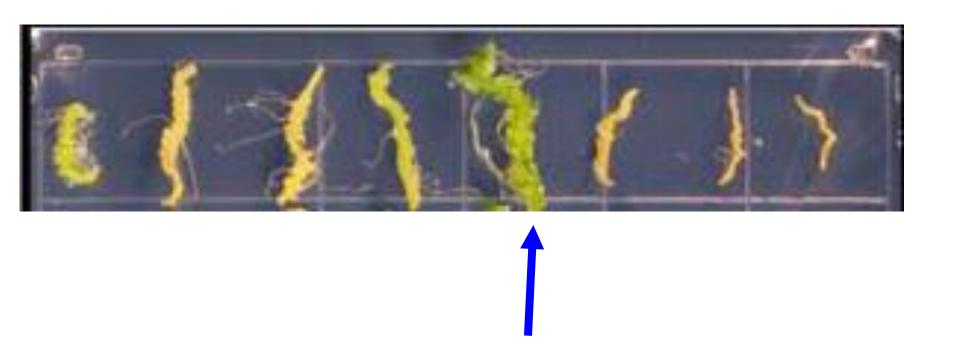
Role of cytokinin in plant development

Cytokinin – substance crucial for sustain of cell proliferation



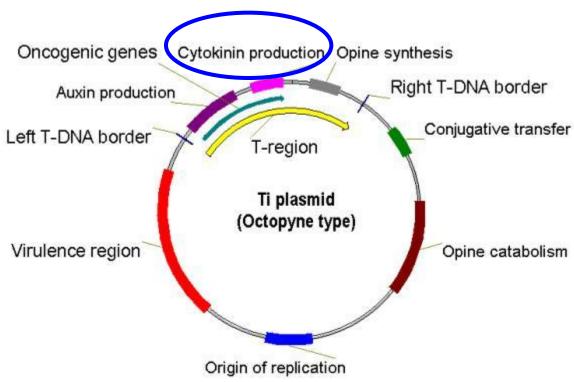
Cytokinins

Benzyladenine (BA)

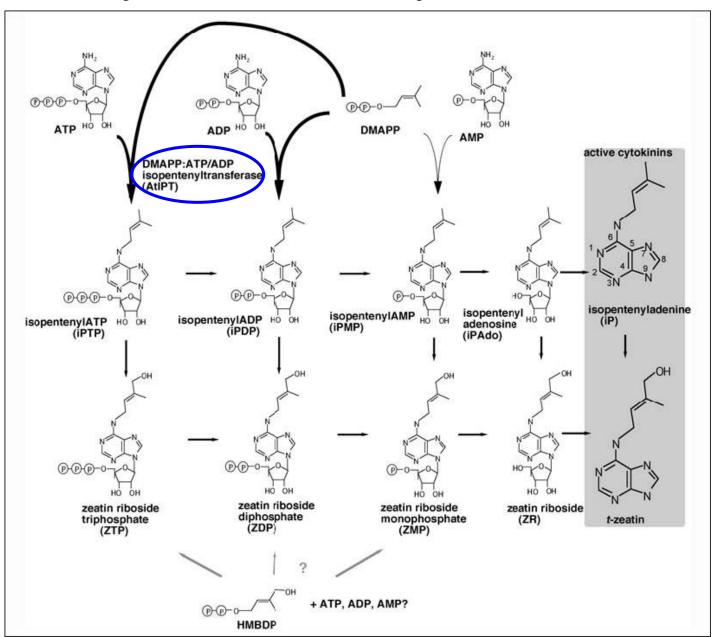
Agrobacterium tumefaciens – IPT (isopentenyltransferase)

gene for cytokinin biosynthesis

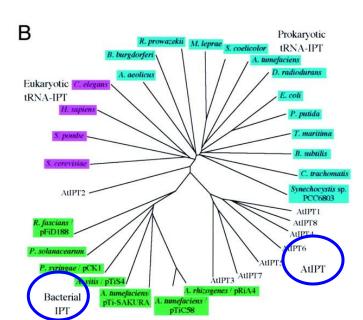




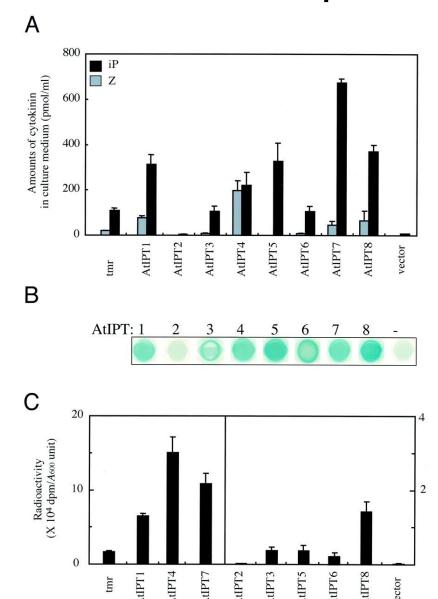
Cytokinin biosynthesis



1 MTELNFHLLPIISDRFTTTTTTSPSFSSHSSSSSLESFTKERENOPLVSSIREDOSRSREND 1 MMMENSENOSIECEKNIKKA 1 MIMKISMAMCKOPEPPSPTLDFPPARAFOPMINILE-PYGER AtIPT4 AtIPT5 AtIPT6 MOOLMTLLSPPLSHSSLLPTVTTKFGSPRLVTTCHGHAGRKHIK AtIPT2 AtIPT3 AtIPT4 AtIPT6 AtIPT1 114 VVSKFLLDDAAEDTEECCADVASVVDQDMVVESVFGRDDLSHGYELLKELDPVAANRIHPNNHRKINQY AtIPT3 AtIPT6 178 -----PRFDPFSG--SCLISSDLRYEC 194 LSLHASRGVLPSKLYQGKTAEVDSFFLTSRONWGCINASRFDY EDFLKIHLSETCAGHLTSLSNDDKVMKENLRKILNFPKDDKLRIMLEEAIDRV 273 PGAD---YTR 198 FSNSD---YSR 176 SKK--APKEPL PFD---RFFRNEQ--BFDDYLKMYKWDND-BLD---EFLRSEMR-AFFAVIORESS --- EARLE TO COULT PRESENTATION OF THE STATE AtIPT4 AtIPT6 416 KSIERDLWTQYV<u>CEACGNKILRGRHEWEHHKOGRTH</u>RKRTTRHKNSQTYKNREVQEAEVN 320 TAVAAAMERELSRCLVA Region h 311 LKRFLSLN



Isolation of Arabidopsis *IPT*s



Arabidopsis *IPT*s (8)

Differential expression patterns

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

mutants, overexpression ???

CYTOKININ - Metabolism

Synthesis – IPT genes

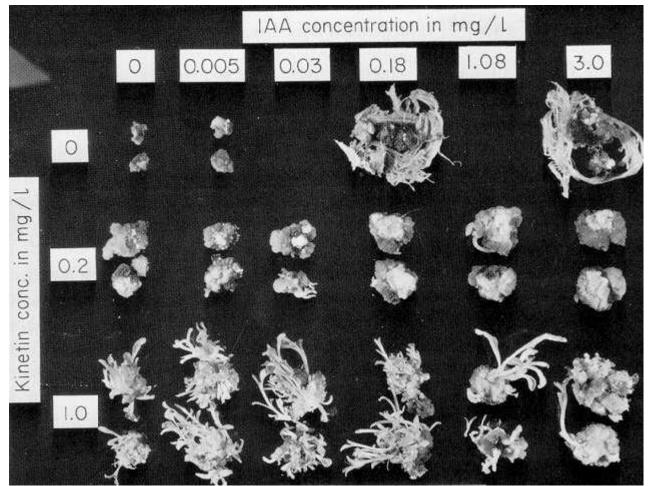
Degradation – CK-oxidase

Conjugation

Cytokinin – role in plant development

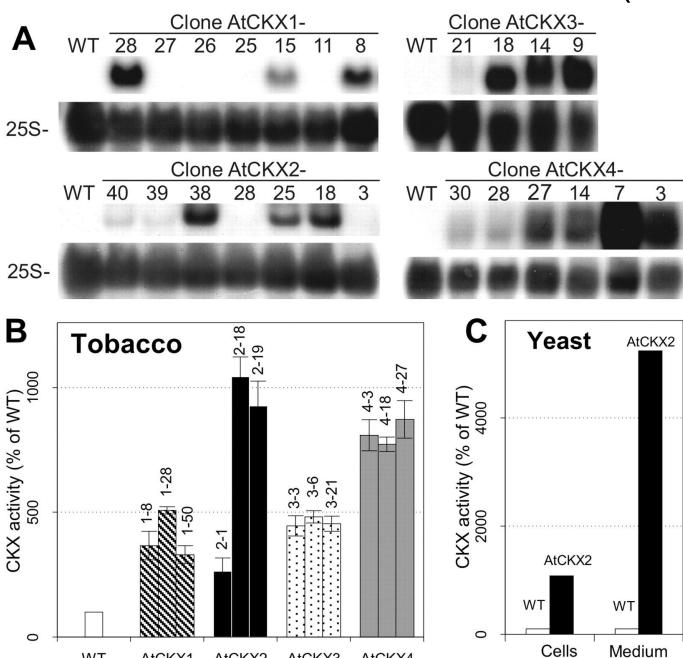
Auxin and cytokinin

roots



shoots

Isolation of CK-oxidase (AtCKX)



WT

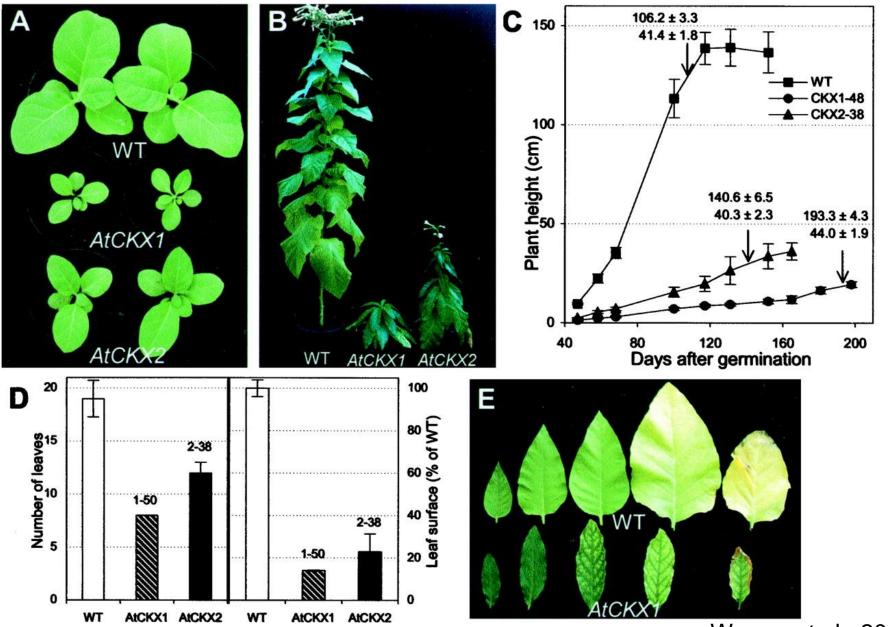
AtCKX1

AtCKX2

AtCKX3

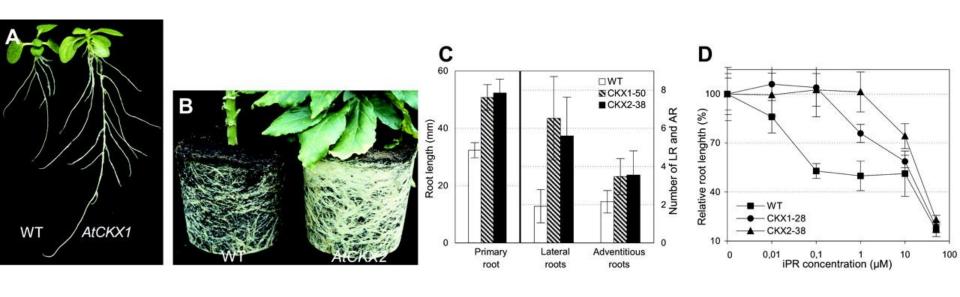
AtCKX4

AtCKXs overexpression in tobacco

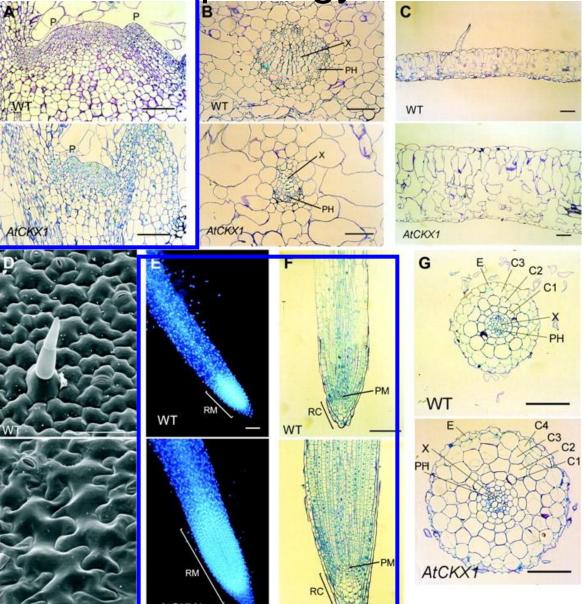


Werner et al., 2001

Effect of AtCKX on tobacco root

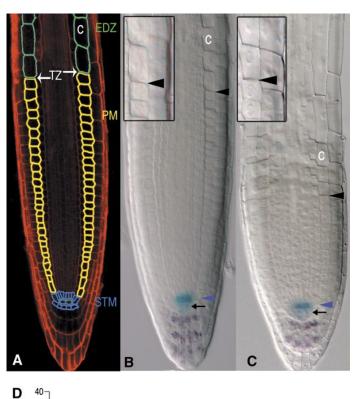


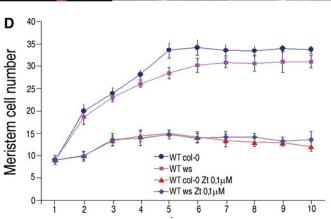
Morphology of AtCKX tobacco plants

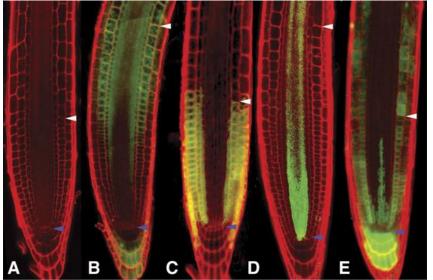


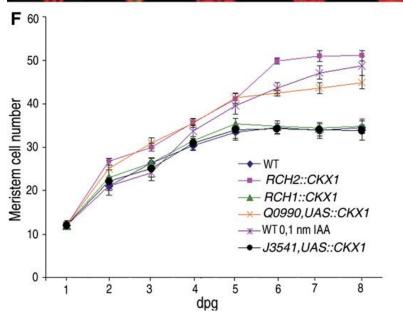
CK in shoot – positive regulato CK in root – negative regulator

Cytokinin – root meristem development

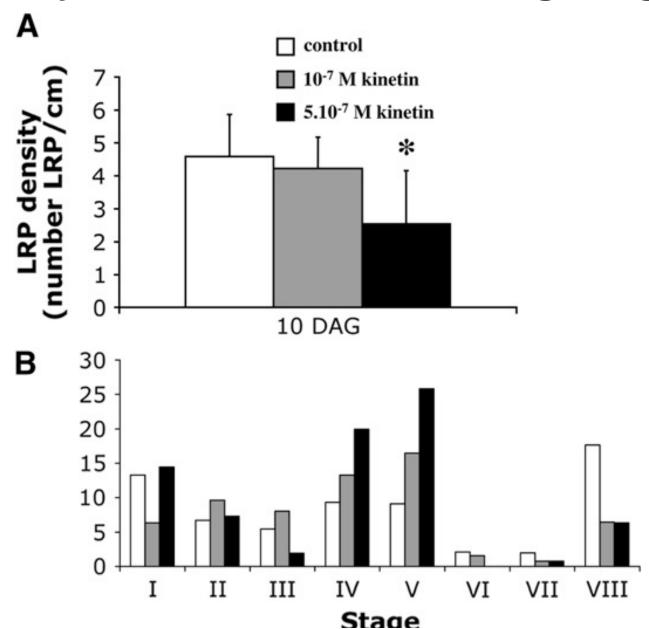






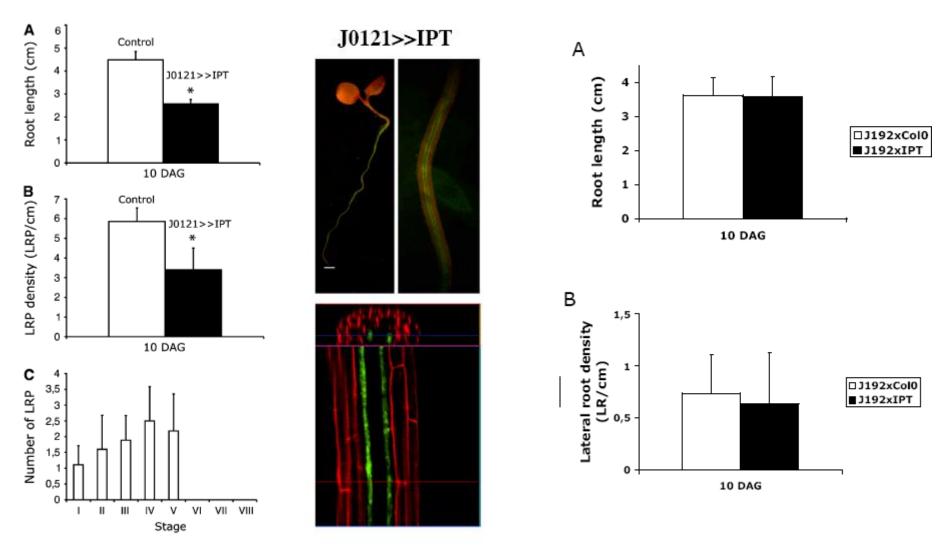


Cytokinin - lateral root organogenesis



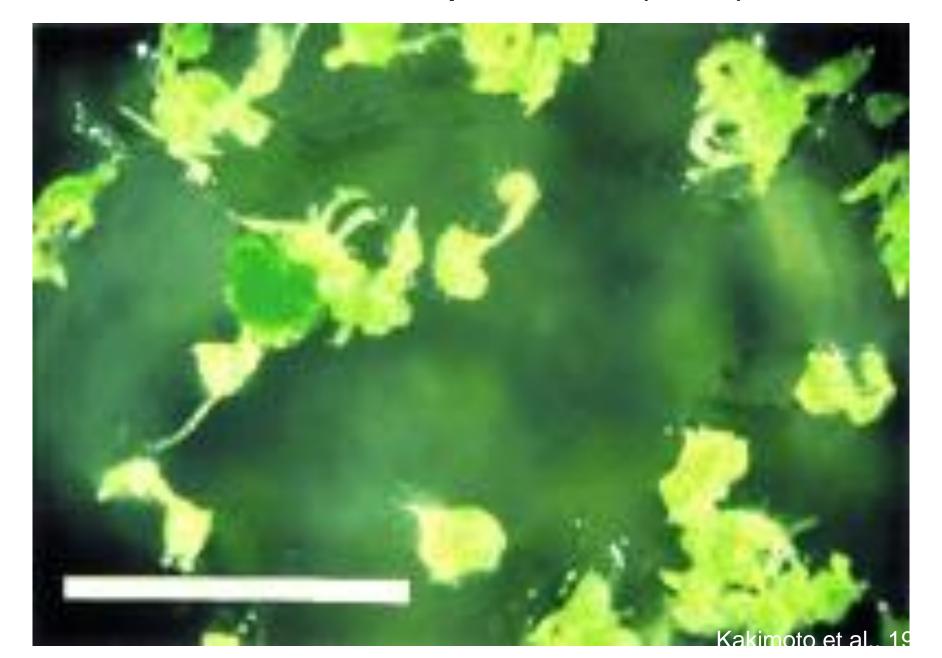
Laplaze et al., 2007

Cytokinin effect is stage specific

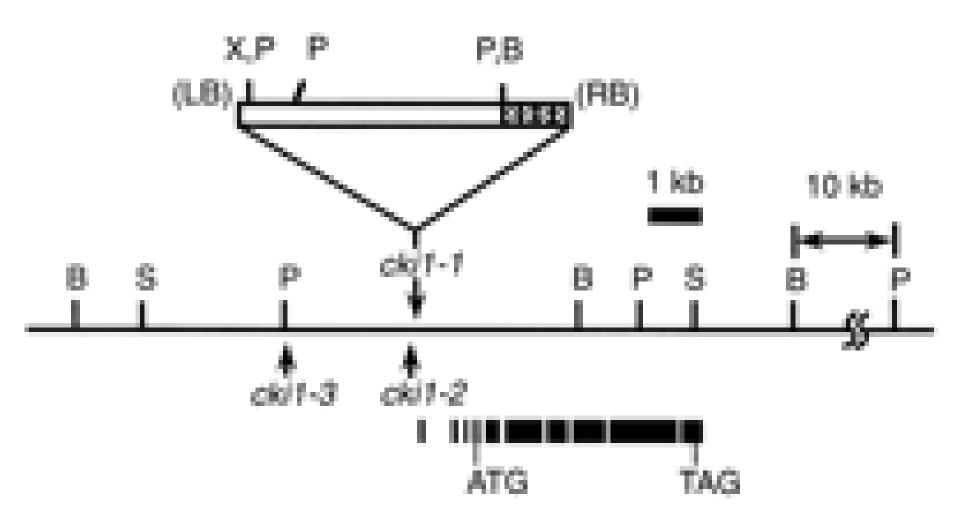


Cytokinin -signal perception and transduction

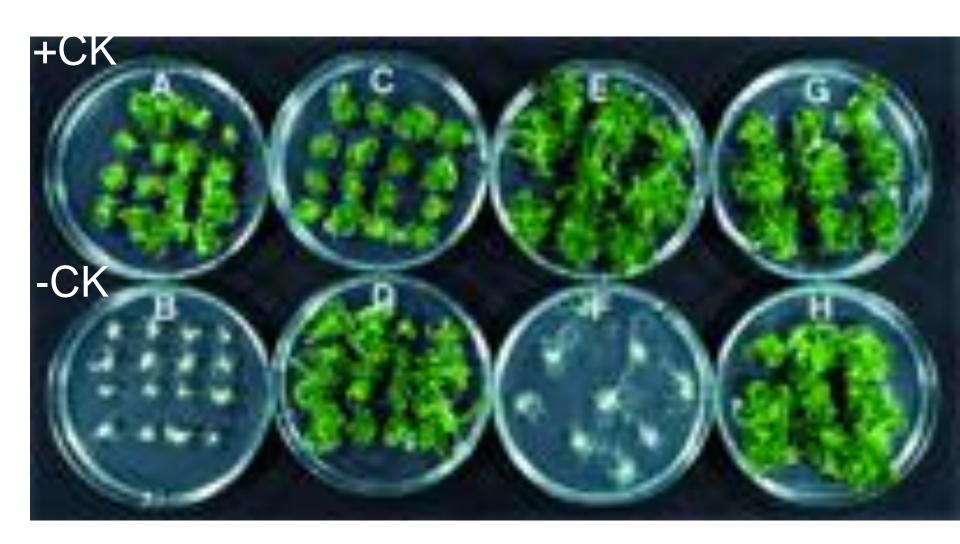
Isolation of CK independent (cki1) mutant

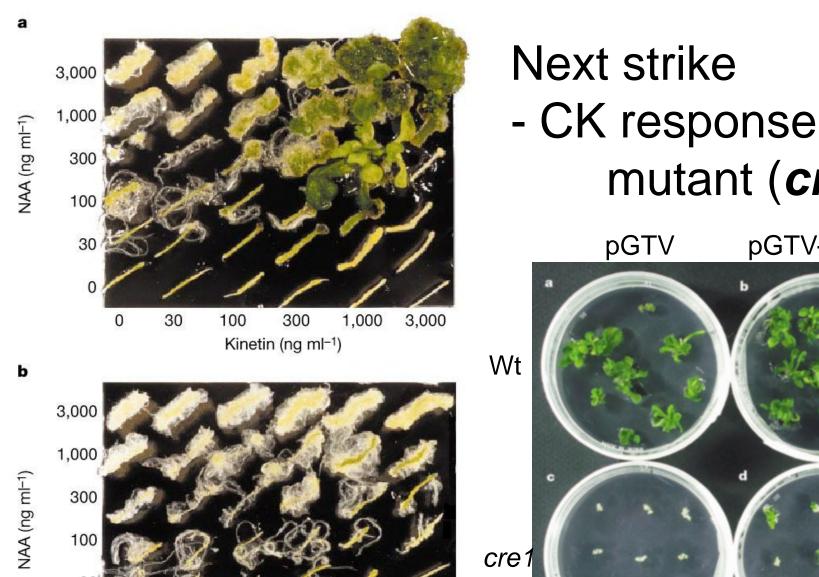


Identification of CKI1 gene



Verification - 35S::CKI1 transgene





1,000

3,000

0

30

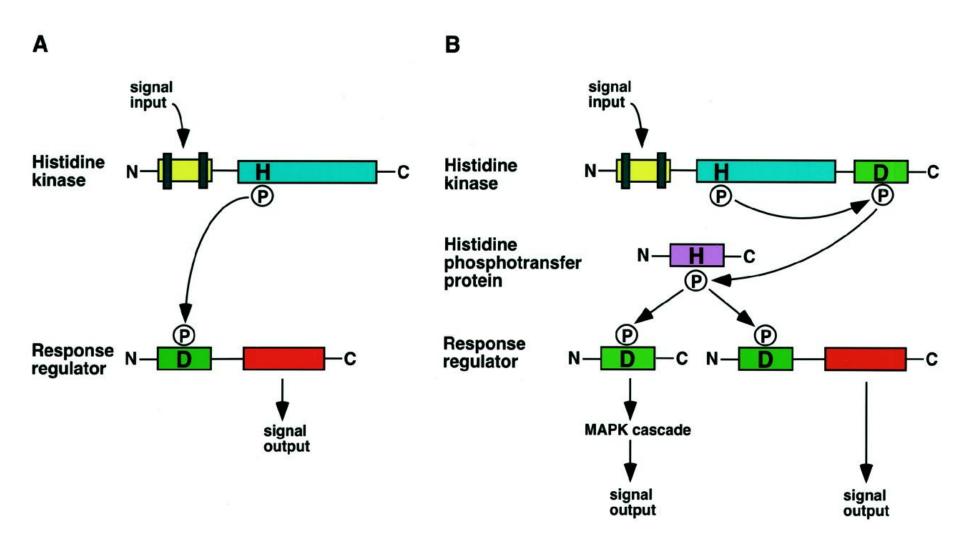
100

300

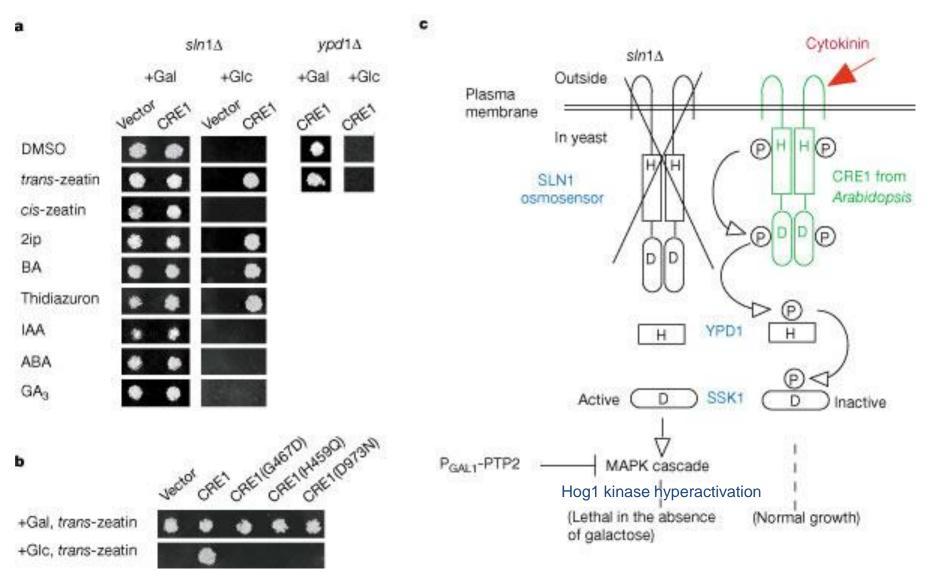
Kinetin (ng ml-1)

mutant (cre1) pGTV-CRE1 pGTV

His kinase transduction pathway



Piece of genius - complementation

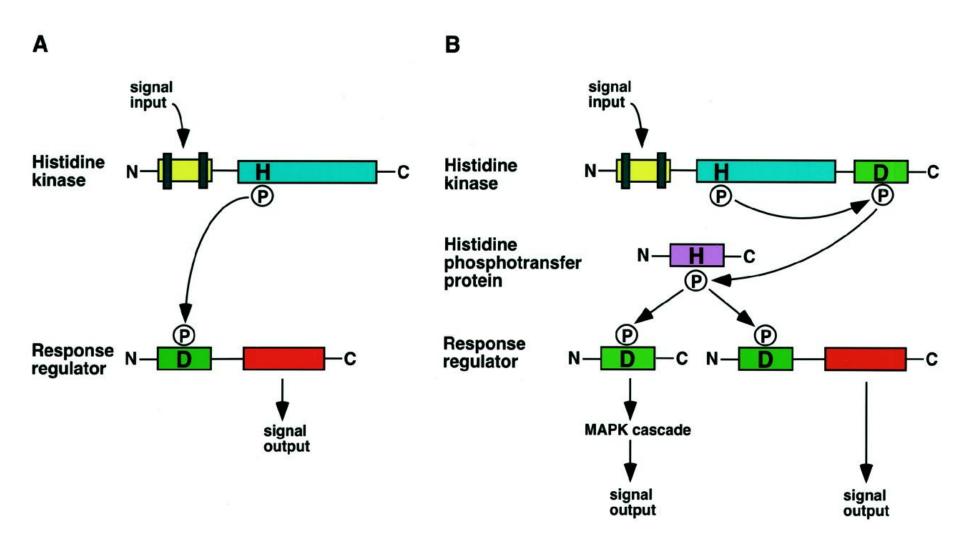


Cytokinin receptors – what else?

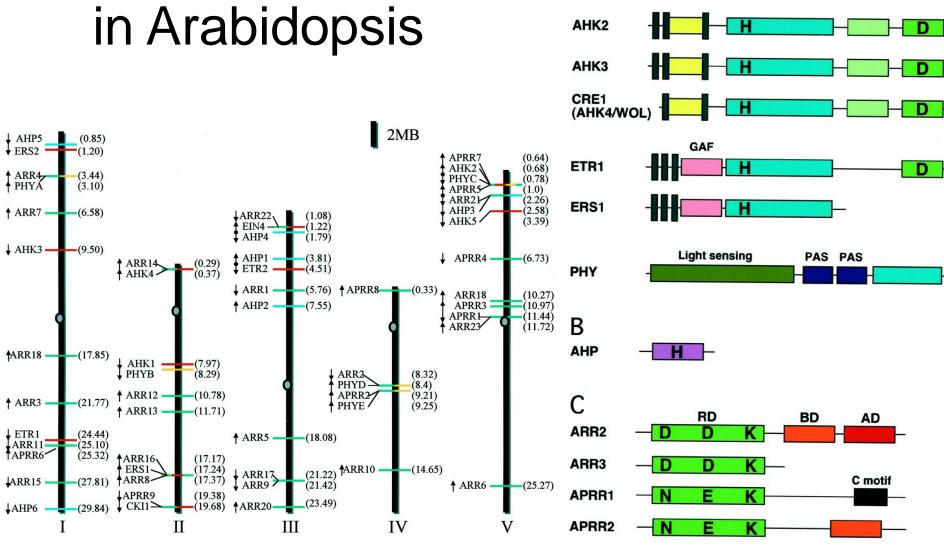
3 CRE1 homologous proteins (AHKs) multiple mutant phenotypes – additive (not lethal)

What does CKI1?

His kinase transduction pathway



His kinase pathway components in Arabidopsis



Α

CKI1

CKI2

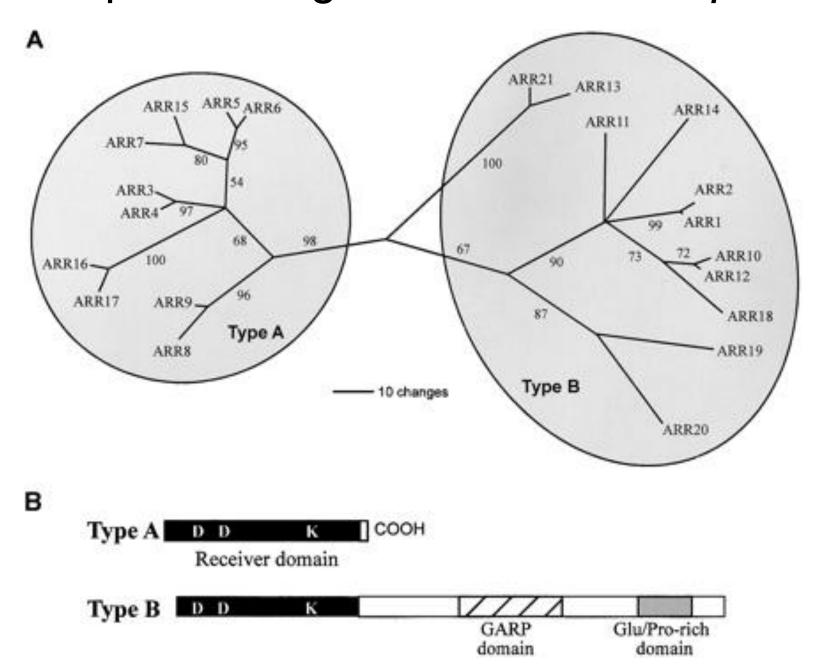
(AHK5) AtHK1 TM ED TM

KD

RLD

RD

Response Regulators in Arabidopsis



CK responsive genes ARR type A Fold induction 12.5 7.5 -10-Fold induction В 15 7.5-10 2.5 -Fold induction 2.5 10 100 1000 100 10 1000 Time (min) Time (min) D CHX C 7.5 3 Fold induction Fold induction 5. 2 -2.5-787 BA CHX CHX

BA

Fold induction

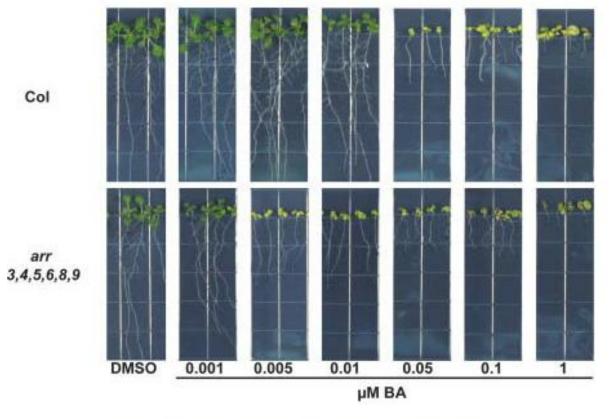
C

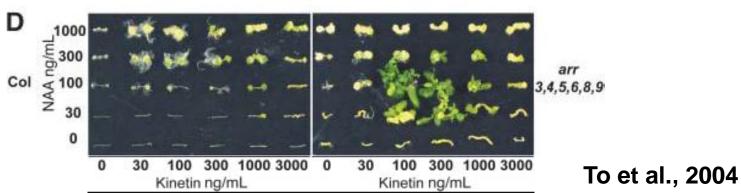
Fold induction

BA

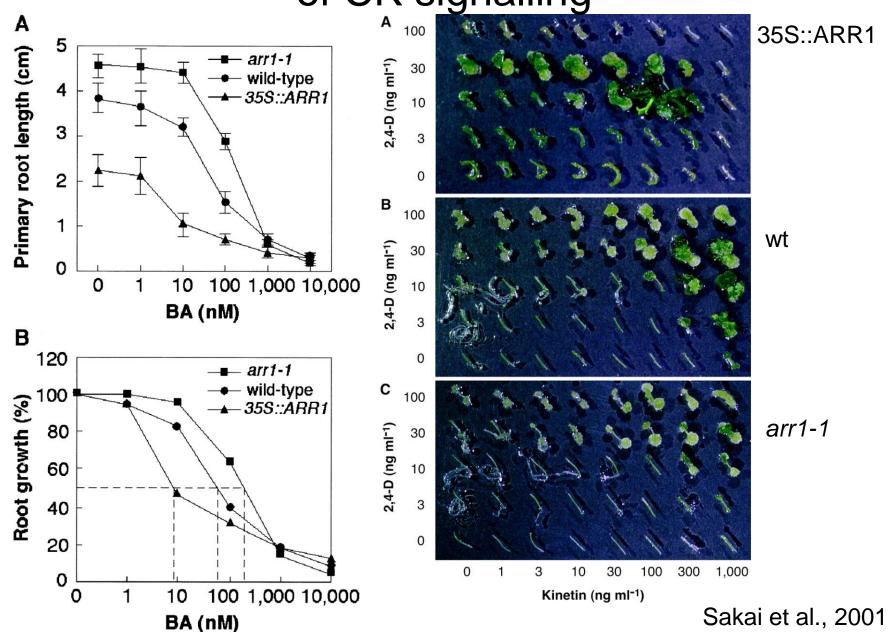
Brandstatter and Kieber, 1999

Response regulator ARR type Anegative regulators of cytokinin signalling

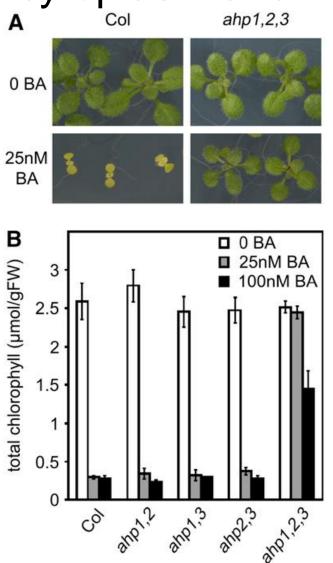




Phenotypes of *arr* type B positive regulator of CK signalling

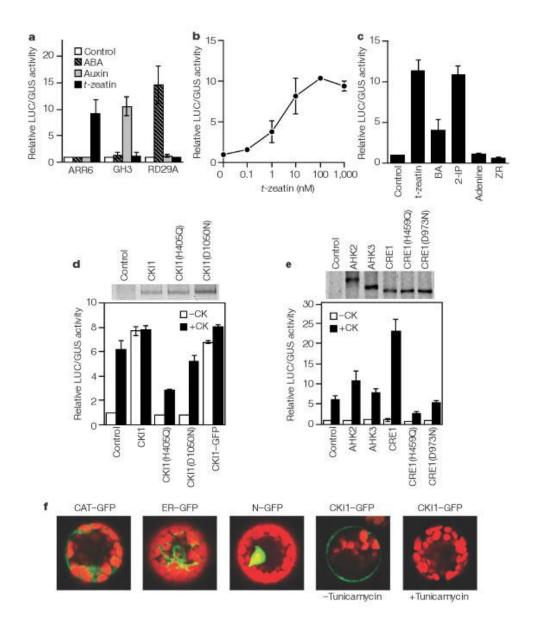


AHPs mediate transfer of cytokinin signal between cytoplasm and nucleus

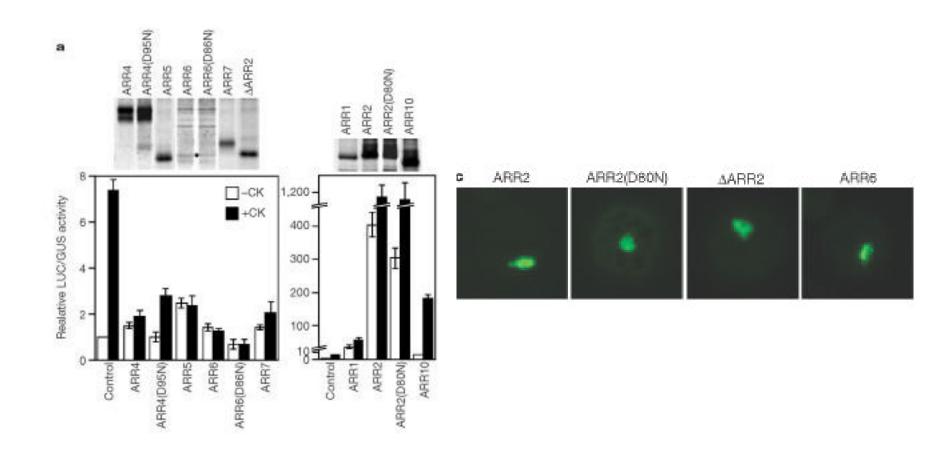


Hutchison et al.,2006

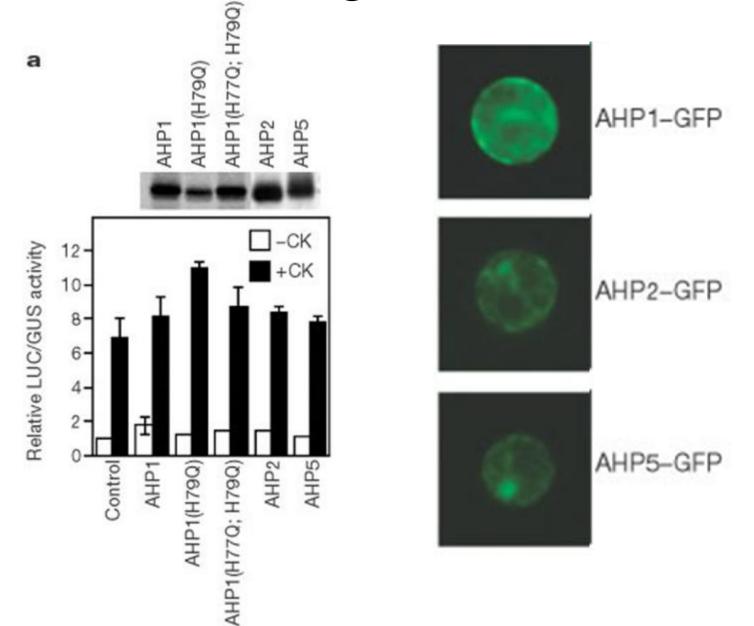
Games with protoplasts

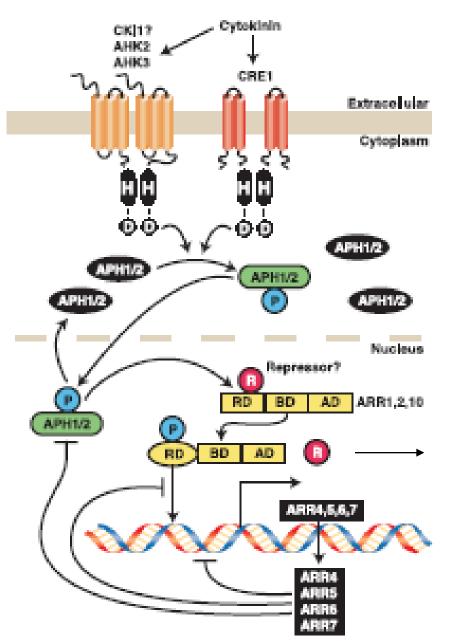


Opposite effects of two classes of ARRs on CK signalling



AHPs – signal to nucleus



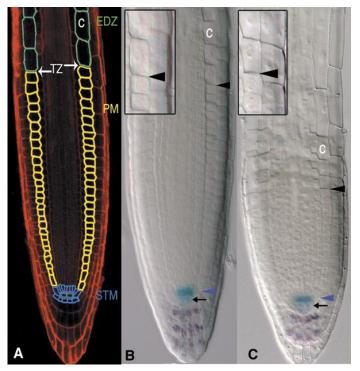


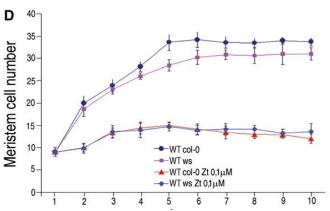
Hextuple of type A ARRs confirms role as negative regulators

e.g. Root elongation assay

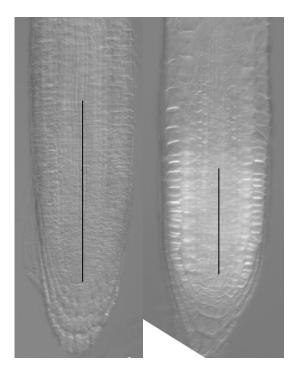
Transcritpion
Cell division
Shoot formation
Delayed senescence
Vascular develppment

Cytokinin – root meristem development

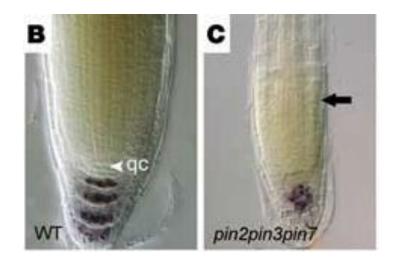




Auxin related mutants with short meristem



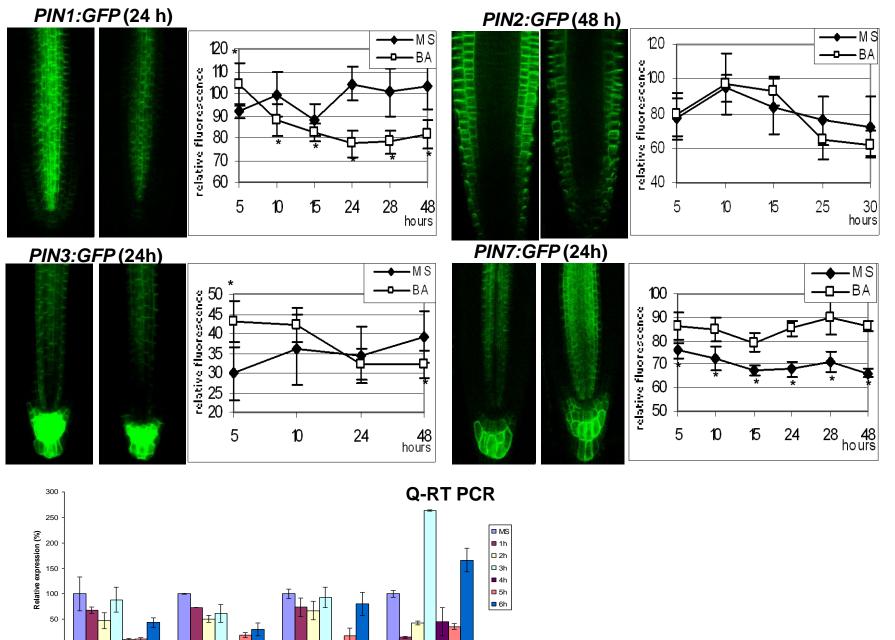
MS cytokinin



Blilou et al.,2005

Auxin transport mutants

Cytokinin modulates auxin transport



PIN7

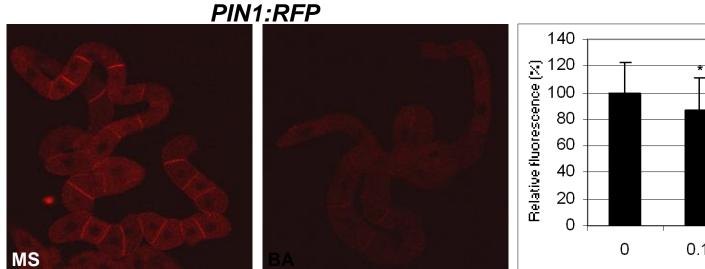
PIN2

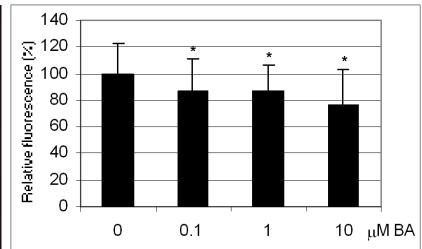
PIN3

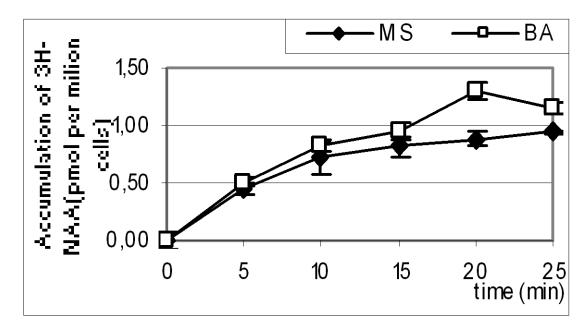
PIN1

Ruzicka et al., 2009

Cytokinin reduces auxin efflux in tobacco BY2 cells

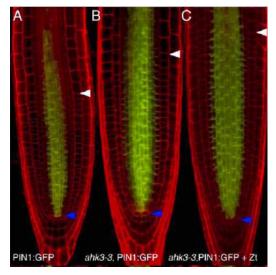




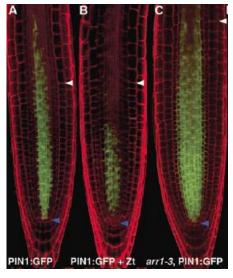


Ruzicka et al., 2009

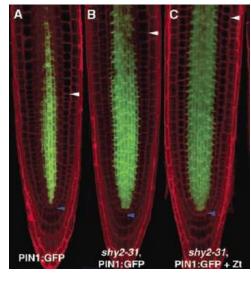
CK – auxin signalling pathways interaction to modulate auxin transport



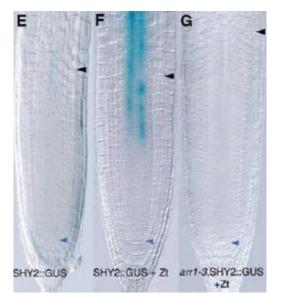
CK receptor



CK response regulator



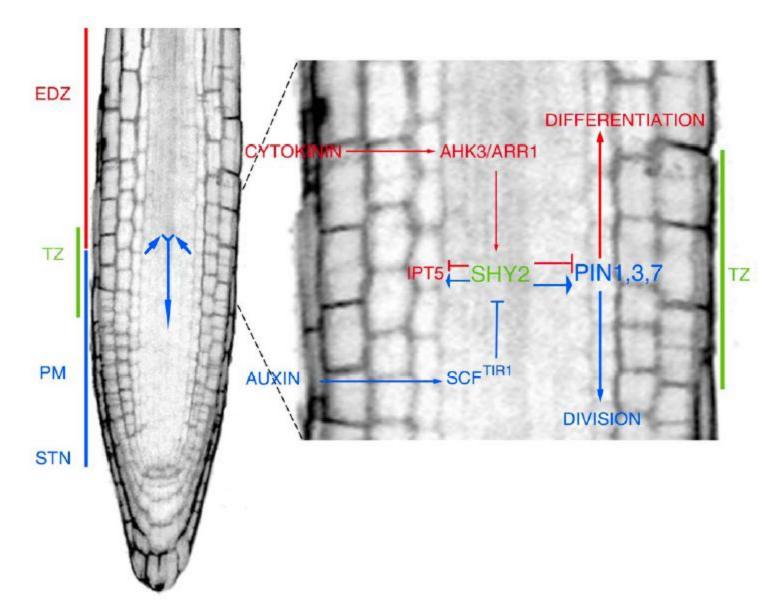
AUX/IAA negative regulator



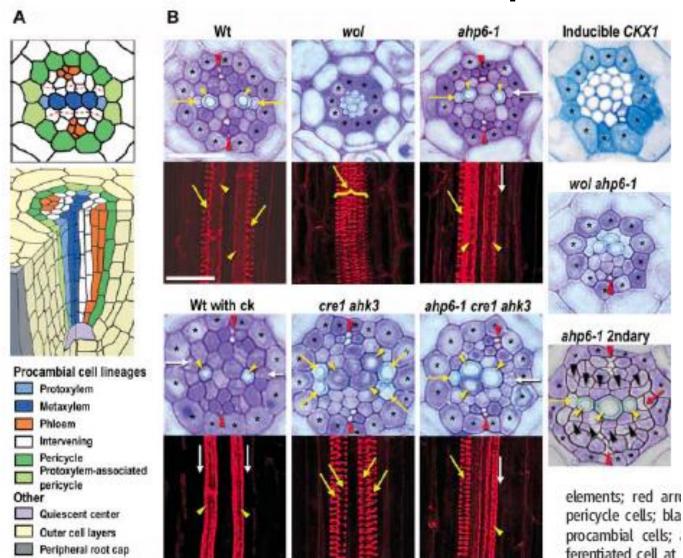
Expression of IAA3/SHY2 is CK regulated

Dello loio et al., 2008

Model of cytokinin and auxin interaction in root meristem

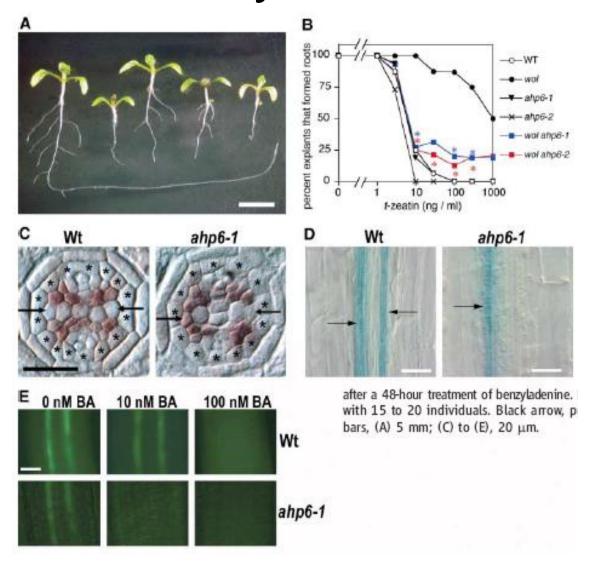


Cytokinin signalling regulates vascular development

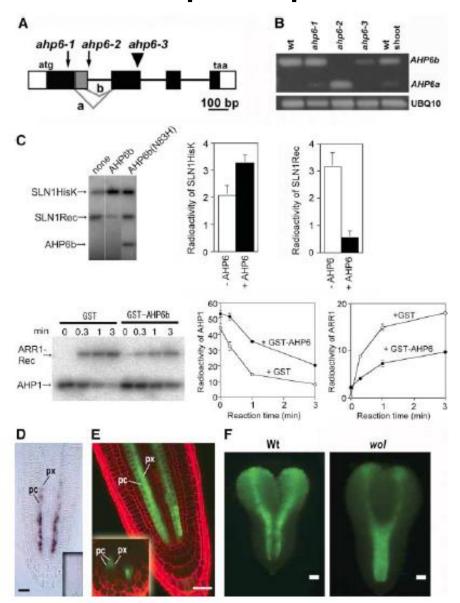


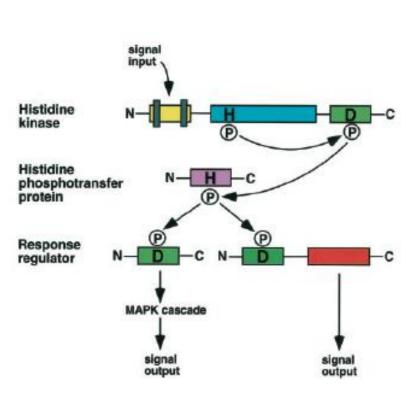
ferentiated cell at Mahonen et al., 2006

ahp6 suppress wol insensitivity to cytokinin



AHP6 codes for histidine phosphotransfer protein 6





AHP6 is negative regulator of cytokinin signalling

