MACQUARIE University

Amino acid metabolism - 1

Shoba Ranganathan

Applied Biosciences

T: 02 9850 6262; E: shoba.ranganathan@mq.edu.au

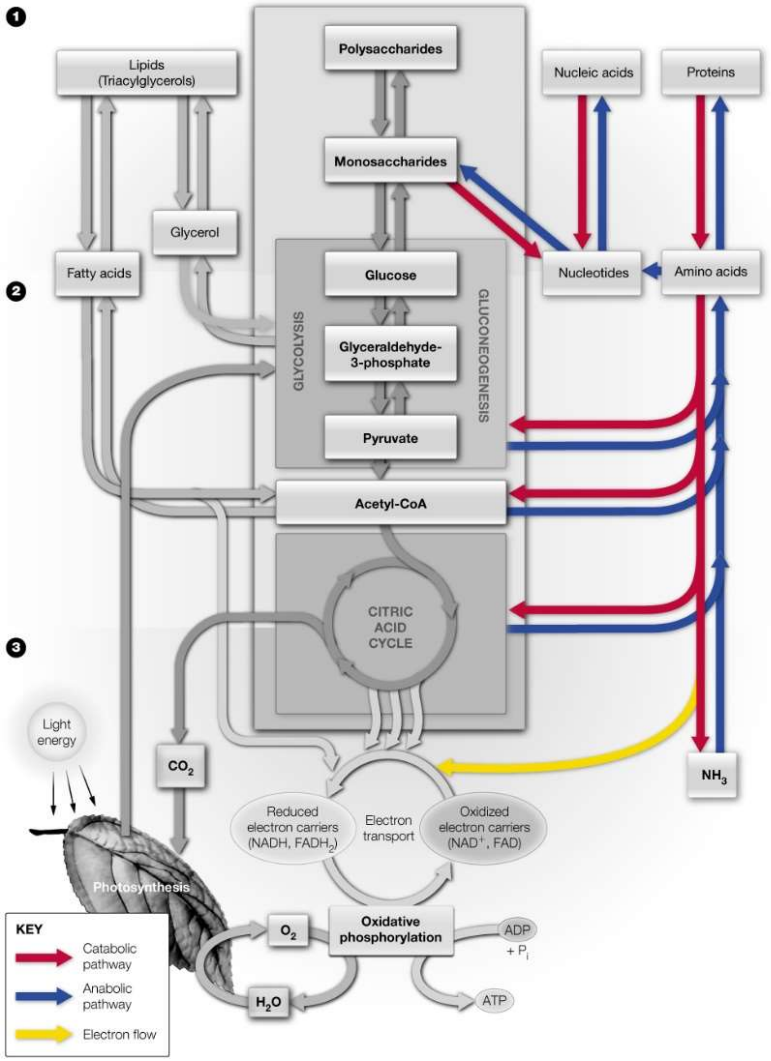
Objectives

- How is the metabolism of aa different from carbohydrates or lipids?
  - The presence of N makes aa very different from other biomolecules
- How are proteins from dietary sources and protease degradation broken down
  - ➢Special treatment for N removal and disposal
  - ➢Rest of the C chain recycled
- **No storage for aa and proteins:** hence excess dietary aa must be converted to precursors of glucose, fatty acids and other metabolic fuels: e.g. ketone bodies
- Introduction to amino acid synthesis

Textbook Chapter 18

Pathways of Nitrogen Metabolism

- The catabolic pathways of protein, amino acid, nucleic acid, and nucleotide metabolism are shown in red, while the anabolic pathways are indicated in blue.
- The electron flows from amino acid oxidation is highlighted in yellow



Nitrogen Economy and Turnover

- Rudolf Schoenheimer studied nitrogen metabolism in rats in the 1930s by feeding them ^{15}N-labeled Tyr:
  - ~50% of the ^{15}N was excreted in urine,
  - the rest was incorporated into proteins:
    - of these a small fraction was recovered in Tyr,
    - *most* was incorporated into other amino acids.
- Importantly, the amount of unlabeled protein nitrogen excreted was equal to the amount of labeled nitrogen incorporated into rat proteins; thus a nitrogen equilibrium was maintained in the rat
- Animals do not have polymeric nitrogen compounds or other nitrogen deposits for storage and must continuously replenish nitrogen supplies through diet.
- A healthy 70-kg adult requires about **8 g of nitrogen per day** in his or her diet to remain in nitrogen balance.
  - This corresponds to a daily intake of about **52 g of protein**.

Nitrogen Demand and Supply

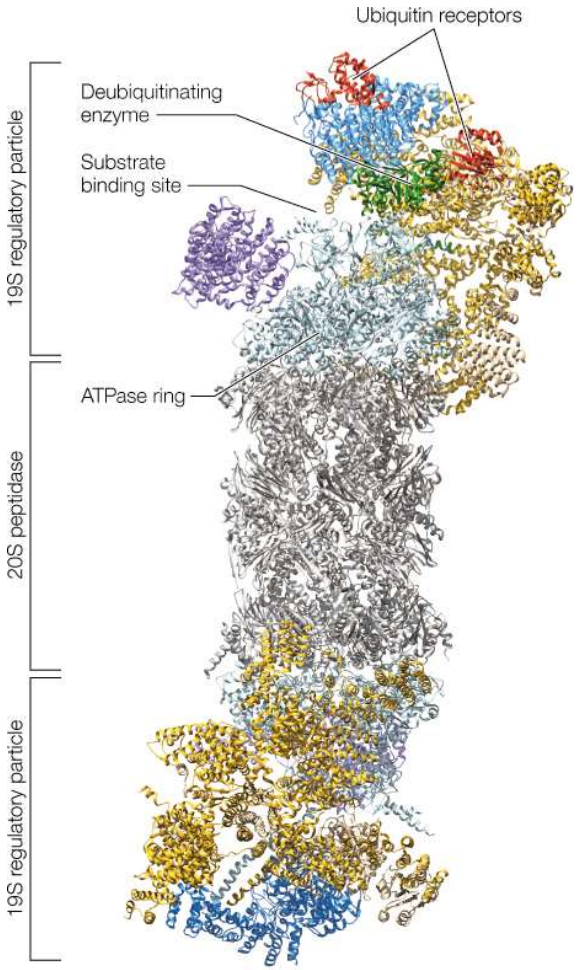
- In animals that do not consume a sufficient amount of nitrogen, proteins (mostly **muscle proteins**) are ***broken down and not replaced***
- Generally proteins are subject to continuous biosynthesis and degradation (**protein turnover**)
- This allows replacement of damaged proteins and biological regulation
- The average half-life of a protein in rat is 1 or 2 days
- Ubiquitin and the 20S proteasome play important roles in intracellular protein degradation

Getting aa from proteins: protein degradation

- Extracellular and intracellular proteins may be digested by **lysosomal proteases**.
  - Lysosomes degrade many proteins with **~50 hydrolytic enzymes**: non-selective in well-nourished cells
  - Extracellular leakage of lysosomal enzymes leads to inflammatory diseases: rheumatoid arthritis
- Other proteins to be degraded are first conjugated to the protein ubiquitin.
- The proteasome, a barrel-shaped complex, unfolds ubiquitinated proteins in an ATP-dependent process and proteolytically degrades them

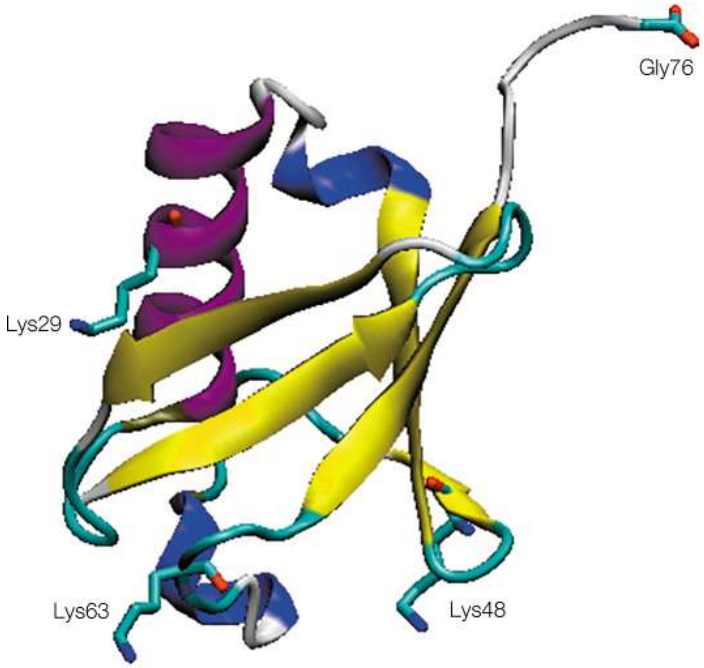
The 20S Proteasome

- is a nonlysosomal ATP-dependent protease
- present in the cytosol,
- proteasome action is tightly controlled
- targets and degrades only proteins tagged with ubiquitin

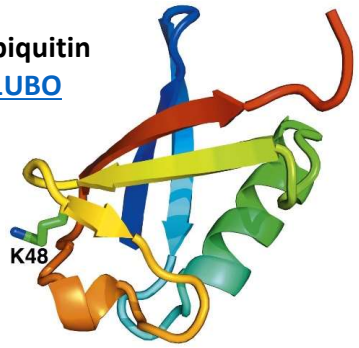


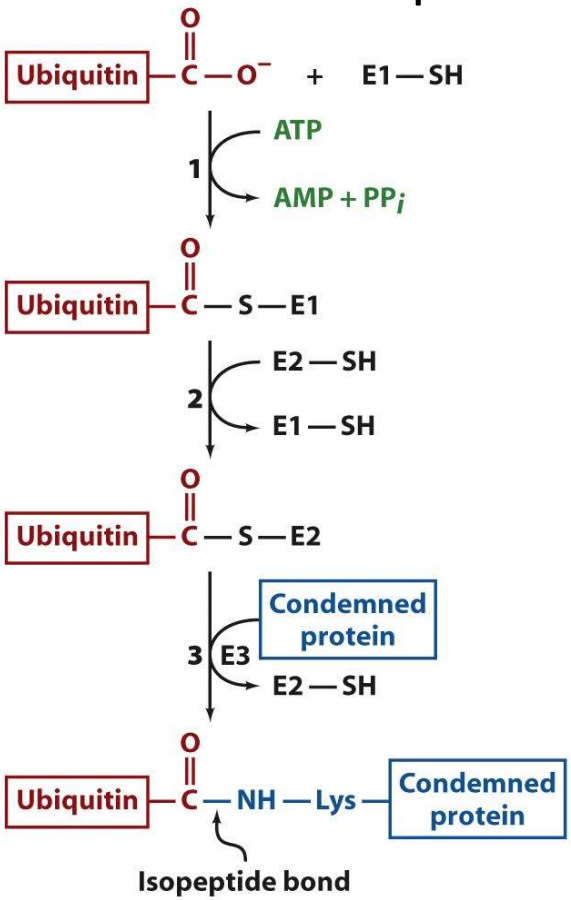
Ubiquitin

- Human ubiquitin is a 76 residue protein expressed in all cells
- Proteins targeted for degradation are covalently bonded to ubiquitin via an isopeptide bond; this reaction is catalyzed by a ubiquitin ligase
- Ubiquitin ligase also catalyzes the addition of ubiquitin to a previously conjugated ubiquitin
- Polyubiquitin chains are recognized by the proteasome resulting in degradation of the tagged protein



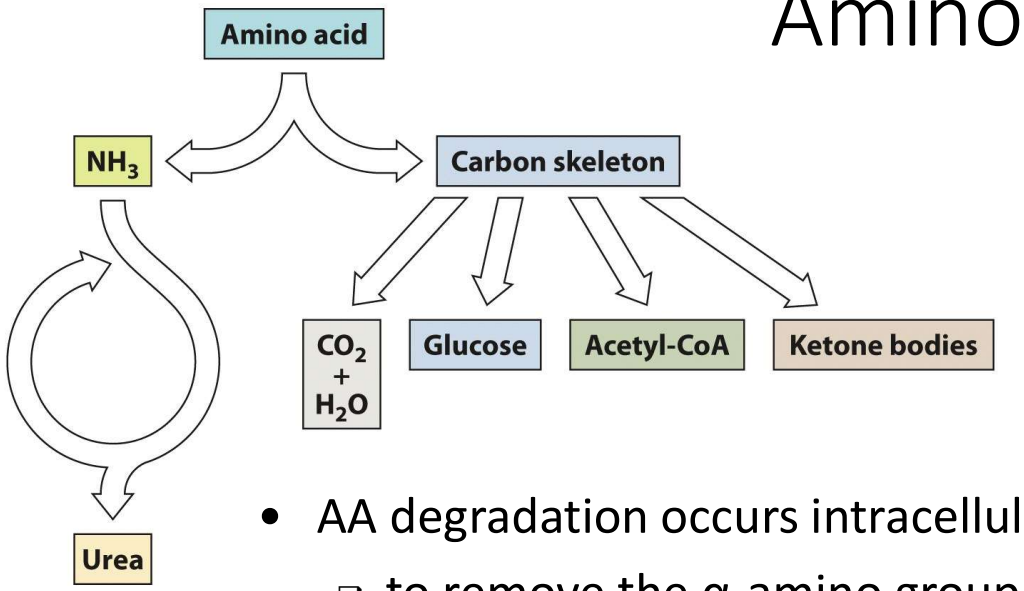
Non-lysosomal protein break-down requires ubiquitination





- ATP-dependent tagging of the extremely conserved 76-aa monomeric protein, ubiquitin
- 3-step process: E1: UQ-activating enzyme; E2s: UQ-conjugating enzymes; E3: UQ-protein ligase: binding to Lys of target protein
- Degradation requires min. **4 tandem UQs**
  - *Not a random process*
  - Both housekeeping and regulatory functions
  - UQ-tagged proteins get "recycled": proteasome

Amino acid deamination

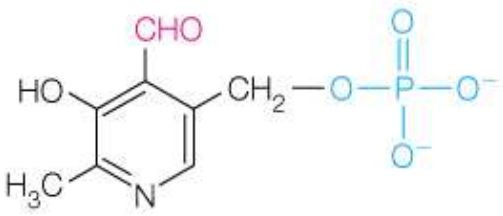


- Free aa come from both degradation of cellular proteins and from digestion of dietary proteins (pepsin, trypsin, chymotrypsin, elastase + endo- and exo- peptidases)

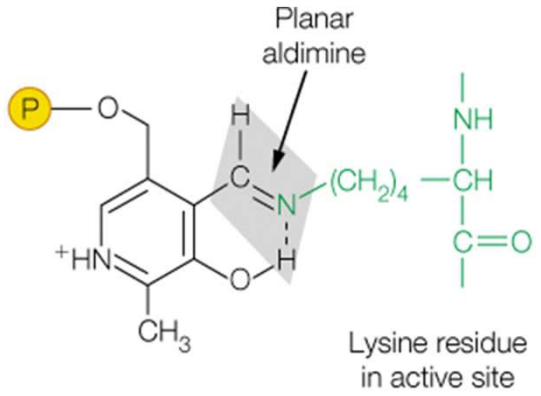
- AA degradation occurs intracellularly:
  - to remove the α-amino group and make urea for excretion
  - to breakdown and recycle the α-keto acid
- Transamination interconverts an amino acid and an α-keto acid (occurs both in aa synthesis and breakdown) – needs cofactor pyridoxal phosphate (PLP)
- Oxidative deamination of glutamate releases ammonia (toxic to the cell) for disposal.

Pyridoxal Phosphate (PLP)

- Vitamin B_6
- serves as a coenzyme for the majority of enzymes that catalyze some chemical change at the α-, β-, or γ-carbons of the common amino acids, including:
  - decarboxylations
  - eliminations
  - racemizations
  - retro-aldol reactions



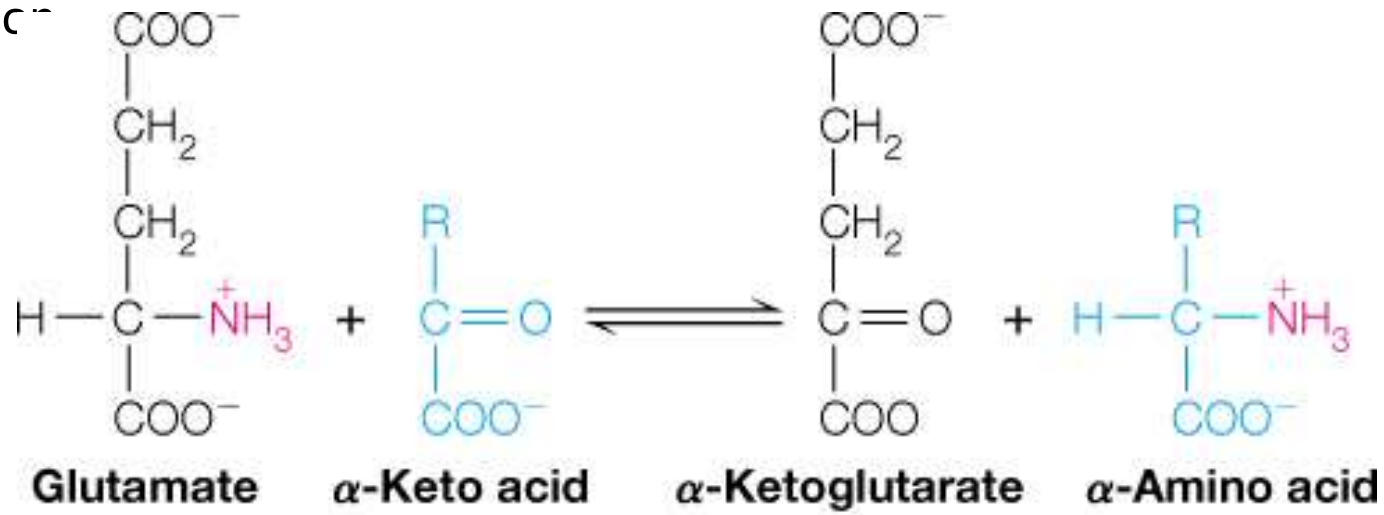
Pyridoxal phosphate (PLP)



Enzyme-bound pyridoxal phosphate

Transamination Reactions in Amino Acid Degradation

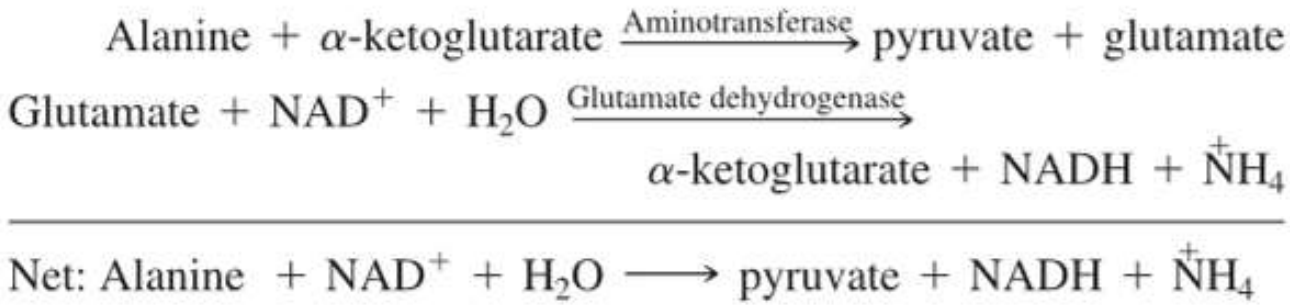
- Amino acid degradation usually begins with conversion to the corresponding α-keto acid by transamination or oxidative deamination
- The α-amino group usually from **glutamate**, is transferred by **transamination** of the corresponding **α-keto acid** to form **α-ketoglutarate** and the **α-amino acid**
- Aminotransferases use a **PLP cofactor**; the reaction has a K_{eq} near 1
- Transamination plays a central role in the citric acid cycle and in ammonia assimilatic



MACQUARIE University

Degradation of amino acids

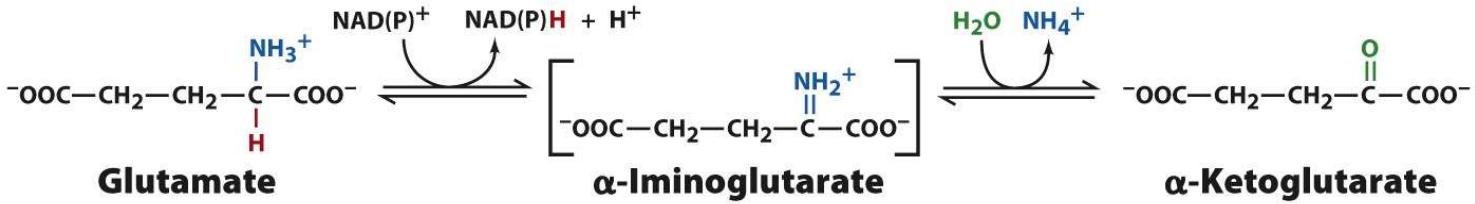
- In degradation, the aminotransferase works in concert with glutamate dehydrogenase, as exemplified by the degradation of alanine:



- In animals, **glutamate dehydrogenase** is located in the **inner mitochondrial membrane**, consistent with a primary role in energy generation. Moreover, the animal enzyme is allosterically controlled; α-ketoglutarate synthesis is inhibited by GTP and ATP and stimulated by ADP. Thus, the enzyme is activated under conditions of **low energy charge**.

Glutamate is oxidatively deaminated

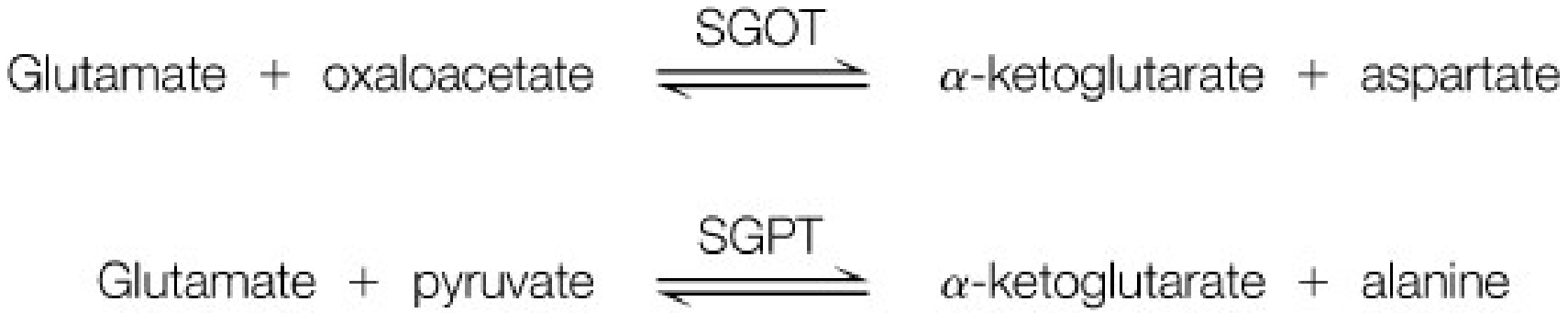
- In the mitochondria, by **glutamate dehydrogenase** (GDH), using NAD^+ or $NADP^+$ as cofactor



- GDH allosterically inhibited by **GTP** and NADH (high energy charge) and activated by **ADP** and NAD^+ **(Prac 5)**
- Mutations in GDH lead to insensitivity to GTP inhibition, resulting in **hyperammoneamia:** elevated levels of ammonia in the blood
- **Ammonia** released by GDH reaction is a cellular toxin and needs to be removed by the urea cycle

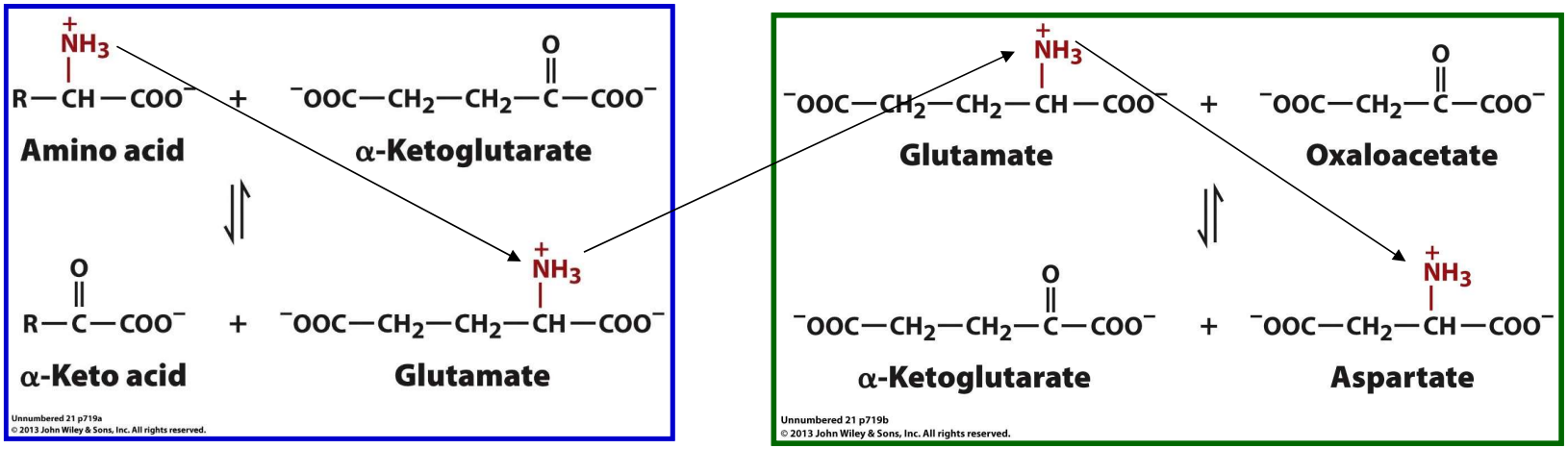
SGOT and SGPT Aminotransferases

- Most aminotransferases use α-ketoglutarate and as one of the two α-keto/α-amino acid pairs involved
- Serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) are important in clinical diagnoses of heart and liver damage



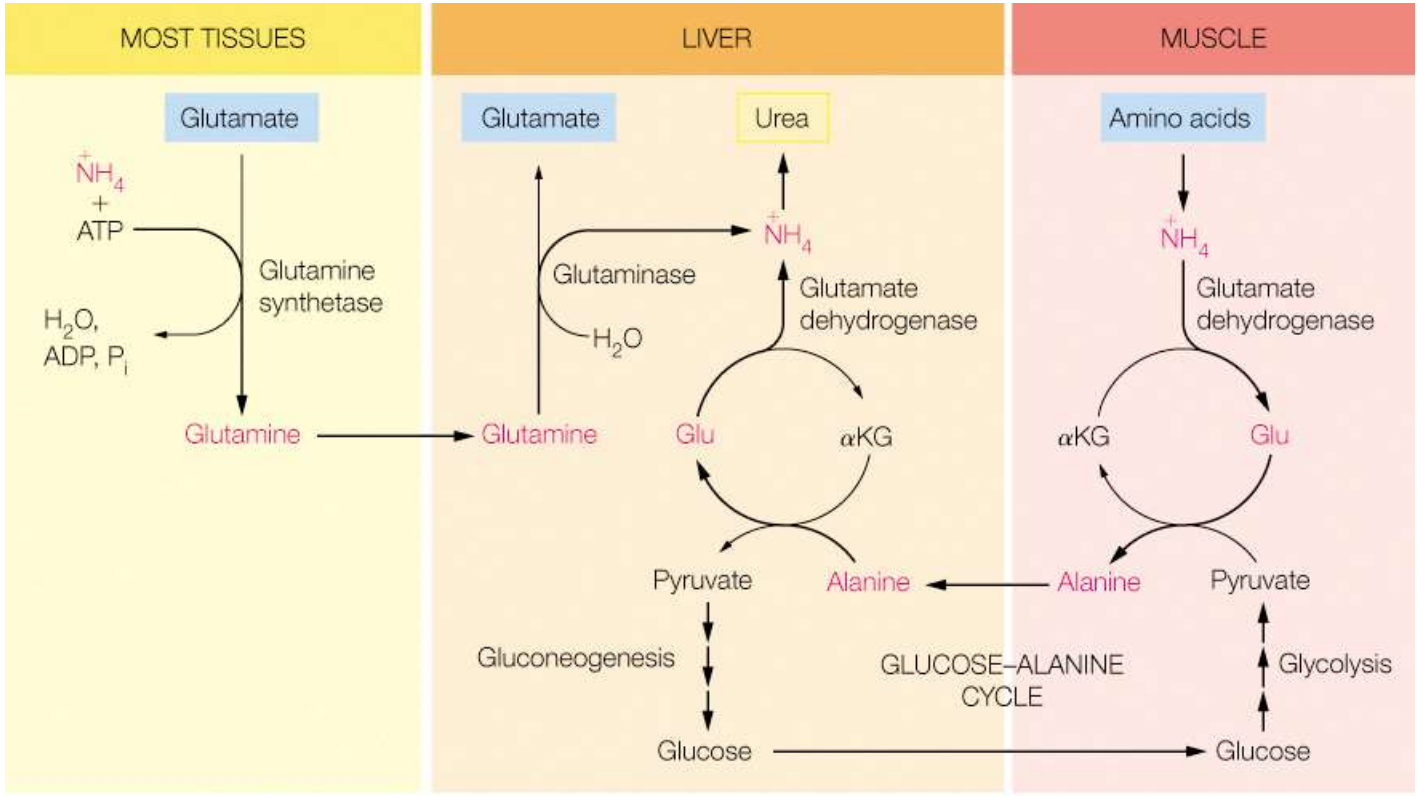
Transaminases use PLP (from Vitamin B_6)

- aka **aminotransferases**: transfer amino group to α-keto acid (predominantly α-ketoglutarate) forming glutamate and an α-keto acid from the aa



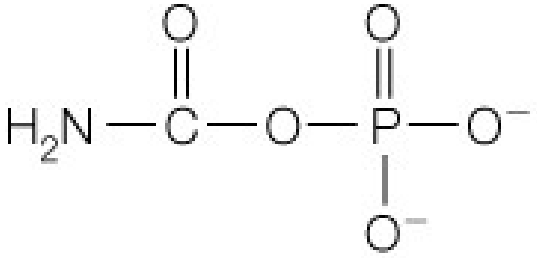
- PLP (pyridoxal-5'-phosphate) cofactor –from pyridoxine (Vitamin B_6)
  - **All aa <u>except lysine</u>** are deaminated thus by specific transaminases
  - ❖The **α-keto acid** however is mostly **α-ketoglutarate**, followed by ***oxaloacetate***, leading to **glutamate** and ***aspartate*** as the <u>main products of aa degradation</u>

Export of Excess Ammonia to the Liver for Excretion as Urea



Detoxifying ammonia by carbamoyl phosphate synthetase: CPS I

- Ammonia is toxic at high levels
- Animals assimilate NH_3 via reactions yielding carbamoyl phosphate
- CPS I in the mitochondria generates carbamoyl phosphate, an intermediate for arginine synthesis
- either NH_3 or glutamine can serve as the nitrogen donor in this reaction

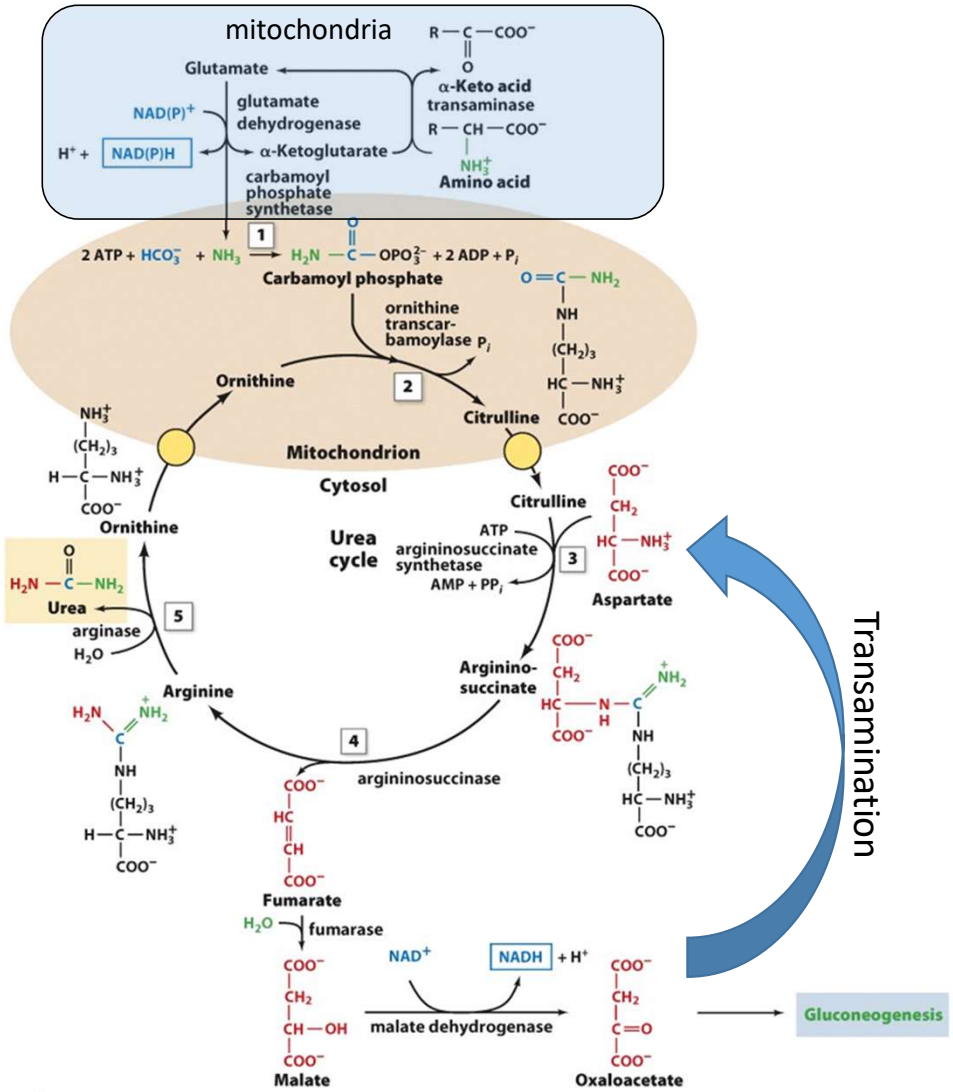


Carbamoyl phosphate

$$NH_3 + HCO_3^- + 2\ ATP \rightarrow \text{carbamoyl phosphate} + 2\ ADP + P_i$$

$$\text{Glutamine} + H_2O + HCO_3^- + 2\ ATP \rightarrow$$

$$\text{carbamoyl phosphate} + 2\ ADP + P_i + \text{glutamate}$$

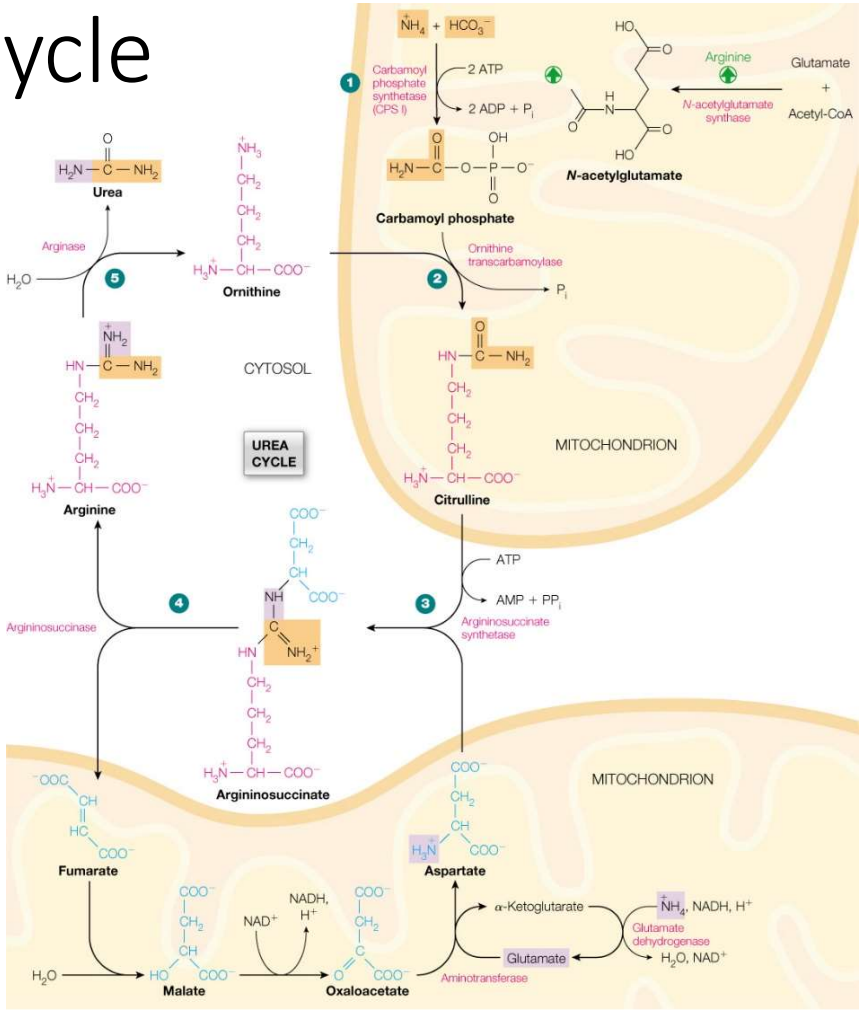


The urea cycle

- **first known metabolic cycle**: 1932
- 5 enzymatic reactions: 2 in the mitochondria; 3 cytosolic
- *Note similarities to CAC*
- Two intermediates: ornithine and citrulline must enter and exit the mitochondria
- The 2 amino groups come from glutamate and aspartate, while the C in urea comes from CO_2 as soluble bicarbonate ion
- Arginine is synthesized!

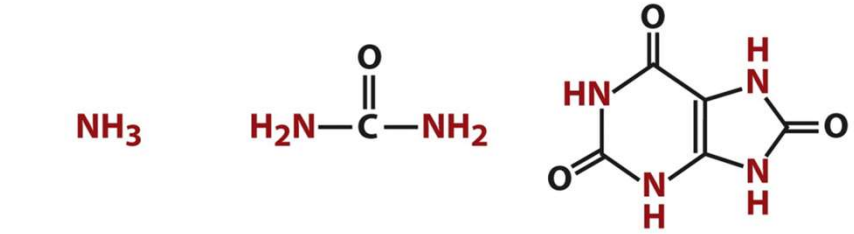
The Krebs–Henseleit Urea Cycle

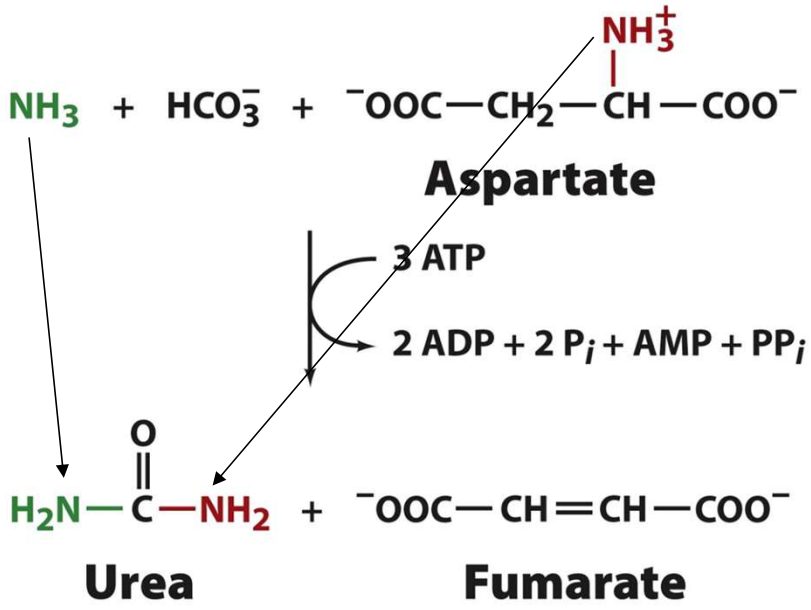
- Urea (upper left) contains a carbon and a nitrogen (orange) derived from carbamoyl phosphate and a nitrogen (purple) derived from aspartate
- NH_4^+ and CO_2 were incorporated by carbamoyl phosphate synthetase (upper right) and glutamate dehydrogenase (lower right)
- *N*-acetylglutamate, synthesized from glutamate and acetyl-CoA, allosterically activates carbamoyl phosphate synthetase

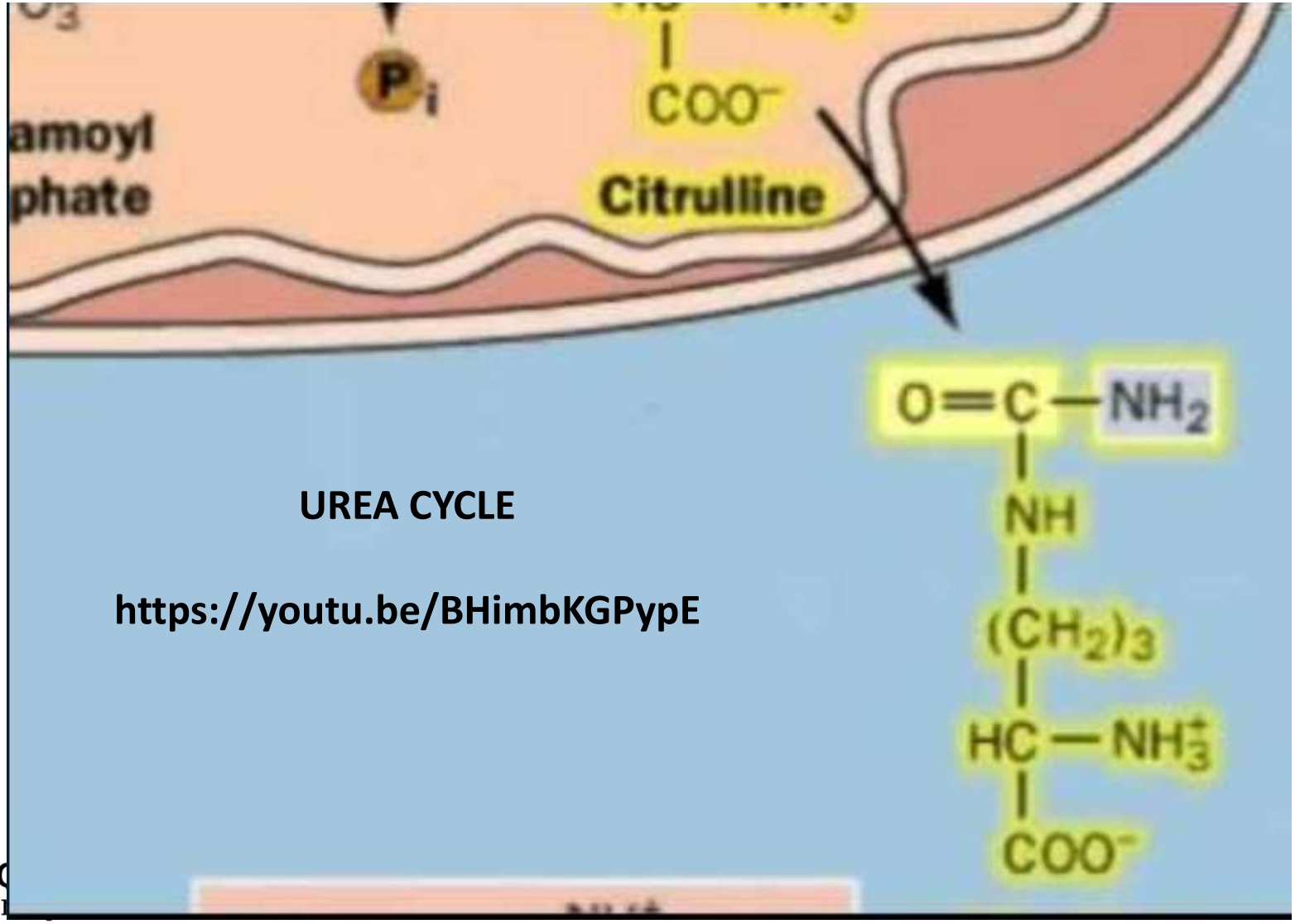


Converting ammonia into less toxic forms

- Terrestrial vertebrates remove ammonia as urea
  - Aquatic animals simply excrete ammonia
  - Birds and reptiles make uric acid instead.
- Urea is made in the **liver**, then secreted into the bloodstream and captured and excreted from the kidneys






amoyl
phate
Citrulline
UREA CYCLE
https://youtu.be/BHimbKGPypE

The urea cycle is linked to gluconeogenesis and CAC

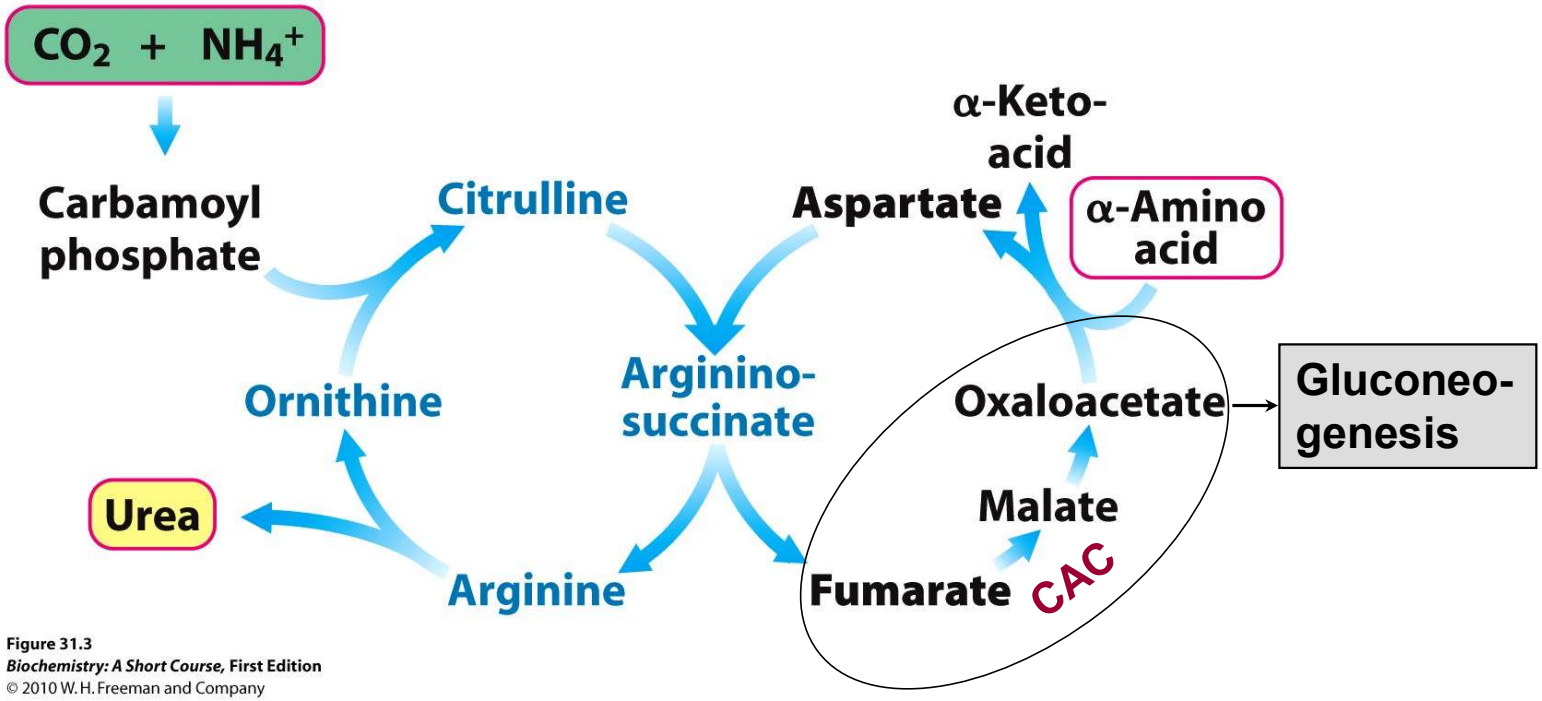
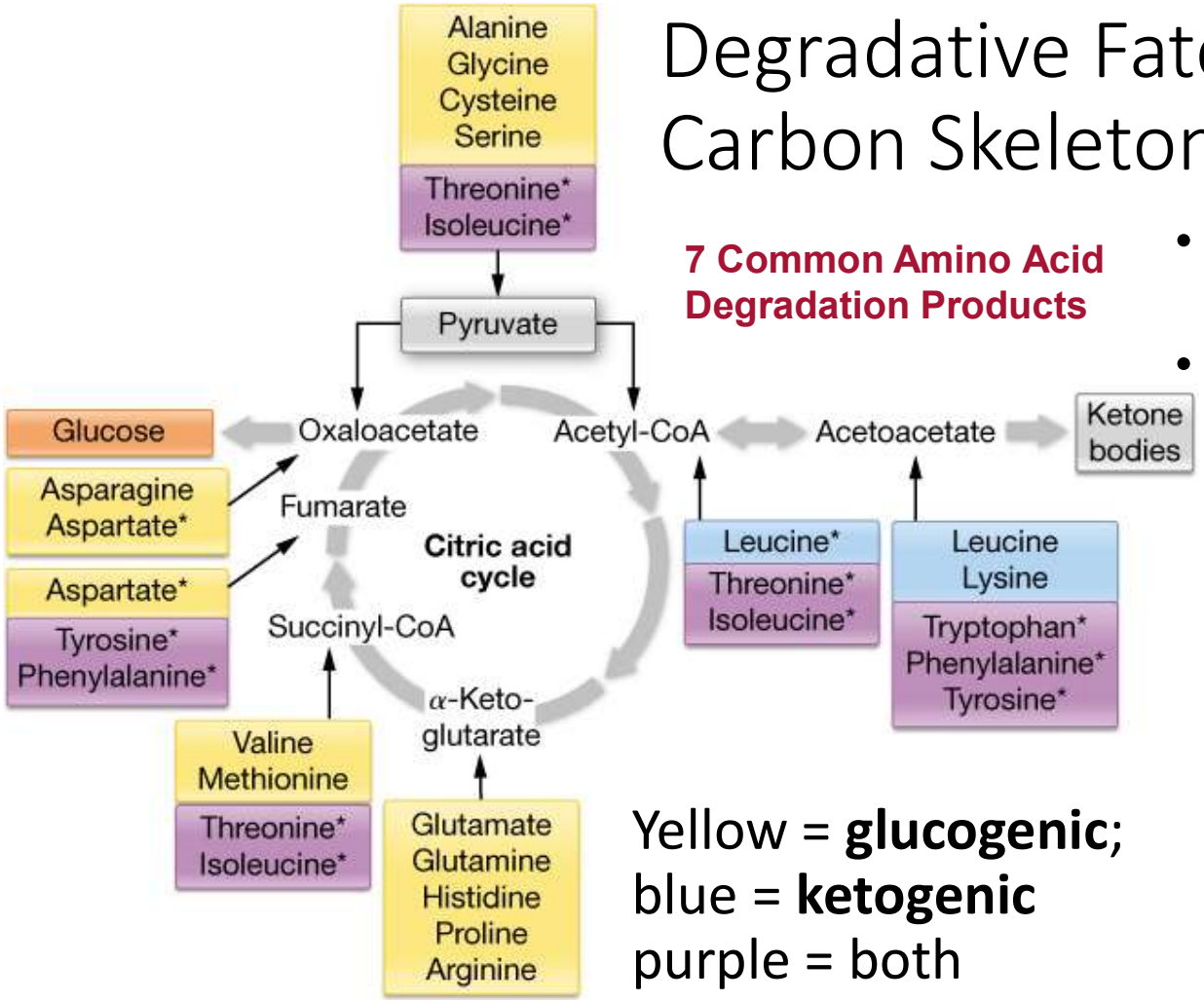


Figure 31.3
Biochemistry: A Short Course, First Edition

Degradative Fates of Amino Acid Carbon Skeletons



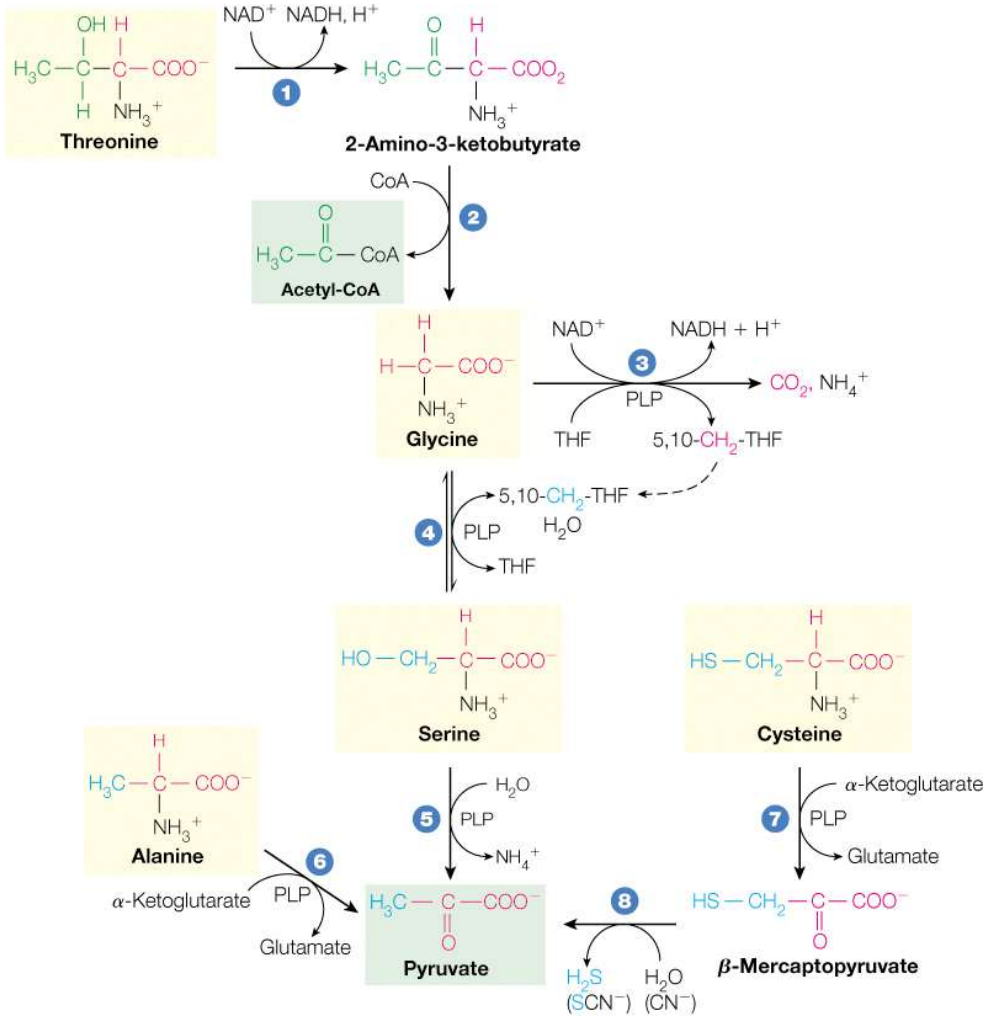
7 Common Amino Acid Degradation Products

Yellow = **glucogenic**;
blue = **ketogenic**
purple = both

- C skeleton is degraded to compounds metabolized to CO_2 and H_2O
- 10-15% of metabolic energy comes from aa's
  - Glucogenic aa's: pyruvate, α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate: (5) glucose precursors
  - Ketogenic aa's: acetyl-CoA and acetoacetate: (2) fatty acid or ketone body precursors
  - Some are both: Ile, Phe, Thr, Trp, Tyr (IFTWY)

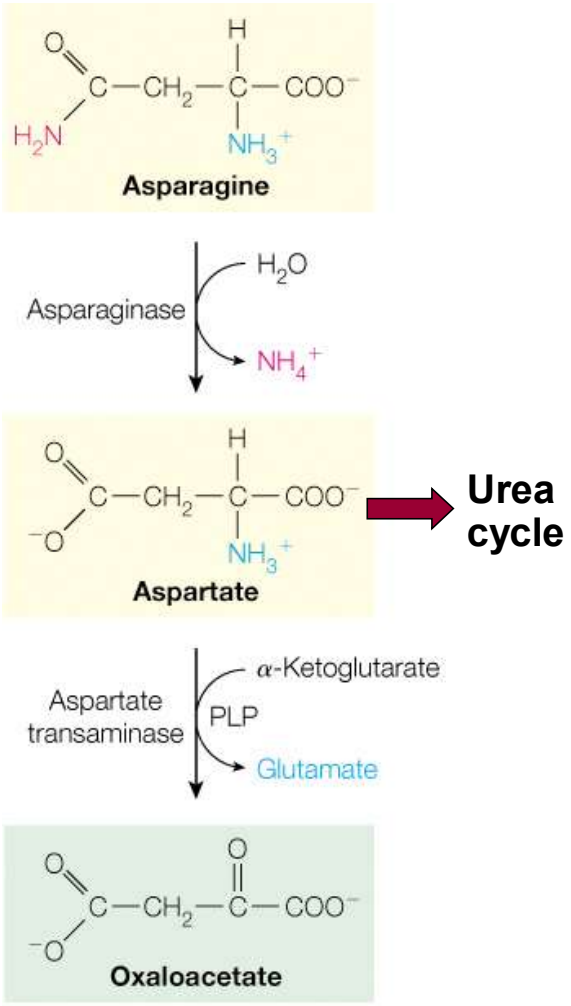
Degradation to Pyruvate

- Alanine, cysteine, glycine, serine, and threonine are degraded to pyruvate in mammals
- Cofactor THF (tetrahydrofolate) derived from folic acid (Vit. B group) required to provide single-carbon functional groups:
  - ➢methyl
  - ➢methylene
  - ➢formyl



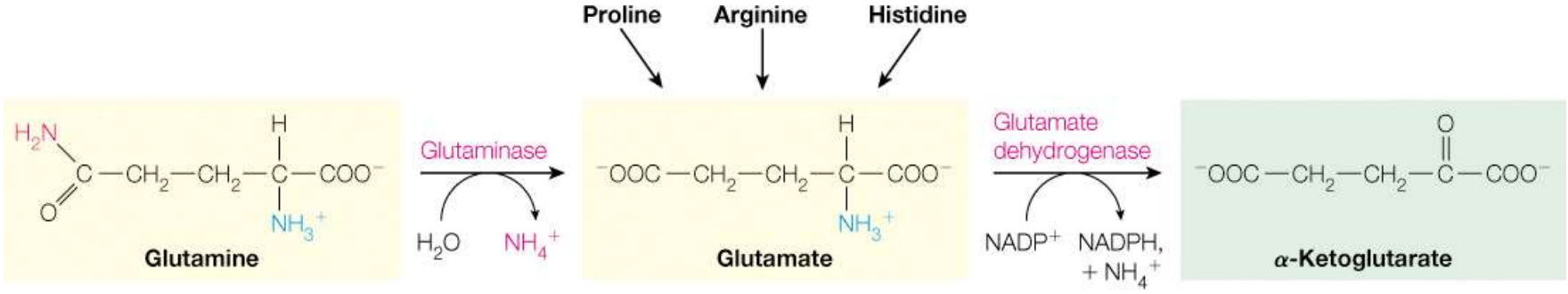
Degradation to Oxaloacetate

- Asparagine and aspartate are converted to oxaloacetate
- Normal and malignant lymphocytes depend on the uptake of asparagine from the blood for growth, and asparaginase depletes circulating asparagine
- Asparaginase is an example of enzyme-based chemotherapy
- Asparaginase treatment leads to complete remission in many cases
- The *E. coli* enzyme is widely used in the treatment of childhood **acute lymphoblastic leukemia (ALL)**

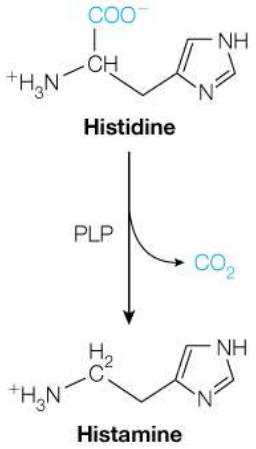


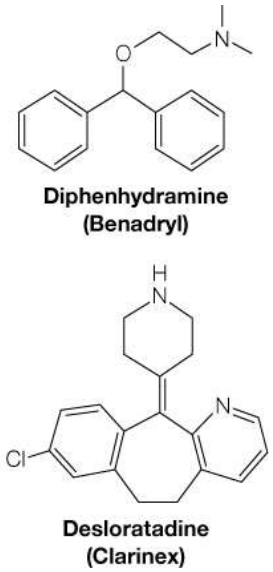
Degradation to Glutamate

- Glutamine, proline, arginine, and histidine are all degraded to glutamate



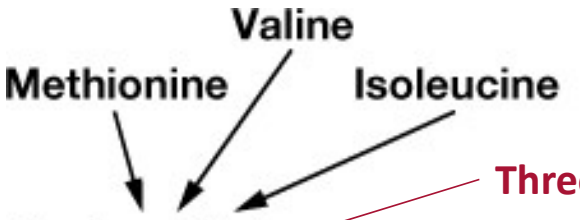
- Histidine also undergoes a PLP-dependent decarboxylation to generate **histamine**, a potent vasodilator
- Antihistamines are used to treat inflammation and allergic reactions





Degradation to Succinyl-CoA

- Methionine, valine, threonine and isoleucine are degraded to succinyl-CoA
- Biotin and Vit. B_{12} are essential cofactors.
  - The cyanide in B_{12} is replaced by either a methyl or a 5'-deoxyadenosyl group in bioactive forms

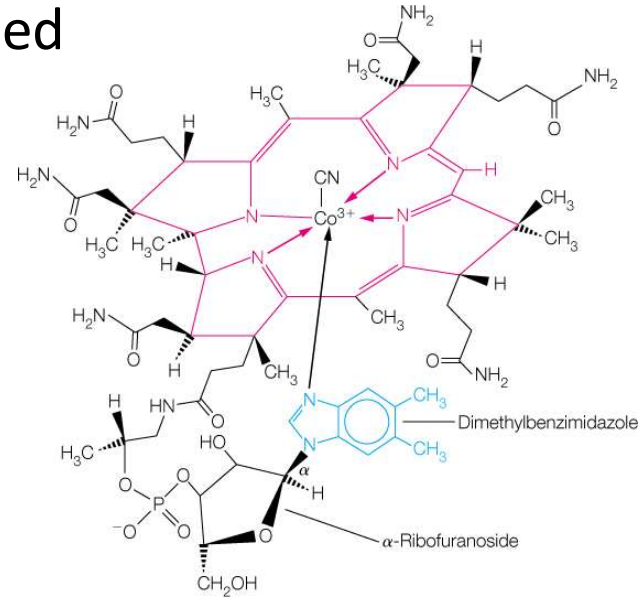


Threonine

Propionyl-CoA

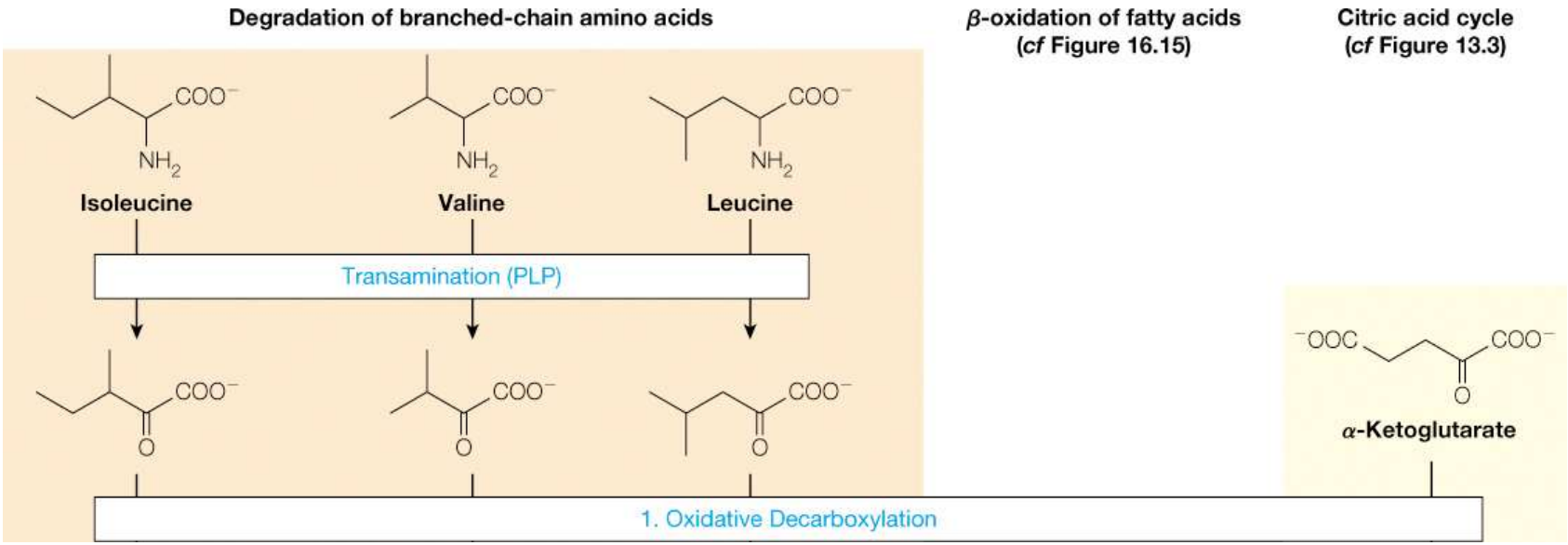
D-Methylmalonyl-CoA

L-Methylmalonyl-CoA $\xrightarrow{B_{12}}$ Succinyl-CoA

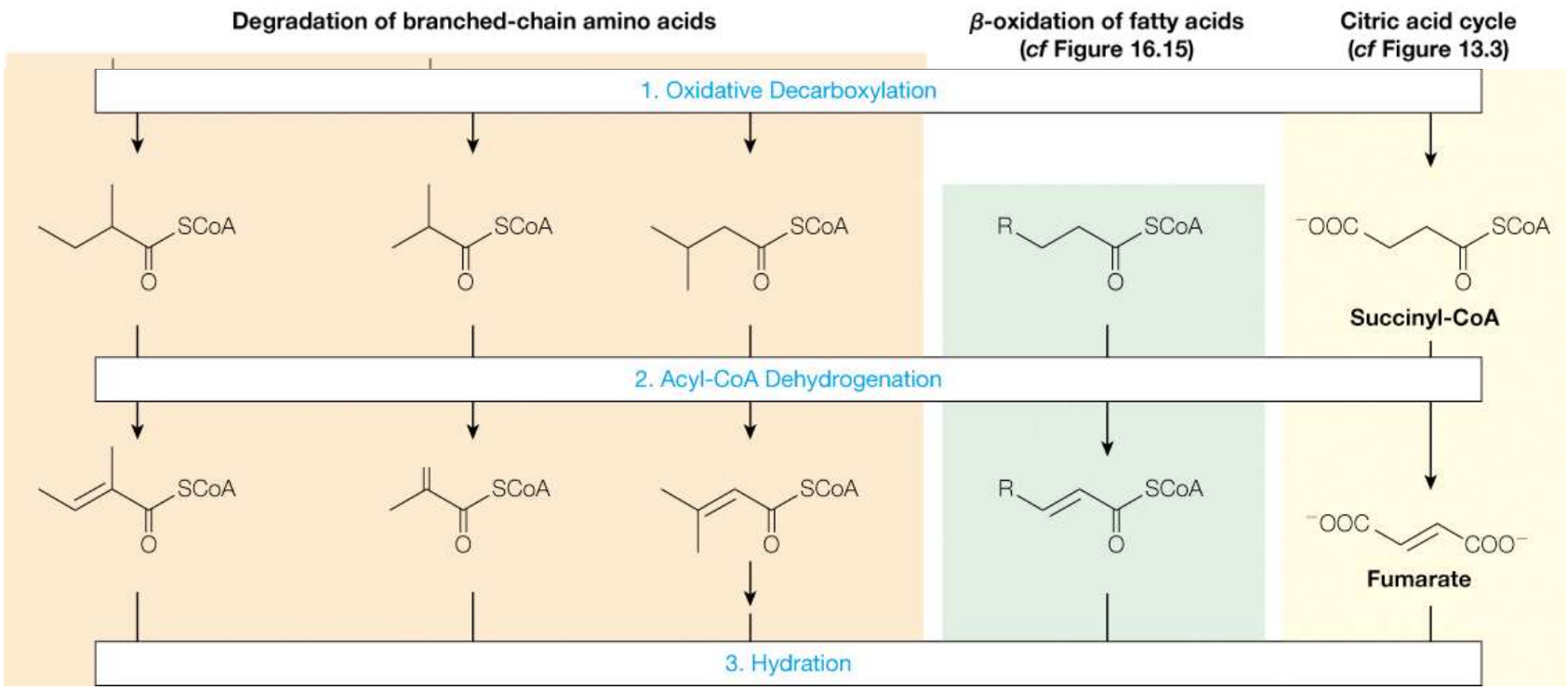


Degradation of Branched-Chain Amino Acids -1

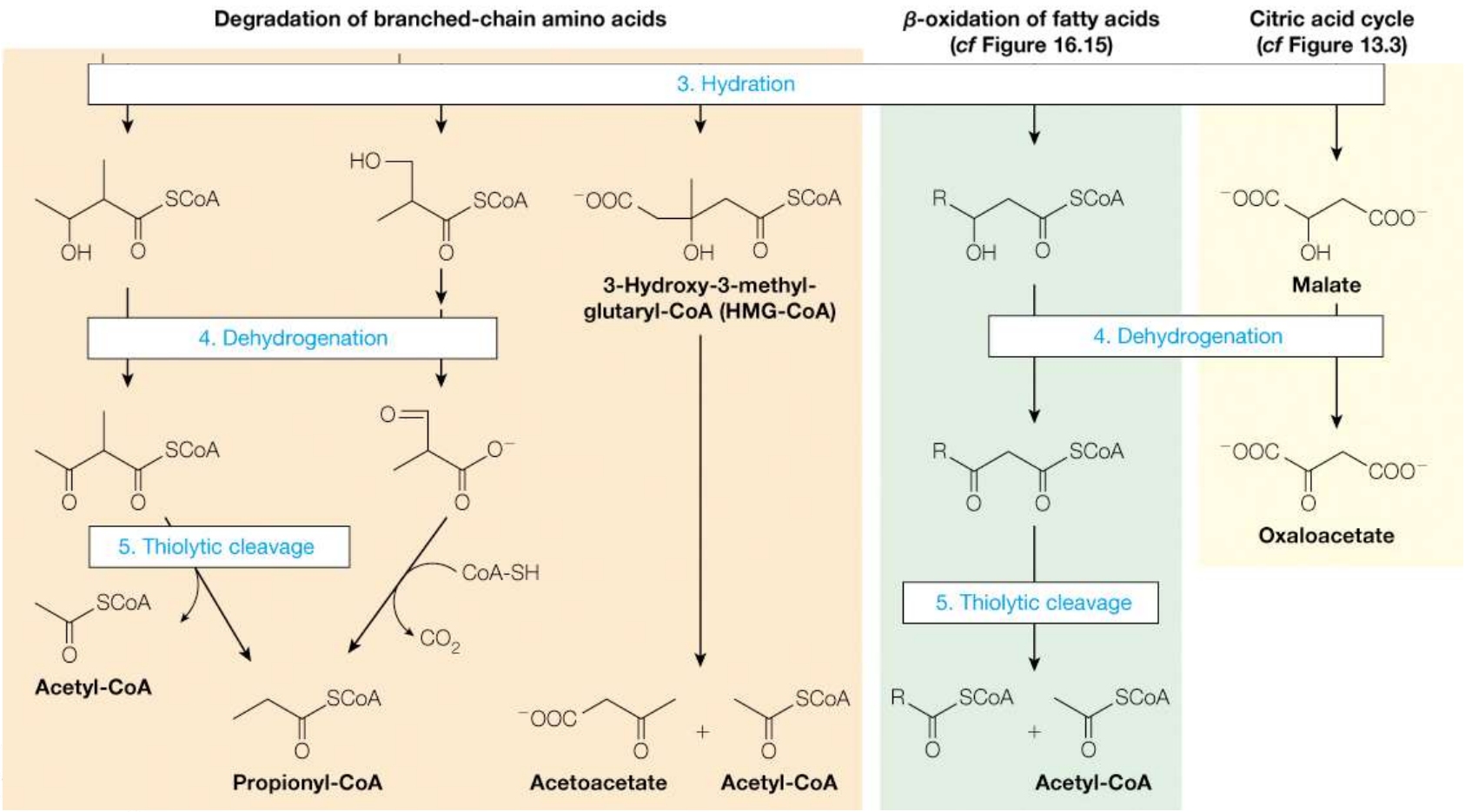
- Branched-chain amino acid oxidation, fatty acid β-oxidation, and the citric acid cycle share a common chemical strategy



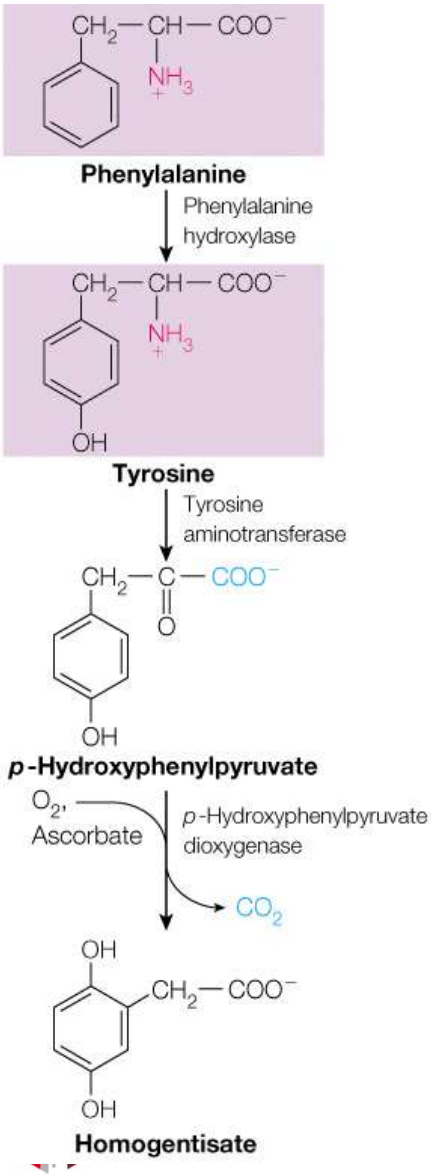
Degradation of Branched-Chain Amino cids -2

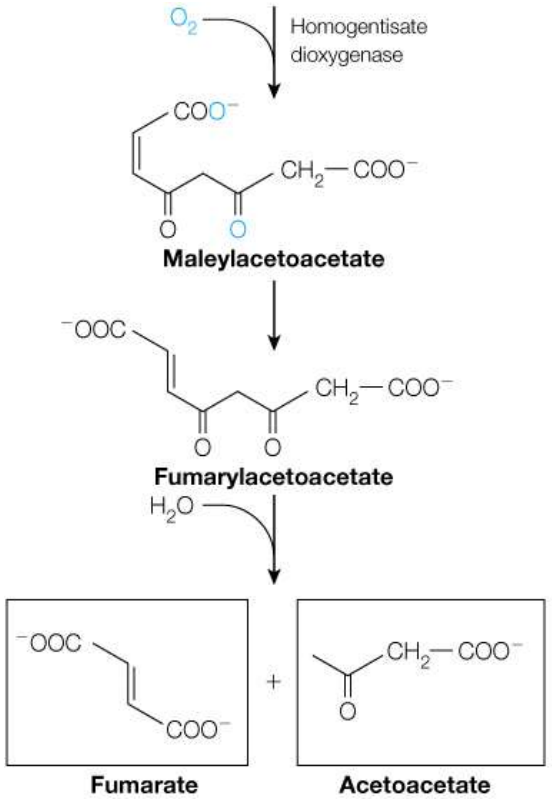


Degradation of Branched-Chain Amino Acids -3



Degradation of Phenylalanine and Tyrosine





- Tyrosine and phenylalanine are degraded to acetoacetate and fumarate

Diseases from defective amino acid degradation

- Best known is **phenylketonuria:**
  - phenylalanine breakdown affected
  - absence or deficiency of phenylalanine hydroxylase
  - Leads to excess of phenylpyruvate
  - Results in severe mental retardation a few months after birth

Table 31.1 Inborn errors of amino acid metabolism

Disease	Enzyme deficiency	Symptoms
Citrullinema	Argininosuccinase	Lethargy, seizures, reduced muscle tension
Tyrosinemia	Various enzymes of tyrosine degradation	Weakness, self-mutilation, liver damage, mental retardation
Albinism	Tyrosinase	Absence of pigmentation
Homocystinuria	Cystathionine β-synthase	Scoliosis, muscle weakness, mental retardation, thin blond hair
Hyperlysinemia	α-Aminoadipic semialdehyde dehydrogenase	Seizures, mental retardation, lack of muscle tone, ataxia

Summary

- AA breakdown Involves both synthetic and degradative steps
  - To make precursors of polypeptides
  - To recover metabolic energy
- Urea cycle in the mitochondrion
  - ✓Safe N removal and disposal as urea
  - Arg is synthesized in the urea cycle
  - Linked to CAC and gluconeogenesis
- AA grouped into the categories based on C products
  - Ketogenic (aromatic and long chain hydrophobic aa's)
  - Glucogenic (charged, polar and short chain hydrophobic aa's)
  - A few are both (Ile, Phe, Thr, Trp, Tyr: IFTWY)

Amino acid synthesis: To make or not to make aa's

- Plants and microorganisms can synthesize all 20 amino acids.
- Humans synthesize only 10/20 common amino acids – "nonessential"
- What we cannot make comes from food – "essential"
  - Pathways to synthesize these have been lost due to evolution – we can get them anyway, so why make them?
  - Making them would probably be inefficient.

TABLE 18.1 Nutritional requirements for amino acids in mammals

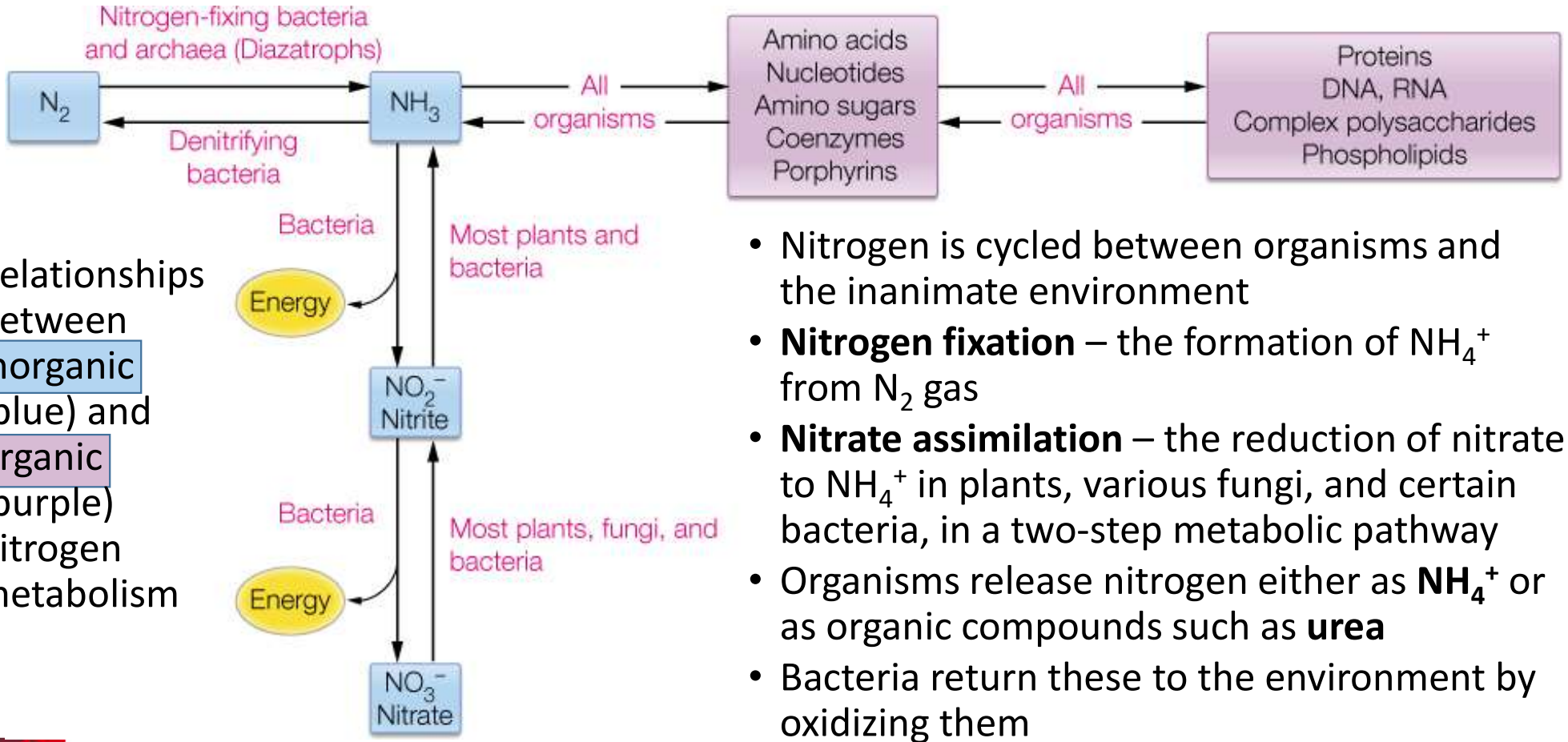
Essential
Arginine,* histidine, isoleucine, leucine, lysine, methionine,* phenylalanine, threonine, tryptophan, valine
Nonessential
Alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine**

* Although mammals can synthesize arginine and methionine, the use of these amino acids for the production of urea and methyl groups, respectively, is greater than the capacity of their biosynthetic pathways.
** Tyrosine is considered nonessential because mammals can produce it during phenylalanine degradation via the phenylalanine hydroxylase reaction.

We need atmospheric nitrogen to make glutamate!

- 3% of the human body mass is nitrogen (4th most abundant)
  - Amino acids and nucleotides, the building blocks of proteins and nucleic acids contain nitrogen
  - Here, nitrogen is in a reduced state.
- The major forms of naturally occurring nitrogen are inorganic:
  - Elemental N_2: 78% of air
  - Inorganic Nitrate (NO_3^-): saltpetre, Chile saltpetre.
- Nitrogen can be incorporated into organic molecules as ammonium ions (NH_4^+).
- **Plants and microorganisms can synthesize all 20 amino acids** from inorganic forms: NO_3^- and NH_4^+
- ***Animals need preformed glutamate***!

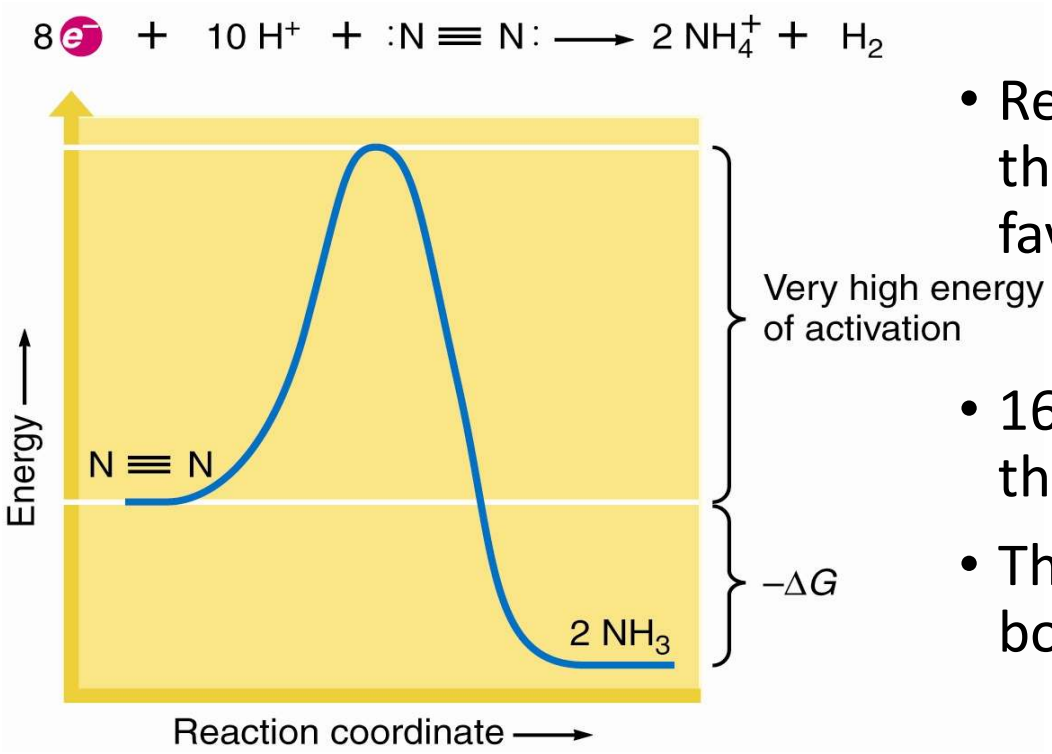
The Nitrogen Cycle



Relationships between inorganic (blue) and organic (purple) nitrogen metabolism

- Nitrogen is cycled between organisms and the inanimate environment
- **Nitrogen fixation** – the formation of NH_4^+ from N_2 gas
- **Nitrate assimilation** – the reduction of nitrate to NH_4^+ in plants, various fungi, and certain bacteria, in a two-step metabolic pathway
- Organisms release nitrogen either as **NH_4^+** or as organic compounds such as **urea**
- Bacteria return these to the environment by oxidizing them

The nitrogen triple bond is hard to break



- Reaction however is thermodynamically favourable.
- 16 **ATP**s required to provide the activation energy
- The triple bond in N_2 has a bond energy of **~940 kJ/mol**

Nitrogen Fixation by symbiotic bacteria

- Possible only in certain **prokaryotes** – anaerobic.
  - < 1% of the inorganic nitrogen converted into organic compounds by this pathway but essential to use the vast atmospheric N_2 reserves.
  - N_2-fixing bacteria are free living or symbionts with higher plants, supporting plant growth and agriculture.
- Biological reduction of N_2 is carried out by the enzyme **nitrogenase**:

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

- Industrially, this reduction is carried out by the Haber–Bosch process:

$$N_2 + 3H_2 \xrightarrow[450^\circ C,\ 270\ atm]{\text{catalyst}} 2NH_3$$

The Haber–Bosch process

The Nitrogenase Structure

- The structure of the molybdenum-dependent nitrogenase from *Azotobacter vinelandii* shows the flow of electrons from reduced ferrodoxin or flavodoxin (Fd) to N_2
- Note: similar iron-S complexes are present in the mammalian ETC!
- A special Fe-Mo (molybdenum) complex is essential!
- Anaerobic!