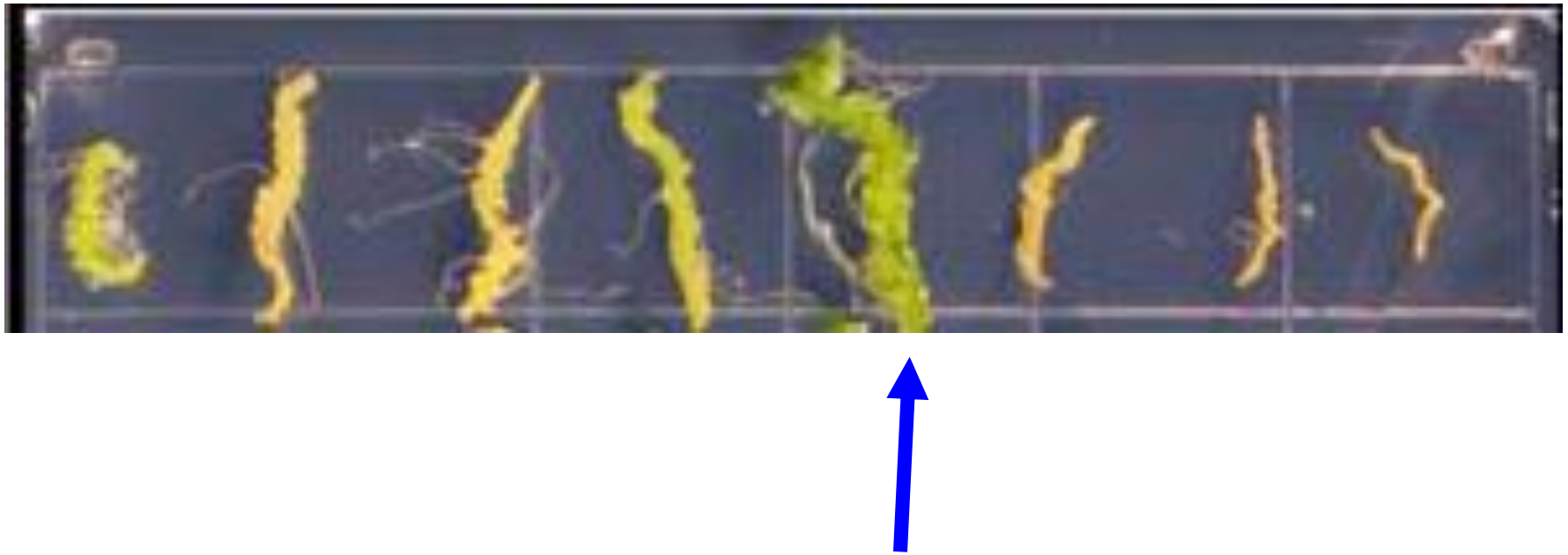
CYTOKININ - Metabolism

Synthesis – *IPT* genes

Degradation – CK-oxidase

Conjugation

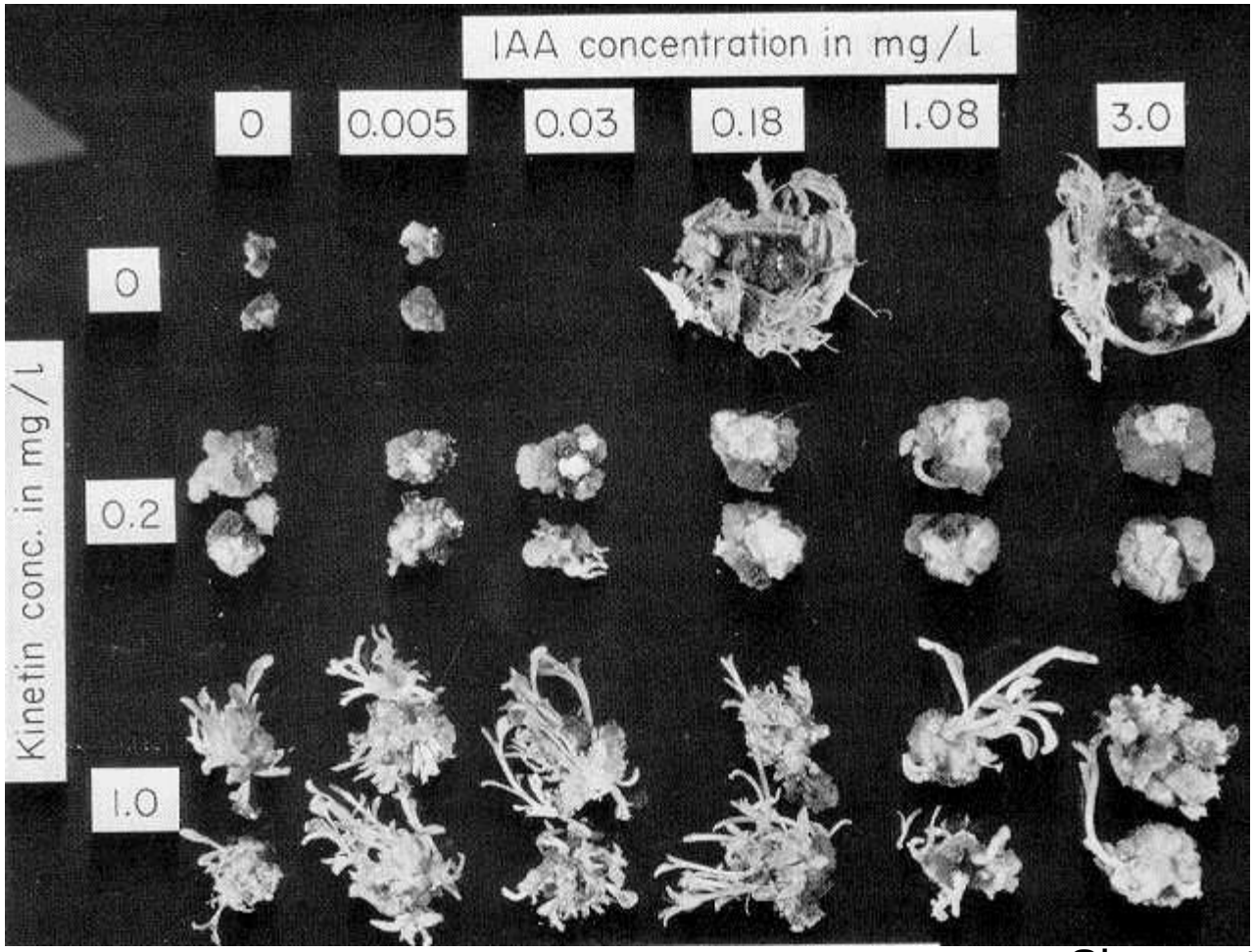
Cytokinin – substance crucial for sustain of cell proliferation



Auxin and cytokinin

roots

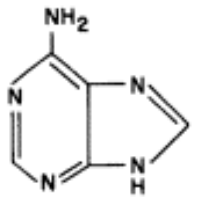
shoots



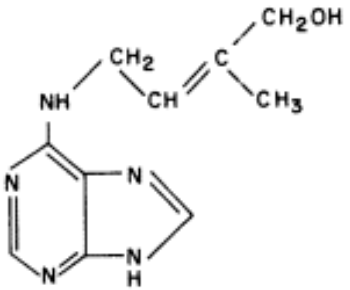
Skoog *et* Miller, 1957

Cytokinins

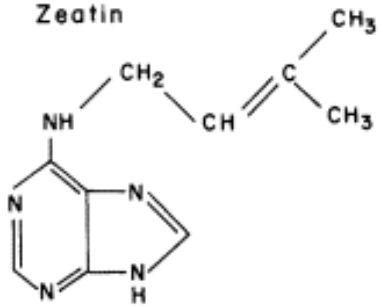
Native



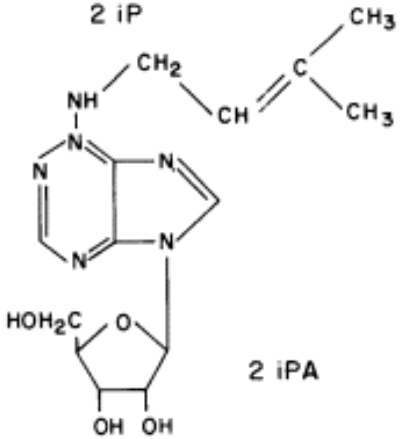
Adenine



Zeatin

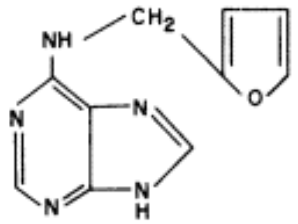


2 iP

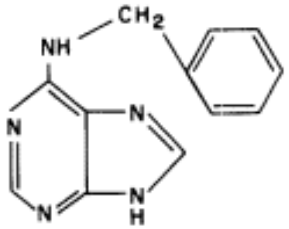


2 iPA

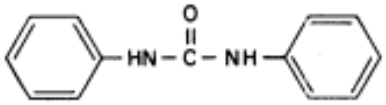
Synthetic



Kinetin

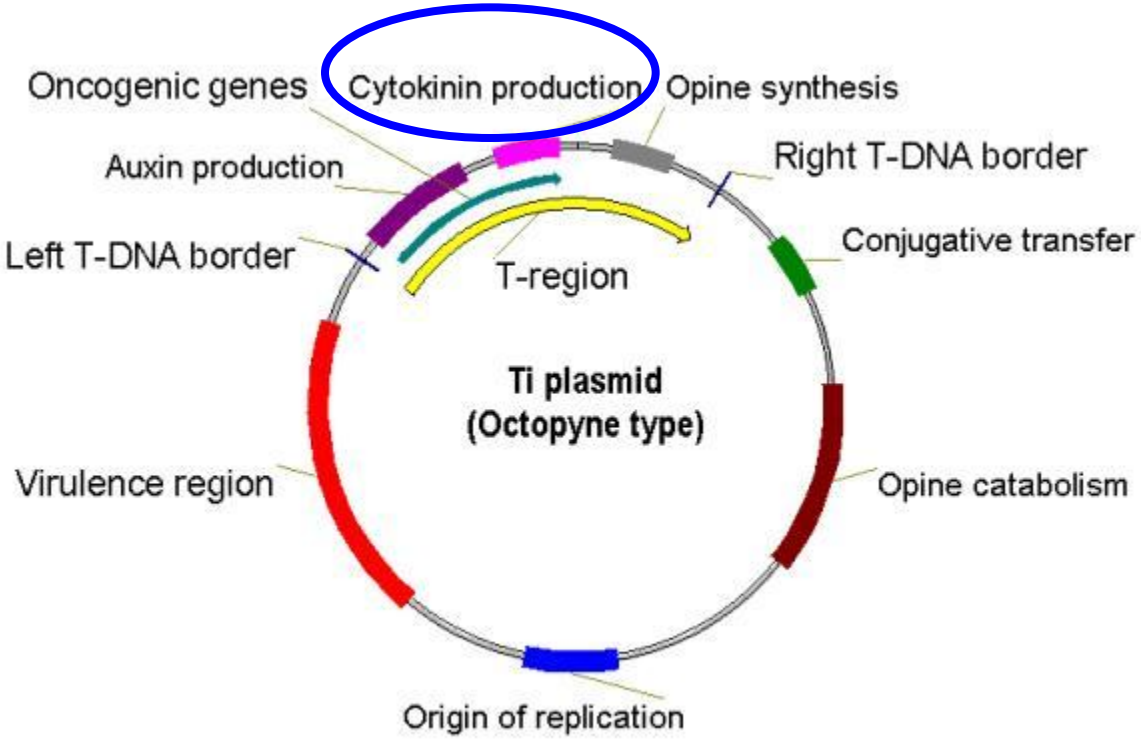


Benzyladenine (BA)

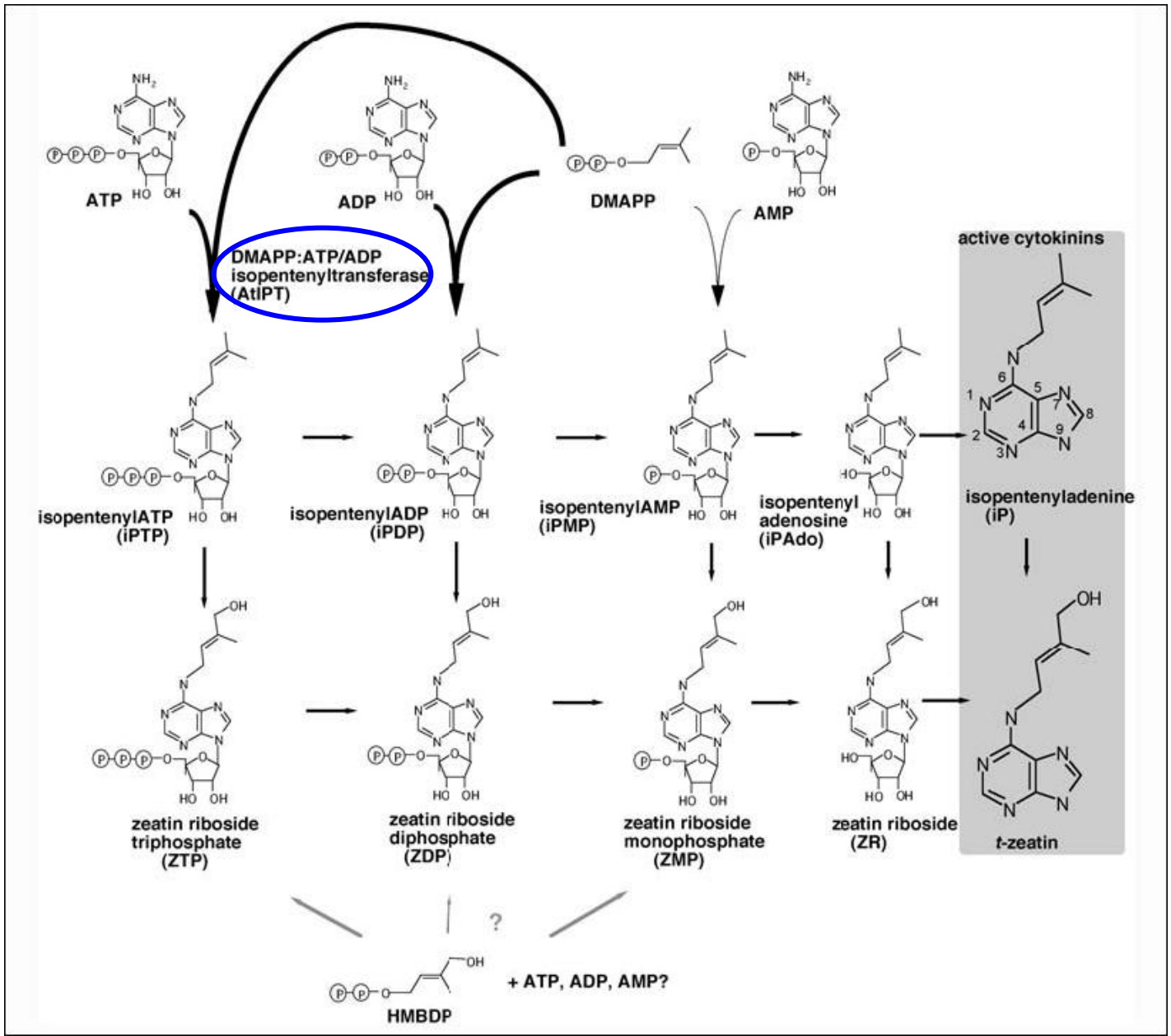


Diphenylurea

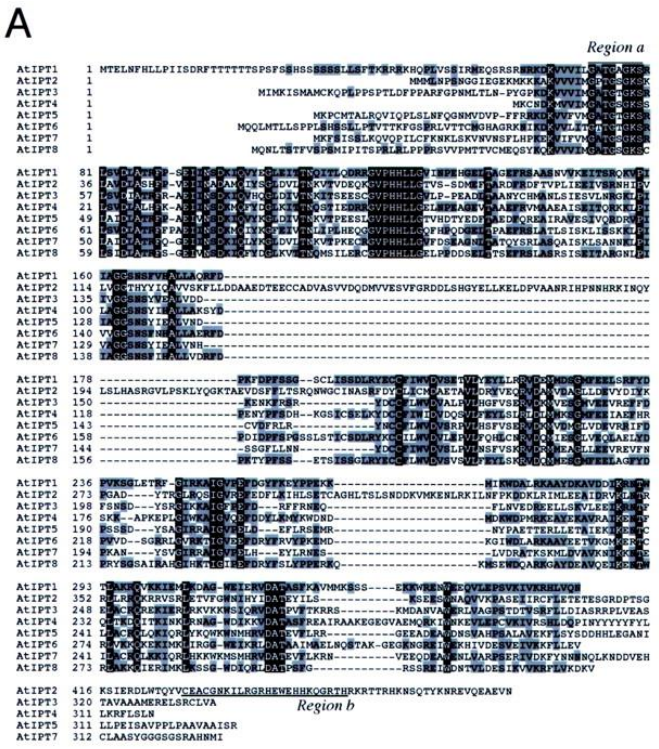
Agrobacterium tumefaciens – IPT (isopentenyltransferase) gene for cytokinin biosynthesis

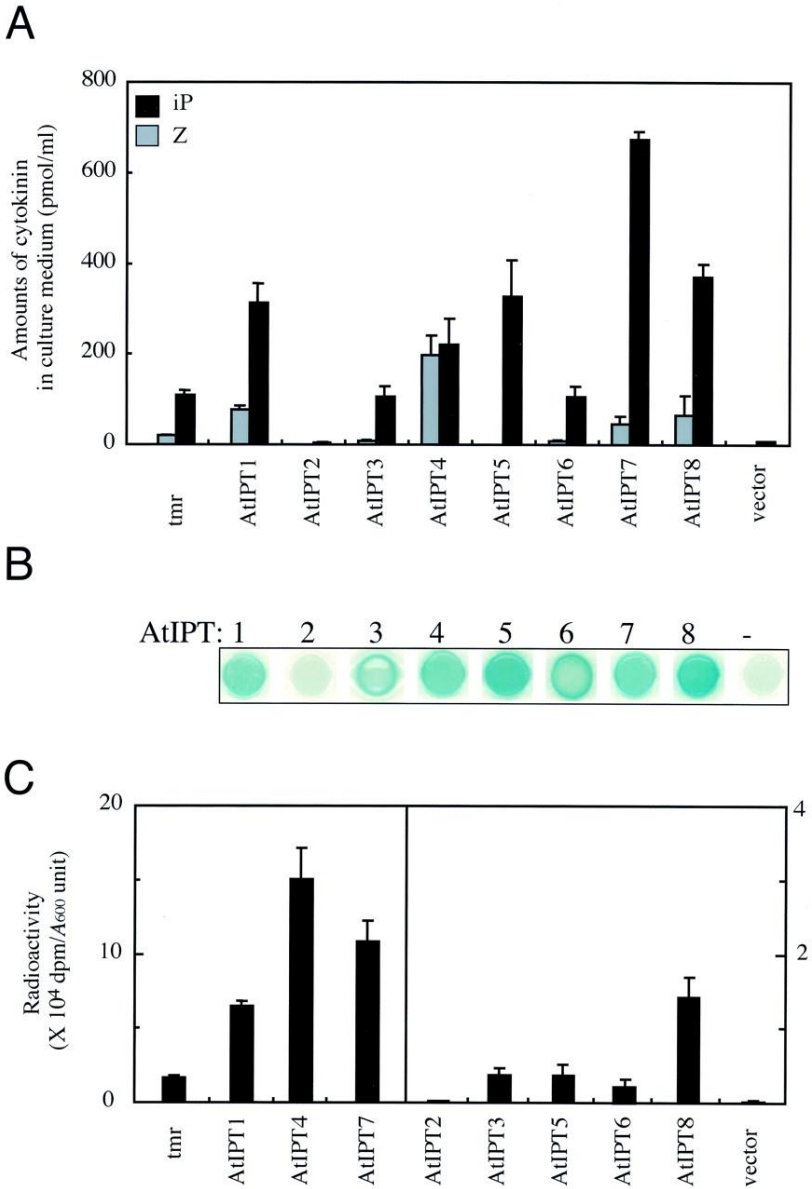


Cytokinin biosynthesis



Isolation of Arabidopsis *IPT*s





Arabidopsis *IPT*s (8)

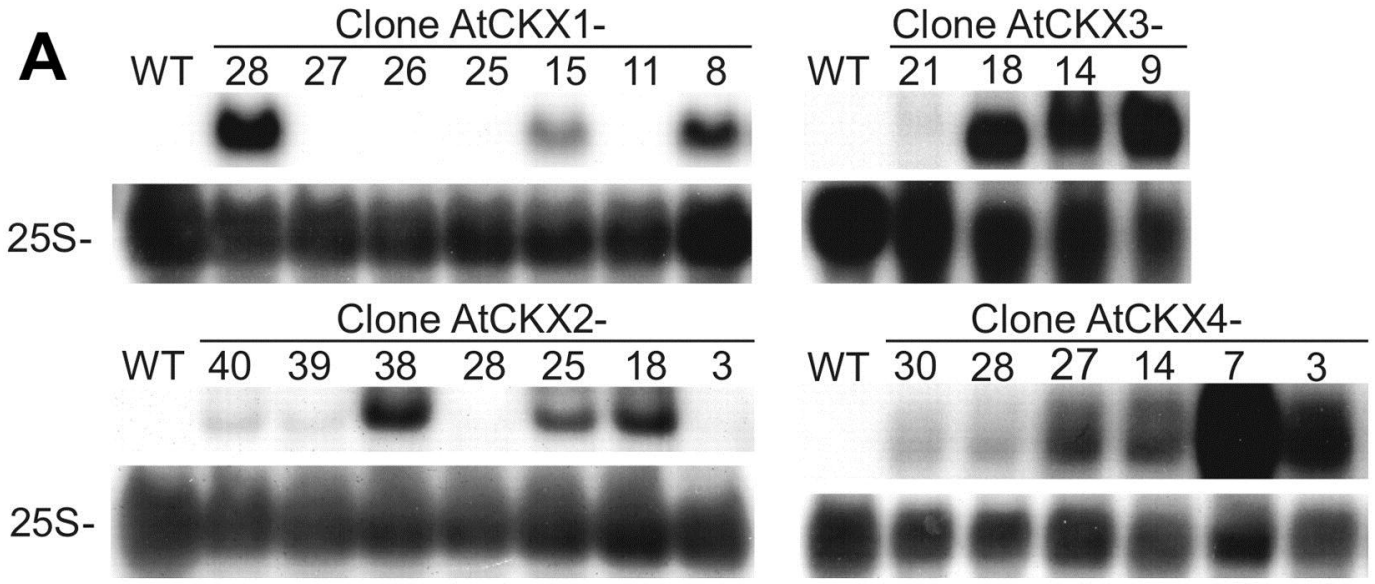
Differential expression patterns

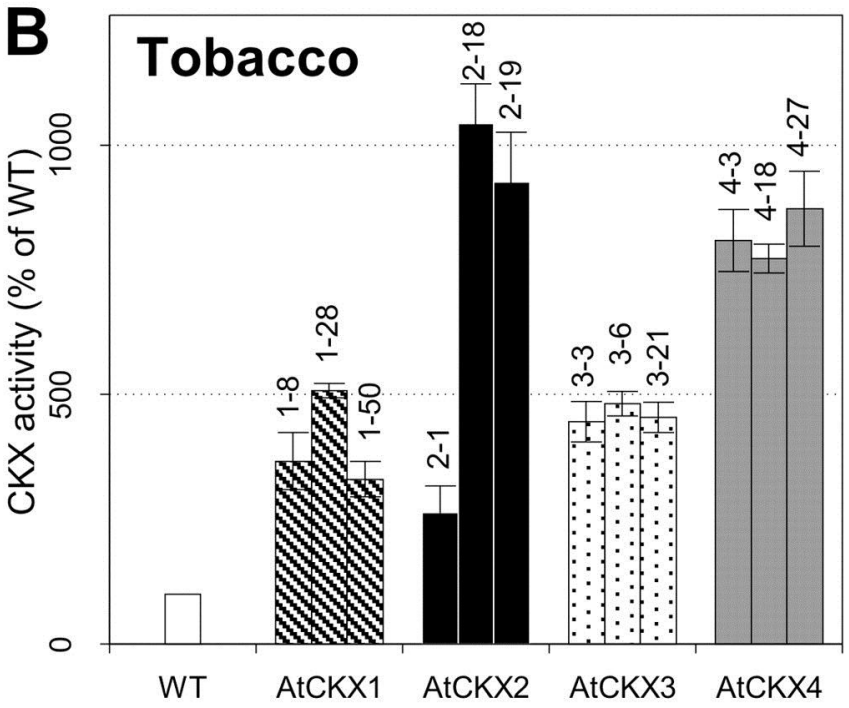
Differently responsive promotors
(cytokinin, auxin, nitrate, combinations)

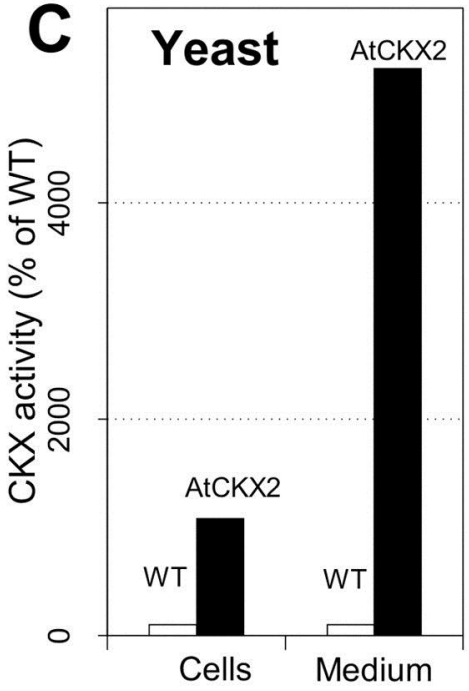
mutants, overexpression ???

Kakimoto 2004

Isolation of CK-oxidase (AtCKX)

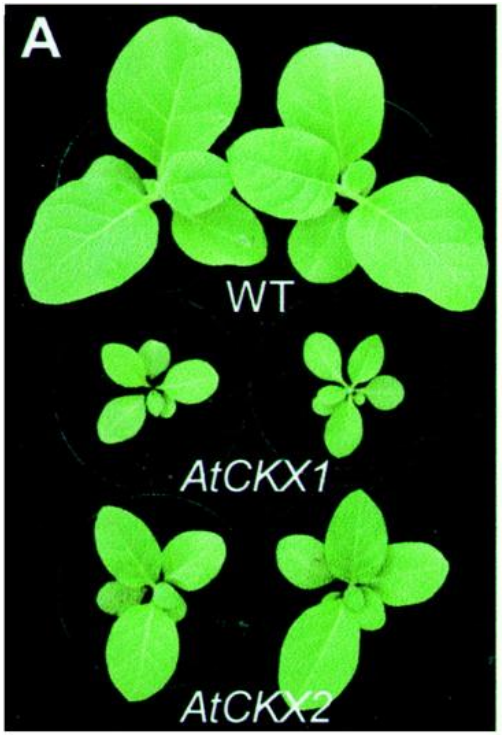


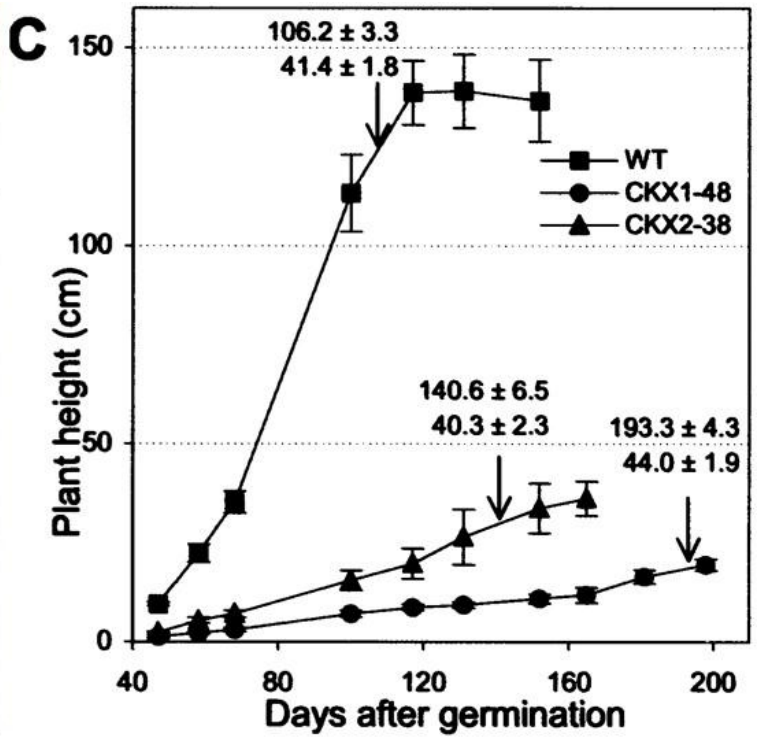


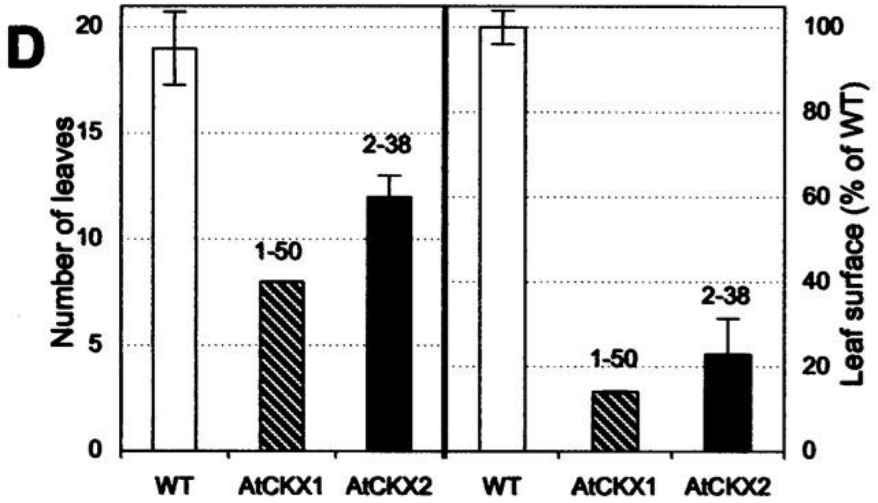


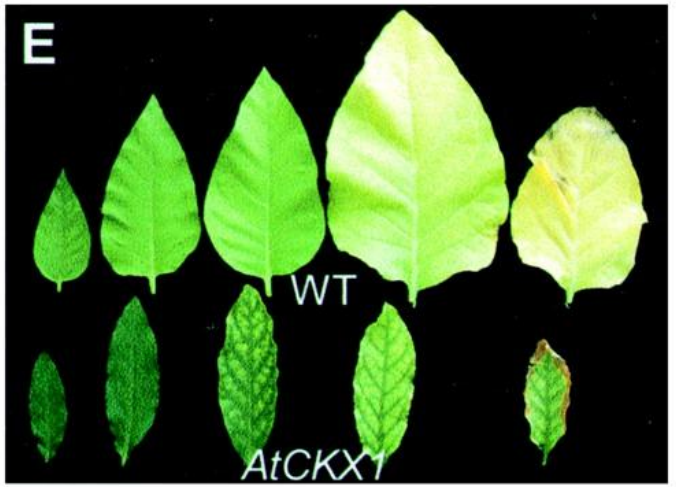
Werner et al., 2001

*AtCKX*s overexpression in tobacco



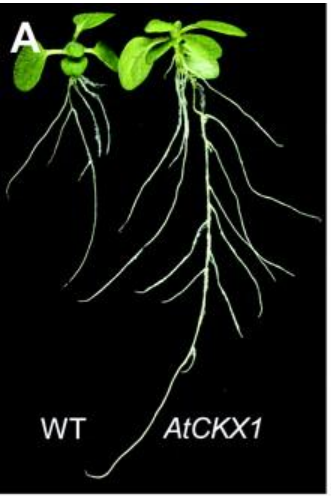


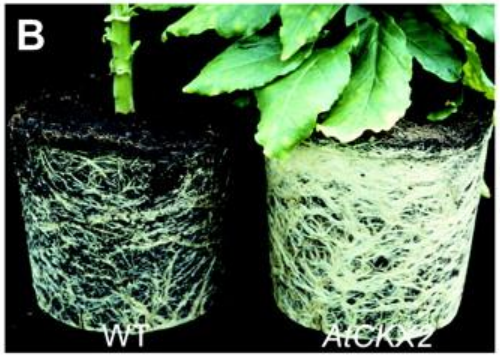


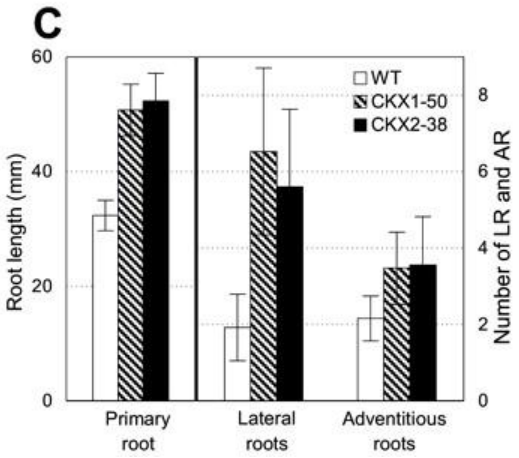


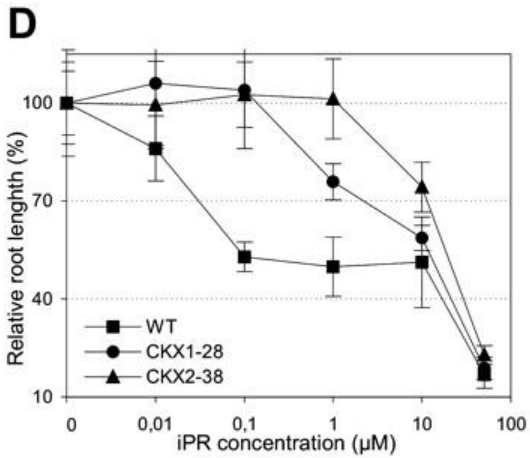
Werner et al., 2001

Effect of AtCKX on tobacco root





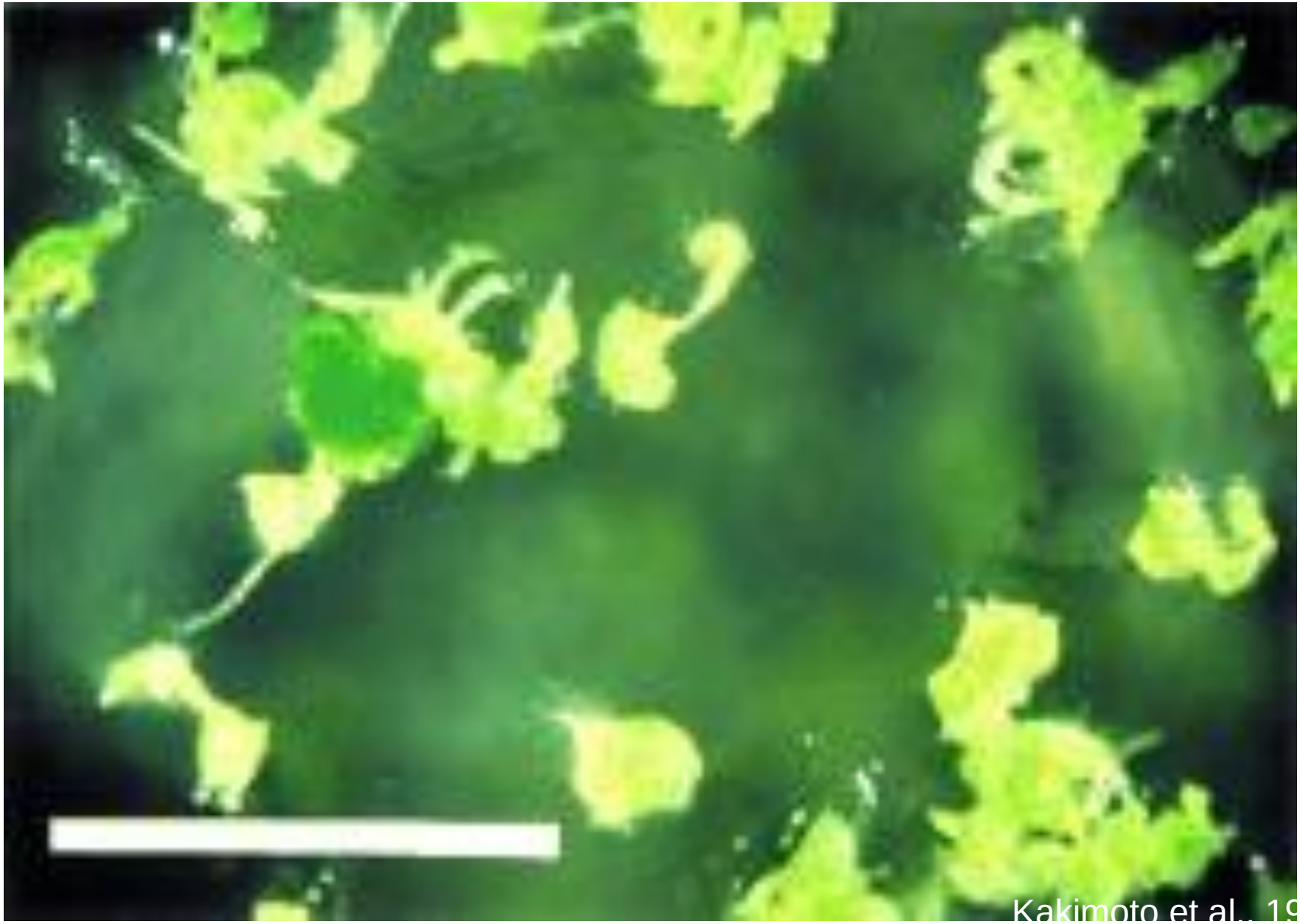




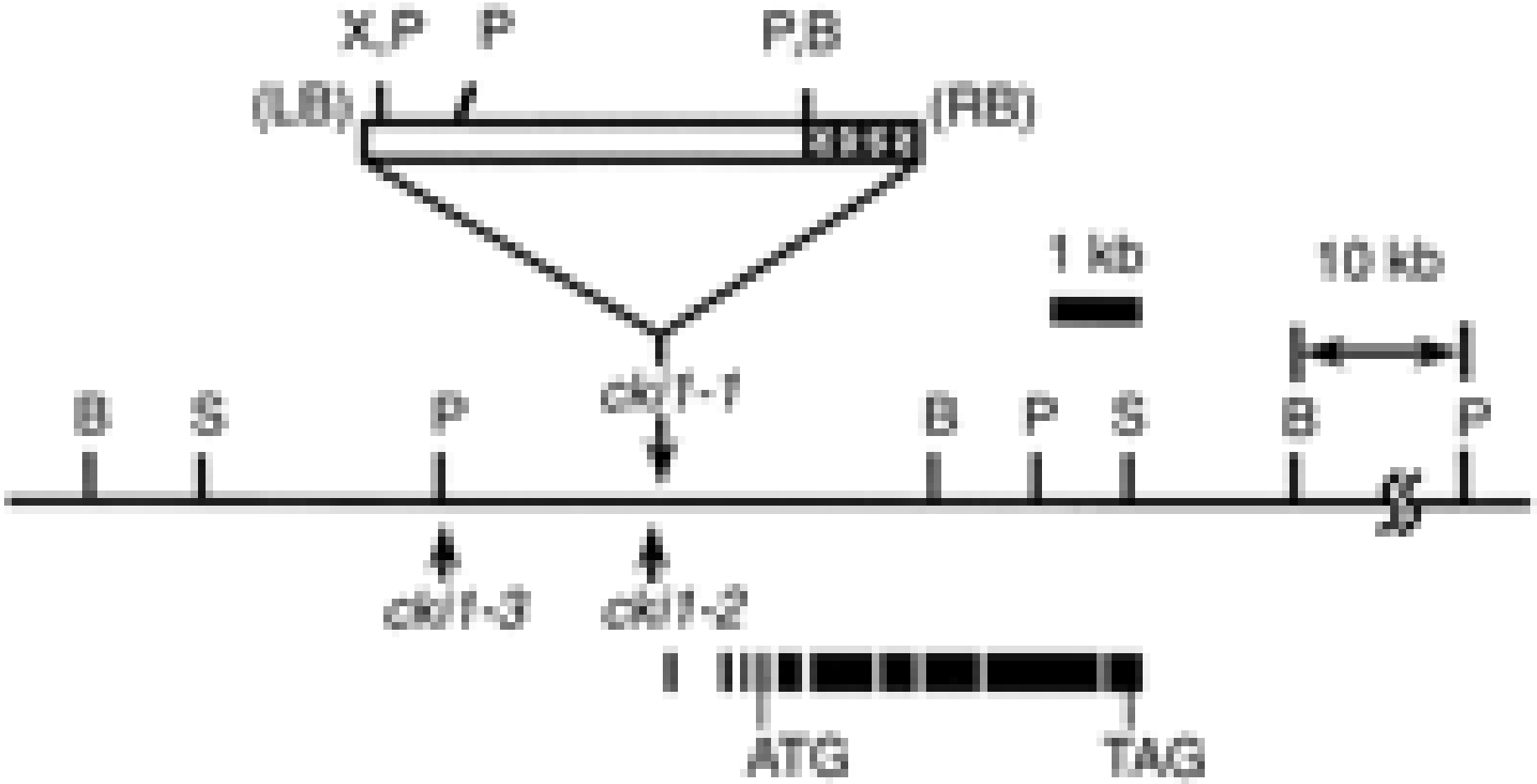
Werner et al., 2001

Cytokinin
–signal perception and transduction

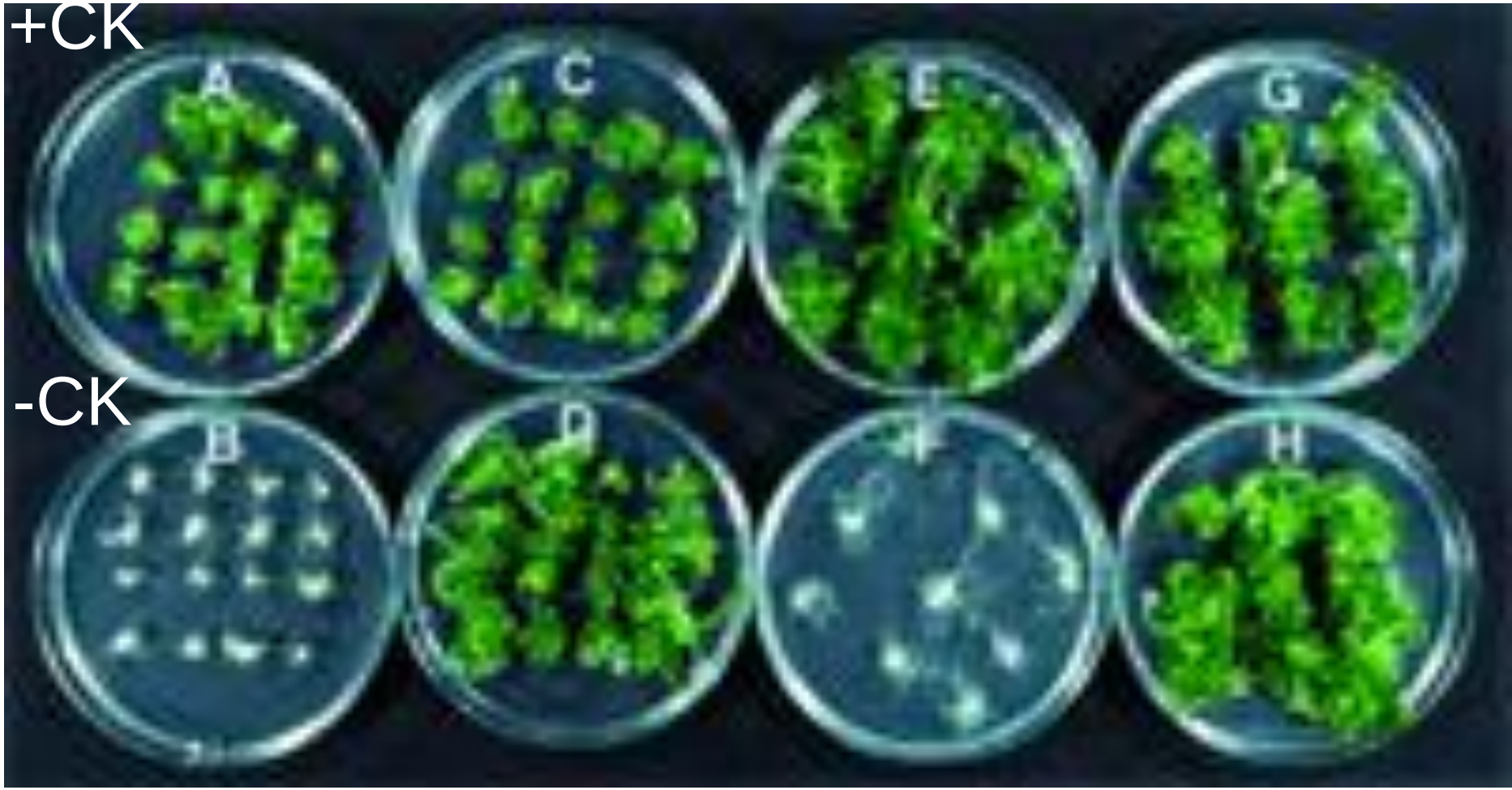
Isolation of CK independent (*cki1*) mutant



Identification of *CKI1* gene

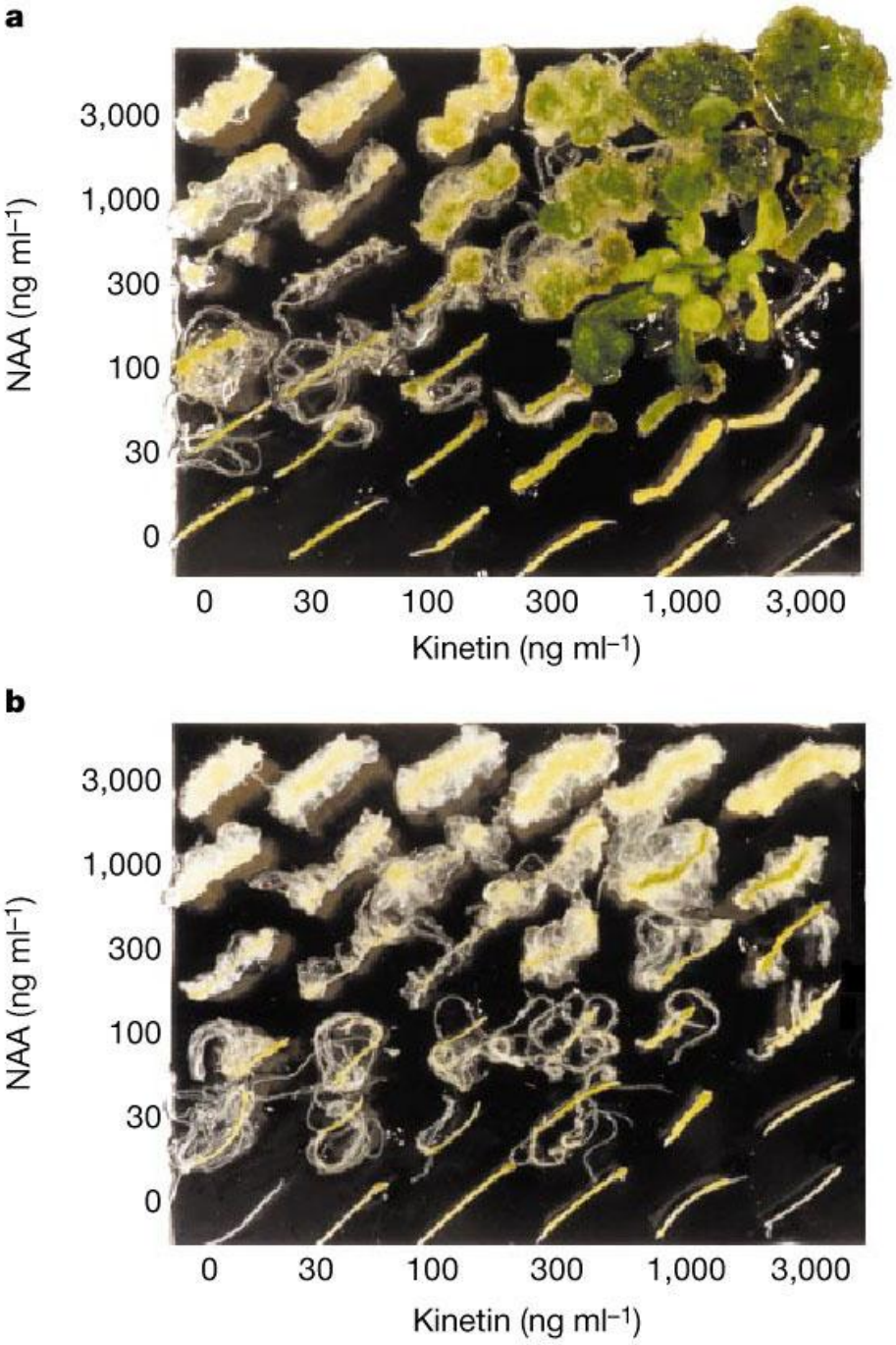


Verification - *35S::CKI1* transgene



Kakimoto et al., 1996

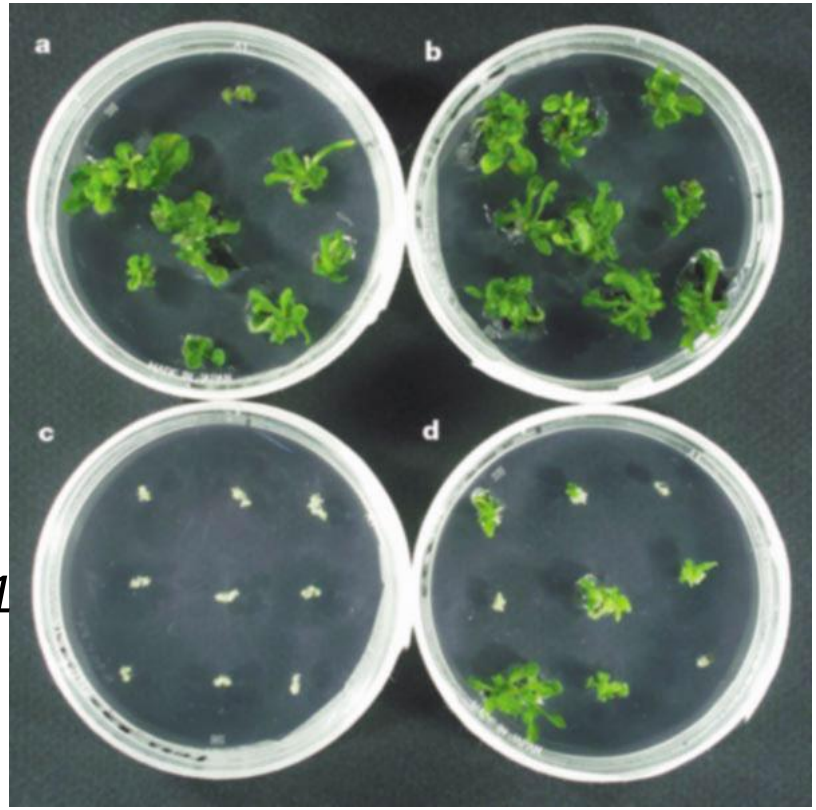
Next strike
- CK response mutant (***cre1***)



pGTV pGTV-CRE1

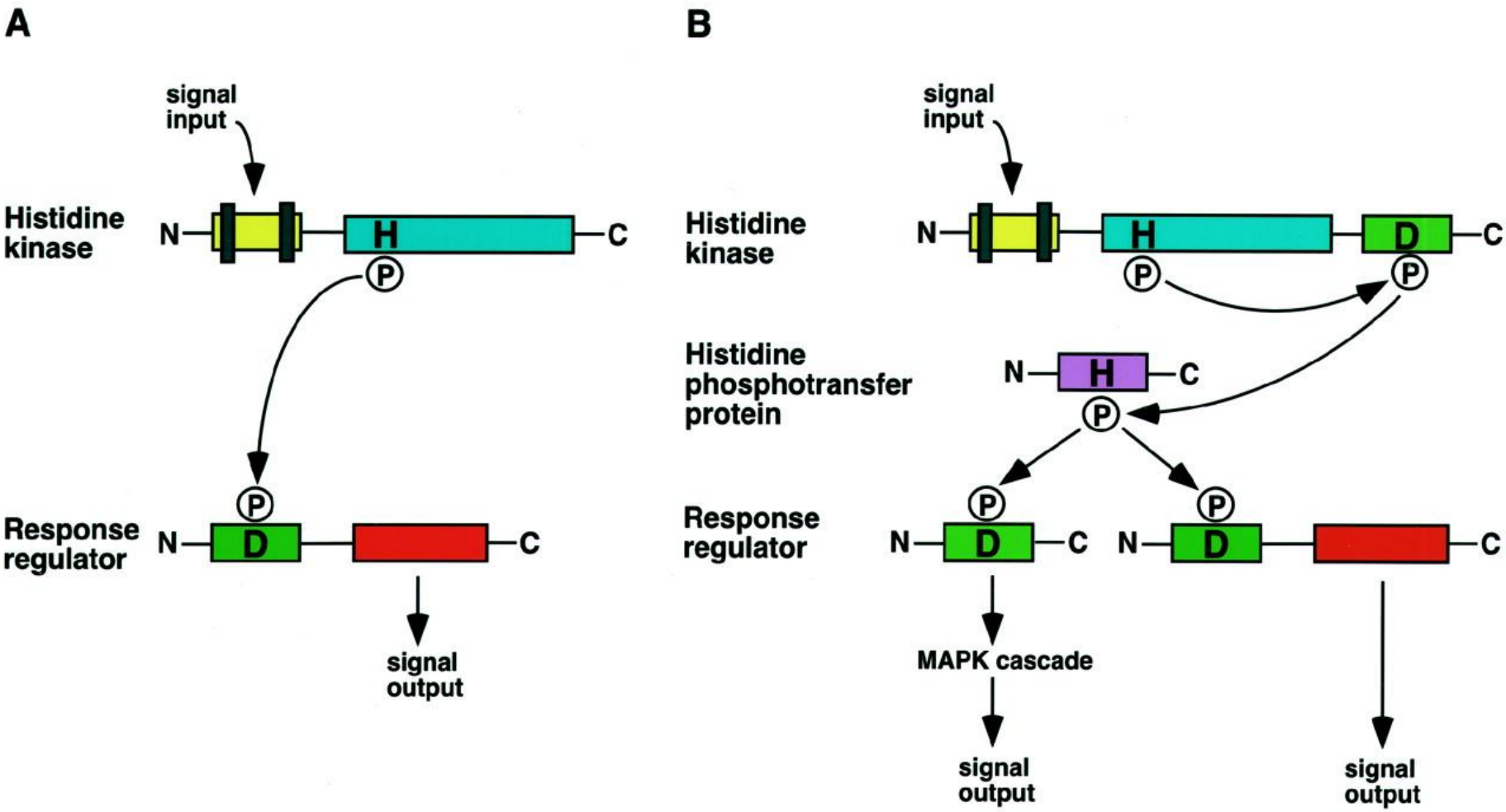
Wt

cre1

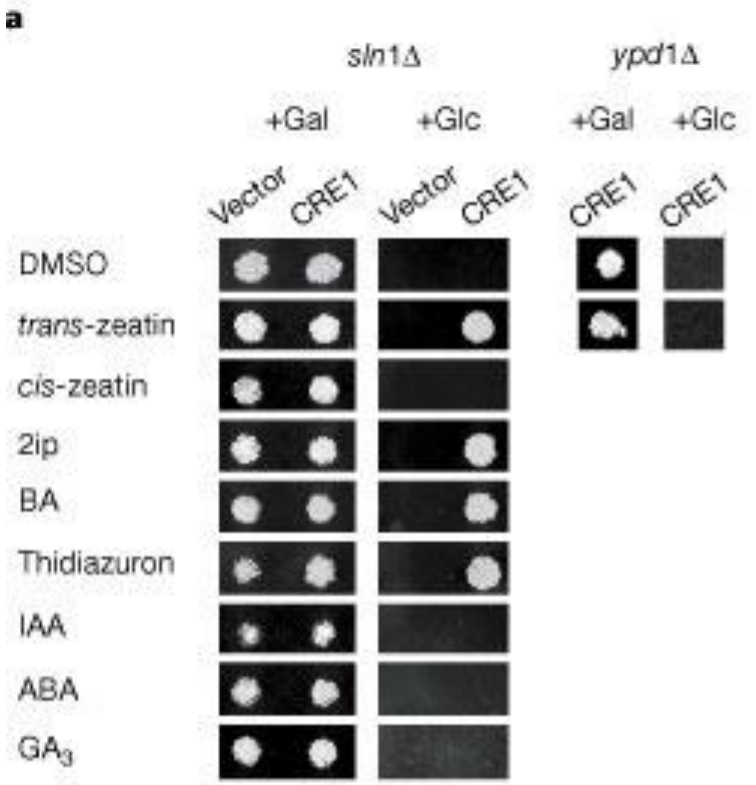


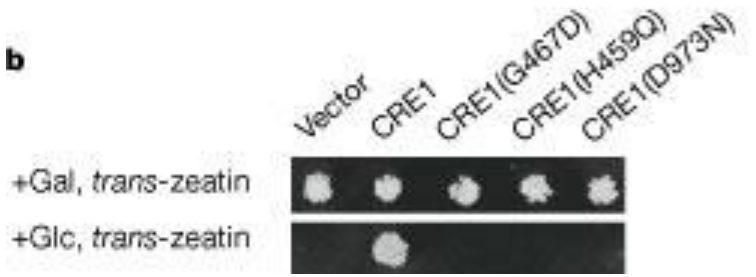
Inoue et al., 2001

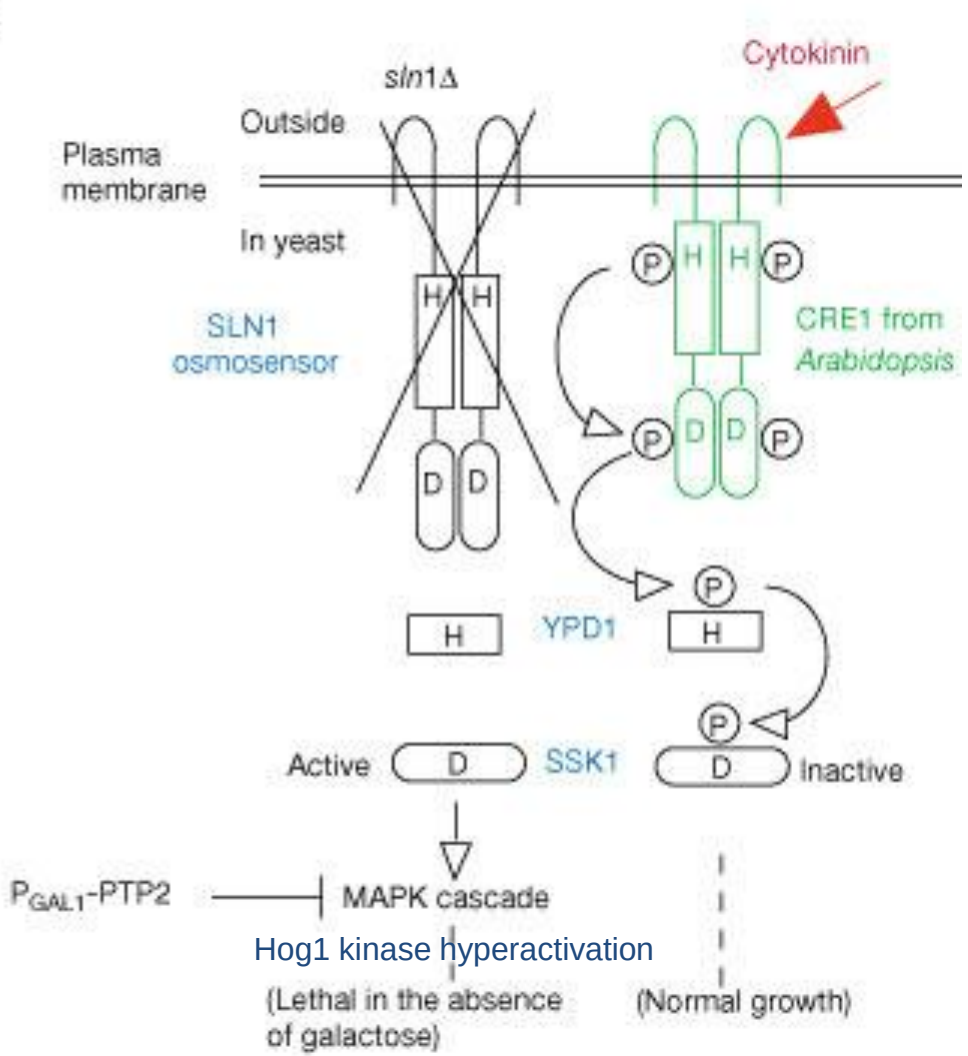
His kinase transduction pathway



Piece of genius - complementation







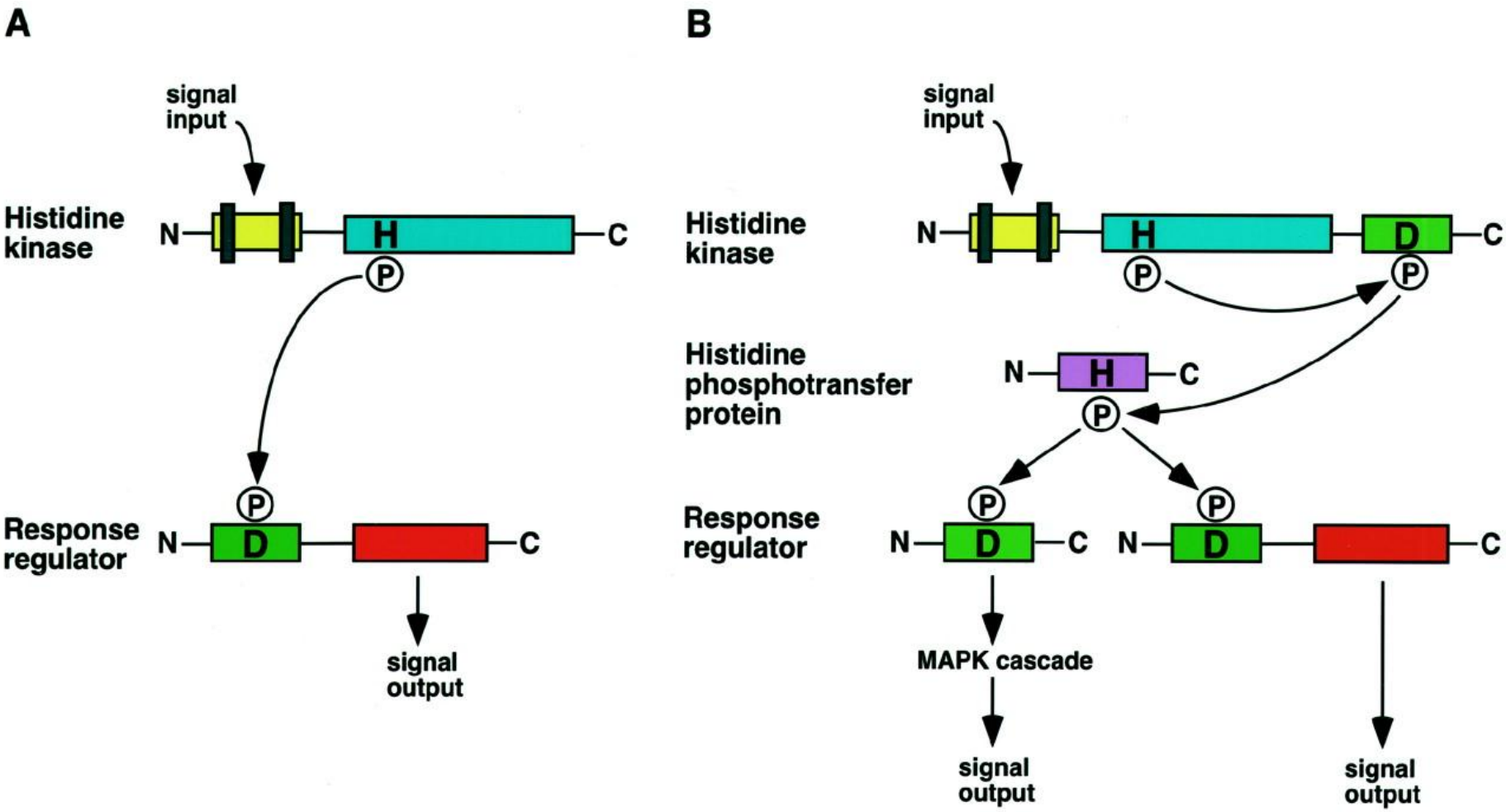
Inoue et al., 2001

Cytokinin receptors – what else?

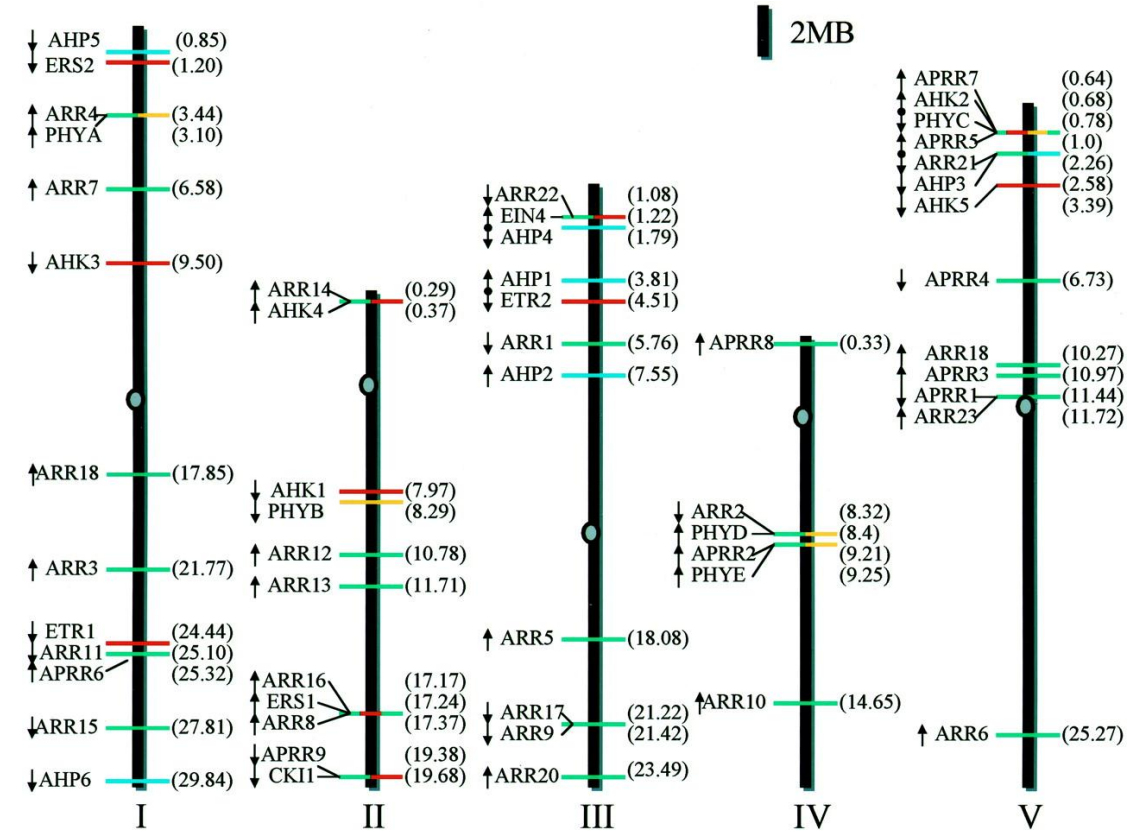
3 CRE1 homologous proteins (AHKs)
multiple mutant phenotypes – additive
(not lethal)

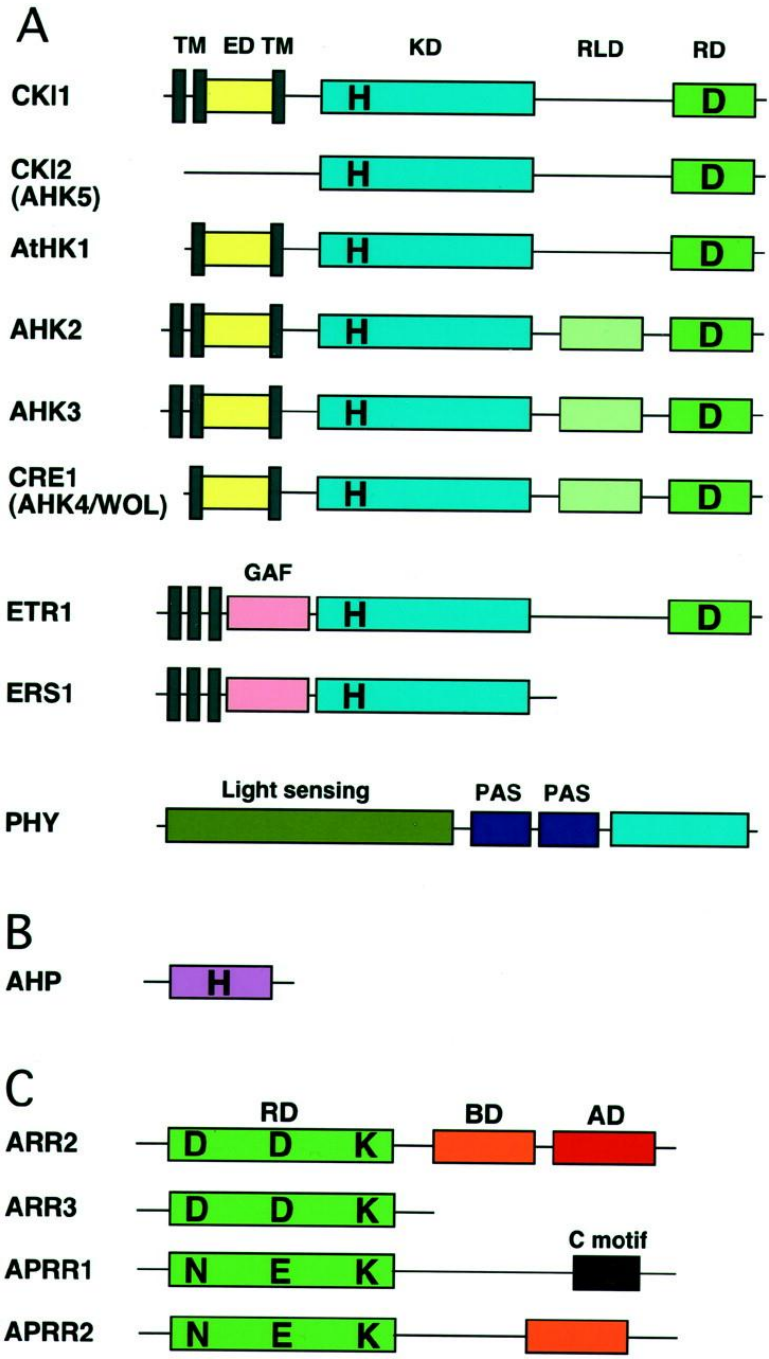
What does CKI1?

His kinase transduction pathway

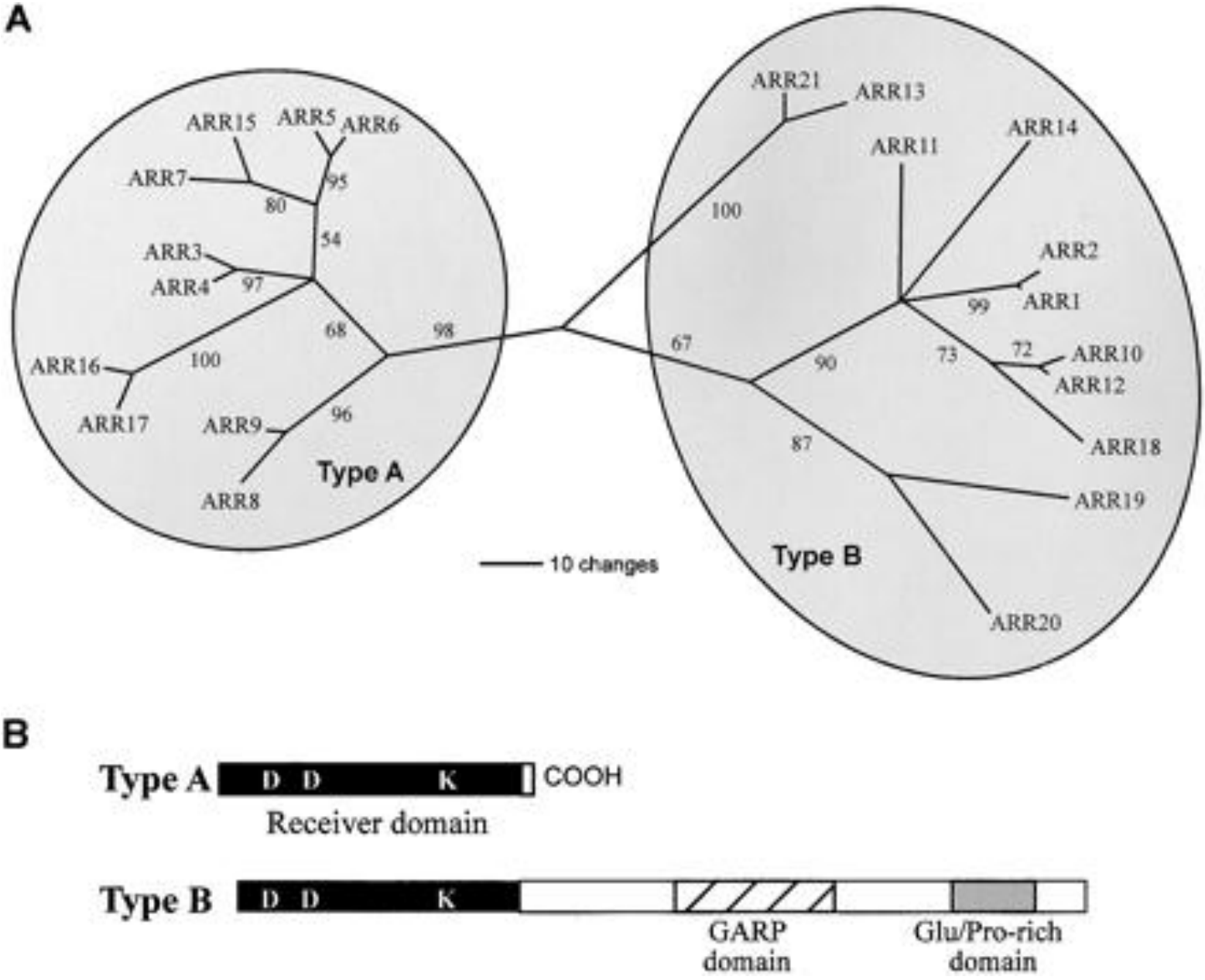


His kinase pathway components in Arabidopsis

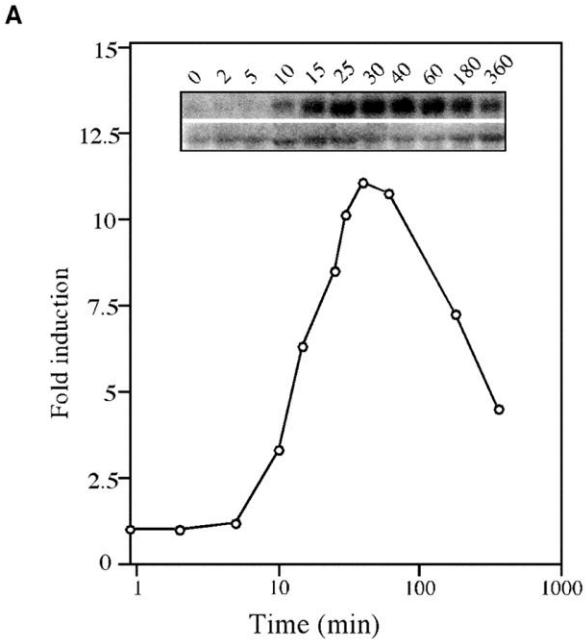


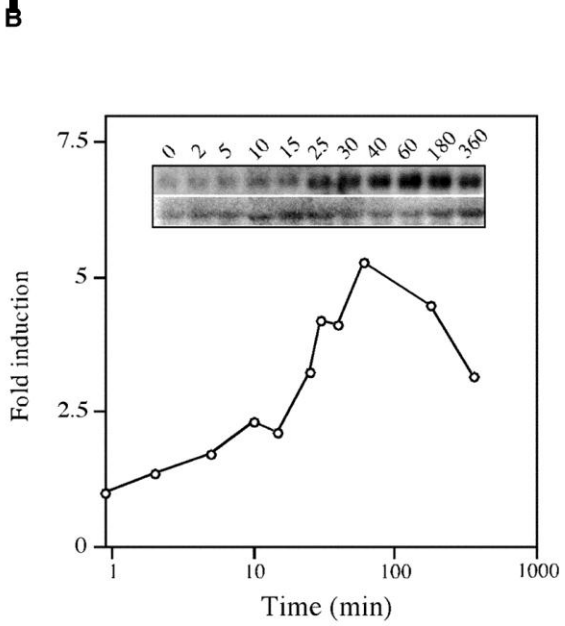


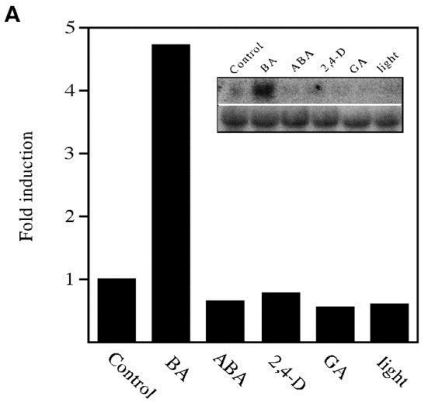
Response Regulators in *Arabidopsis*

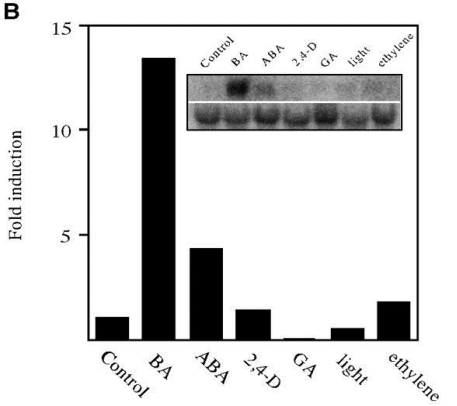


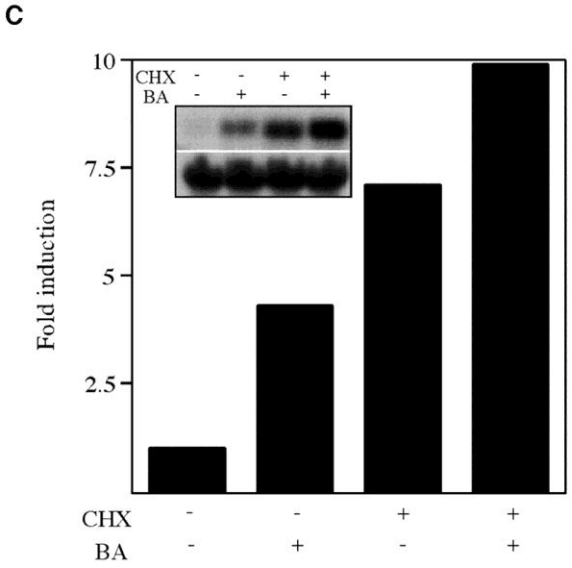
CK responsive genes – *ARR* type A

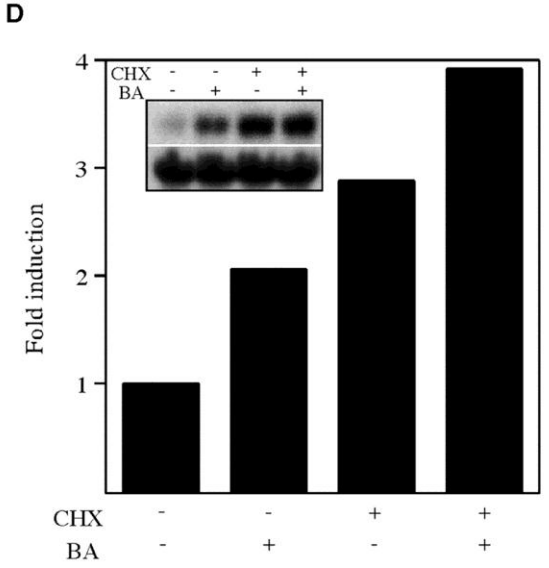


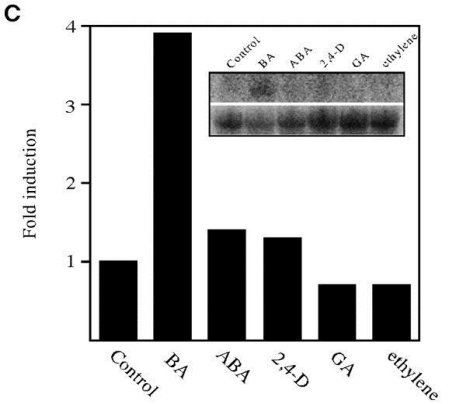






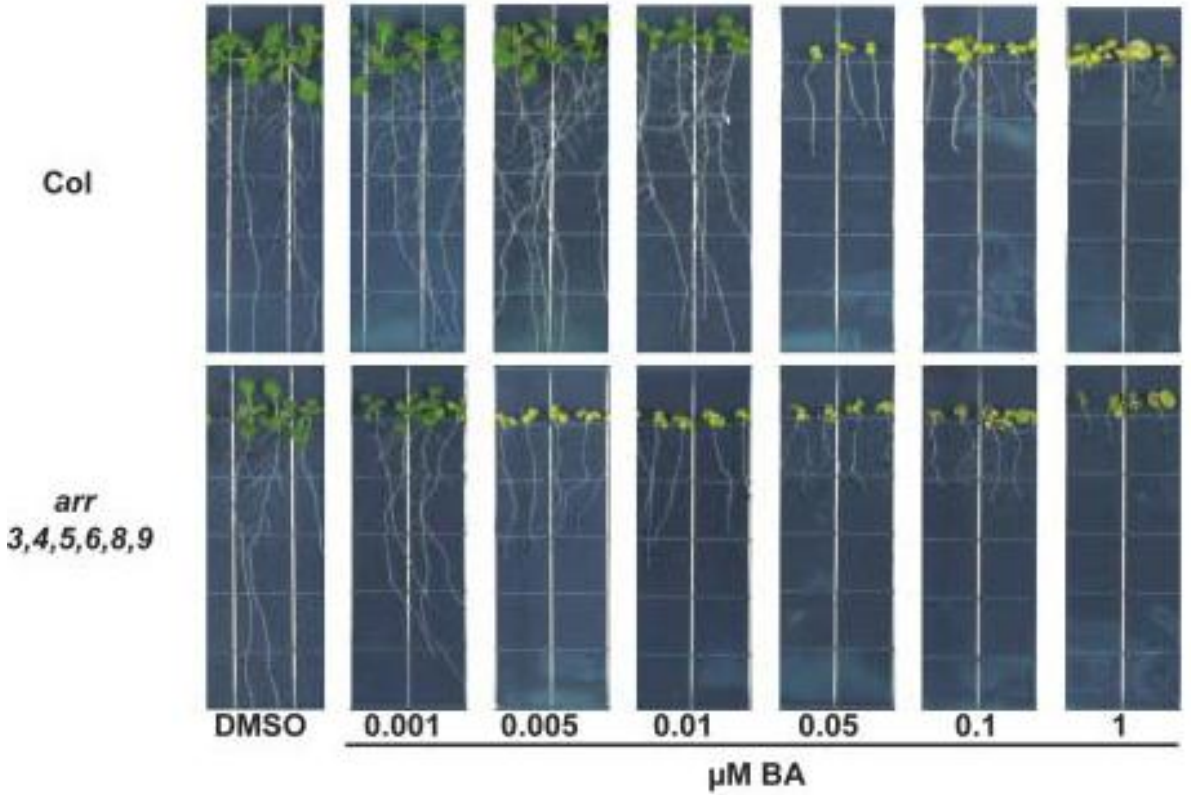


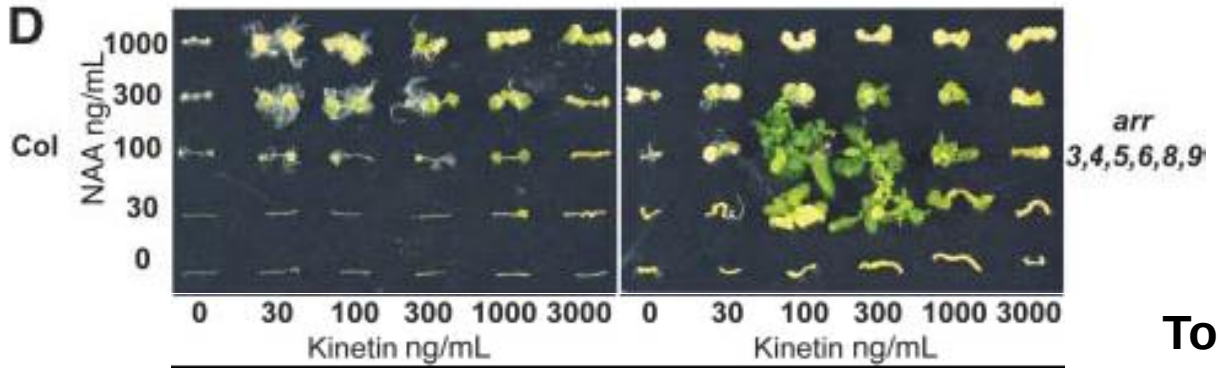




Brandstatter and Kieber, 1999

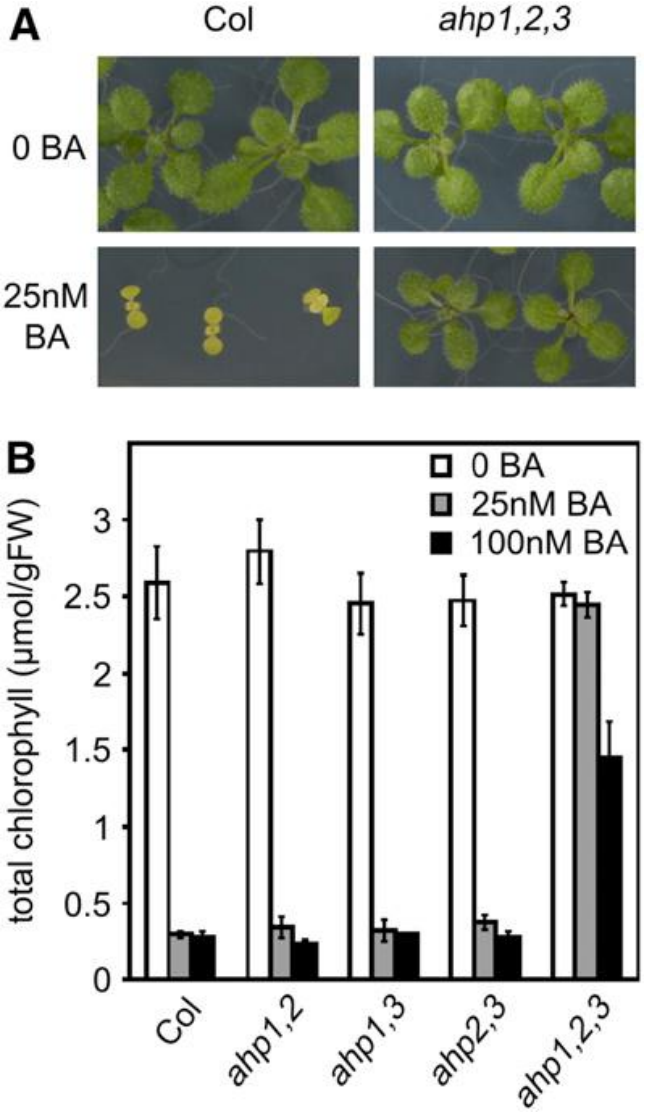
Response regulator ARR type A-negative regulators of cytokinin signalling





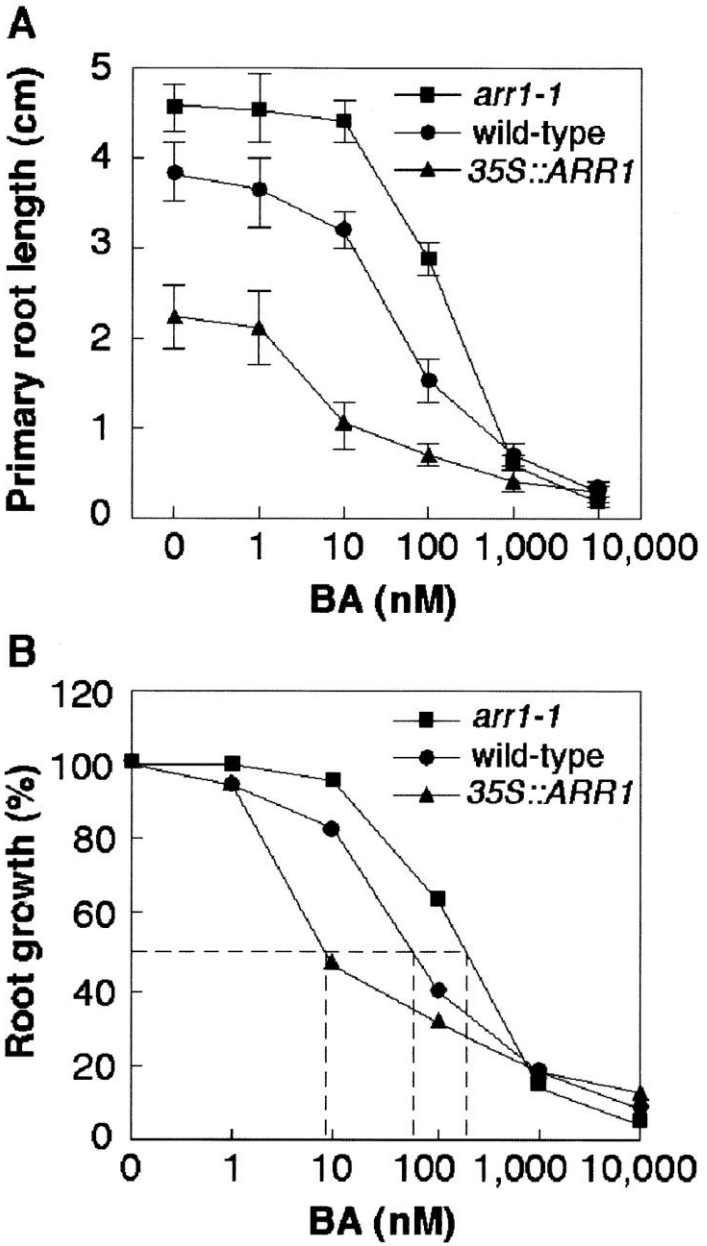
To et al., 2004

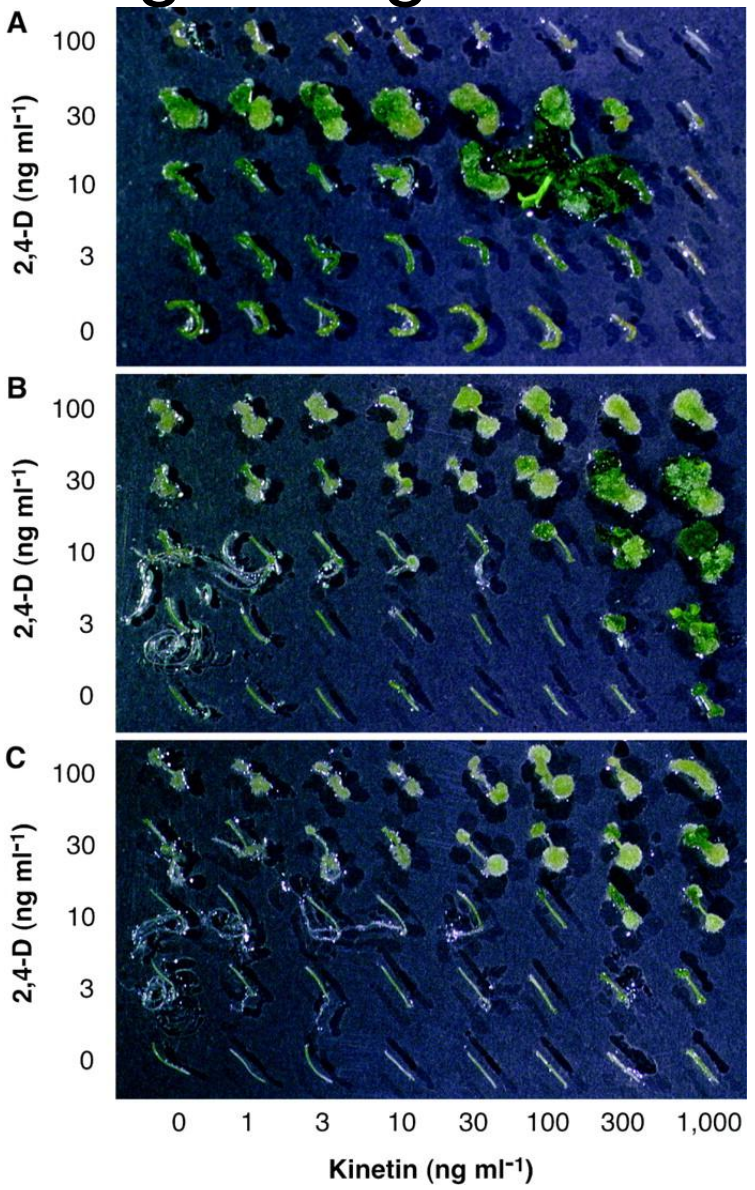
AHPs mediate transfer of cytokinin signal between cytoplasm and nucleus



Hutchison et al.,2006

Phenotypes of *arr* type B positive regulator of CK signalling





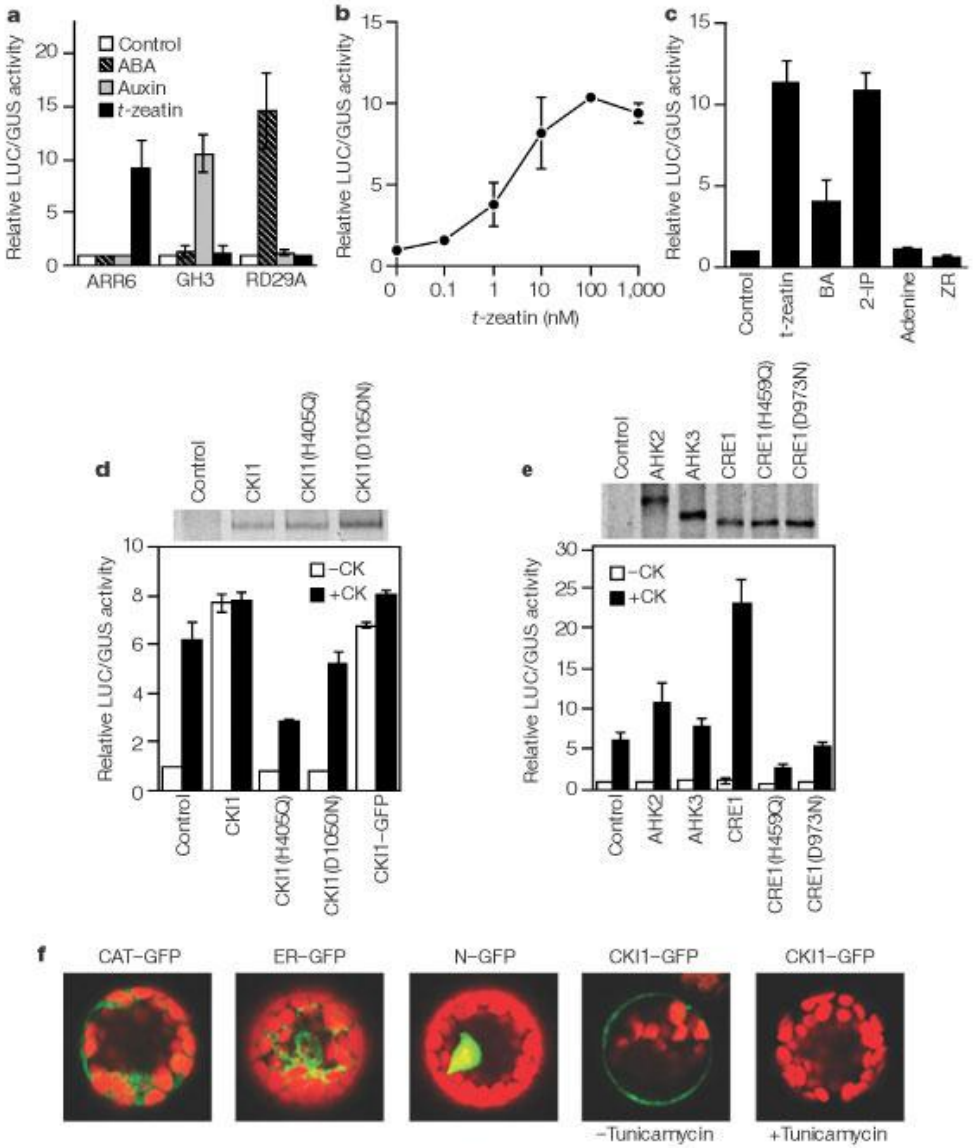
35S::ARR1

wt

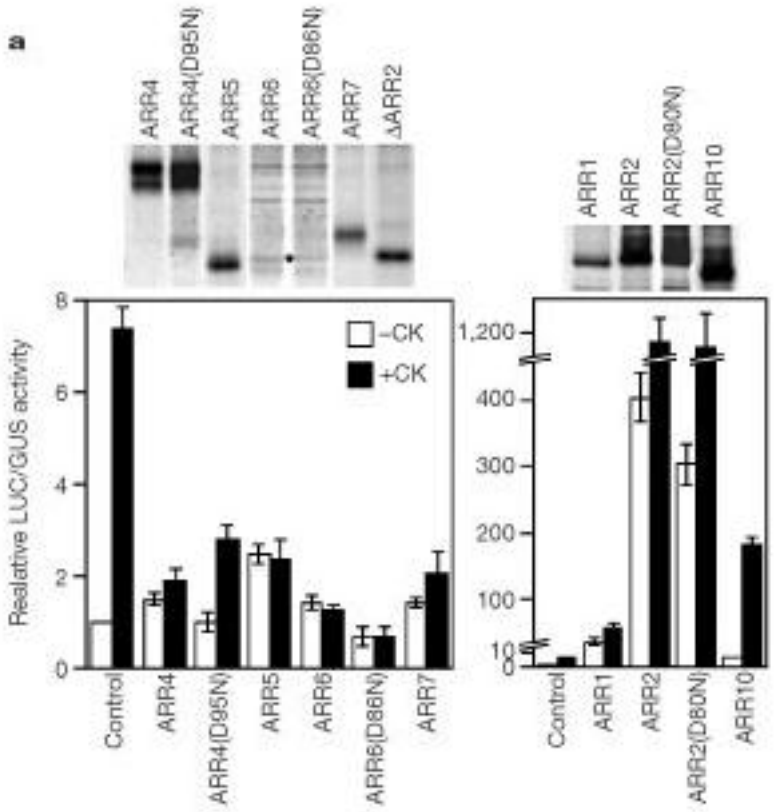
arr1-1

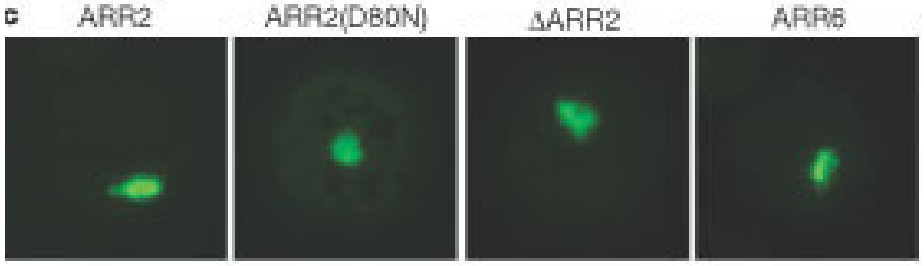
Sakai et al., 2001

Games with protoplasts

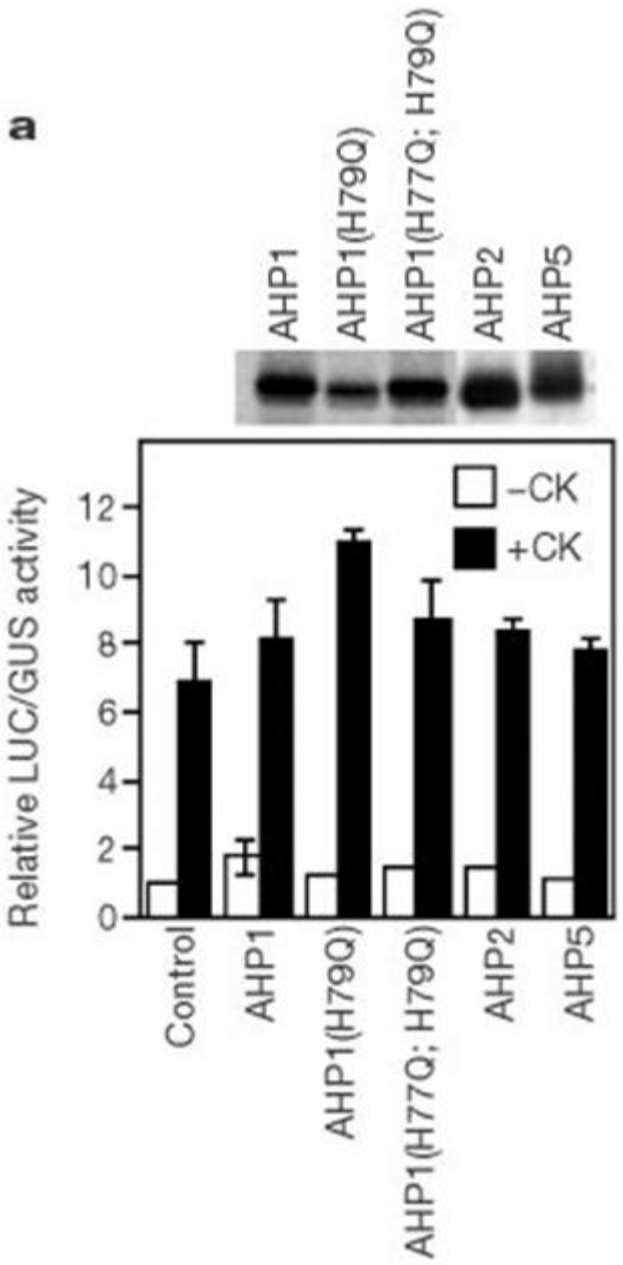


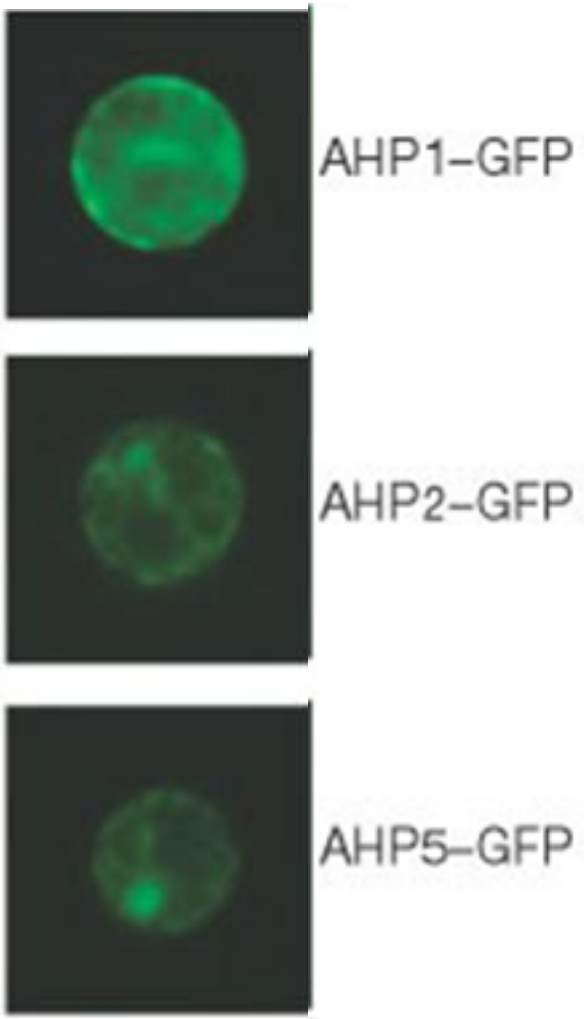
Opposite effects of two classes of ARRs on CK signalling

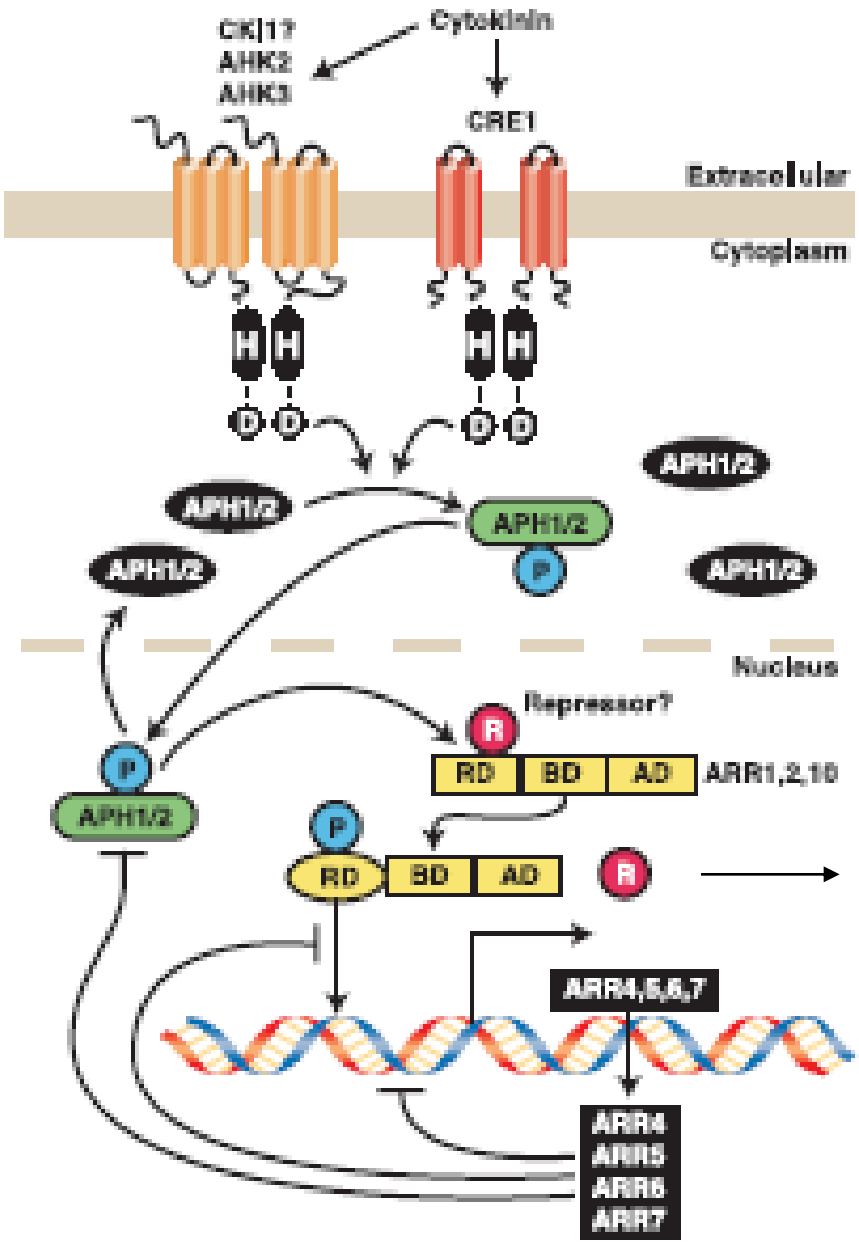




AHPs – signal to nucleus







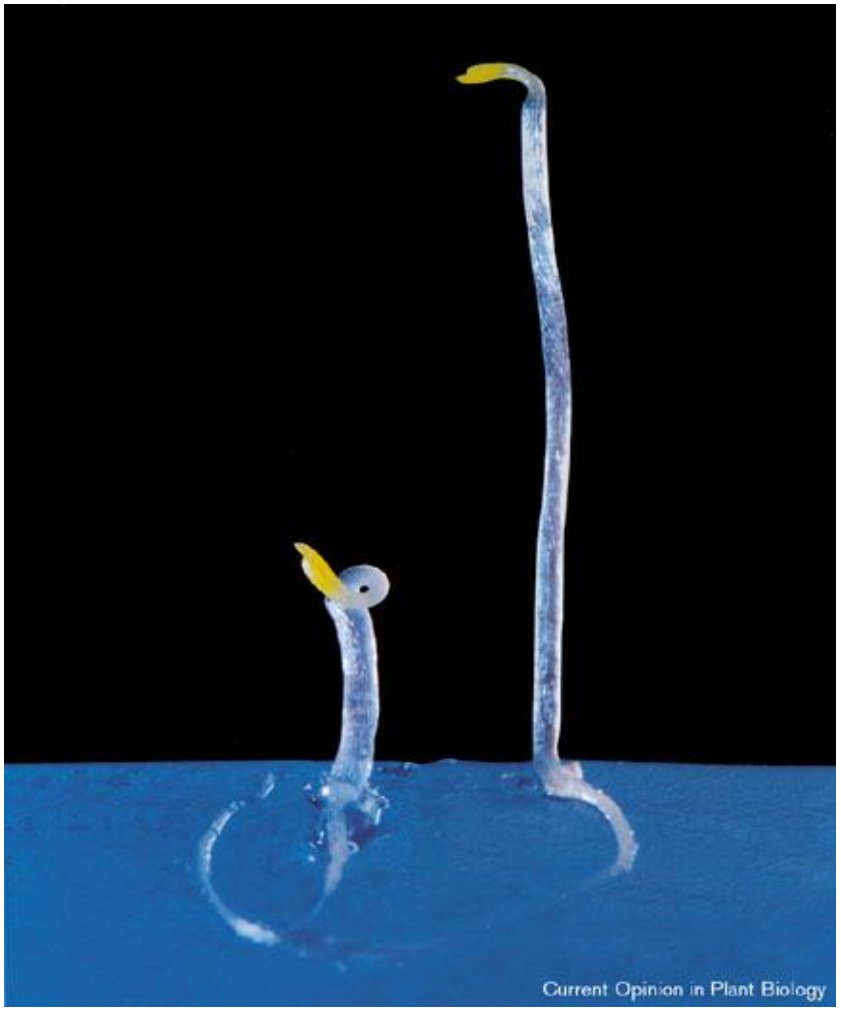
Hextuple of type A ARRs confirms role as negative regulators

e.g. Root elongation assay

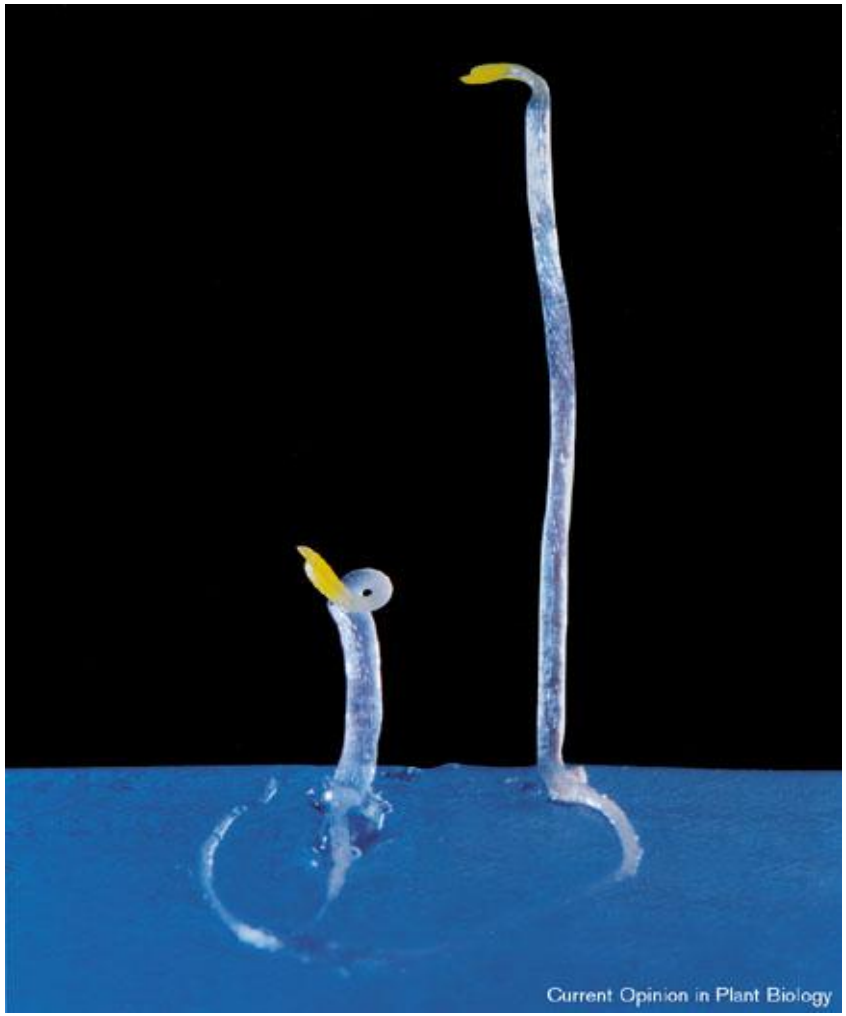
Transcritpion
Cell division
Shoot formation
Delayed senescence
Vascular develppment

Regulation of plant development by ethylene

Mutant screens for ethylene pathway genes

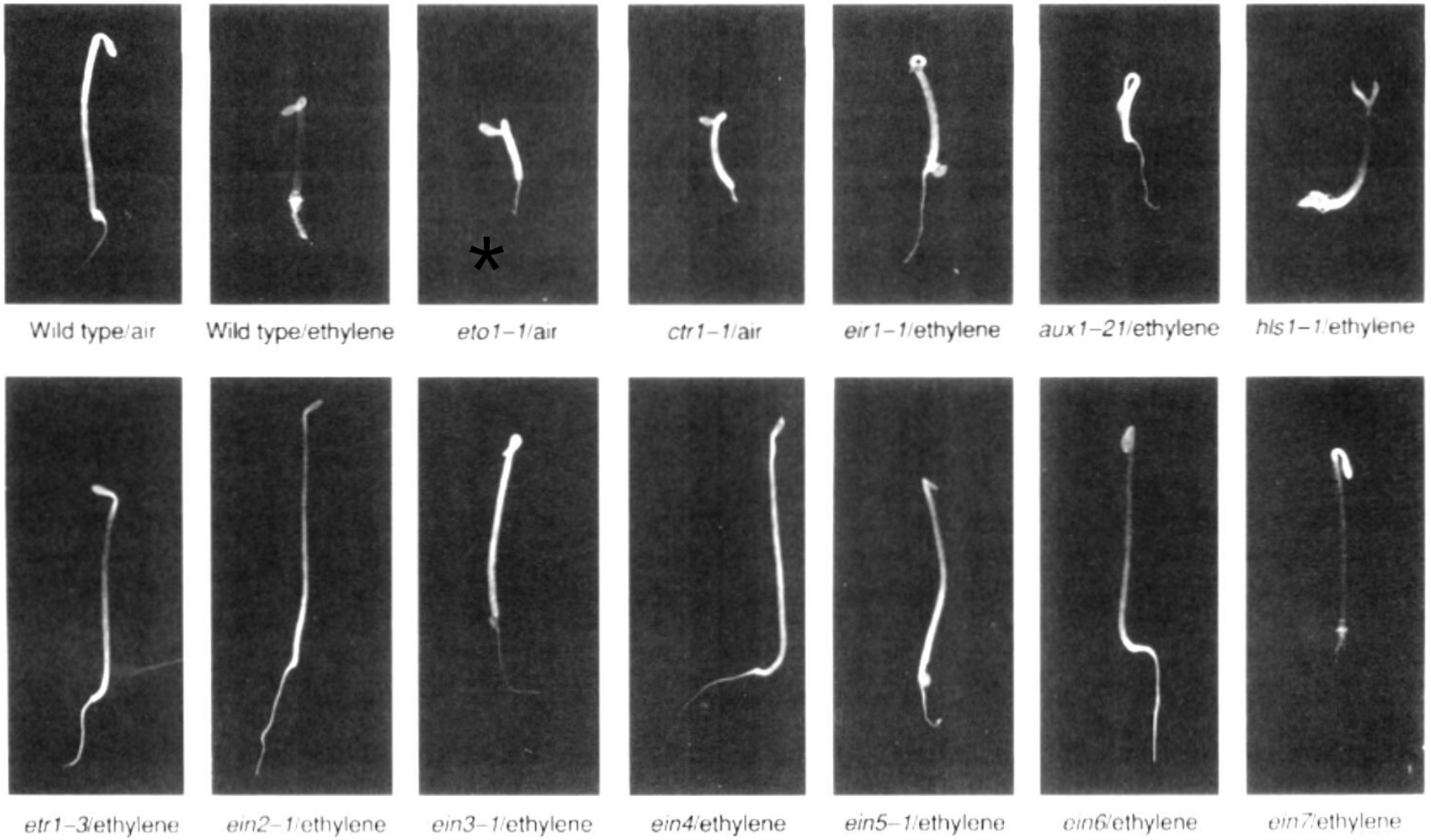


C_2H_4 air



air C_2H_4

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

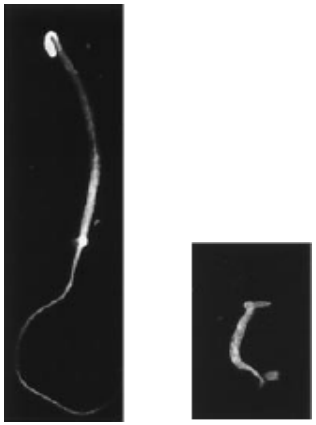


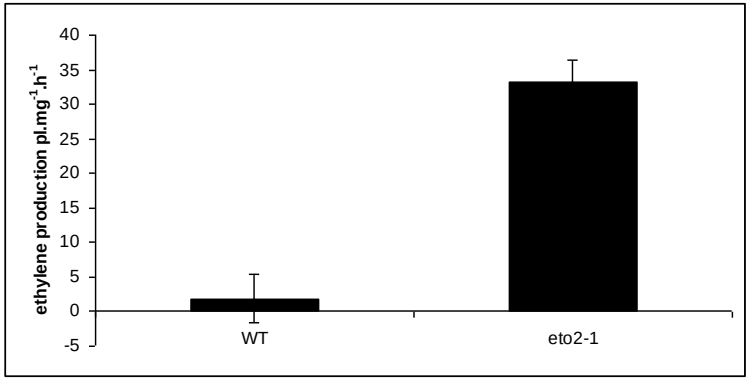
Roman et al., 1994

* phenotype rescued by inhibitor of ethylene biosynthesis

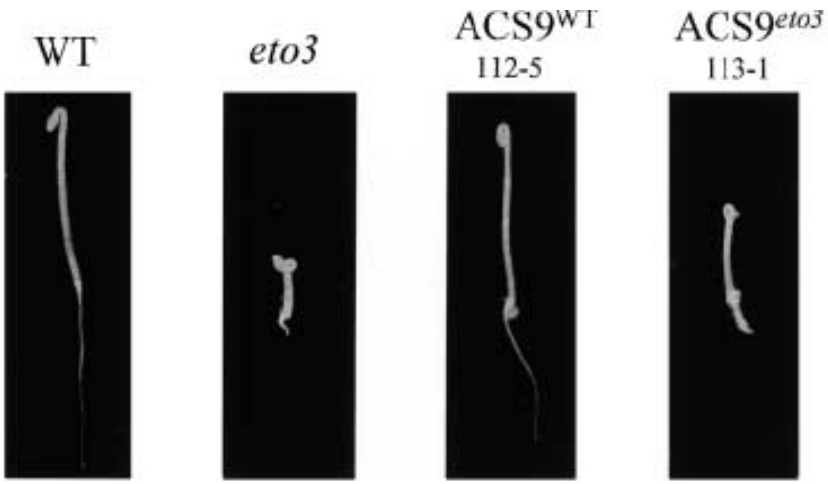
eto mutants – constitutive triple response

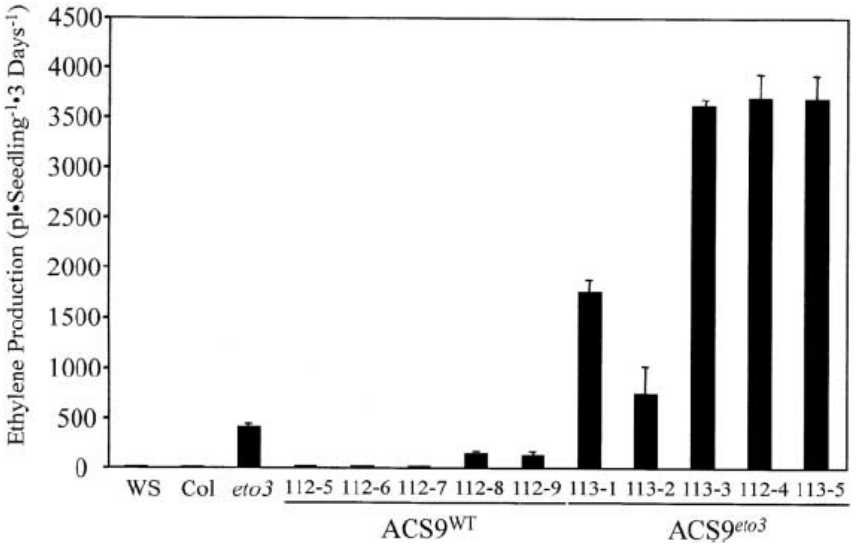
eto2





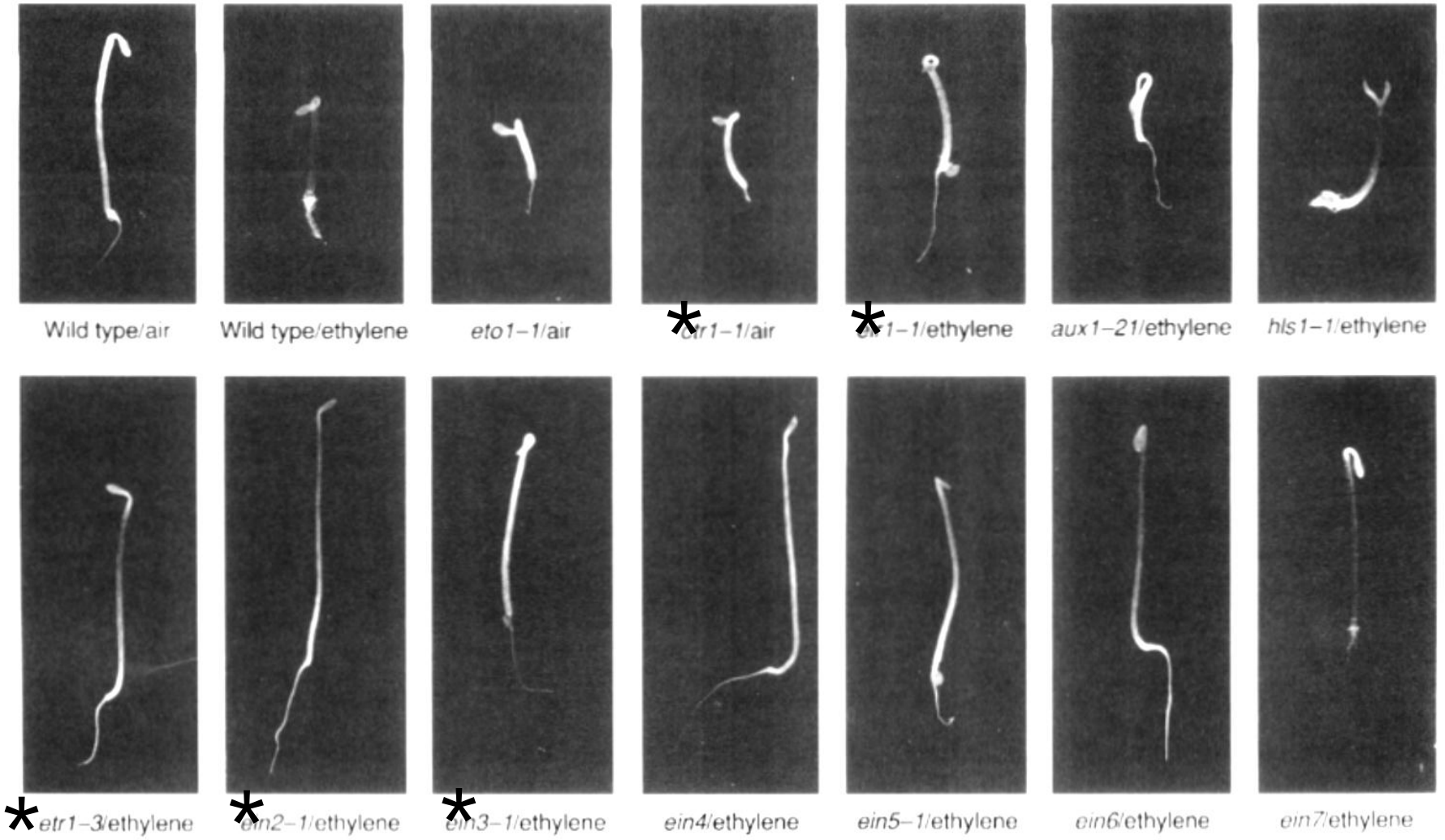
eto3





- overproduction of ethylene

Screen for ethylene mutants
II. Genes invoved in signalling pathway

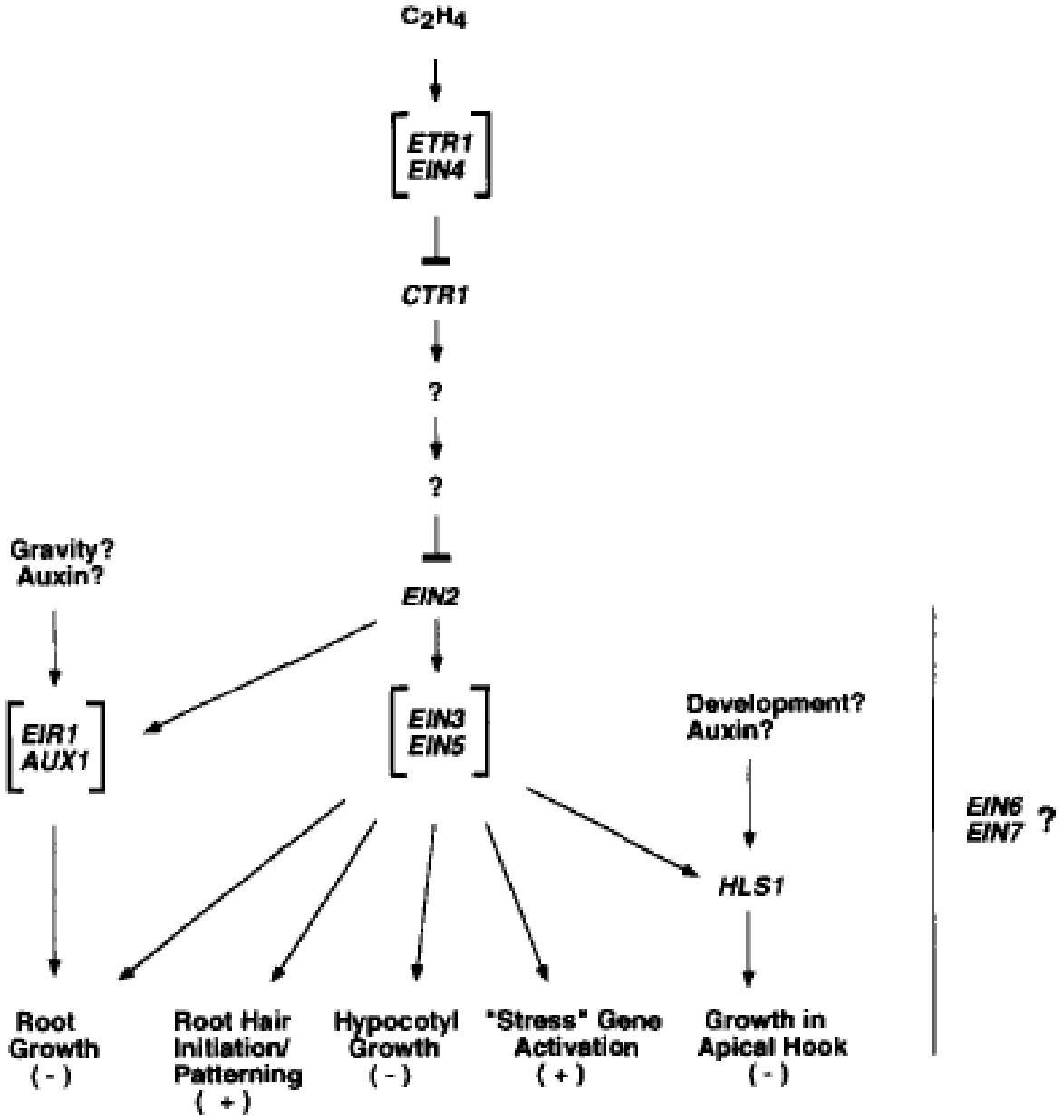


Roman et al., 1994

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

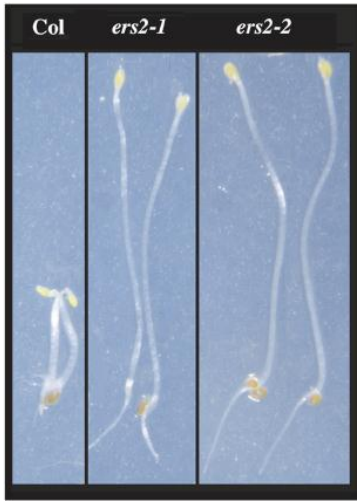
ETR1 codes for histidine kinase

etr1

Bleecker et al.,1988

ein4

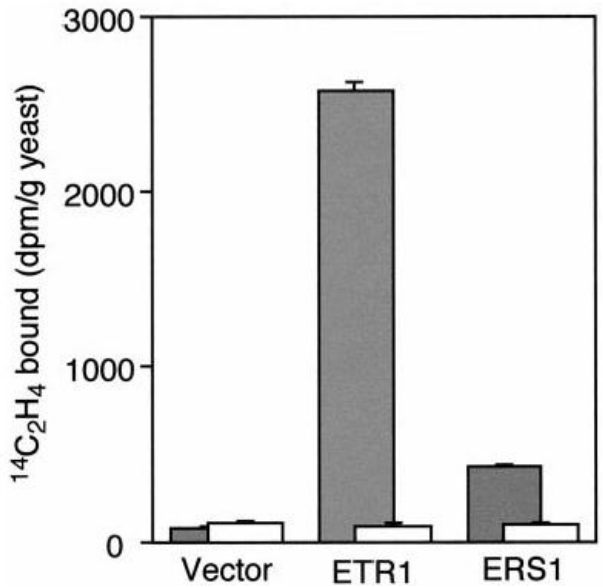
ers2

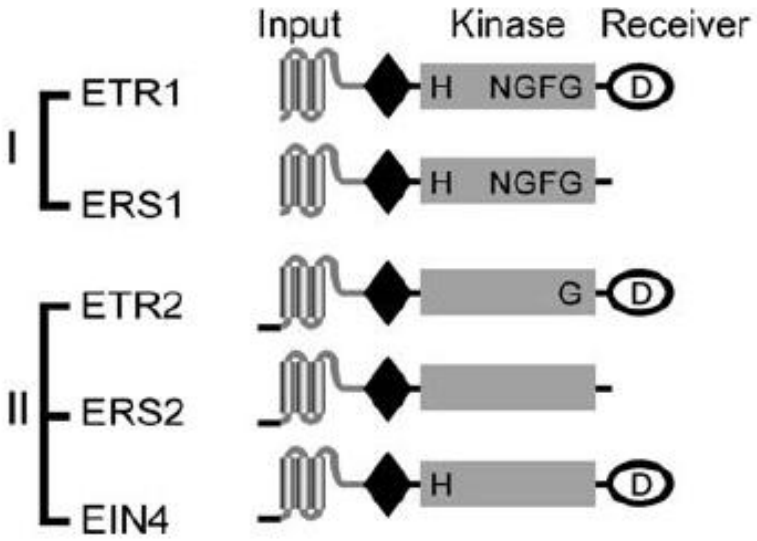


Hua et al., 1998

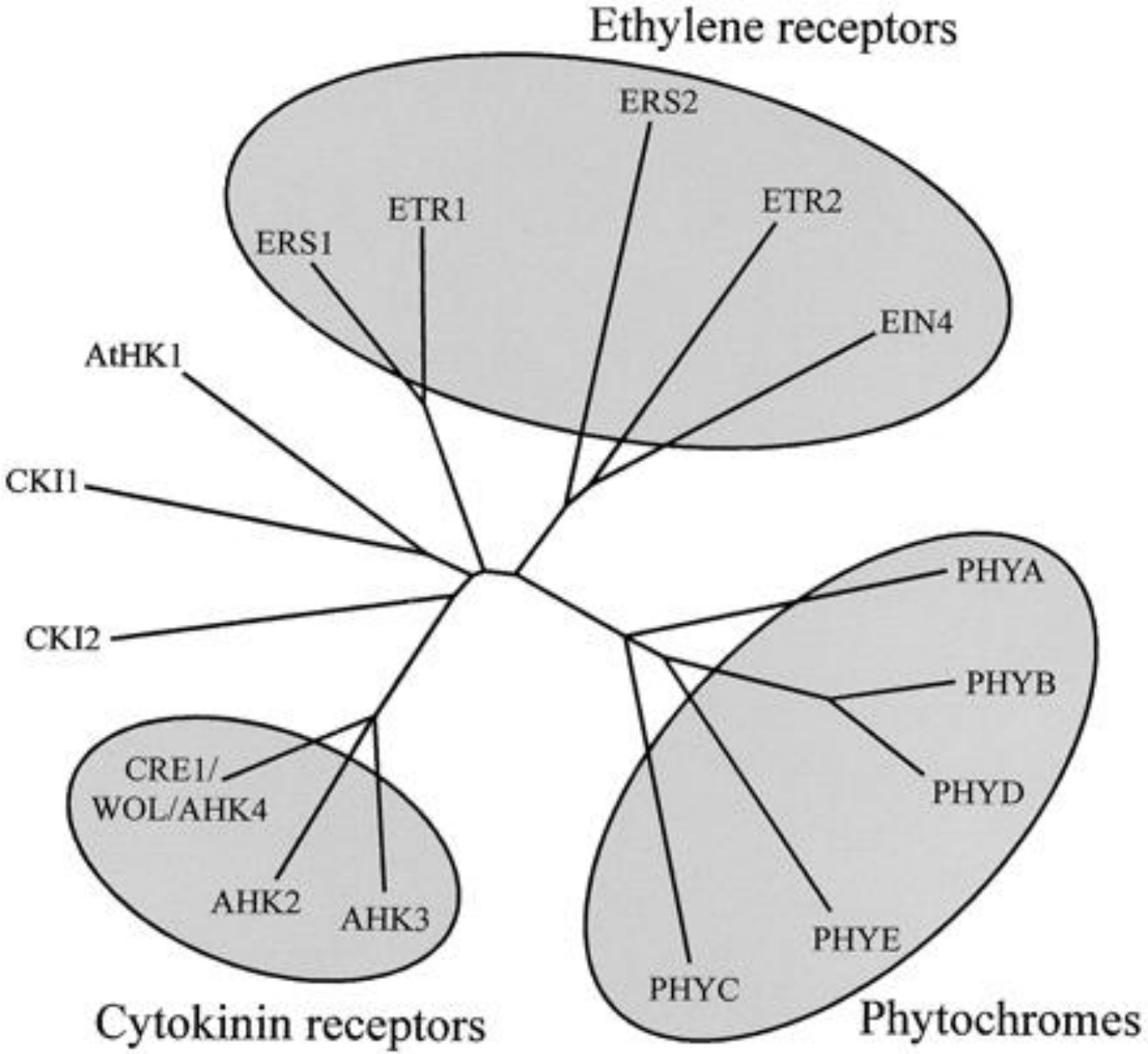
etr2

Sakai et al., 1998

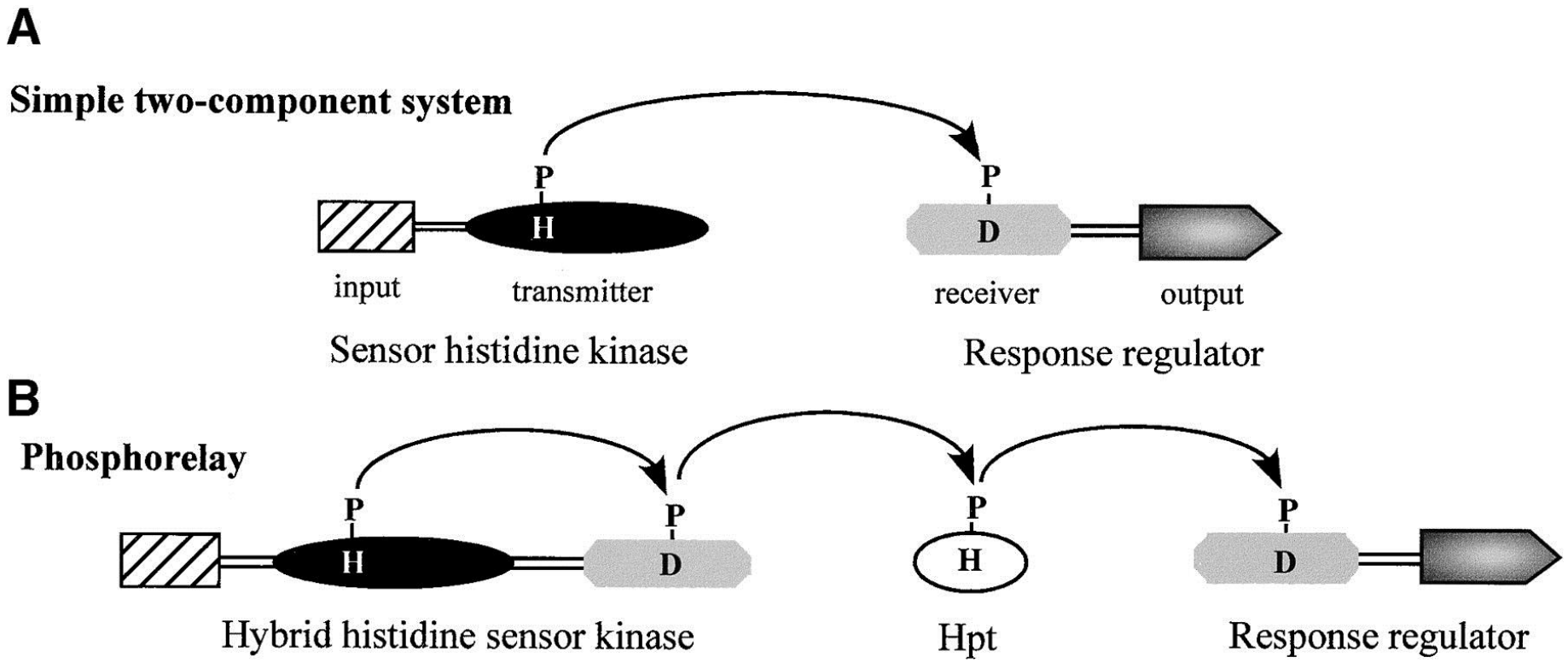


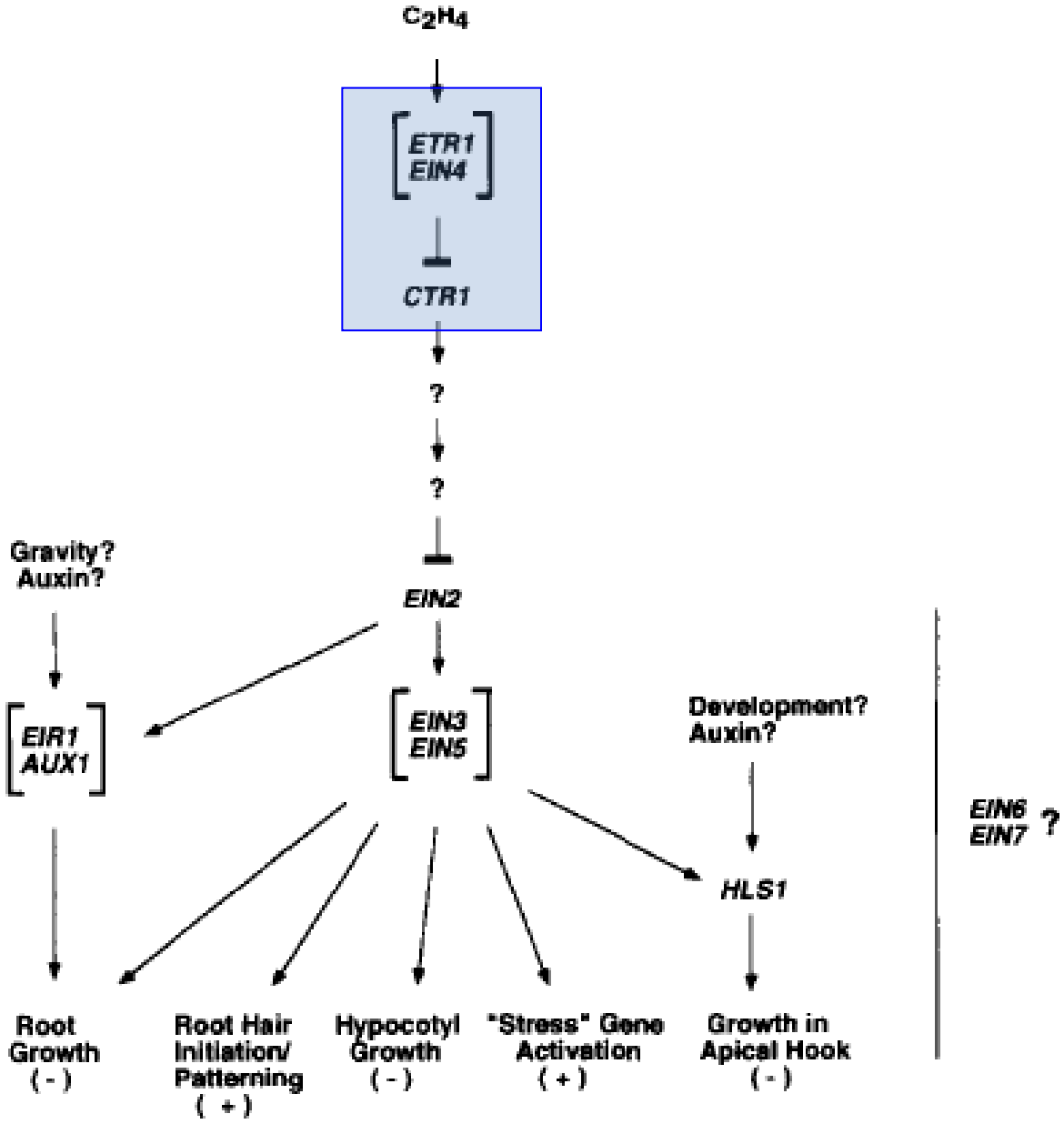


His-kinases in Arabidopsis

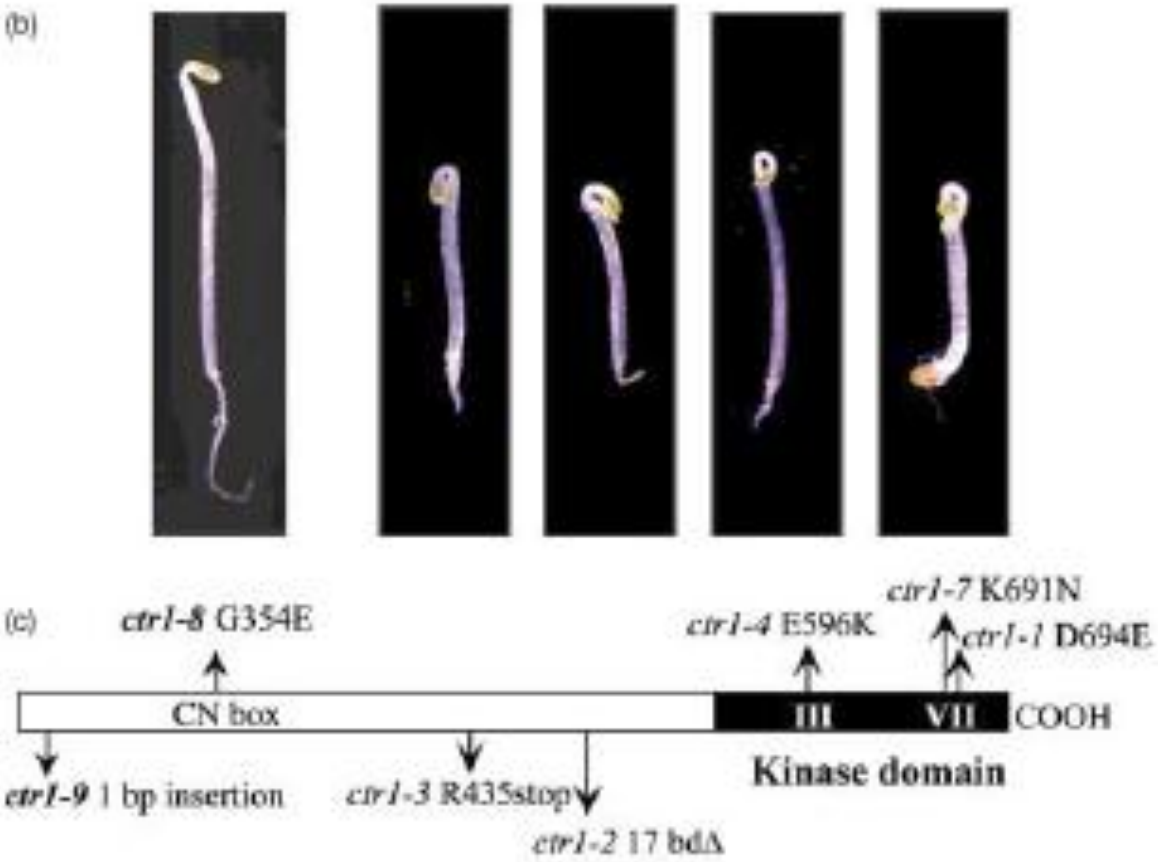


Ethylene signalling
– homology to two component system ?

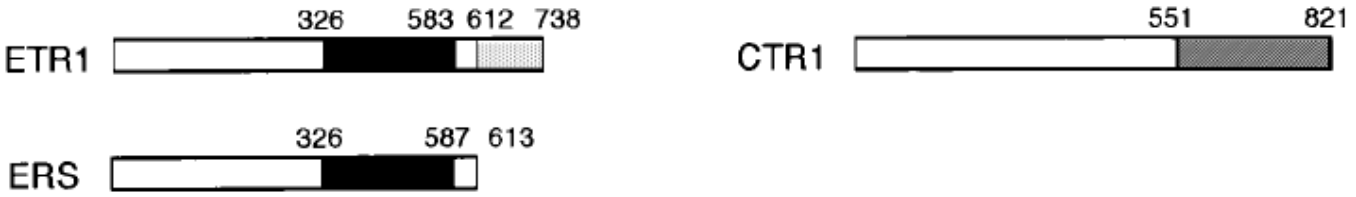



C_2H_4
ETR1
EIN4
CTR1
?
?
Gravity?
Auxin?
EIN2
EIR1
AUX1
EIN3
EIN5
Development?
Auxin?
EIN6
EIN7
?
HLS1
Root
Growth
(-)
Root Hair
Initiation/
Patterning
(+)
Hypocotyl
Growth
(-)
"Stress" Gene
Activation
(+)
Growth in
Apical Hook
(-)

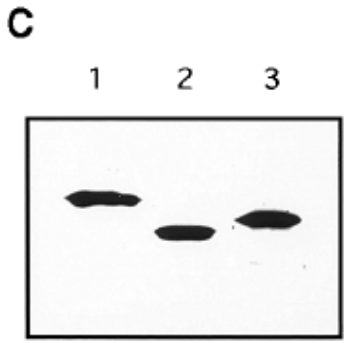
Ctr1 – codes for protein kinase of Raf family



ETR1 interacts with CTR1

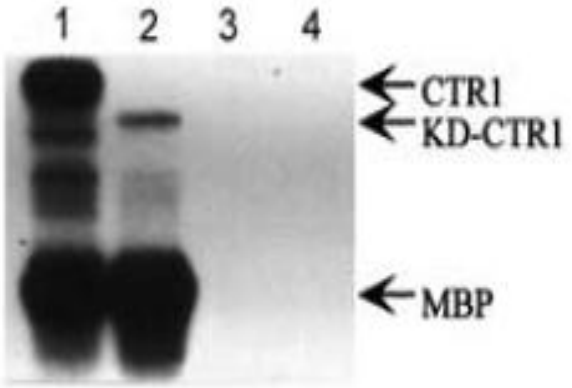


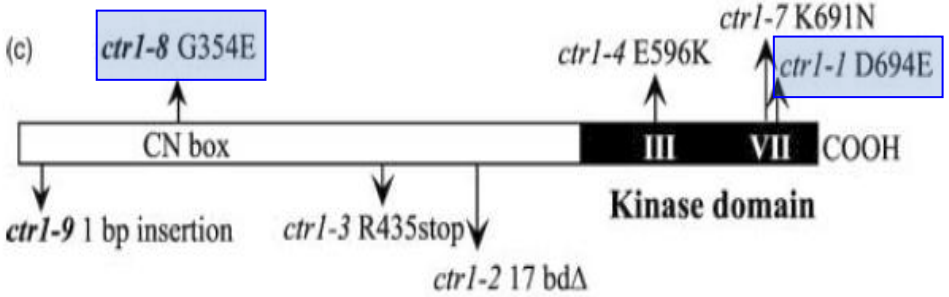
	DB FUSION	AD FUSION	HIS	lacZ	β-gal units
1	ETR1 293-729	CTR1 53-568			71 ± 5.0
	"	CTR1 538-821			0.10 ± 0.02
	"	vector			0.07 ± 0.02
2	ETR1 293-610	CTR1 53-568			0.10 ± 0.02
	"	vector			0.04 ± 0.00
3	ERS 261-613	CTR1 53-568			4.4 ± 0.20
	"	vector			0.05 ± 0.01
	lamin	CTR1 53-568			0.05 ± 0.01

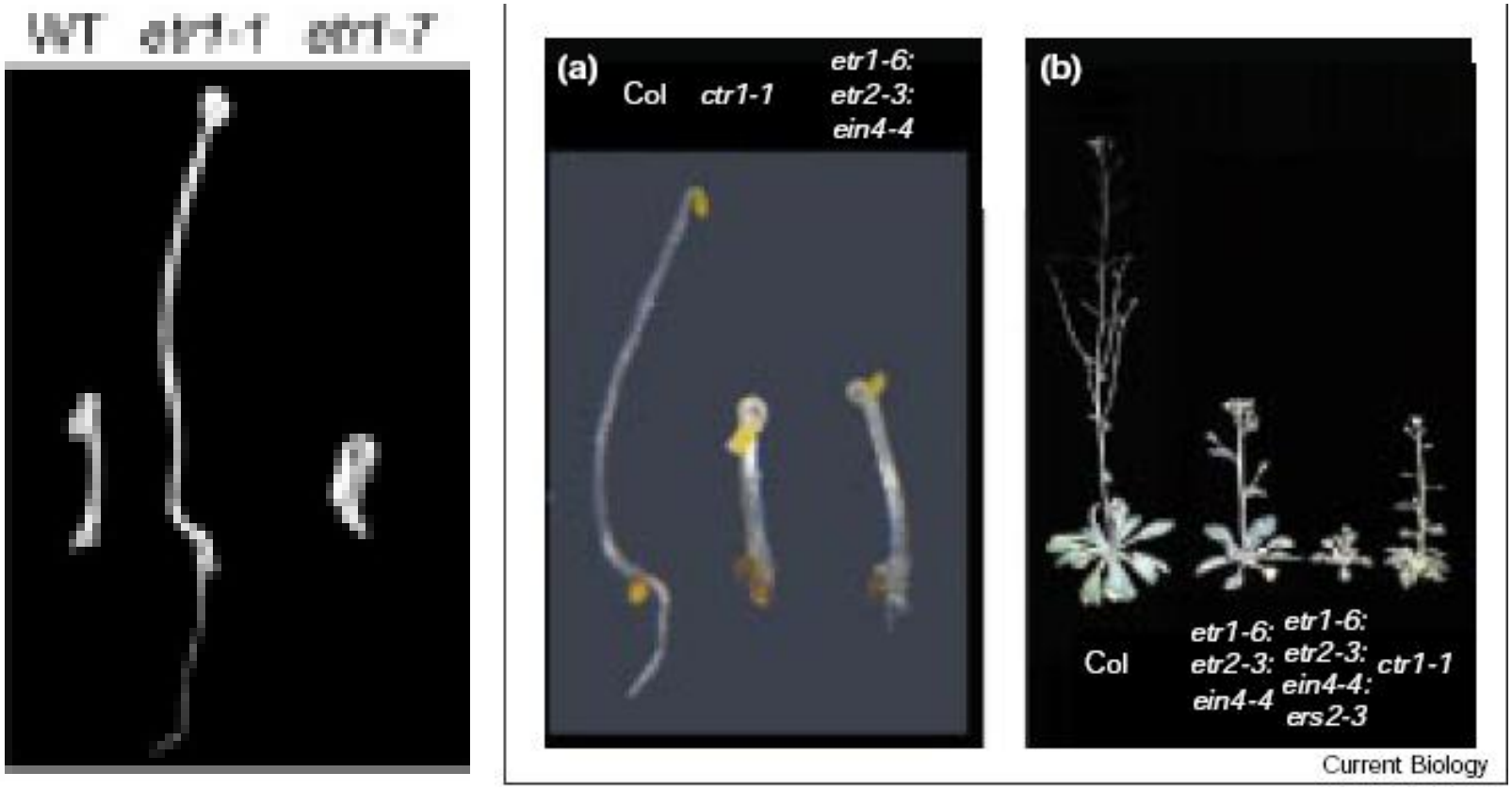


Clark et al., 1998

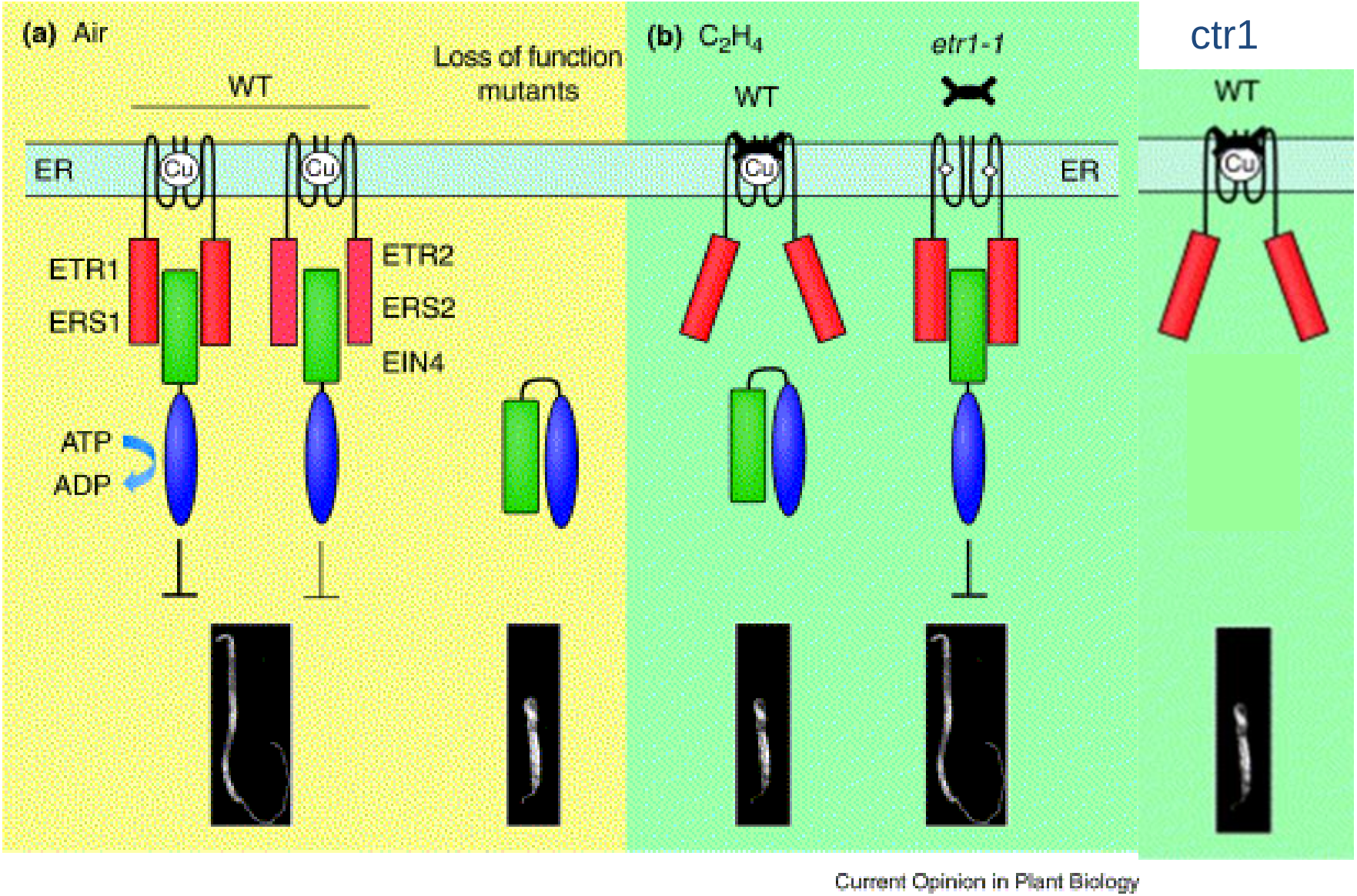
CTR1 has protein kinase activity





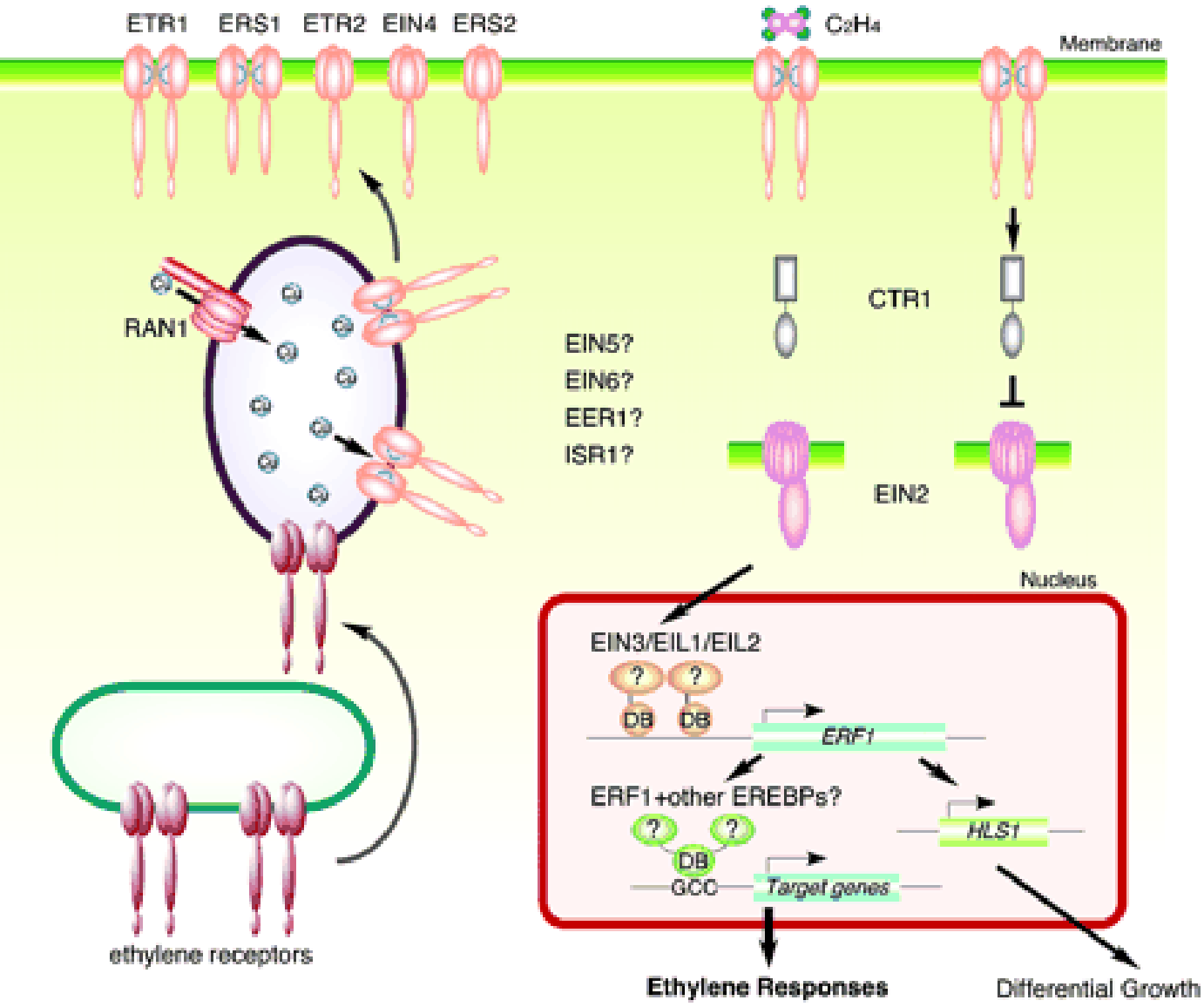


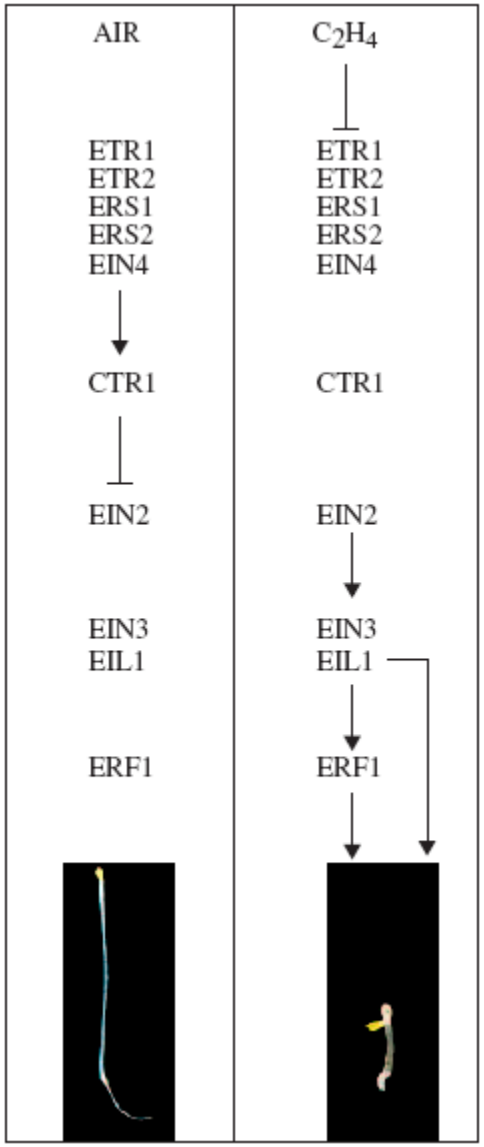
-ethylene receptor dominant mutation > ethylene insensitivity
-ethylene receptor loss of function mutation > constitutive ethylene response
-*ctr1* loss of function mutation > constitutive ethylene response



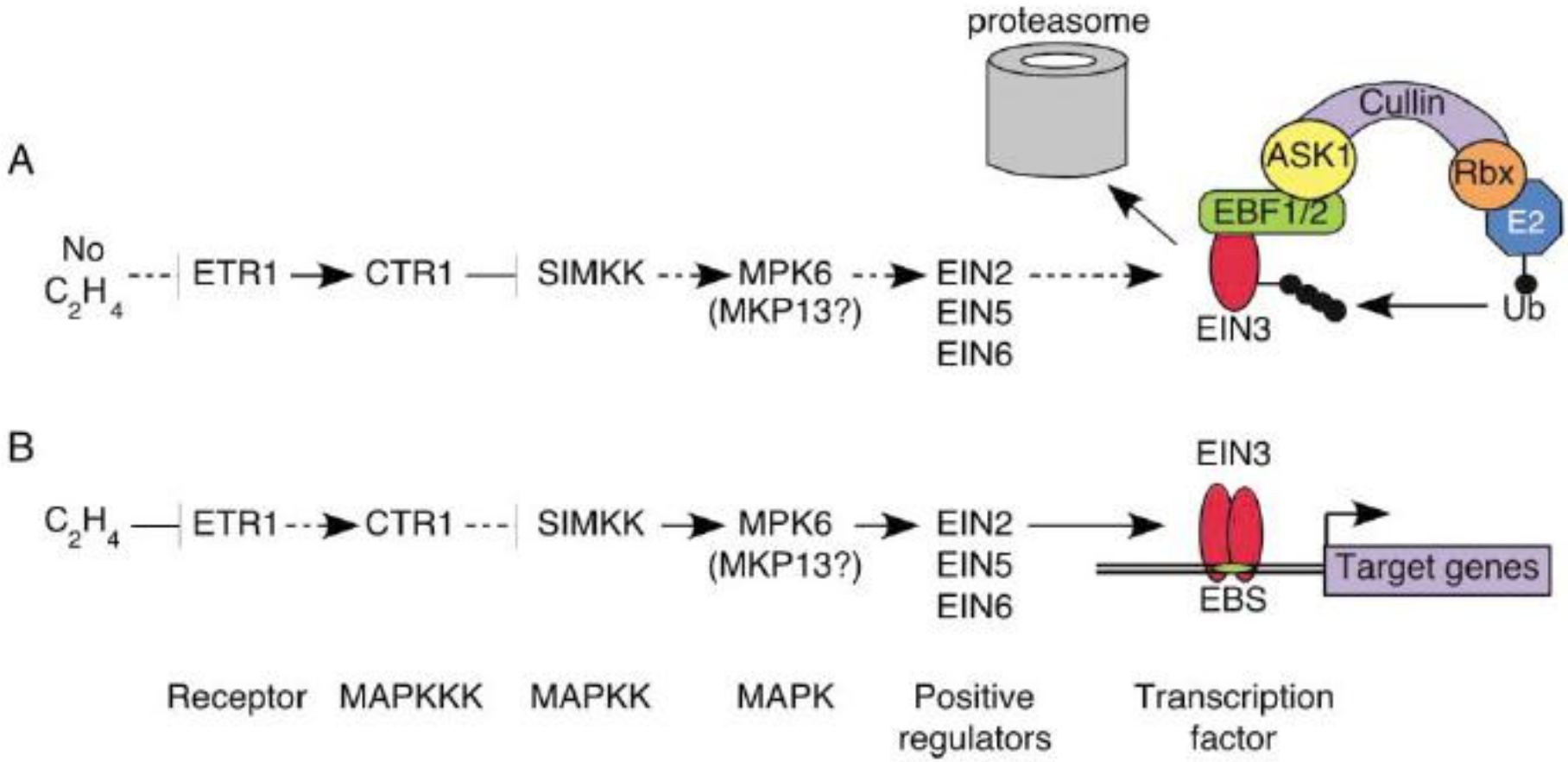
Current Opinion in Plant Biology

Ethylene signal transduction pathway





MODELS



Guo and Ecker, 2003