

from Johnson and Ecker (Ann. Rev. Genet. 32, 227-254, 1998)

Genetic Analysis of Ethylene-triggered Signalling in Arabidopsis

types of mutants:

mutants that are insensitive to application of exogenous ethylene

mutants that constitutively exhibit phenotypes associated with ethylene-treated plants

mutants show other physiological alterations ("triple-response")

genes identified: *etr1*, *etr2*, *etr3*, *ein2*, *ein3*, *ein4*, *ein5*, *ein6*, *ein7*, *ctr1*

epistasis: *ctr1* is downstream from *etr1* and *ein4*; *ein2*, *ein3*, *ein5*, *ein6*, *ein7* downstream from *ctr1*

all *etr1*, *etr2*, and *ein4* mutant alleles are dominant; *ctr1* and *ein2* are recessive -> phenotypes suggest that *ctr1* is a negative regulator of *ein2* (active *ctr1* turns off *ein2*)

ETR1 - ethylene receptor

polypeptide shows similarities to two-component regulators - possess a histidine kinase domain and a response regulator domain

yeast that express ETR1 can bind ethylene in a saturable manner, with constants consistent with in vivo studies -> ETR1 is the ethylene receptor

yeast that express ETR1 mutants do not bind ethylene

mutational analysis suggests that the N-terminus of ETR1 has the binding site

ETR1 probably functions as a dimer (possible explanation for dominance of *etr1* alleles?)

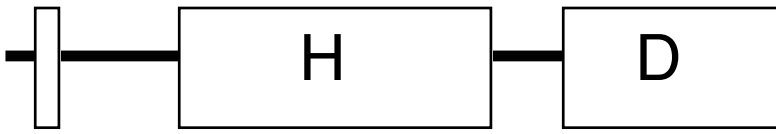
ETR1 is related to other proteins (ETR2, EIN4, ERS1, and ERS2) that may also be ethylene receptors

other probable ethylene receptors lack the response-regulator domain of ETR1

the histidine kinase domains of ETR1 and ERS1 interact with CTR1

an alternative model to explain dominance of *etr1* alleles: ethylene binding inactivates ETR1, leading to inactivation of CTR1, etc.

questions - role of response regulator domain(s), phosphotransfer from ETR1 or ERS1 to CTR1? why so many receptors? are there histidine relays, response regulators apart from ETR1?

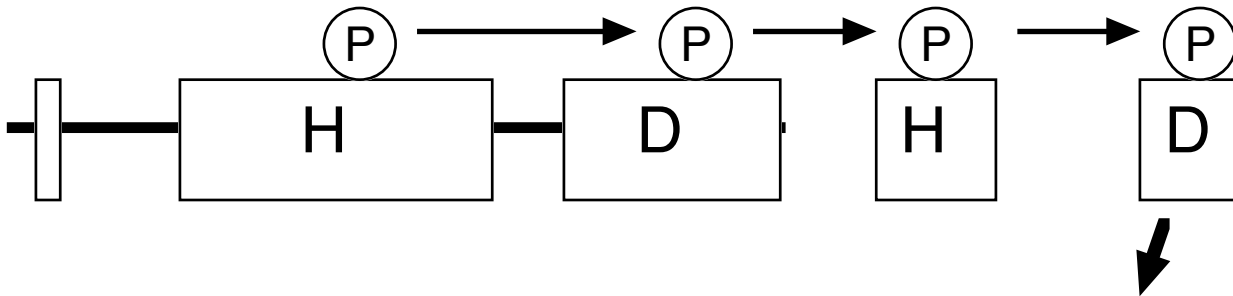


ETR1



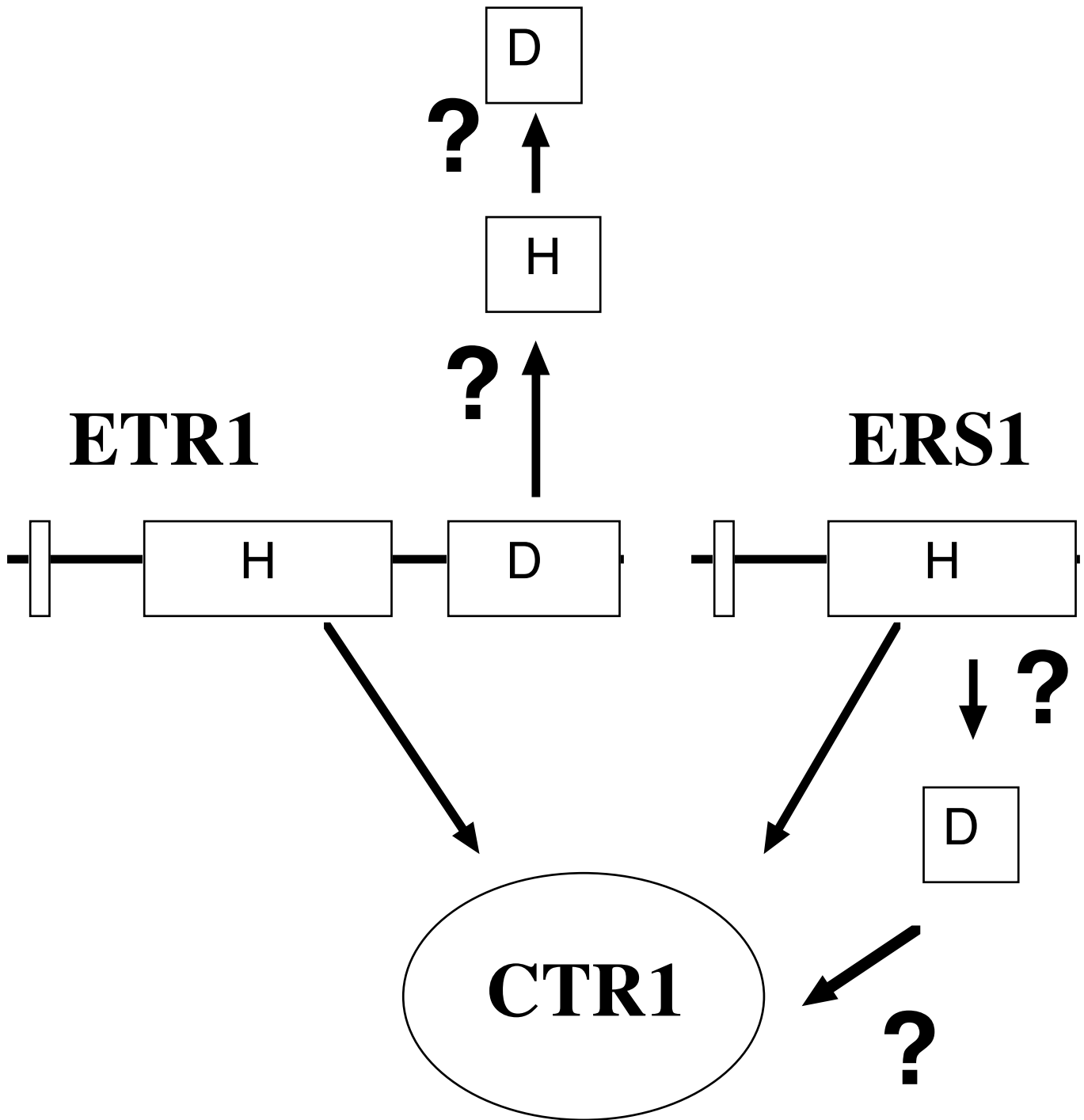
ERS1

phosphorelay system in prokaryotes, eukaryotes



**gene activation
(bacteria)**

**MAPKKK
regulation
(eukaryotes)**



EIN3

similar in organization to transcription factors

nucleus-localized

overexpression can overcome effects of ein2 mutants

overexpression leads to a constitutive ethylene response phenotype

related to other Arabidopsis genes (EIL's)

model - activates transcription factors that bind ethylene-responsive DNA elements

in absence of ethylene, EIN3 is degraded by F-box proteins EBF1 and EBF2

HLS1 - "hookless"

[mRNA] increased by ethylene treatment, decreased in ein2 mutants

constitutive expression leads to constitutive hook curvature

similar to N-acetyltransferases

hls1 mutants are phenocopied by inhibitors of auxin transport, exogenous auxin

EIN2

integral membrane protein

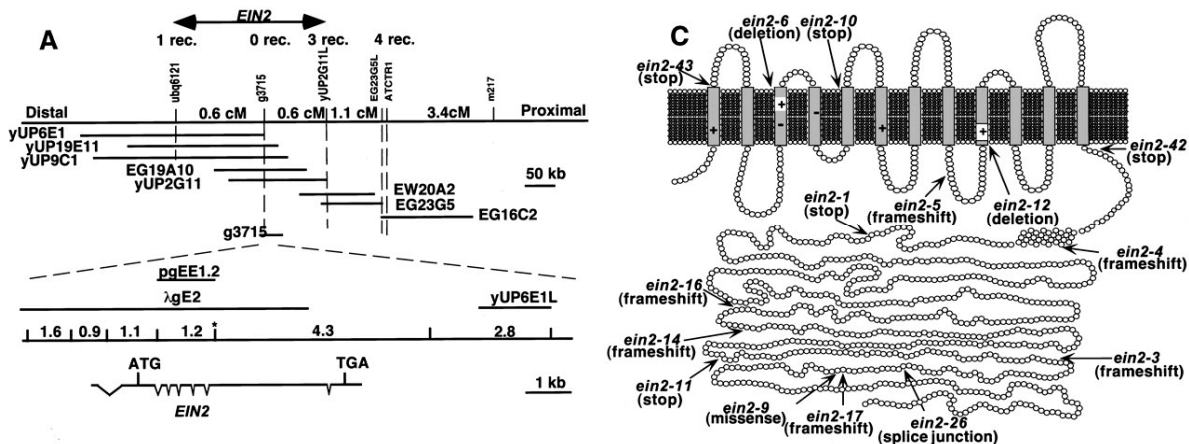
similar to mammalian disease-related metal ion transporters

has a coiled-coil domain (suggestive of protein-protein interactions)

overexpression of the C-terminal (soluble) domain leads to a constitutive ethylene response phenotype

overexpression phenotype requires EIN3

questions - does the metal ion transporter similarity indicate a role for metal ions in ethylene signaling (second messengers?)? how does CTR1 regulate EIN2? how does EIN2 regulate downstream players?



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CTR1

821 amino acid protein

**related in sequence to raf-type protein kinases
(MAPKKK)**

**baculovirus-expressed protein phosphorylates serine
and threonine residues on model substrates**

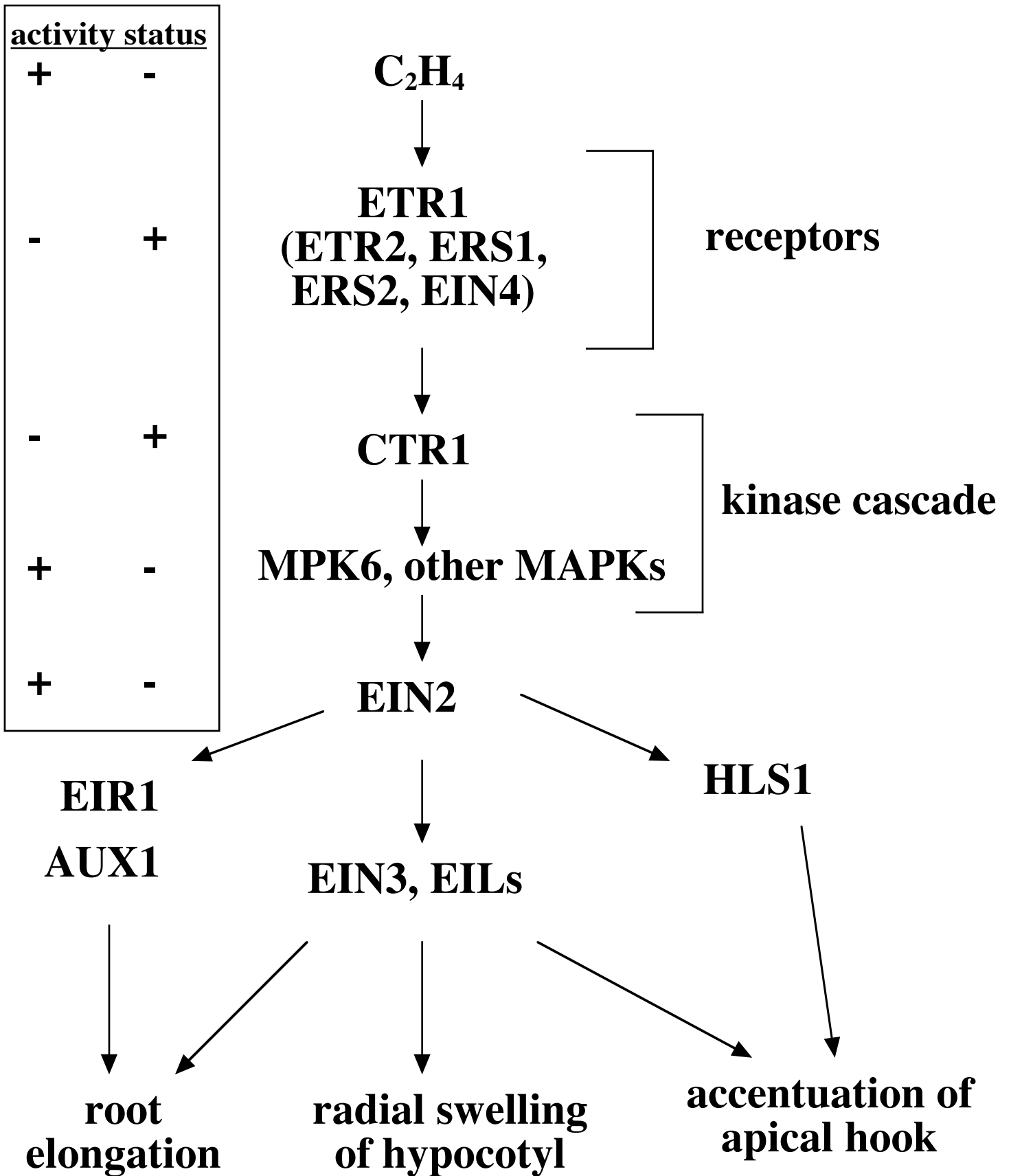
ctr1 mutants lack kinase activity

**->CTR1 activity inhibits downstream events in
ethylene signaling**

**CTR1 controls the activity of at least two MAPKs in
Arabidopsis**

**the MAPKs lie in between CTR1 and EIN2 in the
signalling pathway**

CTR1 inhibits the activity of the MAPKs



EIN5

mutants are insensitive to ethylene

mutation of ein5 partially suppresses constitutive triple response phenotype of ctr1 mutants - EIN5 acts downstream of CTR1

map-based cloning (Olmedo et al., PNAS Aug 18 early edition) -> EIN5 = XRN4

XRN4 - a 5'->3' exonuclease related to yeast XRN1

XRN4 degrades mRNAs (generally) in plants, and may be recruited to miRNA targets in the course of miRNA-mediated turnover (Souret et al., Mol. Cell 15, 173-183, 2004; Gazzani et al., Science 306, 1046-1048, 2004)

EIN5/XRN4 acts to decrease the levels of the F-box proteins that degrade EIN3 by degrading their mRNAs; mutations in ein5 lead to higher EBF1, EBF2 levels, lower EIN3 levels, diminished ethylene responses