BIOL2009 Lecture:

Mechanisms of hormone reception and signal transduction

OR

Why do we learn all this material??

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www.cilr.uq.edu.au for pdf files of material and research background

Mechanisms of hormone reception and signal transduction

Good reviews:

Chang, C. and J.A. Shockey (1999) The ethylene response pathway: Signal transduction to gene regulation. *Current Opinion in Plant Biology,* vol. 5, pg. 352-358

Also Gresshoff, P.M. (2004) Genetics and genomics of nodulation and nitrogen fixation. *Handbook of Plant Biotechnology*, Wiley and Sons, Eds. H. Klee and P. Christou

(get it from the CILR website as PDF)

Previous lectures:

1) measurement of eukaryotic transcription:

Northern blots, RT-PCR, Quantative RT-PCR, micro-array,

2) control of eukaryotic transcription:

transcriptional factors, recruitment,cis-trans elements, promoters, silencers

NOW:

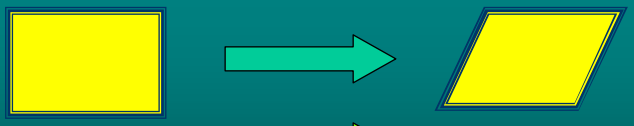
how are genes connected to phenotype?

Basic learning principles:

1. external and internal signals need to go across the membrane and affect the biochemistry / gene expression of the cell
2. This is achieved by receptor proteins
3. The receptor activity needs to be transmitted through a signal transduction cascade
4. This process often involves of protein phosphorylation

Basic learning principles:

Signal reception and transduction results from changes in molecular shape



no function → function

OR

function → no function

Basic learning principles:

Molecular shape is influenced by molecular interactions with small or large molecules (such as a peptide (e.g., insulin, human growth factor), a medium size molecule (estrogen; a steroid) or a really simple molecule such as nitric oxide (NO; Nobel Prize 2001) or ethylene (banana ripening)

Protein phosphorylation

Requires protein kinases

Kinases move a phosphate group from ATP to a protein at a serine, threonine or tyrosine residue. *Protein phosphatases reverse!!!*

Two types:

1) soluable (e.g., calcium dependent protein kinase; MAP kinase)

2) membrane bound: LRR receptor kinases

(e.g., GmNARK (see next lecture, EGFR)

Two examples of membrane receptors:

1) external regulation of infection and cell proliferation by the hormone ethylene receptor ETR1

2) internal systemic regulation of cell proliferation involving a LRR receptor kinase, GmNARK

1) The biological system

Legume plants form nodules on their roots that convert nitrogen gas to ammonia

fully nodulated root system

developing nodule

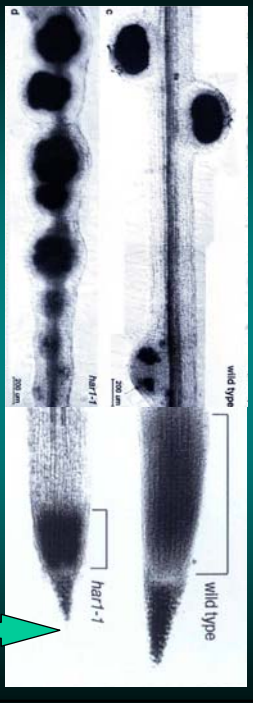
Good review:
Caetano-Anollés, G. and Gresshoff, P.M. (1991) Plant genetic control of nodulation in legumes. *Ann. Rev. Microbiol.* Vol. 45, pg. 345-382

2) Control:

The process is regulated by the plant, although it is induced by the bacterium *Rhizobium*;

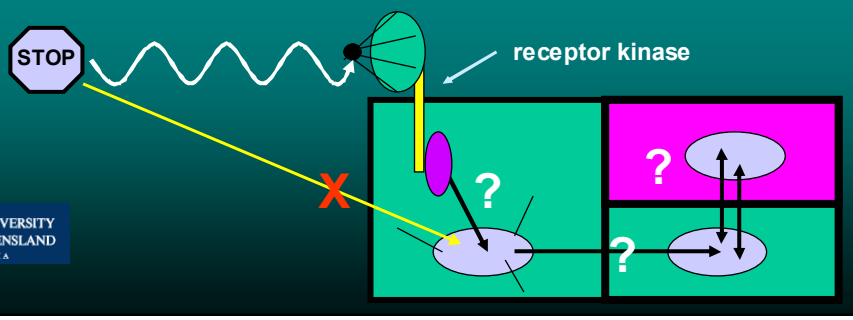
therefore plant gene regulation is essential as part of organ differentiation and function

A single gene defect alters cell division pattern



This implies gene regulation

(via transcriptional control) and the interaction of external signals (elicitors, hormones, growth regulators, metabolites) with receptors



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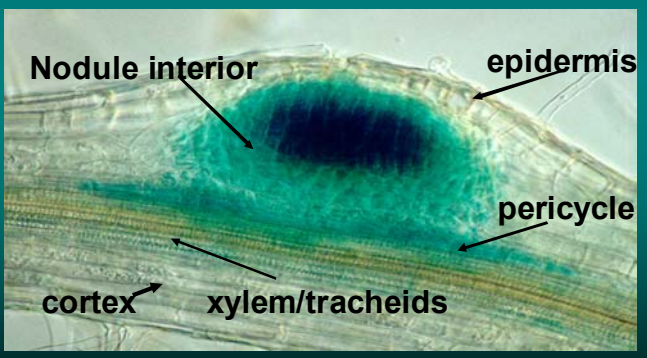
A new organ develops using 'existing' signal transduction pathways



1 mm

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3) The Process of Nodulation:

regulation of cell division and differentiation

Root

Nodule

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Rhizobium bacterium

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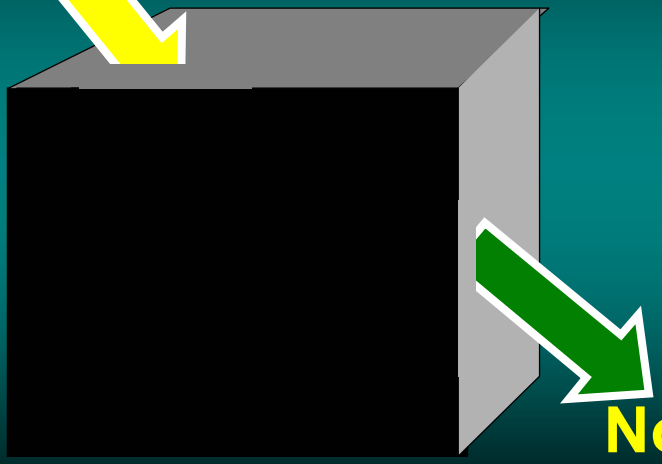
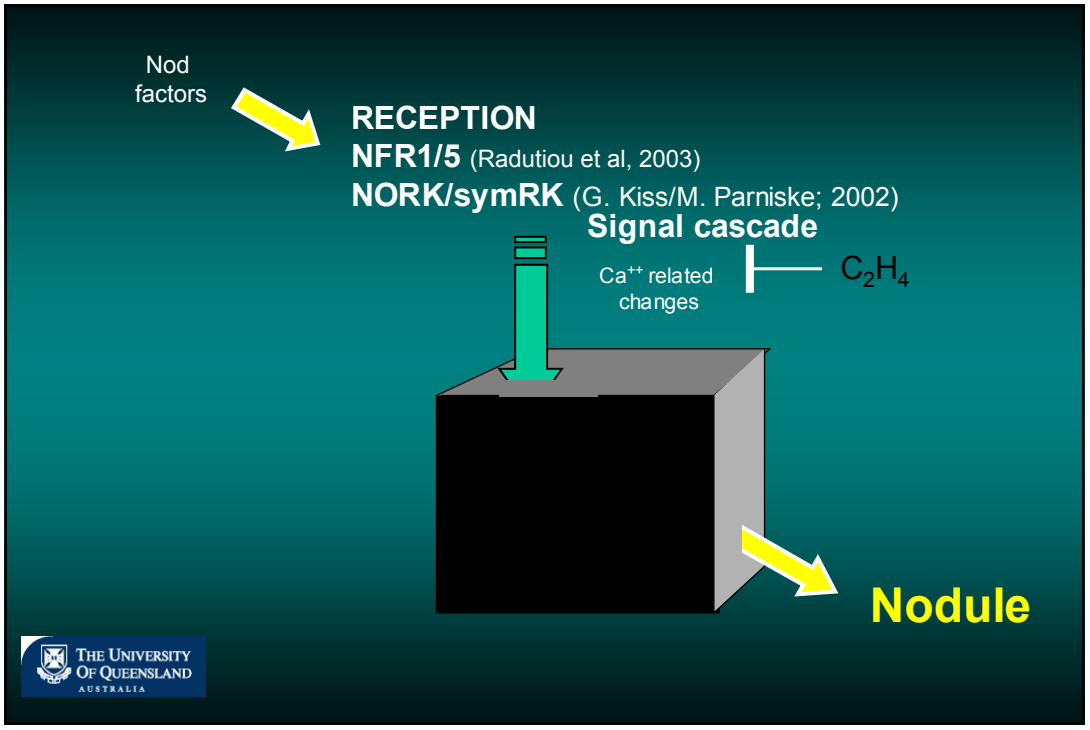
Nod factors

(lipo-oligo-saccharides; J. Denarie, 1990)



Nodule


Nod factors
RECEPTION
NFR1/5 (Radutiou et al, 2003)
NORK/symRK (G. Kiss/M. Parniske; 2002)
Signal cascade
Ca++ related changes
C2H4
Nodule
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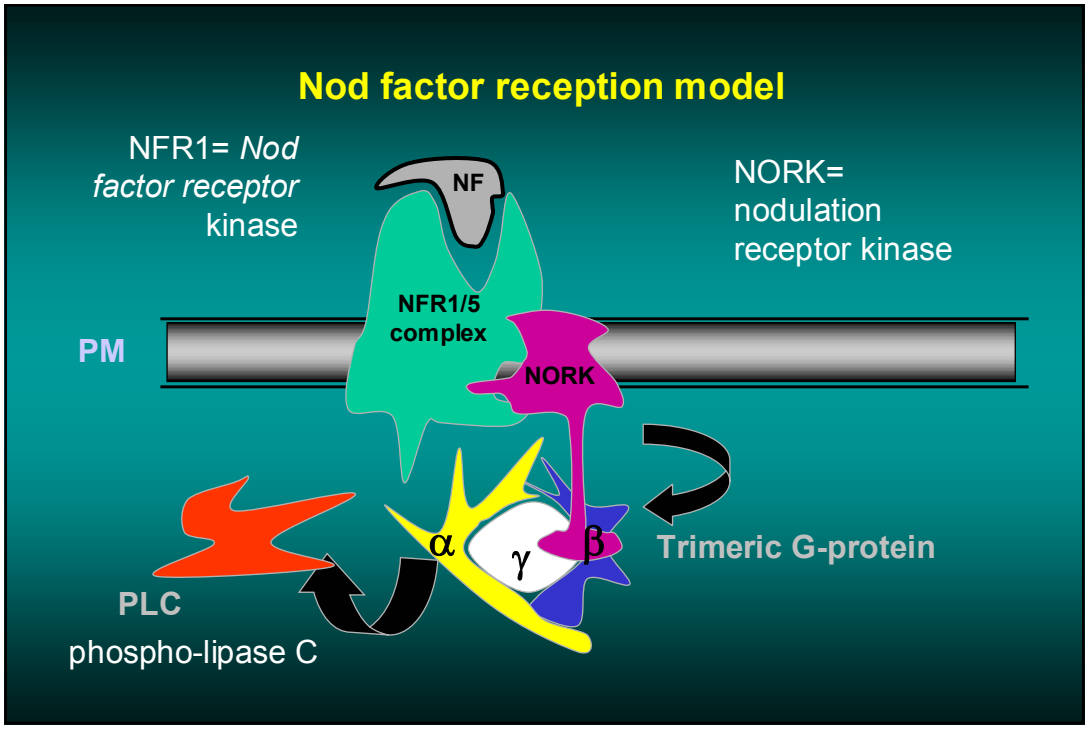
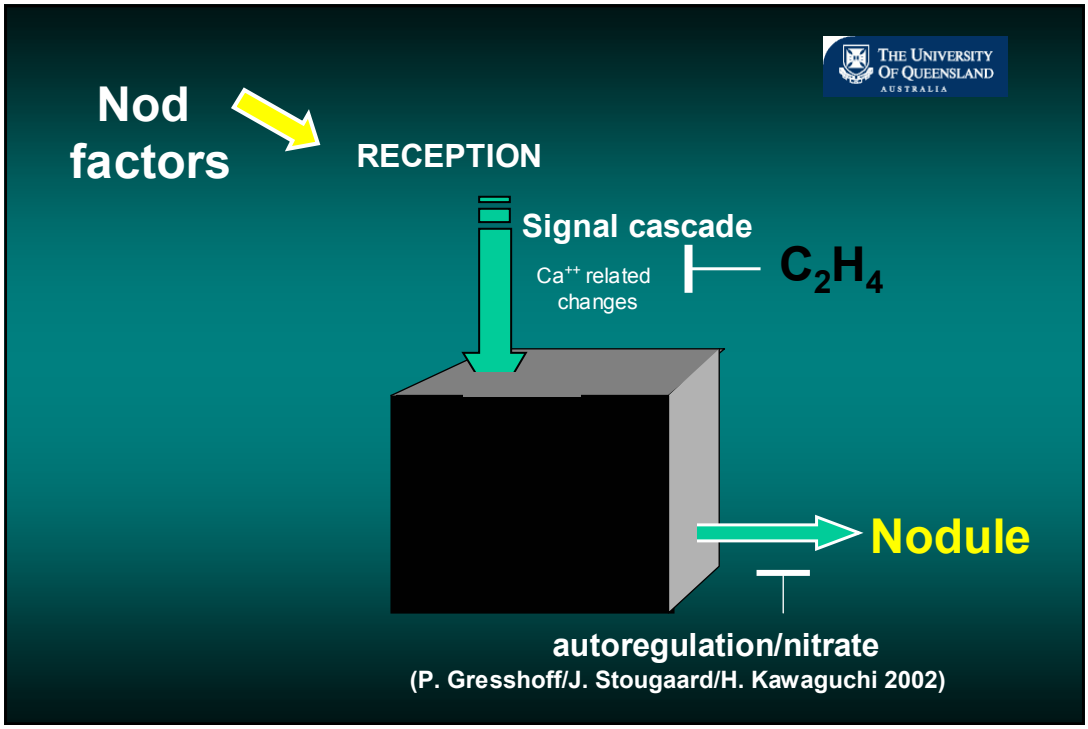
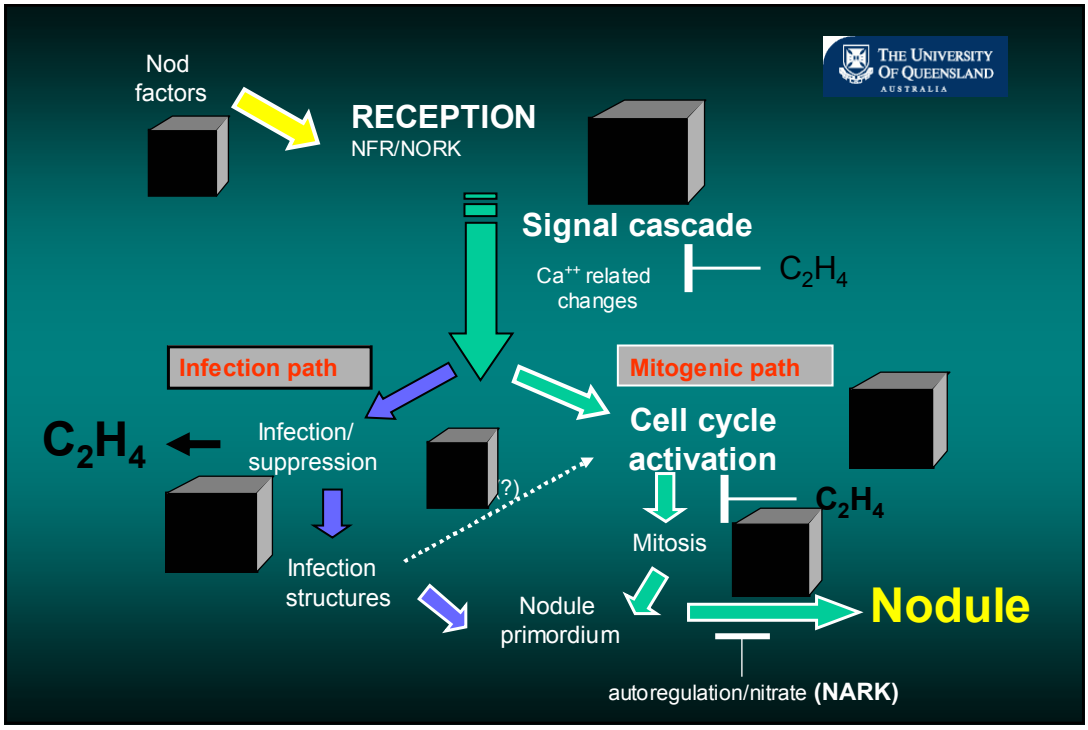

Nod factor reception model
NFR1= Nod factor receptor kinase
NF
NORK= nodulation receptor kinase
NFR1/5 complex
PM
NORK
α
γ
β
Trimeric G-protein
PLC
phospho-lipase C


Nod factors
RECEPTION
Signal cascade
Ca++ related changes
C2H4
Nodule
autoregulation/nitrate
(P. Gresshoff/J. Stougaard/H. Kawaguchi 2002)


Nod factors
RECEPTION
NFR/NORK
Signal cascade
Ca++ related changes
C2H4
Infection path
Mitogenic path
C2H4
Infection/ suppression
Cell cycle activation
(?)
C2H4
Mitosis
Infection structures
Nodule primordium
Nodule
autoregulation/nitrate (NARK)

Many plant mutations, representing key genes, have been isolated that govern:

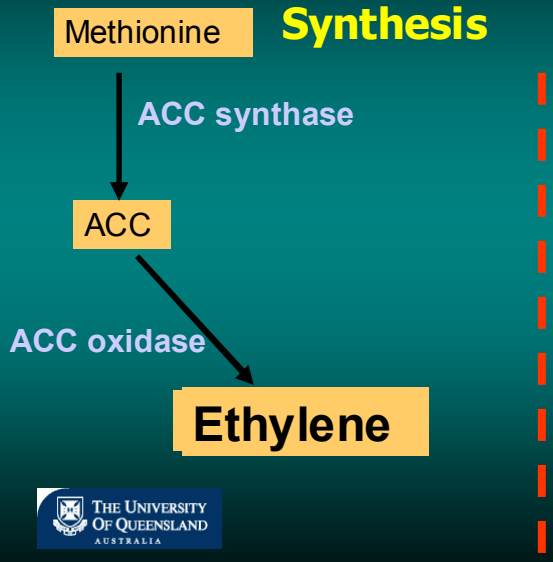
1) Reception
2) Signal transduction
3) Mitotic activation
4) Nodule growth
5) Nodule function

1) **Mutations in the ethylene synthesis or perception should affect legume development**

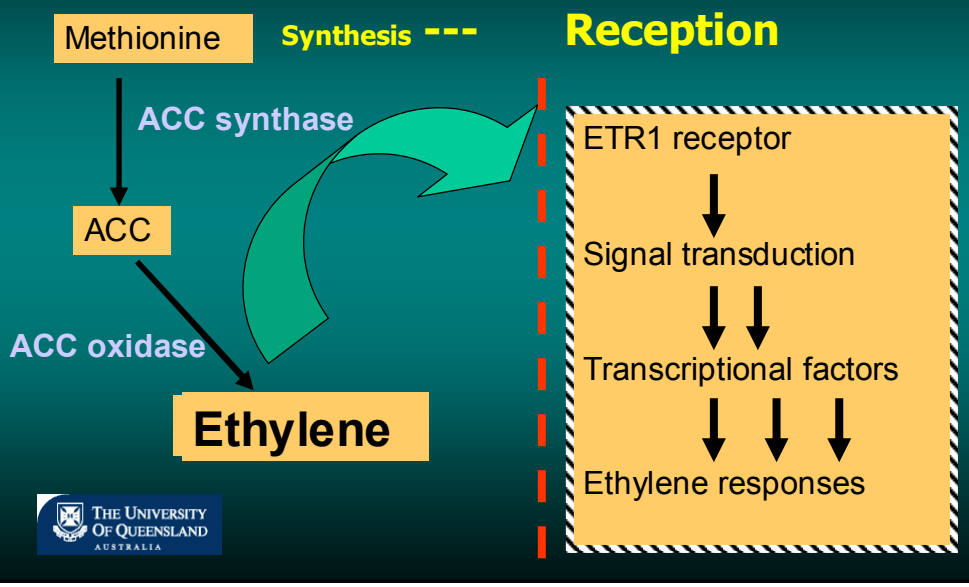
2) **Ethylene is a gaseous hormone related to nitric oxide**

3) **Ethylene or its precursor ACC should develop positional developmental gradients**

Ethylene action in all plants:



Ethylene action in all plants:



Mutations also alter the ethylene response

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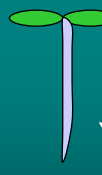
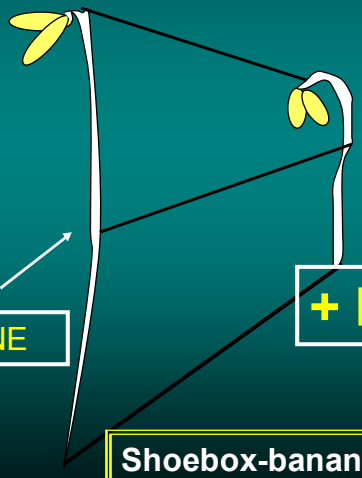
Triple Response Selection

Germination in the dark

Light control





Short thick root

Short hypocotyl

Increased hook

+ ETHYLENE

- ETHYLENE

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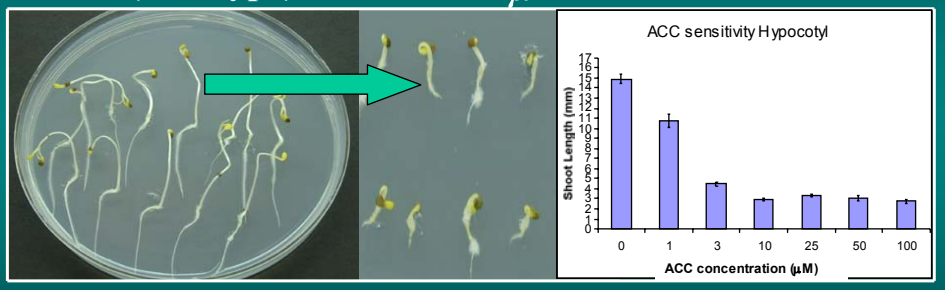
Shoebox-banana peel experiment

Triple Response in the dark

Test organism: *Lotus japonicus* (a legume)

Gifu (wild type)

Gifu
ACC 50 μM



Allowed easy selection of mutants and transgenics

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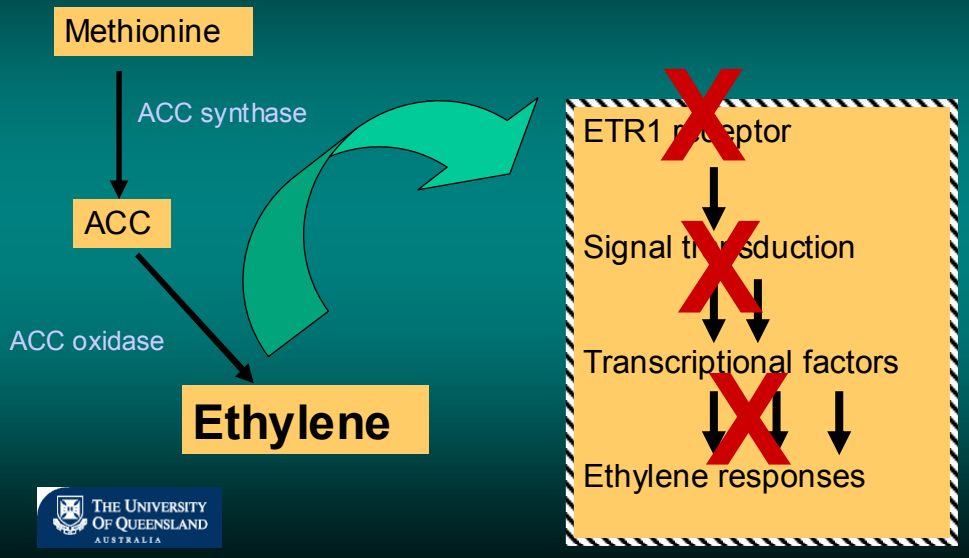
Ethylene action altered by mutation



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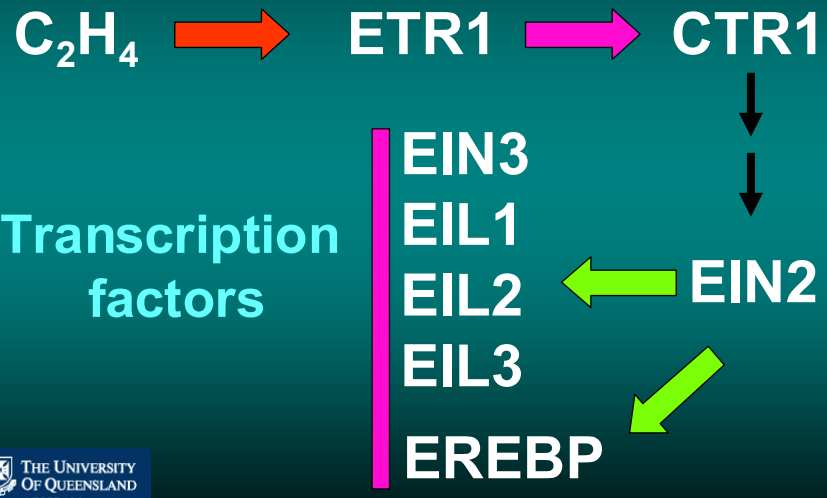
Ethylene signal cascade is defined by mutants in ethylene perception



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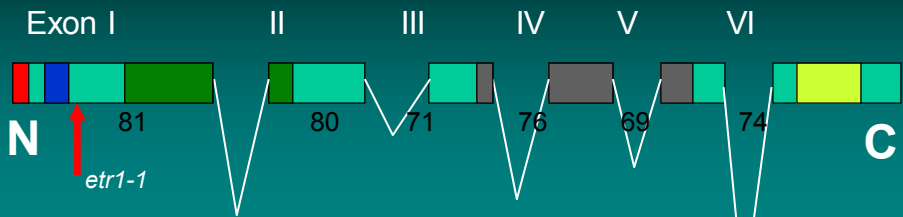
Lotus japonicus Ethylene receptor *ETR1*



- ethylene binding
- three transmembrane/Cu^{++} binding
- GAF domain
- histidine protein kinase
- aspartate receiver (-OH)

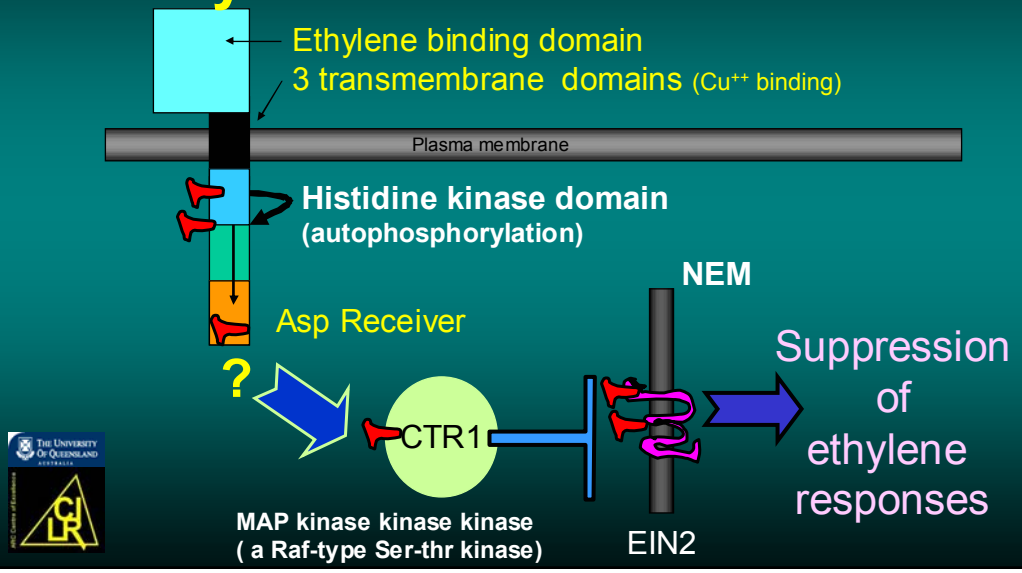
4.30 kb Lj
2.65 kb At

(GAF: cGMP phosho-diesterase, adenylate cyclase, FhlA protein)

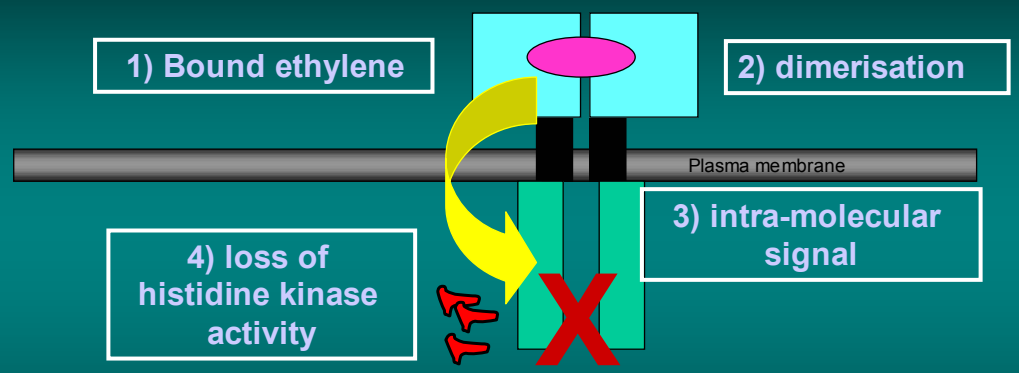
- same exon intron position as AtEtr1
- all but two intron same size as AtEtr1

Ethylene Receptor ETR1

No ethylene! - a two-component receptor kinase



Ethylene Reception



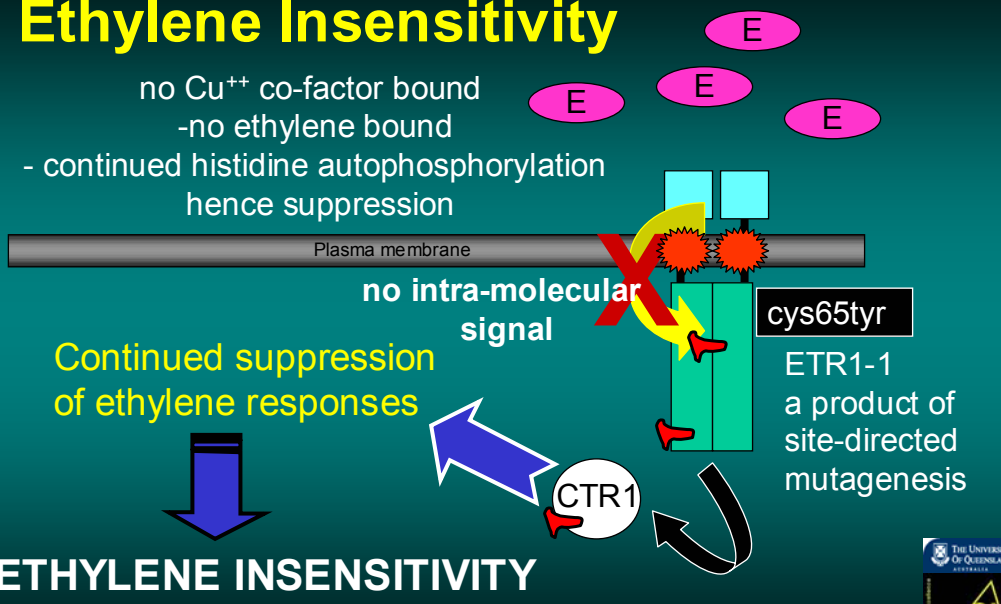
no signal to CTR1

NO suppression of ethylene responses

NO suppression of EIN2

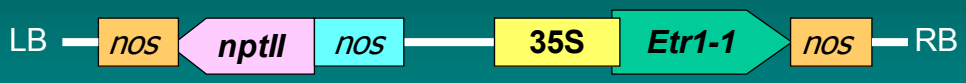
Ethylene Insensitivity

no Cu^{++} co-factor bound
-no ethylene bound
- continued histidine autophosphorylation
hence suppression



Genetic Engineering of Ethylene Insensitivity

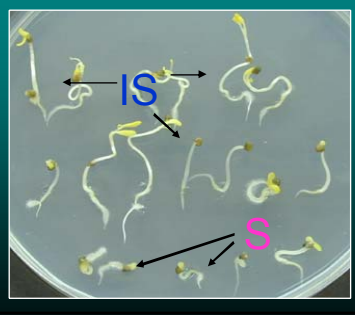
- use *Agrobacterium* gene transfer



selection gene

transgene under constituitive control

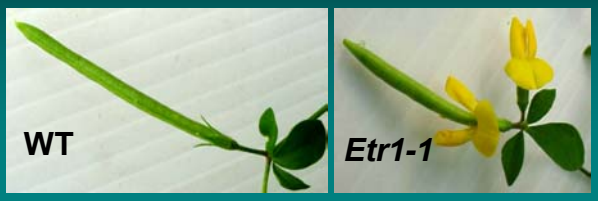
- *Agrobacterium tumefaciens* LBA4404
- hypocotyl transformation
- (Stiller *et al*, 1997; Lohar *et al*, 2001)
- geneticin selection



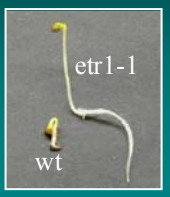
e.g., 1EG4-23 (ACC 50 μM)

Characteristics of *L. japonicus* Ate*tr1-1*

(i) Delayed flower/fruit maturation



(iii) No triple response

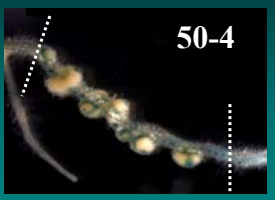


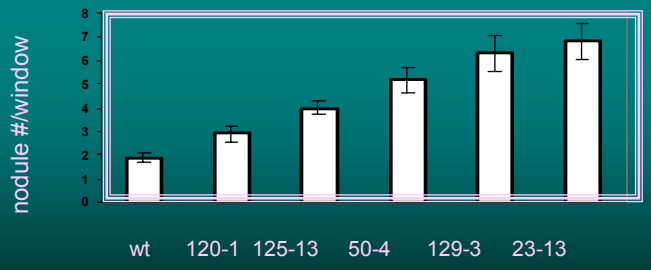
(ii) Dominant inheritance

Lines	Ins	Sen	Ins/Sens	χ^2
G23	102	34	3.0:1	0.00
G4	79	22	3.6:1	0.56
G125	108	37	2.9:1	0.02

(iv) More nodulation

Ethylene insensitivity changes the 'hits per window'

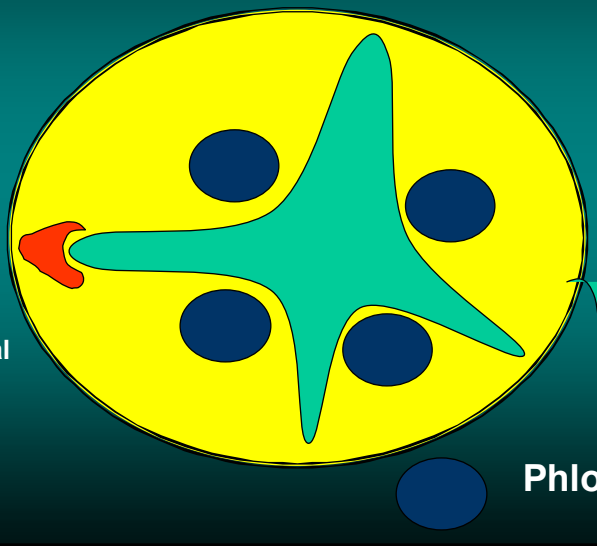




Conclusion: more nodules per window, but still subject to autoregulation by NARK

Positional Information and Plant Vasculature

Zone of cell divisions in the pericycle close to xylem pole leading to nodule or lateral root formation



Origin of Nodule Foci Relative to Vasculature

line	number (%) of nodule foci opposite xylem	in between
Gifu	105 (96)	5 (4)
23-13	103 (68)	48 (32)
129-3	34 (69)	15 (31)
15-1	49 (73)	18 (27)

- **ACC oxidase high close to phloem**
- **20° sector of meanline (= 40°total)**

AtETR1-1 causes 4% -> 30% shift

Conclusions:

1. Receptors transmit information
2. Shape change is facilitated by phosphorylation
3. Proteins interact
4. Soluble and membrane receptors exist
5. Transcription is regulated after signal transduction
6. Ethylene is a well-researched signal system
7. Ethylene signalling has parallels in animals and bacteria

8. How do we discover and transfer such genes?
9. How is cell division regulated once it is induced?

NEXT LECTURE