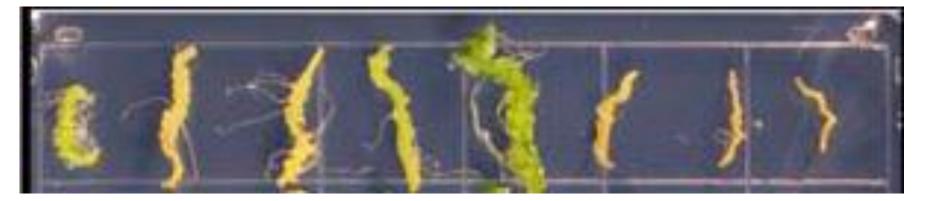
### **CYTOKININ - Metabolism**

### Synthesis – *IPT* genes

### Degradation – CK-oxidase

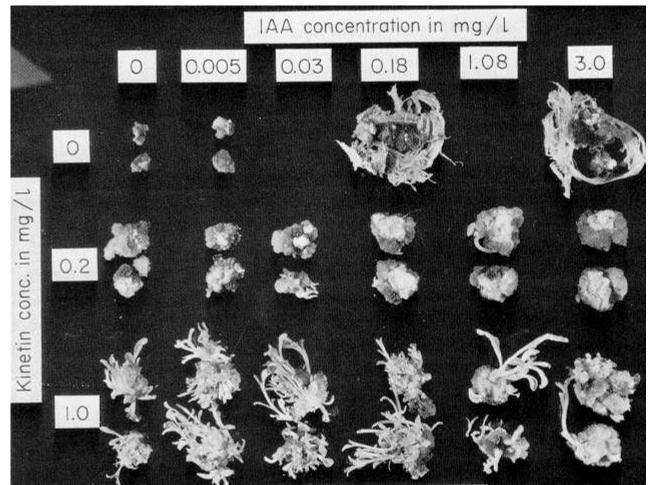
Conjugation

# Cytokinin – substance crucial for sustain of cell proliferation



### Auxin and cytokinin

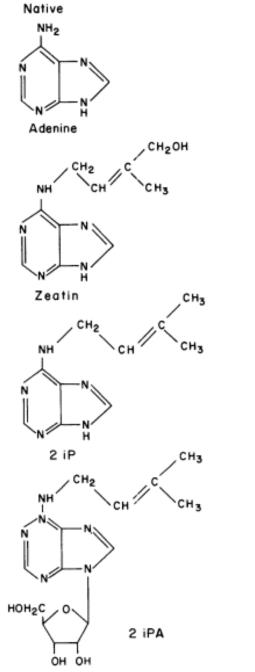
#### roots

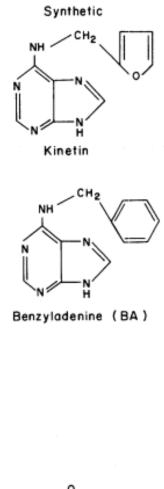


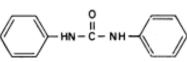
shoots

Skoog et Miller, 1957

# Cytokinins



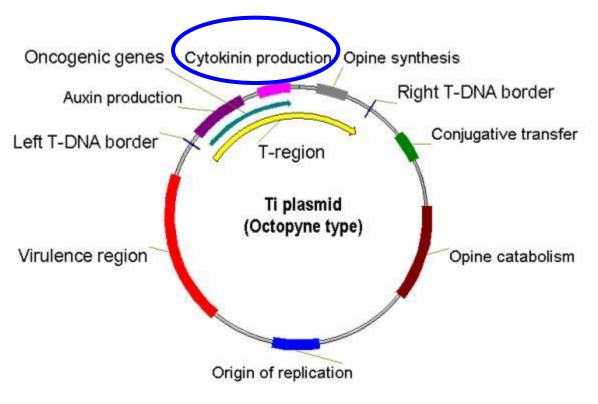




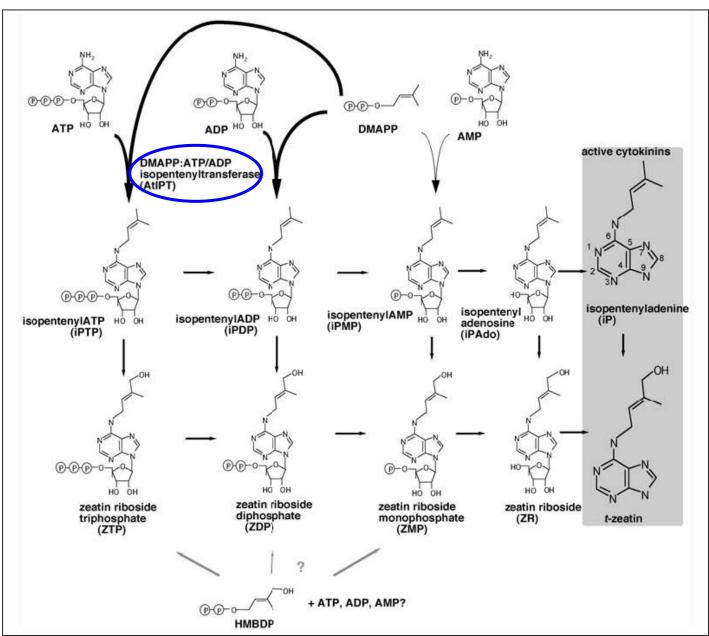
Diphenylurea

#### Agrobacterium tumefaciens – IPT (isopentenyltransferase) gene for cytokinin biosynthesis





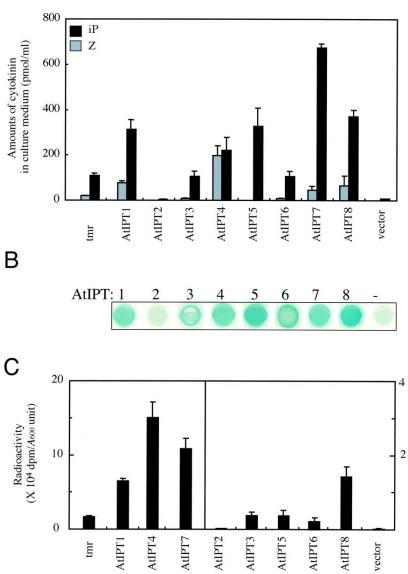
### Cytokinin biosynthesis



#### Isolation of Arabidopsis IPTs

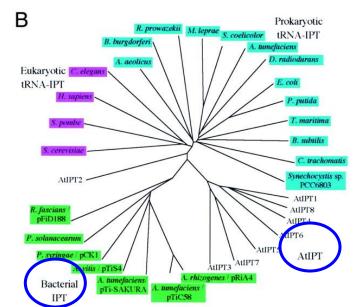
Α

Region a



		3
AtIPT1	1	MTELNFHLLPIISDRFTTTTTTSPSFSSHSSSSSSLLSFTKEREKBOPLVSSIREOSRSR FREDEVUVIL
AtIPT2	1	MMML NPB NGG I EGEKMKKE A WWW I MED BESEKKK
AtIPT3	1	MINKISMAMCKOPLPPSPTLDFPPARFGPNMLTLN-PYGPKDKVVVIMEA CETEER
AtIPT4	1	MTELNFHLLPIISDRFTTTTTTSPSFSHSSENELLSTTKIRKOFVSIIRHKOVVIIDTO NOOGSIENKOKKAVAVID MINKISMAMCKOPEPSSTLDFYSNOOIDOKKAVKAVID SKCHOVVID MIRKISMACKOPEPSSTLDFYSNOOIDOKAVID SKCHOVVID MIRCHTALSPULHIGELWTTKIGGRAVITGGRAAKHING VLITE MQQLMTLLSPULHIGELWTTKIGGRAVITGGRAAKHING VLITE MQQLMTLSPULHIGELWTTKIGGRAVIS
AtIPT5	1	MKPCMTALBOYTOPLSLNFOGNMUDUP_FFPBFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
AtIPT6	1	MOOLMTLLSPPLEHESTLPTVTTKFGSPRLVTTCHGHAGRKETED WT. TTTTTTTTTT
AtIPT7	1	MKFS ISSILKOVOPILCEKNKLSKVNVNSELHPER UTFUMGA SEGUED
AtIPT8	ĩ	MONLTSTFVSPSMIPITSPELSLPPPRSVVPMTTVCMEOSYKO/ WVV INGATESCKSC
AtIPT1	81	
AtIPT2	36	AVELASHER-VELTNARAM TV SCOVITSKUTUDEOKSUPHI ALETVS-SOME DARDERDETURI TETUSENH TEV
AtIPT3	57	AND TATES R-AND INSERTION HOLD IN ATTSEES COMPANY OF PEADERANY CHIMANISTESUINE OF
AtIPT4	21	POURTALLESK - ASH TAREKARY OF KTOWN STIEDER WARHING FELVICE ARE UNA ARE UNA ARA TSTTORY IT T
AtIPT5	49	A TELATIEP - ASTUNDED OVER DIVER DIVERSION FRANCE VIDE TO A FOR ONE A TRAVEST VORDENET
AtIPT6	61	PROPERTY OF PARTICIPACITY OF THE PROPERTY OF THE PARTY OF
AtIPT7	50	A TELATING - GET IN SEK TOLING DUT TO KUT PKEC ROUPHE A SUF DSTAINING TOYSRI AS OA TSKISANNET T
AtIPT8	59	IS IDDATES - GETWISDATCEY CLAVETNOWS ILERCEVPHENDEL PODDSED TS TREASES ISET ABONI PT
	0.00	
AtIPT1	160	IAGCSNSFVHALLAORPD
AtIPT2	114	LVGCTHYYIOPVVSKFLLDDAAEDTEECCADVASVVDQDMVVESVFGRDDLSHGYELLKELDPVAANRIHPNNHRKINOY
AtIPT3	135	IVGCBH8YVPALVDD
AtIPT4	100	LACCSNSY IHALLAKSYD
AtIPT5	128	IAGGENEYIEALVND
AtIPT6		VVCCSNSF NHALLAFRFD
AtIPT7		
AtIPT8	138	IAGESNSFIH-LLVDRFD
AtIPT1	178	PKFDPFSSGSCLISSDLRYECFIWYDVSETVDYEYLLREVEDUNDSGNFEELSRFYD
AtIPT2	194	LSLHASRGVLPSKLYQGKTAEVDSFTLTSRQNWGCINASRFDYELICMEAETAWDRYVEQEVEAUVDAELLDEVYDIYK
AtIPT3	150	YDCCFLWYDVALPWIHGFVSERVDKVVESGHVEEVFD
AtIPT4	118	PENYPFSDHKGSICSEEKYDCC/IWIDVDQSWFFYLSLEIEIEISMKEGHTEEIAEFHR
AtIPT5	143	YNCHLINVEVSRPWIHSFVSERVENU VDMELVDEVRRIFD
AtIPT6	158	PDIDPPSPGSSLSTICEDLRVKCCILWUDVLEPULPQHECNEVECSIEEGLVEQLAELWD
AtIPT7 AtIPT8	144	
ACIPTS	120	LSLHASROVLPSKLYQGKTAVEN I FOR THE SCHOOL OF
AtIPT1	236	PYAGGLETEY - DIRAC TO'LD'DO'LY ACYPYA'R RAADYTRE JOGE NAME LOFIN HILS R'CAGRIESLS NODKYNKENLER HLEYR MORKINHLES HIB NO DI GRUP FSILDYSRE JONG DI CHART - FFRM
At IPT2	273	DGADYTP T. BOST OUD FEDEL THILS TO AGHL TSISNON FUMERAL BY TINED KINY FINITE THE PRATE OF THE TO BE TH
AtIPT3	198	
AtIPT4	176	
At IPT5	190	PSSBDYSANTERALGUET.DFFI.PSTMPNYDAFTTFI.IFTATEVTTFT
At IPT6	218	
AtIPT7	194	PKANYSUGTER ALGUEST.HFVI.DNESTUDRATKSKMT.DVAUKNTWK.EP
AtIPTS	213	PRYSGSAIRAHGIHKTIGIPPDRYFSLYPPERKO
AtIPT1	293	THAKEVKKIEMEKDAG-WEIERVEAASPKAVMMKSSSEKKWRENTEOVIEPSVKIVKRHLVOB
AtIPT2	352	RELERCKRRVSREETVFGWNIHYIDADEYILSKSEESWNAOVVKPASEIIRCFLETETESGRDPTSG
AtIPT3	248	ELACRERETERERKVKKWSIORVDANPVTKRRSKMDANVAZERLVAGPSTDTVSRFLDDASRRPLVEAS
AtIPT4	232	OTTED ITEINKERNAG-WOIKKVEATASFREAIRAAKEGEGVAEMORKINNKEVLEPCVKIVRSHLDOPINYYYYFYL
AtIPT5	241	LEACERLOKIOREYKOWKWNMHRWDAREVTLRRGEEADEADDNSWAHPSALAVEKFLSYSDDHHLEGANI
AtIPT6	274	REVEKEKIMKHIRGG-WEIKRLDATAAIMAELNOSTAK-GEGKNGREIMEKHIVDESVEIVKKFLLEV
AtIPT7	241	I CREIKKIOREHKKWKMSMHRVDAREVTLKRNVEEODEALENLVARPSERIVDKFYNNINOLKNDDVEH
At IPTS	273	TO BLEV OR LETER DIDAL THE E TO CALL AND A WARKES - LER WE NOT AVAILABLE CONTRACTOR AND A C
AtIPT2 AtIPT3		KSIERDLWTQYV <u>CEACGNKILRGRHEWEHHKOGRTH</u> RKRTTRHKNSQTYKNREVQEAEVN
AtIPT3		TAVAAAMERELSRCLVA Region b
AtIPT5		LLPEISAVPPLPAAVAAISR
Atip15		CLAASYGGGSGSRAHNMI
ACAFA!	346	

Α



#### Arabidopsis *IPT*s (8)

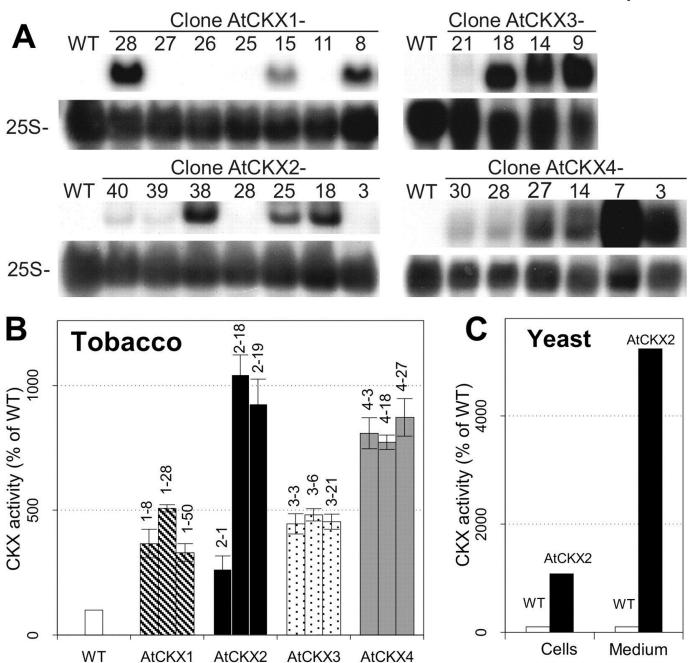
**Differential expression patterns** 

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

mutants, overexpression ???

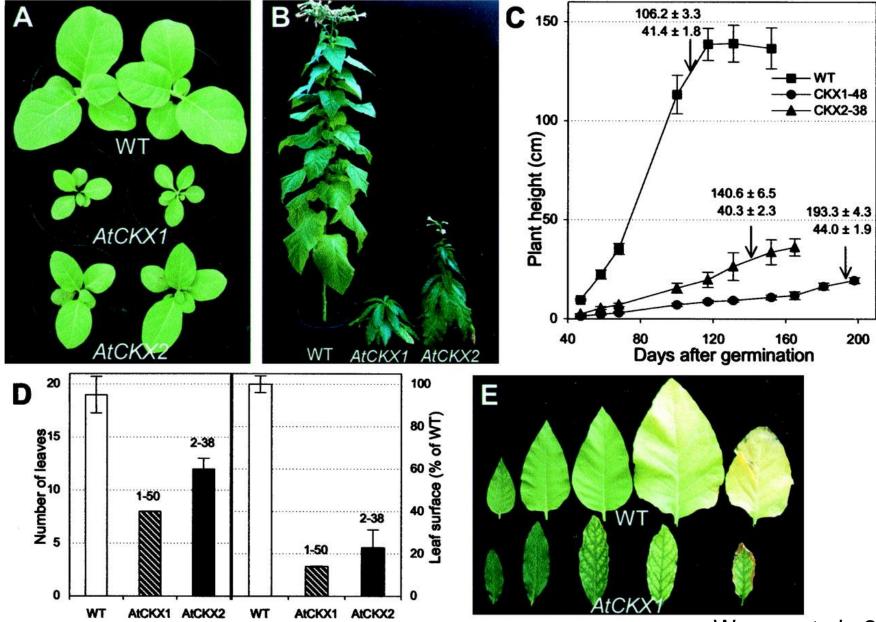
Kakimoto 2004

#### Isolation of CK-oxidase (AtCKX)



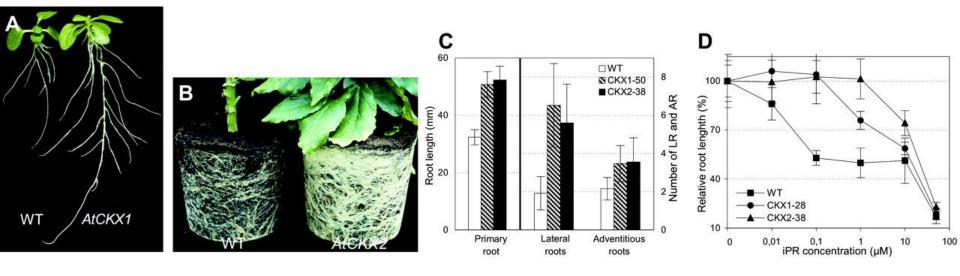
Werner et al., 2001

#### AtCKXs overexpression in tobacco



Werner et al., 2001

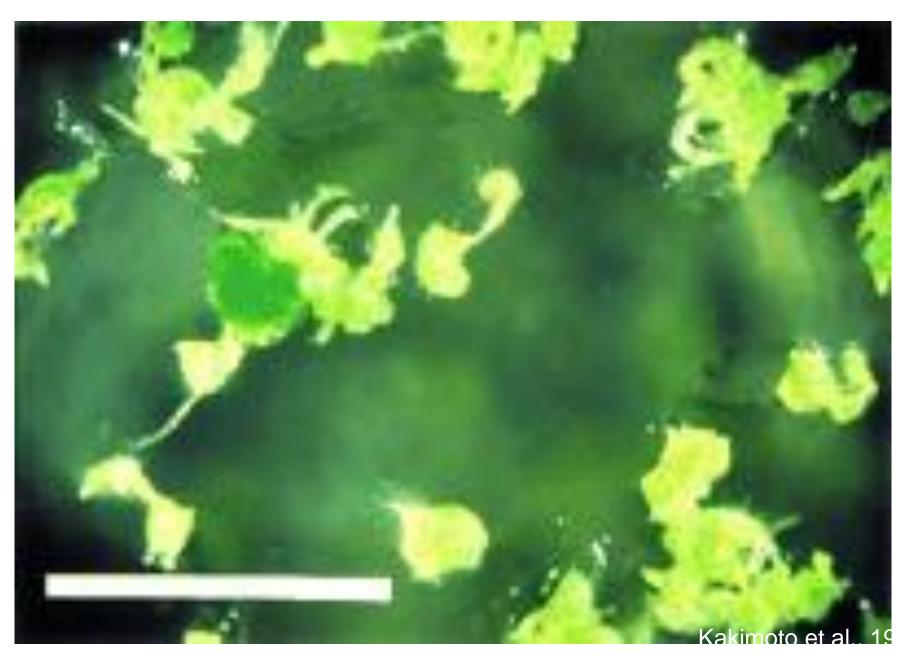
#### Effect of AtCKX on tobacco root



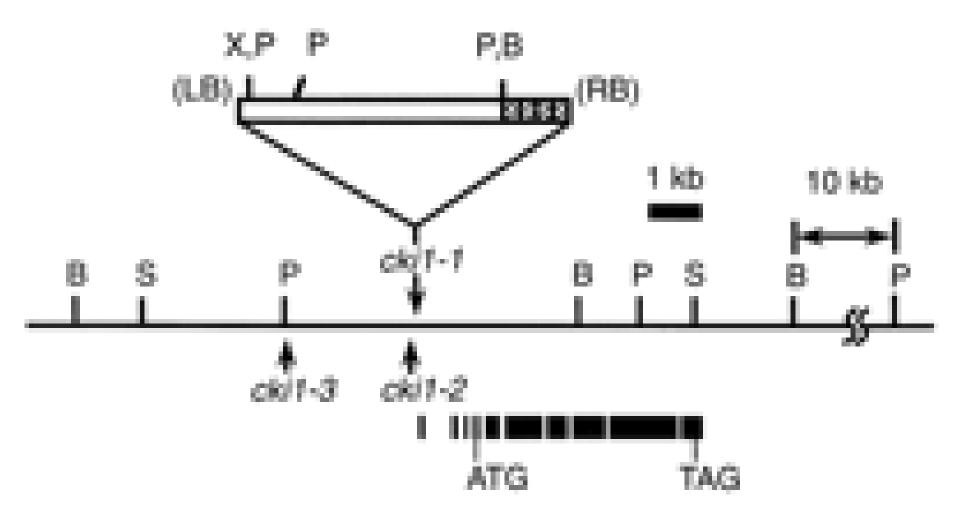
Werner et al., 2001

### Cytokinin –signal perception and transduction

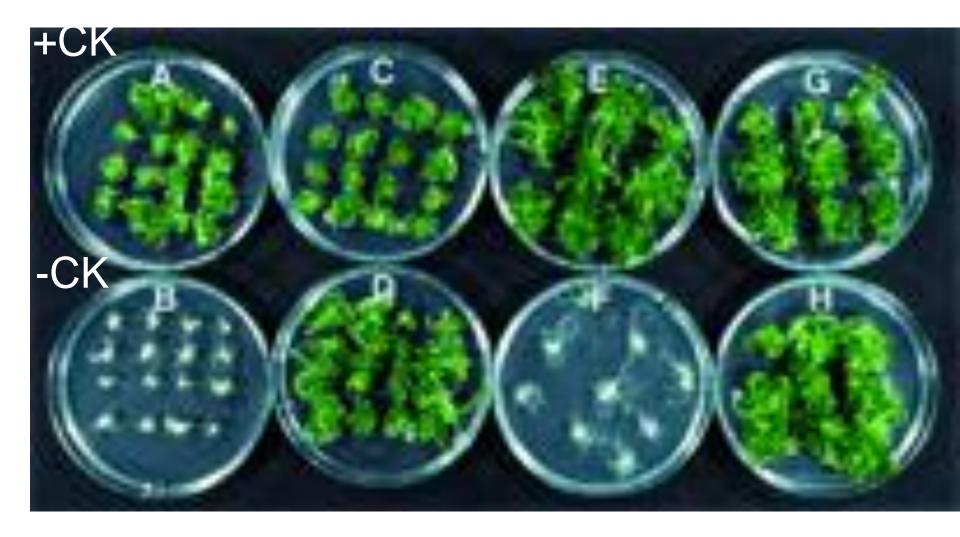
### Isolation of CK independent (cki1) mutant



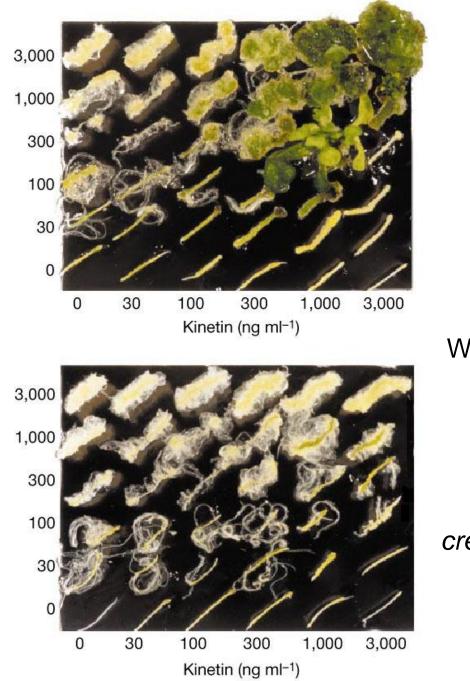
### Identification of CKI1 gene



### Verification - 35S::CKI1 transgene



Kakimoto et al., 1996



а

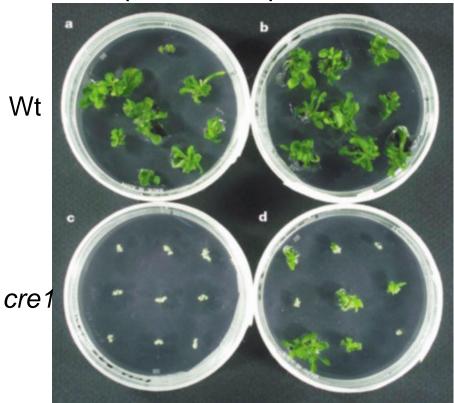
NAA (ng ml-1)

b

NAA (ng ml-1)

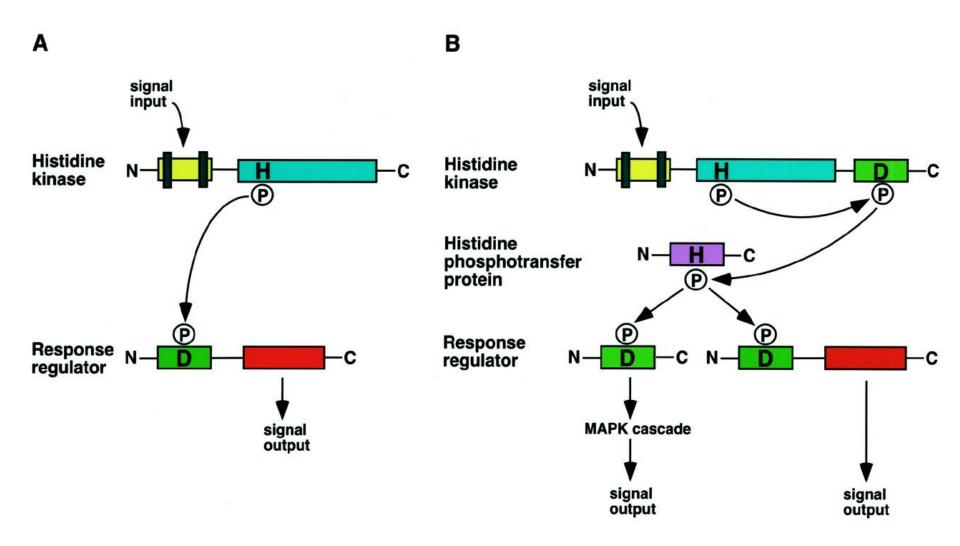
# Next strike - CK response mutant (*cre1*)

#### pGTV pGTV-CRE1

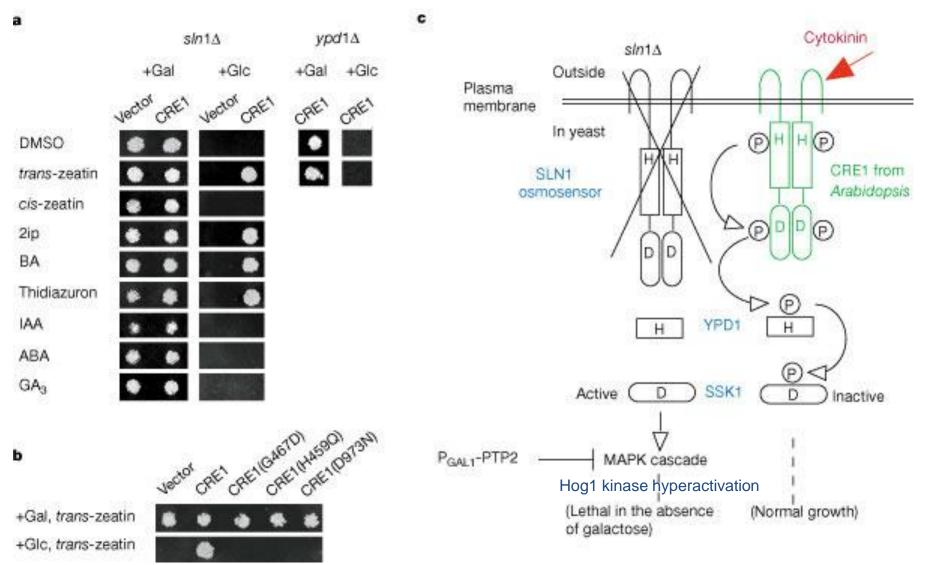


#### Inoue et al., 2001

### His kinase transduction pathway



# Piece of genius - complementation



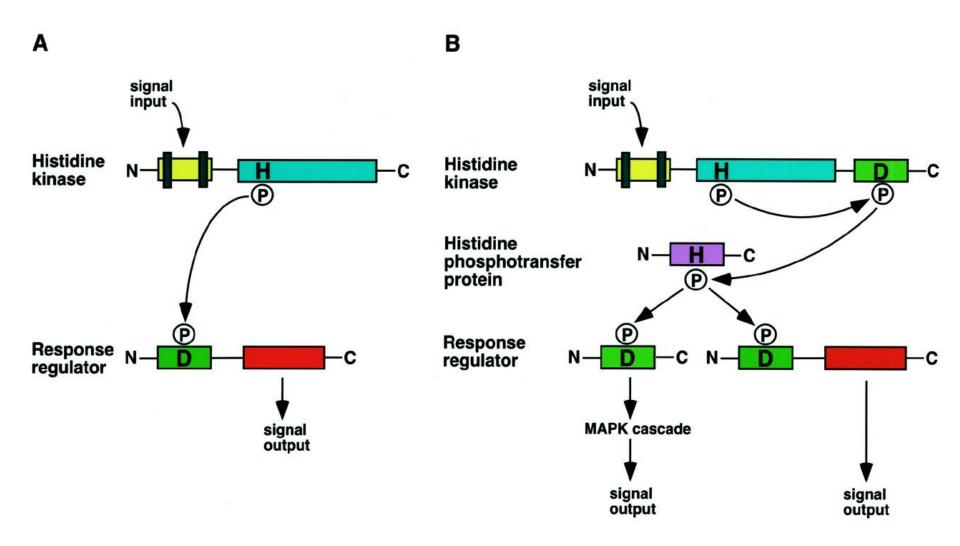
Inoue et al., 2001

## Cytokinin receptors – what else?

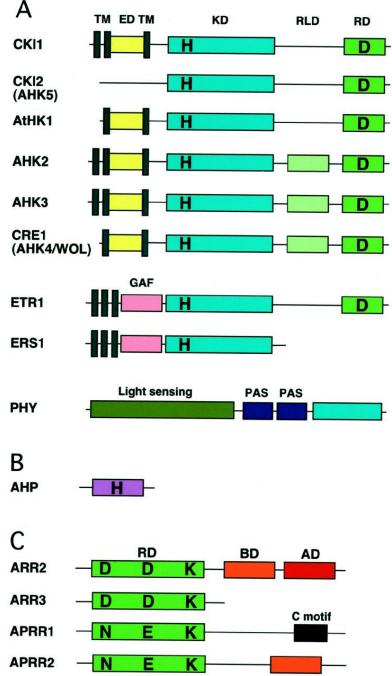
### 3 CRE1 homologous proteins (AHKs) multiple mutant phenotypes – additive (not lethal)

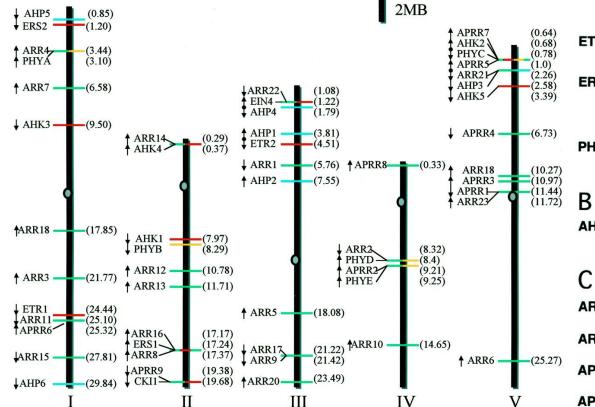
What does CKI1?

### His kinase transduction pathway

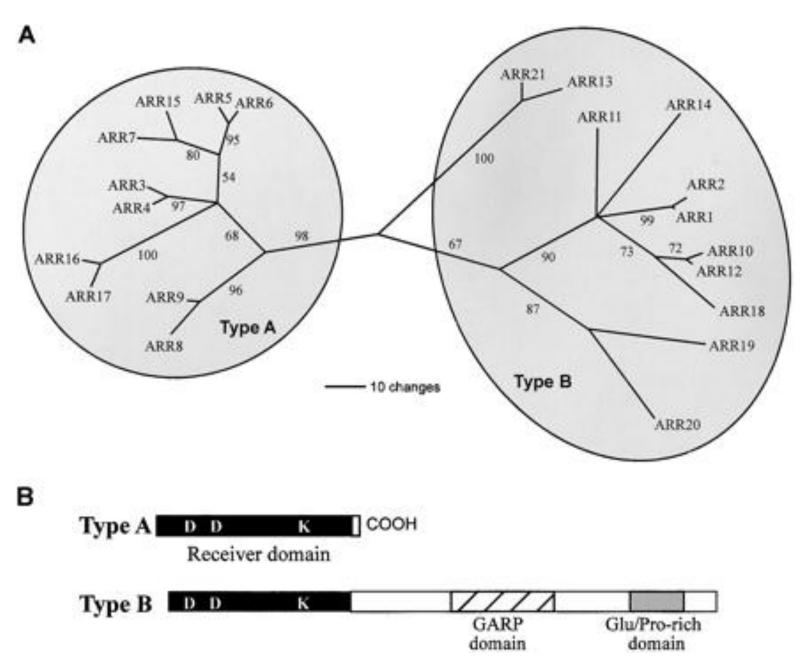


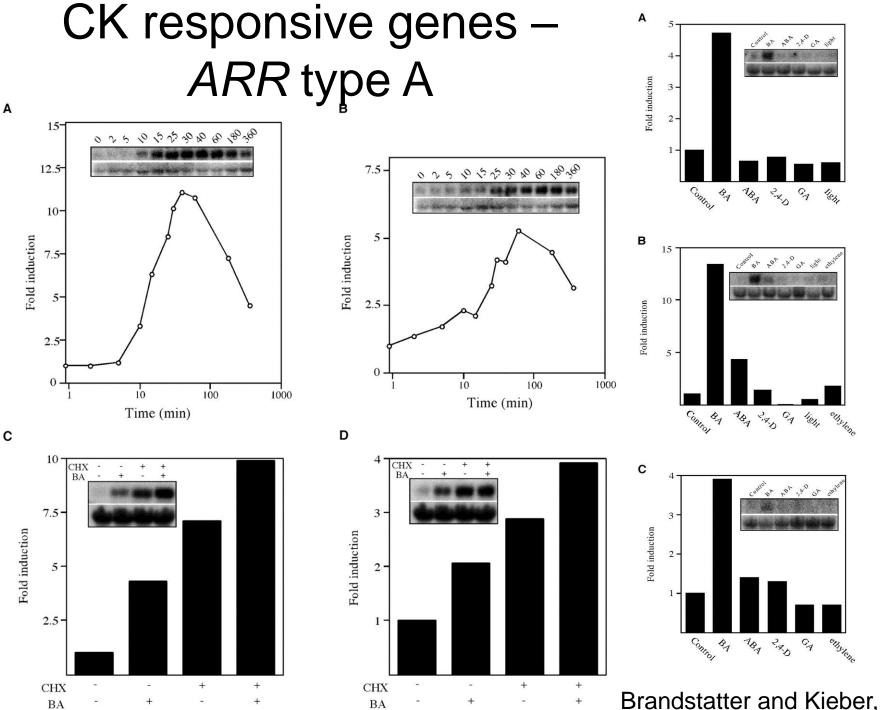
### His kinase pathway components in Arabidopsis





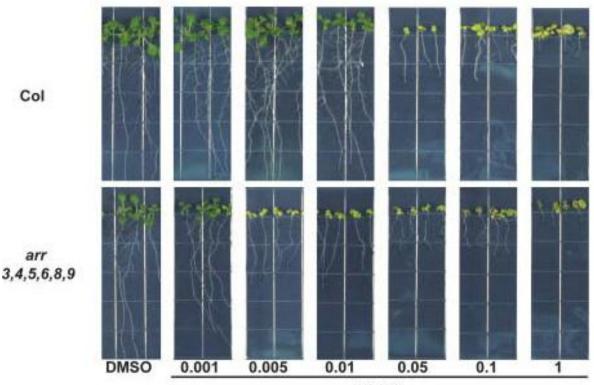
### Response Regulators in Arabidopsis



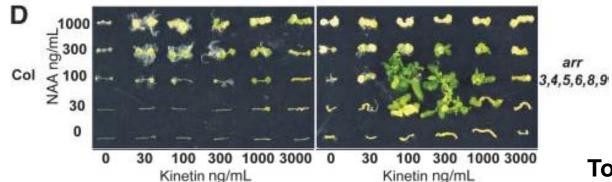


Brandstatter and Kieber, 1999

### Response regulator ARR type Anegative regulators of cytokinin signalling

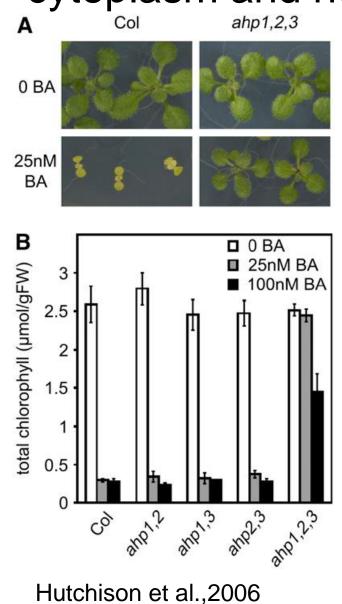


µM BA

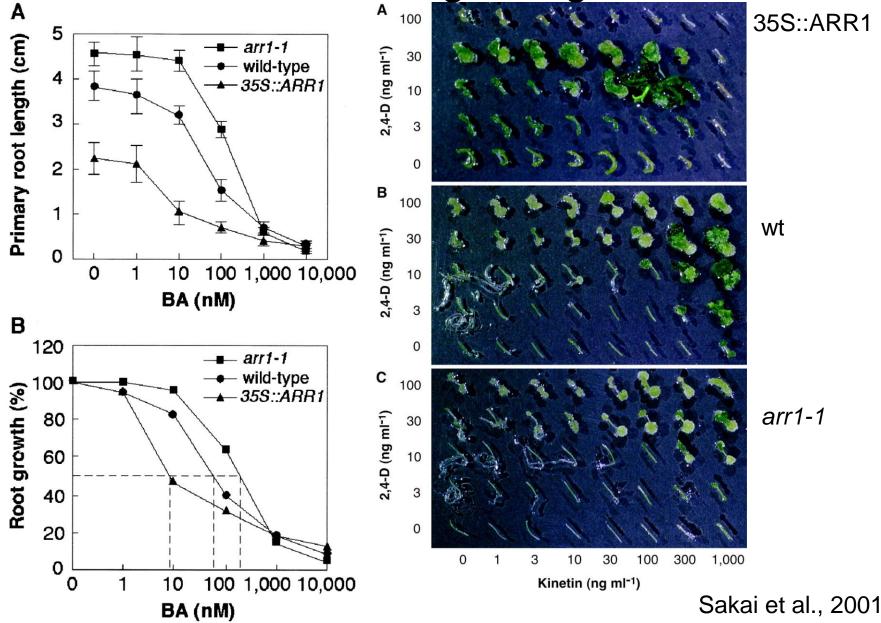


To et al., 2004

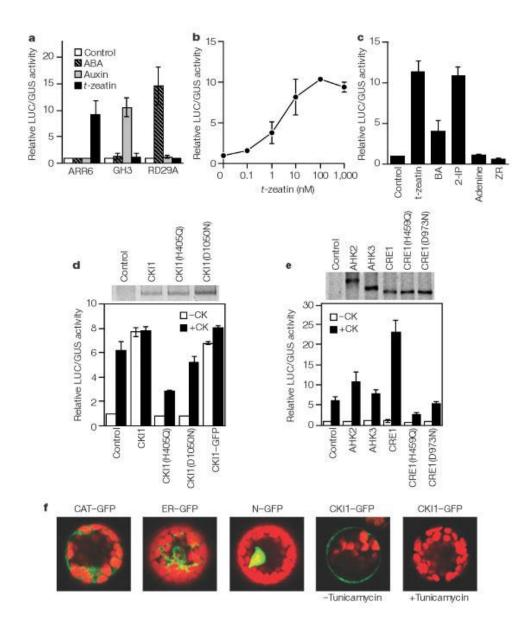
# AHPs mediate transfer of cytokinin signal between cytoplasm and nucleus



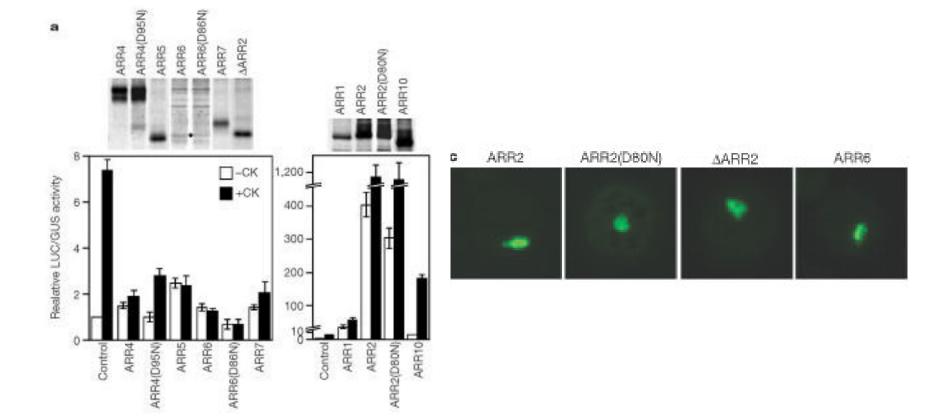
#### Phenotypes of *arr* type B positive regulator of CK signalling

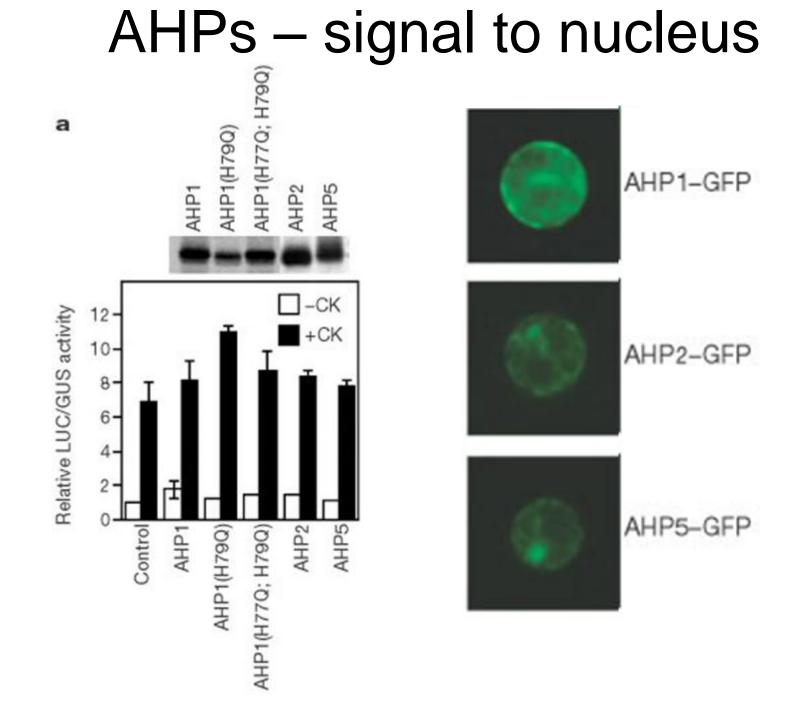


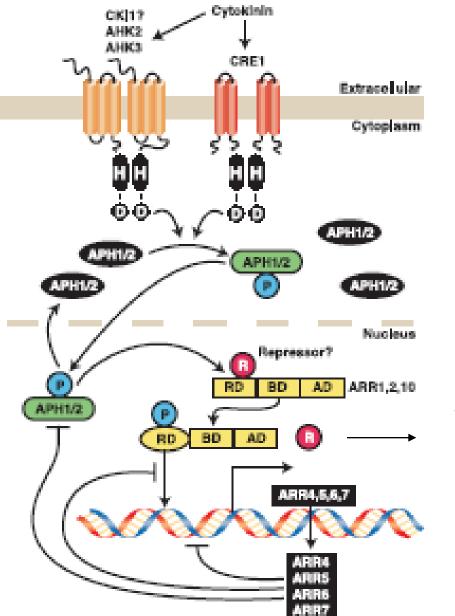
### Games with protoplasts



# Opposite effects of two classes of ARRs on CK signalling







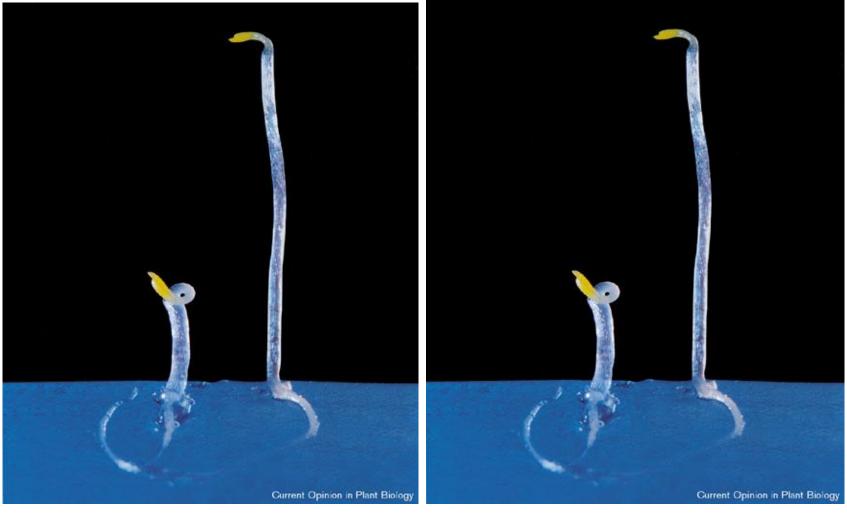
Hextuple of type AARRs confirms role as negative regulators

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



 $C_2H_4$ 

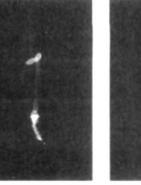


air

 $C_2H_4$ 

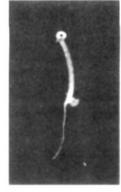
### Screen for ethylene mutants I. Genes involved in regulation of biosynthesis















Wild type/air

Wild type/ethylene

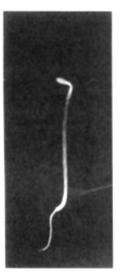
eto1-1/air

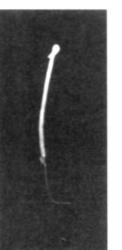
ctr1-1/air

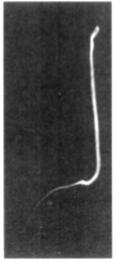
eir1-1/ethylene

aux1-21/ethylene

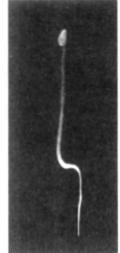
hls1-1/ethylene













etr1-3/ethylene

hylene ein2

ein2-1/ethylene ein3-1/ethylene

ein4/ethylene

ein5-1/ethylene

ein6/ethylene

ein7/ethylene

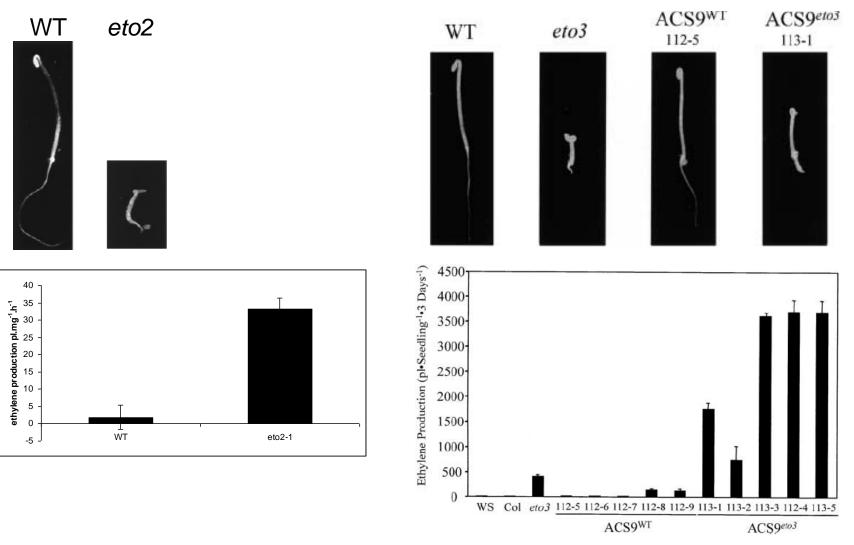


\* phenotype rescued by inhibitor of ethylene biosynthesis

#### eto mutants – constitutive triple response



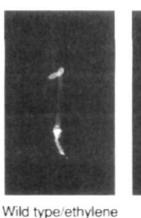
eto3



- overproduction of ethylene

#### Screen for ethylene mutants II. Genes invoved in signalling pathway









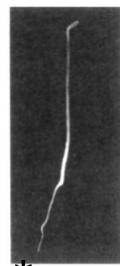


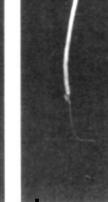




hls1-1/ethylene















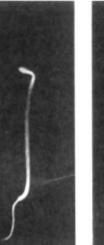


ein7/ethylene

ein6/ethylene

#### Roman et al., 1994

Wild type/air



★ etr1-3/ethylene

ein2-1/ethylene

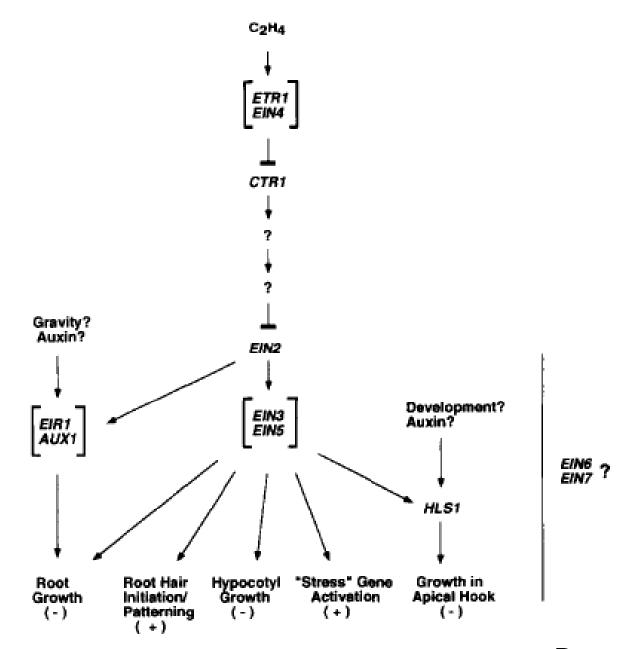
ein3-1/ethylene

ein4/ethylene

ein5-1/ethylene

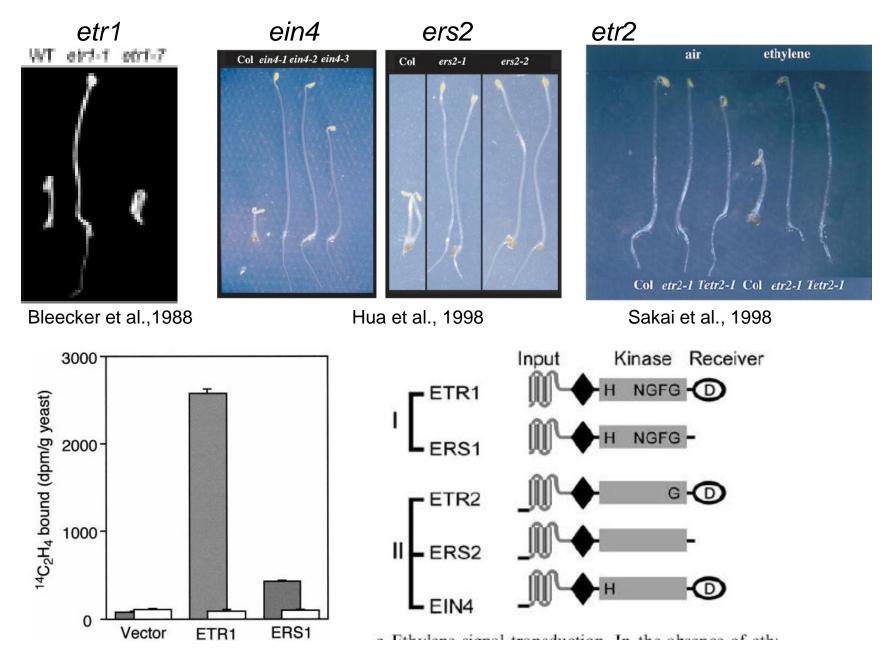
B. Double mutants <sup>a</sup>	
aux1-21 ctr1-1	Aus-, $Ctr^{-c}$
aux1-21 eir1-1	Aux <sup>-</sup>
ctr1-5 ein 2-1	Ein <sup>-</sup>
ctr1-1 ein3-2	Ein <sup>-</sup>
ctr1-1 ein5-1	Ein
ctr1-1 ein7	Ein <sup>-</sup>
ctr1-1 eir1-1	$Eir^{-c}$ , $Ctr^{-c}$
ctr1-1 etr1-3	Ctr
ctr1-1 hls1-1	Hls <sup>-</sup> , Ctr <sup>-</sup>
ein2-1 eir1-1	Ein <sup>-</sup> , Eir <sup>-</sup>
ein2-6 eir1-1	Ein <sup>-</sup> , Eir <sup>-</sup>
ein2-1 eto1-1	Ein <sup>-</sup>
ein2-6 eto 1-1	Ein <sup>-</sup>
ein2-1 etr1-3	Ein
ein2-1 hls1-1	Ein <sup>–</sup> , Hls <sup>–</sup>
ein3-1 eir1-1	Ein <sup>-</sup> , Eir <sup>-</sup>
ein5-1 eir1-2	Ein <sup>-</sup> , Eir <sup>-</sup>
eir1-1 hls 1-1	Eir <sup>-</sup> , Hls <sup>-</sup>

Roman et al., 1994

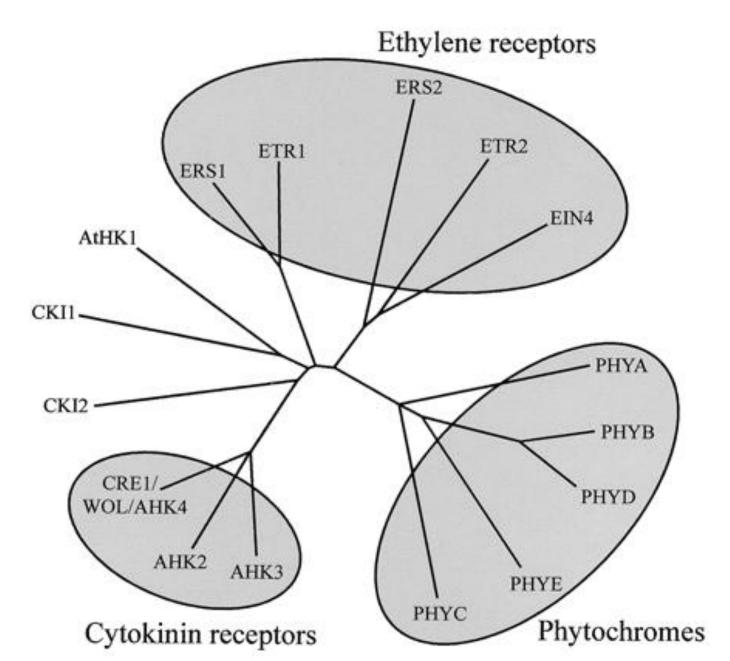


Roman et al., 1994

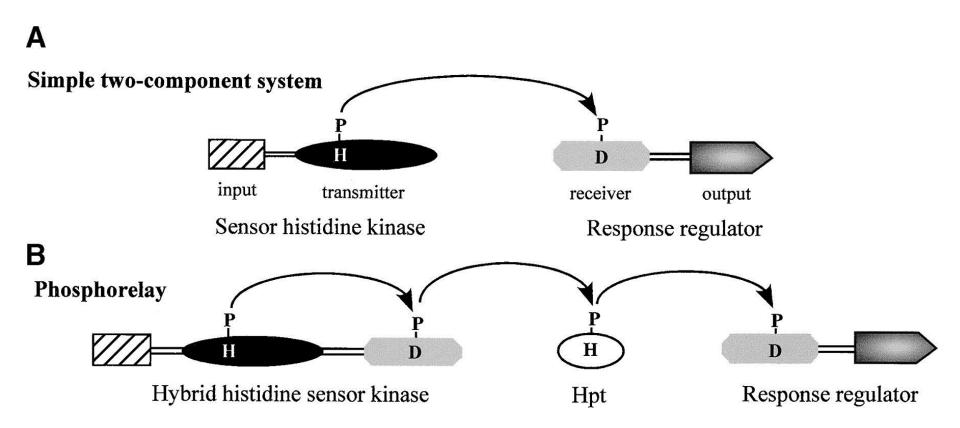
# ETR1 codes for histidine kinase

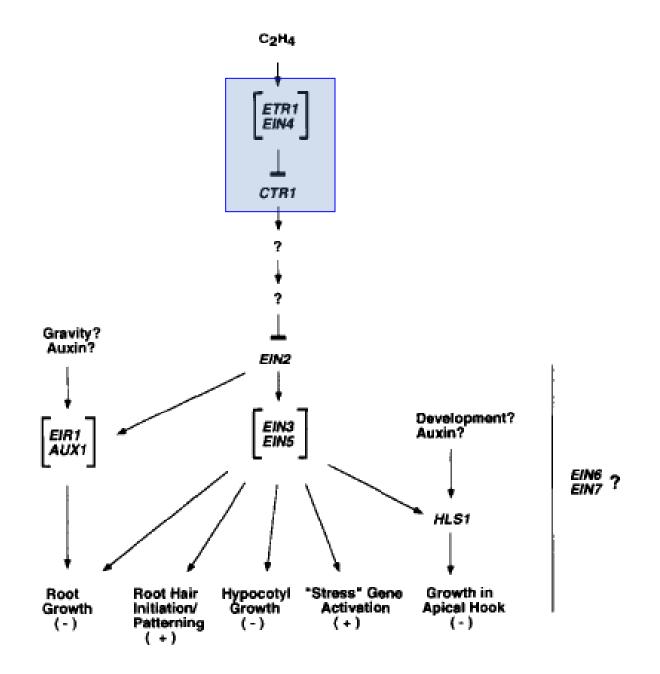


# **His-kinases in Arabidopsis**

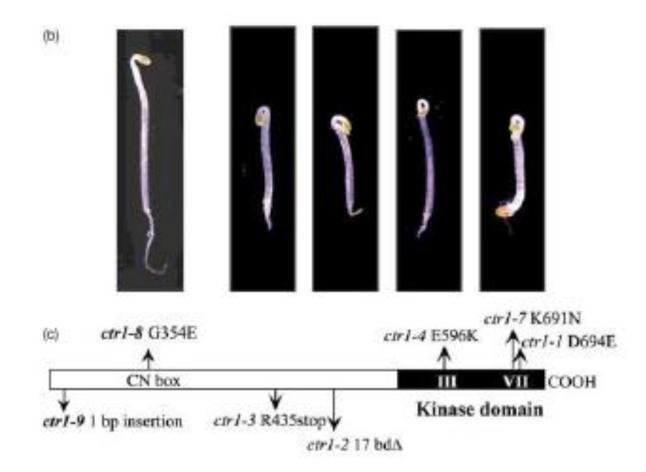


# Ethylene signalling – homology to two component system ?



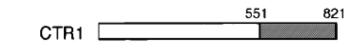


## Ctr1 – codes for protein kinase of Raf family



# ETR1 interacts with CTR1





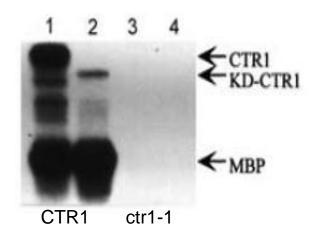
	DB FUSION	AD FUSION	HIS	lacZ	β-gal units
1	ETR1 293-729	CTR1 53-568		-	71 ± 5.0
		CTR1 538-821	\$70 F		$0.10 \pm 0.02$
		vector	100 P	2011	$0.07 \pm 0.02$
2	ETR1 293-610	CTR1 53-568	22 8	03	$0.10 \pm 0.02$
		vector	Antiputar Const		$0.04 \pm 0.00$
3	ERS 261-613	CTR1 53-568		a 0	4.4 ± 0.20
		vector	- 11 - ···	1	0.05 ± 0.01
	lamin	CTR1 53-568	3 3		0.05 ± 0.01

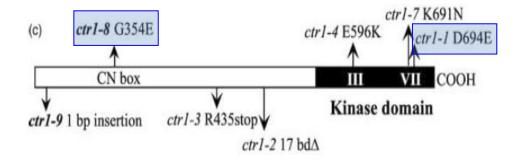


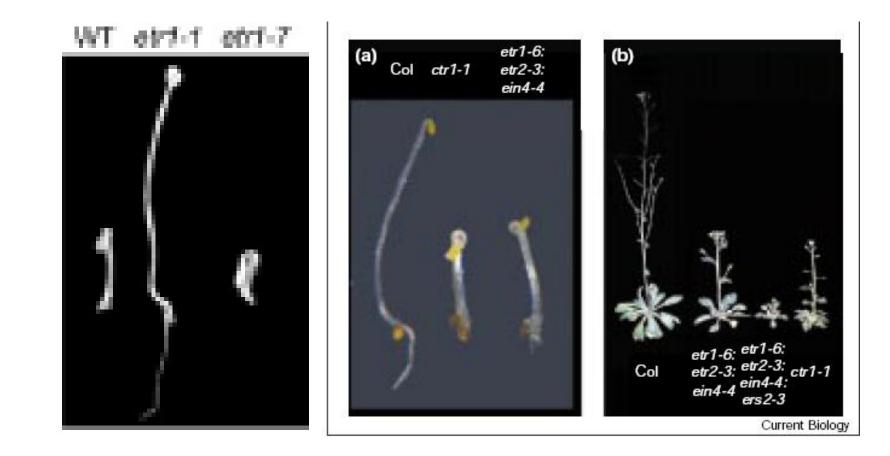


#### Clark et al., 1998

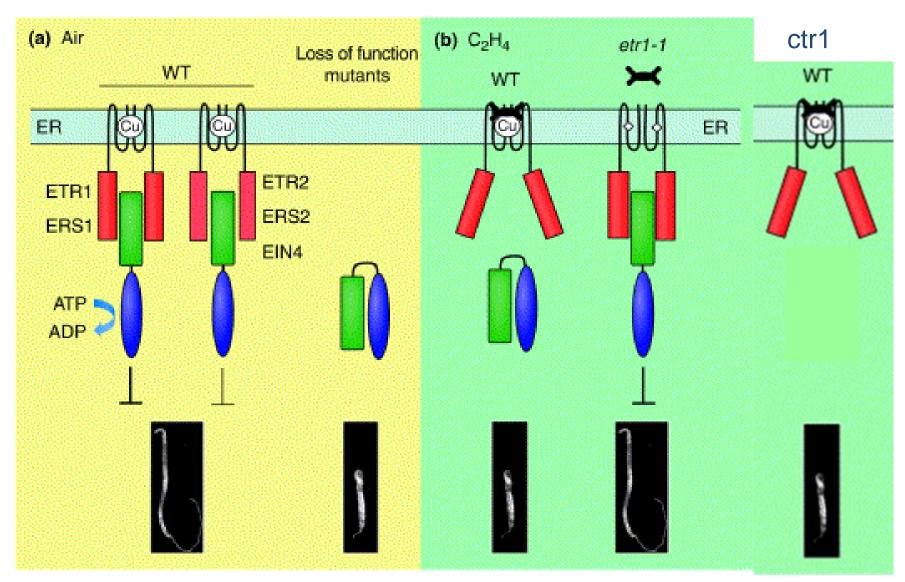
### CTR1 has protein kinase activity





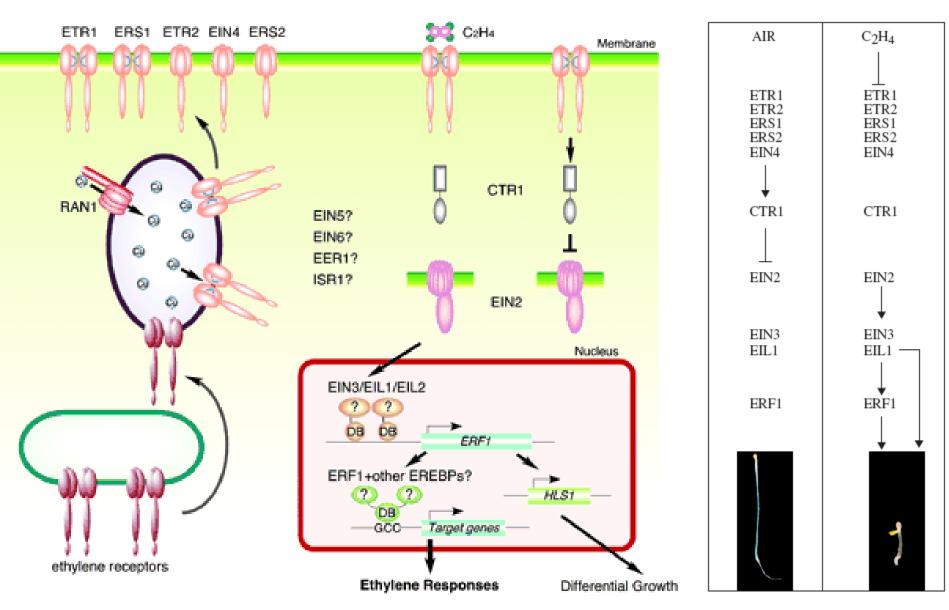


-ethylene receptor dominant mutation > ethylene insensitivity
-ethylene receptor loss of function mutation > constitutive ethylene response
-ctr1 loss of function mutation > constitutive ethylene response

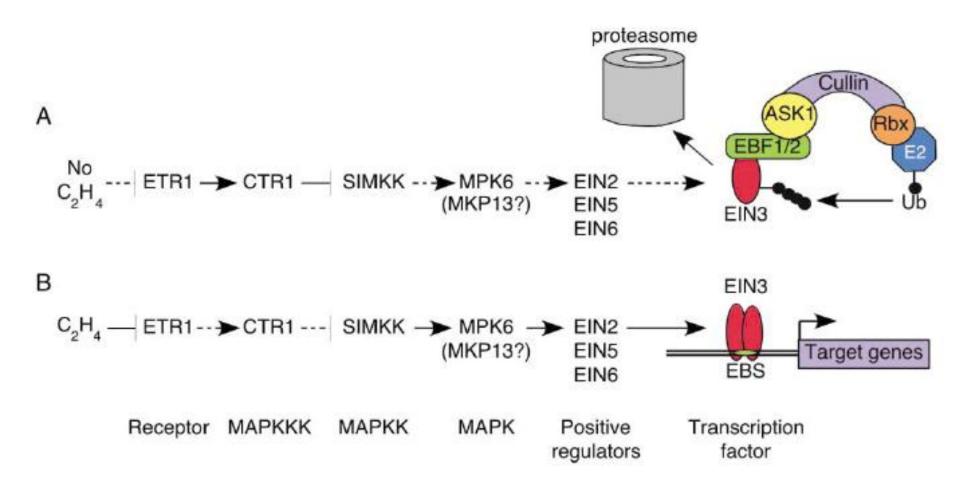


Current Opinion in Plant Biology

# Ethylene signal transduction pathway



# MODELS



Guo and Ecker, 2003