N_2 FIXATION

CC-12
UNIT-7

DR. ARINDAM MANDAL
Assistant Professor
Bejoy Narayan Mahavidyalaya
Itachuna, Hooghly
West Bengal

INTRODUCTION

- **N_2 is an essential constituent of all biomolecules, both in plants and animals.**

- **Most of the plants obtain N2 from soil in the form of nitrate or ammonia ions, but is limited.**

- **Atmosphere consist 78% of molecular N2 but plants unable to convert this molecular N2 into a useful form because the lack of the enzyme Dinitrogenase.**

- **Only prokaryotic species possess this enzyme.**

What is N_2 Fixation?

The process of reducing dinitrogen to ammonia is known as nitrogen fixation or dinitrogen fixation.

Nitrogen fixation is a prokaryote domain, because only prokaryote organisms have the enzyme complex, called dinitrogenase, that cata- lyzes the reduction of dinitrogen to ammonia.

Prokaryotes that fix nitrogen, called nitrogen- fixers, include both free-living organisms and those that form symbiotic associations with other organisms.

N_2 Fixing Organisms

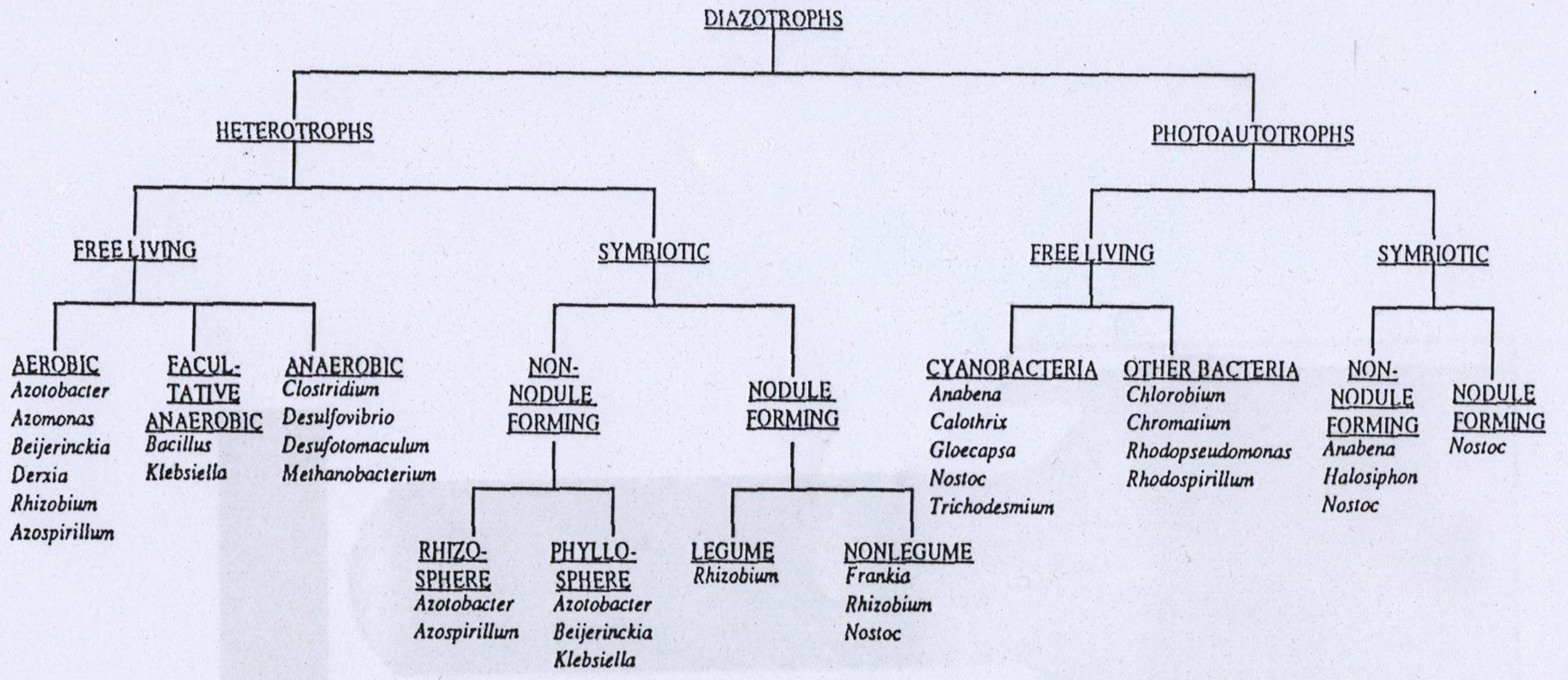


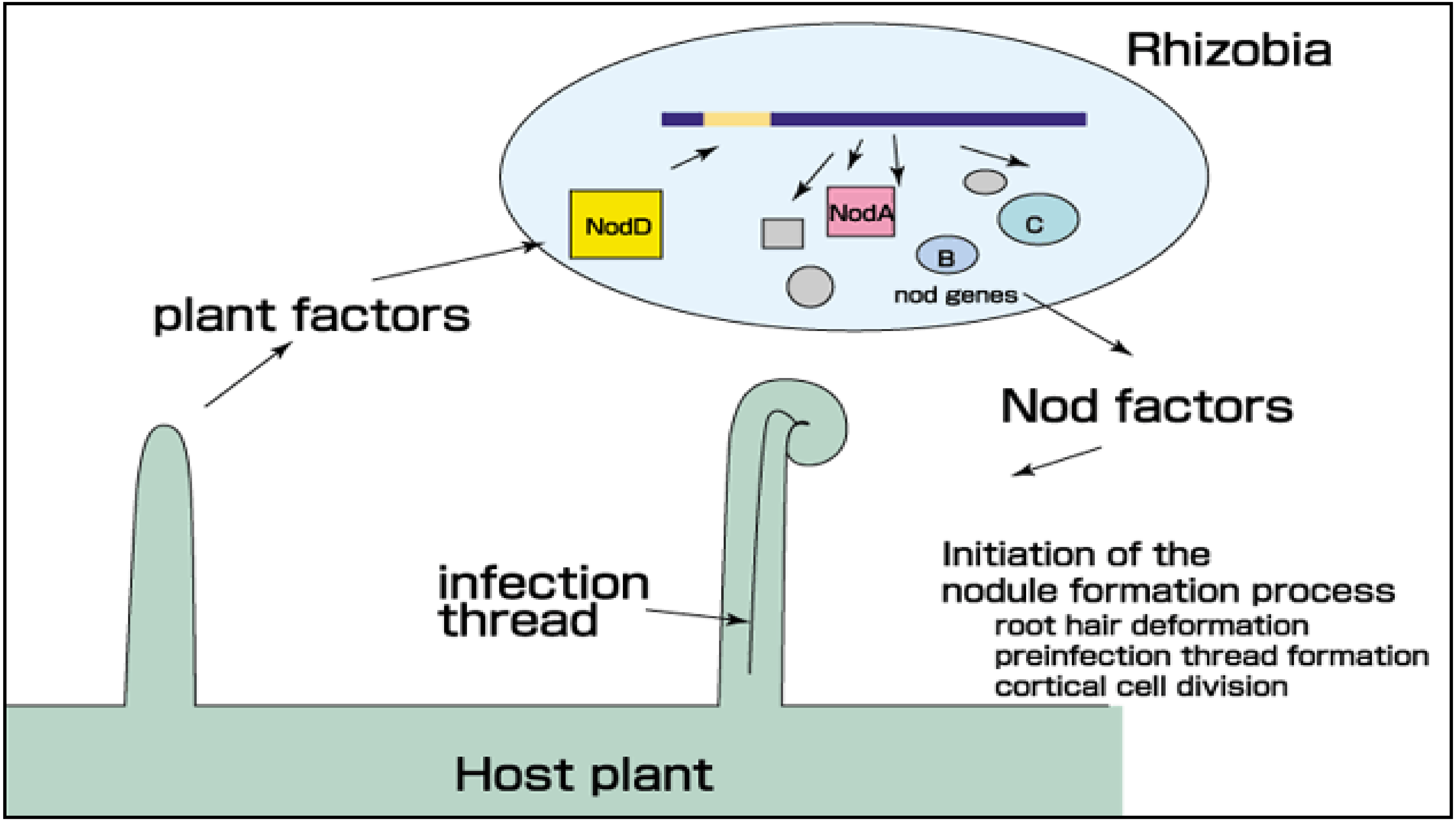
Figure 2 Diagrammatic representation of the primary nitrogen-fixing genera.

Rhyzobial Symbiosis

- **In rhyzobial type of symbiotic associations the plant is identified as the host and the microbial partner is known as the microsymbiont.**
- **The most common form of symbiotic association results in the formation of enlarged, multicellular structures, called nodules, on the root (or occasionally the stem) of the host plant.**
- **In the case of legumes,1 the microsymbiont is a bacterium of one of three genera: *Rhizobium, Bradyrhi- zobium*, or *Azorhizobium*. Collectively, these organisms are referred to as rhizobia.**
- **The rhizobia are further divided into species and subgroups called biovars (a biological variety) according to their host range.**

Nodulation

- **Rhizobia initially occur as free-living organisms in the soil.**
- **The sequence of events begin with bacterial infection of the root and ending with formation of mature, nitrogen-fixing nodules**
- **Overall the process involves a sequence of multiple interactions between the bacteria and the host roots.**
- **This signaling, the subsequent infection process, and the development of N_2-fixing nodules involve specific genes in both the host and the symbionts.**


Rhizobia
NodD
NodA
B
C
nod genes
plant factors
Nod factors
infection thread
Initiation of the nodule formation process
root hair deformation
preinfection thread formation
cortical cell division
Host plant

Steps of Nodulation

(A) ***Rhizobia*** **bind to an emerging root hair in response to chemical attractants sent by the plant.**

↓

(B) In response to factors produced by the bacteria, the root hair exhibits abnormal curling growth, and *Rhizobia* cells proliferate within the coils.

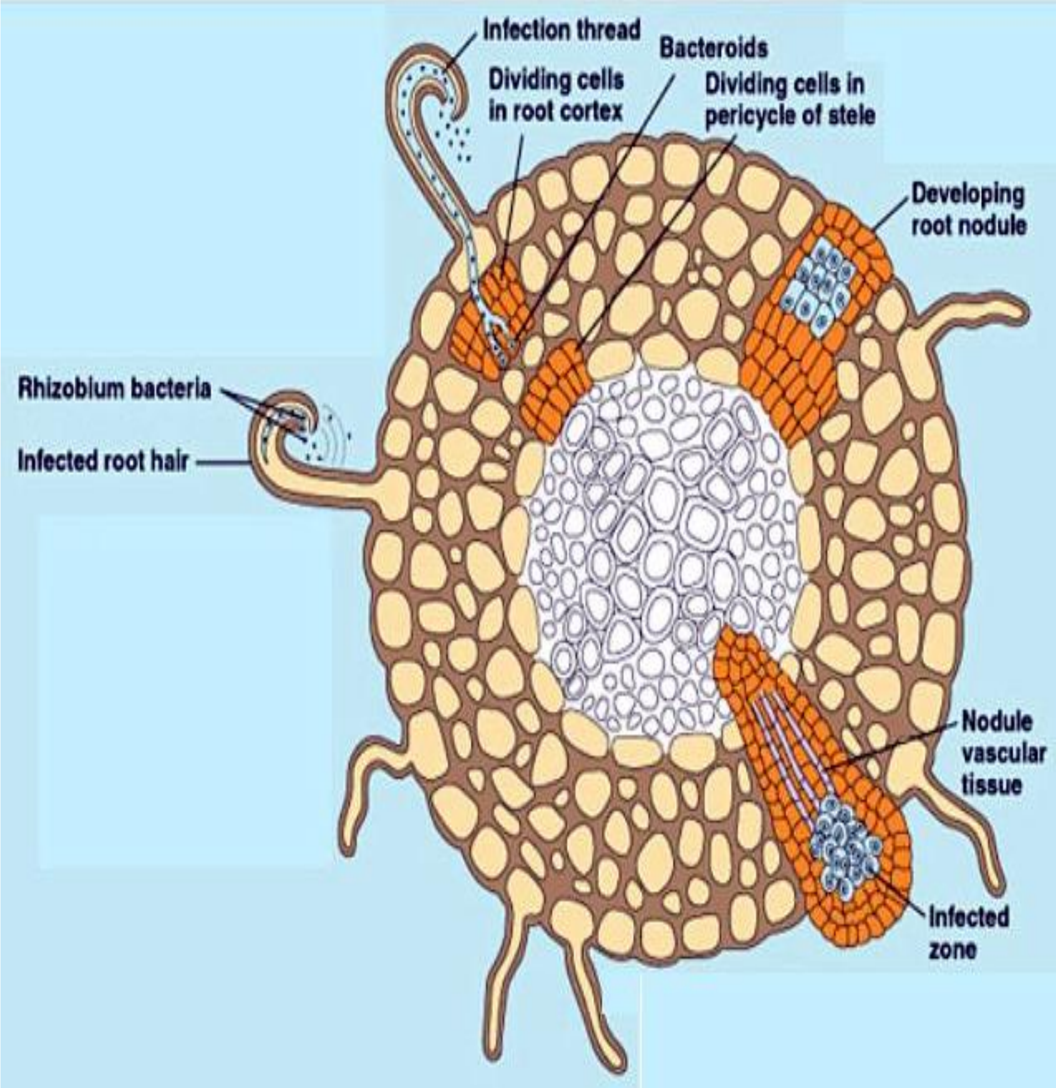
↓

(C) Localized degradation of the root hair wall leads to infection and formation of the infection thread from Golgi secretory vesicles of root cells.

↓

(D) The infection thread reaches the end of the cell, and its membrane fuses with the plasma membrane of the root hair cell.

↓



Steps of Nodulation

↓

(E) *Rhizobia* are released into the apoplast and penetrate the compound middle lamella to the sub-epidermal cell plasma membrane, leading to the initiation of a new infection thread, which forms an open channel with the first.

↓

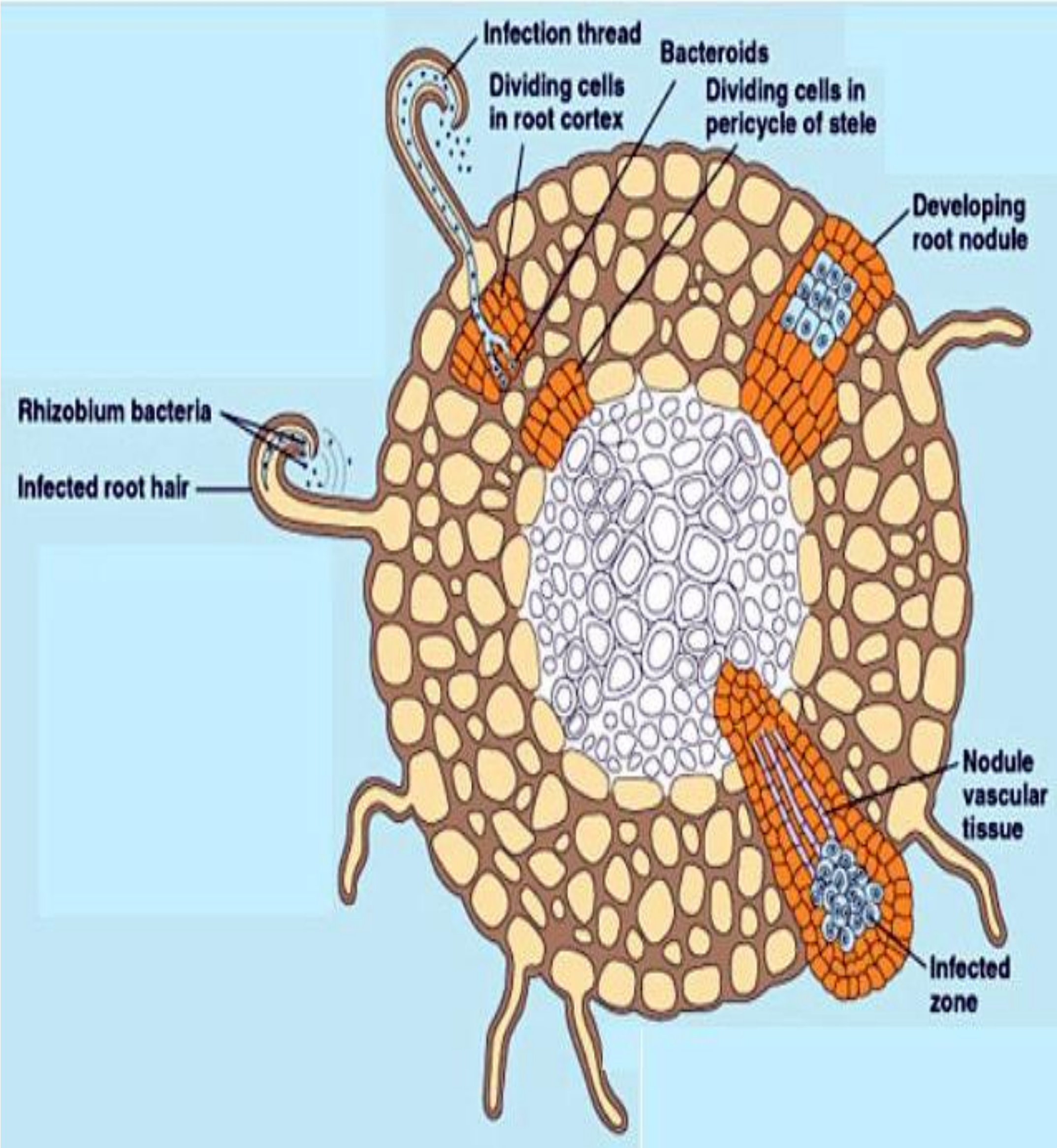
(F) Deeper into the root cortex, near the xylem, cortical cells of host root dedifferentiate and start dividing, forming a distinct area within the cortex, called a ***nodule primordium***, from which the nodule will develop.

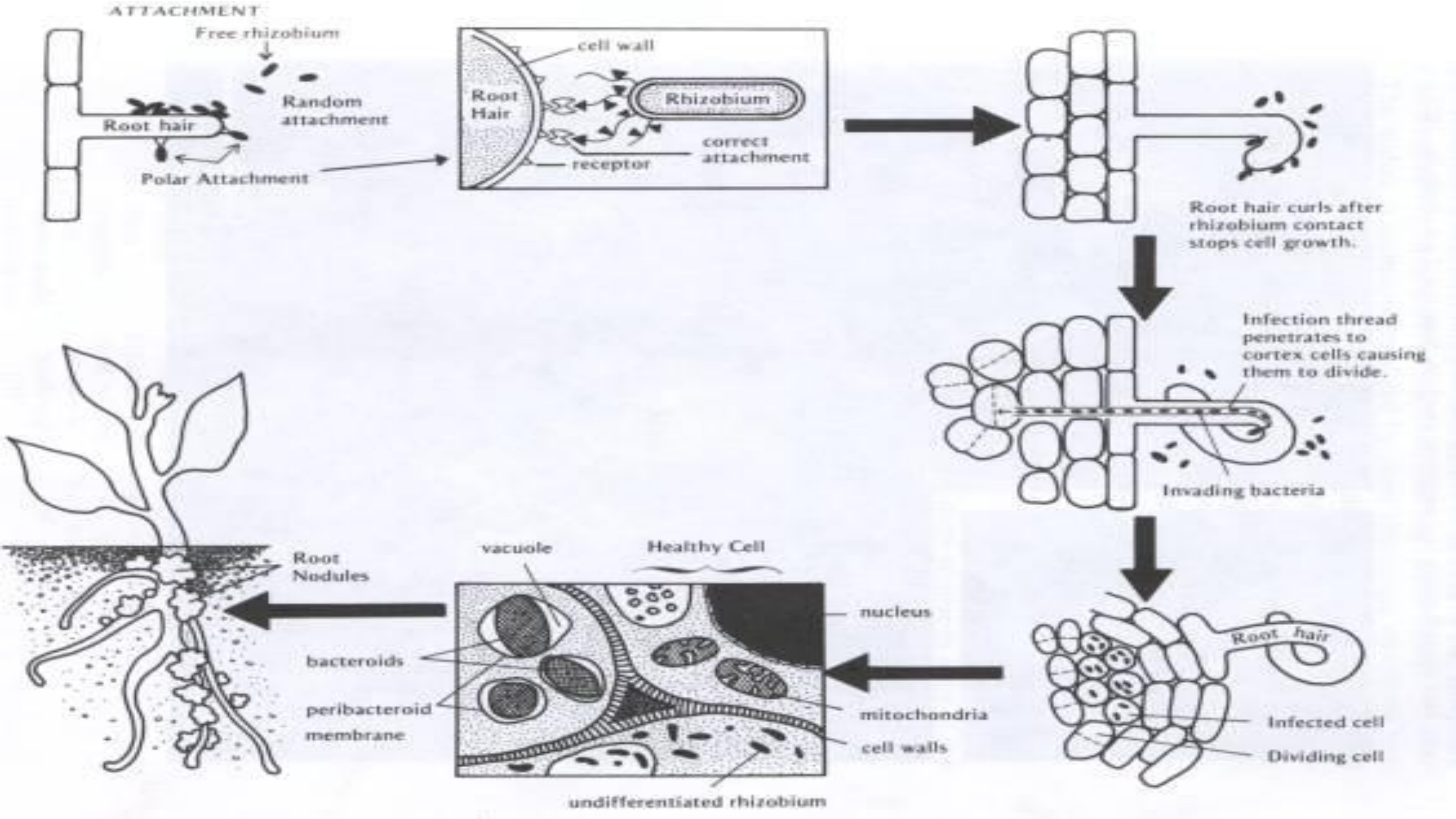
↓

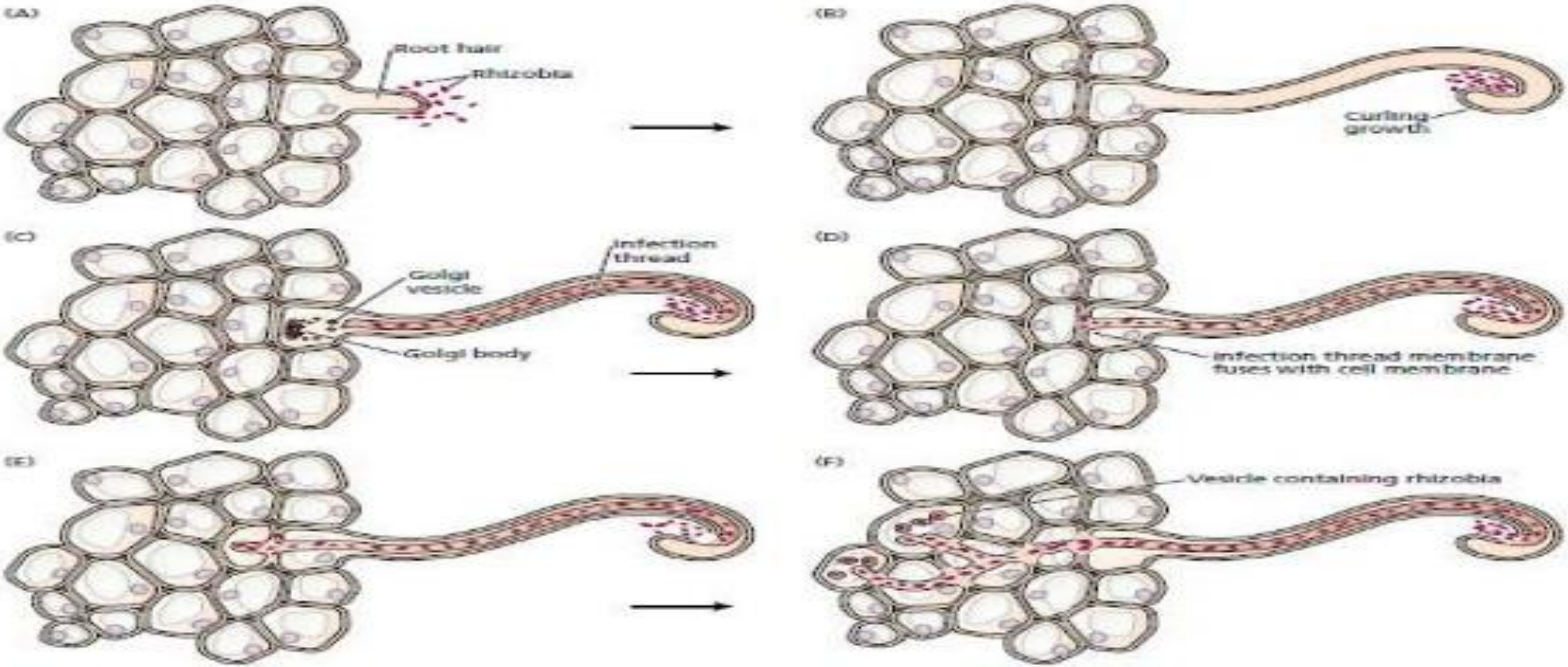
(G) The infection thread extends and branches until it reaches target cells, where vesicles composed of plant membrane that enclose bacterial cells are released into the cytosol.

↓

(H) The bacteria stop dividing and begin to enlarge and to differentiate into N_2-fixing endosymbiotic organelles called **bacteroids**. The membrane surrounding the bacteroids is called the **peribacteroid membrane**.




ATTACHMENT
Free rhizobium
Random attachment
Root hair
Polar Attachment
cell wall
Root Hair
Rhizobium
correct attachment
receptor
Root hair curls after rhizobium contact stops cell growth.
Infection thread penetrates to cortex cells causing them to divide.
Invading bacteria
Root hair
Infected cell
Dividing cell
Healthy Cell
vacuole
nucleus
bacteroids
peribacteroid membrane
mitochondria
cell walls
undifferentiated rhizobium
Root Nodules



(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

Biochemistry of N_2 Fixation

- **Biological nitrogen fixation,produces ammonia from molecular nitrogen. The overall reaction is-**

$$N_2 + 8\,e^- + 8\,H^+ + 16\,ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

- **The reduction of N_2 to $2NH_3$, a six-electron transfer, is coupled to the reduction of two protons to evolve H_2.**

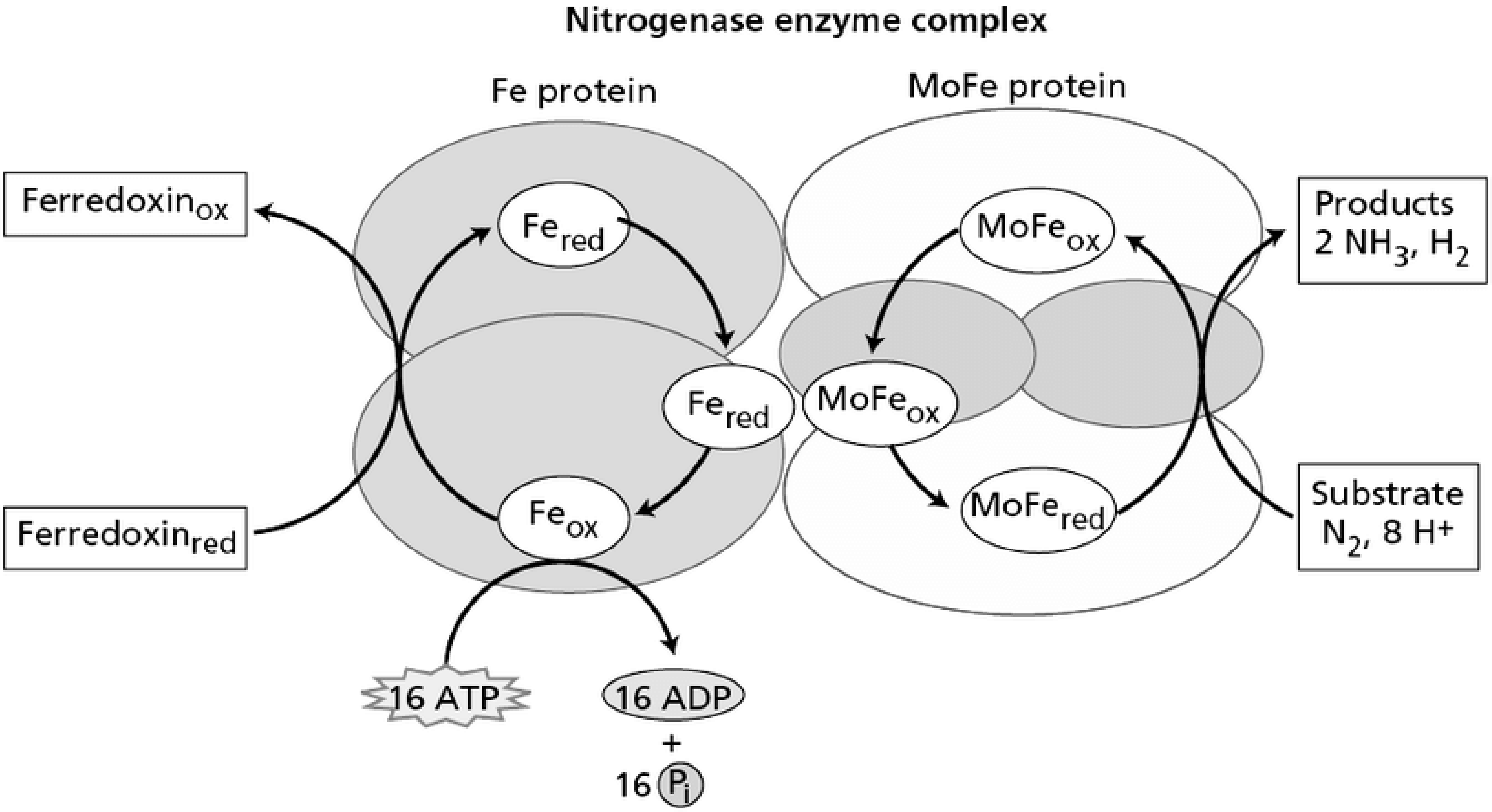
- **The Dinitrogenase enzyme complex catalyzes this reaction.**

The Dinitrogenase

- **The nitrogenase enzyme complex can be separated into two components—the Fe protein and the Mo-Fe protein—neither of which has catalytic activity by itself.**
- **The Fe protein is the smaller of the two components and has two identical subunits of 30 to 72 kDa each. Each subunit contains an iron–sulfur cluster that participates in the redox reactions involved in the conversion of N_2 to NH_3.**
- **The Mo-Fe protein has four subunits, with a total molecular mass of 180 to 235 kDa. Each subunit has two Mo–Fe–S clusters.**
- **Both the subunits-Fe protein and Mo-Fe protein are inactivated by oxygen.**

Process of N_2 reduction

- **In the reduction reaction, ferredoxin serves as an electron donor to the Fe protein; Ferredoxin is a small (14 to 24 kD) protein containing an iron-sulphur group;electrons are carried by the iron moiety.**
- **In the second step, Fe protein hydrolyzes ATP and reduces the MoFe protein.**
- **The MoFe protein then catalyzes the reduction of both dinitrogen gas and hydrogen.**
- **The energetics of nitrogen fixation is complex. The production of NH_3 from N_2 and H_2 is an exergonic reaction**
- **ATP in the reaction react with reduced Fe protein and to cause a conformational change in this protein that alters its redox potential. This facilitates the transfer of electrons between the Fe protein and the MoFe protein.**


Nitrogenase enzyme complex
Fe protein
MoFe protein
Ferredoxin$_{ox}$
Ferredoxin$_{red}$
Fe$_{red}$
Fe$_{red}$
Fe$_{ox}$
MoFe$_{ox}$
MoFe$_{ox}$
MoFe$_{red}$
Products
2 NH_3, H_2
Substrate
N_2, 8 H^+
16 ATP
16 ADP
+
16 P_i

Strategies for regulating oxygen level

- **One of the more critical problems facing nitrogen-fixing organisms is the sensitivity of dinitrogenase to molecular oxygen.**
- **Several strategies for regulating oxygen level have developed to resolve this conflict.**

1. First, many free-living bacterial nitrogen fixers have retained an anaerobic lifestyle or, if facultative, fix dinitrogen only under anaerobic conditions.

2. Second, certain species of nitrogen-fixing cyanobacteria have structurally isolated the nitrogen-fixing apparatus. The nitrogen-fixing cells of the cyanobacteria are specialized cells called heterocysts.

3. Third, the oxygen supply is regulated to a large extent by an oxygen-binding protein called leghemoglobin in legume nodules.

Leghaemoglobin

- **Leghemoglobin is present in the cytoplasm of infected nodule cells at high concentrations and gives the nodules a pink color.**
- **The host plant produces the globin portion of leghemoglobin in response to infection by the bacteria; the bacterial symbiont produces the heme portion.**
- **Leghemoglobin has a high affinity for oxygen,about ten times higher than the β chain of human hemoglobin.**
- **Recent studies indicate that it stores only optimum oxygen to support nodule respiration for a few seconds. Its function is to help transport oxygen to the respiring symbiotic bacterial cells.**