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

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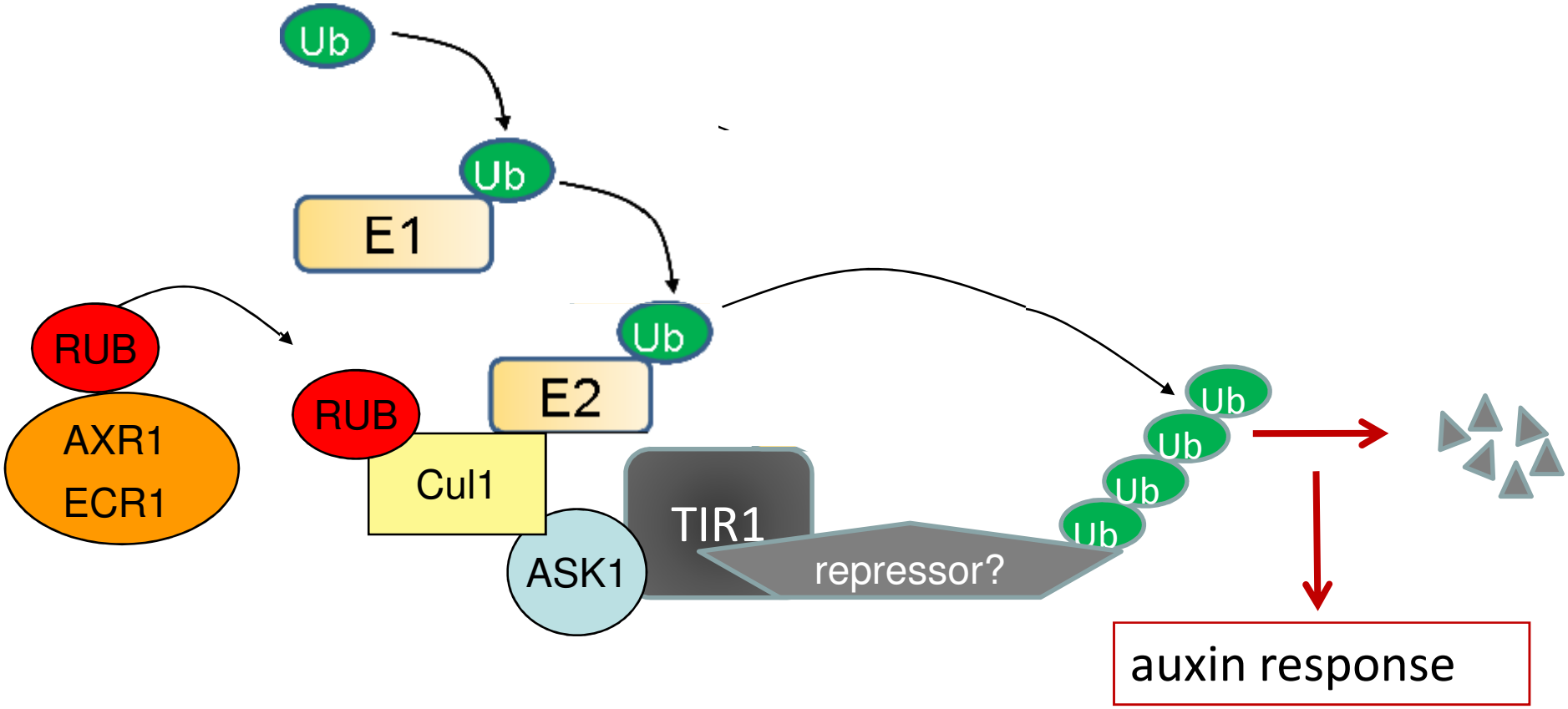
F. Scheibner, O. Lindecke 27.05.2009

Modell von 1999 (Gray et al.)



GUS = β-Glucoronidase

-spaltet Glycoside → Abbau
- hydrolysiert durch Ammoniumsalz X-Gluc → BLAUFÄRBUNG

Analyse der AUX/IAA-stabilität mit Hilfe von GUS-Fusion

GUS- Fusionsprotein-Modell

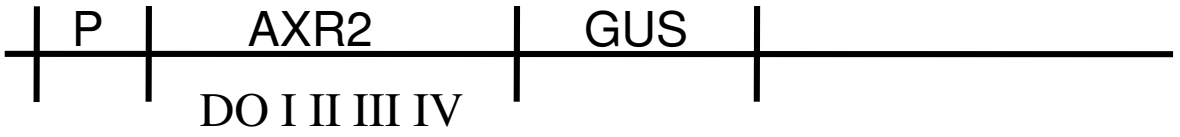
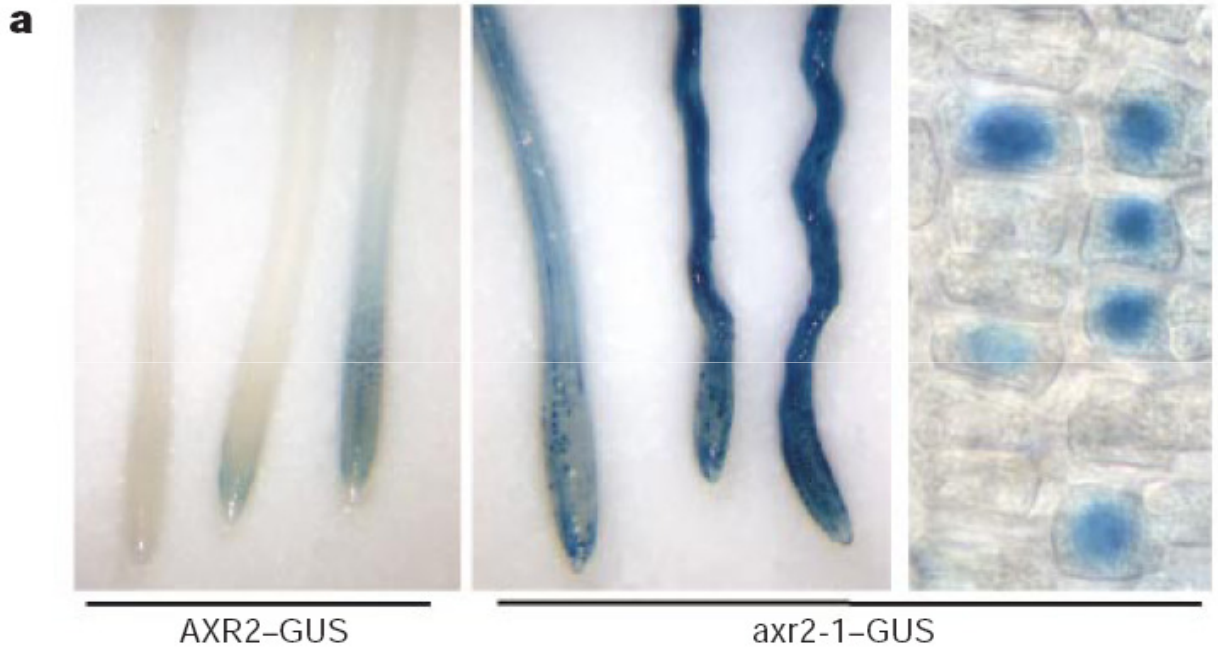


Fig. 1

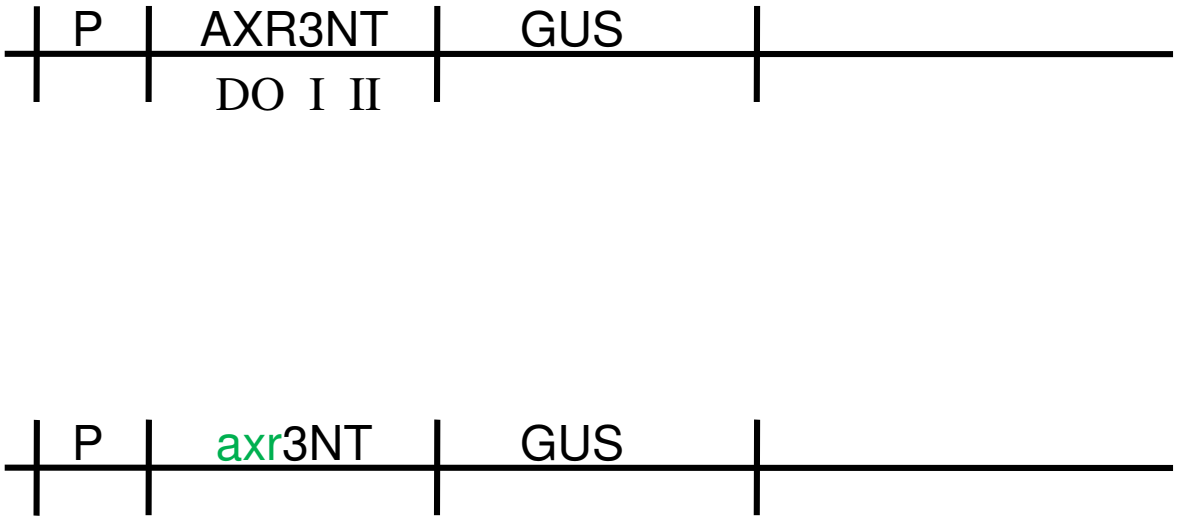
Analyse der AUX/IAA-stabilität mit Hilfe von GUS-Fusion



axr2-1-GUS in
Wurzelmeristemzellen

Analyse von AUX/IAA-GUS Fusionskonstrukten

Erweitertes GUS- Fusionsprotein-Modell



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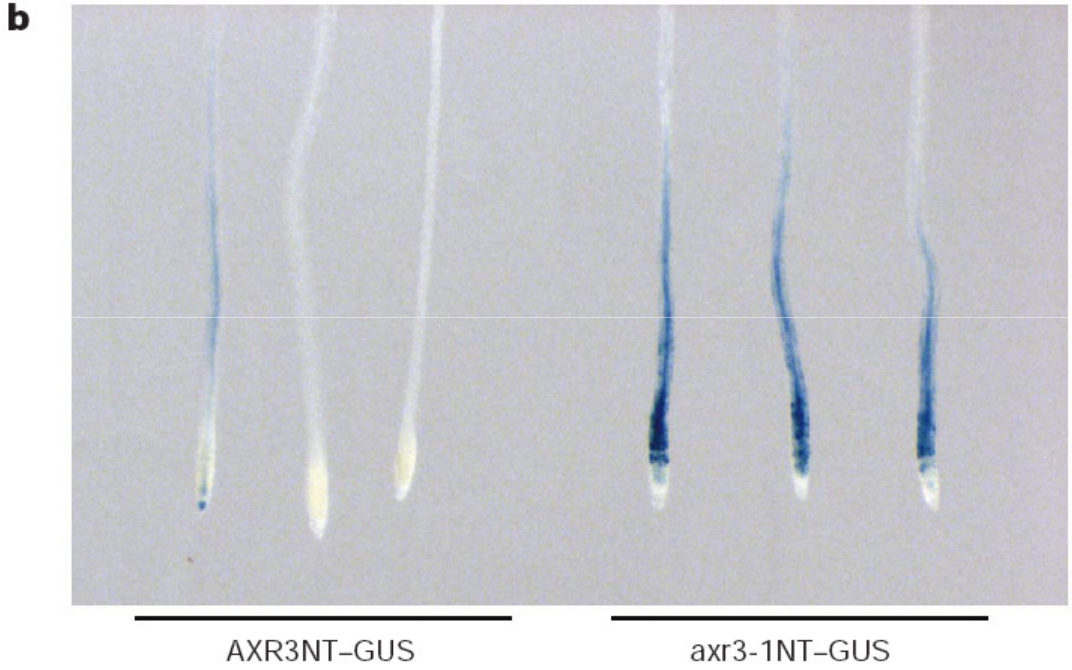
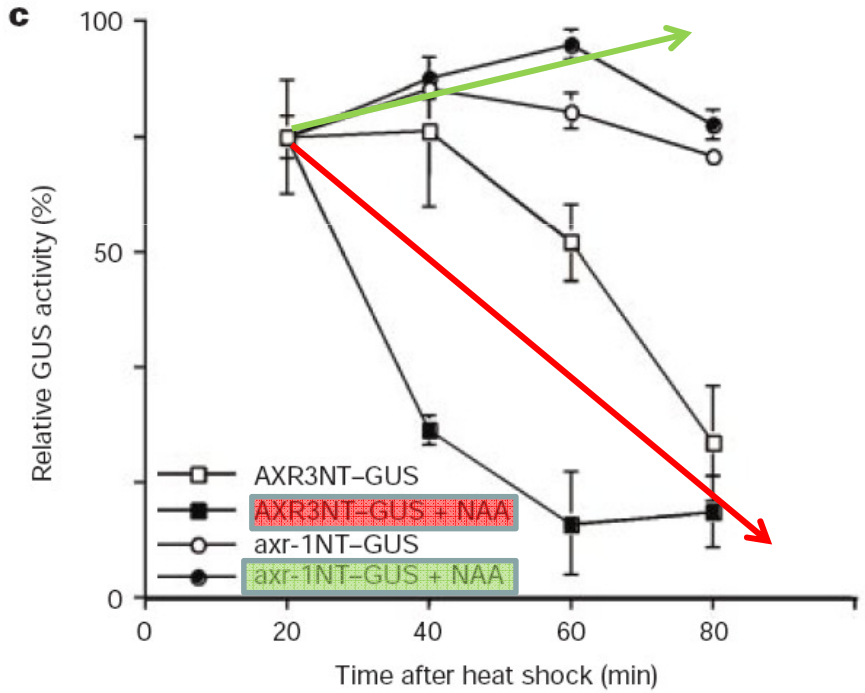


HS für 2h und GUS-Aktivität 60min nach HS

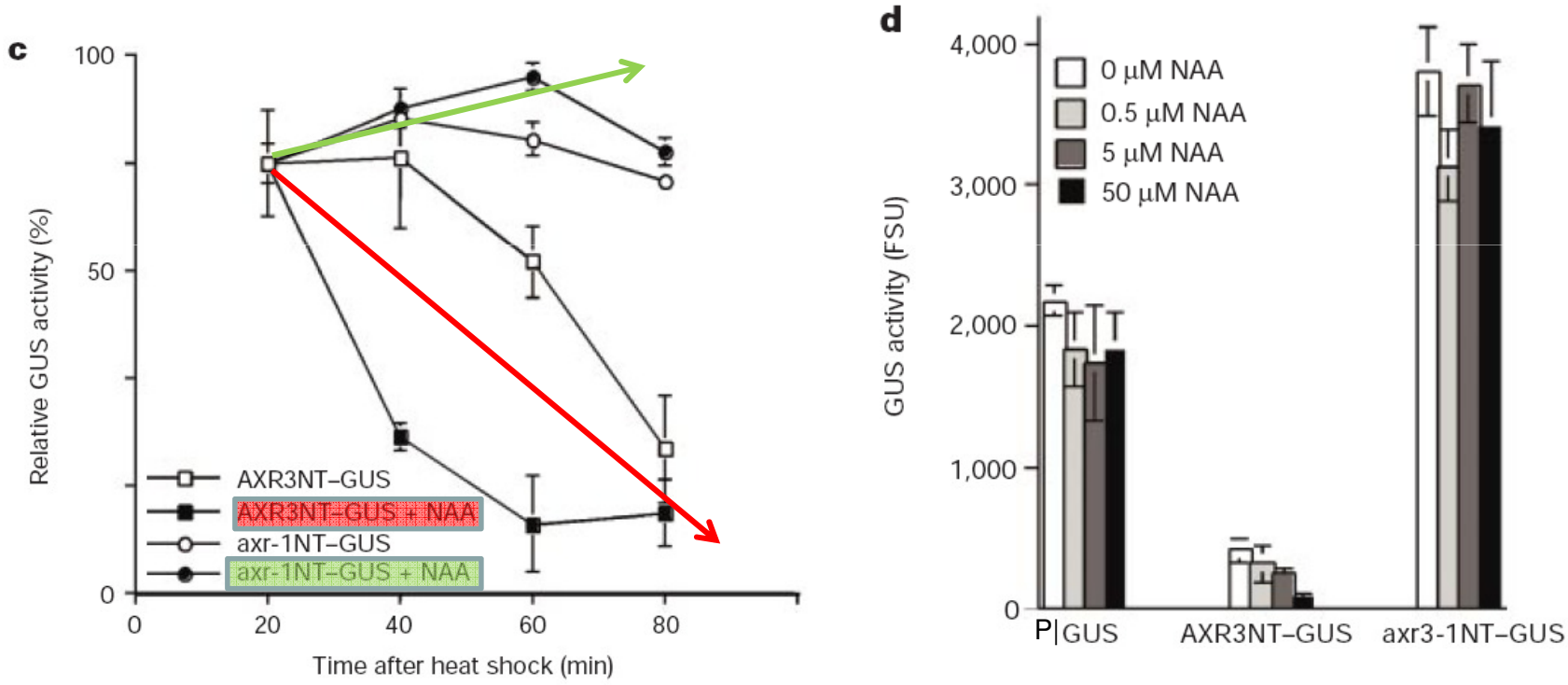
Reguliert Auxin AUX/IAA -Abbau?

Fig. 1



Einfluß der Auxindosis auf die Stabilität?

Fig. 1



Ist es möglich das der Ubiquitin-Proteasomen-Weg
am AUX/IAA-Abbau beteiligt ist?

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MG132 = Proteasom- Inhibitor

→ Kann MG132 den auxininduzierten Abbau von AXR2-GUS bzw. AXR3NT-GUS verhindern?

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Fig. 2

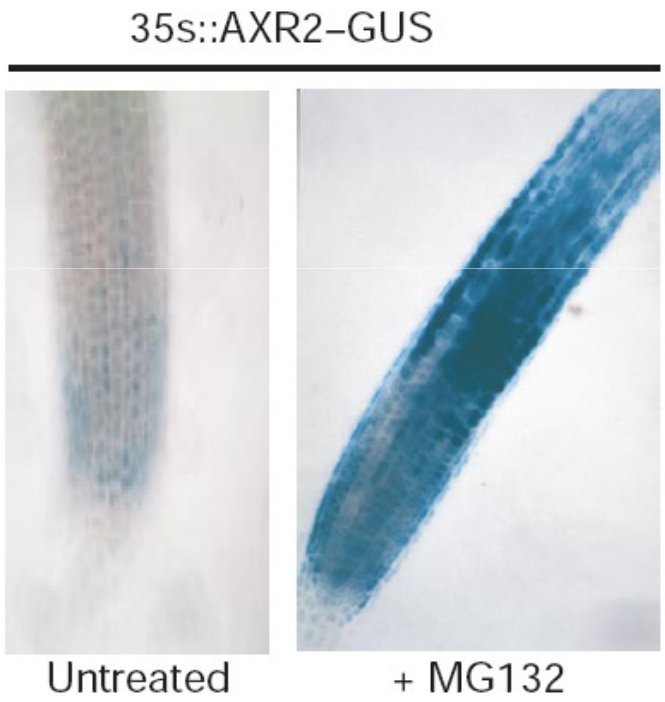


Fig. 2

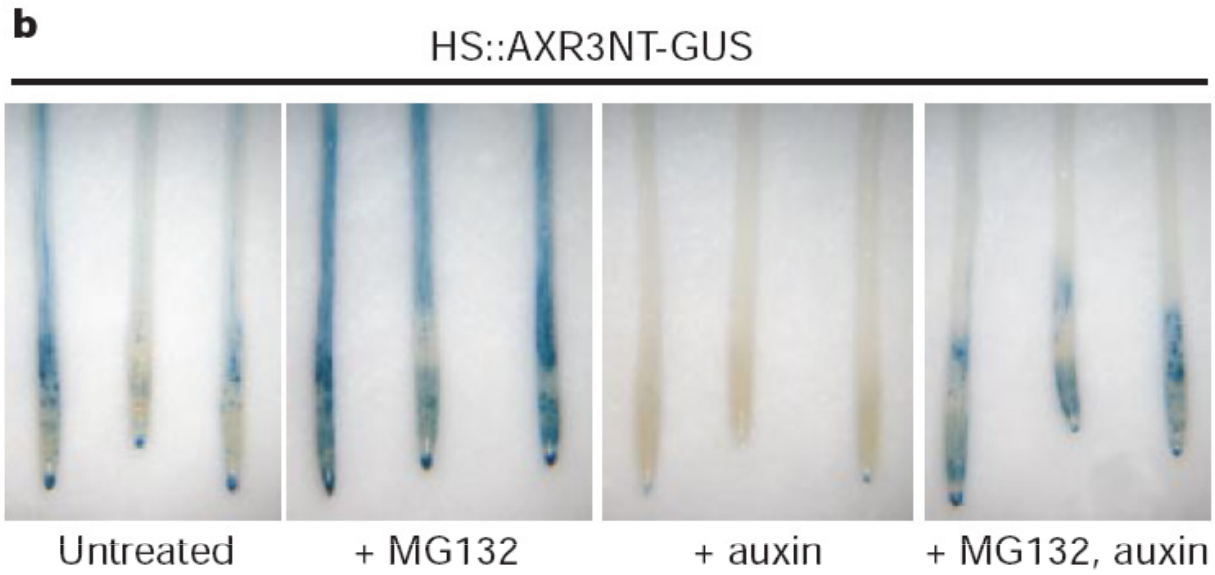


Figure 2 The proteasome inhibitor MG132 increases AUX/IAA protein stability. **a**, Seven-day-old seedlings were treated with 10 μM MG132 for 2 h and stained for GUS activity. **b**, Nine-day-old seedlings were heat shocked for 2 h. Where indicated, seedlings were treated with 10 μM MG132 after 1 h, and 5 μM 2,4-D was added at the end of the heat-shock period. Sixty minutes later, seedlings were stained overnight to detect GUS activity.

Fig. 2 **b**

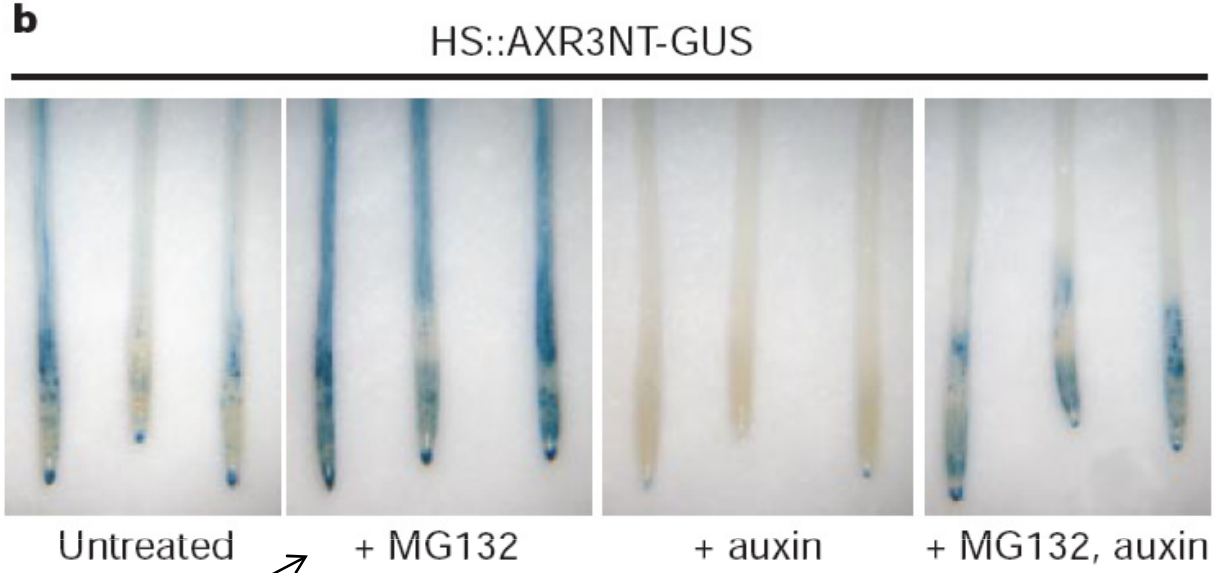
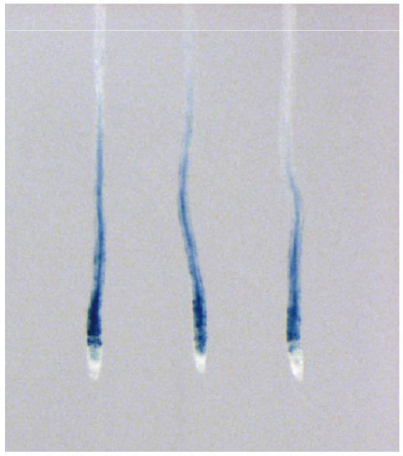


Fig. 1b



Phänokopie

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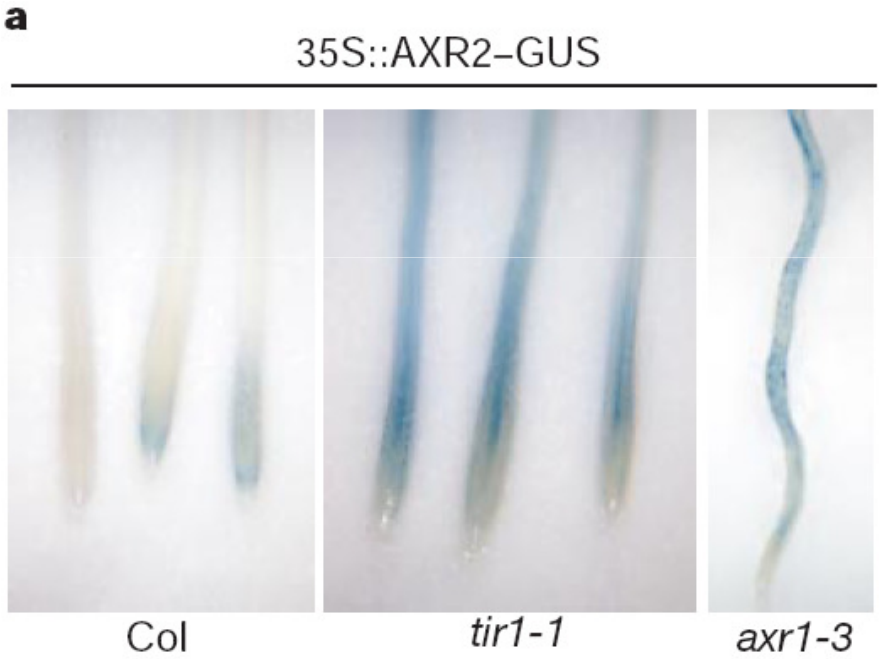
bis hierhin biochemischer Nachweis – ab hier genetischer Nachweis

Untersuchung der Effekte von axr1- und tir1-Genmutationen auf die AUX/IAA- Stabilität

Fig.3



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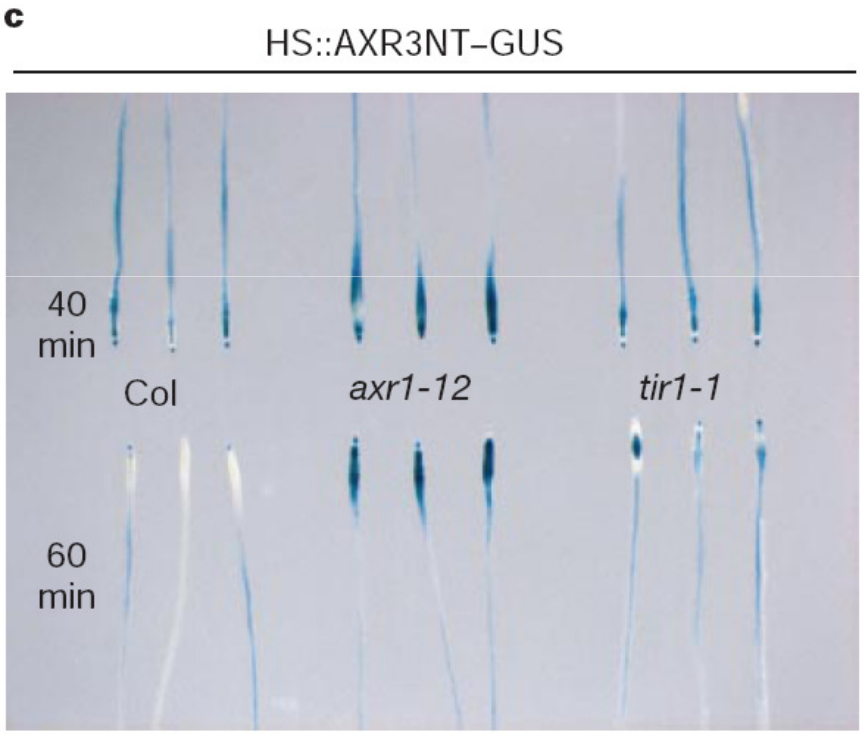

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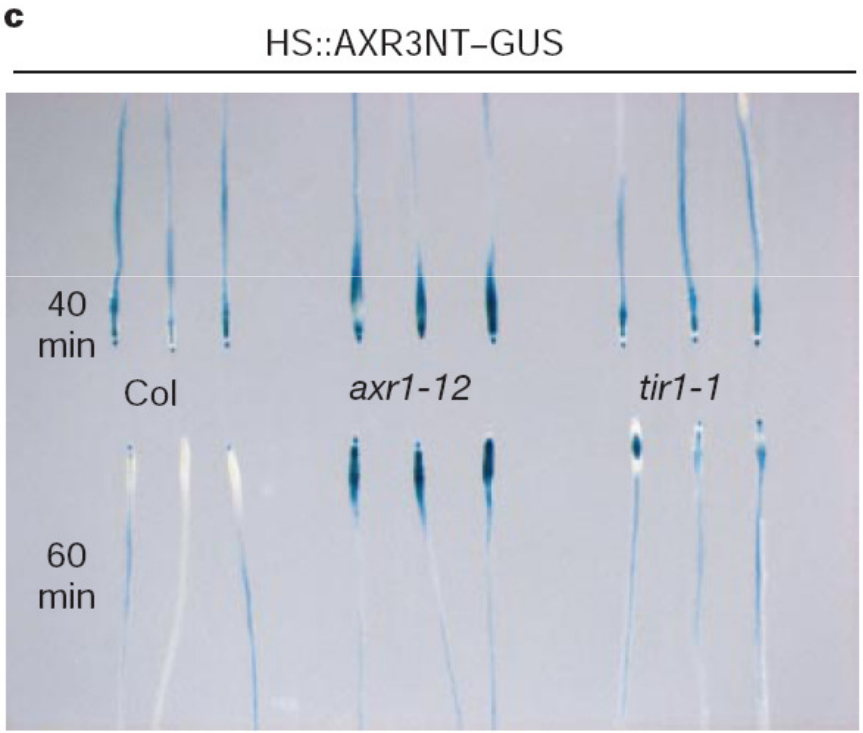


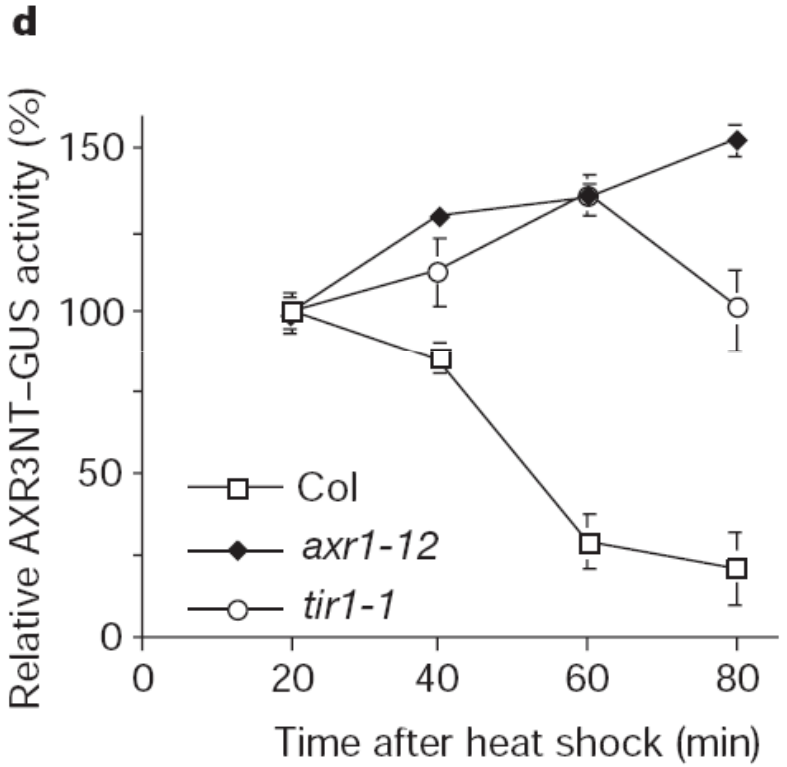
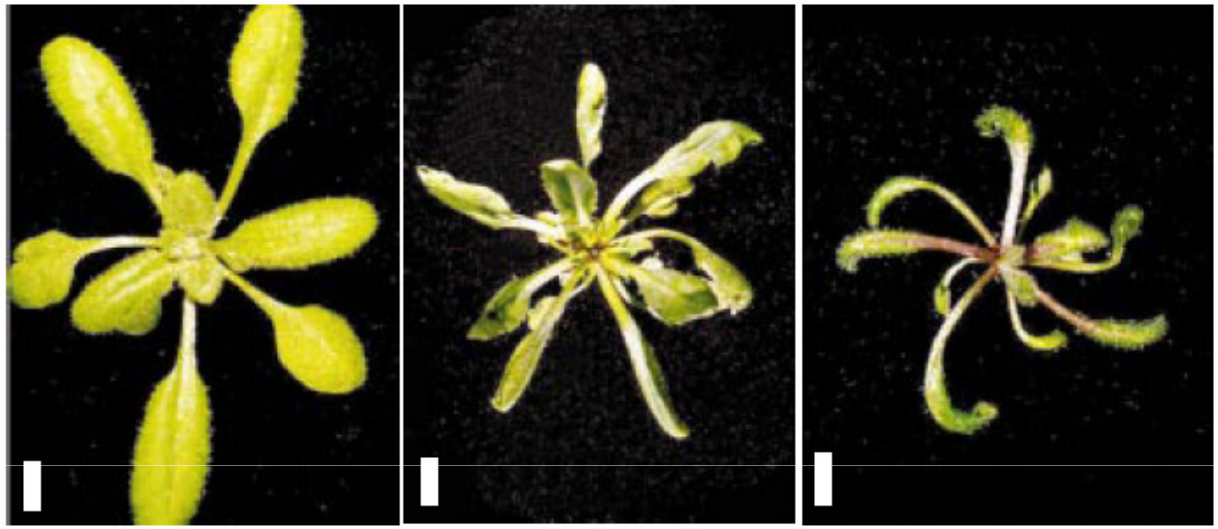


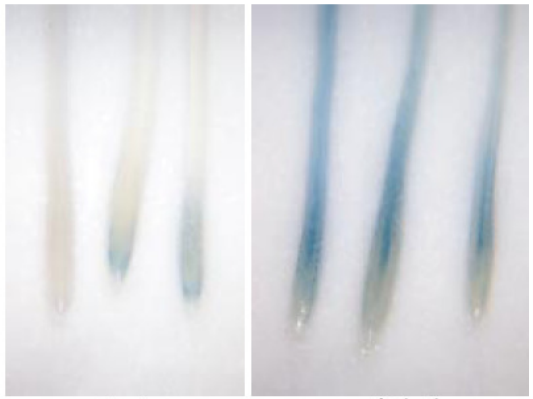
Fig.3 **b**

35s Col[AXR2–GUS] *tir1-1*[AXR2–GUS] Col[axr2-1–GUS]



a

35S::AXR2–GUS



Col *tir1-1*

axr2-1–GU

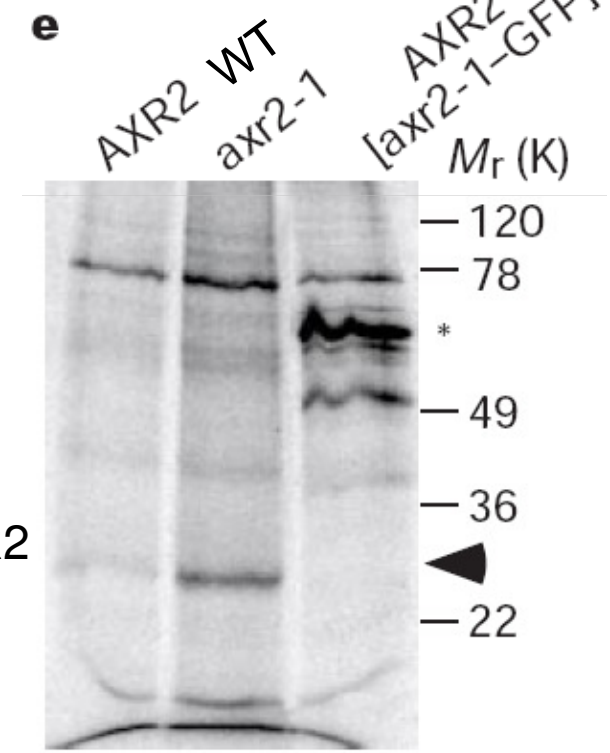
Fig.1a

Spiegeln die GUS-Fusionsproteine (AUX/IAA) wirklich die Protein-Stabilität wider?

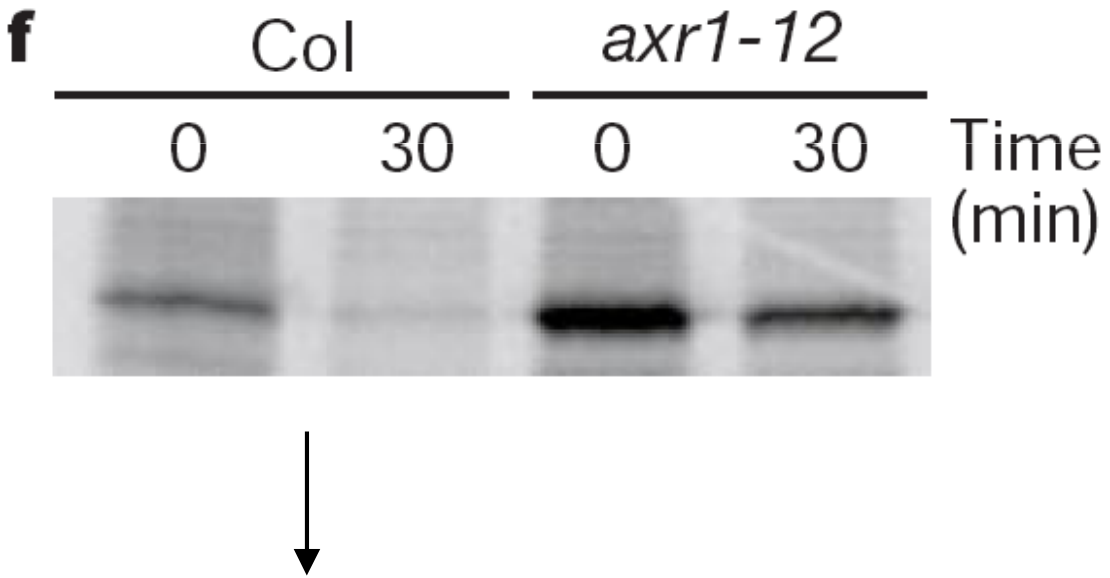
AB αAXR2

Gel

Western-Blot



Pulse-Chase-Experiment



schneller turn-over

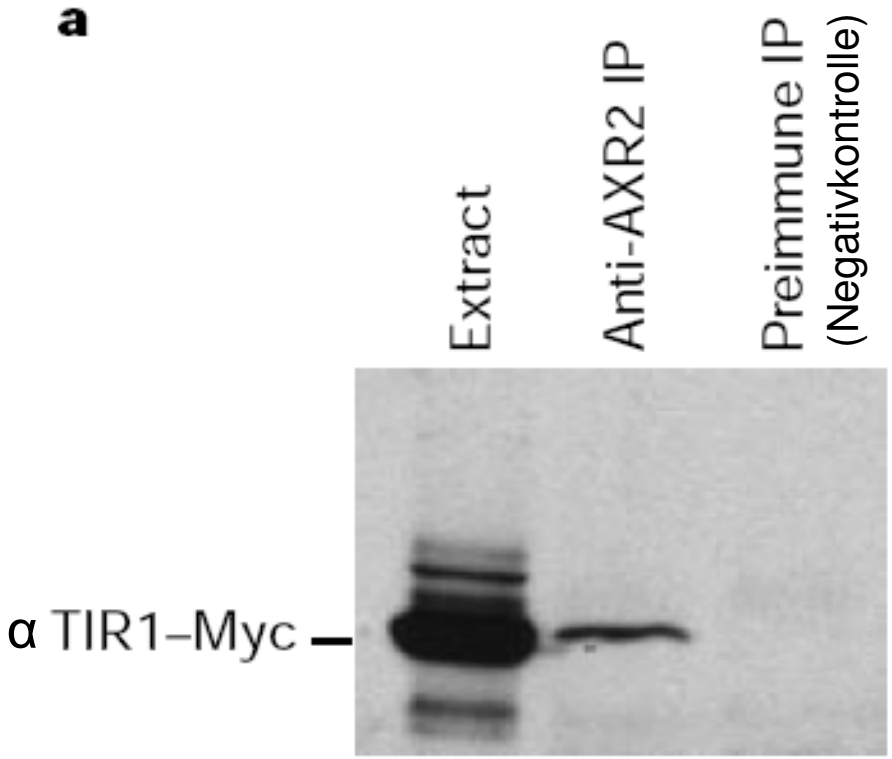
Interagiert SCF^{TIR1} physisch mit AUX/IAA-Proteinen?

Pulldown

= Protein-Protein-Interaktionsnachweis

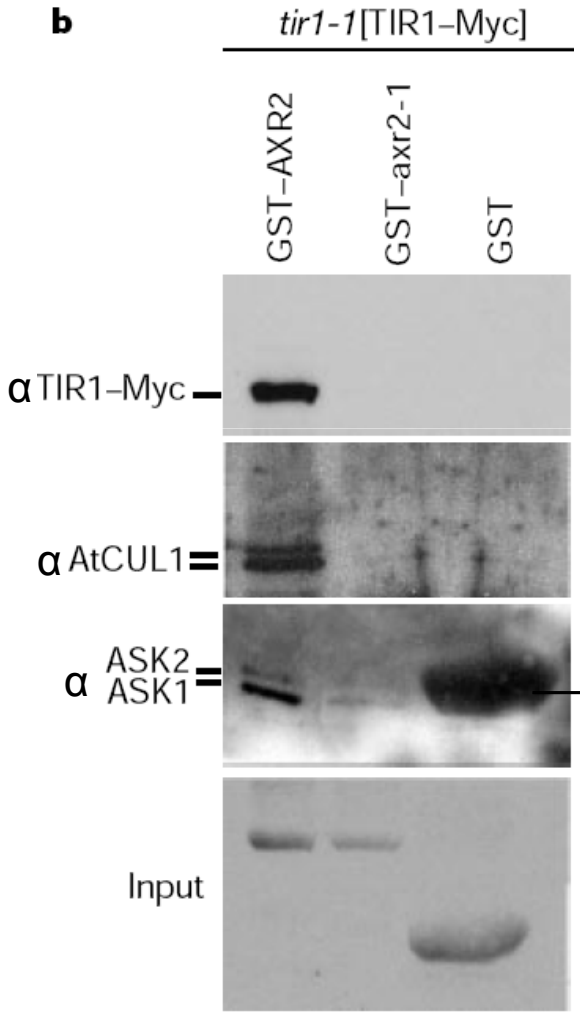
→ Protein mittels AB mitsamt seiner Interaktionspartner präzipitiert
→ Nachweis im Westernblot

myc-tag



TIR1-MYC in anti-AXR2 Immunopräzipitat

in vitro pull-down assay (Immuno-Blot)

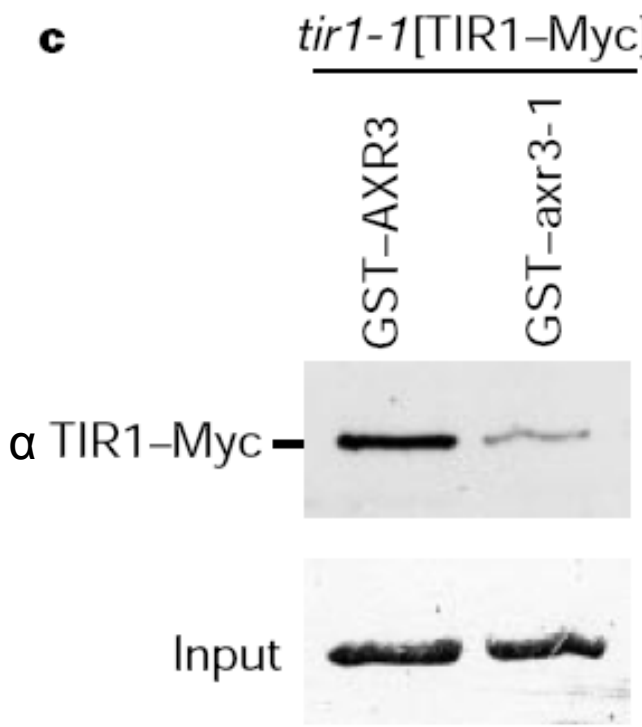


Künstlicher WT mit *myc*-tag

SCF-Box bindet GST-AXR2

Kreuzreaktion?!

Kontrolle mit anderen „Familienmitgliedern“

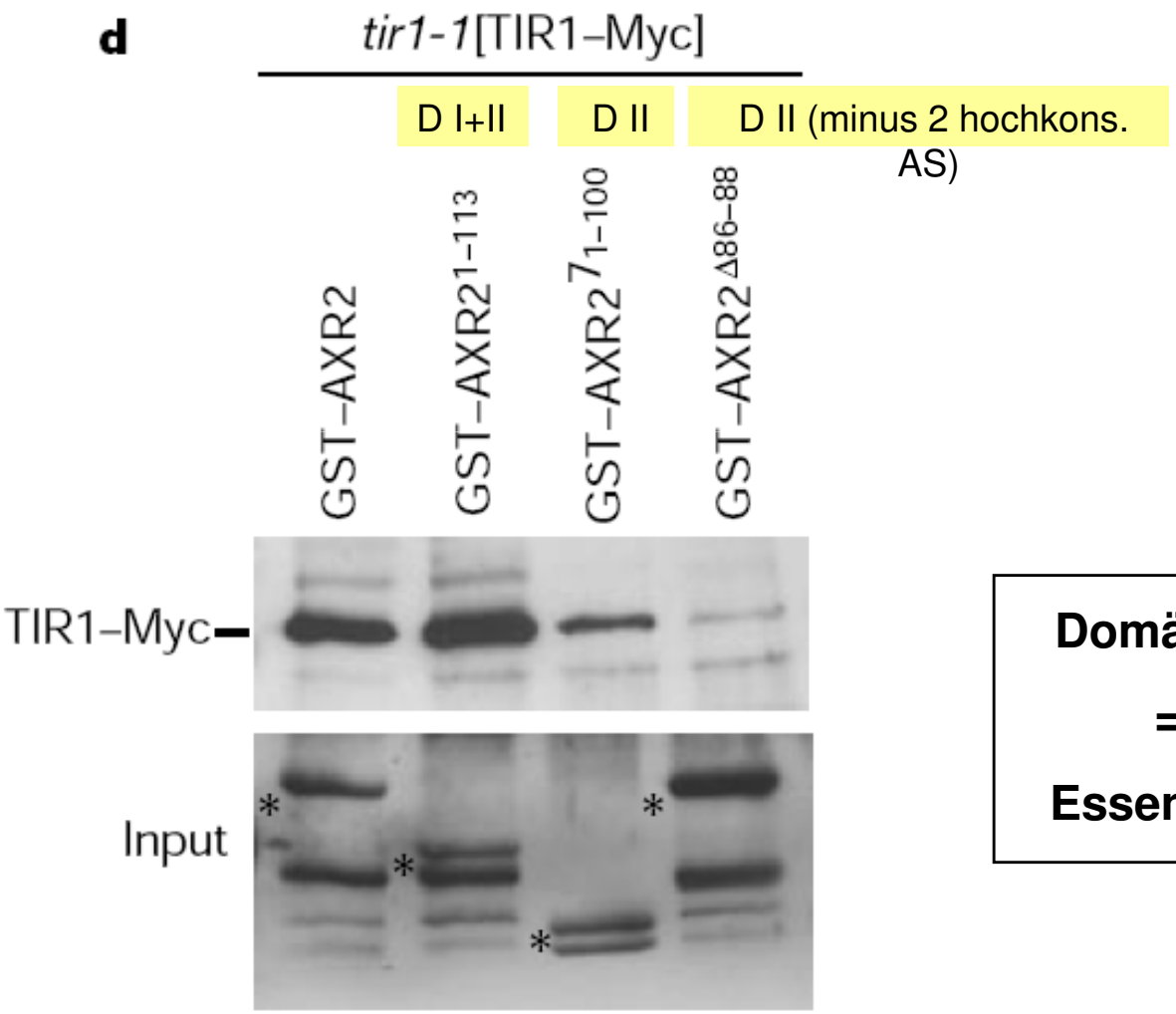


Mutation zeigt schwache Interaktion

→ **gleiches Ergebnis: SCF^{TIR1} bindet AUX/IAA-Proteine**

Welche Bedeutung hat Domäne II der AUX/IAA-Proteine?

Domäne II



Domäne II

=

Essentiell!

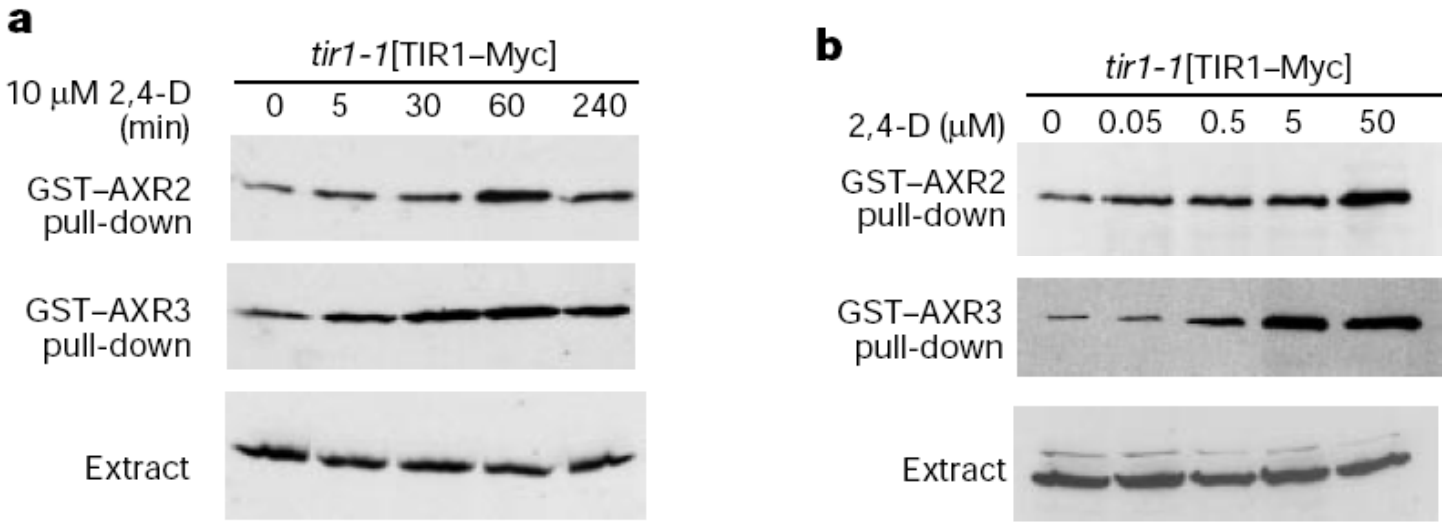
Wie ist der Einfluss von Auxin einzuschätzen?

Auxin-Einfluss

synth. 2,4-D

Konzentration konst.

Wirkungszeit konst. (60 min)



Auxin fördert F-Box-AUX/IAA-Bindung konzentrations- & zeitabhängig!

Modell von 2001 (Gray et al.)