Seminar
Molekulare Mechanismen der Signaltransduktion

02.05.10 – MQ

- Background Auxin

- Einstieg in genetische Studien zum Auxin Signaling:

1. Estelle and Somerville, (**1987**) Auxin resistant mutants of *Arabidopsis thaliana* with an altered morphology. **Molecular and General Genetics** 206:200

2. Lincoln et al., (**1990**) Growth and development of the axr1 mutants of Arabidopsis. **Plant Cell** 2:1071

3. Leyser et al., (**1993**) Arabidopsis auxin-resistance gene AXR1 encodes a protein related to ubiquitin-activating enzyme E1. **Nature** 364:161

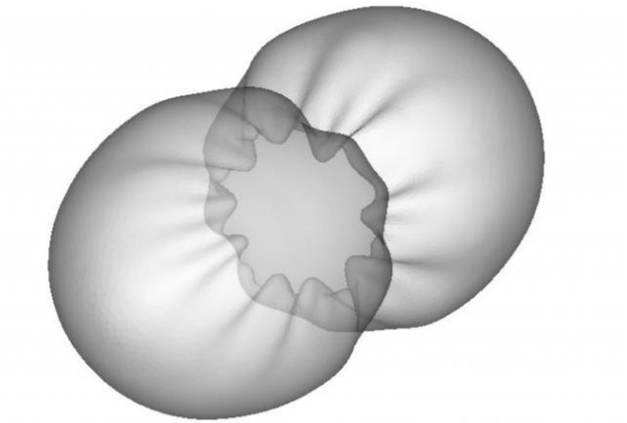
- Terminvergabe

Introduction

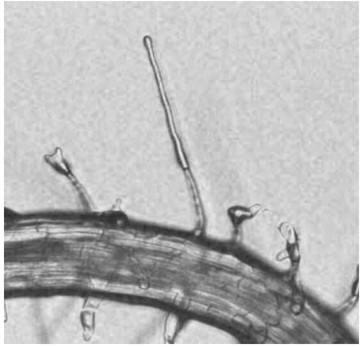
Definition:
Hormones are chemical signals that are produced in one part of the body, transported to other parts, bind to specific receptors, and trigger responses in target cells and tissues.

- Hormone (greek) → to excite

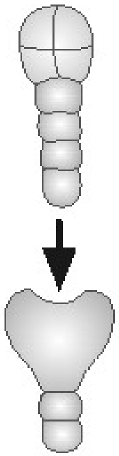
- In general, plant hormones control plant growth and development by affecting the division, elongation, and differentiation of cells.



cell division



cell elongation

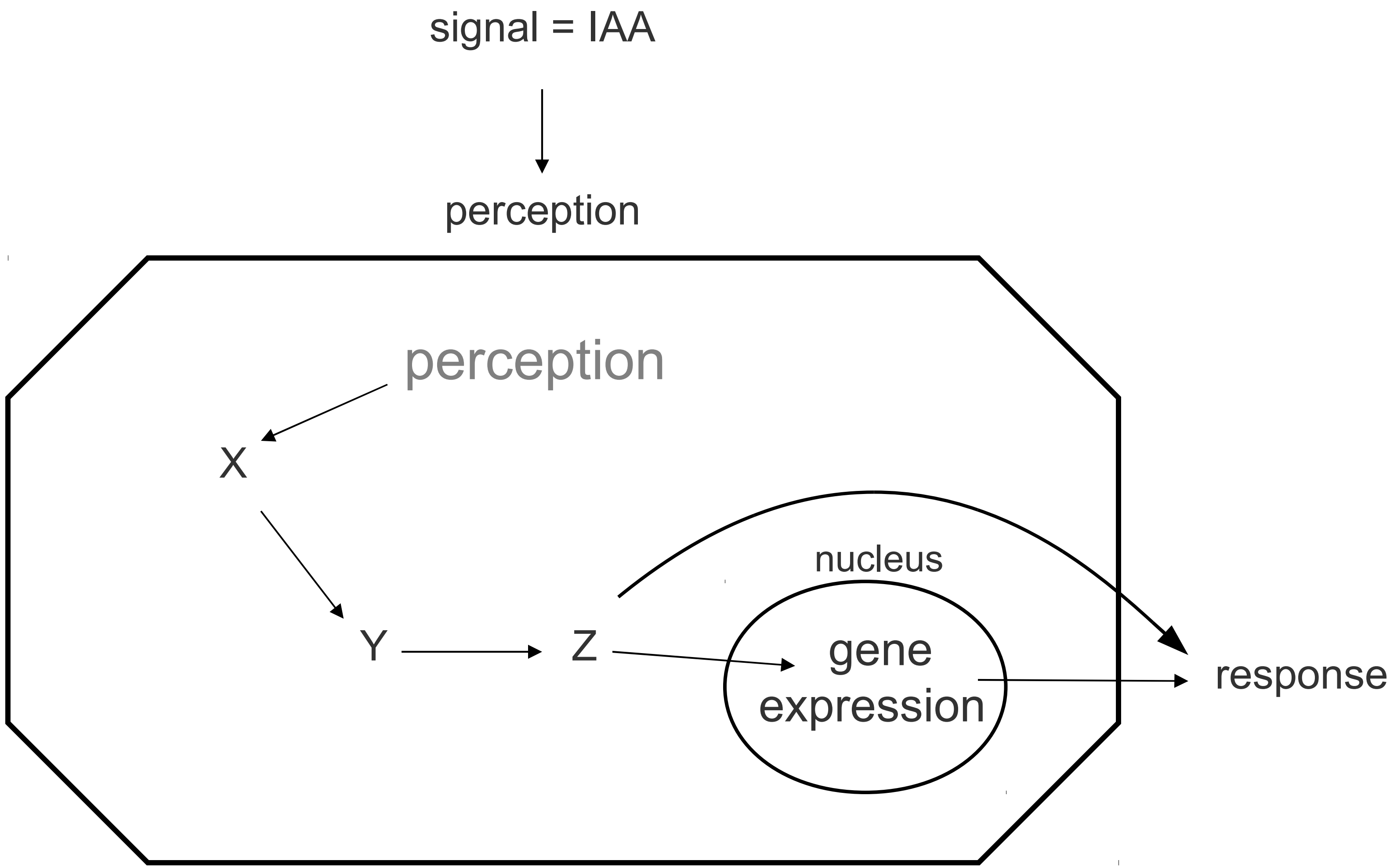


cell differentiation

→ **Aim of this seminar**: Elucidate the transduction pathway of the signaling molecule/hormone auxin from perception to cellular/morphological responses

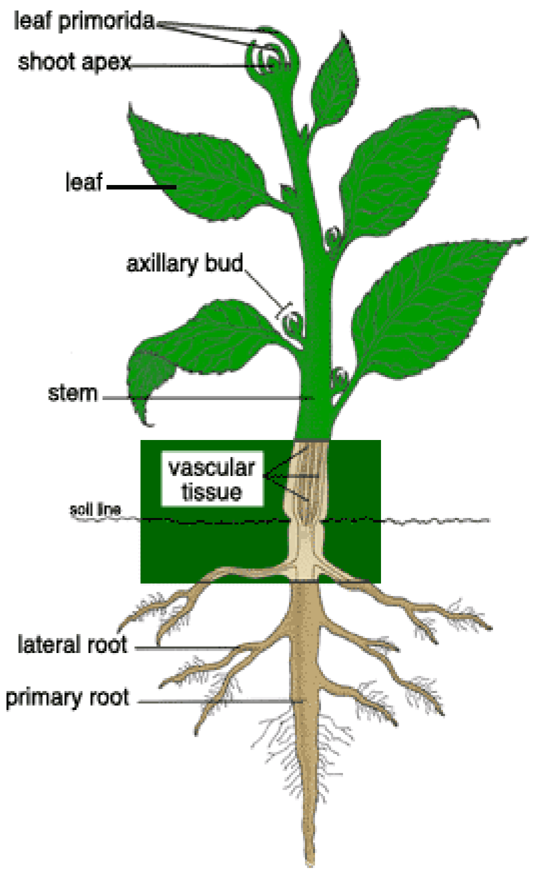
Excluding: biosynthesis, metabolism, transport

Black box of signaling



Auxin History I - 1758-1880

By definition hormones need to move between organs
→ transport system in plants?



1758

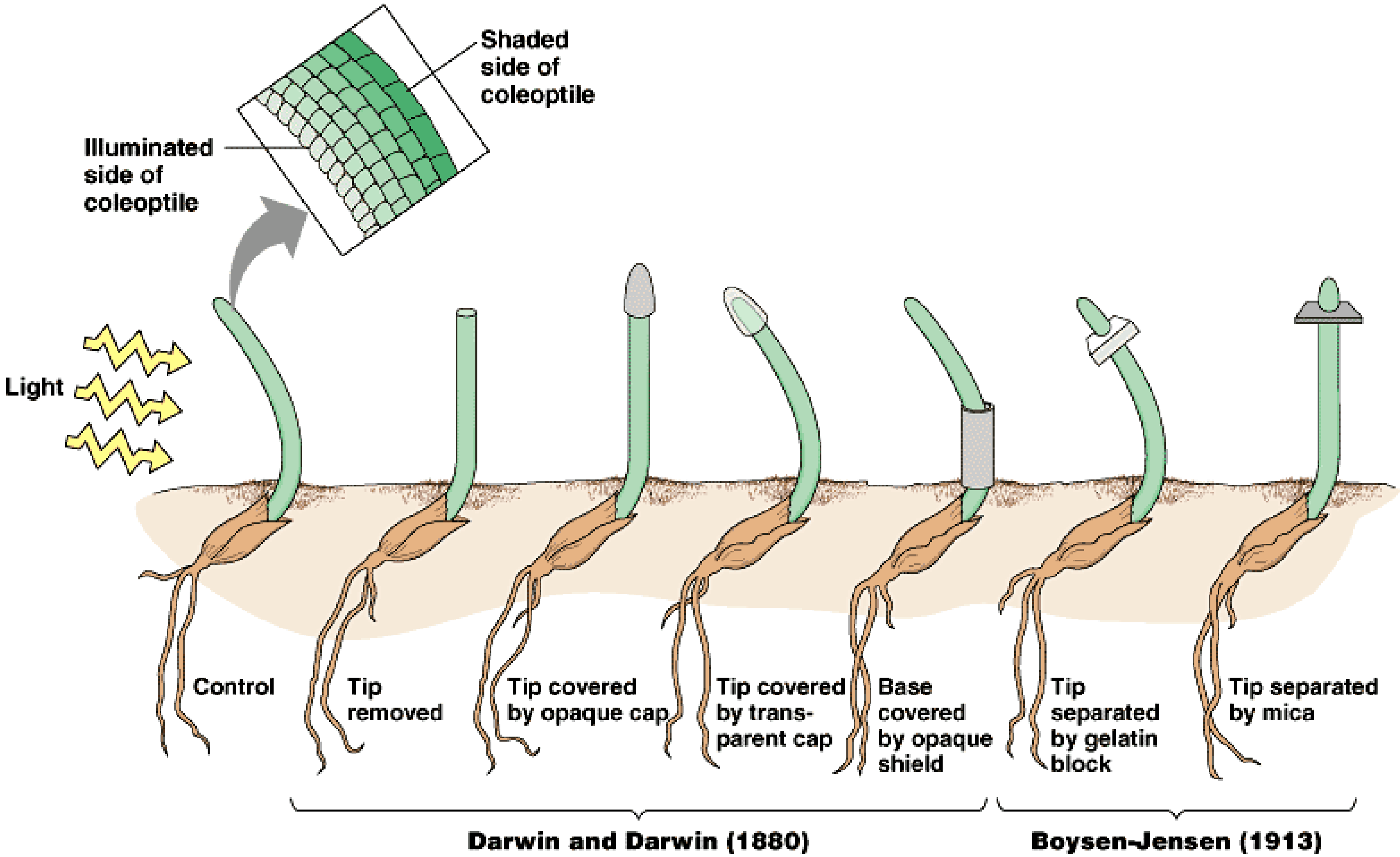
Henri Louis Duhamel du Monceau

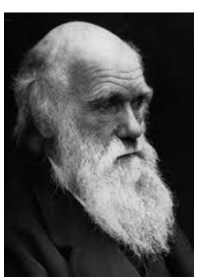
1880

Julius von Sachs

The vasculature!
Alternatively: movement by diffusion

Auxin History II - 1880-1935



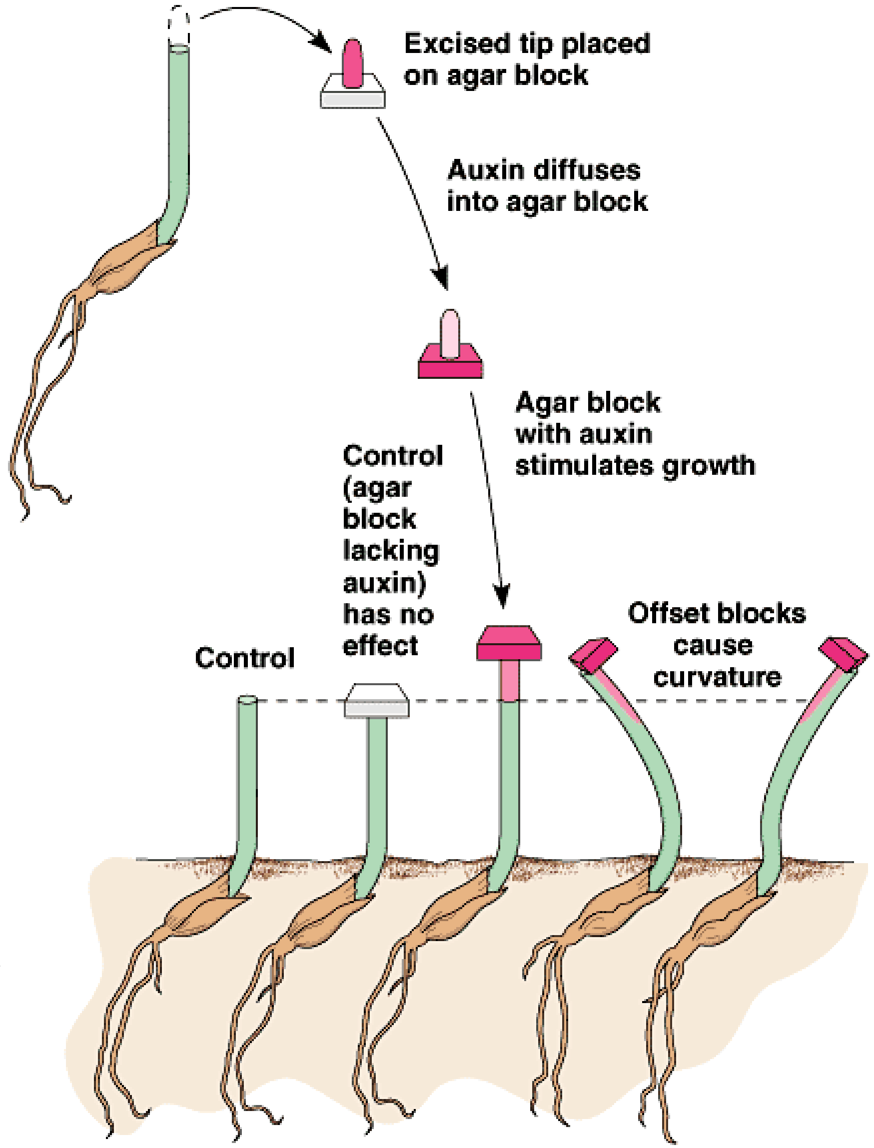


"When seedlings are freely exposed to a lateral light some influence is transmitted from the upper part of the coleoptile that acts on the lower part of the coleoptile"
"*The Power of Movement in Plants*"
(1880) by Darwin and Darwin.

Peter Boysen-Jensen demonstrated that the signal was a mobile chemical substance

Auxin History II - 1880-1935

Frits W. Went - 1958



In 1926, F.W. Went extracted the chemical messenger for phototropism, naming it **auxin** → *auxano* (greek) = to grow

→ an asymmetrical distribution of auxin moving down from the coleoptile tip causes cells on the dark side to elongate faster than cells on the brighter side.

Auxin History II - 1880-1935

James F. Bonner

The Journal of General Physiology

THE ACTION OF THE PLANT GROWTH HORMONE

By JAMES BONNER

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena)

(Accepted for publication, June 1, 1933)

TABLE II

Growth of Coleoptile Sections in Growth Substance Solution and in Water
(Each value is a mean from twelve–fifteen sections)

Solution	Growth in 2 hrs.	Growth in 4 hrs.	Growth in 24 hrs.
	per cent	*per cent*	*per cent*
Water	3	4	9
Water	3	5	7
Water	3	5	12
Growth substance	15	24	55
Growth substance	13	26	45
Growth substance	14	29	48

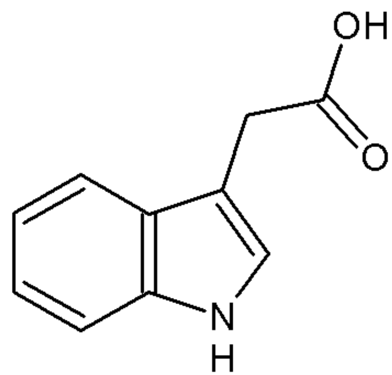
→ Auxin induces rapid growth of coleoptile segments by cell enlargement

Auxin History II - 1880-1935

Kenneth V. Thimann
1960

Supplement to "Nature," January 19, 1935

Identity of the Growth-Promoting and Root-Forming Substances of Plants



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

→ the principal auxin in all plant species

Auxin History III - 1935-1985

THE

NEW PHYTOLOGIST

Vol. XXXIV, No. 5 4 December, 1935

ACTIVATION OF CAMBIAL GROWTH BY PURE HORMONES

By R. Snow
Fellow of Magdalen College, Oxford

+ IAA

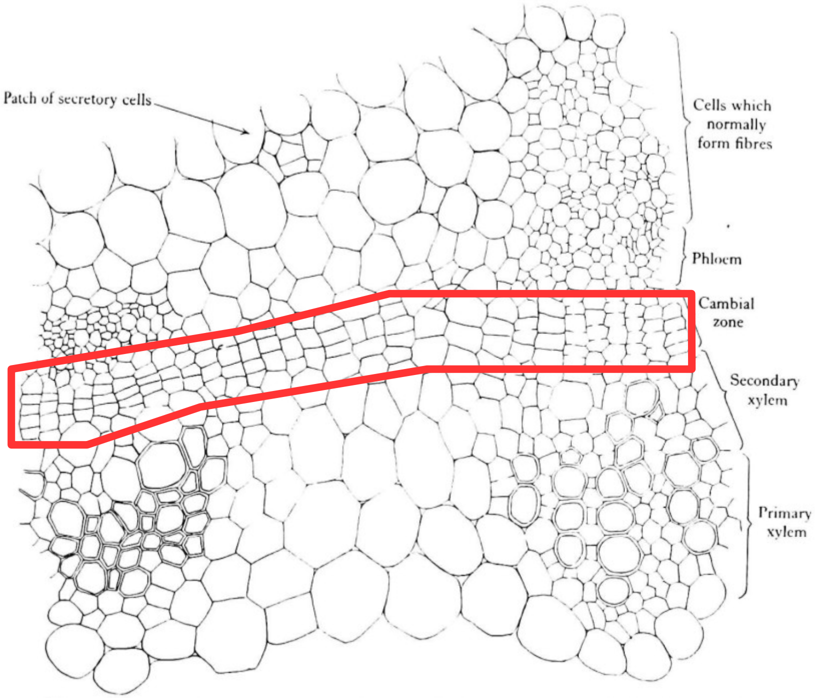


Fig. 4, Exp. 3. Experimental plant no. 1. Section at 10 mm. below top, and 5 mm. below zone covered by gelatins and auxin-*a*. ×202.

- IAA

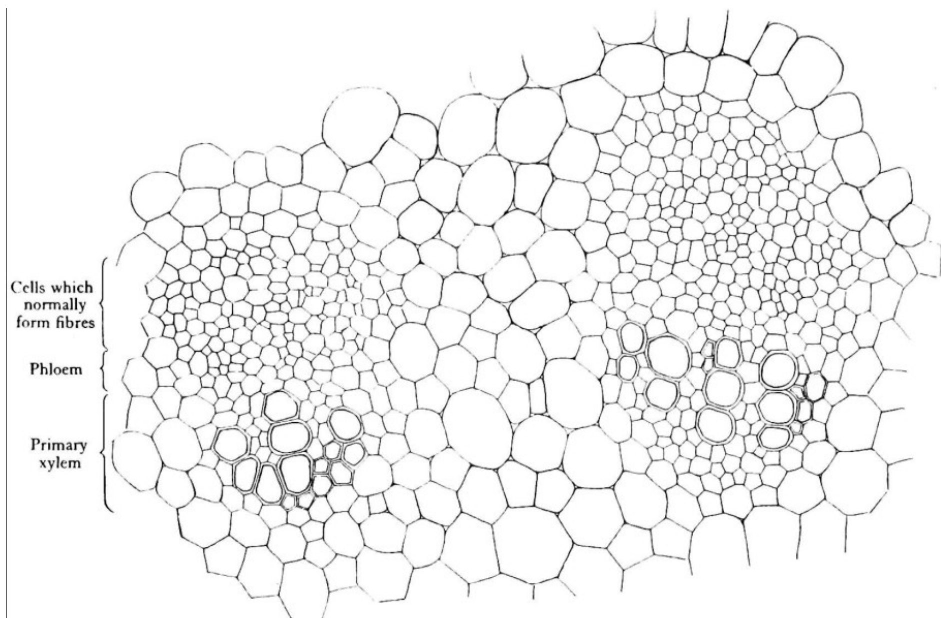


Fig. 5, Exp. 3. Decapitated control plant no. 1. Section at 15 mm. below top. ×202.

→ Auxin induces cell extension, division and differentiation

Auxin History III - 1935-1985

Changes Induced by Indoleacetic Acid in Nucleic Acid Contents and Growth of Tobacco Pith Tissue[1]

Julius Silberger, Jr., and Folke Skoog
Department of Botany, University of Wisconsin, Madison

SCIENCE, Vol. 118
October 16, 1953

Auxin treatment increases the level of nucleic acids!

↓

Gene activation?

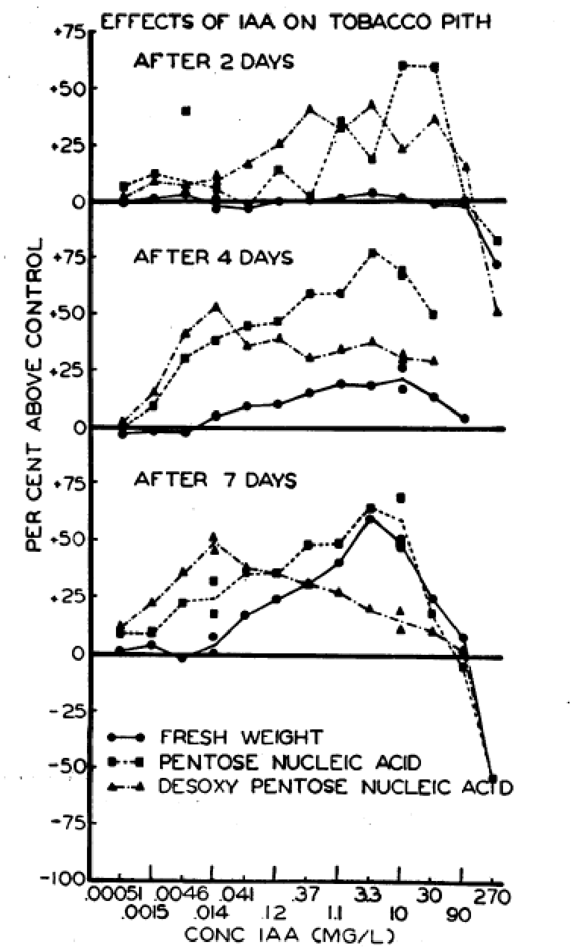


FIG. 1. Changes as per cent of controls in fresh weight, DNA content, and PNA content in excised tobacco pith tissue disks cultured on a sucrose agar medium with serial concentrations of IAA for 2, 4, and 7 days.

Support: Inhibitors of RNA and protein biosynthesis prevent cell elongation (Key, **1969**, Annu Rev Plant Physiol)

→ **Gene activation hypothesis**: Auxin regulates the synthesis of specific RNAs required for cell growth

Auxin History III - 1935-1985

THE JOURNAL OF GENERAL PHYSIOLOGY · VOLUME 53 · 1969

Timing of the Auxin Response in Coleoptiles and Its Implications Regarding Auxin Action

MICHAEL L. EVANS and PETER M. RAY

From the Division of Natural Sciences, University of California, Santa Cruz, California 95060. Dr. Evans's present address is Department of Biology, Kalamazoo College, Kalamazoo, Michigan 49001. Dr. Ray's present address is Department of Biological Sciences, Stanford University, Stanford, California 94305

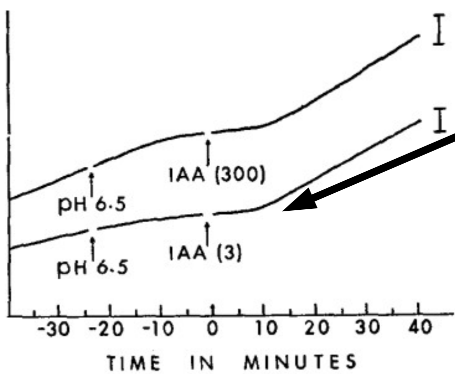


Figure 4. Effect of widely different IAA concentrations on timing of elongation response of oat coleoptile segments. At the first arrow water in chamber was replaced with Na citrate buffer, 10^{-3} M, pH 6.5; at the second arrow this was replaced with the same medium containing either 3 or 300 μg/ml IAA as indicated. The vertical bar by each curve in this and subsequent figures represents 1.0 mm of elongation, for that particular record, for the entire row of coleoptile segments.

Growth response 10 min after IAA application

↓

Too rapid for gene activation?

↓

Acid growth hypothesis

Auxin History III - 1935-1985

J. Mol. Biol. (1985) **183**, 53–68

Rapid Induction of Specific mRNAs by Auxin in Pea Epicotyl Tissue

Athanasios Theologis†, Thanh V. Huynh and Ronald W. Davis

Department of Biochemistry
Stanford School of Medicine
Stanford University, Stanford, CA 94305, U.S.A.

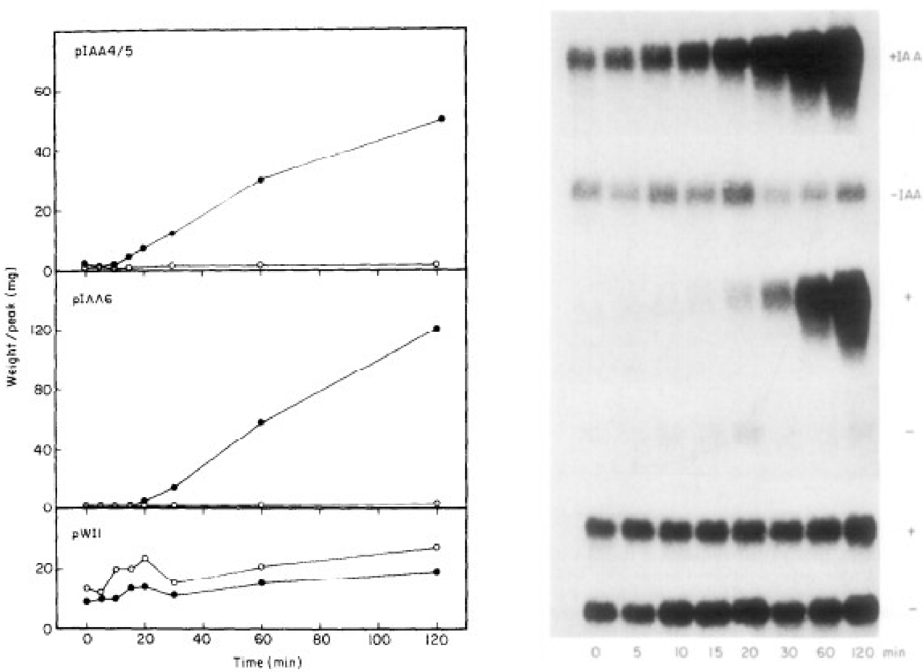


Figure 3. Induction kinetics of the IAA-inducible mRNAs. Endogenous IAA-depleted pea segments were treated with or without 20 μM-IAA. Poly(A)$^+$ RNA was isolated at various time intervals from 2 g fresh weight (100 segments) of control or IAA-treated tissue: 20 μg of poly(A)$^+$ RNA from 0, 5, 10, 15, 20, 30, 60 and 120 min incubations were electrophoretically separated and transferred to aminophenylthioether paper as described in Materials and Methods. Time-points are indicated at the bottom of respective lanes on the right-hand side of the Figure. Two RNA papers were prepared, one contained the IAA mRNAs (+IAA) and the other the control mRNAs (−IAA). The filters were successively hybridized to ^{32}P-labeled pIAA4/5, pIAA6 and pWI1 plasmid DNAs (top, middle, bottom right) after previous removal of the radioactive probe as described in Materials and Methods. The autoradiograms of these papers are shown on the right-hand side of the Figure, and were scanned in a Joyce–Loebl recording densitometer. The areas under the curves were quantitated by weighing, and the results are shown on the left-hand side of the Figure. (●) With IAA; (○) without IAA.

Rapid induction of mRNAs after 10-15 min of IAA application

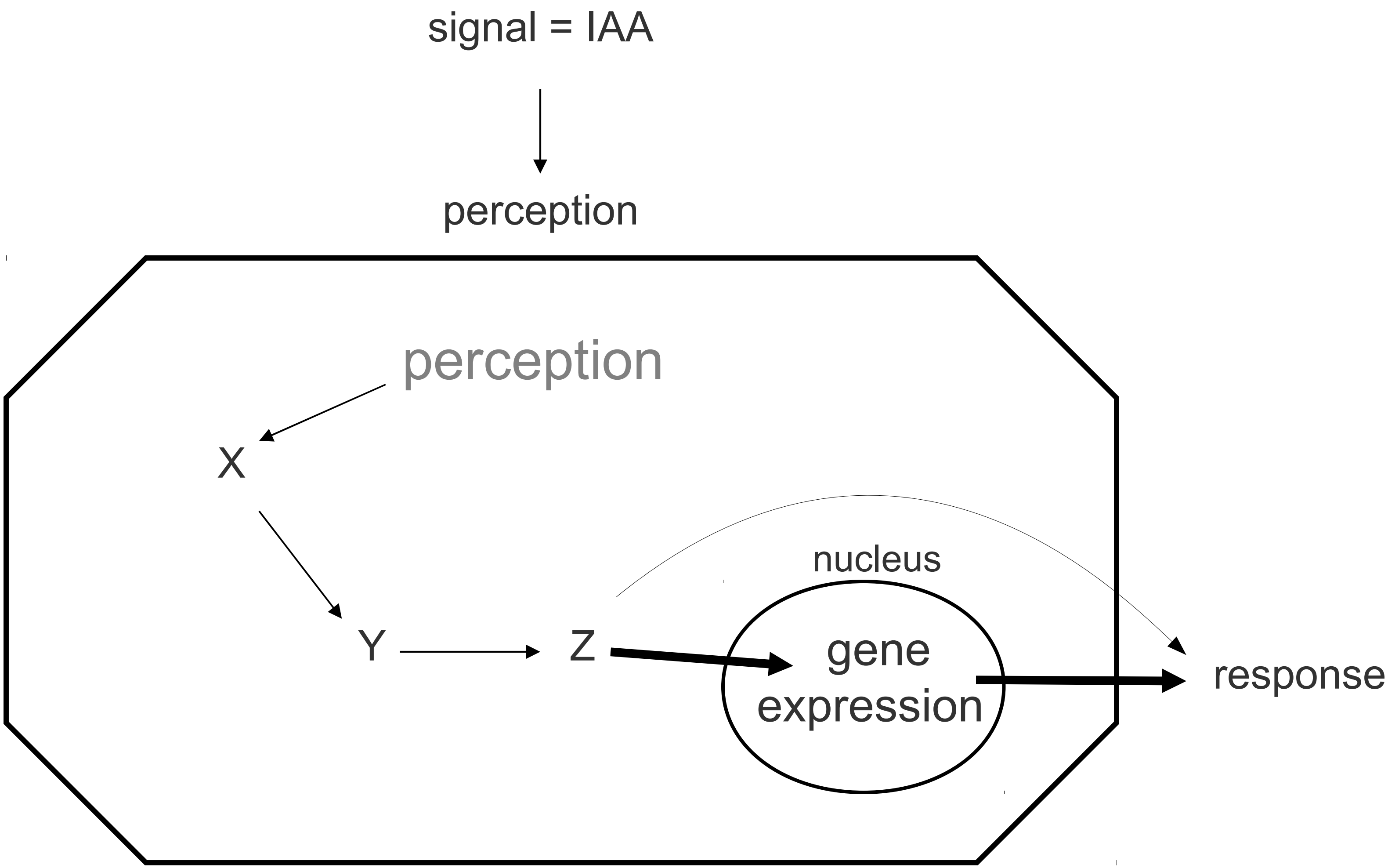
↓

Matches the reported elongation kinetics (protein synthesis?)

↓

The most likely scenario:
Auxin regulates cell growth indirectly by controling *de novo* expression of genes required for that process.

Black box of signaling



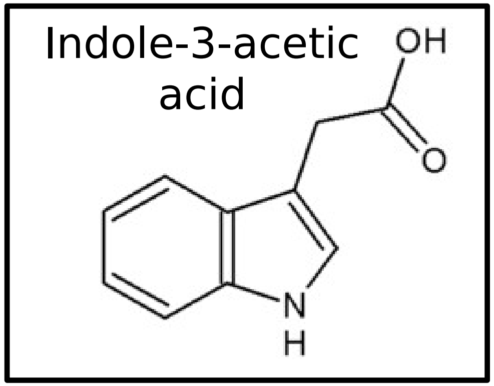
Gene activation hypothesis suggests the 'nuclear route'

Arabidopsis thaliana

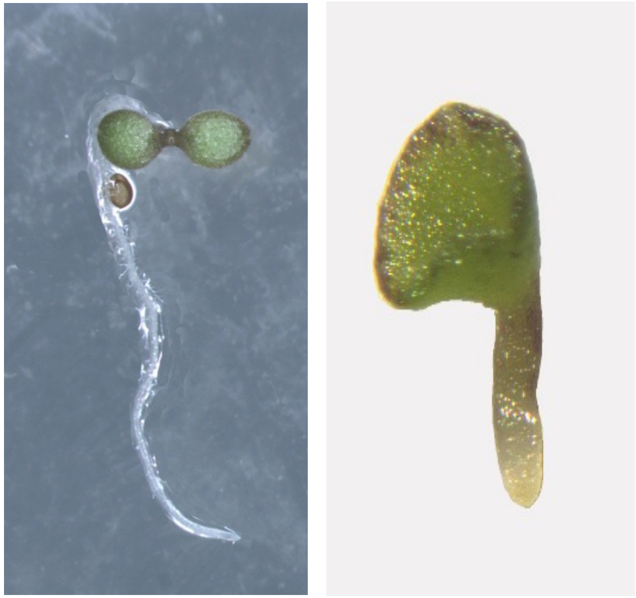
Small size	(30 cm)
Rapid life cycle	(6-8 weeks)
Prolific seed production	(5000 seeds/plant)
Sequenced genome	(125 Mb; ~28,000 genes)
Easily transformable	
Tremendous community resources	

A power multicellular eukaryotic model system

Auxin regulates plant development



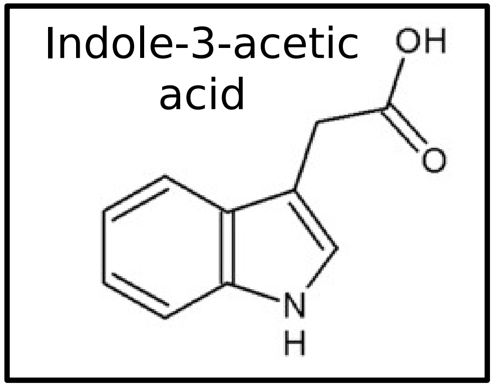
- Embryonic patterning
- Growth & Apical dominance
- Root development
- Tropic growth responses



wild-type

bdl axr1 mutant

Auxin regulates plant development



Embryonic patterning

Growth & Apical dominance

Root development

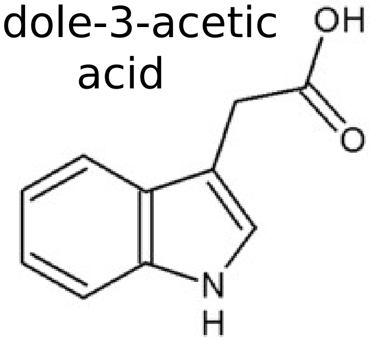
Tropic growth responses

wild-type

axr6-3 mutant

Auxin regulates plant development

Indole-3-acetic acid

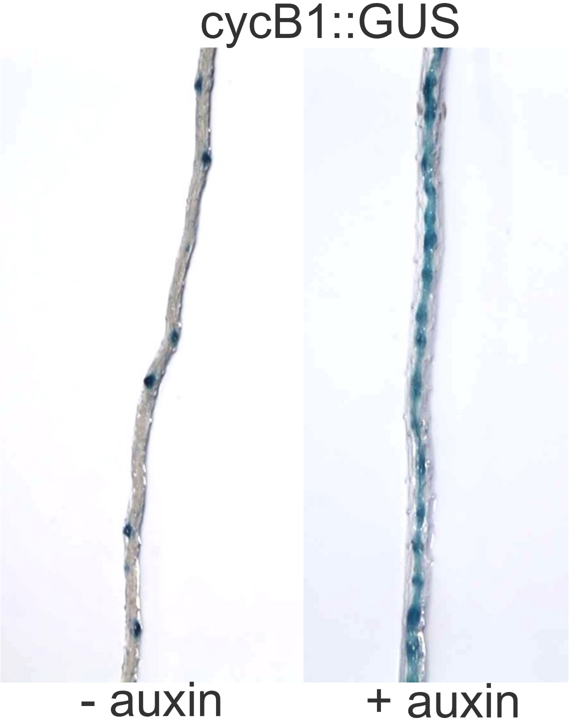


Embryonic patterning

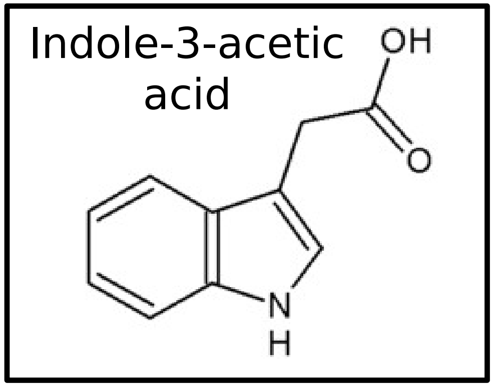
Growth & Apical dominance

Root development

Tropic growth responses



Auxin regulates plant development

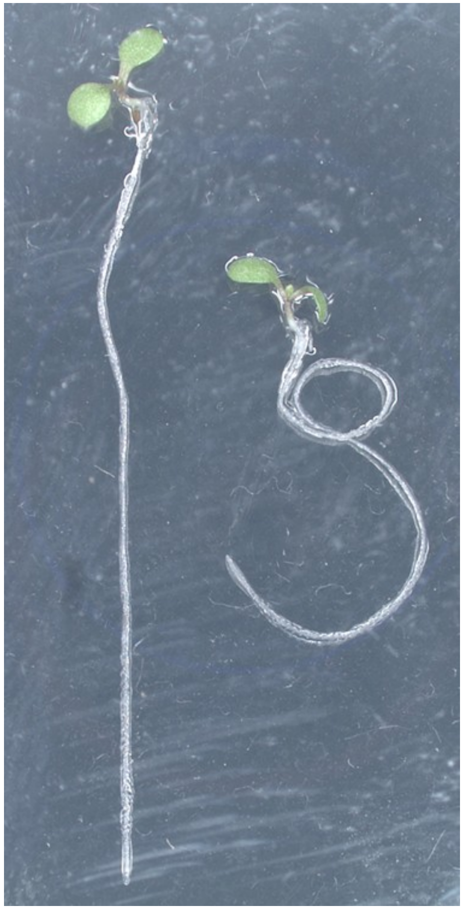


Embryonic patterning

Growth & Apical dominance

Root development

▷ Tropic growth responses



wild-type *aux1* mutant

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

Aim:
- Isolation of mutants with increased auxin resistance
- Identification of signaling elements that regulate auxin response

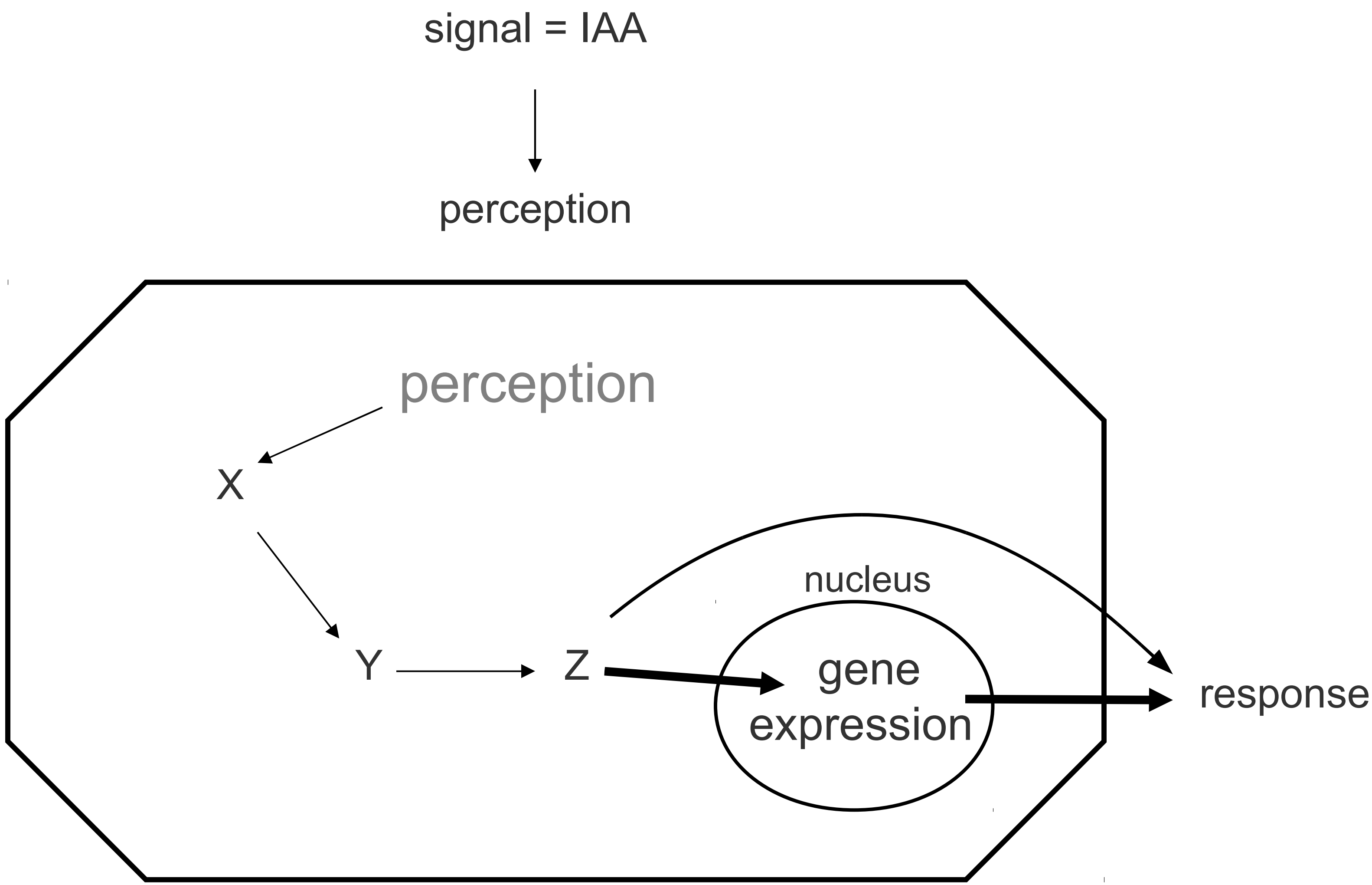
What was known?
- Auxin induces cell extension
- Auxin induces cell division
- Auxin induces cell differentiation

} Depends most likely on *de novo* gene expression/protein synthesis

What was NOT known?
- enzymes of auxin biosynthesis
- auxin transport facilitators
- auxin signaling elements

- Arabidopsis genome sequence

Black box of signaling

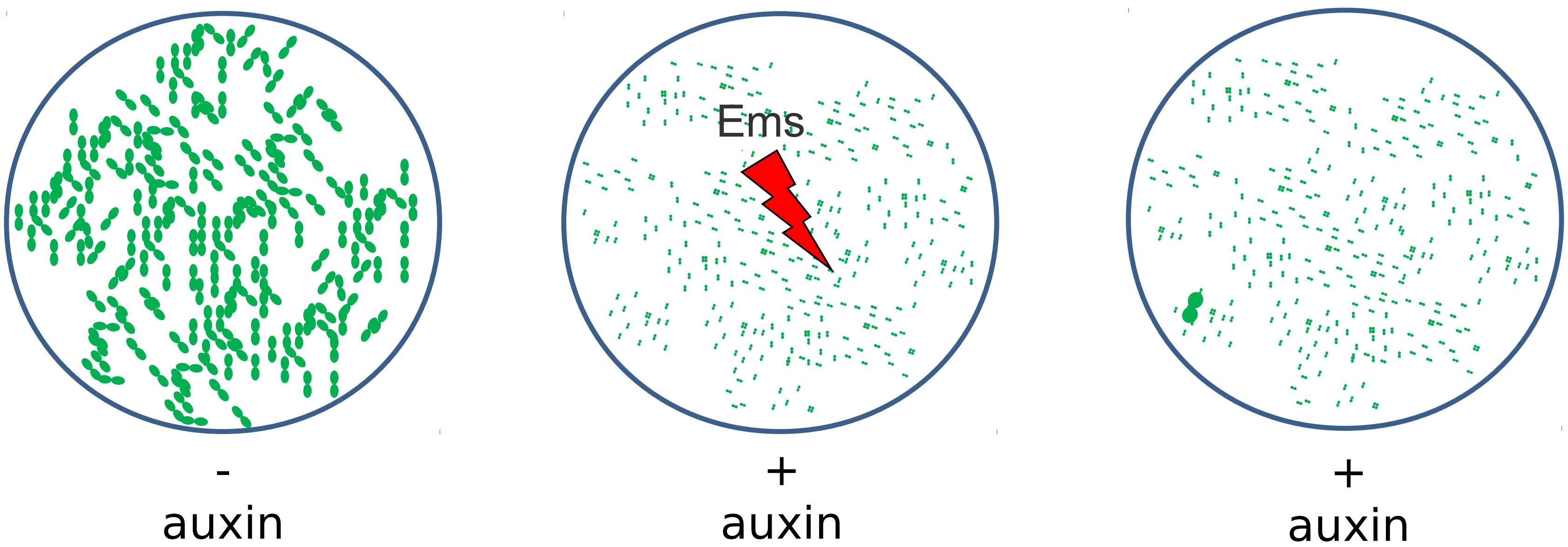


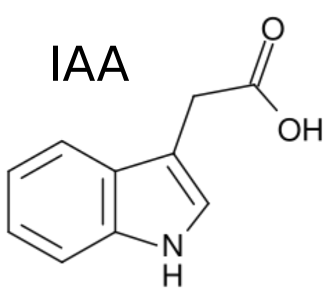
Genetic approach?

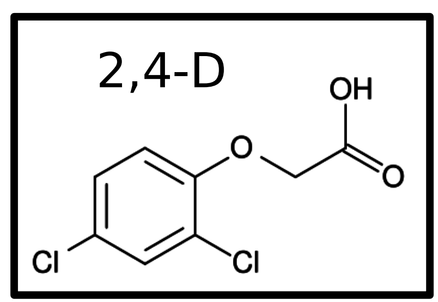
Mutagenesis

Prerequisite:
- Phenotype associated with trait of interest

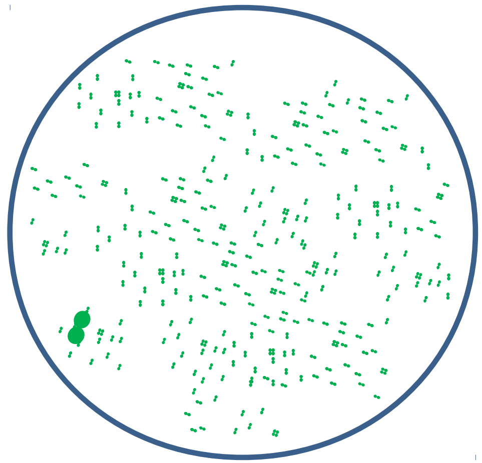
→ Seedling development → screen for mutants defect in wild-type response







Mutant/Gene nomenclature



\+
auxin

Same mutant screen, different genes:
axr1
axr2
axr3
axrx

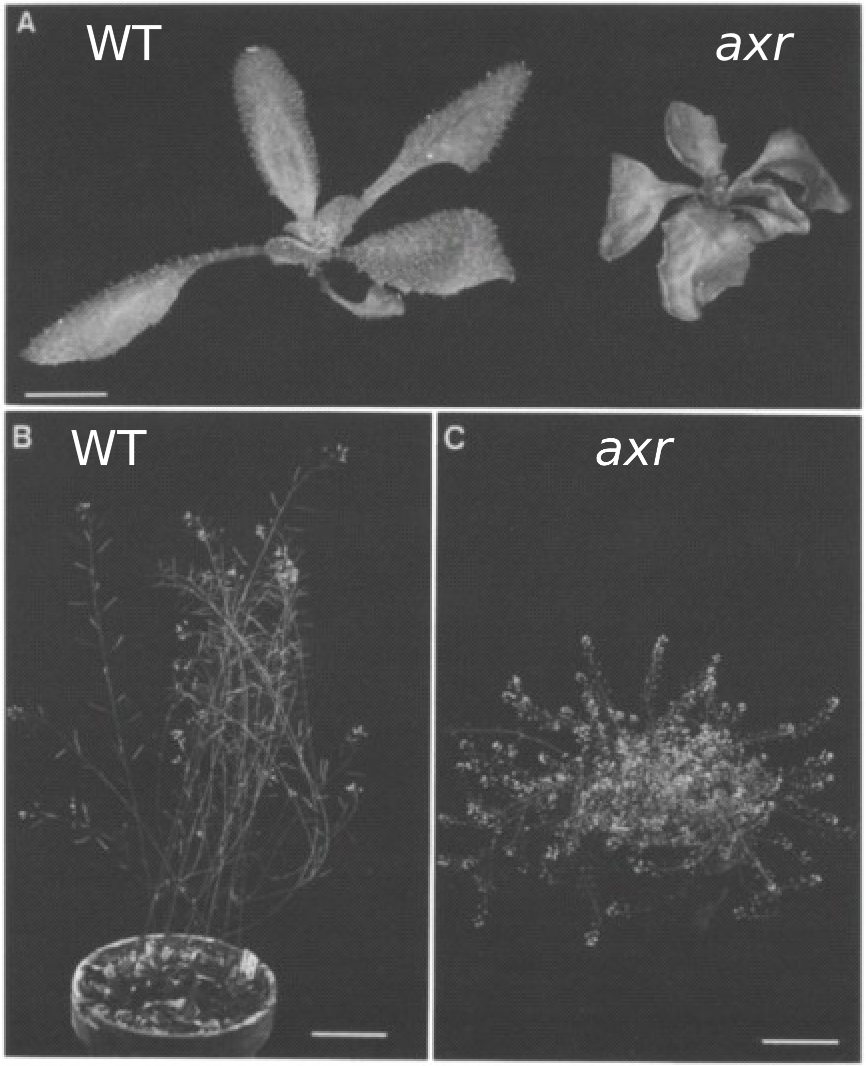
Name of the mutant: *auxin resistant 1 - axr1*

alleles:
axr1-1
axr1-2
axr1-3
axr1-x

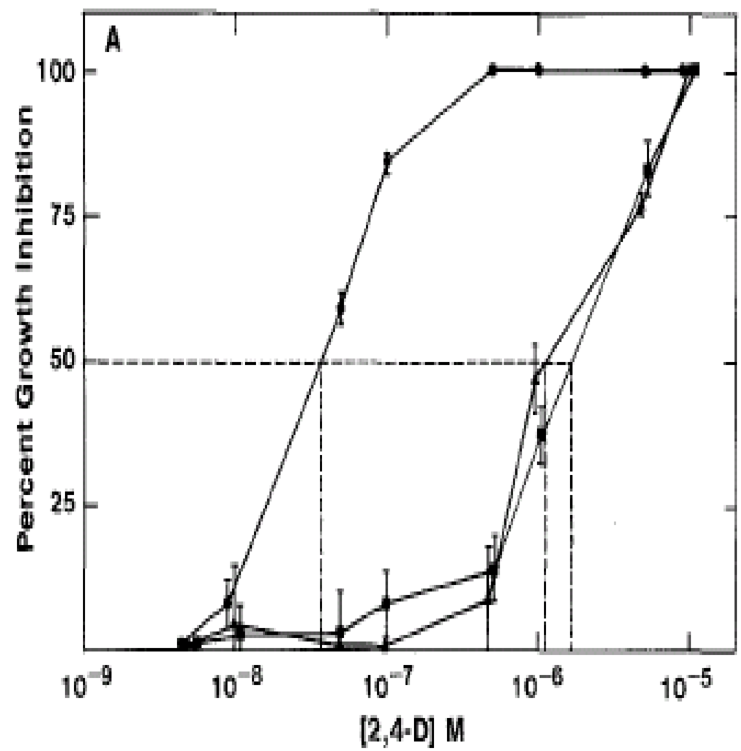
Name of the gene: *AUXIN RESISTANT 1 - AXR1*

Name of the protein: AUXIN RESISTANT 1 - AXR1

Phenotypes



- rosette smaller, short petioles, crinkled leaves
- roots thinner, not as highly branched
- 25-30 inflorescences compared to 1-5 wild type
→ apical dominance
- pollen fertility reduced → stamen shorter



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

Conclusion:
- Defects associated to auxin action at every developmental stage
→ AXR1 must be elemental for auxin response across tissues and developmental stages
→ but mutants viable → loss-of-function, not null alleles!

“...An attractive possibility is that the *AXR1* gene codes for an auxin receptor...“

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

Aims:
- **Further characterization of *axr* mutants**
- **Genetic mapping of the underlying gene**

The Plant Cell, Vol. 2, 1071–1080, November 1990 © 1990 American Society of Plant Physiologists

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

Isolation of additional mutants with *axr1*-like phenotype:

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*

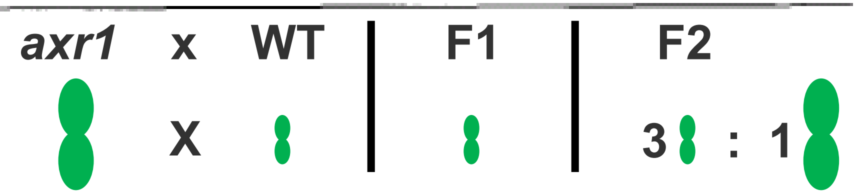
[a] A total of 470,000 seeds from four distinct M2 populations was screened for mutants that were able to elongate roots on either 5 μM 2,4-D or 50 μM IAA.
[b] Estelle and Somerville (1987).
[c] This study.

Segregation analysis:

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] $P > 0.05$.



***axr1*-like mutant phenotypes are inherited recessively**
→ typical for loss-of-function alleles

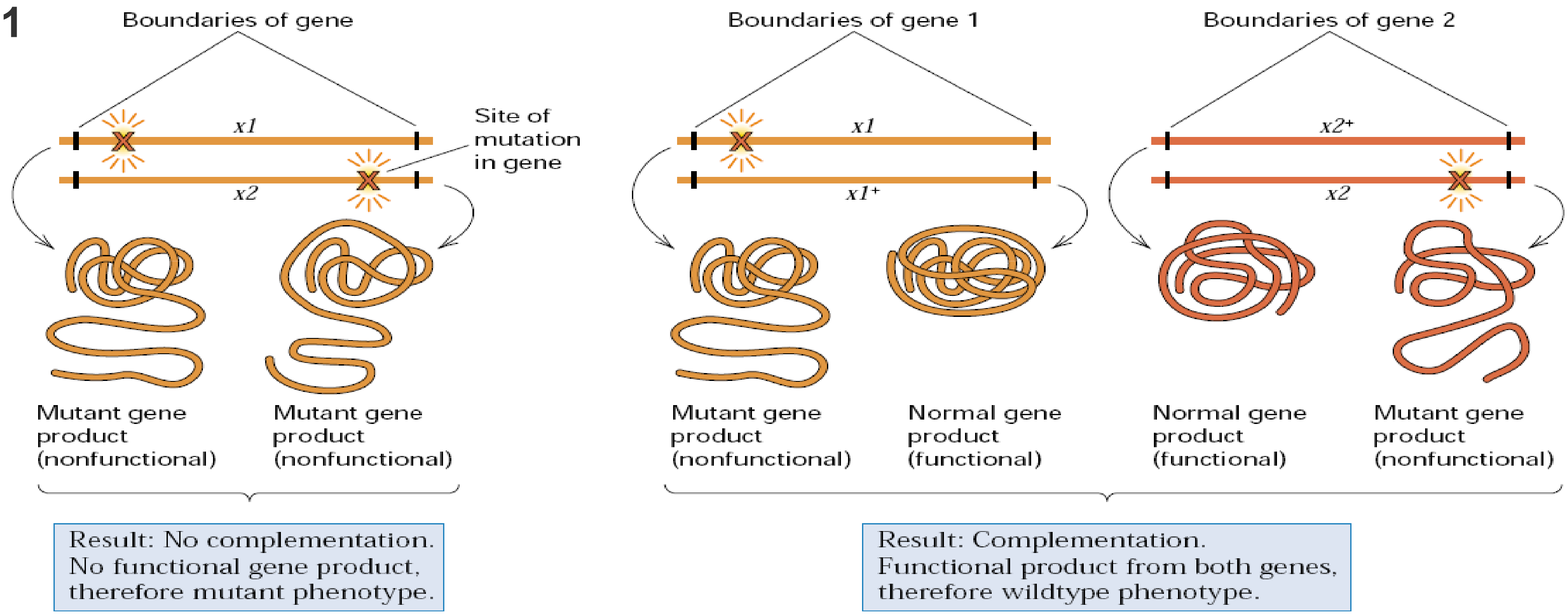
axr mutations in the same gene?

P

X

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

F1



Mutant phenotype → same gene/allelic

Wild type phenotype → different genes

Complementation test

Table 3. Complementation Analysis of *axr1* Lines

	Number of Plants	
Cross	Resistant	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

axr1-x mutations are alleles in the same gene

Morphology:

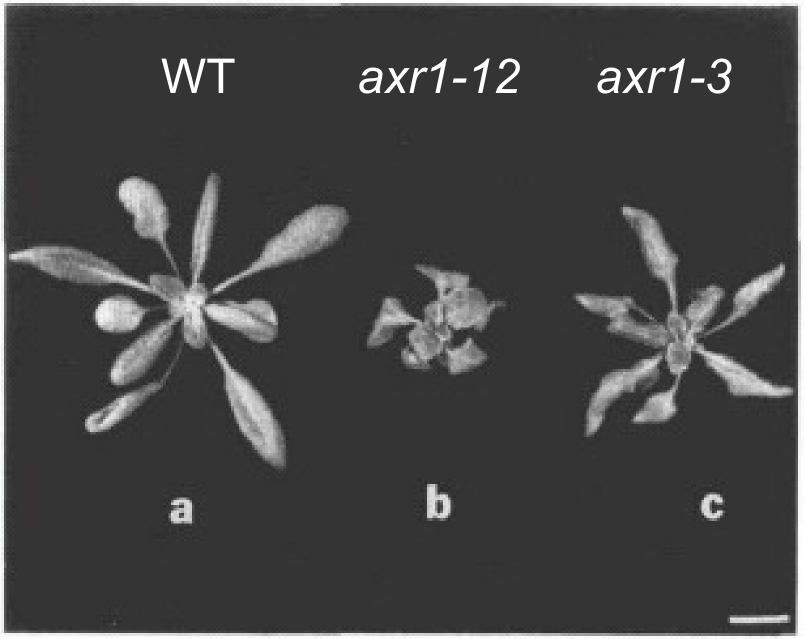


Figure 1. Phenotype of Wild-Type and Mutant Rosettes.

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.

→ Allelic differences also in root growth assays

Alleles show different expression of morphological and response defects

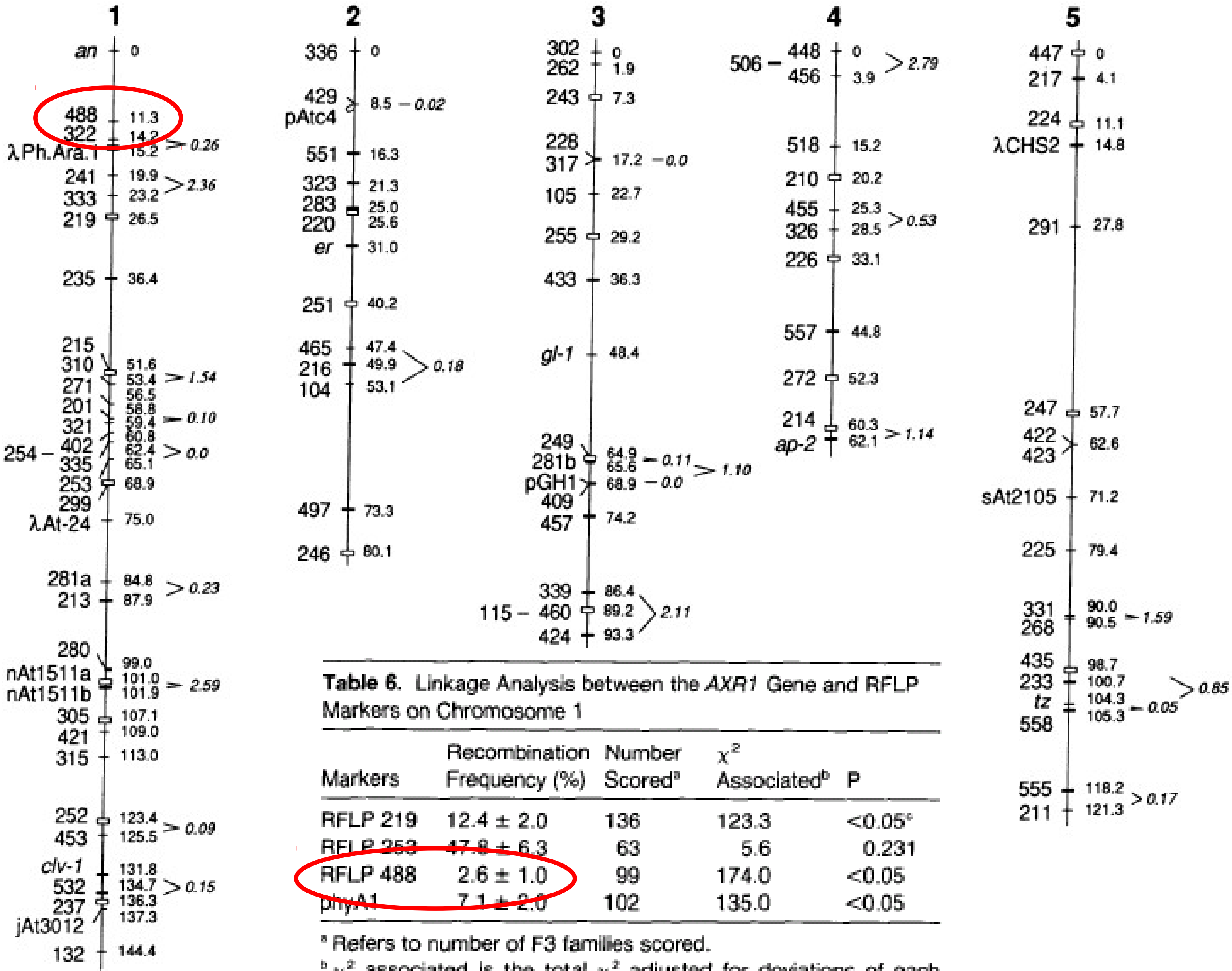


Table 6. Linkage Analysis between the *AXR1* Gene and RFLP Markers on Chromosome 1

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).

The Plant Cell, Vol. 2, 1071–1080, November 1990 © 1990 American Society of Plant Physiologists

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

Conclusion:

- ***axr1*** **mutants represent different alleles in the same gene**

- **'weak and strong' alleles**

- **essential function in growth related processes throughout developmental stages is confirmed**

- ***AXR1*** **gene maps to the top of chromosome 1**

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

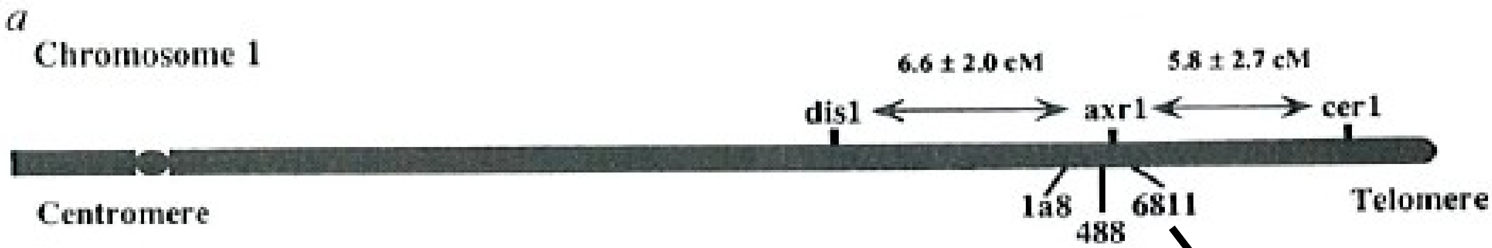
H. M. Ottoline Leyser, Cynthia A. Lincoln*, Candace Timpte, Douglas Lammer, Jocelyn Turner & Mark Estelle†

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

Aim:

- Fine-mapping and cloning of *AXR1*

AXR1 - Chromosome Walking



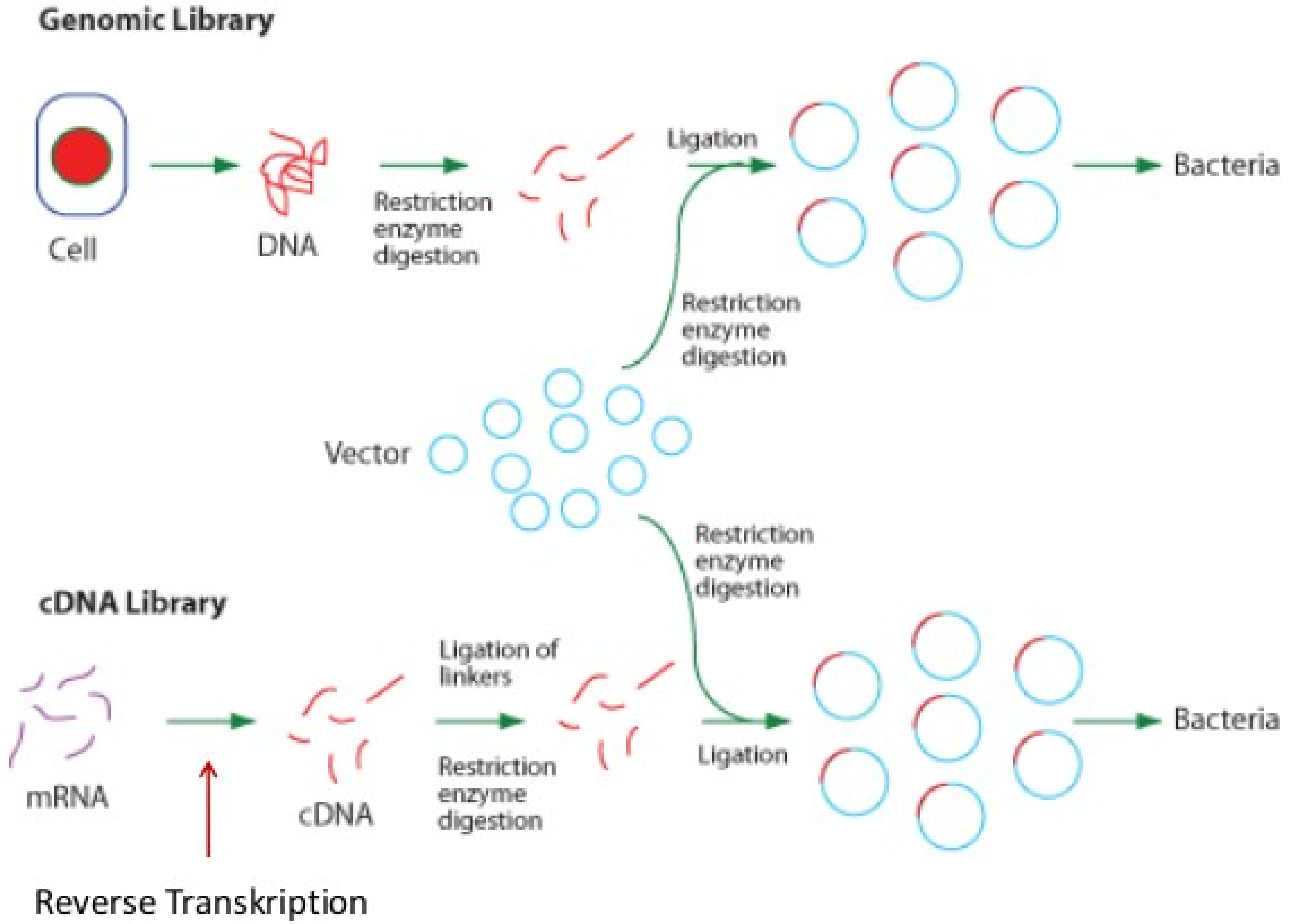
Very large region (physically)

↓

Target region in smaller DNA fragments → subcloning

↓

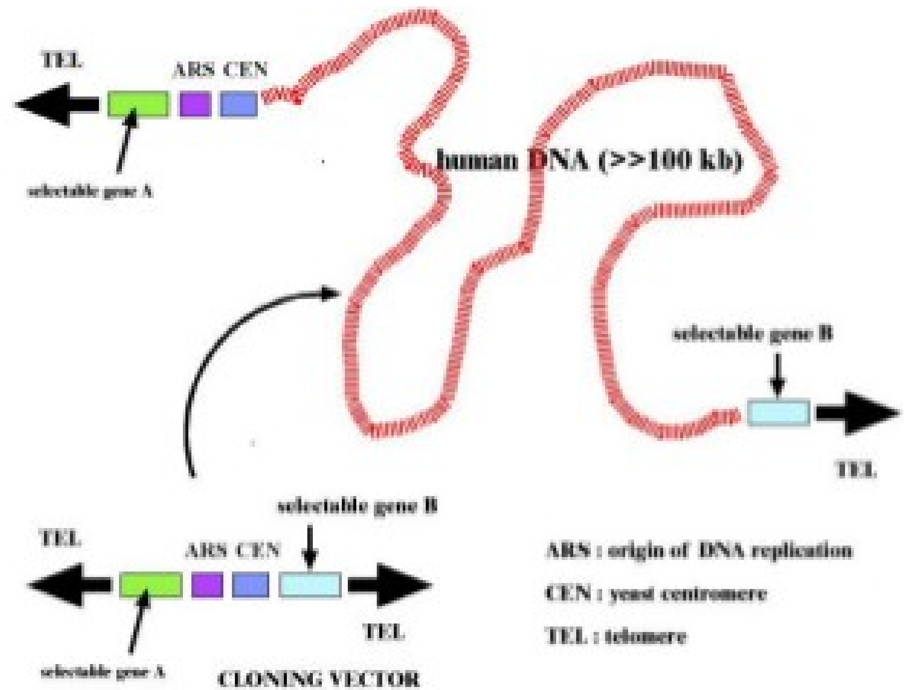
Genomic DNA libraries


Genomic Library
Cell
DNA
Restriction enzyme digestion
Ligation
Bacteria
Restriction enzyme digestion
Vector
Restriction enzyme digestion
cDNA Library
Ligation of linkers
mRNA
cDNA
Restriction enzyme digestion
Ligation
Bacteria
Reverse Transkription

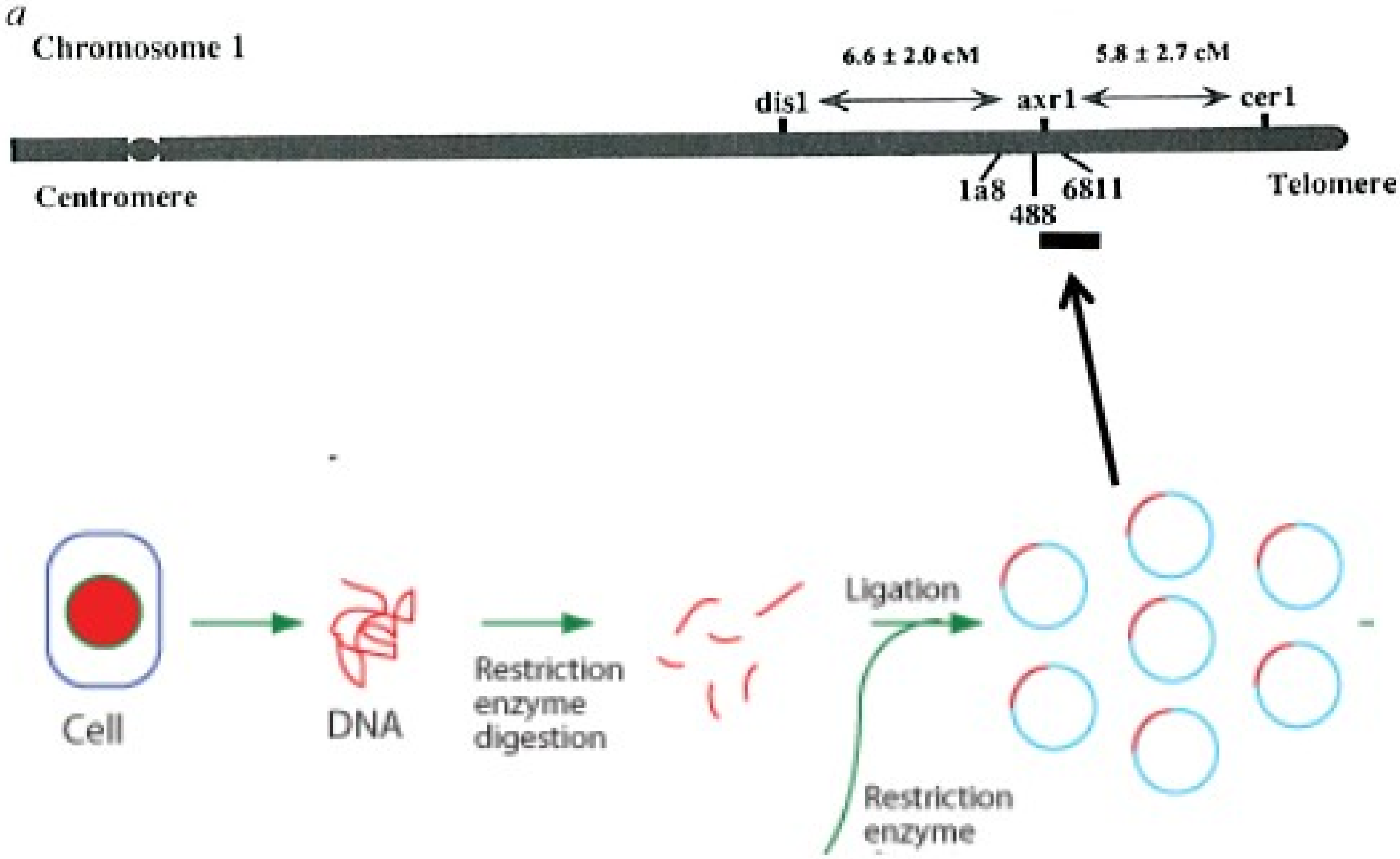
Vectors for cloning of large DNA fragments – YACs, cosmids, ...

Approximate maximum length of DNA that can be cloned into vectors

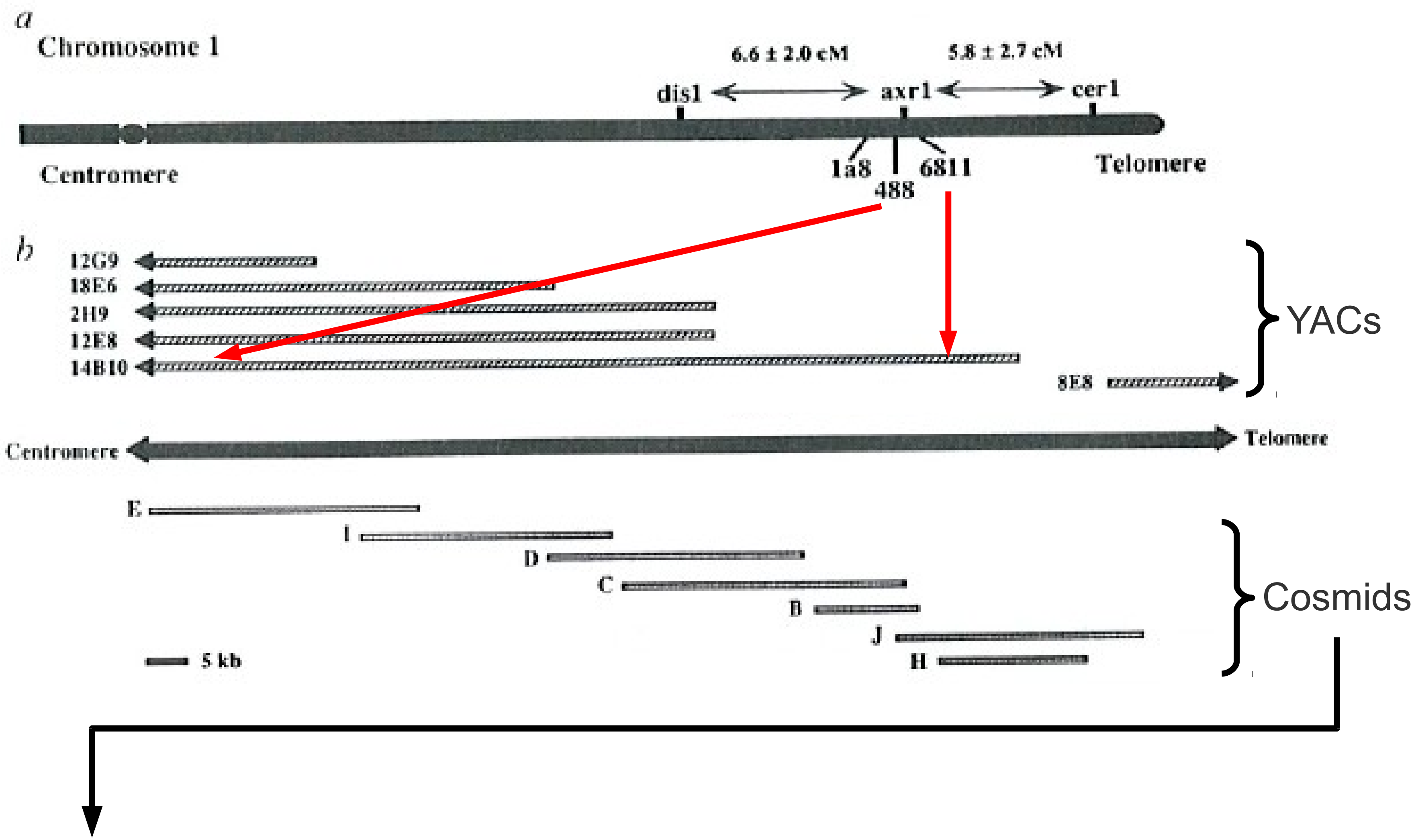
Vector type	Cloned DNA (kb)
Plasmid	20
lambda phage	25
Cosmid	45
BAC (bacterial artificial chromosome)	300
YAC (yeast artificial chromosome	1000



AXR1 - Chromosome Walking



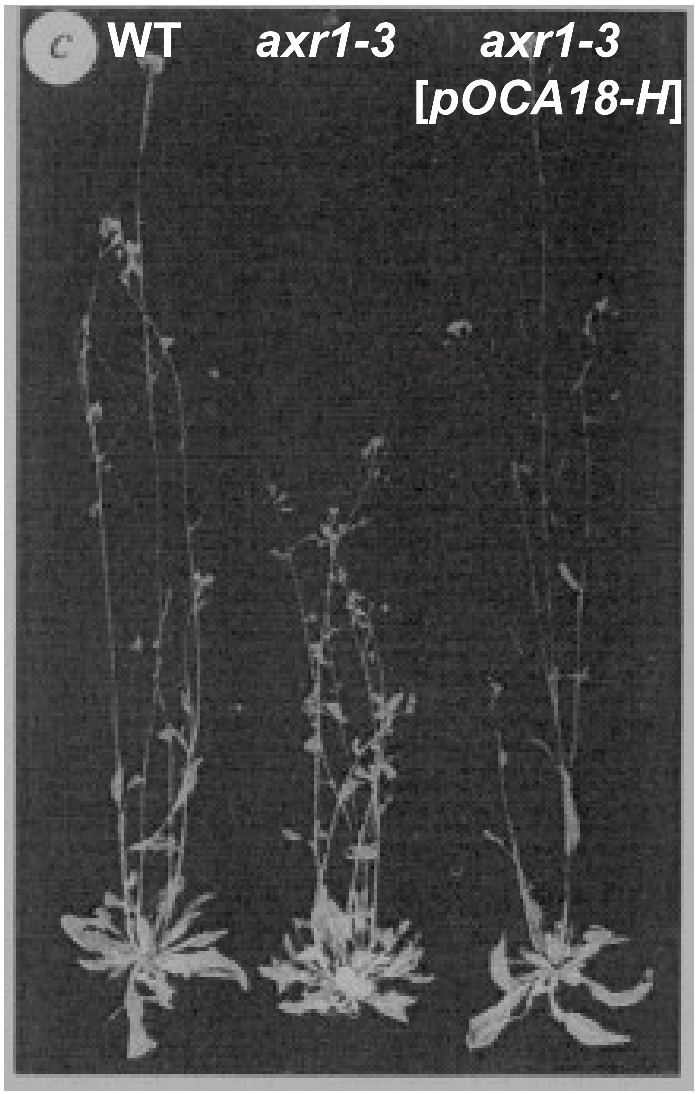
AXR1 - Chromosome Walking



Transform cosmid clones E – H by agrobacterium transformation into *axr1-3* mutants

Check for restoration of the wild type phenotype

Transgenic complementation analysis



Cosmid H can restore the wild type phenotype in the mutant background

Cosmid H contains the *AXR1* gene

Transgenic complementation analysis

Cosmid H contains the *AXR1* gene

↓

Screen cDNA library with cosmid H → Which genes are located on cosmid H?

↓

2 cDNAs identified → *AXR1* candidates

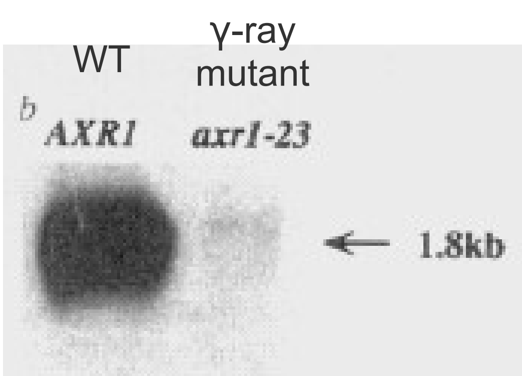
↓

Northern Blot → How much transcript (1.8 kb mRNA) ist present in the *axr1-23* deletion mutant?

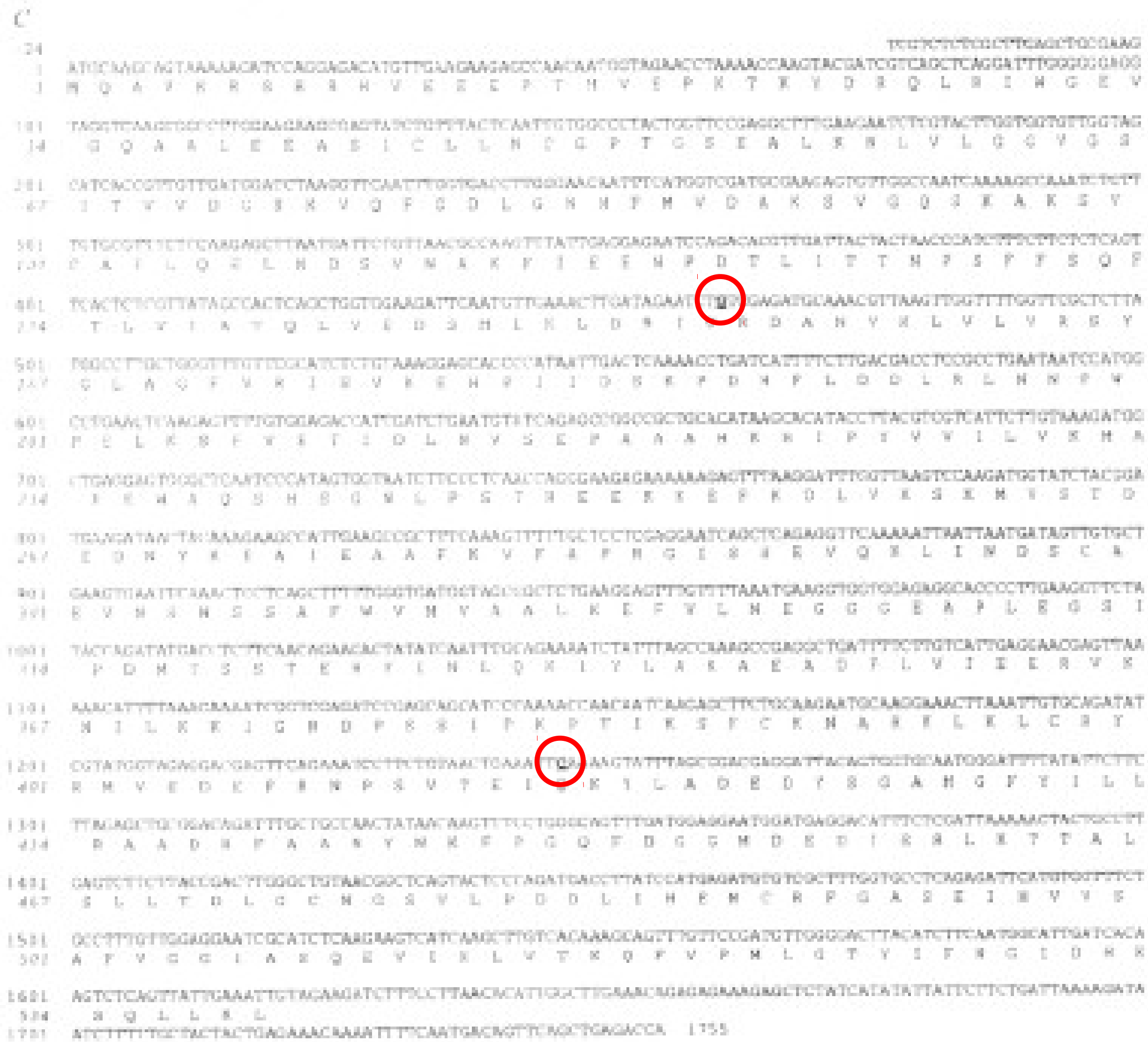
↓

None!

→ 1.8 kb transcript represents the *AXR1* gene



Sequencing of the *AXR1* gene in *axr1-3* and *axr1-12* mutants



axr1-3 = G461A
Cysteine → Tyrosine

axr1-12 = C1246T
Glycine → STOP

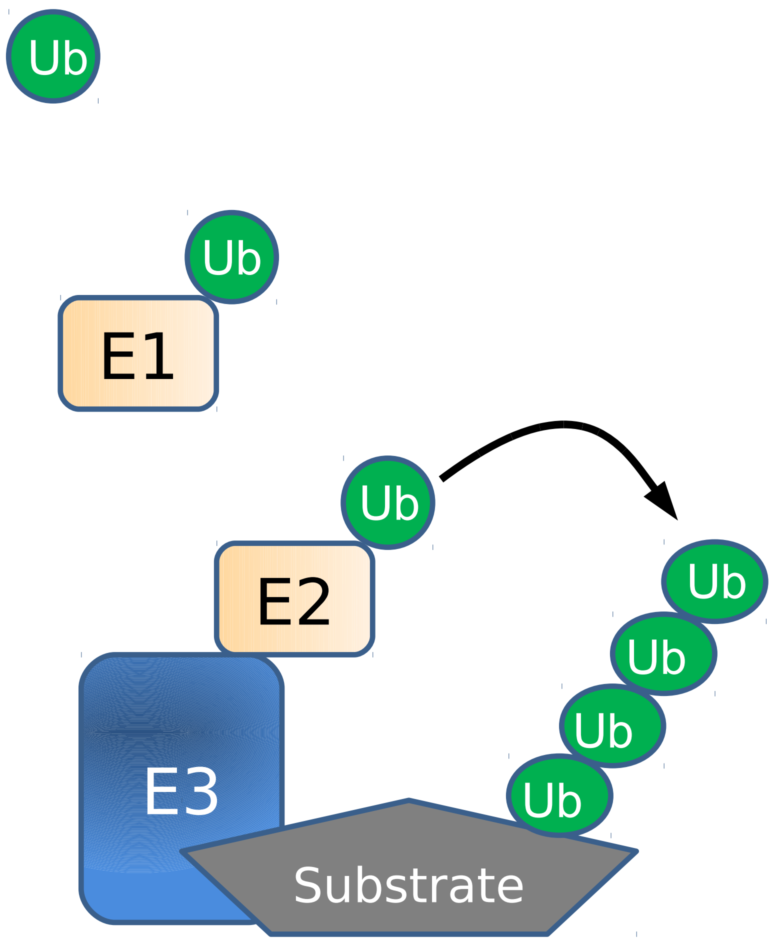
AXR1 function?

Sequence similarity searches with Genbank database:

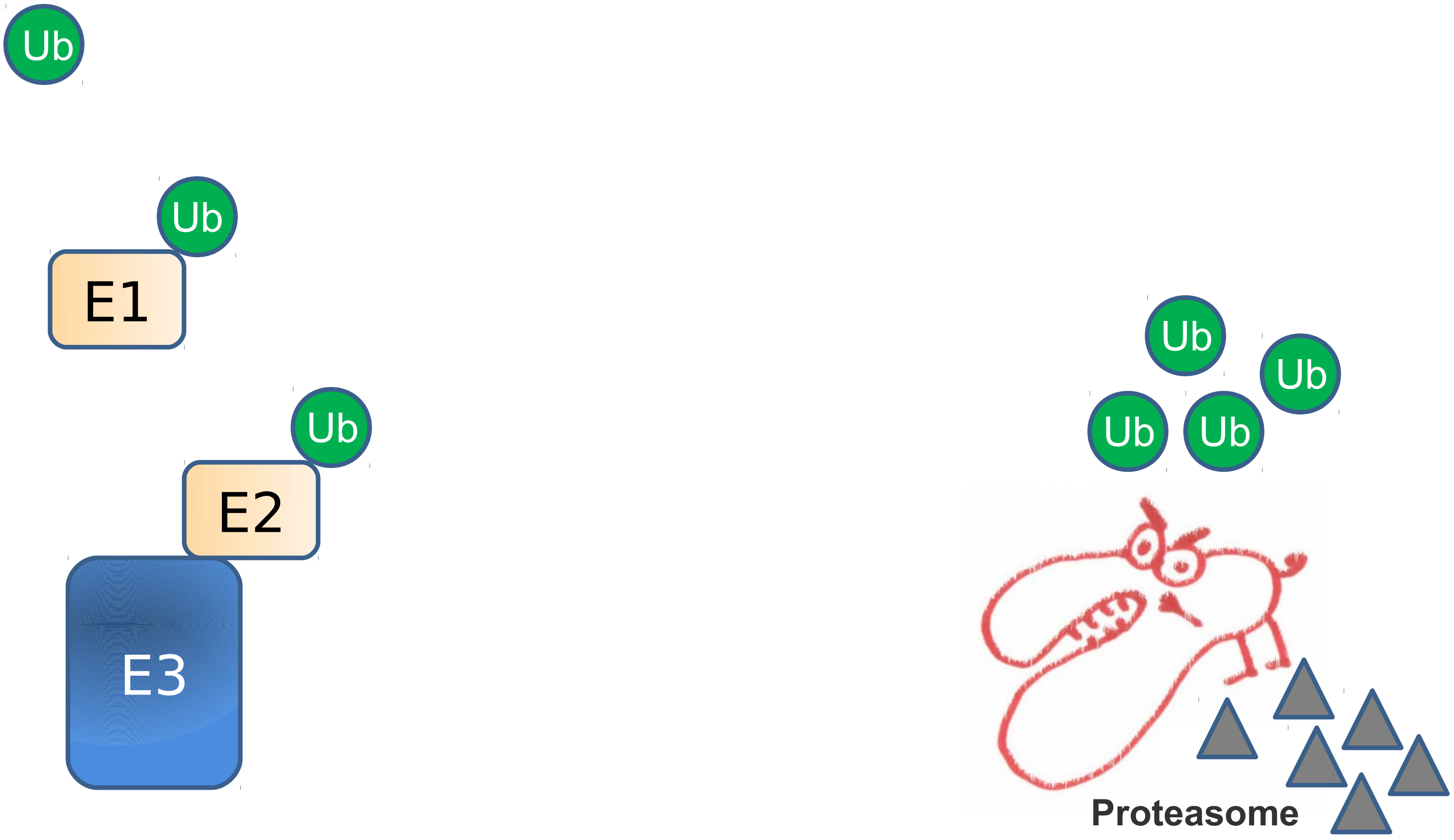
AXR1 Protein: 540 AS, ca. 60 kD

→ Similarity to human and yeast E1 ubiquitin activating enzymes

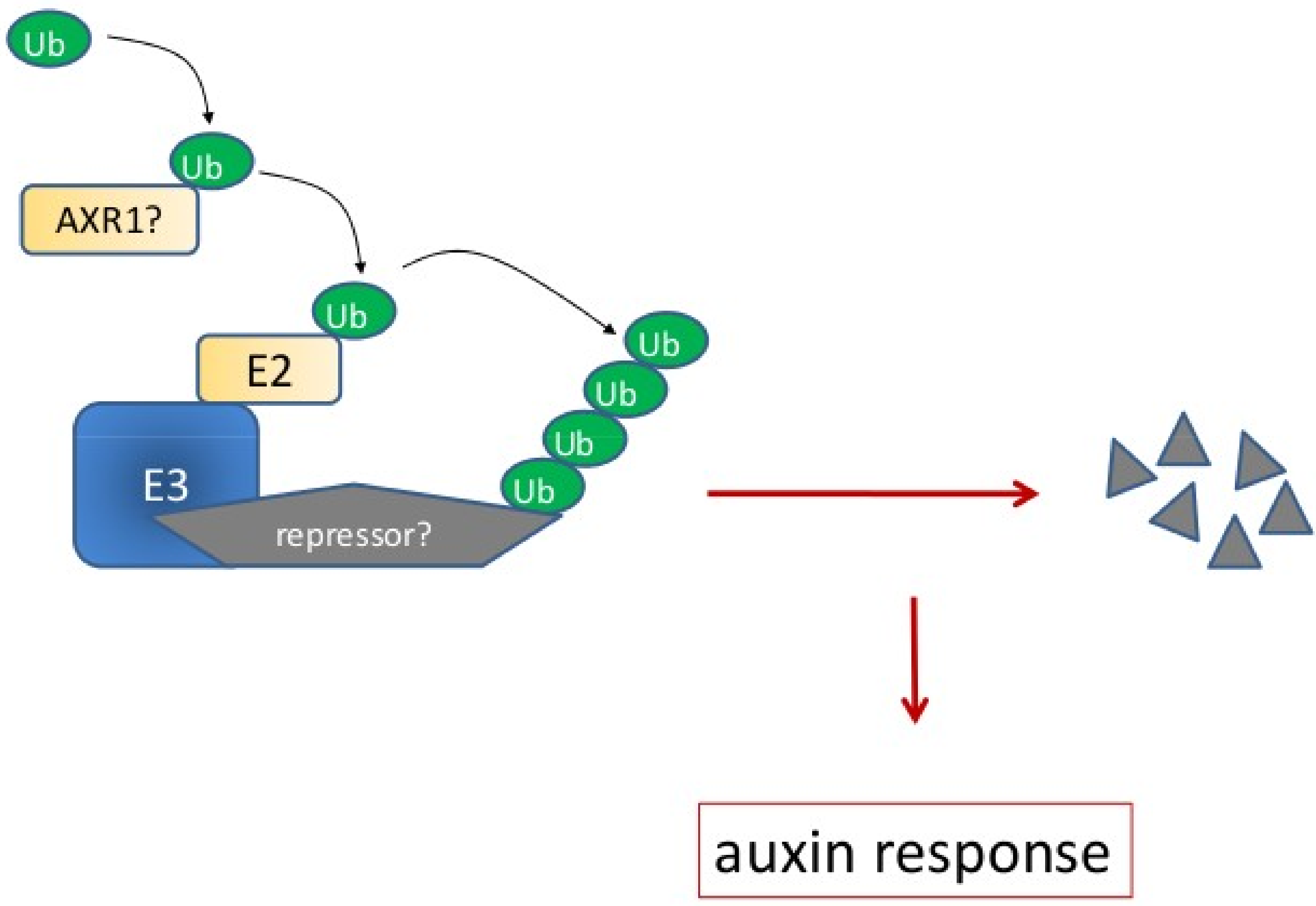
The ubiquitin system



The ubiquitin system



Preliminary model of auxin signaling



How could protein degradation be associated with the gene activation hypothesis?

Allgemeines zum paper Aufbau / zur paper Präsentation

- Abstract
  - Zusammenfassung des papers
- Introduction
  - Einleitung / Vorstellung der Hintergründe
- Materials/Methods
  - was wurde wie gemacht
- Results
- Diskussion
  - Ergebnisse werden evaluiert und in Zusammenhang mit Daten aus der Literatur gebracht
- Literature cited

- Hintergrund / Einleitung /Ausgangsmodel

- Zielstellung!
- Ergebnisse (anhand der Abbildungen)
- Diskussion
- Fazit: welche Erkenntnisse sind dazu gekommen

Model erweitern

Präsentationen werden nach dem jeweiligen Seminar alle auf die Webseite gestellt!
http://quintlab.openwetware.org/Teaching.html