

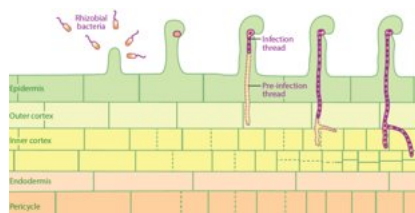
BIOL 377 – Cell-cell communication

Week 13

Readings – see papers uploaded to canvas

Oldroyd GED. 2007. Nodules and Hormones. *Science* 315:52-53.

Murray JD, Karas BJ, Sato S, Tabata S, Amyot L, Szczylowski K. 2007. A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. *Science* 315:101-4.



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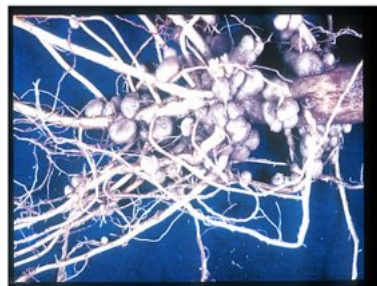
Nodulation in Legumes – how plants co-operate with nitrogen fixing rhizobia

legumes (soybean, pea, peanut) - fix nitrogen

formation of nitrogen-fixing root nodules

- convert N_2 to ammonia
- root hairs - recognition system
 - allow entry of rhizobia
 - exclude other microbes
- signal from rhizobia
 - Nod (nodulation) factors
- receptors on plant root hairs

Figure 6. Picture of a nodulated soybean root.

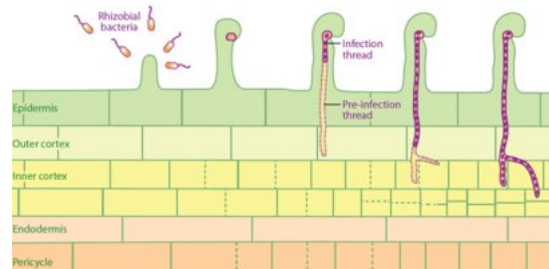


<http://www.soyroothair.org/biology-of-nodulation.html>

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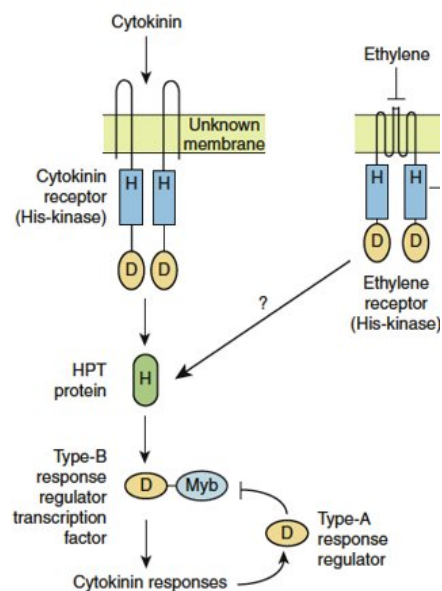
Screens for Nodulation mutants revealed components of the cell-cell communication pathway

- mutant screens
 - non-nodulating
 - hyper-nodulation
- pea (*Pisum sativum*), soybean (*Glycine max*)
- *Lotus japonicus*, alfalfa (*Medicago truncatula*)
 - model organisms - mapping, genome sequencing
 - map and isolated mutant genes



Oldroyd et al. 2011. The Rules of Engagement in the Legume-Rhizobial Symbiosis. *Annu. Rev. Genet* 45:119–44

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Screens for Nodulation mutants uncovered kinases!

- hypernodulation mutants (*L. japonicus*)
 - e.g. **HAR1** - LRR Receptor kinase
 - excessive nodulation - detrimental to host plant
 - feedback control suppress nodules in younger tissues
- non-nodulating mutants (*L. japonicus*)
 - e.g. **NFR1 and NFR5** - LysM Receptor kinases
 - e.g. **SYMRK** – LRR receptor kinase
- many other signalling components also identified through these screens

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Hyper-nodulation mutant makes too many nodules

- Soybean hypernodulation mutant
- mutation in **NARK** gene
- (soybean version of **HAR1**)

Figure 1. Searle et al (2003).
Long-distance signaling in
nodulation directed by a
CLAVATA1-like receptor
kinase. *Science* 299:109-12.



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Non-Nodulating mutants show defects in root hair response to rhizobia inoculation

- non-nodulating mutants - *Lotus japonicus*
 - *nfr1* and *nfr5*
 - *symRK*

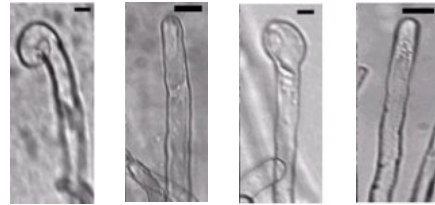


Figure 1. Root hair response after *M. loti* inoculation.
(*nfr5-1* mutant looks the same as *nfr1-1*)

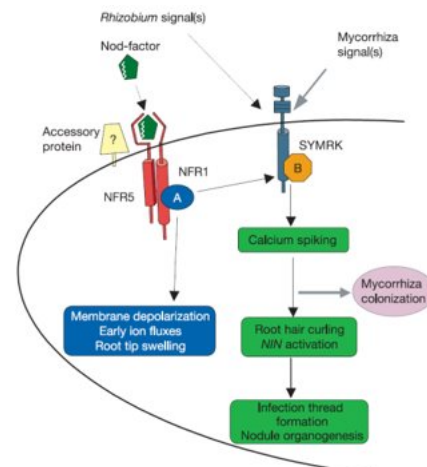
Radutoiu et al. (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425:585-92.

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LysM Receptor Kinases have an extracellular domain that recognizes N-acetylglucosamine

- NFR1 and NFR5 - LysM receptor kinase family
- LysM domain
 - enzymes - bind polymers with N-acetylglucosamine
- Nod-factors = lipochito-oligosaccharides
 - N-acetylglucosamine backbone

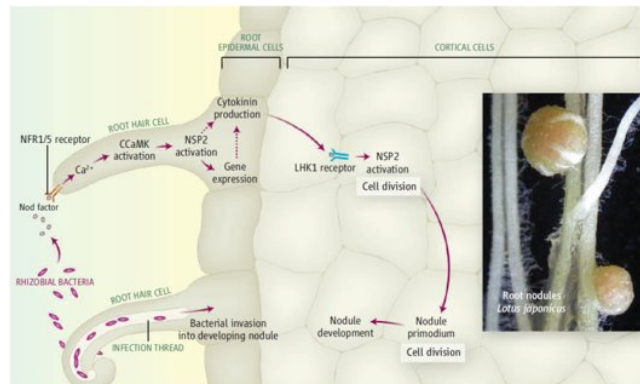
Fig 7. Working model for the functional role of NFR1 and NFR5 in plant perception of rhizobial and mycorrhizal signals.



Radutoiu et al. (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425:585-92.

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Nodulation requires two pathways that work in parallel



- 1) bacterial infection thread formation – root epidermis
- 2) root nodule organogenesis – root cortical cells
- mutants have uncoupled these pathways (e.g. *hit1-1*)

Oldroyd GED. 2007. Nodules and Hormones. *Science* 315:52-53.

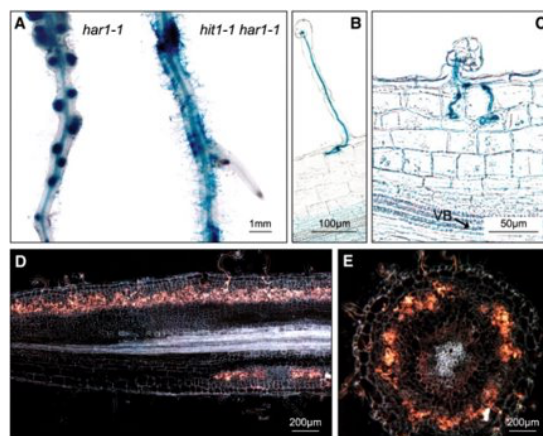
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Infection and nodulation events in wild-type and mutant plants

Fig 1. Root segments stained for β -galactosidase (LacZ)

activity 10 dai (**A to C**)
and

14 dai (**D and E**) with *M. loti*



Murray et al. 2007. *Science* 315:101-4.

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Infection and nodulation events in wild-type and mutant plants

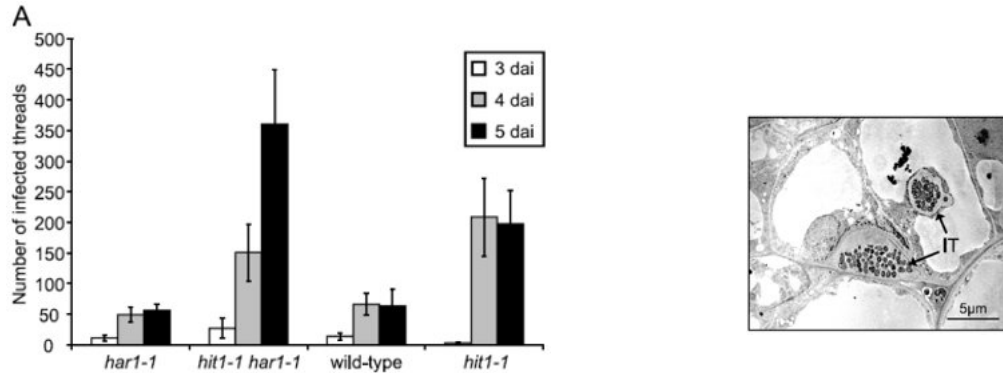


Fig S1. (A) Infection threads were counted at 3, 4 and 5 days after infection with *M. loti* carrying a hemA::lacZ reporter gene fusion.

(D) TEM image of a 2 µm thin section of *hit1-1* root showing a large pocket of *M. loti* that are confined to infection threads

Murray et al. 2007. Science 315:101-4.

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Nodulation phenotype of the *hit1-1* mutant

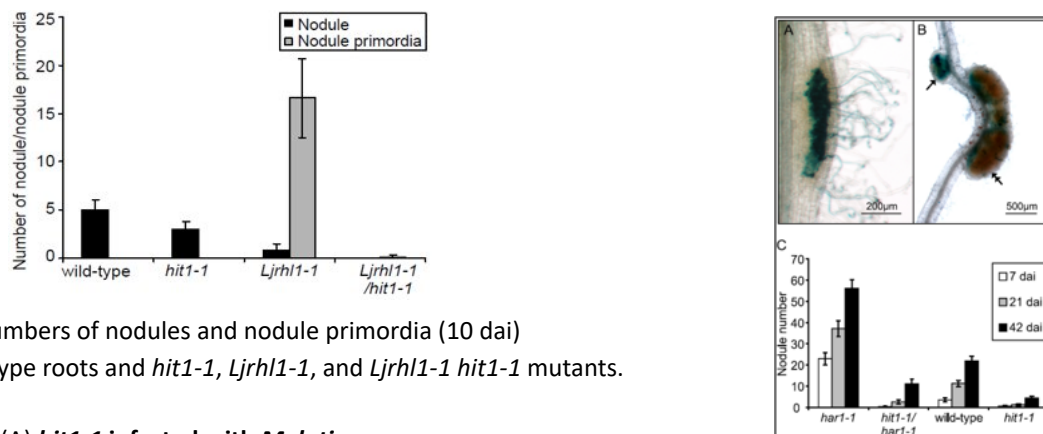


Fig. 2. Numbers of nodules and nodule primordia (10 dai) on wild-type roots and *hit1-1*, *Ljrh1-1*, and *Ljrh1-1 hit1-1* mutants.

- Fig S2. (A) *hit1-1* infected with *M. loti*.
- Abundant infection events 14 dai. (large number of IT descend toward root cortex).
- (B) *hit1-1* infected with *M. loti*. Rare successful infection events (14 dai) lead to formation of grossly enlarged and deformed nodules (double arrowhead) and only intermittently to wild-type like nodules (arrow).
- (C) Number of nodules formed on the root of *har1-1*, *hit1-1 har1-1*, wild-type, *hit1-1*

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HIT1 gene structure, expression, & yeast complementation

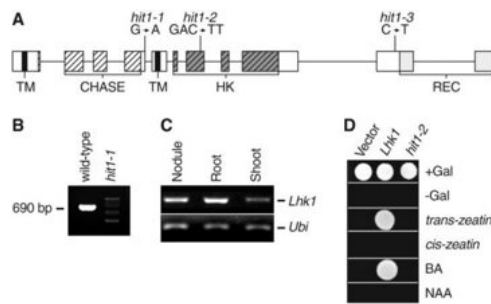


Fig. 3. (A) The exon (box)/intron (line) structure of the *Lhk1* gene
(B) RT-PCR of *hit1-1* mutant
(C) RT-PCR of *Lhk1* in various *L. japonicus* tissues
(D) *Lhk1* complementation of yeast *sln1* mutant

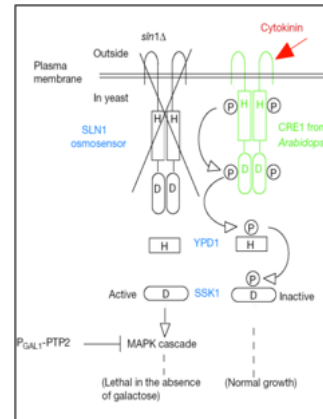


Fig 4. Yeast complementation system with cytokinin Histidine Kinase.

Inoue et al. 2001. Identification of CRE1 as a cytokinin receptor from Arabidopsis. *Nature* 409:1060-3.

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Cytokinin responses in the model for nodulation

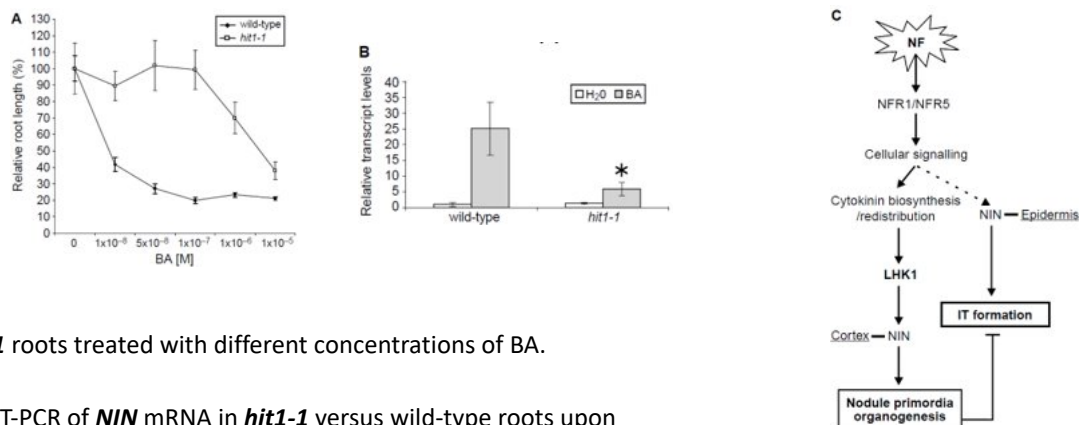


Fig 4.

hit1-1 roots treated with different concentrations of BA.

(B) qRT-PCR of *NIN* mRNA in *hit1-1* versus wild-type roots upon exogenous application of 50 nM BA

(C) The proposed role of **LHK1** in NF induced nodule organogenesis

Murray et al. 2007. *Science* 315:101-4.

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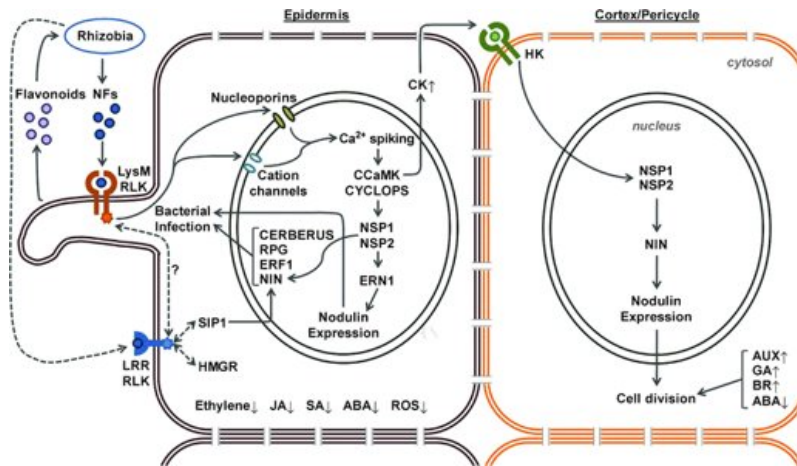


Figure 2. Molecular events associated with the early stages of nodulation. Legume roots initiate nodulation by exuding flavonoids into the rhizosphere. This attracts compatible rhizobia to the root and stimulates them to produce nod factors (NF). NF is reported to be perceived at the epidermis by a leucine-rich repeat receptor-like kinase (LRR RLK) which triggers a number of downstream events involved in bacterial infection. Concomitantly, NF is also reported to be perceived by two LysM RLKs, which leads to the NF signaling cascade, cortical/pericycle cell divisions, and bacterial infection events. A mobile signal (possibly the phytohormone cytokinin) is presumed to relay NF perception from the epidermis to the cortex where it initiates cell divisions that give rise to the nodule primordia. The levels of many other hormones are also tightly controlled throughout nodule organogenesis and have roles in regulating nodule initiation and development.

Ferguson et al. 2010. Molecular analysis of legume nodule development and autoregulation. *J Integr Plant Biol* 52:61-76

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Can we make cereals that fix nitrogen?

Table 1
Common SYM genes in rice

Legume sym	Rice sym	Phenotype of legume root symbiosis mutant transformed with rice orthologue
SymRK	OsSymRK ^a	Myc+, Nod-
NUP85	OsNUP85	n.d.
NUP133	OsNUP133	n.d.
NENA	OsNENA	n.d.
CASTOR	OsCASTOR	Myc+, Nod+
POLLUX	OsPOLLUX	Myc-, Type I, Inf-
CCaMK	OsCCaMK	Myc+, Nod+
CYCLOPS	OsCYCLOPS	Myc+, Nod+
VAPYRIN	OsVAPYRIN	n.d.

^a OsSymRK encoding SYMRK version with two LRRs only and a short N-terminal region;

Charpentier M, Oldroyd G.
2010. How close are we
to nitrogen-
fixing cereals? *Curr Opin Plant
Biol* 13:556-64

- “Engineering nitrogen-fixing cereals is essential for sustainable food production for the projected global population of 9 billion people in 2050.”
- After *NFR1/NFR5*: at least 8 genes functioning in both root nodule symbiosis and arbuscular mycorrhizal (AM) symbiosis (interaction between fungi and most plants)
- What genetic engineering is needed for recognition of nitrogen-fixing bacteria, bacterial colonisation, and nodule organogenesis?

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