BIOCH 765: Biochemistry II

Spring 2014

Amino Acid Metabolism (Chapter 21)

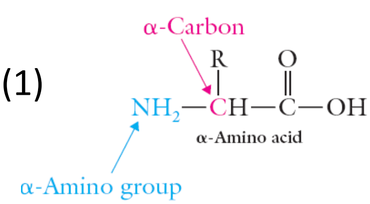
Jianhan Chen

Office Hour: MF 1:30-2:30PM, Chalmers 034

Email: jianhanc@ksu.edu

Office: 785-2518

Overview



- Introduction to amino acids and proteins (1)
- Protein degradation (1)
- Amino acid deamination (1)
- The urea cycle (1)
- Breakdown of amino acids (2)
- Amino acid synthesis (1)
- Nitrogen fixation (1)

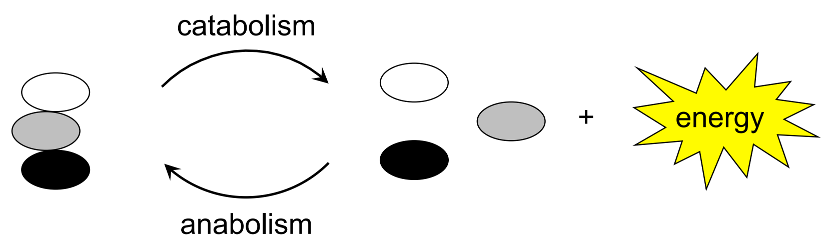
- Key reference: Chapter 21 of Voet, Voet & Pratt (and this lecture note/google)

Overview of Metabolism

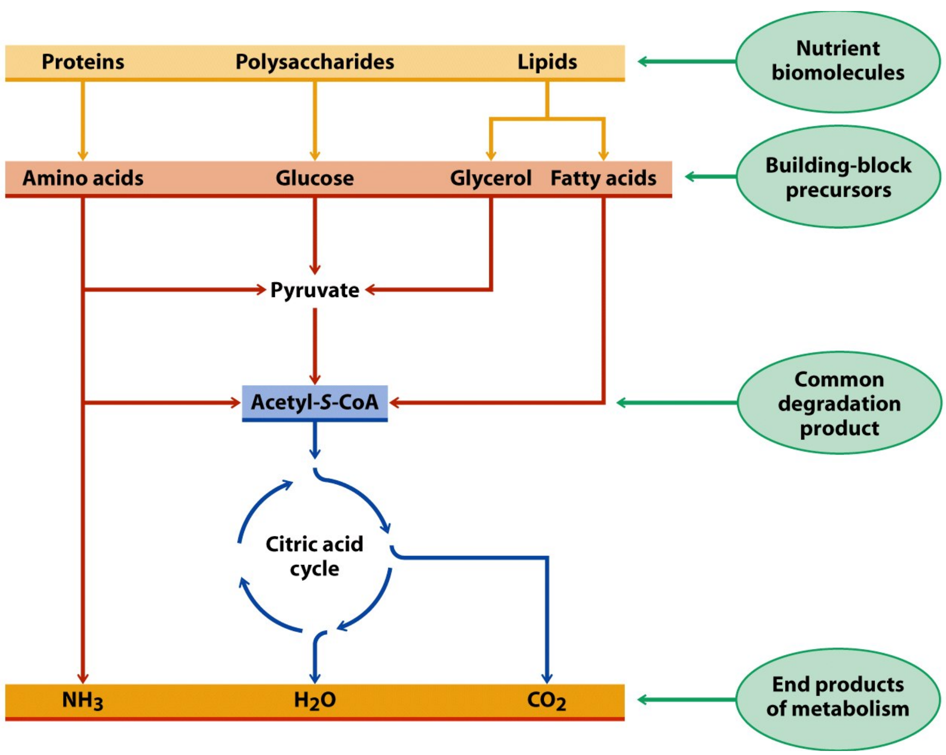
• *Metabolism* is the sum/total of all the biochemical reactions that take place in a living organism.

• *Catabolism* is all metabolic reactions in which large biochemical molecules are broken down to smaller ones, thus generating energy.

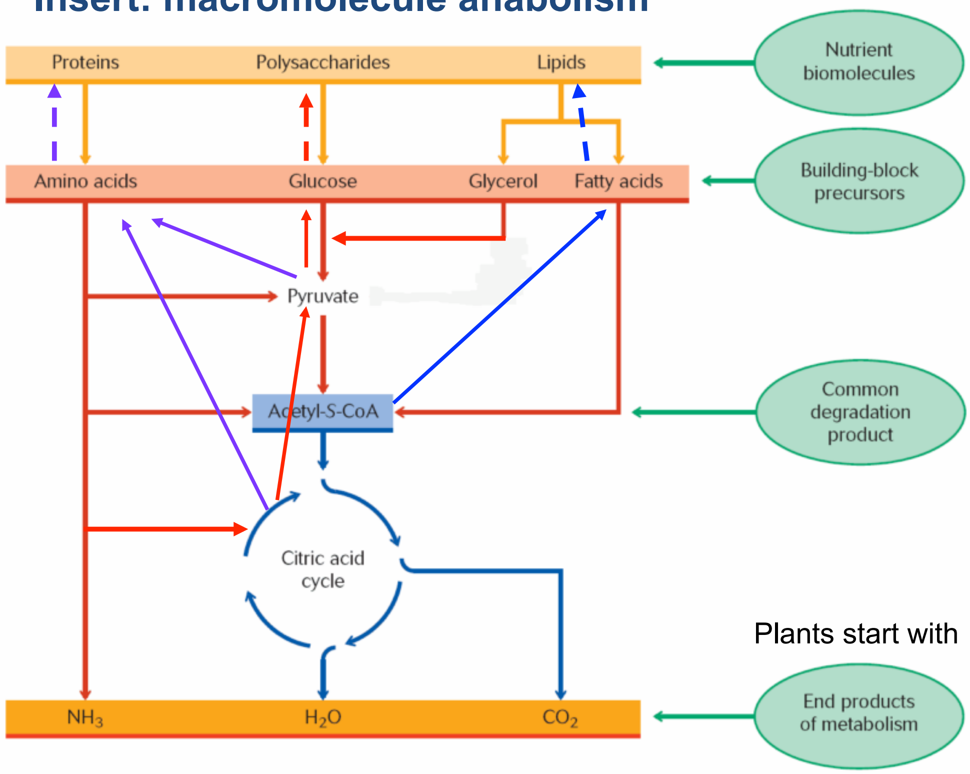
• *Anabolism* is all metabolic reactions in which small biochemical molecules are joined to form larger ones through consumption of energy.



Major Stages of Catabolism



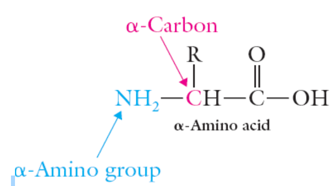
Insert: macromolecule anabolism

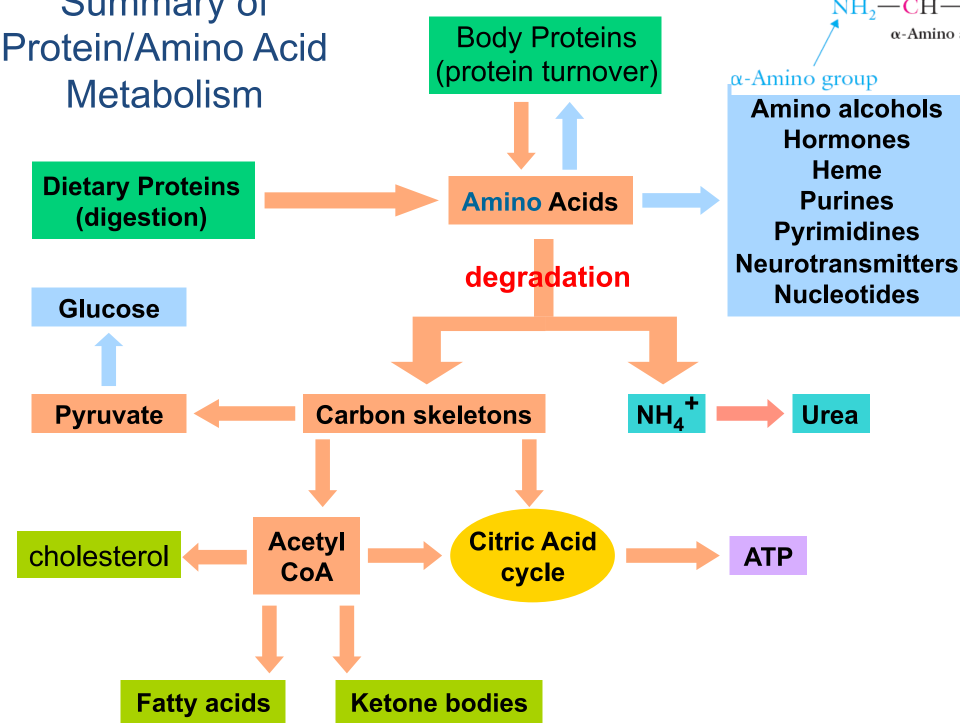


Plants start with

Biosynthetic pathways always differ from catabolic pathways.

Summary of Protein/Amino Acid Metabolism





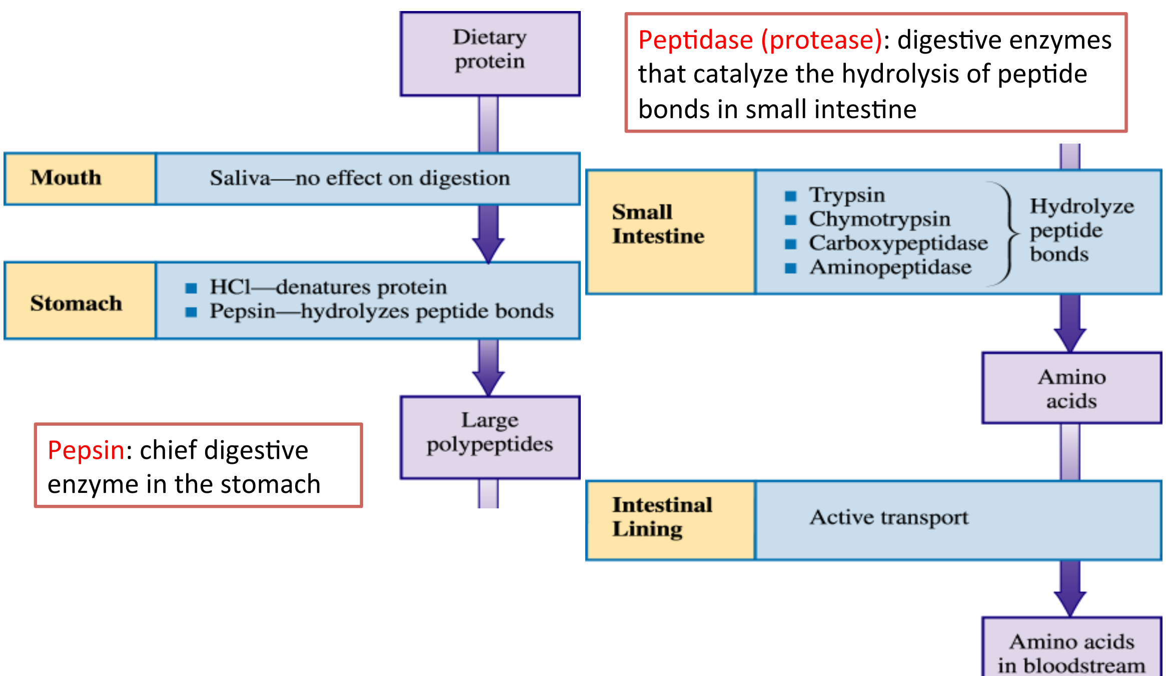
Chapter 21 - 1

1. PROTEIN DEGRADATION

Key Concepts 21.1

- Extracellular and intracellular proteins may be digested by lysosomal proteases.
- 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.

Protein Digestion



Peptidase (protease): digestive enzymes that catalyze the hydrolysis of peptide bonds in small intestine

Pepsin: chief digestive enzyme in the stomach

Protein Turnover

- Proteins are constantly being turned over in cell
  - Clear damaged proteins
  - Part of cell regulation
  - Metabolic needs
- Correlation of enzyme lifetime and the need to regulate
- The turnover rate also depend on cellular and nutritional conditions

TABLE 21-1 Half-Lives of Some Rat Liver Enzymes

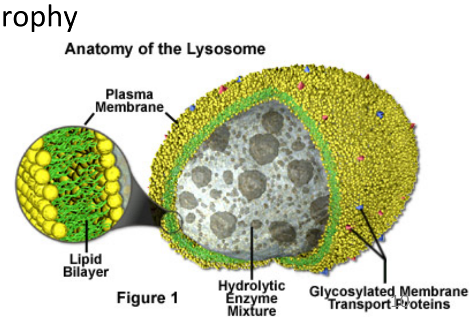
Enzyme	Half-Life (h)
Short-Lived Enzymes	
Ornithine decarboxylase	0.2
RNA polymerase I	1.3
Tyrosine aminotransferase	2.0
Serine–threonine dehydratase	4.0
PEP carboxylase	5.0
Long-Lived Enzymes	
Aldolase	118
GAPDH	130
Cytochrome *b*	130
LDH	130
Cytochrome *c*	150

Source: Dice, J.F. and Goldberg, A.L., *Arch. Biochem. Biophys.* **170**, 214 (1975).

Table 21-1

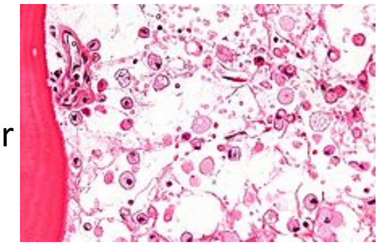
Lysosomes: non-selective degradation

- Cellular organelles that contain acid hydrolase enzymes
- pH ~ 5
  - Lysosomal enzymes inactive @ neutral pH: protection from lysosome leakage
- Feed by endocytosis and autophagy
- Selective pathway activated after a prolonged fast
  - Imports and degrades cytosolic proteins contain KFERQ or a closely related sequence
  - Not proteins from tissues that do not atrophy (e.g., brain and testes)
- Regression of uterus after childbirth
  - 2000g -> 50g in nine days



Lysosomal Storage Diseases (LSD)

- Genetic malfunction of lysosomal enzymes
- 1 in 5000 live births
- Accumulation of specific macromolecules or monomeric compounds inside the endosomal–autophagic–lysosomal system, leading to abnormal signaling
- Gaucher's disease
  - deficiency of glucocerebrosidase
  - Glucosylceramide lipid accumulation
  - affects spleen, liver, kidneys, lungs, brain and bone marrow.
  - bruises, fatigue, anaemia, low blood platelets, osteoporosis, and enlargement of the liver and spleen

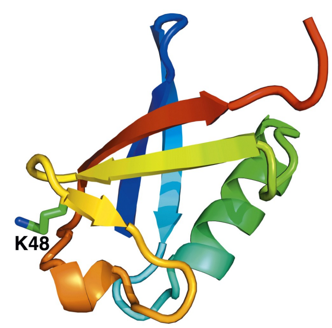


“crinkled paper” macrophages

Acid beta-glucosidase

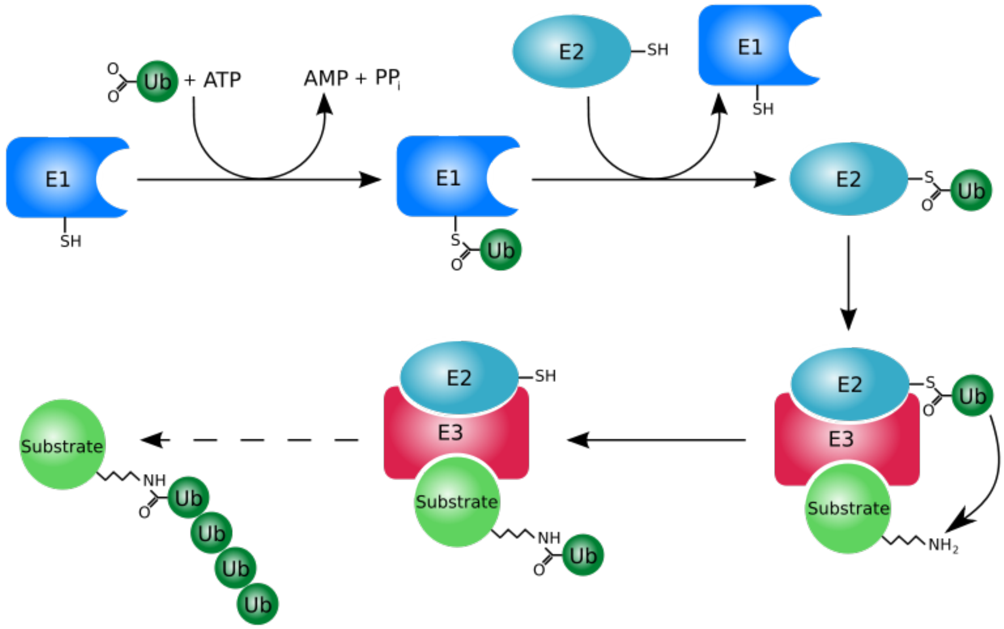
http://en.wikipedia.org/wiki/Gaucher%27s_disease

Ubiquitin Dependent Degradation

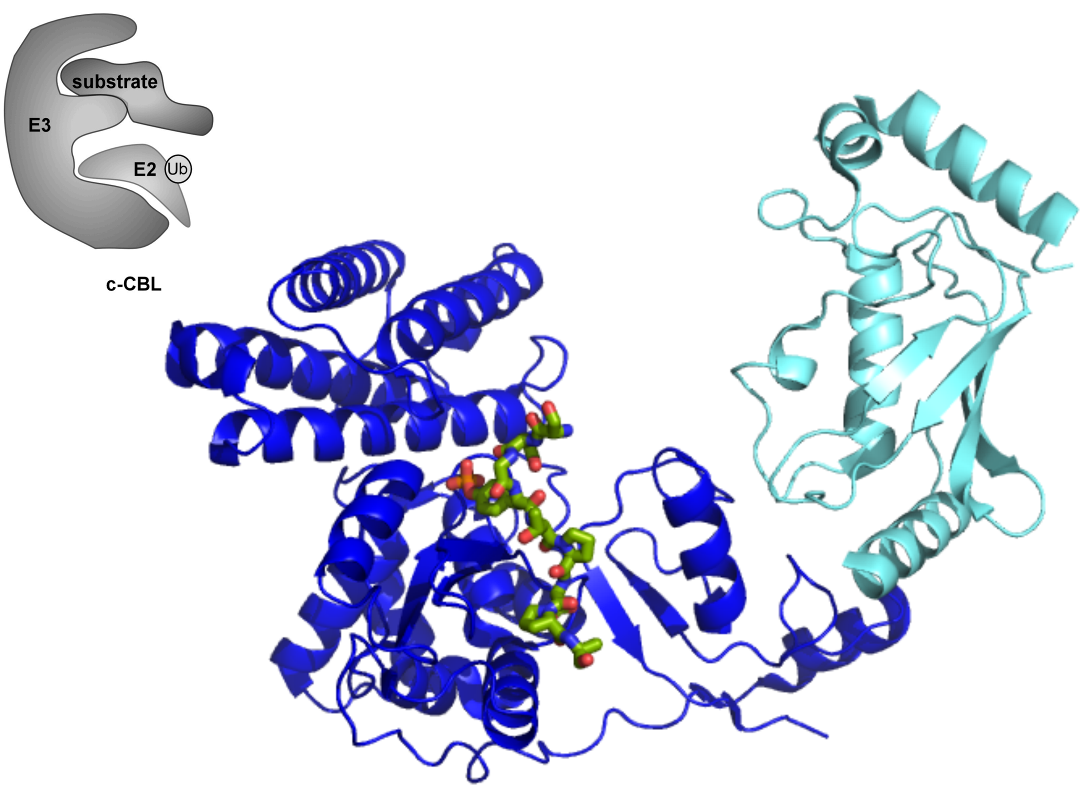


- ATP-dependent process
- Required ubiquitination
  - Ubiquitin: 76-residues, highly conserved
  - Involves three types of enzymes
    - E1: ubiquitin activating enzyme (one): consume ATP
    - E2's: ubiquitin conjugating enzymes (>20 in mammals)
    - E3: ubiquitin-protein ligases (many): transfer ubiquitin from E2 to Lys sidechains (responsible for recognizing proteins to clear!)
  - At least four ubiquitin units
    - Some poly-ubiquitin > 50
  - Recognition rules yet to be fully understood
- Both housekeeping (maintain protein balance and remove damaged proteins) and regulation
- Ubiquitinated proteins processed by proteasome

Ubiquitin Dependent Degradation



http://en.wikipedia.org/wiki/Ubiquitin



Proteasome

- Consume ATP to unfold and hydrolyze ubiquitinated proteins
- Multi-protein assembly (~2100 KD, 26S)
  - Jeroen Roelofs in Biology is an expert on proteasome assembly

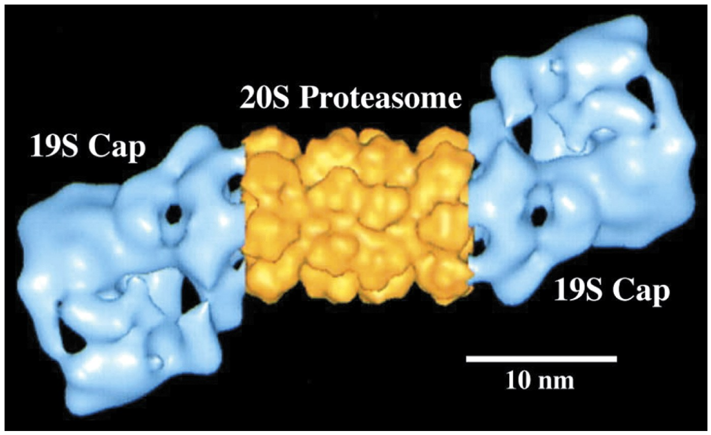


Figure 21-3
Courtesy of Wolfgang Baumeister, Max-Planck-Institut für Biochemie, Martinsried, Germany

Proteasome

http://www.youtube.com/watch?v=4DMqnfrzpKg

20S Proteasome (Core Particle)

- 7 alpha and beta subunits; catalytic activity in beta-rings
- Narrow, hydrophobic chamber: accessible only by unfolded proteins

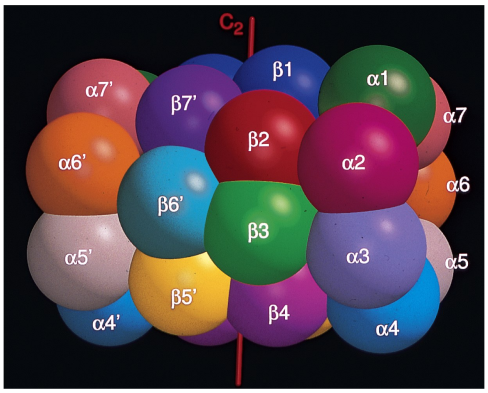


Figure 21-4a
Courtesy of Robert Huber, Max-Planck-Institut für Biochemie, Martinsried, Germany

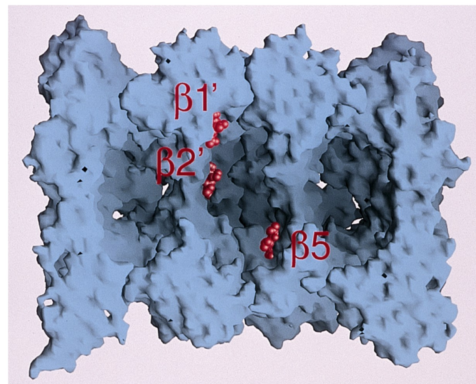


Figure 21-4b
Courtesy of Robert Huber, Max-Planck-Institut für Biochemie, Martinsried, Germany

Yeast 20S proteasome
PDBid 1RYP

20S Proteasome (Core Particle)

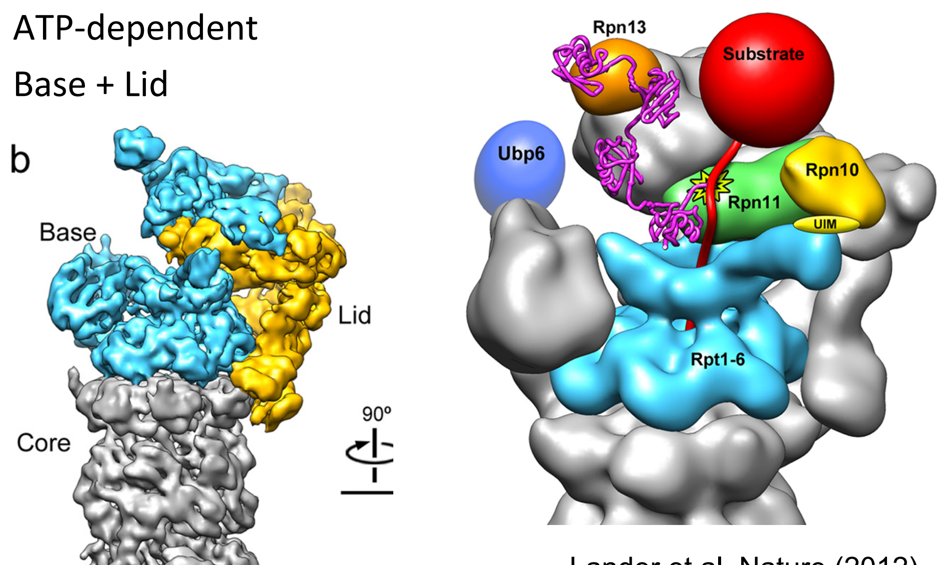
- 7 alpha and beta subunits; catalytic activity in beta-rings
- Narrow, hydrophobic chamber: accessible only by unfolded proteins
- Only three beta-subunits are catalytically active
  - N-terminal Thr residues as catalytic nucleophiles
  - Located in the center of 20S chamber
- Three active beta-subunits have different substrate specificities, cleaving after acidic (beta1), basic (beta2; trypsin-like) and hydrophobic (beta3; Chymotrypsin-like) residues.
  - Lead to ~8 residue fragments, which are degraded further by cytosolic peptidase
- Ubiquitin not degraded; they are released for reuse

Open Discussion: why ~8 residue fragments?

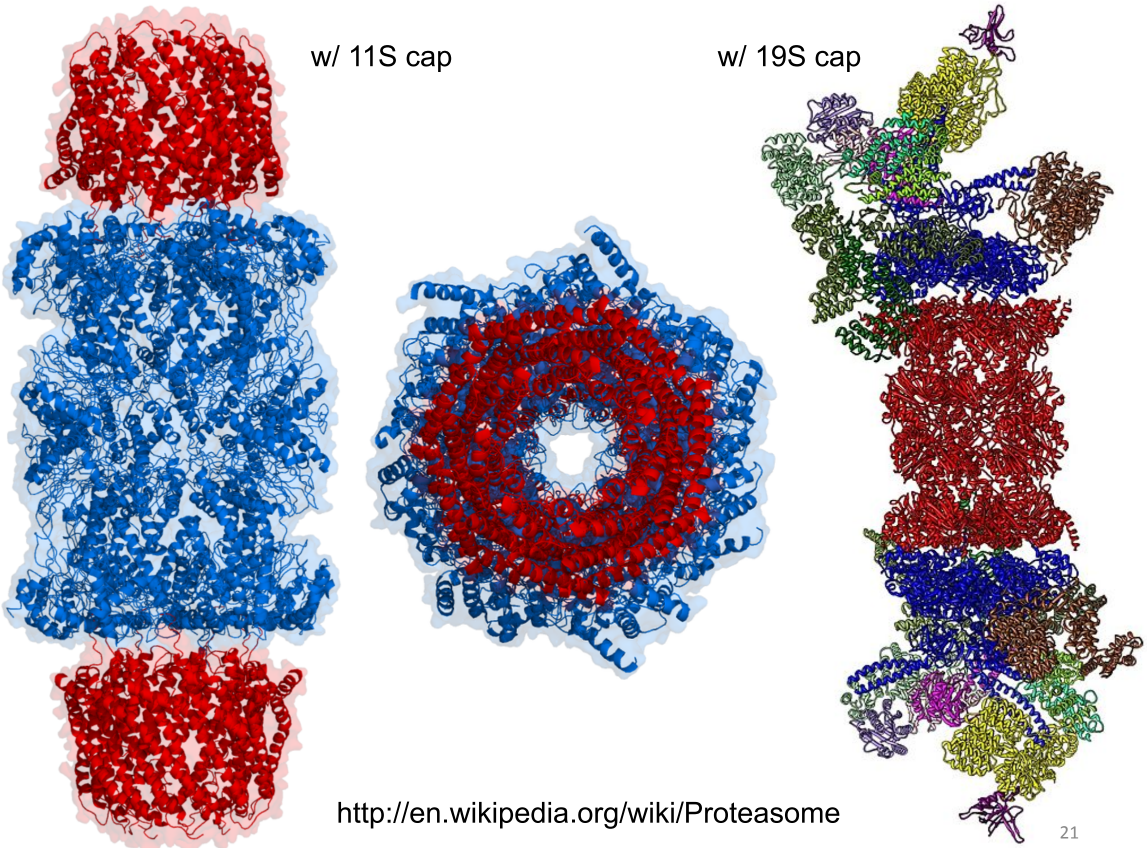
- Biochemical limit: not possible to make an enzyme that can cut every peptide bond
- Speed limit: too slow to cut shorter fragments; would require many more ribosomes
- No need: abundant peptidase in cell
- Functional need: e.g., short fragments for antigen presenting in immune response

19S Cap/Regulatory Particles

- Recognize ubiquitinated proteins, unfold them and feed to the 20S core protease particle
- ATP-dependent
- Base + Lid



Lander et al, Nature (2012)



http://en.wikipedia.org/wiki/Proteasome

Protease ClpP

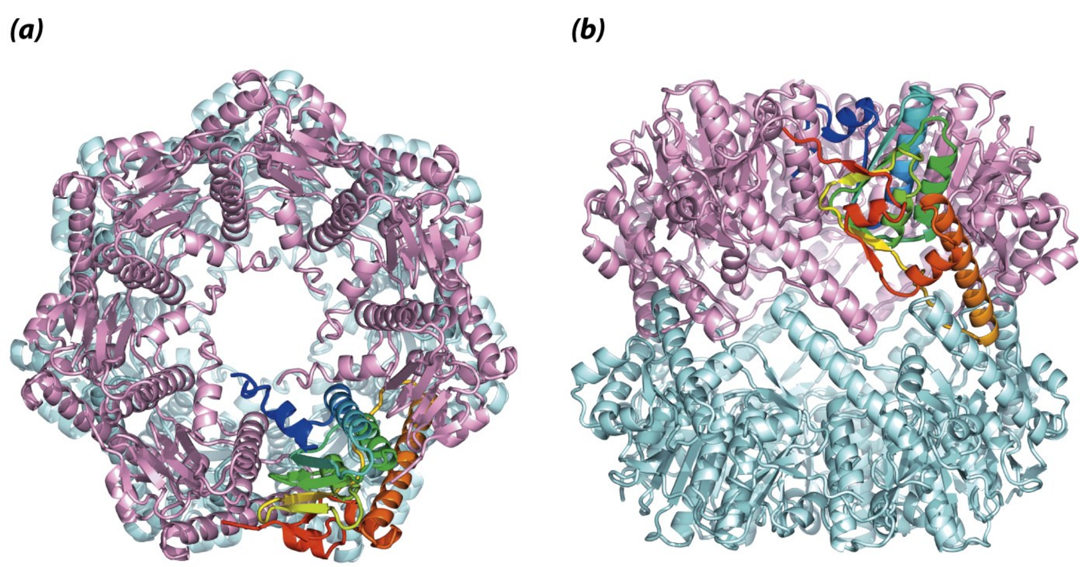
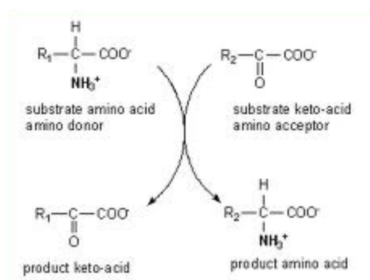


Figure 21-5

Subtopic Summary

- What is the role of the lysosome in degrading extracellular and intracellular proteins?
- Why must protein degradation be somewhat selective?
- Describe the steps of protein ubiquitination;
  - What is the difference between mono- and polyubiquitination?
- Describe the pathway for proteasome-mediated protein degradation, including the roles of ubiquitin and ATP.
- What is the advantage of the proteasomal active sites having different substrate specificities?

This is also your future study guide …



Chapter 21-2

AMINO ACID DEAMINATION

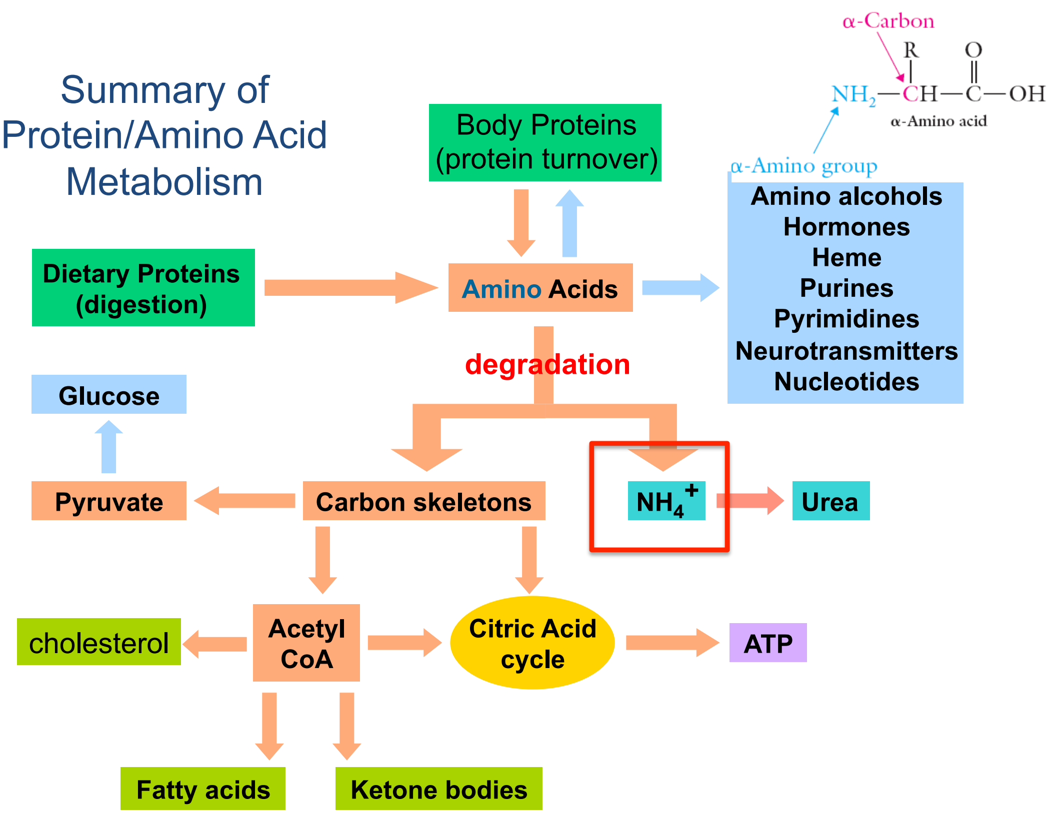
Key Concepts 21.2

- Transamination interconverts an amino acid and an α-keto acid.
- Oxidative deamination of glutamate releases ammonia for disposal.

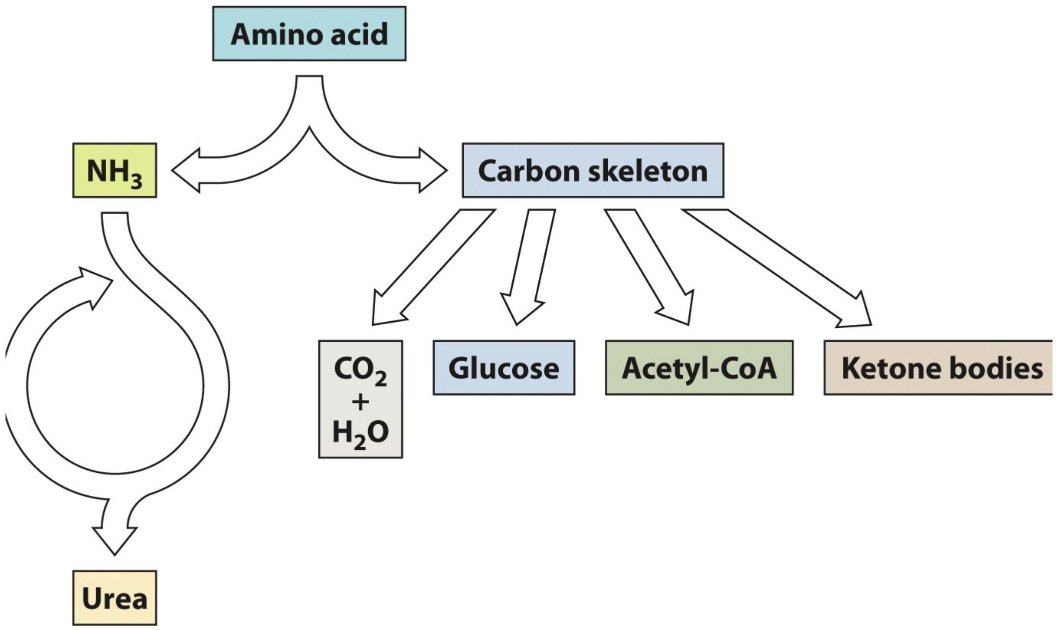
Amino acid utilization

- No long-term storage of amino acids besides muscle proteins
- Proteins from diet, protein synthesis and *turnover* contribute to the amino acid pool.
  - Most important usage: protein synthesis (~75%).
  - Synthesis nonessential amino acids and other of nitrogen-containing compounds
  - Production of energy (catabolism)
- The *amino acid pool* is the total supply of free amino acids (GLN and GLU represent 50% of the aa pool) available for use in cell. **(why?)**
- (positive and negative) *Nitrogen balance* is the state that results when the amount of nitrogen taken into the human body as protein equals the amount of nitrogen excreted from the body in waste materials.
  - Each day a 75 kg person synthesizes about 400 g protein as tissues turn over
  - 50-100 g protein is consumed in the diet. So, each day equivalent of 50-100 g of protein must be excreted in some way

Summary of Protein/Amino Acid Metabolism



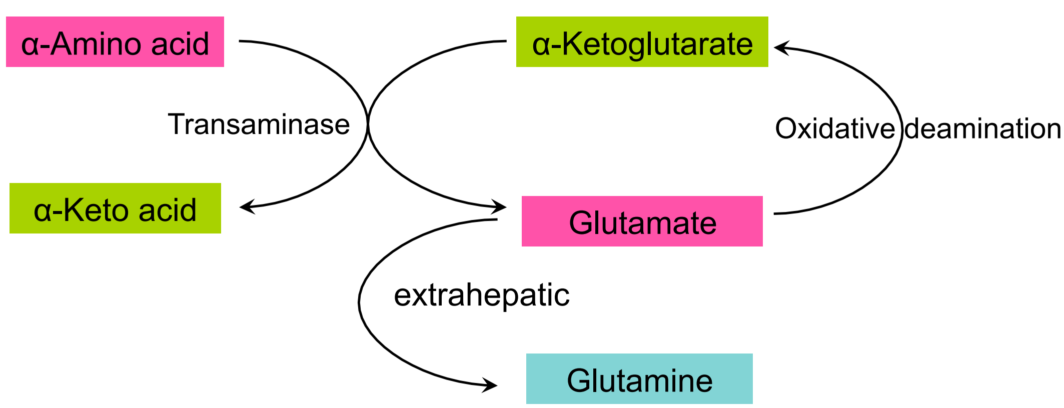
Amino Acid Catabolism

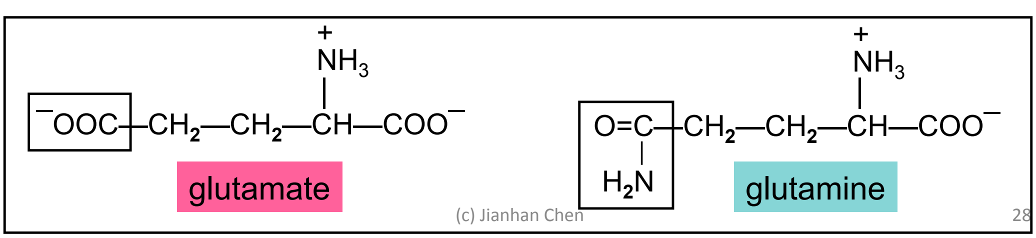


igure 21-6

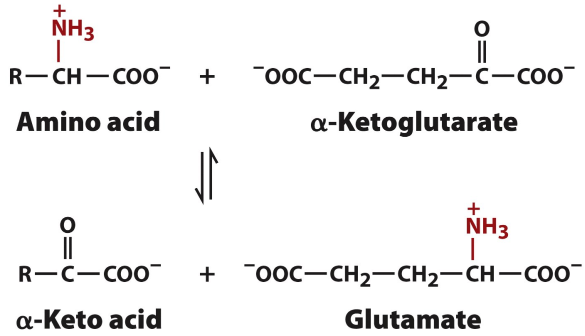
Transamination and Oxidative Deamination

- Removal of amino group: 1st step to amino acid catabolism





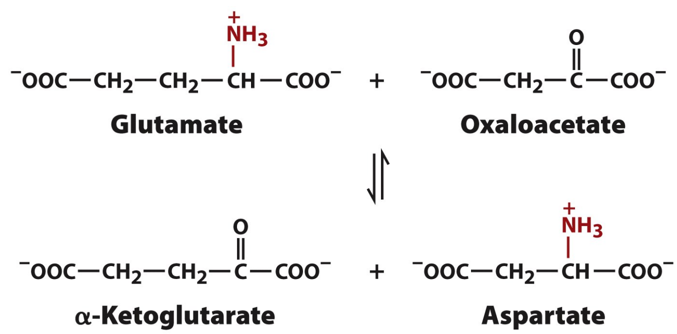
Transamination



Transamination: the transfer of the amino group to an alpha-keto acid

← α-Ketoglutarate is main amino group acceptor

↓ Glutamate can under 2nd transamination to produce aspartate



Occurs mostly in liver (Ala aminotransferase) and heart (Asp aminotransferase); presence of those activities in the blood are used as diagnostic tool to detect liver and heart damage.

Co-enzyme PLP

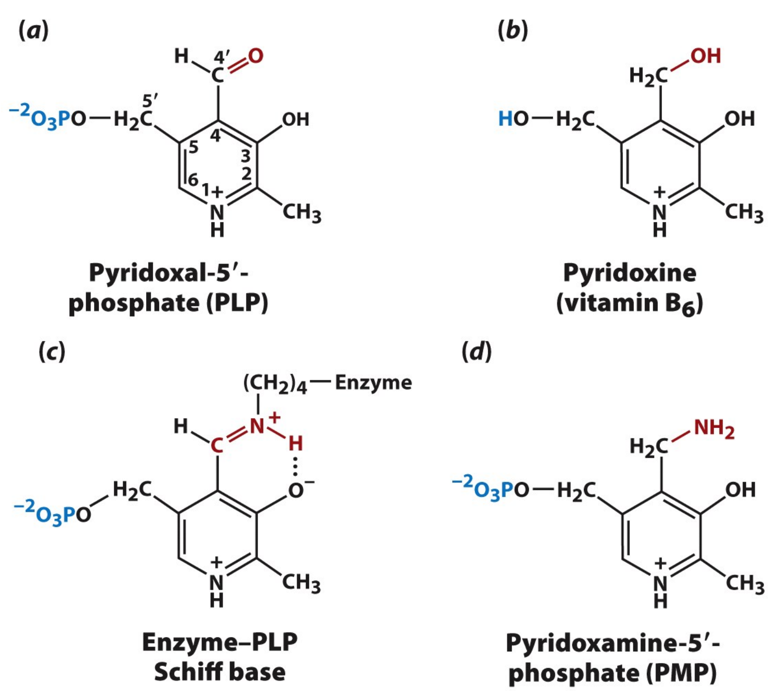
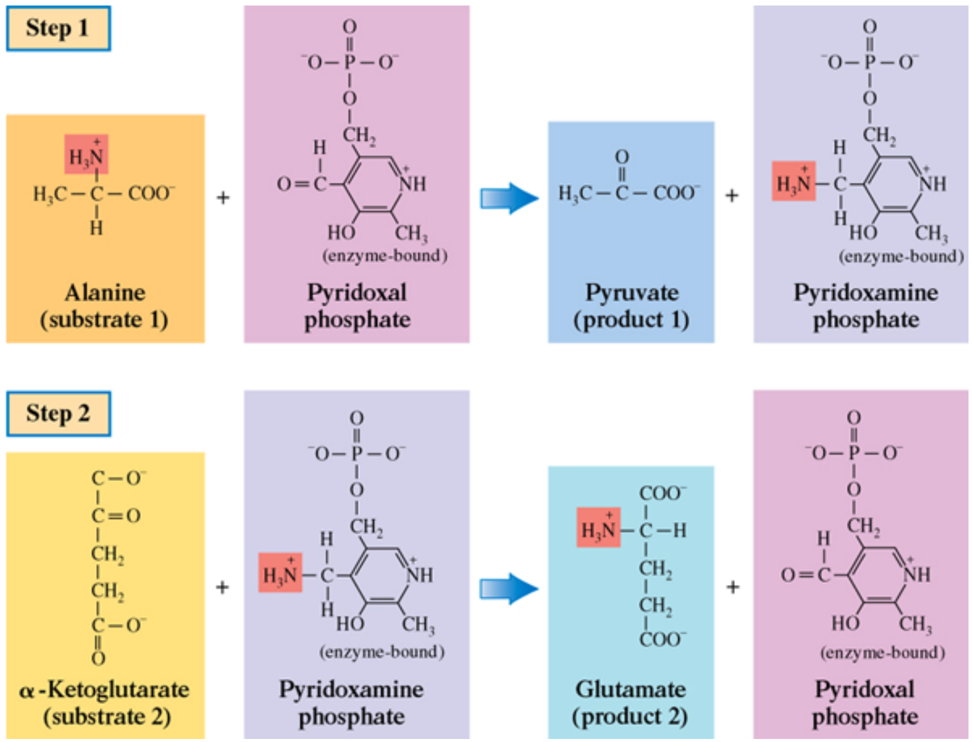


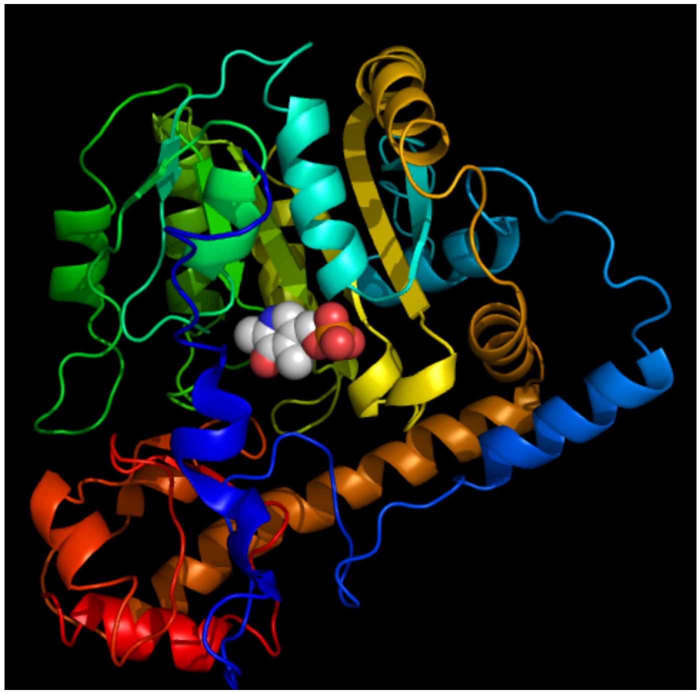
Figure 21-7



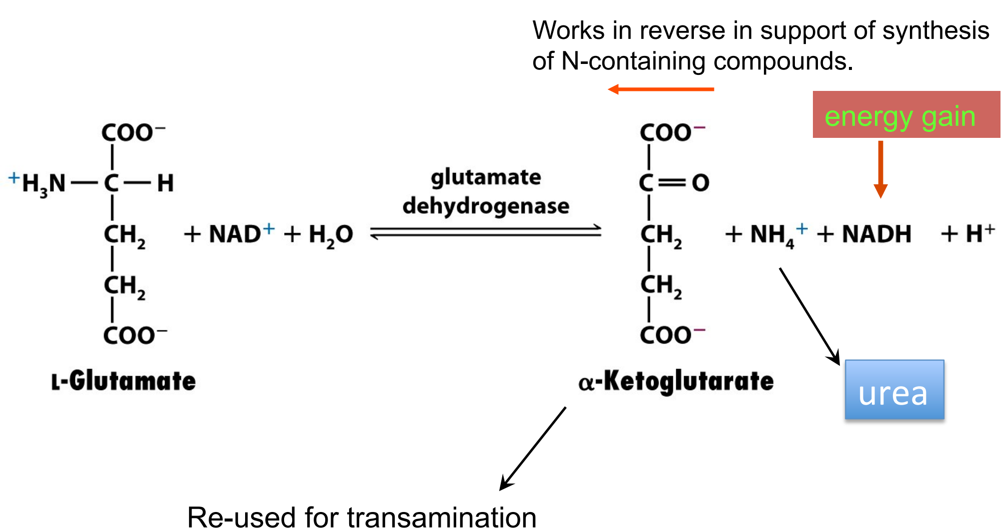
Transaminases

- Different transaminase for different amino acids, but most only accept α-Ketoglutarate and to a less degree oxaloacetate
  - collect the amino groups onto a single amino acid, glutamate ("amino group storage")
  - Reversible (both synthesis and degradation)
  - Lysine not transaminated
- α-ketoglutarate (intermediate of citric acid cycle) is the main amino group acceptor; pyruvate in the amino group acceptor in muscles
- Transamination occurs mostly in liver (ala aminotransferase) and heart (aspartate aminotransferase); presence of those activities in the blood are used as diagnostic tool to detect liver and heart damage.

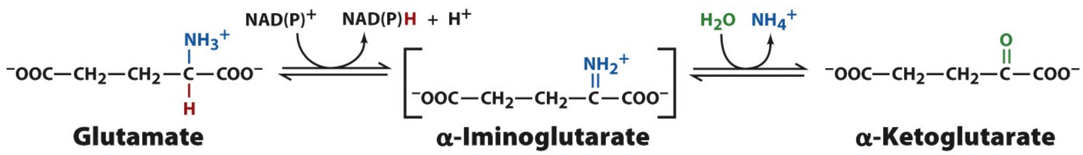
Aspartate Transaminase with PLP



Oxidative Deamination



Oxidative Deamination



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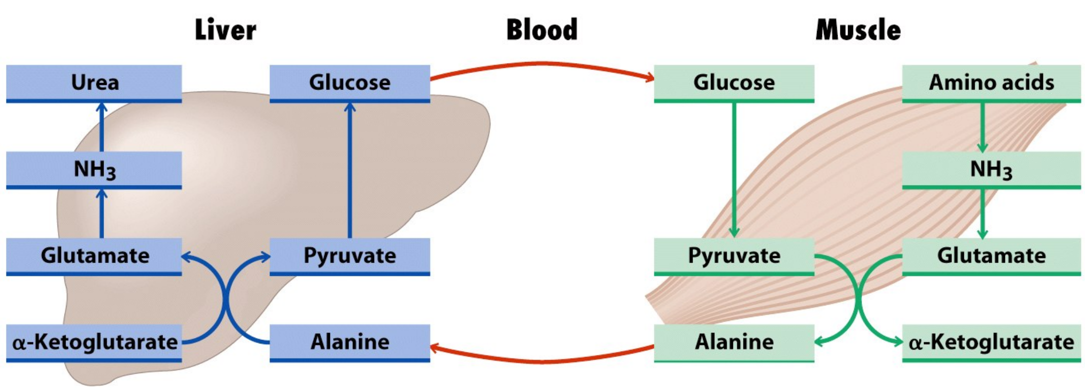
- Glutamate dehydrogenase (GDH): mitochondrial enzyme, uses both NAD+ and NADP+
  - Allosterically inhibited by GTP & NADH, activated by ADP & NAD+
  - α-Ketoglutarate part of citric acid cycle
- Used to be thought as a possible route for clearing ammonia
  - Reversed reaction
  - Now ruled out
- Ammonia must be cleared, through the urea cycle

Glucose-Alanine Cycle



- Kill two birds with one stone:
  - pyruvate created in muscle converted back into glucose
  - Amonium ions removed from muscle cells and converted into urea

Use of muscle protein (wasting) during starvation.

Our only storage form of amino acids is muscle protein and this must be used under conditions of starvation to make glucose and support essential protein synthesis.

Amino acid degradation in the muscle first involves the standard transamination to produce glutamate and all gluconeogenic α-keto acids produced are converted to pyruvate.

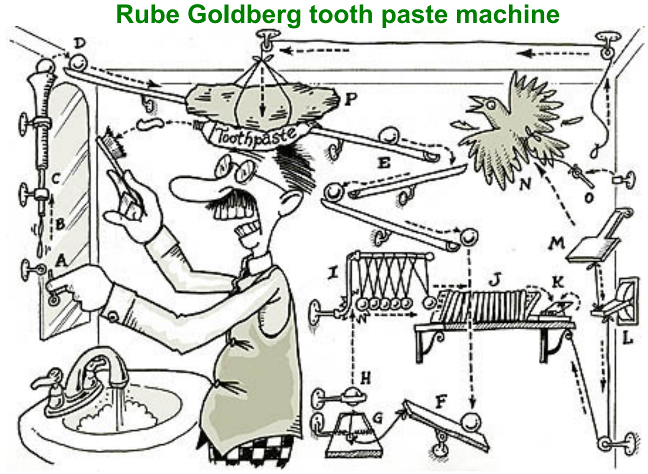
Then by transamination the amino group of glutamate is passed to pyruvate to produce **alanine**. Alanine is then transferred in blood to the liver.

In the liver, the urea cycle gets rid of ammonia. Pyruvate is converted to glucose for export back to the brain or if fat is depleted to muscle.

Quick Summary

- Describe how α-ketoglutarate and oxaloacetate participate in amino acid catabolism.
- Why Glu/Gln represent ~50% of the amino acid pool?
- What is the role of PLP in transamination?
- Summarize the reactions that release an amino acid's amino group as ammonia.

Rube Goldberg tooth paste machine



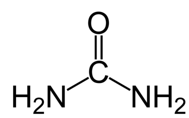
Chapter 21-3

UREA CYCLE

Key Concepts 21.2

- Five reactions incorporate ammonia and an amino group into urea.
- The rate of the urea cycle changes with the rate of amino acid breakdown.

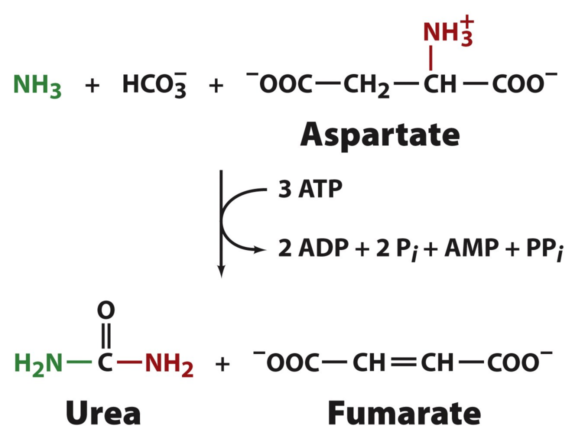
$2\ NH_3 + CO_2 \rightarrow$



urea

Overall Urea Cycle Reaction

- 1st metabolic cycle known: outlined in 1932
- Urea synthesized in liver, secreted into blood stream, and sequestered by kidneys for excretion in urine
- Nitrogen atoms in urea come from NH3 and aspartate
- 2 NADH produced, which is equivalent to 5 ATPs (i.e., a net gain of 2 ATPs!).
- Involve 5 key enzymes!



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Urea Cycle

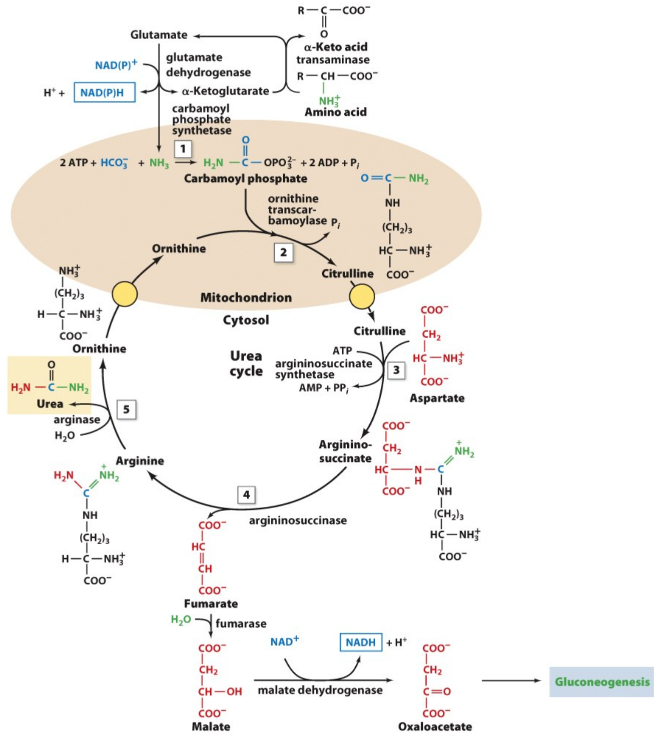


Figure 21-9

The Urea Cycle

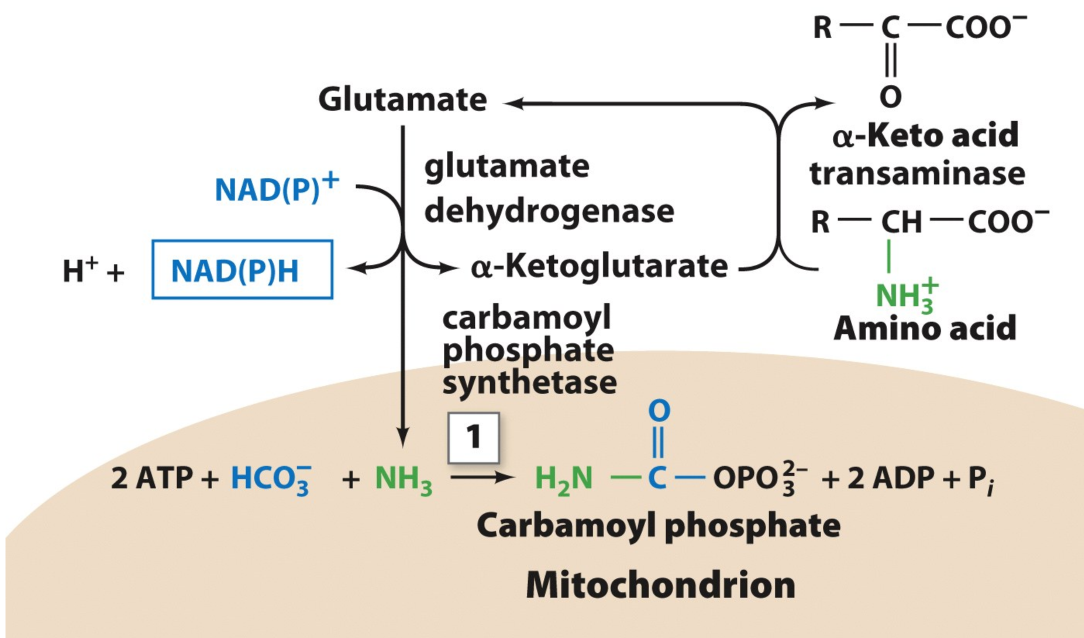


Figure 21-9 part 1

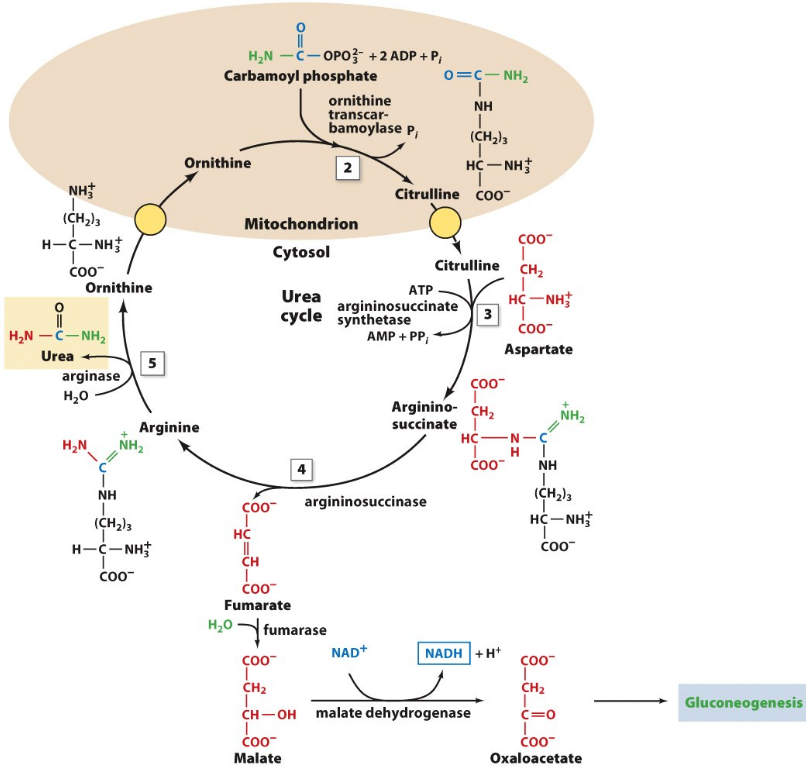
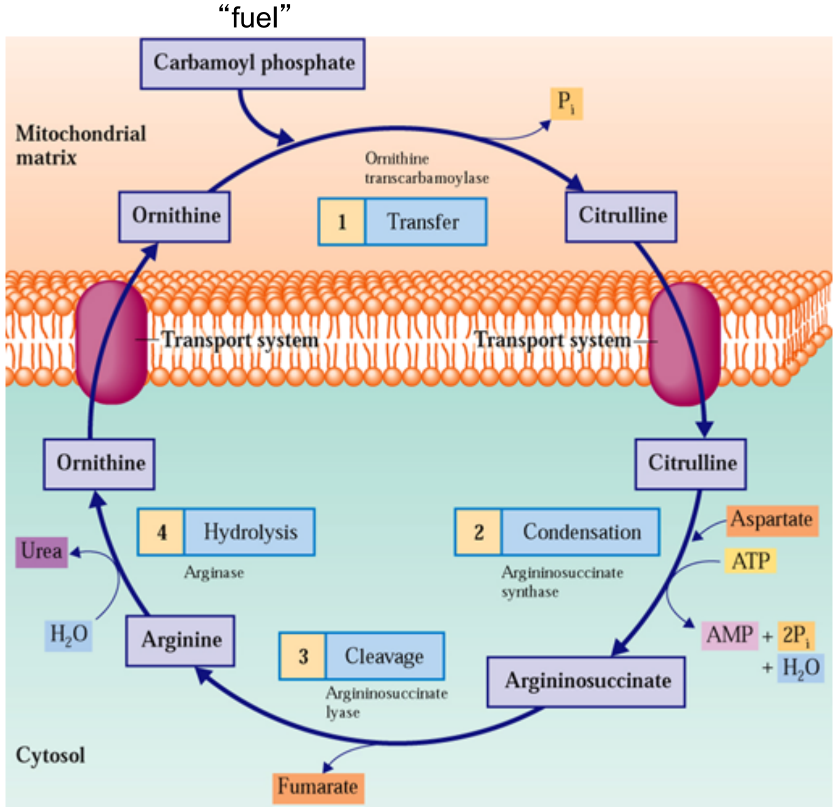
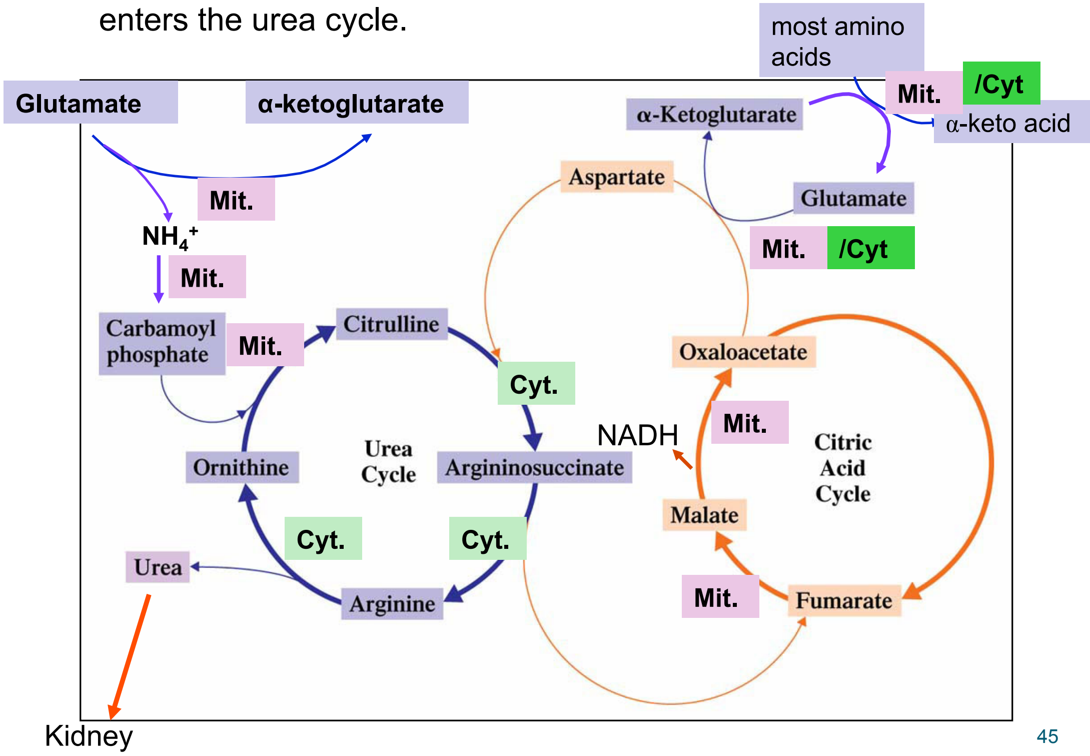


Figure 21-9 part 2

“fuel”



Fumarate from the urea cycle enters the citric acid cycle, and aspartate produced from oxaloacetate of the citric acid cycle enters the urea cycle.



Carbamoyl Phosphate Synthase (CPS)

- Condensation and activation of NH3 and CO2
- Rate limiting irreversible reaction
  - Also involved in primidine and arginine biosynthesis
- Three steps: catalyzed by the same CPS
  - Mitochondria CPS I: NH3 as nitrogen donor
  - Cytosolic CPS II: glutamine as nitrogen donor

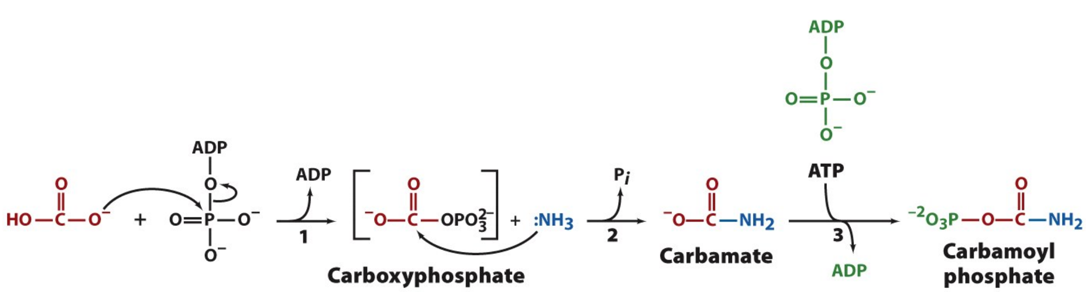


Figure 21-10

CPS II

Channeling: three active sites connected by a narrow 96 A long tunnel

NH3 travels ~45A to react with carboxyphosphate and resulting carbamate travels 35 A to reach carbamoyl phosphate synthesis site

Dramatically increased efficiency of the overall reaction!

Critical for CPS as the intermediates are short-lived (<100 ms)

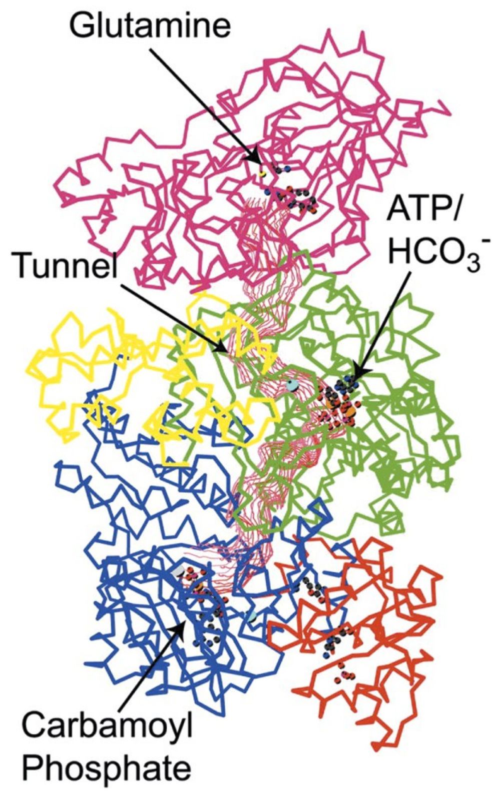


Figure 21-11
(Courtesy of Hazel Holden and Ivan Rayment, University of Wisconsin

CPS II

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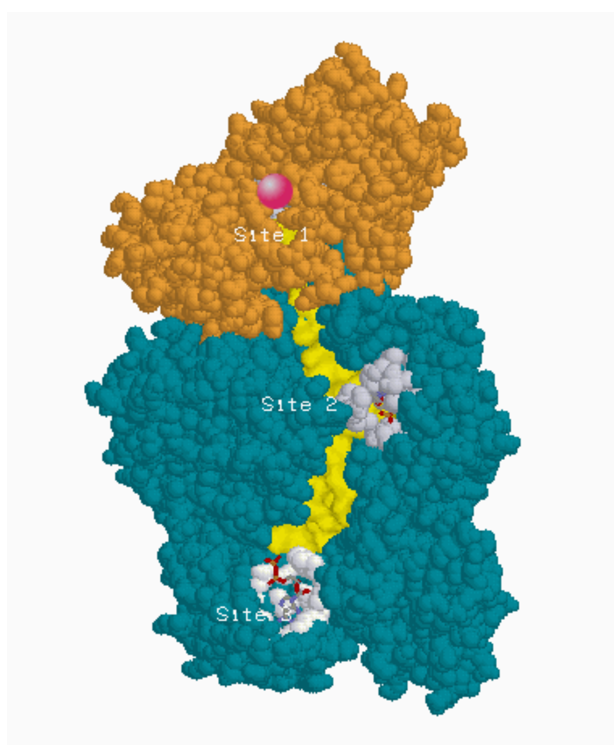
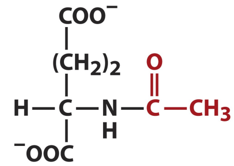


Regulation of Urea Cycle



***N*-Acetylglutamate**

- CPS: key regulation point
  - Allosterically regulated by N-acetylglutammate
  - Formed by glutamate and acetyl-CoA
  - Amino acid breakdown increases glutamate concentration and subsequently activate CPS
- Other enzymes of urea cycle are all regulated by the substrate concentration
- Hyperammonemia:
  - Urea cycle enzyme deficiency
  - Mental retardation and lethargy
- Ammonia toxicity: brain and central nerve system

Quick Summary

- Summarize the steps of the urea cycle. How do the amino groups of amino acids enter the cycle?
- What is rate limiting step?
- Where are three ATPs used?
- What are the advantages of channeling?
- How is the rate of amino acid deamination linked to the rate of the urea cycle?

The energy consumed in the urea cycle can be recovered from metabolism associated with this cycle.

A. True
B. False
C. Can't be determined

Open discussion: why the mess?

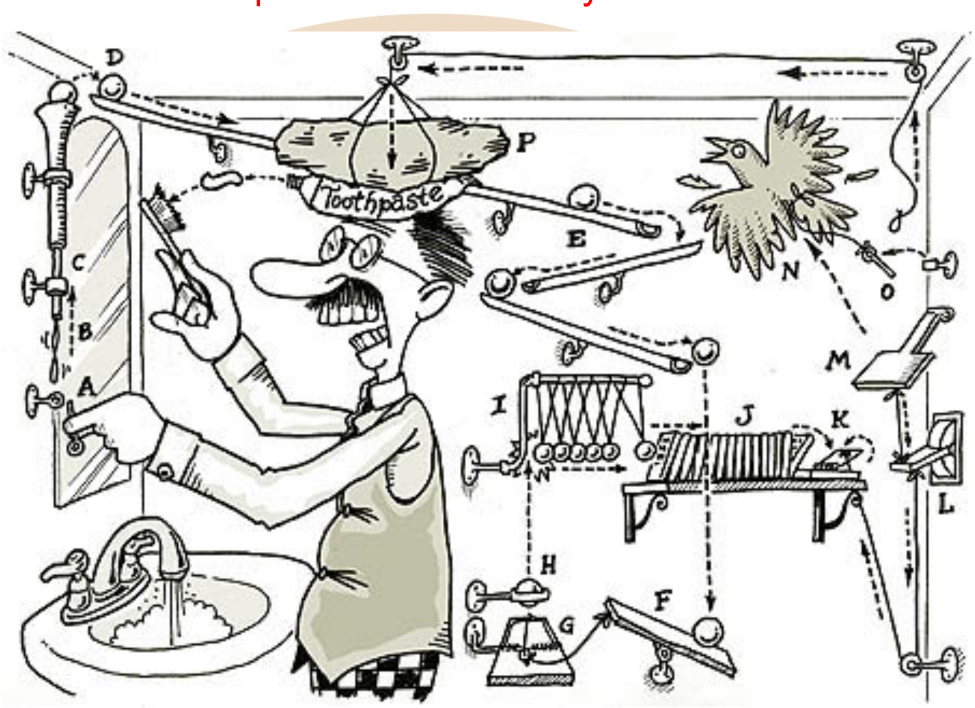
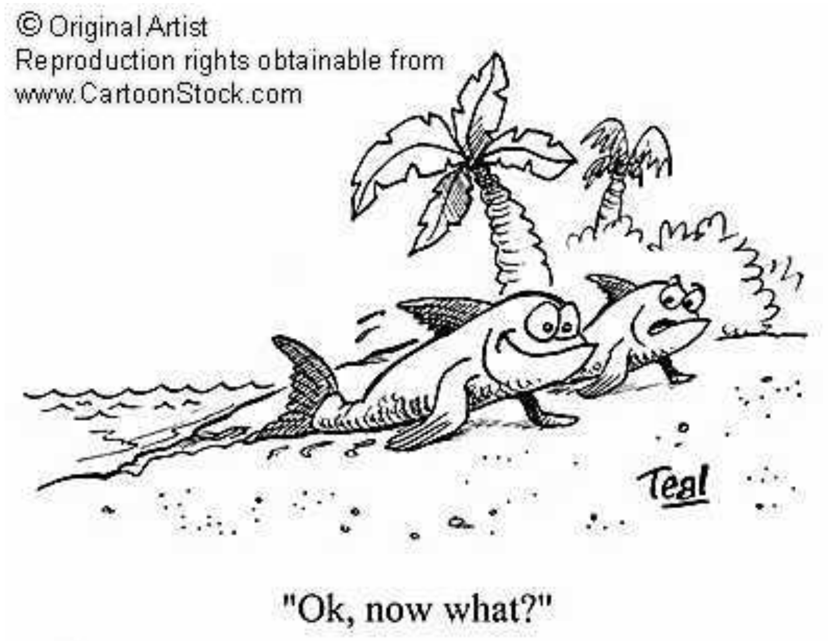
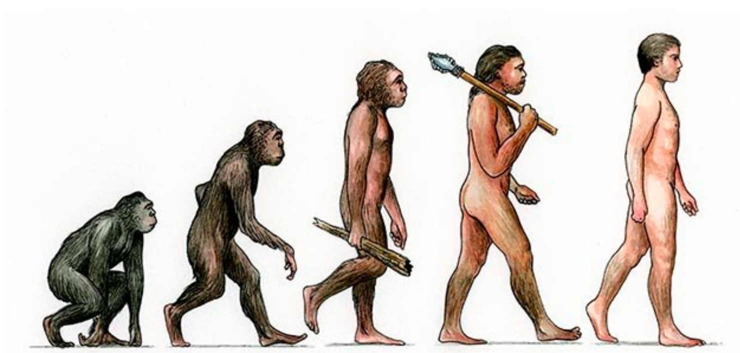


Figure 21-9 part 2

http://hhe.wikispaces.com/Rube+Goldberg+Machines

NH_4^+ not a problem for our aquatic ancestors, until …





From hiccups to wisdom teeth, our own bodies are worse off than most because of the differences between the wilderness in which we evolved and the modern world in which we live. (The Print Collector / Corbis)

The Top Ten Daily Consequences of Having Evolved

From hiccups to wisdom teeth, the evolution of man has left behind some glaring, yet innately human, imperfections

By **Rob Dunn**
SMITHSONIANMAG.COM
NOVEMBER 19, 2010

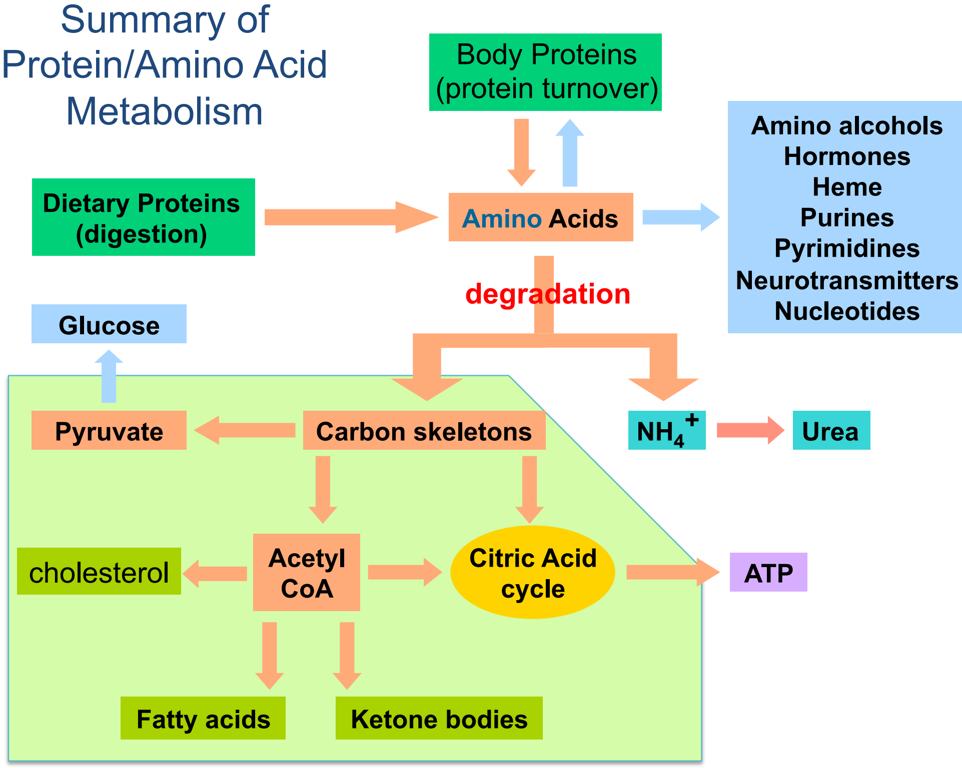
Chapter 21-4

BREAKDOWN OF AMINO ACIDS

Key Concepts 21.4

- Alanine, cysteine, glycine, serine, and threonine are broken down to pyruvate.
- Asparagine and aspartate are broken down to oxaloacetate.
- α-Ketoglutarate is produced by the degradation of arginine, glutamate, glutamine, histidine, and proline.
- Isoleucine, methionine, threonine, and valine are converted to succinyl-CoA.
- Leucine and lysine degradation yields acetyl-CoA and acetoacetate.
- Tryptophan is degraded to acetoacetate.
- Phenylalanine and tyrosine yield fumarate and acetoacetate.

Summary of Protein/Amino Acid Metabolism



Summary of Amino Acid Degradation

- 10-15% of energy usage
- 7 metabolic intermediates
- Grouping
  - 2 aa only ketogenic: Lys, Leu
  - 13 aa only* glucogenic: Gly, Ser, Val, His, Arg Cys, Pro, Ala, Glu, Gln, Asp, Asn, Met
  - 5 aa both glucogenic and ketogenic: ILE, Thr, Phe, Tyr, Trp

* all AA can be eventually used for generating acetyl-CoA and thus fatty acids

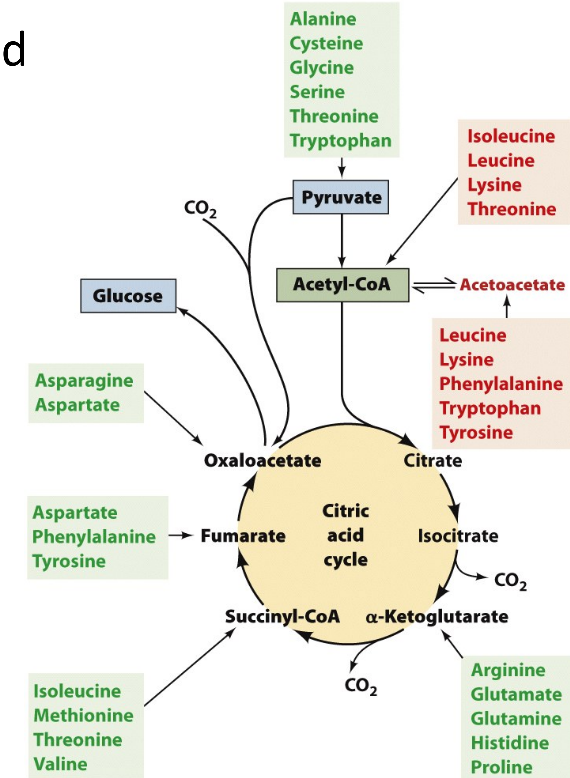


Figure 21-13

1). A, C, G, S & T to Pyruvate

- A: directly to pyruvate (transamination)
- S: dehydration (similar to deamination)
  - Serine-Threonine Dehydratase
- C: multiple routes for removing –SH, released in salts + NH3
- G: first converted to serine
  - Serine Hydroxymethyltransferase
  - Co-enzyme THF
- T: acyetyl-CoA + glycline

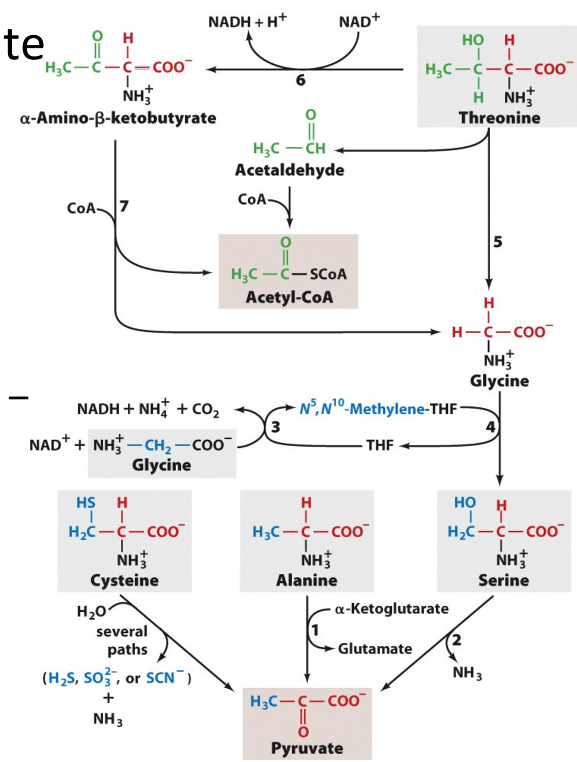
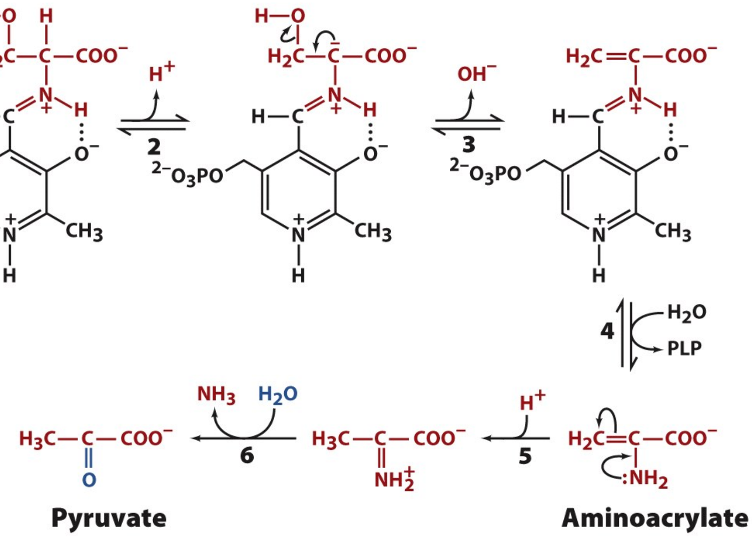
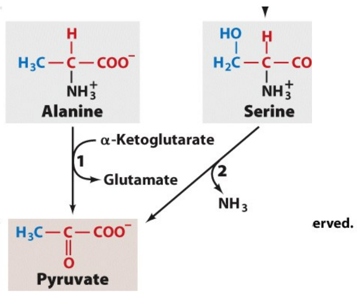


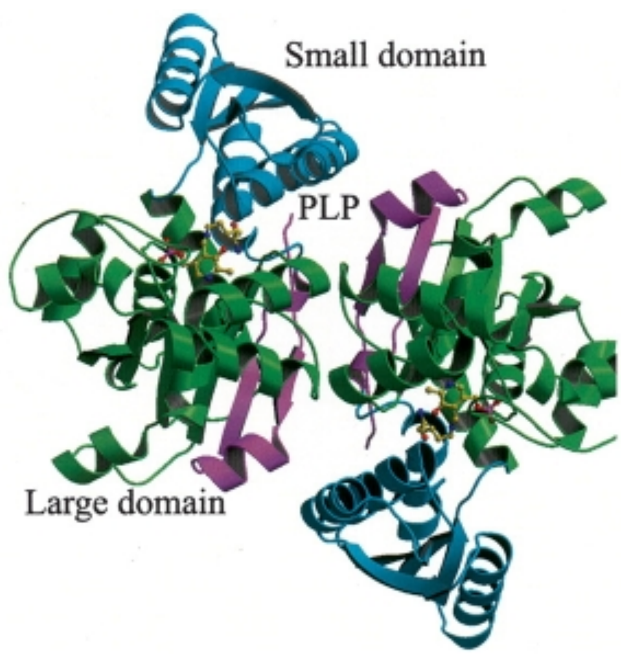
Figure 21-14

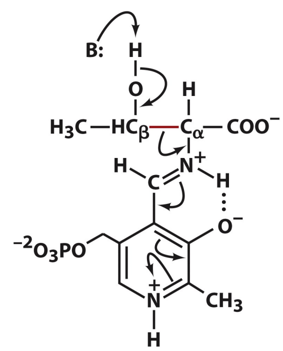
Serine-Threonine Dehydratase Reaction





Serine-Threonine Dehydratase Reaction





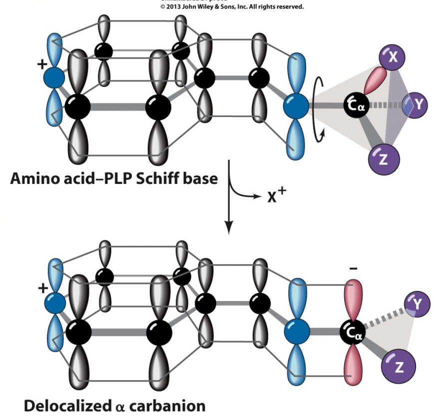
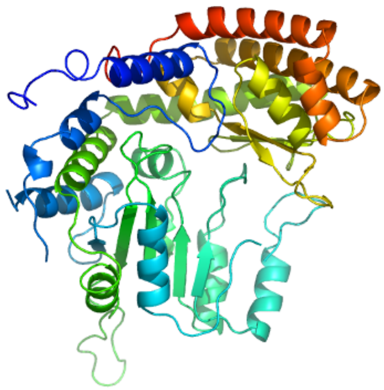
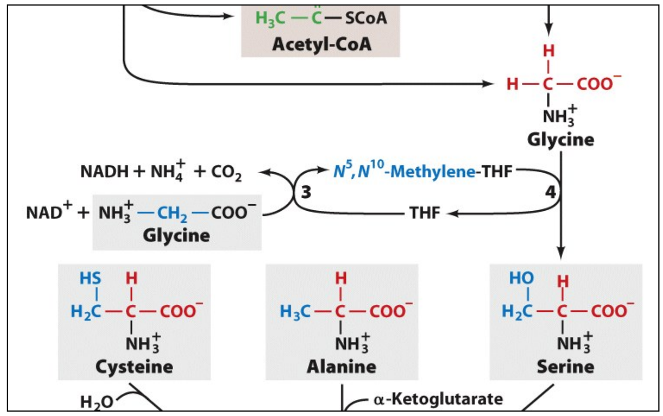


Figure 21-16

Serine Hydroxymethyltransferase

PDB: 3OU5





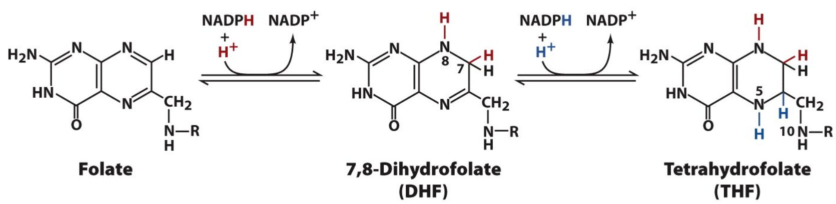
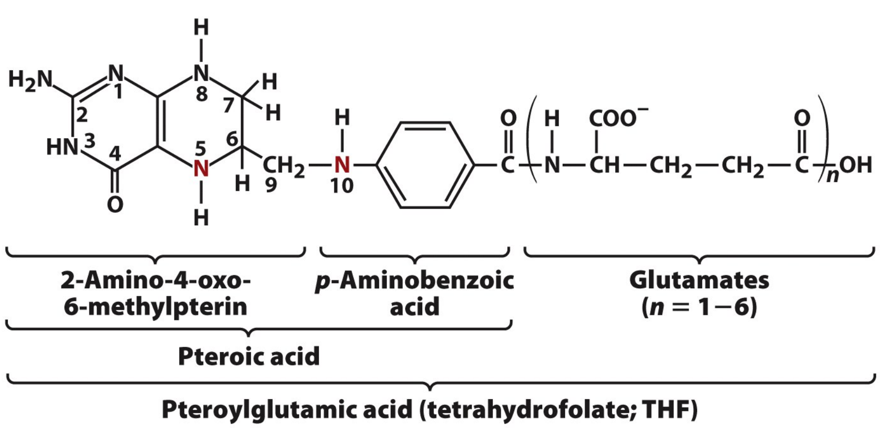


Figure 21-19

One-Carbon Carrier THF



Unnumbered 21 p734a

- Biosynthesis often involves addition of one carbon
- Poly glutamate track
- Carries C1 at either N5 or N10 position

Various C1 Unit Carried by THF

TABLE 21-2 Oxidation Levels of C_1 Groups Carried by THF

Oxidation Level	Groups Carried	THF Derivative(s)
Methanol	Methyl ($-CH_3$)	N^5-Methyl-THF
Formaldehyde	Methylene ($-CH_2-$)	N^5,N^{10}-Methylene-THF
Formate	Formyl ($-CH{=}O$)	N^5-Formyl-THF, N^{10}-formyl-THF
	Formimino ($-CH{=}NH$)	N^5-Formimino-THF
	Methenyl ($-CH{=}$)	N^5,N^{10}-Methenyl-THF

Table 21-2

- C1 units carried by THF can interconvert
- THF analogs can work antibiotics to inhibit bacteria synthesis of THF, thereby blocking THF-requiring reactions (mammals do not synthesize folic acids and thus unaffected).

THF C_1 Unit Interconversion

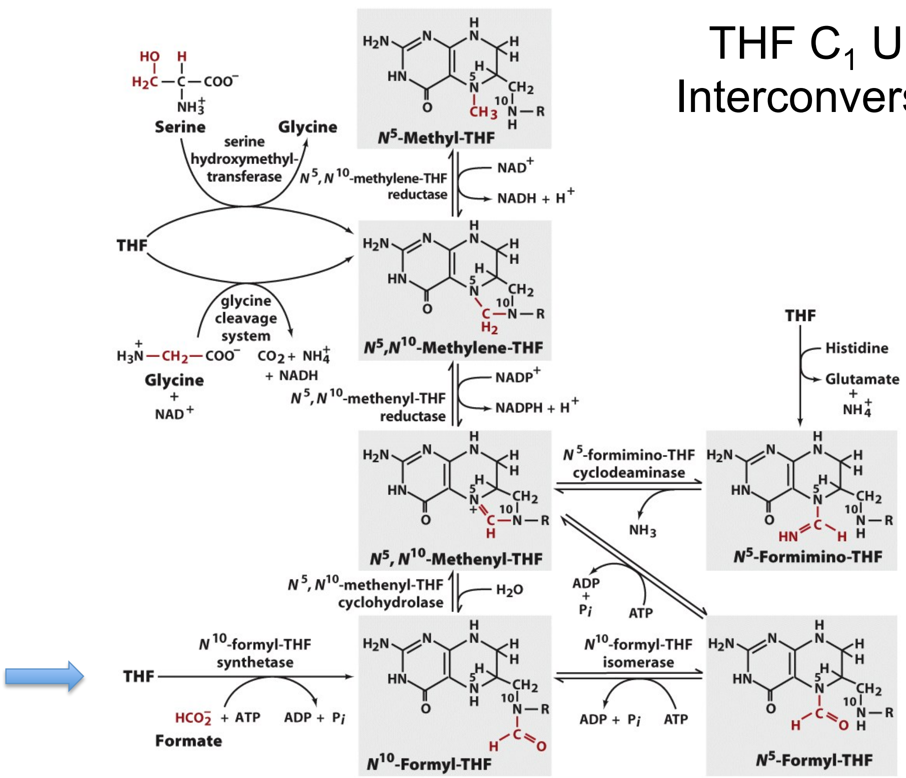
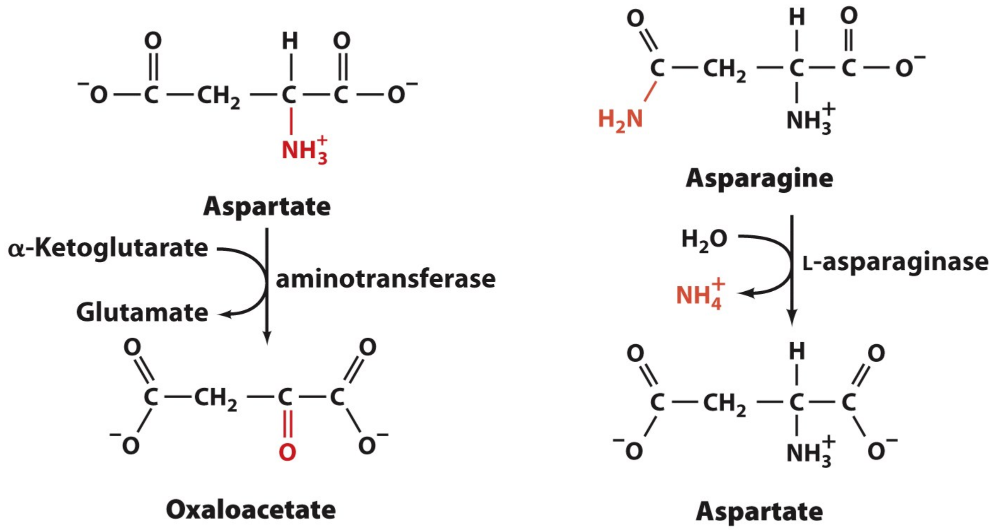


Figure 21-20

2). D & N to oxaloacetate

- Transamination; N->D via hydrolysis



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3). R, E, Q, H & P to α-Ketoglutarate

All of them are converted to Glu before transamination to produce α-Ketoglutarate

Involve either transamination or hydrolysis reactions

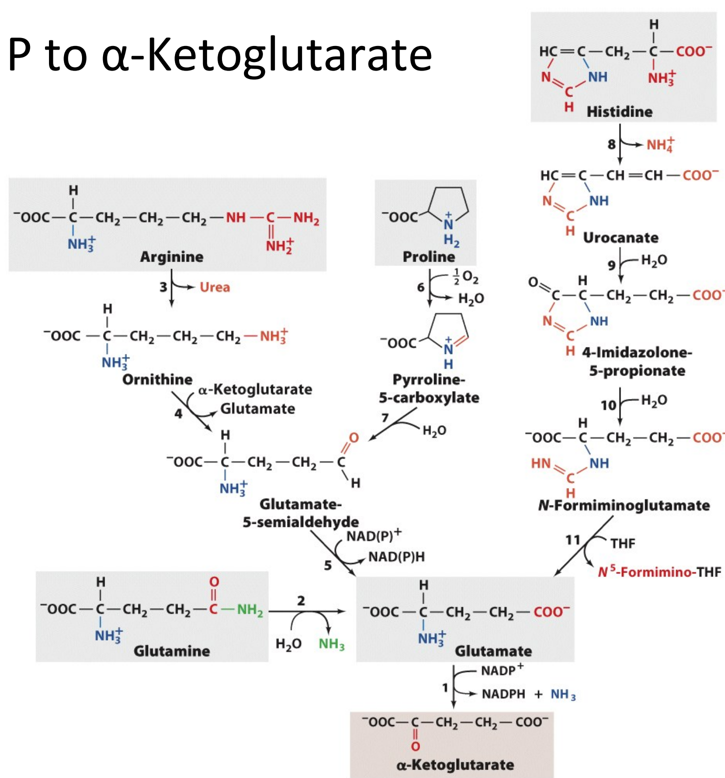


Figure 21-17

4). M/T degradation

Complex pathways to produce propionyl-CoA (also from odd-chain fatty acid degradation), which is then converted to succinyl-CoA

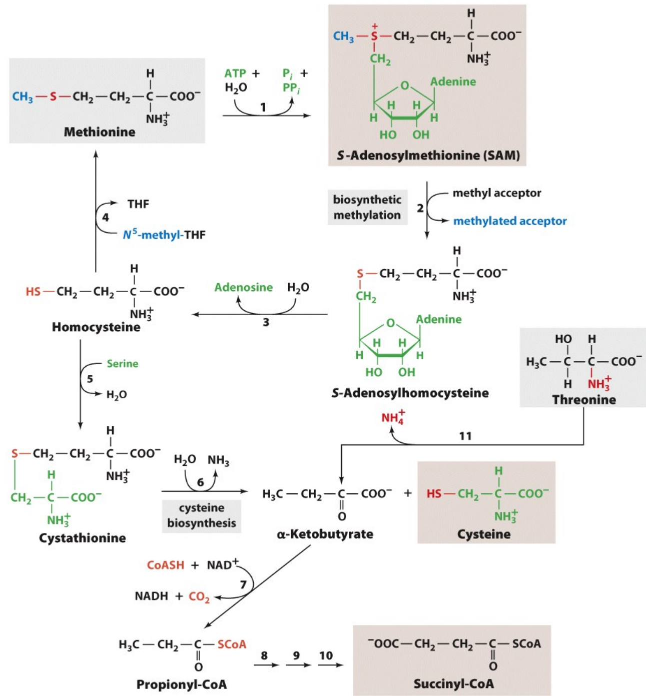


Figure 21-18

5. Branched Amino Acid

- ILE, VAL and LEU
- Three main steps
  - Transamination
  - Oxidative decarboxylation
  - Dehydrogenation
- Products
  - Succinyl-CoA (ILE,VAL)
  - Acetyl-CoA (Leu, ILE)
  - Acetoacetate (Leu)

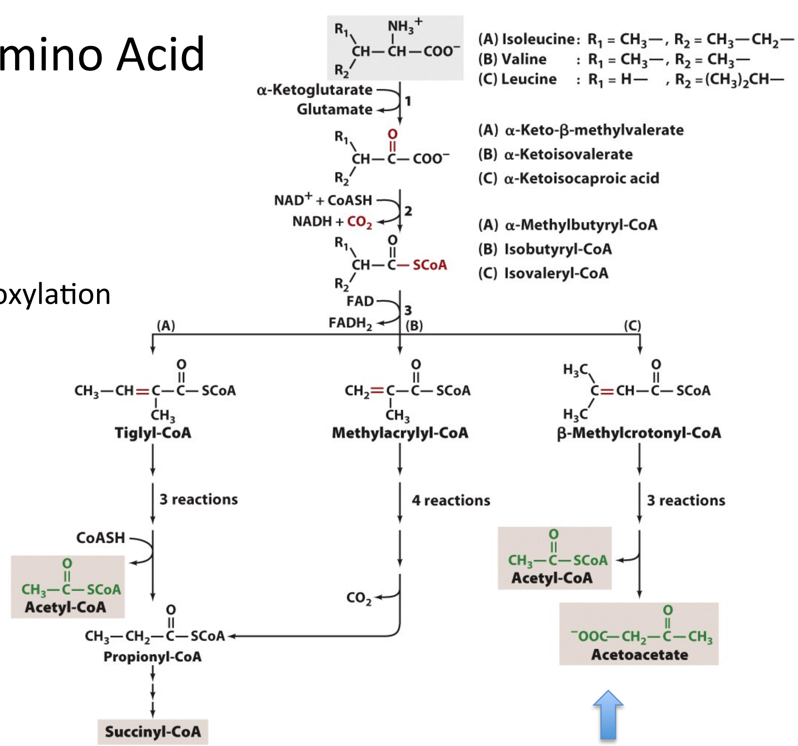


Figure 21-21

Leu is ketogenic only

6. Lys to Acetyl-CoA and Acetoacetate

- 2 CO_2 as by products
- Involves 11 enzymes!
- Three main types of reactions
  - Transamination
  - Oxidative decarboxylation
  - Dehydrogenation
- Consumes 1 NADPH but produces 4 NAD(P)H + $FADH_2$

Lys is also ketogenic only

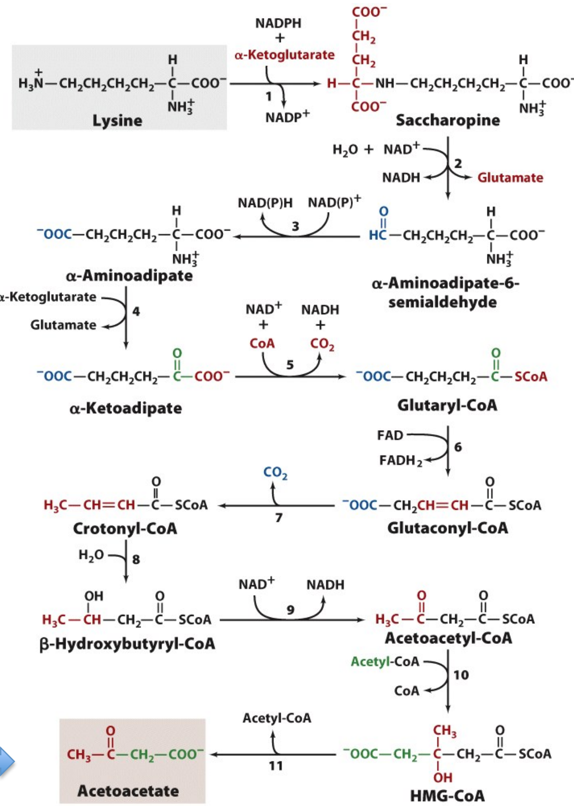


Figure 21-22

7. Trp to Ala and Acetoacetate

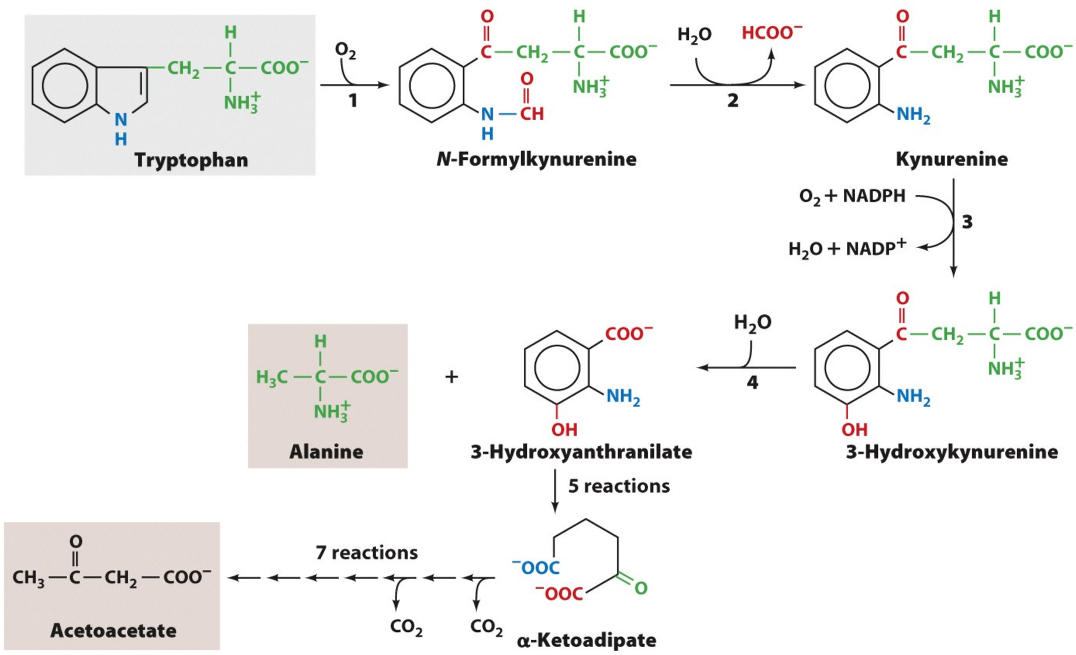


Figure 21-23

8. Phe/Tyr to Fumarate and Acetoacetate

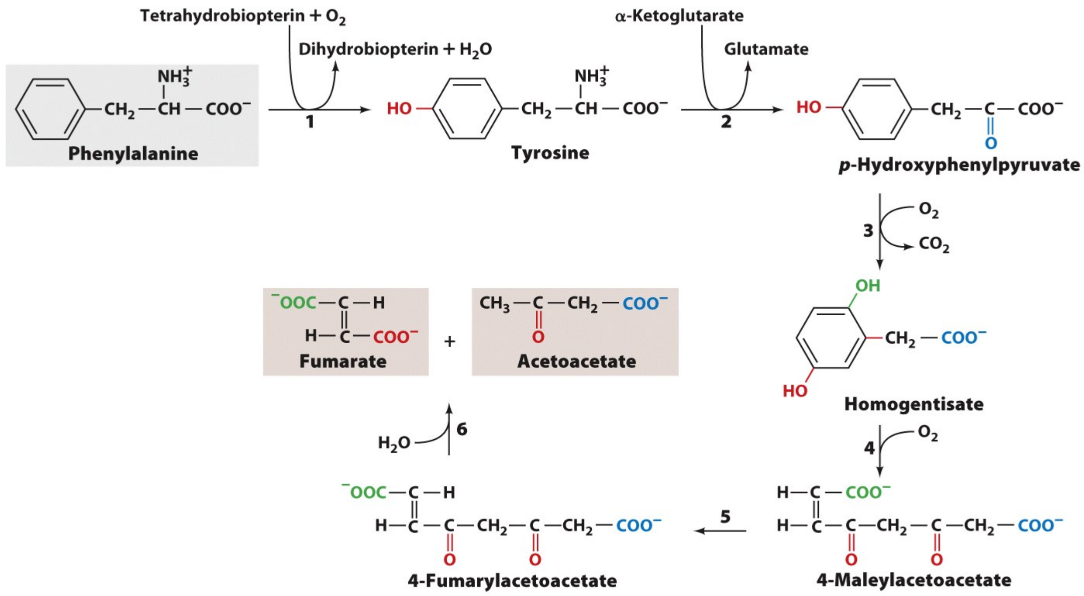


Figure 21-24

Summary of Amino Acid Degradation

- 10-15% of energy usage
- 7 metabolic intermediates
- Grouping
  - 2 aa only ketogenic: Lys, Leu
  - 13 aa only* glucogenic: Gly, Ser, Val, His, Arg Cys, Pro, Ala, Glu, Gln, Asp, Asn, Met
  - 5 aa both glucogenic and ketogenic: ILE, Thr, Phe, Tyr, Trp

* all AA can be eventually used for generating acetyl-CoA and thus fatty acids

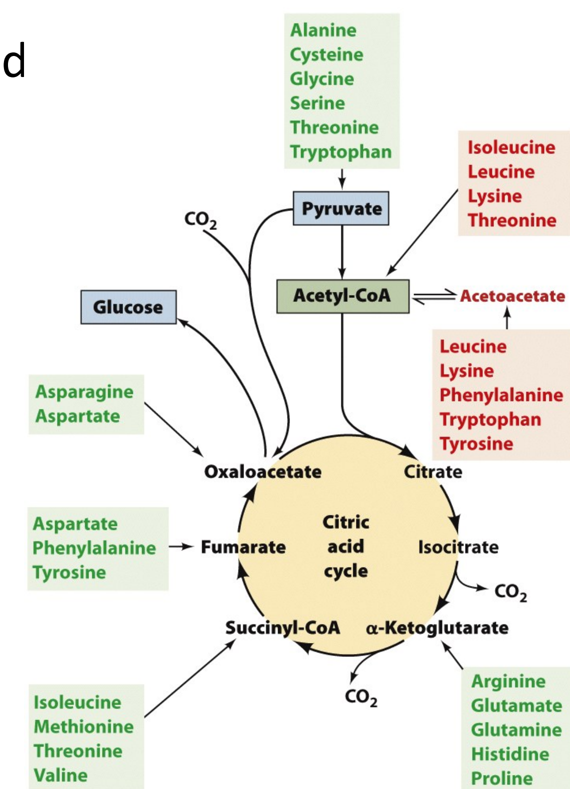


Figure 21-13

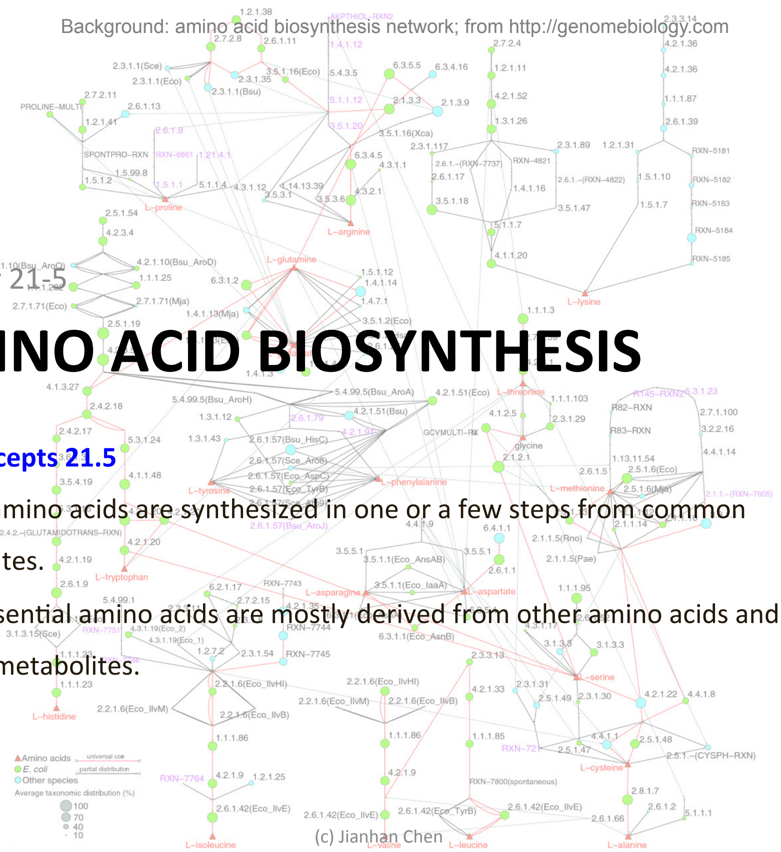
The carbon skeleton of amino acids may not be used to:

A. Generate acetyl-CoA.
B. Generate glucose.
C. Generate urea.
D. Generate ketone bodies.

Which of the following enzymes require ATP?

A. Carbamoyl phosphate synthetase I
B. Methionine synthase.
C. Arginosuccinate synthetase.
D. A and C.
E. All require ATP.

Background: amino acid biosynthesis network; from http://genomebiology.com



Chapter 21-5

AMINO ACID BIOSYNTHESIS

Key Concepts 21.5

- Some amino acids are synthesized in one or a few steps from common metabolites.
- The essential amino acids are mostly derived from other amino acids and glucose metabolites.

Essential vs non-essential Amino Acids

TABLE 21-3 Essential and Nonessential Amino Acids in Humans

Essential	Nonessential
Arginine[a]	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

[a]Although mammals synthesize arginine, they cleave most of it to form urea (Section 21-3A).

Table 21-3

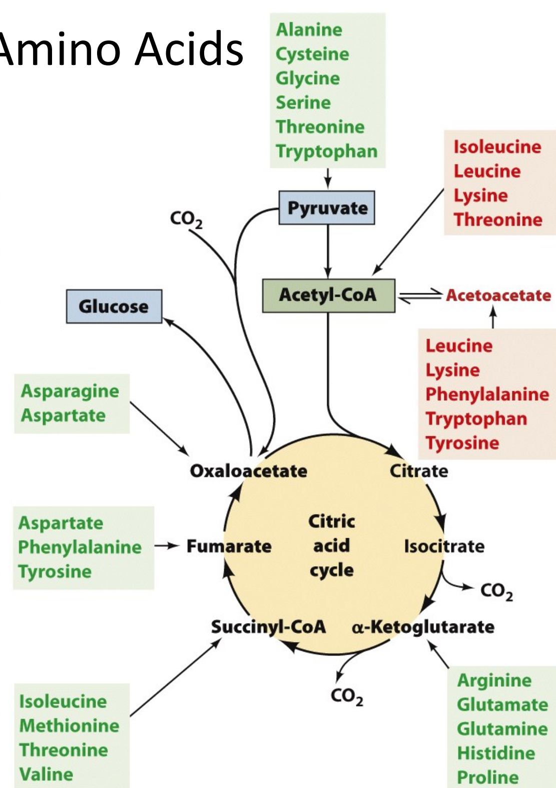


Figure 21-13

1. A, D, E, N, & Q Synthesis

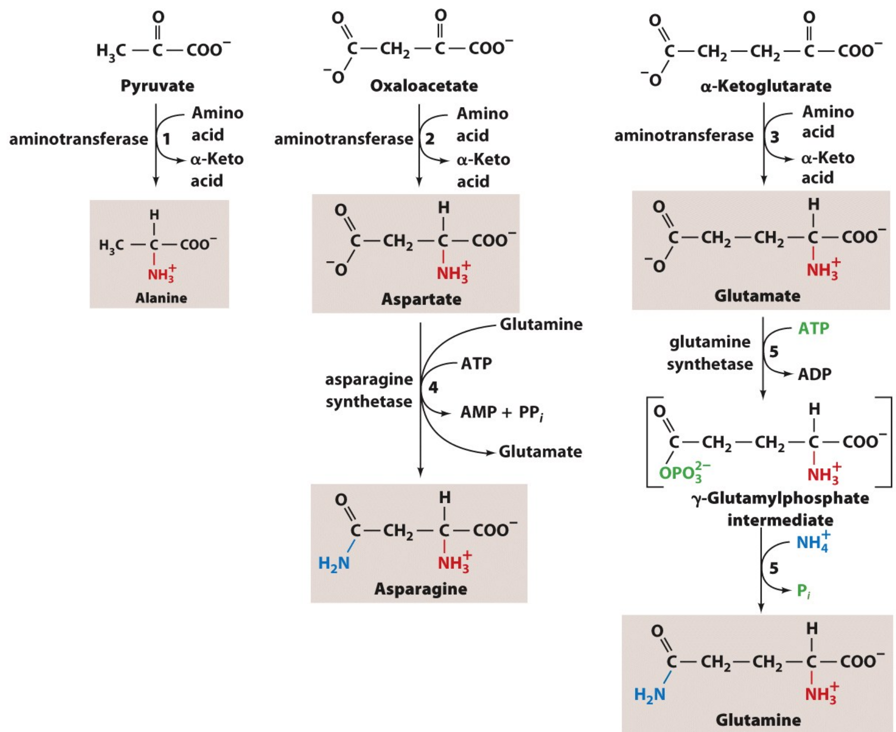
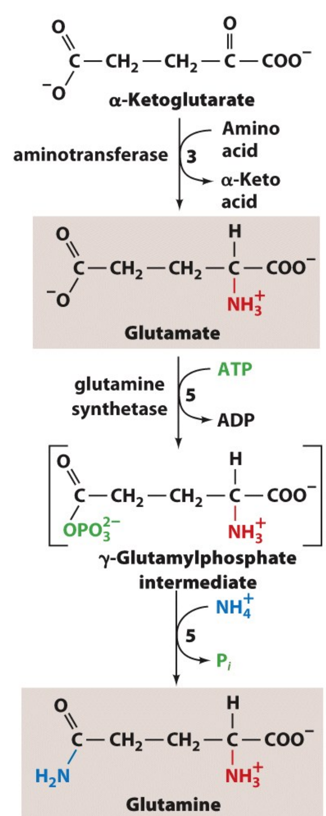


Figure 21-27

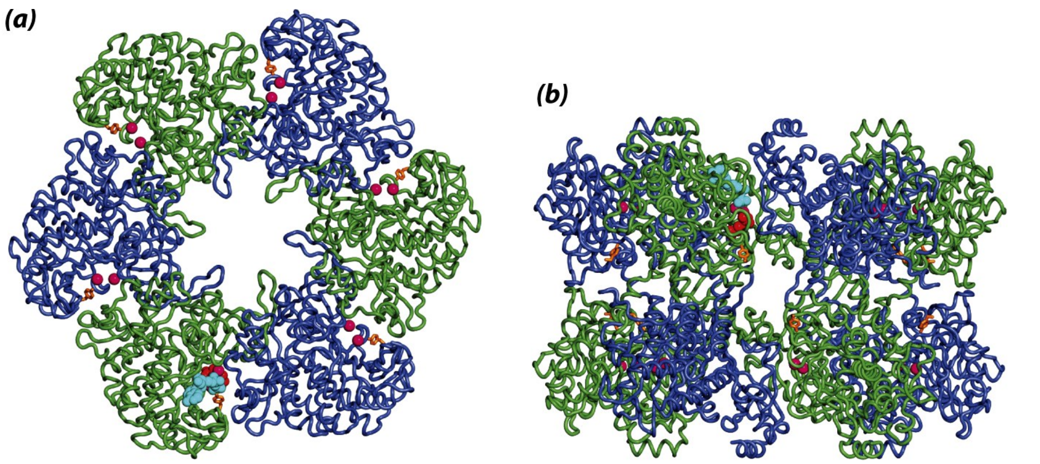
Glutamine Synthetase

- A central control point for nitrogen metabolism
- Temporary ammonia storage
- Activated by α-Ketoglutarate (product of deamination)
- Further regulated by several allosteric regulators
  - Either products of pathways leading from Gln/Glu
  - Ala/Ser/Gly: indicators of N level in cell
- 12-mer enzyme complex



Glutamine Synthetase

- Key features: 12-mer; two Mn^{2+} per interface; Try 397 (adenylylation site);
  - ADP (cyan) and phosphinathricin (red): competitive inhibitors



PDB: 1FPY

Adenylylation

- Cumulative inhibition of glutamine synthetase
- Complex cascade catalyzed by Adenylyltransferase and P_{II}.
- Reversible
- P_{II} in turns regulated by uridylylation (for deadenylylation)
- Uridylyltransferase activated by α-Ketoglutarate and ATP; inhibited by glutamine and Pi.

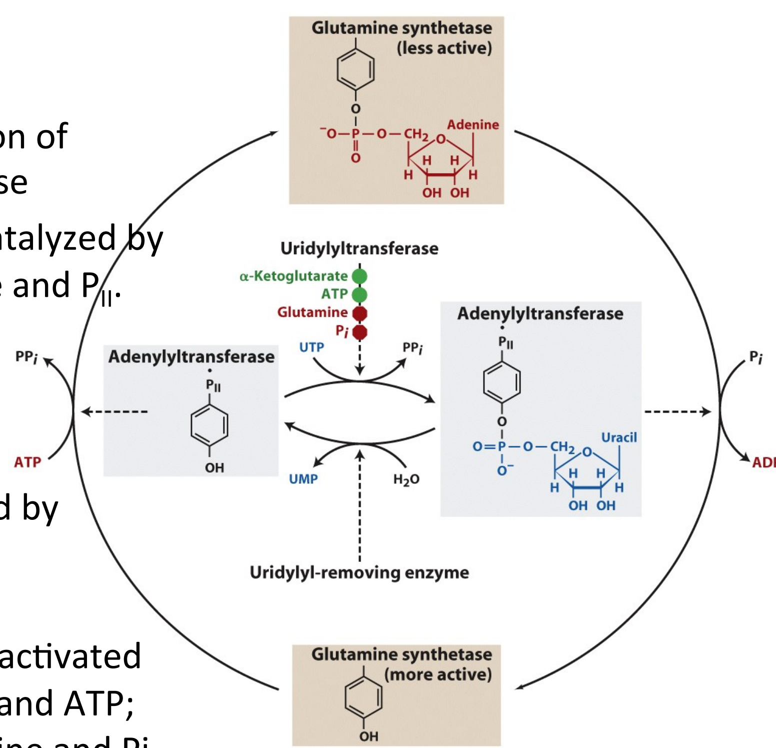


Figure 21-29

2. Biosynthesis of Glutamate Family (Pro, Arg)

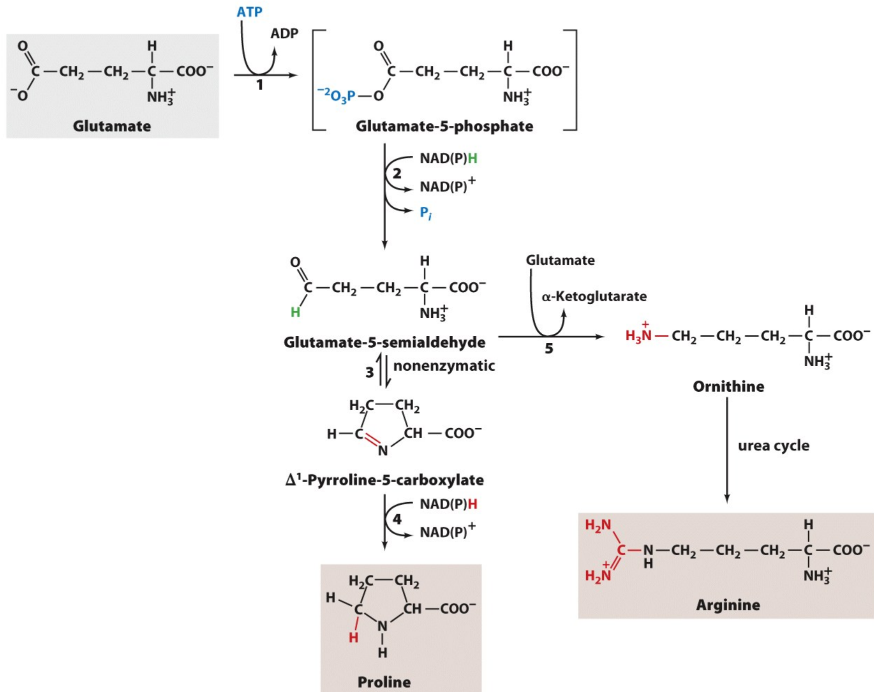
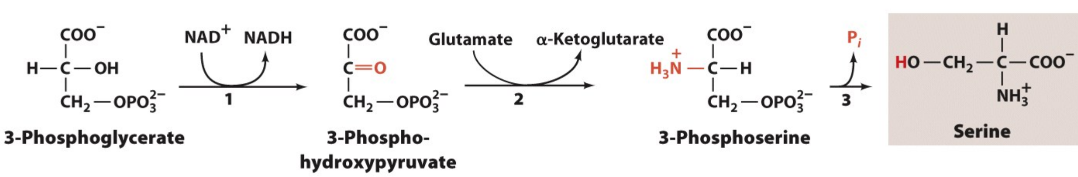
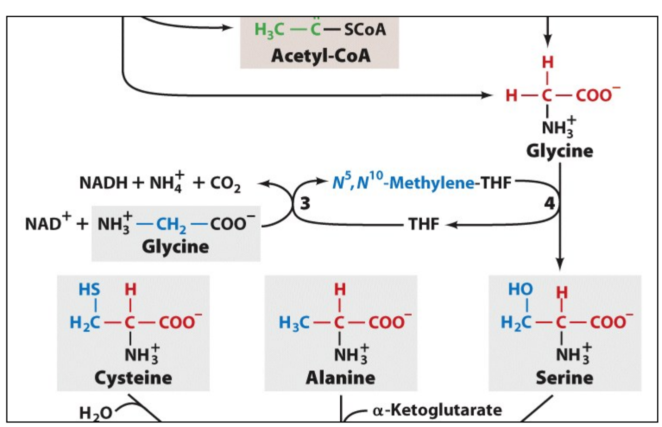


Figure 21-30

3. Ser, Cys & Gly: from 3-Phosphoglycerate



Two routes for serine to glycine:



Serine to cysteine:

Requires methionine (essential AA)

Reactions 5 & 6:

"trans-thiol reaction"

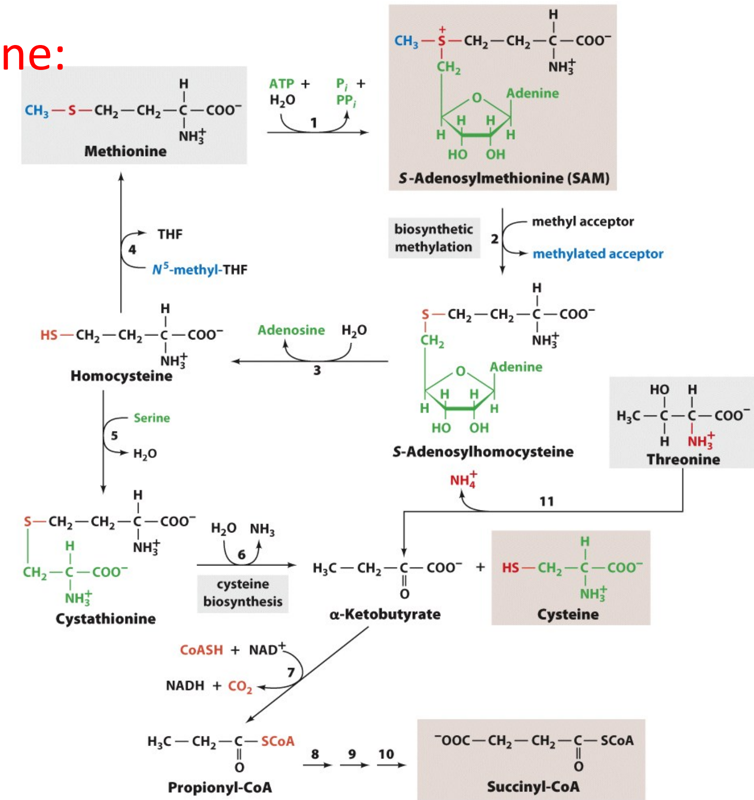


Figure 21-18

Which of the following is an essential amino acids in humans?

A. Alanine

B. Asparagine

C. Aspartate

D. Arginine

Essential Amino Acids Biosynthesis

- In plants and microorganisms
- Typically more steps

TABLE 21-3 Essential and Nonessential Amino Acids in Humans

Essential	Nonessential
Arginine[a]	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

[a] Although mammals synthesize arginine, they cleave most of it to form urea (Section 21-3A).

Table 21-3

1. Aspartate Family: Lys, Met, Thr

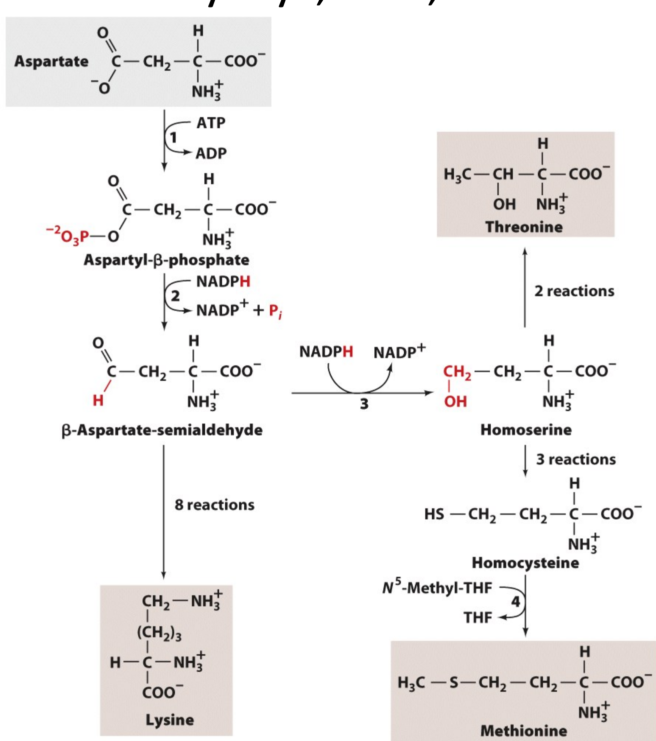


Figure 21-32

2. Pyruvate Family: Leu, ILE, Val

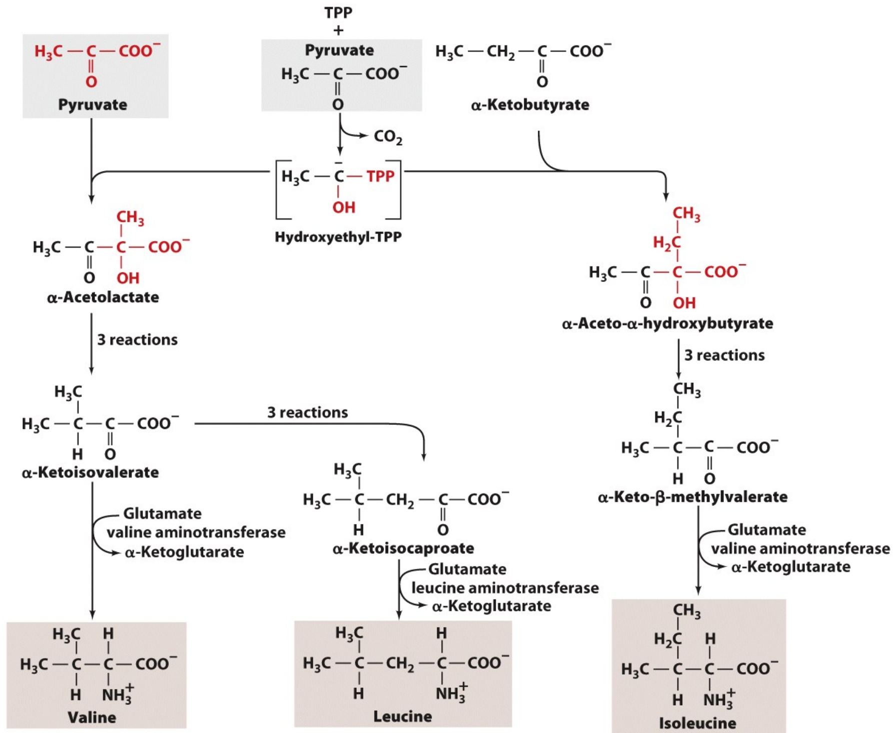


Figure 21-33

3. Phe, Trp, & Tyr: from glucose derivatives

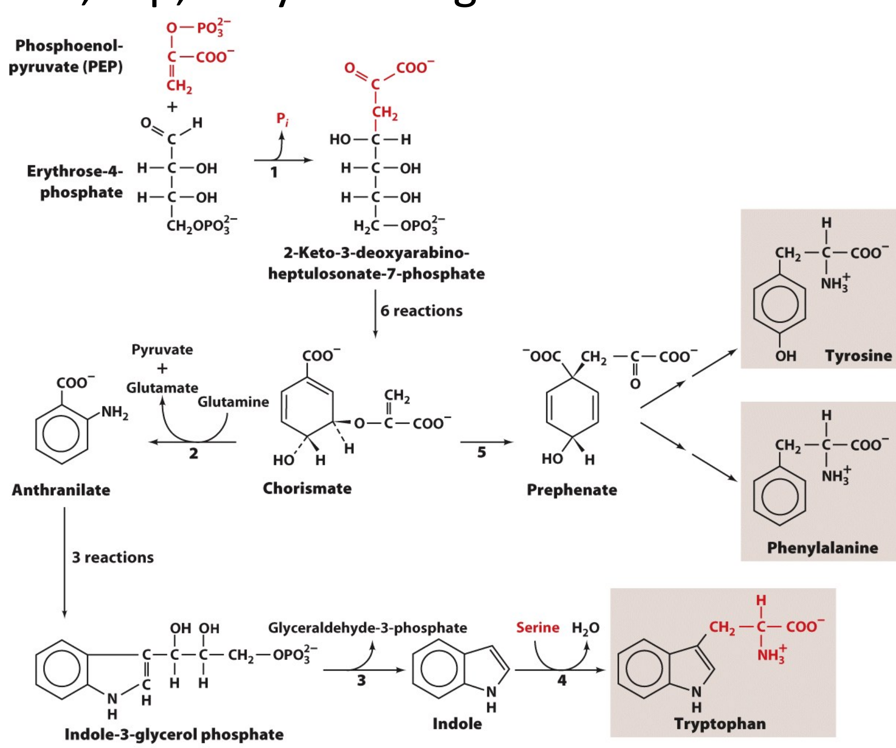


Figure 21-34

Tryptophan Synthase

Solvent filled wide channel

Regulation of entrance/ exit of the channel

Allosteric regulation that senses the chemical reactions

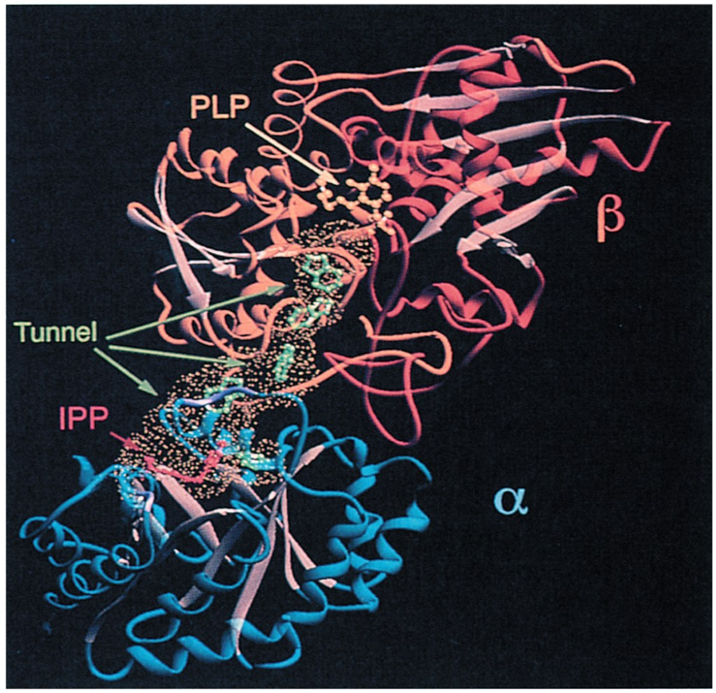


Figure 21-35
Courtesy of Craig Hyde, Edith Miles, and David Davies, NIH

4. His: involves intermediates form purine synthesis (evidence of an RNA world?)

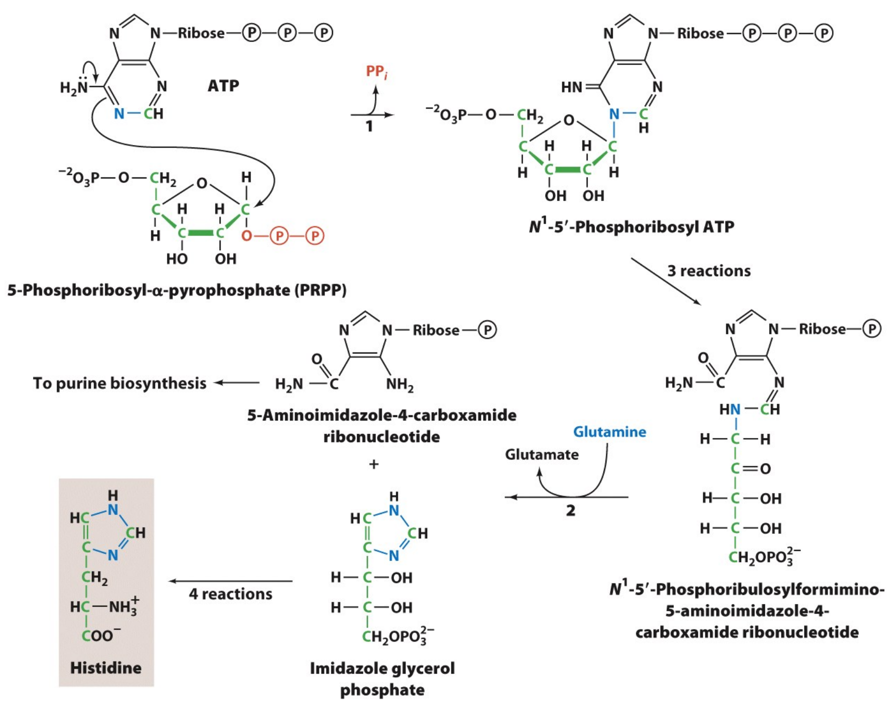


Figure 21-36

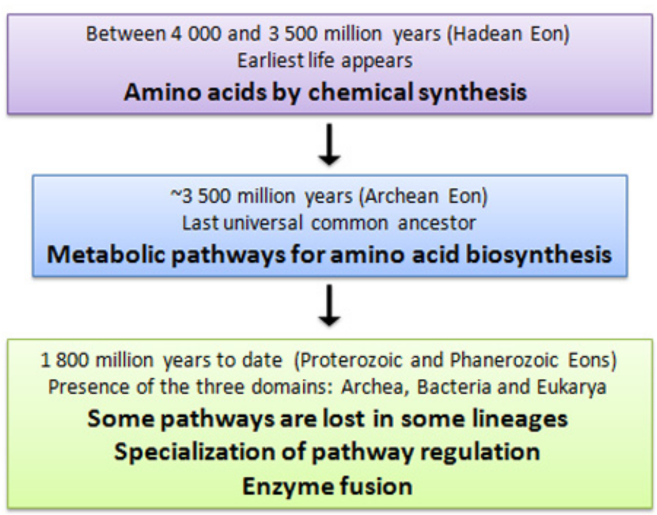
Summary

- What are the metabolic precursors of the nonessential amino acids (4 of them)?
- Summarize the types of reactions required to synthesize the nonessential amino acids.
- List the compounds that are used to synthesize the essential amino acids in plants and microorganisms.
- Compare the catabolic and anabolic pathways for one or more of the amino acids. In which pathways do oxidation reactions or reduction reactions predominate?

Optional Reading Assignment

- **An Evolutionary Perspective on Amino Acids**

http://www.nature.com/scitable/topicpage/an-evolutionary-perspective-on-amino-acids-14568445



Chapter 21-6

OTHER PRODUCTS OF AMINO ACID METABOLISM

Key Concepts 21.5

• Heme is synthesized from glycine and succinyl-CoA and is degraded to a variety of colored compounds for excretion.

• The synthesis of bioactive amines begins with amino acid decarboxylation.

• Arginine gives rise to the hormonally active gas nitric oxide.

1. HEME: from glycine and Succinyl-CoA

- All C/N from Gly & acetate
- Mitochondria + cytosol
- Liver and Erythroid cells: ~85% of heme
- Several NH_4 and CO_2 produced
- Acute lead poisoning:
  - Inhibition of PBG synthetase
  - Accumulation of ALA in blood
- ALA synthetase: key regulation point

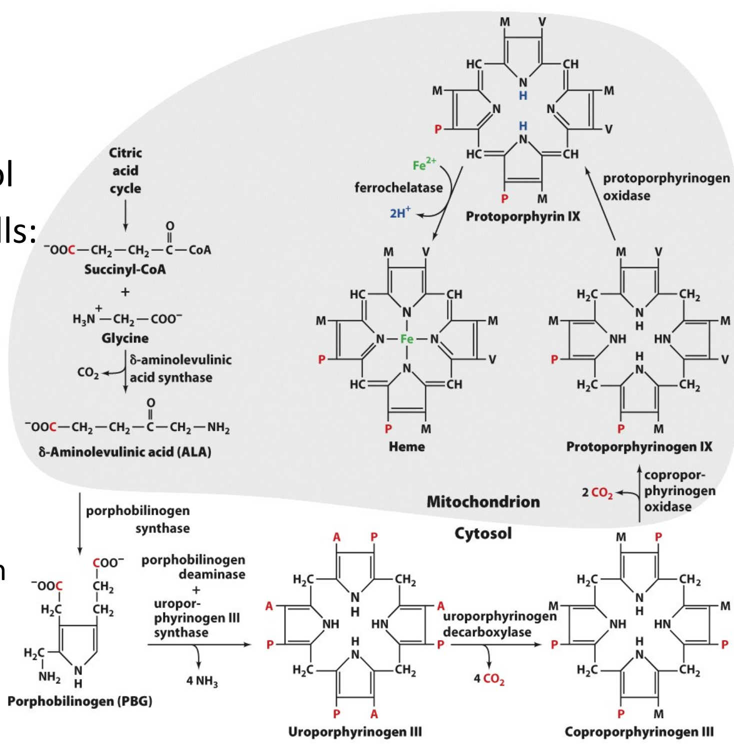


Figure 21-37

Heme Degradation

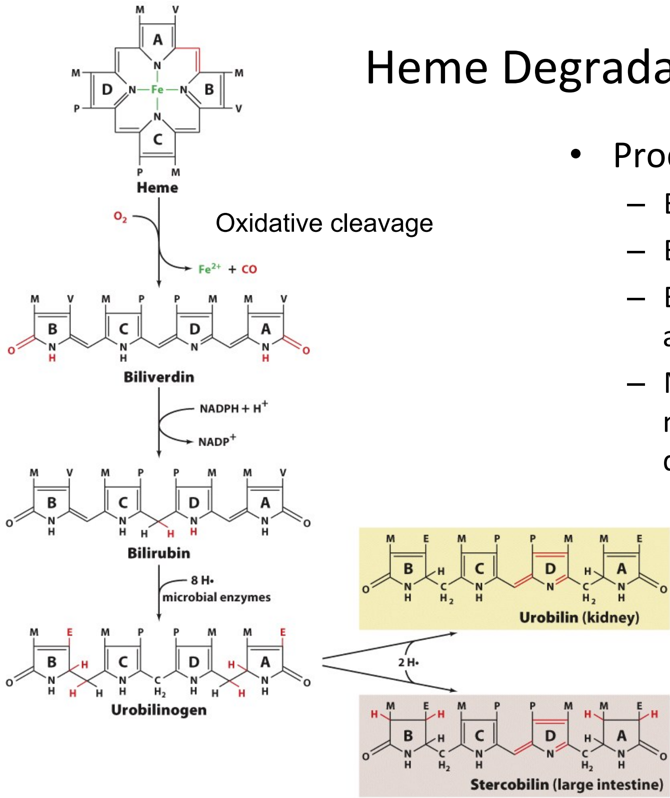
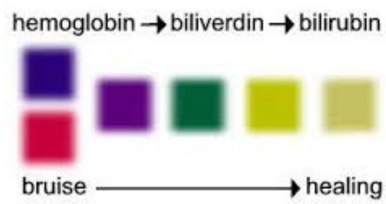


Figure 21-38

- Products must be excreted!
  - Biliverdin: green
  - Bilirubin: red-orange
  - Bilirubin: require microbe assistance!
  - Most urobilinogen is microbially converted to deeply red-brown stercobilin

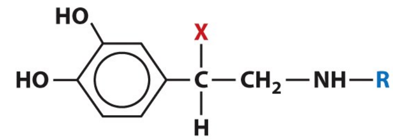


(Infant) Jaundice

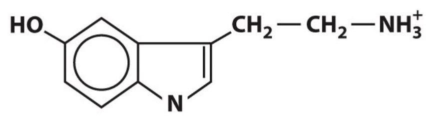
- Accumulation of bilirubin: under skin & whites of the eyes
- Liver dysfunction, bile duct obstruction: excessive red cell destruction
- Infant: lack an enzyme that breaks down bilirubin
  - Fluorescent bath: photochemically conversion to soluble isomers
  - Excessive high juandice could damage brain once breaching BBB

2. Physiologically active amines

- Hormones & neurotransmitters
- Adrenaline: "fight and flight"
- Dopamine: Parkinson's disease
  - Shaking palsy: loss of motor skills
  - >50 years old
  - Dopamine precursor supplement (L-DOPA)
- Histamine
  - Allergic responses
  - Anti-histamine drugs



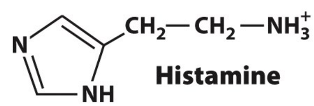
X = OH, R = CH_3 **Epinephrine (adrenalin)**
X = OH, R = H **Norepinephrine**
X = H, R = H **Dopamine**



Serotonin (5-hydroxytryptamine)

$^{-}OOC-CH_2-CH_2-CH_2-NH_3^+$

γ-Aminobutyric acid (GABA)

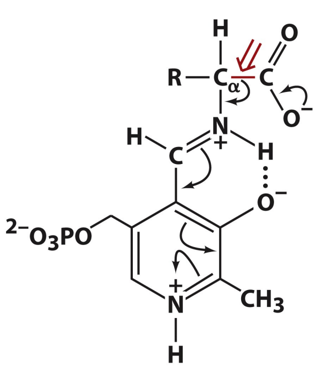


Histamine

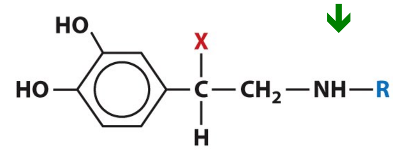
(c) Jianhan Chen

PLP-dependent decarboxylation

- Biosynthesis of active amines involves decarboxylation of corresponding precursor amino acids
- PLP as a co-enzyme
  - Stabilize the intermdiate carbanion upon CA-COO- cleavage

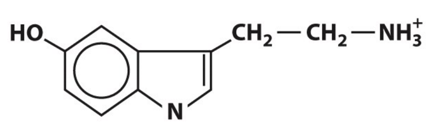


Tyr ➔



X = OH, R = CH_3 **Epinephrine (adrenalin)**
X = OH, R = H **Norepinephrine**
X = H, R = H **Dopamine**

Trp ➔

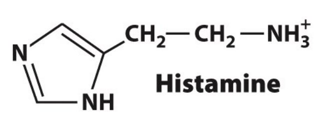


Serotonin (5-hydroxytryptamine)

Glu ➔ $^{-}OOC-CH_2-CH_2-CH_2-NH_3^+$

γ-Aminobutyric acid (GABA)

His ➔



Histamine

(c) Jianhan Chen

Sequential Synthesis of l-DOPA, Dopamine, Norepinephrine, & Epinephrine

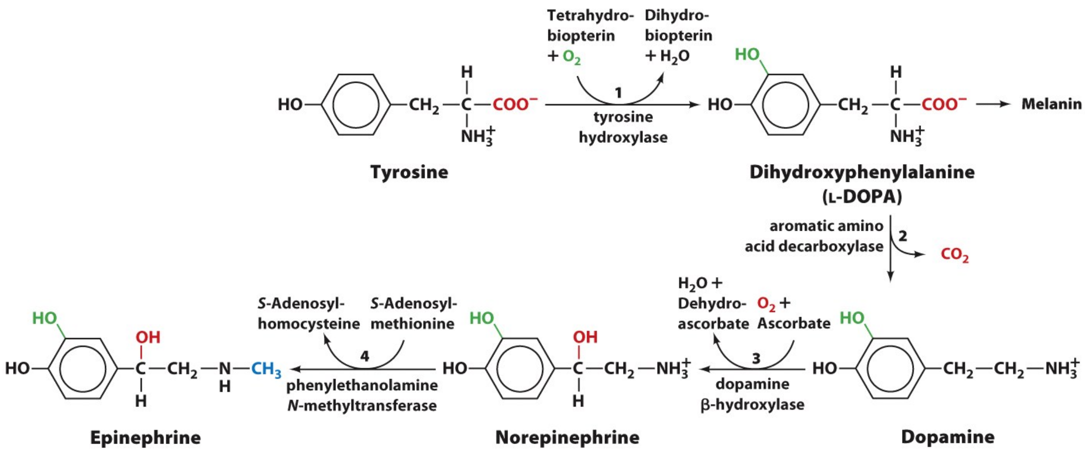
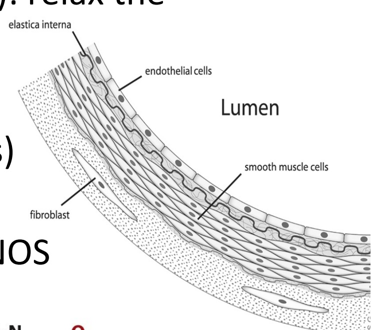
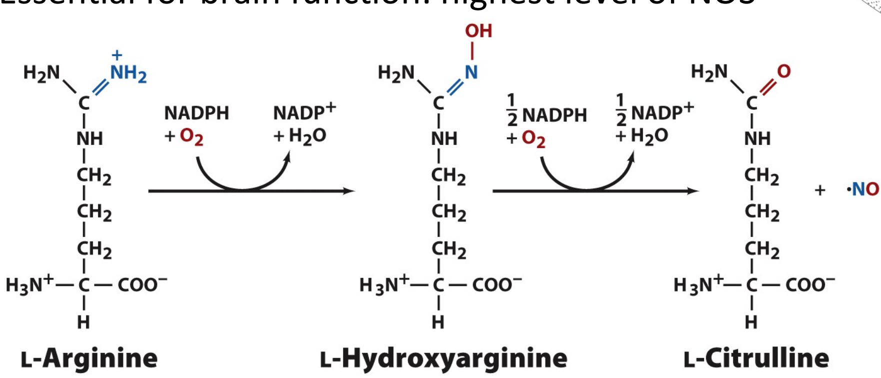


Figure 21-39

3. Nitric Oxide: derived from Arg

- Endothelium-derived relaxation factor (EDRF): relax the smooth muscle via stable nitric oxide (NO)
- Nitric oxide synthetase (NOS)
- NO has half-life ~5s: thus local (<1mm effects)
  - Made locally by endothelial cells
- Essential for brain function: highest level of NOS





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5 electron oxidization

Nitric oxide synthetase

- Homodimer
- Require several co-enzymes: FMN + FAD + BH4 + heme

PDB:1NSI

"Laughing Gas"

http://www.nyhq.org/wtn/page.asp?PageID=WTN000228

Quick Summary

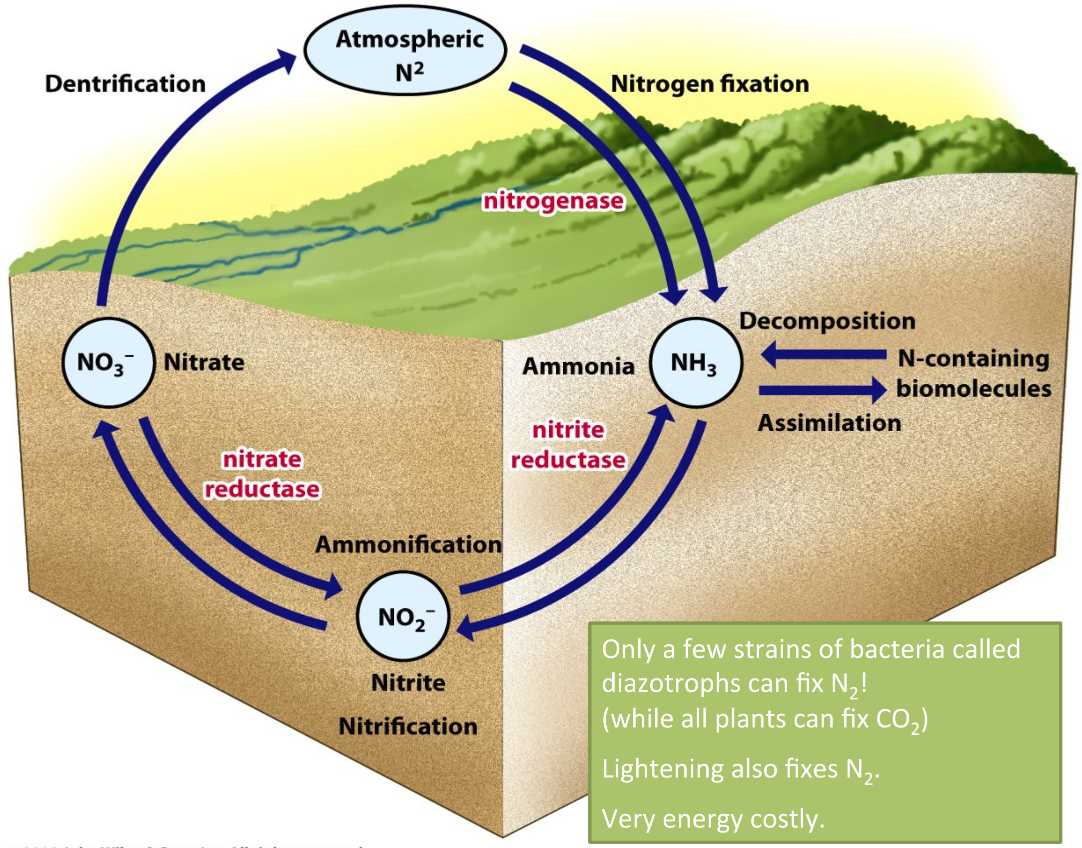
- What are the starting materials for heme biosynthesis?
- What are the end products of heme metabolism?
- Identify the amino acids that give rise to catecholamines, serotonin, GABA, and histamine.
- What are the substrates and products of the nitric oxide synthase reaction?
- How does NO differ from signaling molecules such as the catecholamines?

Chapter 21-7

NITROGEN FIXATION

Key Concepts 21.5

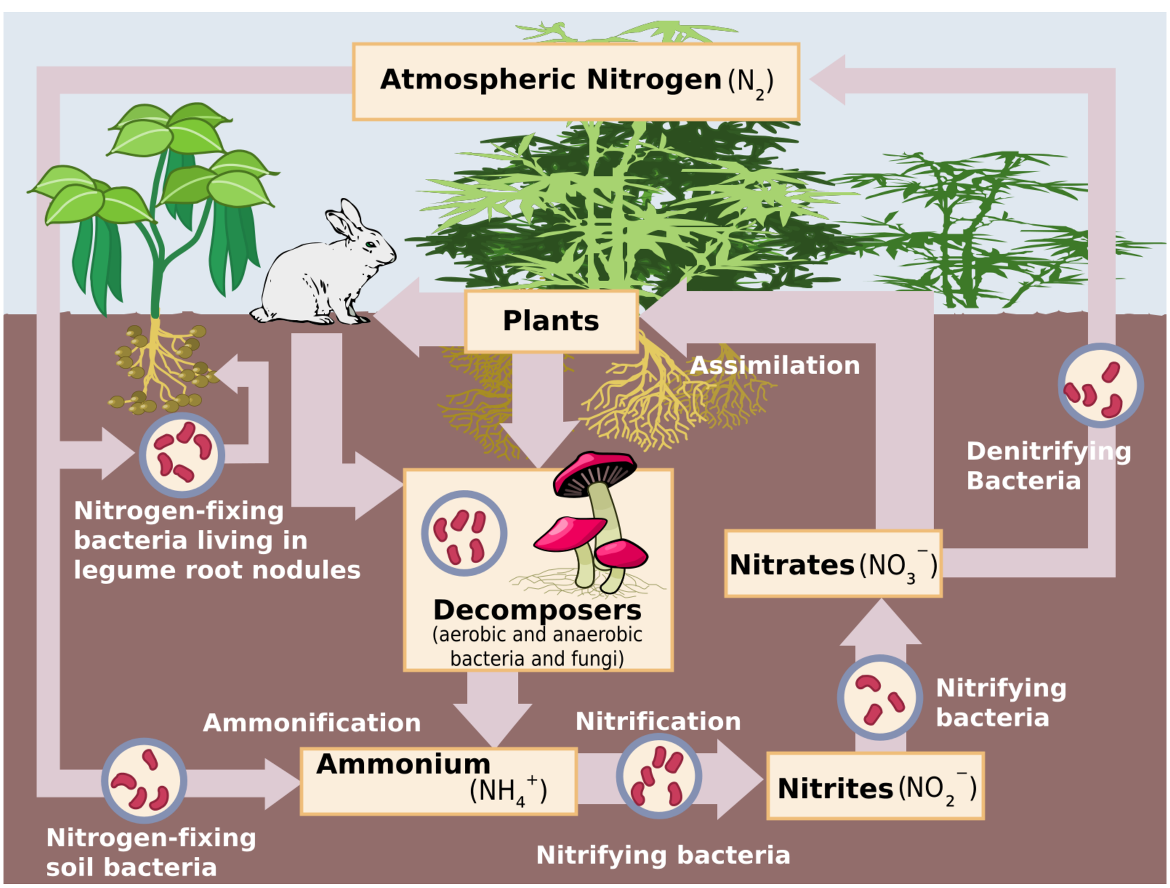
- The reduction of N_2 to NH_3 by nitrogenase is an energetically costly process.
- Ammonia is incorporated into amino acids by the action of glutamate synthase.



Only a few strains of bacteria called diazotrophs can fix N_2!
(while all plants can fix CO_2)

Lightening also fixes N_2.

Very energy costly.



Diazotrophs

- Nitrogen fixation
- Marine cyanobacteria and bacteria in root nodules of legumes (the pea family: beans, clover and alfalfa)
- Nitrogenase

Root nodules of soybean plants

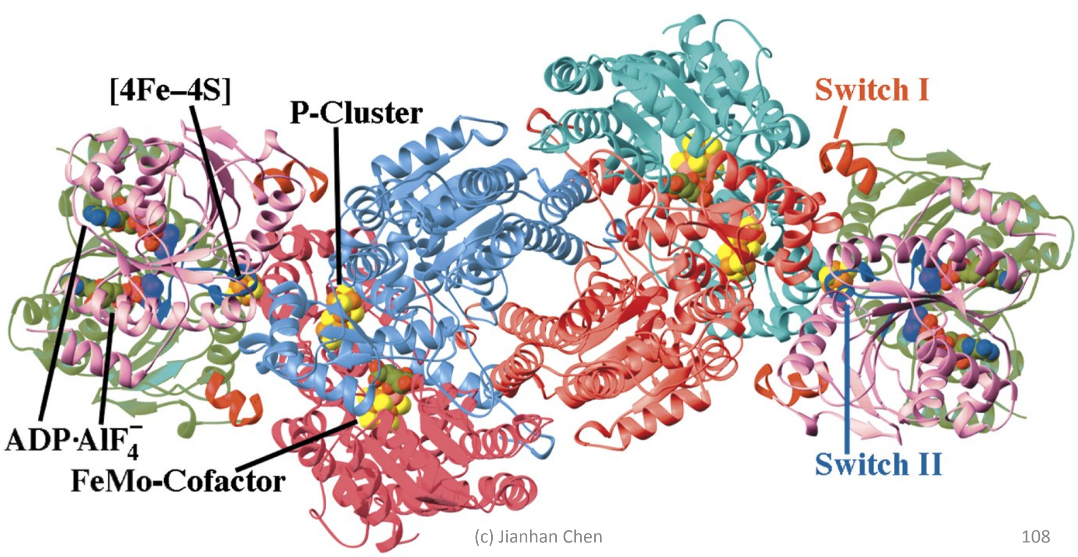
Figure 21-40
David M. Dennis/Peter Arnold, Inc.

Nitrogenase

- Catalyzes the reduction of N_2 to NH_3

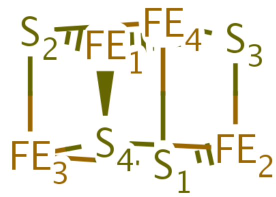
$$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$$

- 2 Fe-protein homodimer and 1 MoFe-protein heterotetramer

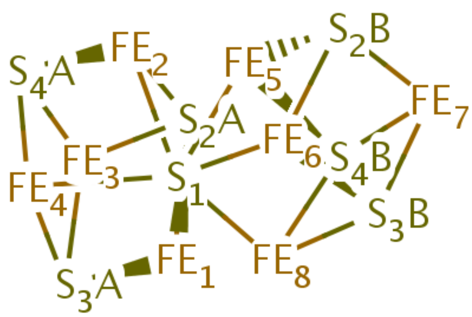


Nitrogenase: redox centers

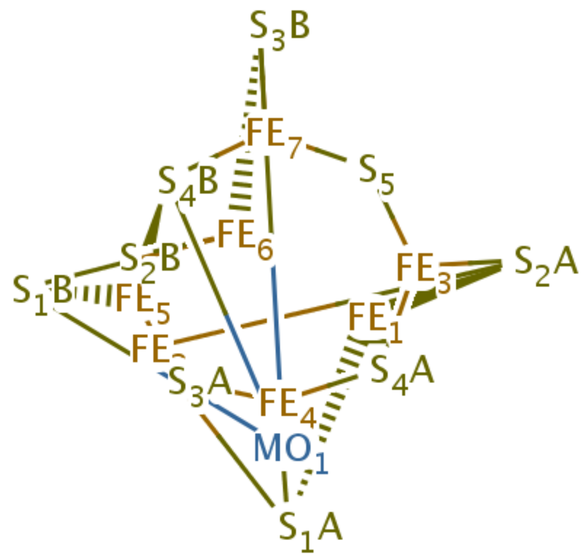
4Fe-4S cluster



P-cluster

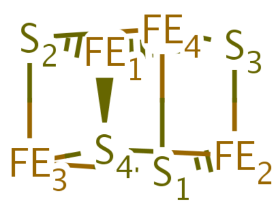


Fe-Mo cofactor:



Nitrogenase: redox centers

4Fe-4S cluster



P-cluster:

Two 4Fe-3S linked by sulfide ion

Coordinated by 7 Cys thiol side chains

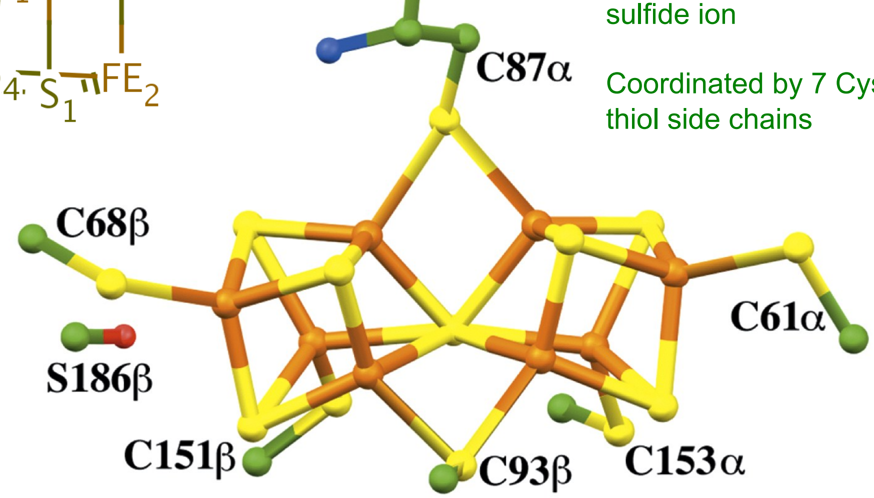
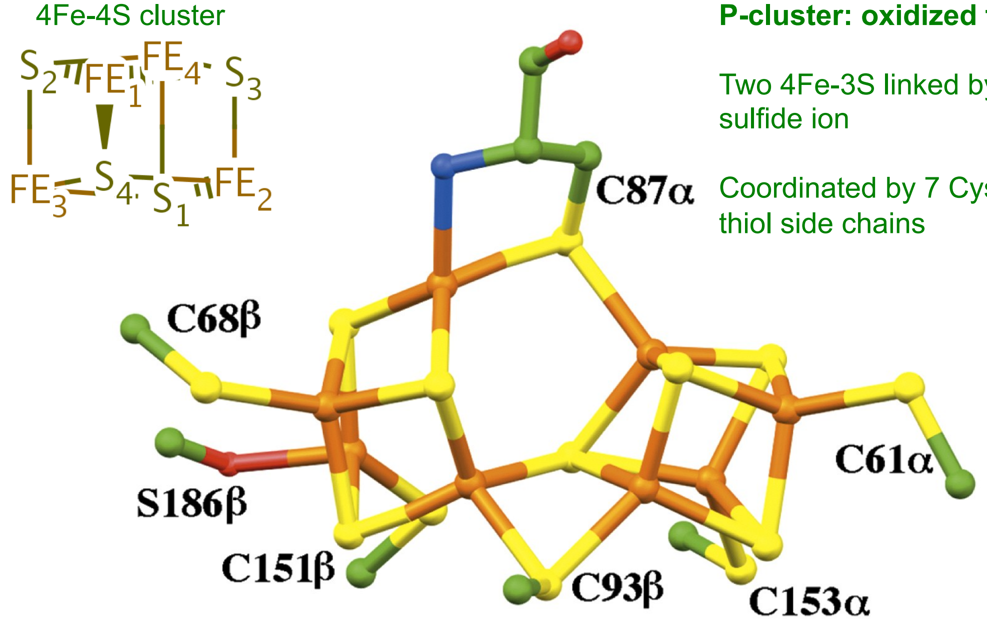


Figure 21-42a

Nitrogenase: redox centers

4Fe-4S cluster



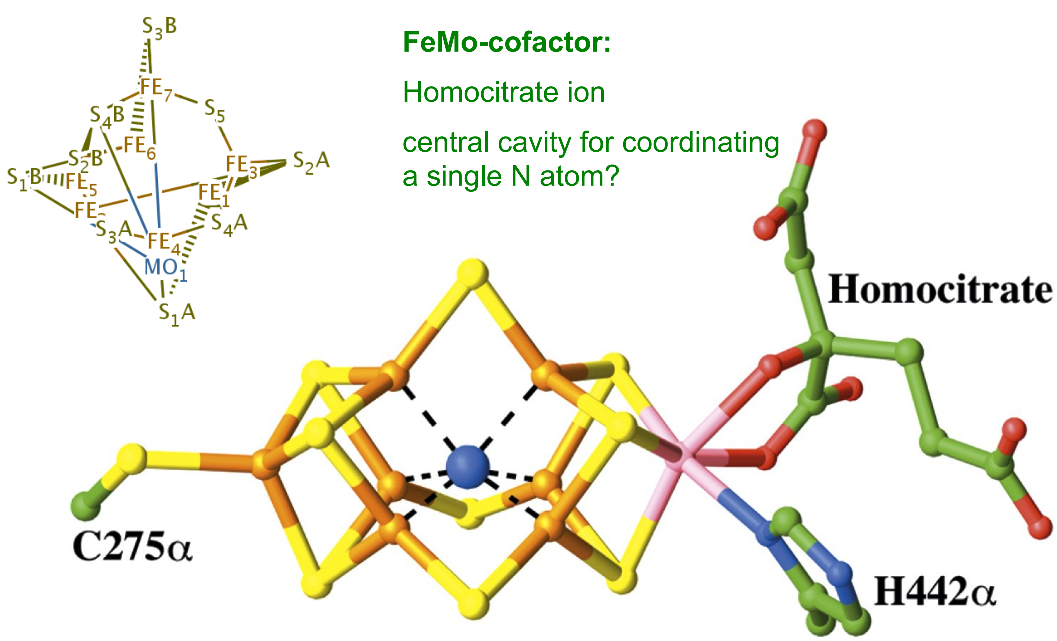
P-cluster: oxidized form

Two 4Fe-3S linked by sulfide ion

Coordinated by 7 Cys thiol side chains

Figure 21-42b

Nitrogenase: redox centers



FeMo-cofactor:

Homocitrate ion

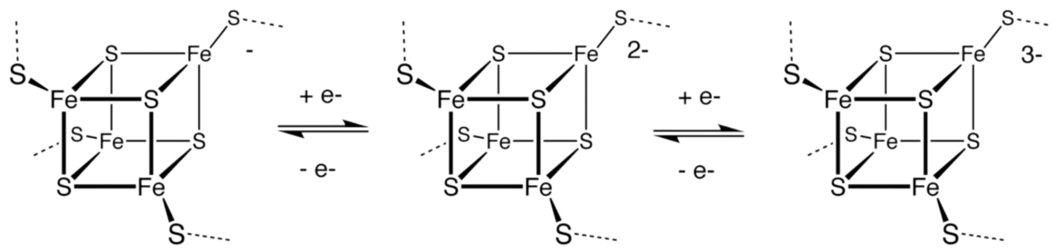
central cavity for coordinating a single N atom?

Figure 21-42c

Nitrogenase: Electron Transfer and Conformational Switches

$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$

- Require electron source: generated either oxidatively or photosynthetically and carried by 4Fe-4S ferrodoxin
- Require ATP hydrolysis driven conformational changes and disassociation of Fe- and MoFe-protein components after each electron transfer!

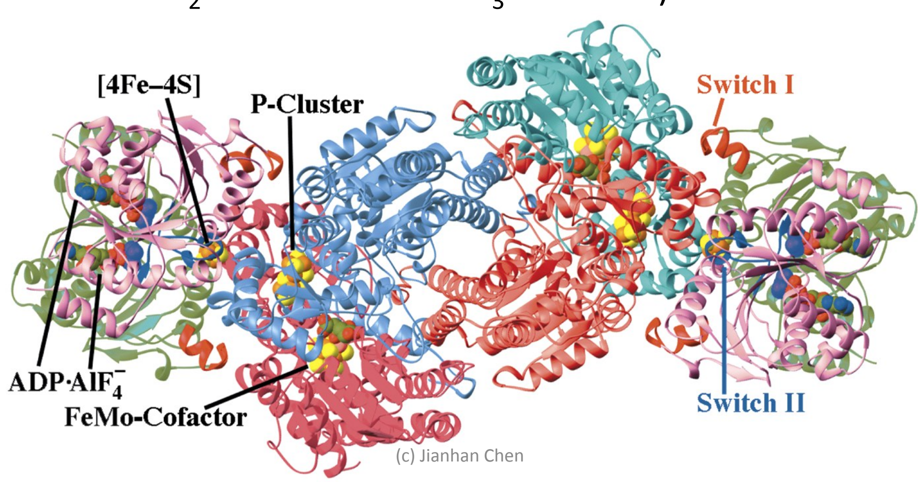


http://en.wikipedia.org/wiki/Ferredoxin

Nitrogenase: Electron Transfer and Conformational Switches

$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$

- Each electro transfer cycle involves two ATP hydrolysis, which induced conformational switches in Fe-protein to reduce its redox potential from -0.29 to -0.40 V (sufficient to drive N2 reduction: $N_2 + 6\ H^+ + 6e^- \rightarrow NH_3$ is -0.34 V)



Nitrogenase: Electron Transfer and Conformational Switches

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- Switch I: drive disassociation from MoFe-protein complex (rate limiting)
- Swtich II: modify 4Fe-4S environment (and thus redox potential); also bring 4Fe-4S closer to P-cluster (to facilitate electron transfer)

- ATP hydrolysis occur only when Fe-protein is in complex MoFe-protein: allow coupling of ATP hydrolysis with electron transfer (to MoFe-cofactor

Nitrogenase: Electron Transfer and Conformational Switches

$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$

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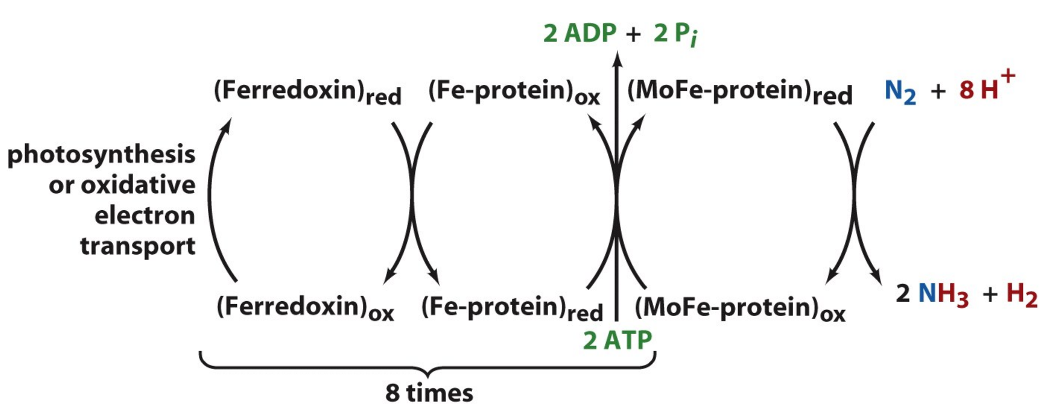
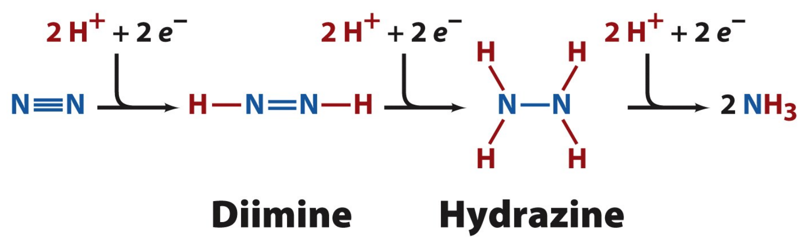


Figure 21-43

Nitrogenase: N_2 Reduction Reactions

$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$

- Energetically very costly
- 6 electrons for reducing N_2 + 2 electrons for reducing H_2O
- Diimine can react with H_2 and produces N_2!
- Actual cost: 20-30 ATP per N_2



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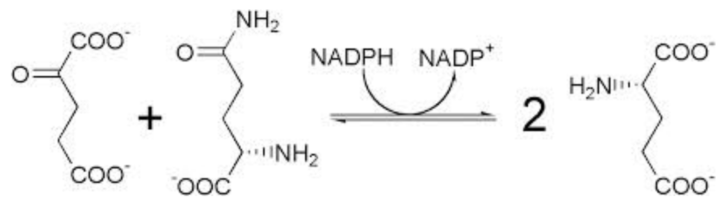
Moonlighting of Nitrogenase

- HC≡CH → H2C=CH2
- N−=N+=O → N2 + H2O
- N=N=N− → N2 + NH3
- C≡N− → CH4, NH3, H3C–CH3, H2C=CH2 (CH3NH2)
- N≡C–R → RCH3 + NH3
- C≡N–R → CH4, H3C–CH3, H2C=CH2, C3H8, C3H6, RNH2
- O=C=S → CO + H2S [8][9]
- O=C=O → CO + H2O [8]
- S=C=N− → H2S + HCN [9]
- O=C=N− → H2O + HCN, CO + NH3 [9]

http://en.wikipedia.org/wiki/Nitrogenase

Assimilation

- Critical process of incorporating fixed nitrogen into biomolecules
- Glutamine synthetase
- Glutamate synthase: bacteria and plants



- Net reaction (combining glutamine synthetase and glutamate synthase):

α-Ketoglutarate + NH_4^+ + NADPH + ATP → glutamate + $NADP^+$ + ADP + Pi

Glutamate Synthase Reaction

- Channeling!

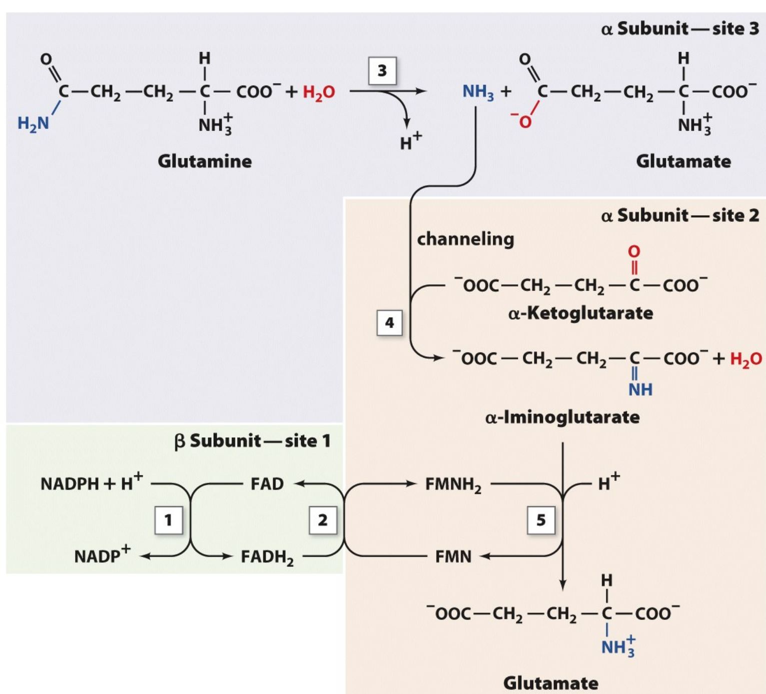
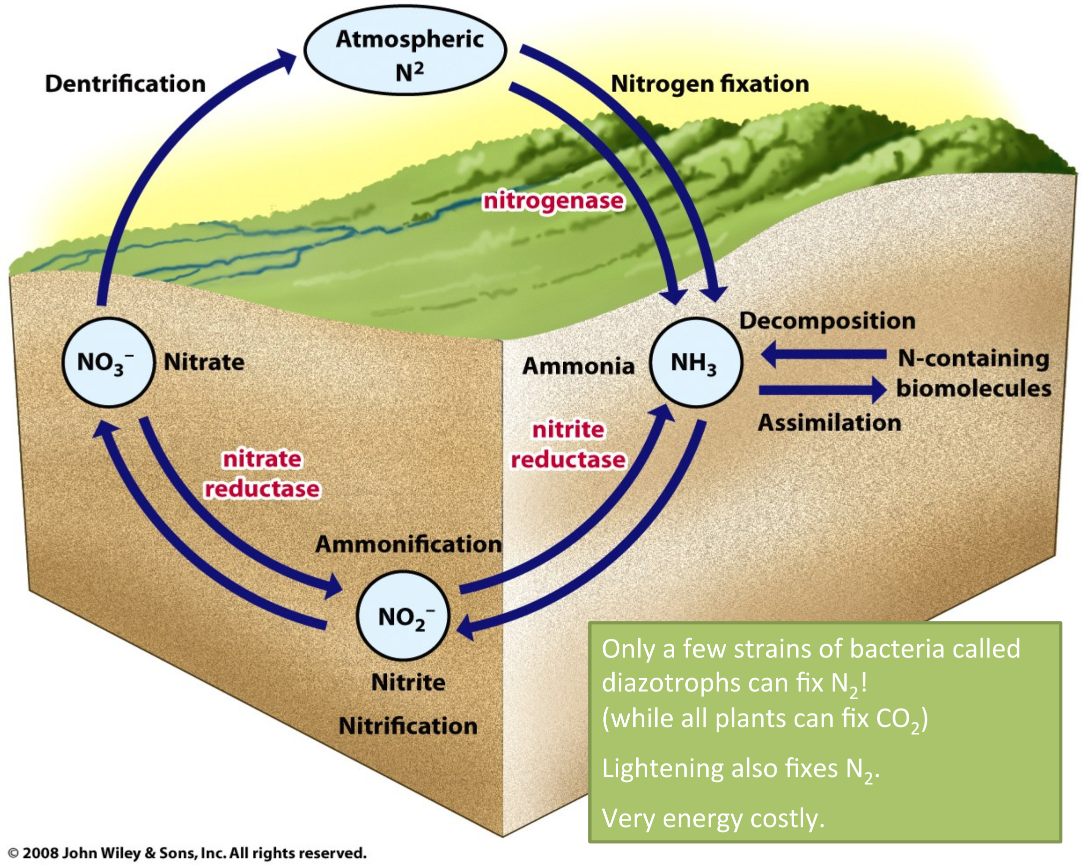


Figure 21-44



Only a few strains of bacteria called diazotrophs can fix N_2!
(while all plants can fix CO_2)

Lightening also fixes N_2.

Very energy costly.

Quick Summary

- Summarize the mechanistic role of ATP in nitrogen fixation.
- Why is nitrogen fixation so energetically costly?
- Describe the reactions that assimilate NH3 into amino acids. Which reactions occur in mammals?
- List the enzymes of amino acid metabolism in which channeling occurs (three so far).
- How is fixed nitrogen recycled in the biosphere?

The combined effect of glutamine synthetase and glutamate synthase is to incorporate fixed nitrogen into an organic compound and to produce an amino acid.

A. True
B. False

Which of the following enzymes is not part of the nitrogen cycle?

A. Nitrogenase
B. Nitrate oxidase
C. Nitrite reductase
D. Nitrate reductase