- Plants assimilate most of the nitrate absorbed by their roots into organic nitrogen compounds.

- The first step of this process is the reduction of nitrate to nitrite in the cytosol (Oaks 1994).
- Catalysed by **Nitrate Reductase**

$$NO_3^- + NAD(P)H + H^+ + 2\,e^- \rightarrow NO_2^- + NAD(P)^+ + H_2O$$

- The nitrate reductases of higher plants are composed of two identical subunits, each containing three prosthetic groups:

1. FAD (flavin adenine dinucleotide),
2. Heme, and
3. A molybdenum complexed to an organic molecule called a *pterin*

(Mendel and Stallmeyer 1995; Campbell 1999).

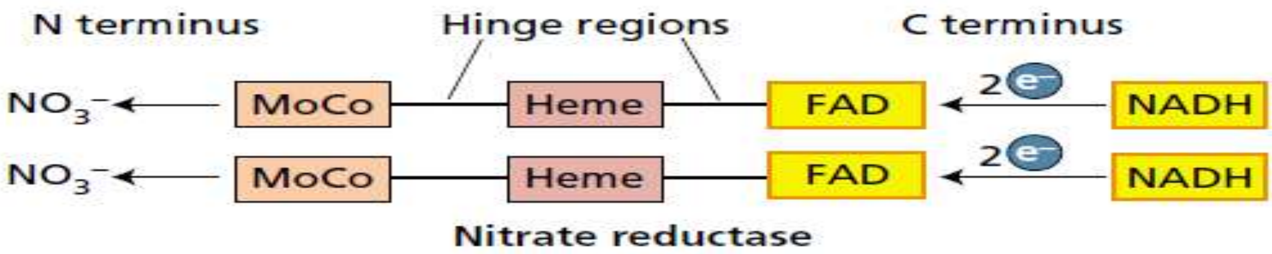


FIGURE 12.3 A model of the nitrate reductase dimer, illustrating the three binding domains whose polypeptide sequences are similar in eukaryotes: molybdenum complex (MoCo), heme, and FAD. The NADH binds at the FAD-binding region of each subunit and initiates a two-electron transfer from the carboxyl (C) terminus, through each of the electron transfer components, to the amino (N) terminus. Nitrate is reduced at the molybdenum complex near the amino terminus. The polypeptide sequences of the hinge regions are highly variable among species.

Taiz , 3rd Edition

CSIR NET QUESTION

101. Nitrate reductase is an important enzyme for nitrate assimilation. Given below are some statements on nitrate reductase enzyme:

(A) Nitrate reductase of higher plants is composed of two identical subunits.
(B) One subunit of nitrate reductase contains three prosthetic groups.
(C) One of the prosthetic groups attached to both subunits is heme.
(D) One of the prosthetic groups complexed with pterin is magnesium.

Which one of the following combination of statements on nitrate reductase mentioned above is correct?

1. A, B and C
2. A, C and D
3. B, C and D
4. A, B and D

Ans. 1

Nitrite Reductase Converts Nitrite to Ammonium

- Nitrite (NO2 –) is a highly reactive, potentially toxic ion.
- Plant cells immediately transport the nitrite generated by nitrate reduction from the cytosol into chloroplasts in leaves and plastids in roots.
- In these organelles, the enzyme nitrite reductase reduces nitrite to ammonium according to the following overall reaction

$$NO_2^- + 6\ Fd_{red} + 8\ H^+ + 6\ e^- \rightarrow NH_4^+ + 6\ Fd_{ox} + 2\ H_2O$$

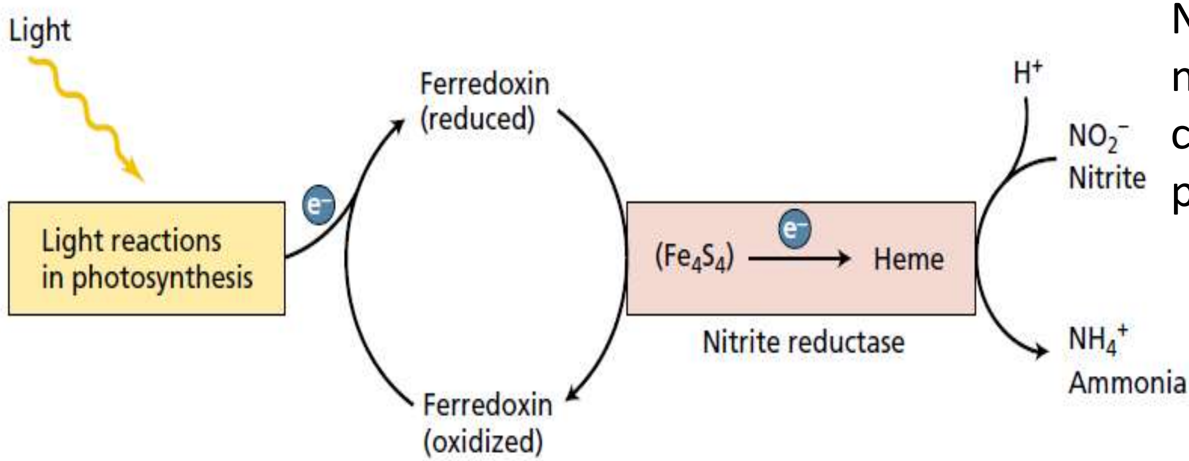


FIGURE 12.5 Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe_4S_4 and heme, which participate in the reduction of nitrite to ammonium.

Nitrite reductase is encoded in the nucleus and synthesized in the cytoplasm with an N-terminal transit peptide that targets it to the plastids

Ammonium assimilated by GS/GOGAT Pathway

- Ammonium, readily available to plants but also quite toxic, inhibit dinitrogenase and interfere with energy metabolism.

- The general pathway for ammonium assimilation was carried and found that initial organic product is glutamine.

- Assimilation of ammonium into glutamine by legume nodule is accomplished by Glutamine synthase cycle, a pathway involving two sequential enzymes

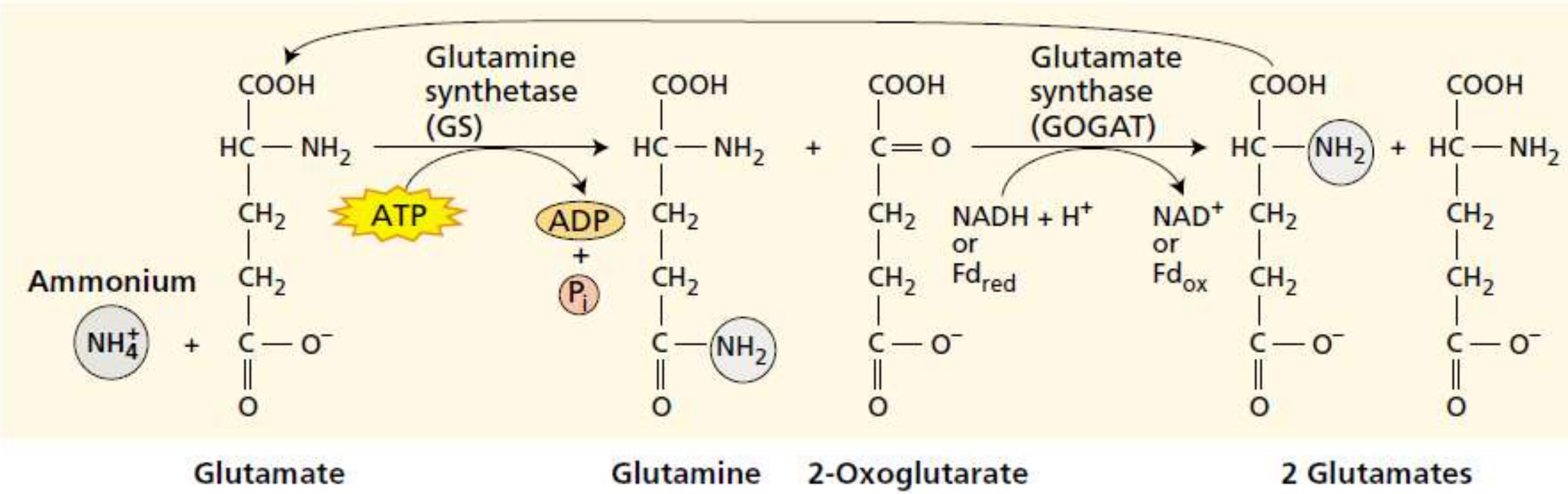
1. **GS (Glutamine Synthase)** two classes of GS, one in the cytosol and the other in root plastids or shoot chloroplasts

$$\text{Glutamate} + NH_4^+ + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i$$

2. **GOGAT(Glutamate Synthase)** Plants contain two types of GOGAT: One accepts electrons from NADH; the other accepts electrons from ferredoxin (Fd).

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{NADH} + H^+ \rightarrow 2\ \text{glutamate} + \text{NAD}^+$$

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{Fd}_{red} \rightarrow 2\ \text{glutamate} + \text{Fd}_{ox}$$



- PSII Proteins regulate the GS/GOGAT pathway
- Senses ATP status and α-ketoglutarate levels by allosterically.
- Cellular nitrogen is checked by glutamine availability.
- PII+ uridine triphosphate→PII-UMP (uridylylation)
- Activates the GS/GOGAT
- Conversely, when glutamine levels are high, PII-UMP converts back to PII, inactivates the GS/GOGAT hence slows down the $NH4^{+}$ assimilation

BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation accounts for most of the fixation of atmospheric N2 into ammonium, thus representing the key entry point of molecular nitrogen into the biogeochemical cycle of nitrogen.

Establishing Symbiosis Requires an Exchange of Signals

- Under nitrogen-limited conditions, the symbionts seek out one another through an elaborate exchange of signals.

- The first stage in the formation of the symbiotic relationship between the nitrogen-fixing bacteria and their host is migration of the bacteria toward the roots of the host plant.

- This migration is a chemotactic response mediated by chemical attractants, especially (iso)flavonoids and betaines, secreted by the roots.

- These attractants activate the rhizobial NodD protein, which then induces transcription of the other *nod* genes (Phillips and Kapulnik 1995).

- Binding of the activated NodD to the *nod* box induces transcription of the other *nod* genes.

Plant genes specific to nodules are called **nodulin** (*Nod*) genes;

Rhizobial genes that participate in nodule formation are called **nodulation** (*nod*) genes (Heidstra and Bisseling 1996).
The *nod* genes are classified as common *nod* genes or host-specific *nod* genes. The **common *nod* genes—*nodA*, *nodB*, and *nodC*—are** found in all rhizobial strains; the **host-specific *nod* genes—such as *nodP*, *nodQ*, and *nodH*; or *nodF*, *nodE*, and *nodL*—differ** among rhizobial species and determine the host range.

nodD, is constitutively expressed.

Nod factors are **lipochitin oligosaccharide** signal molecules, all of which have a chitin **β-1→4-linked *N*acetyl- D-glucosamine** backbone

1. NodA is an *N*-acyltransferase that catalyzes the addition of a fatty acyl chain.

2. NodB is a chitin-oligosaccharide deacetylase that removes the acetyl group from the terminal nonreducing sugar.

3. NodC is a chitin-oligosaccharide synthase that links *N*-acetyl-D-glucosamine monomers.

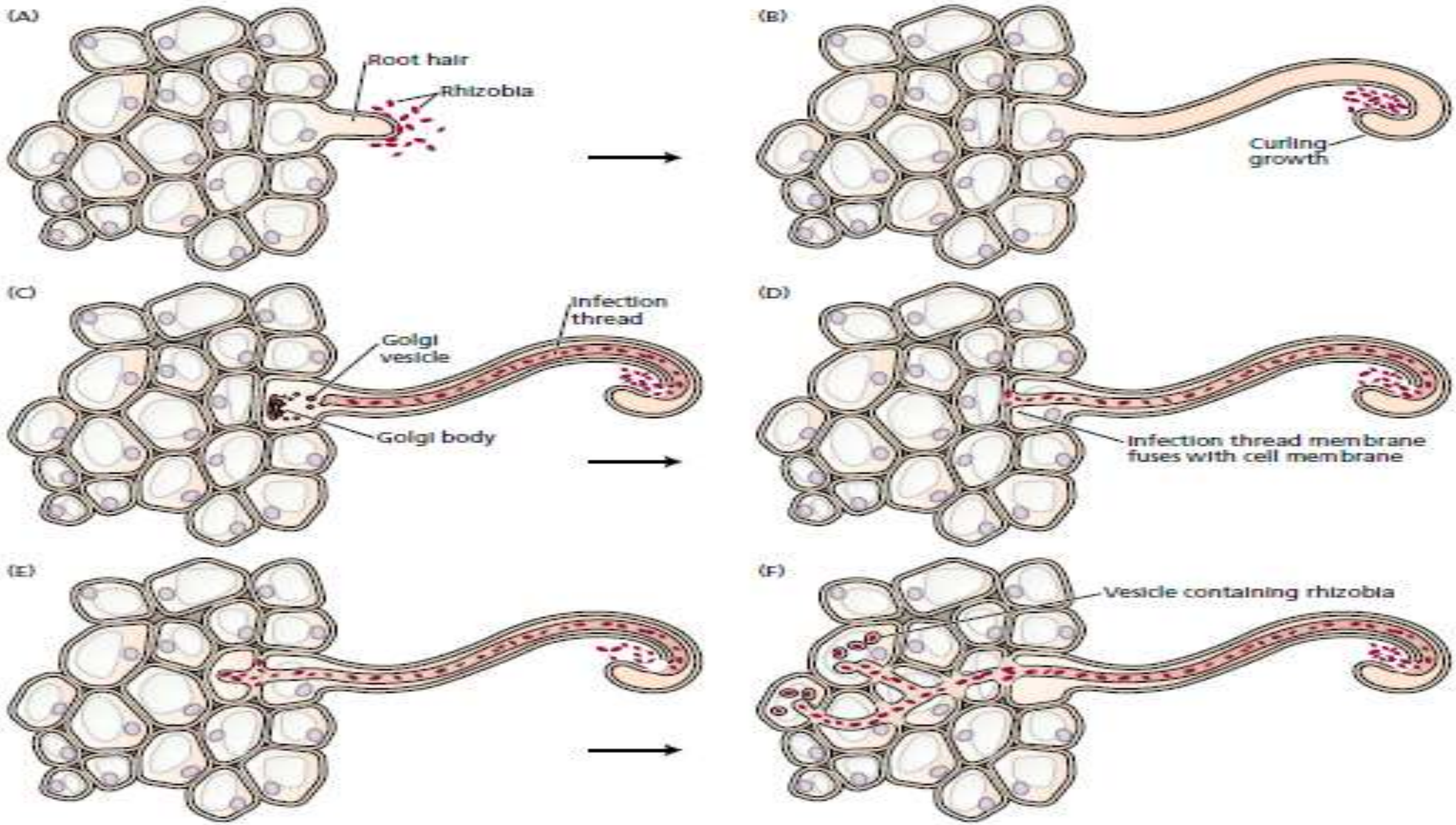
NodE and NodF determine the **length and degree of saturation** of the fatty acyl chain

Other enzymes, such as NodL, **influence the host specificity** of Nod factors through the addition of specific substitutions at the reducing or nonreducing sugar moieties of the chitin backbone.

The rhizobia become enclosed in the small compartment **formed by the curling**. The cell wall of the root hair degrades in these regions, also in response to Nod factors, allowing the bacterial cells direct access to the outer surface of the plant plasma membrane

The next step is formation of the **infection thread** an internal tubular extension of the plasma membrane that is produced by **the fusion of Golgi-derived membrane** vesicles at the site of infection.

The thread grows at its tip by the fusion of secretory vesicles to the end of the tube. Deeper into the root cortex, near the xylem, cortical cells dedifferentiate and start dividing, forming a distinct area within the cortex, called a *nodule primordium*, from which the nodule will develop.



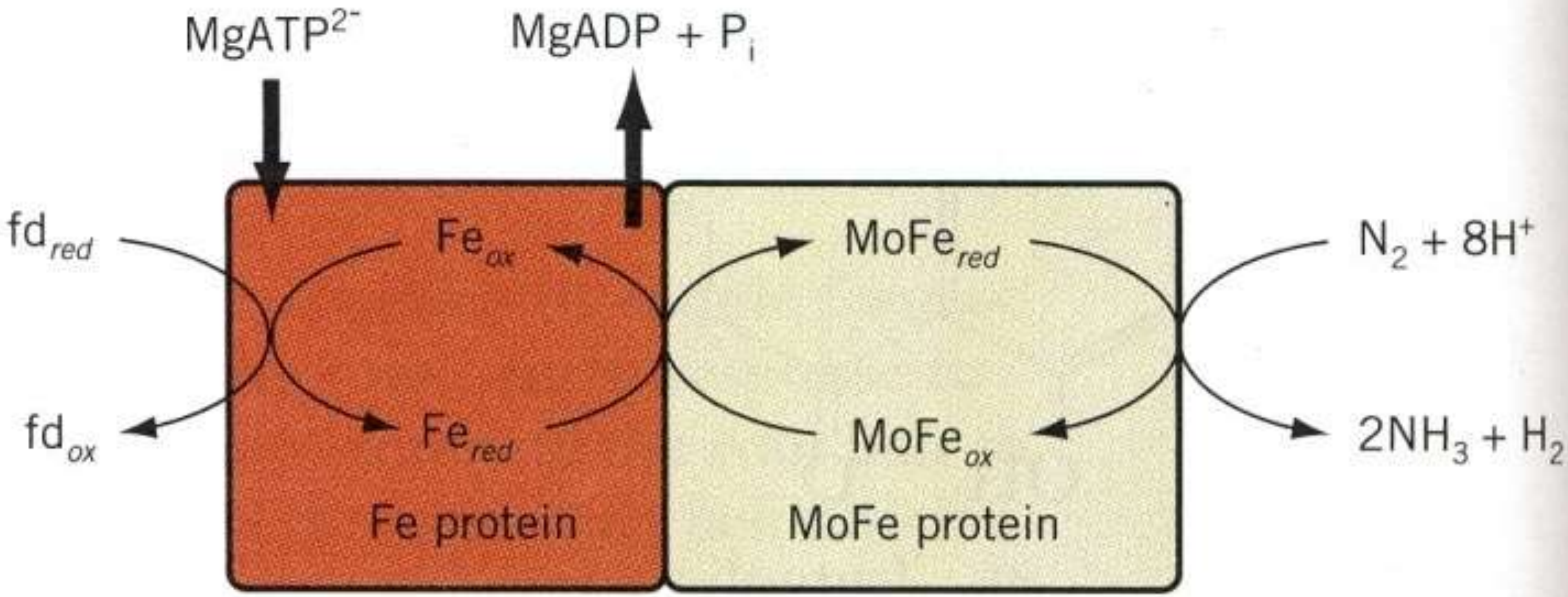
(A)
Root hair
Rhizobia
(B)
Curling growth
(C)
Infection thread
Golgi vesicle
Golgi body
(D)
Infection thread membrane fuses with cell membrane
(E)
(F)
Vesicle containing rhizobia

“Nitrogenase”

•Multimeric complex of two different proteins

- •**Smaller protein** is dimer contains 2 identical subunits (24-36 KDa) called Fe Protein**(Fe_4S_4)**,
- • single cluster of four Fe and S
- • **Larger protein** is called as **MoFe** protein (220KDa), it is a tetramer contains two pairs,
  - • **MoFe contains two molybdenum** atoms in the form of iron-molybdenum-sulphur co-factor
  - •Also contain Fe_4S_4 but number is not clear

- The enzymes in the complex are **nitrogenase reductase & dinitrogenase**
- The dinitrogenase catalyzes the reduction of N_2 to NH_3
- **$N_2 + 8H^+ + 16ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16Pi$**
- Therefore 16 ATP per $2NH_3$
- Two step process
  - Fe protein is reduced by primary electron donor ferredoxin
  - Reduced Fe protein pass electrons **to MoFe** which catalyse reduction of both Nitrogen and hydrogen
  - ATP helps in the transfer of electrons from **FeS to MoFe**



DINITROGENASE