

ARTICLES

The *Arabidopsis* F-box protein TIR1 is an auxin receptor

Stefan Kepinski^{1,2} & Ottoline Leyser¹

¹Department of Biology, University of York, Box 373, York YO10 5YW, UK. ²Umeå Plant Science Centre, SLU, S-90183, Umeå, Sweden.



last week

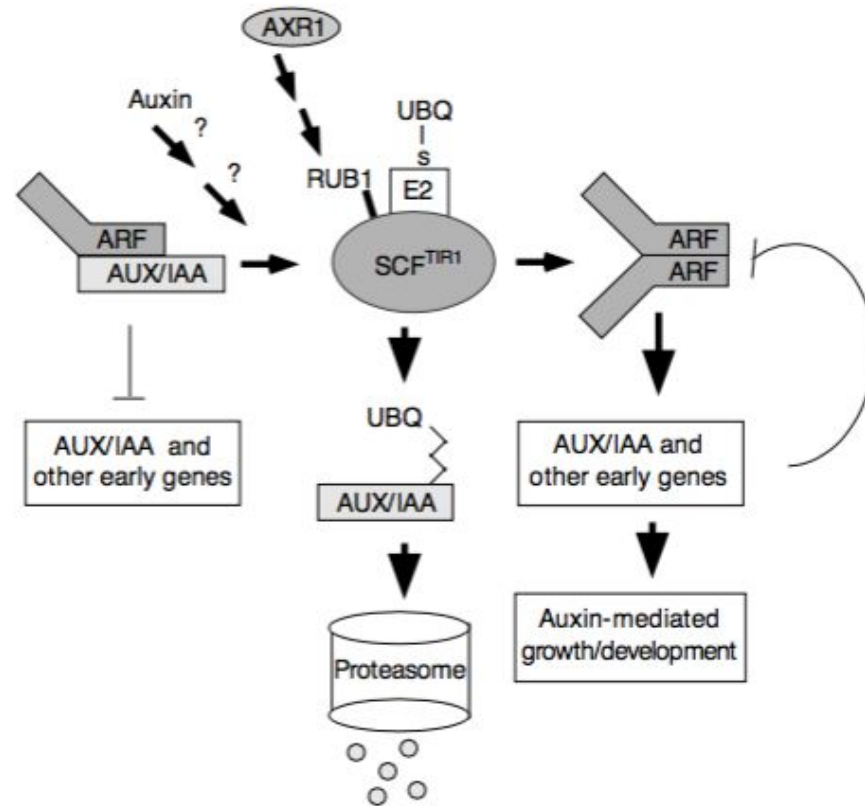


Figure 6 Model for auxin response. AUX/IAA proteins repress the auxin-response pathway by negatively regulating ARF transcription factors. Auxin promotes the ubiquitination of AUX/IAA proteins by targeting them to the SCF^{TIR1} ubiquitin-ligase. The subsequent degradation of AUX/IAA proteins results in activation of ARF and derepression of the auxin-response pathway. Because AUX/IAA genes themselves are rapidly induced by auxin, a negative-feedback loop exists with the newly synthesized AUX/IAA proteins restoring repression upon the pathway. Although the mechanism of AUX/IAA action is unclear, one possible mechanism is by preventing the formation of ARF-ARF dimers.

...meanwhile

The best- characterized auxin binding protein: ABP1
Receptor Protein? → No, Since 2015 (no working
complementation assay)

plant extracts from which membranes have been
removed, indicating that the site of auxin perception in
this response might be soluble

Question?

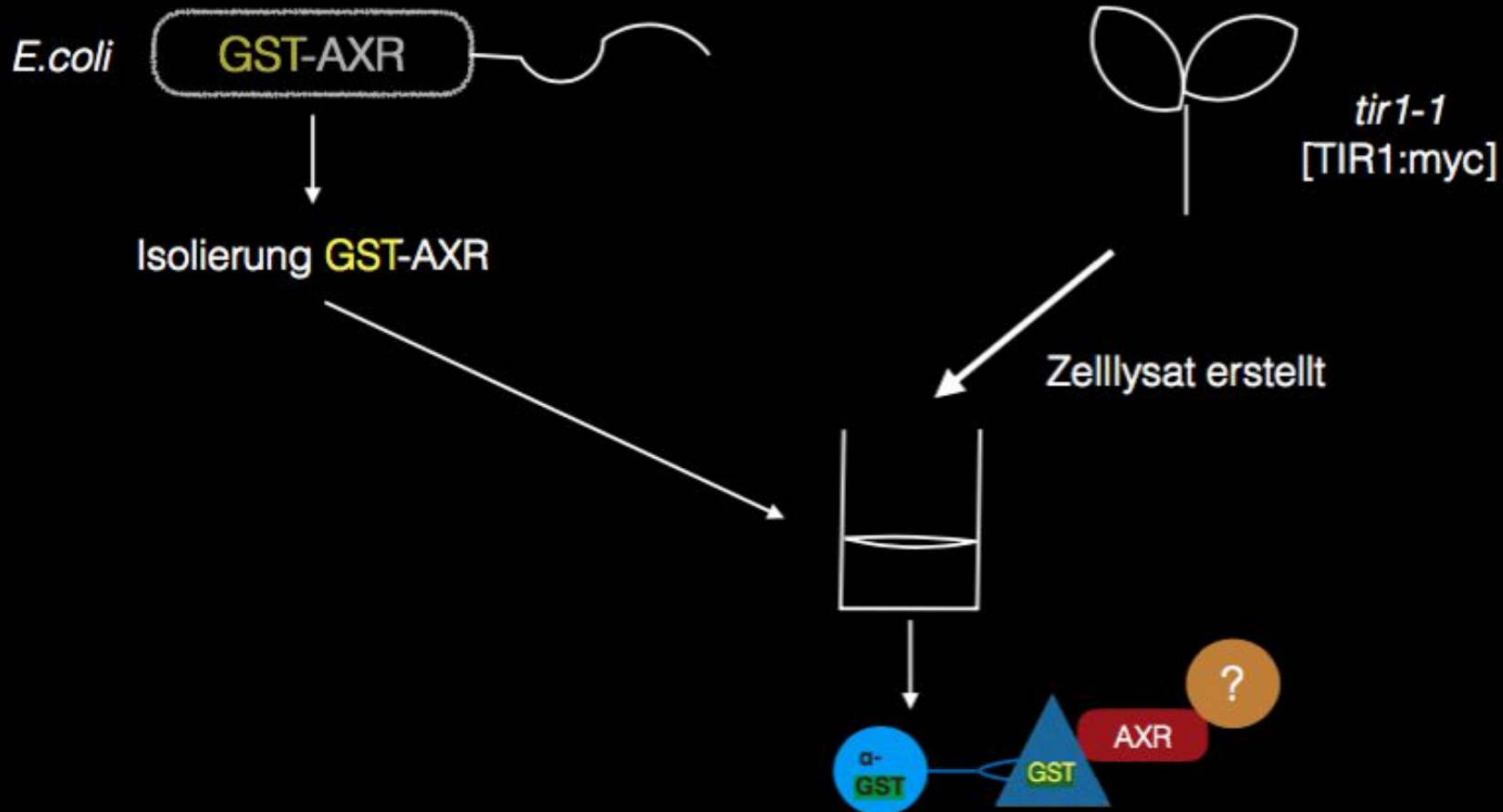
Is TIR1 a receptor?

Or is there a short signal pathway?

Plant materials

3. Interagiert GST mit AXR/IAA Proteinen:

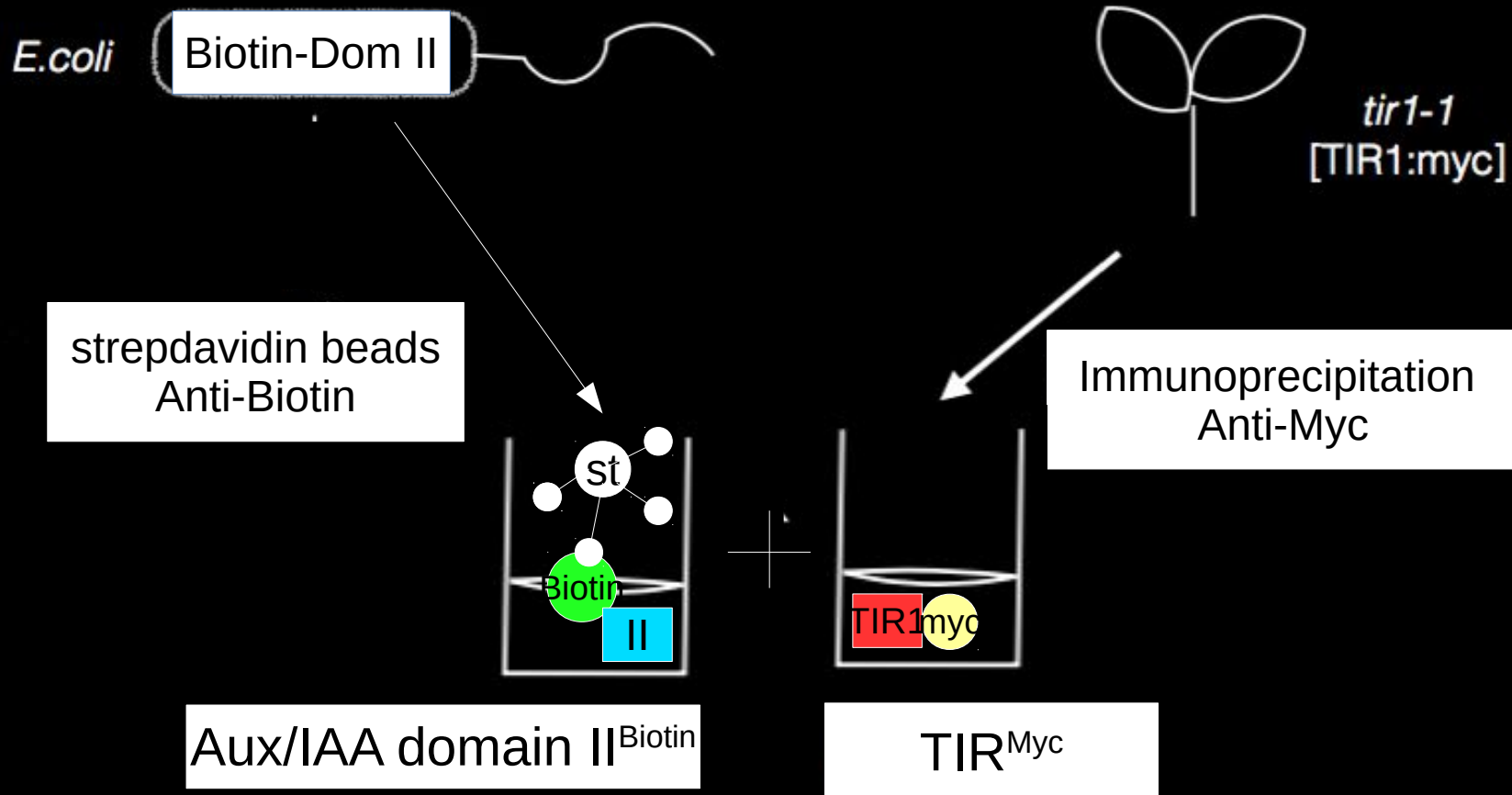
Herstellung GST getaggtter AXR Proteine



Plant materials

3. Interagiert mit dem Komplex mit AUX/IAA Proteinen:

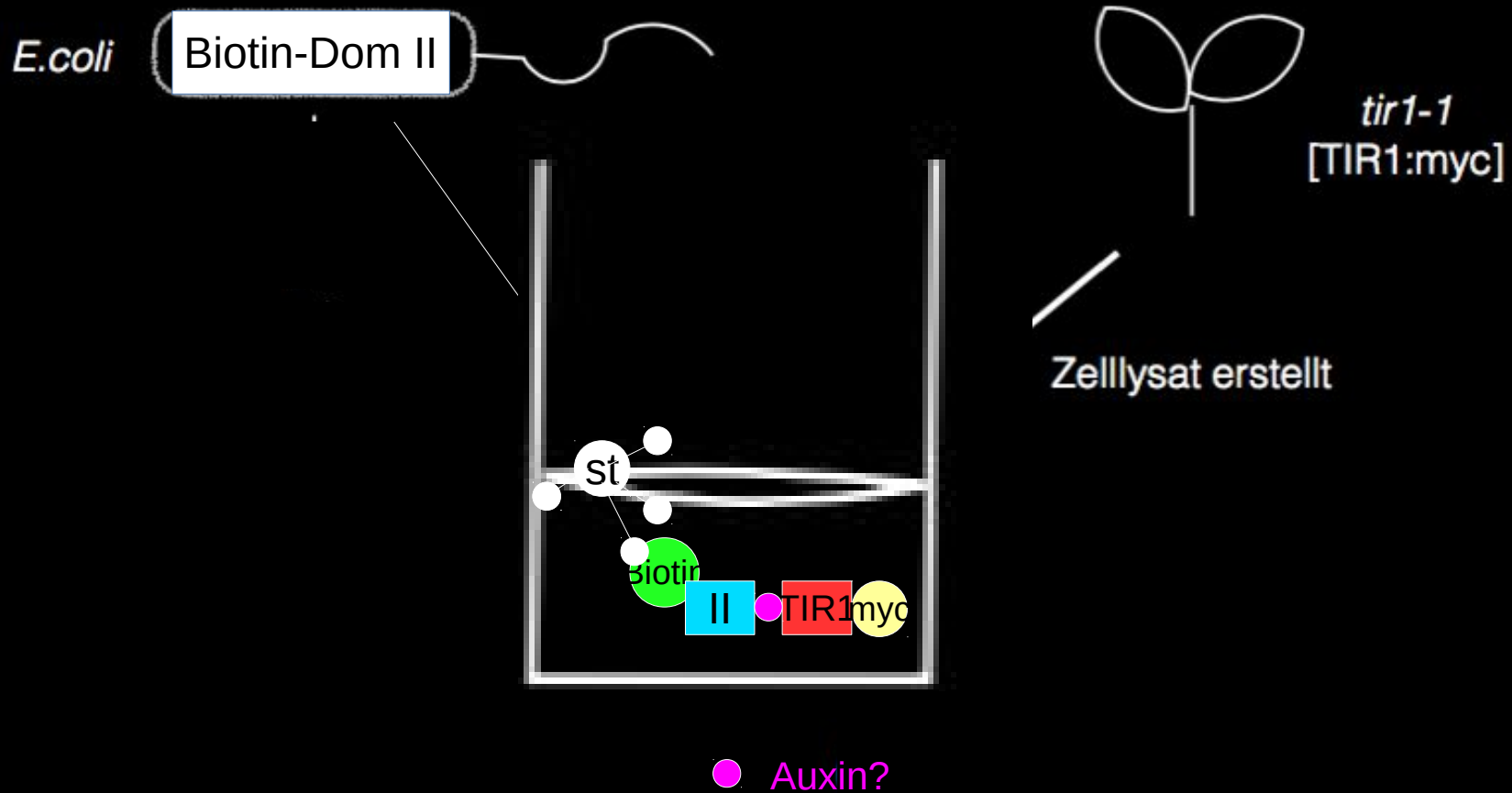
~~Herstellung GST getaggtter AXR Proteine~~



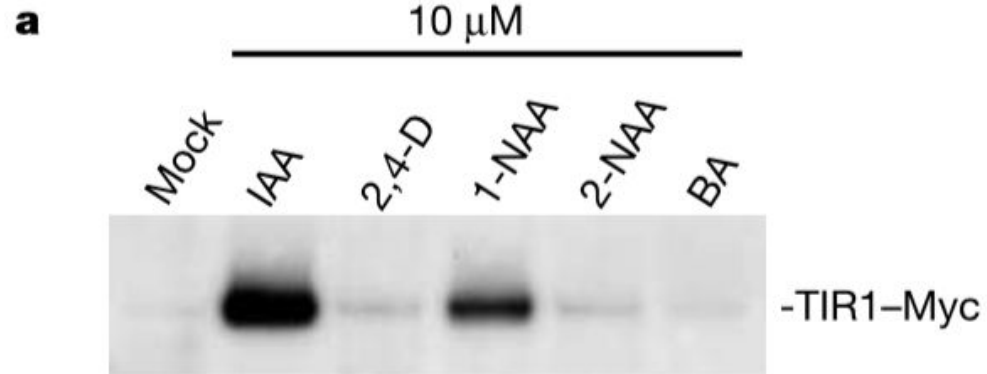
Plant materials

3. Interagiert mit dem Komplex mit AUX/IAA Proteinen:

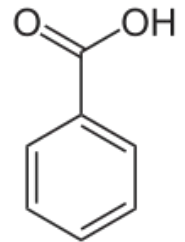
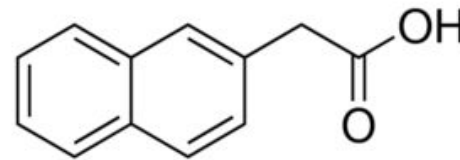
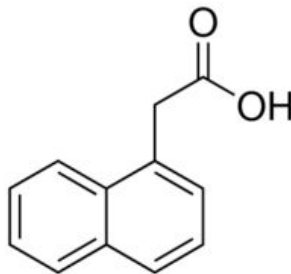
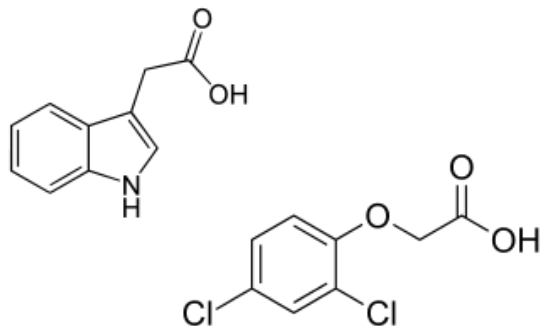
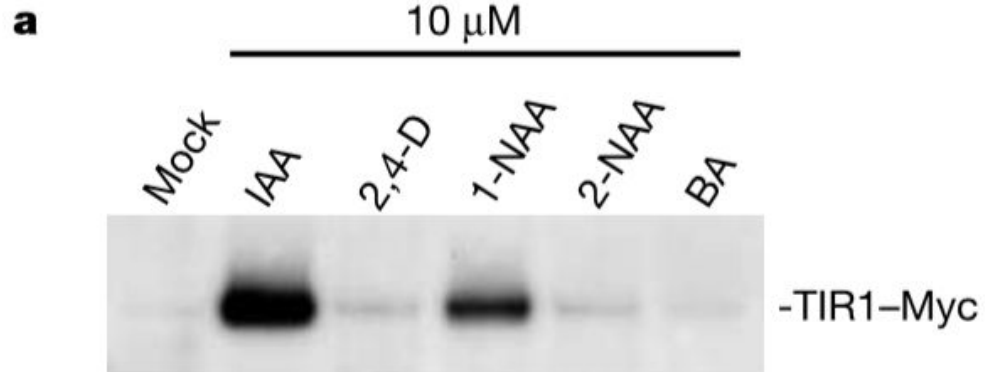
~~Herstellung GST getaggtter AXR Proteine~~



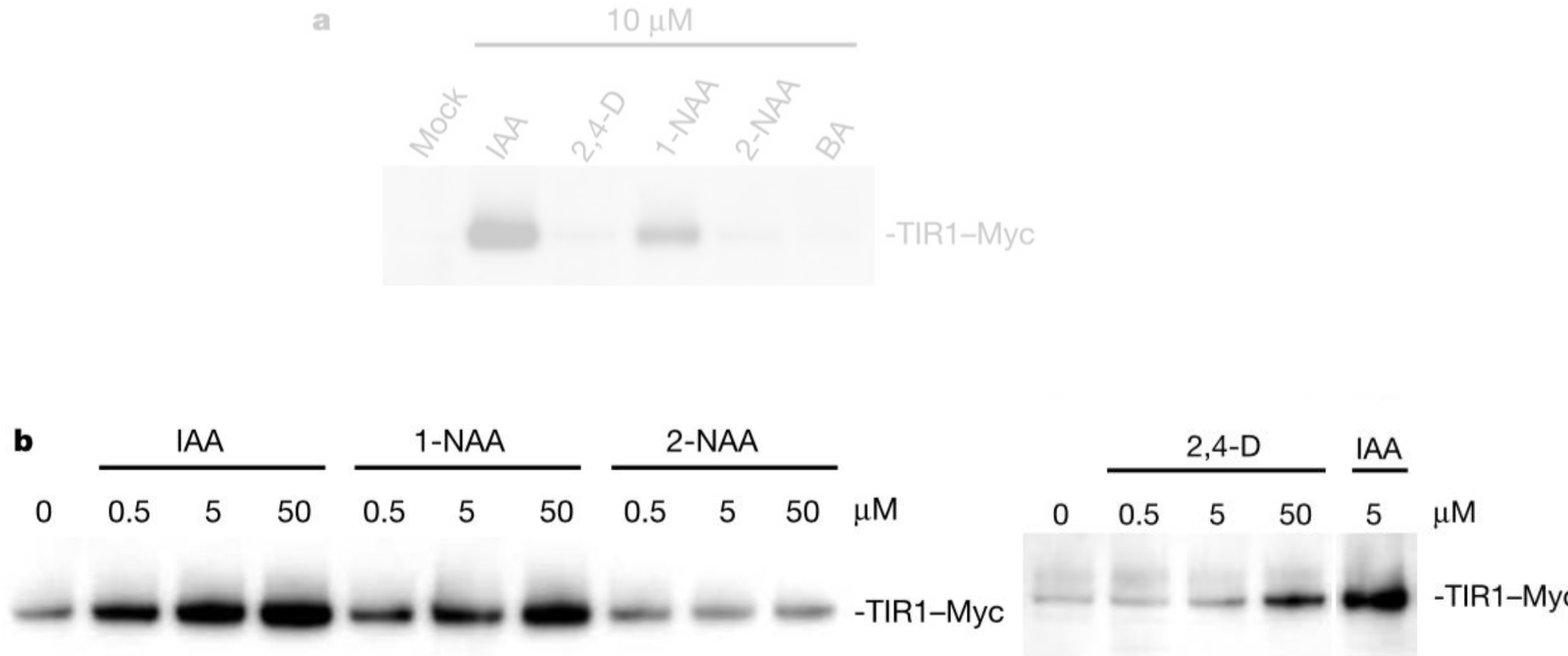
An auxin receptor activity copurifies with **TIR1** (Fig.1)



An auxin receptor activity copurifies with **TIR1** (Fig.1)



An auxin receptor activity copurifies with TIR1 (Fig.1)



An auxin receptor activity copurifies with TIR1

immunoprecipitate must include the auxin receptor for this response

1-naphthalene- acetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were also able to promote the interaction, but with lower activity.

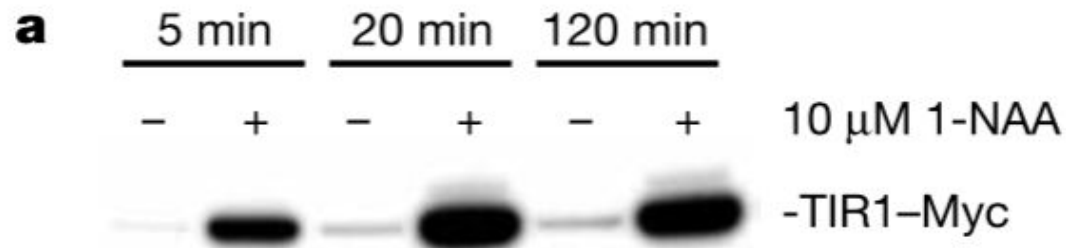
An auxin receptor activity copurifies with TIR1

dose-response profiles mirror those observed for similar pull-down assays performed on plant extracts

Concentration-dependent!

But also time-dependent?

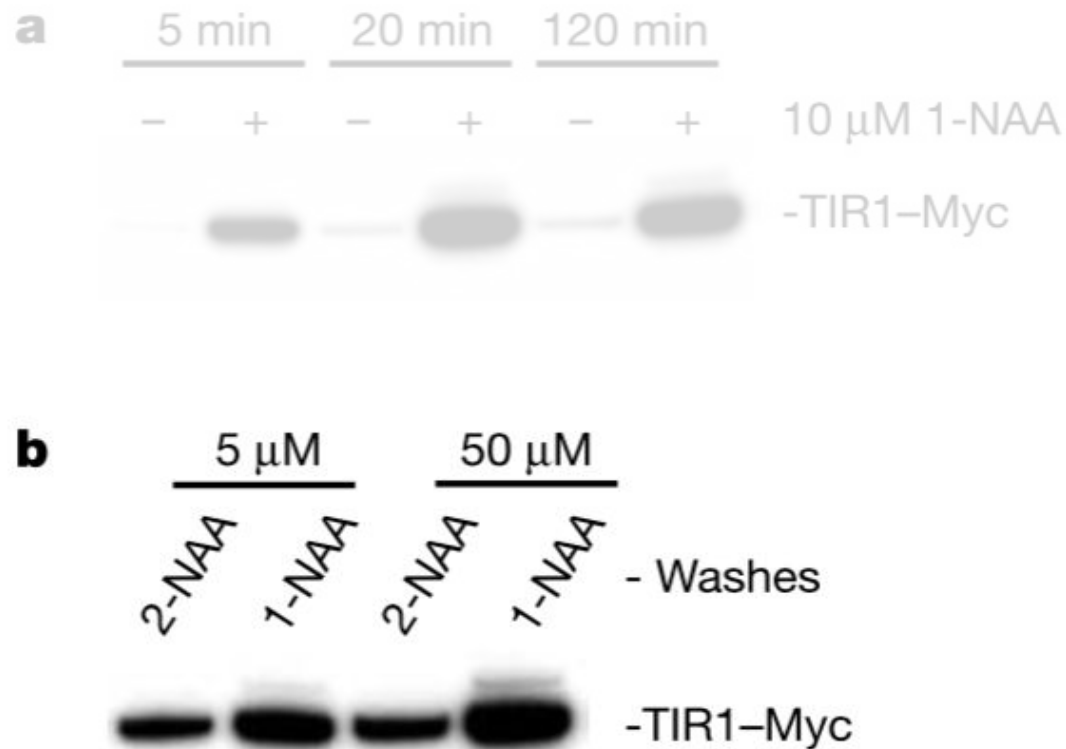
An auxin receptor activity copurifies with TIR1 (Fig.2)



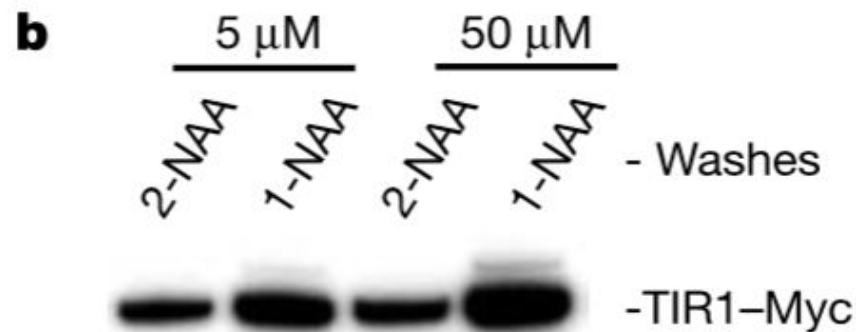
An auxin receptor activity copurifies with TIR1

If auxin is continuously required to sustain the interaction, washing with active auxin (1-NAA) should preserve the interaction better than washing with inactive auxin (2-NAA).

An auxin receptor activity copurifies with TIR1 (Fig.2)

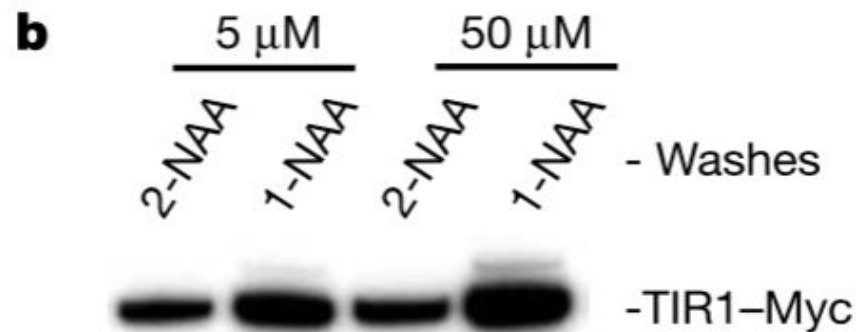
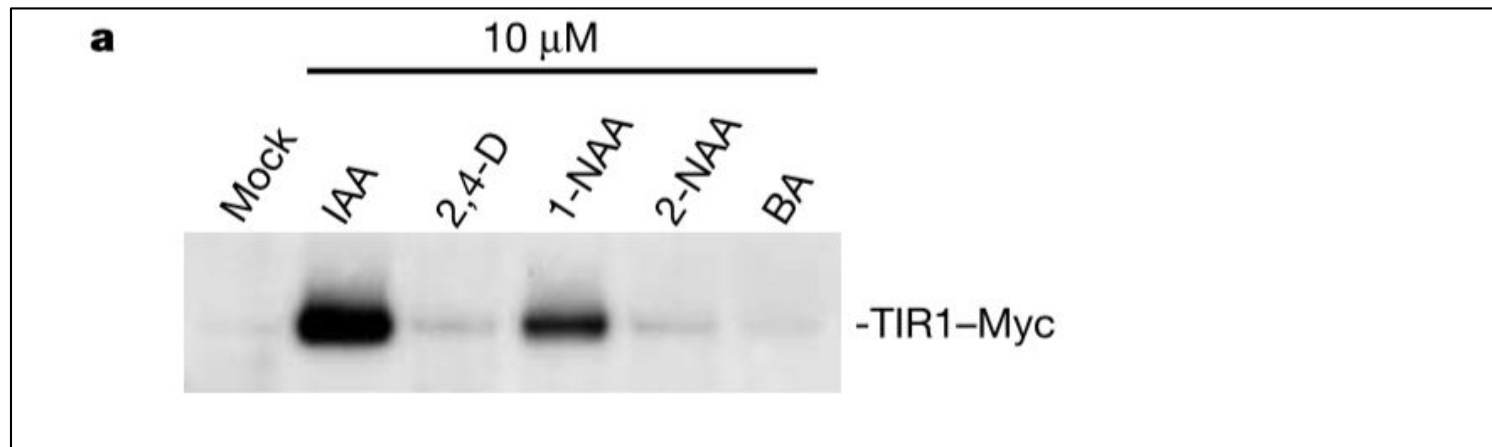


An auxin receptor activity copurifies with TIR1 (Fig.2)



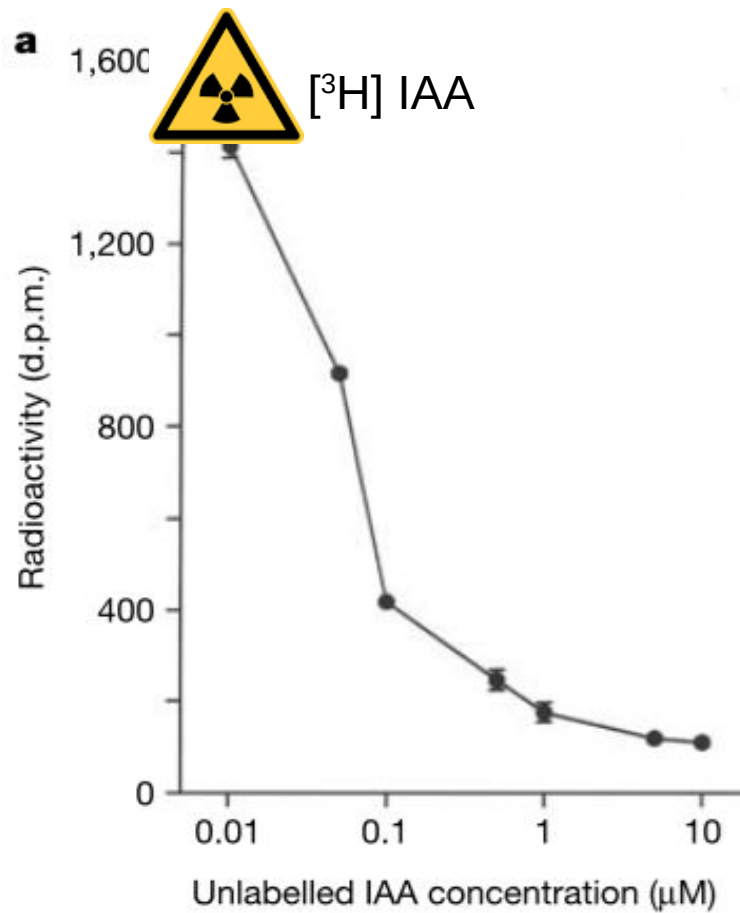
True! But why not washed with IAA or 2,4-D?

An auxin receptor activity copurifies with TIR1 (Fig.2)

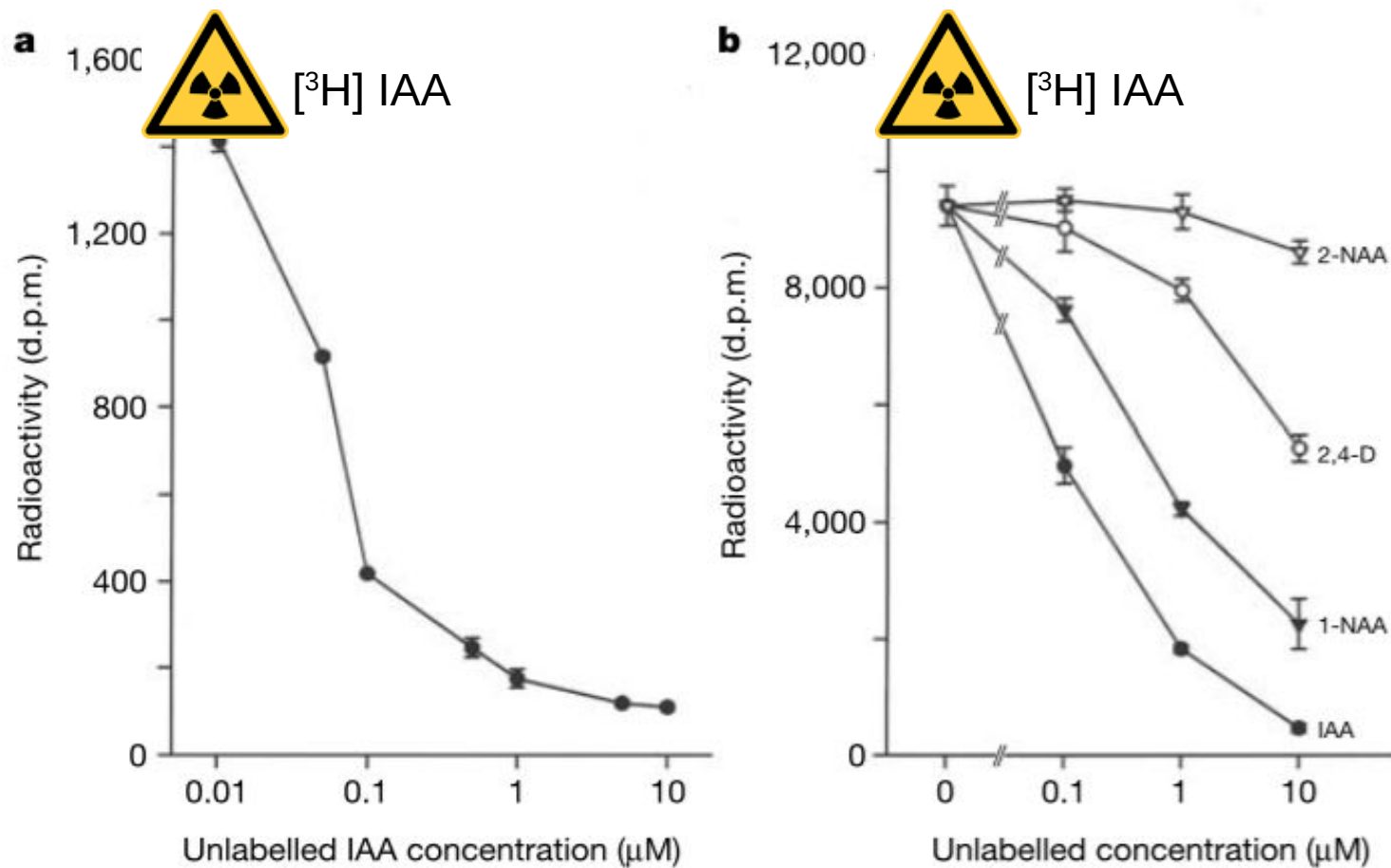


True! But why not washed with IAA or 2,4-D?

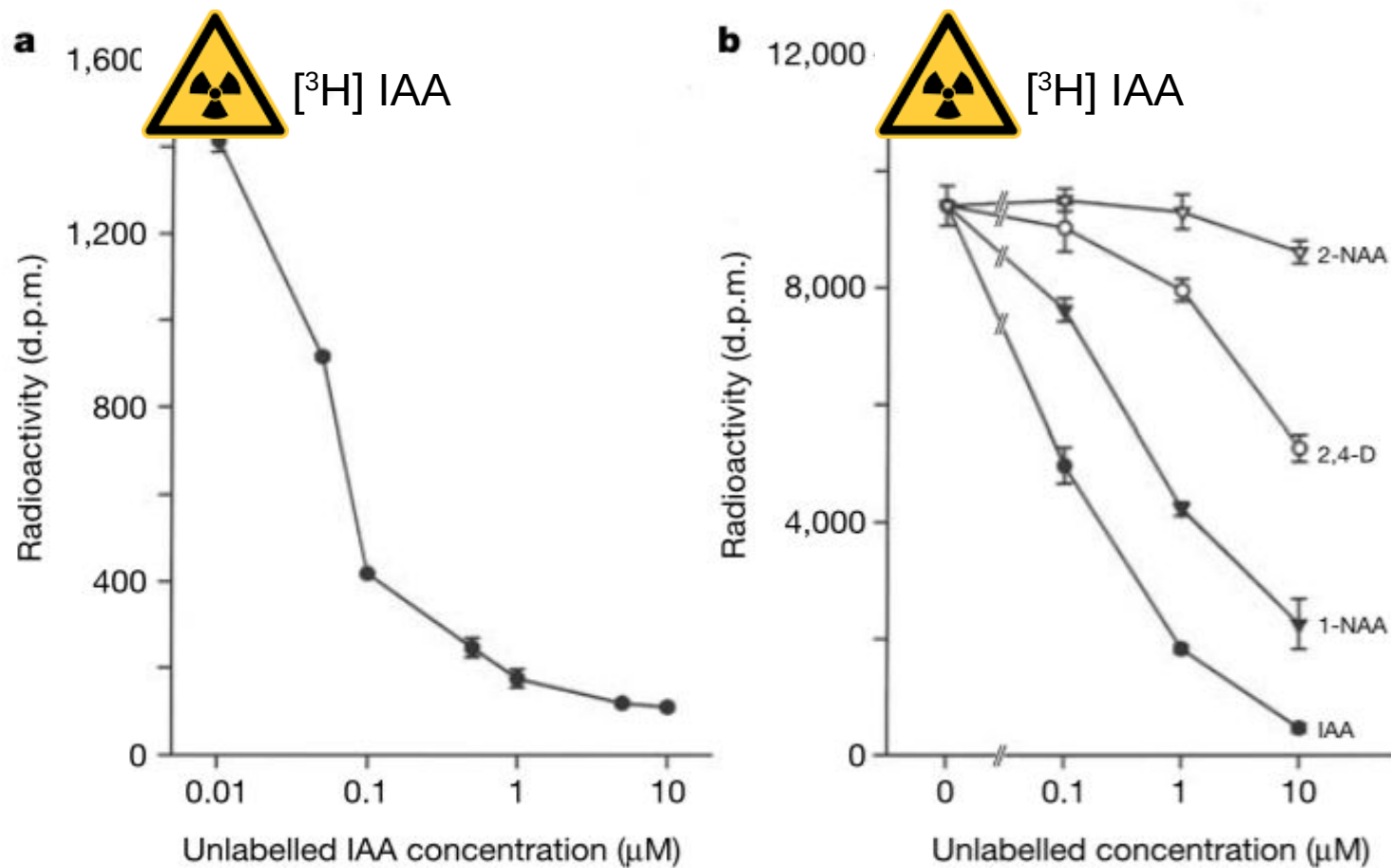
Binds SCF^{TIR1} auxin directly? (Fig.3)



SCF^{TIR1} binds auxin directly (Fig.3)



SCF^{TIR1} binds auxin directly (Fig.3)



Which Protein binds?

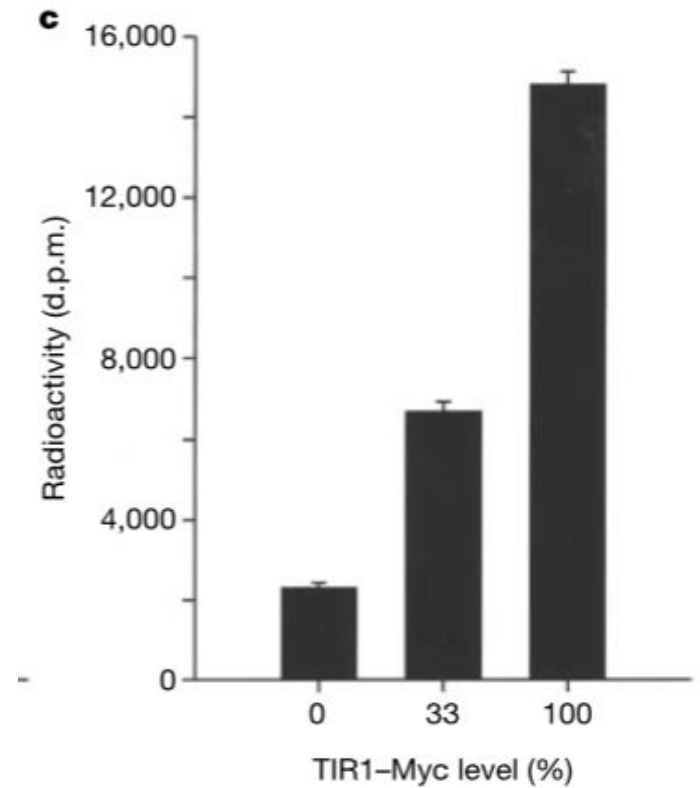
SCF^{TIR1} binds auxin directly

the data show that significant binding occurs at physiologically relevant concentrations of auxin

The ability of each auxin to compete with IAA quantitatively mirrors the ability of the auxins to enhance the SCF^{TIR1}–Aux/IAA interaction

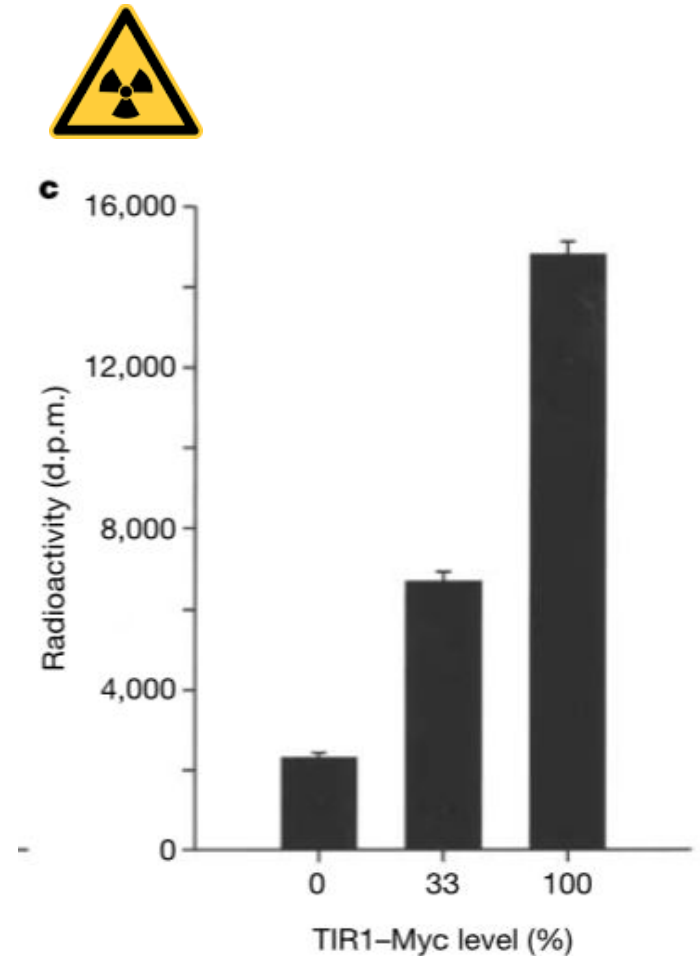
auxin binds to the SCF^{TIR1} complex rather than to the Aux/IAA. (control assays with seedlings)

SCF^{TIR1} binds auxin directly (Fig.3)



SCF^{TIR1} binds auxin directly (Fig.3)

This indicates quantitative [³H]IAA binding with respect to TIR1–Myc dose and strongly indicates that SCF^{TIR1} binds auxin.



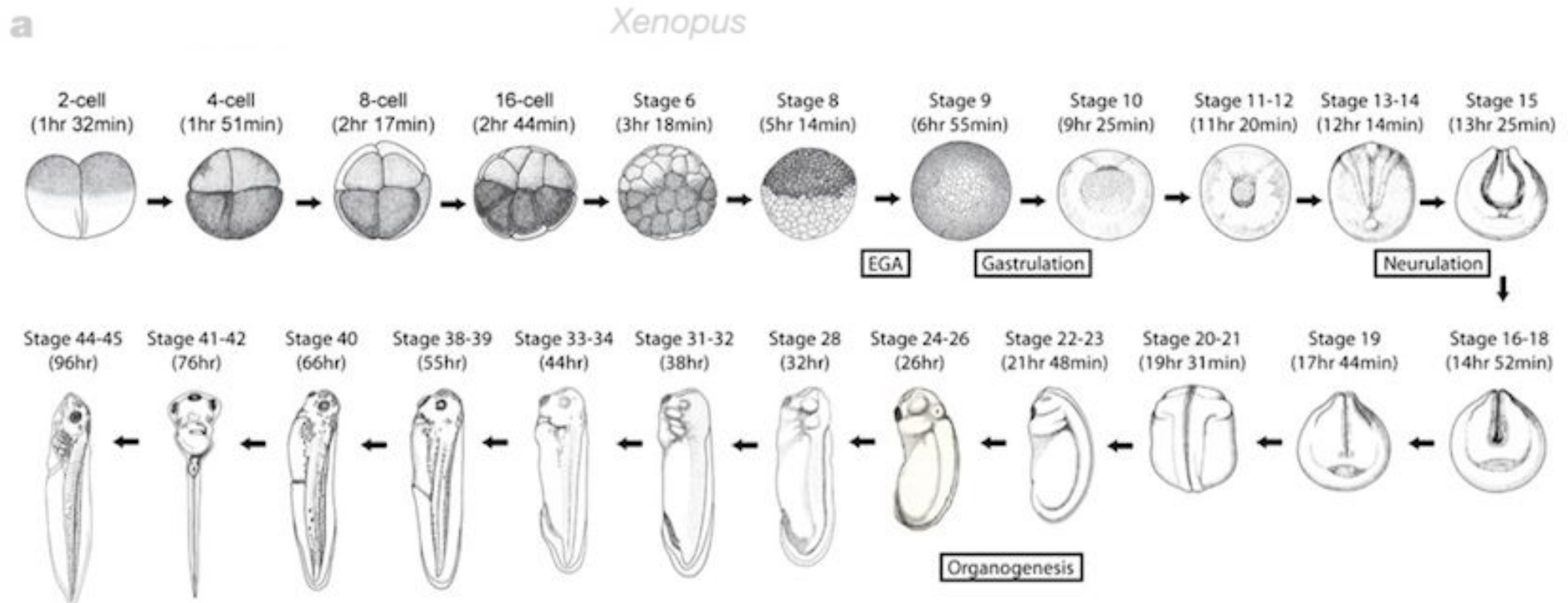
SCF^{TIR1} binds auxin directly

auxin binds directly to the SCF^{TIR1} complex

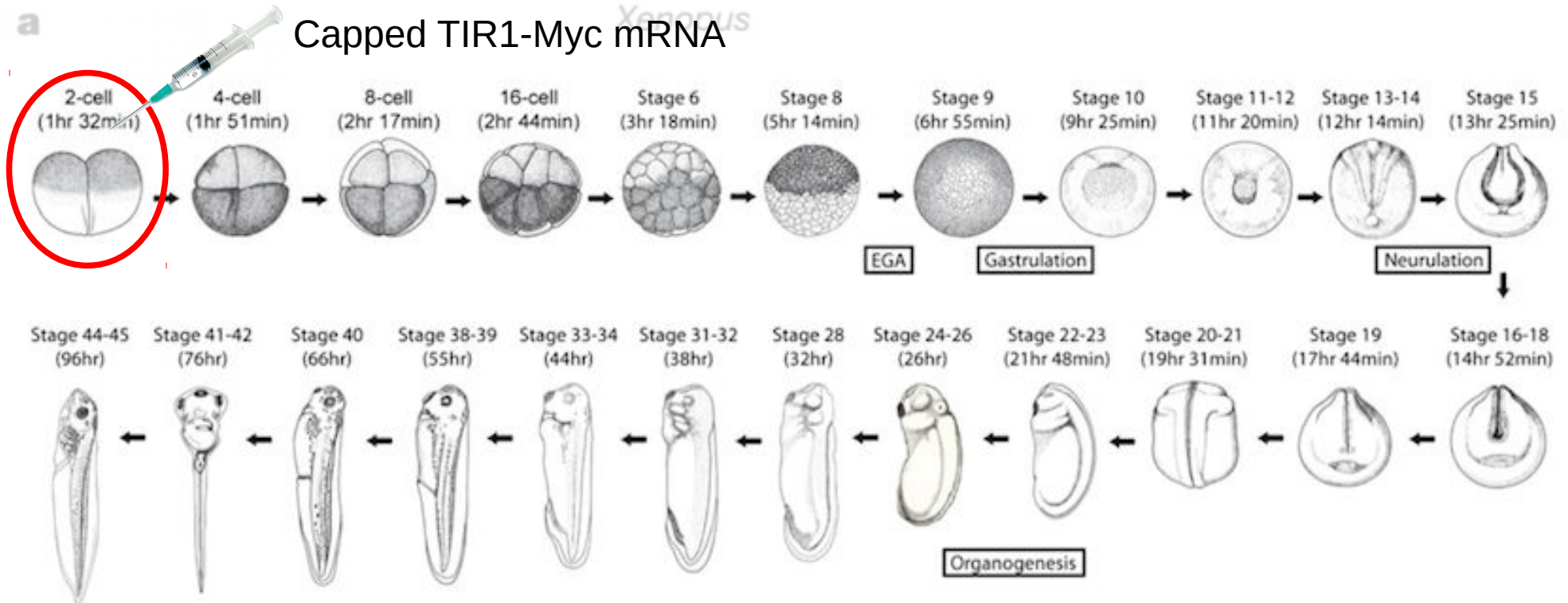
only known auxin-specific subunit of the complex is
TIR1

expressing TIR1 in a heterologous, non- auxin-
responsive system

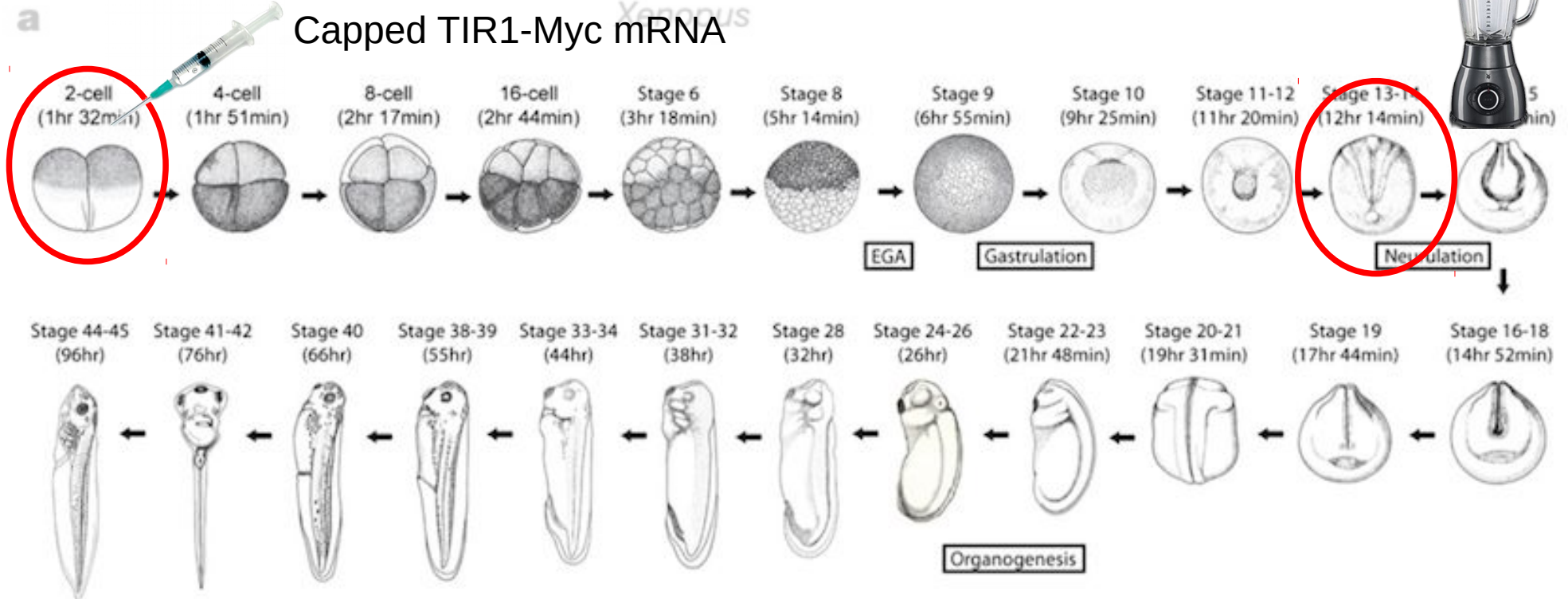
TIR1 is an auxin receptor



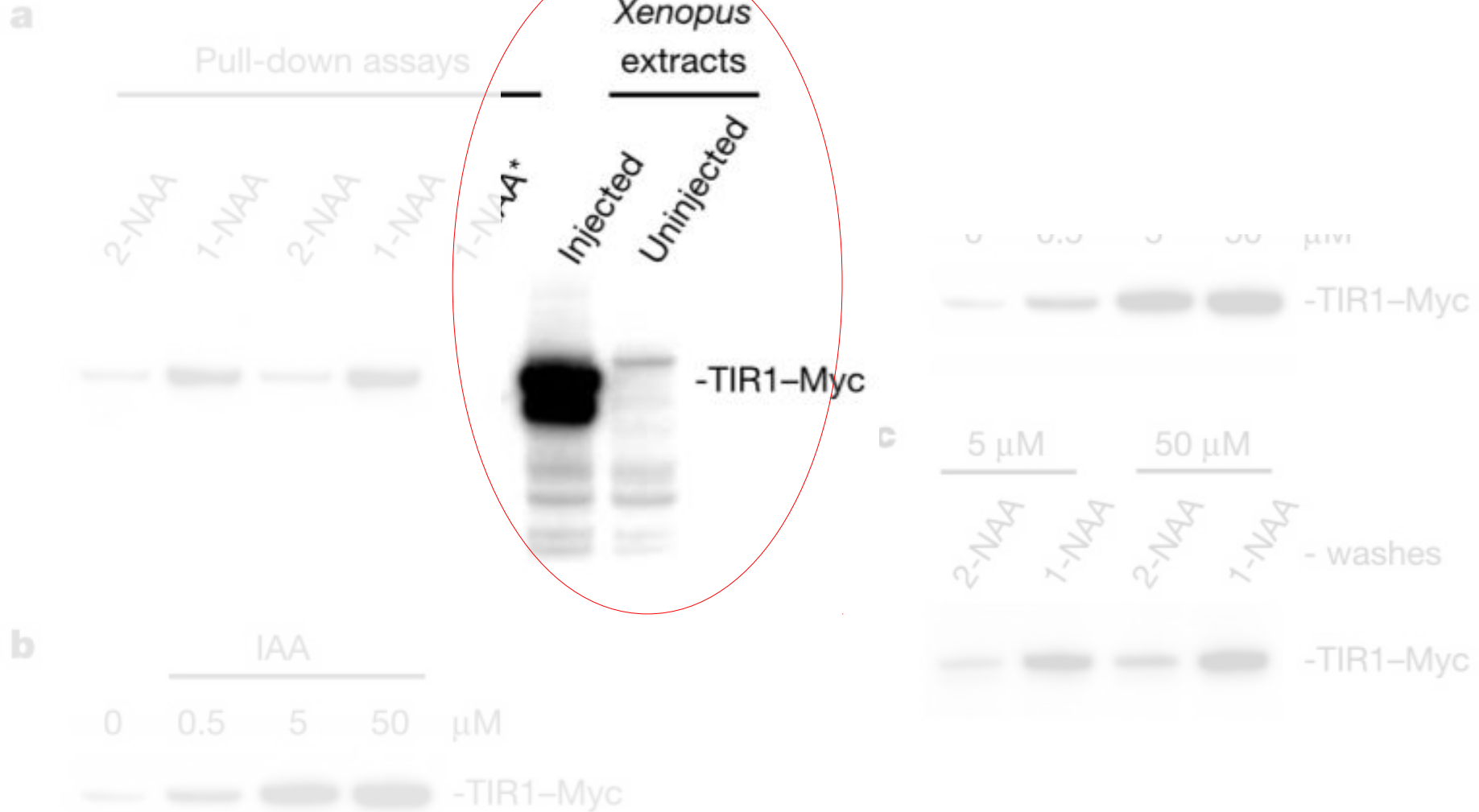
TIR1 is an auxin receptor



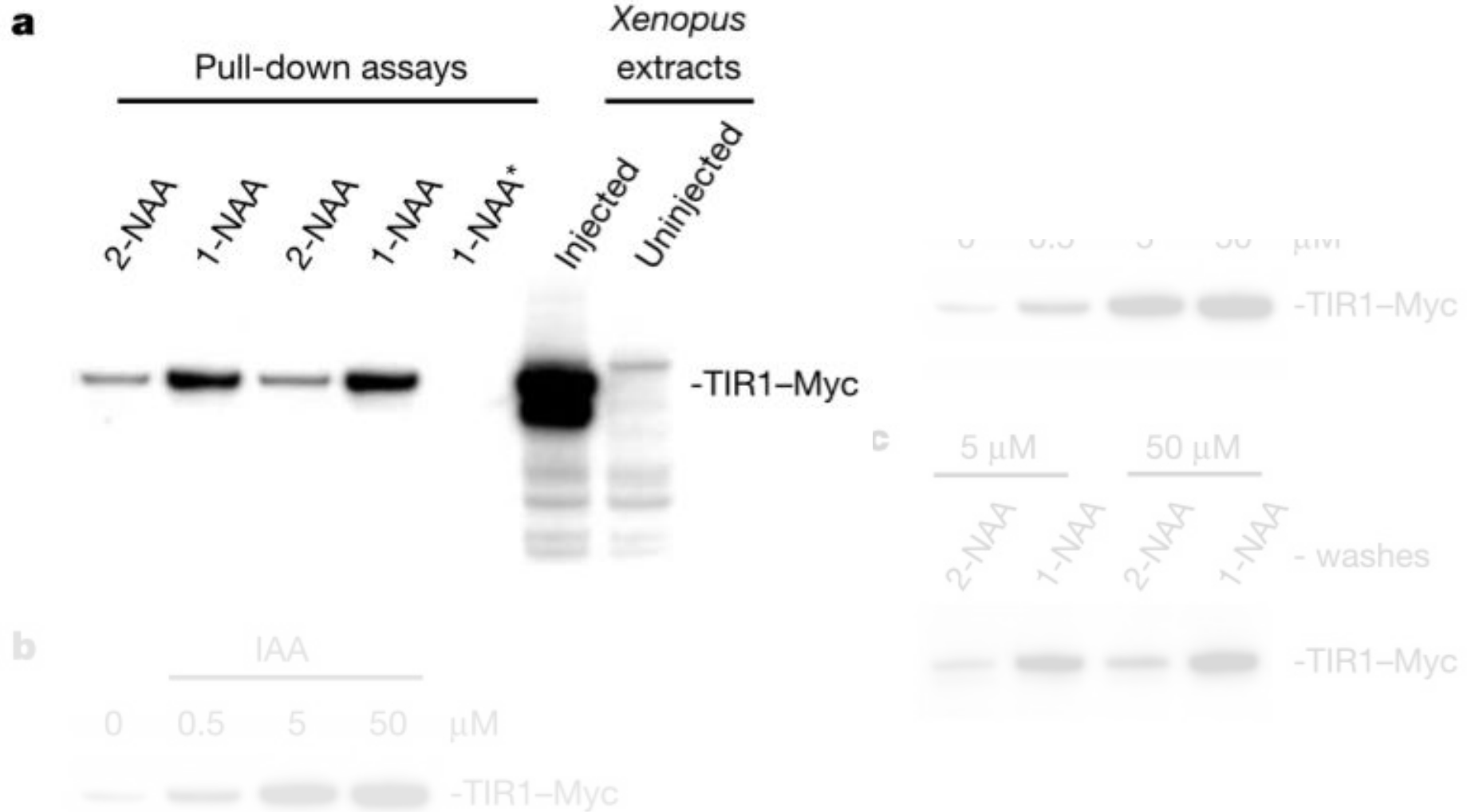
TIR1 is an auxin receptor



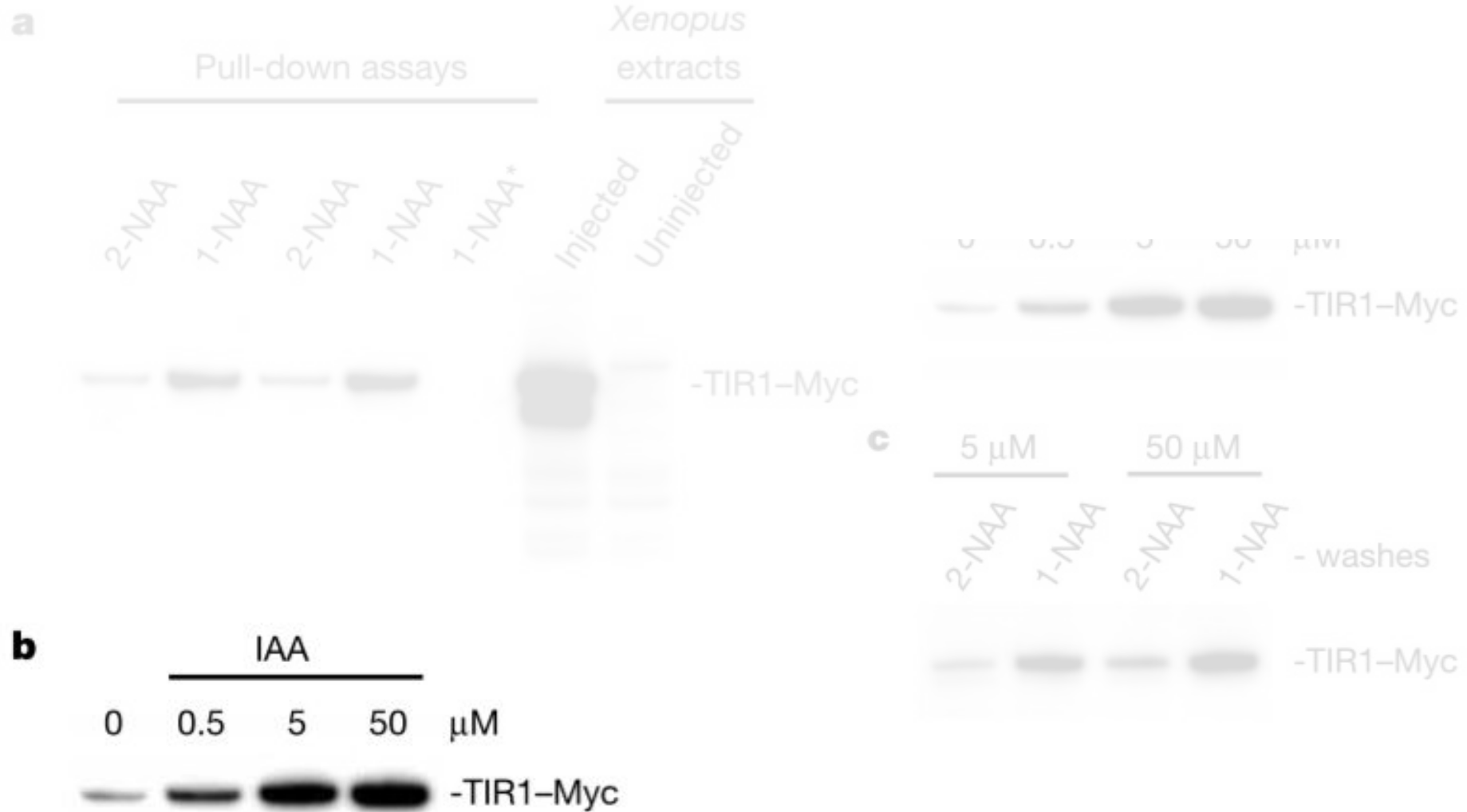
TIR1 is an auxin receptor (Fig.4)



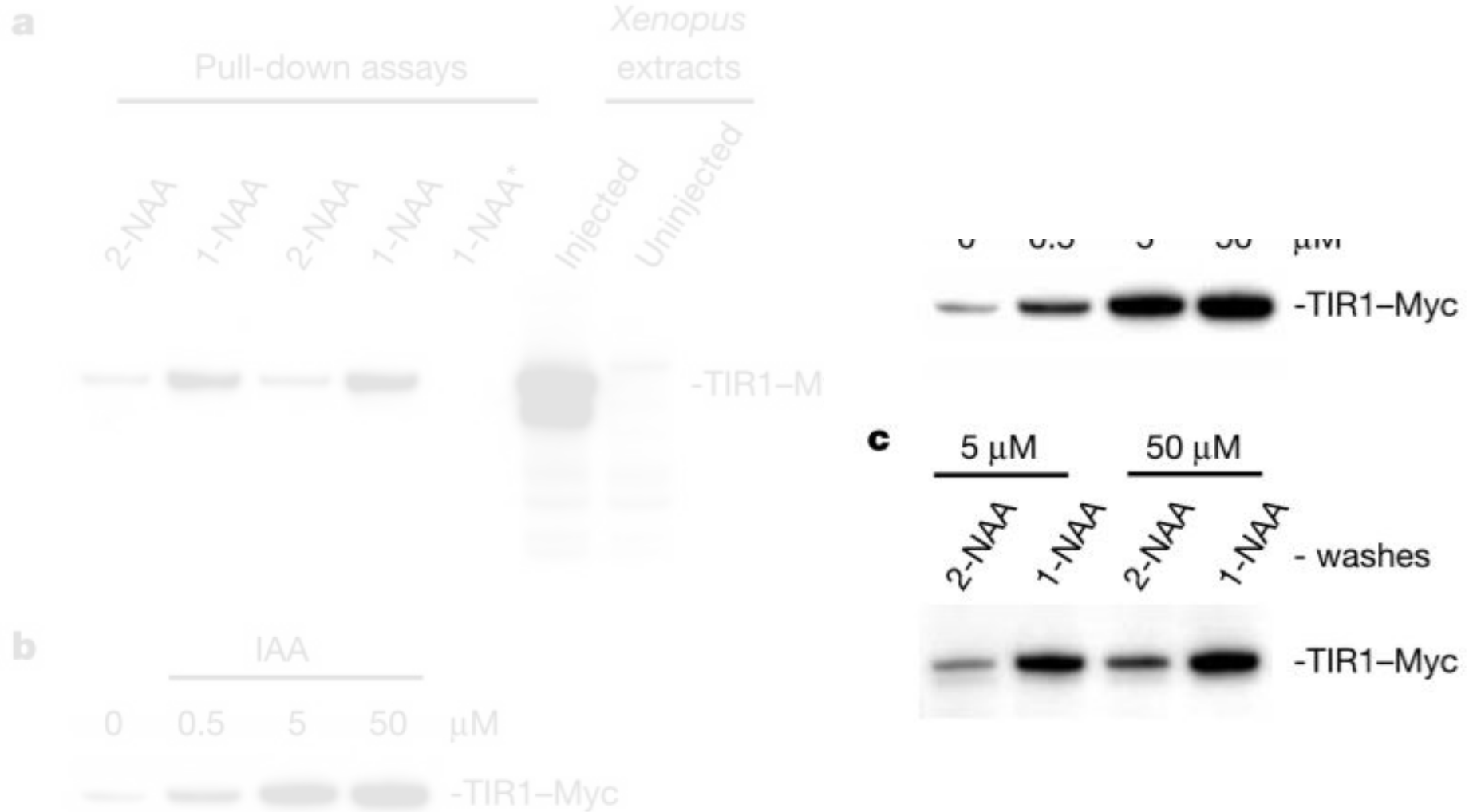
TIR1 is an auxin receptor (Fig.4)



TIR1 is an auxin receptor (Fig.4)



TIR1 is an auxin receptor (Fig.4)

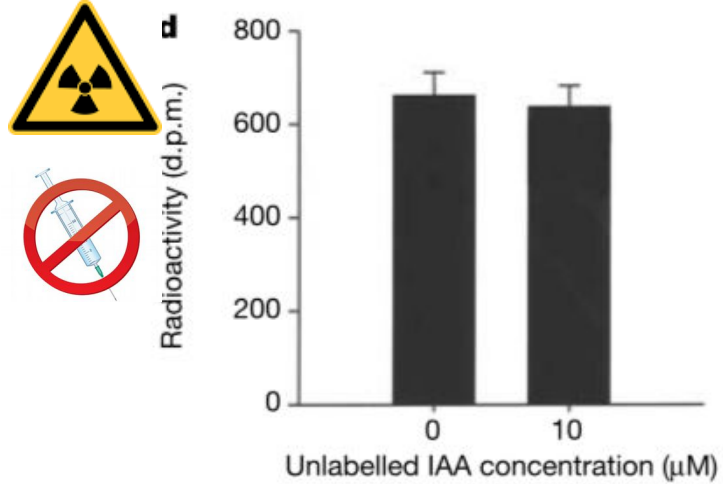


TIR1 is an auxin receptor

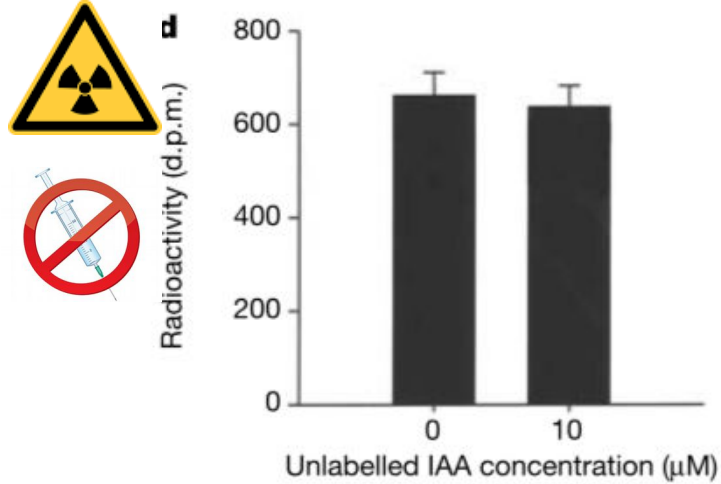
Xenopus-expressed TIR1–Myc was found to interact with Aux/IAA domain II peptide in an auxin-regulated and dose-dependent way

this result indicates that TIR1 is the auxin receptor for this response

TIR1 is an auxin receptor (Fig.4)

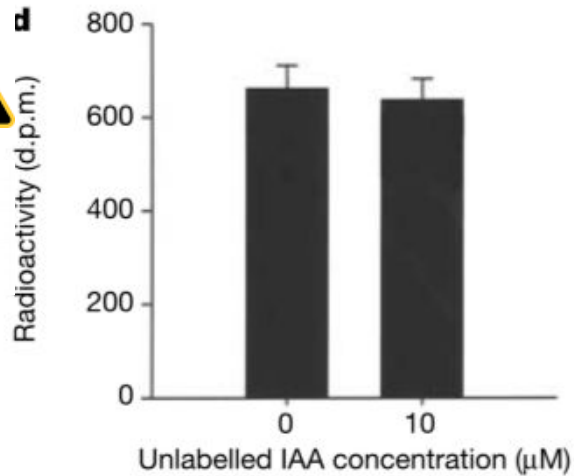


TIR1 is an auxin receptor (Fig.4)

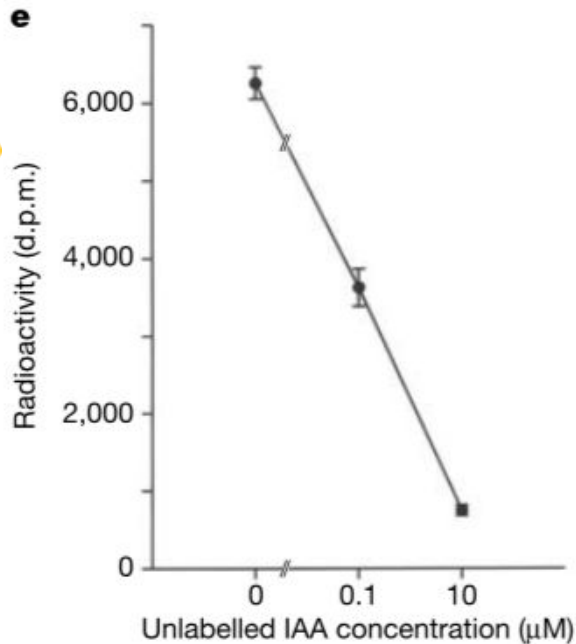


Control -
No specific [^3H]IAA binding

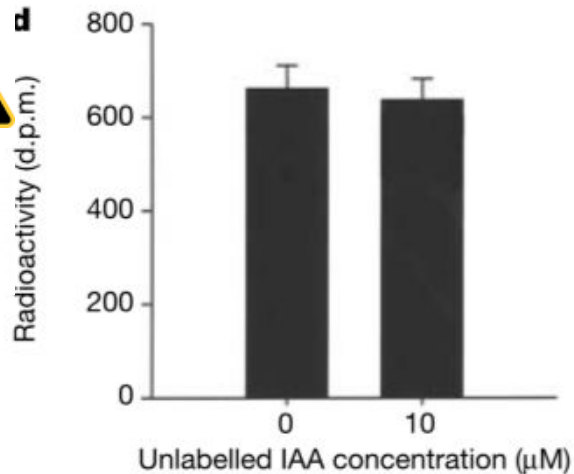
TIR1 is an auxin receptor (Fig.4)



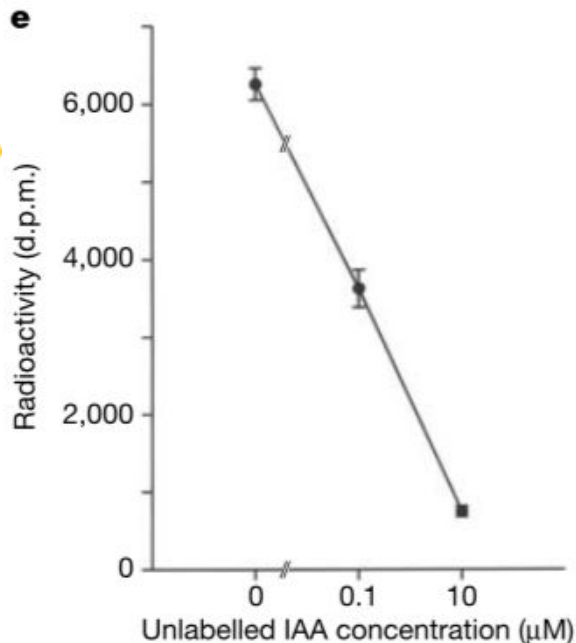
Control -
No specific [^3H]IAA binding



TIR1 is an auxin receptor (Fig.4)



Control -
No specific [3^{H}]IAA binding



clear evidence of saturable
binding of auxin as judged by the
ability of unlabelled IAA to
displace [3^{H}]IAA

Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box

The Aux/IAA proteins, including domain II, are specific to plants; they have no homologues in animals, nor is there any evidence that IAA is active as a signal in animal systems.

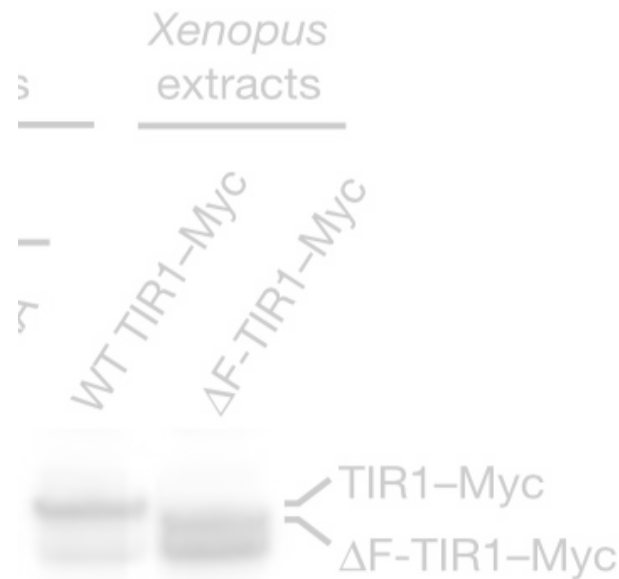
Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box

F-box proteins are brought into SCFs by the interaction of the F-box motif with SKP1

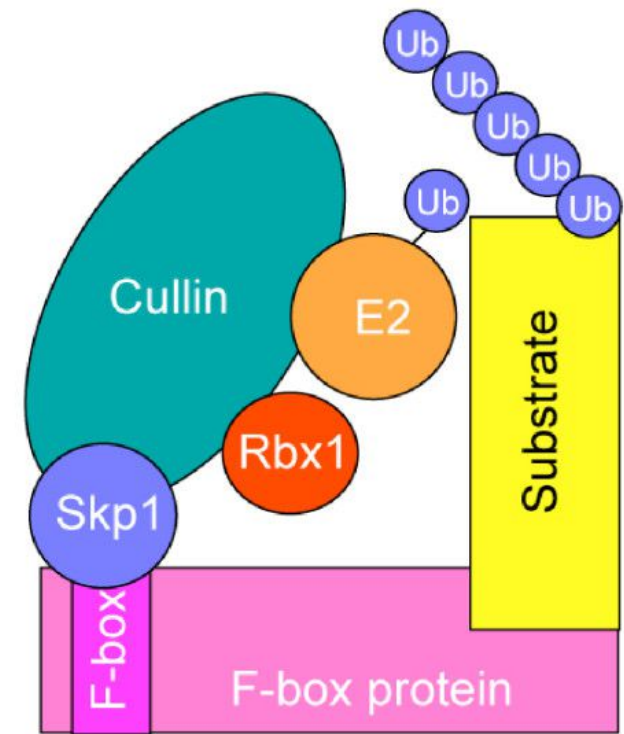
TIR1 $\leftrightarrow$ SKP1-like protein ASK1

ASK1 shares significant homology with the *X. laevis* Skp1 protein

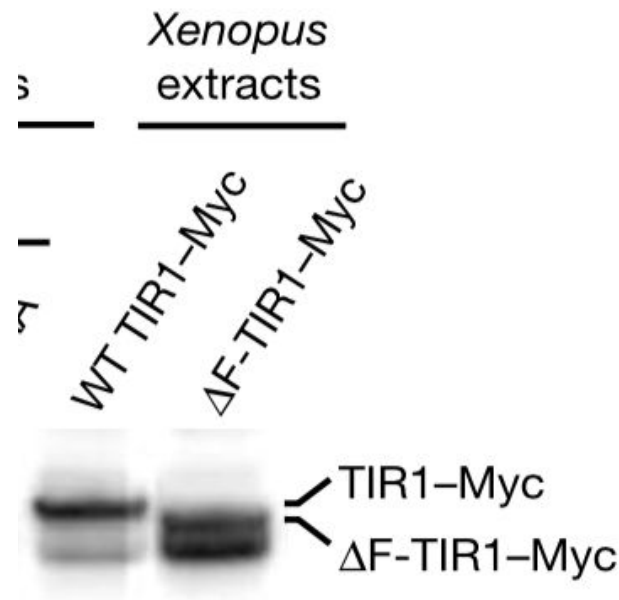
Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



A The SCF ubiquitin ligase complex

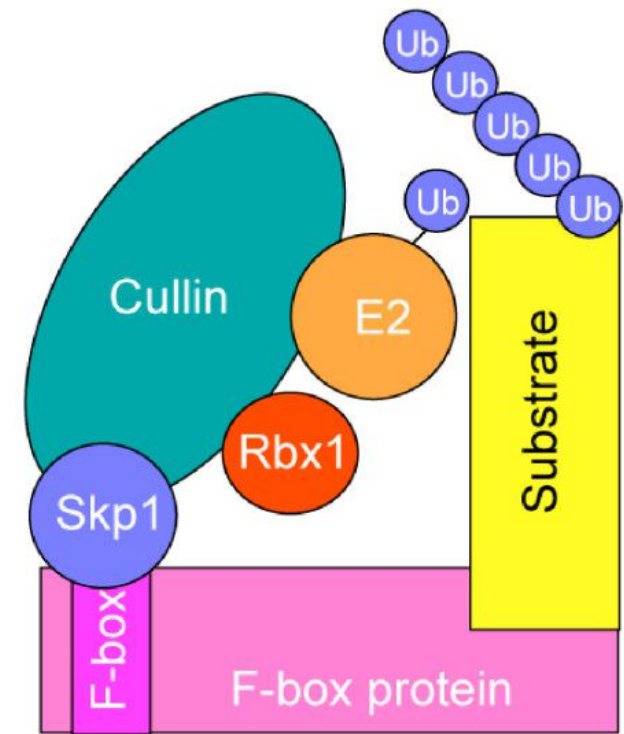


Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



Effect of the deletion?

A The SCF ubiquitin ligase complex

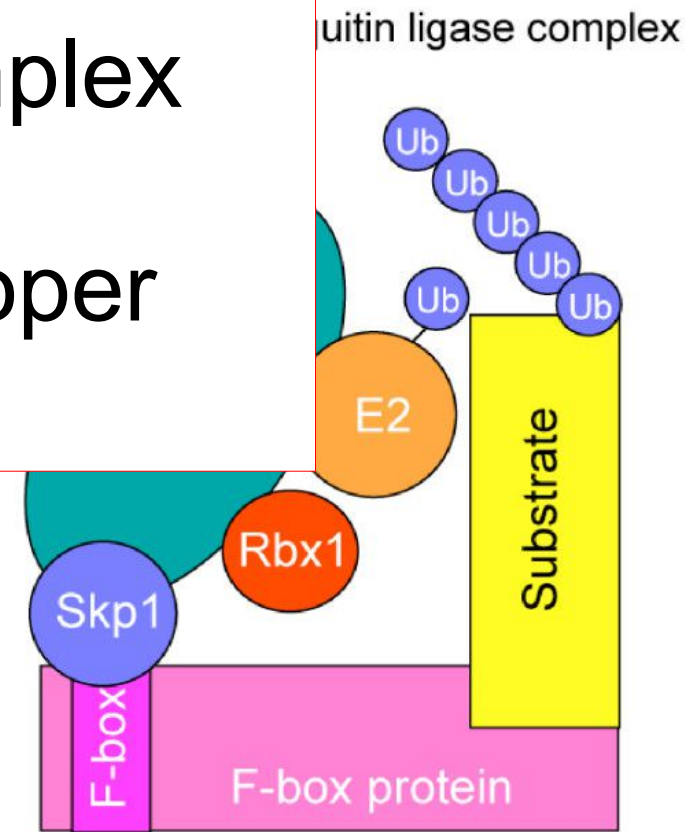


Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)

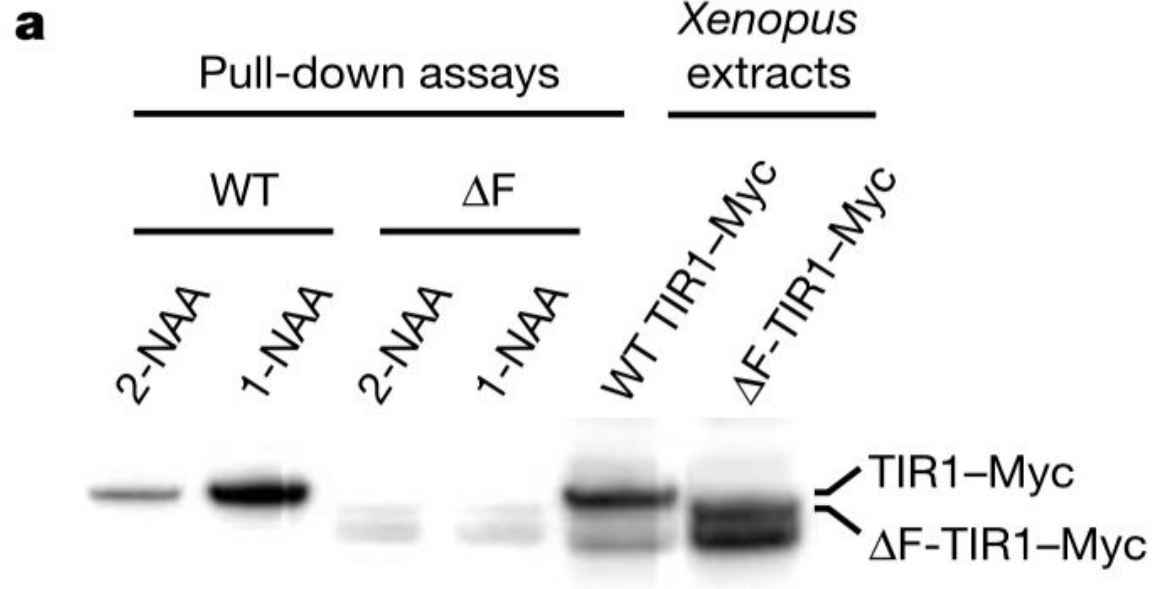
1. not able to form SCF complex
2. Folding dose not work proper

 ΔF-TIR1-Myc

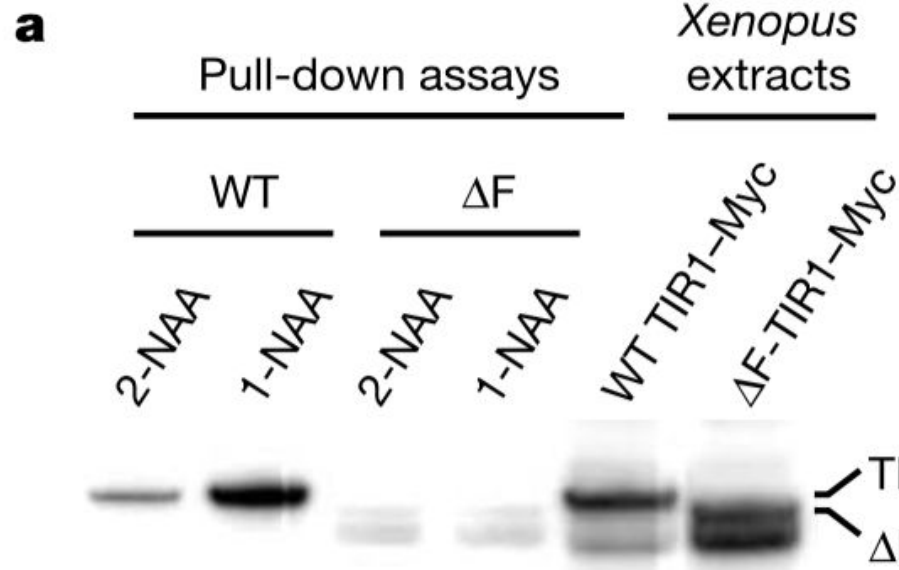
Effect of the deletion?



Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)

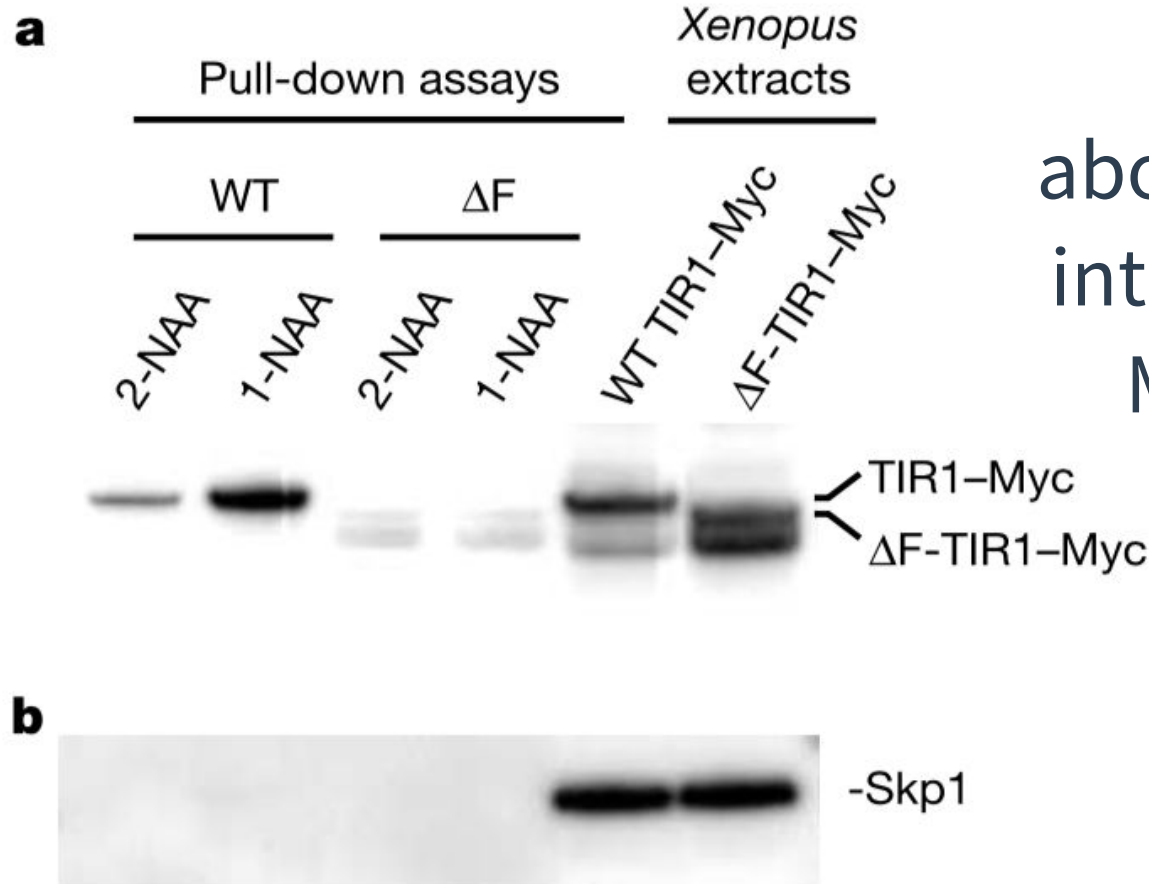


Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



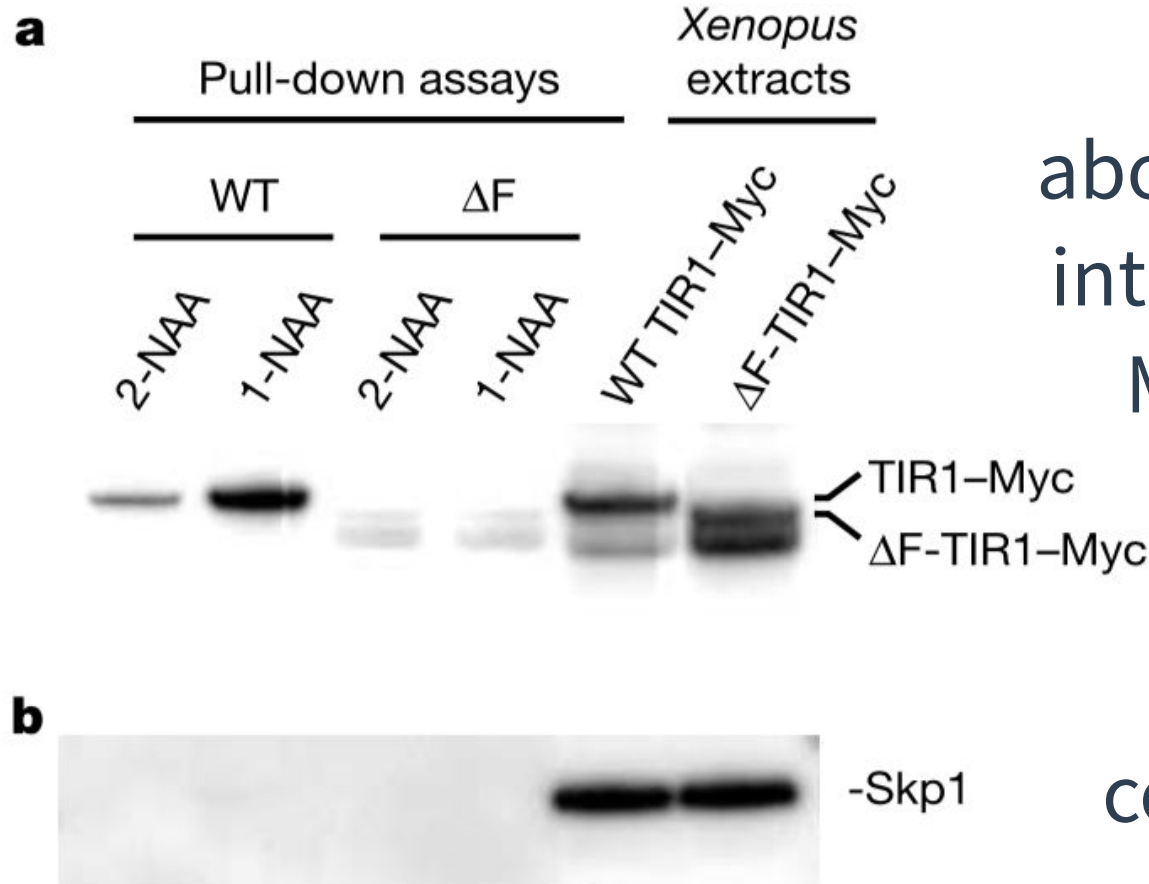
deletion of the F-box
abolishes auxin-stimulated
interaction between TIR1–
Myc and the domain II
peptide

Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



deletion of the F-box
abolishes auxin-stimulated
interaction between TIR1–
Myc and the domain II
peptide

Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



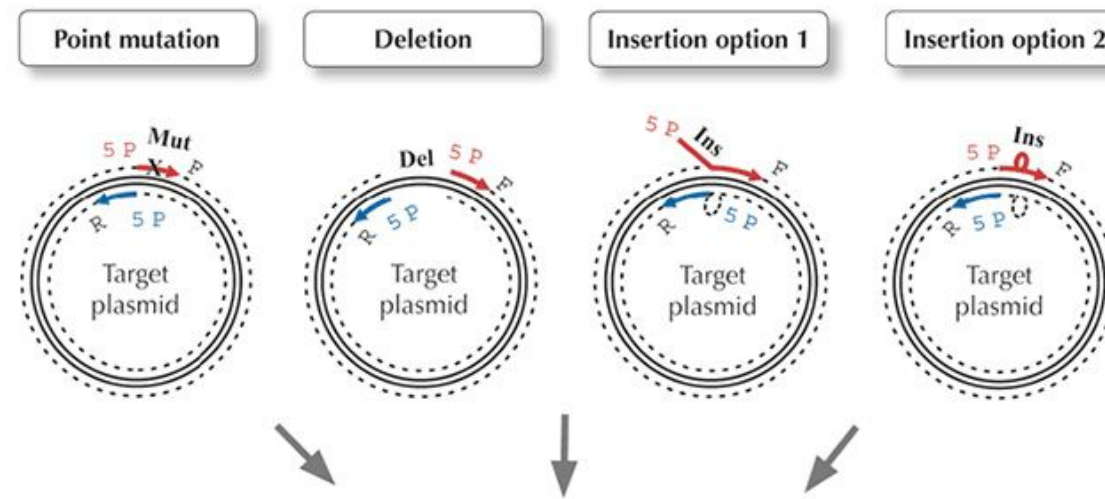
deletion of the F-box
abolishes auxin-stimulated
interaction between TIR1–
Myc and the domain II
peptide

little or no Skp1
copurified with TIR1 in
these experiments

Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)

This indicates that the incorporation of TIR1 into an SCF might not be required for auxin binding or Aux/IAA interaction.

Auxin-inducible system

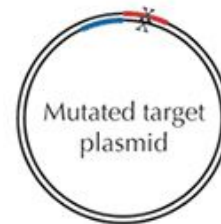


Linear amplified target plasmid with desired mutation



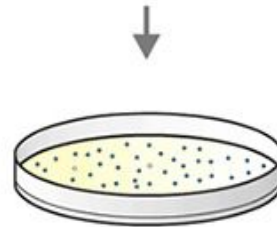
Step 1.

Amplification of target plasmid with two phosphorylated primers.



Step 2.

Plasmid circularization by ligation.



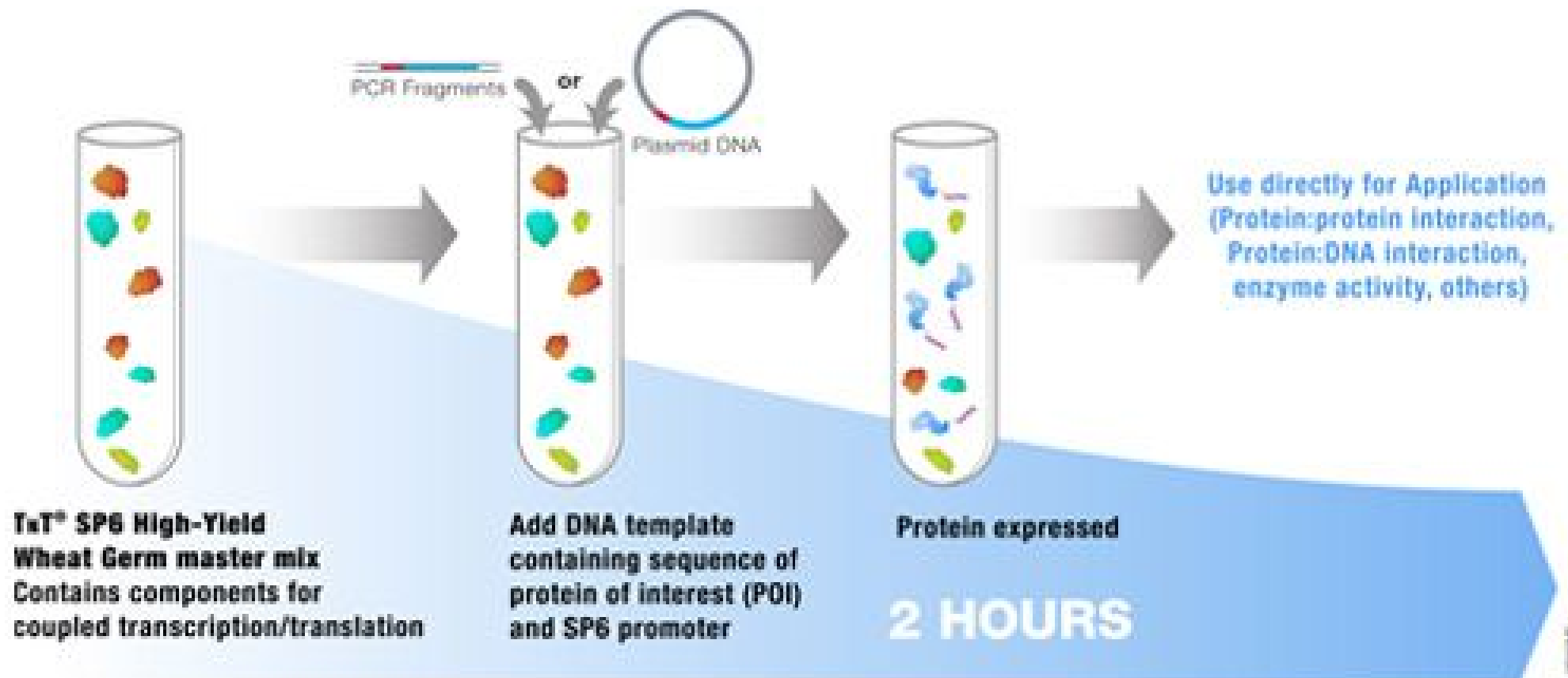
Step 3.

Transformation into *E. coli*.

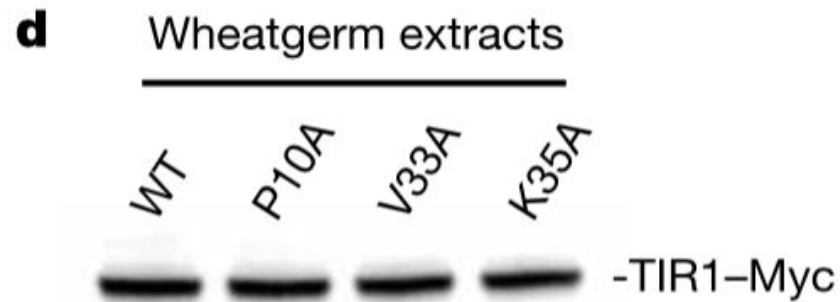
Site directed mutagenesis kit (Stratagene)

Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)

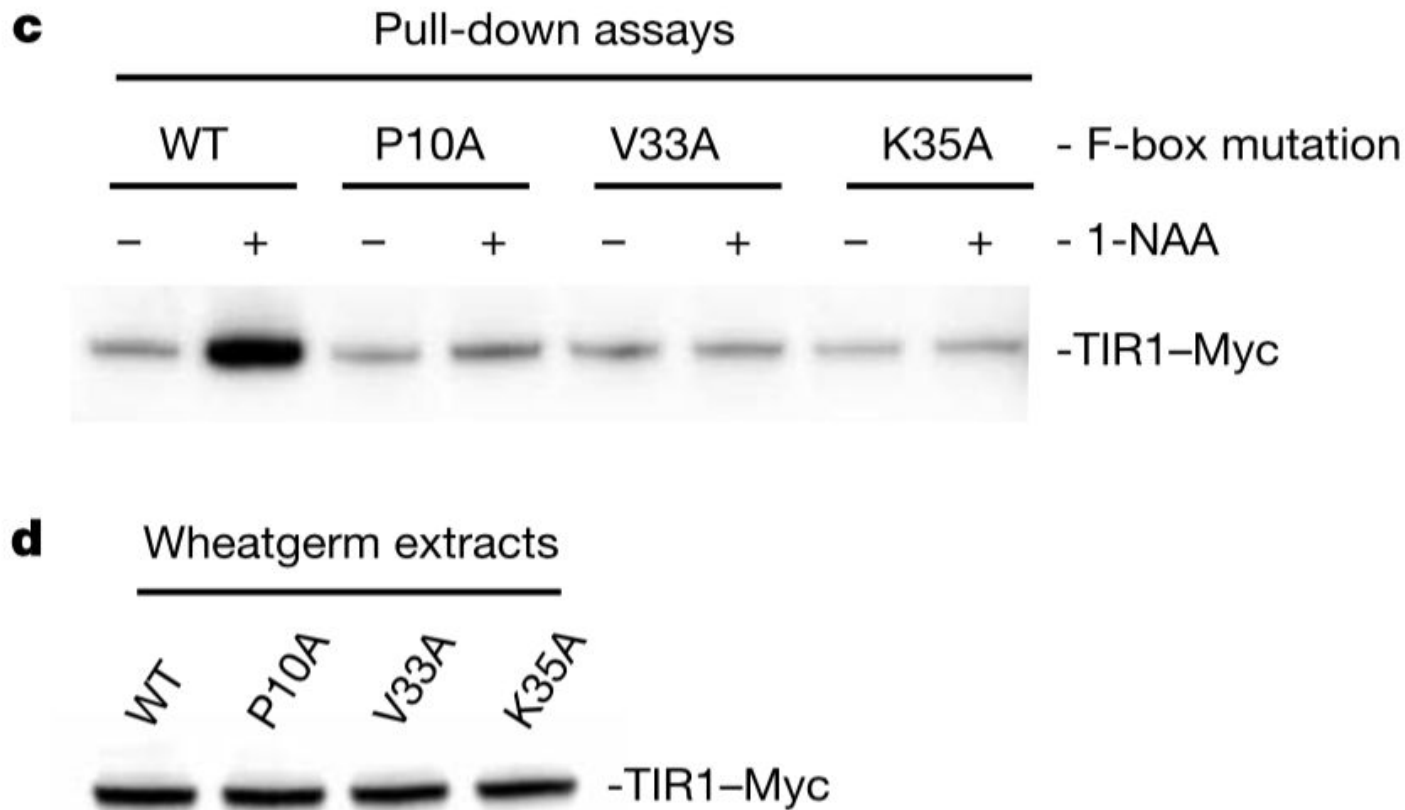
Wheatgerm extracts



Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box (Fig.5)



Auxin-induced TIR1–Aux/IAA binding requires the TIR1 F-box

these results provide some support for the idea that the F-box of TIR1 has a specific function in addition to SKP1 binding, such as auxin or Aux/IAA binding.

Latest model

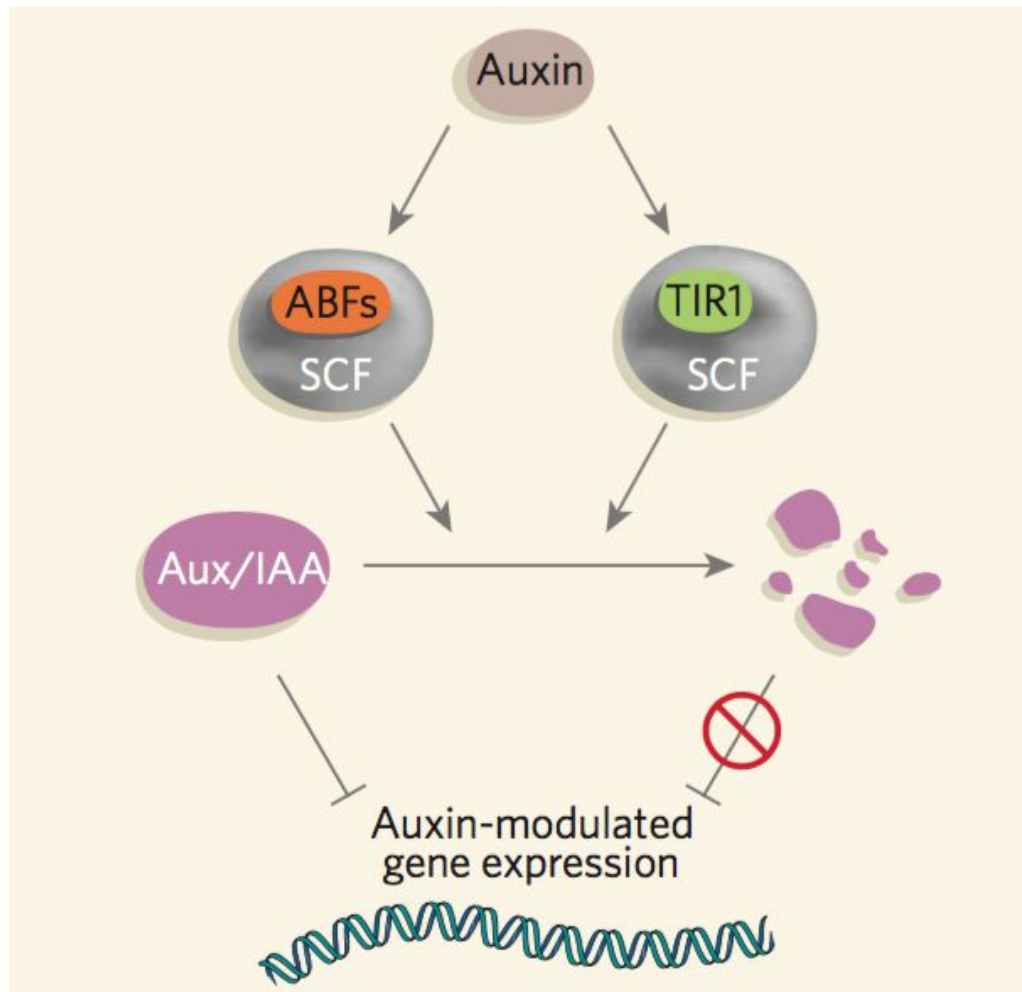


Figure 2 | Model of auxin action. Auxins act directly with SCF complexes containing either transport inhibitor response protein 1 (TIR1) or the related auxin-binding factors (ABFs). This catalyses the destruction of Aux/IAA proteins, which directly inhibit the genes that carry out the auxin response. The inhibitory effect of Aux/IAA is thus relieved, allowing auxin responses to occur.