Symbiotic Nitrogen Fixation (in legumes)

Define: *The process by which molecular nitrogen (N_2) is reduced ("fixed") directly to NH_4^+*

Reaction: $N_2 + 10H^+ + 8e^- \rightarrow 2\ NH_4^+ + H_2$

- requires large **energy input** (see Haber-Bosch process: 30% of energy input for maize is N fertilizer

Why is N fixation important?

- net nitrogen input into soil (5-10 % of global N input)
- legume-Rhizobium interaction is a model system for plant-microbe signaling

Nitrogenase is a **prokaryotic** enzyme found only in some bacteria, but plants often benefit:

- free-living **cyanobacteria** (*Nostoc*) (associates with in Azolla water ferns in rice paddies)
- symbiotic bacteria: - *Frankia*, an *actinomycete* (alder trees (*Alnus*), also *Causuarina*)
- lichens often contain cyanobacteria as symbionts -> net N input into **forest ecosystems**
- *Rhizobium* and relatives (*Bradyrhizobium*, *Azorhizobium*) form nodules leguminous plants of great importance for agriculture: *NB: plants can have a specific bacterial biovar - high specificity!*

The plant provides energy and carbon (sucrose-> malate), bacteria provides NH_4^+

Features of the Legume-*Rhizobium* symbiosis:

1. - orchestrated formation of nodules, a new organ with distinct development
2. - plant & *Rhizobium* communicate via chemical signals
3. - the biochemistry of nitrogen fixation, metabolism and regulation

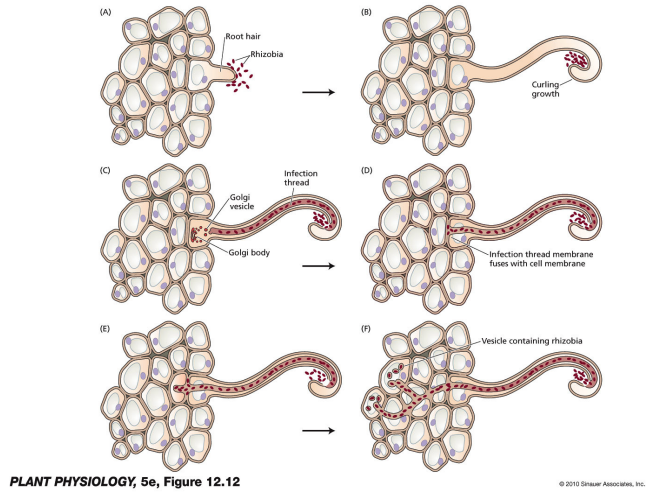
1. Root nodule formation is a '*controlled infection*'

i) - root hairs curl, ultimately leads to invagination of membranes, and CW hydrolysis in crook.
ii) - bacteria enter via infection thread and migrate into root cortex where they enter a cell
iii) - root cortex cells undergo division / morphogenesis, begin to make a nodule
iv) - bacteria eventually develop in special vacuoles (**symbiosomes**)

=> how is all this orchestrated??

Mature nodule structure: **bacteroid** + **peribacteroid membrane** =**symbiosomes**.

- nodulins = plant proteins required for the formation / functioning of symbiotic nodules
- early nodulins function in nodule formation,
- late nodulins in functions (metabolism, fixation, assimilation)



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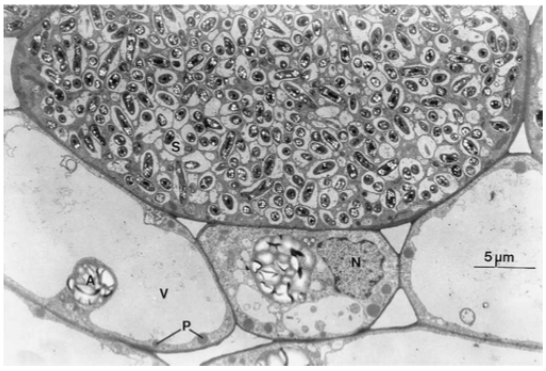
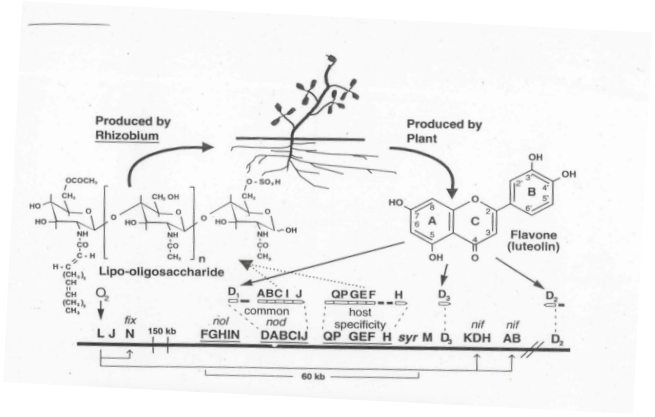


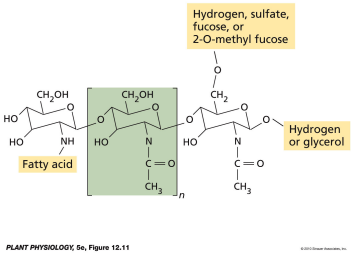
2. Plant-*Rhizobium* communication (= chemical ecology of this interaction)

- roots release flavonoid signals (ie., **flavone**) which stimulate Rhizobium **nod genes**
- **nod genes** can be either *'common'* or '*host-specific*', and the corresponding enzymes synthesize **nod factors** (see below).
- **nodD** is the flavonoid receptor & activator that regulates other rhizobial nod genes

Nod factors are **lipo-oligosaccharide** signals
- chitin-like, different from plant CW etc
- symbiotic specificity derives from nod factor structure (= 'key' for invasion)
- perceived at cell membrane of plant

Summary: Both plant and bacterial signals are involved

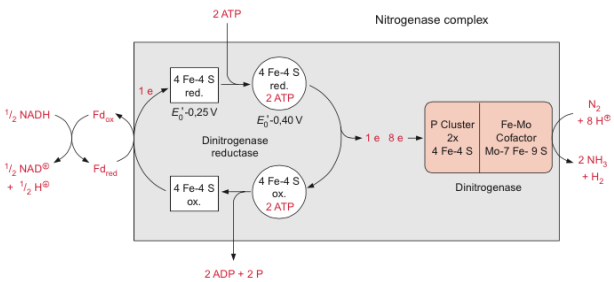


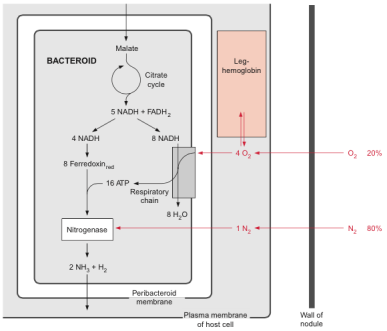


3. Nodule function and nitrogen fixation:

recall: rhizobia changed to **bacteroids**, and a completely new set of genes/pathways are expressed. This includes **nitrogenase**, as well enzymes of the **citric acid cycle** and **respiratory chain** (energy)

Nitrogenase enzyme (= nitrogenase complex) is bacterially encoded (prokaryotic enzyme)
- contains Fe-protein dimer and **MoFe** tetramer
- highly abundant, inactivated by **oxygen** (see leghemoglobin)
- electrons move from NADH --> ferredoxin --> dinitrogen reductase--> nitrogenase --> N_2
- high energy requirement 16 ATPs, 4 NADH for each N_2 reduced.
- H2 is also formed as a side reaction [nitrogenase also reduces acetylene]





Other nodule cell components

Leghemoglobin: (a late nodulin) - has high oxygen affinity, functions to reduce oxygen tension and protect nitrogenase from being inactivated.

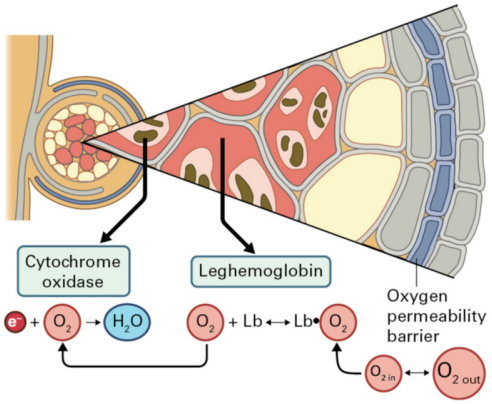
Peribacteroid membrane: specialized for exchange between plant cell cytoplasm and bacteroid

Metabolic Considerations:

- N assimilatory enzymes similar to those seen previously (**glutamine synthetase, glutamate synthase, aspartate aminotransferase**). These are found in host cell cytoplasm, and utilize the NH4 released from bacteroid.
- Amino acids as well as **allantoic acid** / **allantoin** are products that are exported via phloem.
- in exchange, plant is providing malate as carbon skeleton for amion acids, and for respiratory substrates. It is imported into the host cell as sucrose.

Regulation of nitrogen fixation

- nitrogenase is down-regulated by soil nitrogen (no nodules at high N in soil).
- regulation of bacterial N fixation genes (fix genes) via oxygen levels in nodule.
- fixation requires high ATP/ADP, so energy supply from the plant (malate) also regulates process.



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