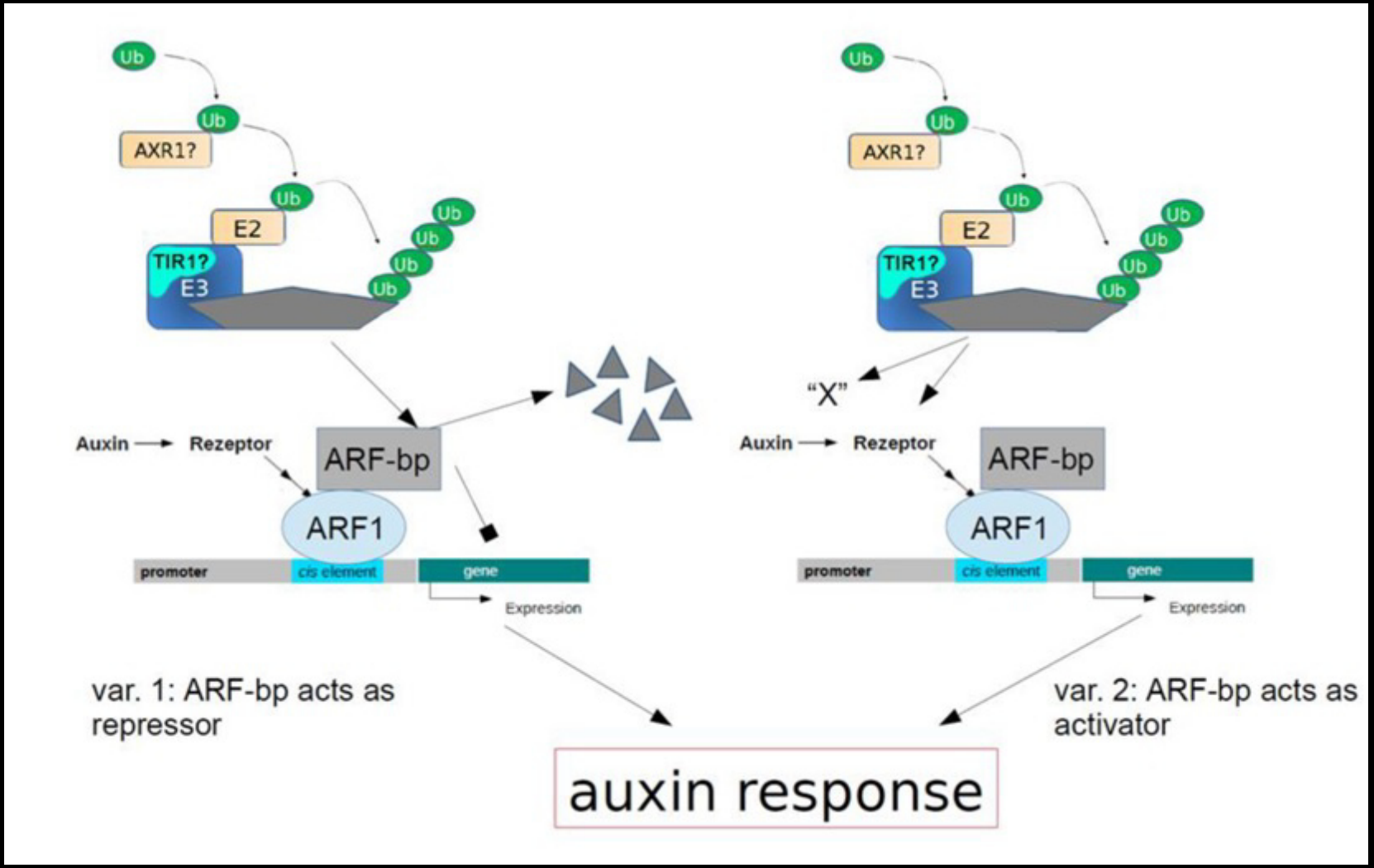
Ruegger et al. 1998

- F-Box Protein TIR1 – Auxin response
- TIR1 Teil eines SCF-ubiquitin-ligase Komplex?

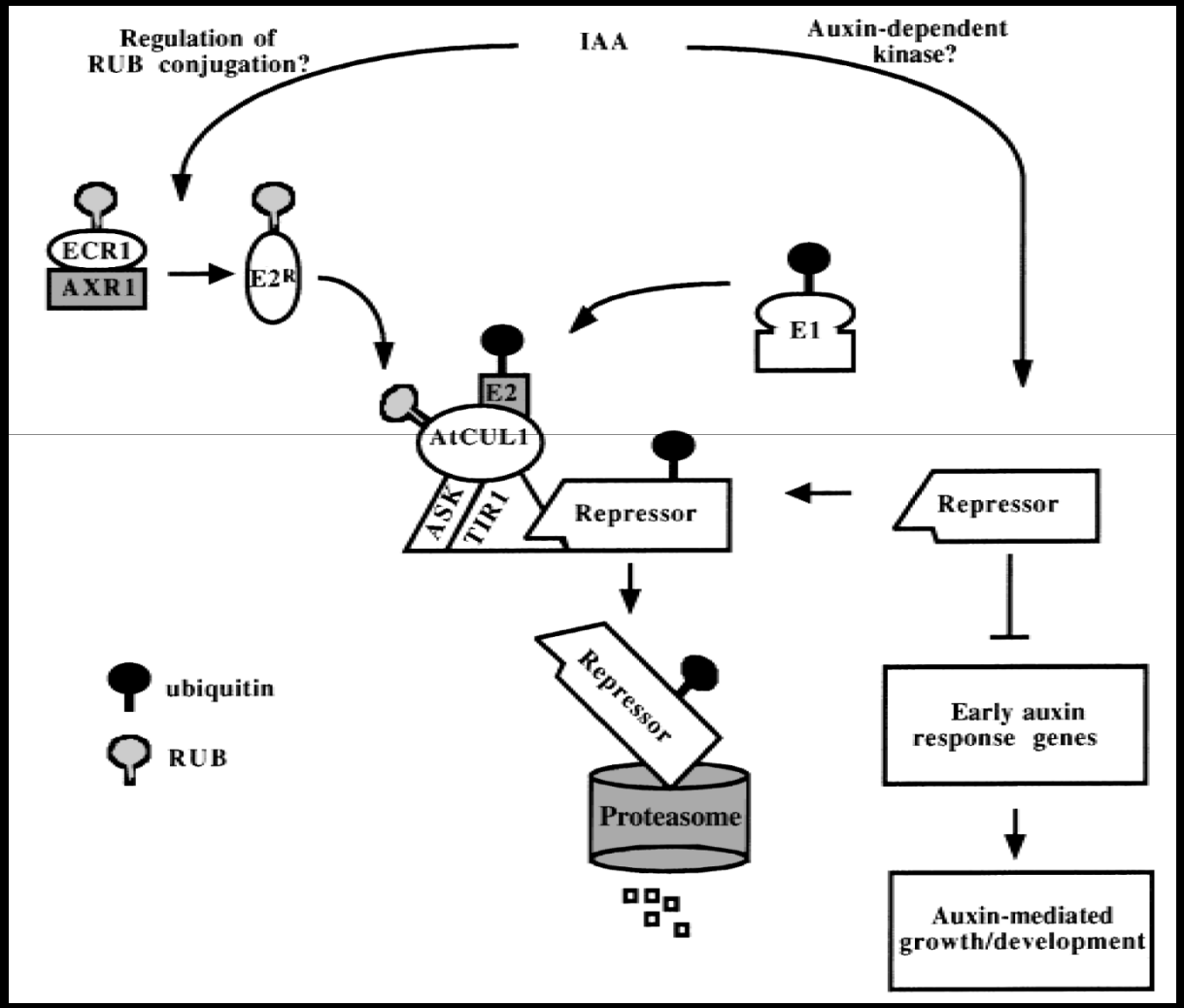
Neue Ergebnisse:

- 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^{TIR1}-dependent degradation of AUX/IAA proteins

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