Nitrogen-fixing symbiosis

1. Overview of symbiosis
2. Bacterial symbiosis genes
3. Plant response to signals

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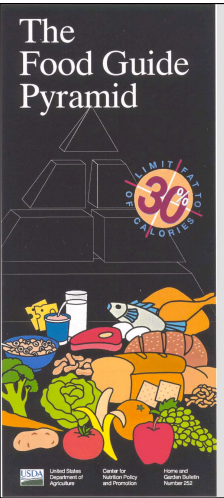
Stanford University

Nitrogen-fixing symbiosis:

1. Overview of symbiosis
   (a) root nodule formation and function



Carbohydrates

Fats

Proteins

Vitamins

Fiber

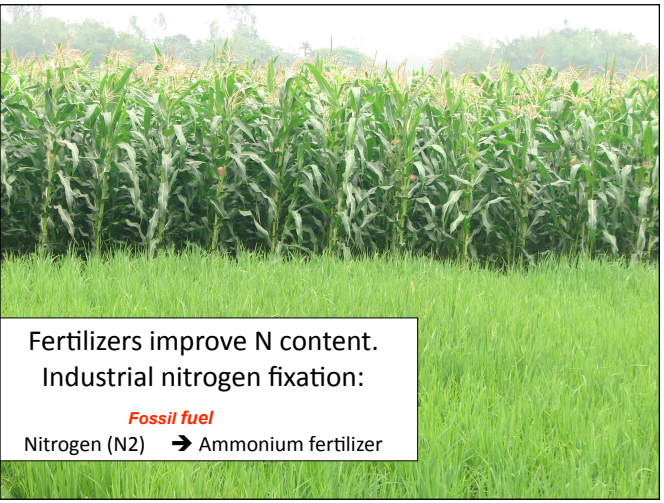
Protein malnutrition is a devastating worldwide problem

PROTEIN

Nitrogen (N) is a critical element

Where do animals get N?
From plants

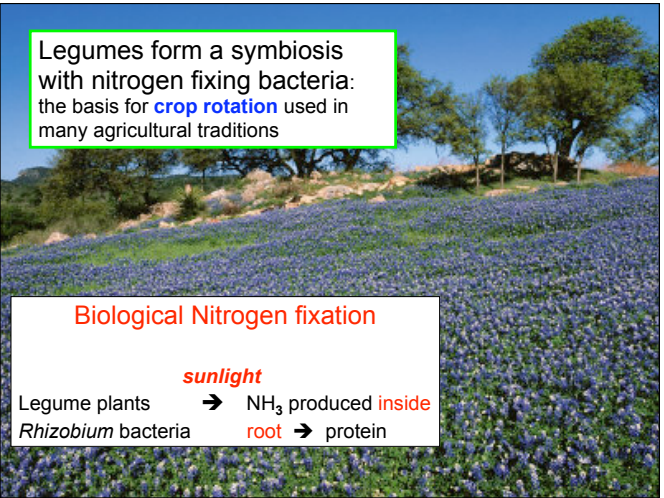
Where do plants get nitrogen?
A: Usually, from soil minerals. However, soil N becomes depleted over time. In poor soil, plants struggle to grow.



Nitrogen is everywhere in the air; but it is inert and unavailable to plants

Can be chemically combined by “Haber process”

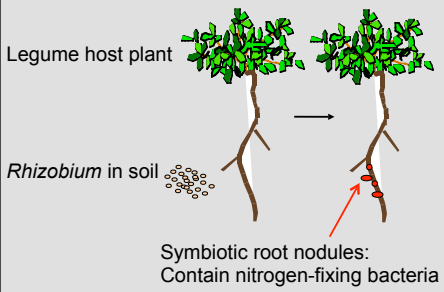
Historical note - process developed by German chemist Fritz Heber, to allow Germany to make explosives from nitrate



NH3 ammonium produced by bacteria

Plant assimilates this into amino acids, and can make protein

Legumes form a symbiosis with nitrogen fixing bacteria



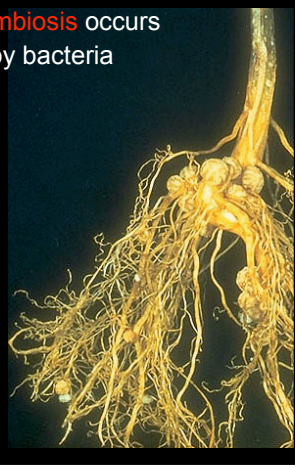
Replaces need for N fertilizer

How unique is this? In terms of plants, the events and the pattern of occurrence are remarkable

But symbiosis as a general phenomenon happens elsewhere

Rhizobium-legume N_2 fixing symbiosis occurs in root nodules stimulated by bacteria

- Basis for crop rotation, decrease need for fertilizer
  - Bacteria fix Nitrogen
  - Plant Photosynthesis provides energy
- Occurs in root nodules
- Specific and complex
- Restricted to legume plant family



Note bacteria can be free living

Legumes: agriculture and ecosystem

Food and forage:
legume crops provide high protein seeds and leaves

- Soybean, cowpea
- Common bean
- Pea
- Alfalfa, Clover

Legumes are important in both agriculture and in natural environments

Here are a few examples from agriculture

Legumes: agriculture and ecosystem

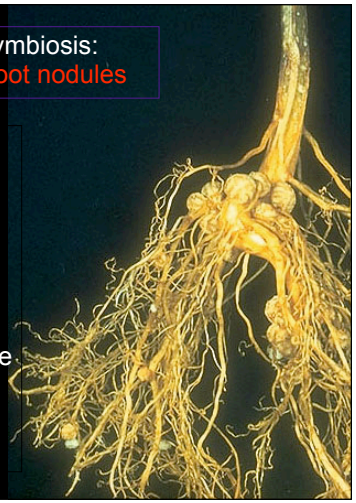
Legume trees, vines, and shrubs provide nutrition and stability in harsh environments

Rhizobium-legume symbiosis: N_2 fixation happens in root nodules

Complex developmental process requires genes of both bacteria and plant.

It is species-specific.

Bacterium and plant each respond to signals from the other.



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Can see roots – this is soybean

Large growths are nodules

Rhizobium-legume symbiosis: ***specificity***

Plant	Bacteria
Soybean	*Bradyrhizobium japonicum*
Soybean	*Sinorhizobium fredii*
Pea	*Rhizobium leguminosarum*
Trefoil	*Mesorhizobium loti*
Alfalfa	*Sinorhizobium meliloti*

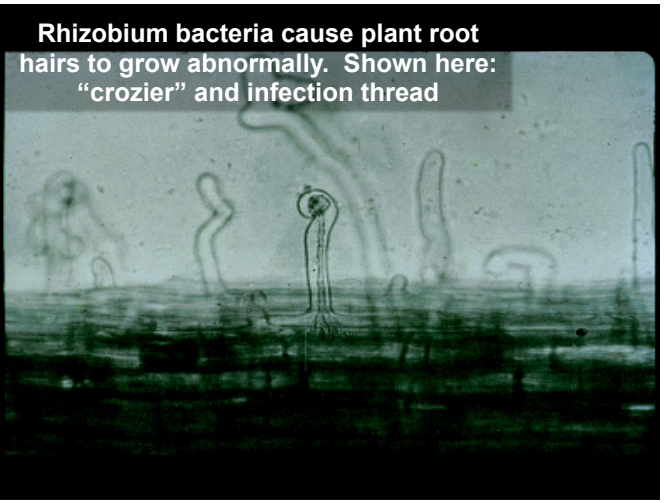
Not only is symbiosis restricted to legumes, but within legumes there is an additional level of selectivity

Nodule formation: complex development

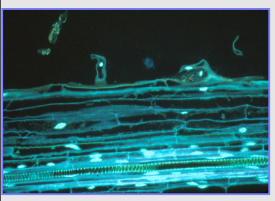
- Novel events occur in both bacteria and plants:
  - Cells, tissues, organs
  - Physiology and biochemistry
  - Gene expression
- Study by multiple approaches
  - Microscopy
  - Genetics
  - Biochemistry

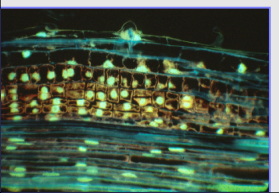
Plant never makes a nodule except in presence of bacterium

Look at some of the remarkable events that happen when a plant senses its correct symbiotic partner

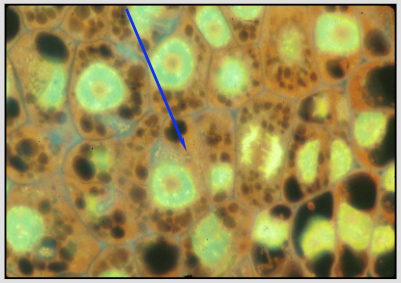


Nodule development: early nodulation.
Rhizobium stimulates plant cell divisions:
➔ nodule primordium



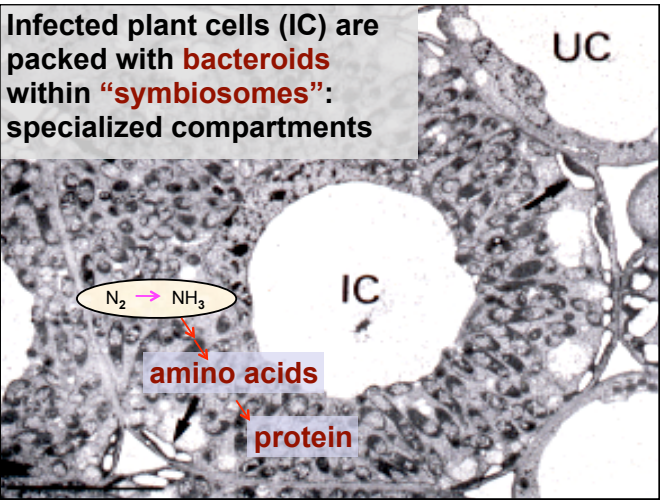


Nodule development: infection.
Infection thread delivers bacteria to newly formed plant host cells.

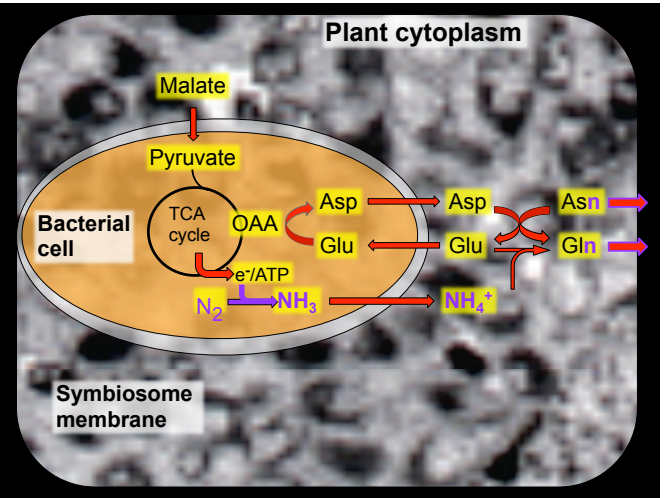


Bacteria are released into membrane sacs derived from *plant plasma membrane*

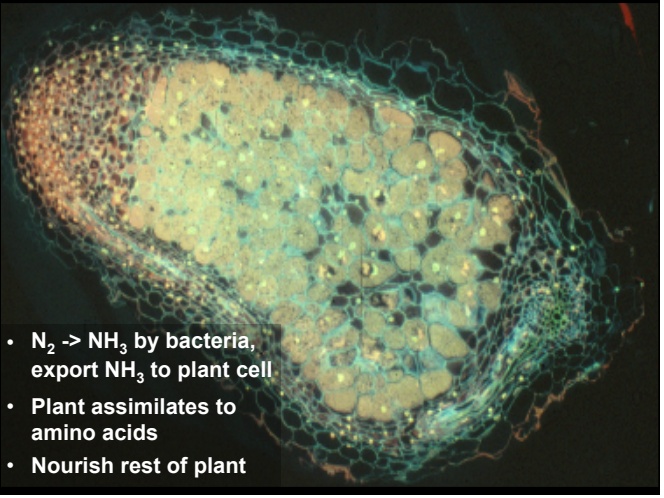
Cell walls and membranes – big unknowns

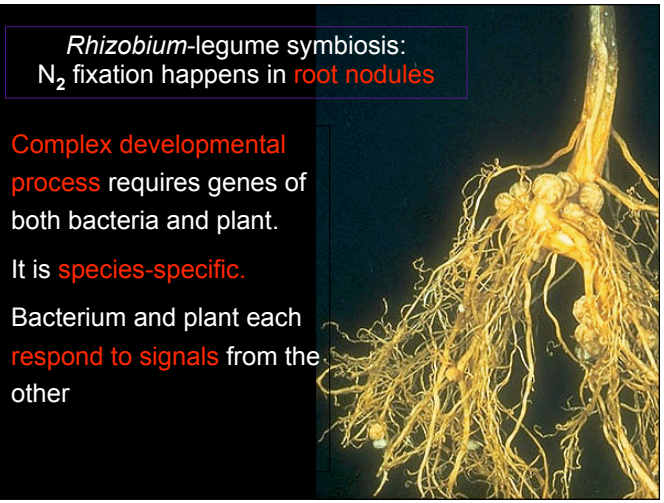


Differentiation of "peribacteroid membrane" creates specialized compartment to support nitrogen fixation by bacteroids inside infected plant cell



Infected root nodule cells have some of the most exquisitely engineered compartments and metabolics links anywhere in the living world. This infected cell is a masterpiece. Anyone who is interested in cell biology topics such as protein trafficking and compartmentation signals would do well to check out this system of study.





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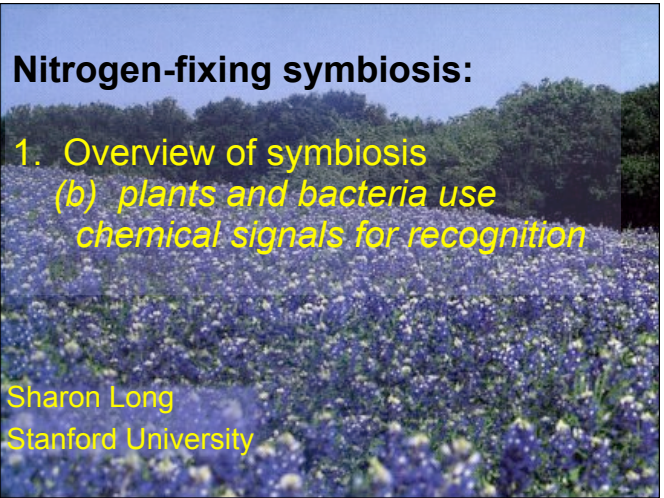
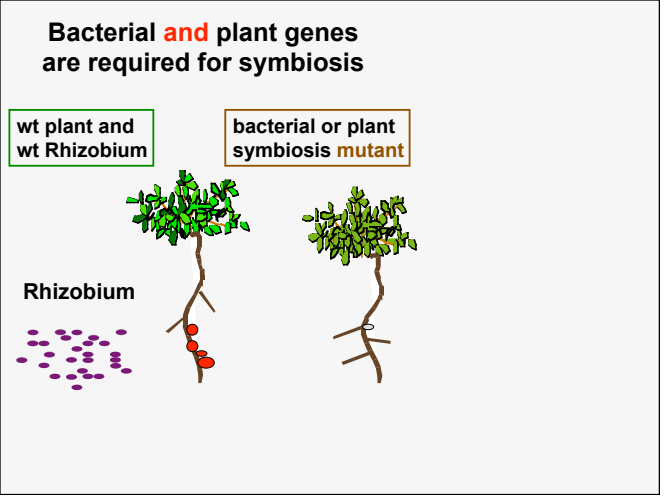
Can see roots – this is soybean

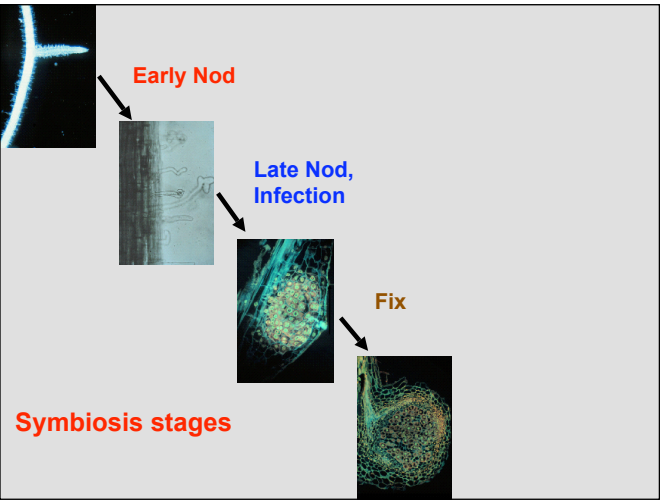
Large growths are nodules

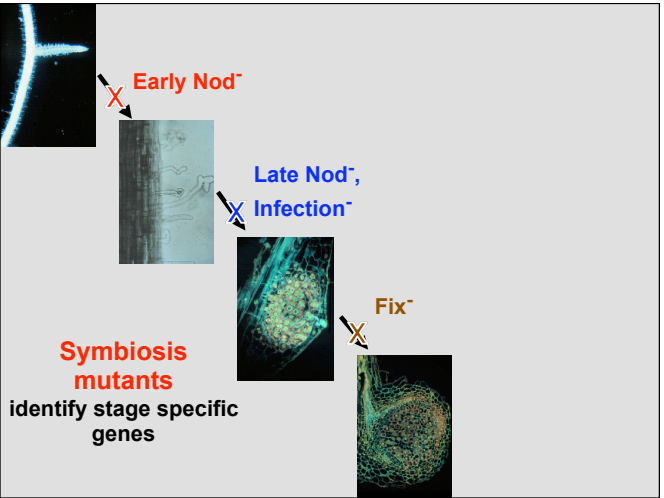


We have just seen that nodules are highly complex, and species specific. In the next segment, I will provide some background on how the two partners signal each other in the very initial stages of symbiosis.

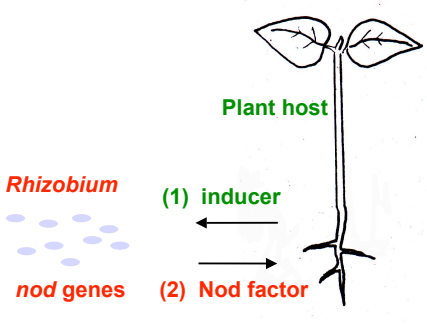
Plants without N get yellow – chlorophyll is made from an amino acid precursor,



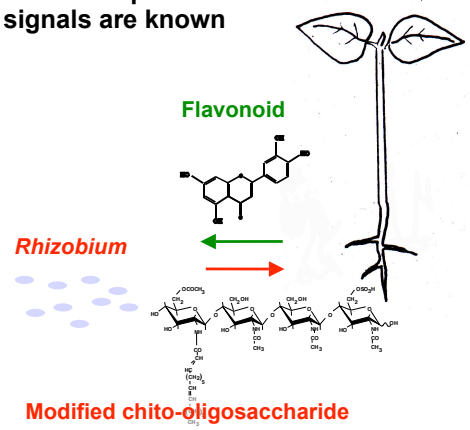


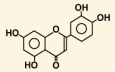
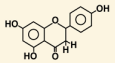
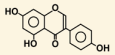


Overview: early nodulation involves signal exchange



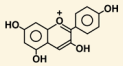
The initial plant and bacterial signals are known



Plant	Example of Flavonoid	
Alfalfa (*Medicago sativa*)		luteolin
Pea (*Pisum sativum*)		naringenin
Soybean (*Glycine max*)		genistein

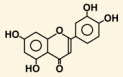
Plant flavonoids: thousands of different compounds with diverse - and incompletely understood - functions

glass of red wine →



anthocyanin pigment

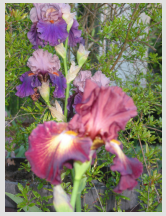
legume root →



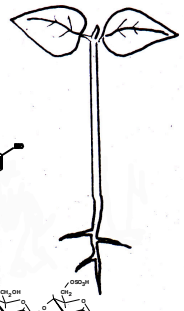
signal to *Rhizobium*

Plants and chemistry

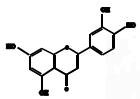
- Plants have no nervous system, motility
- How to attract pollinators? - pigments, aroma
- Recognize, defend against predators? - toxins, inhibitors



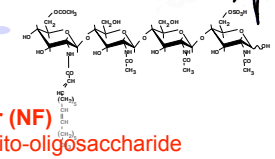
The initial plant and bacterial signals are known



Flavonoid

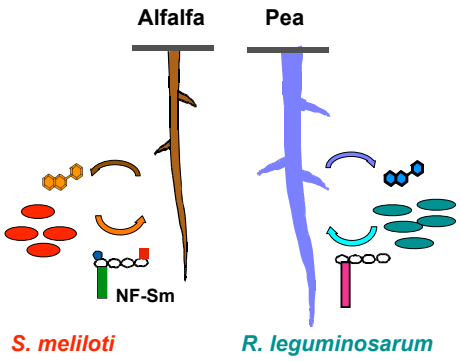


Rhizobium

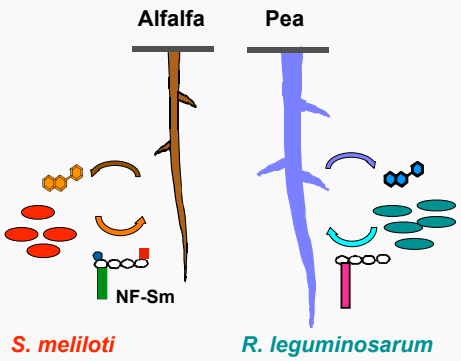


Nod Factor (NF)
Modified chito-oligosaccharide

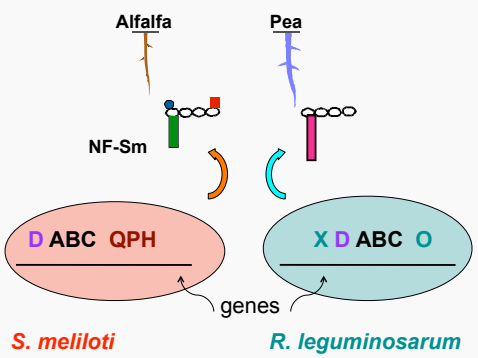
Early signals are host specific

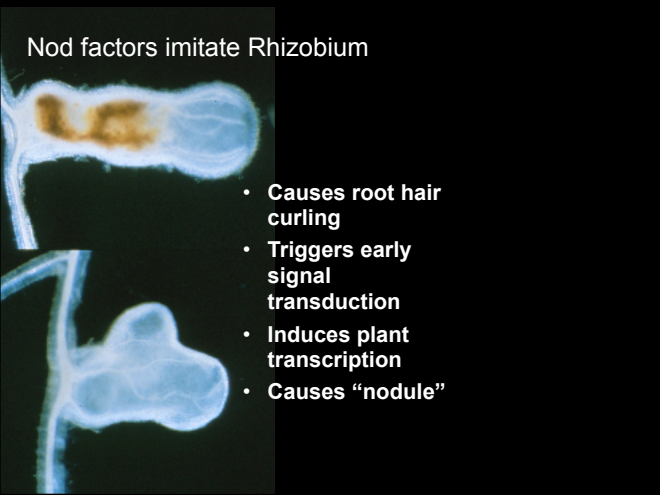


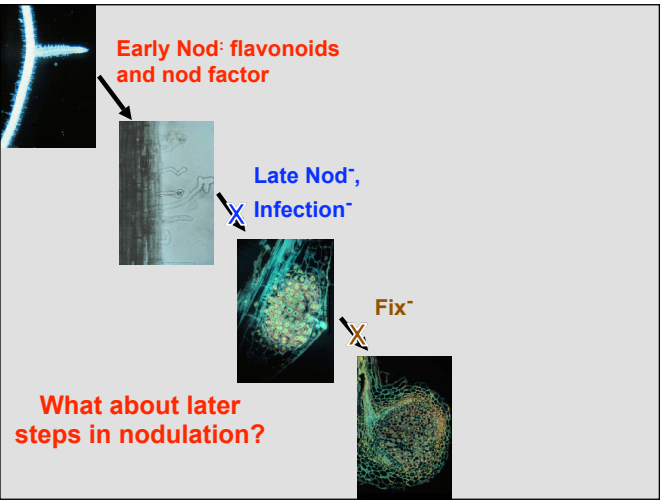
Early signals are host specific



Nod factor structure determined by common and host specific nod genes

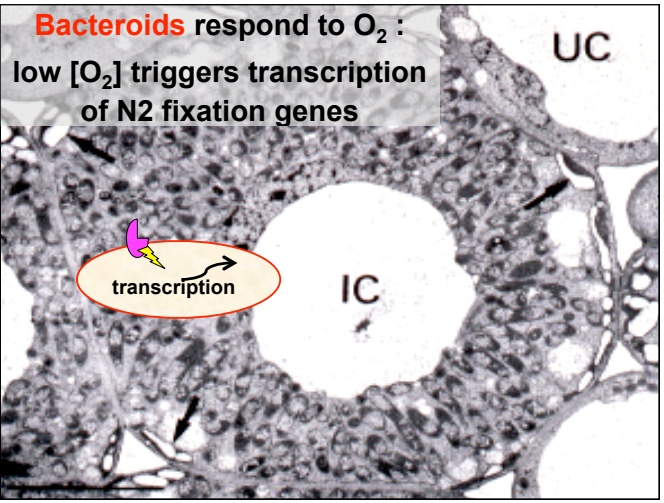




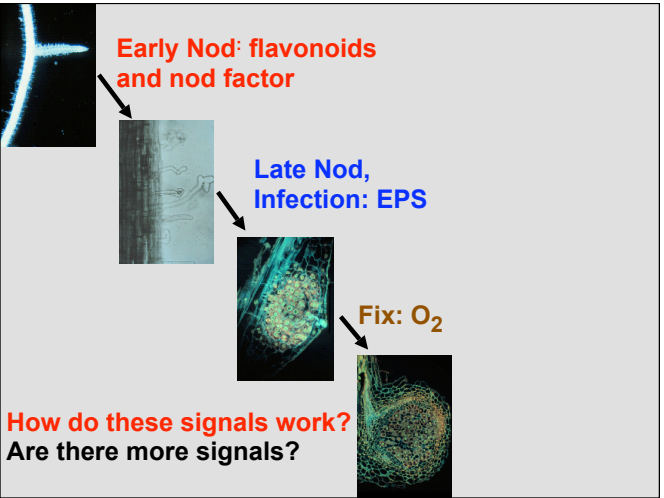


Mutants unable to make EPS (succinoglycan) form nodules on alfalfa, but cannot invade the host

Exopolysaccharides protect cell in soil and are required for successful plant invasion



Differentiation of "peribacteroid membrane" creates specialized compartment to support nitrogen fixation by bacteroids inside infected plant cell



Research on symbiosis can make a difference

What are key factors for effective nodulation/fixation?

How, in molecular terms, do the two partners get from [free living, top] to nodules [bottom}??

For example, what genes are involved, and how do the bacteria and plants respond to each other signals?

Can this symbiosis be optimized to assist agriculture?

Much progress has been made using model organisms, especially Lotus japonicus and Medicago truncatula, and a great deal of work is carried out on crops such as soybean.

Groups in Japan (genetics and genomics of Lotus), Australia (biochemistry, genetics and physiology of Medicago), France, Germany, The Netherlands, Denmark, the United Kingdom, Canada, Mexico and the United States. And that's just the model plant systems.

Other laboratories in countries throughout the world study a wide variety of topics related to symbiosis, including China, India, Morocco, Senegal and South Africa, Italy, Sweden and Poland, Argentina, Uruguay and Brazil. If you live in those countries, or any nearby, chances

Nodule formation: complex development

Novel events occur in both bacteria and plants:

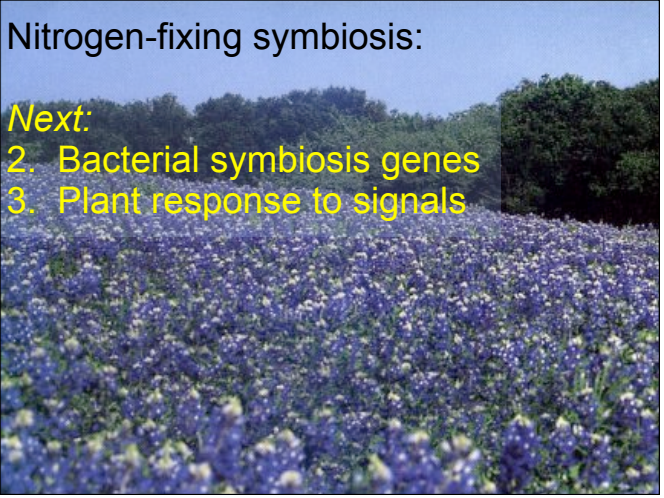
- Cells, tissues, organs
- Physiology and biochemistry
- Gene expression

Study by multiple approaches

- Microscopy
- Genetics
- Biochemistry

In these two introductory segments, I have shown how complex nodules are, and provided a brief overview of the initial stages of signaling between plants and bacteria.

To reach a deeper understanding of the mechanism of nodule formation – what makes it succeed or fail – researchers use approaches such as genetics (to define the heritable factors governing symbiosis), together with molecular and biochemical analysis of proteins and signaling, and microscopy and other cell biology approaches. In my next two talks, I will show some of our work on two subjects:



Studying symbiosis - research questions

- Genes
- Signals
- Cells