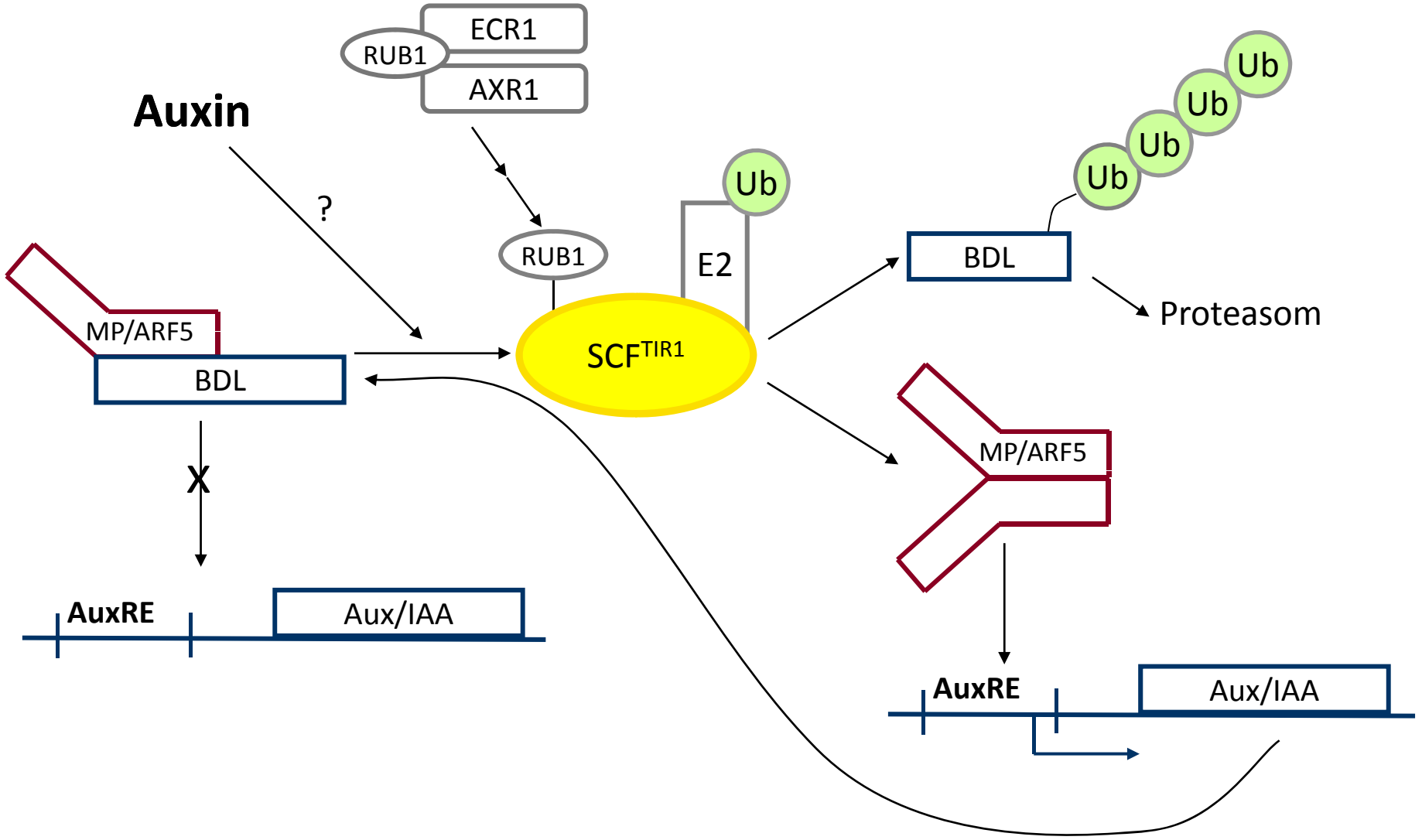
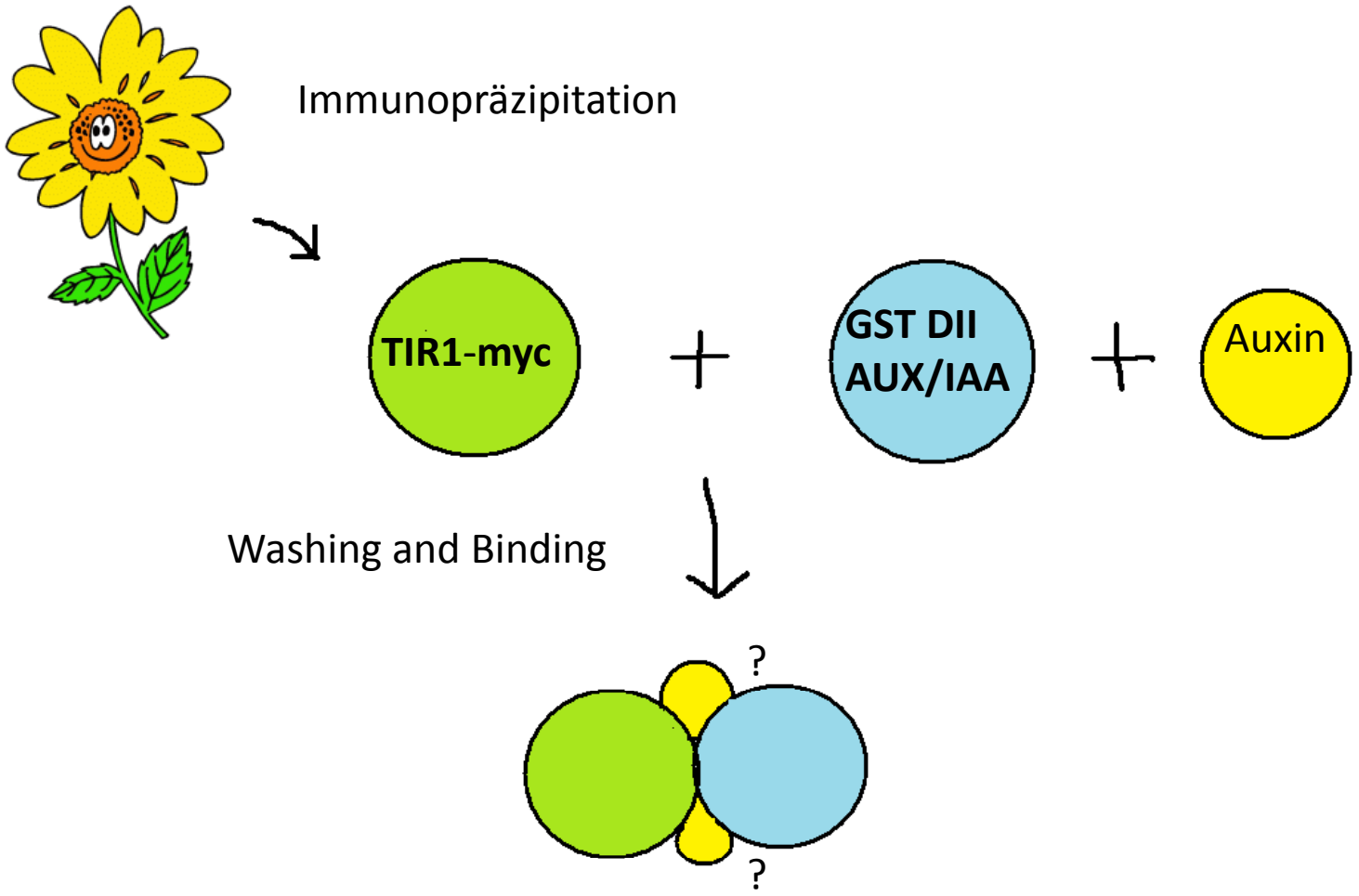
The *Arabidopsis* F-box protein TIR1 is an auxin receptor

Stefan Kepinski & Ottoline Leyser

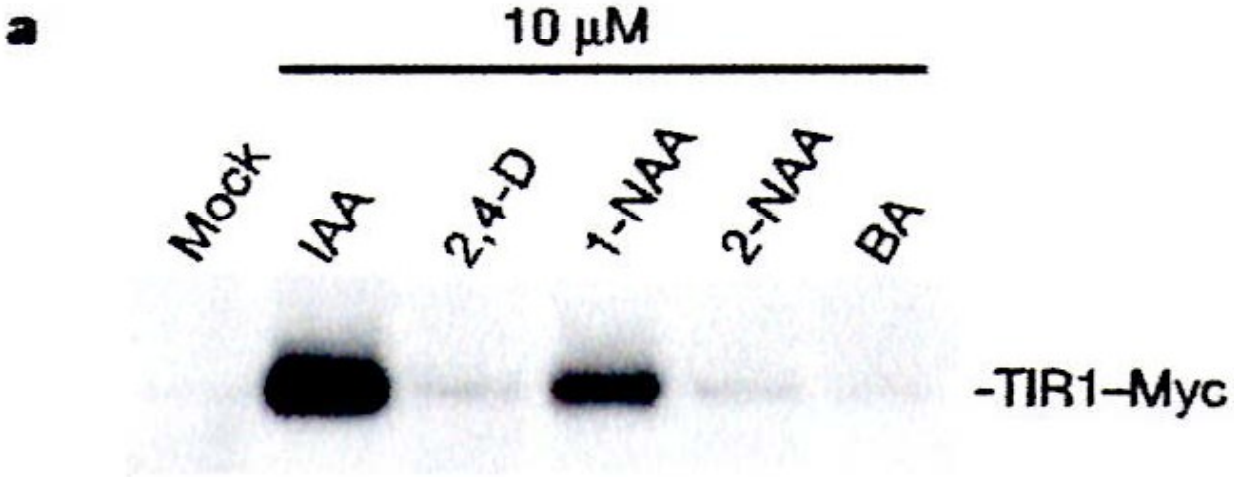
Modell

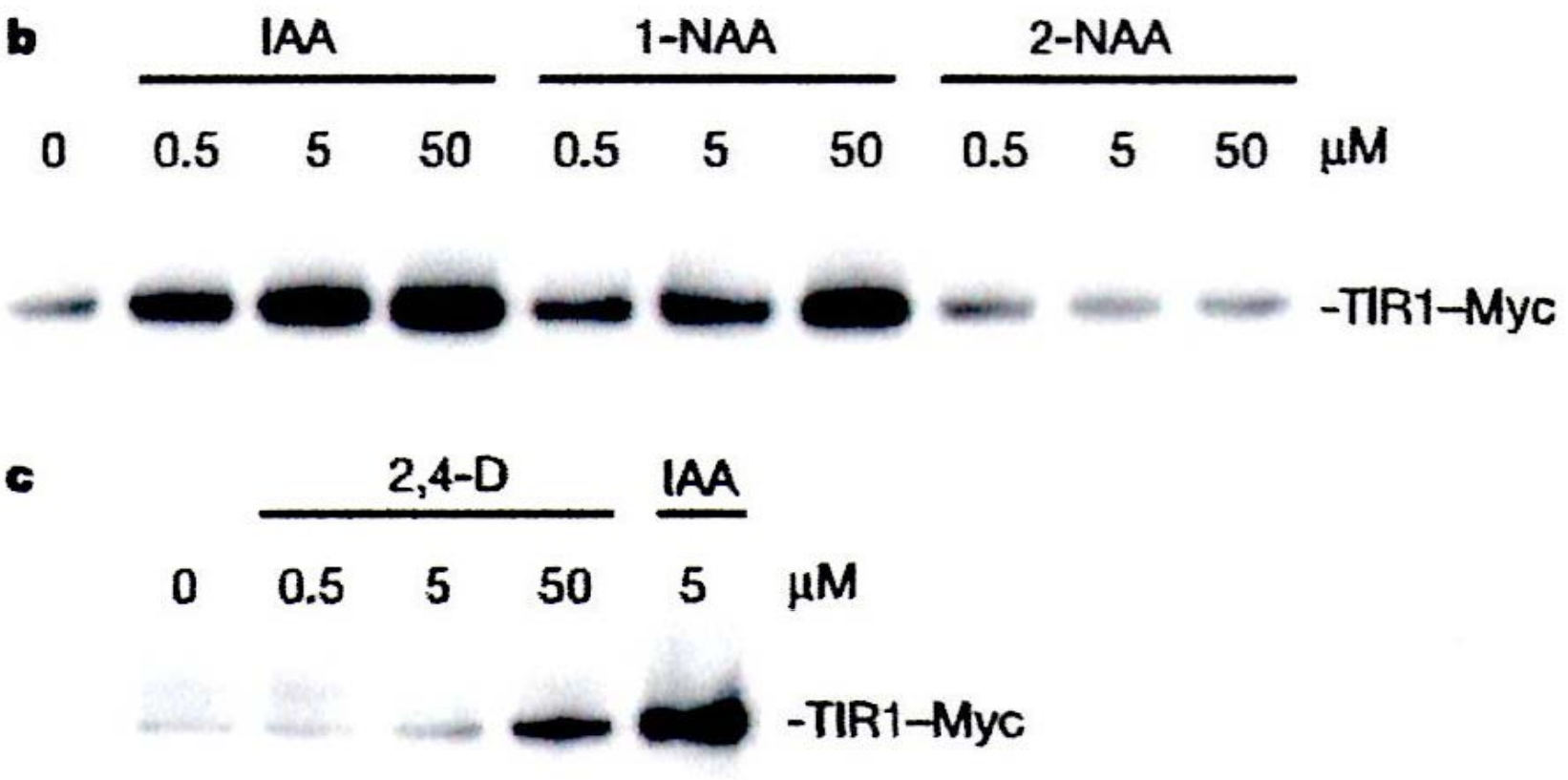

ECR1
RUB1
AXR1
Auxin
?
RUB1
Ub
E2
Ub
Ub
Ub
Ub
BDL
Proteasom
MP/ARF5
BDL
SCF^TIR1
MP/ARF5
X
AuxRE
Aux/IAA
AuxRE
Aux/IAA

Pull-down assays



Domain II peptide pull-down assays with immunopurified TIR1-Myc




b
IAA
1-NAA
2-NAA
0
0.5
5
50
0.5
5
50
0.5
5
50
μM
-TIR1–Myc
c
2,4-D
IAA
0
0.5
5
50
5
μM
-TIR1–Myc

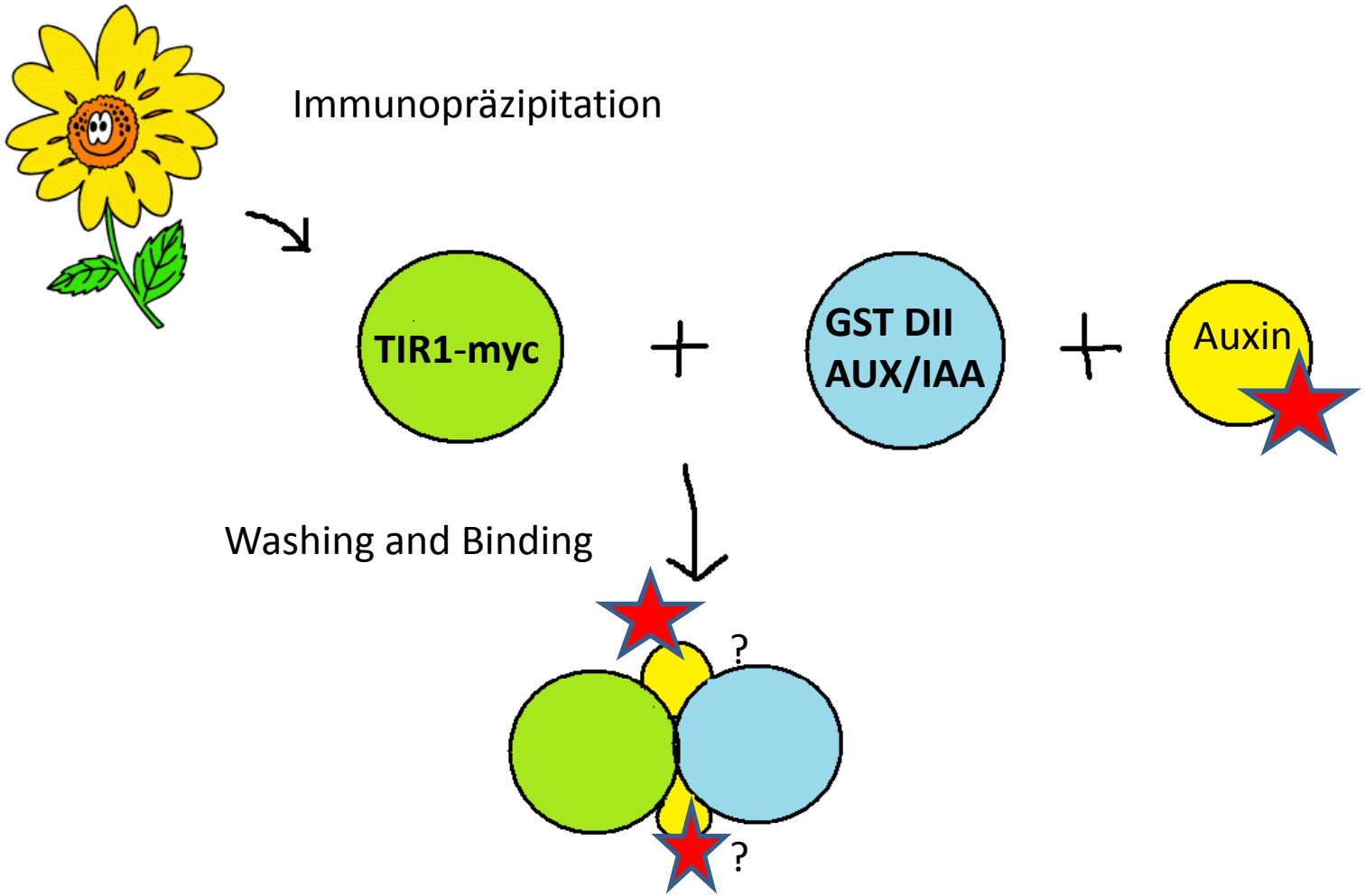

a
5 min
20 min
120 min
- + - + - +
10 μM 1-NAA
-TIR1–Myc

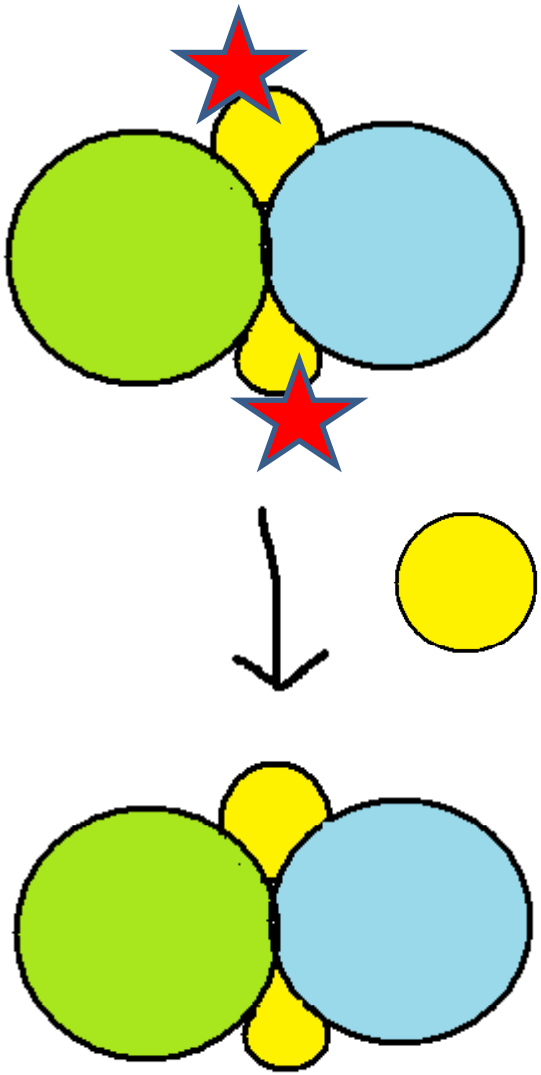

b
5 µM
50 µM
2-NAA
1-NAA
2-NAA
1-NAA
- Washes
-TIR1–Myc

Ergebnis

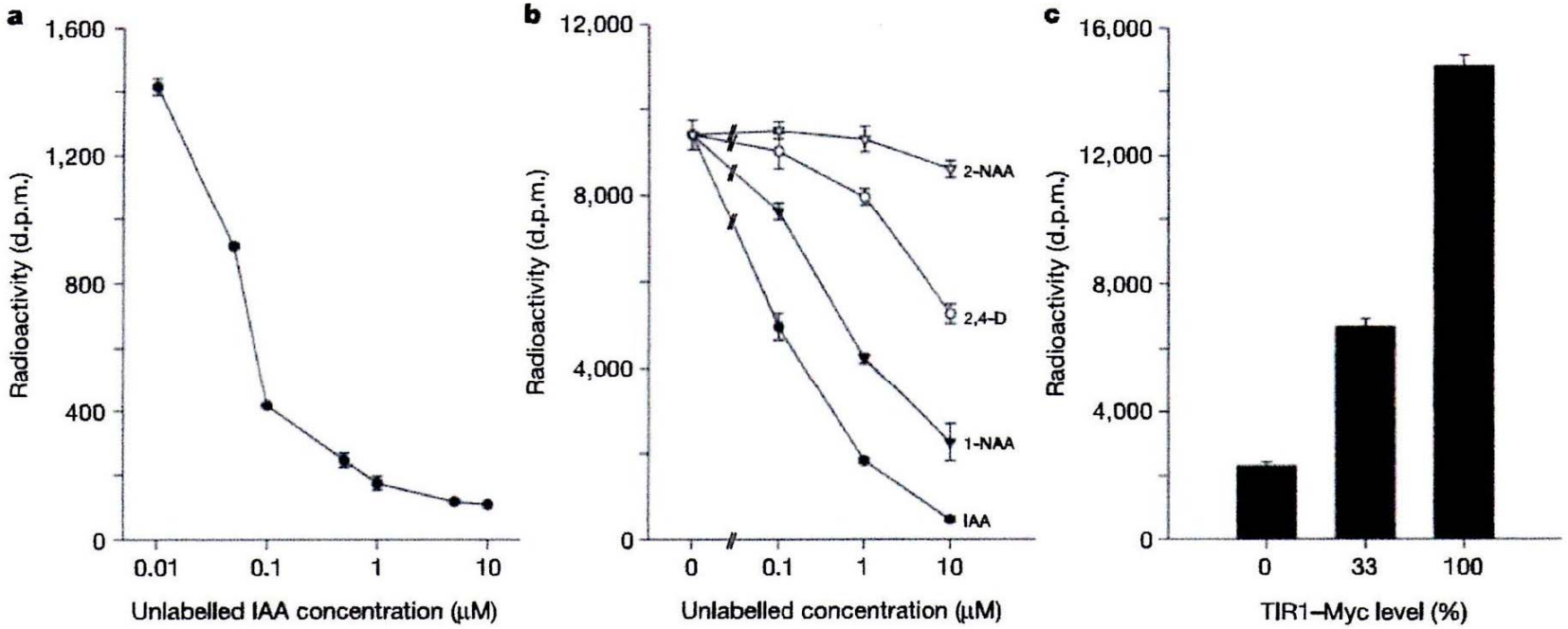
- In vitro: Dosis- und Zeitabhängigkeit
- Auxin Teil der Bindung
- Konstante Auxin-Konzentration um Interaktion aufrechtzuerhalten bzw. starke Bindung zu erreichen
- Noch kein Nachweis dafür, dass Auxin Teil des SCF-Komplexes

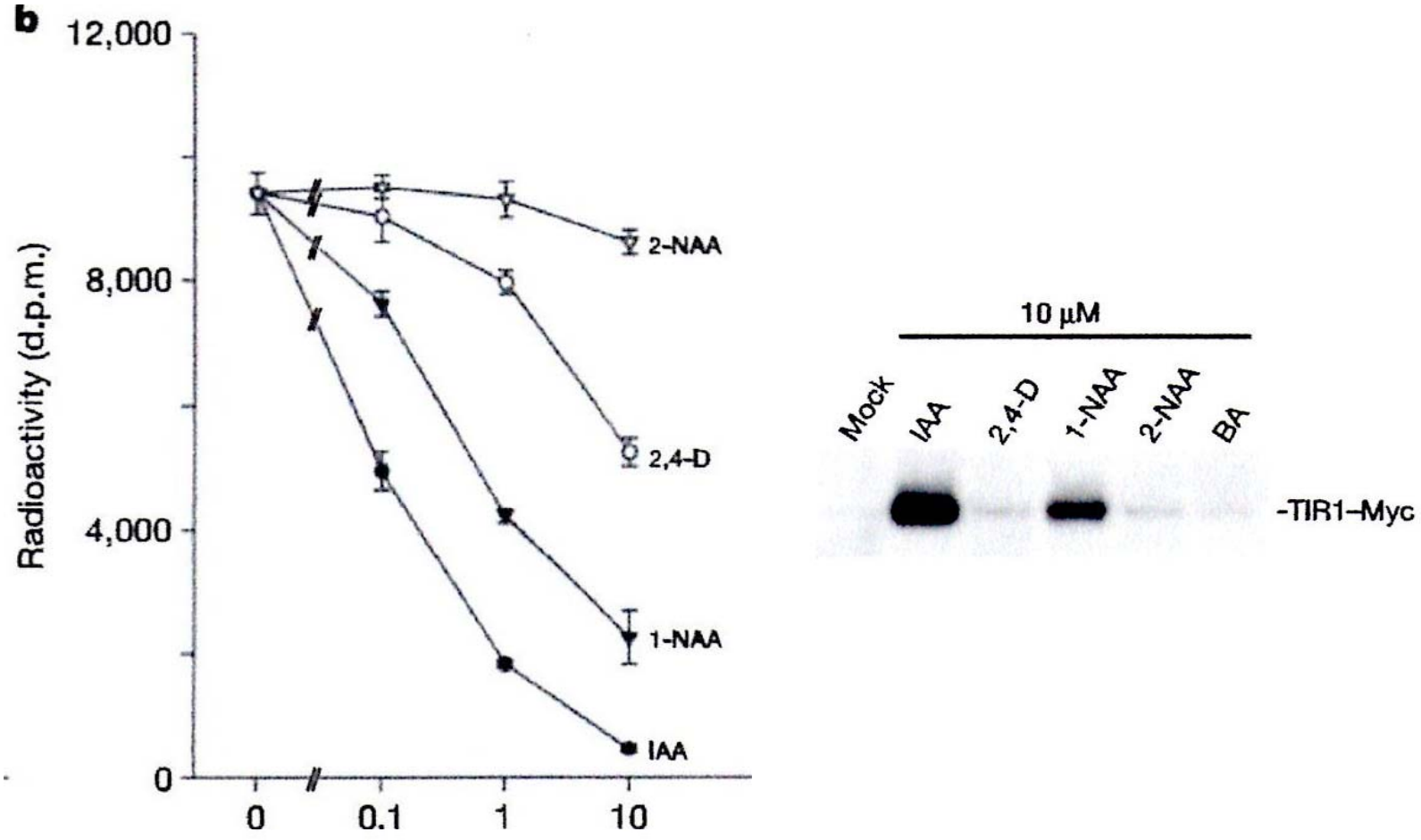
Pull-down assays





TIR1-AUX/IAA interaction involves direct auxin binding



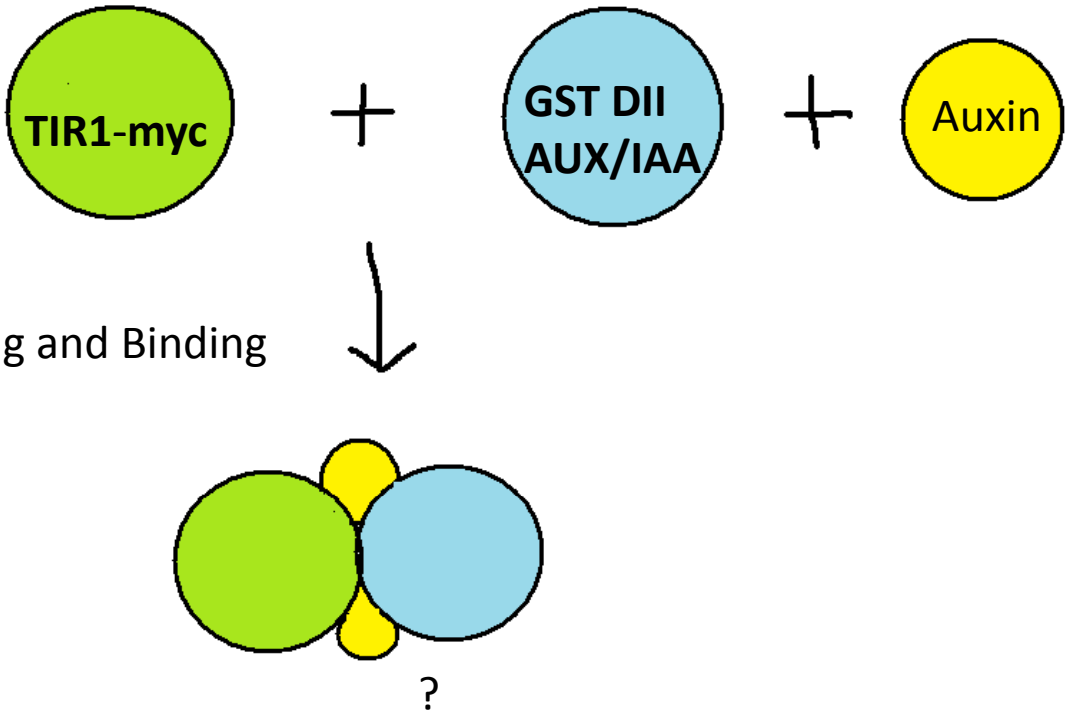

b
12,000
8,000
4,000
0
Radioactivity (d.p.m.)
0
0.1
1
10
2-NAA
2,4-D
1-NAA
IAA
10 μM
Mock
IAA
2,4-D
1-NAA
2-NAA
BA
-TIR1-Myc

Ergebnis

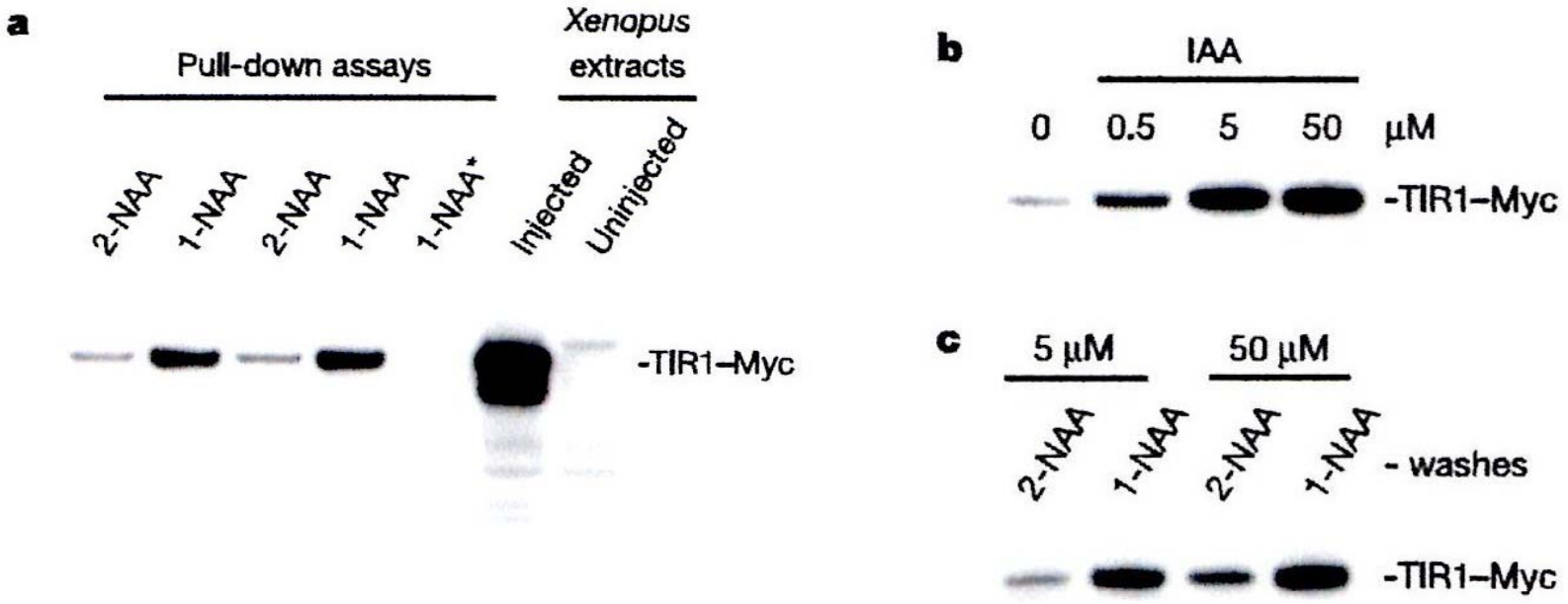
- Direkte Bindung von Auxin an den SCF-Komplex
- Auxin ist Teil des Komplexes

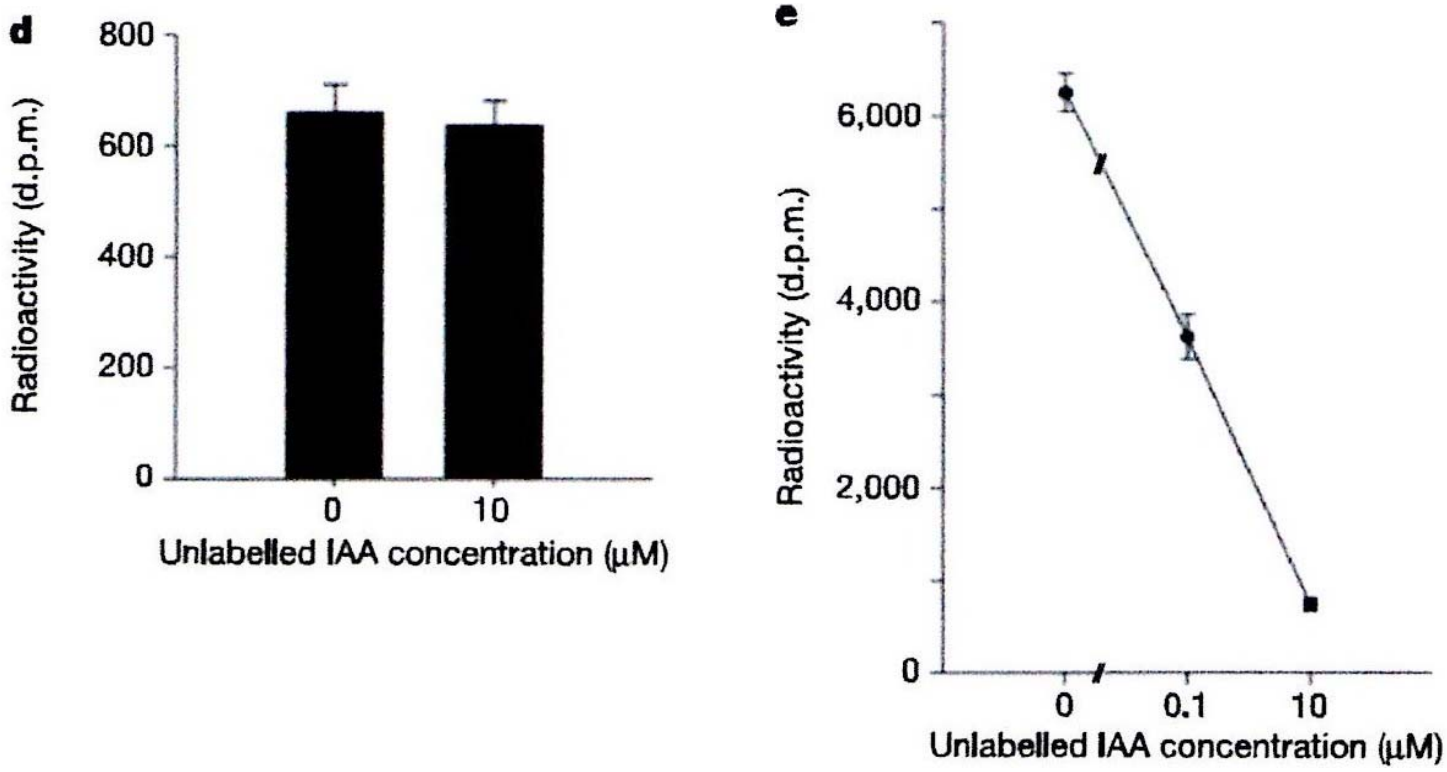
Pull-down assays

Immunopräzipitation



TIR1-Myc expressed in *Xenopus laevis* embryos

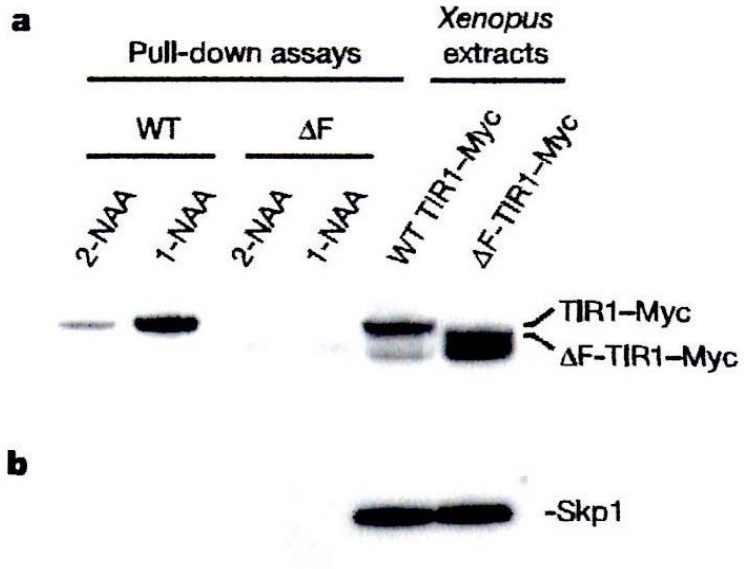


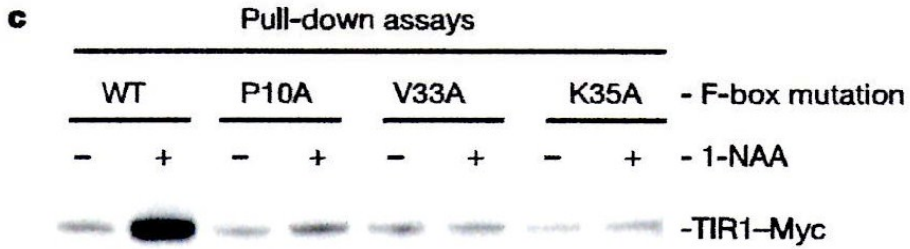

d
800
600
400
200
0
Radioactivity (d.p.m.)
0
10
Unlabelled IAA concentration (μM)
e
6,000
4,000
2,000
0
Radioactivity (d.p.m.)
0
0.1
10
Unlabelled IAA concentration (μM)

Die Überraschung

TIR1 ist ein Auxin-Rezeptor

The F-box motif of TIR1 is required for auxin-induced TIR1-Aux/IAA interaction





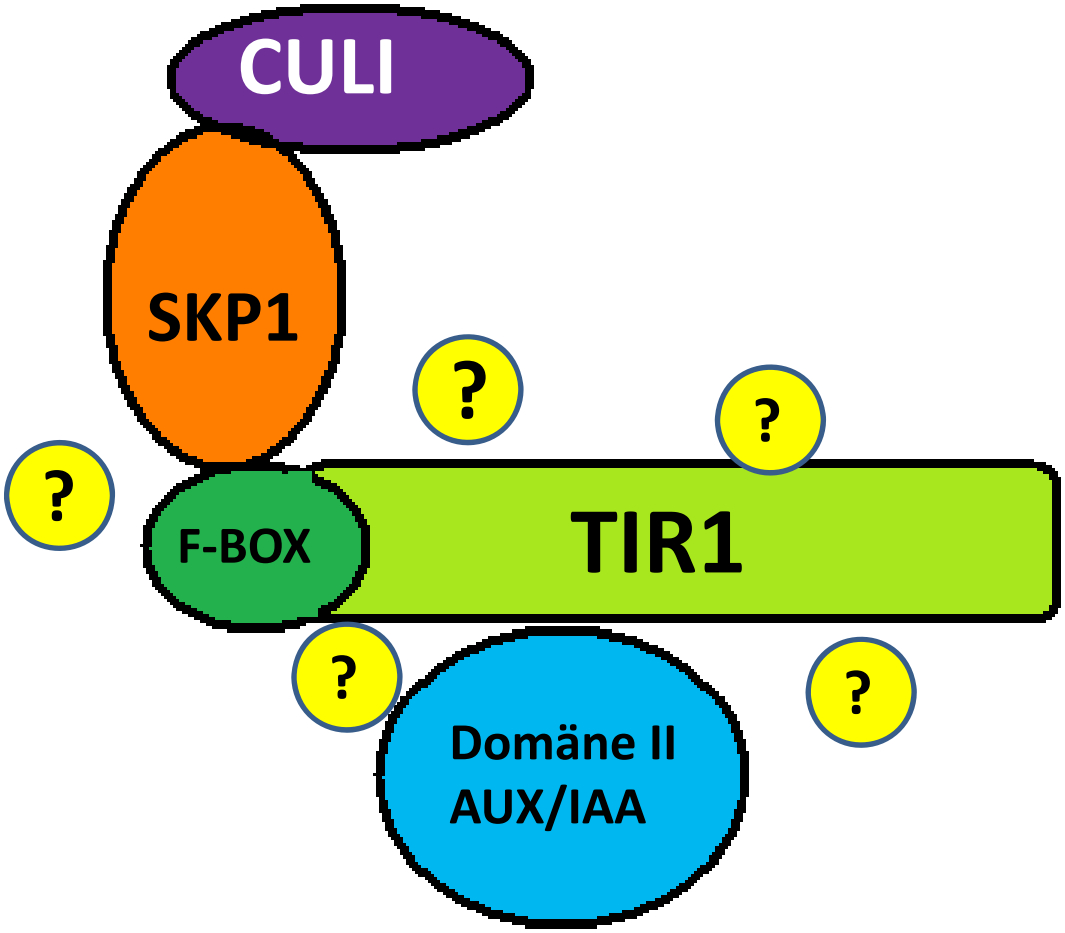
Ergebnis

- F-Box ist für die Interaktion von Auxin mit dem SCF-Komplex notwendig

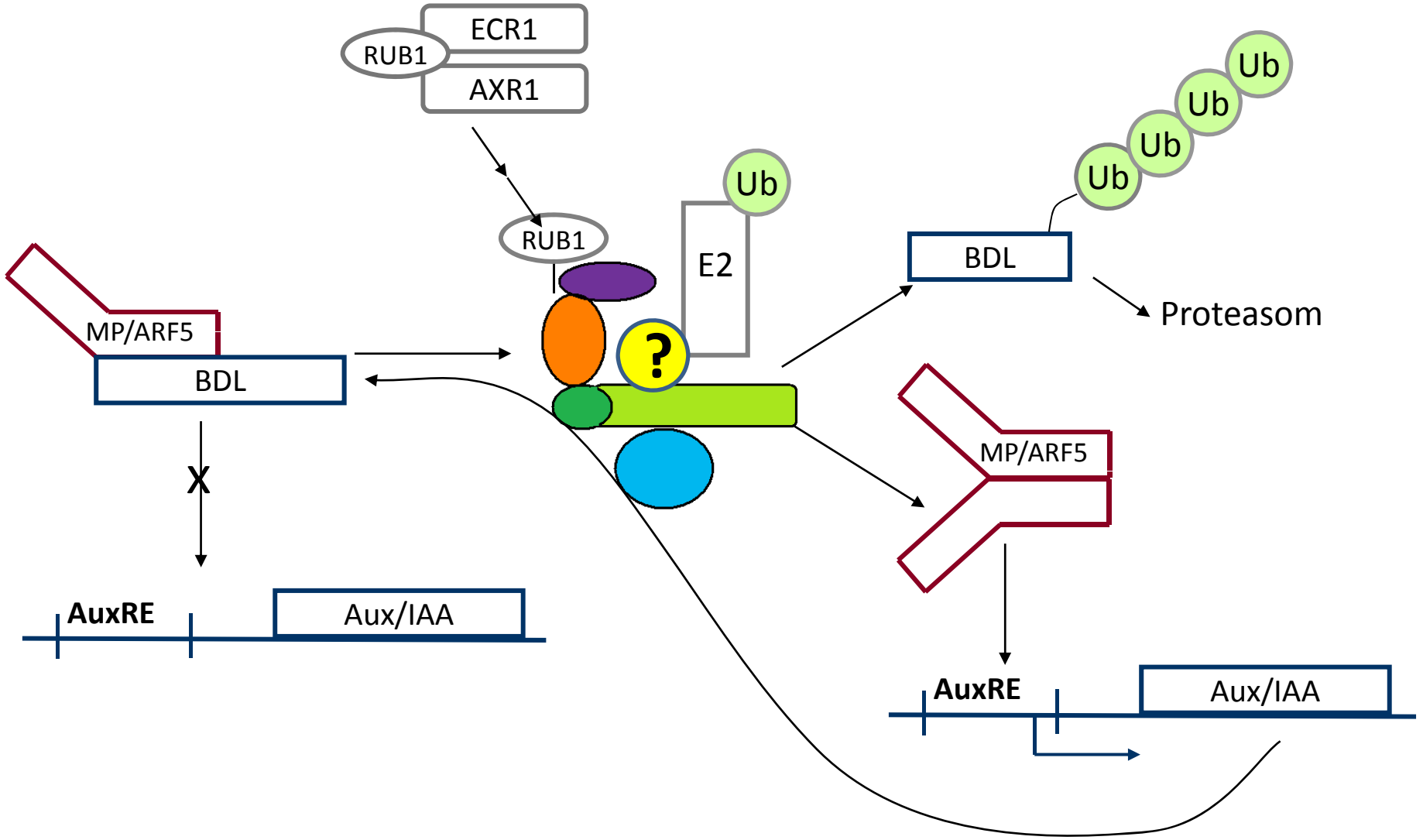
Zusammenfassung

1) AUX/ IAA binden direkt an TIR1
2) Auxin muss vorhanden sein -> Teil des Komplexes
3) Für die Bindung von AUX/IAA an TIR1 ist die Domäne II verantwortlich (nur 17 AS)

Möglichkeiten der Komplexbildung



Modell


ECR1
RUB1
AXR1
RUB1
Ub
E2
?
MP/ARF5
BDL
X
AuxRE
Aux/IAA
Ub
Ub
Ub
Ub
BDL
Proteasom
MP/ARF5
AuxRE
Aux/IAA

Offene Fragen

- Genau Wechselwirkungen zwischen SCF und AUX/IAA ?
- Gehen von dem SCF-Komplex alle Auxin-Antworten aus ?
- Wie bindet Auxin an TIR1? Durch Konformationsänderung?