

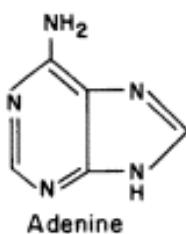
Role of cytokinin in plant development

Cytokinin – substance crucial for sustain of cell proliferation

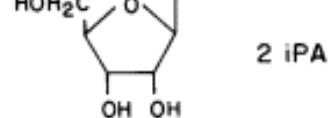
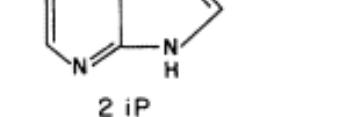
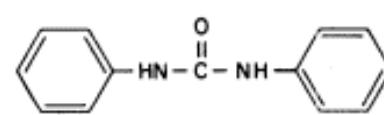
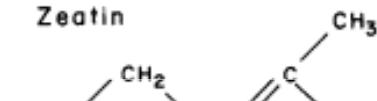
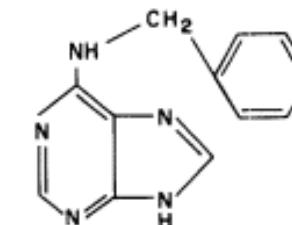
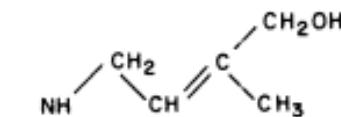
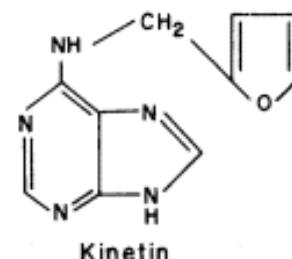


Cytokinins

Native



Synthetic



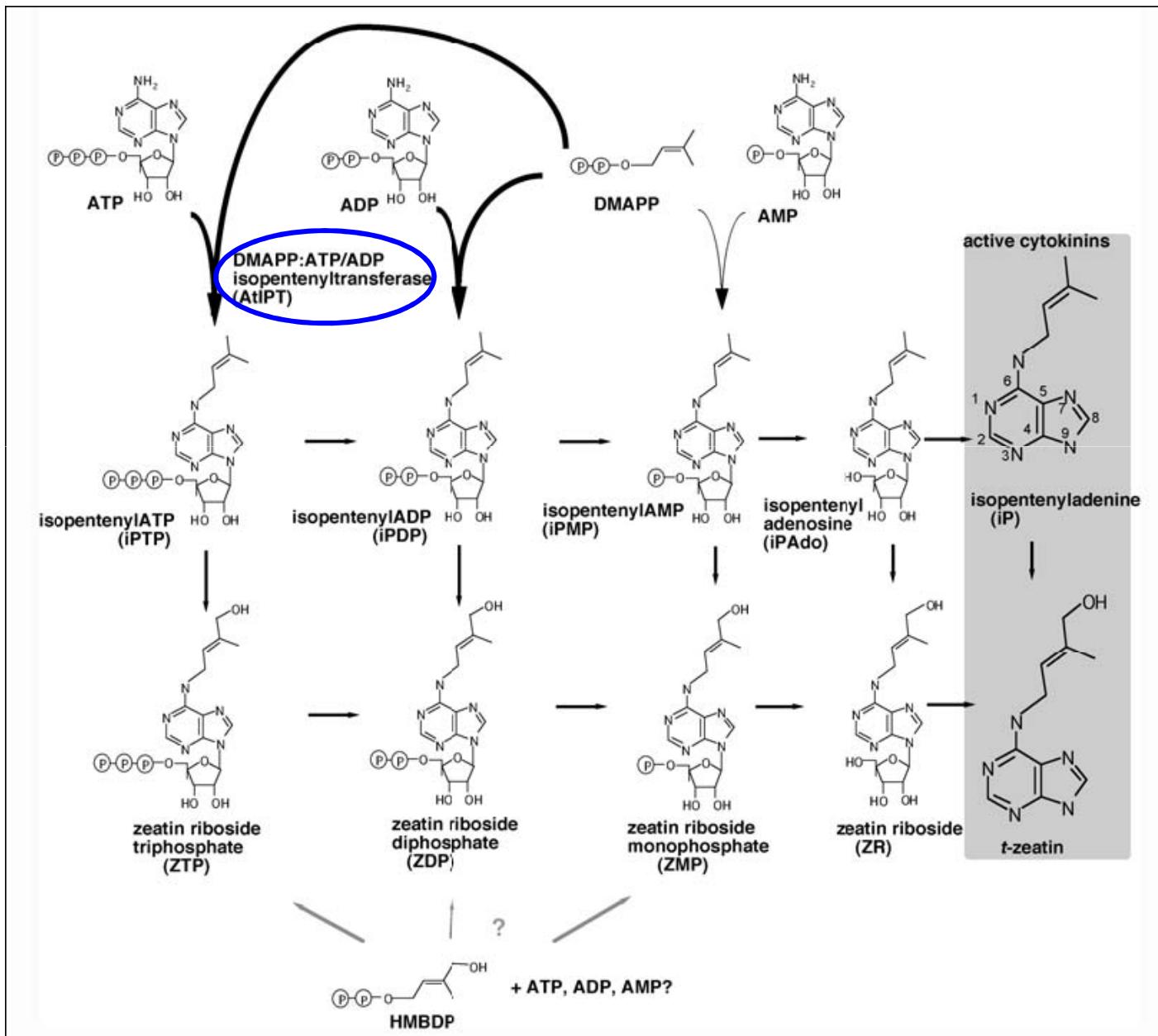
CYTOKININ - Metabolism

Synthesis – *IPT* genes

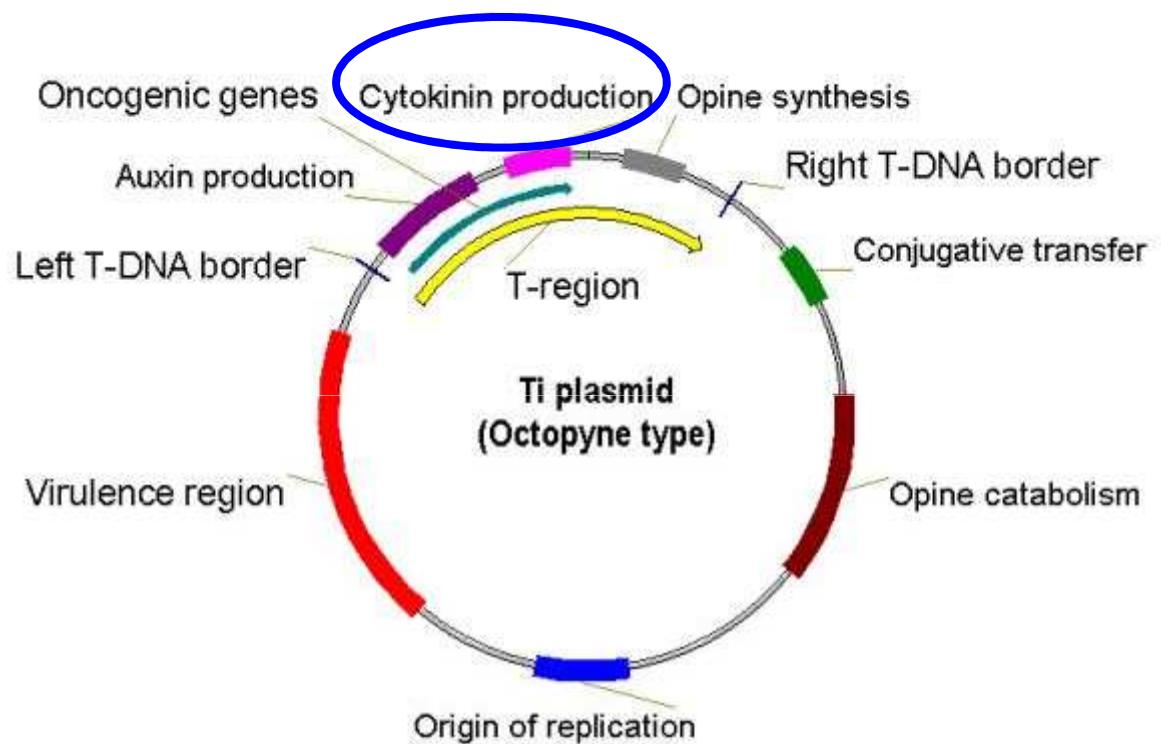
Degradation – CK-oxidase

Conjugation

Cytokinin biosynthesis



Agrobacterium tumefaciens – *IPT* gene for cytokinin biosynthesis



Isolation of *Arabidopsis* *IPTs*

A

Region a

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AtIPT1  1 MTELNFHLLPIISDRFTTTTSPGSFHSQG-----LLEPPVGRKQHDFYVSEIHRGSRPRLRQWVWVIAK
AtIPT2  1 M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M
AtIPT3  1 MIMKISMAMCKQPPPSPTLDFPPARFGPNMLTLN-PYGRDKEVVFVNGTGTGKES
AtIPT4  1 MKCND-----VVVING-TGTGKES
AtIPT5  1 MRPCMTALRGQVQPLSLNFGQNMVDP-FFRKEKQAVVFNNGTGTGKES
AtIPT6  1 MQQLTLLSPV-----ELVTTKGGS-----LVTTCG-----GAGA-----ELVLT-----TGTGKES
AtIPT7  1 M-----M-----LKVQVQPLLCFKNRSLRVRVNSFLHPEK-----LVT-----TGTGKES
AtIPT8  1 M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M-----M

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AtIPT1  81 D-----A-----S-----E-----L-----C-----V-----C-----V-----C-----V-----C-----V-----C-----C
AtIPT2  36 A-----A-----S-----E-----L-----C-----V-----C-----V-----C-----V-----C-----V-----C-----C
AtIPT3  51 A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----C
AtIPT4  21 A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----C
AtIPT5  49 A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----C
AtIPT6  61 L-----V-----A-----P-----A-----L-----A-----A-----A-----A-----A-----A-----A-----A-----C
AtIPT7  50 A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----A-----C
AtIPT8  59 L-----V-----A-----P-----A-----L-----A-----A-----A-----A-----A-----A-----A-----A-----C

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AtIPT1 160 L-----C-----V-----V-----C-----V-----C-----V-----C-----V-----C-----V-----C-----C-----C
AtIPT2 114 V-----G-----T-----Y-----I-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C
AtIPT3 135 V-----G-----S-----E-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C
AtIPT4 100 L-----G-----S-----E-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C
AtIPT5 120 V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C
AtIPT6 129 V-----C-----V-----I-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C
AtIPT7 138 V-----C-----S-----E-----V-----V-----V-----V-----V-----V-----V-----V-----V-----V-----C

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AtIPT3 143 -----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT4 118 -----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT5 144 -----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT6 158 -----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT7 156 -----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P

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AtIPT3 198 F-----S-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT4 176 S-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT5 157 S-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT6 218 S-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT7 194 P-----A-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT8 213 P-----Y-----S-----V-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P

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AtIPT3 248 P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
AtIPT4 222 C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C
AtIPT5 241 L-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C-----C
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AtIPT7 241 R-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P-----P
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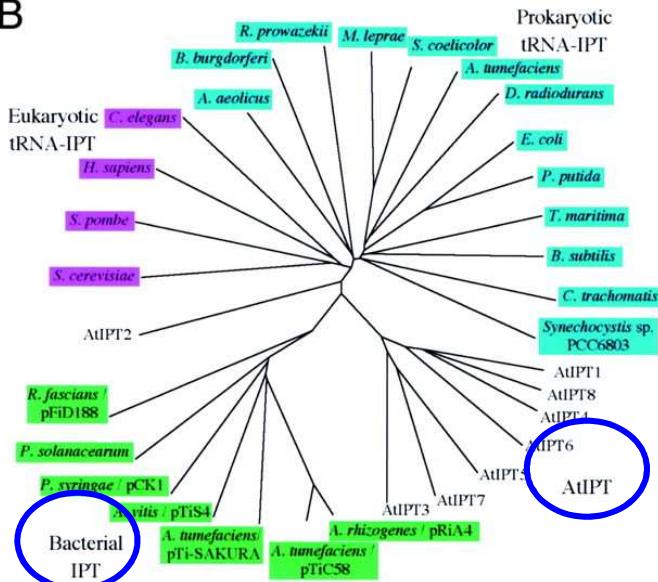
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AtIPT4 311 L-----P-----I-----S-----A-----P-----L-----P-----A-----S-----I-----R-----C-----V-----R-----V
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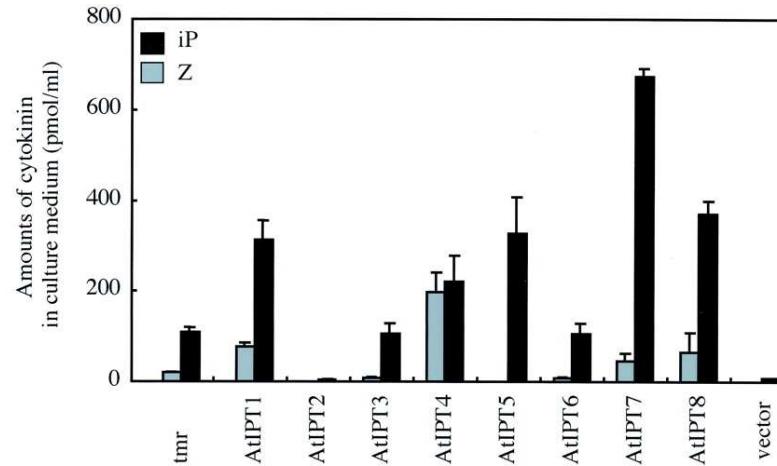
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Region b

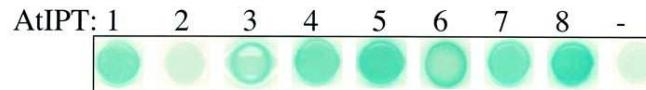
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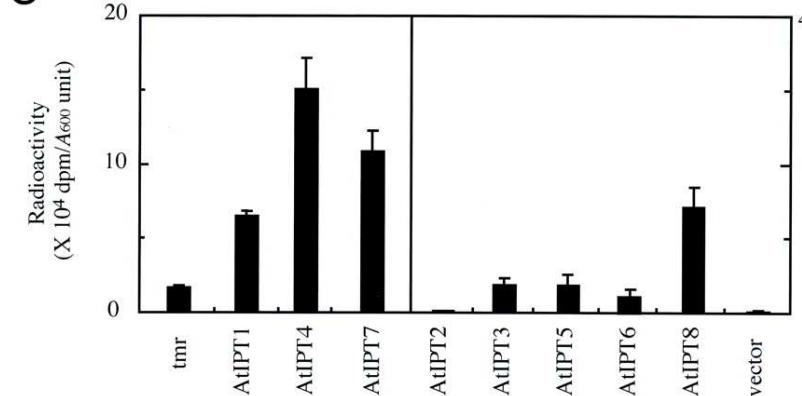
A



B



C



Arabidopsis IPTs (8)

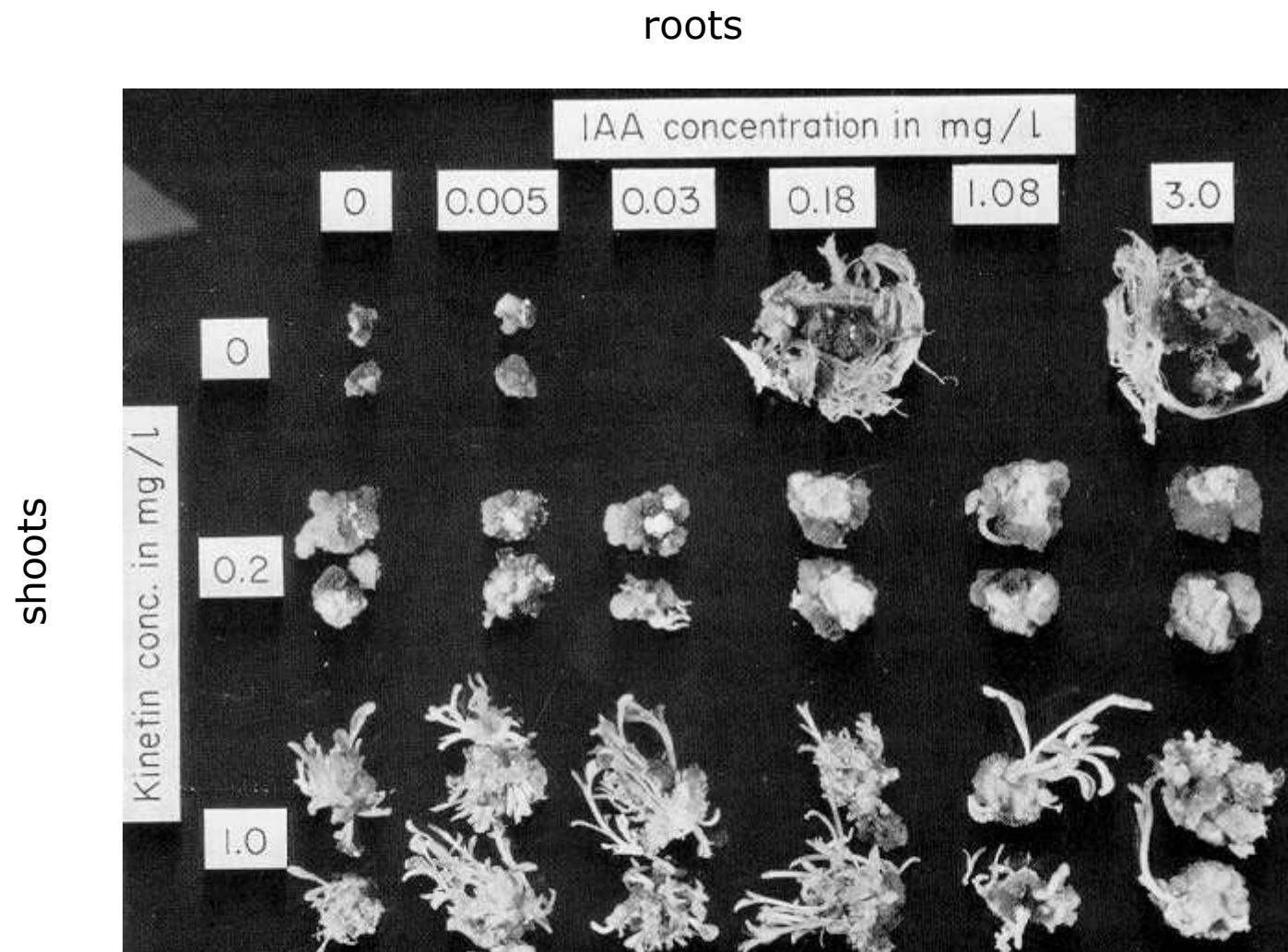
Differential expression patterns

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

mutants, overexpression ???

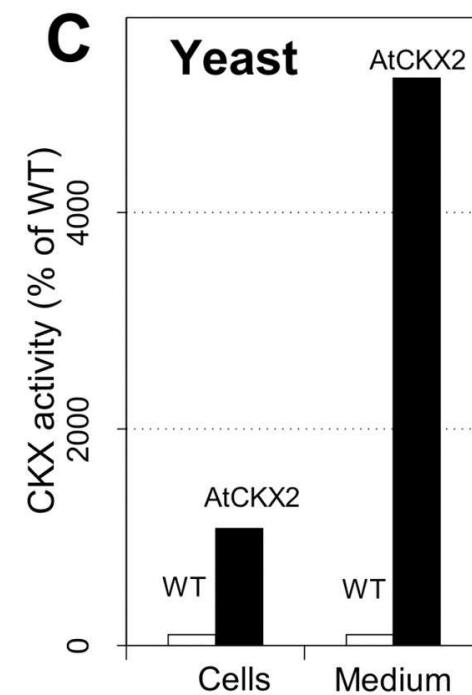
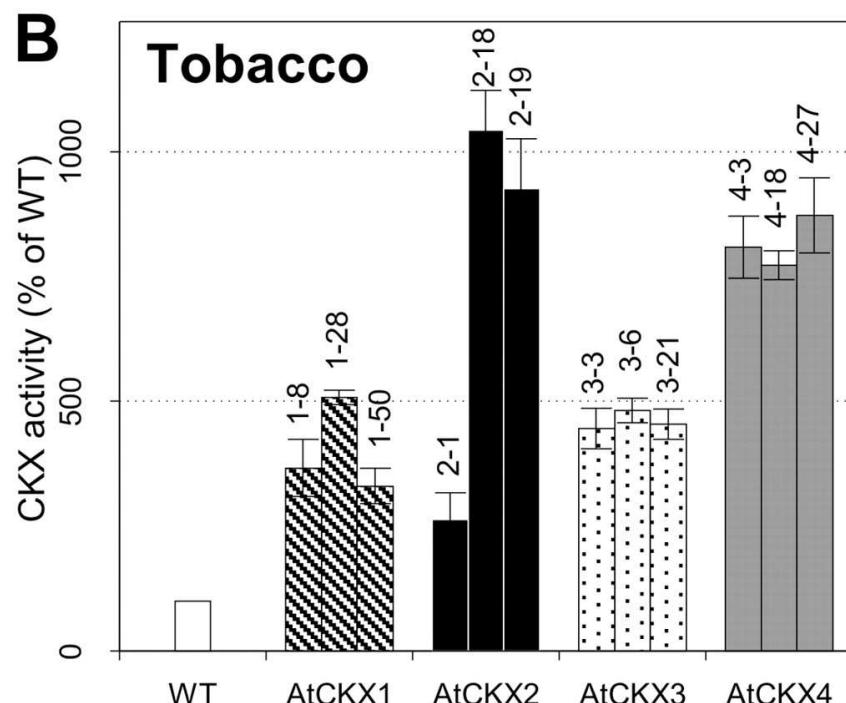
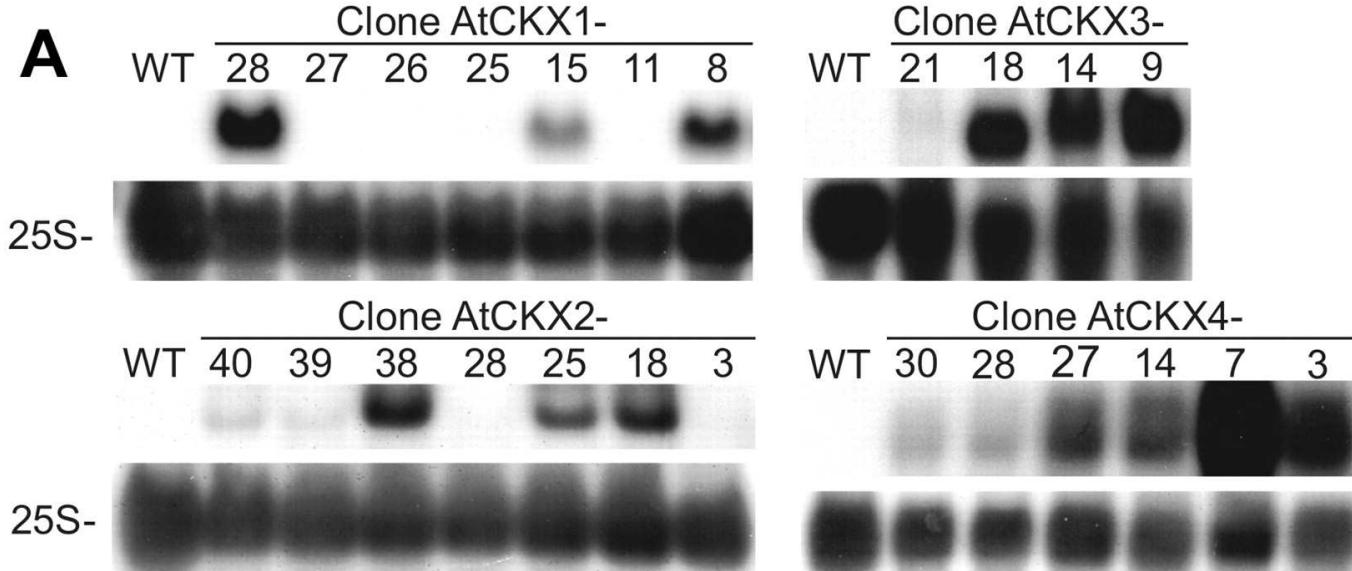
Cytokinin – role in plant development

Auxin and cytokinin



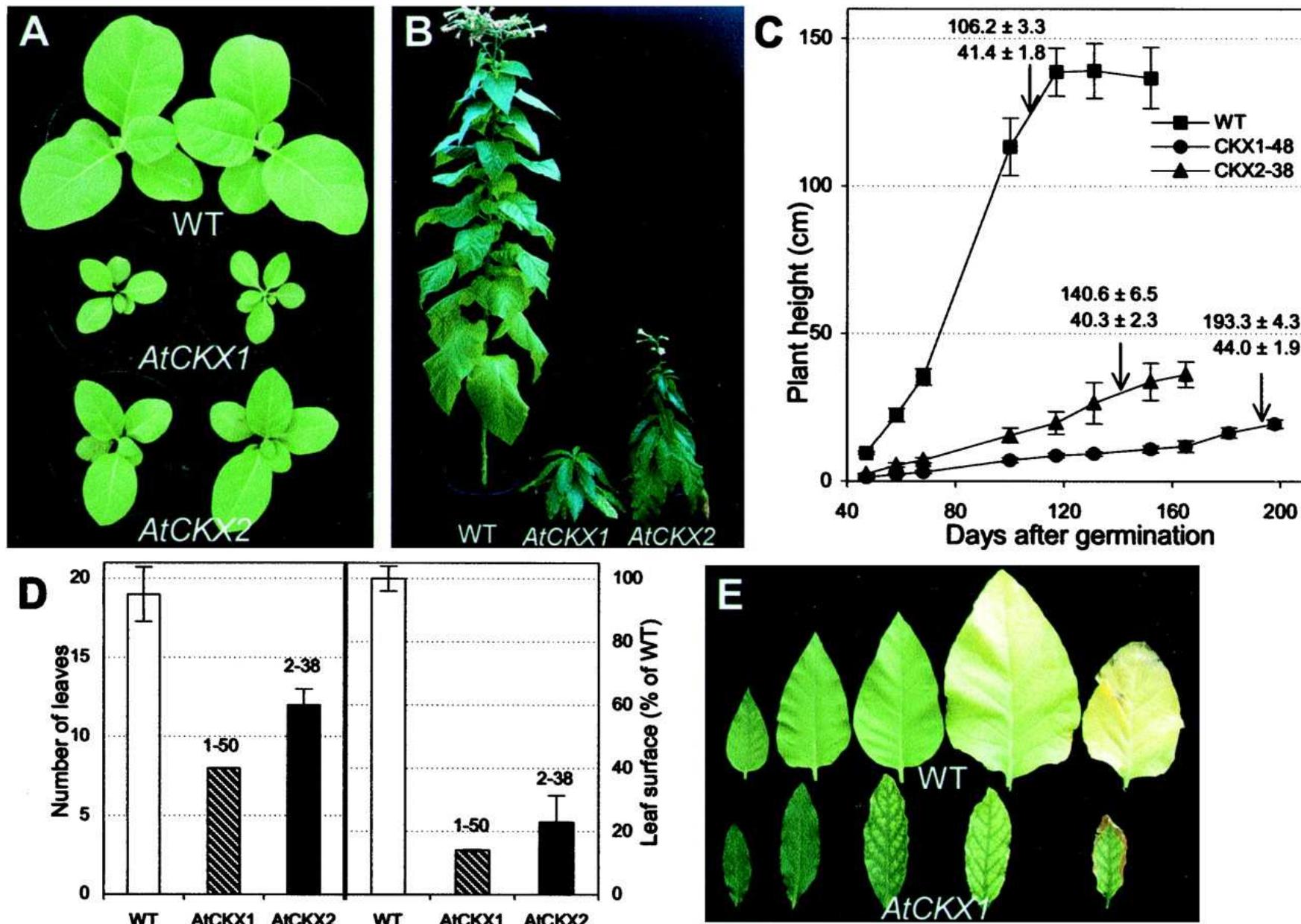
Skoog et Miller, 1957

Isolation of CK-oxidase (AtCKX)



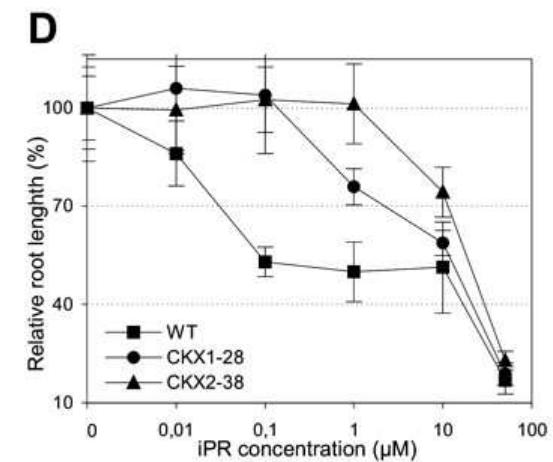
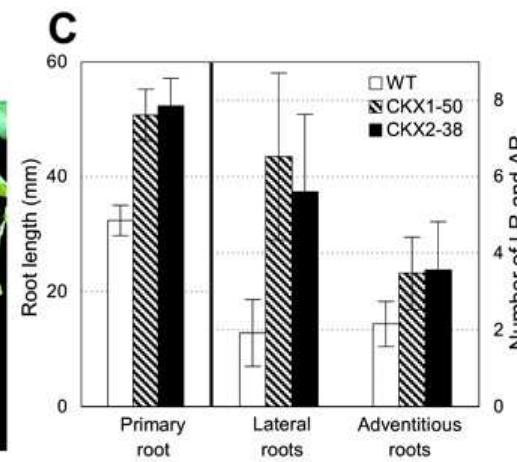
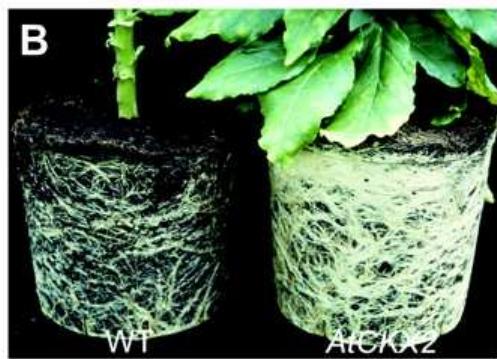
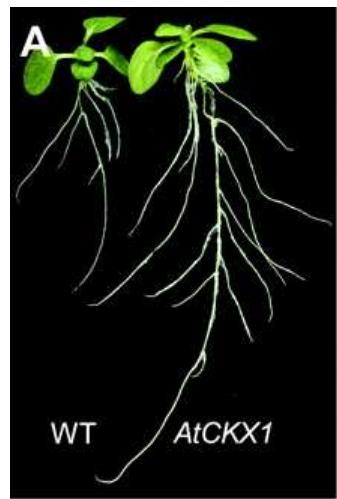
Werner et al., 2001

AtCKXs overexpression in tobacco



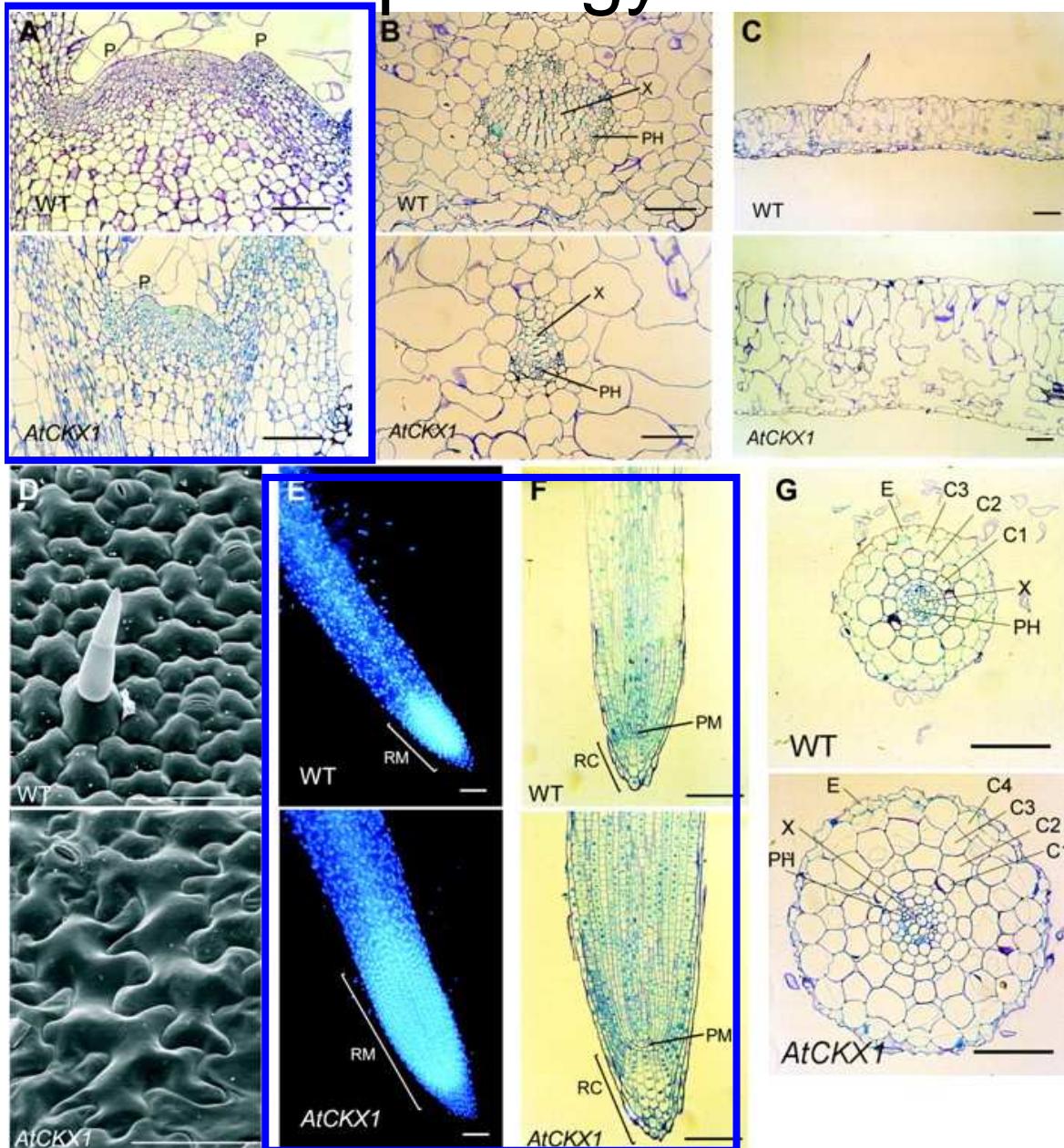
Werner et al., 2001

Effect of AtCKX on tobacco root



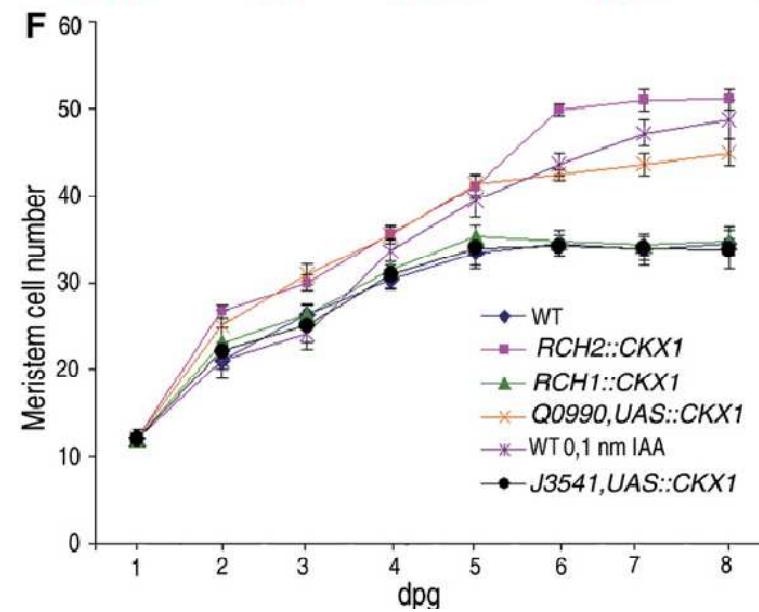
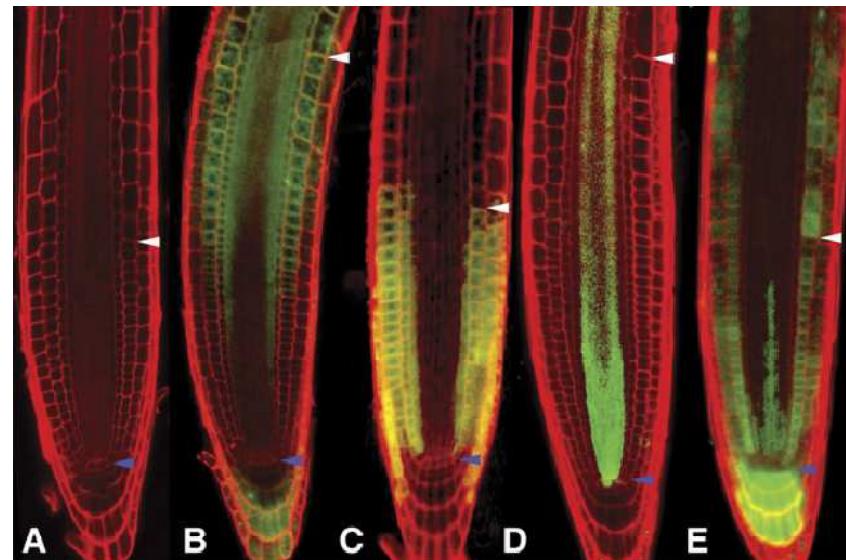
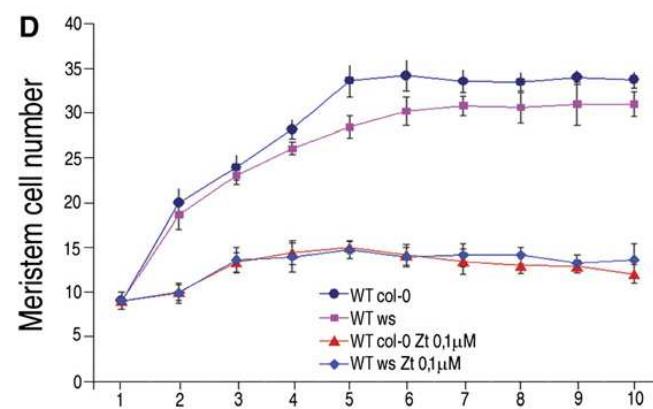
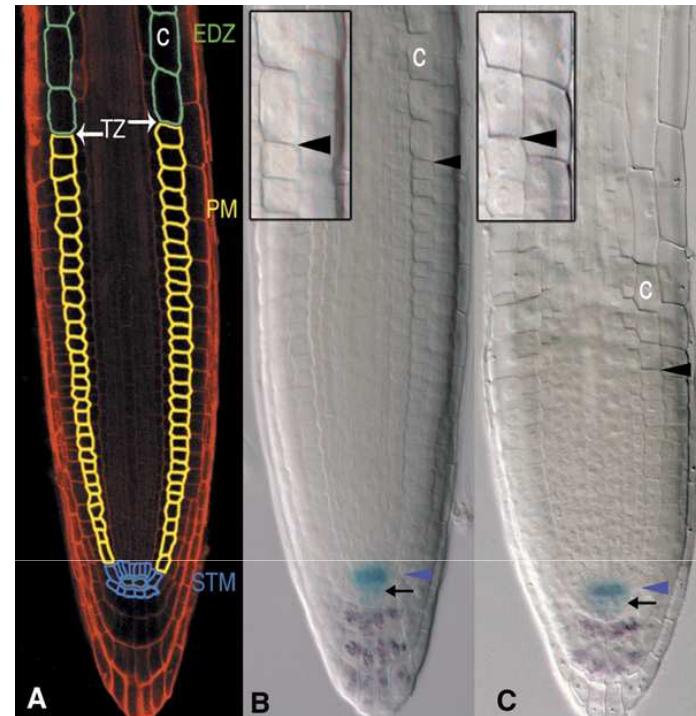
Werner et al., 2001

Morphology of AtCKX tobacco plants



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

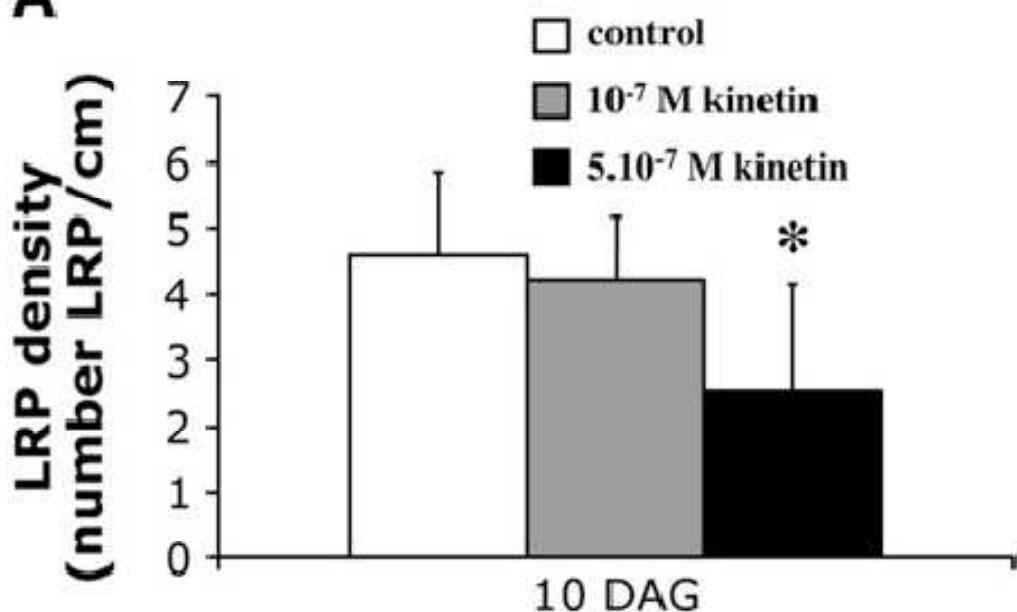
Cytokinin – root meristem development



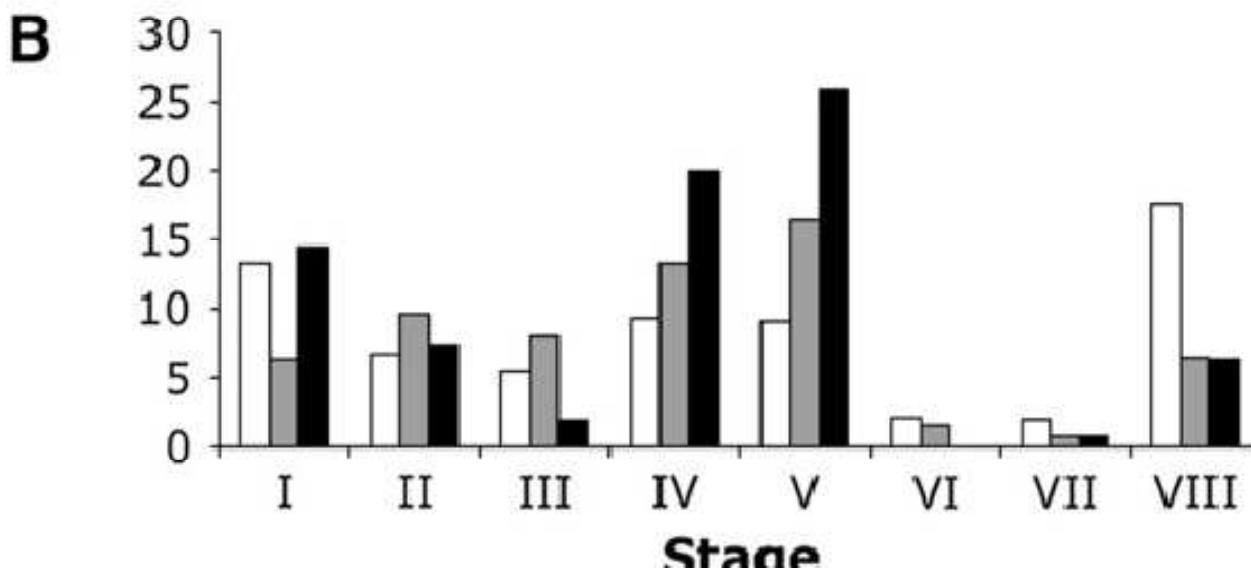
Dello Orio et al., 2007

Cytokinin - lateral root organogenesis

A

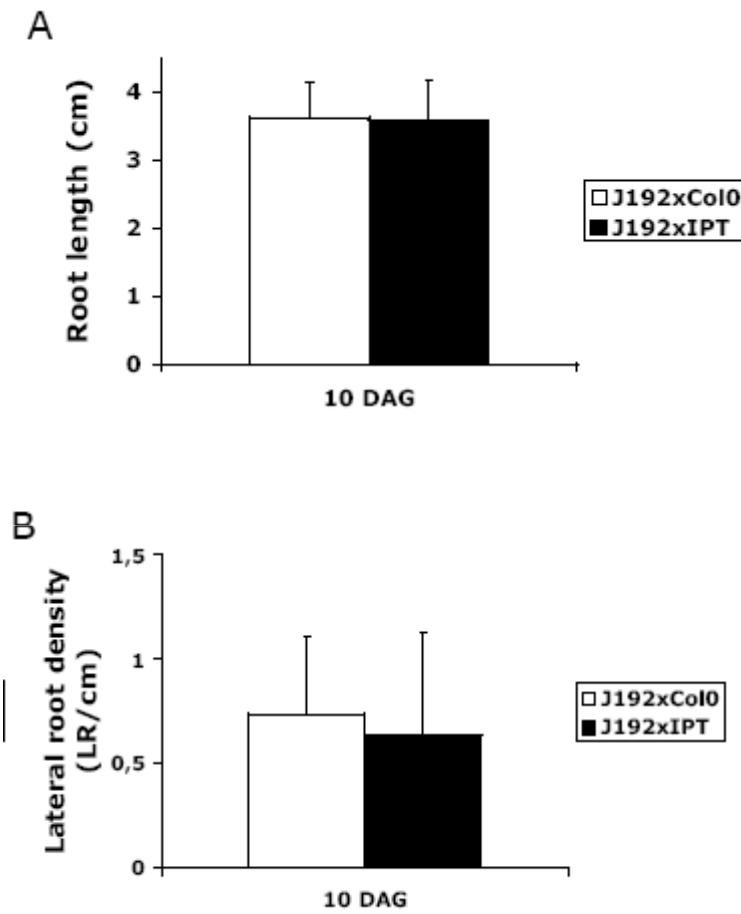
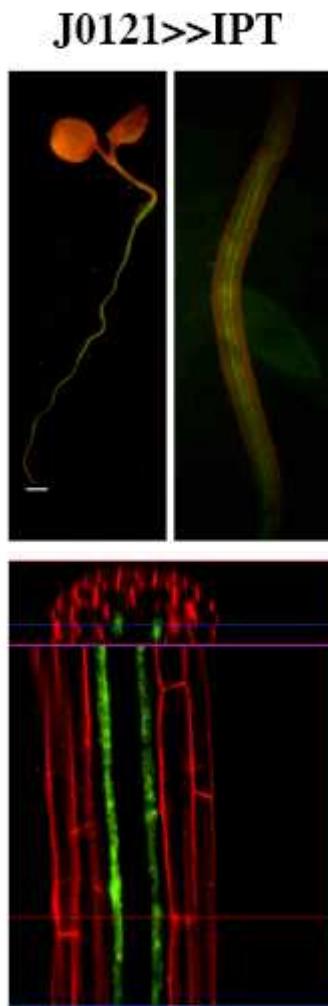
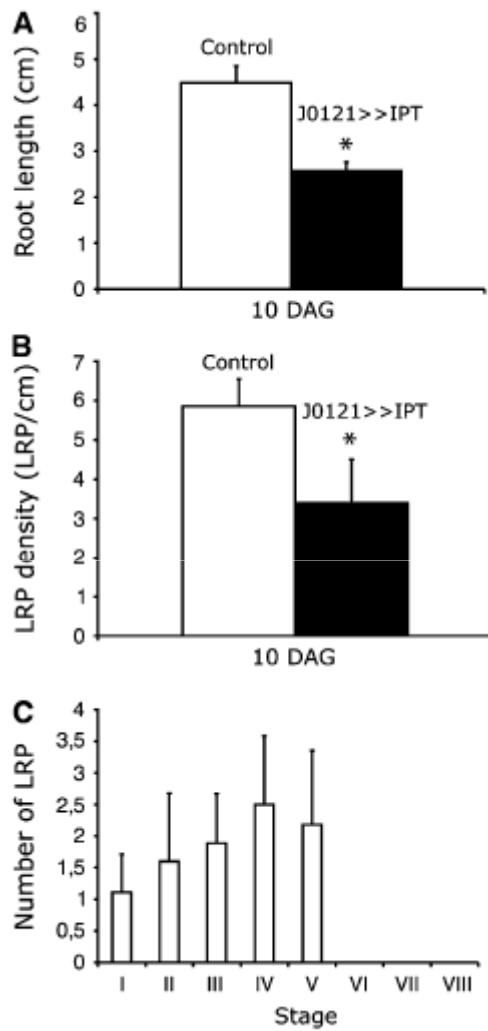


B



Laplace et al., 2007

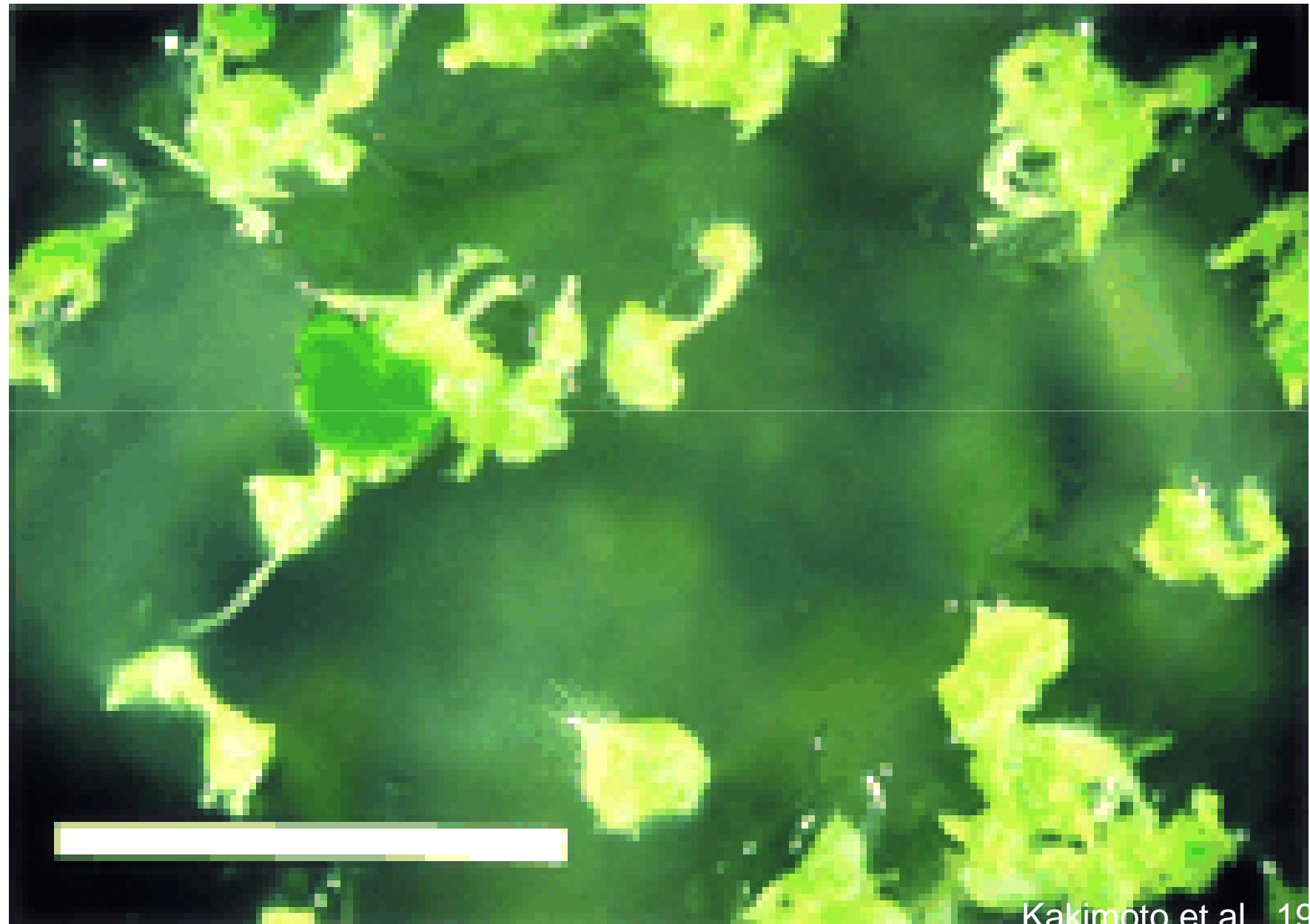
Cytokinin effect is stage specific



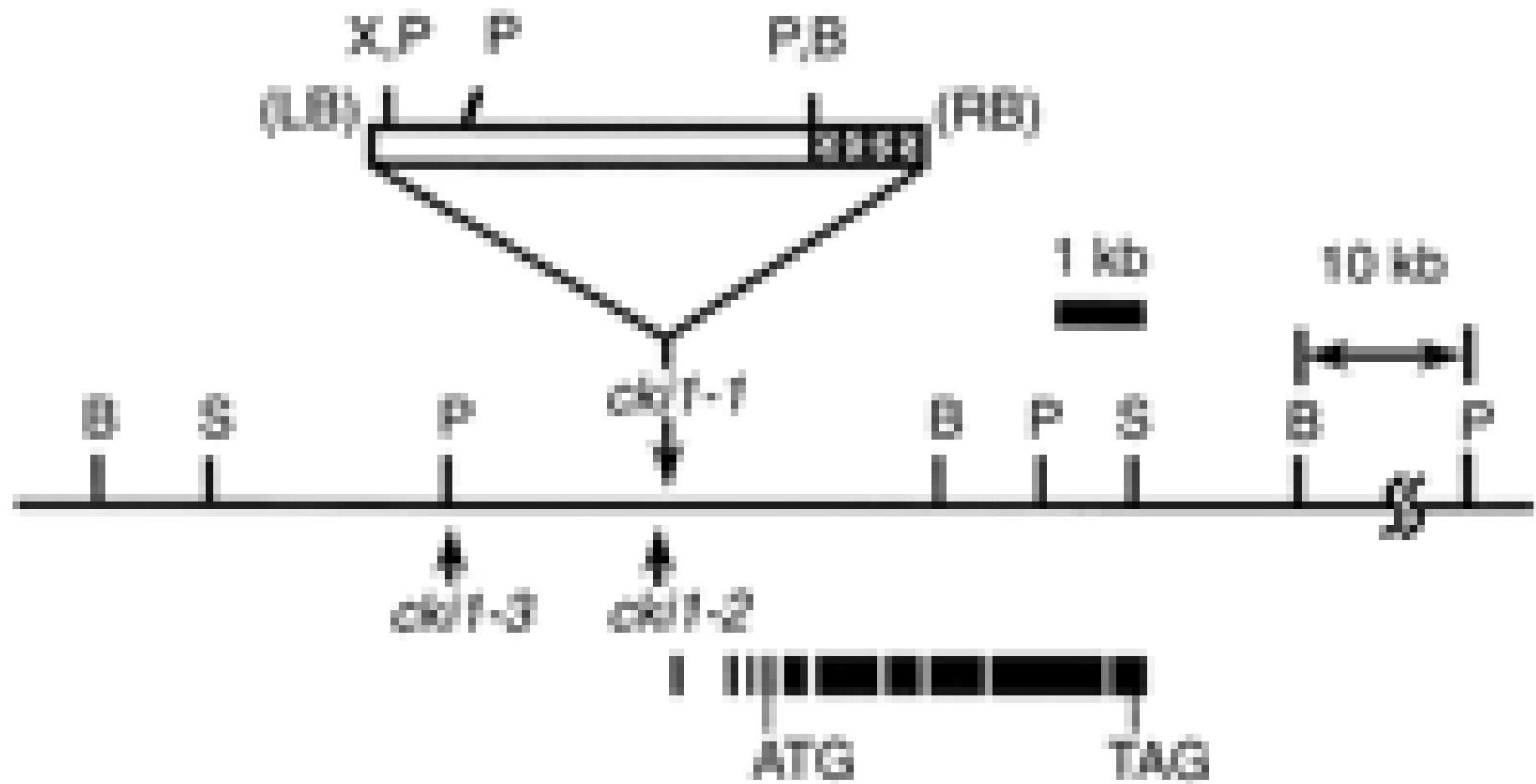
Laplaze et al., 2007

Cytokinin –signal perception and transduction

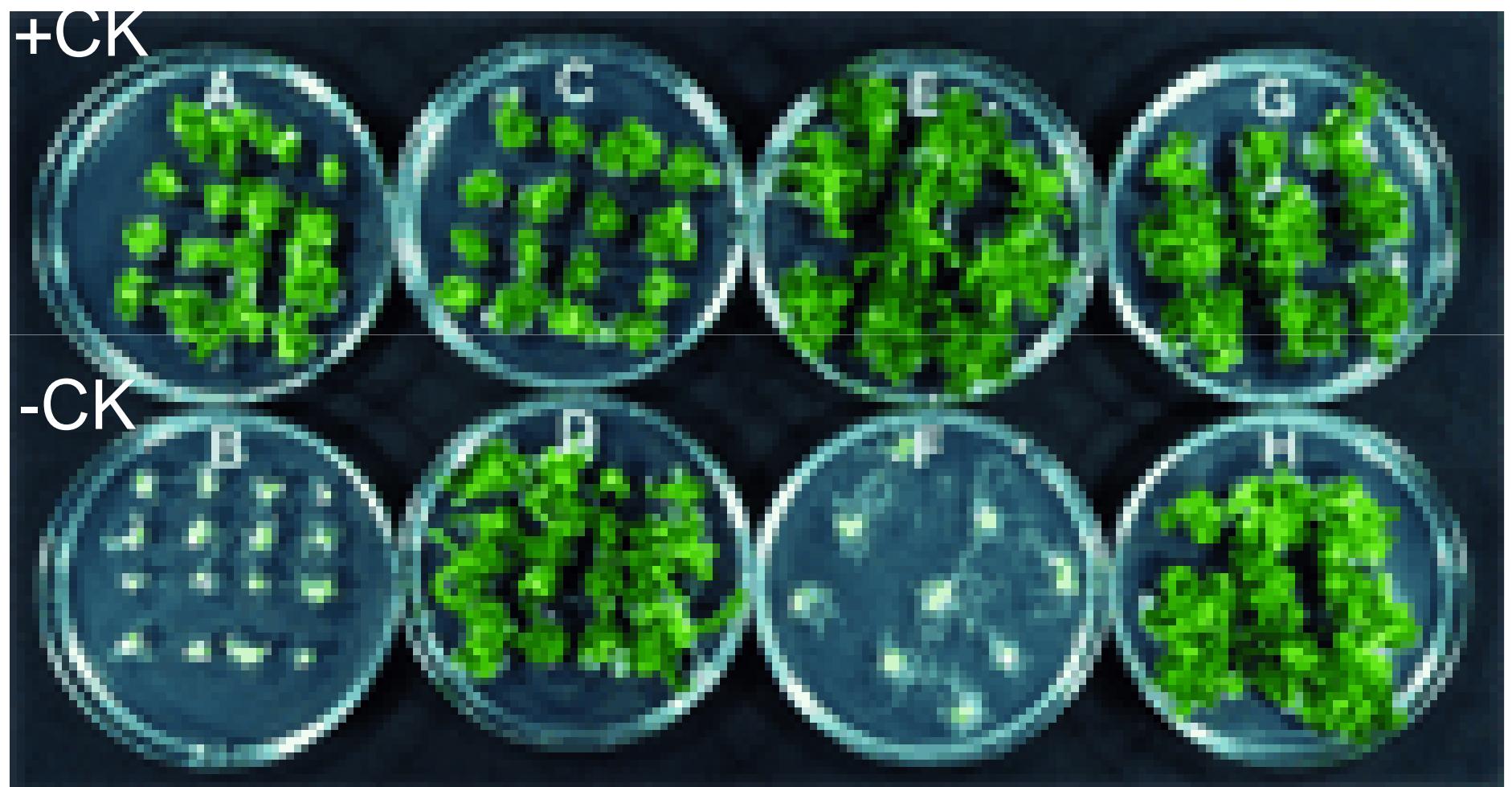
Isolation of CK independent (*cki1*) mutant



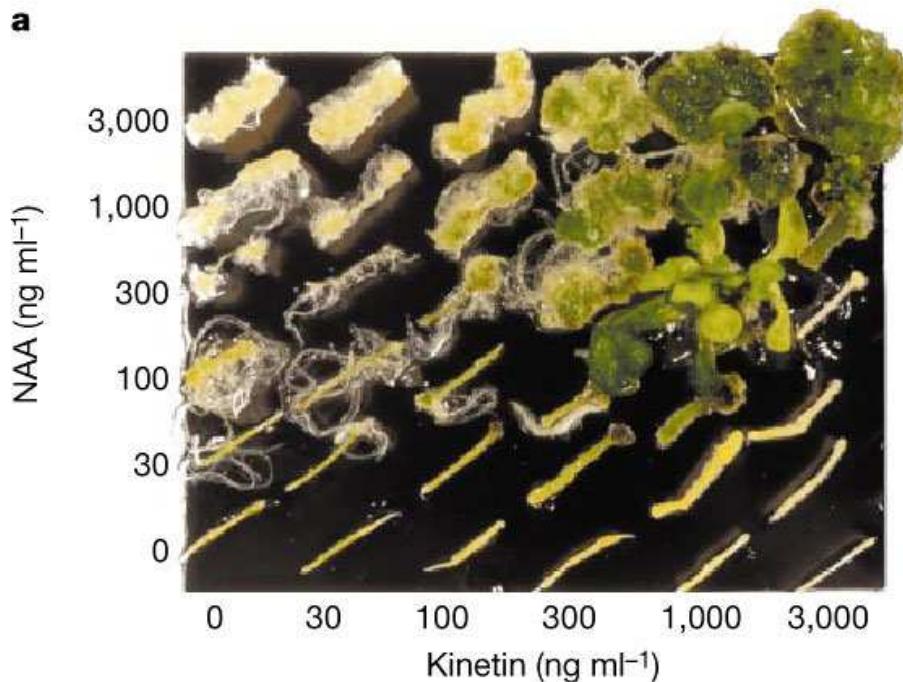
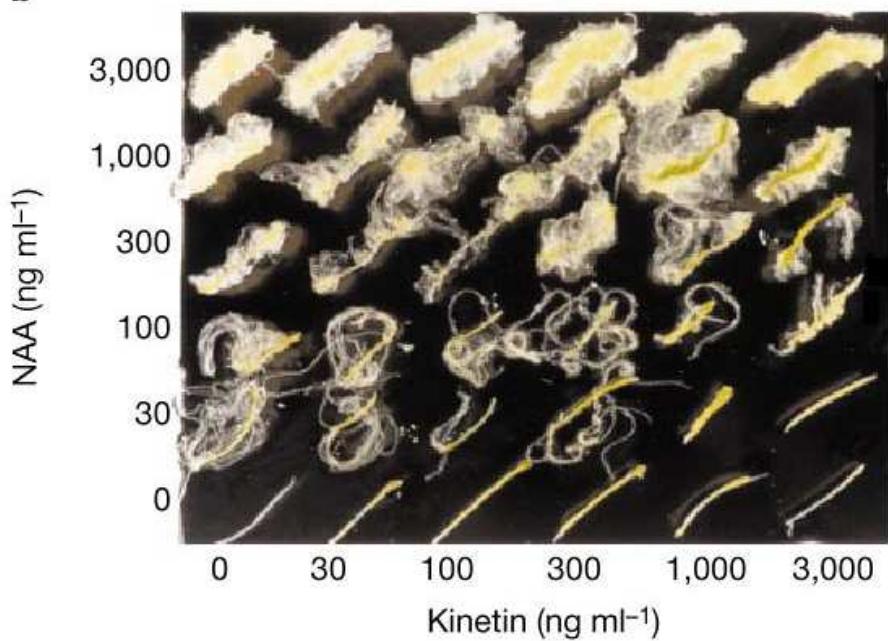
Identification of *CKI1* gene



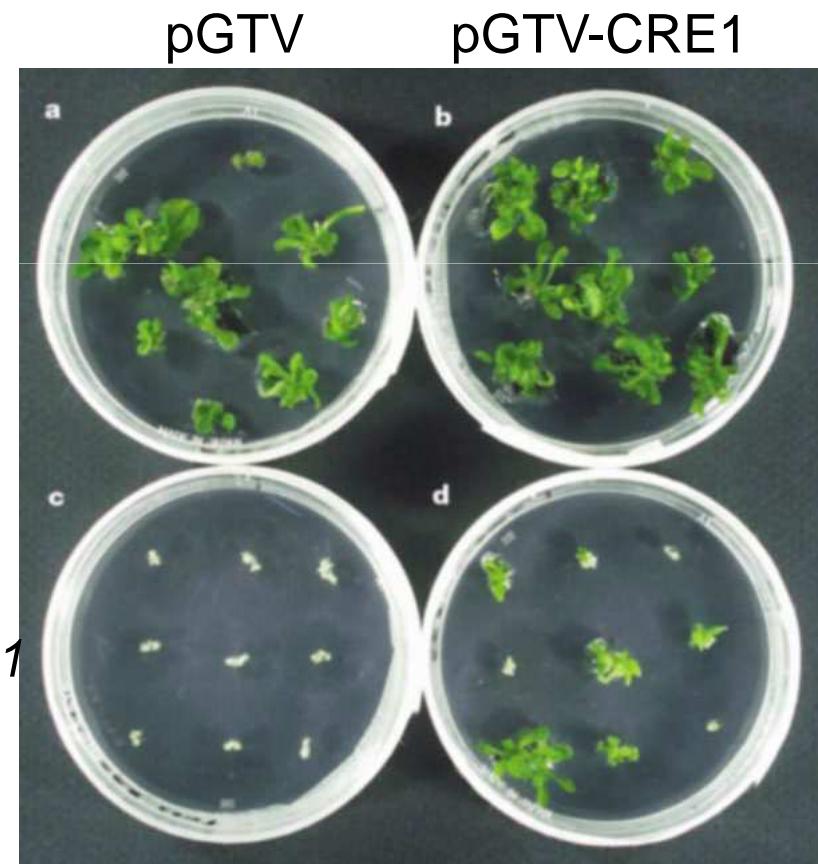
Verification - 35S::CKI1 transgene



Kakimoto et al., 1996

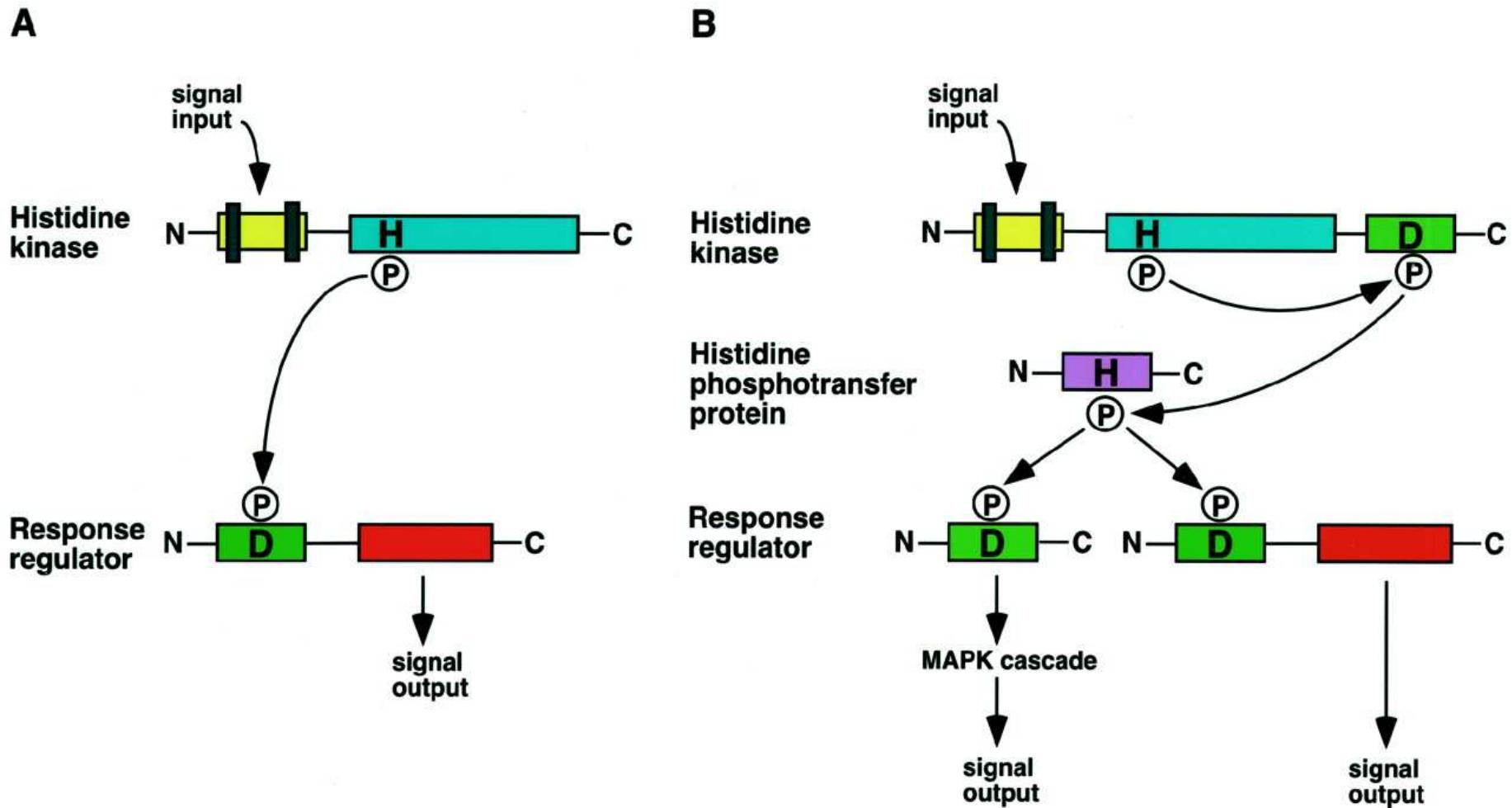
a**b**

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

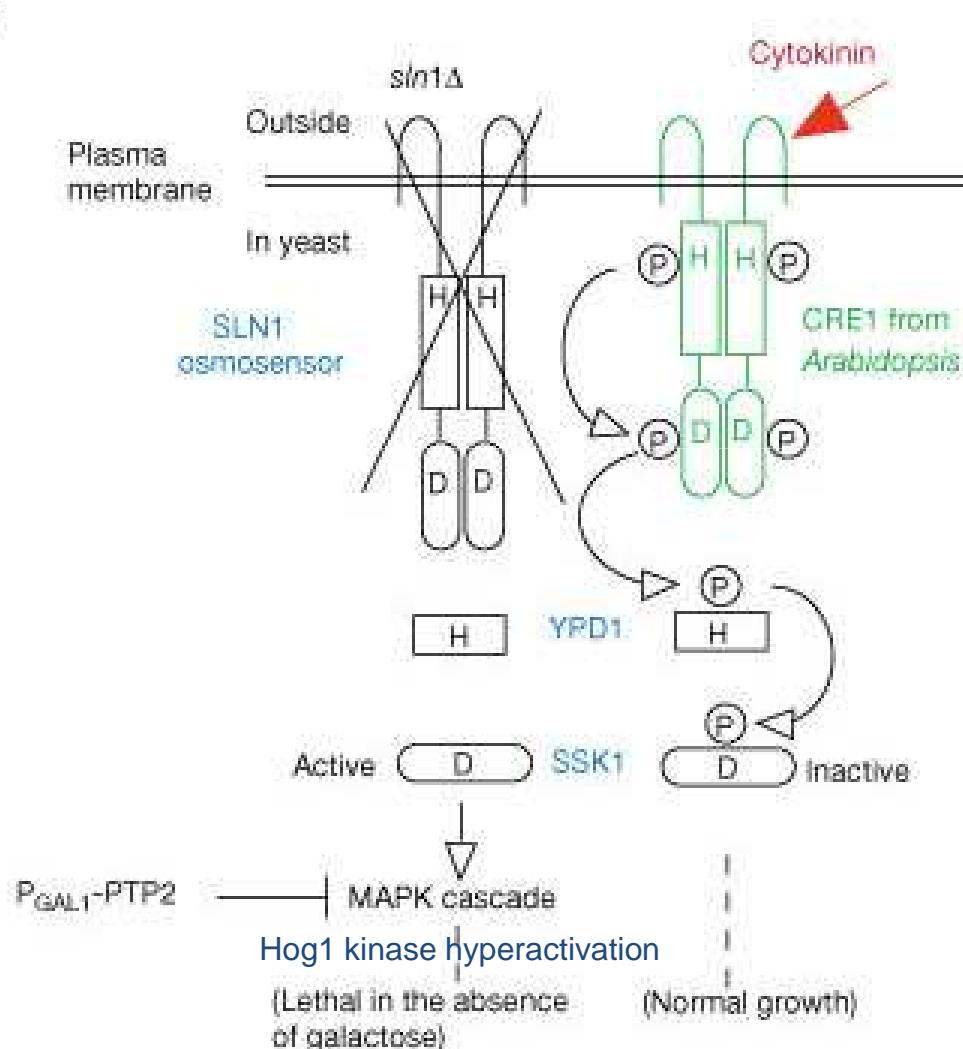
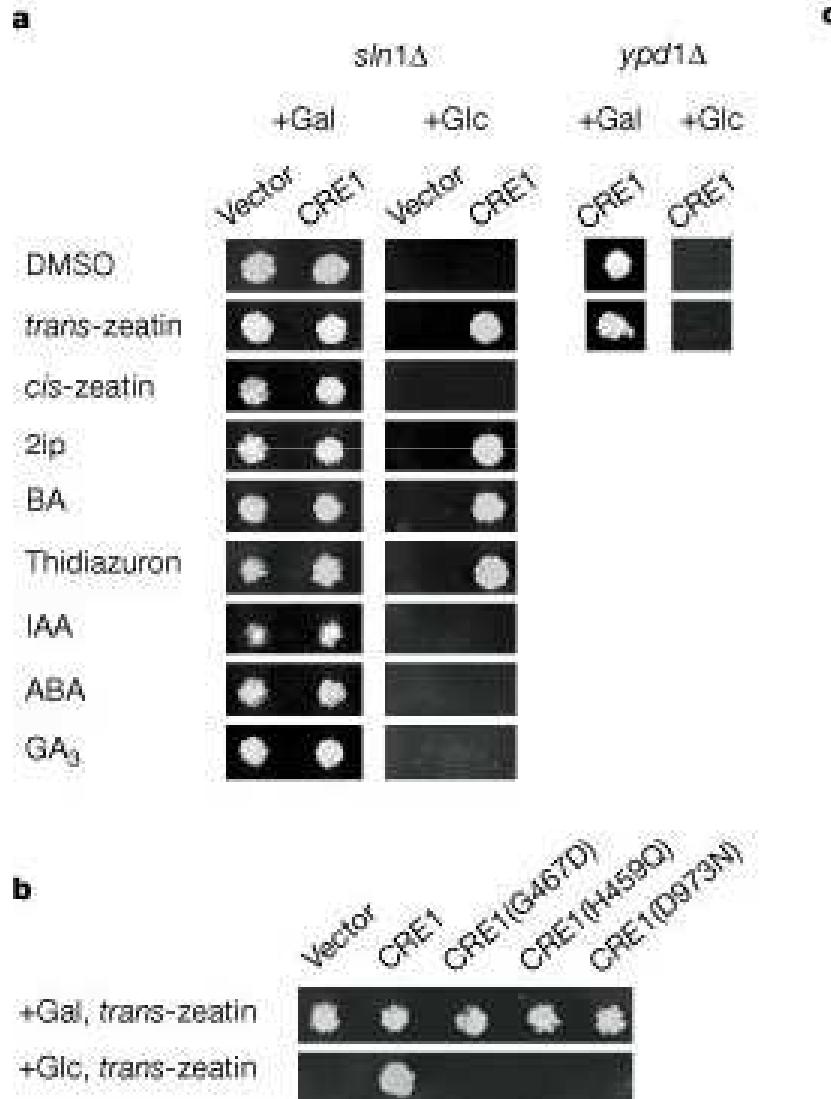


Inoue et al., 2001

His kinase transduction pathway



Piece of genius - complementation



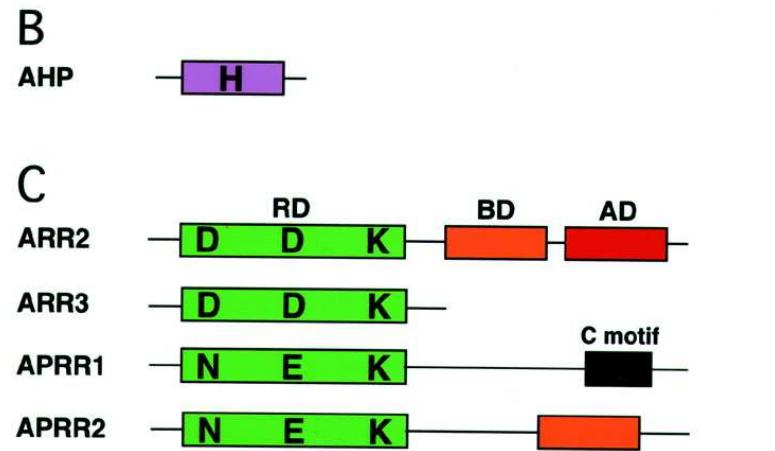
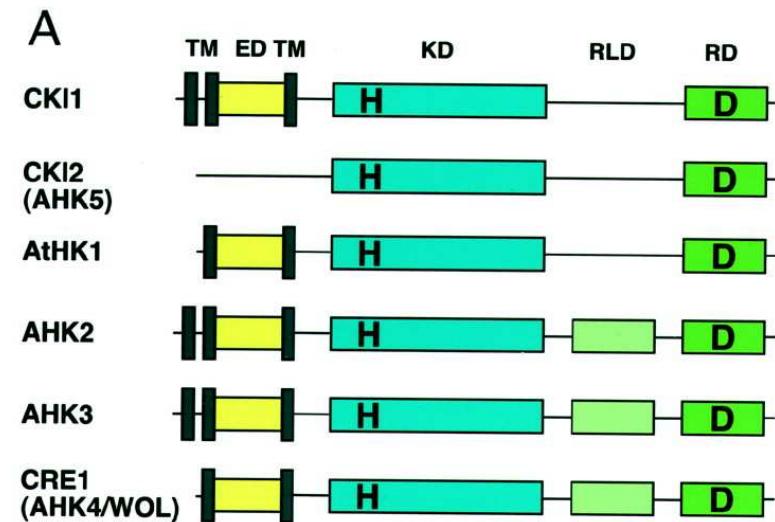
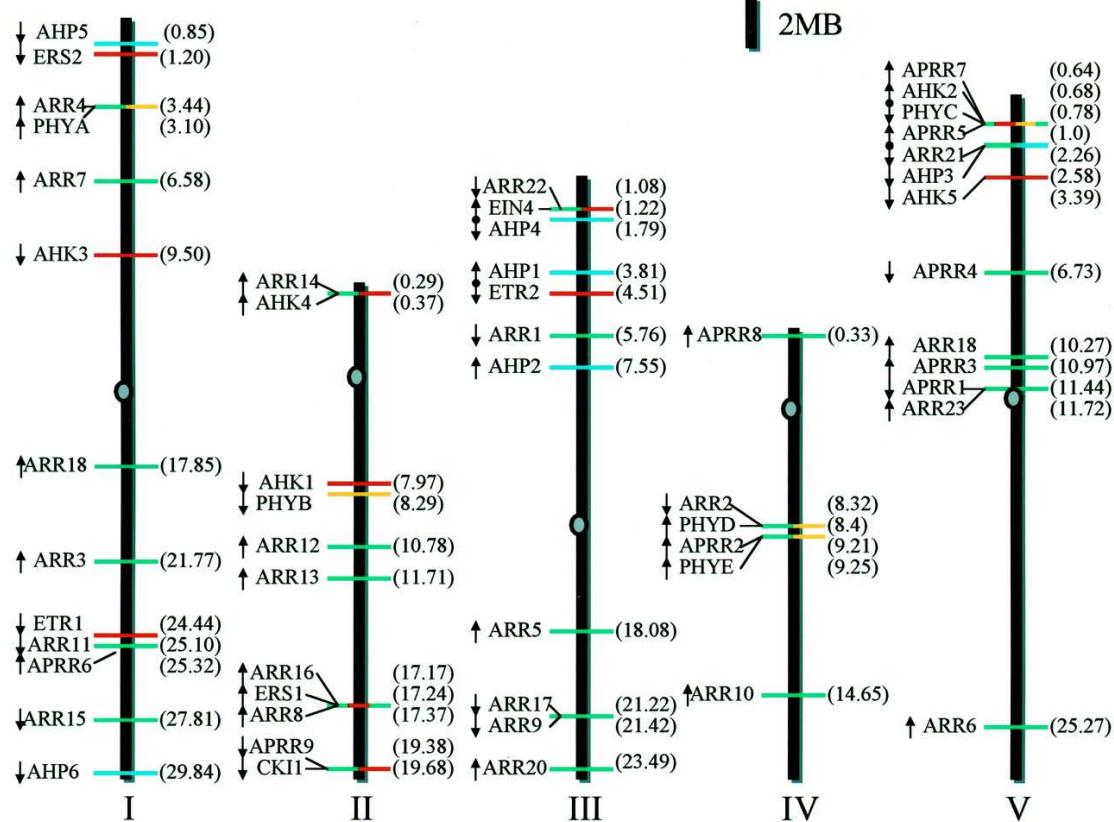
Inoue et al., 2001

Cytokinin receptors – what else?

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

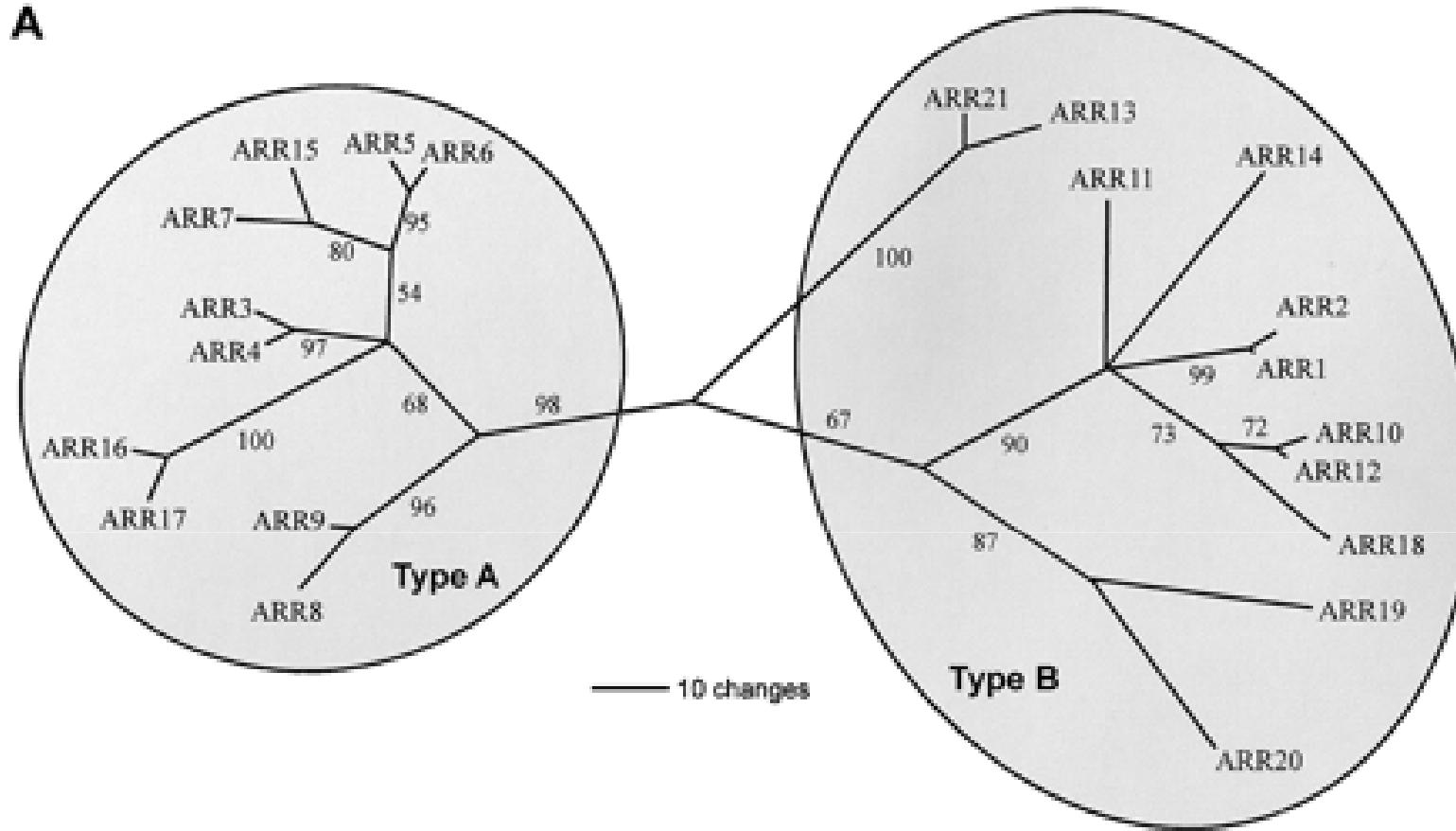
What does CKI1?

His kinase pathway components in *Arabidopsis*

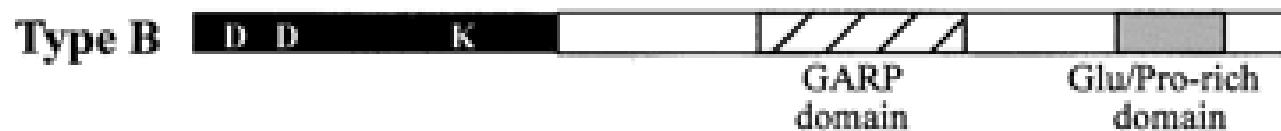
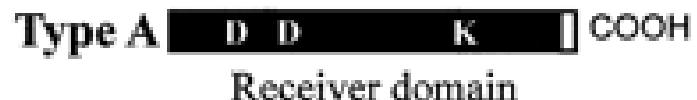


Response Regulators in *Arabidopsis*

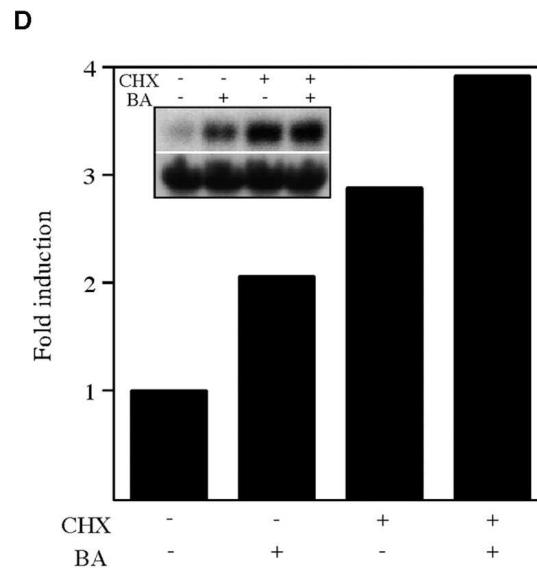
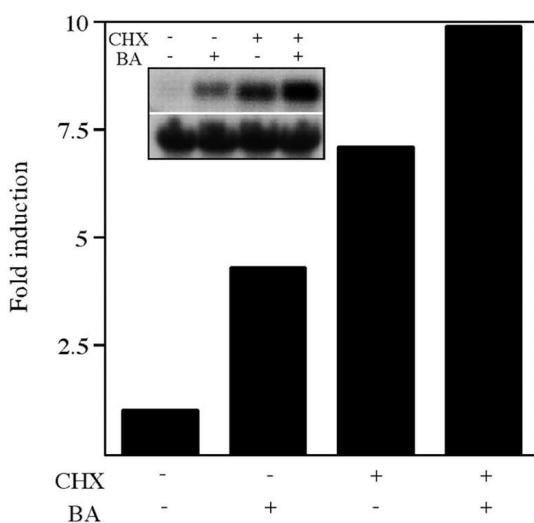
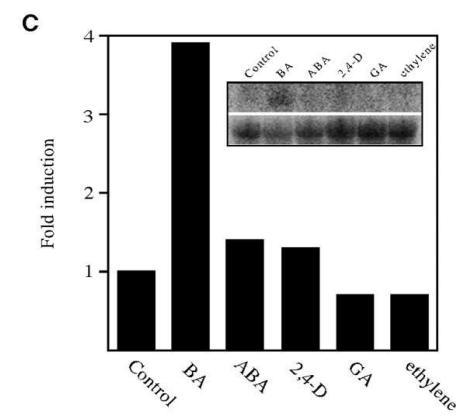
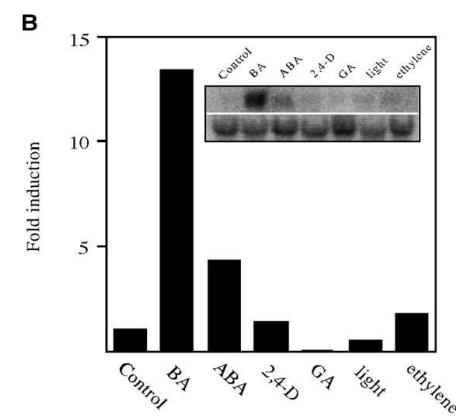
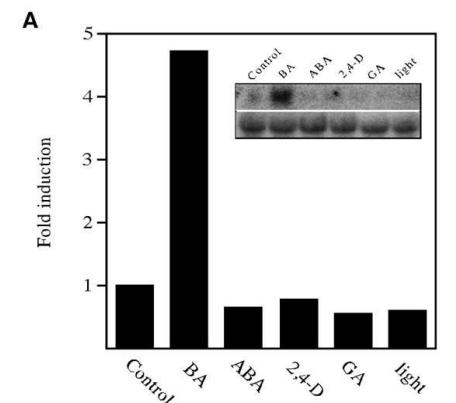
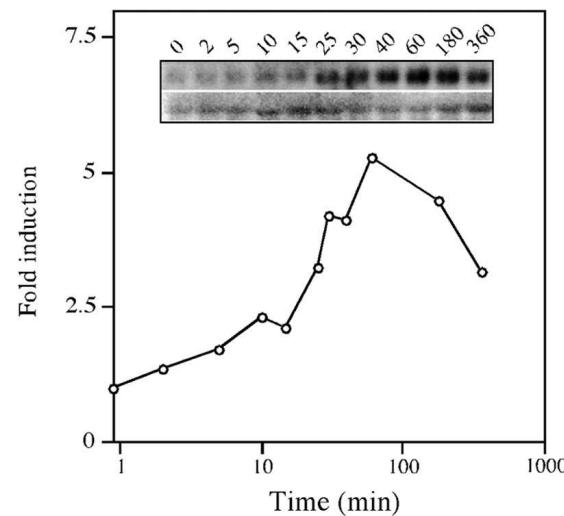
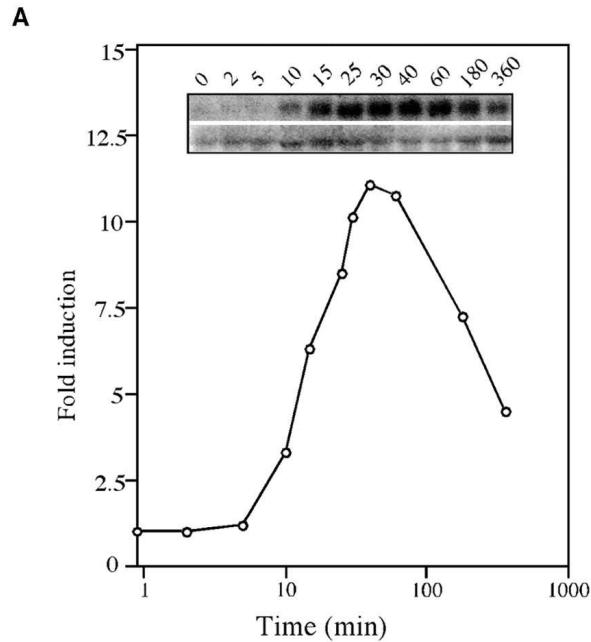
A



B

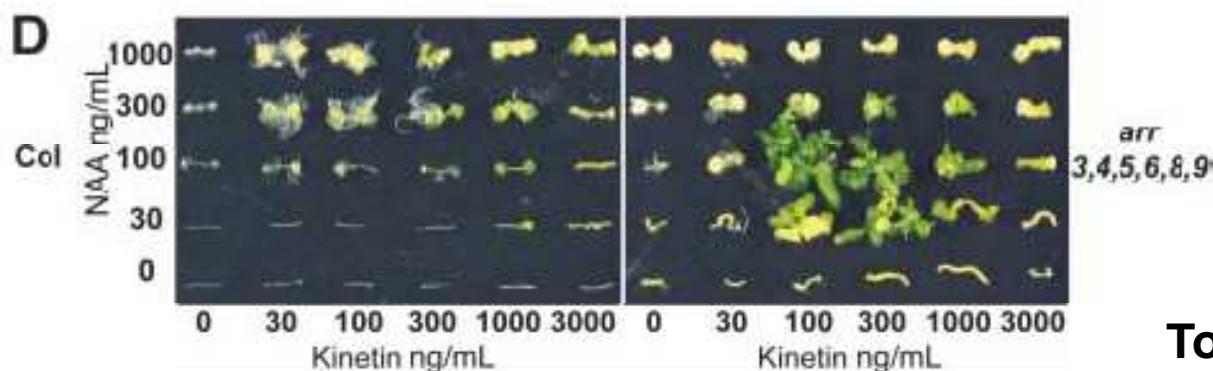
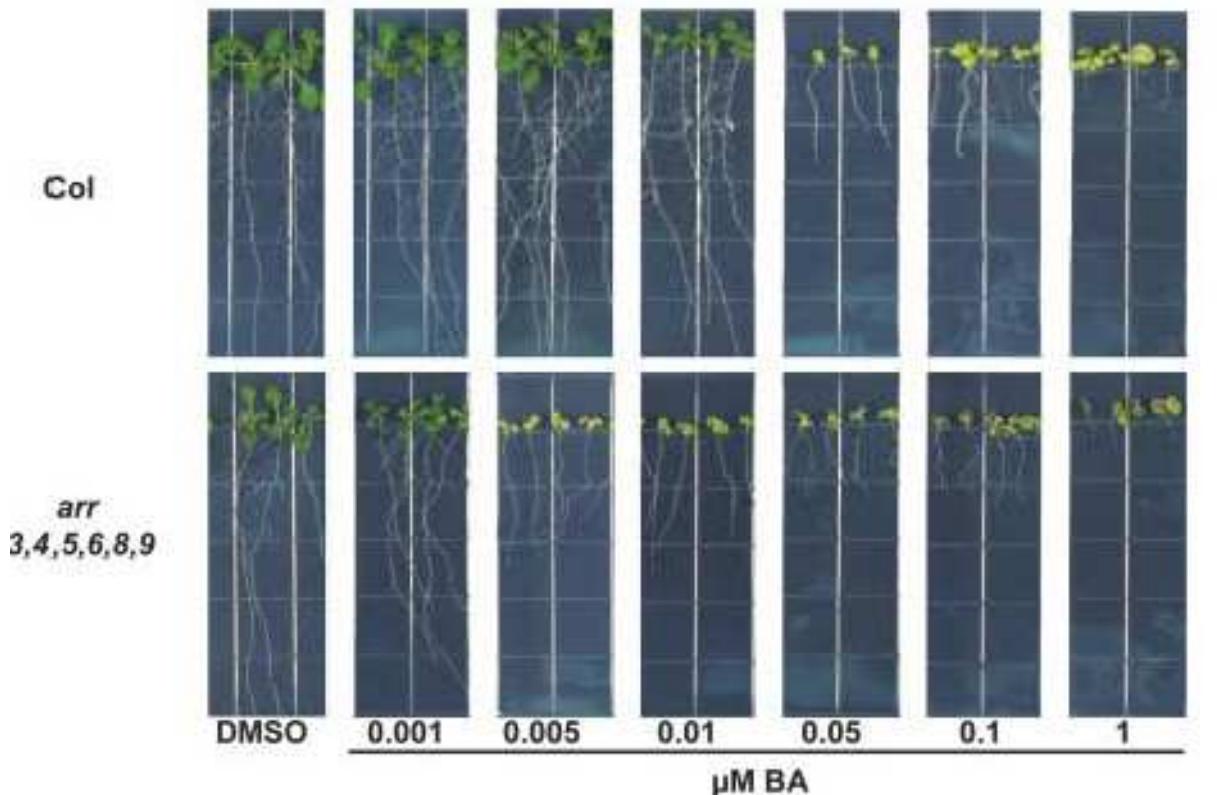


CK responsive genes – *ARR* type A



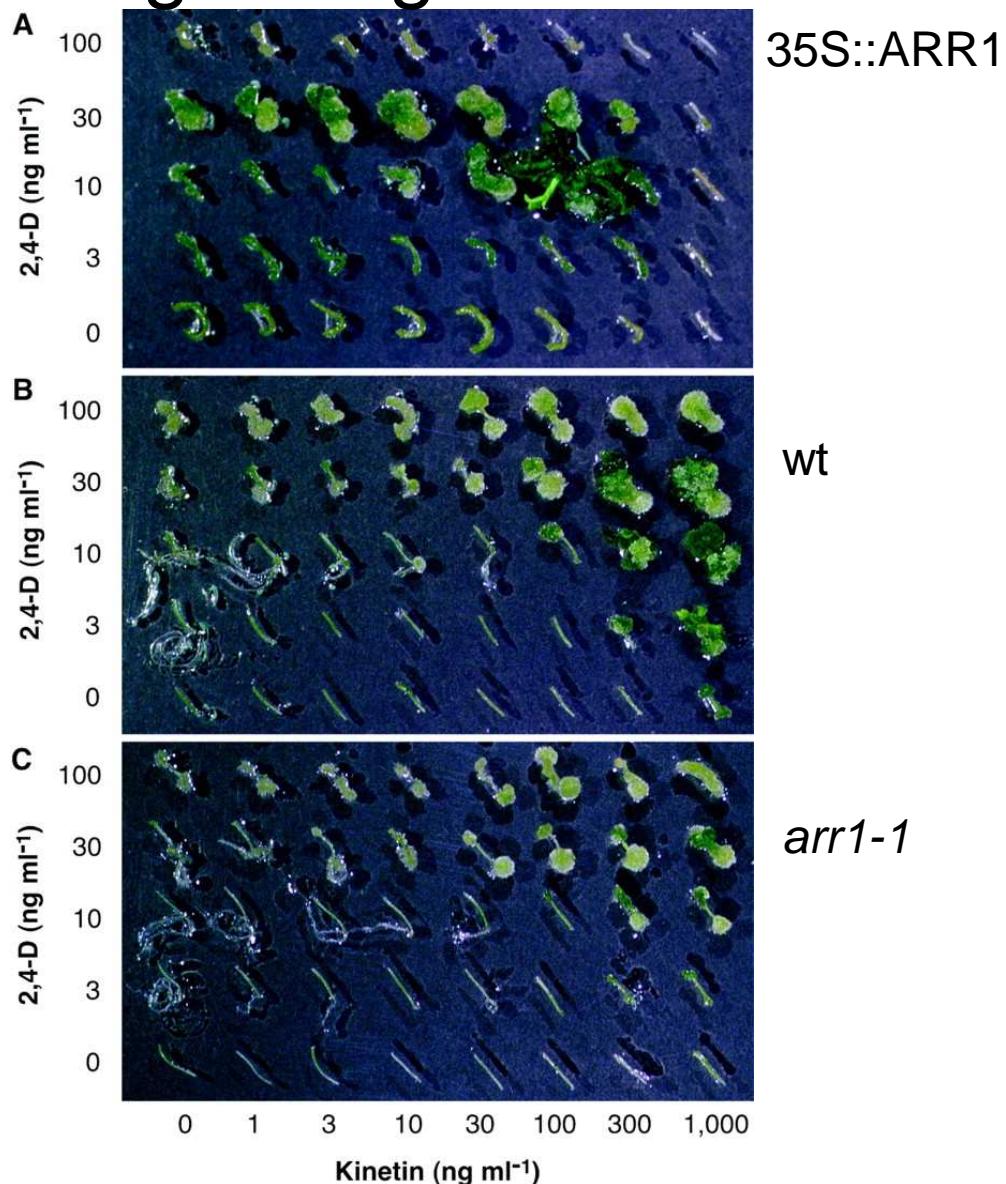
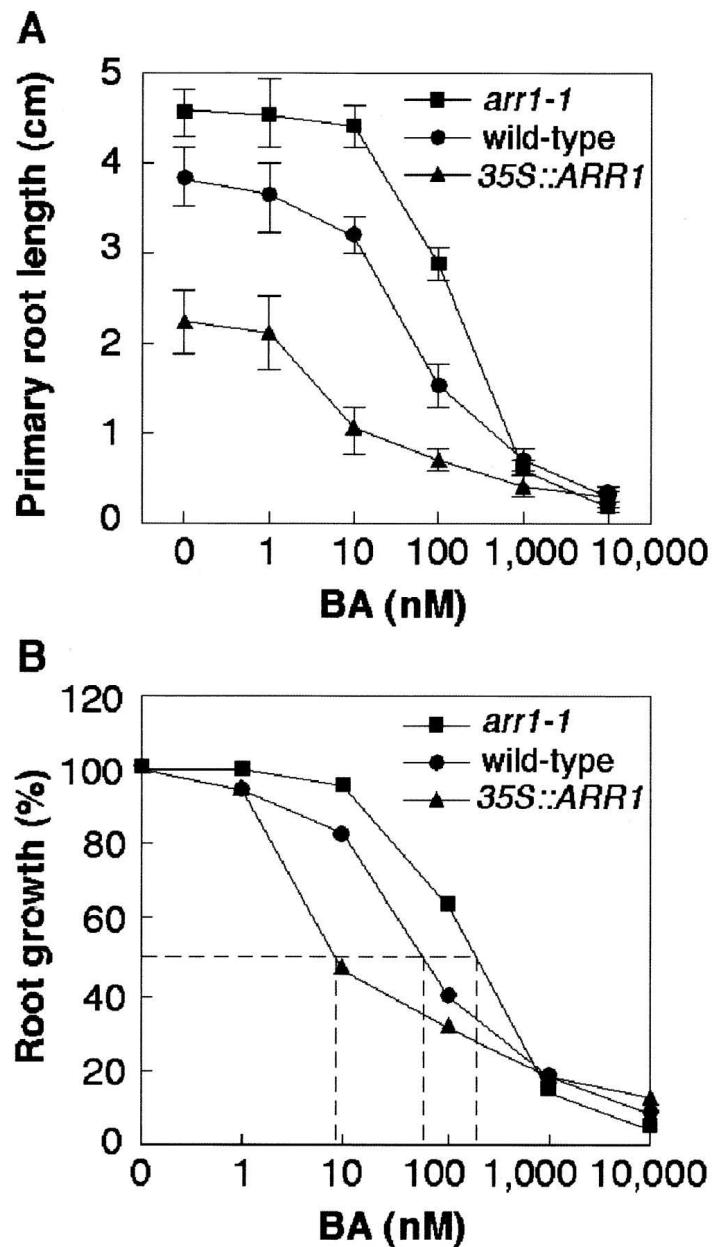
Brandstatter and Kieber, 1999

Response regulator ARR type A-negative regulators of cytokinin signalling



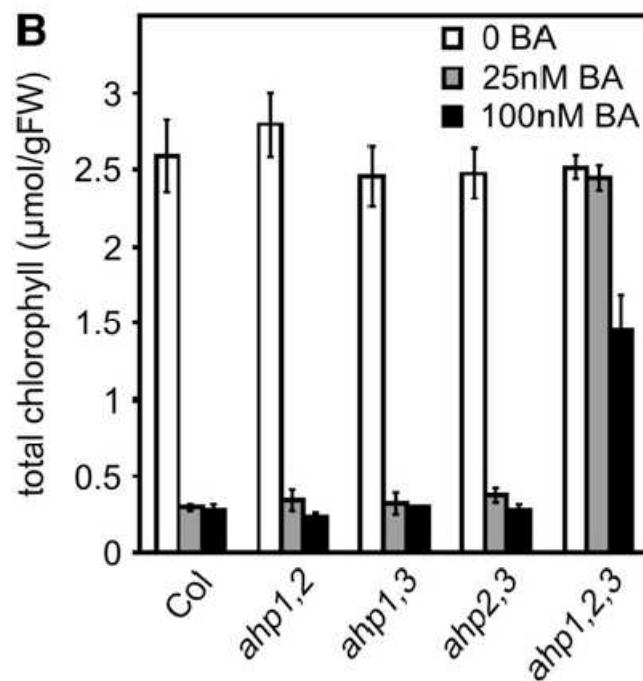
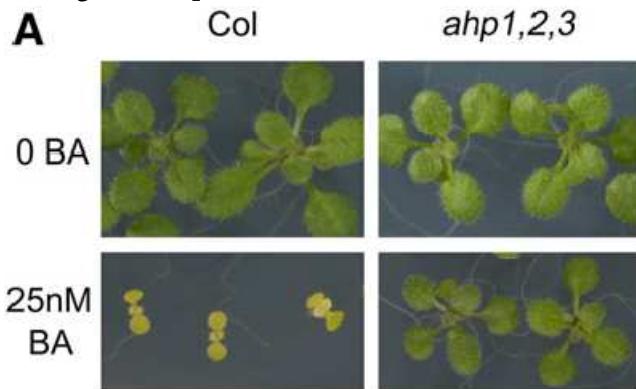
To et al., 2004

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



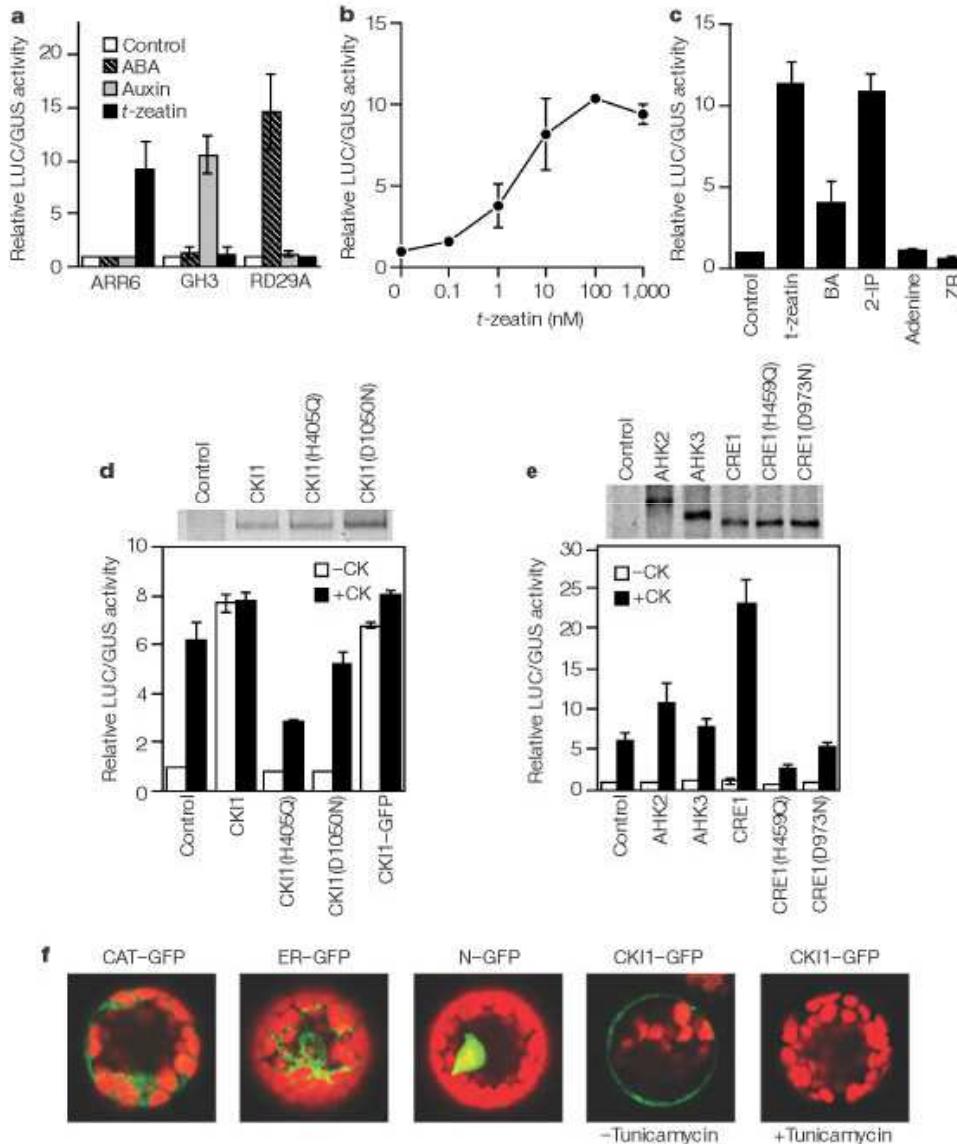
Sakai et al., 2001

AHPs mediate transfer of cytokinin signal between cytoplasm and nucleus

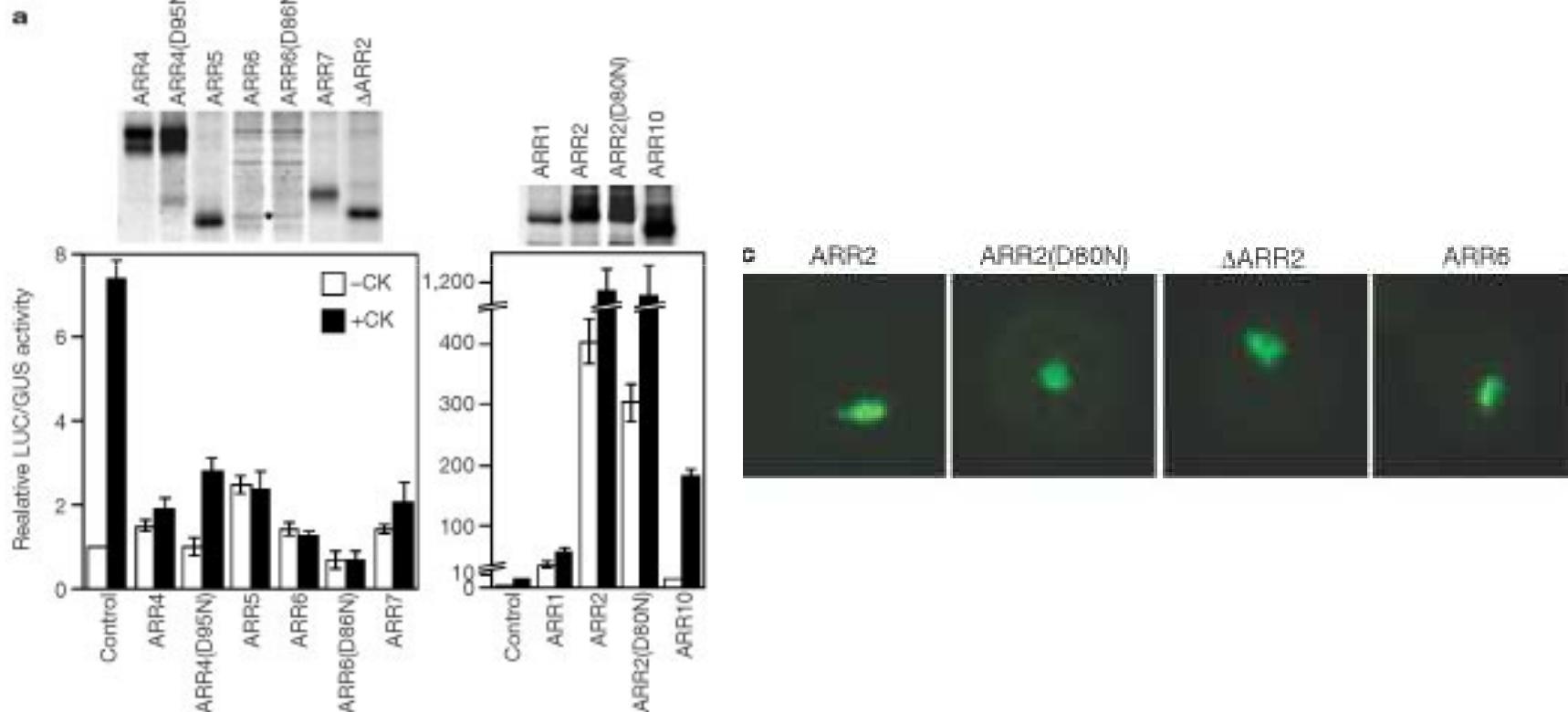


Hutchison et al., 2006

Games with protoplasts

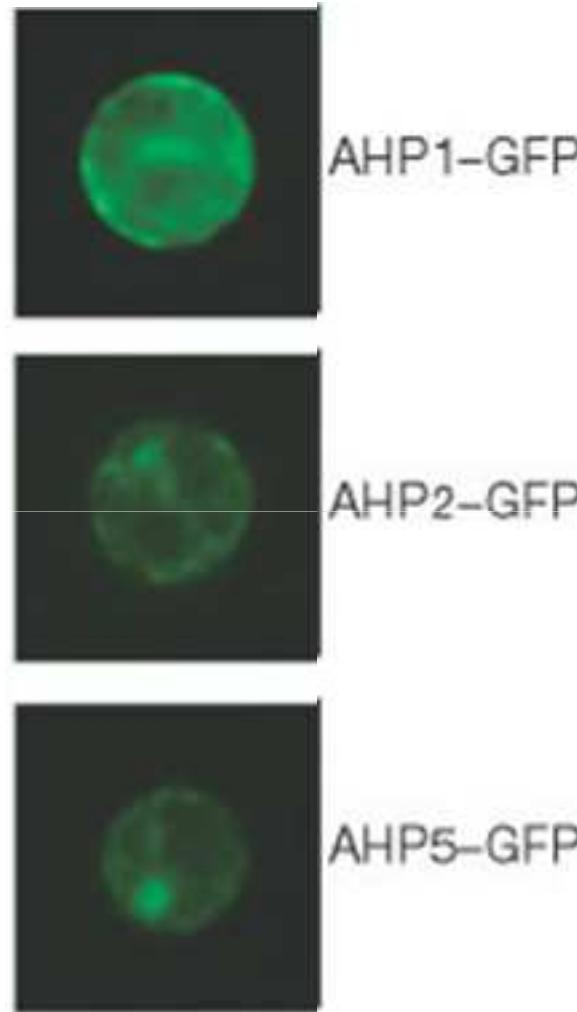
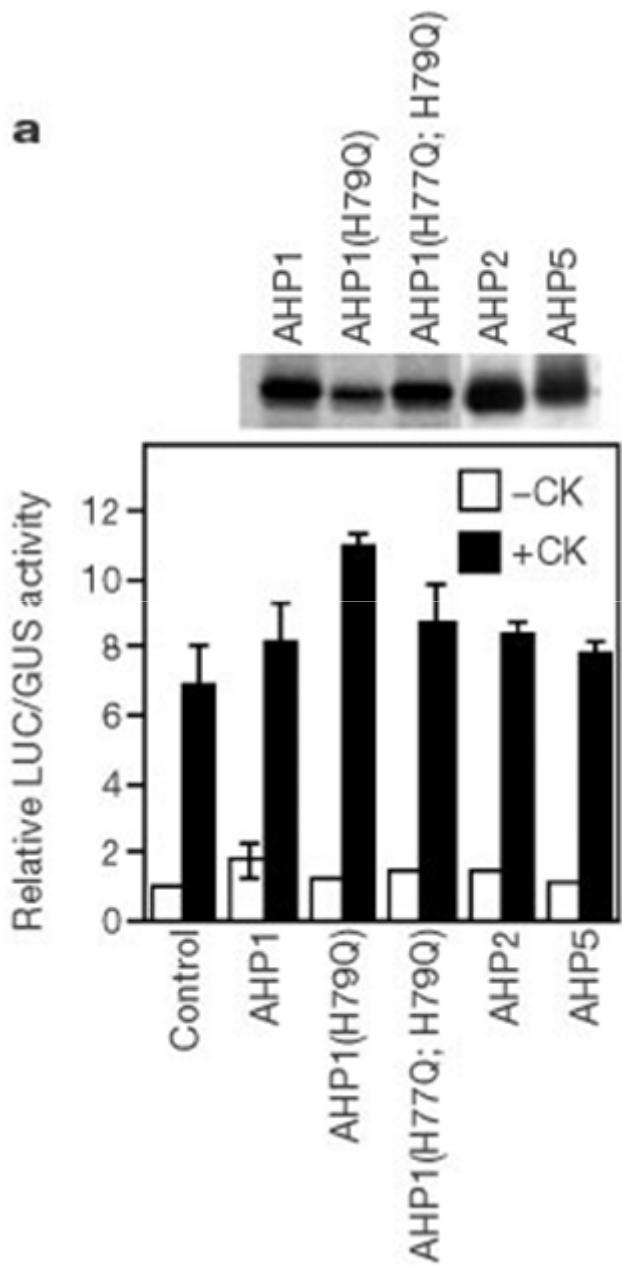


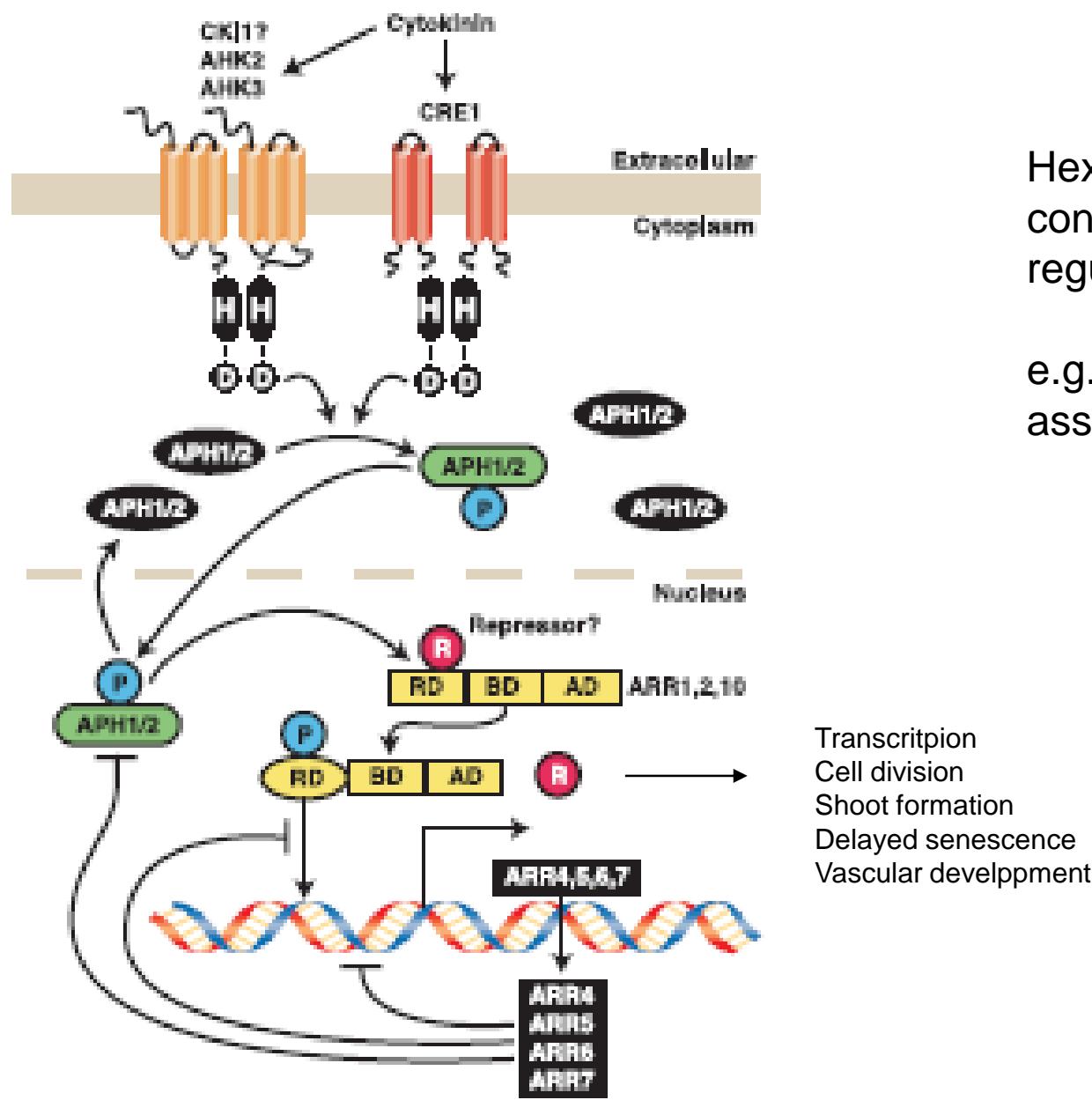
Opposite effects of two classes of ARRs on CK signalling



AHPs – signal to nucleus

a



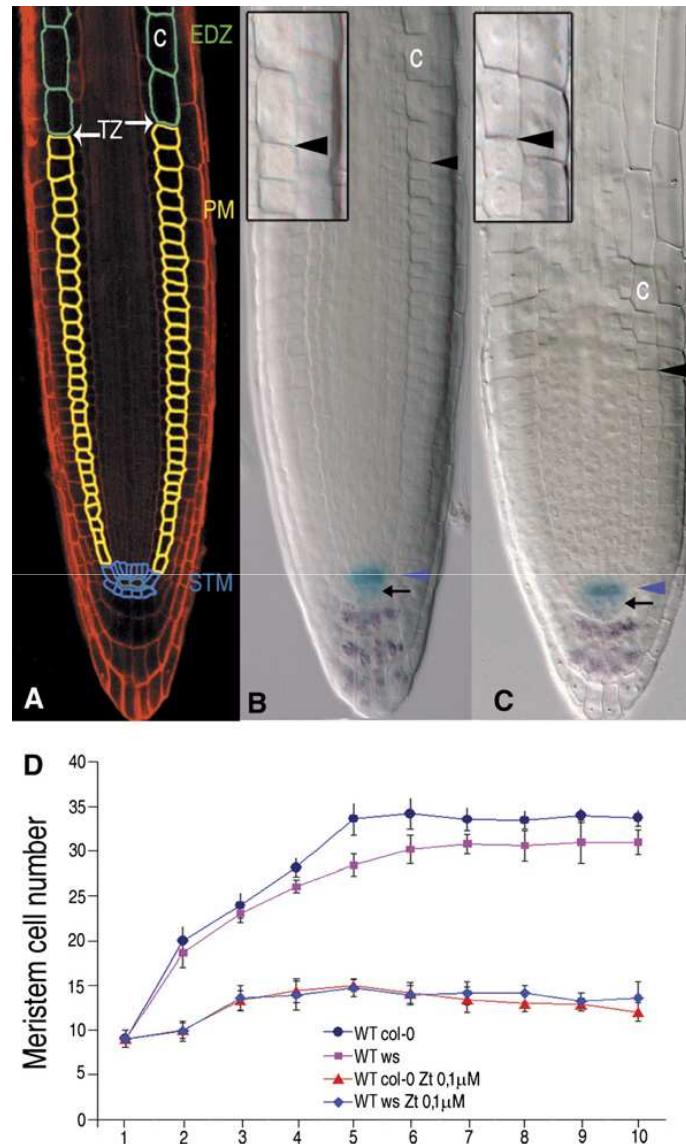


Hexuple of type A ARRs confirms role as negative regulators

e.g. Root elongation assay

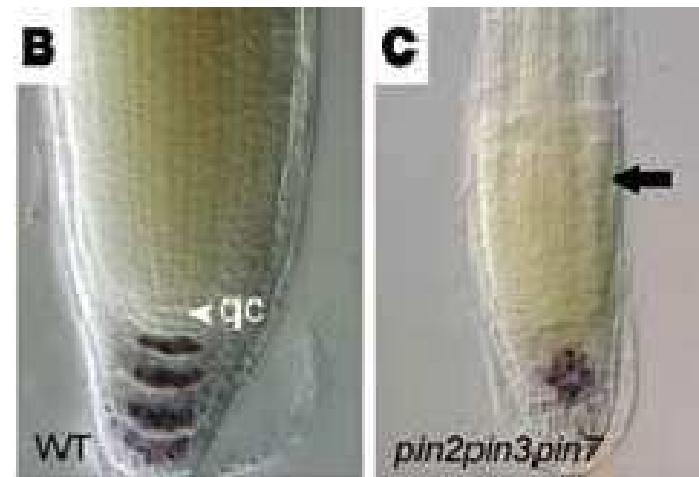
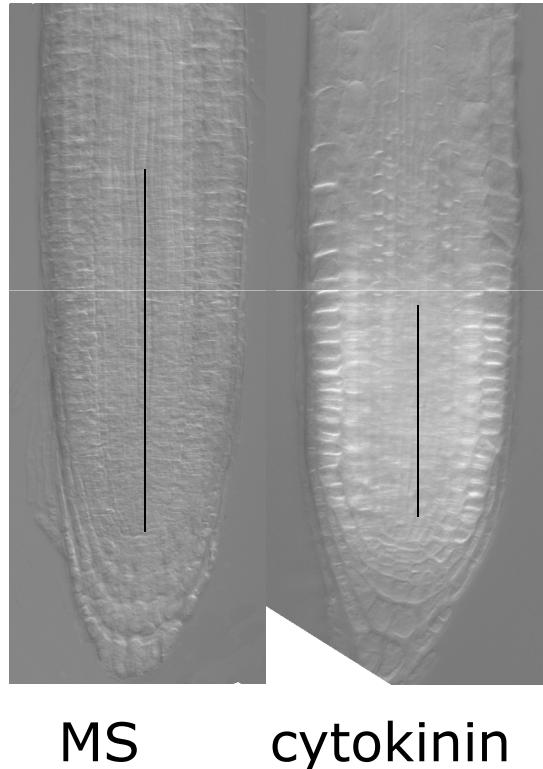
Transcription
Cell division
Shoot formation
Delayed senescence
Vascular development

Cytokinin – root meristem development



Dello Ioio et al., 2007

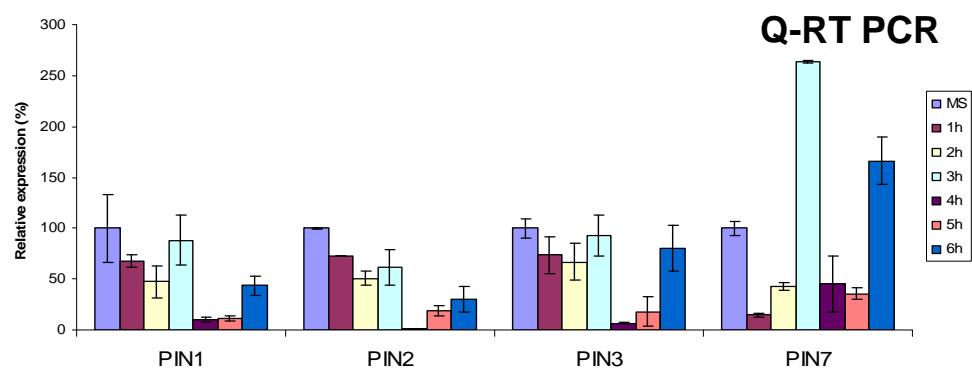
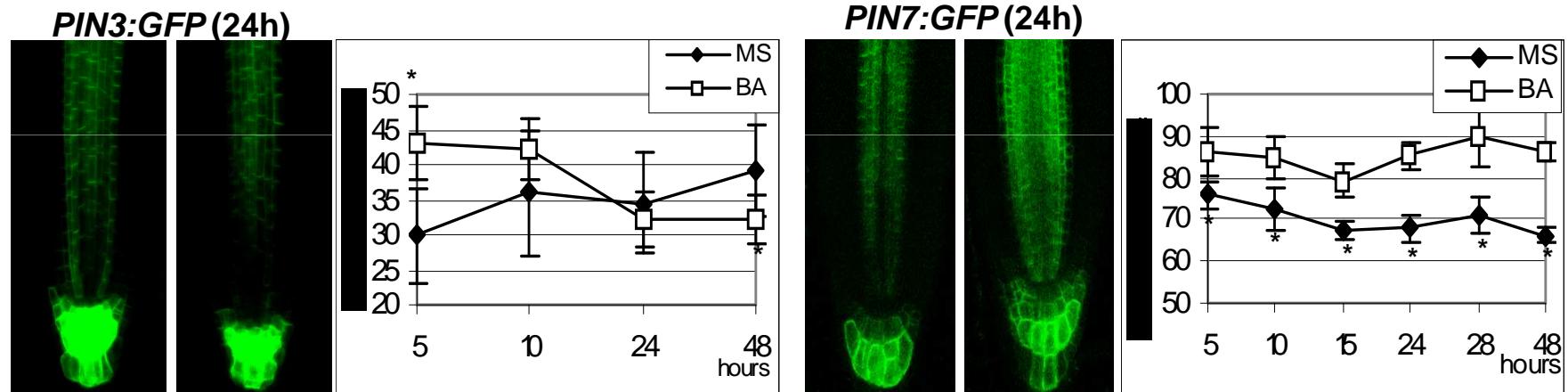
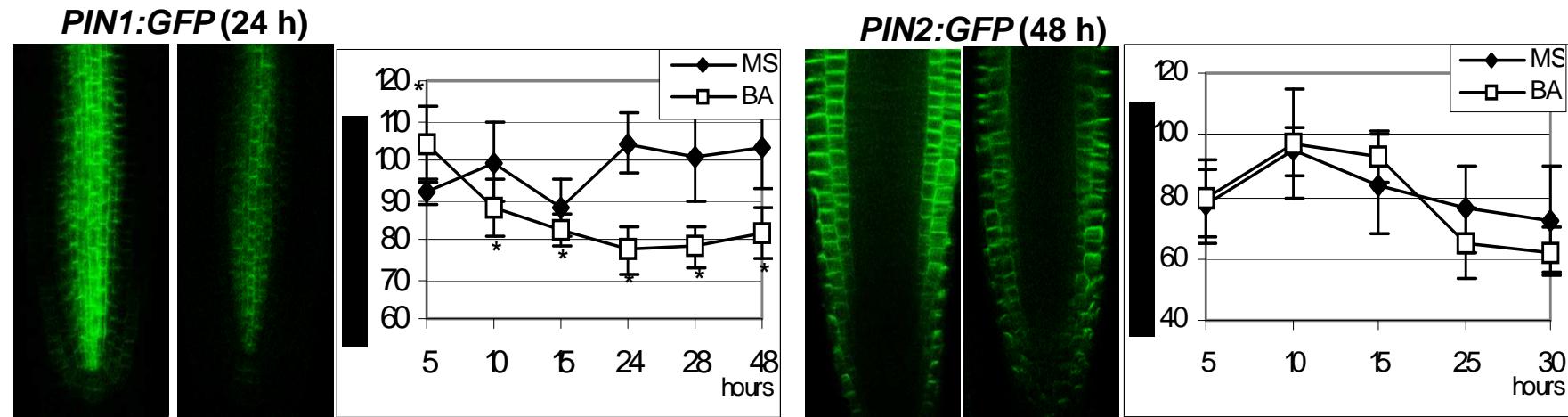
Auxin related mutants with short meristem



Blilou et al., 2005

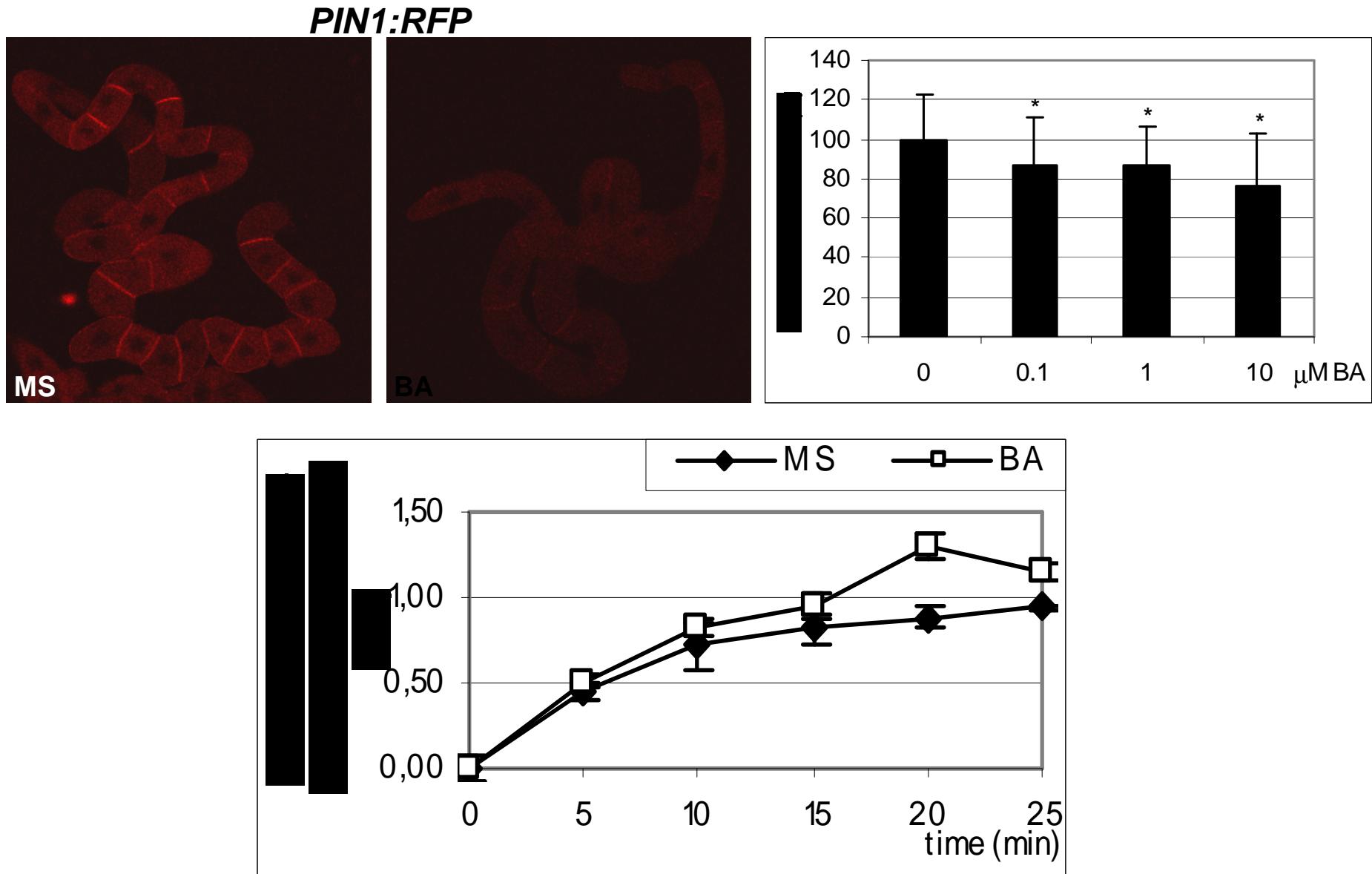
Auxin transport mutants

Cytokinin modulates auxin transport



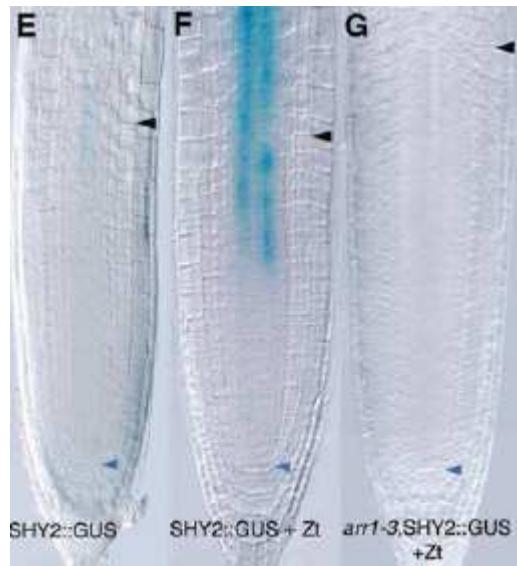
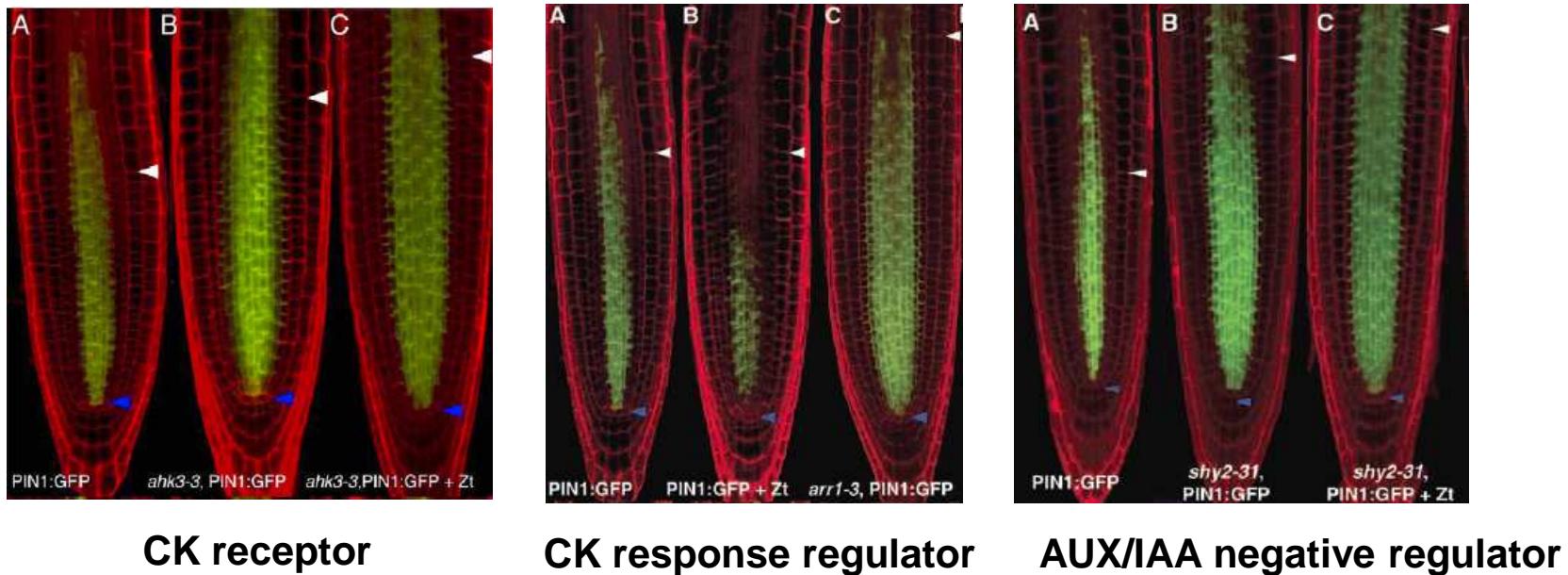
Ruzicka et al., 2009

Cytokinin reduces auxin efflux in tobacco BY2 cells



Ruzicka et al., 2009

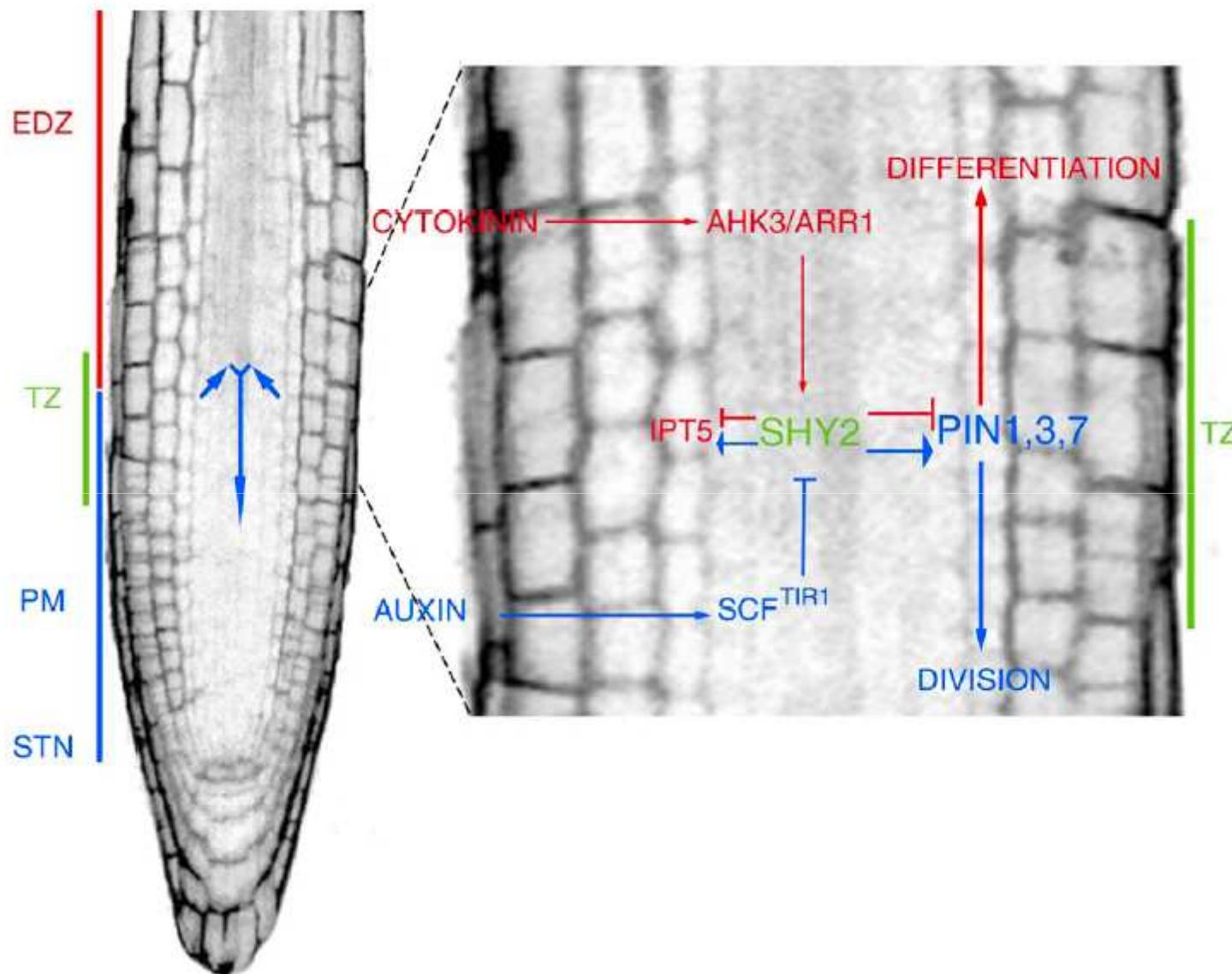
CK – auxin signalling pathways interaction to modulate auxin transport



Expression of IAA3/SHY2
is CK regulated

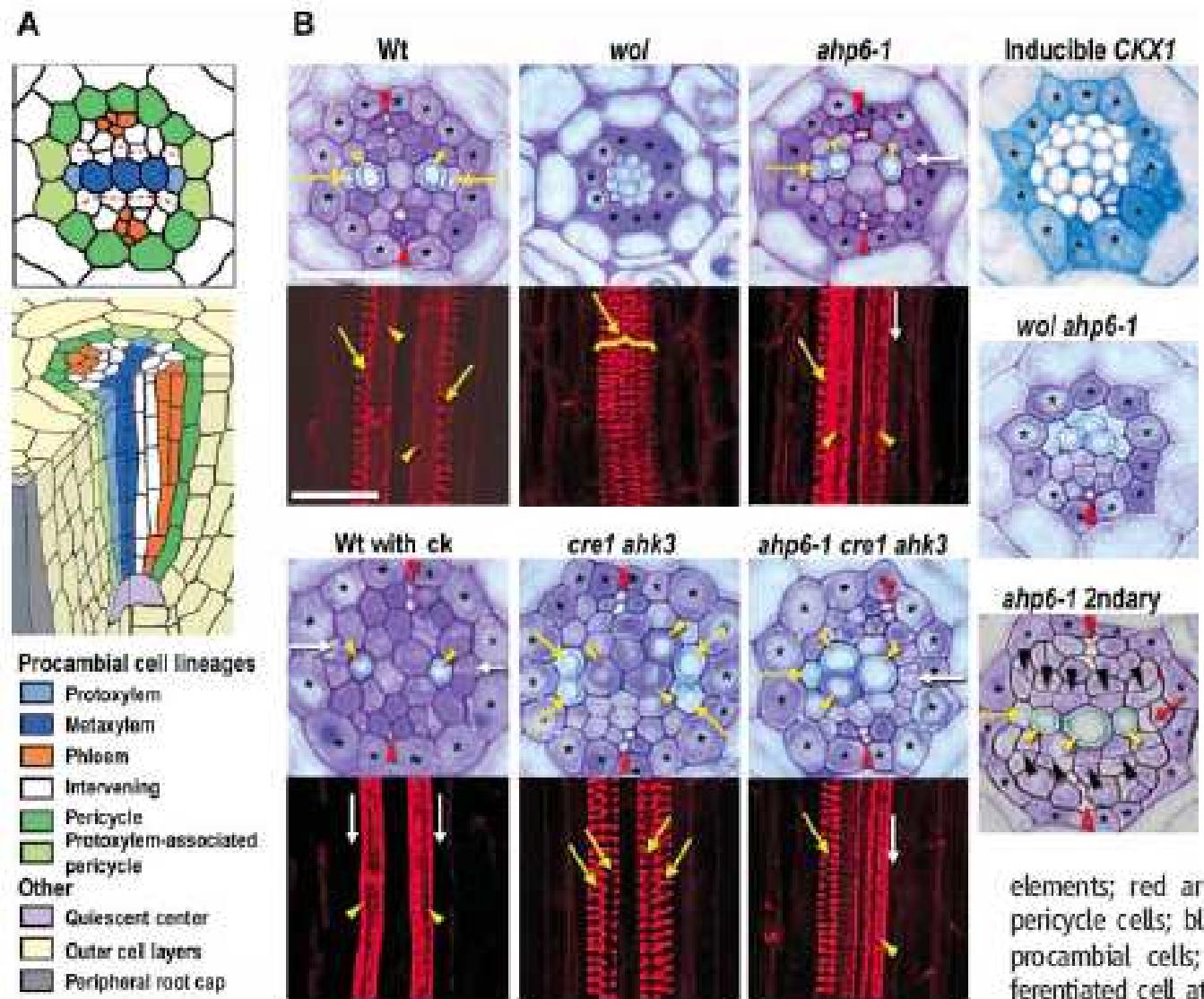
Dello Ioio et al., 2008

Model of cytokinin and auxin interaction in root meristem

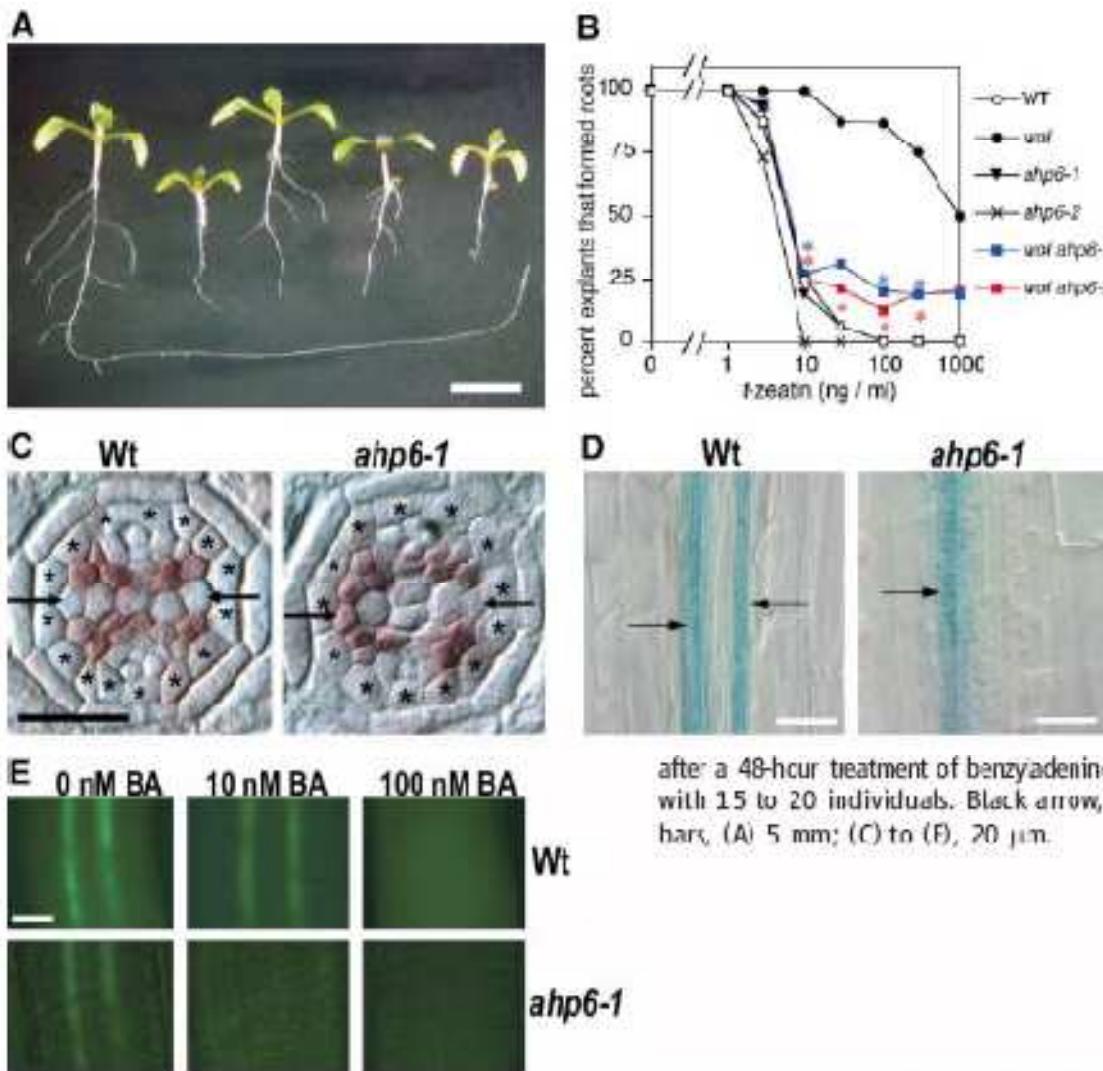


Dello Ioio et al., 2008

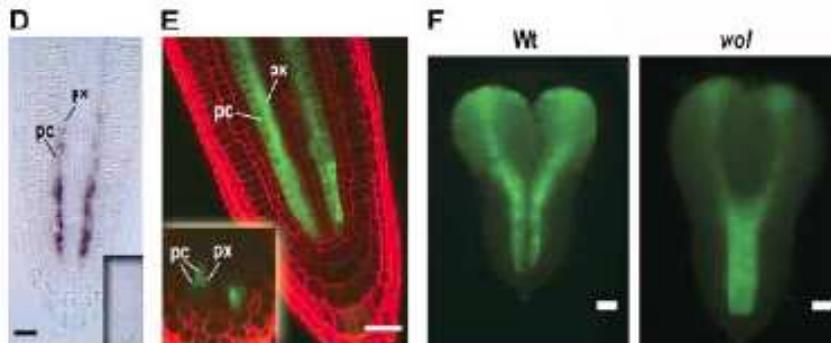
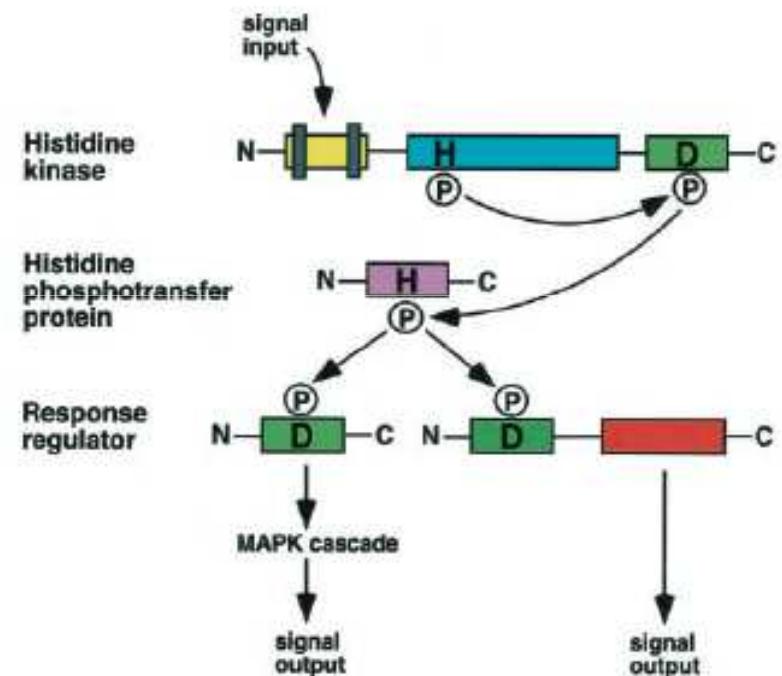
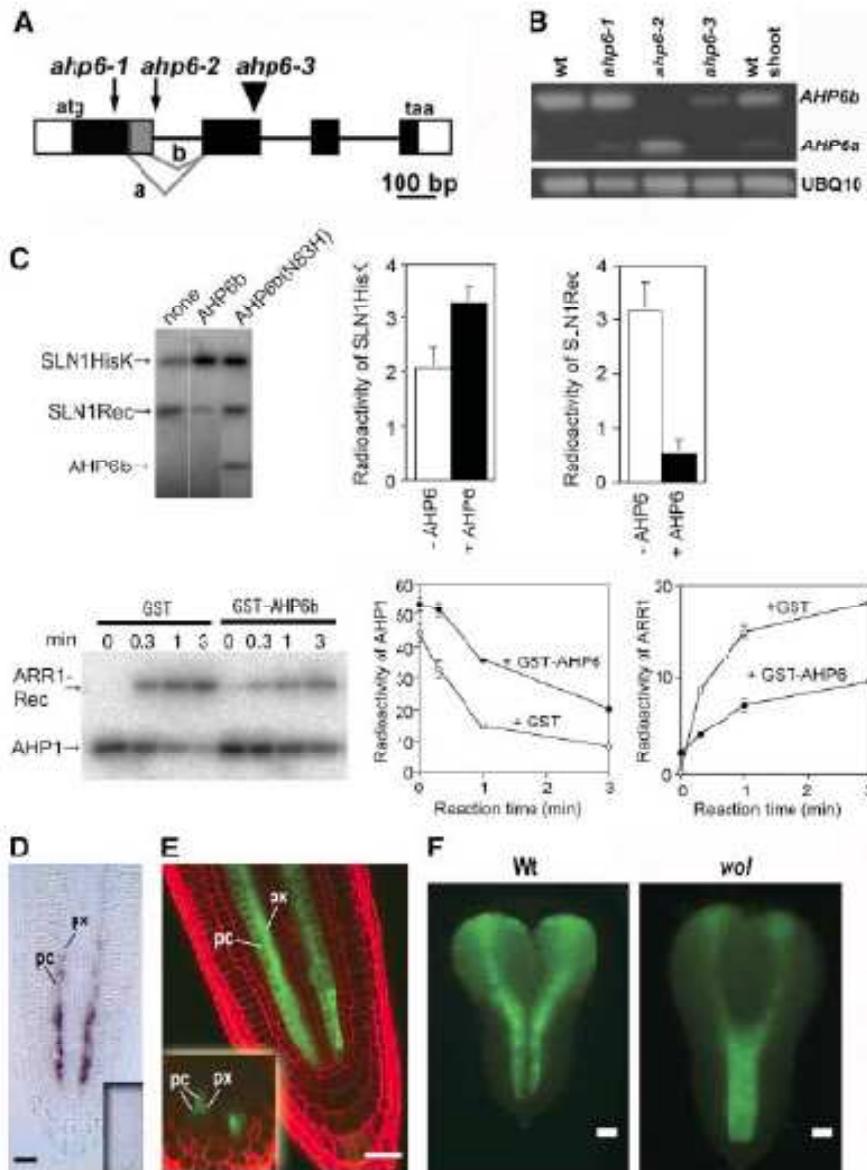
Cytokinin signalling regulates vascular development



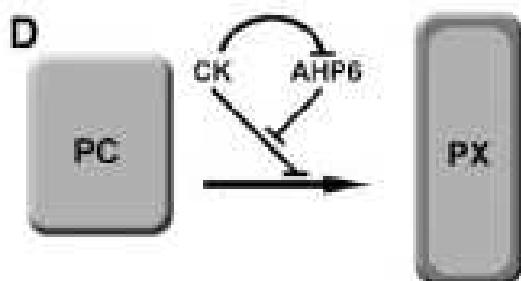
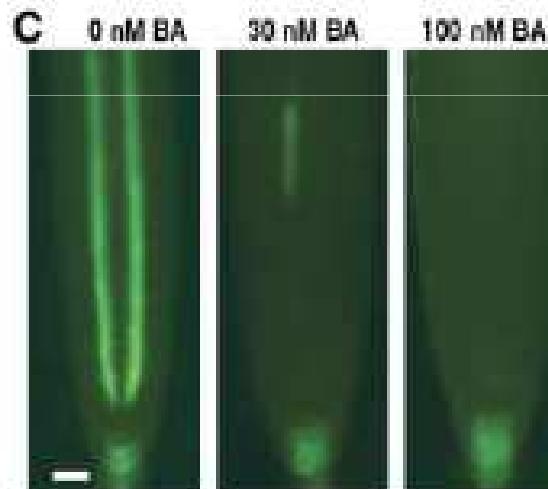
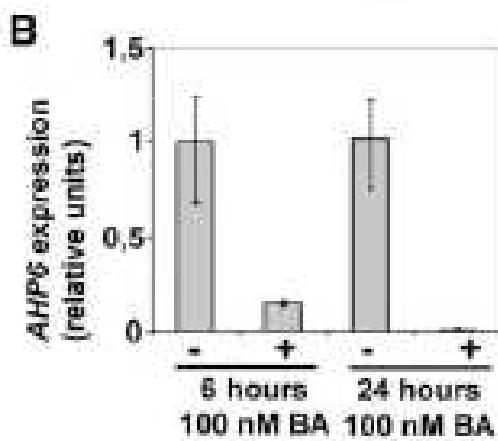
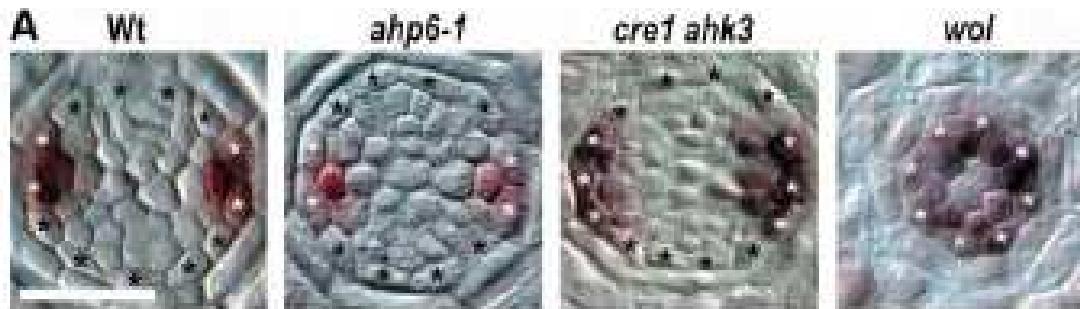
ahp6 suppress *wol* insensitivity to cytokinin



AHP6 codes for histidine phosphotransfer protein 6



AHP6 is negative regulator of cytokinin signalling



in a sporadic manner. Panels shown formed with 15 to 20 individuals. (I reciprocal interaction of cytokinin regulating the balance between the m cell identity (PC) and the differentiation (PX). Scale bars, 20 μ m.