

# 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

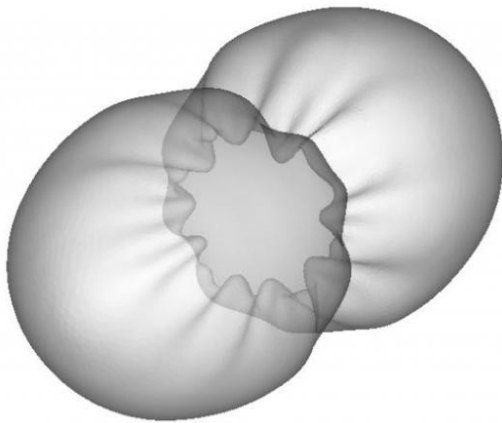
- Terminvergabe

# Introduction

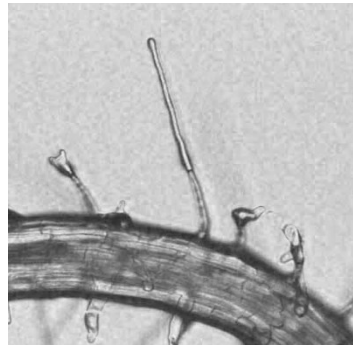
## Definition:

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.

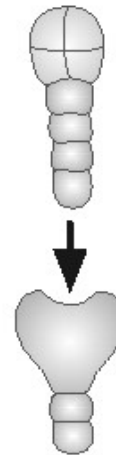
- Hormone (greek) → to excite
- In general, plant hormones control plant growth and development by affecting the division, elongation, and differentiation of cells.



cell division



cell  
elongation

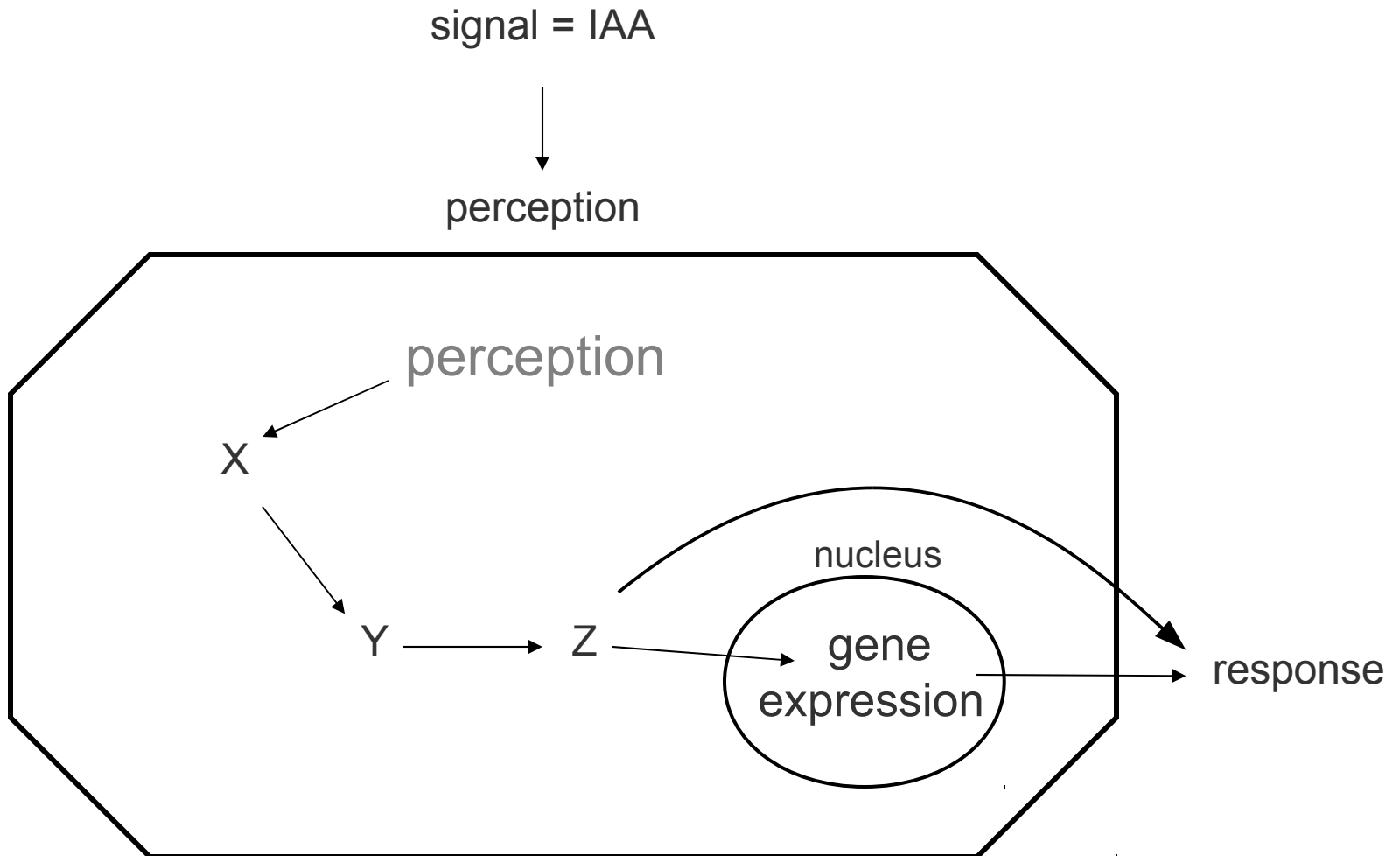


cell  
differentiation

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

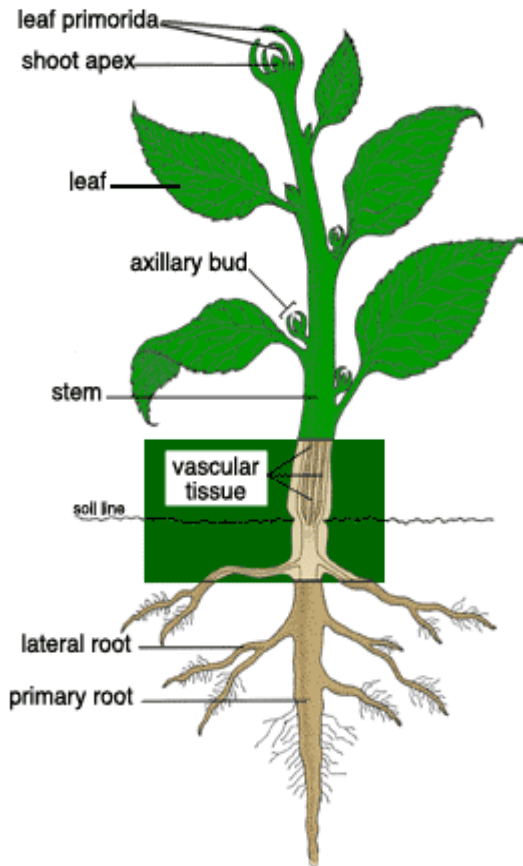
Excluding: biosynthesis, metabolism, transport

# 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

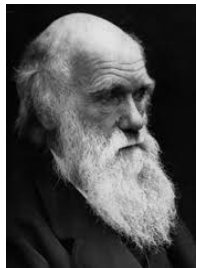
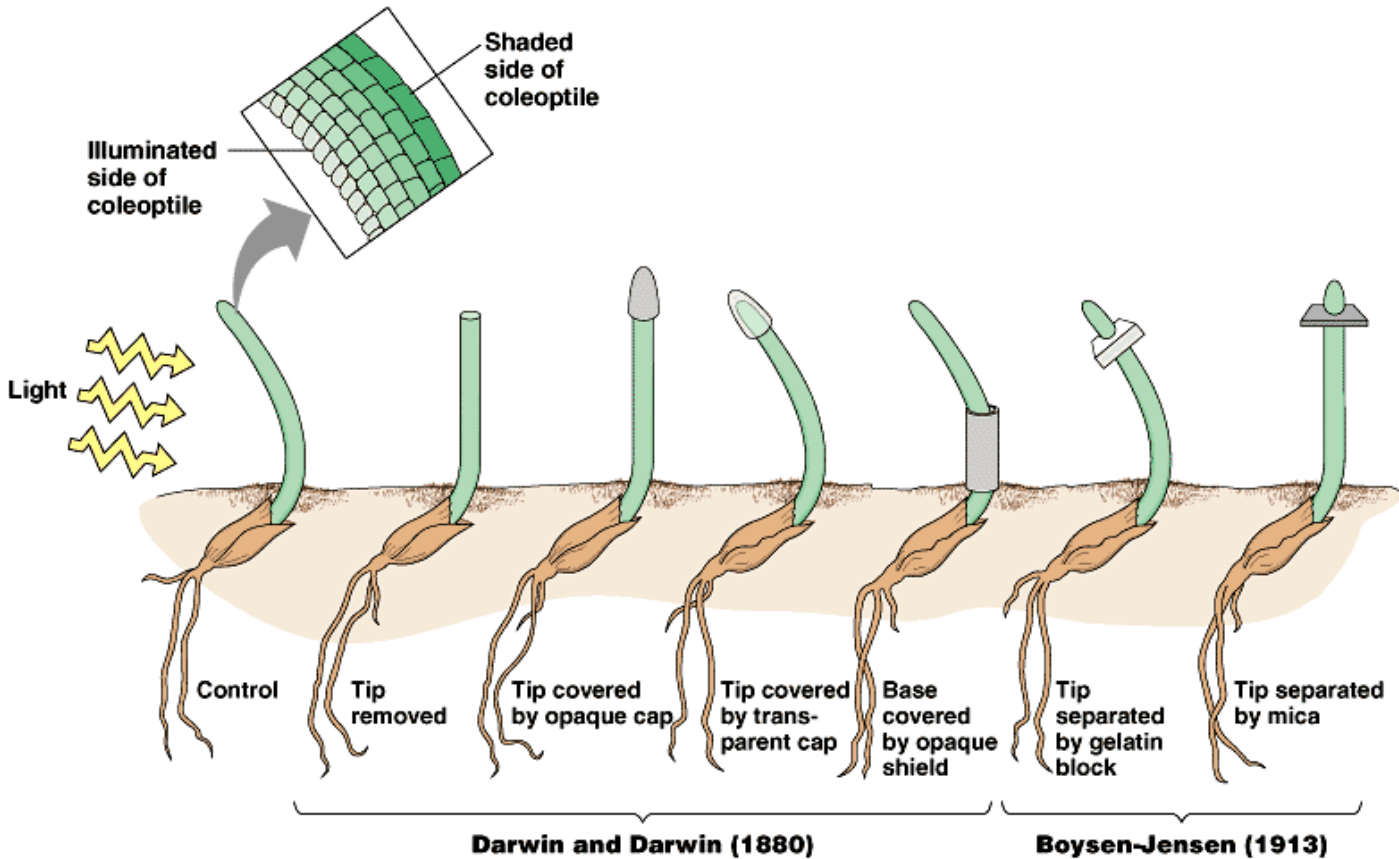


**1880**

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

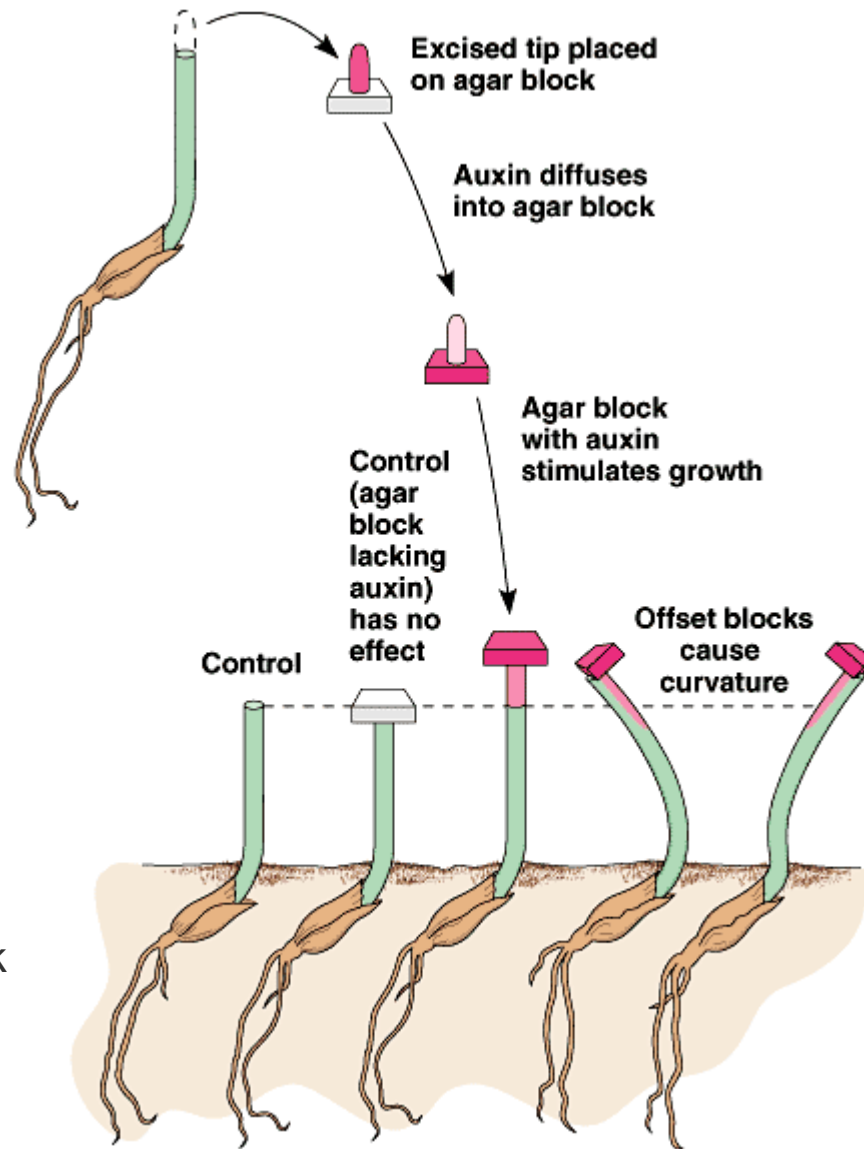
# Auxin History II - 1880-1935



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.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
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

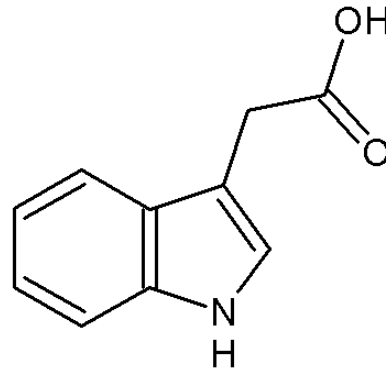
# Auxin History II - 1880-1935



Kenneth V. Thimann  
1960

*Supplement to "Nature," January 19, 1935*

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



IAA  
**indole-3-acetic acid**

→ 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

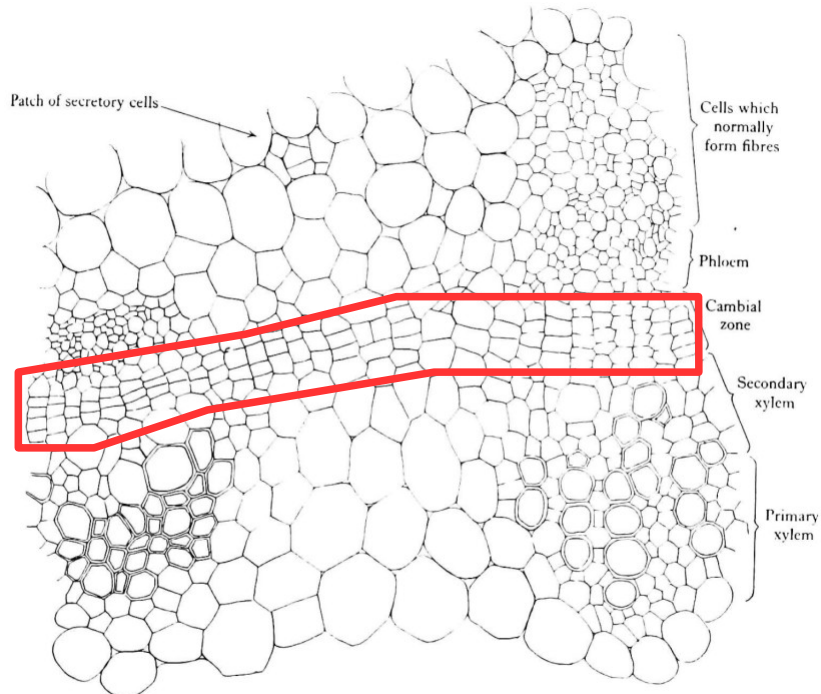


Fig. 4, Exp. 3. Experimental plant no. 1. Section at 10 mm. below top, and 5 mm. below zone covered by gelatins and auxin-*a*.  $\times 202$ .

- IAA

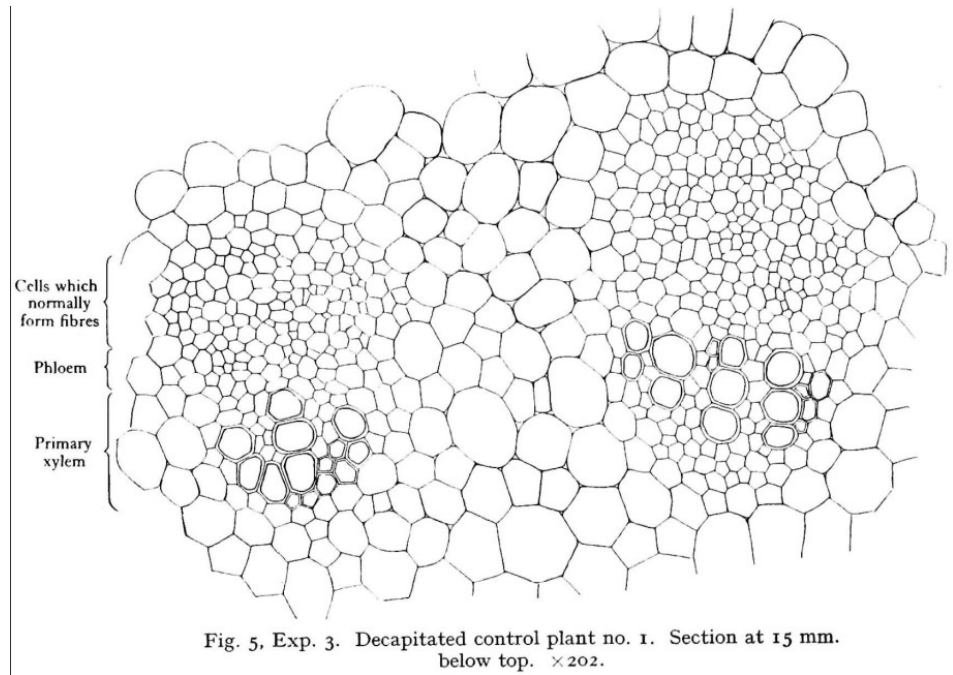


Fig. 5, Exp. 3. Decapitated control plant no. 1. Section at 15 mm. below top.  $\times 202$ .

→ 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<sup>1</sup>

Julius Silberberger, 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?

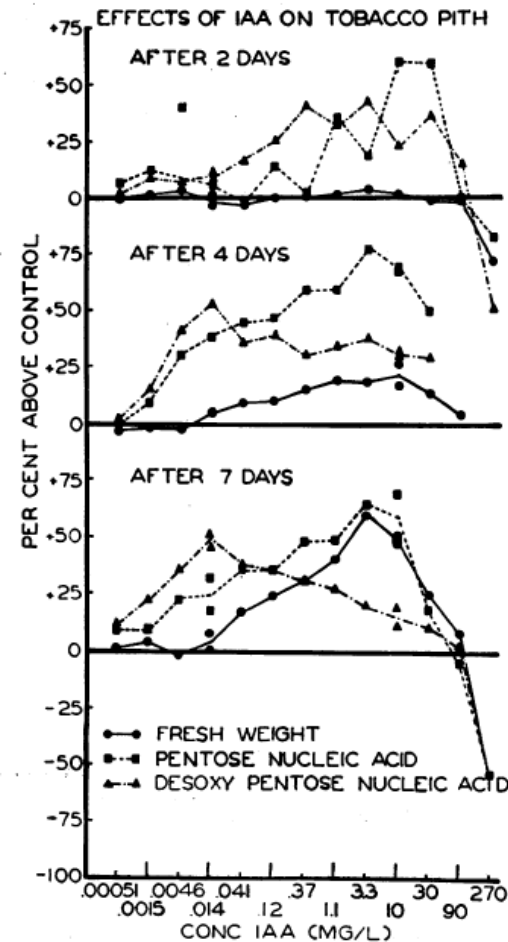


FIG. 1. Changes as per cent of controls in fresh weight; DNA content, and PNA content in excised tobacco pith tissue disks cultured on a sucrose agar medium with serial concentrations of IAA for 2, 4, and 7 days.

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<sup>1</sup>

## 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

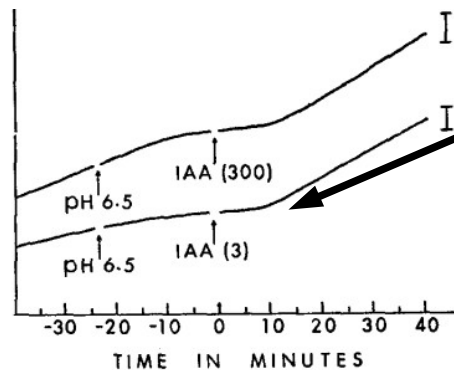


FIGURE 4. Effect of widely different IAA concentrations on timing of elongation response of oat coleoptile segments. At the first arrow water in chamber was replaced with Na citrate buffer,  $10^{-3}$  M, pH 6.5; at the second arrow this was replaced with the same medium containing either 3 or 300  $\mu\text{g}/\text{ml}$  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

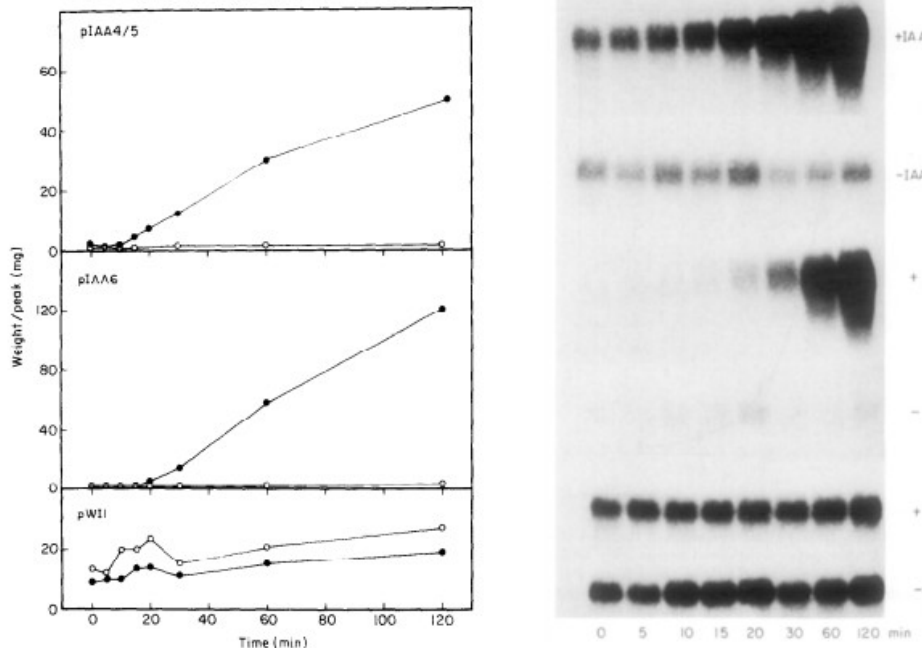
# Auxin History III - 1935-1985

*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.*



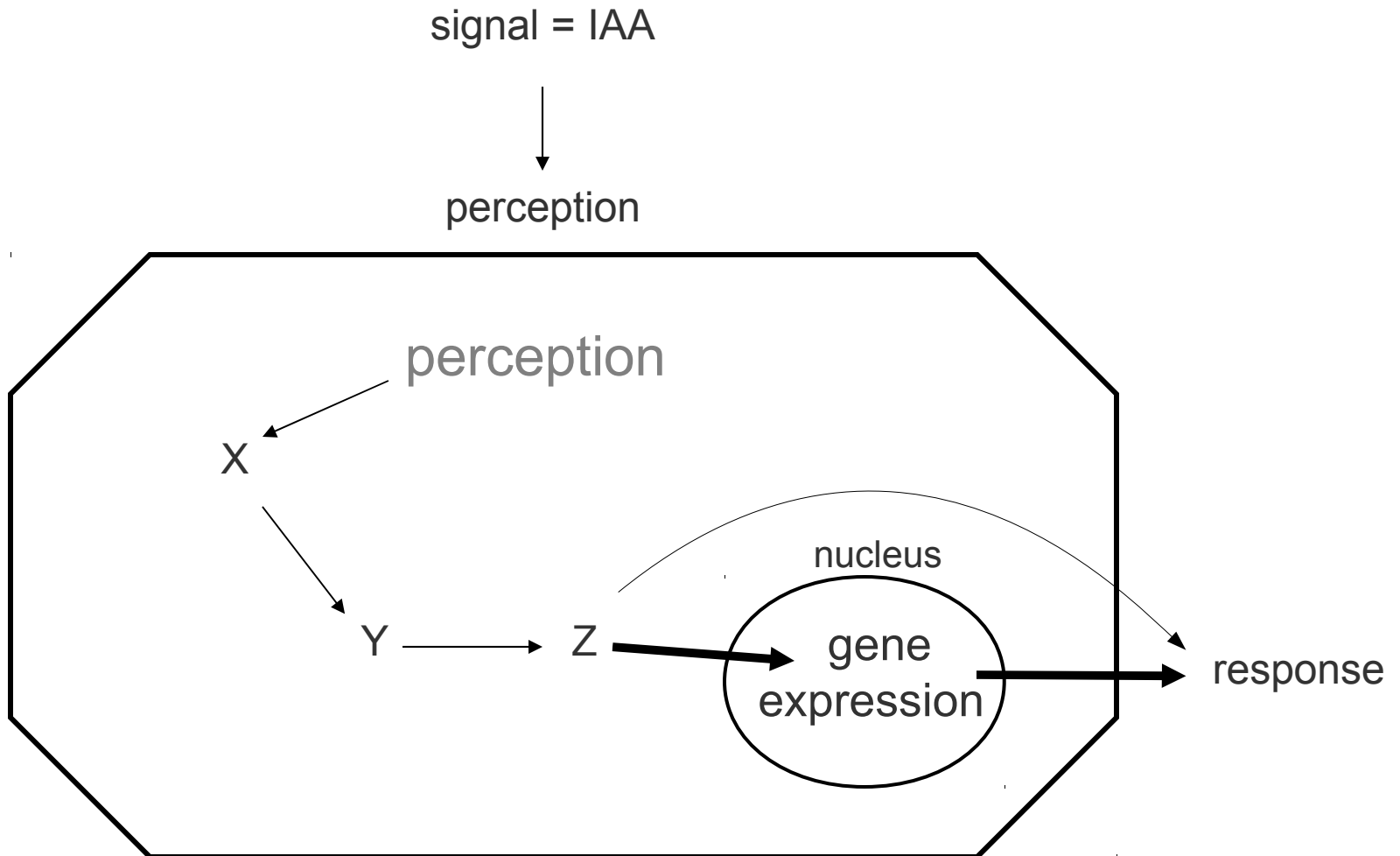
**Figure 3.** Induction kinetics of the IAA-inducible mRNAs. Endogenous IAA-depleted pea segments were treated with or without 20  $\mu$ M-IAA. Poly(A)<sup>+</sup> RNA was isolated at various time intervals from 2 g fresh weight (100 segments) of control or IAA-treated tissue: 20  $\mu$ g of poly(A)<sup>+</sup> 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 <sup>32</sup>P-labeled pIAA4/5, pIAA6 and pW11 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 controlling *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)

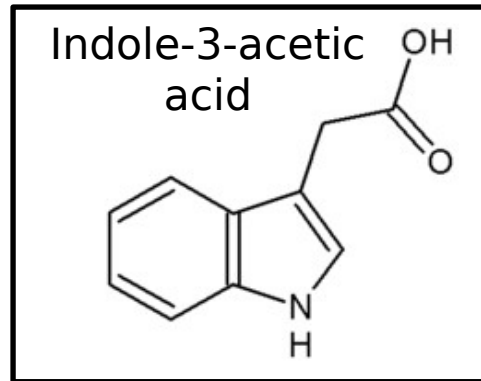
Easily transformable

Tremendous community resources

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**A power multicellular eukaryotic model system**

# Auxin regulates plant development

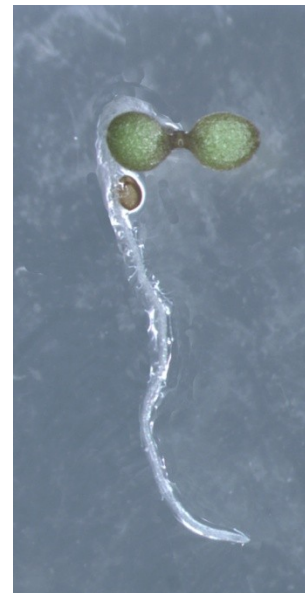


▷ Embryonic patterning

Growth & Apical dominance

Root development

Tropic growth responses

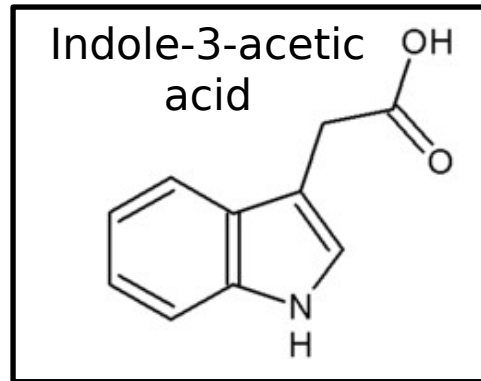


wild-type



*bdl axr1*  
mutant

# Auxin regulates plant development



Embryonic patterning

▷ Growth & Apical dominance

Root development

Tropic growth responses



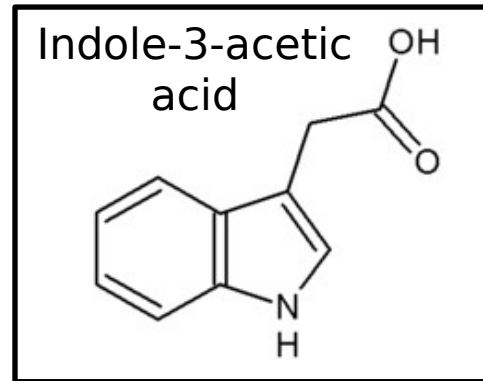
wild-type



*axr6-3* mutant



# Auxin regulates plant development

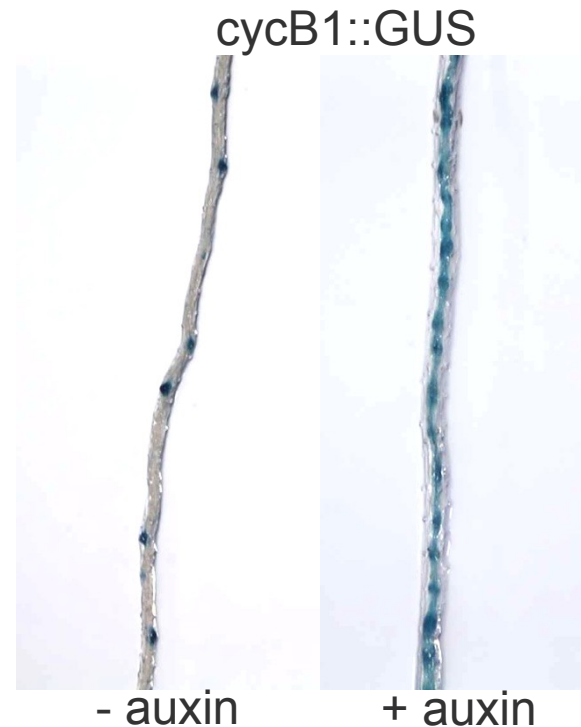


Embryonic patterning

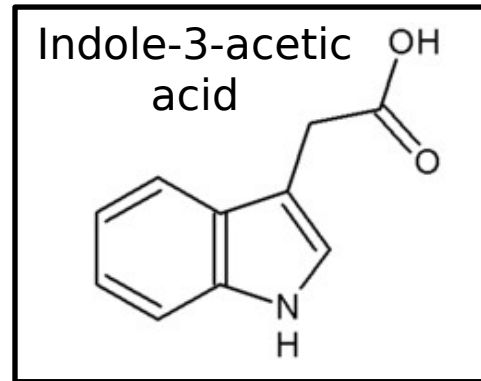
Growth & Apical dominance

▷ Root development

Tropic growth responses



# Auxin regulates plant development



Embryonic patterning

Growth & Apical dominance

Root development

▷ Tropic growth responses



wild-type *aux1* mutant

## **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

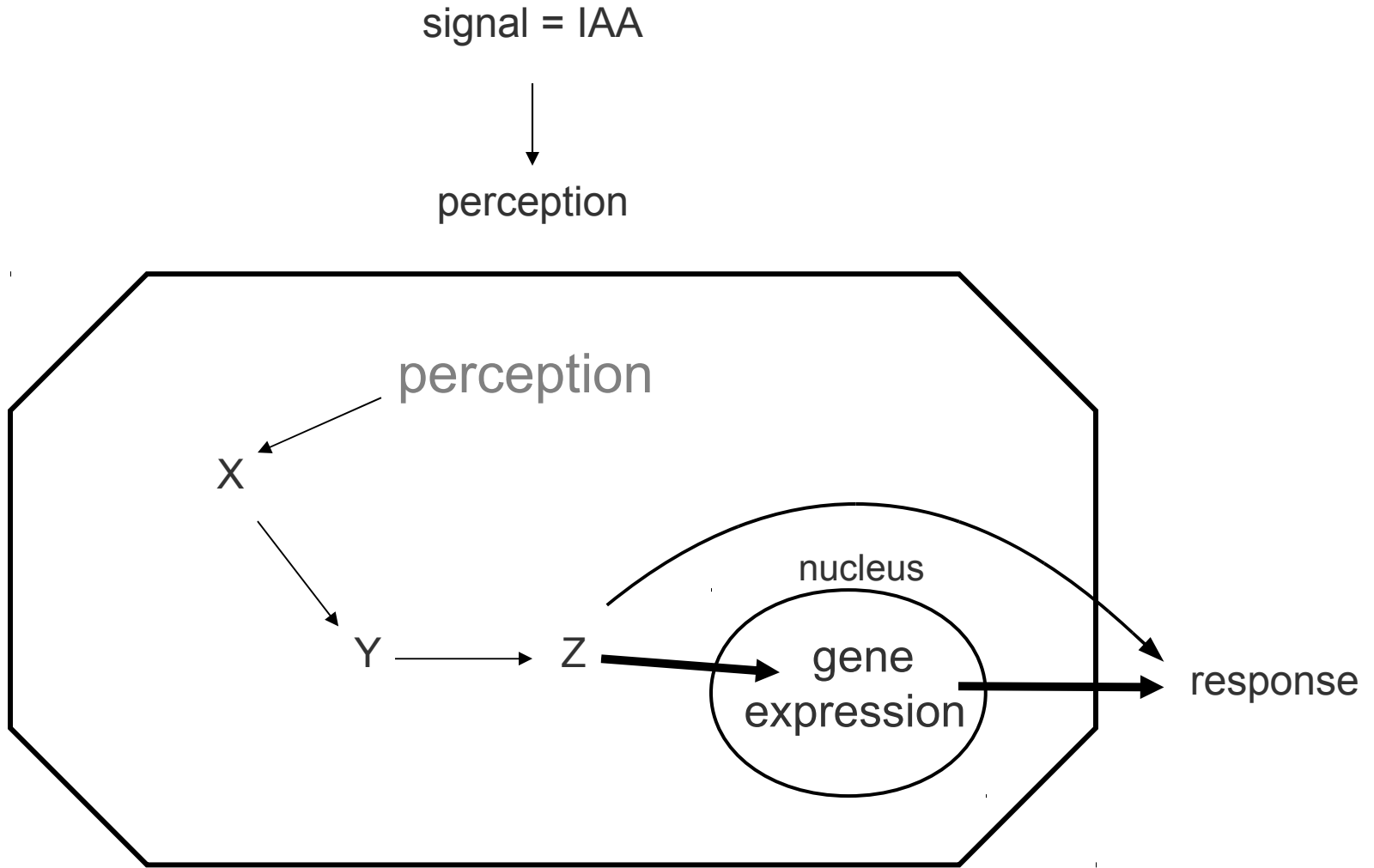


Depends most likely on *de novo* gene expression/protein synthesis

What was NOT known?

- enzymes of auxin biosynthesis
- auxin transport facilitators
- auxin signaling elements
  
- Arabidopsis genome sequence

# Black box of signaling



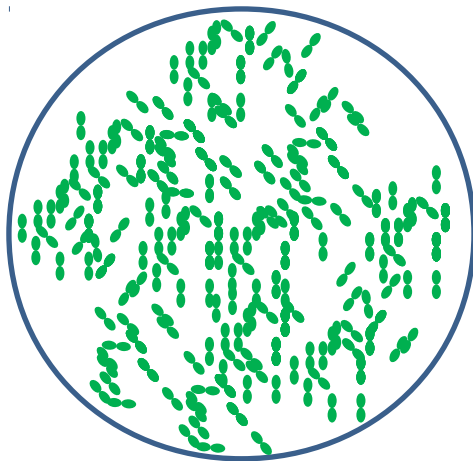
**Genetic approach?**

# Mutagenesis

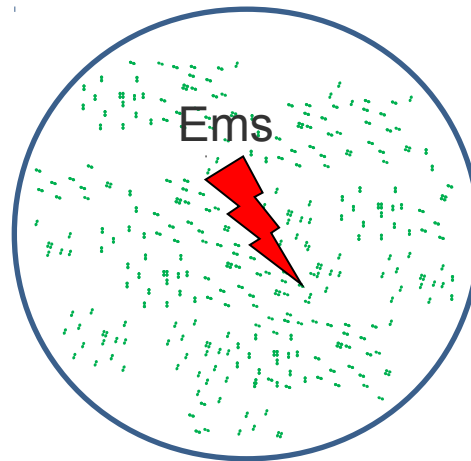
Prerequisite:

- Phenotype associated with trait of interest

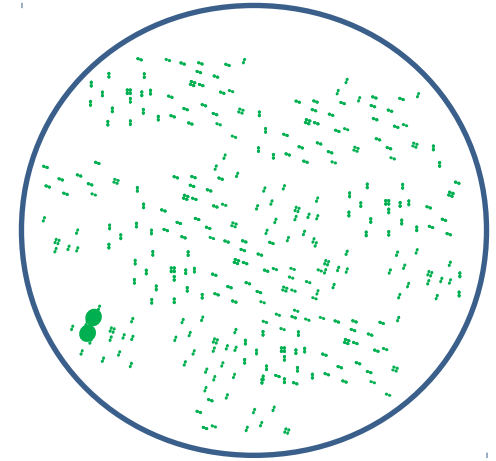
└─ Seedling development → screen for mutants defect in wild-type response



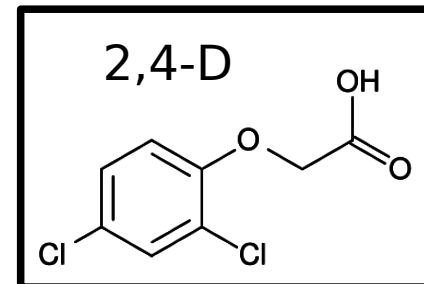
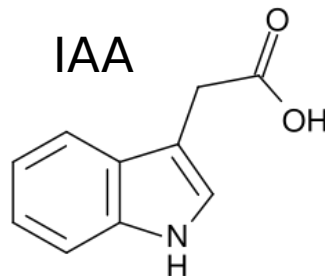
-  
auxin



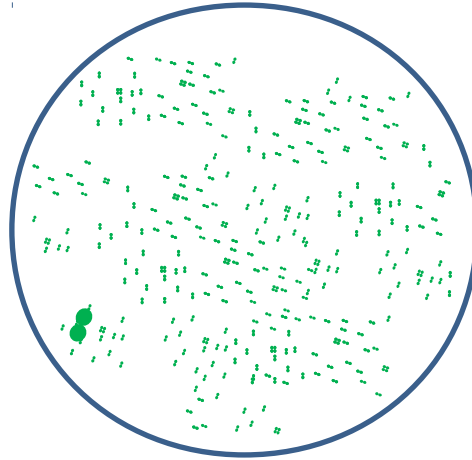
+  
auxin



+  
auxin



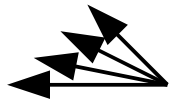
# Mutant/Gene nomenclature



+  
auxin

Same mutant screen,  
different genes:

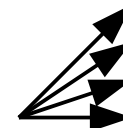
*axr1*  
*axr2*  
*axr3*  
*axrx*



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

alleles:

*axr1-1*  
*axr1-2*  
*axr1-3*  
*axr1-x*

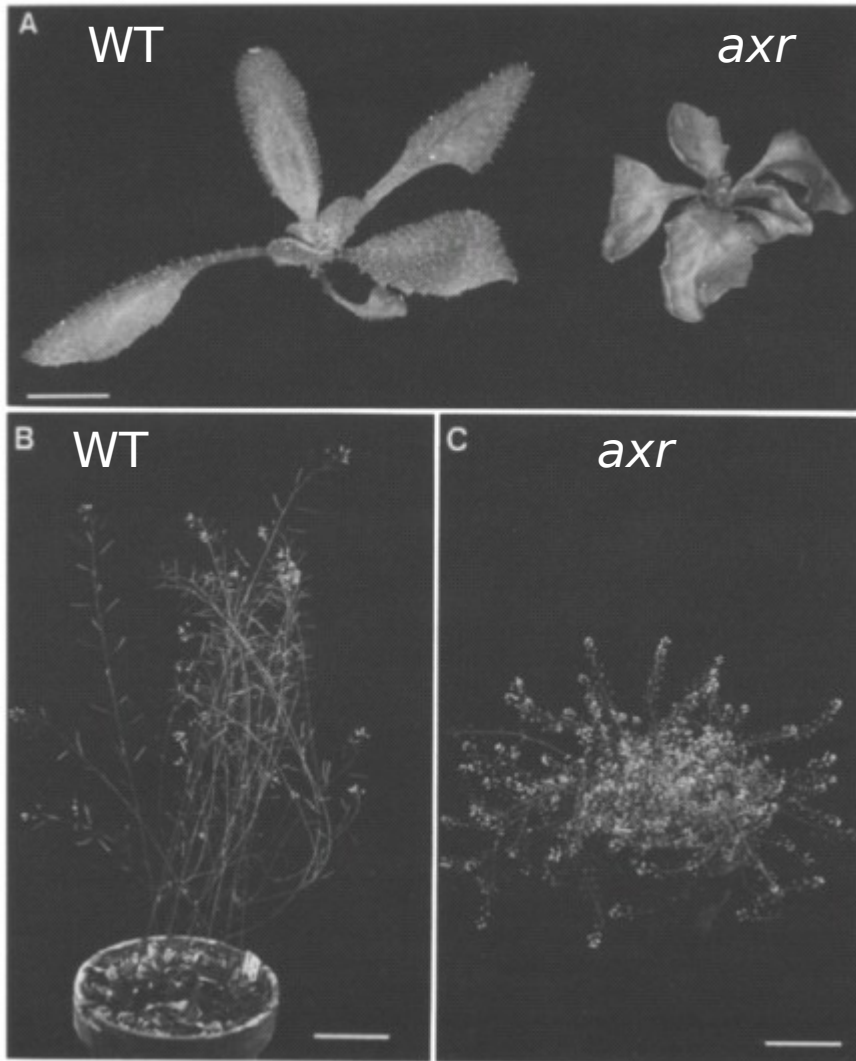


Name of the gene: *AUXIN RESISTANT 1 - AXR1*

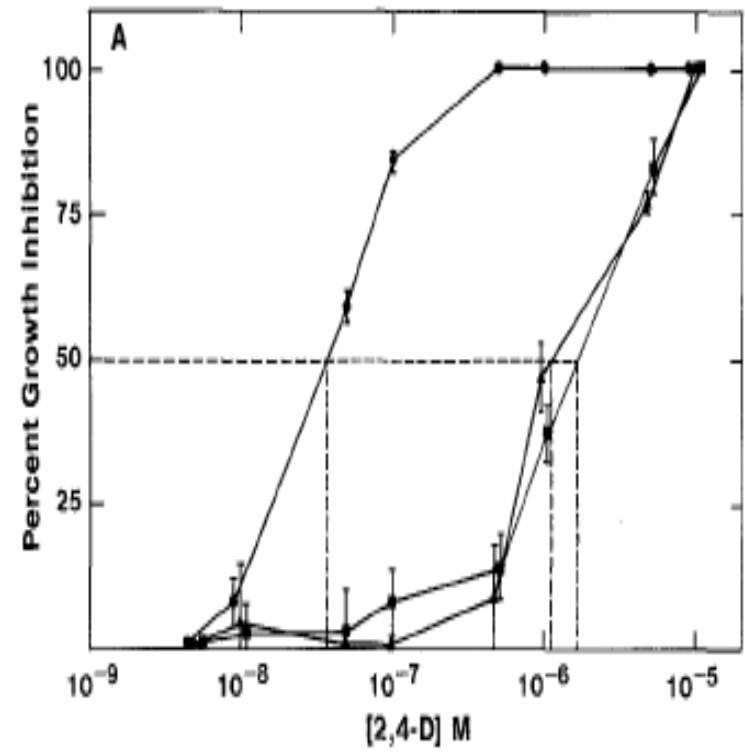


Name of the protein: *AUXIN RESISTANT 1 - AXR1*

# 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



## **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...”**



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

**Cynthia Lincoln, James H. Britton, and Mark Estelle<sup>1</sup>**

Department of Biology, Indiana University, Bloomington, Indiana 47405

## **Aims:**

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

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

Cynthia Lincoln, James H. Britton, and Mark Estelle<sup>1</sup>

Department of Biology, Indiana University, Bloomington, Indiana 47405

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

**Table 1.** Recovery of *axr1* Mutants<sup>a</sup>

M2 population	Mutagen	Selection	Mutants recovered
A <sup>b</sup>	EMS	2,4-D	<i>axr1-1</i> <i>axr1-2</i> <i>axr1-3</i> <i>axr1-4</i> <i>axr1-5</i> <i>axr1-6</i>
B <sup>b</sup>	EMS	2,4-D	<i>axr1-7</i> <i>axr1-8</i> <i>axr1-9</i> <i>axr1-11</i> <i>axr1-12</i> <i>axr1-15</i>
C <sup>c</sup>	EMS	2,4-D	<i>axr1-16</i> <i>axr1-17</i> <i>axr1-18</i> <i>axr1-19</i> <i>axr1-20</i> <i>axr1-21</i>
C <sup>c</sup>	EMS	IAA	<i>axr1-22</i>
D <sup>c</sup>	$\gamma$	2,4-D	<i>axr1-23</i>

<sup>a</sup> A total of 470,000 seeds from four distinct M2 populations was screened for mutants that were able to elongate roots on either 5  $\mu$ M 2,4-D or 50  $\mu$ M IAA.

<sup>b</sup> Estelle and Somerville (1987).

<sup>c</sup> This study.

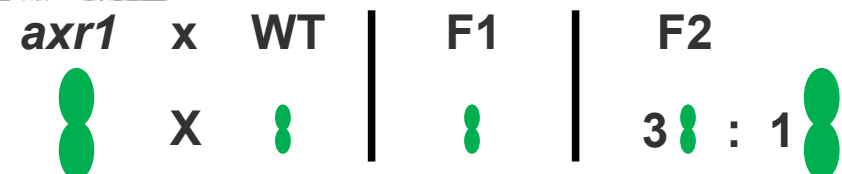
Segregation analysis:

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

Cross	Number of Plants		$\chi^2$ <sup>a</sup>
	Resistant	Sensitive	
<i>axr1-19</i> × wild-type F1	0	23	
F2	186	493	2.07 <sup>b</sup>
<i>axr1-21</i> × wild-type F1	0	51	
F2	82	281	1.12 <sup>b</sup>
<i>axr1-22</i> × wild-type F1	0	22	
F2	56	216	2.83 <sup>b</sup>
<i>axr1-23</i> × wild-type F1	0	33	
F2	117	383	0.683 <sup>b</sup>

<sup>a</sup>  $\chi^2$  was calculated based on an expected ratio of three sensitive to one resistant.

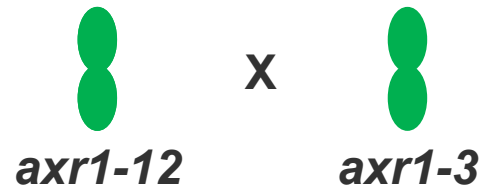
<sup>b</sup>  $P > 0.05$ .



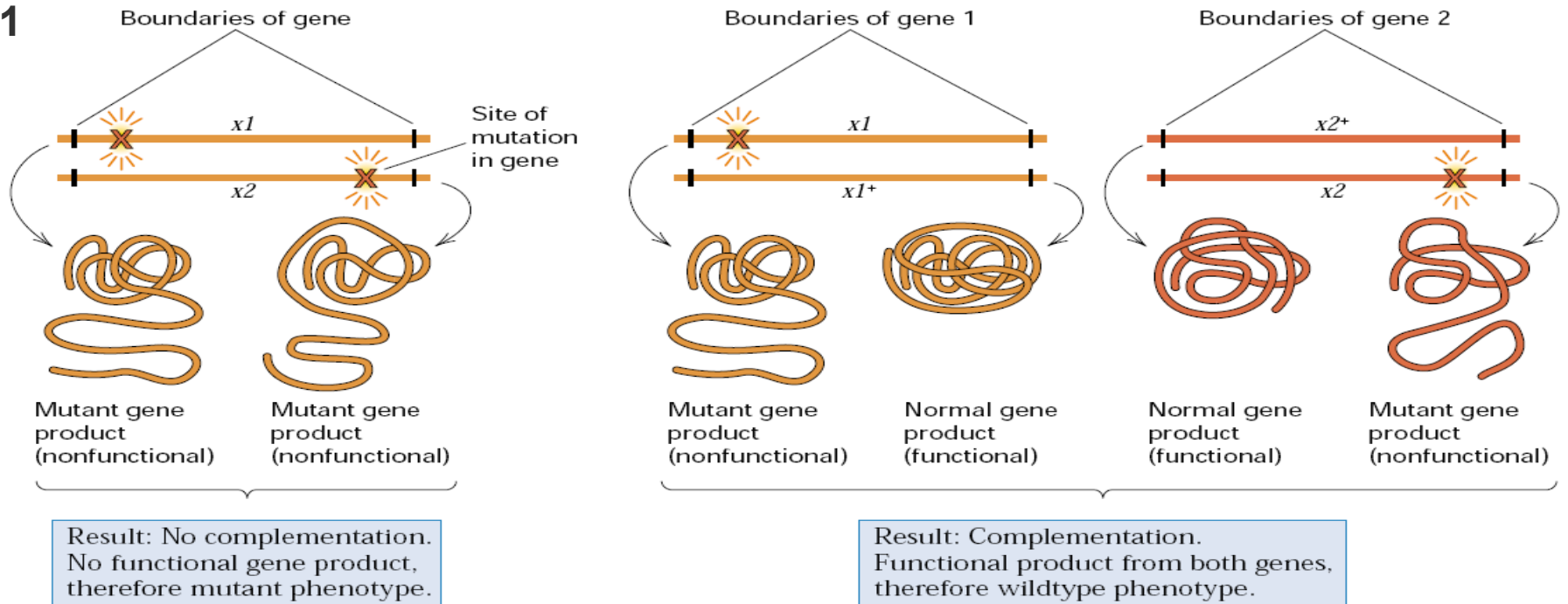
*axr1*-like mutant phenotypes are inherited recessively  
 → typical for loss-of-function alleles

# *axr* mutations in the same gene?

P



F1



**Mutant phenotype**  
→ same gene/allelic



**Wild type phenotype**  
→ different genes

# Complementation test

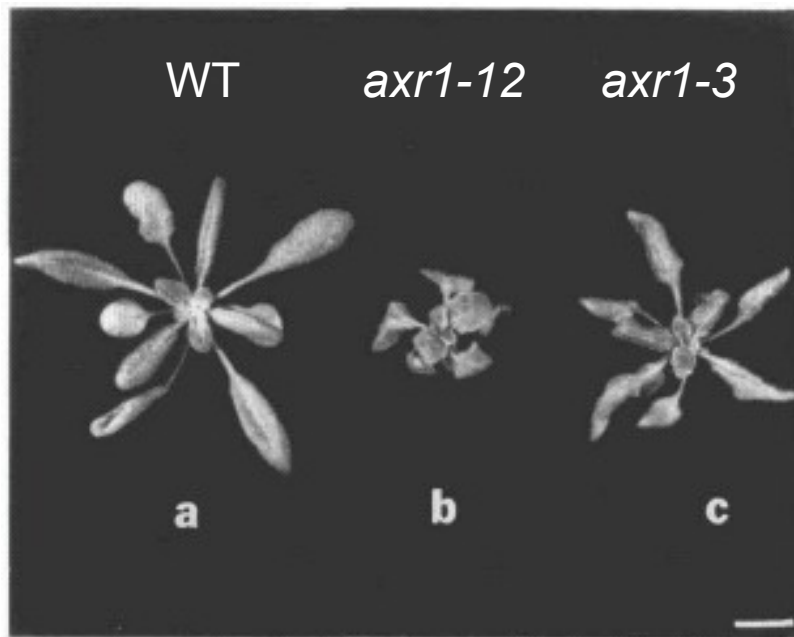
**Table 3.** Complementation Analysis of *axr1* Lines

Cross	Number of Plants	
	Resistant	Sensitive
<i>axr1-12</i> × <i>axr1-3</i>	33	0
<i>axr1-19</i> × <i>axr1-3</i>	21	0
<i>axr1-20</i> × <i>axr1-3</i>	13	0
<i>axr1-22</i> × <i>axr1-3</i>	24	0
<i>axr1-23</i> × <i>axr1-3</i>	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.

→ Allelic differences also in root growth assays

**Alleles show different expression of morphological and response defects**



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

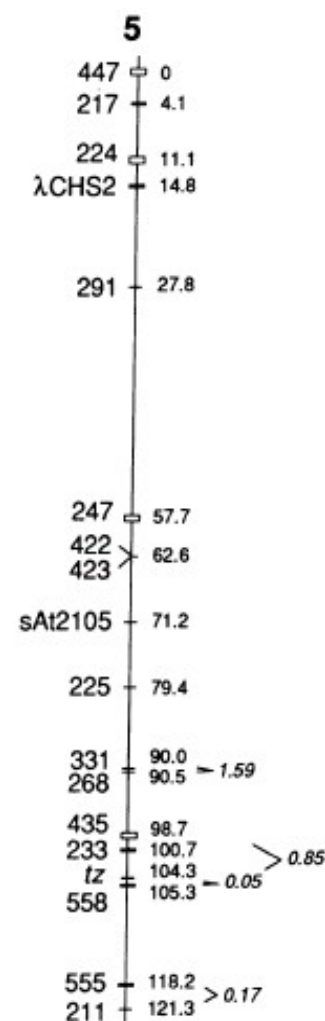
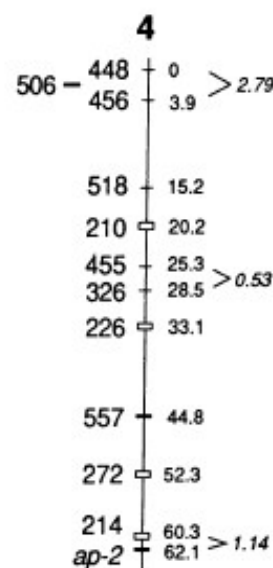
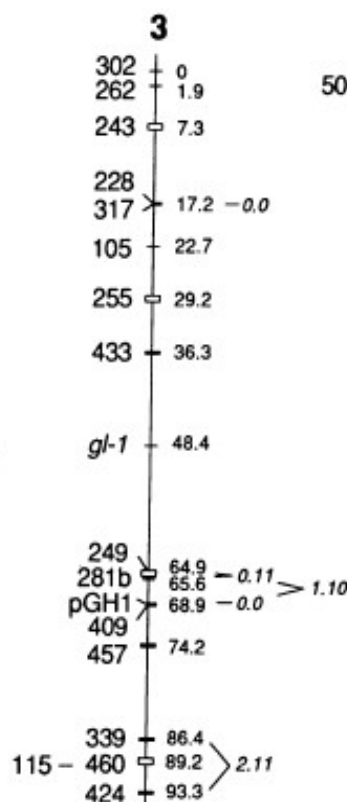
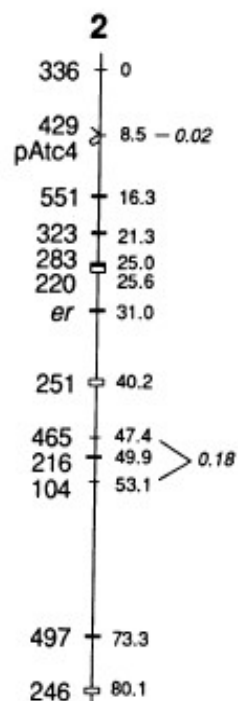
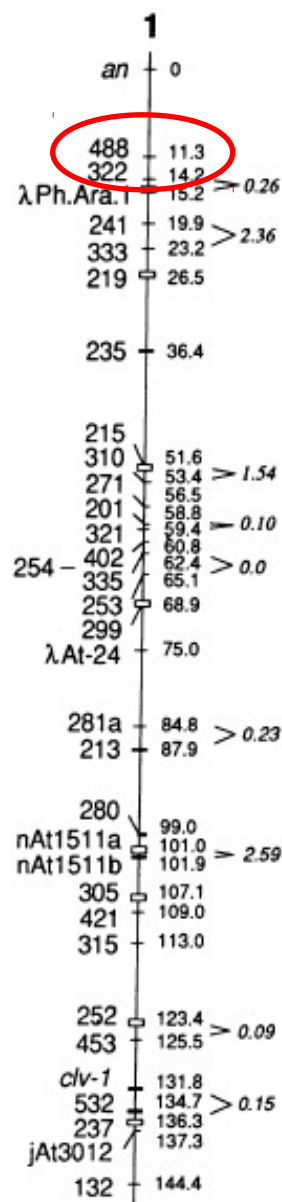
Wild-type and mutant plants were photographed when 7 weeks old.

(a) Wild type.

(b) *axr1-12/axr1-12*.

(c) *axr1-3/axr1-3*.

Bar = 3 cm.



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

Markers	Recombination Frequency (%)	Number Scored <sup>a</sup>	$\chi^2$ Associated <sup>b</sup>	P
RFLP 219	12.4 ± 2.0	136	123.3	<0.05 <sup>c</sup>
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

<sup>a</sup> Refers to number of F3 families scored.

<sup>b</sup>  $\chi^2$  associated is the total  $\chi^2$  adjusted for deviations of each individual marker from Mendelian segregation.

<sup>c</sup> P value < 0.05 indicates deviation from nonlinkage (i.e., linkage).

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

**Cynthia Lincoln, James H. Britton, and Mark Estelle<sup>1</sup>**

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 Timpfe, Douglas Lammer,  
Jocelyn Turner & Mark Estelle†**

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

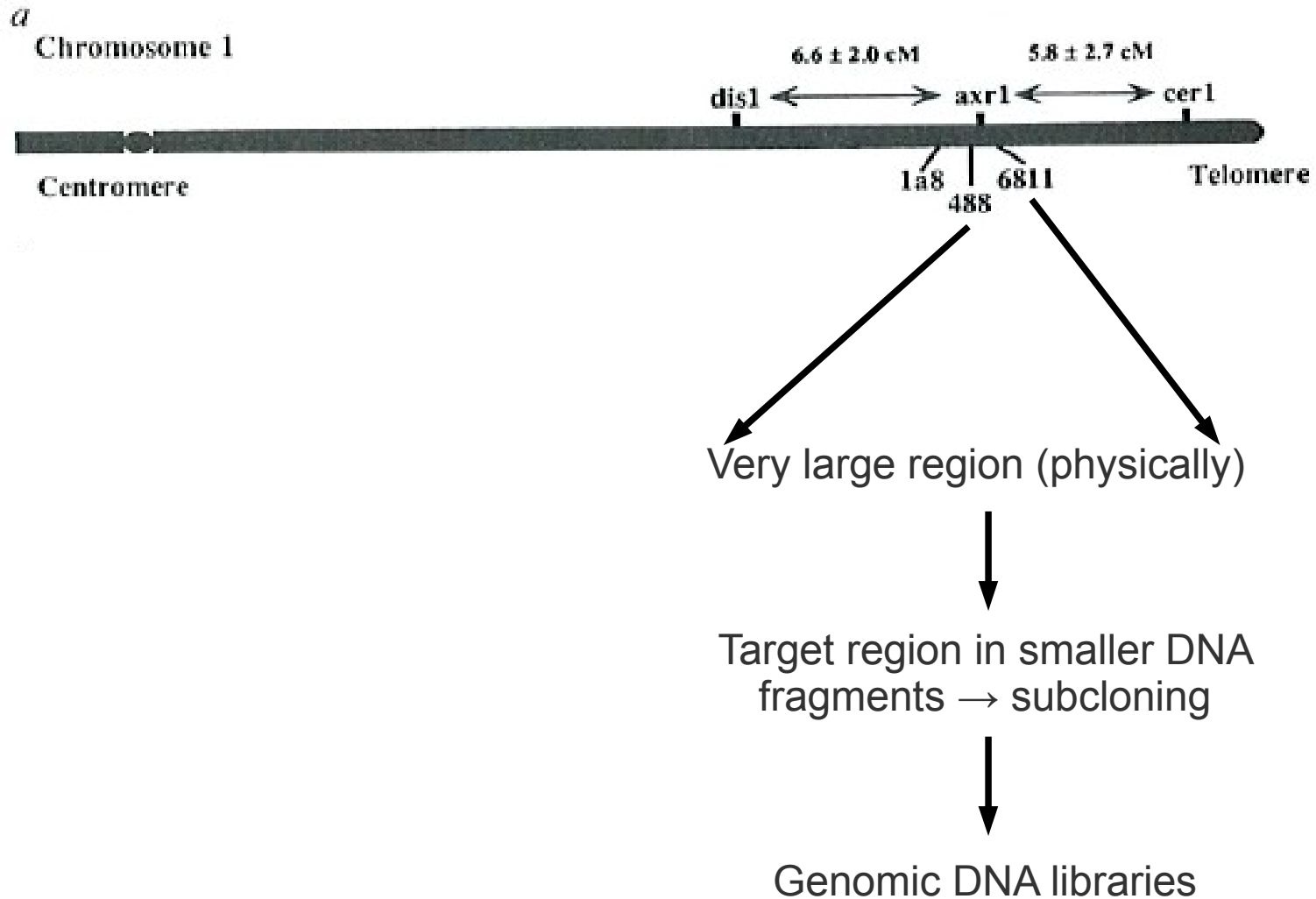
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**Aim:**

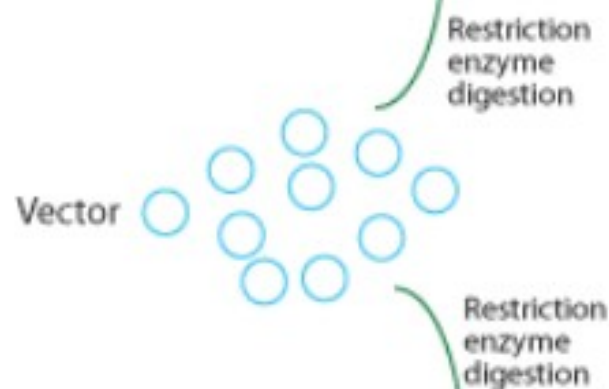
**- Fine-mapping and cloning of *AXR1***



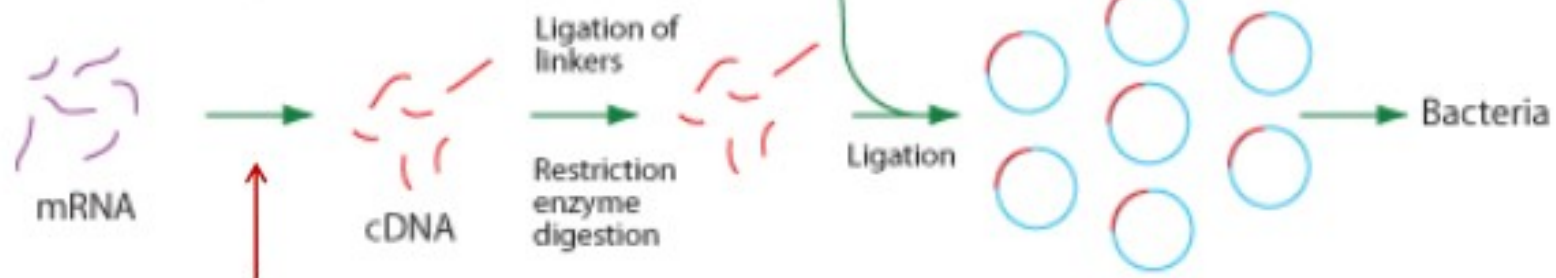
# *AXR1* - Chromosome Walking



## Genomic Library



## cDNA Library

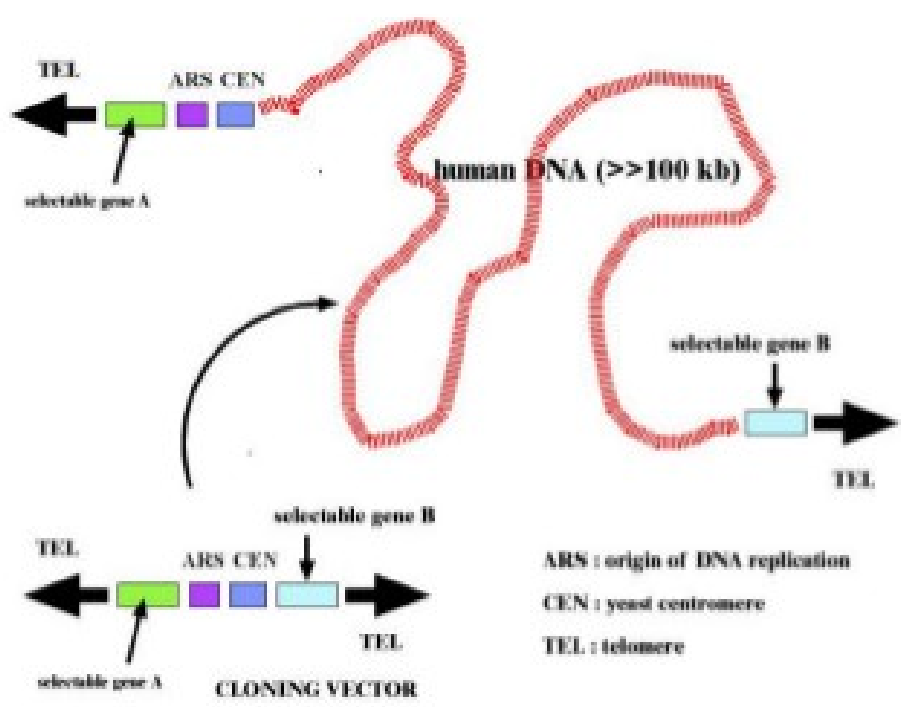


Reverse Transkription

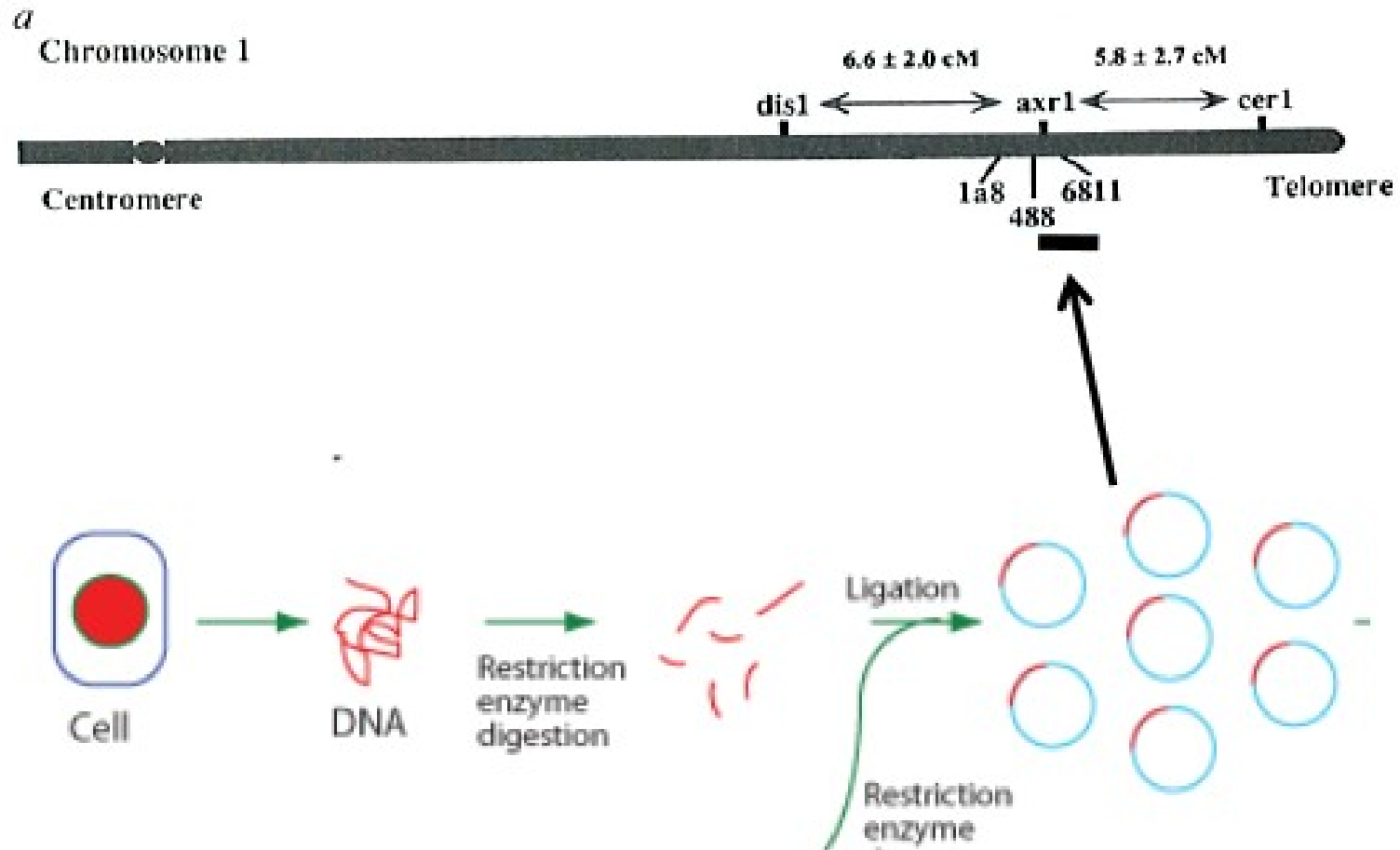
# Vectors for cloning of large DNA fragments – YACs, cosmids, ...

Approximate maximum length of DNA that can be cloned into vectors

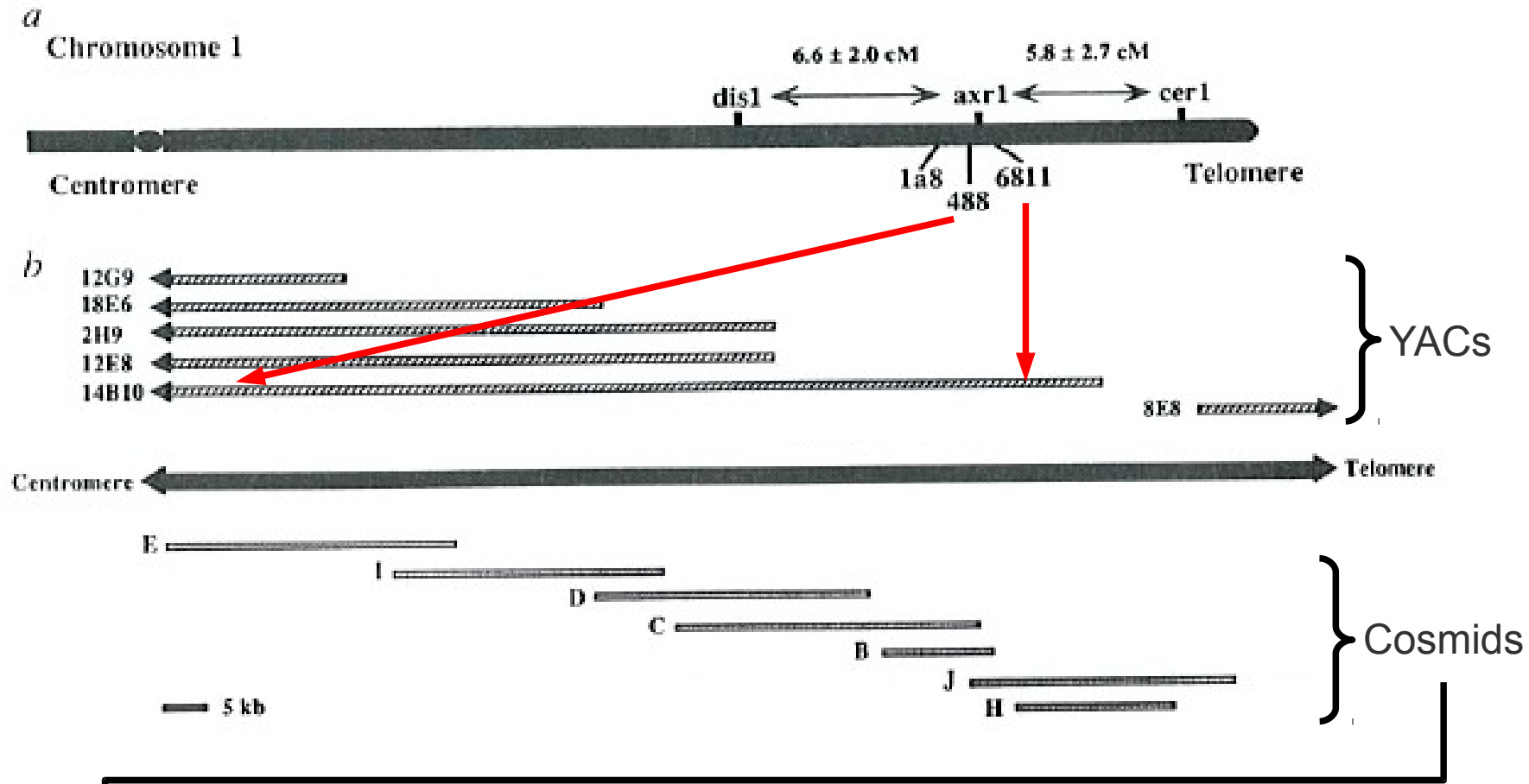
Vector type	Cloned DNA (kb)
<a href="#">Plasmid</a>	20
<a href="#">lambda phage</a>	25
<a href="#">Cosmid</a>	45
<a href="#">BAC</a> (bacterial artificial chromosome)	300
<a href="#">YAC</a> (yeast artificial chromosome)	1000



# *AXR1* - Chromosome Walking



# AXR1 - Chromosome Walking

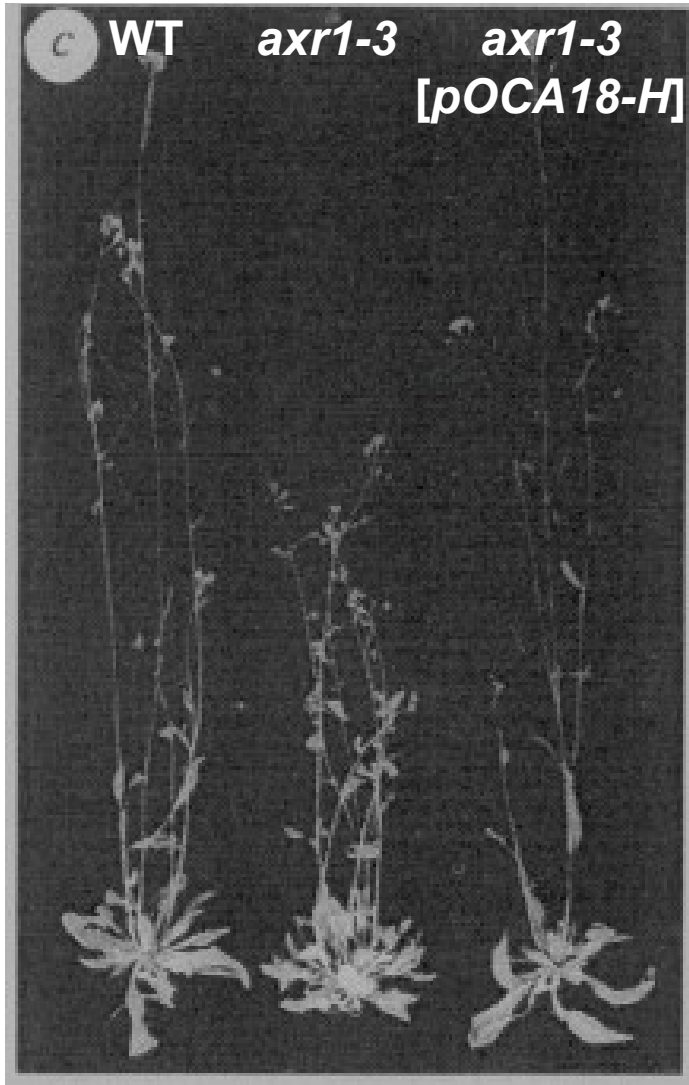


Transform cosmid clones E – H by agrobacterium transformation into *axr1-3* mutants



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



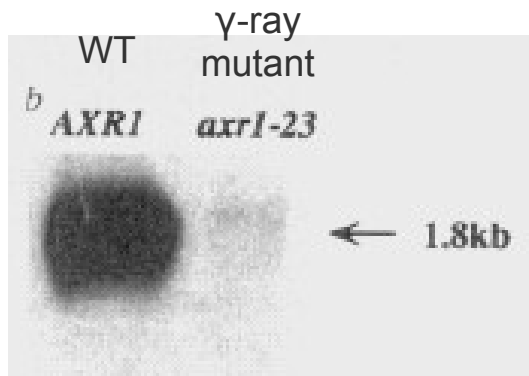
Northern Blot

→ How much transcript (1.8 kb mRNA) is 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

C

```

24      1      2      3      4      5      6      7      8      9      10     11     12     13     14     15     16     17     18     19     20     21     22     23     24
1  ATGCAAGTGGTAAAGACATCCAGGACACATGTTGAGGAGAGAGCCAAACATGCTAGAACCTAAAGCCAGGTACGATCGTTCGCTCAGGATTTTGGGGGGAAG
3  M Q A V K R S R S H V E E E P T H V E P K T R Y D S Q L R I H G E V

181     182     183     184     185     186     187     188     189     190     191     192     193     194     195     196     197     198     199     200     201     202     203     204
1  TAGCTCAAGGCGCTTCGCAAGAGAGGAGCTATCTGTTTACTCAATGTTGCGGCTTACTGCTTCGAGGCTTTCAAGCAATCTGTACTTGGTGGTGTGTTGGTAG
3  Q Q A A L E E A S I C L L H C G P T G S E A L R R L V L G G V G S

281     282     283     284     285     286     287     288     289     290     291     292     293     294     295     296     297     298     299     300     301     302     303     304
1  CATCACCGTGTGTTGATGGATCTAAAGTTCAATTTGCTACAGCTTGGGAAACATTTCTATGGTGGATGCGAAGAGCTGTTGGCCCAATCAAAAGCTAAATCTGTT
3  I T V V D G S R V G F C D L G H H P M V D A K S V G Q S R A K E Y

381     382     383     384     385     386     387     388     389     390     391     392     393     394     395     396     397     398     399     400     401     402     403     404
1  TGTGCGTTTCTTCAGGAGCTTATATATCTGTTAAGAGCAAGTTTATTTGAGGAGAGATGCGACACAGTTGATTTACTACTTAACCGAAGCTTCTCTCTCACT
3  P A F L Q S L H D S V M A K P I E E H P D T L I T T M F S F F S Q F

481     482     483     484     485     486     487     488     489     490     491     492     493     494     495     496     497     498     499     500     501     502     503     504
1  TCACCTCTGTTATAGCCACTCAAGCTGCTGTAAGATTTCAATTTTCAAGCTTCAAGATTTCTGAGAGTGGAAAGCTTAAGTTGGTTTGGTTGCTCTTA
3  T L Y I A T Q L V E D S H L K L D R I L R D A H Y K L V L V R E Y

581     582     583     584     585     586     587     588     589     590     591     592     593     594     595     596     597     598     599     600     601     602     603     604
1  TGGGCTTGTGGGTTTGTGCTGCTCTCTGTAAGGAGGACCCCATATTTGACTCAAAAGCTGATCATTTCTCTGACGAGCTTGGGCTGAAATATGATGG
3  V L A G F V K I E V E E H R I I D S E P D H F L D D L R L H N P W

681     682     683     684     685     686     687     688     689     690     691     692     693     694     695     696     697     698     699     700     701     702     703     704
1  CTTGAATTTAAGAGCTTTTGTGGAGACATTTGATCTGTAATCTGTCAGAGCGGCGCGGCTGCACATAGGCACATACCTTACCTGCTGATTTTGTAAAGATGG
3  P E L K R E F Y E T S D L M V S E P A A A H K R I P Y V Y I L V E H A

781     782     783     784     785     786     787     788     789     790     791     792     793     794     795     796     797     798     799     800     801     802     803     804
1  CTGAGGACTTGGGCTCAATCCATAGTGGTATCTTCTCTCAAGGAGGAGGAAAGAAAGATTTAAGGATTTGGTTAAGTCTGAAGATGTTATCTACGGA
3  F E H A G Q S H S G W L P S T R E E E E E E R D L V E S E H S T D

881     882     883     884     885     886     887     888     889     890     891     892     893     894     895     896     897     898     899     900     901     902     903     904
1  TGAAGATATATTAAGAAAGAGCAATTAAGCGGCTTTTCAAGATTTTCTCTCTCAGGAAATCAGCTCAGAGGTTCAAAAATTAATTAATGATAGTTGTGCT
3  E D N Y K E A I E A A F K V F T A P H G E S H E V Q R L I N D S C A

981     982     983     984     985     986     987     988     989     990     991     992     993     994     995     996     997     998     999     1000    1001    1002    1003    1004
1  GAAGTTAAATTAAGATCTCTCAGCTTTTGGGTTAAGTACGCGCTCTGAAGAGGTTTGGTTTAAATGAAGGTTGCTGAGAGGCAAGCCCTTGAAGCTTCTA
3  E V M A H S S A F M V H Y A A L K E F Y L N E G G G E A P L E G S I

1081    1082    1083    1084    1085    1086    1087    1088    1089    1090    1091    1092    1093    1094    1095    1096    1097    1098    1099    1100    1101    1102    1103    1104
1  TACTAGATATGATCTCTTCAAGAGAGAGCTATATCAATTTGCGAGAAATCTTCTTTACCCAGGCGGAGGCTGATTTTCTTGTCTATGAGGAAAGAGTTAA
3  P D H T S S T E R Y L H L Q K I Y L A R A E A D F L V I E E R V K

1181    1182    1183    1184    1185    1186    1187    1188    1189    1190    1191    1192    1193    1194    1195    1196    1197    1198    1199    1200    1201    1202    1203    1204
1  AAGCATTTTAAACAAATCTCTGCGAGAGCTCTGAGGAGGATCTCAAAAGCTCAACATCAAGACCTTTCTGCAAGAAATGCAAGGAAACTTAAATTTGTCAGATAT
3  N I L K K I G H D P K S I P K P T I E S F C K N A R E L E L C R Y

1281    1282    1283    1284    1285    1286    1287    1288    1289    1290    1291    1292    1293    1294    1295    1296    1297    1298    1299    1300    1301    1302    1303    1304
1  CGTATGGTACAGGAGGAGTTCTGAGAAAGCTTTCTGGAAGTCAAAATTCAGAAAGTATTTAGCGAGAGGAGATTACAGTGGTGGCAATGGAGTTTATATTTCTTC
3  R M V E D K F E N P S V T E I L K I L A D E D Y S G A H G F Y I L L

1381    1382    1383    1384    1385    1386    1387    1388    1389    1390    1391    1392    1393    1394    1395    1396    1397    1398    1399    1400    1401    1402    1403    1404
1  TTAGAGTGTGGAGAGTTTGTGCTGCAAGTATATCAAGTTTCTGCGAGGTTTGAAGAGGAAATGAGATGAGGAGATTTCTGCAATTAAGAAAGTACTGCTTCT
3  P A A D H F A A N Y M K E P G Q F D G G M D E D I S R L E T T A L

1481    1482    1483    1484    1485    1486    1487    1488    1489    1490    1491    1492    1493    1494    1495    1496    1497    1498    1499    1500    1501    1502    1503    1504
1  GATCTCTCTTCAAGCAATTTGGGCTGGTAAGGCTCTAGTACTCTCTAGATCAAGCTTATGATGAGATGAGTGGCTTTGGTGGCTCAGAGATTCATGTTGTTTCT
3  S L L T D L G C H G S V L P D D L I H E M C R P G A S E I E V Y S

1581    1582    1583    1584    1585    1586    1587    1588    1589    1590    1591    1592    1593    1594    1595    1596    1597    1598    1599    1600    1601    1602    1603    1604
1  GCTTTTGTGGAGGAATCGCATCTCAAGAGTCAATCAAGCTTTGTCACAAAGCAGTTTGTGTCGAGCTTGGGCACTTACATCTTCAATGACATGATCAACA
3  A F V C C I A X Q E Y E R L V T K Q F V P M L G T Y I F R G I D H R

1681    1682    1683    1684    1685    1686    1687    1688    1689    1690    1691    1692    1693    1694    1695    1696    1697    1698    1699    1700    1701    1702    1703    1704
1  AGTCTCAGTTATTAAGATTTGTAGAGATCTTTGCTTAAACATTTGATTTGAAGAGGAGAGAGGCTCTATCTATTTATTTCTTCTGATTAAAGATATA
3  R G L L S L

1781    1782    1783    1784    1785    1786    1787    1788    1789    1790    1791    1792    1793    1794    1795    1796    1797    1798    1799    1800    1801    1802    1803    1804
1  ATCTTTTCTGACTACTGAGAAACAAATTTTCAATGACAGTTTCAAGTGGAGACCA 1755
    
```

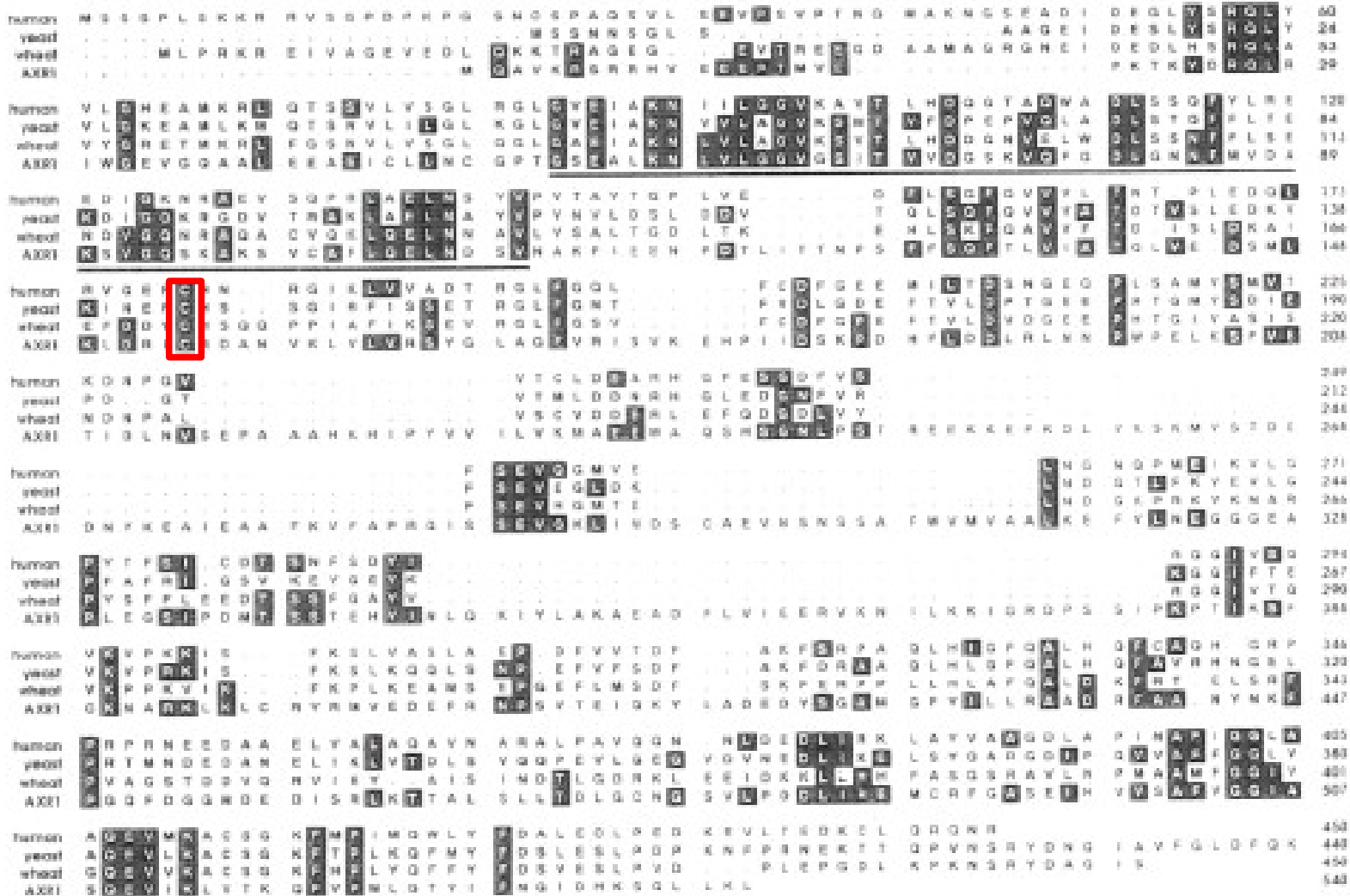
*axr1-3* = G461A  
Cysteine → Tyrosine

*axr1-12* = C1246T  
Glycine → STOP



# AXR1 function?

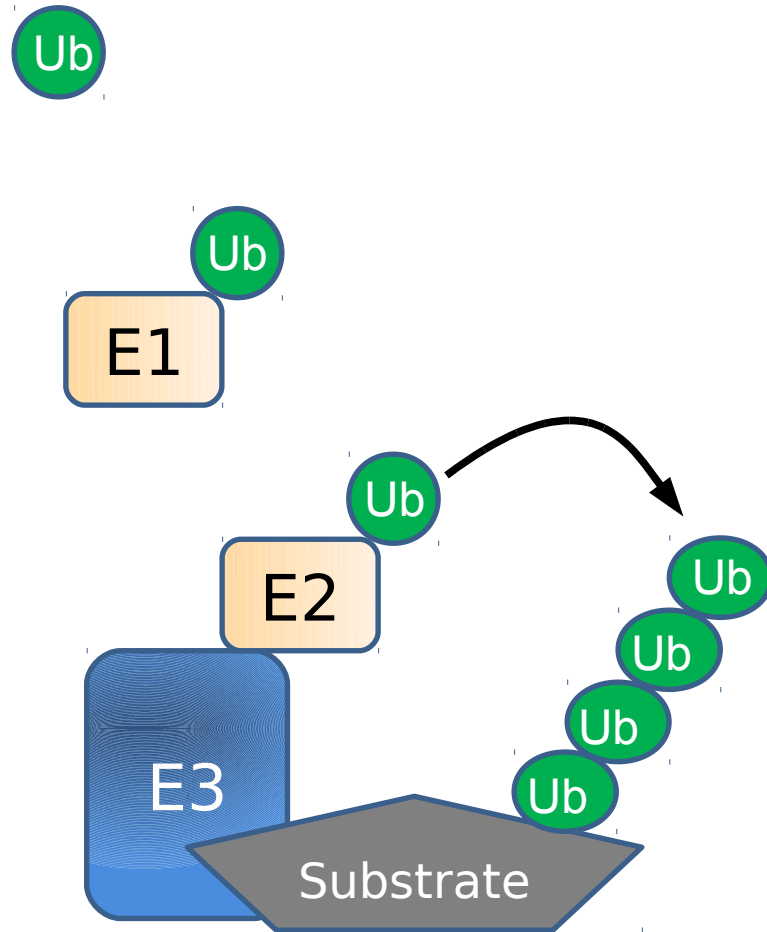
## Sequence similarity searches with Genbank database:



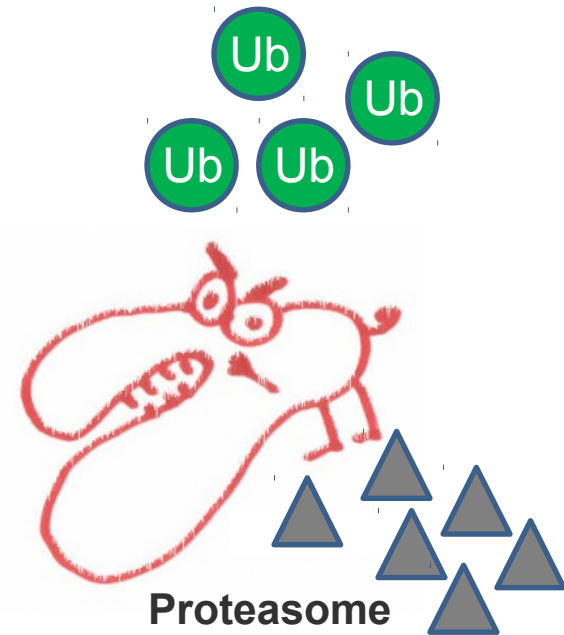
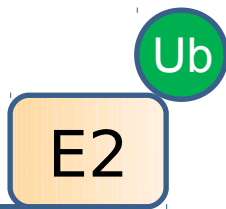
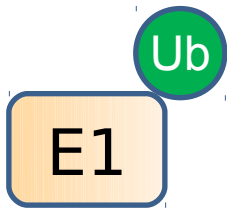
AXR1 Protein: 540 AS, ca. 60 kD

## Similarity to human and yeast E1 ubiquitin activating enzymes

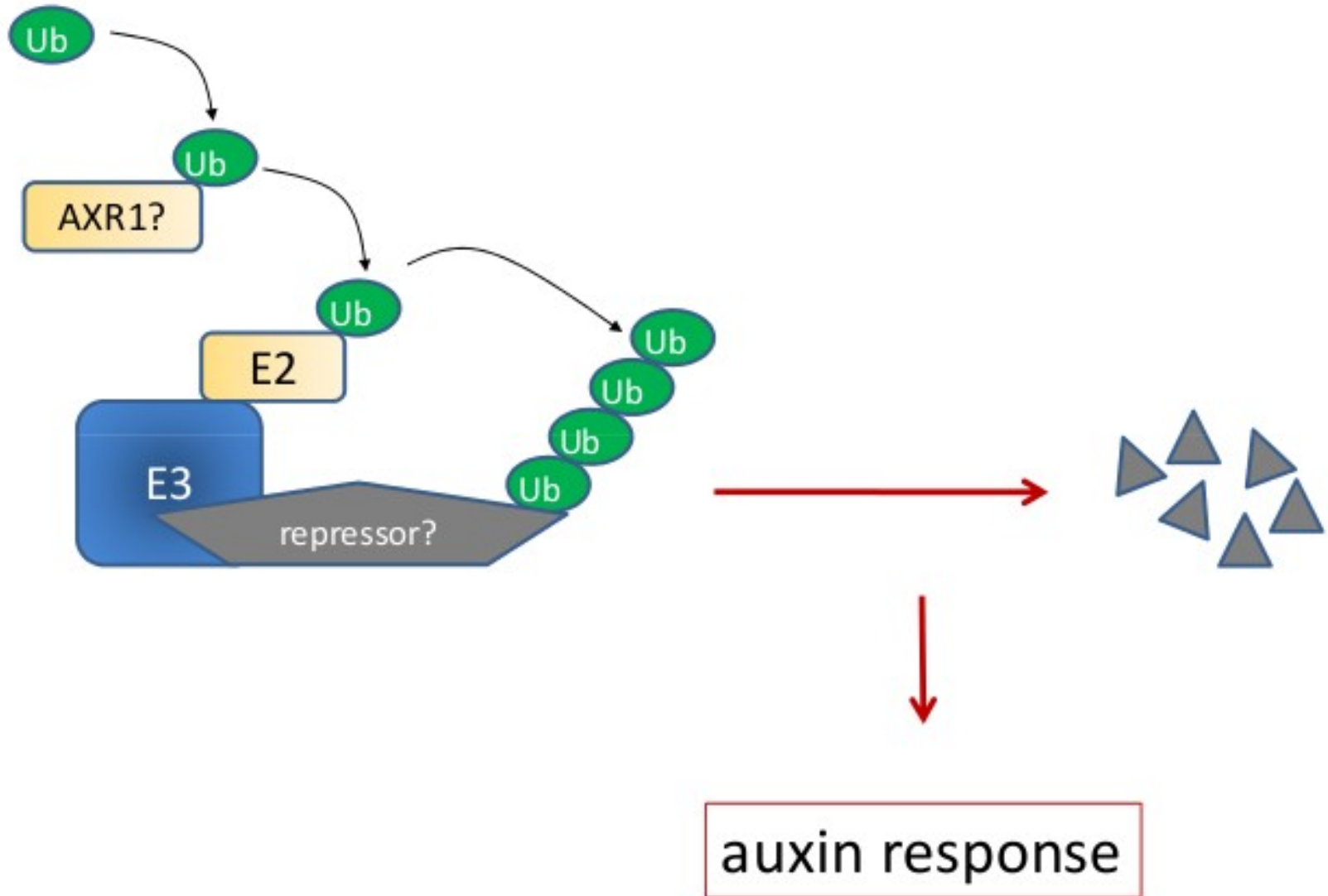
# The ubiquitin system



# 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

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- 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>