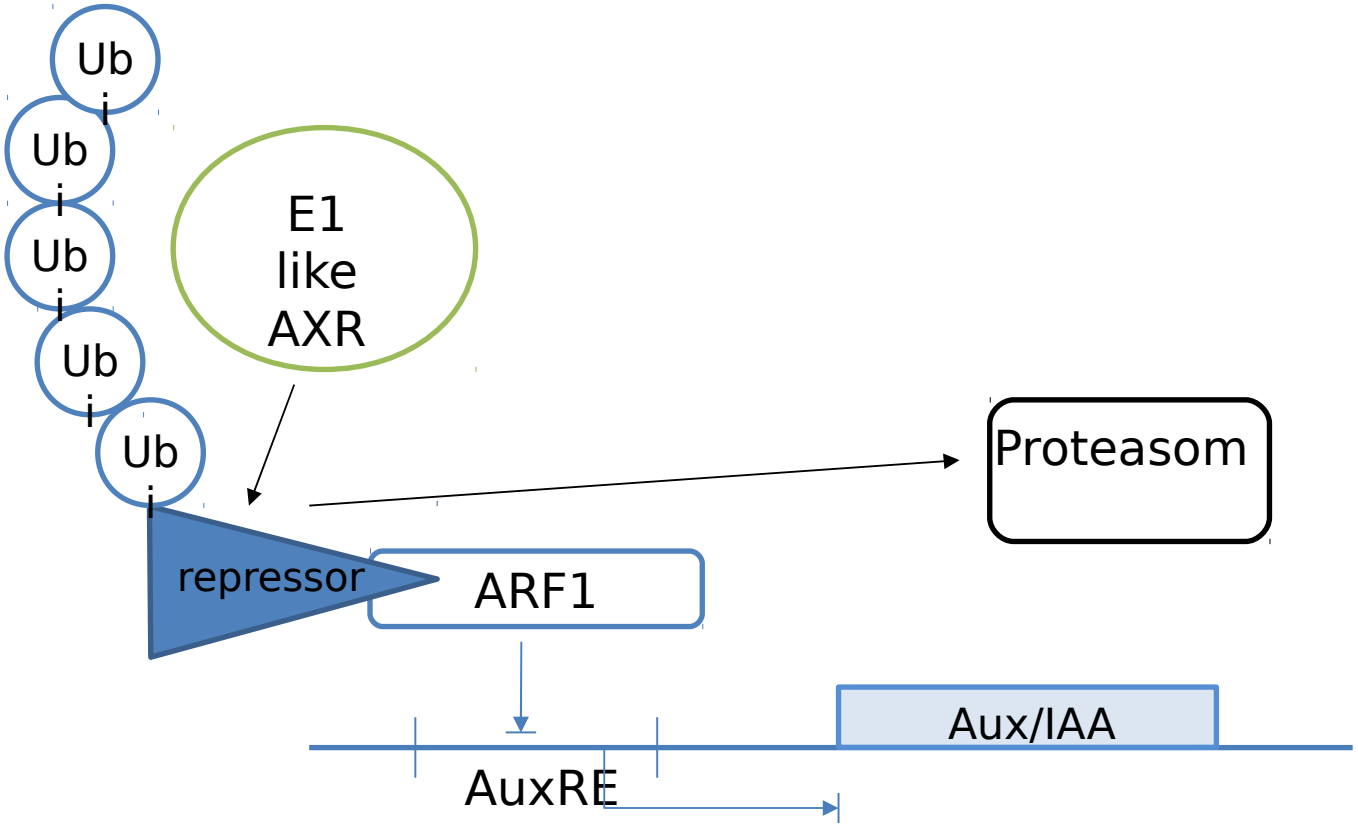
The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast Grr1p

Max Ruegger, Elizabeth Dewey, William M. Gray, Lawrence Hobbie, Jocelin Turner and Mark Estelle


Ub
i
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E1
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AXR
Proteasom
repressor
ARF1
Aux/IAA
AuxRE

Ziel:

Identification of a new gene called TRANSPORT INHIBITOR RESPONSE 1 (TIR1)

Table 1. *Segregation of CPD resistance in a* tir1-2 × *wild-type F_2 population*

F_2 root length (mm)	F_2 genotype[1]		
	tir1/tir1[2]	*tir1/+*[2]	+/+[2]
17	2		
16	5		
15	1		
14			
13			
12		1	
11			
10		6	
9		6	
8		7	6
7		3	4
6		2	4
5			
4		1	
3		1	
Totals	8	27	14

[1]Genotype determined by analyzing CPD resistance in F_3 plants.
[2]Number of seedlings.

Vergleich der Wurzellängen von homo- und heterozygoten Pflanzen auf CPD-Medium

Mutation wirkt semidominant

Table 2. *CPD resistance in* tir *homozygous and heterozygous plants*

Line	Root length (mm)	±S.E.	No.
+/+	7.8	0.1	32
tir1-1/tir1-1	15.7	0.3	33
tir1-3/tir1-3	15.8	0.4	34
tir1-7/tir1-7	13.0	0.5	33
tir1-1/+	10.9	0.3	15
tir1-3/+	12.5	0.3	17
tir1-7/+	12.6	0.2	17

Vergleich von Mutationen von unterschiedlichen Allelen

Bei allen Allelen ist die Semidominanz nachweisbar

Besteht die Resistenz auf Grund eines veränderten Transportwegs, oder tritt generell keine Auxinantwort auf??

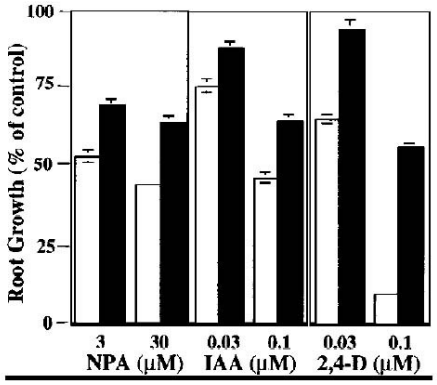


Figure 1. The roots of wild-type (open bars) and *tir1-1* (solid bars) seedlings are resistant to the growth-inhibiting properties of NPA, IAA, and 2,4-D. Seeds were germinated on nutrient medium. After 3 days, seedlings were transferred to media containing the indicated compound. Five days later, new root growth was measured and plotted as a percentage of root growth on medium without compound. Bars represent standard errors. Absence of bar indicates error less than thickness of line ($n = 12$).

Vergleich des Wurzelwachstums von Wildtyp mit Mutante

- Signifikatnte Unterschiede erkennbar

- Beim Mutant liegt ein genereller Defekt in der Auxinantwort vor

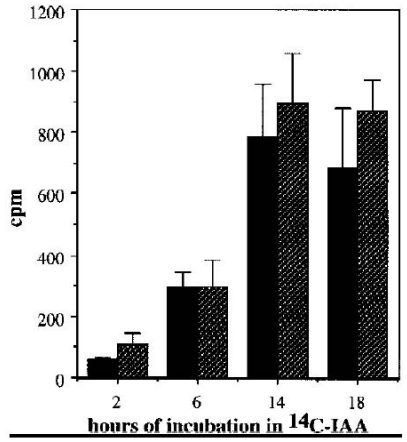
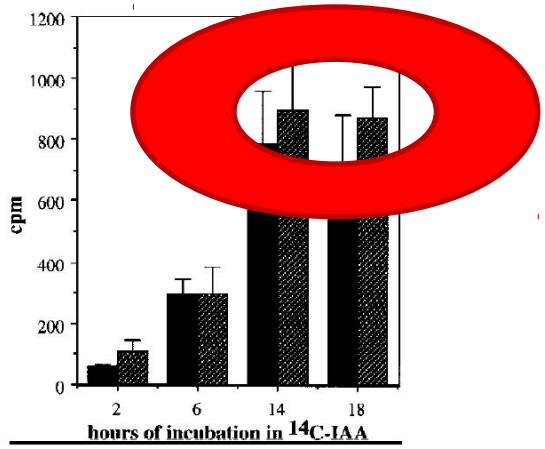


Figure 2. Polar auxin transport is similar in wild-type (solid bars) and *tir1-1* (hatched bars) inflorescence stems. Two and one-half centimeters of stem was excised, and the apical end was placed in a nutrient solution containing 1 μM [^{14}C]IAA for the times indicated. The amount of radioactive IAA transported to the basal end of the stem was assayed by liquid scintillation. Each column represents the mean of three replicates; the bar represents the standard error of the mean.

Vergleich des polaren Auxintransport von Wildtyp und Mutante

- Keine signifikanten Unterschiede erkennbar

- Es liegt ein genereller Defekt in der Auxinantwort vor



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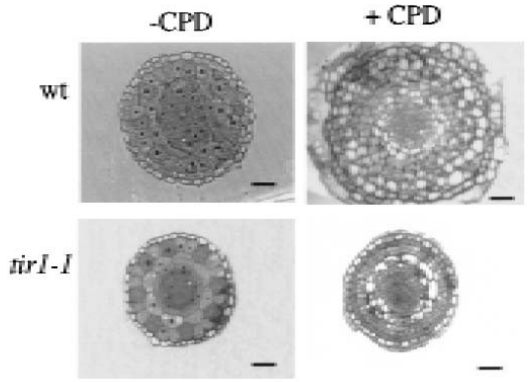


Figure 3. Cell proliferation in response to CPD is reduced in the roots of *tir1-1* seedlings. Seedlings were grown for 7 days on nutrient medium plus or minus 5 μM CPD, fixed, and embedded in Spurrs (Ruegger et al. 1997). Root cross sections 300 μm from the root tip are shown. Bars, 20 μm.

Vergleich von Zellteilungsraten in der Wurzel

- Bei unbehandelten Pflanzen kein Unterschied erkennbar
- Bei behandelten Pflanzen weißt Wildtyp vermehrte Zellzahlen auf

- *tir1-1* Mutante reagiert nicht auf Auxin als Zellteilungssingnal

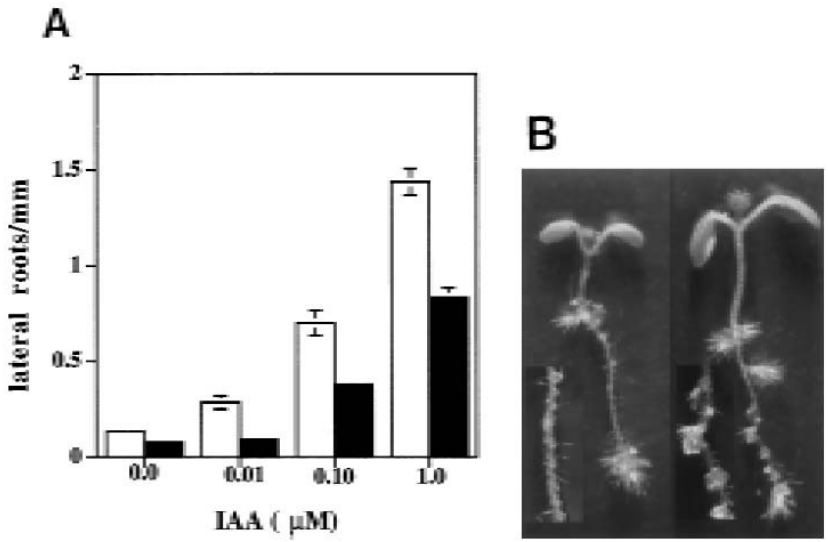


Figure 4. The *tir1-1* mutant is deficient in IAA-induction of lateral roots. (*A*) Eight-millimeter root segments were excised from 5-day-old wild-type (open bar) and *tir1-1* (solid bar) seedlings grown on nutrient medium and transferred to medium with IAA. Lateral roots were counted after 5 days by use of a dissecting microscope. Bars represent standard errors. Absence of bar indicates error less than thickness of line ($n = 10$). (*B*) Ten-day-old wild-type (*left*) and *tir1-1* (*right*) seedlings grown on 0.5 μM 2,4-D. (*Insets*) Higher magnification images of roots.

Vergleich von Seitenwurzelbildung bei Mutante und Wildtyp unter IAA-Einfluss

- Wildtyp bildet mehr Seitenwurzeln

- Mutante reagiert nicht auf Auxin als Zellteilungs- und Zelldifferenzierungssignal

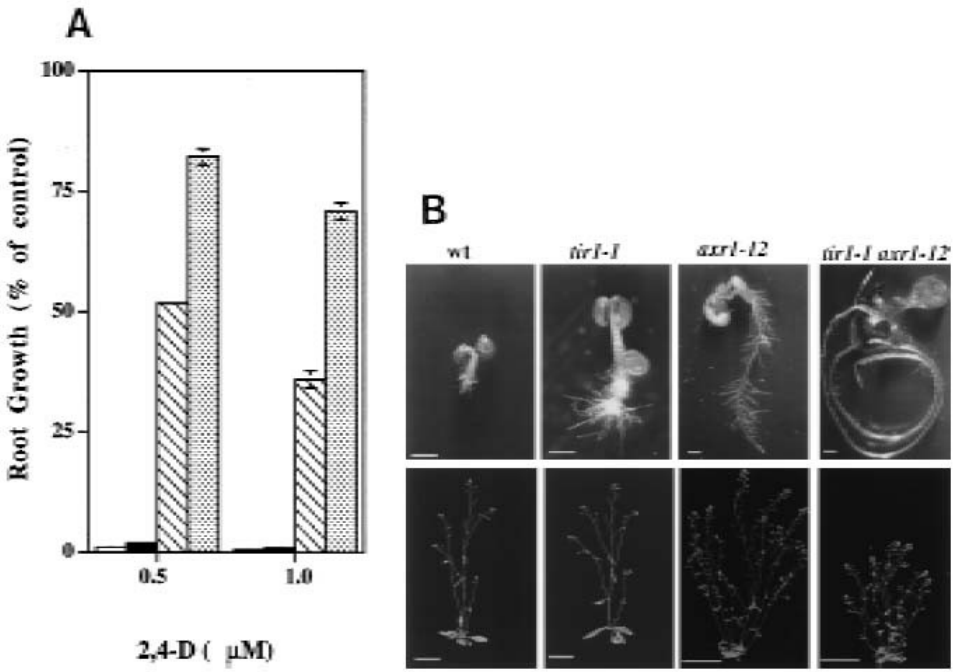
Figure 5. Hypocotyl elongation in response to elevated temperature is reduced in *tir1-1* seedlings compared with wild-type seedlings. Seedlings were grown on vertically oriented agar medium for 9 days at the two temperatures.

Vergleich von Hypocotyl-Elongation zwischen Wildtyp und Mutante

- Wildtyp zeigt mehr Hypocotyl-Elongation

- Mutante reagiert nicht auf Auxin als Zellstreckungssignal

Figure 6. *tir1-1* and *axr1-12* have synergistic effects on auxin response and plant morphology. (*A*) The *tir1-1* mutation decreased auxin response in an *axr1-12* background at a concentration at which *tir1-1* has no effect by itself. Seedlings were grown and treated as described for Fig. 1. (Open bars) Wild type; (solid bars) *tir1;* (hatched bars) *axr1;* (stippled bars) *axr1 tir1.* Bars represent standard error and absence of bar indicates error less than thickness of line. (*B*) Seedlings (*top* row) were photographed after 12 days of growth on 1 μM 2,4-D. Bar, 2.5 mm. Mature plants (*bottom* row) were photographed 35 days after germination in soil. Bar, 5 cm.



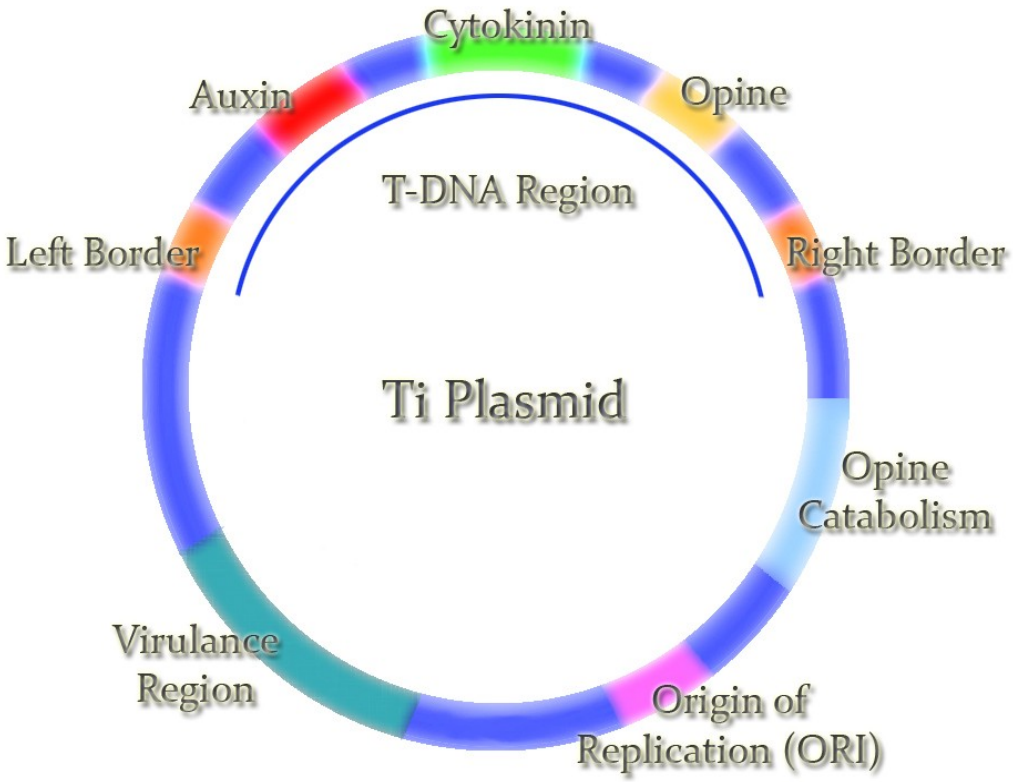
TIR1 ist am AXR1 vermittelten Prozess beteiligt

T-DNA

- stammen aus Agrobacterium

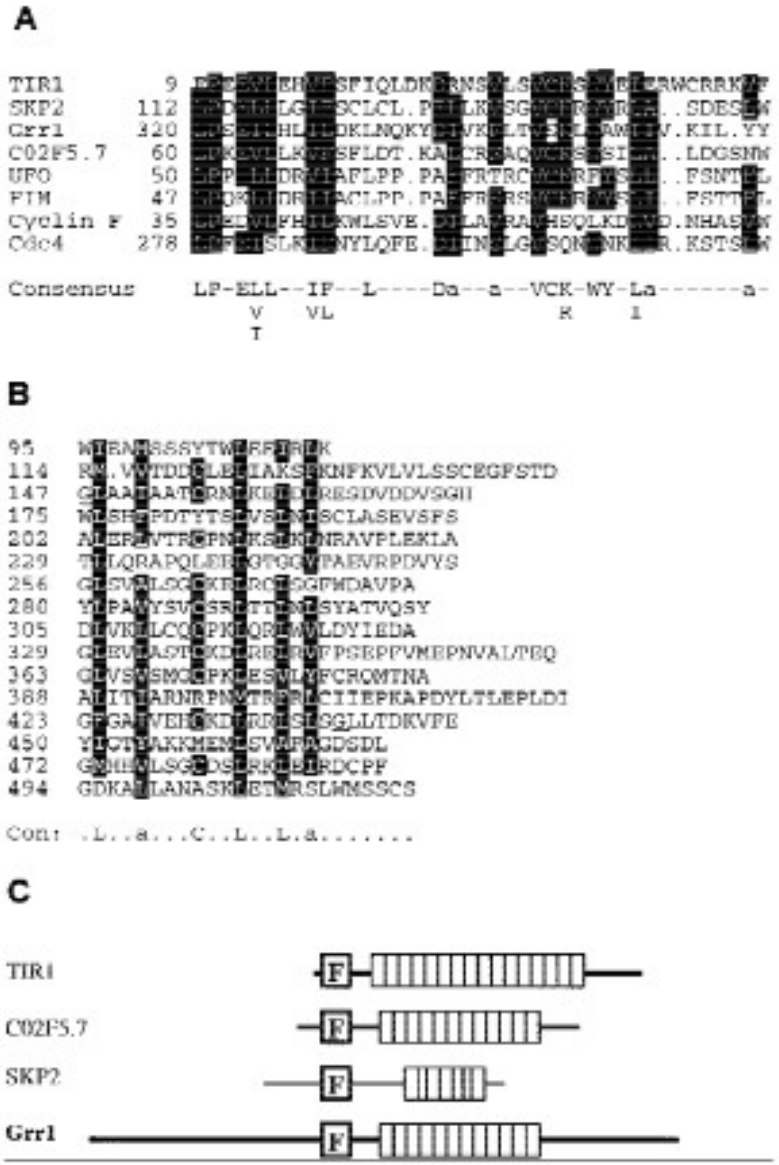
- führen zum knockout des Genes

- mittlerweile alle Insertions-stellen bekannt



Quelle: wikipedia.org

Ruegger et al.

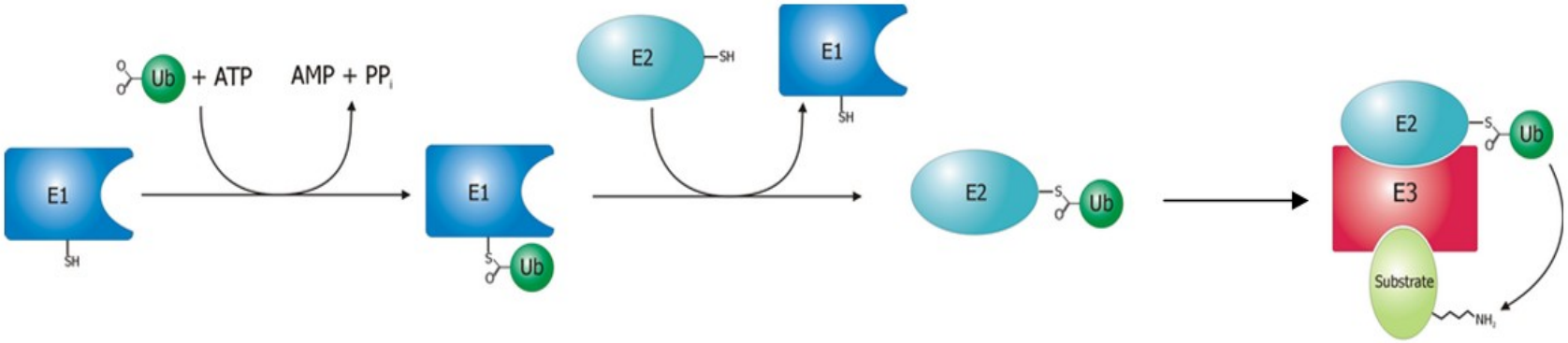


- F-Box und LRR (LEUCIN-RICH-REPEATS)

- Ähnlichkeiten zu Grrp1 (Hefe), SKP2 (Mensch)

- F-Box und LRR bilden Protein-Protein-Interaktionen

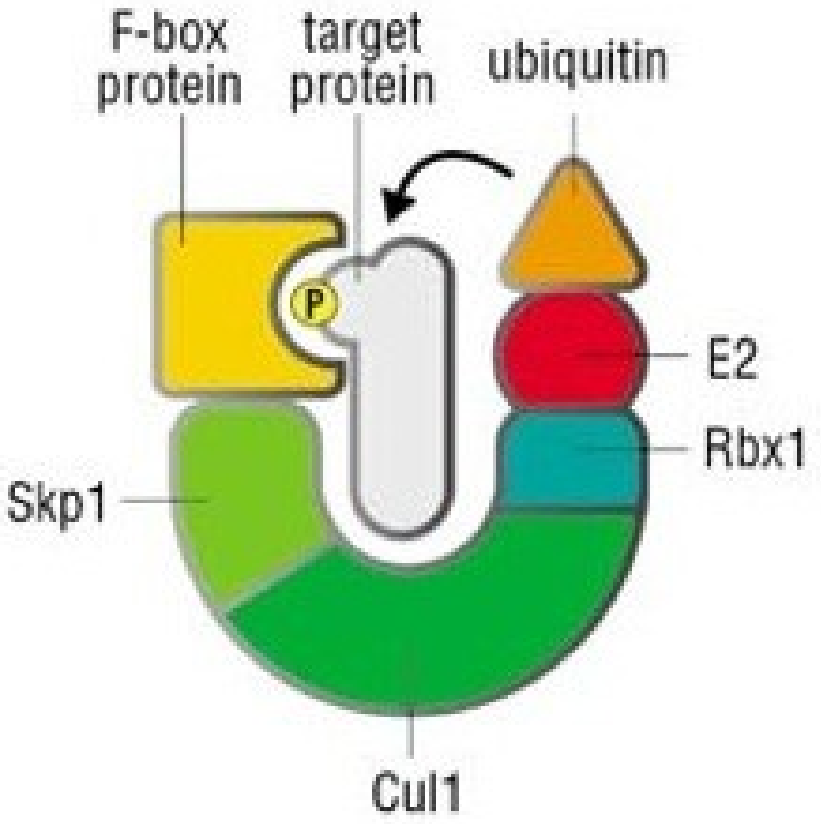
Übersicht Ubiquitinierung



- schon bekannt beim Menschen

SCF Komplex

- **S**kp, **C**ullin, **F-box** containing complex



Hypothese

TIR1 ist Teil der Ubiquitin-protein Ligase (E3) und bewirkt Transfer von Ubiquitin auf Zielprotein.