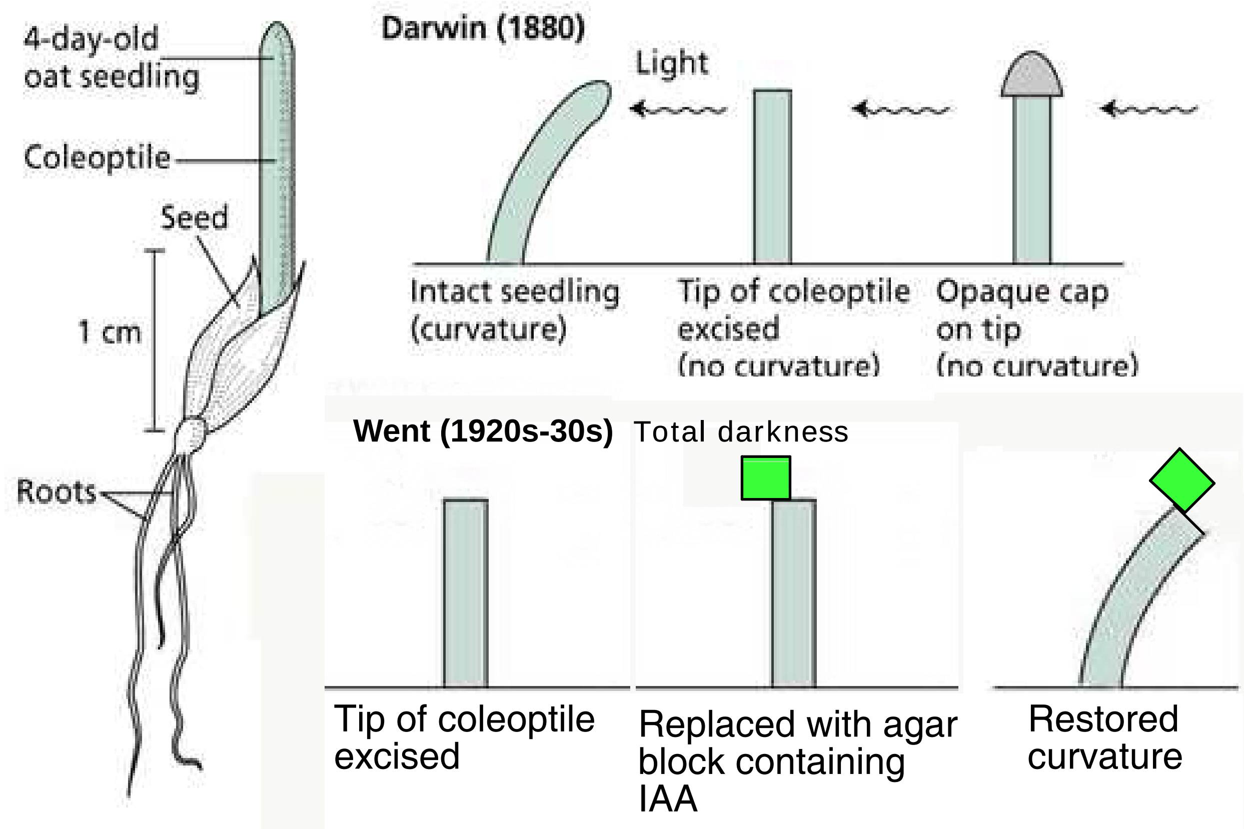
Seminar
Molekulare Mechanismen der Signaltransduktion

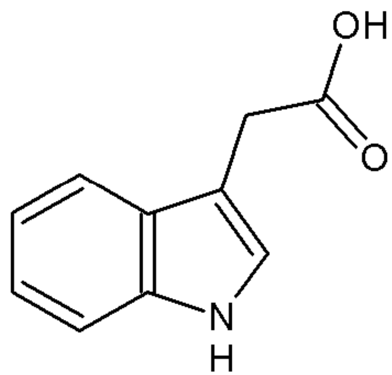
15.04.08 – MQ

1. Estelle and Somerville, (**1987**) Auxin resistant mutants of *Arabidopsis thaliana* with an altered morphology. **MGG** 206:200
2. Lincoln et al., (**1990**) Growth and development of the axr1 mutants of Arabidopsis. **PC** 2:1071
3. Leyser et al., (**1993**) Arabidopsis auxin-resistance gene AXR1 encodes a protein related to ubiquitin-activating enzyme E1. **N** 364:161

Auxin - history and pioneering experiments



"When seedlings are freely exposed to a lateral light some influence is transmitted from the upper part of the coleoptile that acts on the lower part of the coleoptile"
"*The Power of Movement in Plants*" (1880) by Darwin and Darwin.



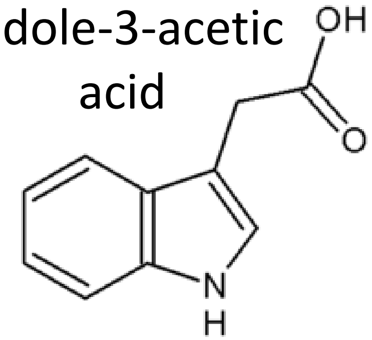
IAA

indole-3-**a**cetic **a**cid

- *auxein* (greek) = to grow
- first phytohormone to be identified

Auxin regulates plant development

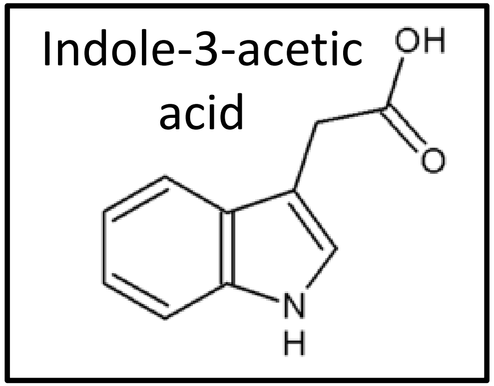
Indole-3-acetic acid



- Embryonic patterning
- Growth & Apical dominance
- Root development
- Tropic growth responses

wild-type   *bdl axr1* mutant

Auxin regulates plant development



Embryonic patterning

⇨ Growth & Apical dominance

Root development

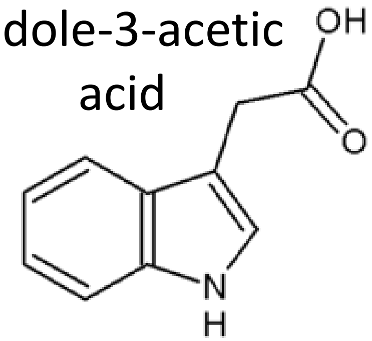
Tropic growth responses

wild-type

axr6-3 mutant

Auxin regulates plant development

Indole-3-acetic acid



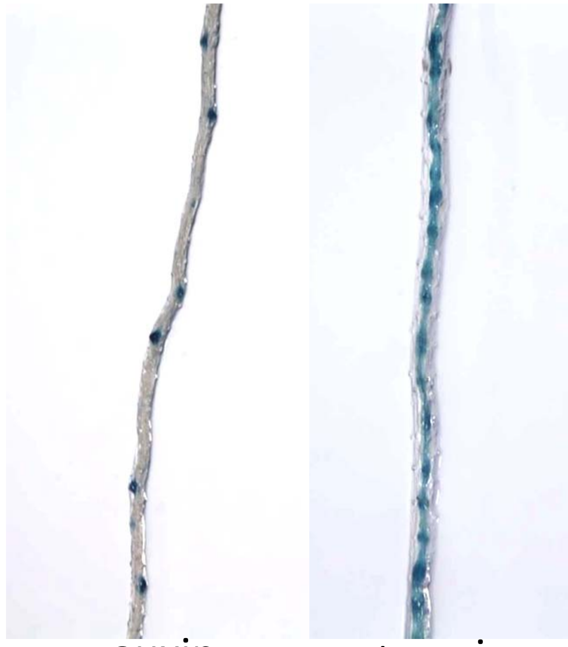
Embryonic patterning

Growth & Apical dominance

⇨ Root development

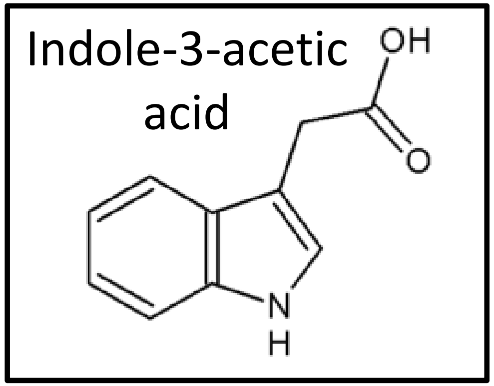
Tropic growth responses

cycB1::GUS



- auxin + auxin

Auxin regulates plant development

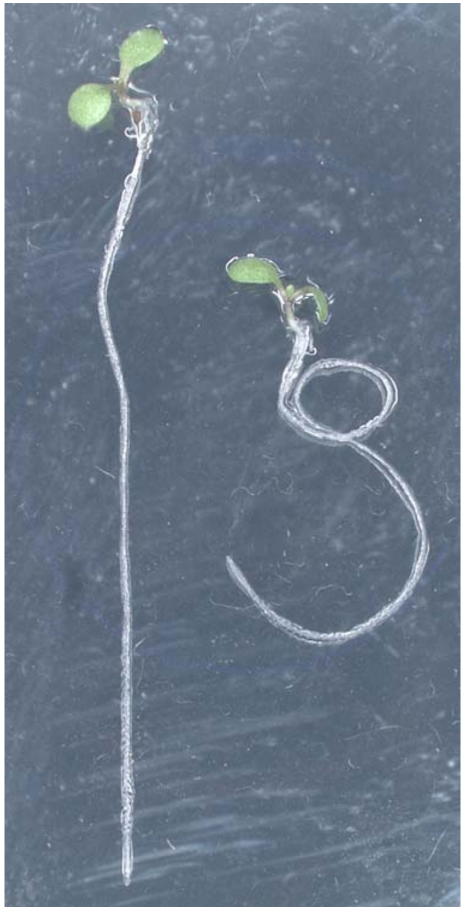


Embryonic patterning

Growth & Apical dominance

Root development

⇨ Tropic growth responses



wild-type *aux1* mutant

Arabidopsis thaliana

Small size	(30 cm)
Rapid life cycle	(6 weeks)
Prolific seed production	(5000 seeds/plant)
Sequenced genome	(125 Mb; ~26,000 genes)
Easily transformable	
Tremendous community resources	

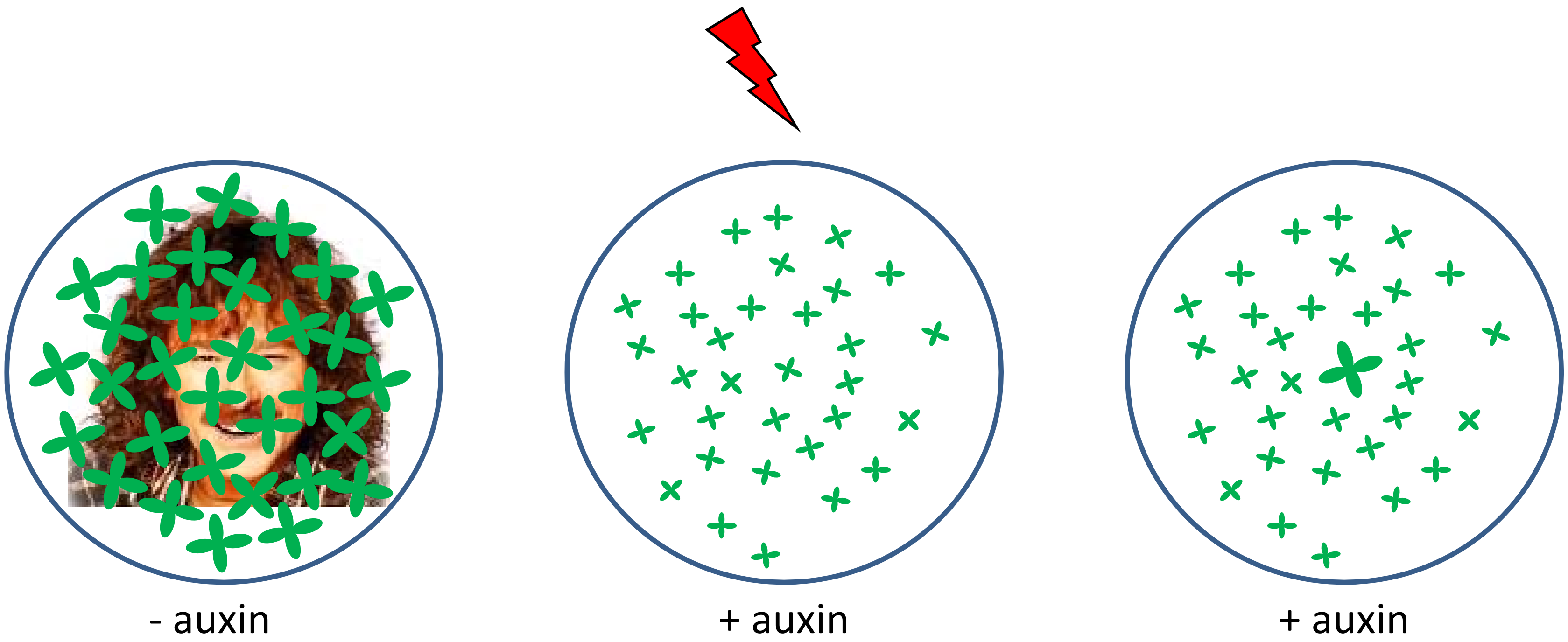
A power multicellular eukaryotic model system

Mol Gen Genet (1987) 206:200–206

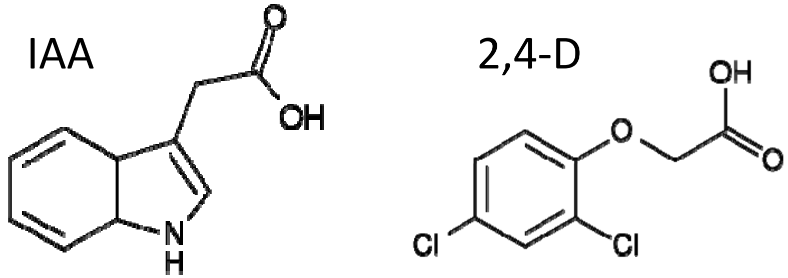
MGG

Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology

Mark A. Estelle* and Chris Somerville
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA


- auxin
+ auxin
+ auxin

IAA 2,4-D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OH
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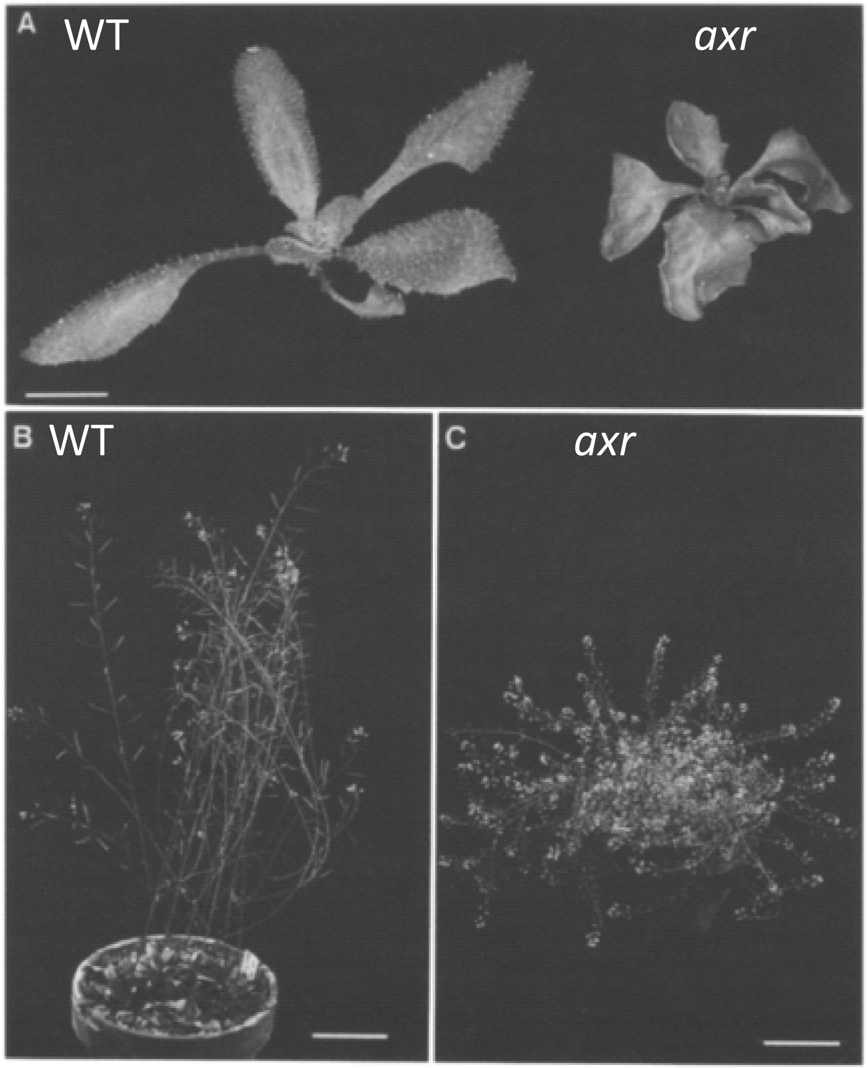
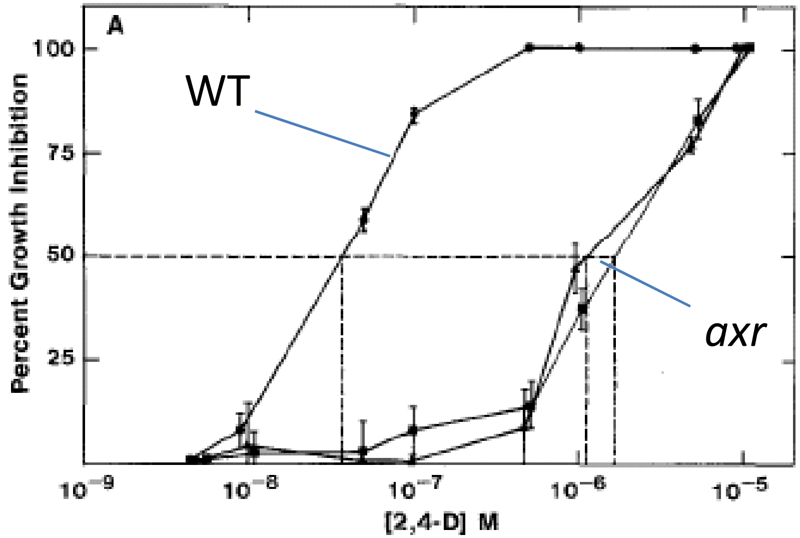
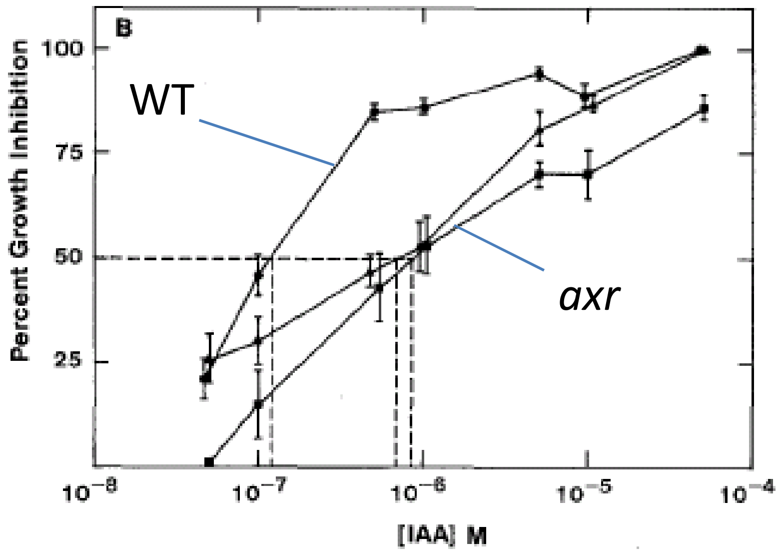
Cl
Cl


A
WT
axr
B
WT
C
axr


A
100
75
50
25
Percent Growth Inhibition
WT
axr
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
[2,4-D] M


B
100
75
50
25
Percent Growth Inhibition
WT
axr
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
[IAA] M

The Plant Cell, Vol. 2, 1071–1080, November 1990

Growth and Development of the *axr1* Mutants of *Arabidopsis*

Cynthia Lincoln, James H. Britton, and Mark Estelle[1]
Department of Biology, Indiana University, Bloomington, Indiana 47405

Table 1. Recovery of *axr1* Mutants[a]

M2 population	Mutagen	Selection	Mutants recovered
A[b]	EMS	2,4-D	*axr1-1*
			axr1-2
			axr1-3
			axr1-4
			axr1-5
			axr1-6
B[b]	EMS	2,4-D	*axr1-7*
			axr1-8
			axr1-9
			axr1-11
			axr1-12
			axr1-15
C[c]	EMS	2,4-D	*axr1-16*
			axr1-17
			axr1-18
			axr1-19
			axr1-20
			axr1-21
C[c]	EMS	IAA	*axr1-22*
D[c]	γ	2,4-D	*axr1-23*

[a] A total of 470,000 seeds from four distinct M2 populations was screened for mutants that were able to elongate roots on either 5 μM 2,4-D or 50 μM IAA.
[b] Estelle and Somerville (1987).
[c] This study.

axr1 **x** **WT**

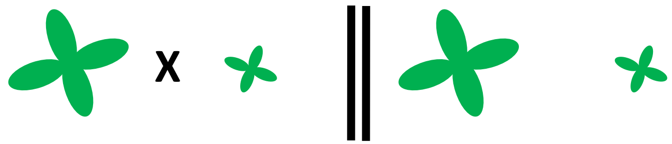
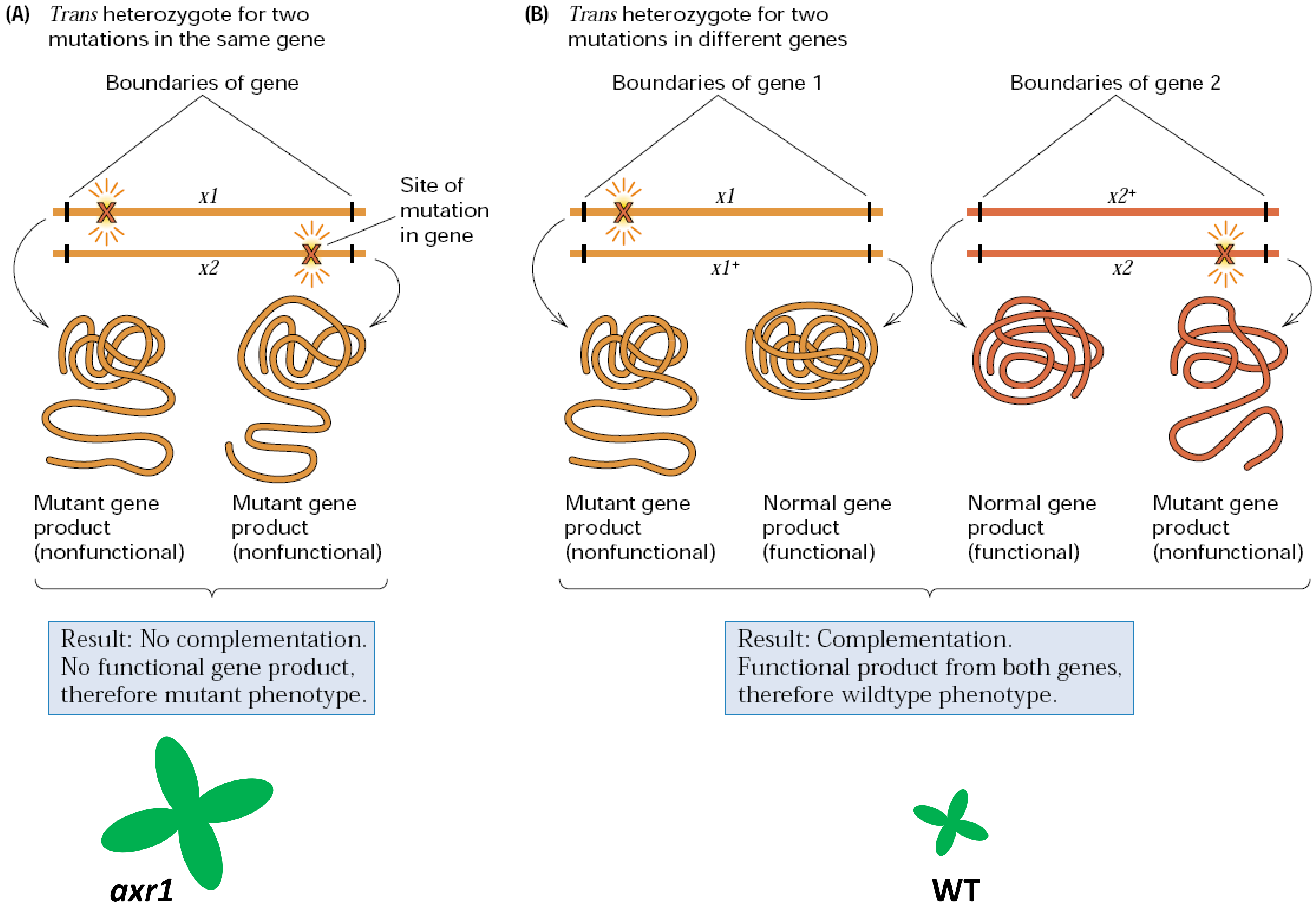


Table 2. Genetic Segregation of 2,4-D Resistance in *axr1* Lines

Cross		Number of Plants		
		Resistant	Sensitive	χ^2 [a]
axr1-19 × wild-type	F1	0	23	
	F2	186	493	2.07[b]
axr1-21 × wild-type	F1	0	51	
	F2	82	281	1.12[b]
axr1-22 × wild-type	F1	0	22	
	F2	56	216	2.83[b]
axr1-23 × wild-type	F1	0	33	
	F2	117	383	0.683[b]

[a] χ^2 was calculated based on an expected ratio of three sensitive to one resistant.
[b] $P > 0.05$.

Mutationen allelisch oder in verschiedenen Genen?



Mutationen allelisch oder in verschiedenen Genen?
→ Komplementationstest auf Auxin

axr1 **WT**

axr1-12/axr1-12 ***axr1-3/axr1-3***

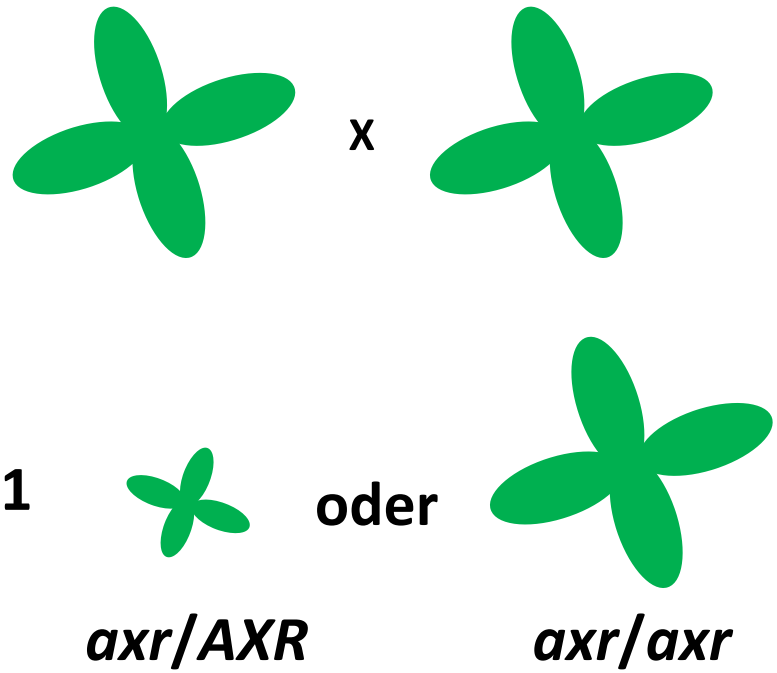


Table 3. Complementation Analysis of *axr1* Lines

Cross	Number of Plants: Resistant	Number of Plants: Sensitive
axr1-12 × *axr1-3*	33	0
axr1-19 × *axr1-3*	21	0
axr1-20 × *axr1-3*	13	0
axr1-22 × *axr1-3*	24	0
axr1-23 × *axr1-3*	39	0

→ Mutationen sind verschiedene Allele imselben Gen!

Morphologie:

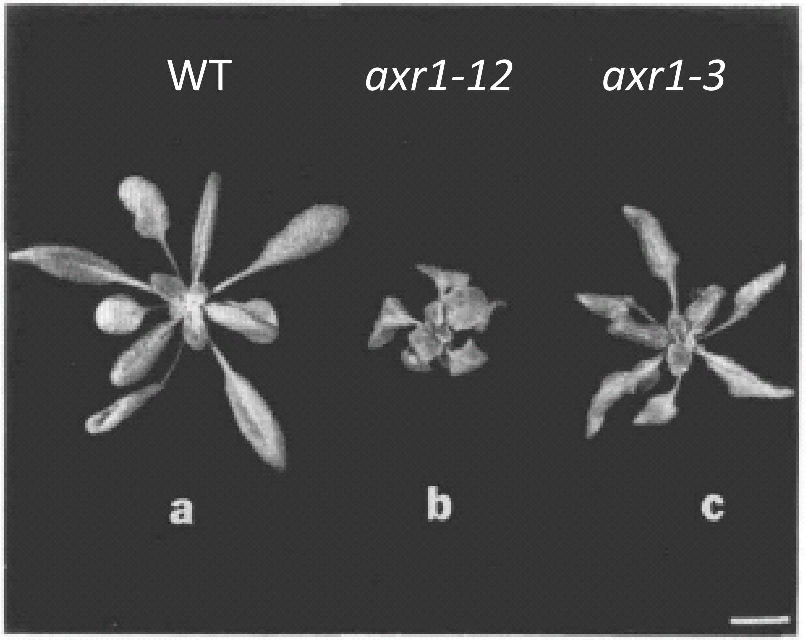


Figure 1. Phenotype of Wild-Type and Mutant Rosettes.

Rosettes were photographed when the plants were 3 weeks old.
(a) Wild type.
(b) *axr1-12/axr1-12*.
(c) *axr1-3/axr1-3*.
Bar = 1 cm.

Figure 2. Comparison of Mature Wild-Type and Mutant Plants.

Wild-type and mutant plants were photographed when 7 weeks old.
(a) Wild type.
(b) *axr1-12/axr-12*.
(c) *axr1-3/axr1-3*.
Bar = 3 cm.

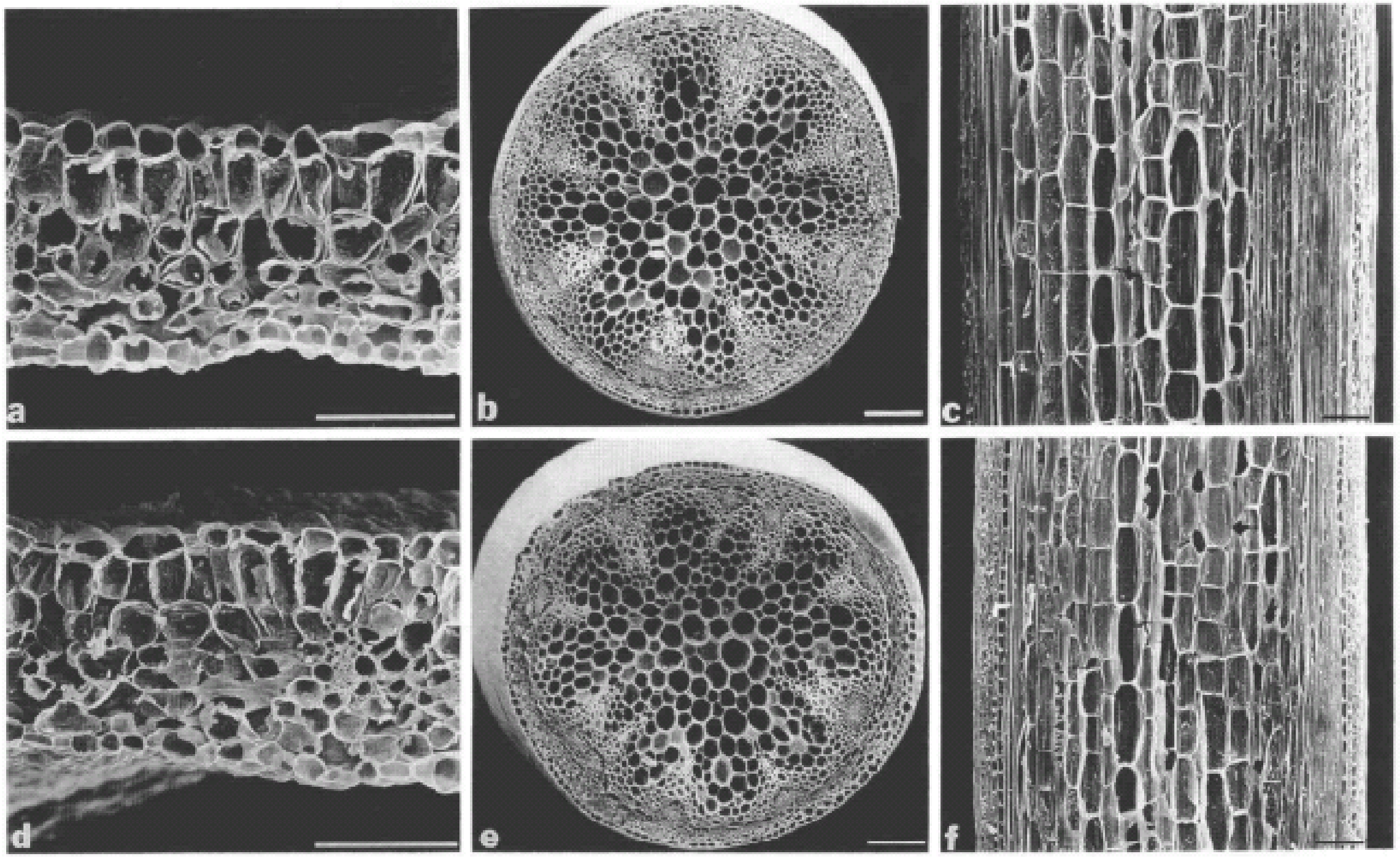
→ Allele zeigen unterschiedliche Ausprägung morphologischer Defekte

Quantifizierung morphologischer Unterschiede:

Table 4. Morphology of Wild-Type and Mutant Plants

	Wild Type	*axr1-3*	*axr1-12*
Height (cm)	50.9 ± 1.0	38.4 ± 1.3	19.2 ± 1.8
No. of inflorescences	5.75 ± 0.25	6.25 ± 1.4	6.6 ± 1.6
No. of lateral branches	46.2 ± 4.5	96.7 ± 21.4	124.0 ± 24.4
Distance between siliques (cm)	0.65 ± 0.04	0.30 ± 0.01	0.32 ± 0.02
No. of siliques	673.75 ± 68.0	526.5 ± 56.0	2.4 ± 1.3
No. of pollen grains/flower	2035.0 ± 500.0	2700.0 ± 337.0	680.0 ± 32.0
Hypocotyl length in etiolated seedlings (cm)	1.40 ± 0.04	1.20 ± 0.05	0.77 ± 0.03

SEM → morphologische Defekte durch unterschiedliche Zellgrößen oder Gewebeorganisation?



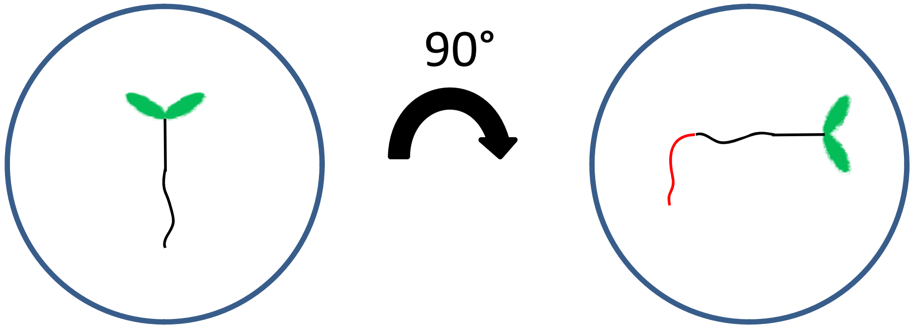
WT

axr1-12

→ keine wesentlichen strukturellen Defekte
→ vaskuläre Strukturen etwas weniger differenziert
→ Zellgrößen in etwa gleich

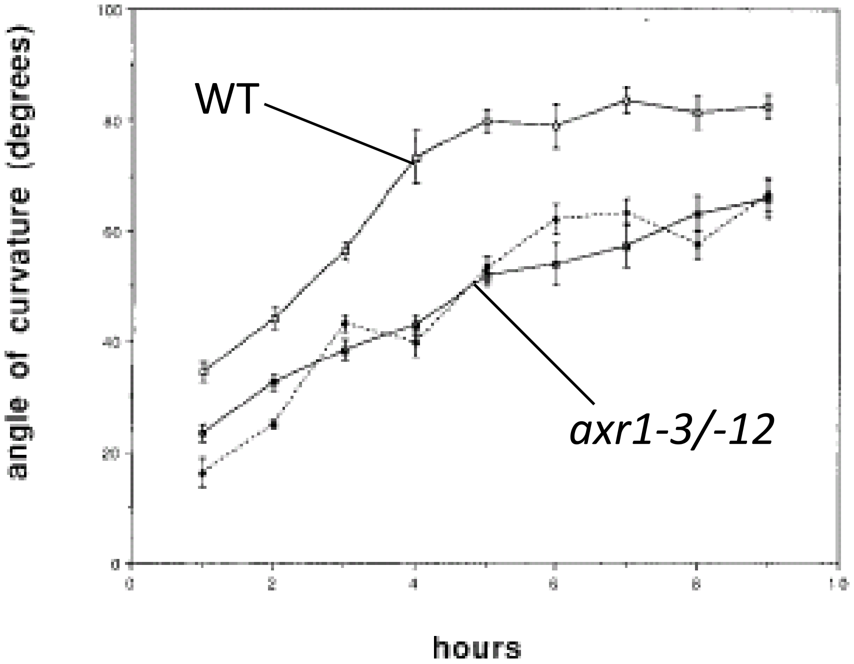
Auxin Response in der Wurzel:

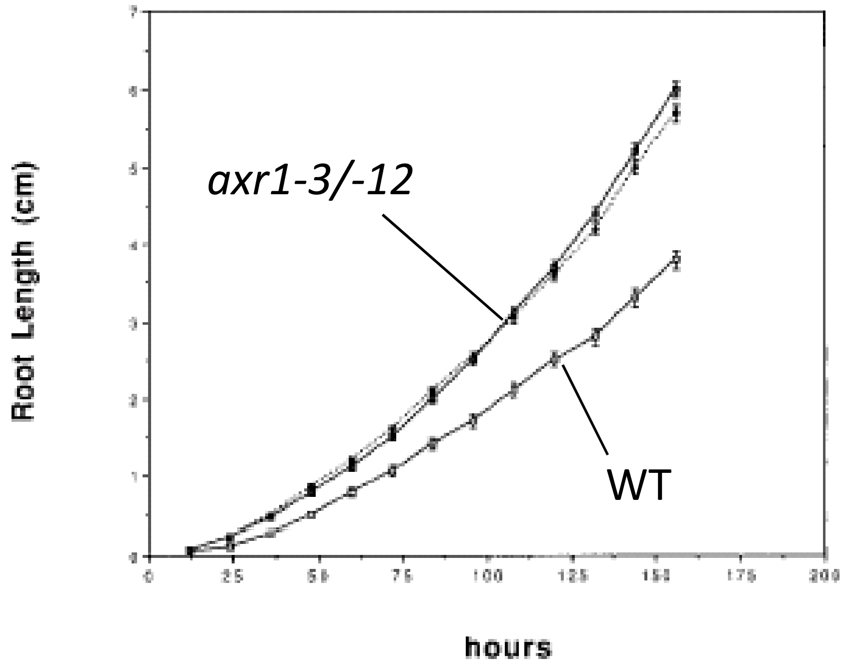
1. Gravitropismus



→Graviresponse in den Mutanten langsamer

→ nicht durch reduziertes Wurzel-wachstum!

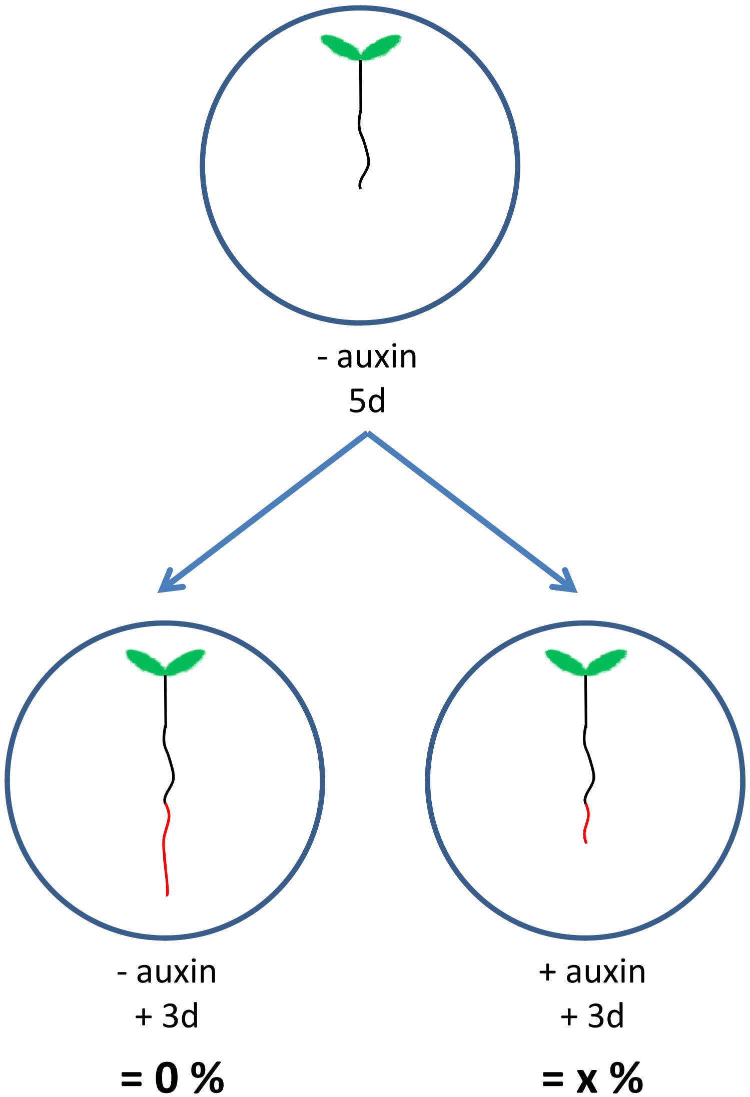


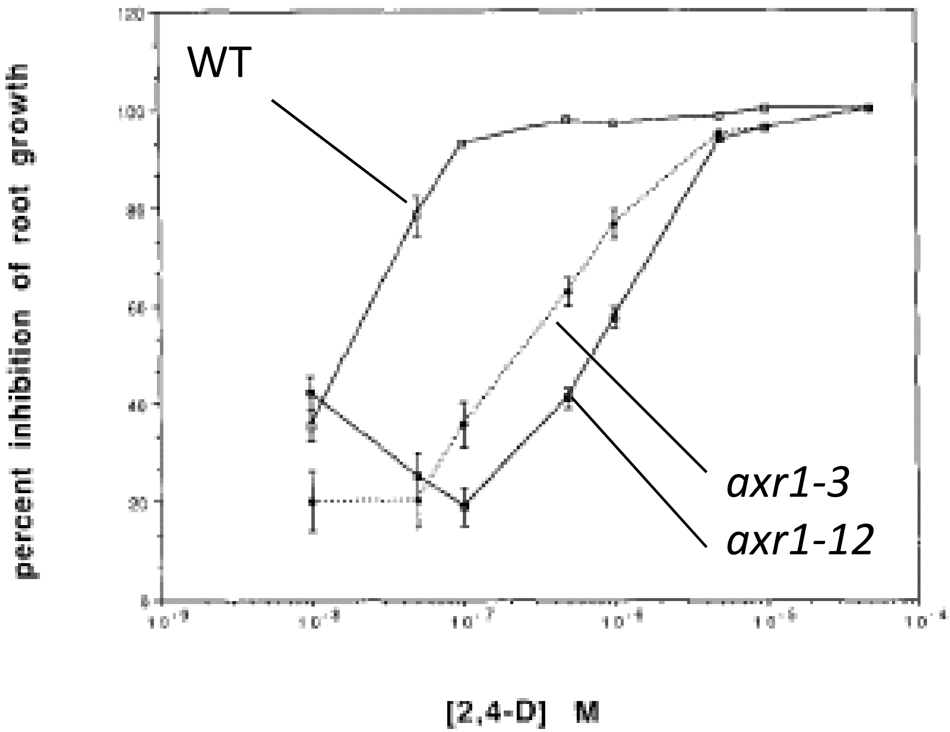


Auxin Response in der Wurzel:

2. Wurzelelongation auf Auxin

→klassischer Auxin Response Defekt





Quantifizierung des Wurzelassays:

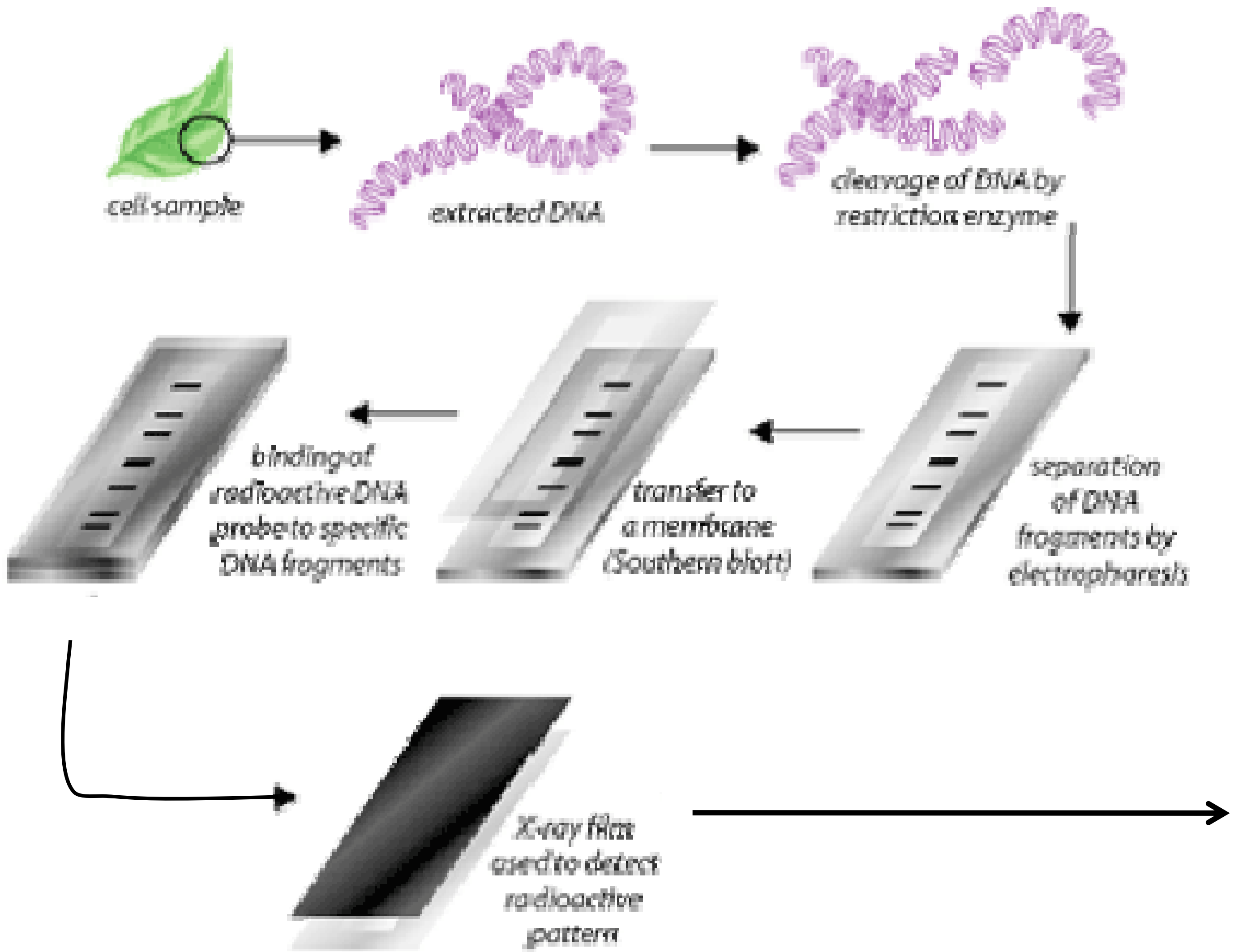
Table 5. Effect of 2,4-D on Rosette Growth of Wild-Type and Mutant Plants

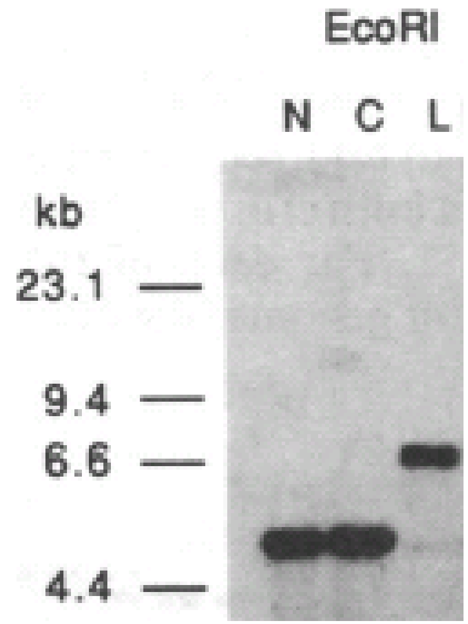
	0 μM 2,4-D		10 μM 2,4-D		100 μM 2,4-D	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Wild type	59.5 ± 2.9	67.8 ± 2.8	46.0 ± 2.5 (23%)	44.5 ± 2.1 (34%)	19.6 ± 0.74 (67%)	21.4 ± 1.0 (68%)
axr1-3	45.2 ± 3.1	54.7 ± 3.0	50.3 ± 2.8	59.7 ± 2.7	26.3 ± 1.5 (42%)	26.5 ± 1.5 (52%)
axr1-12	49.6 ± 2.5	55.5 ± 2.9	47.1 ± 2.3 (5%)	47.3 ± 3.1 (15%)	33.8 ± 2.0 (32%)	38.0 ± 1.8 (32%)

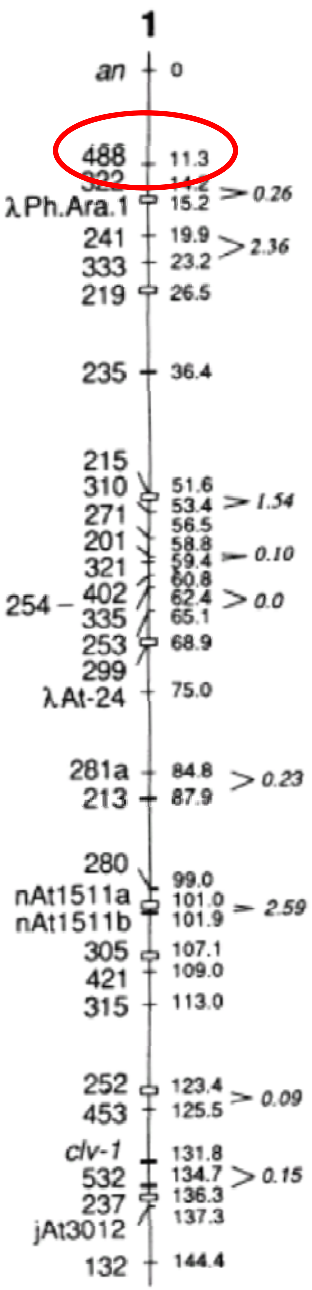
Values are fresh weight in milligrams ± SE. Percentages in parentheses are percent inhibition of growth.

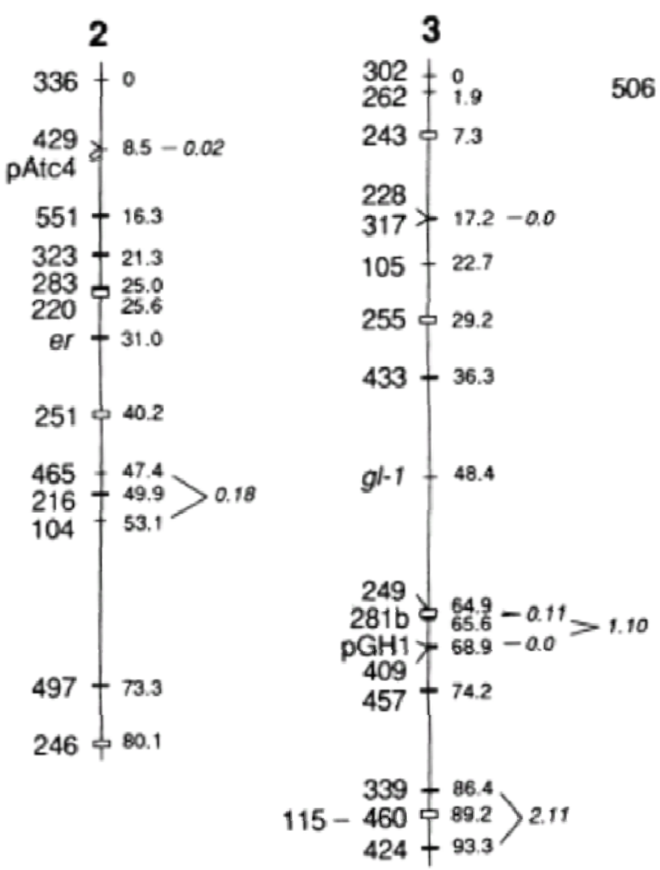
Genetische Kartierung von *AXR1*:

<u>RFLP – Restriktionsfragment-Längenpolymorphismus</u>









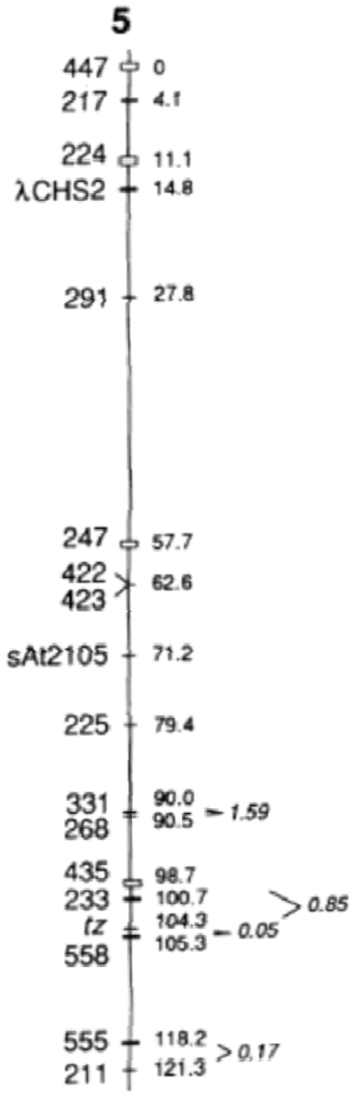


Table 6. Linkage Analysis between the *AXR1* Gene and RFLP Markers on Chromosome 1

Markers	Recombination Frequency (%)	Number Scored[a]	χ^2 Associated[b]	P
RFLP 219	12.4 ± 2.0	136	123.3	<0.05[c]
RFLP 253	47.8 ± 6.3	63	5.6	0.231
RFLP 488	2.6 ± 1.0	99	174.0	<0.05
phyA1	7.1 ± 2.0	102	135.0	<0.05

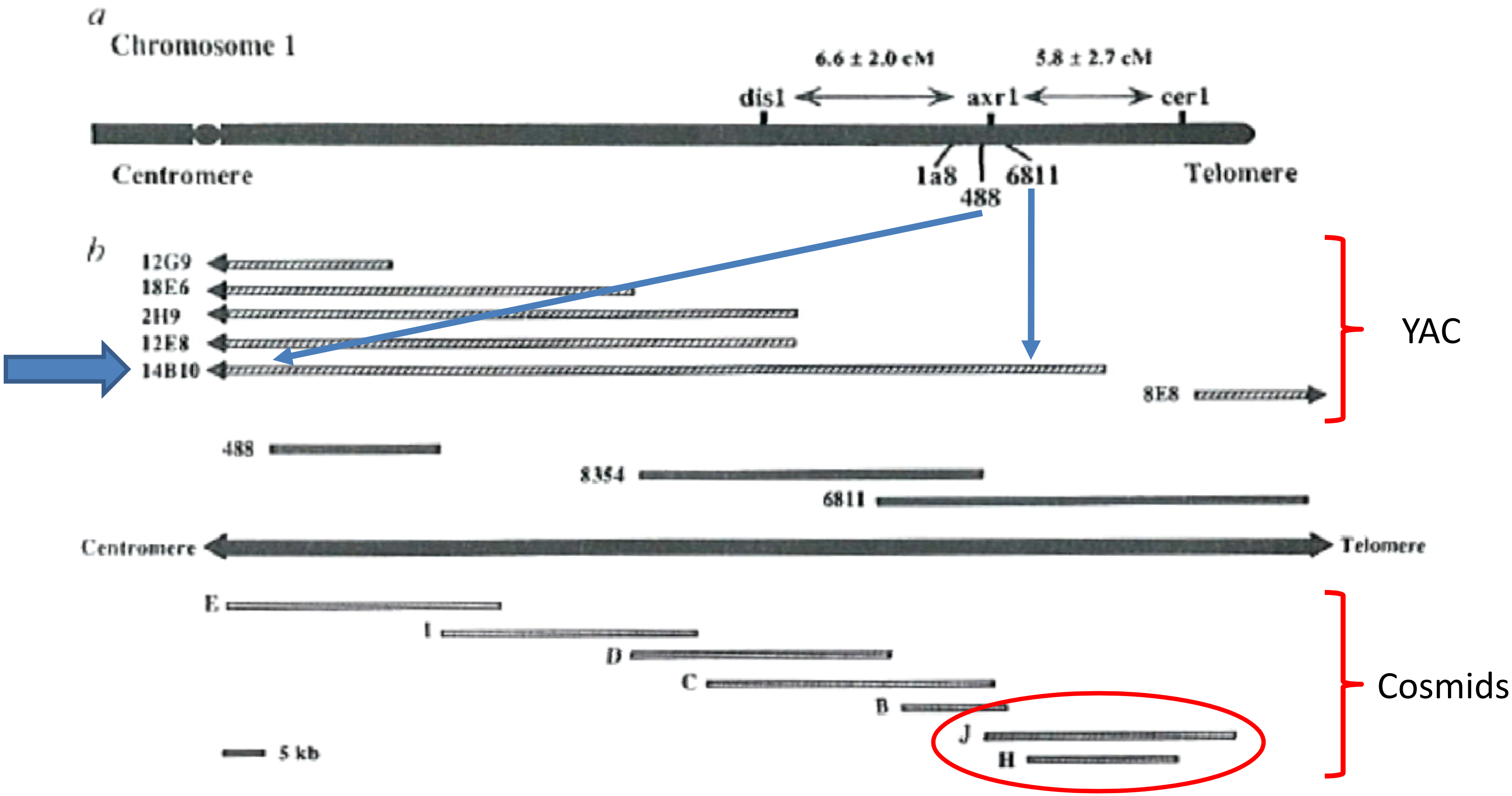
[a] Refers to number of F3 families scored.
[b] χ^2 associated is the total χ^2 adjusted for deviations of each individual marker from Mendelian segregation.
[c] P value < 0.05 indicates deviation from nonlinkage (i.e., linkage).

Arabidopsis auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1

H. M. Ottoline Leyser, Cynthia A. Lincoln*, Candace Timpte, Douglas Lammer, Jocelyn Turner & Mark Estelle†

Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

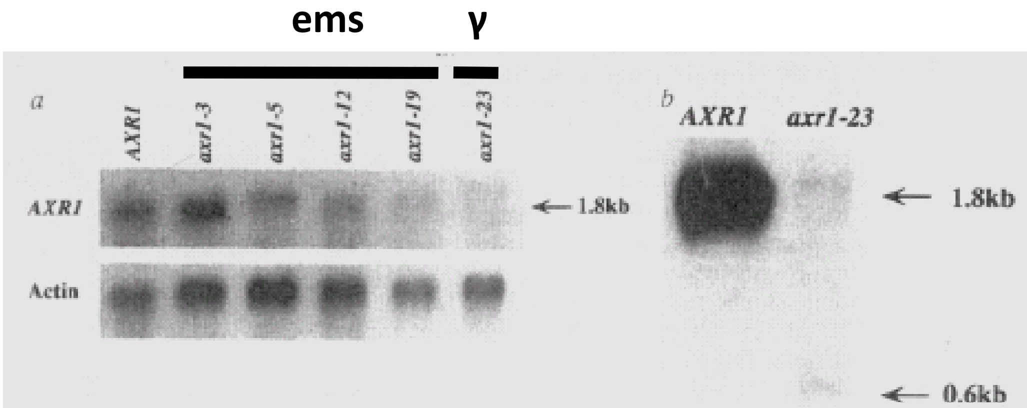
AXR1 - Chromosome Walking



→ Cosmid pOCA18-H enthält das *AXR1* Gen

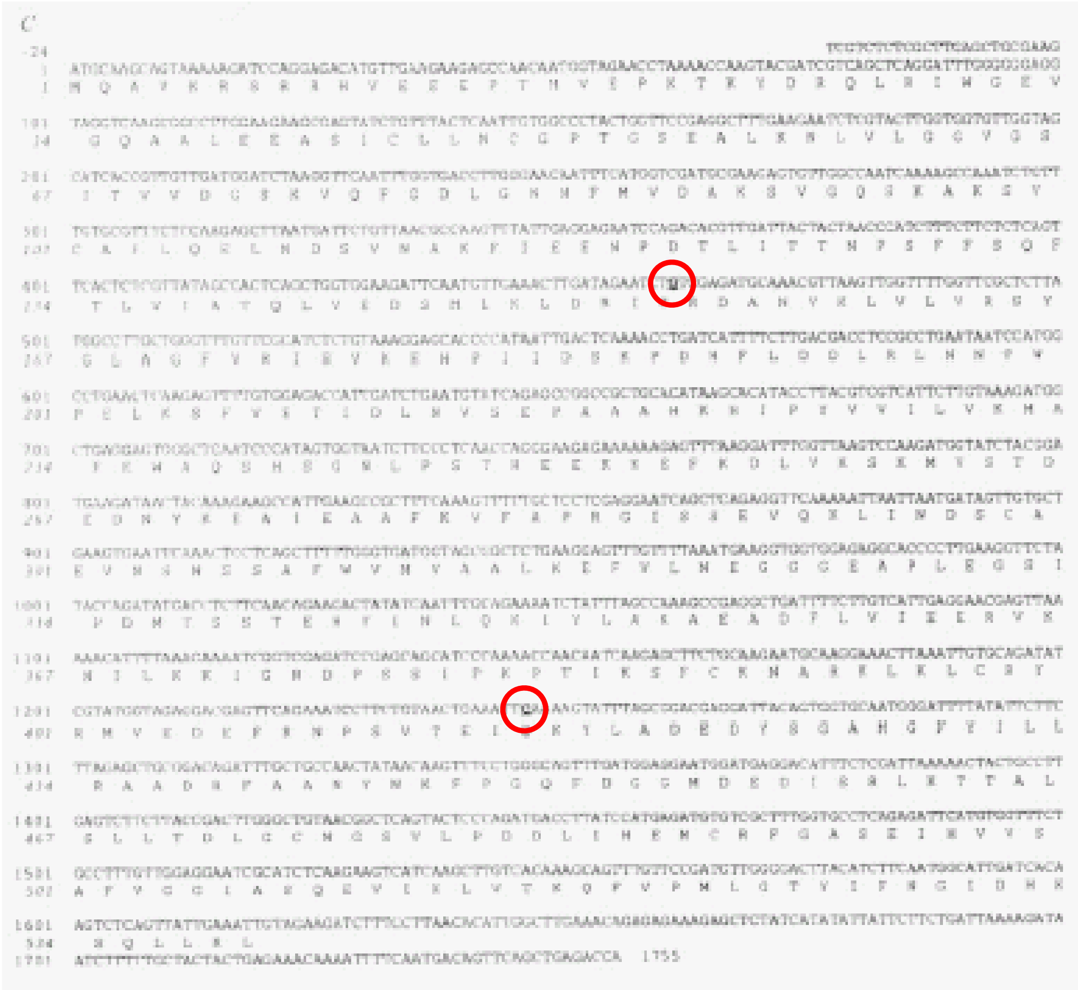
cDNA Bibliothek Screen mit pOCA18-H

2 cDNAs identifiziert → 0.8 und 1.8 kb



1.8 kb cDNA abwesend in *axr1-23*!

AXR1 Intron-Exon Struktur:



axr1-3 = G461A

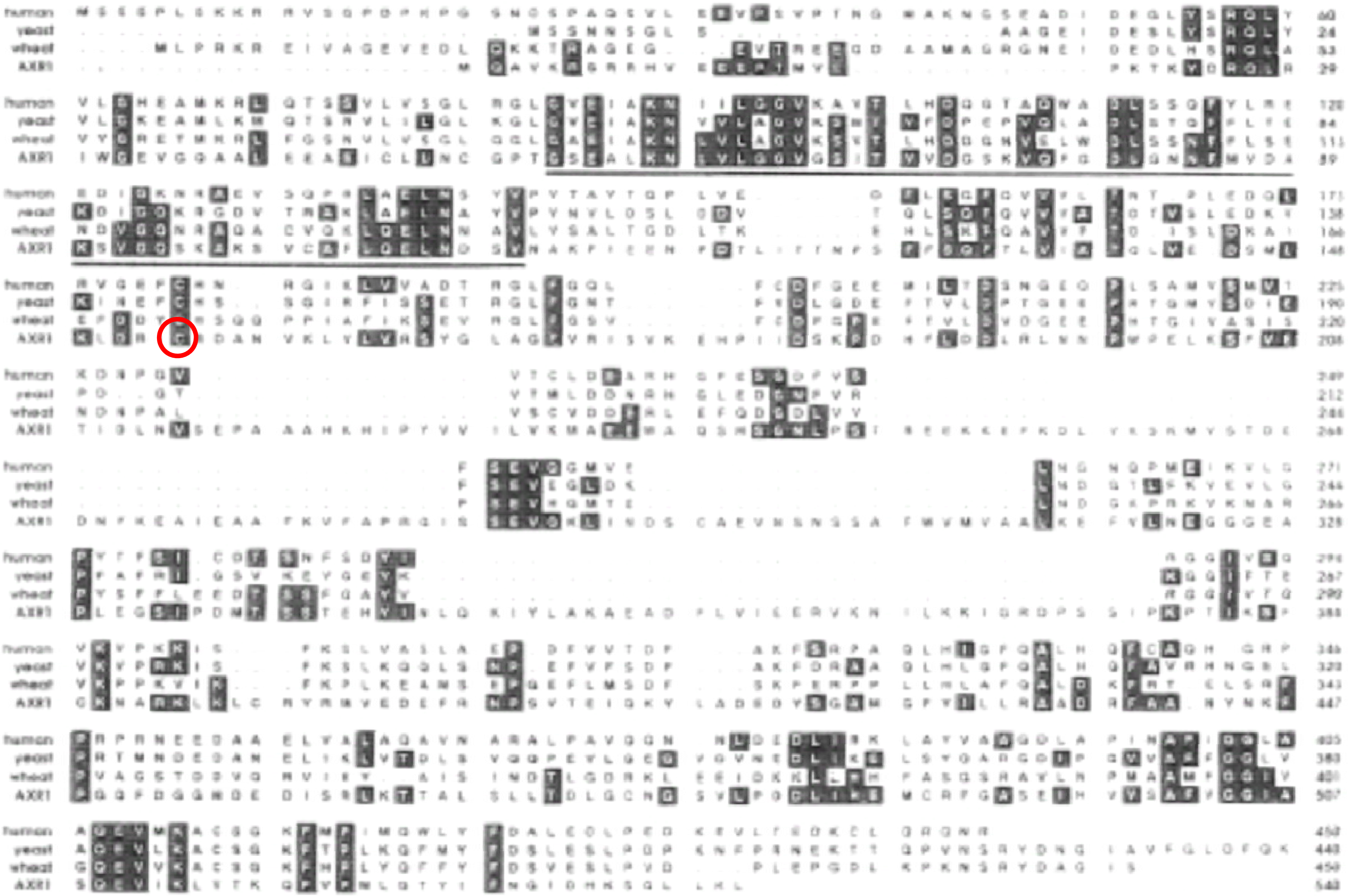
Cystein → Tyrosin

axr1-12 = C1246T

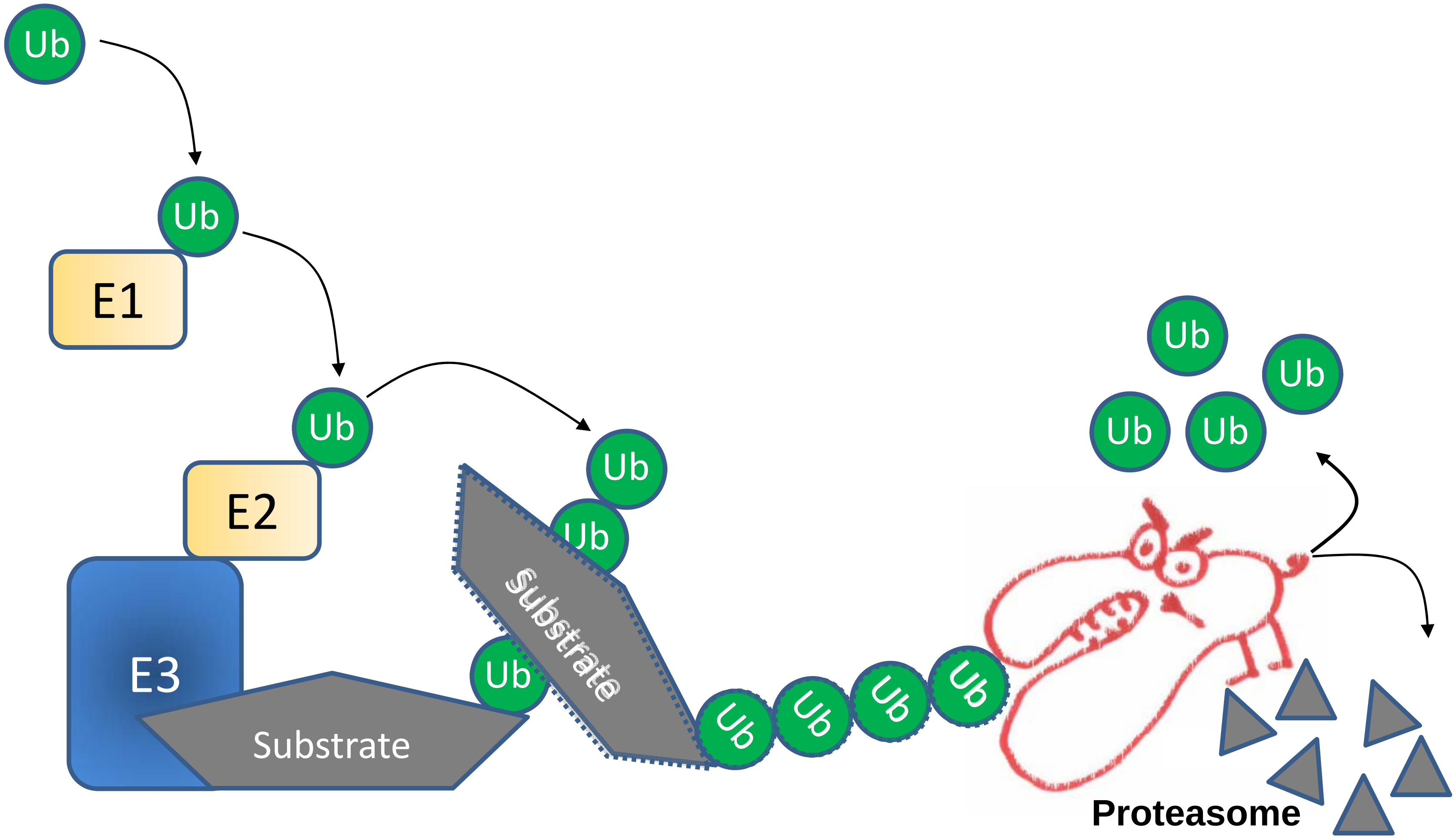
Glycin → STOP

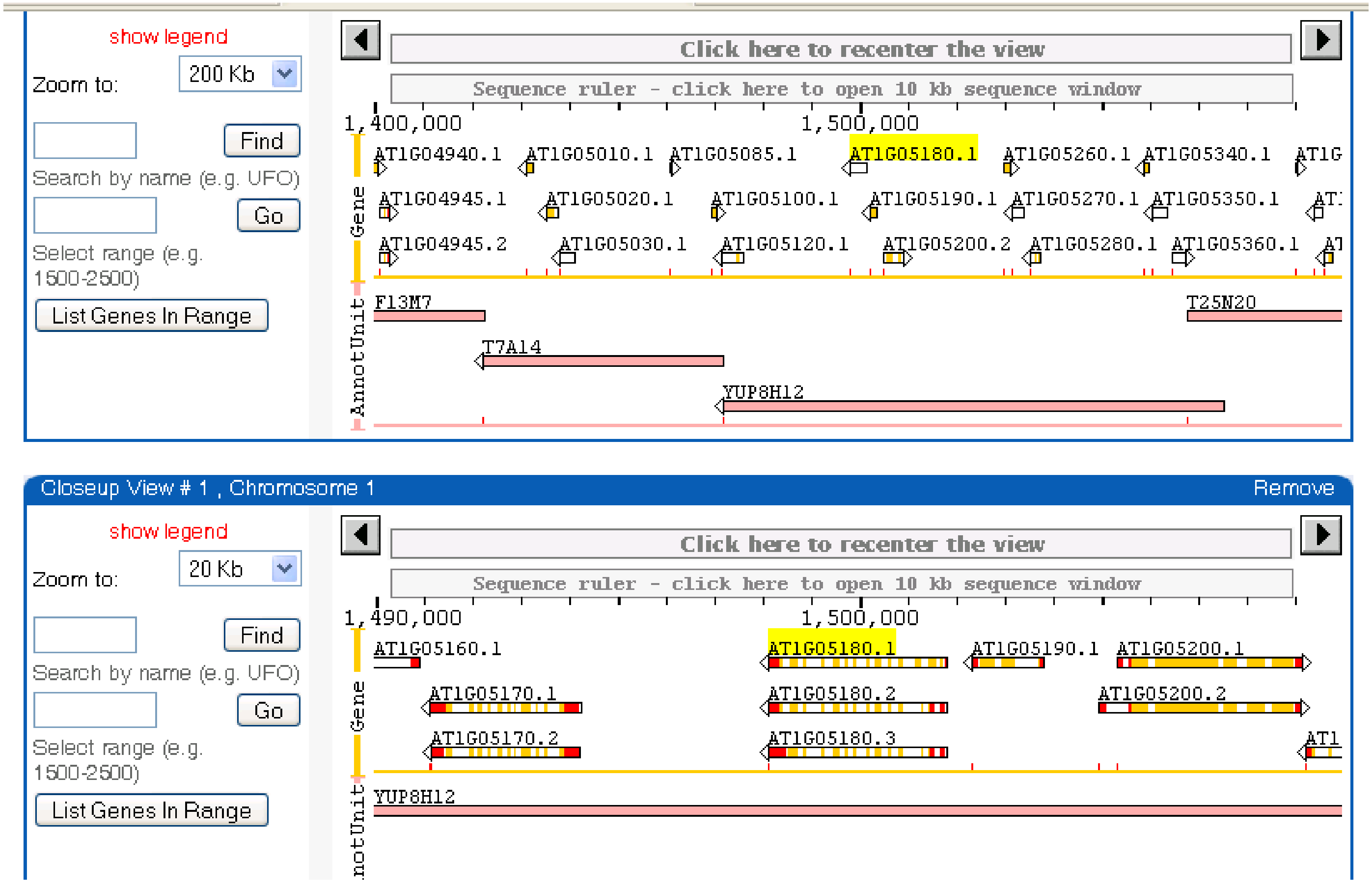
AXR1 Protein: 540 AS, ca. 60 kD

axr1-3
C→Y



The ubiquitin system

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List Genes In Range
Click here to recenter the view
Sequence ruler - click here to open 10 kb sequence window
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20 Kb
Find
Search by name (e.g. UFO)
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Click here to recenter the view
Sequence ruler - click here to open 10 kb sequence window
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AT1G05170.2
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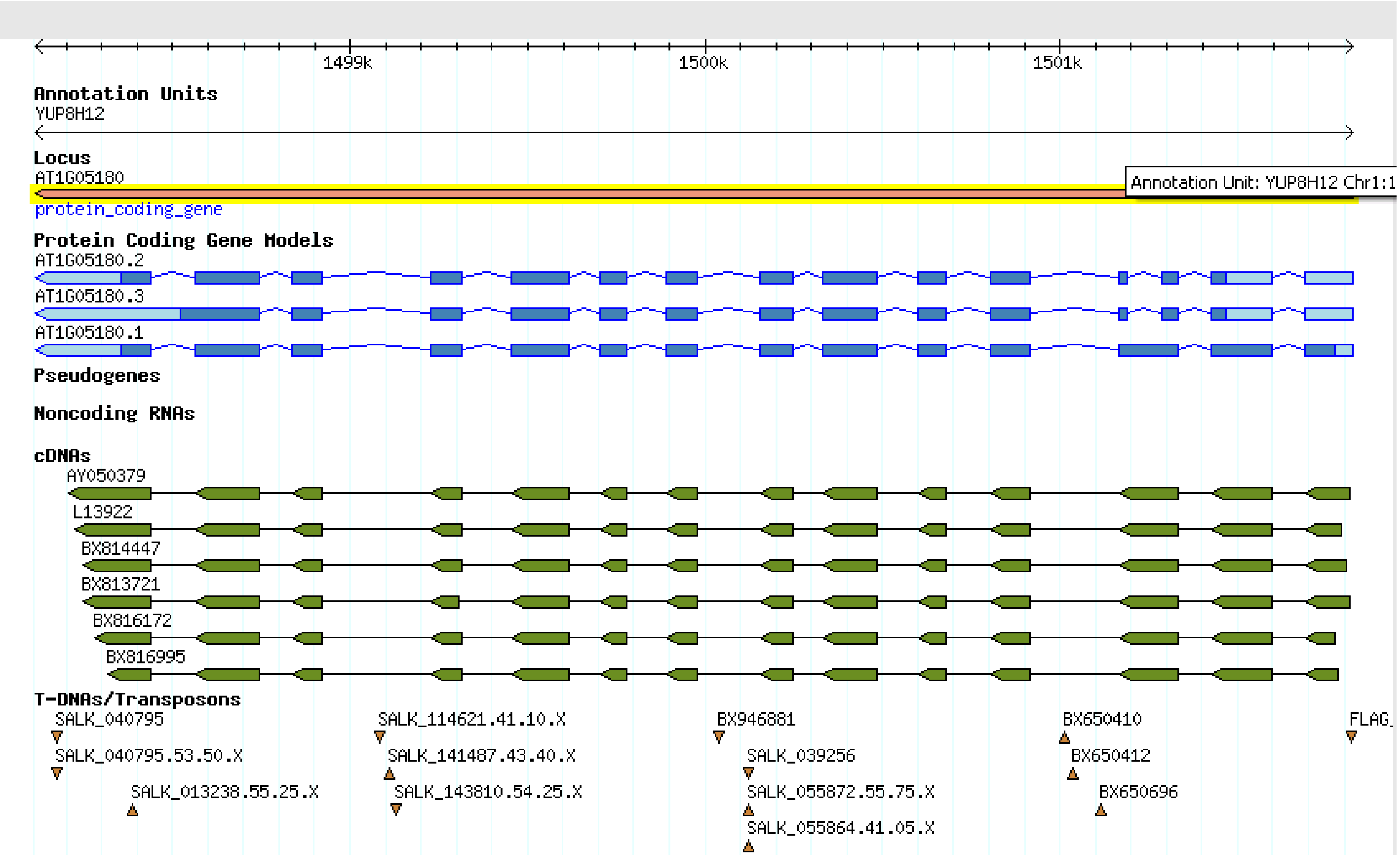
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