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@PuckerLab

Prof. Dr. Boas Pucker

PBPM-BP-04

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - eCampus: PBPM0 - Plant Biochemistry, Physiology and Molecular Biology (LEC)
  - GitHub: https://github.com/bpucker/teaching/PBPM

- Questions: Feel free to ask at any time

- Feedback, comments, or questions: pucker[a]uni-bonn.de

Repetition

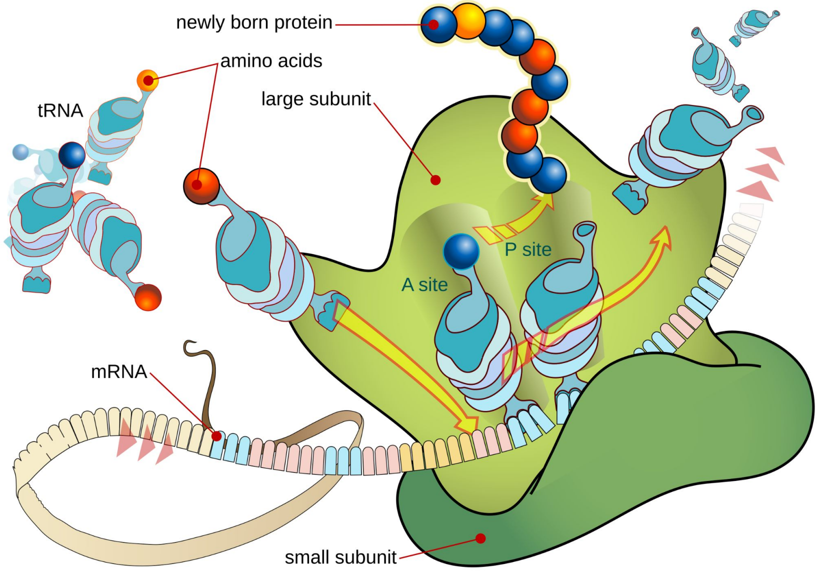
- Components of a plant cell
- Nucleic acids
- DNA replication / PCR
- Transcription & splicing
- Transcription factors and cis-regulatory elements

Protein biosynthesis

(translation)

Translation

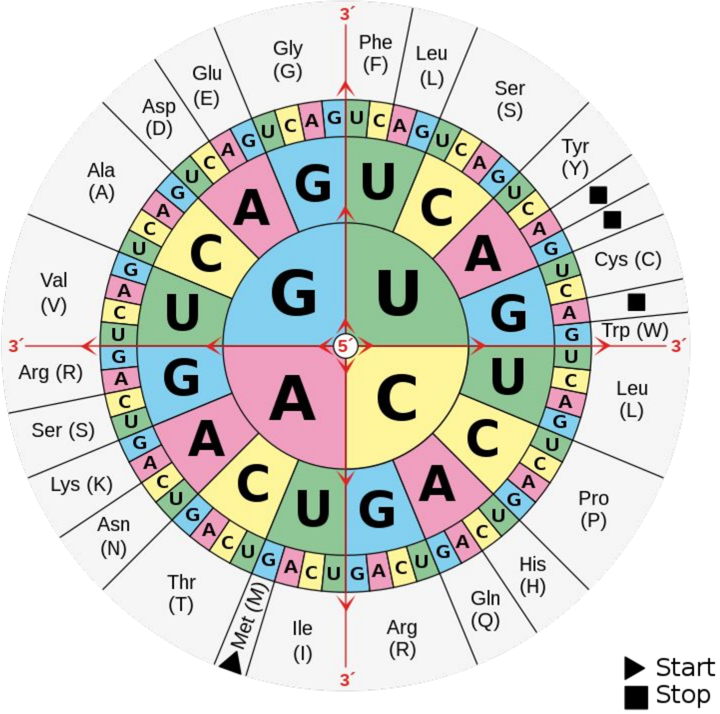
- mRNA contains the instructions for protein synthesis
- Ribosome binding initiates translation
  - 40S + 60S = 80S
- Energy required for translation elongation
- Translation termination at stop codon



https://commons.wikimedia.org/wiki/File:Ribosome_mRNA_translation_en.svg

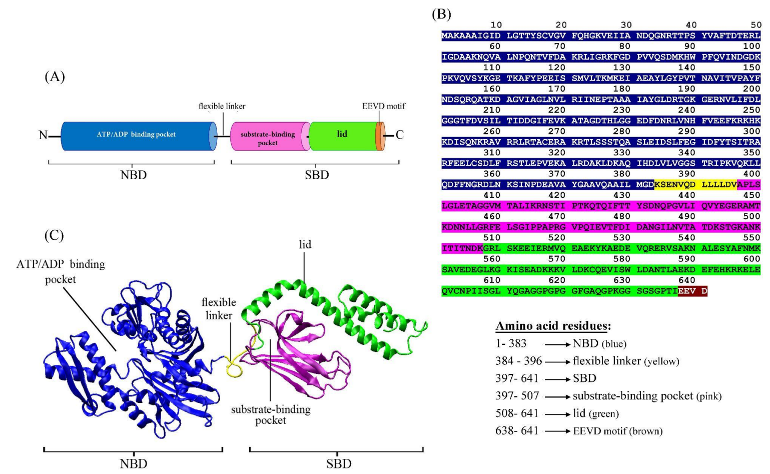
Genetic code

- Generally conserved across all species, but exceptions exist
- Multiple codons for same amino acid
- Codon usage: species-specific usage of certain codons
- Codons are not overlapping



Role of chaperons

- Specialized proteins that assist folding of other proteins by binding hydrophobic regions
- Prevent misfolding under stress conditions
- Facilitates refolding
- HSP = Heat Shock Protein



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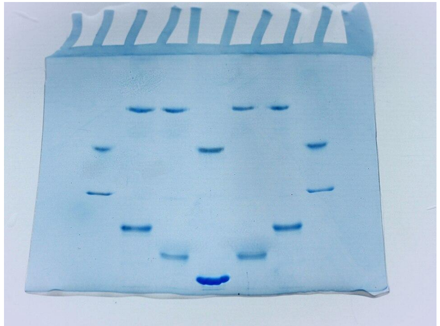
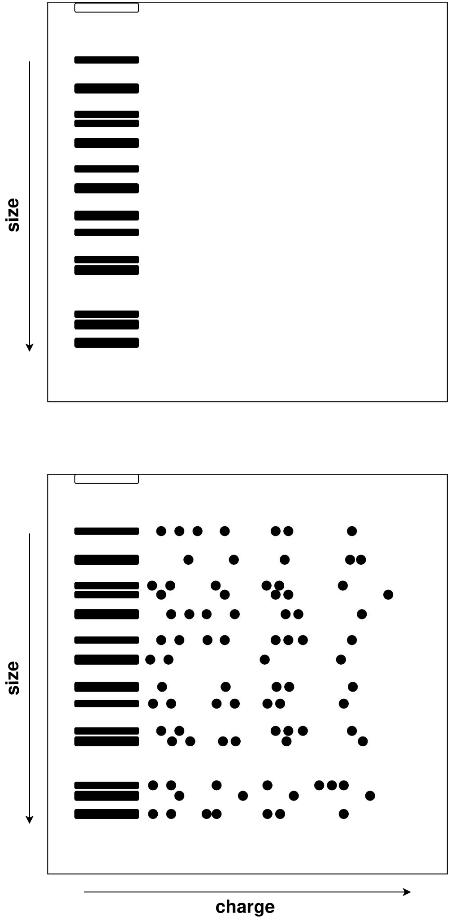
Posttranslational modification

- Phosphorylation: phosphate group at S, T, Y
- Glycosylation: sugar moiety attached to N (N-linked) or S/T (O-linked)
- Ubiquitination: ubiquitin attached to lysine
- Sumoylation: SUMO attachment (Small Ubiquitin-like MOdifier)
- Acetylation: acetyl groups attached to K
- Methylation: methyl groups attached to K or R

Proteomics: 2D SDS PAGE

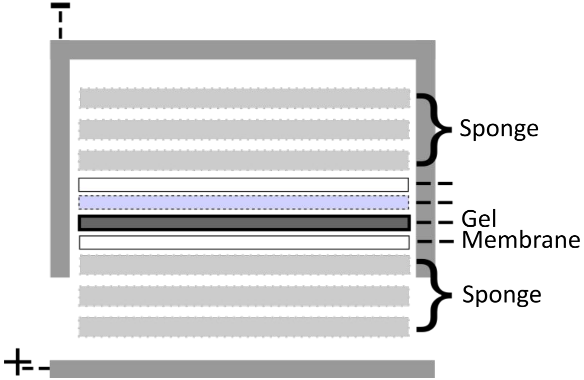
- SDS PAGE: sodium dodecyl sulfate polyacrylamid gel electrophoresis
- Protein separation by size (molecular weight)
- Protein separation by charge (pH gradient)

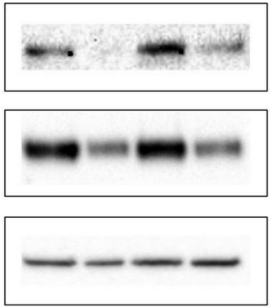




Proteomics: Western blot

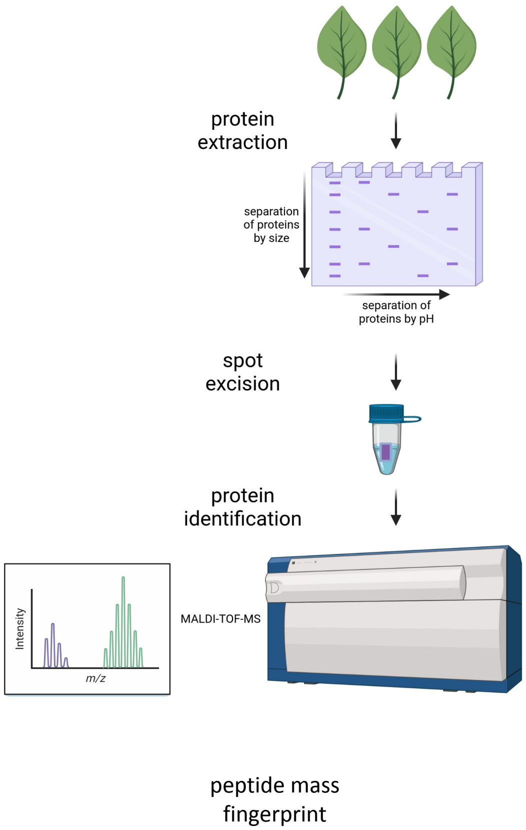
- Separate proteins by SDS PAGE
- Transfer proteins onto blotting membrane
- Add primary antibody that binds to protein of interest
- Add secondary antibody binds to primary antibody and carries reporter
- Reporter is often horseradish peroxidase (HRP)





Proteomics: MALDI-TOF-MS

- Selection of individual spoots from a 2D SDS-PAGE
- Tryptic digestions of protein to generate peptides
- MALDI-TOF-MS analysis of peptides
- Finger print (fragment pattern) of protein can be compared against database



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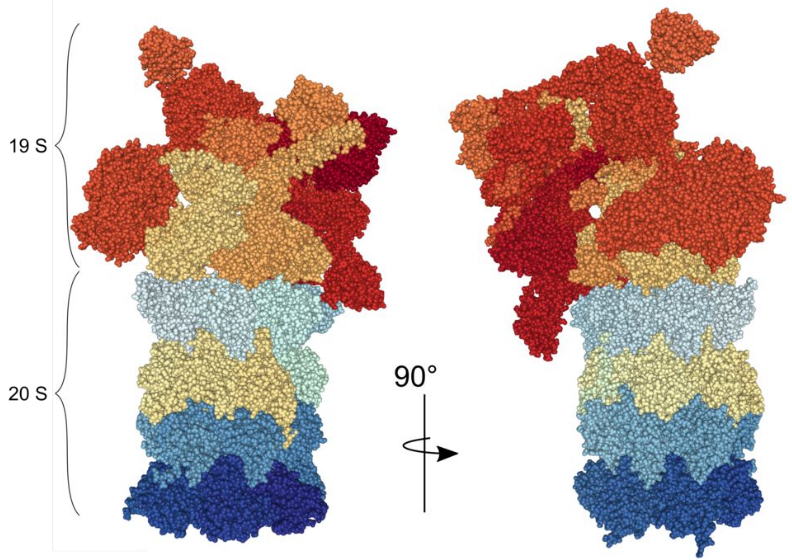
Protein degradations

- Breakdown of proteins into amino acids
- Ubiquitin-Proteasome System (UPS)
- Autophagy-Lysosome/Vacuole Pathway
- Vacuolar proteolysis (non-autophagic)
- Major proteases:
  - cystein proteases: degradation of storage and senescence proteins
  - serine proteases: quality control in organelles
  - metalloproteases: damaged proteins in organelles
  - aspartic proteases: defense and development

26S Proteasome

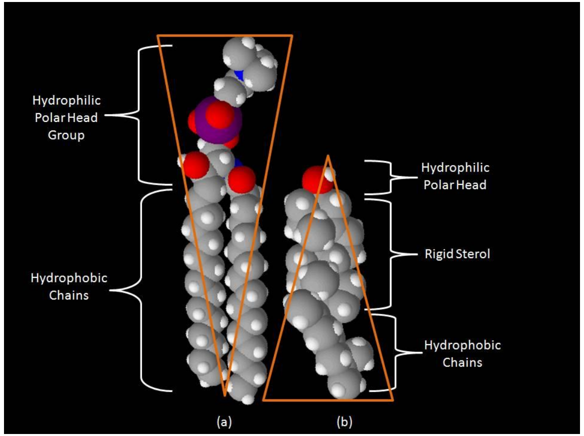
- 26S proteasome = 20S core particle + 19S regulatory particles
- 20S core particle (4 rings)
  - 2 outer alpha-rings: gates
  - 2 inner ß-rings: location of proteolysis
- 19S regulatory particles (caps)
  - Recognizing polyubiquitinated proteins
  - unfolding of substrate proteins (requires ATPases)
  - Feeding of substrate into core structure
  - Ubiquitin recycling



Lipids

Membrane lipids

- Amphipathic molecules that form structural basis of biological membranes
- Different lipid composition in different membranes
- Phospholipids: phosphatidylcholine, phosphatidylserine
- Glycolipids: monogalactosyldiacylglycerol (chloroplast thylakoid membranes)
- Sterols: contribute to membrane fluidity



sphingomyelin, cholesterol

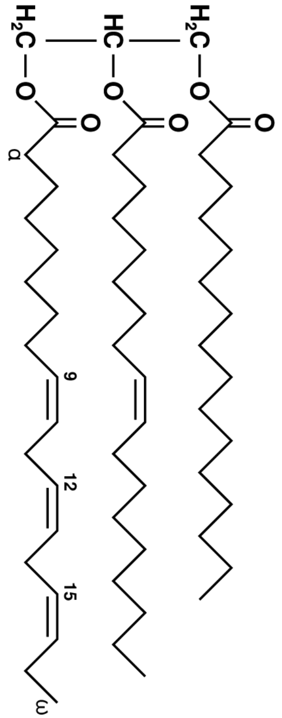
https://commons.wikimedia.org/wiki/File:Space-Filling_Model_Sphingomyelin_and_Cholesterol.jpg

Membrane lipid compositions

- Thylakoid Membranes: Galactolipid Dominance
  - Glycolipids replace phospholipids to conserve phosphate
  - MGDG (non-bilayer-forming) & DGDG (bilayer-forming) stabilize thylakoid lamellar structure
- Cold-Tolerant vs. Cold-Sensitive Plants (canola vs maize chloroplast membranes)
  - Cold-tolerant species have higher levels of polyunsaturated fatty acids
  - Cold-sensitive species have more saturated and monounsaturated fatty acids
- Desert and Halophytic Plants
  - Enhanced sterol and sphingolipid content adds rigidity and ion barrier properties
  - Reduces membrane permeability to Na^+ and Cl^- ions
  - Helps maintain osmotic and ionic homeostasis

Storage lipids

- Energy-dense molecules for long-term energy storage
- Carbon and energy sources for seeds
- Protection against desiccation in seeds
- Primary storage lipids: triacylglycerols (TAGs)
- Waxes: long-chain esters on seed and leaf surfaces (primarily protection)
- Fatty acid synthesis in plastids; TAG assembly in ER via Kennedy pathway

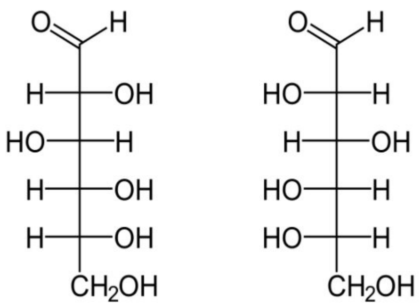


https://upload.wikimedia.org/wikipedia/commons/b/be/Fat_triglyceride_shorthand_formula.PNG

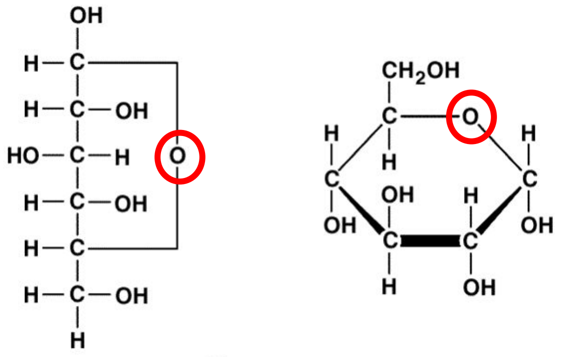
Carbohydrates

Sugar

- Number of carbon atoms in molecule
  - Trioses (C_3): Glyceraldehyde, dihydroxyacetone
  - Pentoses (C_5): Ribose, ribulose (important in nucleotides and Calvin cycle)
  - Hexoses (C_6): Glucose, fructose, mannose, galactose
- Circular/linear forms
- Positions of hydroxy groups (D vs. L)
- Compositions:
  - Monosaccharide (1) Glucose, fructose
  - Disaccharide (2) Sucrose, maltose, lactose
  - Oligosaccharide (3-10) Raffinose, stachyose
  - Polysaccharide (>10) Starch, cellulose



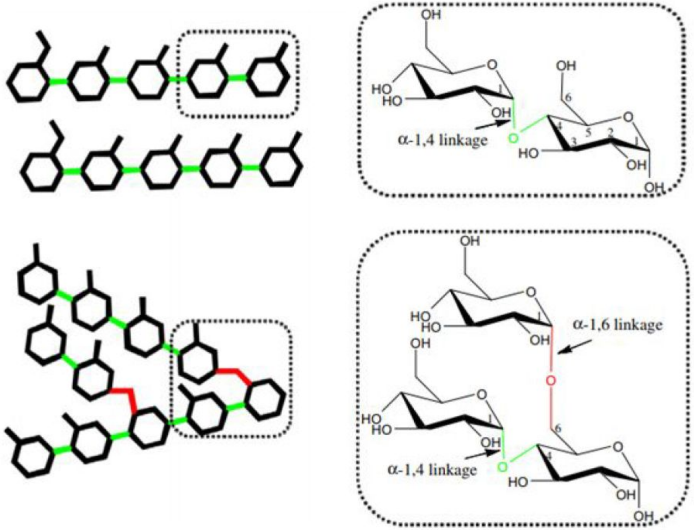
D-Glucose L-Glucose



Glucose

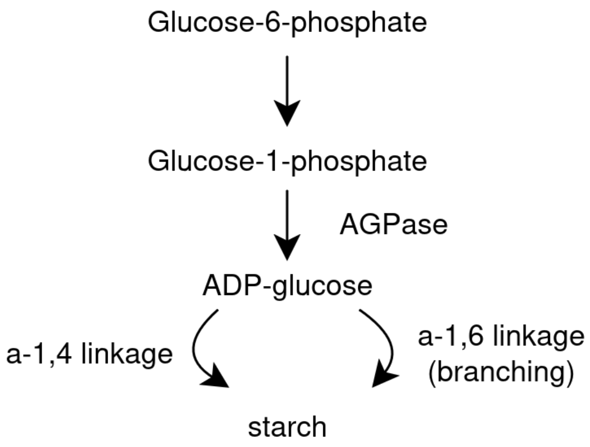
Starch

- Amylose: Linear chains of α(1→4)-linked glucose; forms helical structures; 20-30% of starch
- Amylopectin: Branched polymer: α(1→4) linkages with α(1→6) branches every 20–30 glucose units; 70–80% of starch
- Chloroplasts (leaves): transient starch; synthesized during the day, degraded at night
- Amyloplasts (tubers, seeds): storage starch; long-term carbon reserve



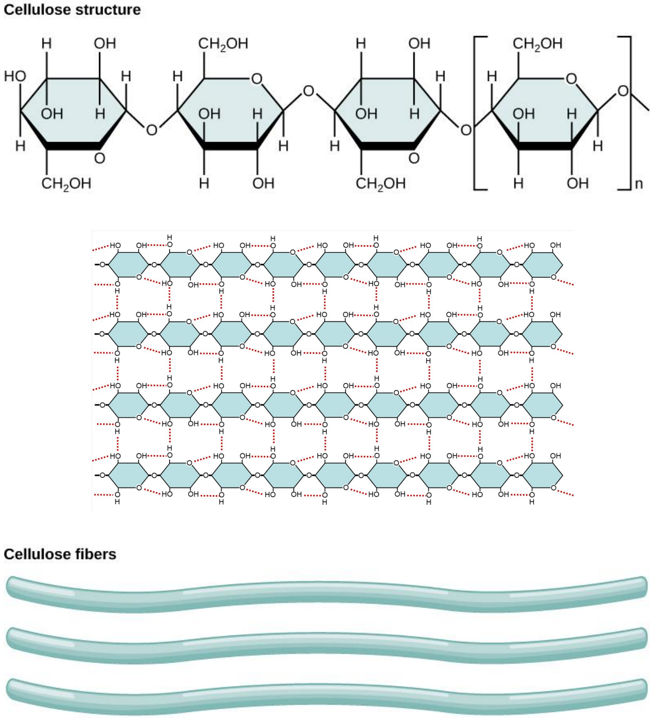
Starch biosynthesis

- Precursor formation: G6P → glucose-1-phosphate → ADP-glucose (via ADP-glucose pyrophosphorylase, AGPase)
- Polymerization: starch synthases (SS) add glucose units (α-1,4 linkages)
- Branching: branching enzymes (BE) create α-1,6 linkages → amylopectin branches
- Debranching (trimming): Isoamylase and pullulanase help organize amylopectin structure



Cellulose

- Linear polysaccharide: β-D-glucose units linked by β (1⟶4) glycosidic bonds
- Every other glucose is rotated 180°; extensive hydrogen bonding between chains
- Cellulose chains forms microfibrils that give plant cell walls mechanical strength
- Most abundant organic compound (wood, cotton, paper)
- Degree of polymerization: 2,000–15,000 glucose residues per chain (species-dependent)
- Synthesized by cellulose synthase complexes at the plasma membrane

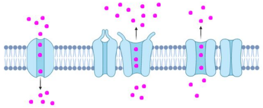


Nitrate assimilation & metabolism

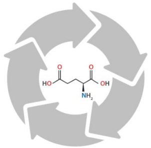
Nitrogen in plants

- Importance of nitrogen

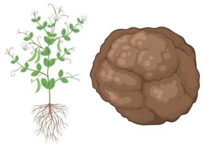
- Nitrogen uptake



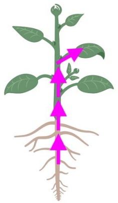
- Nitrogen assimilation



- Biological nitrogen fixation



- Nitrogen transport in plants



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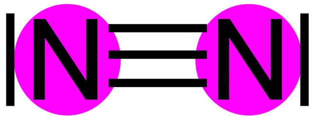
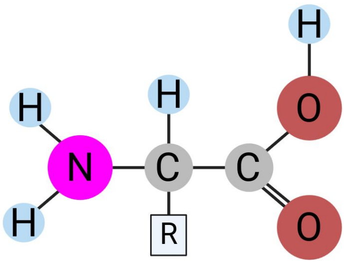
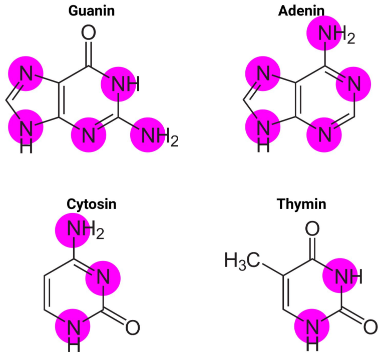
Nitrogen

- Invisible, tasteless, and ubiquitous (78% of air)
- Molecular nitrogen is very stable
- 1-5% of plant dry mass

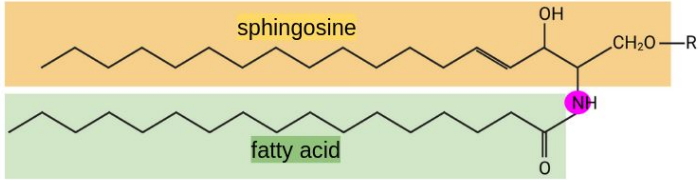




amino acids



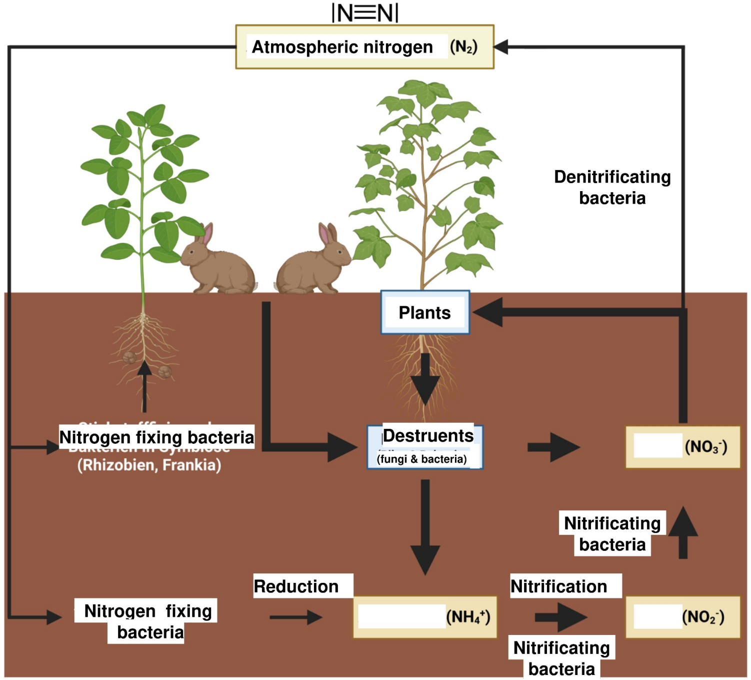
bases



sphingolipids

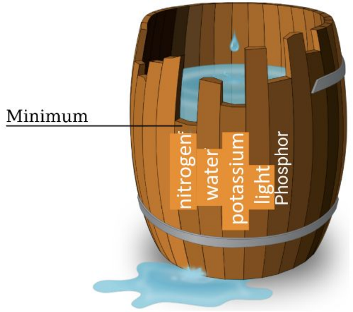
Atmospheric nitrogen cycle

- Atmospheric nitrogen (N_2) not directly accessible
- Bacterial nitrogen fixation into ammonium (NH_4^+) is energy intensive process
- Nitrification converts ammonium into nitrit (NO_2^-) and into nitrate (NO_3^-)
- Denitrification bacteria convert NO_3^- in N_2



Nitrogen as limiting factor for biomass formation

- Liebig's barrel illustrates the limiting factor of plant growth



- Haber–Bosch process enables physicochemical nitrogen fixation (an agricultural revolution at the beginning of the 20th century)

- Ammonia synthesis from nitrogen and hydrogen under high pressure and high temperature

- NPK fertilizer: nitrogen, phosphorus, and potassium are contained in every fertilizer granule

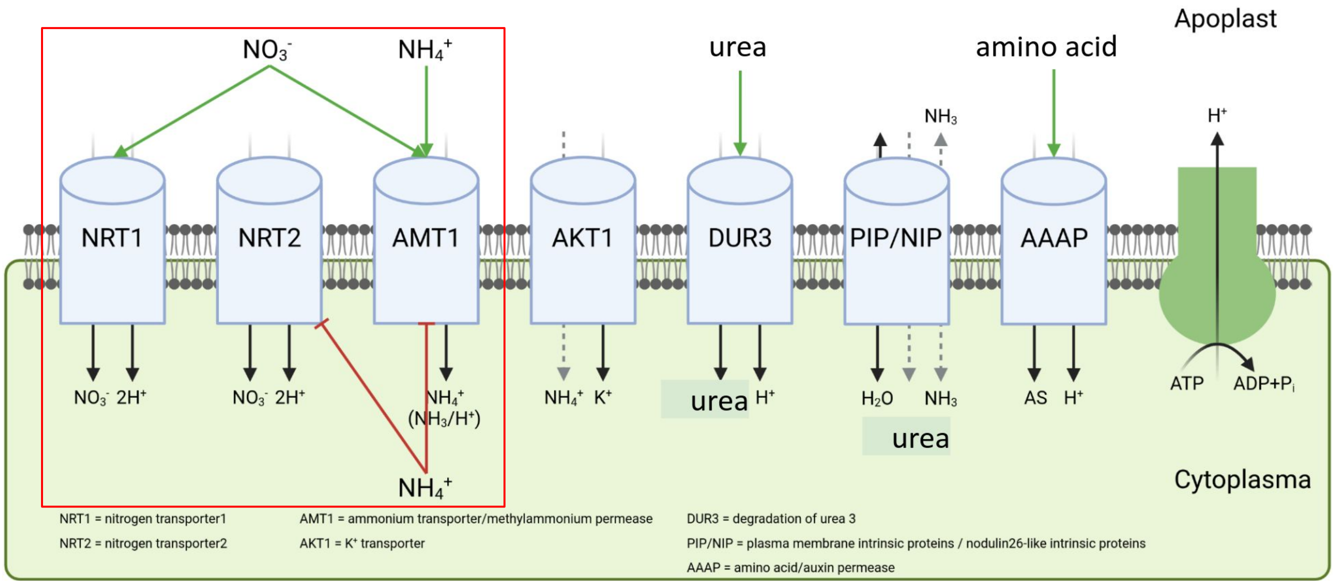
Nitrogen availability in soil

- Nitrate (NO_3^-): 1-5 mM
- Ammonia (NH_4^+): 20-200 µM
- Urea: <70 µM
- Free amino acids: 1-150 µM
- Short peptides
- Plants require a certain ratio of NO_3^- to NH_4^+ for ideal growth

Review von Muratore et al., 2021: 10.3390/plants10040681

Uptake of nitrogen from soil (nitrate)

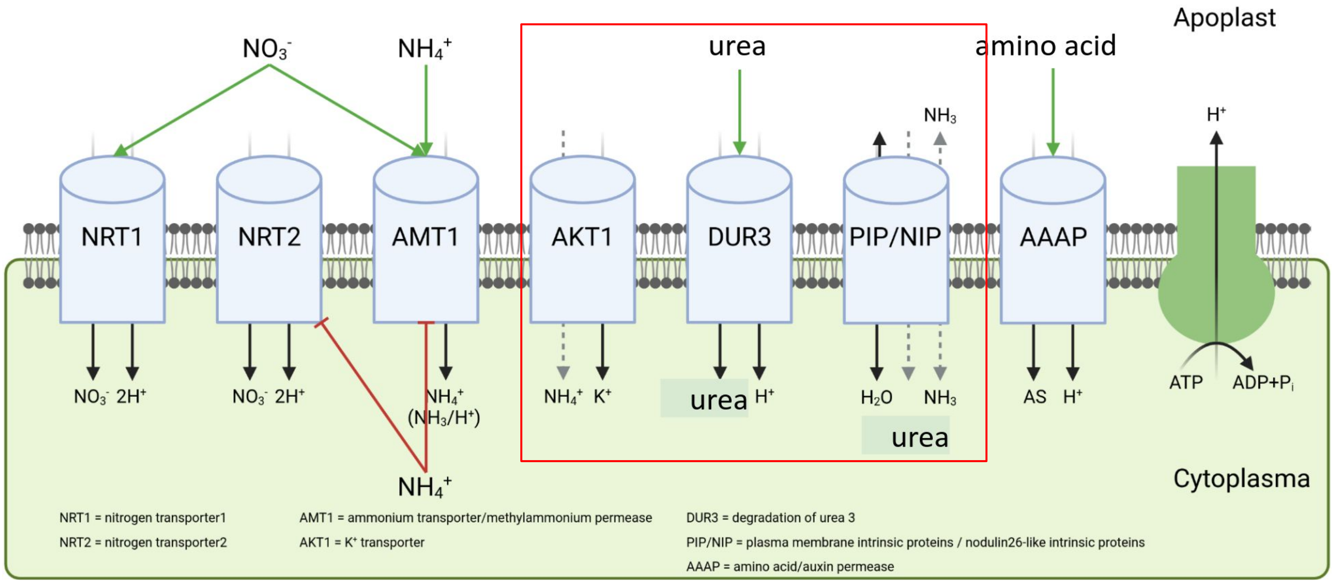
- NRT1 (NPF): nitrate uptake with low affinity
- NRT2: nitrate uptake with high affinity (inducible)



Review von Muratore et al., 2021: 10.3390/plants10040681

Uptake of nitrogen from soil (ammonia)

- AMT1: NH_4^+ uniport and NH_3/H^+ symport (high NH_4^+ is toxic)
- Non-selective channels / aquaporins: NH_3, NH_4^+ diffusion into cell



Review von Muratore et al., 2021: 10.3390/plants10040681

Uptake of nitrogen from soil (urea, amino acids)

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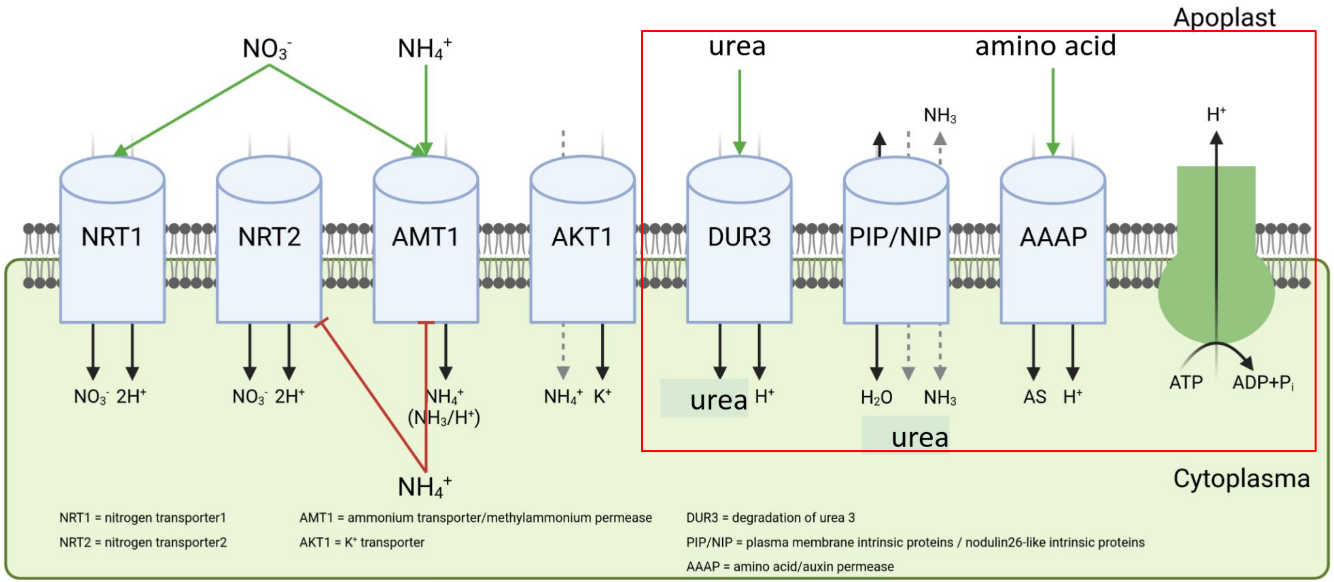
- DUR3 and PIP/NIP: urea transport (H^+ symporter)
- AAAP: potential amino acid transporters



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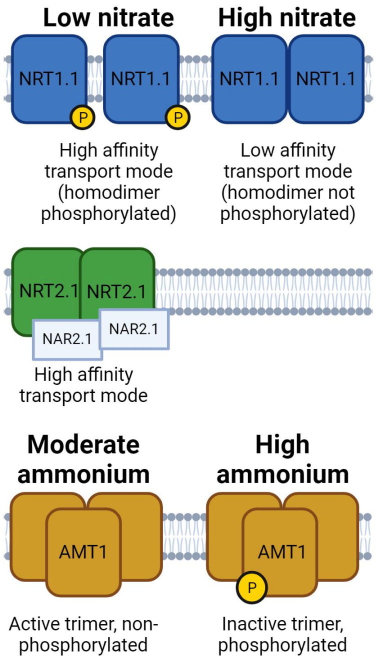
Regulation of nitrogen uptake

- Too much or too little nitrogen poses a challenge
- Phosphorylation of NRT1 nitrogen importers breaks up dimers and increases affinity (at low nitrate concentration)
- NRT1 functions as a transceptor: repressor of NRT2 (at high nitrate concentration)
    - NAR2.1 = Nitrogen Assimilation Related 2.1
- AMT1 is post-translationally regulated (forms an inactive trimer through phosphorylation)

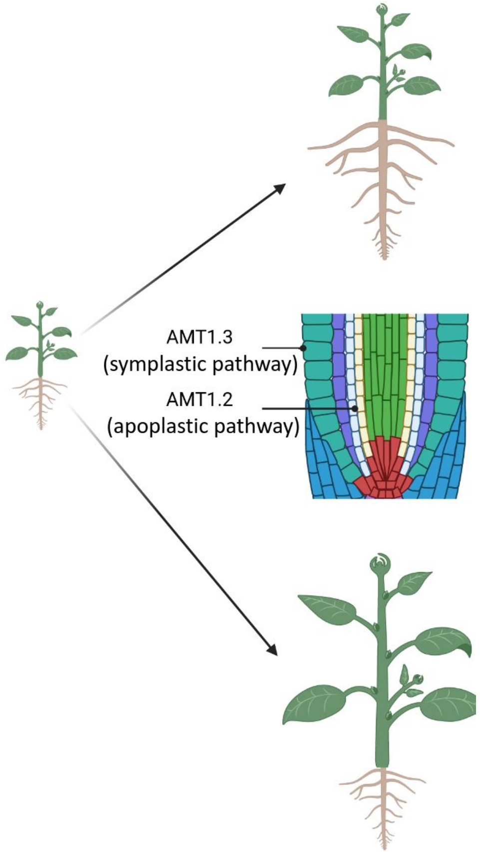


Review von Muratore et al., 2021: 10.3390/plants10040681

Symplastic vs. apoplastic path

- Symplastic pathway:
  - Through plasmodesmata
  - Better under nitrogen deficiency
  - AMT1.3 in the epidermis
  - Promotes root growth

- Apoplastic pathway:
  - Through cell wall spaces
  - Faster under good nitrogen supply
  - AMT1.2 in the endodermis
  - Promotes leaf growth



Review von Muratore et al., 2021: 10.3390/plants10040681

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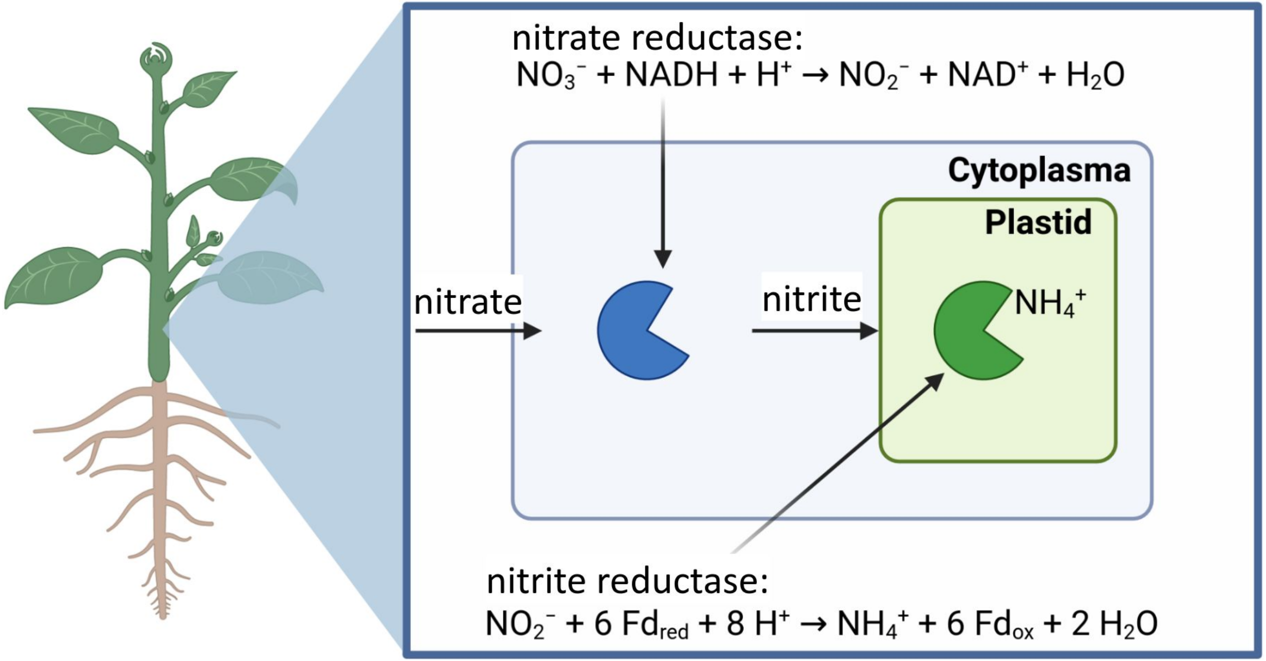
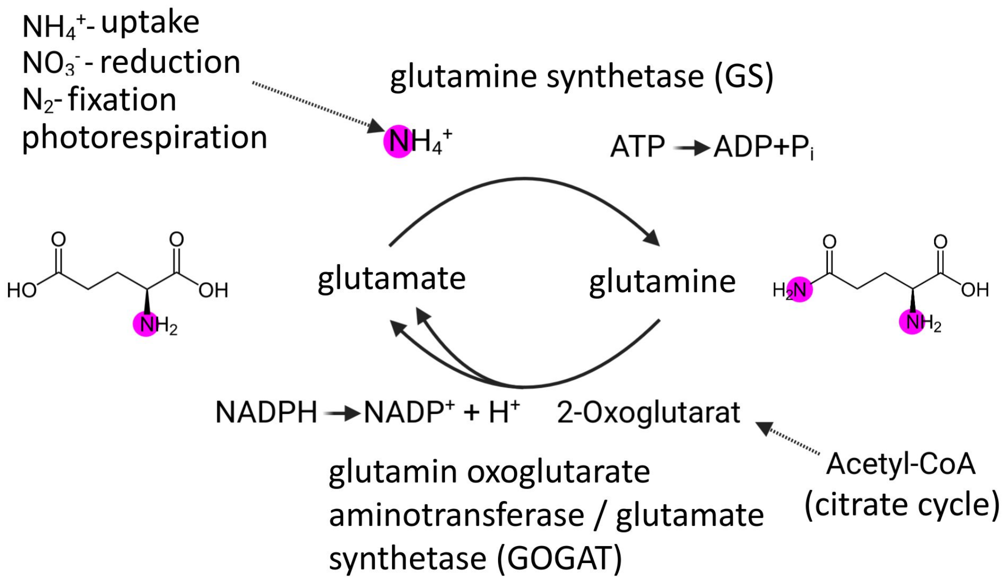
Nitrogen assimilation requires NH_4^+



- Ammonia is assimilated into amino acids (GS/GOGAT cycle)

GS/GOGAT cycle: assimmilation into amino acids



- Transaminations enable the synthesis of various amino acids
- Aspartate & asparagine form the basis for the biosynthesis of DNA bases

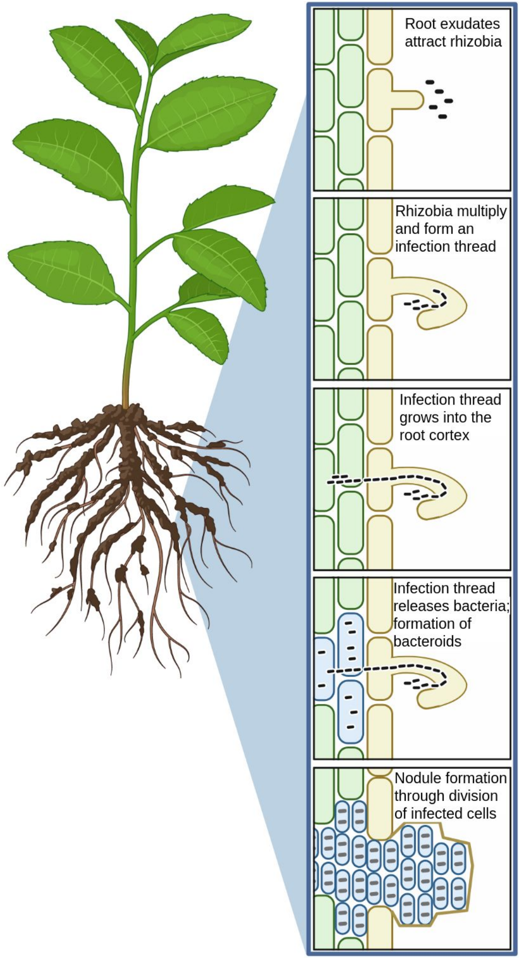
Biological nitrogen fixation

- Rhizobia (root nodule bacteria) fix nitrogen in interaction with legumes
- Cyanobacteria fix nitrogen in oceans and in symbiosis with Azolla
- Alders and casuarinas have a symbiosis with nitrogen-fixing Frankia bacteria
- Free-living bacteria: Azotobacter, Closterium, Klebsiella, Rhodospirillum

Zehr, 2011: 10.1016/j.tim.2010.12.004
Karthikeyan, 2013: 10.1007/s12038-013-9362-3
Sanow et al., 2023: 10.1094/MPMI-10-22-0223-CR

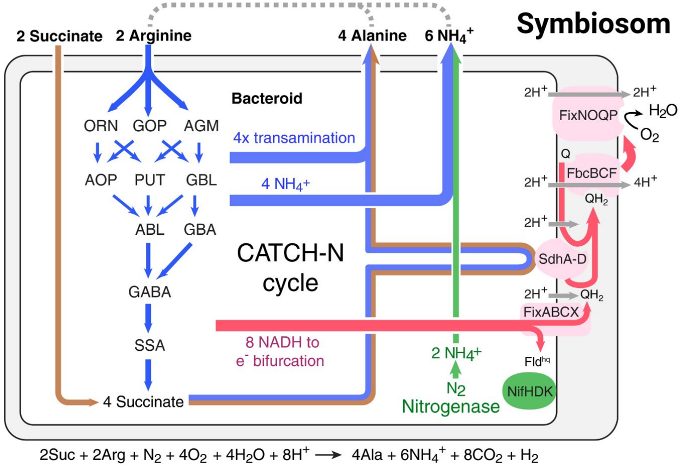
Rhizobia

- Rhizobia are chemotactically attracted by exudates (e.g., luteolin)
- Bacterial strain must be compatible with the plant species
- Root nodules form through cell enlargement associated with polyploidization of the cells
- Bacteria differentiate into bacteroids (symbiosomes)



Rhizobia - a symbiosis?

- Nitrogen fixation is energetically expensive: 16 ATP per N_2 molecule
- Bacteria receive succinate and arginine from plant
- NH_4^+ is delivered to plant (release of excess electrons)
- CATCH-N cycle enables survival of bacteria
- Plant controls bacteria (oxygen deprivation, acidification)

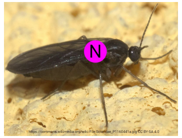


Flores-Tinoco et al., 2020: 10.15252/msb.20199419

Carnivorous plants

- Carnivorous plants mostly capture unicellular organisms, algae, arthropods, or insects as nitrogen source

- Carnivorous plants are only competitive in extreme habitats



Mycorrhiza

- Plants can absorb all nutrients they need in dissolved form through roots

- Symbioses are helpful for nutrients that are limiting (nitrogen, phosphorus, sulfur)

- Mycorrhiza: symbioses with fungi

- Majority of plants live in symbiosis with fungi and obtain nutrients through fungi’s extensive network of hyphae, in exchange for assimilates produced by photosynthesis

Functions of mycorrhiza

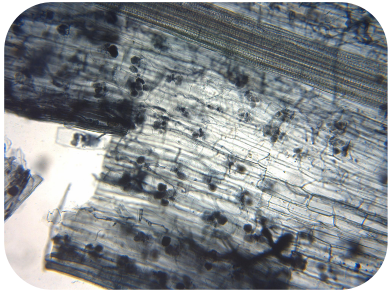
- Up to 30% of a plant's photosynthetic output can be invested in exchange
  - Coniferous forests of northern latitudes: about 15%

- Fungal network supports young seedlings with nutrients, while taking assimilates primarily from older plants

- Two main types: ectomycorrhiza and endomycorrhiza

Types of mycorrhiza

- Endomycorrhiza: e.g. in wheat
  - Evolutionarily very old symbiosis (> 400 million years)
  - Serves primarily for the supply of phosphate



- Ectomycorrhiza: e.g. in poplar
  - An evolutionarily younger form found in more nutrient-rich soils (e.g., forest soils)
  - Serves primarily for the supply of nitrate

- Arbuscular (“tree-like”) mycorrhiza: fungal and plant cells are each surrounded by their own cell membranes
- Each partner retains control over the export and import of nutrients and assimilates

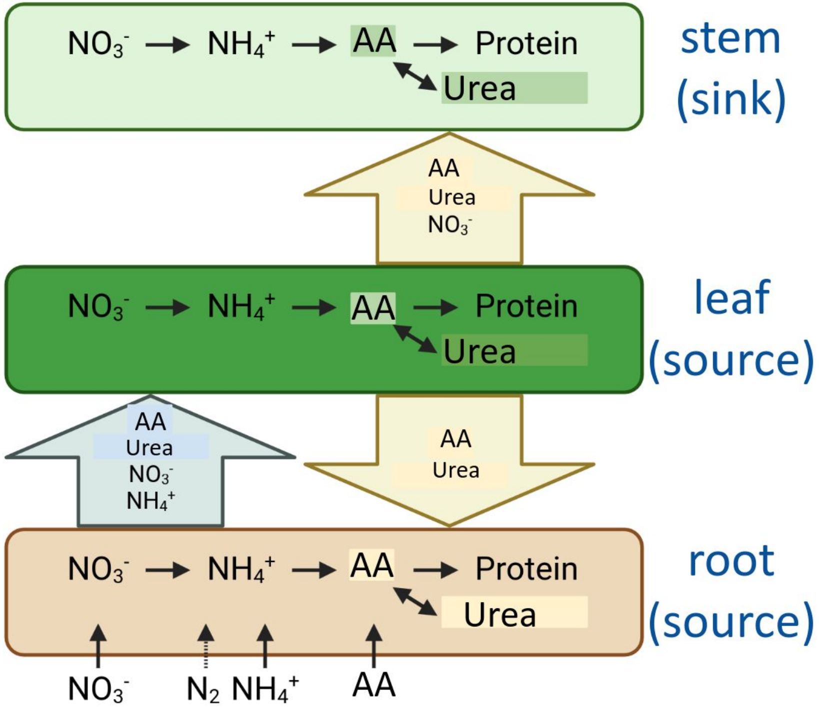
https://commons.wikimedia.org/wiki/File:Arbuscular_mycorrhiza_microscope.jpg

Evolution of mycorrhiza

- Land plants (Embryophyta) originated from algae-like ancestors
- Molecular studies suggest charophyte algae (Zygnematophyceae) are closest relatives
- Colonization of land occurred around 475 million years ago
- First land plants needed protective mechanisms against:
    - Desiccation (drying out)
    - UV radiation
    - Nutrient deficiency (nutrients were no longer available in surrounding water)
- Symbioses with fungi are also found in liverworts and hornworts
- Earliest fungal symbiotic partners were probably Glomeromycota
- Glomeromycota, a distinct phylum of fungi, form arbuscular endomycorrhiza

Transport between plant organs

- Transport via phloem and xylem is possible
- Amino acids (asparagine) and urea are the organic transport forms of nitrogen
- Nitrogen is stored in the central vacuole
- Seeds are an important sink tissue due to storage protein synthesis

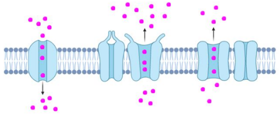


Tegeder & Masclaux-Daubresse, 2017: 10.1111/nph.14876

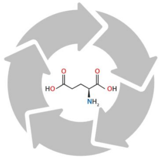
Nitrogen metabolism summary

- Nitrogen as a limiting factor

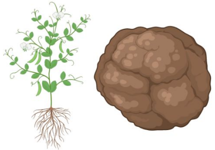
- Uptake from the environment



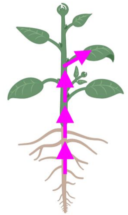
- Nitrogen assimilation (GS/GOGAT cycle, transamination)



- Biological nitrogen fixation (root nodule bacteria)



- Nitrogen transport in plants



Summary

- Translation
- Lipids: membrane vs. storage
- Carbohydrates: sugar, starch, cellulose
- Nitrate assimilation and metabolism

Time for questions!

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Questions

1. Which fundamentally different types of lipids exist in plants?
2. Please name three different carbohydrates!
3. What is the difference between starch and cellulose?
4. What are the major steps in the nitrogen cycle?
5. Which types of nitrogen are taken up by plants?
6. What are the nitrogen assimilation steps?
7. What are rhizobia?
8. Which types of mycorrhiza do you know?