Regulation of plant development by ethylene

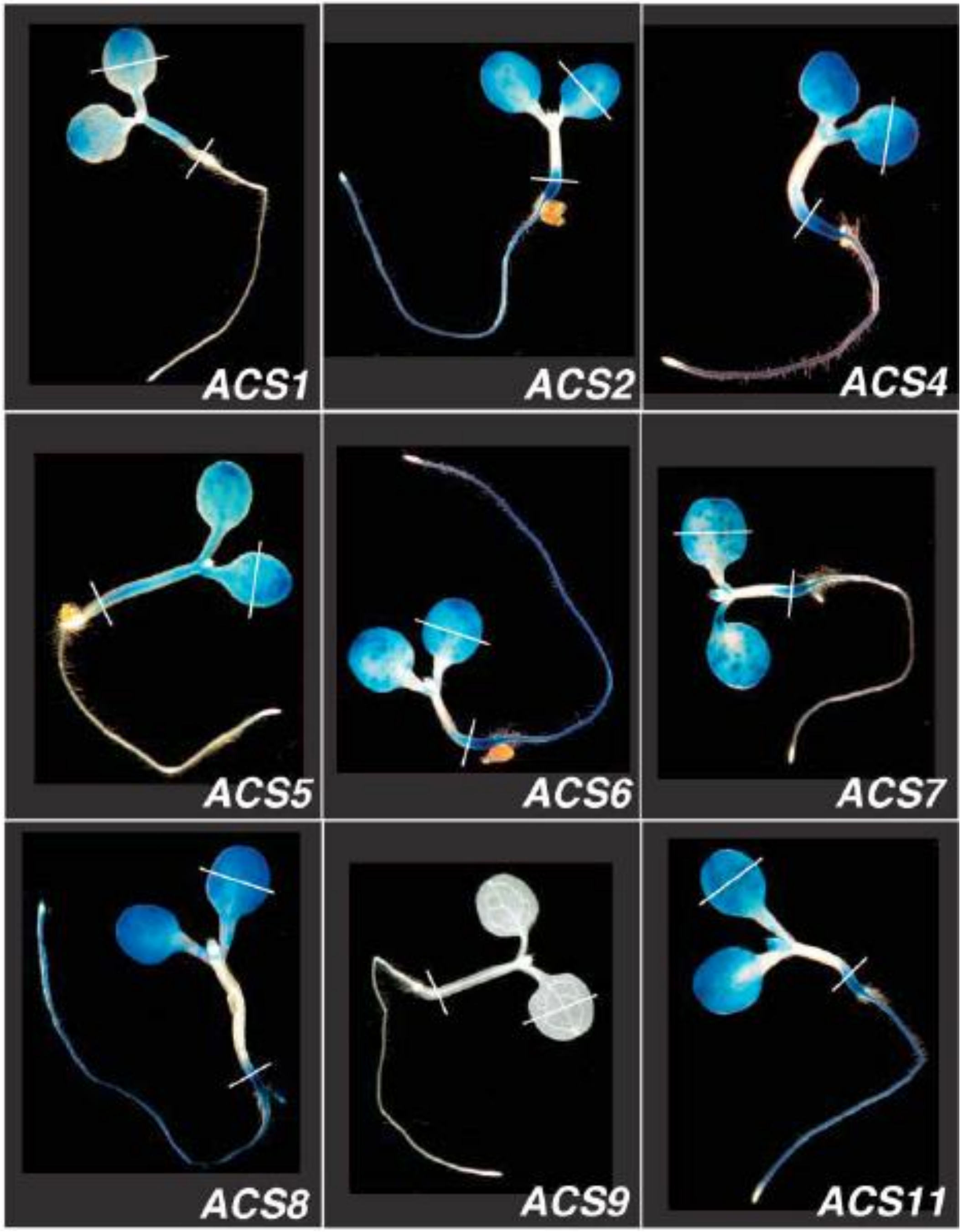
Wild Type

Never-ripe

Ethylene biosynthesis

Compound / Enzyme	Gene in *A. thaliana*: Name (symbol)	Gene in *A. thaliana*: Acc. number	Induction factor
L-methionine			
SAM synthetase	*SAM1*	At1g02500 (T76645)**	–
S-adenosyl-L-methionine			
ACC Synthase	*ACS2*	At1g01480	Ethylene, cycloheximide, wouding, IAA
	ACS6	At4g11280	Ozone, ethylene, cyanide, touching, cycloheximide, IAA
	ACS7	At4g26200	Cycloheximide
1-aminocyclopropane-1-carboxylate			
ACC oxidase	*EAT1(ACO 1)*	At1g05010 (R90435)	Wounding, ethrel, $Fe2^{+}$
	ACO2	At1g62380 (N96535)	–
Ethylene			

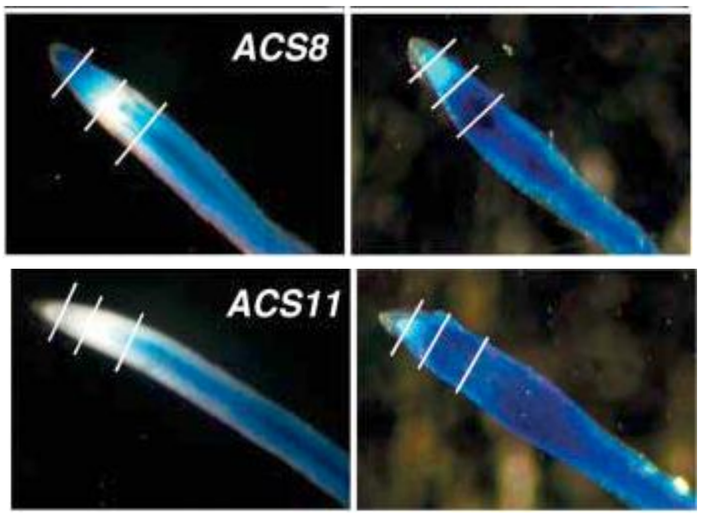
Expression analysis of ACS

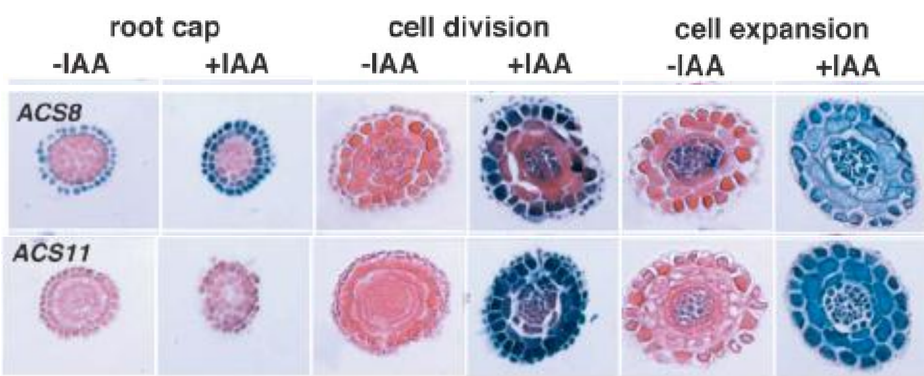


Transcriptional regulation of ACS expression

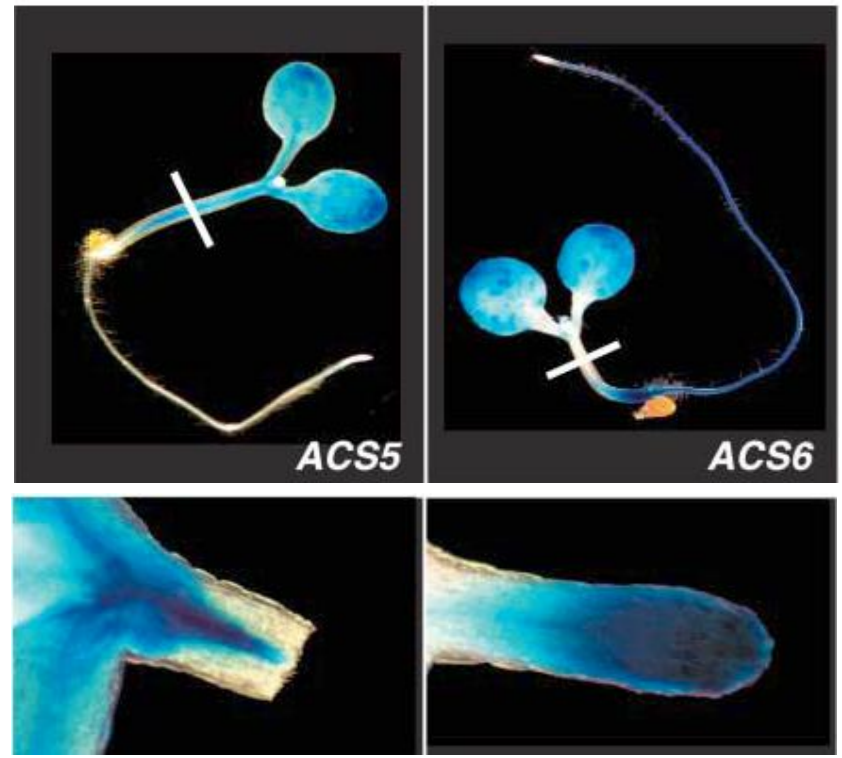
Auxin

-IAA +IAA

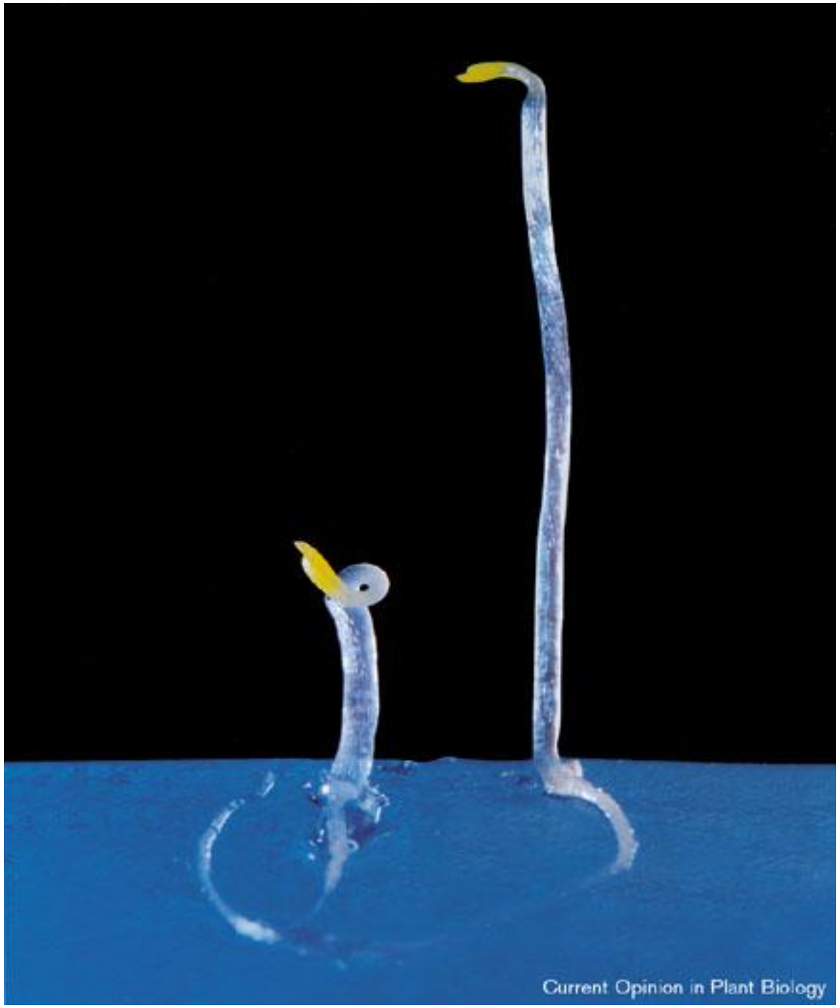


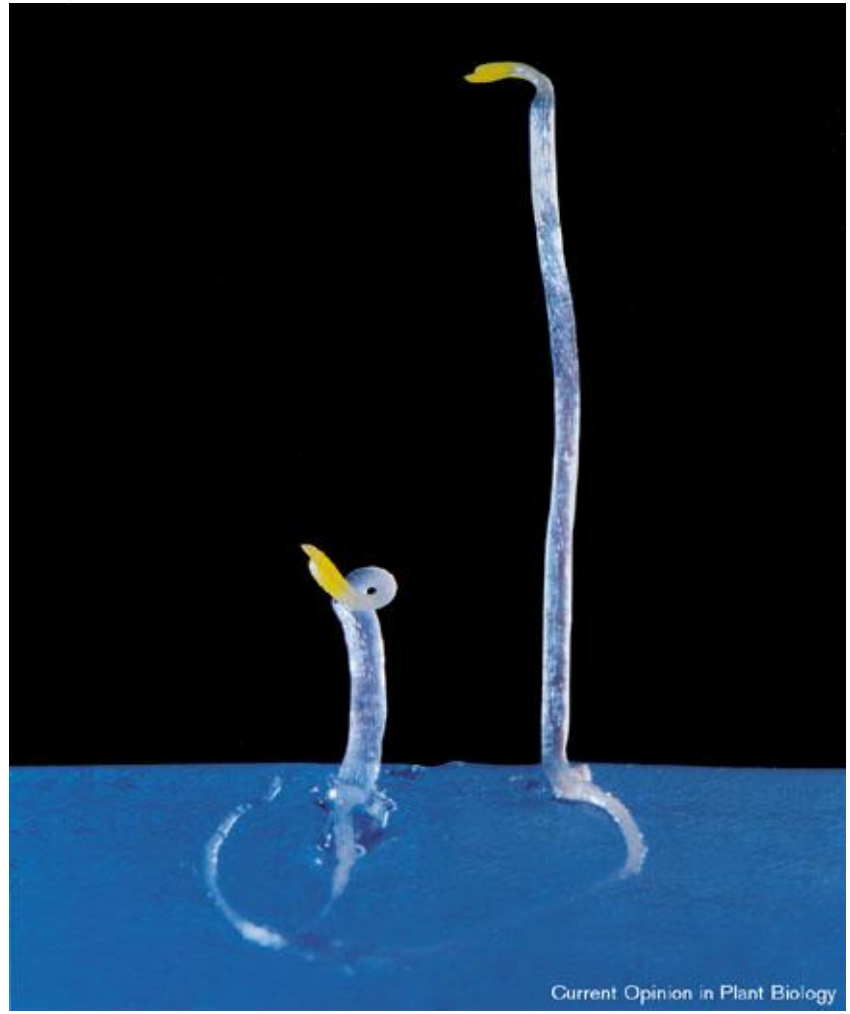


Wounding



Mutant screens for ethylene pathway genes

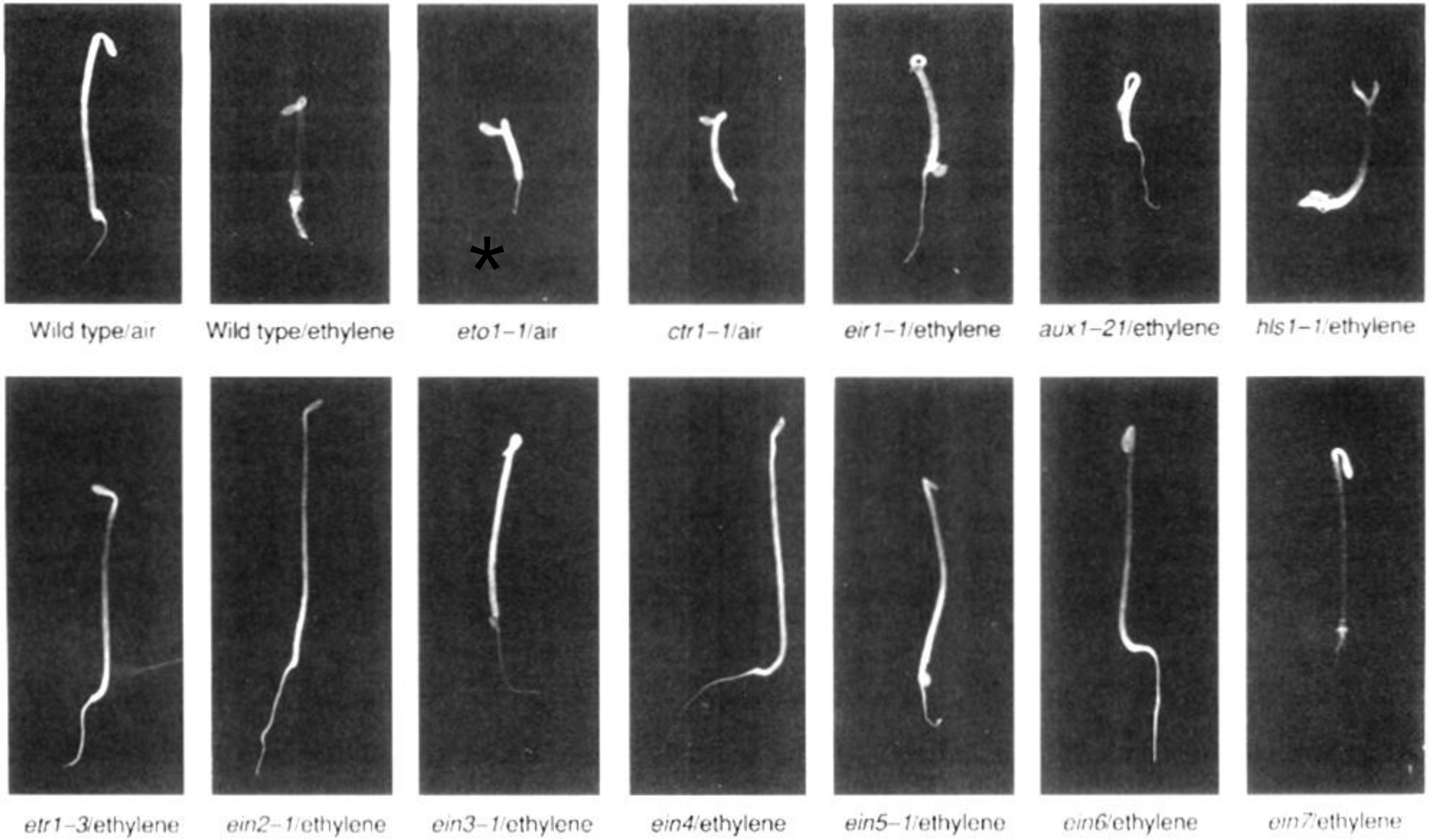




C_2H_4 air

air C_2H_4

Screen for ethylene mutants
I. Genes involved in regulation of biosynthesis



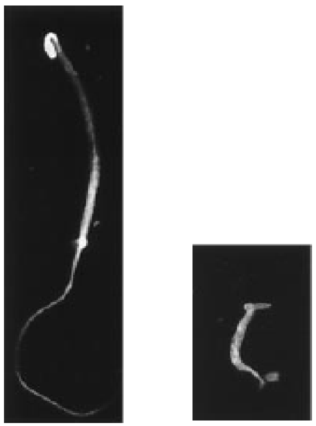
Roman et al., 1994

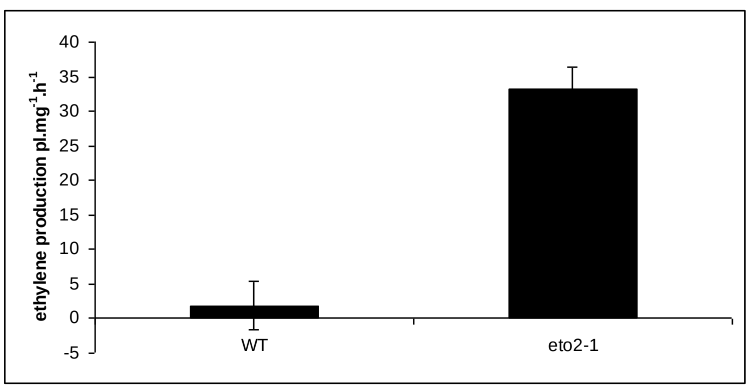
* phenotype rescued by inhibitor of ethylene biosynthesis

eto mutants – constitutive triple response

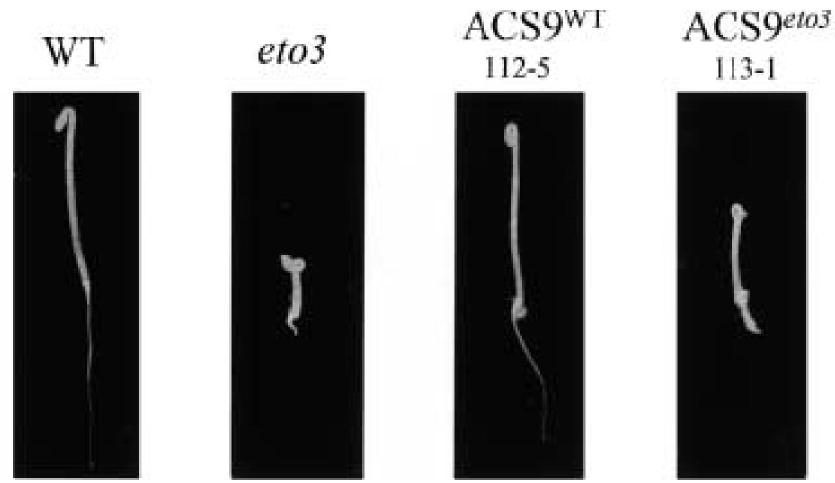
eto2

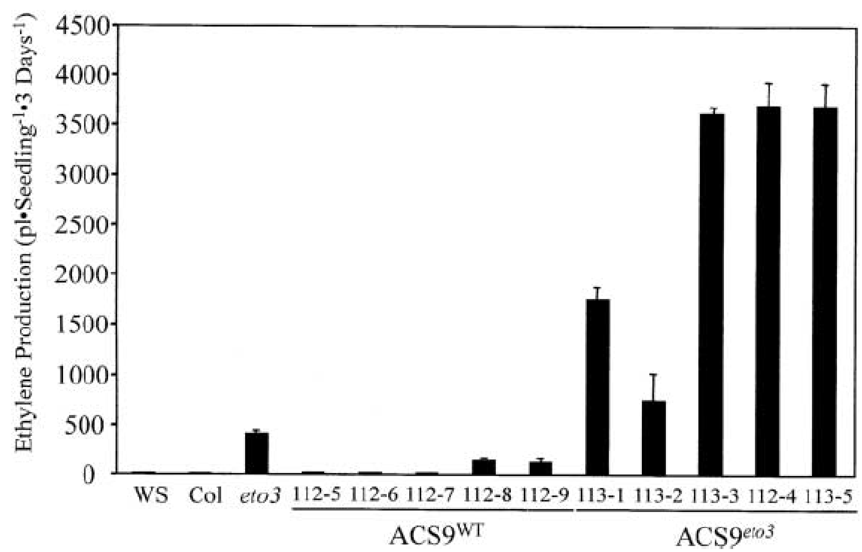
WT *eto2*





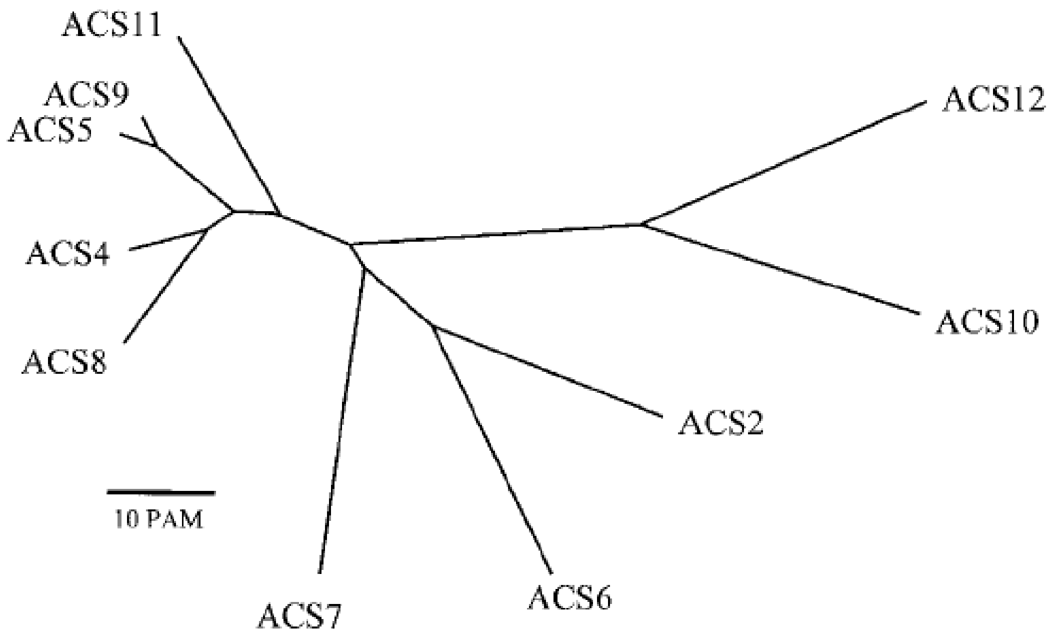
eto3





- overproduction of ethylene

eto2,eto3 dominant mutation results from single amino acid change in the C terminus ACS5,ACS9



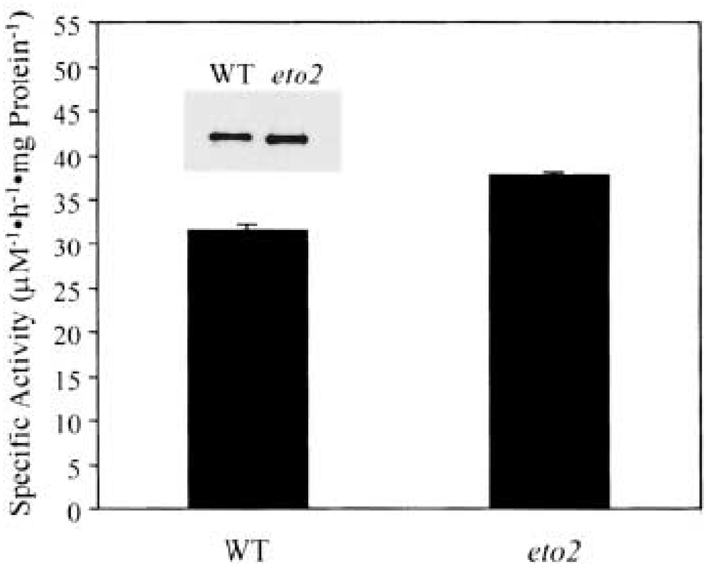
ACS4	457	VSNWVFRLSFHDREAEER
ACS8	452	VSNWVFRLSFHDREPEER
eto2	453	VSNWVFpgfmdrsct
ACS5	453	VSNWVFRVSWTDRVPDER
ACS9	453	VSNWVFRVSWTDRVPDER
eto3	453	VSNWDFRVSWTDRVPDER
		* ↑

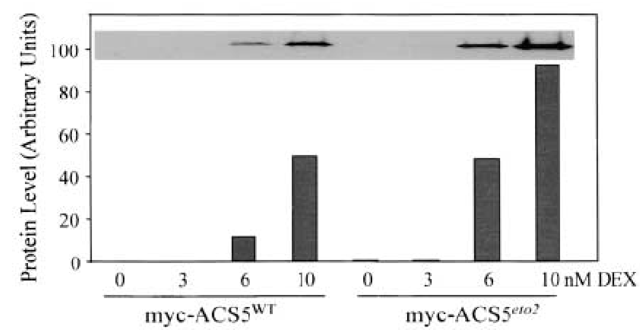
eto3 mutation does not affect level of ACS9 mRNA

Table 1. Levels of *ACS9* mRNA in Wild-Type and *eto3* Seedlings

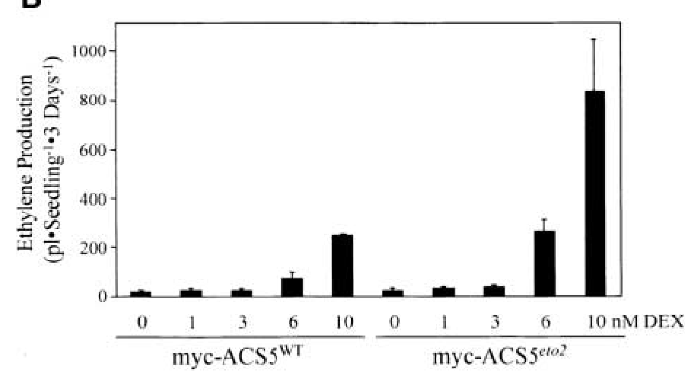
Sample	$C_T$nor (Experiment 1)[a]	$C_T$nor (Experiment 2)	$C_T$nor (Experiment 3)	$C_T$nor (mean ± SD)	*ACS9* mRNA[b]
Wild type	6.95	6.36	6.53	6.61 ± 0.30	1.0
eto2	7.23	6.99	6.07	6.76 ± 0.61	0.9

eto2 mutation does not affect specific activity of ACS5

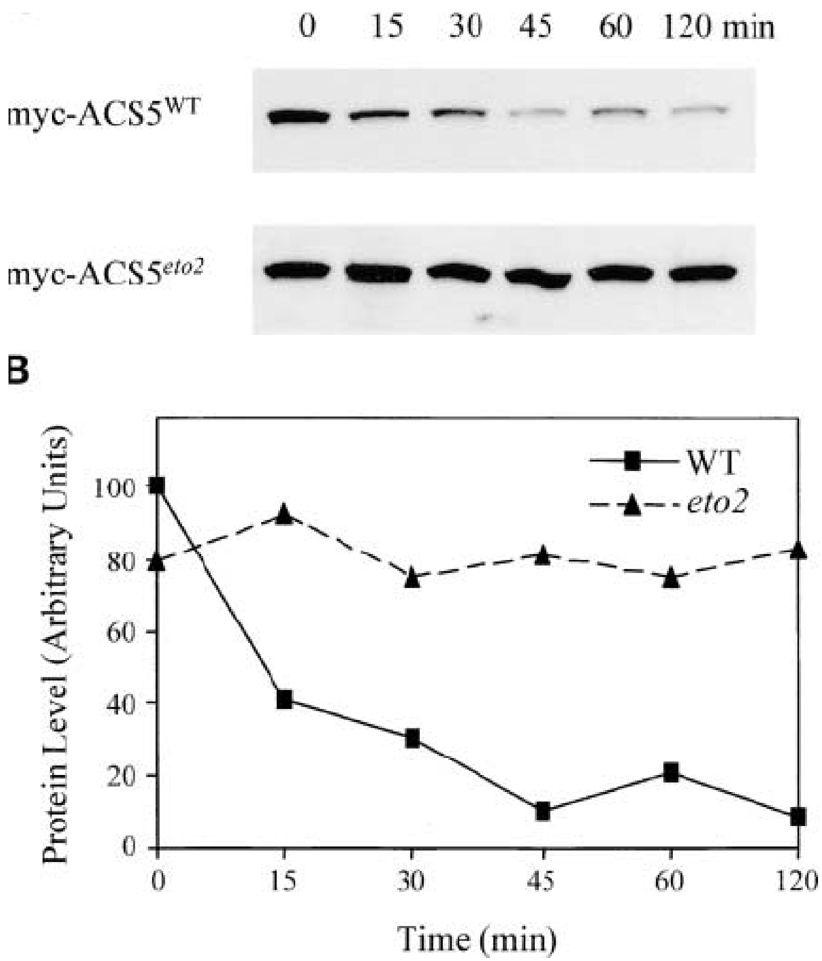


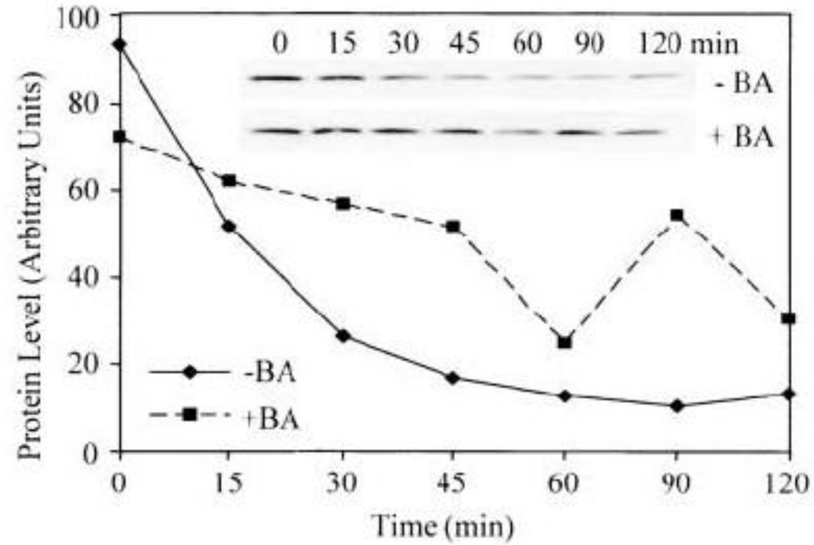


B



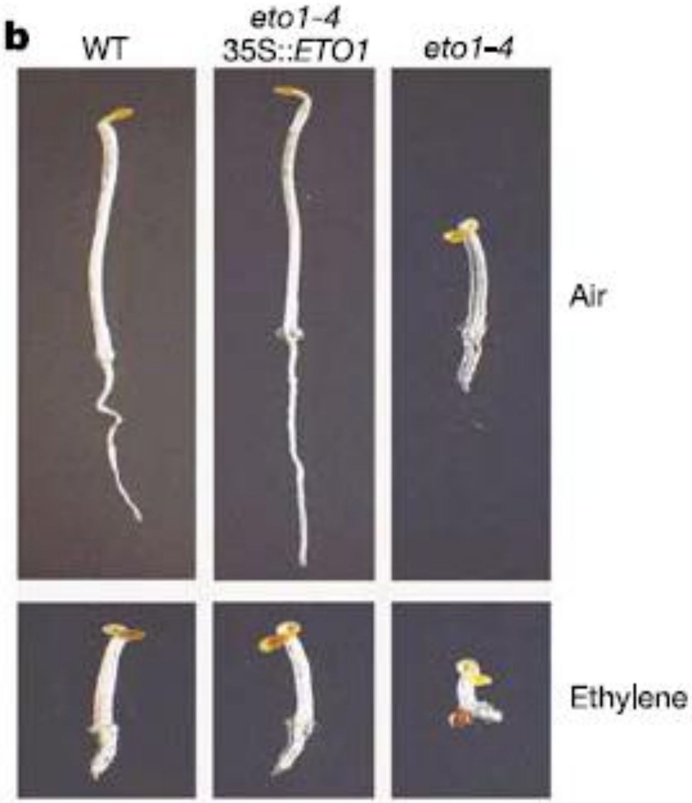
Effect of *eto2* mutation on ACS5 protein stability

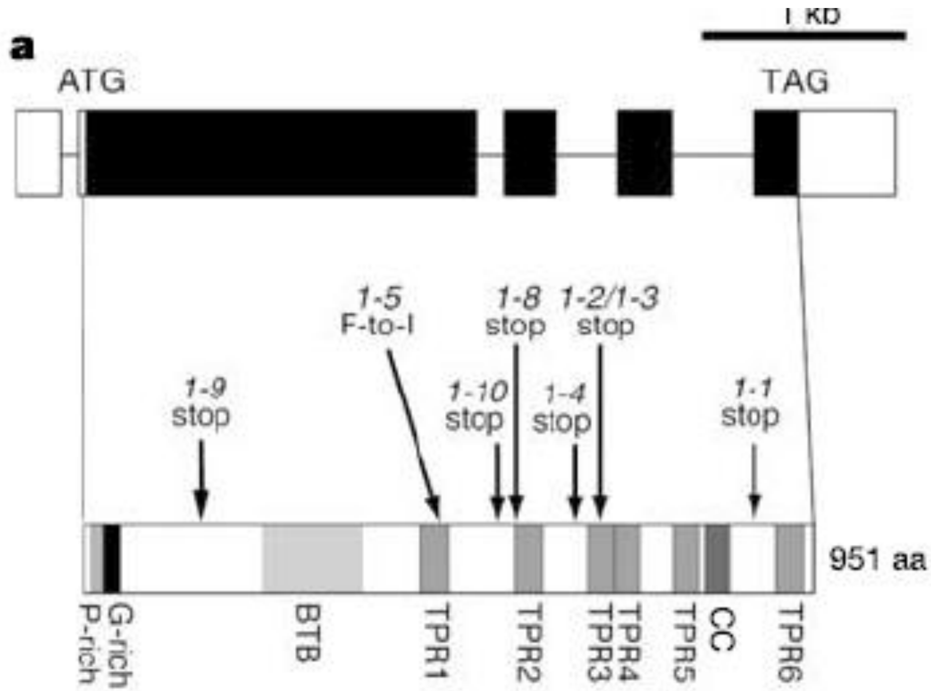




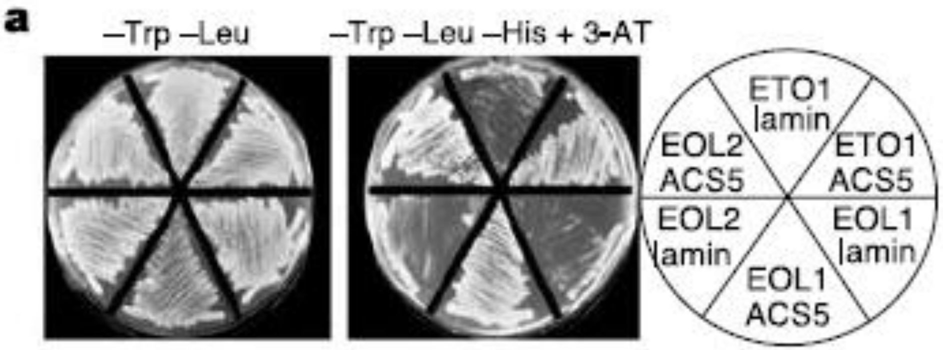
Posttranscritpional regulation of ACS

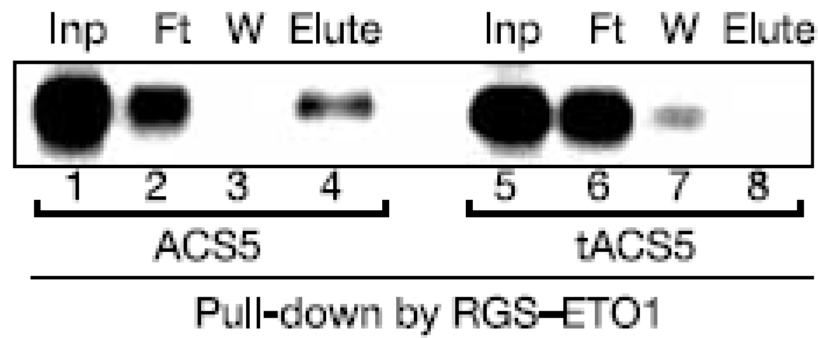
Eto1- recesive mutant with constitutive triple response

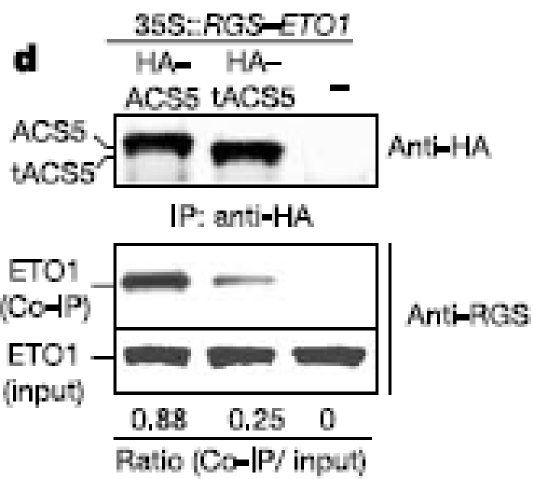


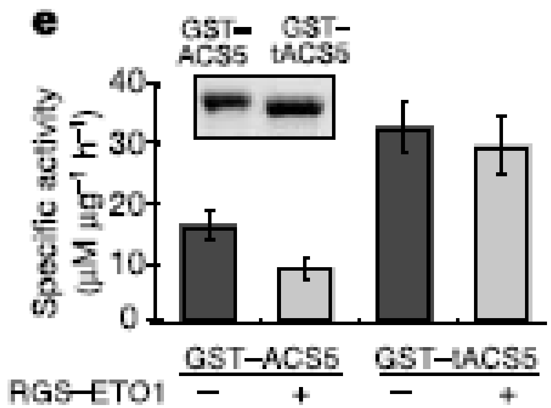


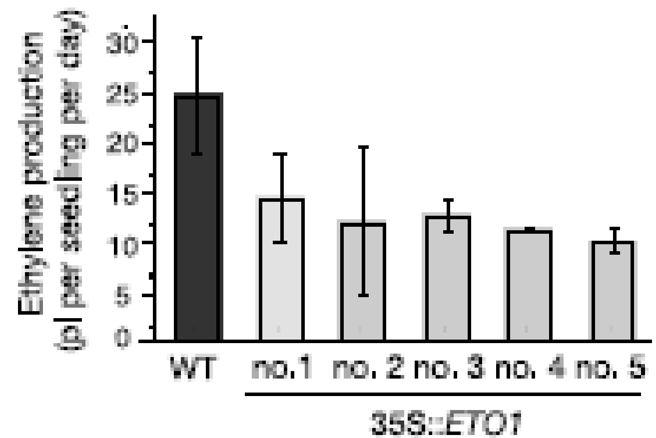
ETO1 interacts with ACS5 and regulates its activity



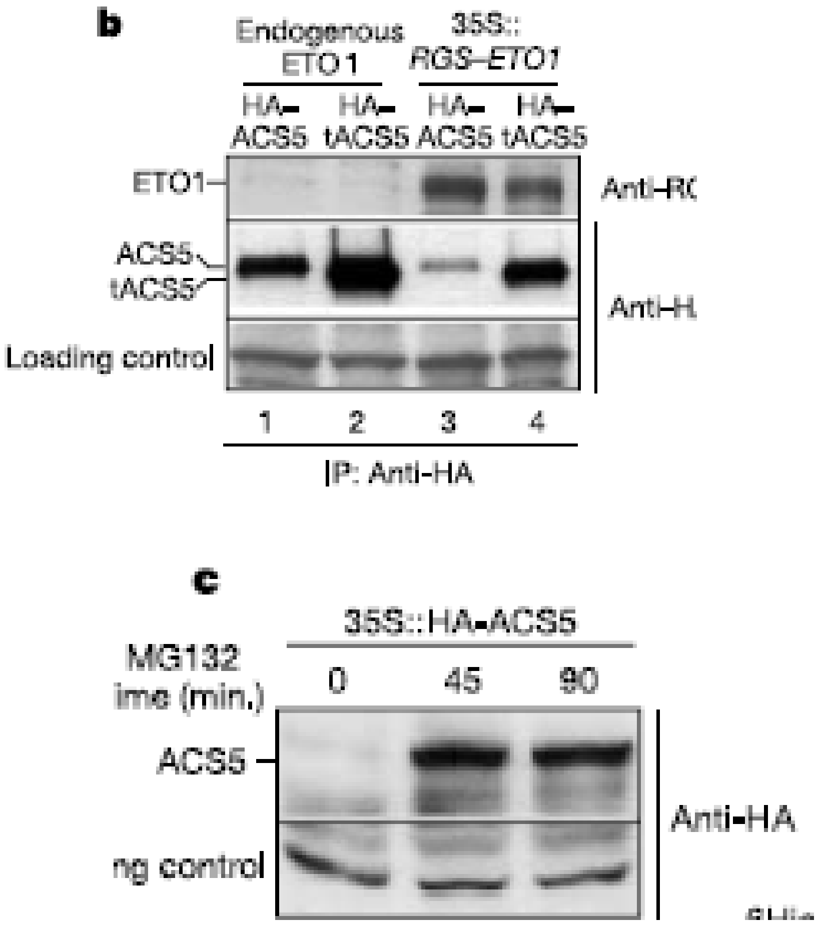




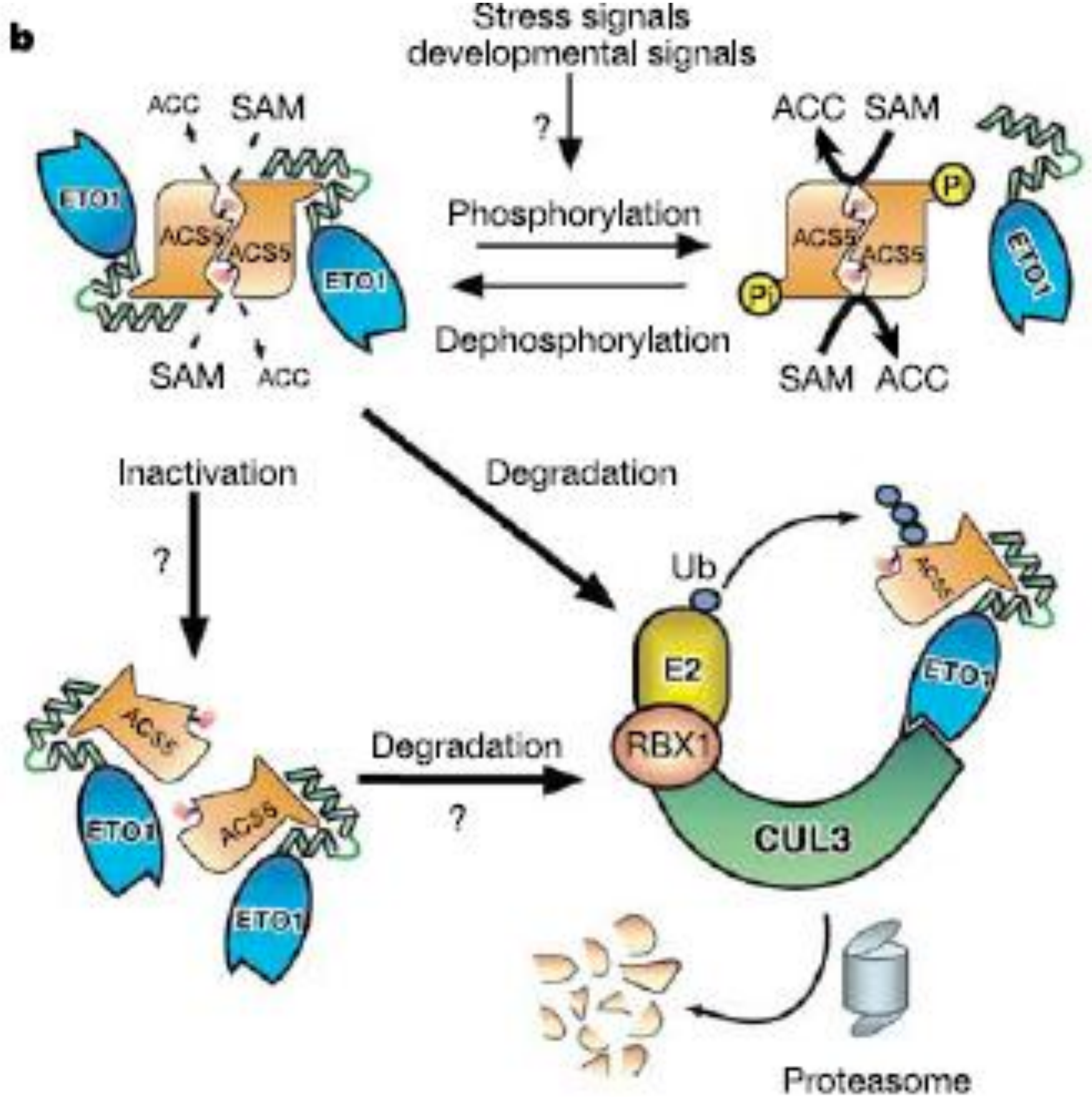




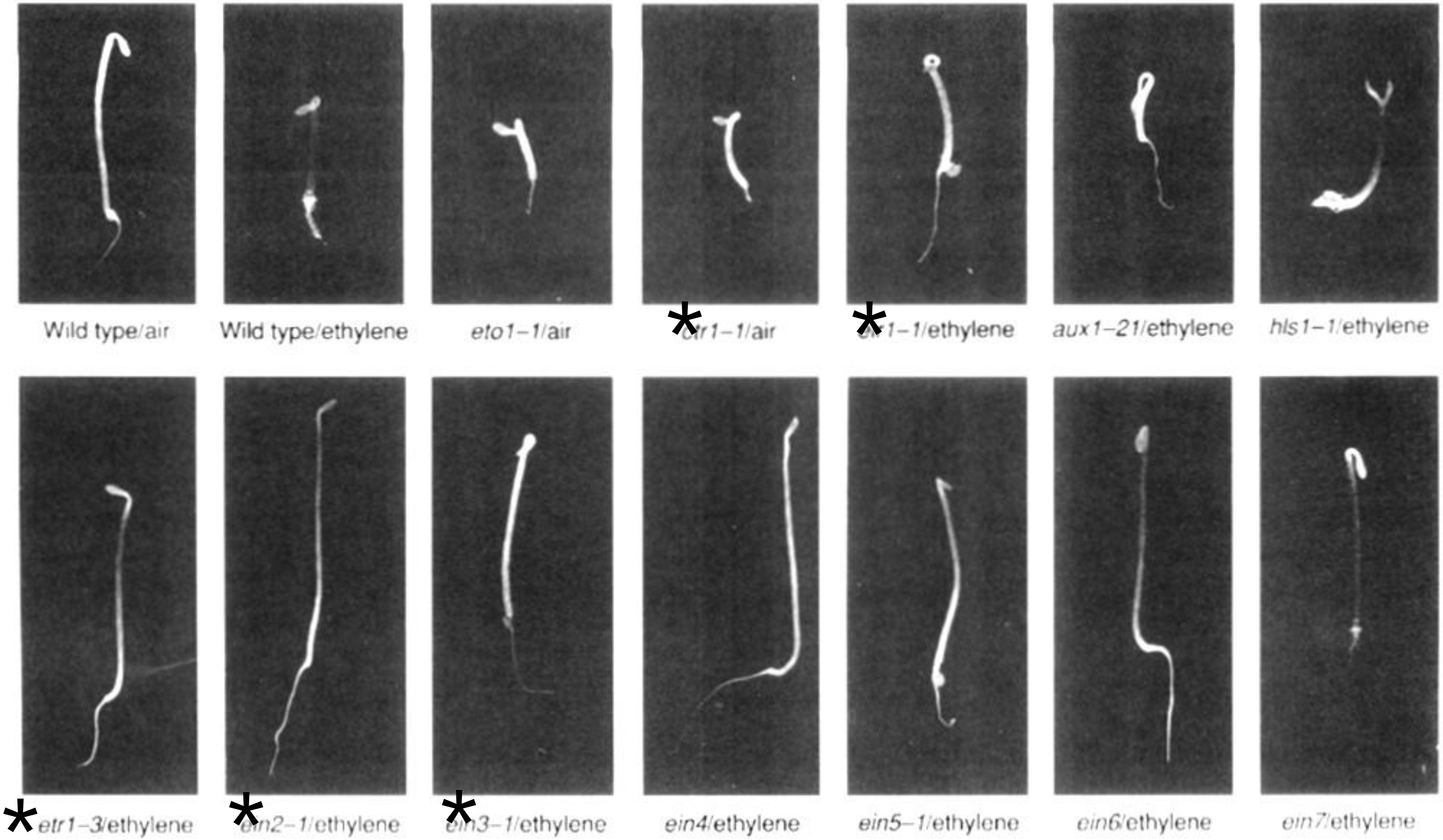
ETO1 promotes ACS5 degradation through proteasome dependent pathway



Model for regulation of ethylene biosynthesis by ETO1



Screen for ethylene mutants
II. Genes invoved in signalling pathway



Roman et al., 1994

	Ecotype	Phenotype[b]
A. Strains[a]		
aux1-7	Columbia	Aux⁻
aux1-21	Columbia	Aux⁻
aux1-22	Columbia	Aux⁻
* *ctr1-1*	Columbia	Ctr⁻
ctr1-5	Wassilewskija	Ctr⁻, kan^r
* *ein2-1*	Columbia	Ein⁻
ein2-6	Wassilewskija	Ein⁻
* *ein3-1*	Columbia	Ein⁻
ein3-2	Wassilewskija	Ein⁻, kan^r
* *ein4*	Columbia	Ein⁻
ein5-1	Columbia	Ein⁻
ein5-2	Columbia	Ein⁻
ein6	Landsberg	Ein⁻
ein7	Columbia	Ein⁻
eir1-1	Columbia	Eir⁻
eir1-2	Columbia	Eir⁻
eto1-1	Columbia	Eto⁻
* *etr1-3*	Columbia	Ein⁻
hls1-1	Columbia	Hls⁻
ein2-1 tt4	Mixed	Ein⁻, Tt⁻
eir1-1 ap1	Mixed	Eir⁻, Ap⁻
DP28	Landsberg	Dis⁻, Clv⁻, Tt⁻
W2	Landsberg	Dis⁻, An⁻
W100	Landsberg	Tt⁻, and more
M10	Landsberg	Ap⁻, Clv⁻

Roman et al., 1994

Quantifying the ethylene response phenotype

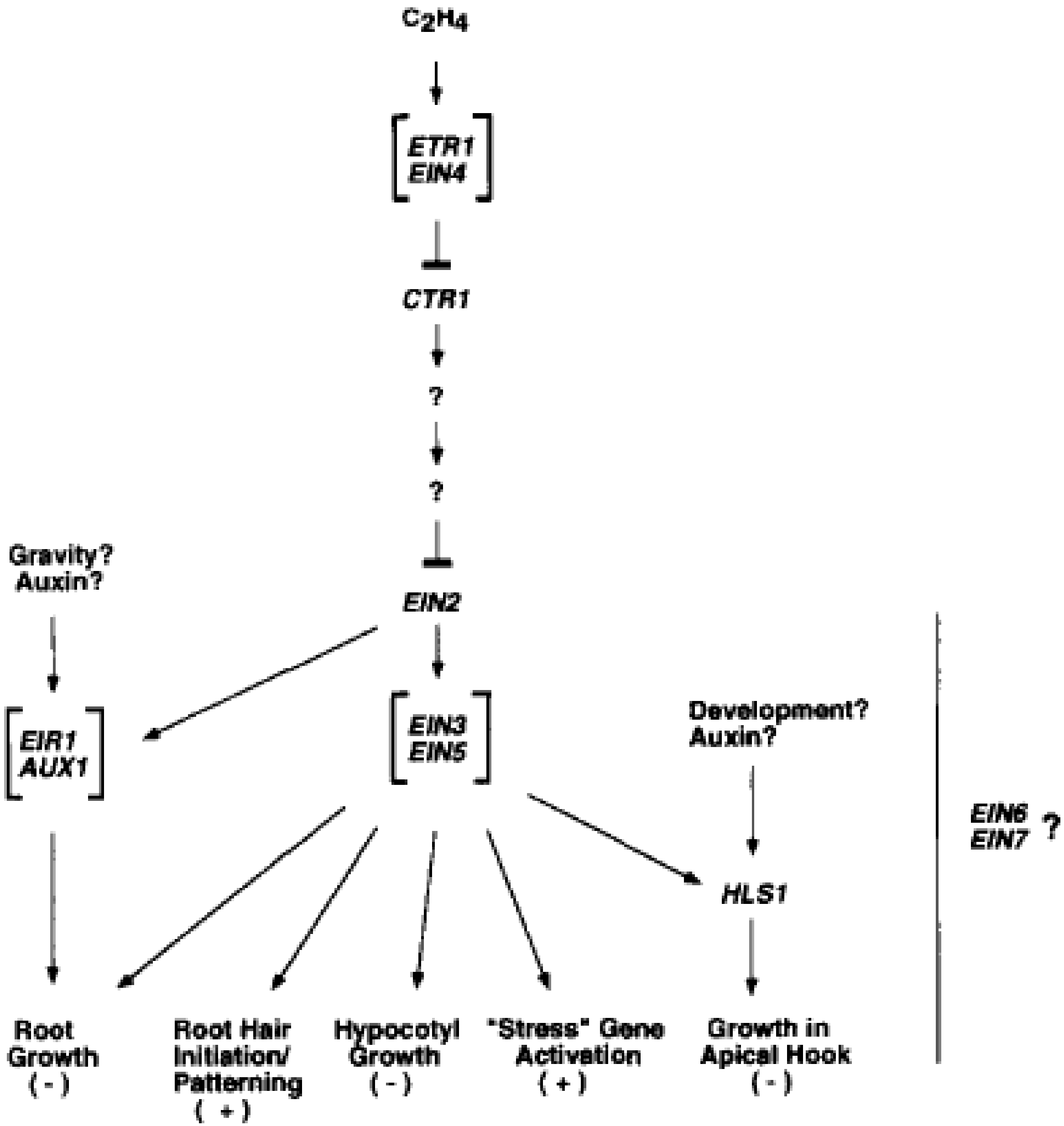
Strain	Ethylene				Air			
	Root length	Hypocotyl length	Total seedling	Hook angle	Root length	Hypocotyl length	Total seeding	Hook angle
Columbia	1.5 ± 0.1	3.0 ± 0.1	4.5 ± 0.1	250 ± 8	3.9 ± 0.2	4.8 ± 0.1	8.7 ± 0.2	114 ± 9
Landsberg	2.1 ± 0.1	2.7 ± 0.1	4.8 ± 0.1	233 ± 18	nd[b]	nd	nd	nd
Wasslewskija	1.0 ± 0.1	3.1 ± 0.1	4.1 ± 0.1	270 ± 6	4.0 ± 0.2	6.1 ± 0.1	10.1 ± 0.2	166 ± 7
aux1-21	4.7 ± 0.2	3.4 ± 0.1	8.1 ± 0.3	197 ± 8	6.3 ± 0.3	6.0 ± 0.1	12.3 ± 0.3	126 ± 6
ctr1-1	0.8 ± 0.0	2.8 ± 0.1	3.6 ± 0.1	247 ± 5	0.9 ± 0.0	3.1 ± 0.1	4.0 ± 01	246 ± 10
ctr1-5	0.4 ± 0.0	1.9 ± 0.1	2.3 ± 0.1	252 ± 5	0.6 ± 0.0	2.4 ± 0.1	3.0 ± 0.1	237 ± 8
ein2-1	6.1 ± 0.2	6.5 ± 0.2	12.6 ± 0.2	39 ± 4	5.8 ± 0.2	6.9 ± 0.2	12.7 ± 0.3	43 ± 7
ein3-1	3.6 ± 0.1	5.2 ± 0.1	8.8 ± 0.2	118 ± 7	5.4 ± 0.3	5.9 ± 0.1	11.3 ± 0.4	77 ± 7
ein3-2	3.1 ± 0.1	5.5 ± 0.1	8.5 ± 0.2	176 ± 6	5.2 ± 0.3	6.3 ± 0.2	11.4 ± 0.3	152 ± 8
ein4	7.1 ± 0.2	7.3 ± 0.3	14.4 ± 0.3	64 ± 9	6.8 ± 0.3	6.9 ± 0.3	13.7 ± 0.4	45 ± 5
ein5-1	2.5 ± 0.1	4.8 ± 0.1	7.3 ± 0.1	144 ± 10	5.6 ± 0.2	5.3 ± 0.2	11.0 ± 0.3	89 ± 8
ein5-2	2.6 ± 0.1	4.6 ± 0.2	7.2 ± 0.2	156 ± 10	4.3 ± 0.2	5.6 ± 0.2	9.9 ± 0.3	113 ± 10
ein6	3.5 ± 0.1	6.2 ± 0.2	9.7 ± 0.2	95 ± 6	7.0 ± 0.2	6.0 ± 0.2	13.0 ± 0.2	47 ± 4
ein7	2.9 ± 0.1	5.2 ± 0.1	8.1 ± 0.2	176 ± 4	5.2 ± 0.2	6.7 ± 0.2	11.9 ± 0.2	137 ± 8
eir1-1	3.4 ± 0.1	3.1 ± 0.1	6.5 ± 0.1	282 ± 7	5.2 ± 0.9	6.2 ± 0.1	11.4 ± 0.1	106 ± 7
eir1-2	3.0 ± 0.1	3.1 ± 0.1	6.1 ± 0.1	261 ± 7	4.8 ± 0.3	5.7 ± 0.2	10.5 ± 0.4	109 ± 10
eto1-1	1.4 ± 0.1	3.3 ± 0.1	4.7 ± 0.2	244 ± 10	1.9 ± 0.1	3.3 ± 0.1	5.3 ± 0.1	239 ± 8
etr1-3	4.6 ± 0.2	6.1 ± 0.1	10.7 ± 0.3	89 ± 7	4.9 ± 0.3	5.5 ± 0.2	10.4 ± 0.3	96 ± 5
hls1-1	0.9 ± 0.1	3.4 ± 0.1	4.3 ± 0.2	5 ± 1	3.3 ± 0.1	5.1 ± 0.1	8.4 ± 0.2	4 ± 1

Roman et al., 1994

B. Double mutants[a]

aux1-21 ctr1-1	Aus−, Ctr^{-c}
aux1-21 eir1-1	Aux^{-}
ctr1-5 ein 2-1	Ein^{-}
ctr1-1 ein3-2	Ein^{-}
ctr1-1 ein5-1	Ein^{-}
ctr1-1 ein7	Ein^{-}
ctr1-1 eir1-1	Eir^{-}, Ctr^{-c}
ctr1-1 etr1-3	Ctr^{-}
ctr1-1 hls1-1	Hls^{-}, Ctr^{-c}
ein2-1 eir1-1	Ein^{-}, Eir^{-}
ein2-6 eir1-1	Ein^{-}, Eir^{-}
ein2-1 eto1-1	Ein^{-}
ein2-6 eto 1-1	Ein^{-}
ein2-1 etr1-3	Ein^{-}
ein2-1 hls1-1	Ein^{-}, Hls^{-}
ein3-1 eir1-1	Ein^{-}, Eir^{-}
ein5-1 eir1-2	Ein^{-}, Eir^{-}
eir1-1 hls 1-1	Eir^{-}, Hls^{-}

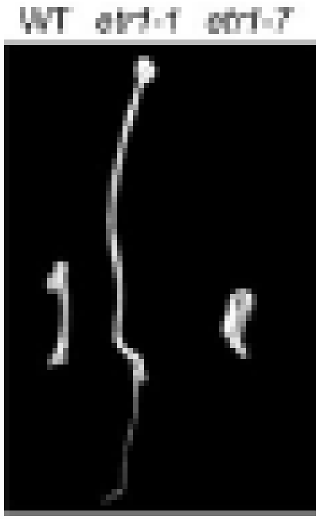
Roman et al., 1994



Roman et al., 1994

ETR1 codes for histidine kinase

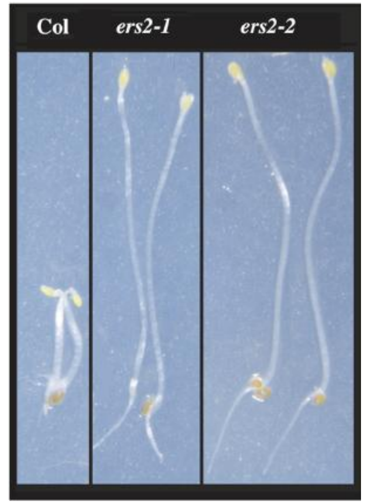
etr1



Bleecker et al.,1988

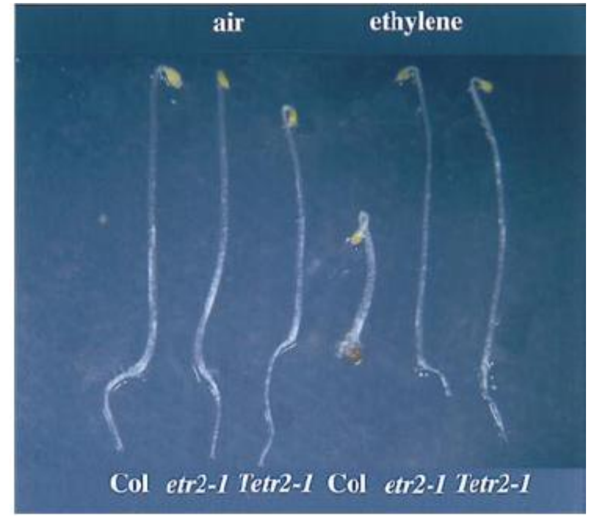
ein4

ers2

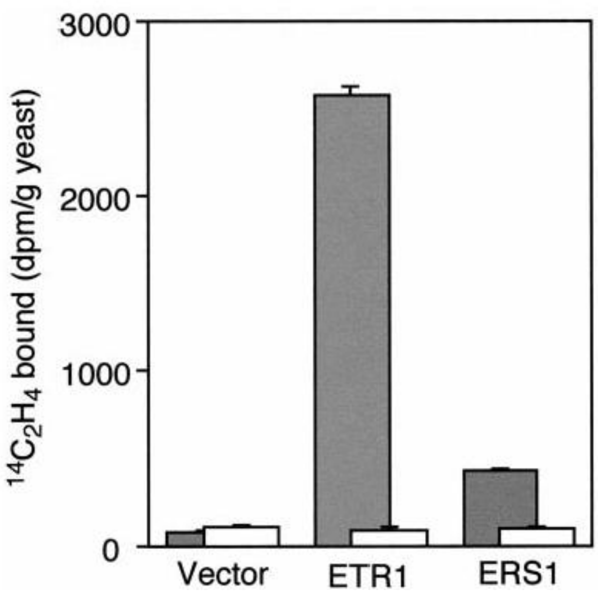


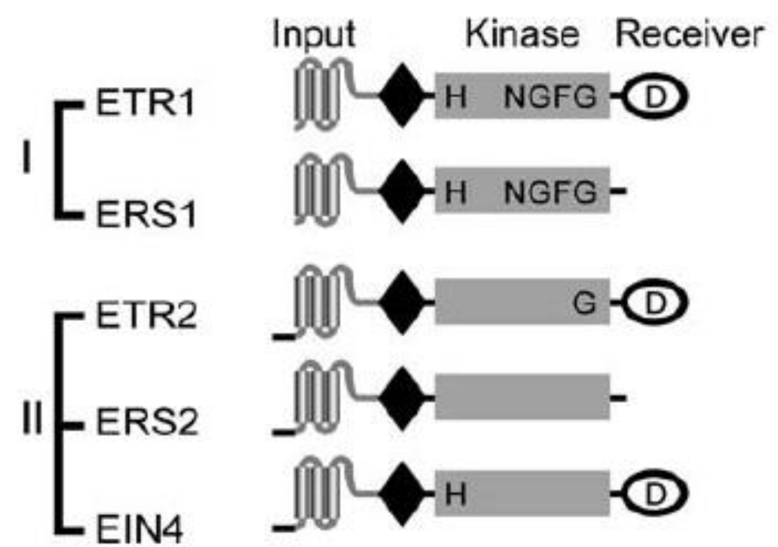
Hua et al., 1998

etr2

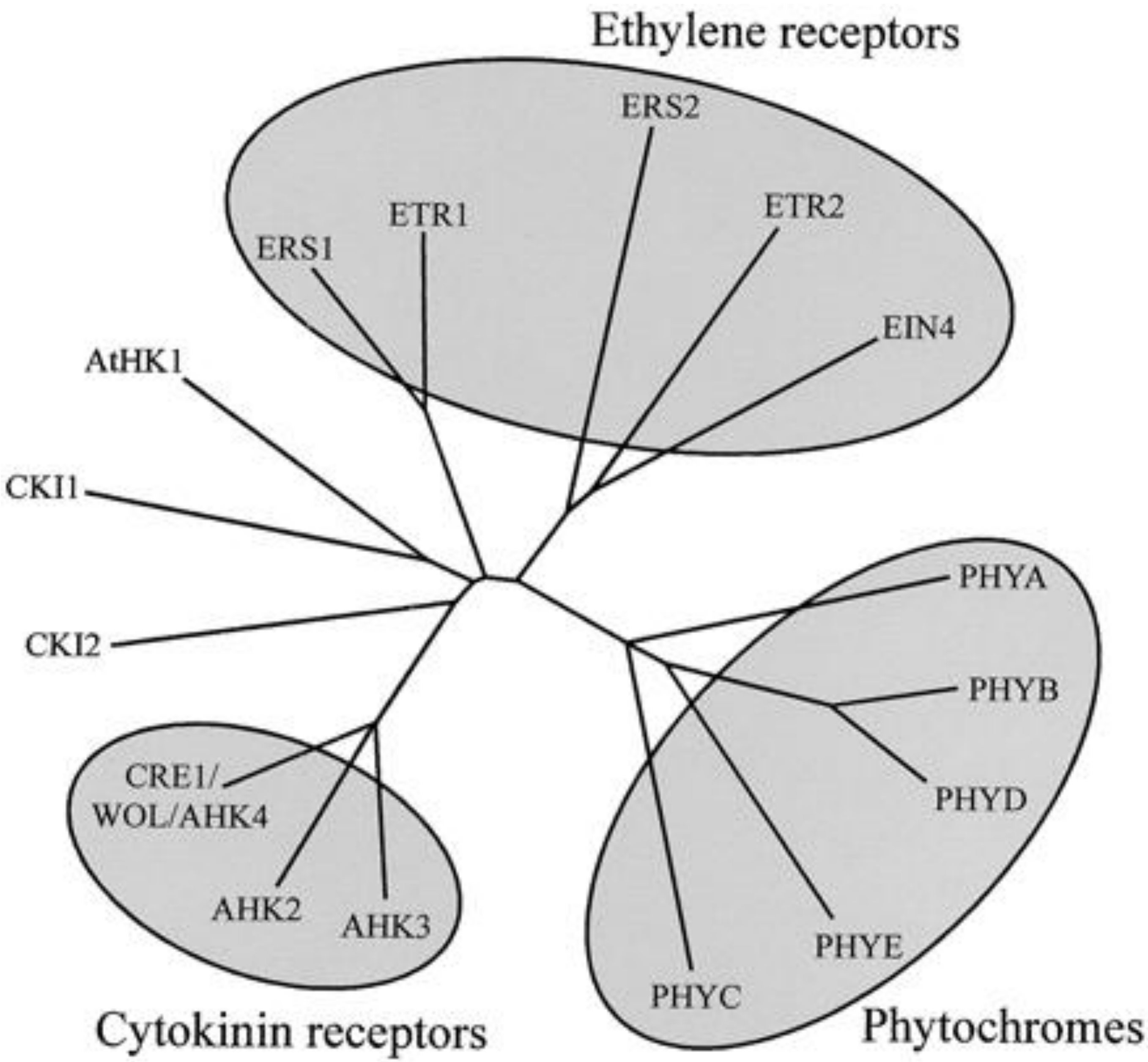


Sakai et al., 1998

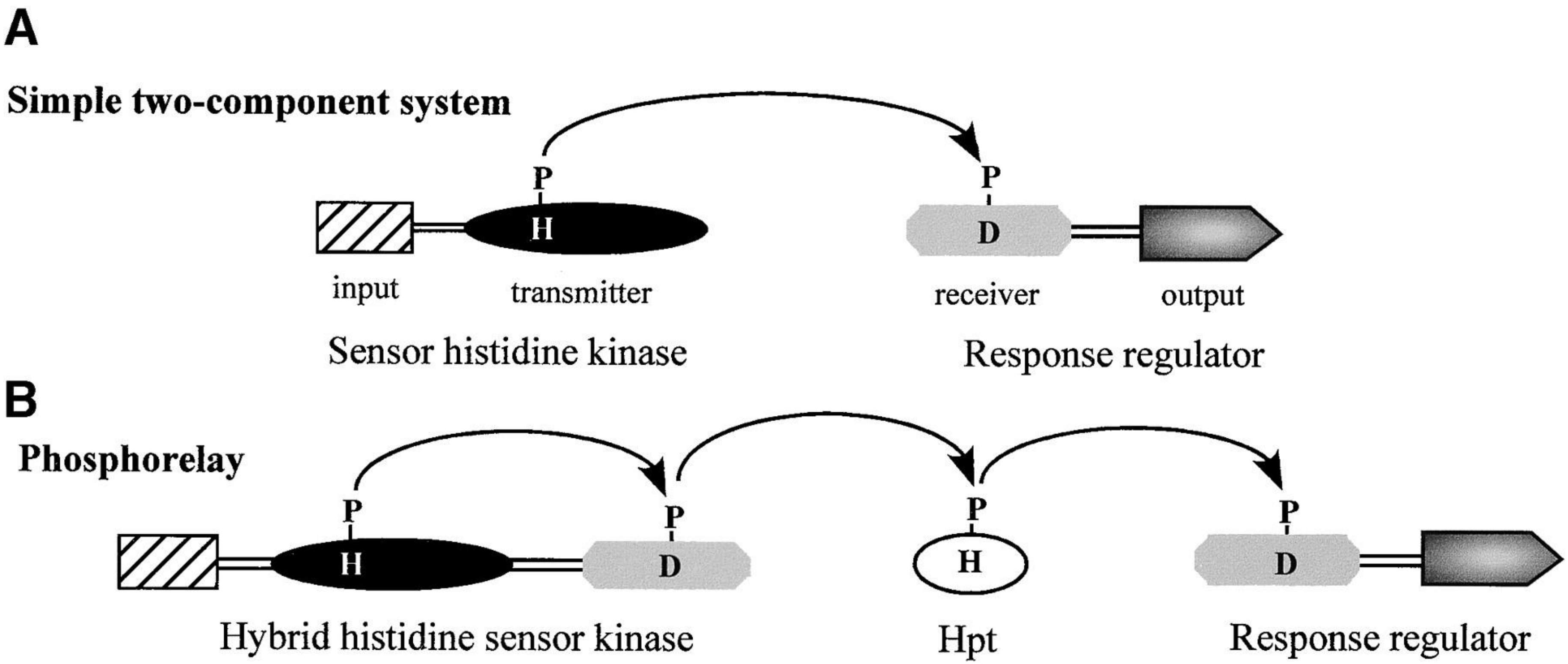


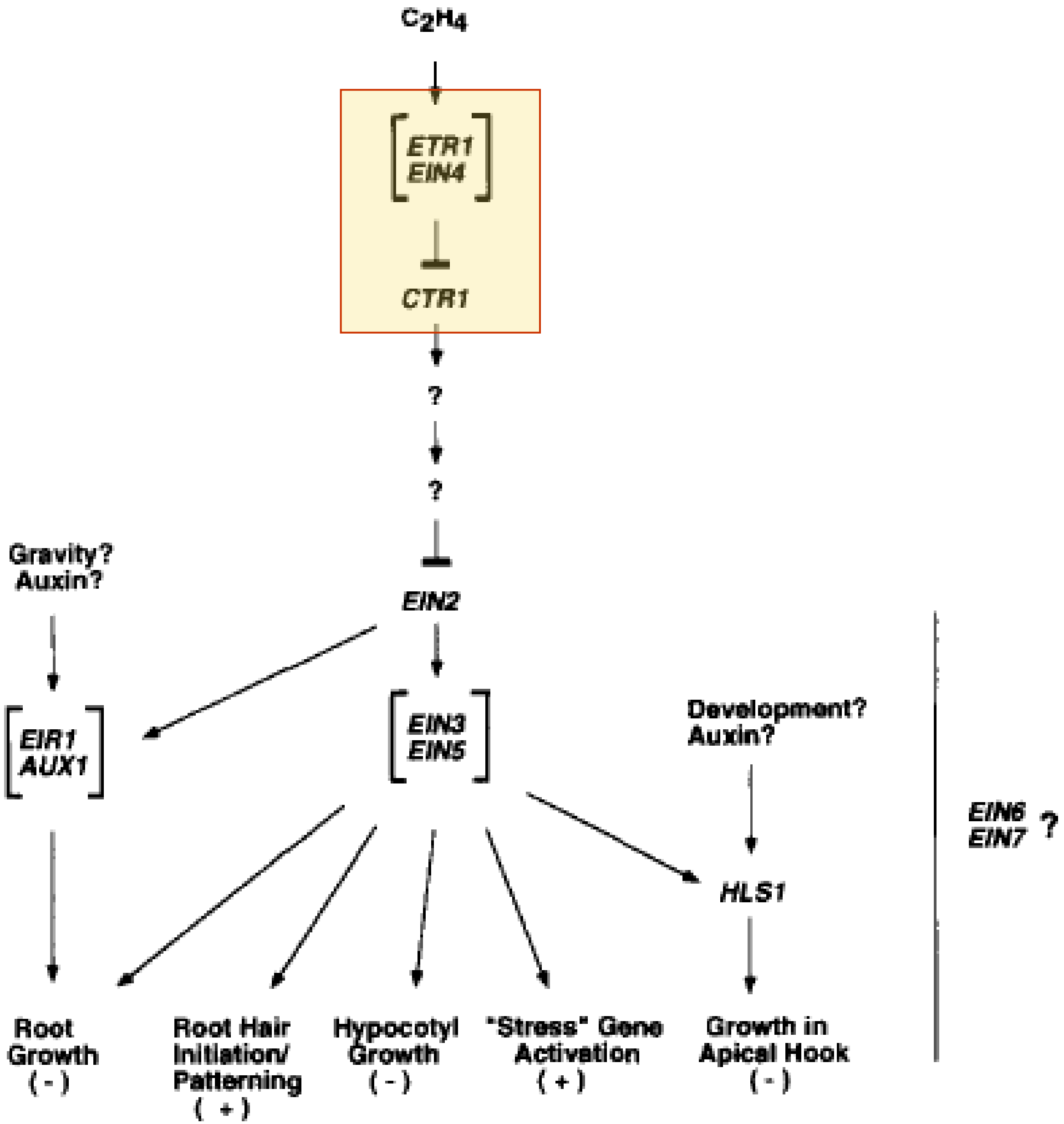


His-kinases in Arabidopsis



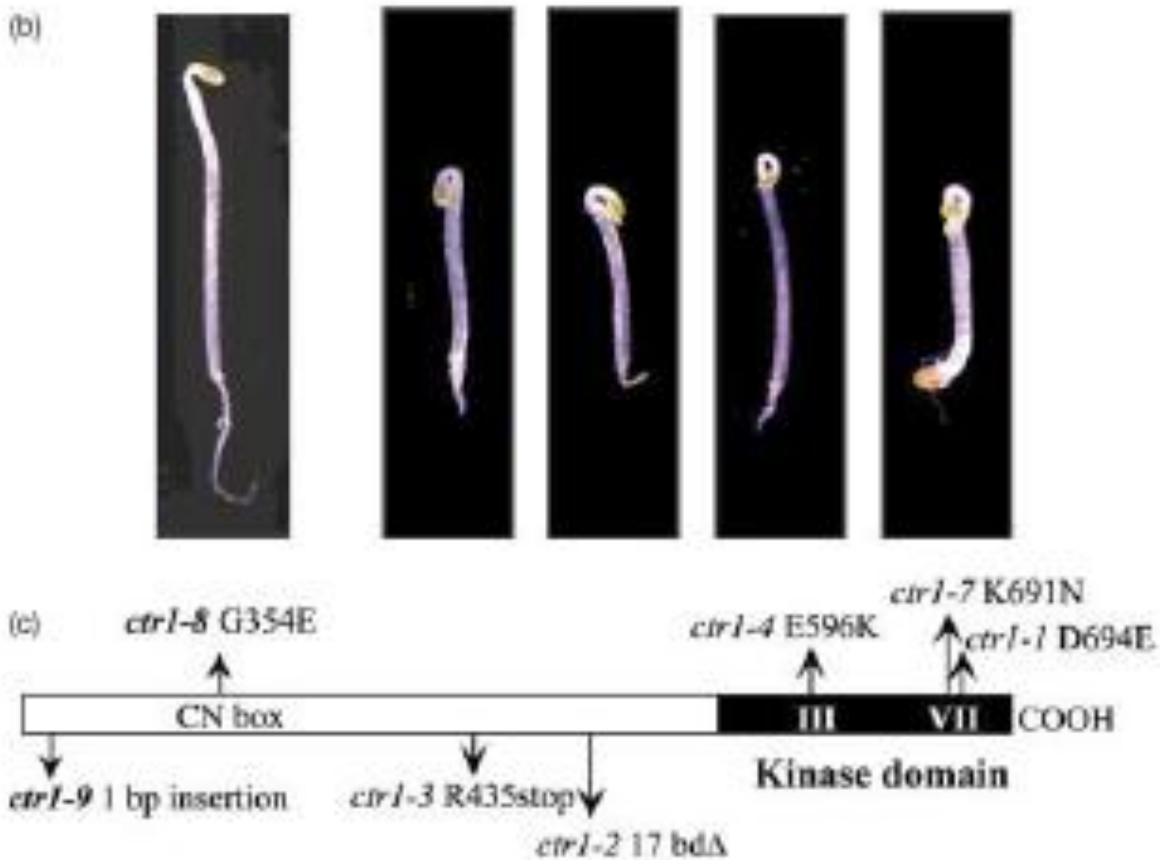
Ethylene signalling
– homology to two component system ?



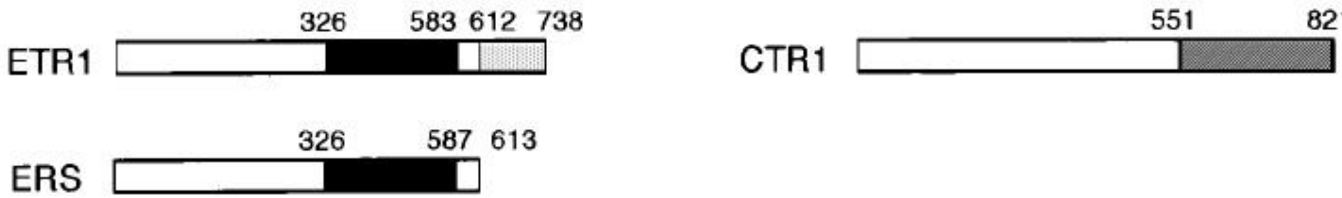


C_2H_4
ETR1
EIN4
CTR1
?
?
EIN2
Gravity?
Auxin?
EIR1
AUX1
EIN3
EIN5
Development?
Auxin?
HLS1
EIN6
EIN7 ?
Root Growth (-)
Root Hair Initiation/ Patterning (+)
Hypocotyl Growth (-)
"Stress" Gene Activation (+)
Growth in Apical Hook (-)

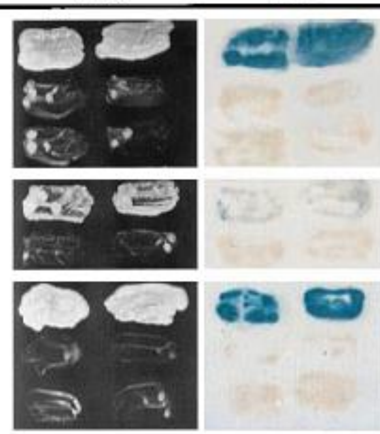
Ctr1 – codes for protein kinase of Raf family



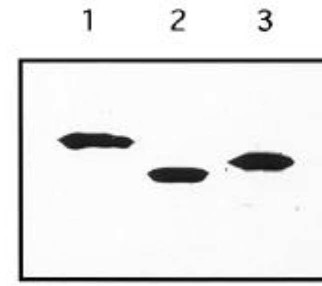
ETR1 interacts with CTR1



	DB FUSION	AD FUSION	HIS	lacZ	β-gal units
1	ETR1 293-729	CTR1 53-568			71 ± 5.0
	"	CTR1 538-821			0.10 ± 0.02
	"	vector			0.07 ± 0.02
2	ETR1 293-610	CTR1 53-568			0.10 ± 0.02
	"	vector			0.04 ± 0.00
3	ERS 261-613	CTR1 53-568			4.4 ± 0.20
	"	vector			0.05 ± 0.01
	lamin	CTR1 53-568			0.05 ± 0.01

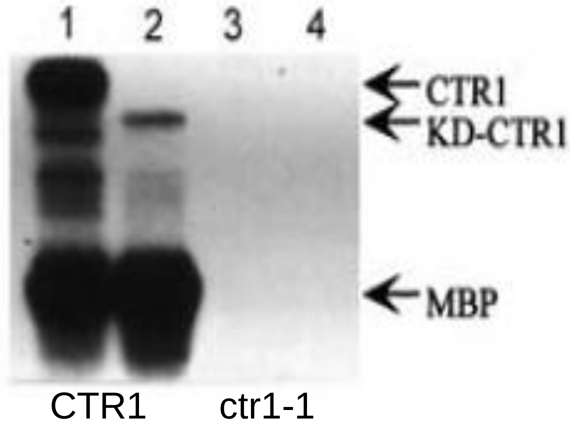


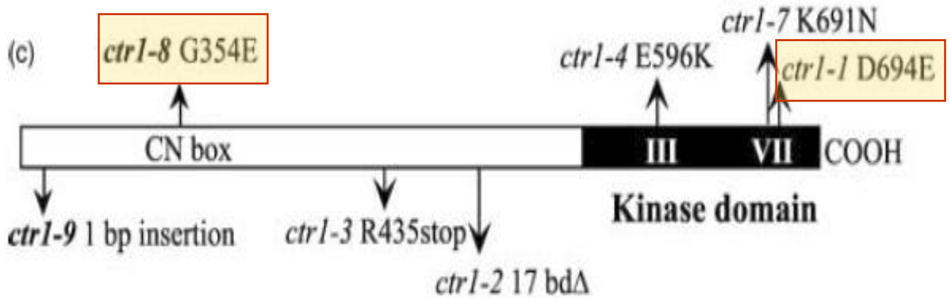
C

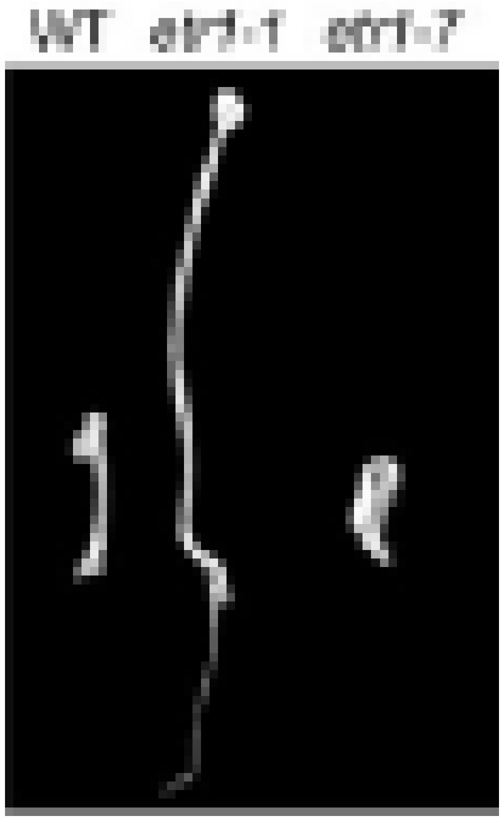


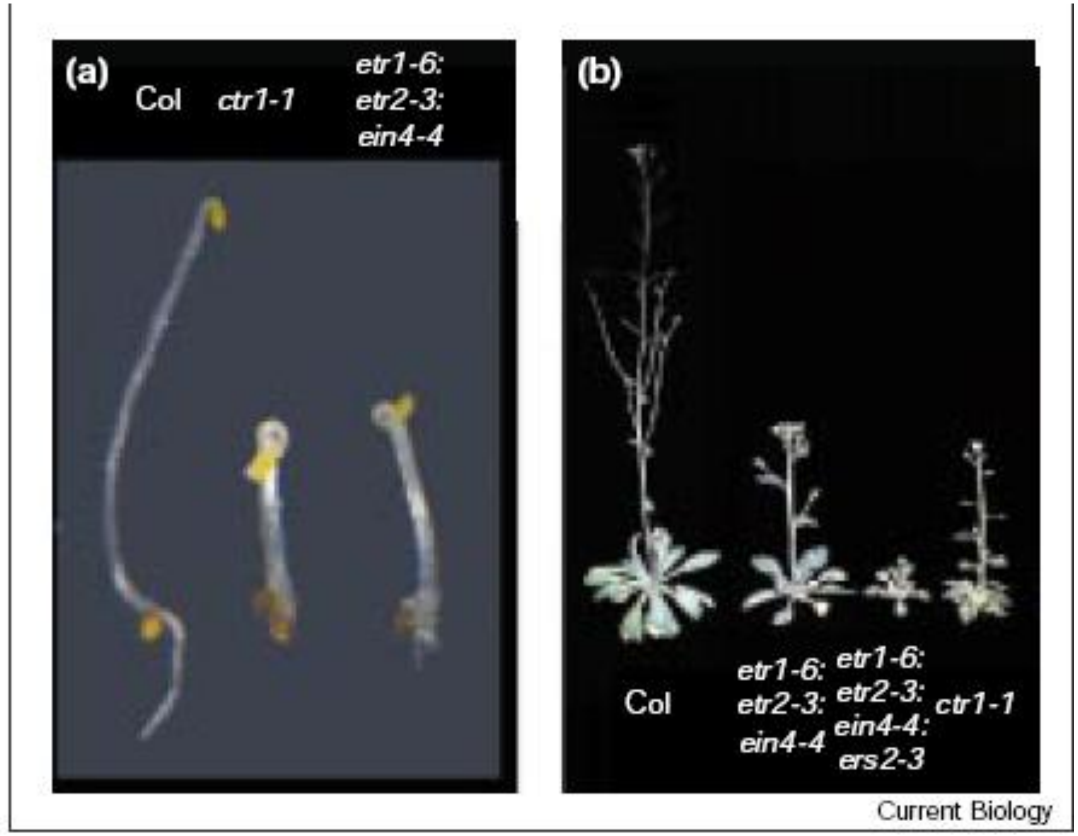
Clark et al., 1998

CTR1 has protein kinase activity





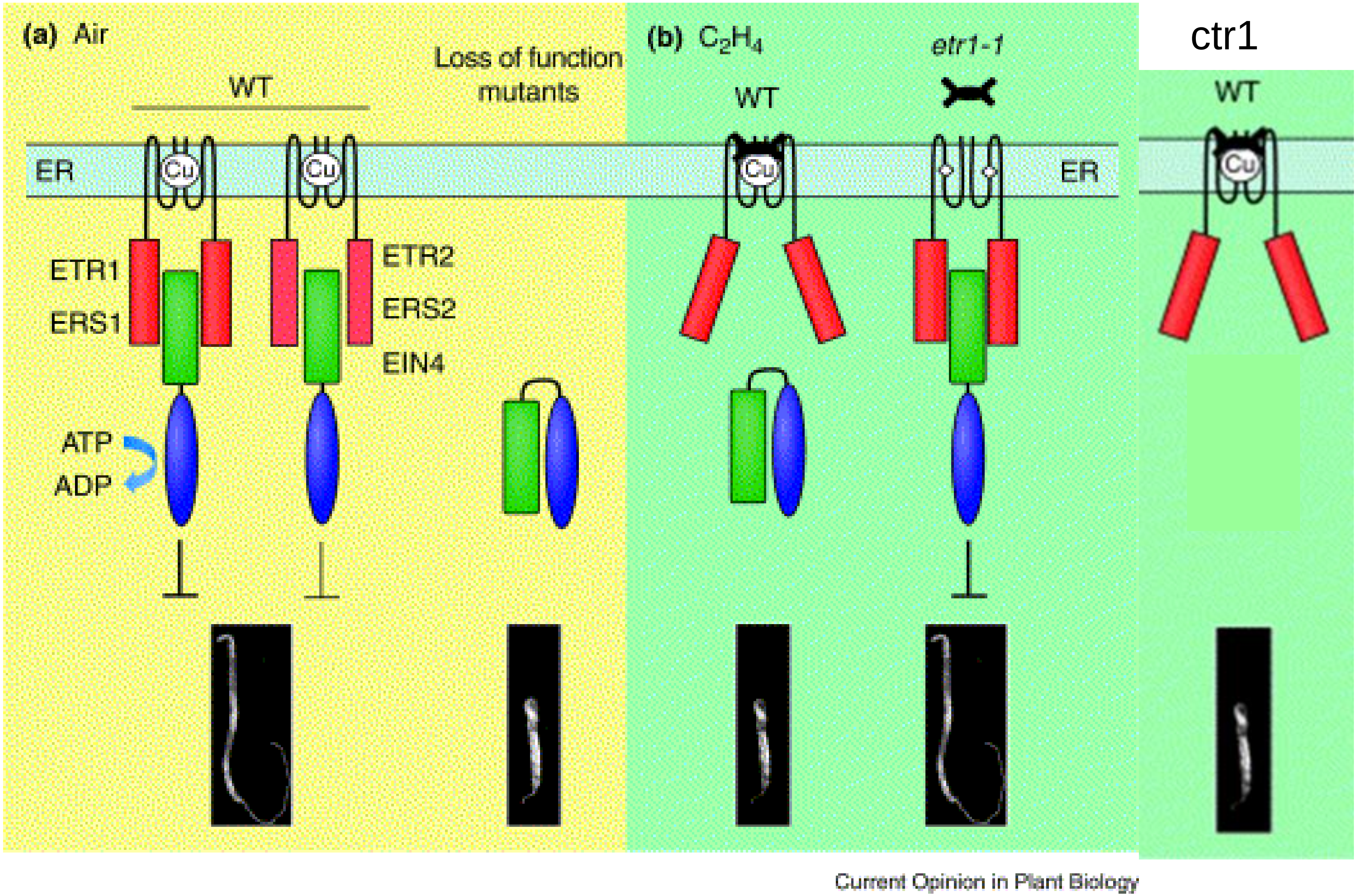


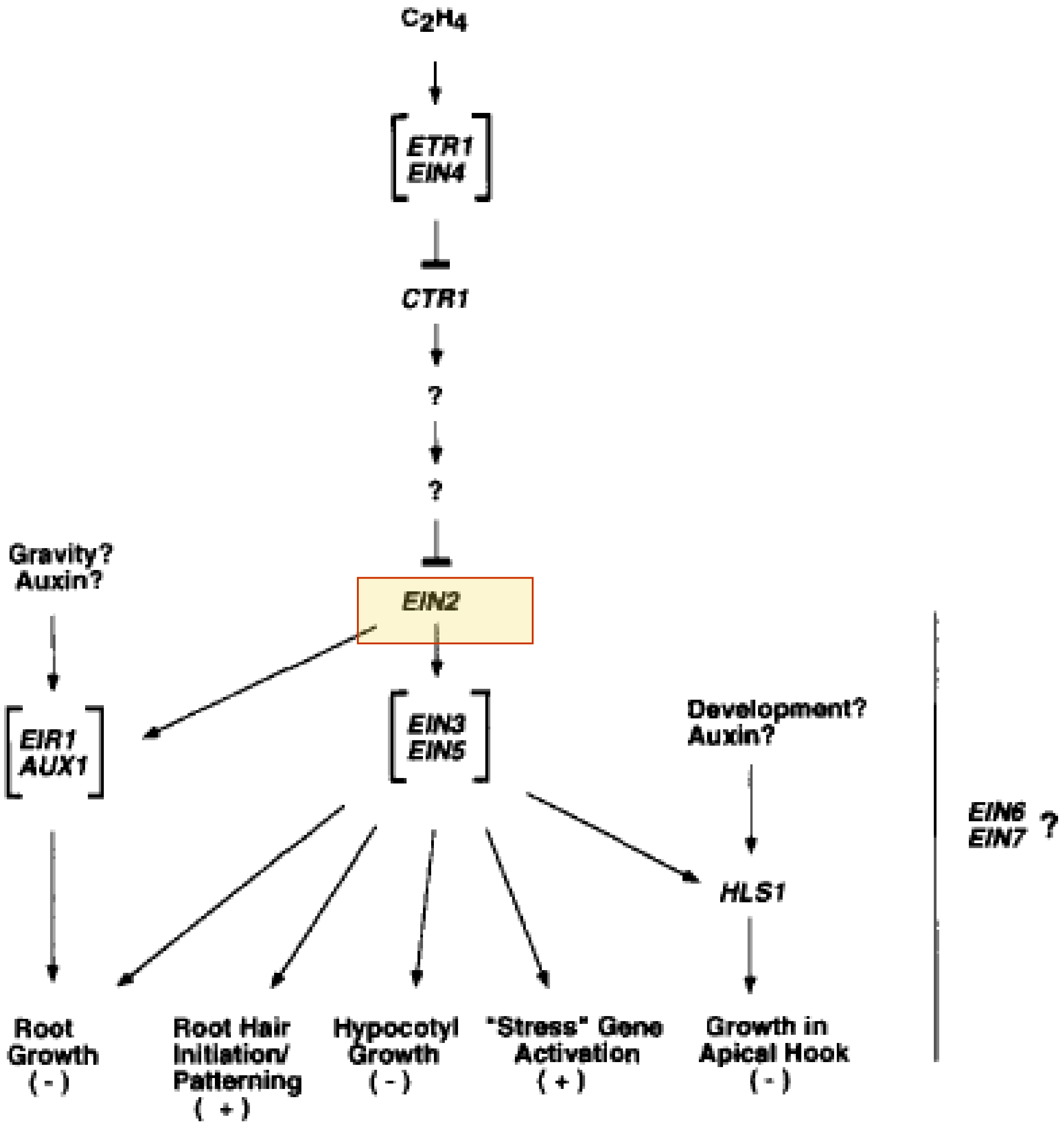


-ethylene receptor dominant mutation > ethylene insensitivity

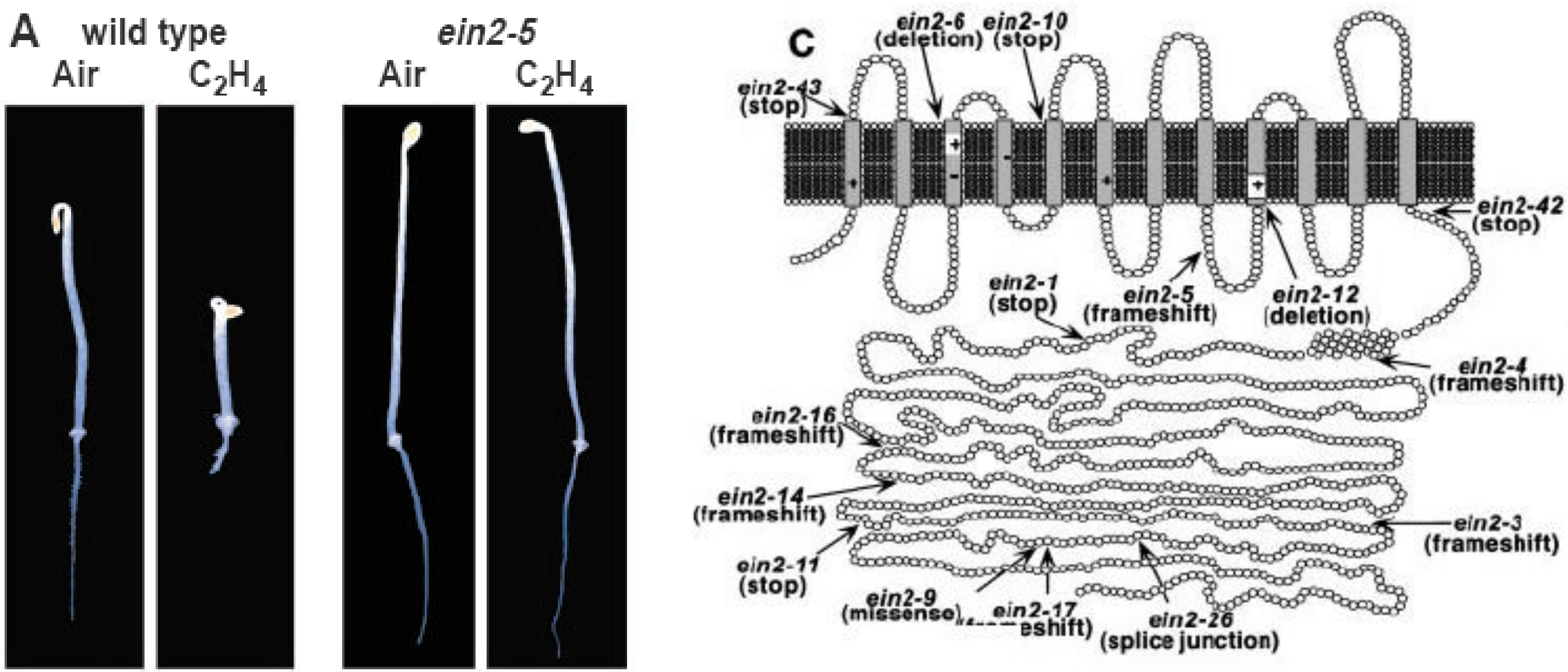
-ethylene receptor loss of function mutation > constitutive ethylene response

-*ctr1* loss of function mutation > constitutive ethylene response

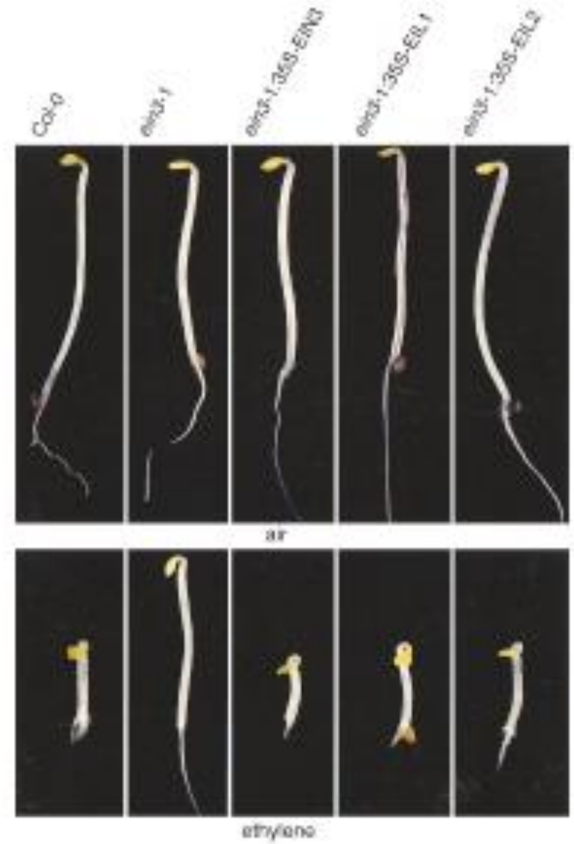

ctr1
(a) Air
WT
Loss of function mutants
(b) C_2H_4
etr1-1
WT
WT
ER
ER
Cu
ETR1
ERS1
ETR2
ERS2
EIN4
ATP
ADP
Current Opinion in Plant Biology

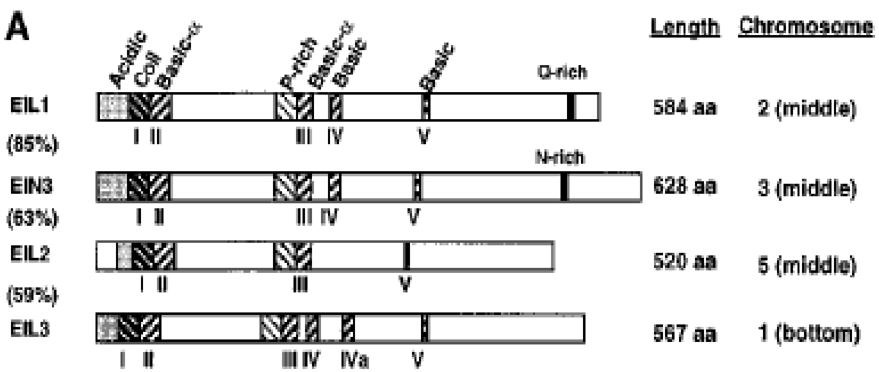

C_2H_4
ETR1
EIN4
CTR1
?
?
Gravity?
Auxin?
EIN2
EIR1
AUX1
EIN3
EIN5
Development?
Auxin?
EIN6
EIN7 ?
HLS1
Root Growth (-)
Root Hair Initiation/ Patterning (+)
Hypocotyl Growth (-)
"Stress" Gene Activation (+)
Growth in Apical Hook (-)

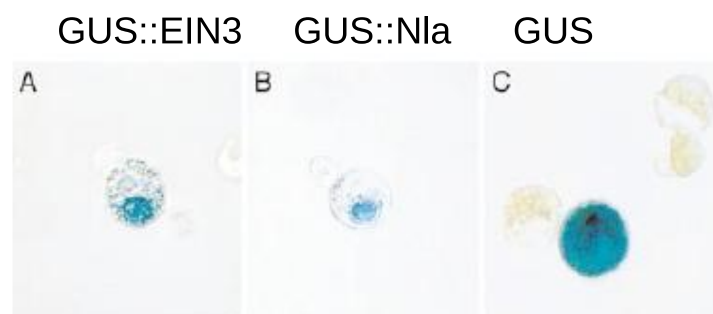
EIN2 - contains domain similar to Nramp metal transporters protein.



EIN3 codes for transcription factor

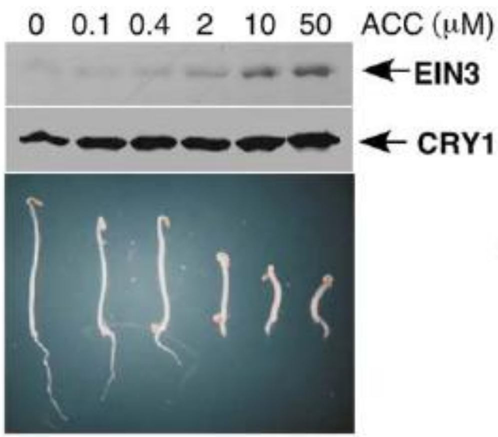


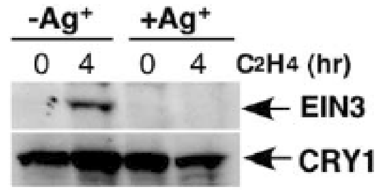


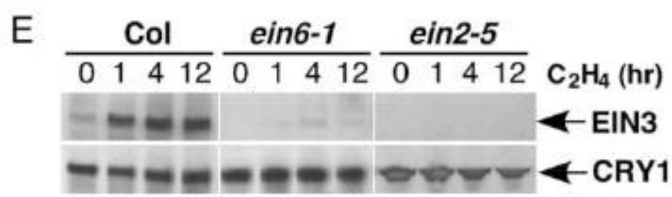


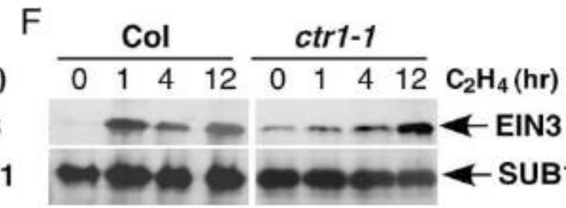
Chao et al., 1997

EIN3 protein level is controlled by ethylene

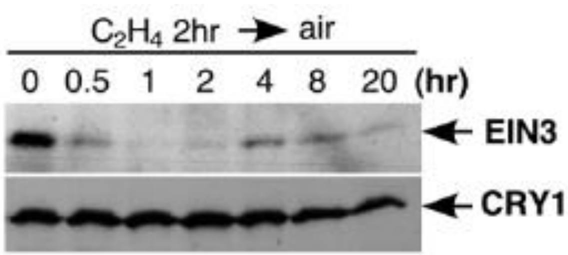


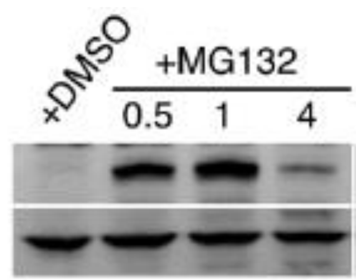






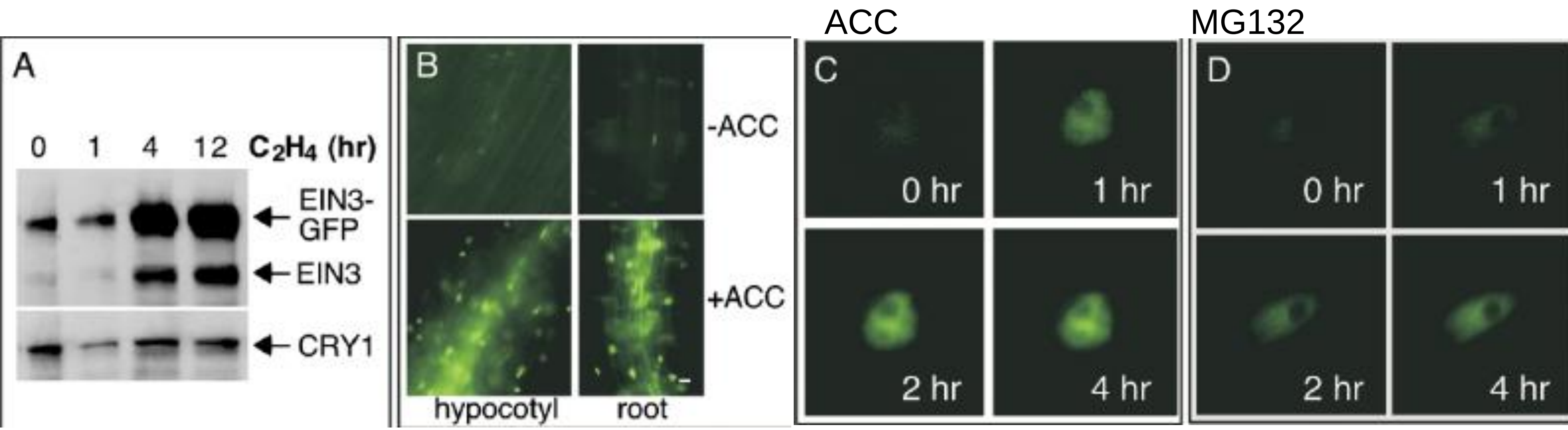
EIN3 is rapidly degraded by a proteasome-mediated pathway



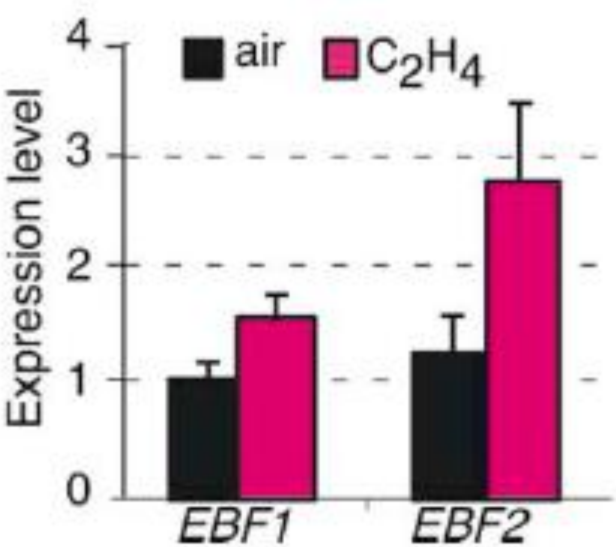


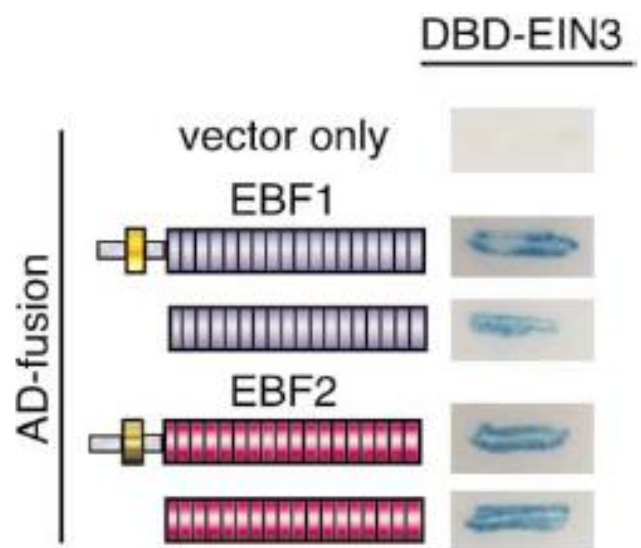
Guo and Ecker, 2003

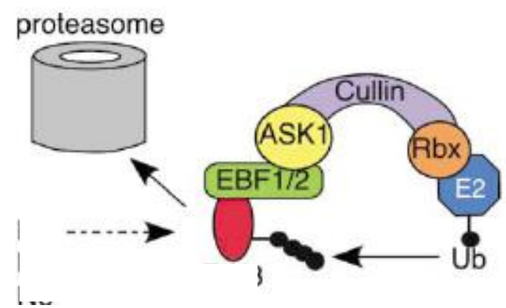
EIN3 accumulates in nucleus upon ethylene or MG132 treatment



EIN3 interacts with F-box proteins

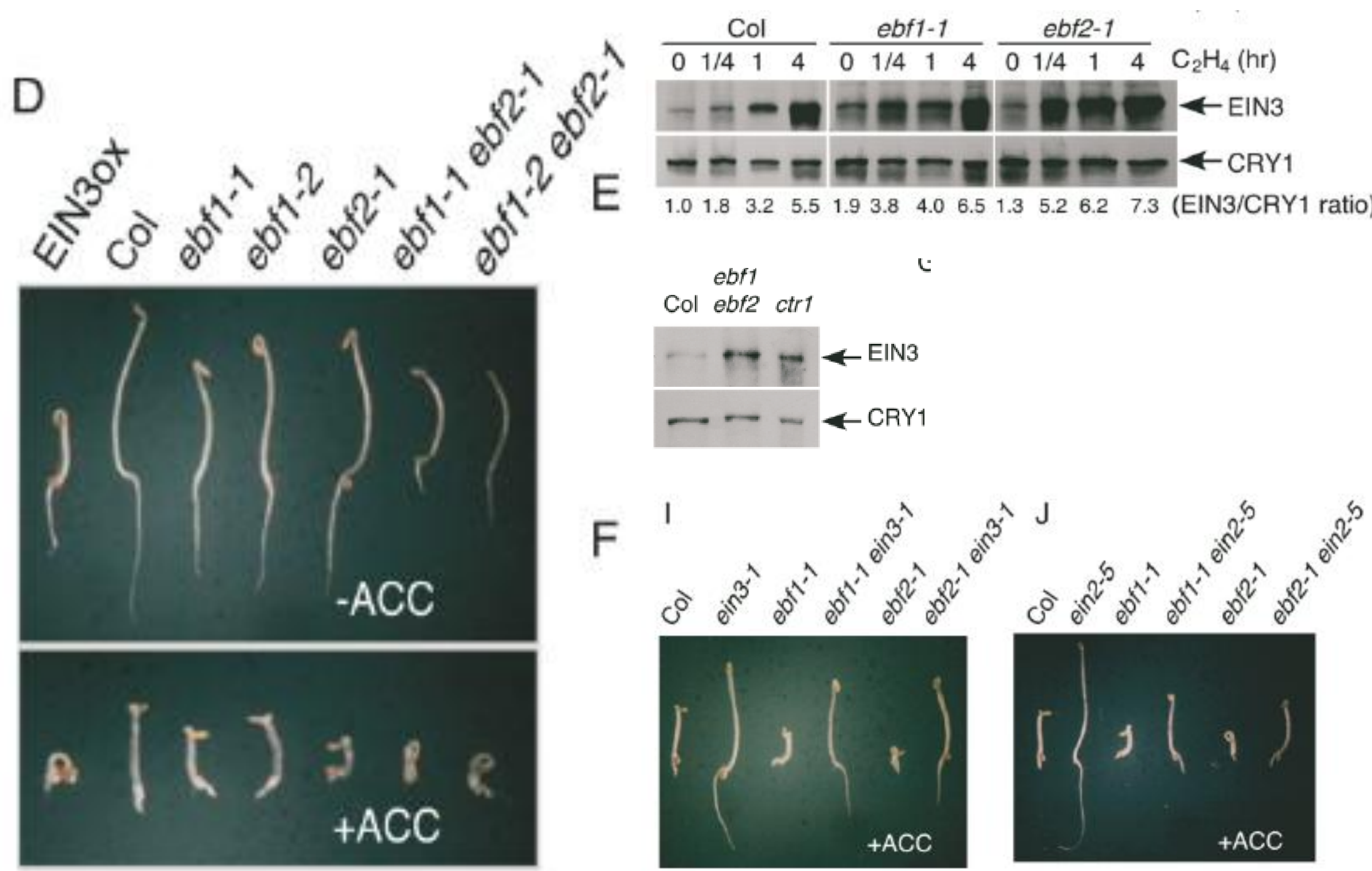






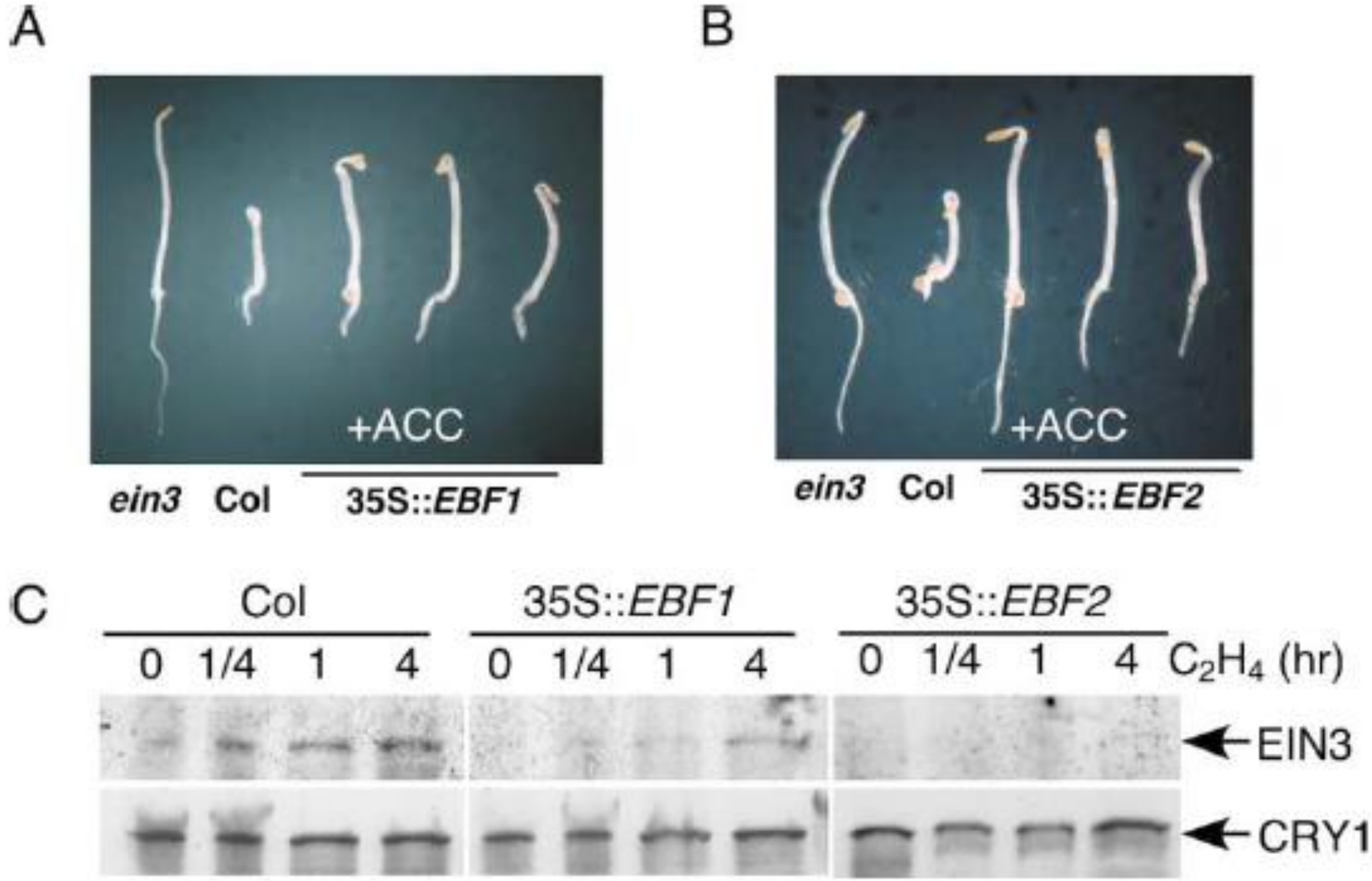
Guo and Ecker, 2003

Mutation in EBF1 and EBF2 results in hypersensitivity to ethylene and accumulation of EIN3 protein



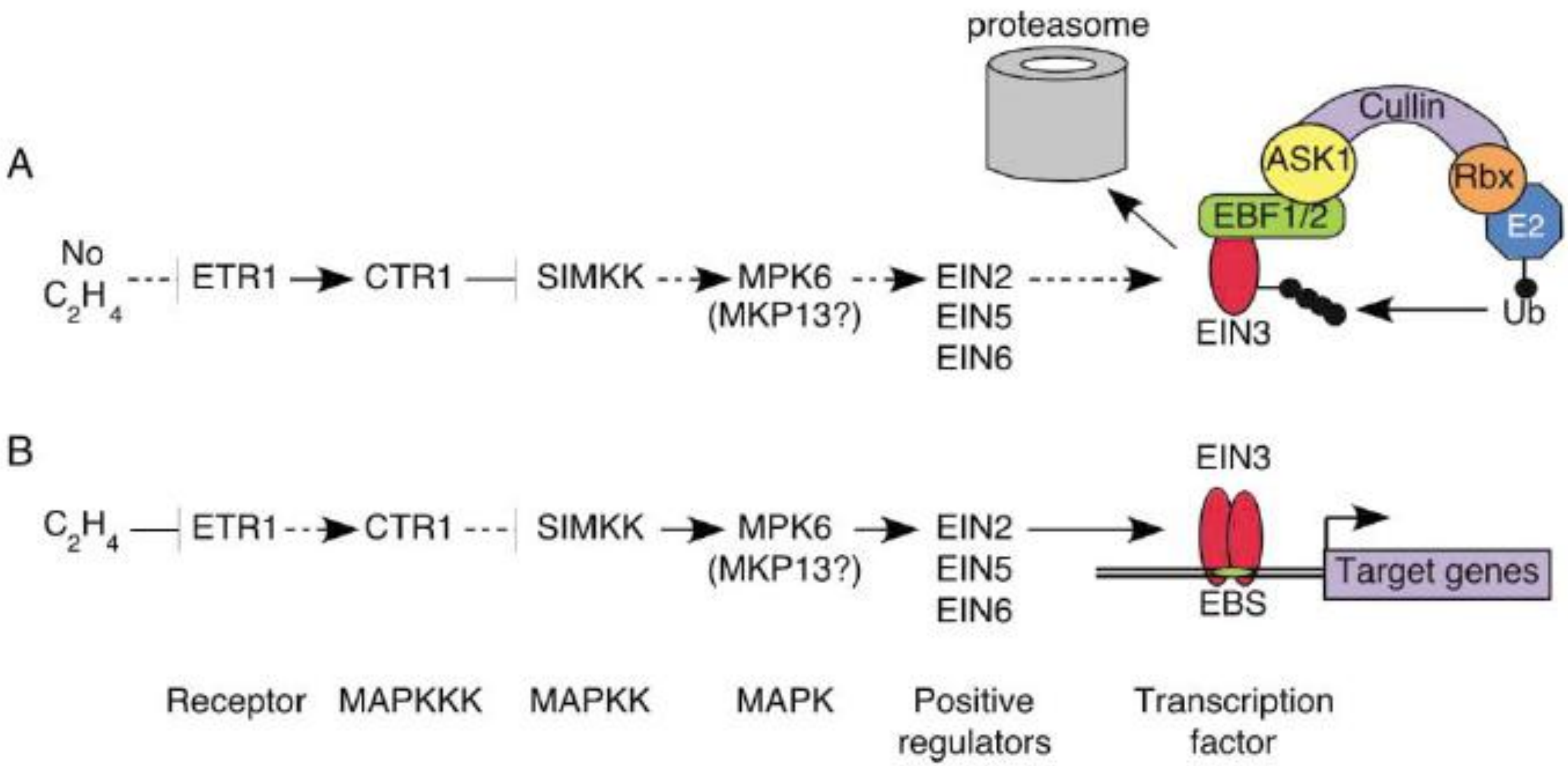
Guo and Ecker, 2003

Overexpression of EBF1 and EBF2 results in ethylene insensitivity and reduced accumulation of EIN3 protein



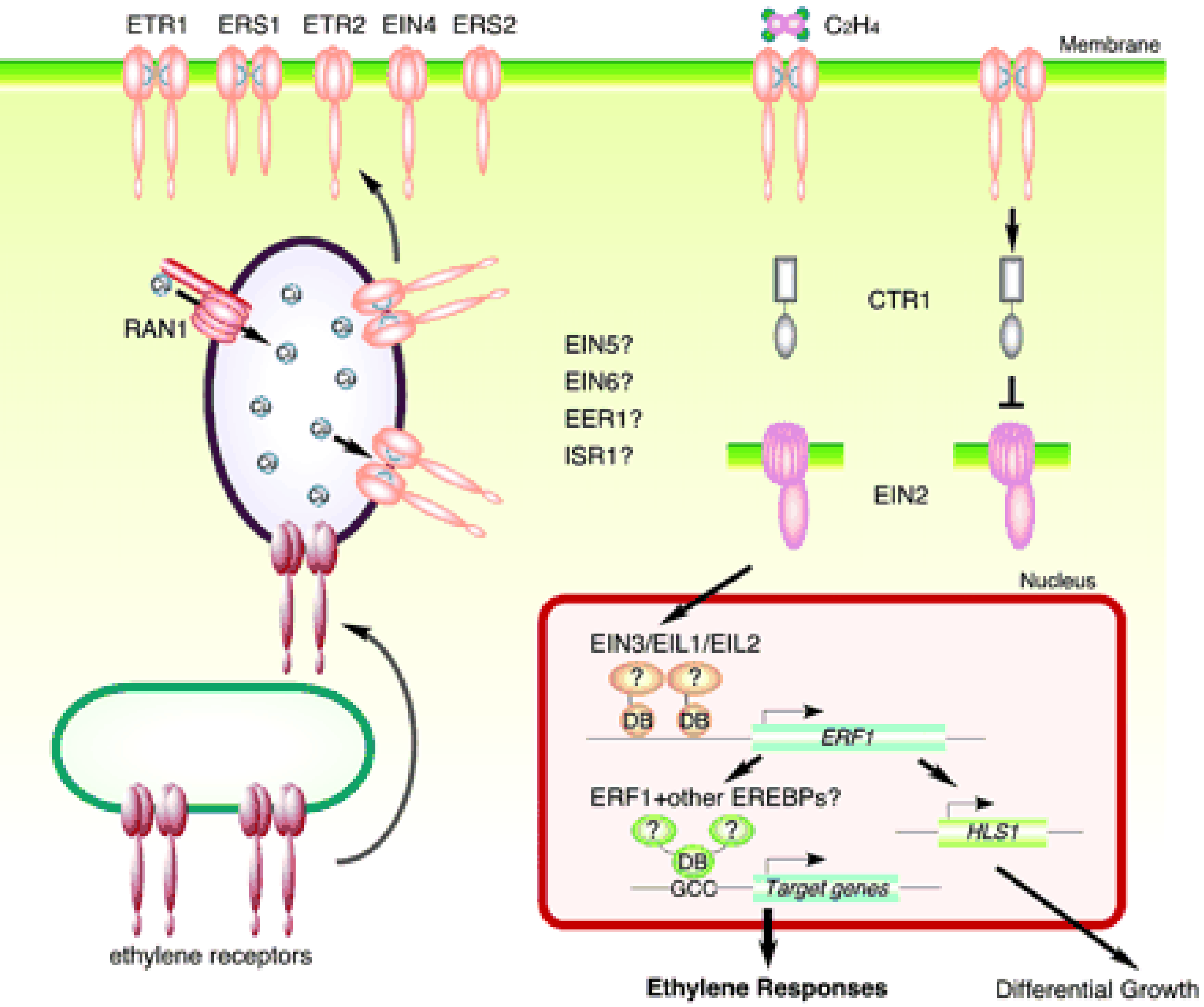
Guo and Ecker, 2003

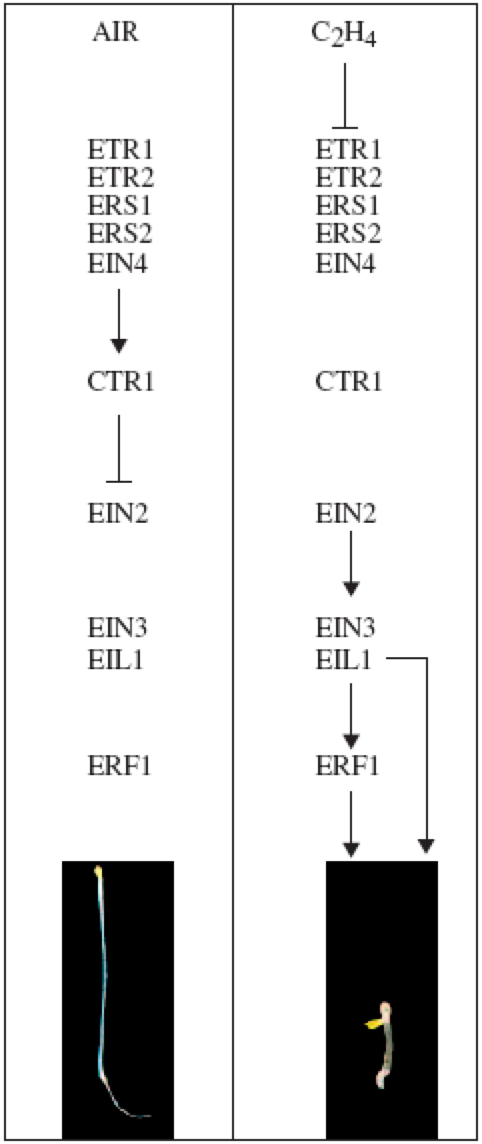
MODELS



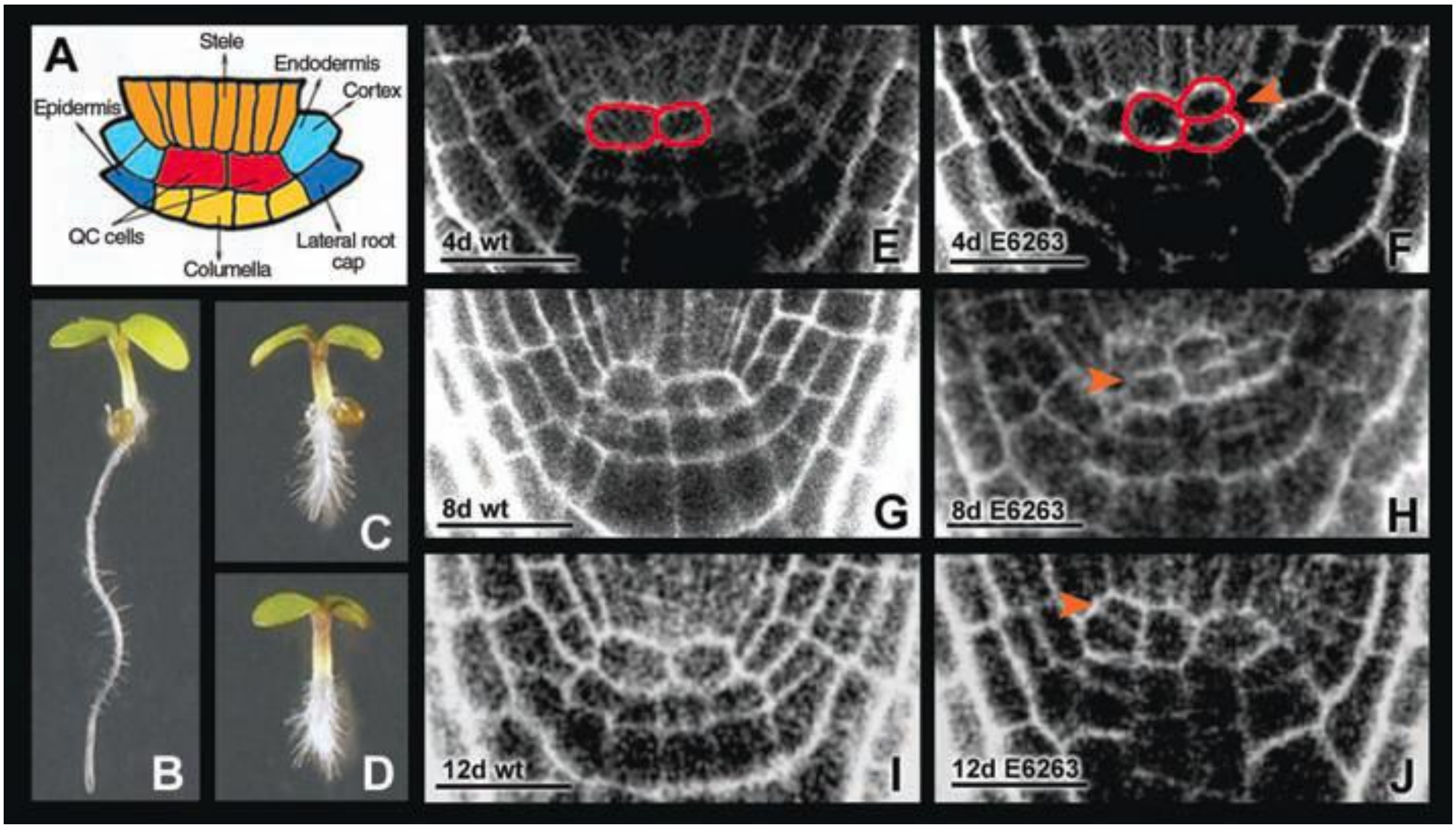
Guo and Ecker, 2003

Ethylene signal transduction pathway



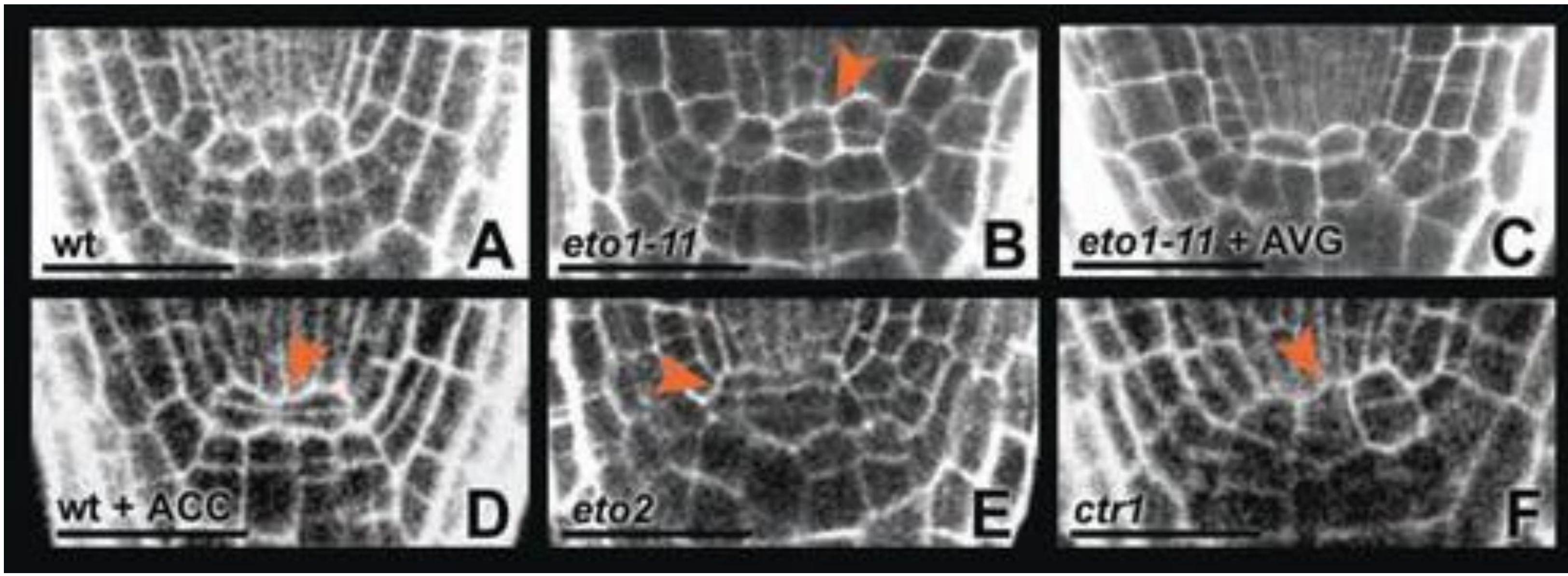


Ethylene Modulates Stem Cell Division in roots

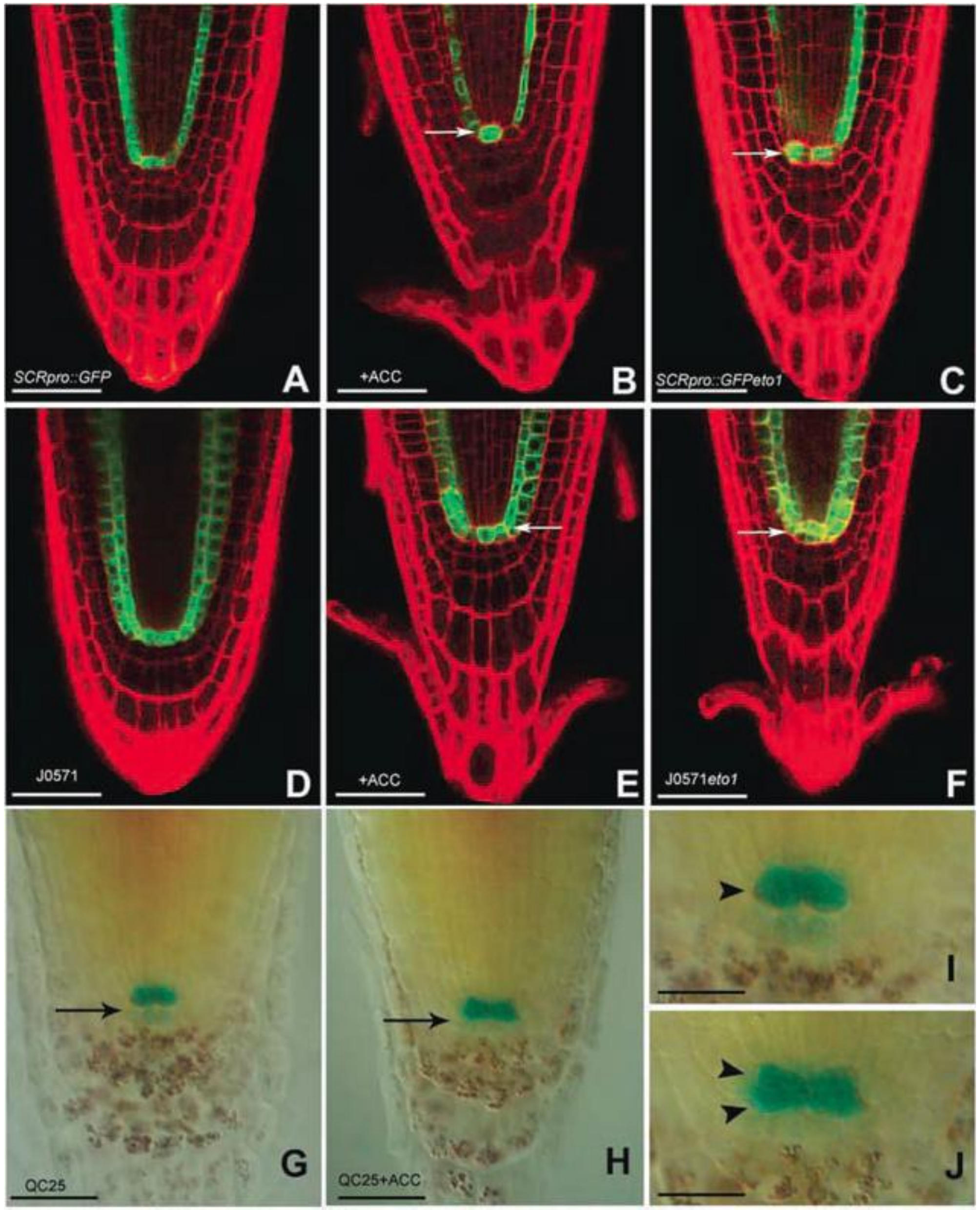


Olga Ortega-Martínez et al., 2007

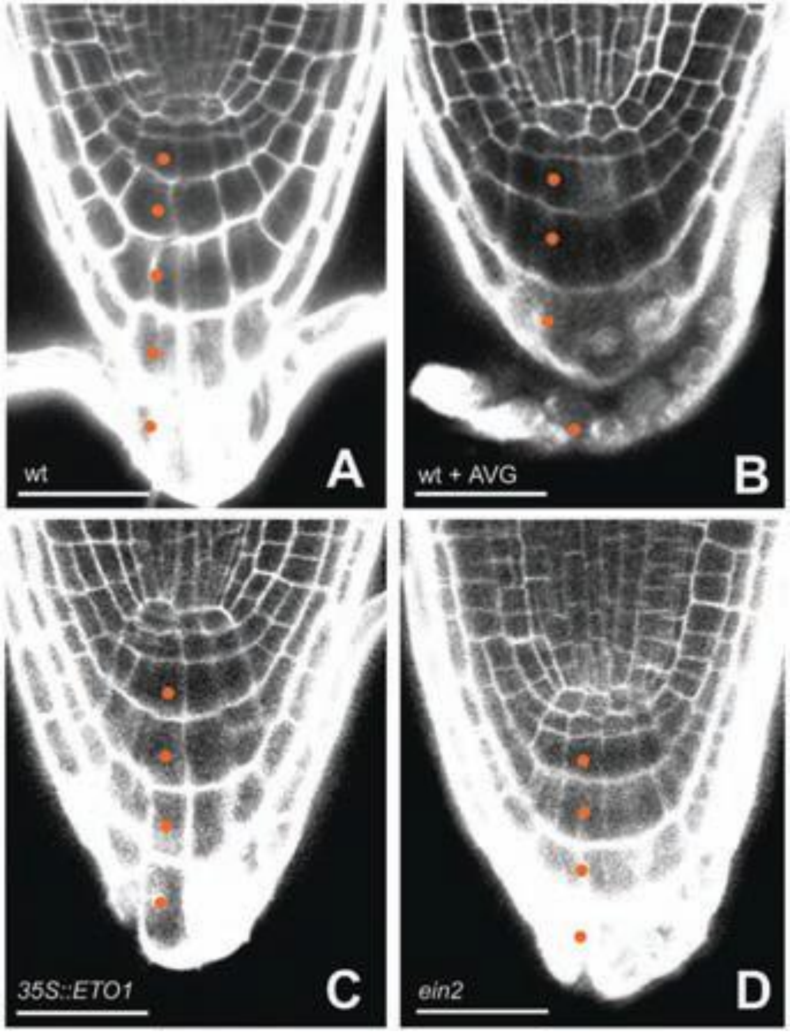
Ethylene promotes QC cell division.



QC cell identity and function are maintained in eto1 mutants



Ethylene stimulates formation of additional columella cell layers



Yeast two hybrid screen

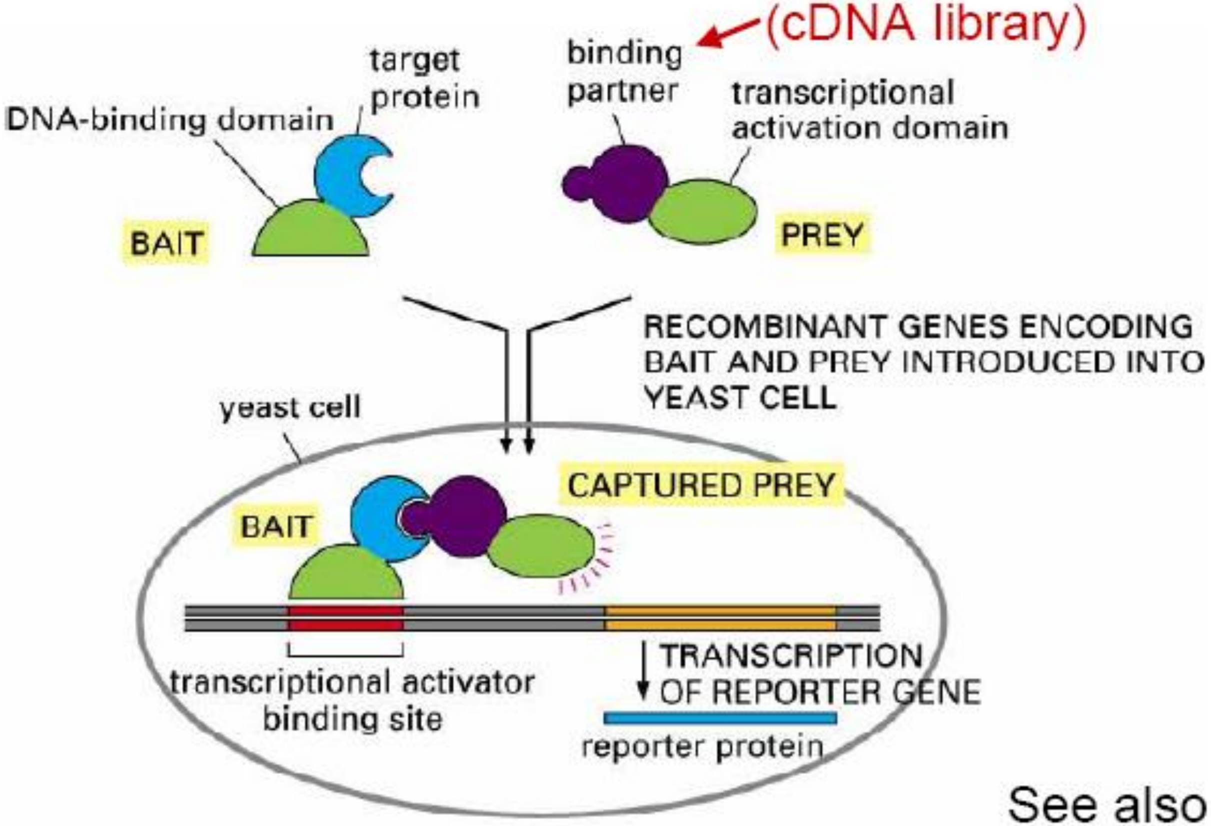


Figure 8–51. Molecular Biology of the Cell, 4th Edition.

See also MCB Fig. 11-39

GST "pull downs"

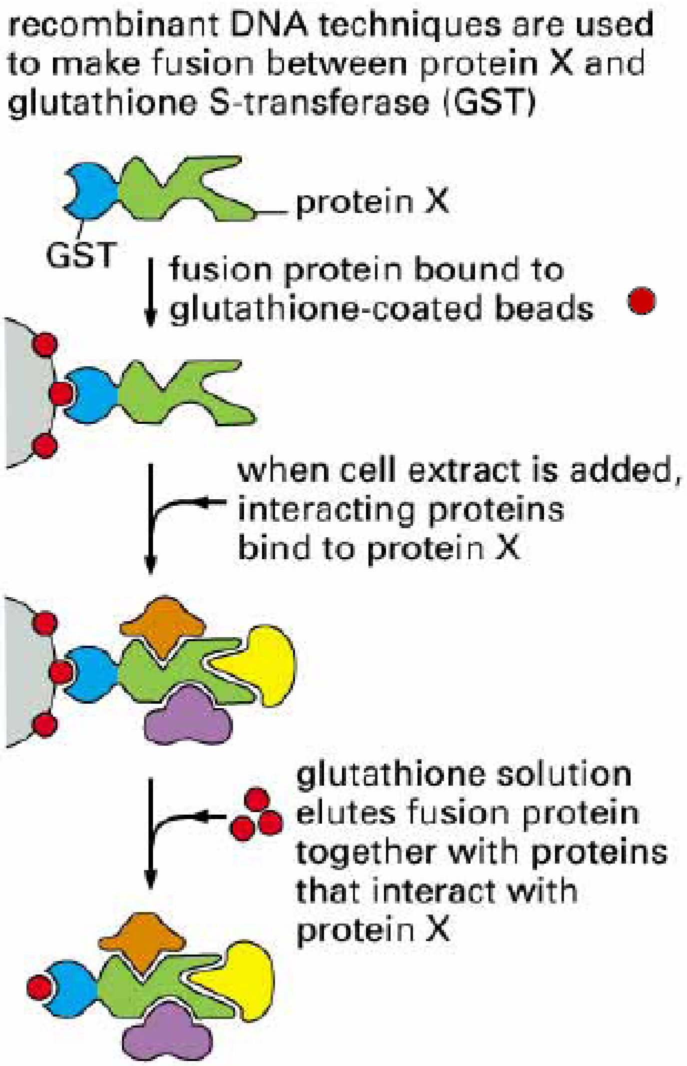
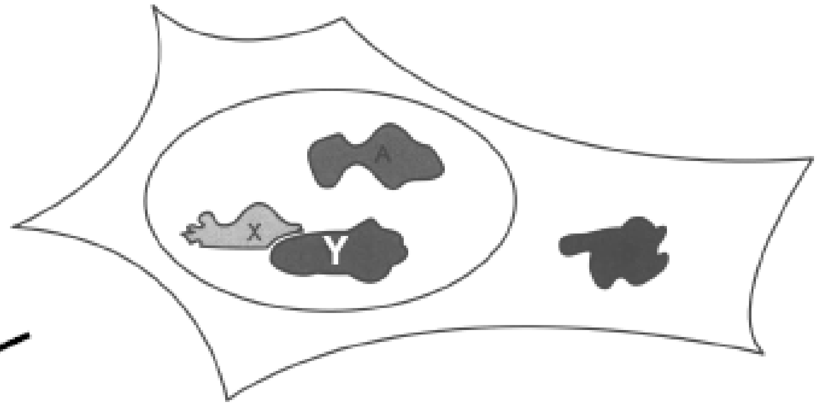


Figure 8–50. Molecular Biology of the Cell, 4th Edition.

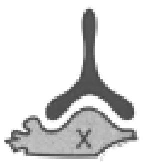
- GST protein is usually expressed in E. coli as microgram quantities are used in typical assays
- Detection of bound proteins are usually by western blotting, using antibody to the putative interactor
- Used extensively with GST-domain fusions in structure function studies
- New proteins can be identified if metabolically labeled cells are used

Co-IP's

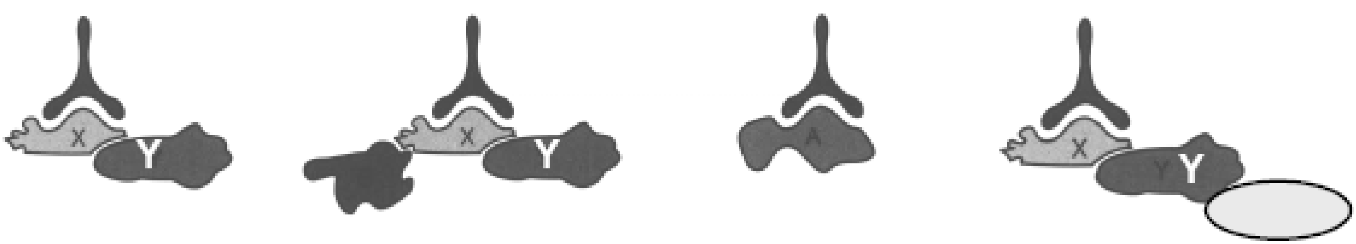
To be implicated in signal transduction, the interaction should depend on activation of the signaling pathway (compare co-IP before and after stimulation/ligand binding)



For regular IP, lyse cells using harsh conditions



Lyse cells and IP protein X using co-IP conditions (1% triton X-100, 1% Brij96, 2% digitonin, or 2% CHAPS, etc.)



IP 1 IP2 IP3 IP4