Systems Microbiology

Wednes Nov 1 - Brock Ch 17, 586-591
Ch 19, 656-66
Ch 31, 989-991

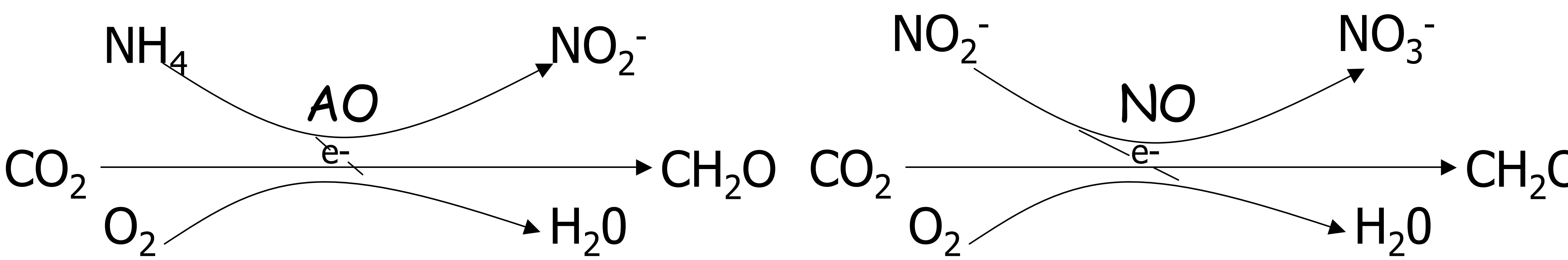
- The Global Nitrogen Cycle
- N_2 fixation - general considerations
- Plant microbial symbioses
  Rhizobium, Agrobacterium

Table and diagram of the key processes and prokaryotes in the nitrogen cycle removed due to copyright restrictions.
See Figure 19-28 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.* 11th ed.
Upper Saddle River, NJ: Pearson PrenticeHall, 2006. ISBN: 0131443291.

Nitrification

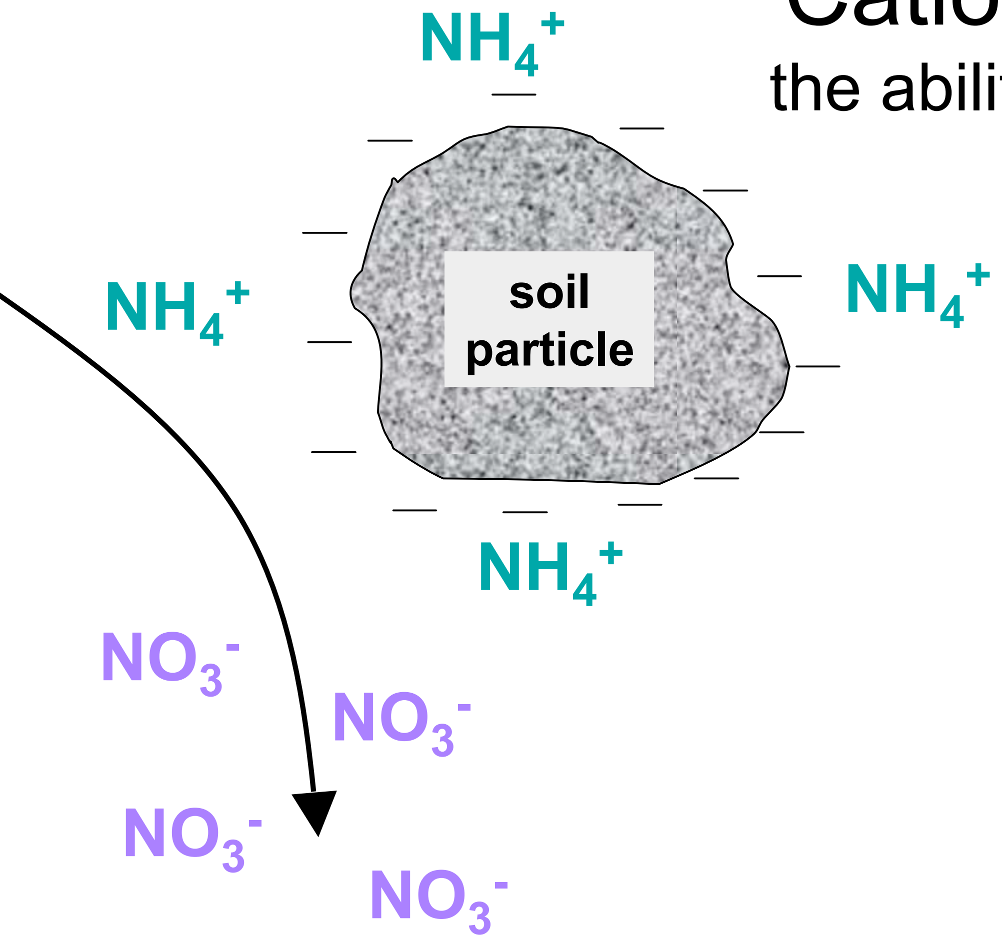
Chemolithoautotrophs (aerobic)

- Ammonia Oxidizers (*Nitrosomonas*, *Nitrosococcus*)
- Nitrite Oxidizers (*Nitrobacter*, *Nitrococcus*)
- Slow growing (less free energy available)
- Enzyme ammonia monooxygenase

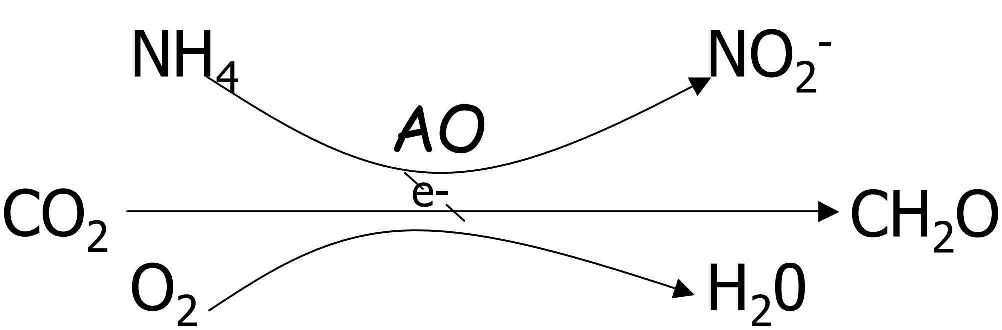


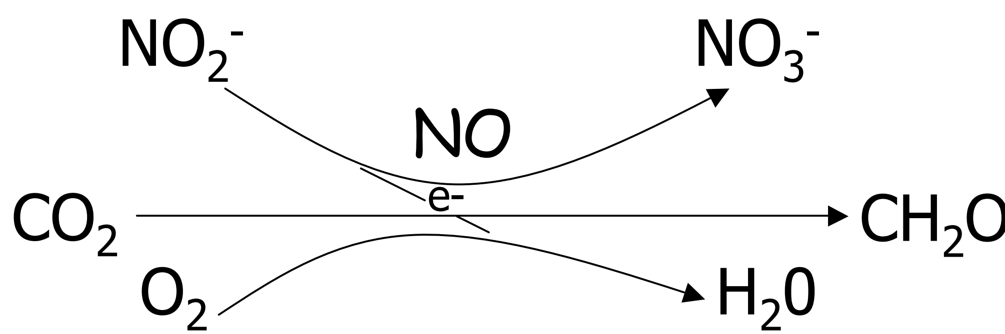
Cation exchange capacity:

the ability of a soil to hold on to cations



Microbial nitrification can effect the retention of nitrogen in soil





NITROGEN CYCLING IN AQUARIA

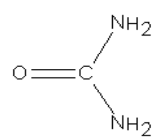
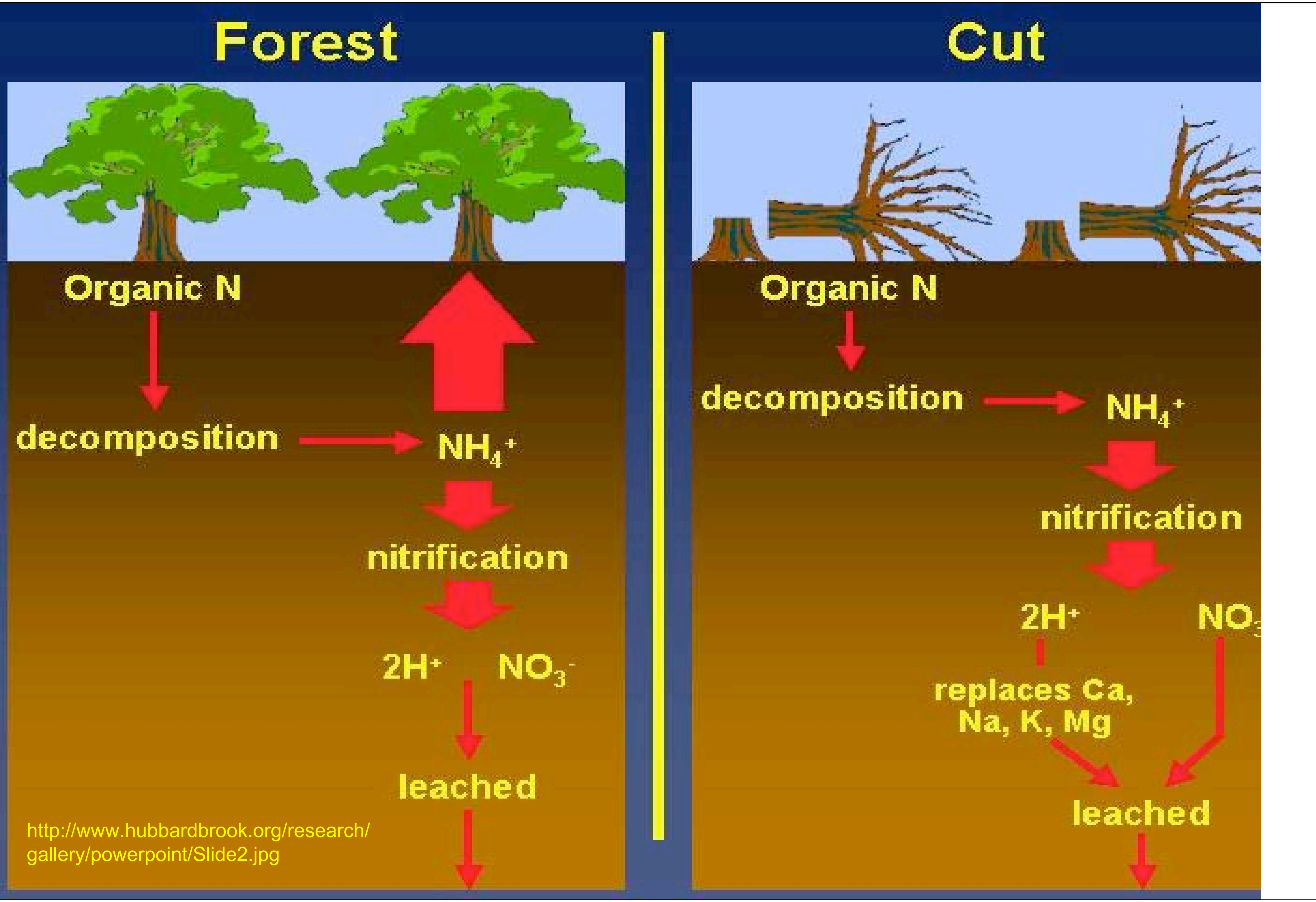


Image of fish swimming in an aquarium removed due to copyright restrictions.


Forest
Cut
Organic N
decomposition
NH4+
nitrification
2H+
NO3-
leached
Organic N
decomposition
NH4+
nitrification
2H+
replaces Ca,
Na, K, Mg
leached
http://www.hubbardbrook.org/research/
gallery/powerpoint/Slide2.jpg

View from above Lake 226 divider curtain in August 1973.
The bright green colour results from Cyanobacteria, which are growing on phosphorus added to the near side of the curtain.

What happen's when you dump lots of phosphate in a lake ???

Aerial view of Lake 227 in 1994. Note the bright green color caused by algae stimulated by the experimental addition of phosphorus for the 26th consecutive year.
Lake 305 in the background is unfertilized.

Aerial photographs removed due to copyright restrictions.

ANABAENA

http://www-biol.paisley.ac.uk/bioref/Eubacteria/Anabaena.jpg

Courtesy of the University of Paisley Biodiversity Reference.

Used with permission.

Image of Microcystis removed due to copyright restrictions.

filamentous cyanobacteria

MICROCYSTIS

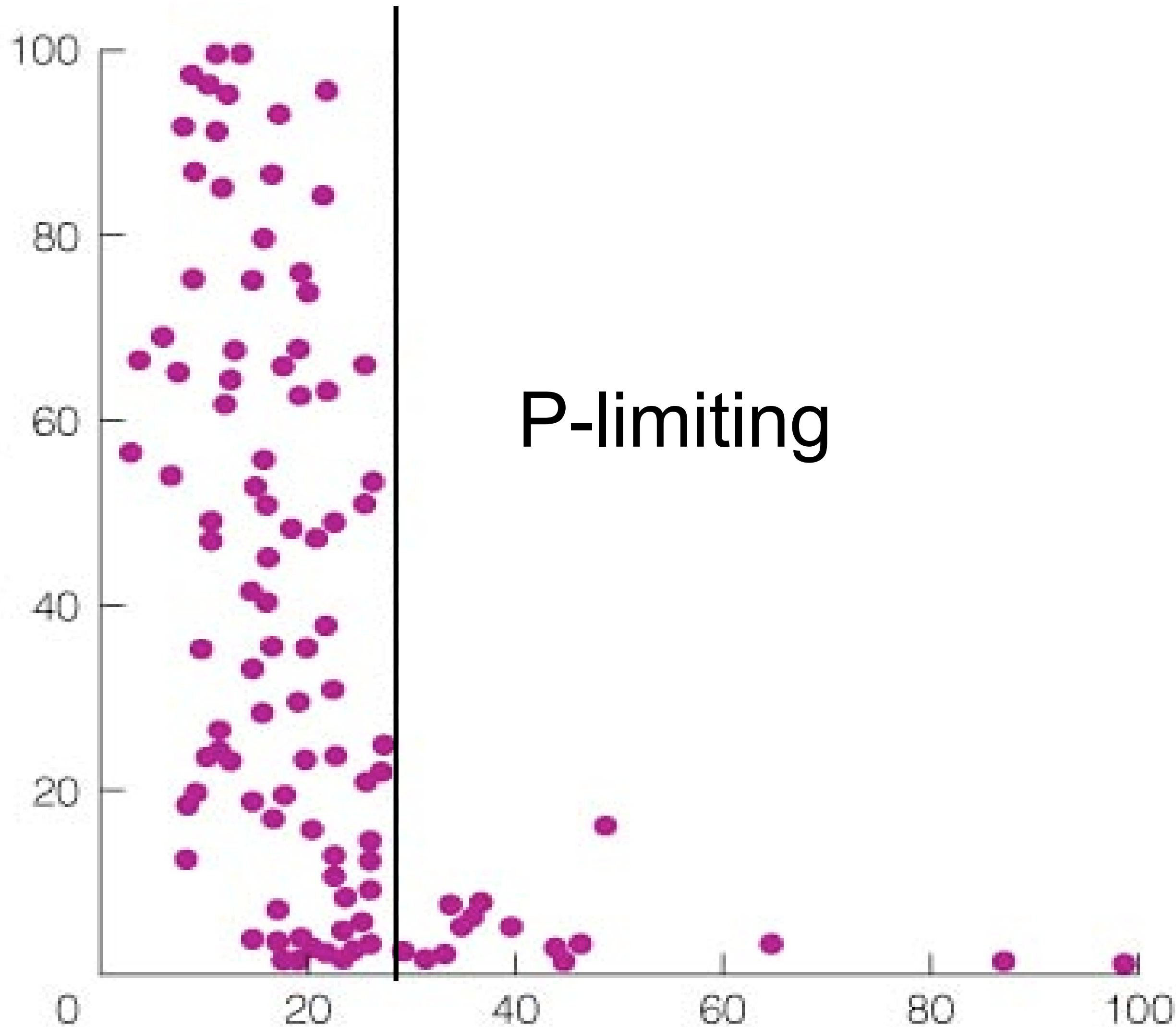
http://silicasecchidisk.conncoll.edu/Pics/Other %20Algae/Blue_Green%20jpegs/Microcystis_Key221.jpg

What happen's when you dump lots of phosphate in a lake ???

N-limiting

P-limiting

% cyano-bacteria (or blue-green algae)



N:P ratio

Nitrogen Fixation

- **Diversity**
  - Cyanobacteria
  - Proteobacteria
  - Archaea
  - But not all species of same group can fix

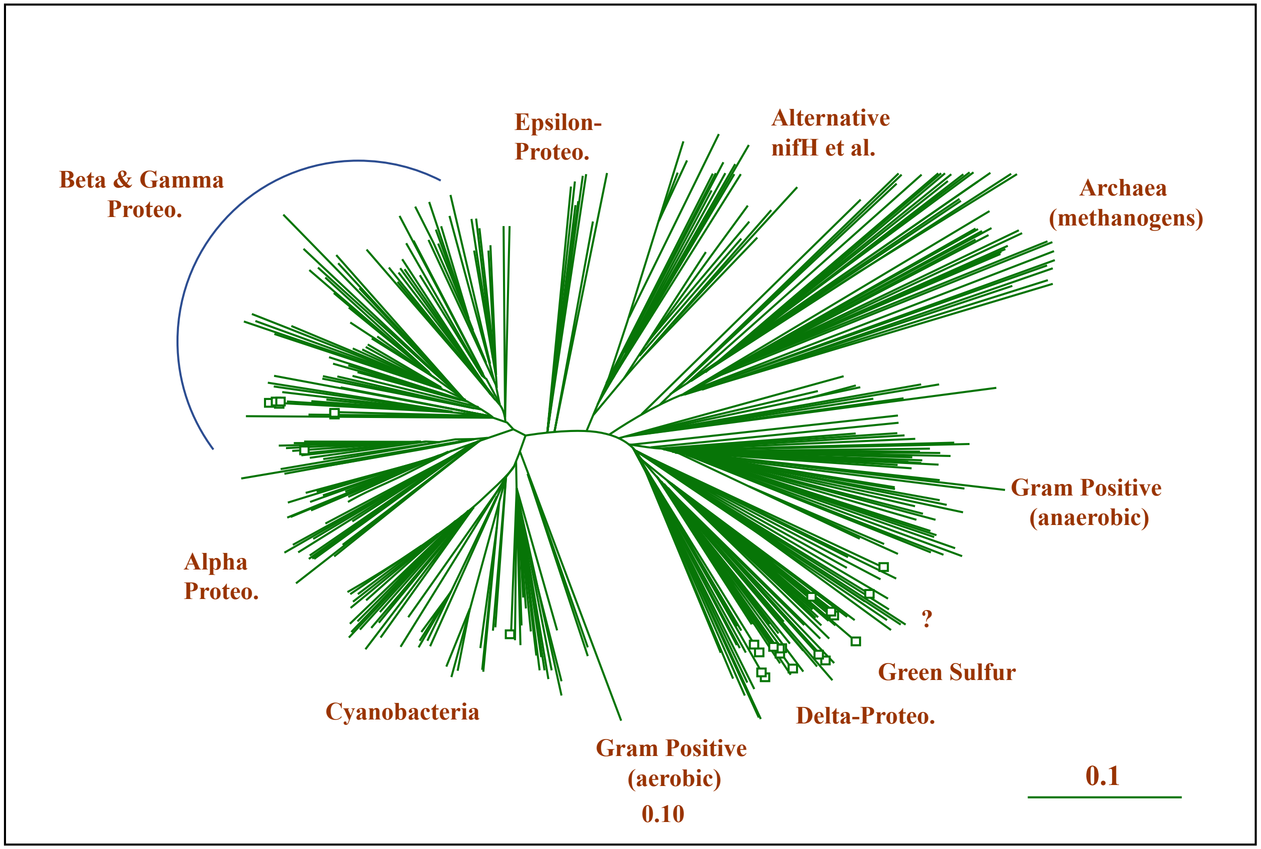


Figure by MIT OCW.

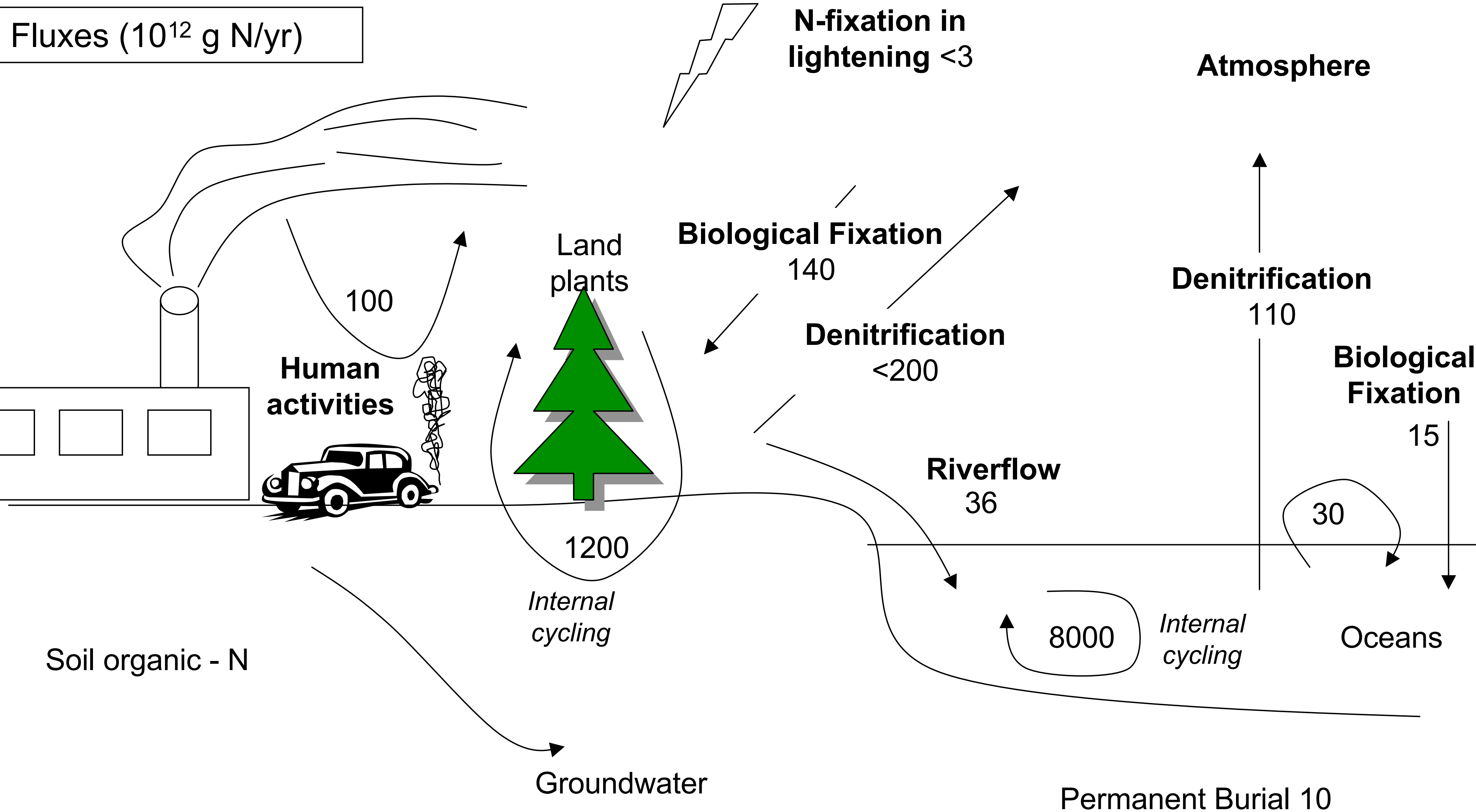
Energetics

Costs 16 ATP per molecule N_2 'fixed'

$$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16\ MgADP + 16\ P_i$$

The Global Nitrogen Cycle

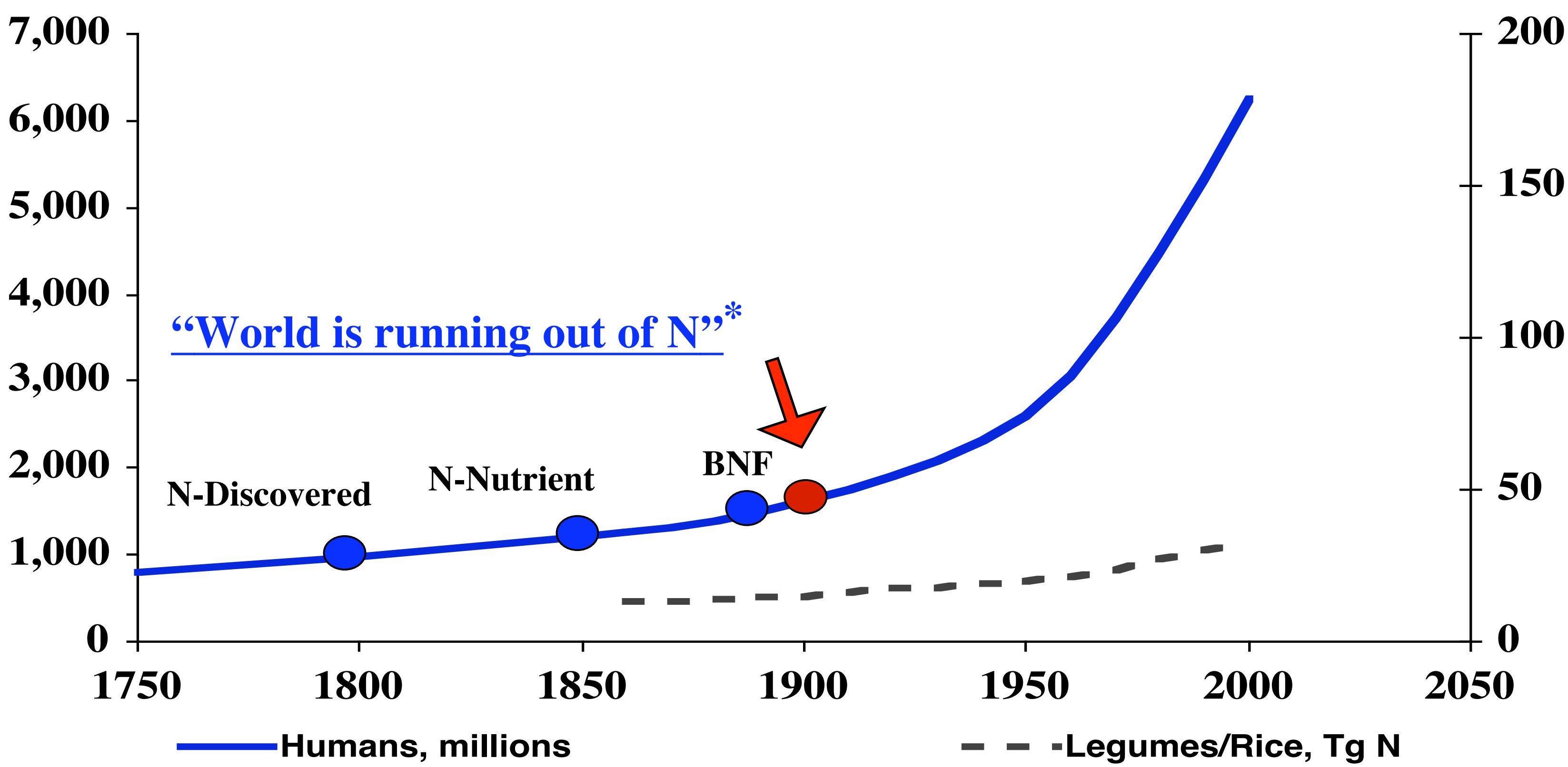
Tg=teragram $=10^{12}$ g



Reference: Schlesinger, 1997

The History of “Nitrogen Science”

--N becomes limiting?--



*1898, Sir William Crookes, president of the British Association for the Advancement of Science

Galloway JN and Cowling EB. 2002; Galloway et al., 2003a

Fritz Haber (1868-1934)

Began work on NH_3, 1904
First patent, 1908
Commercial-scale test, 1909
Developed Cl_2 gas production, 1914
Nobel Prize in Chemistry, 1918
-"for the synthesis of ammonia from its elements"

Photograph of Carl Bosch removed due to copyright restrictions.

Carl Bosch (1874-1940)

The perfect catalyst, 1910
Large-scale production, 1913
Ammonia to nitrate, 1914
Nobel Prize in Chemistry, 1931
-"chemical high pressure methods"

Smil, 2001

Haber-Bosch Process for the Production of Ammonia

$$N_{2(g)} + 3H_{2(g)} \rightleftharpoons 2NH_{3(g)} \qquad \Delta H = -92\ kJ\ mol^{-1}$$

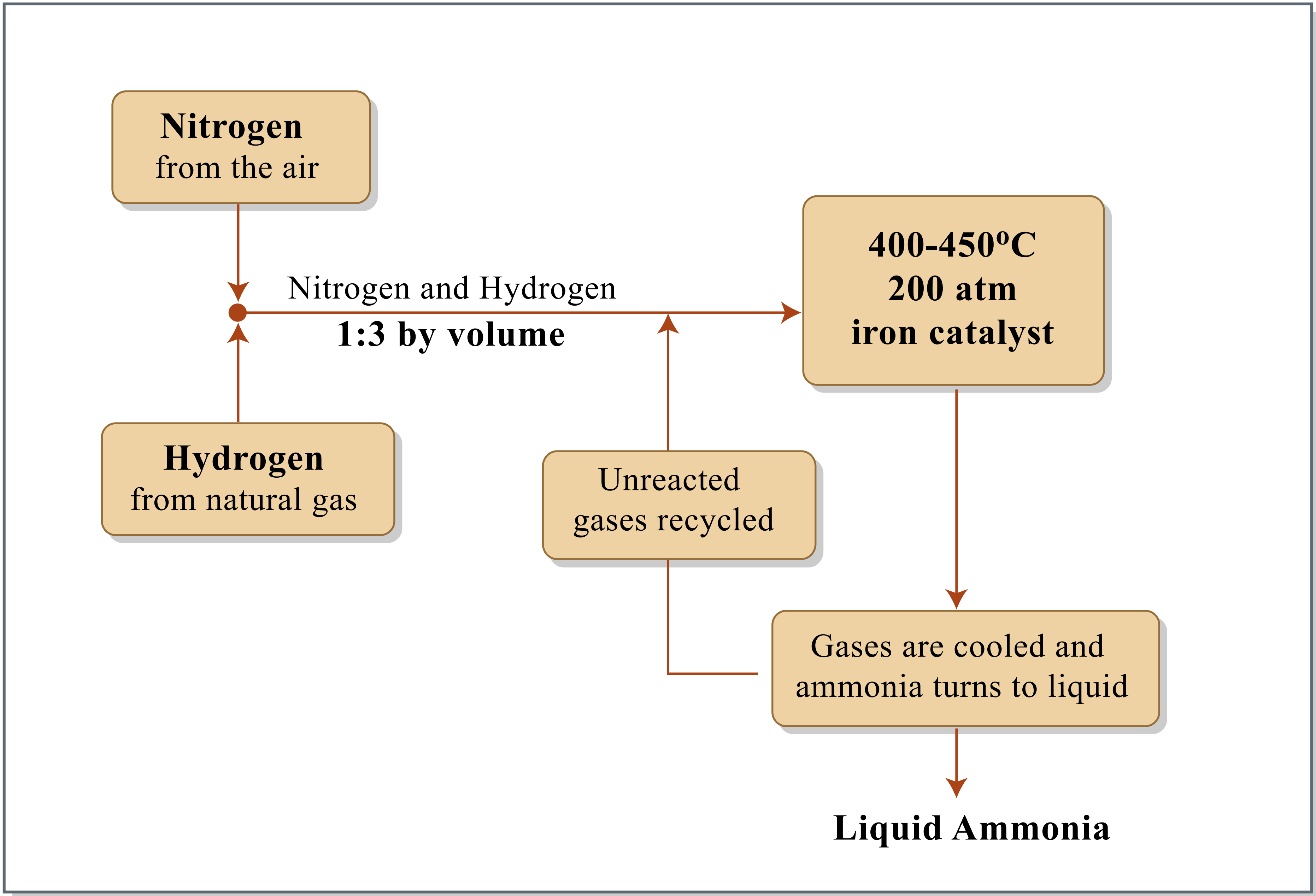
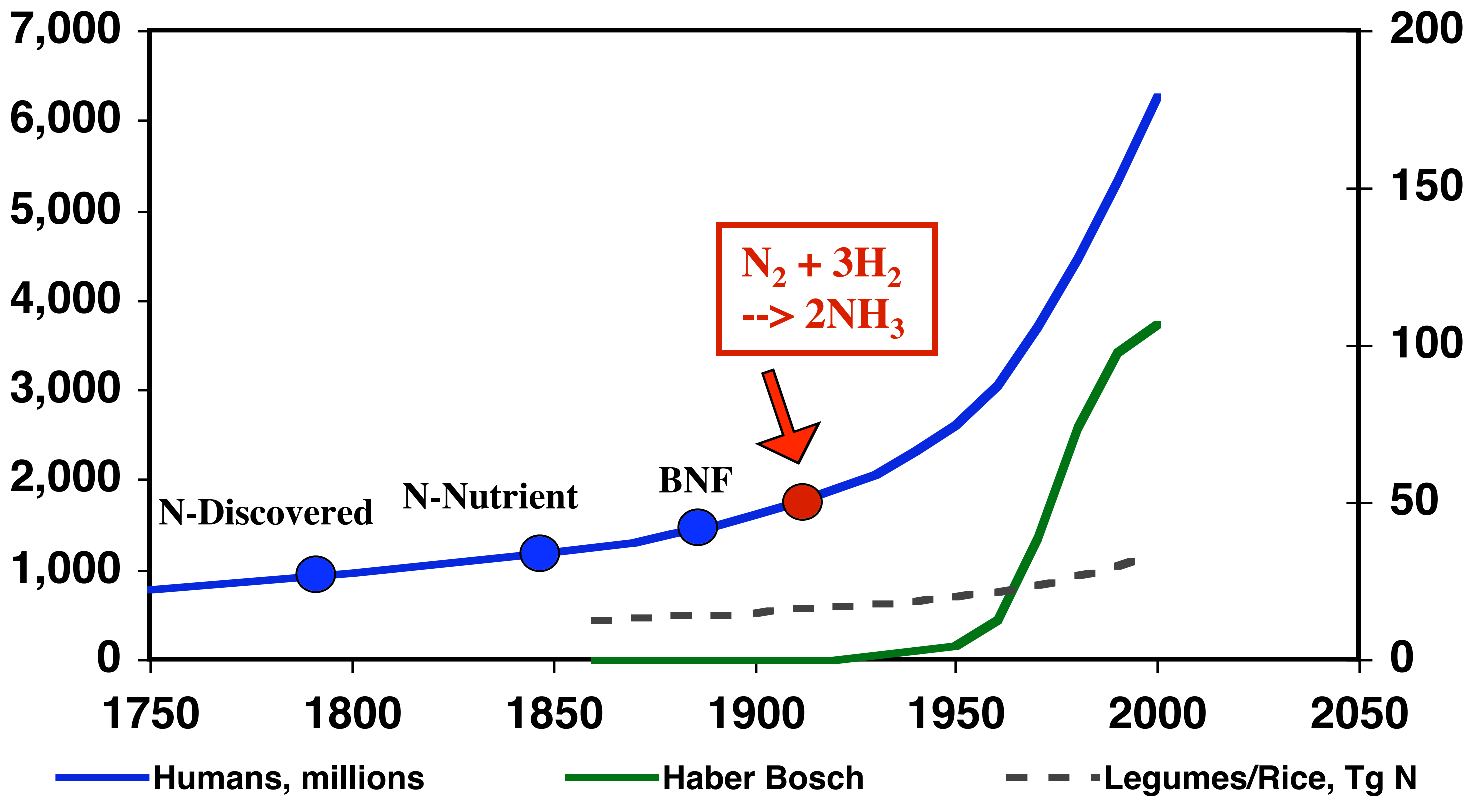


Figure by MIT OCW.

The History of Nitrogen

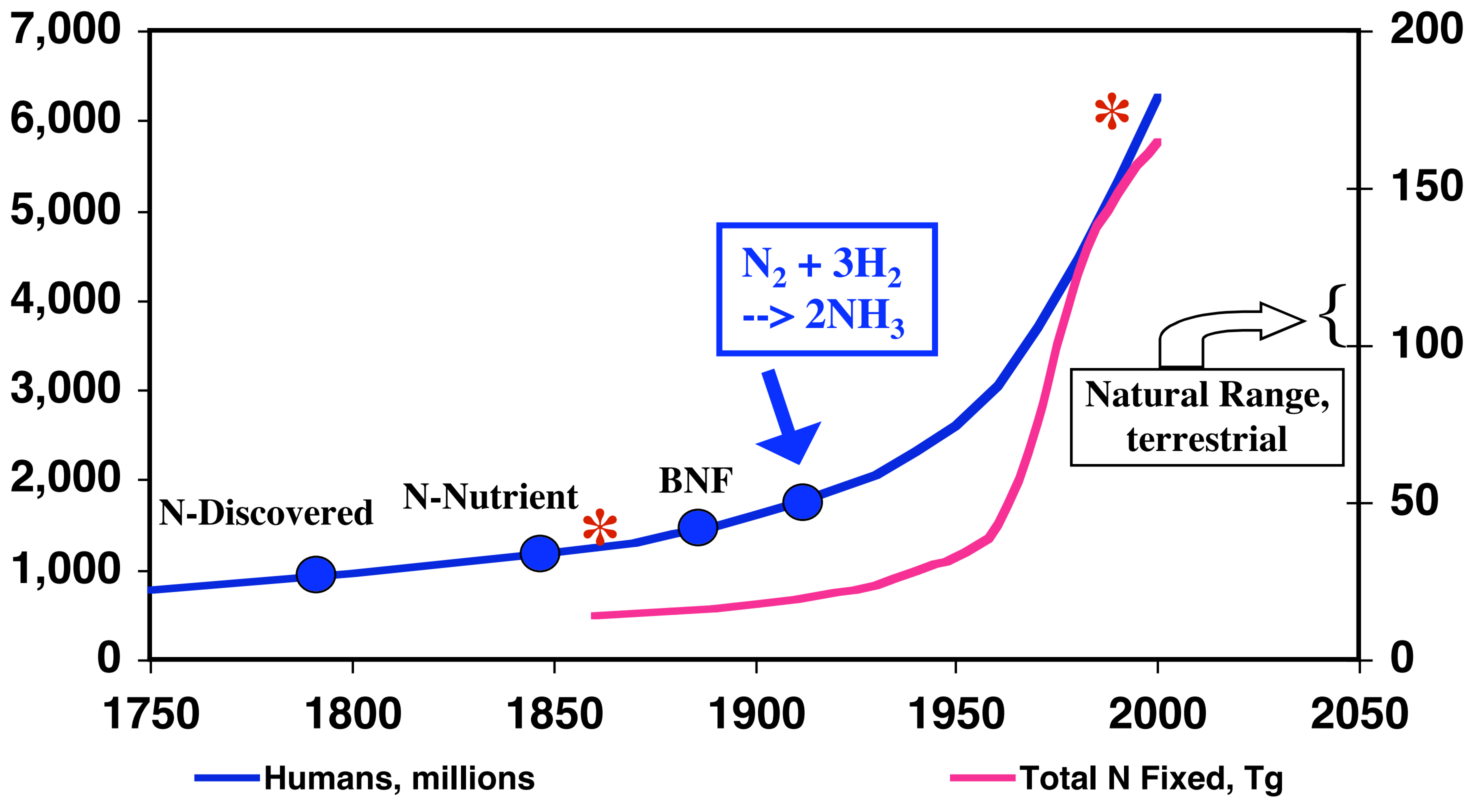
--N_r Creation, Haber Bosch process--



Galloway JN and Cowling EB. 2002; Galloway et al., 2003a

The History of Nitrogen

--N_r Creation, People and Nature--



Galloway JN and Cowling EB. 2002; Galloway et al., 2003a

Nitrogen Drivers in 1860

Photo of a small-scale single farmer grain field.

Grain Production

Photo of one cow.

Meat Production

Photo of trees in a forest.

Energy Production

Nitrogen Drivers in 1860 & 1995

Photo of a small-scale single farmer grain field. → Photo of a massive modern grain farm.

Grain Production

Photo of one cow. → Photo of a large-scale modern cattle farm.

Meat Production

Photo of trees in a forest. → Photo of burning fuel.

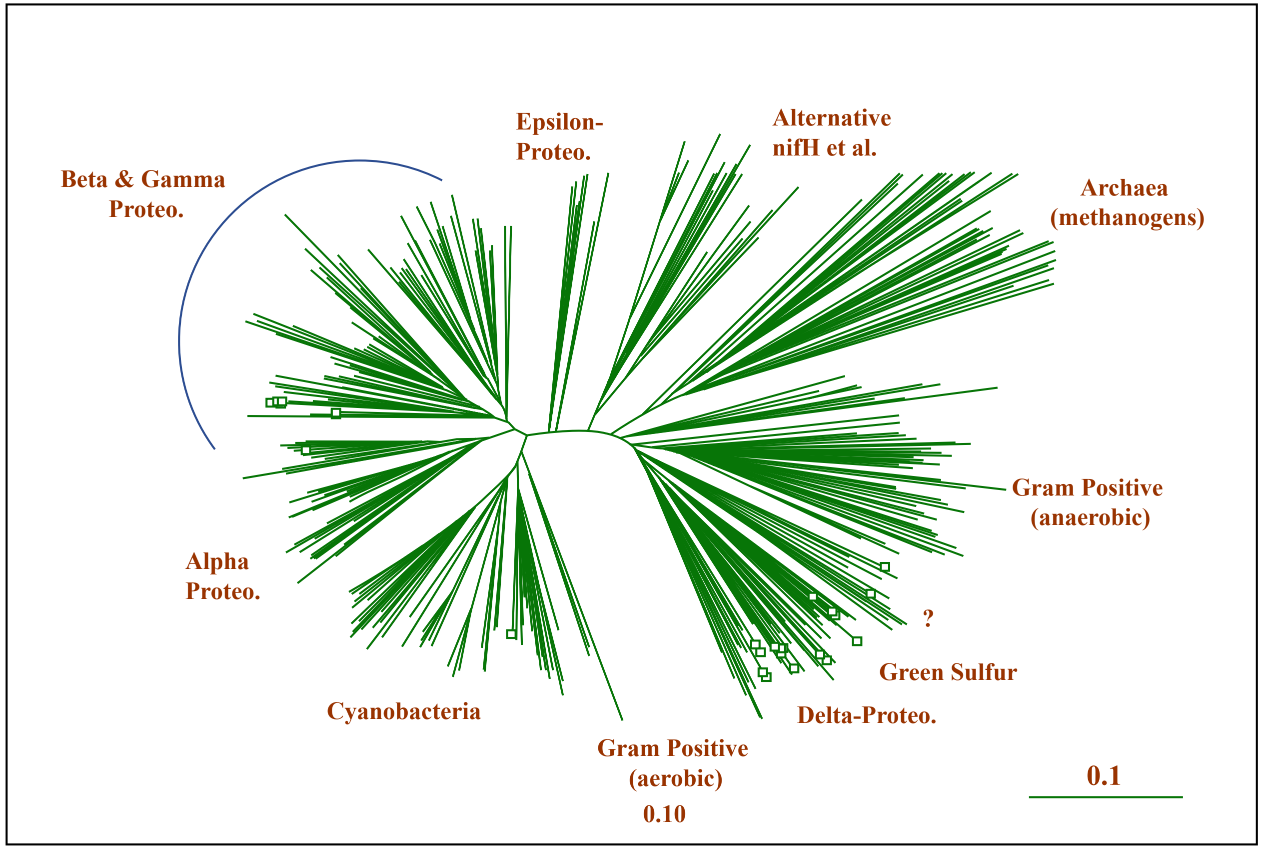
Energy Production

The Global Nitrogen Budget in 1860 and mid-1990s, TgN/yr

Diagram removed due to copyright restrictions.

Nitrogen Fixation

- **Diversity**
  - Cyanobacteria
  - Proteobacteria
  - Archaea
  - But not all species of same group can fix



Energetics

Costs 16 ATP per molecule N_2 'fixed'

$$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16\ MgADP + 16\ P_i$$

Nitrogen Fixation

A) Requires nitrogenase enzyme

- 8 subunits / accessory proteins
- 21 different genes required
- Molybdenum and iron cofactors
- Requires energy to break N-N triple bond

B) Strictly anaerobic process: nitrogenase rapidly inactivated by O_2

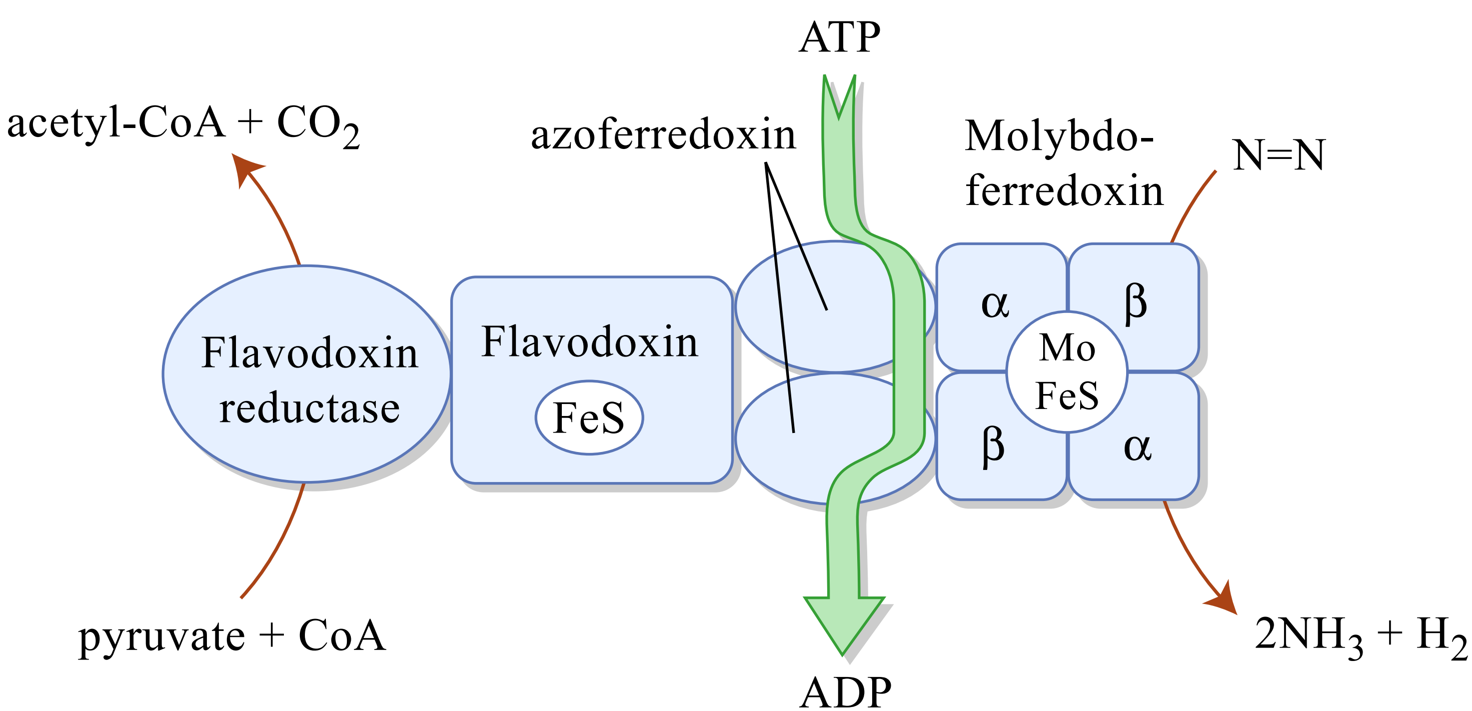


Figure by MIT OCW.

Images and tables removed due to copyright restrictions.

See Figures 17-71, 17-75, 17-73, and Table 17-10 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Nitrogen Fixation

Anabaena heterocyst formation:

- Photosynthetic cyanobacterium
- Filamentous bacterium (chains of cells)
- Under low-nitrogen conditions, every 10th cell becomes an anaerobic heterocyst
- DNA rearrangement allows expression of heterocyst and nitrogenase genes: bacterial development!

Richelia

- N_2 fixing Symbiont in diatom *Rhizosolenia*

Image removed due to copyright restrictions.

Teredo navalis

Image removed due to copyright restrictions.

Nitrogen-fixing bacteria in soya plant root nodules

Image removed due to copyright restrictions.

See Figures 19-58, 19-59, 19-61, and Table 19-8 in Madigan, Michael, and John Martinko.
Brock Biology of Microorganisms. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Rhizobium

- Free-living *Rhizobium* in soil is aerobic (no N_2 fixation)
- Specific species associate with specific legumes
- Both partners undergoes developmental changes
  - Plant responds to bacteria by producing anaerobic nodule
  - Bacteria develop into N_2-fixing anaerobic bacteroid form

Image removed due to copyright restrictions.

Rhizobium

Development of the nodule

- Root hairs of plant release flavonoids
  - Attract *Rhizobium*
  - Signal bacteria to make NodD (transcriptional activator)

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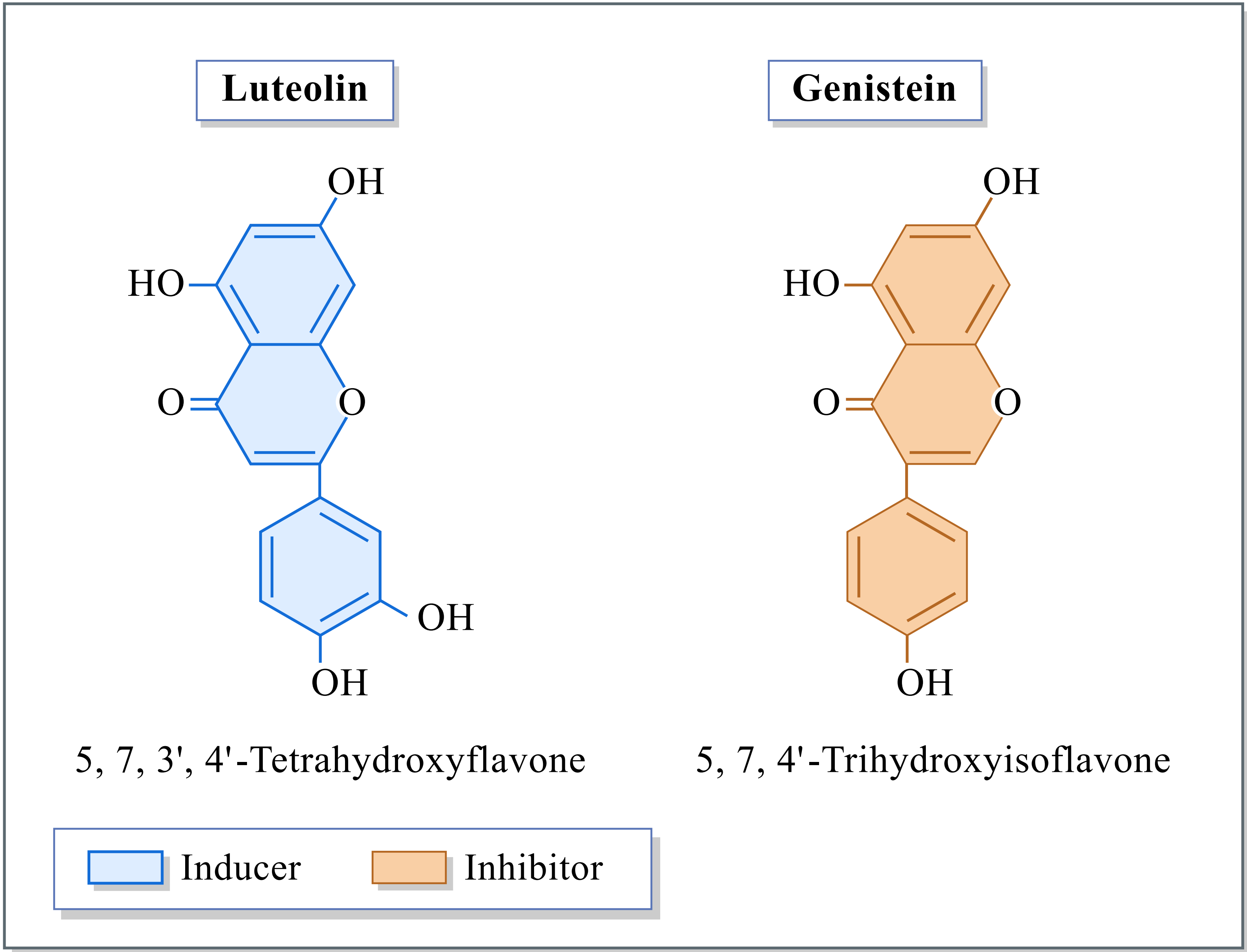


Figure by MIT OCW.

Different flavinoids can either induce or inhibit nodulation

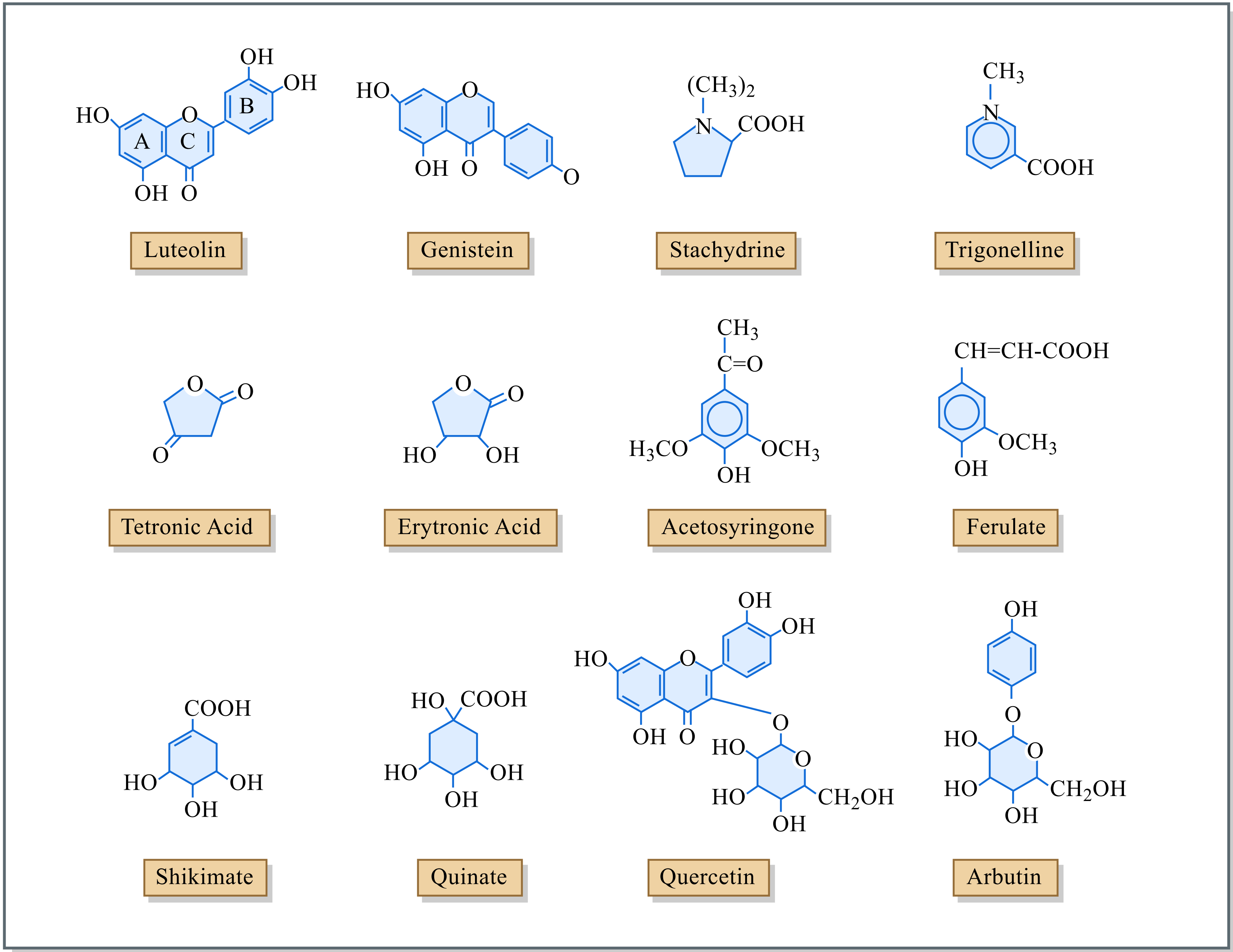


Figure by MIT OCW.

Examples of plant-released molecules that are recognized as signals for induction of specific responses in various plant-associated bacteria

Image removed due to copyright restrictions.
See Figure 19-64 in Madigan, Michael, and John Martinko.
Brock Biology of Microorganisms. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Nod *genes*, nitrogenase *genes*, and host specificity *genes* are on the *Sym* plasmid of *Rhizobium leguminosarum*

Rhizobium

Development of the nodule:

- NodD turns on transcription of *nod* genes

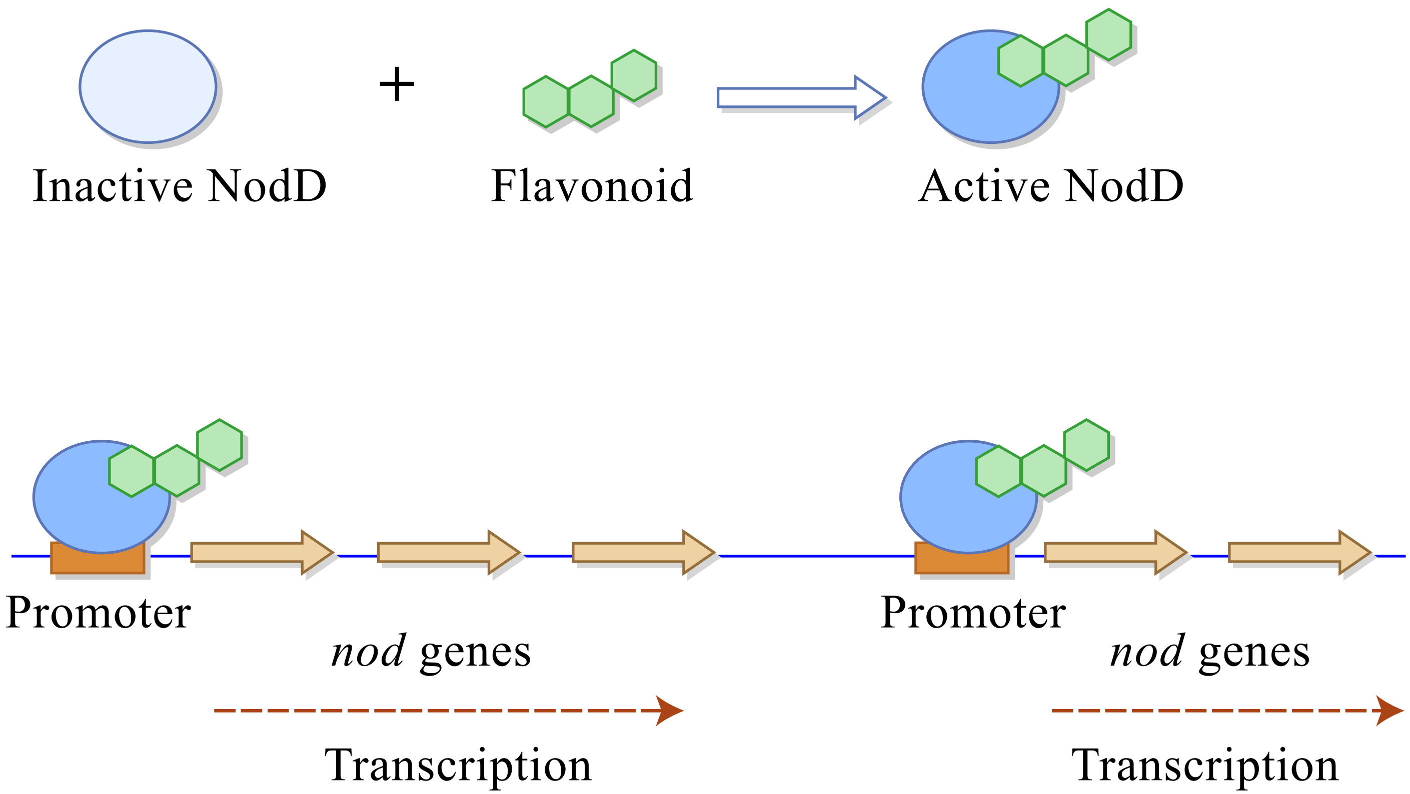
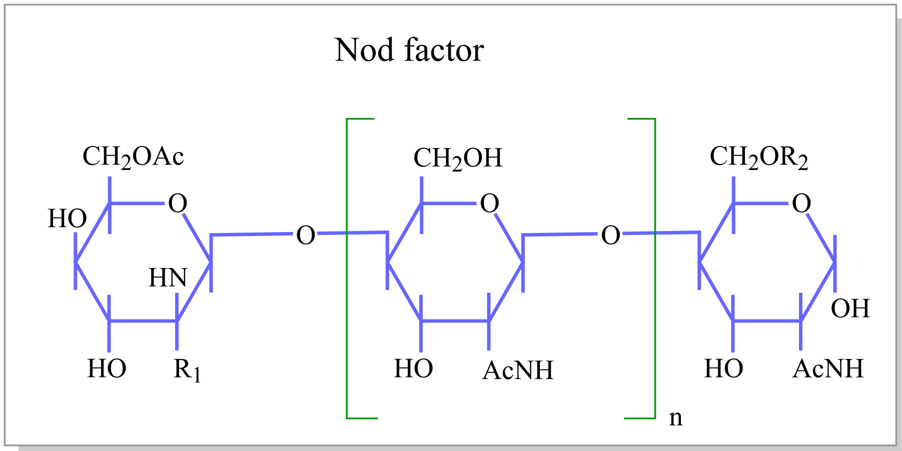
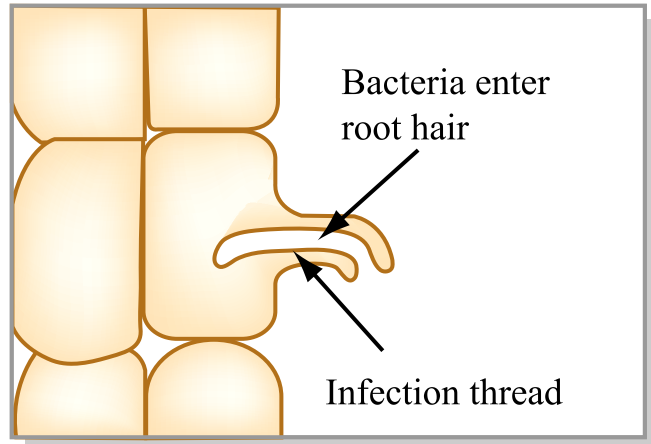


Figure by MIT OCW.

Nod **genes are typically carried on a plasmid - The Sym plasmid - these can encode *nod* genes, host recognition/specificity genes, and *nif* (nitrogen fixation) genes. Can confer host specificity by cross-transforming different rhizopbia with Sym plasmids**

Rhizobium

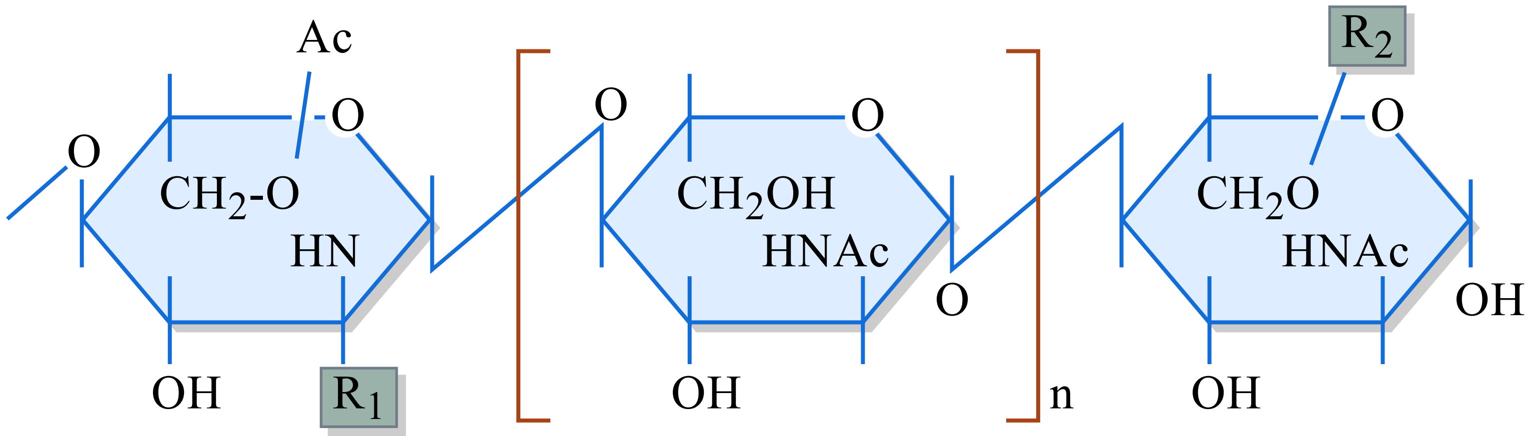




Development of the nodule:

1. *nod* gene products make Nod factors (polysaccharides)
2. Nod factors act as plant hormones
3. Nod factors signal root hair to curl and form an invagination called the infection thread

Figure by MIT OCW.

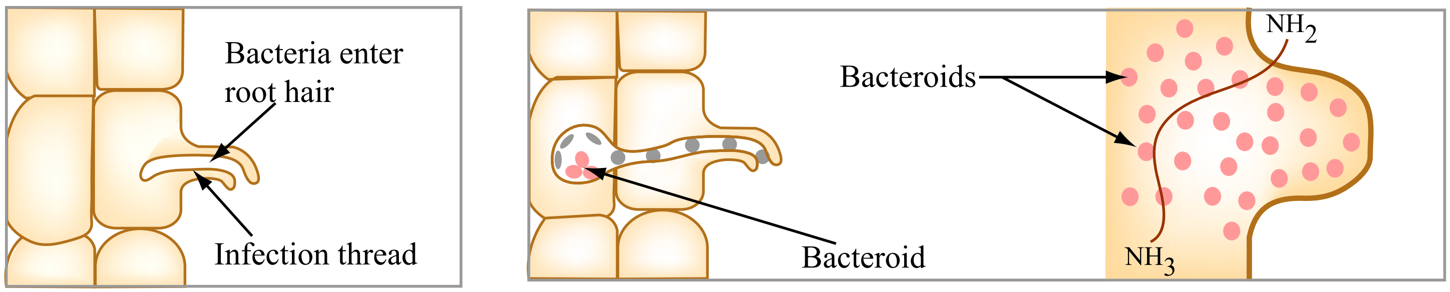


Species	R_1	R_2
Sinorhizobium meliloti	C16:2 or C16:3	SO_4^{2-}
Rhizobium leguminosarum biovar viciae	C18:1 or C18:4	H or Ac

Nod Factors

Figure by MIT OCW.

Rhizobium



Development of the nodule:

1. Bacteria lose flagella, attach to root hair and move along infection thread
2. Bacteria invade root cells
3. Root cells differentiate into nodule, bacteria differentiate into large, spherical bacteroids

Figure by MIT OCW.

Image showing bacteroids and the infection thread removed due to copyright restrictions.

Rhizobium

What happens in the nodule?

- Bacteria leave the infection thread and are inside cells
- Plant cell and bacteria cooperate to make leghemoglobin
  - Plant genes encode the leghemoglobin protein
  - Bacteria produce the heme group
- Leghemoglobin binds O_2 tightly
  - Maintains anaerobic environment for nitrogenase
  - Allows aerobic respiration for bacteria (obligate aerobe!)
- Plant makes malate as carbon/energy source for bacteria
  - Used in TCA to make NADH   ETS to make ATP
- ATP and NADH provide energy and electrons for $N_2 \rightarrow NH_3$

Root nodules

Image removed due to copyright restrictions.
See Figure 19-60 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*.
11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

The nitrogen-fixing nodule hosts symbiotic *Rhizobium* bacteroids

Image removed due to copyright restrictions.

Leghemoglobin O_2:free O_2 ~ 10,000 :1

See Figures 19-67 and 19-55 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*.
11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Ti plasmid & crown gall disease

A portion of the Ti plasmid is inserted into the plant chromosome. These cells grow to form the tumor or gall.

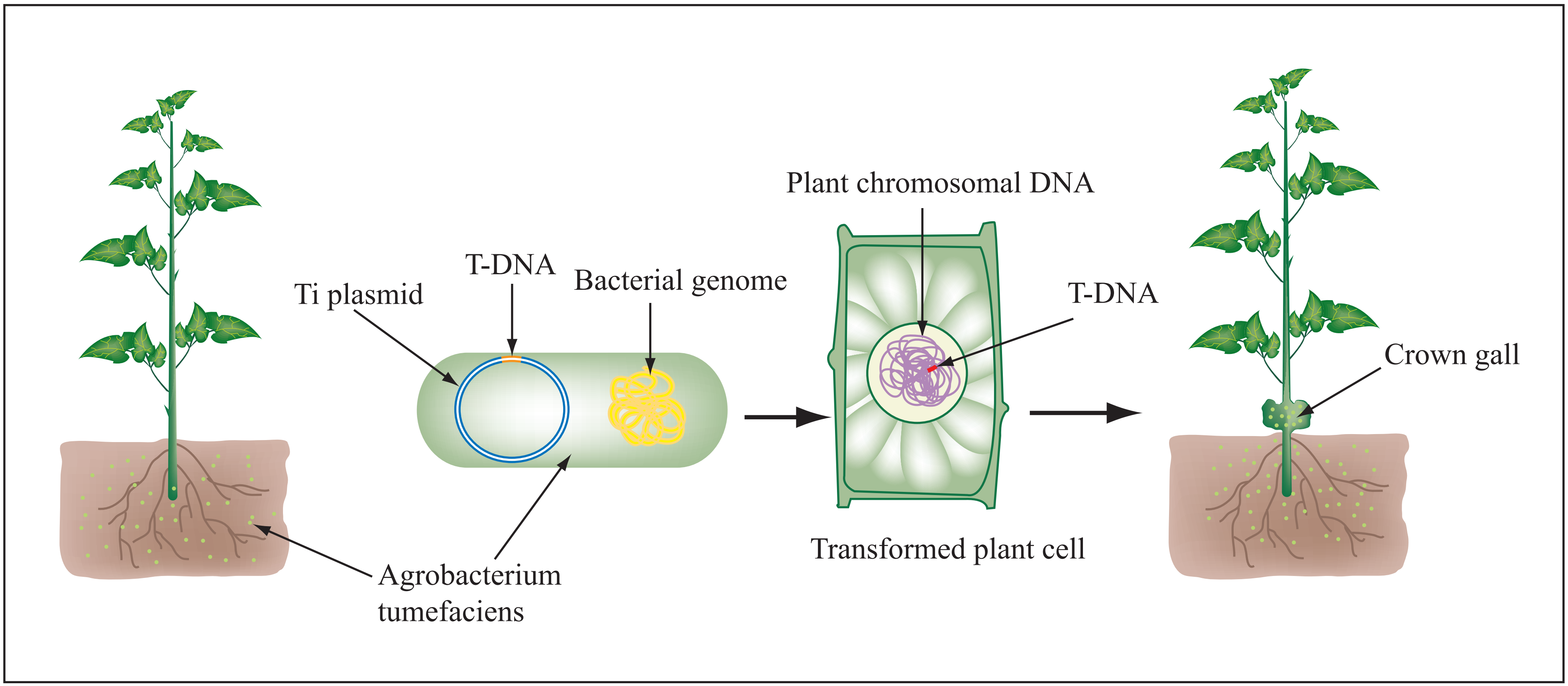


Figure by MIT OCW.

Ti plasmid of *Agrobacterium tumefaciens*

See Figures 19-56 and 19-57 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

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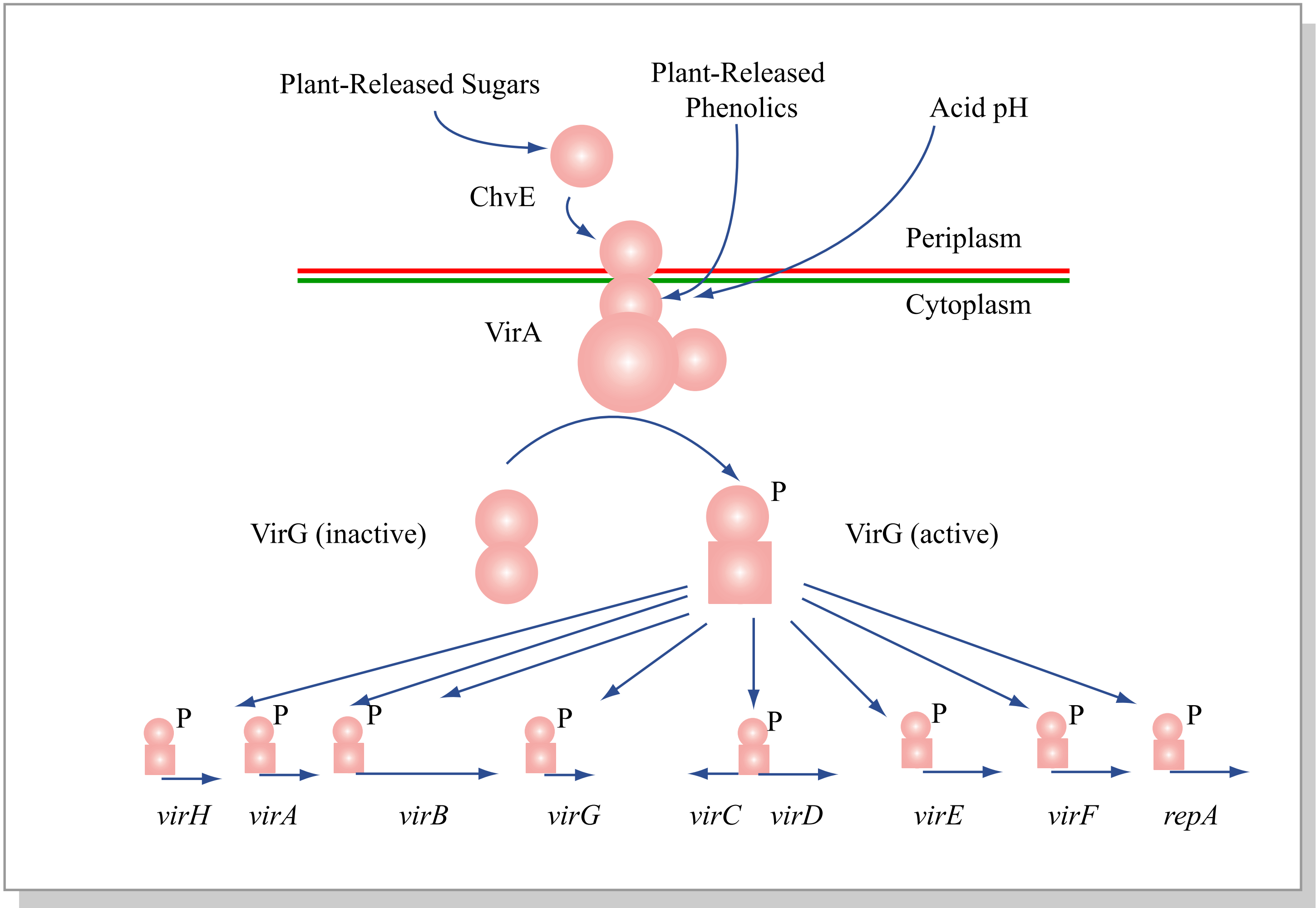

Figure by MIT OCW.

The Ti plasmid

T-DNA transfer functions are encoded in a specific part of the plasmid. Transfer occurs by a mechanism almost identical to bacterial conjugation. Insert a gene into the T-DNA and let the mechanism of DNA transfer take over transfer into plant cells. Ti plasmids are too large to manipulate so a methodology to insert DNA into the T-DNA has been developed.

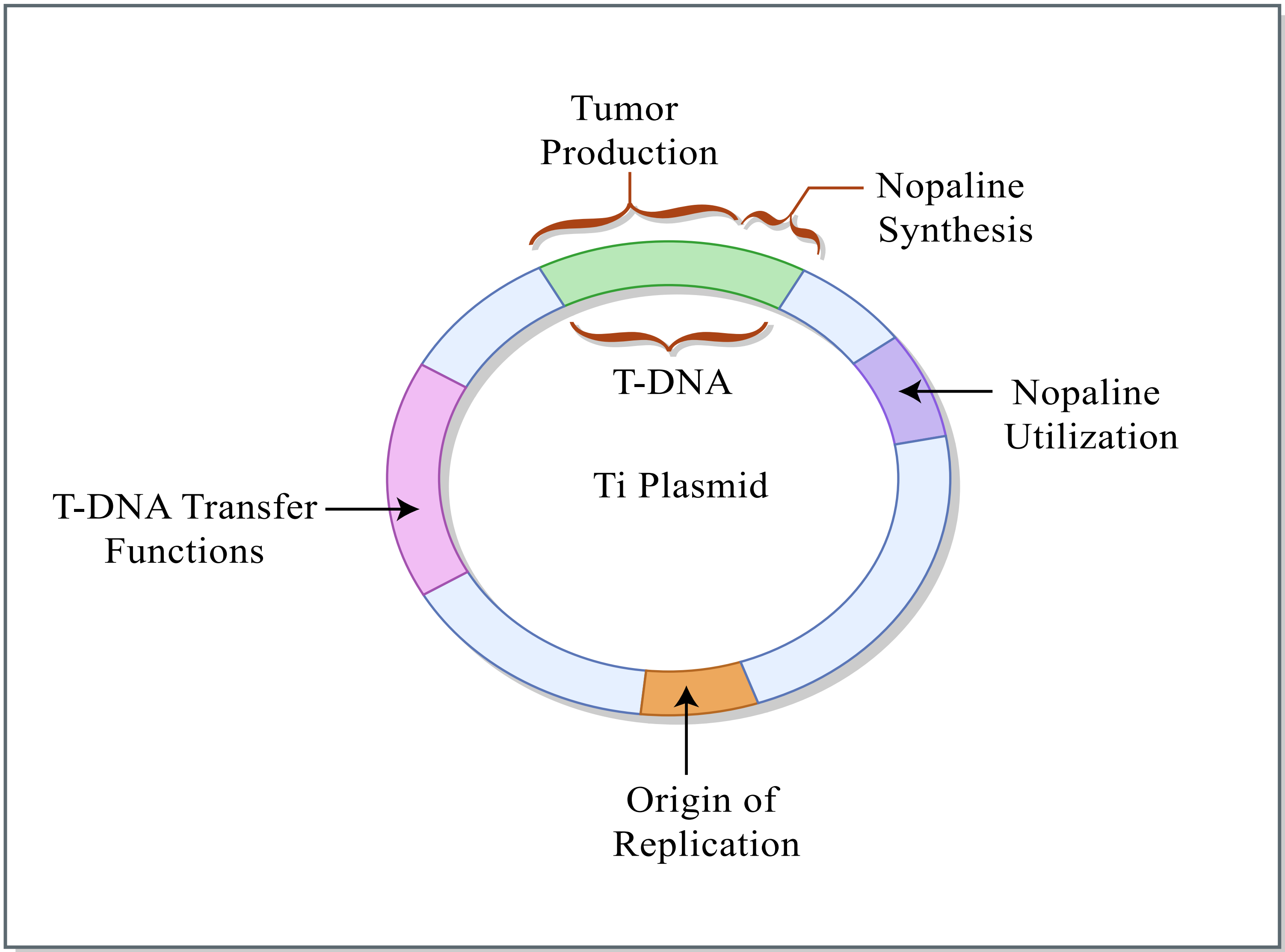

Figure by MIT OCW.

The use of Ti plasmids in engineering transgenic plants (GM plants)

Successfully used for tomatoe potato, soybean, tobacco, cotton - Also trees, including apples & walnuts

Creating a transgene delivery system based on the Ti plasmid

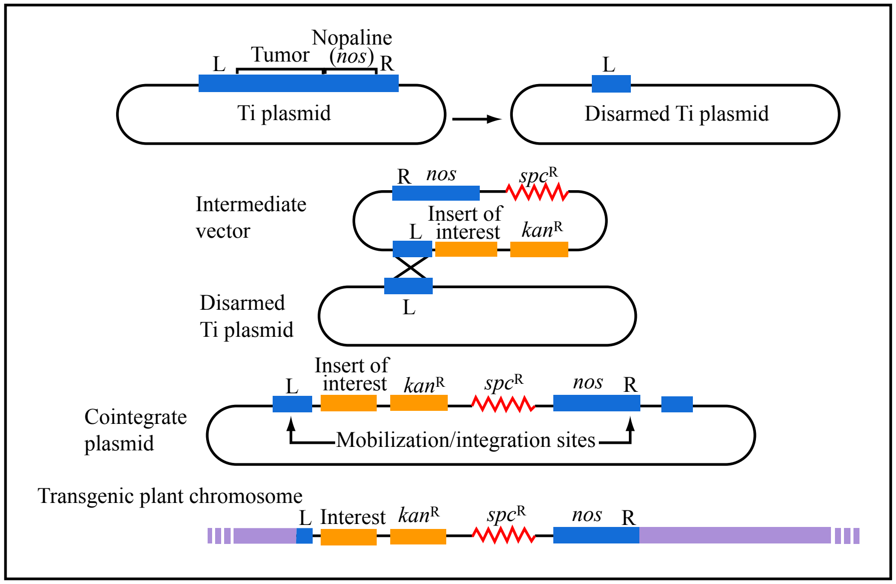

Figure by MIT OCW.

Creating a transgenic plant.

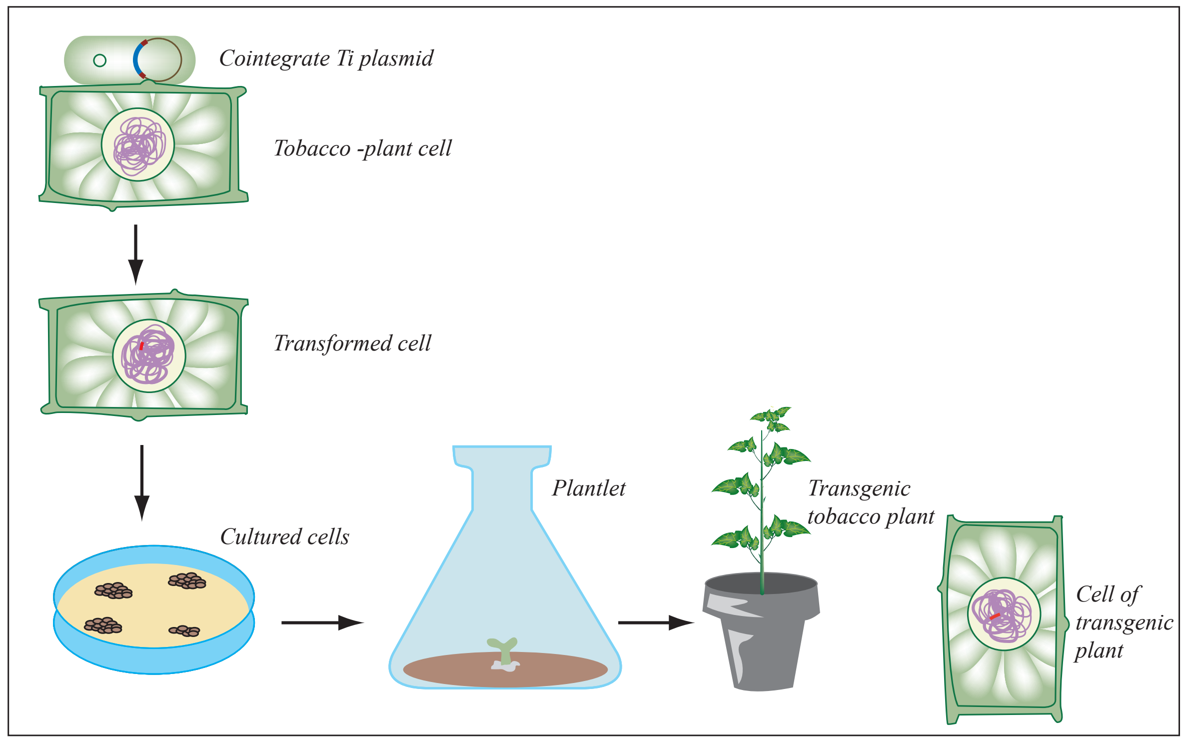

Figure by MIT OCW.

Segregation of the transgene

The transgene segregates at meiosis and mitosis like any normal mendelian gene

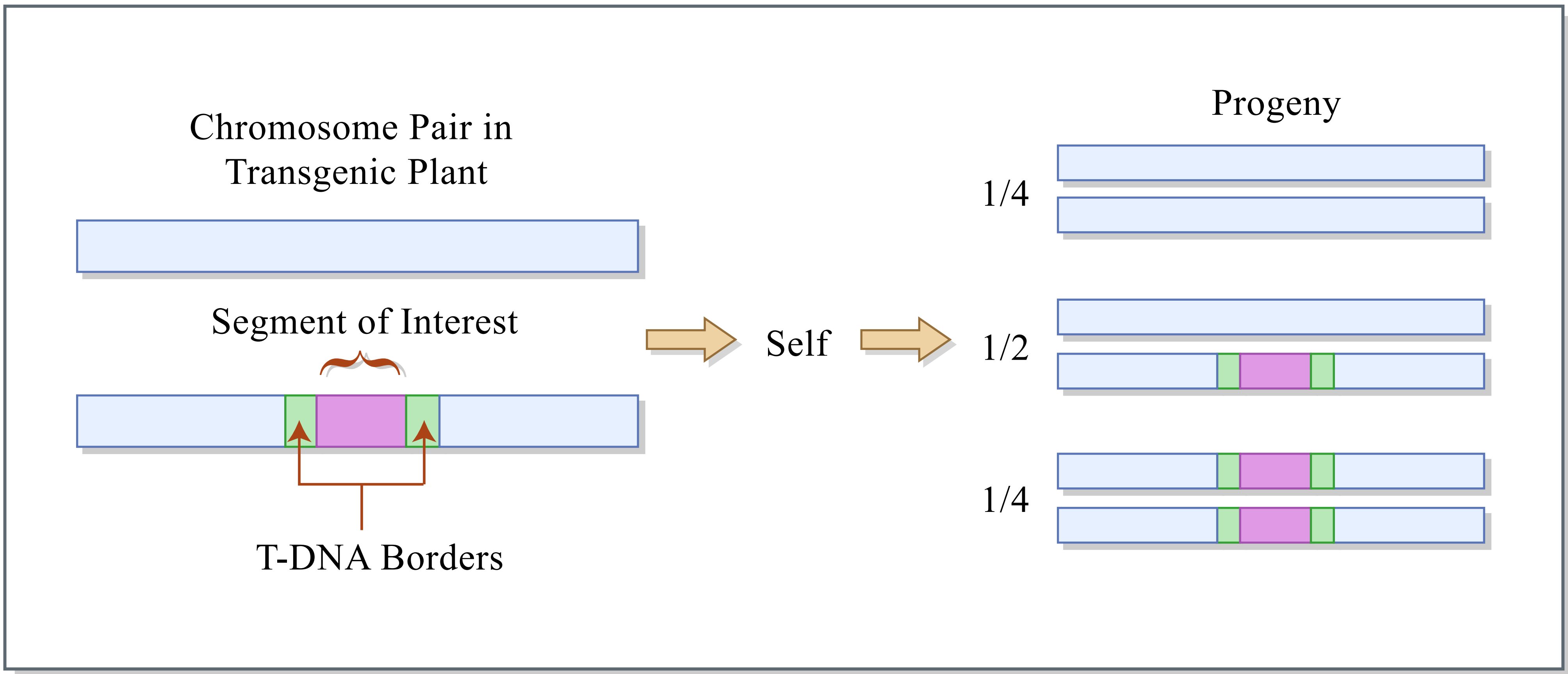

Figure by MIT OCW.

Non-plant species that can be genetically transformed by *Agrobacterium*

Table listing the kingdom, phylum, family, and species of non-plant species that can be genetically transformed by *Agrobacterium* removed due to copyright restrictions.

Trends Genetics 22: 2006
Doi 10.1016/j.tig.2005.10.004

GMOs - societial issues

Photographs removed due to copyright restrictions.

Benefits

- **Crops**
  - Enhanced taste and quality
  - Reduced maturation time
  - Increased nutrients, yields, and stress tolerance
  - Improved resistance to disease, pests, and herbicides
  - New products and growing techniques
- **Animals**
  - Increased resistance, productivity, hardiness, and feed efficiency
  - Better yields of meat, eggs, and milk
  - Improved animal health and diagnostic methods
- **Environment**
  - "Friendly" bioherbicides and bioinsecticides
  - Conservation of soil, water, and energy
  - Bioprocessing for forestry products
  - Better natural waste management
  - More efficient processing
- **Society**
  - Increased food security for growing populations

Controversies

- **Safety**
  - Potential human health impact: allergens, transfer of antibiotic resistance markers, unknown effects Potential environmental impact: unintended transfer of transgenes through cross-pollination, unknown effects on other organisms (e.g., soil microbes), and loss of flora and fauna biodiversity
- **Access and Intellectual Property**
  - Domination of world food production by a few companies
  - Increasing dependence on Industralized nations by developing countries
  - Biopiracy—foreign exploitation of natural resources
- **Ethics**
  - Violation of natural organisms' intrinsic values
  - Tampering with nature by mixing genes among species
  - Objections to consuming animal genes in plants and vice versa
  - Stress for animal
- **Labeling**
  - Not mandatory in some countries (e.g., United States)
  - Mixing GM crops with non-GM confounds labeling attempts
- **Society**
  - New advances may be skewed to interests of rich countries

http://www.ornl.gov/sci/techresources/Human_Genome/elsi/gmfood.shtml