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

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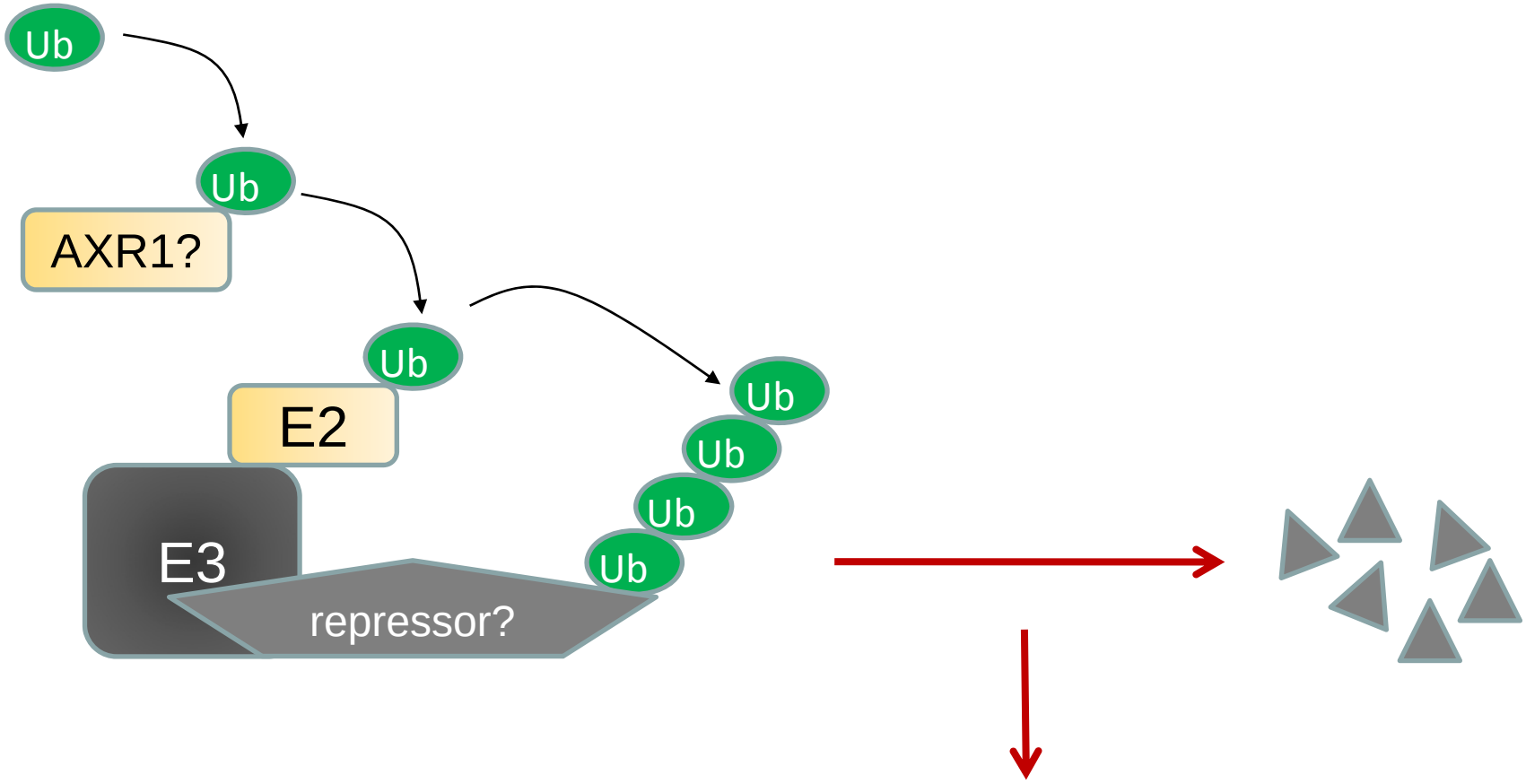
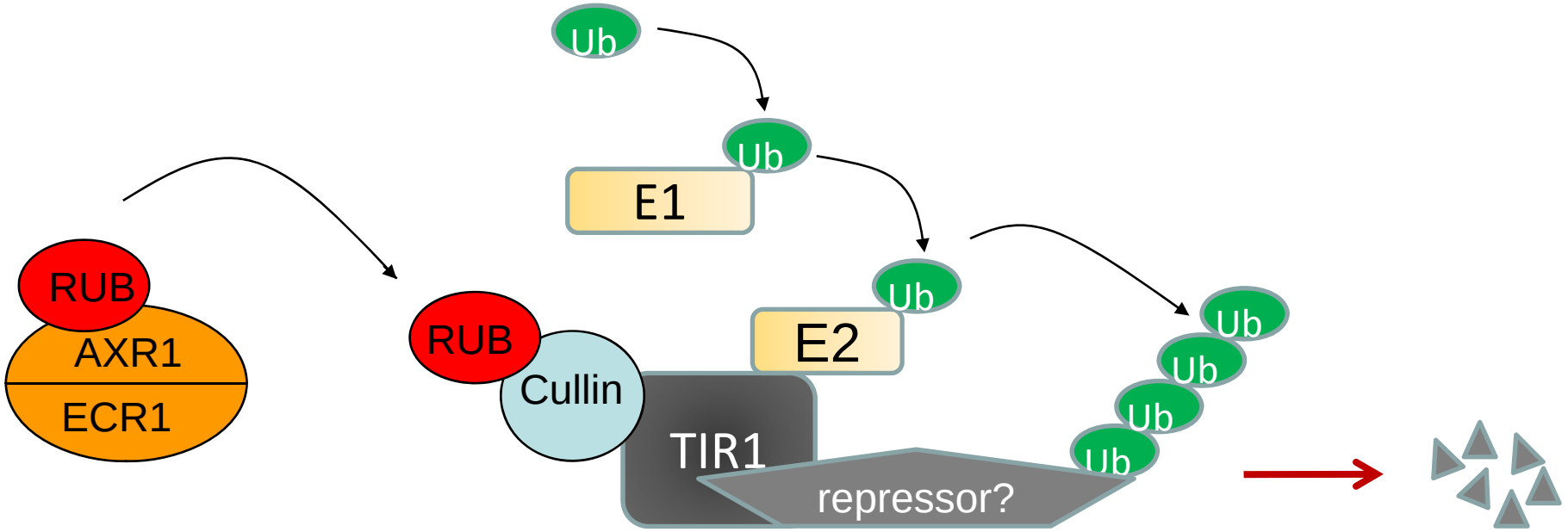
Bisheriges Modell



AXR1 similar to ubiquitin-activating enzyme E1

auxin response



auxin response

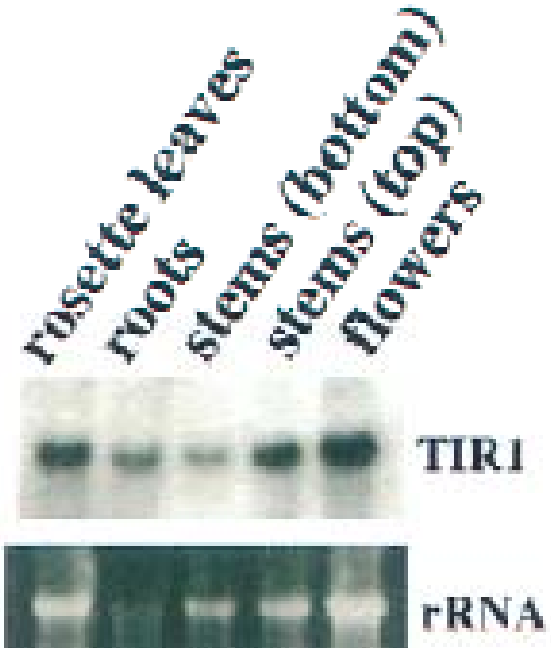
~~AXR1 similar to ubiquitin-activating enzyme E1~~

Kernaussagen dieses Papers

- Beweis, dass *TIR1* mit *ASK1* & *ASK2* (=Skp-like) interagiert
- Mutationen im *ASK1*-Gen → verminderte Auxin-Antwort
- Übermäßige Expression von *TIR1* → morphologische Unterschiede zum WT
- neue Funde weisen darauf hin, dass SCF^{TIR1}-Modell realistisch ist
- → Auxin-Antwort = SCF-vermittelt

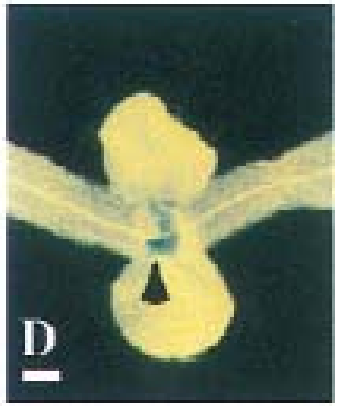
Analyse der TIR1-Expression

Northern Blot



Nachweis der TIR1-Expression

Färbung durch GUS-Reporter



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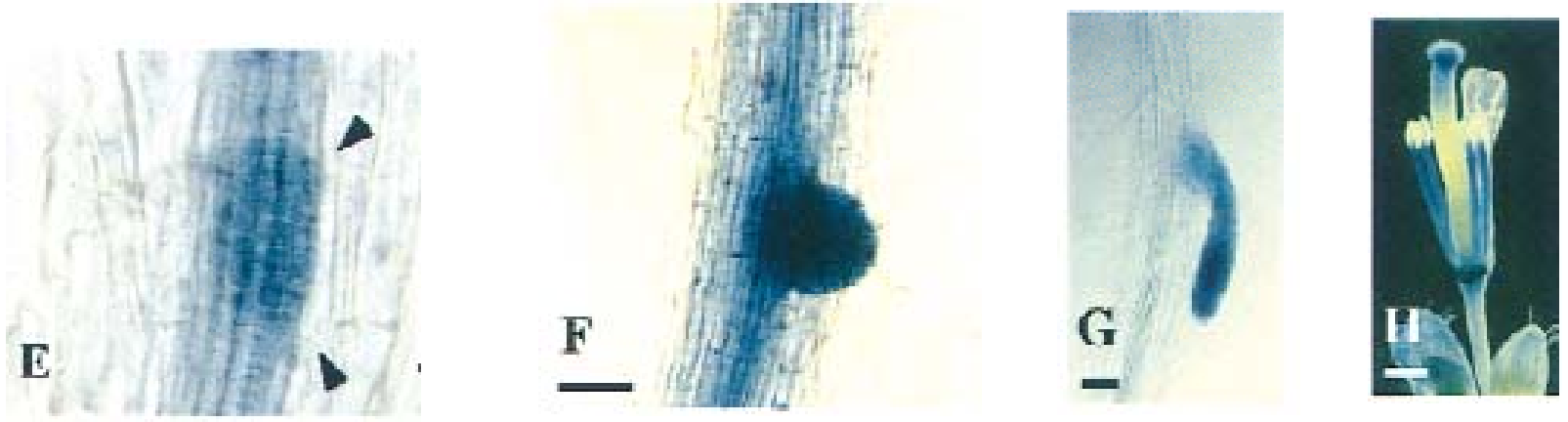


In-situ-Hybridisierung

…TCAT… RNA

…AGTA… Antisense-RNA (markiert)

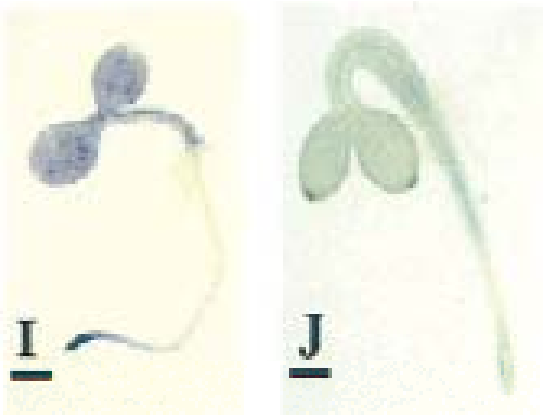
Durch künstlich hergestellte Sonde Komplementarität der Stränge
→ Blaufärbung

…TCAT… Sense-RNA

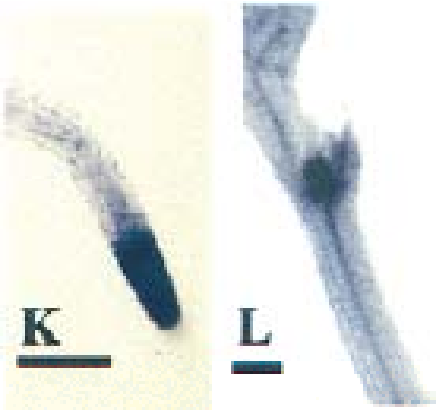
Sense-RNA bindet durch fehlende Komplementarität nicht an RNA
→ keine Färbung

Nachweis der TIR1-Expression

In-situ-Hybridisierung



Antisense Sense



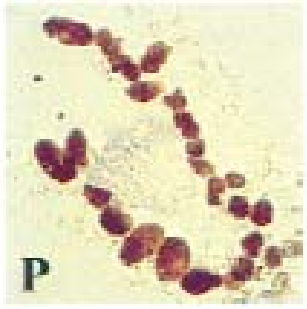
Nachweis der TIR1-Expression

In-situ-Hybridisierung

Antisense

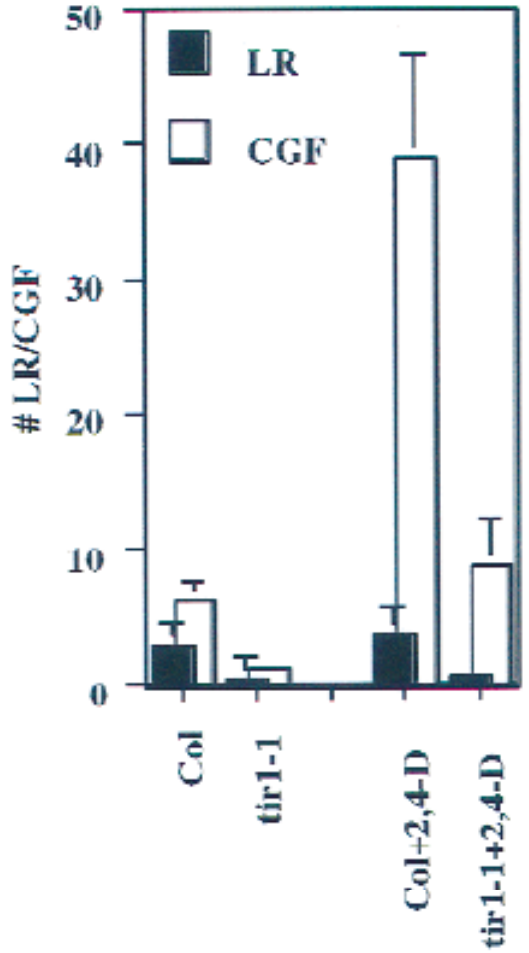
Sense

Antisense



Sense

Auswirkungen von Auxinbehandlung



LR – lateral roots

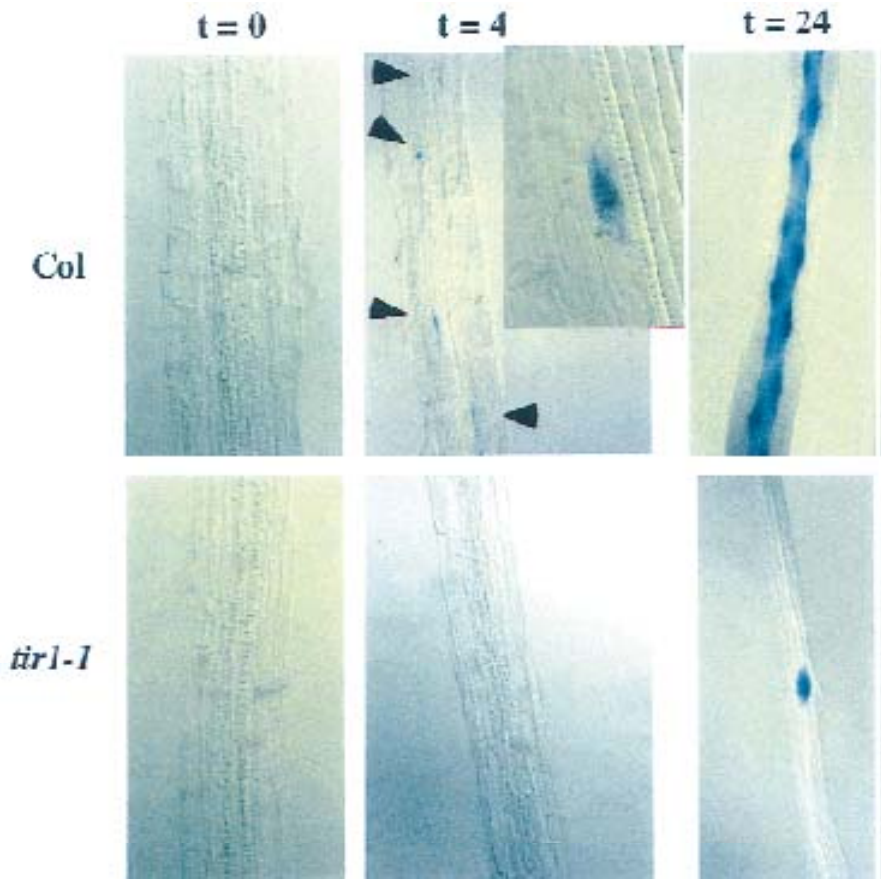
CGF – Cyc1At-GUS-Foci

TIR1-Expression geschieht bevor

Cyc1At exprimiert wird

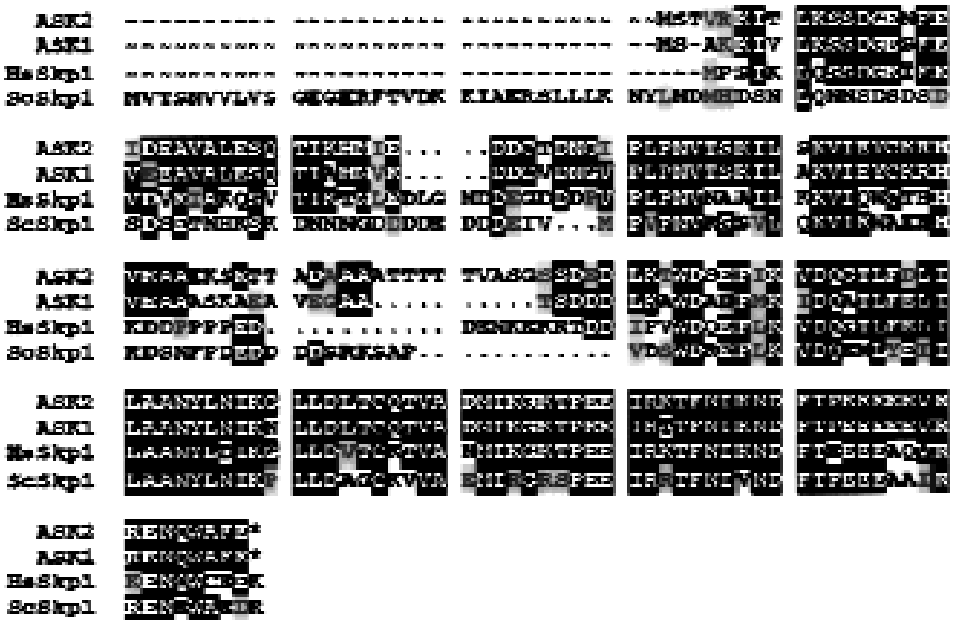
Auswirkungen von Auxinbehandlung

auf Lateralwurzeln



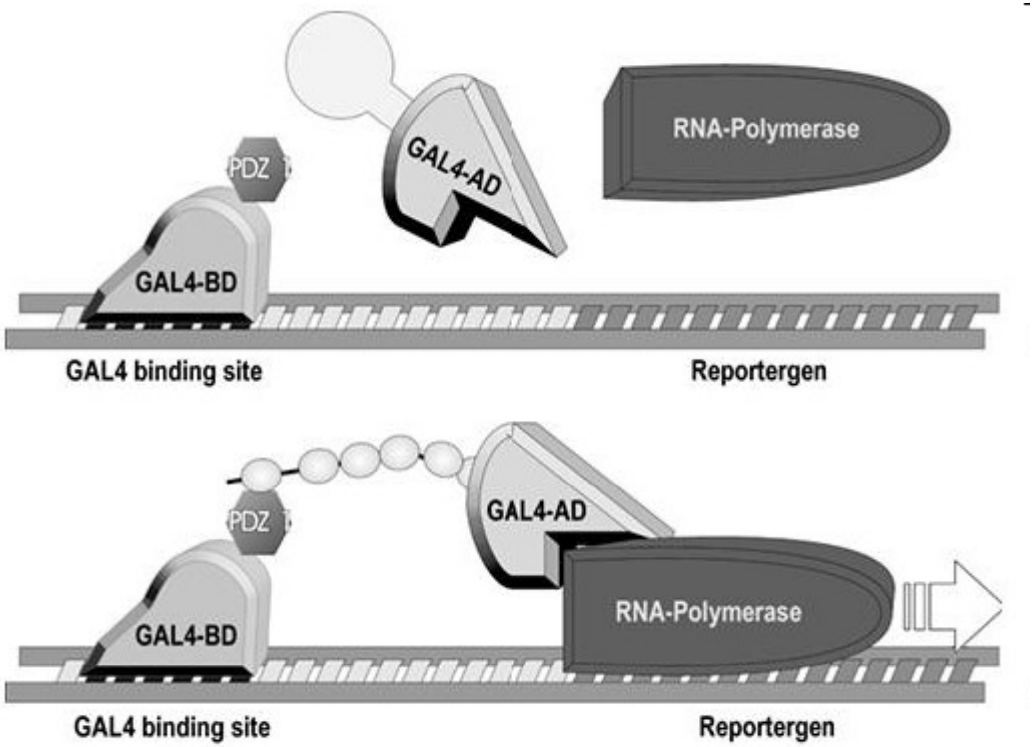
TIR1 notwendig für Teilung von Perizykelzellen

Identifikation der mit TIR1 interagierenden Proteine

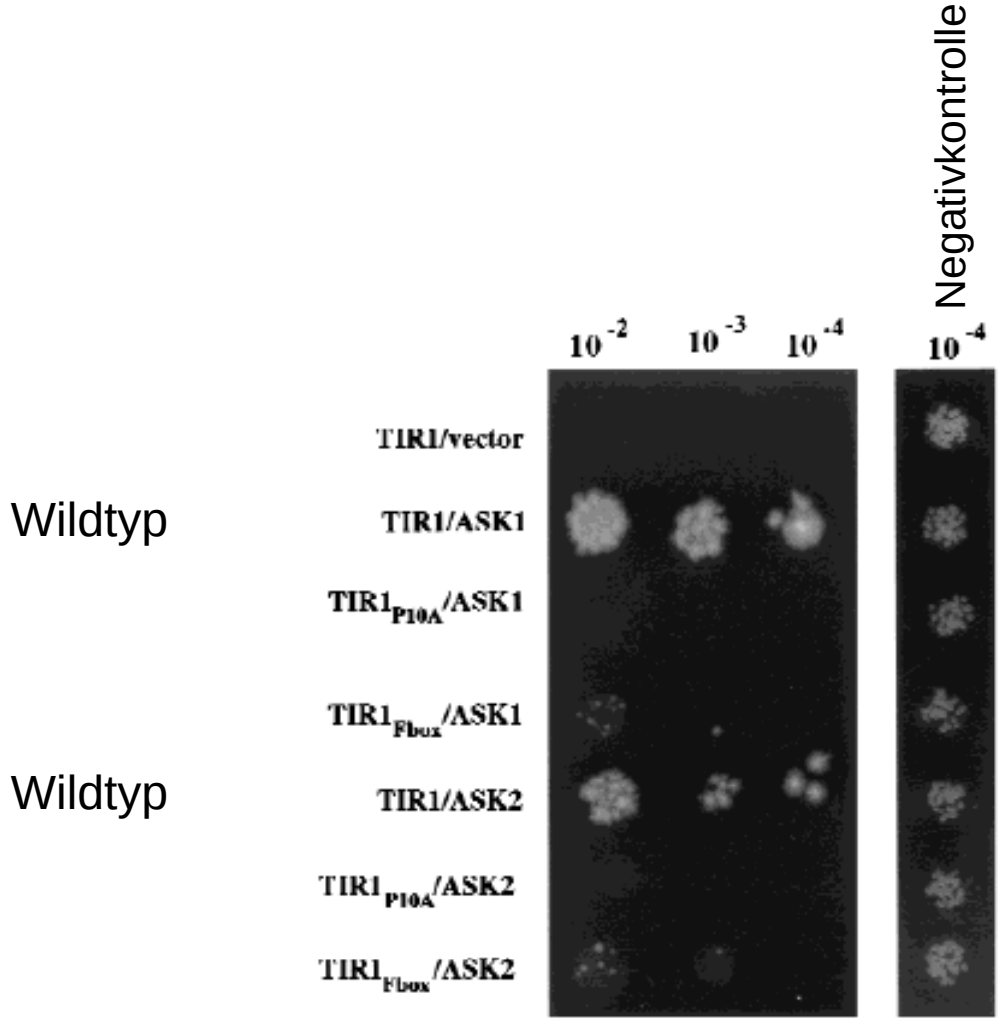


Homologien von ASK1 und ASK2 zu Proteinen in Mensch und Hefe

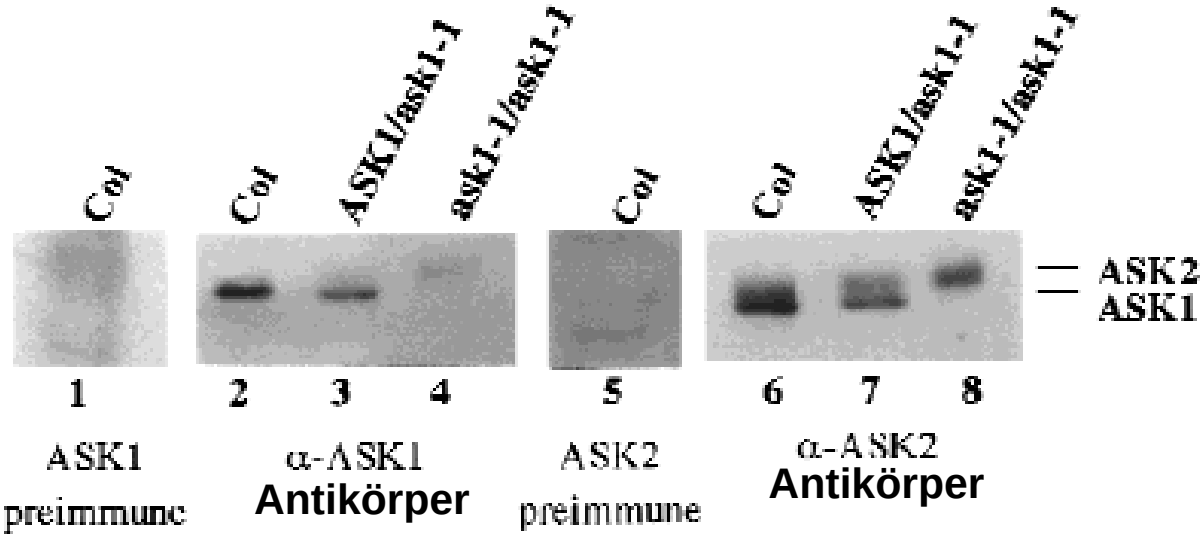
yeast two-hybrid System



Kontrolle in Hefe

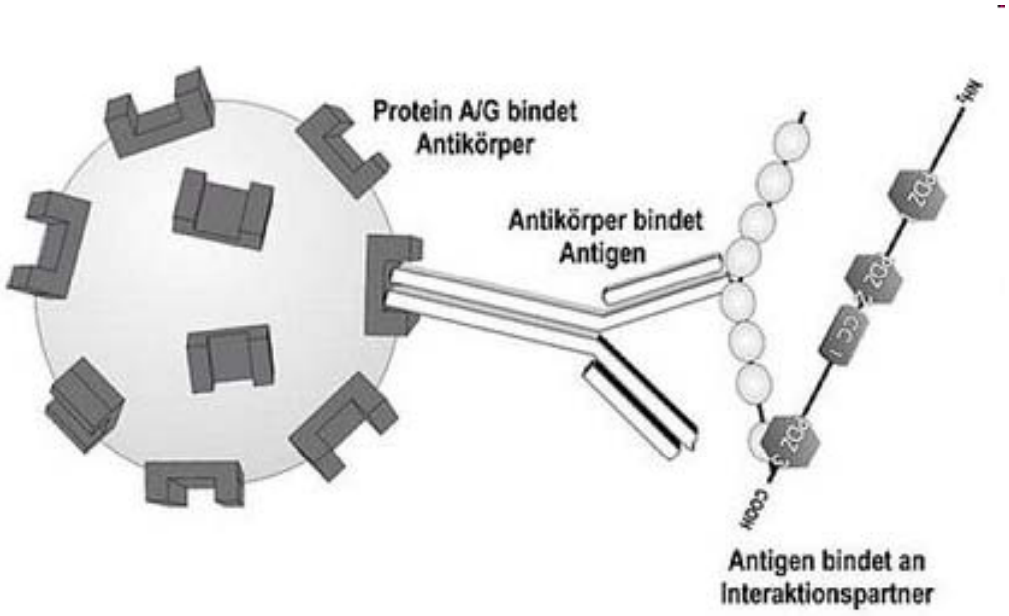


Western Blot



Ladekontrolle fehlt

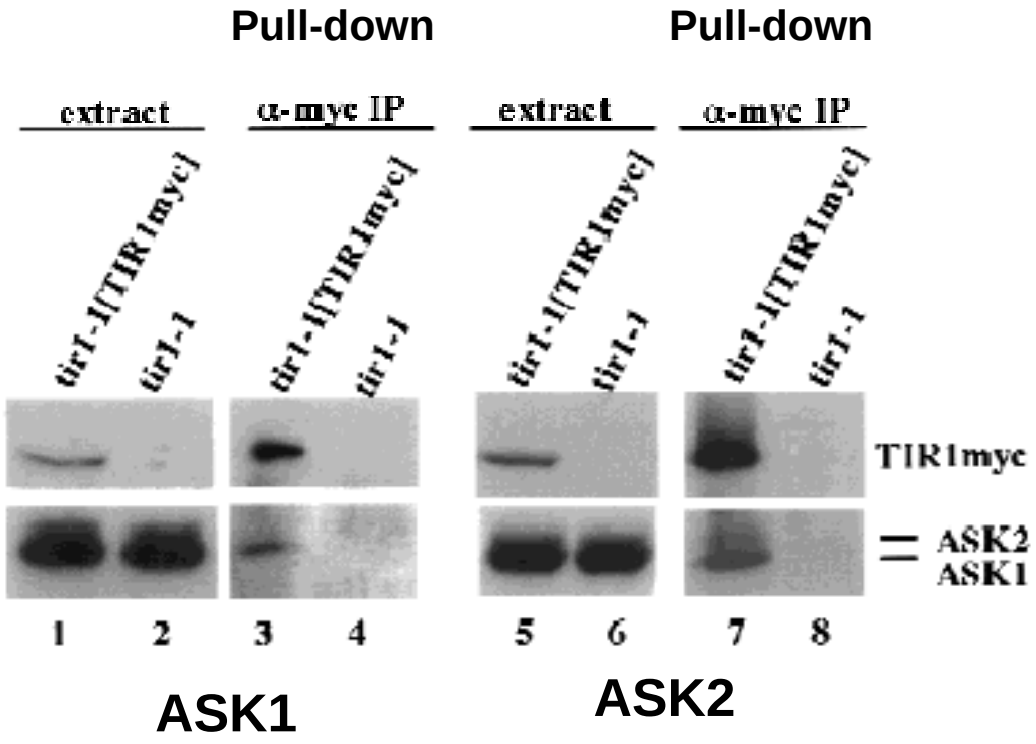
Co-Immunopräzipitation



Nachweis von Protein-Protein-Wechselwirkungen

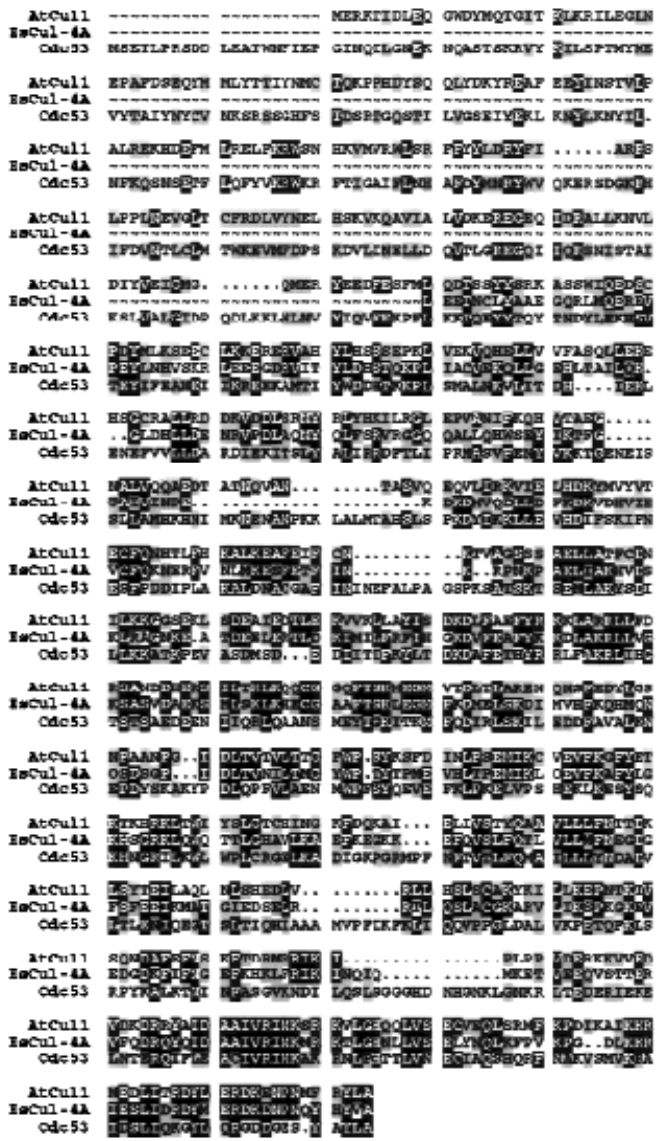
Pull-down

Co-Immunopräzipitation



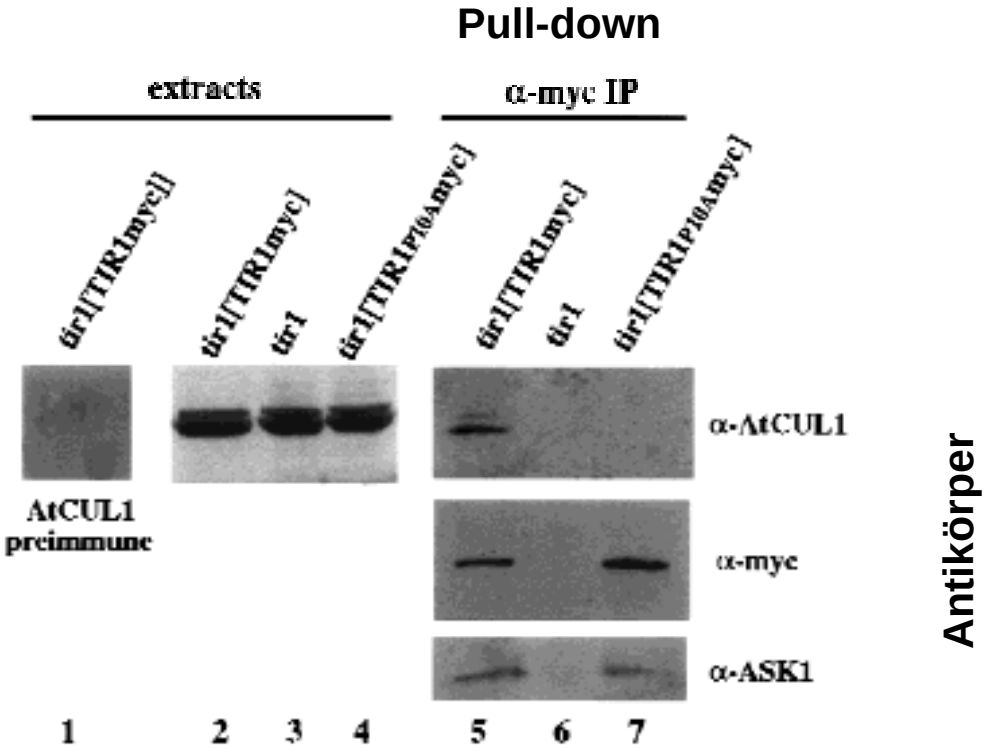
Antikörper

Identifikation von AtCul1

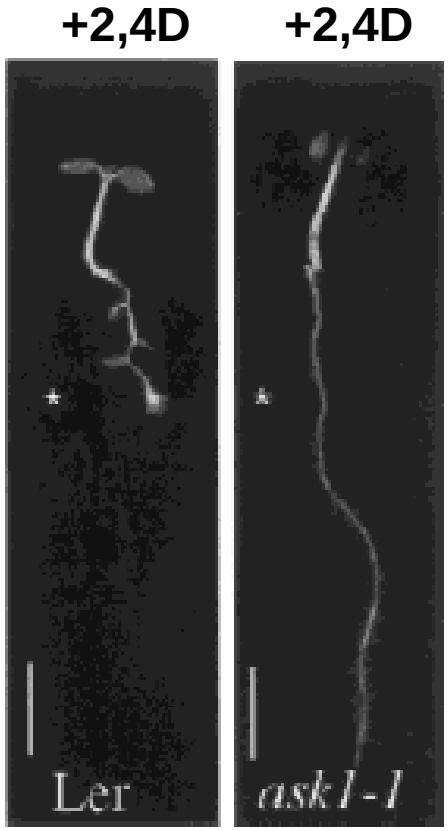


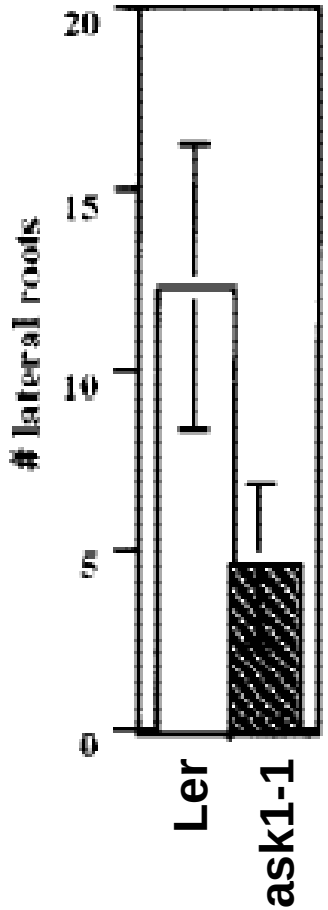
AtCUL1 stark homolog zum menschlichen Cul-4A und zum Cdc53 in Hefe

Pull-down für Cul1

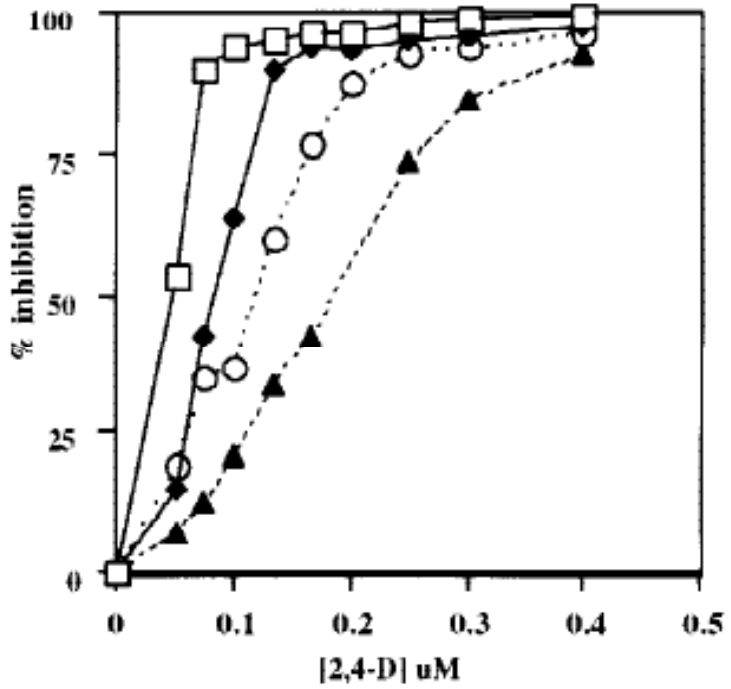


ASK1 für Auxin-response notwendig



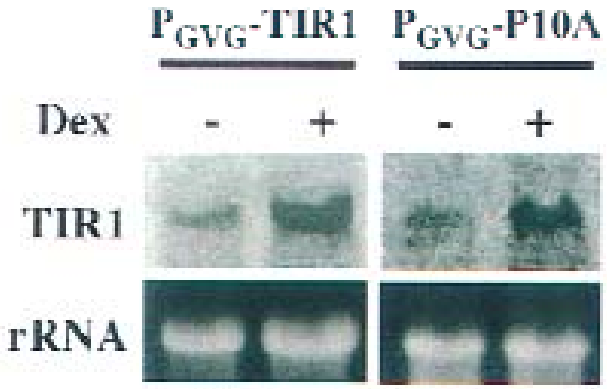


Wachstumshemmung durch Auxin



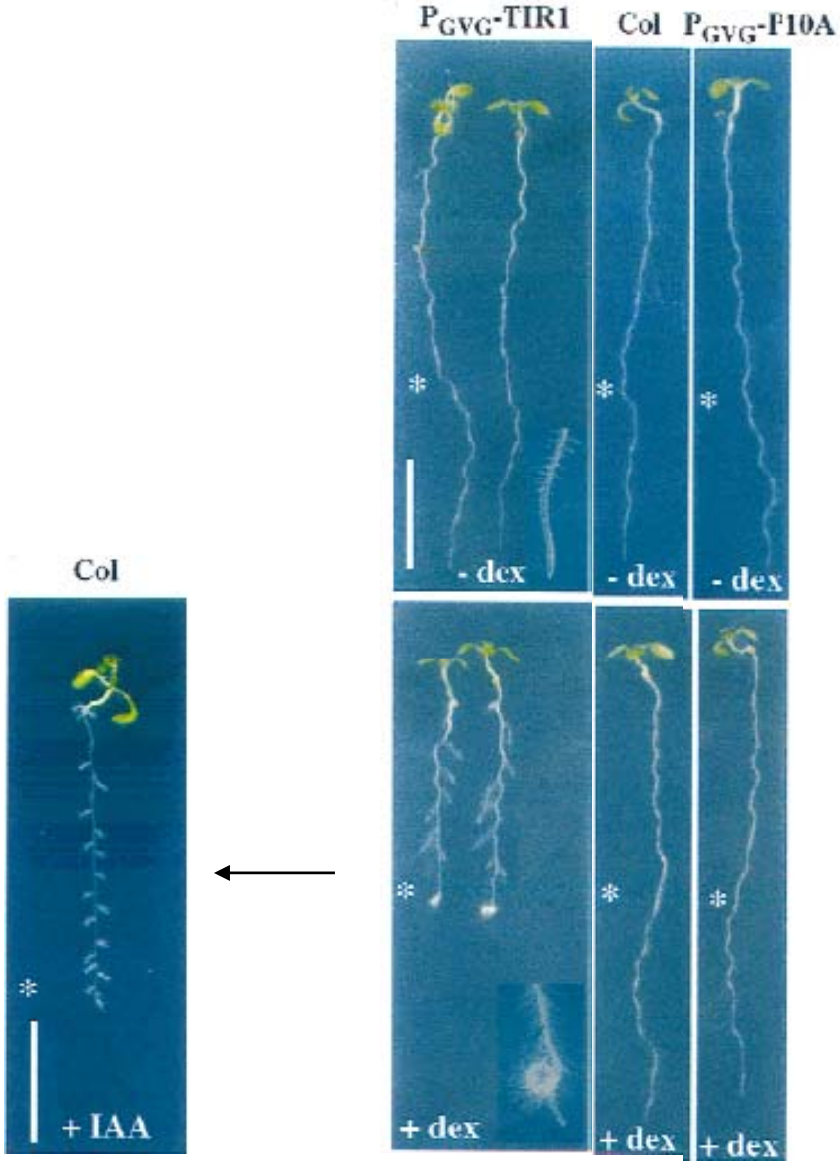
- Wildtyp
- tir1-1
- ask1-1
- tir1-1/ask1-1 (Doppelmutante)

Northern Blot für Dex-Promotor



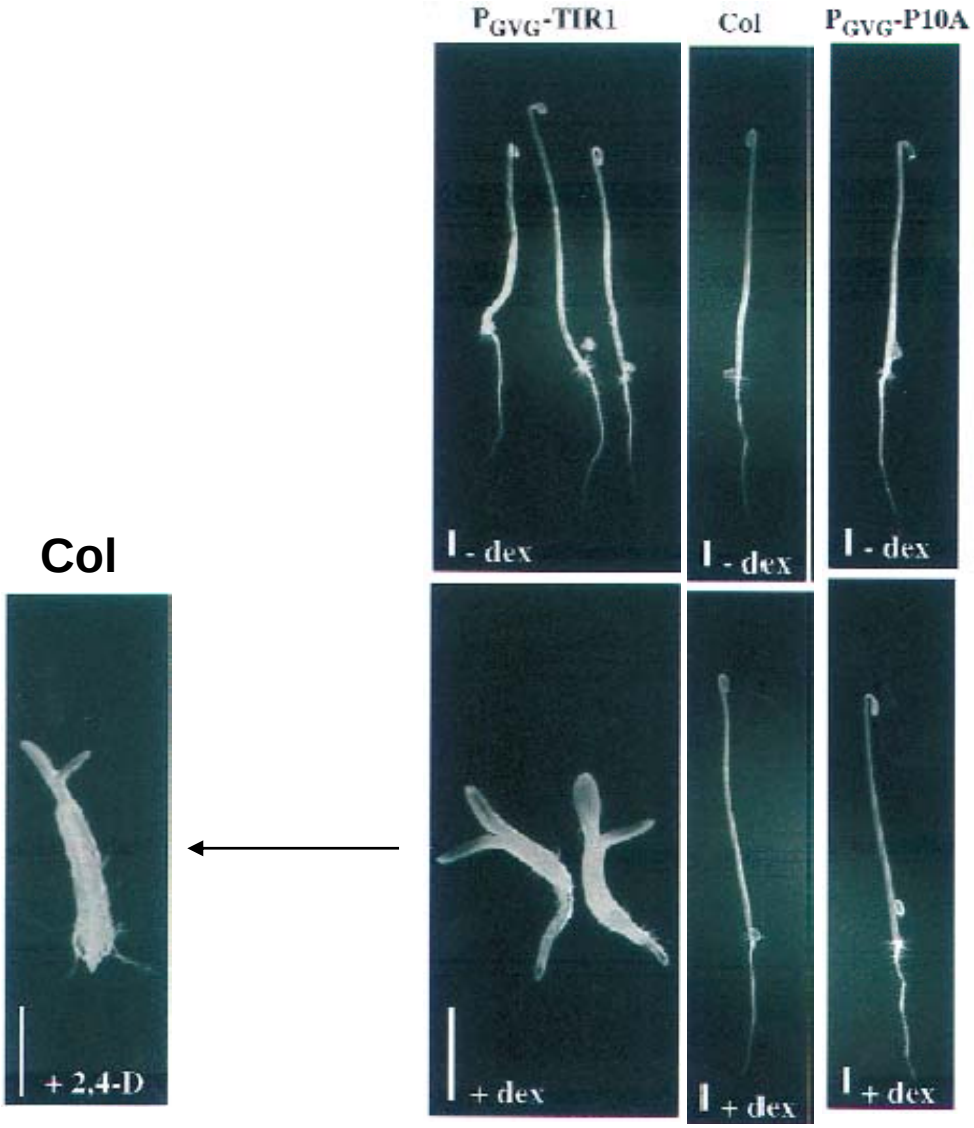
Bei Vorhandensein von Dex und Promotor (P_{GVG}) → Überexpression von TIR1

Analyse der TIR1-Überexpression



Reaktion auf Dex im Licht

Analyse der TIR1-Überexpression



Reaktion auf Dex im Dunkeln

Modell von 1999 (Gray et al.)