Chapter 8-I:
Amino Acid Metabolism

Chapter 20 Opener Fundamentals of Biochemistry, 2/e

Protein degradation

Constant turning over of proteins

(1) *E* storage: muscle

(2) Elimination of abnormal proteins

(3) Regulation of cellular metabolism

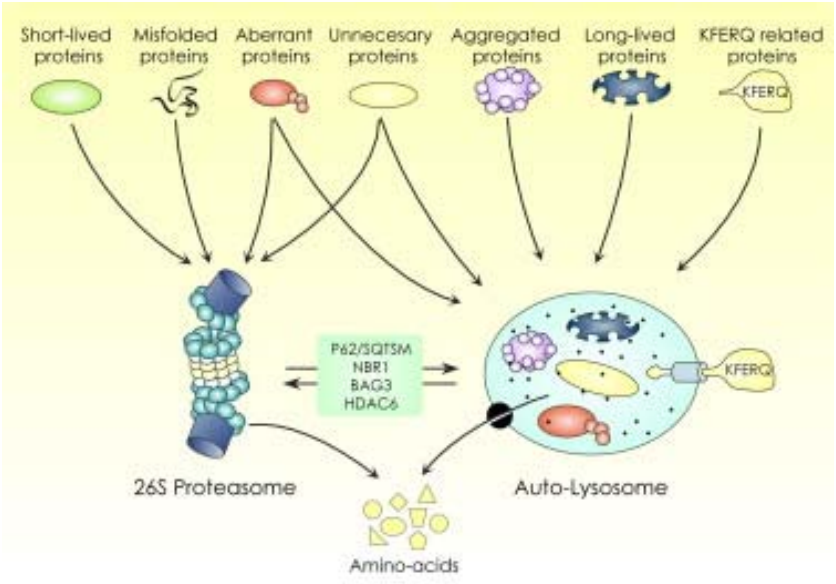
Regulatory role →

Constant catalytic activity →

Table 20-1 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 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).



Catabolic pathways for proteins.The ubiquitin-proteasome system (UPS) substrates include short-lived, misfolded, aberrant and superfluous or unnecessary proteins; whereas substrates for the autophagy-lysosomal system (ALS) include superfluous, aberrant, aggregated and long-lived proteins, as well as a subset of proteins containing a lysosomal-targeting KFERQ motif. Although the UPS and autophagy have long been considered as independent systems, increasing evidence suggests that they interact at several points, for instance at the level of the proteins p62/SQTSM, NBR1, BAG3 and HDAC6.

Cell Calcium Volume 47, Issue 2, February 2010, Pages 112–121

Lysosomal degradation

Lysosomes: ~50 hydrolytic enzymes
Proteases (cathepsins)

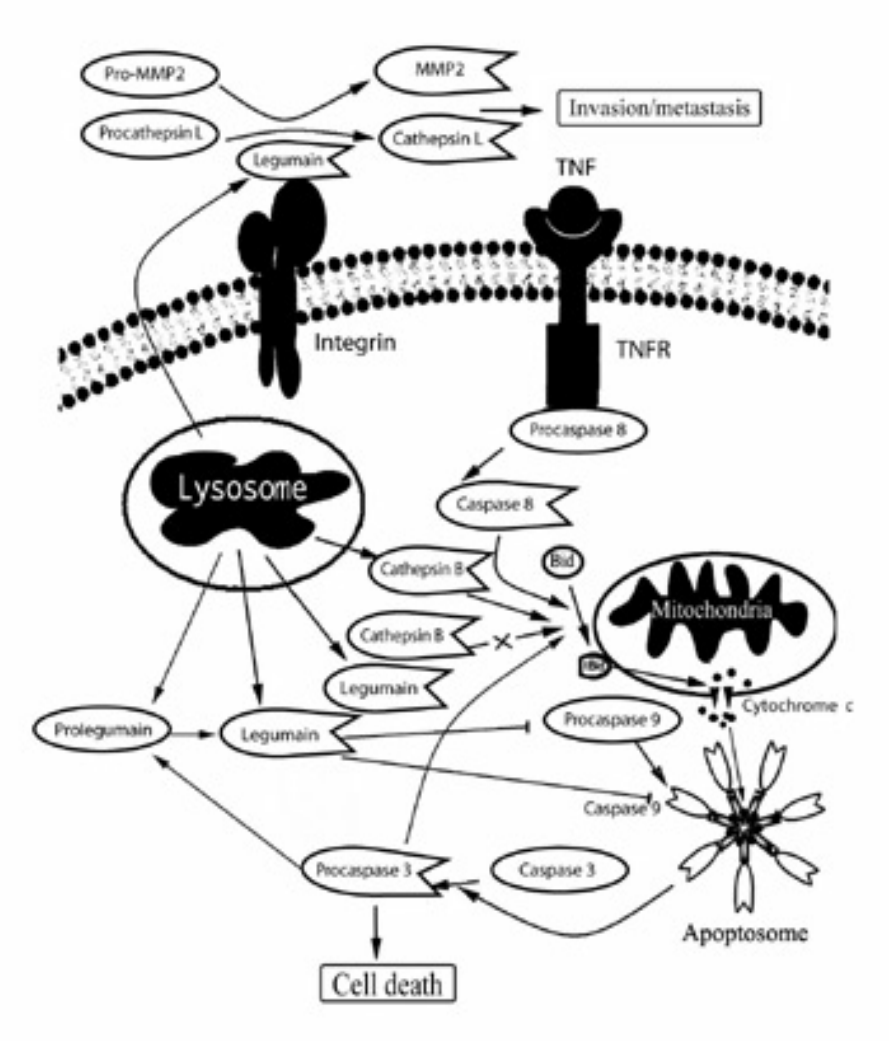
Cathepsins are usually characterised as members of the lysosomal cysteine protease family. In actuality, the cathepsin family also contains members of the serine protease (cathepsin A,G) and aspartic protease (cathepsin D,E) families as well.

Elevated cathepsin enzyme activity in serum or the extracellular matrix often signifies a number of gross pathological conditions.

Selective degradation of cytosolic proteins

KFERQ proteins: under fasting conditions

The Cysteine Protease Network in Tumor Progression and Therapy



Legumain (a cysteine protease) promotes tumor cell invasion and metastasis by binding to cell-surface integrins and activates both matrix metalloproteinase 2 (MMP2) and cathepsin L. It also protects cells from programmed cell death by catalytically inactivating caspase 9. It prevents Bid activation by cathepsin B by binding to and modulating the activity of the cathepsin.

Ubiquitin: highly conserved 76 a.a. proteins

Ubiquitin involving protein breakdown

- ATP-requiring
- Independent of lysosomes

Proteins are marked for degradation

E1: ubiquitin-activating enzymes

E2: ubiquitin-conjugating enzymes

- 11 in yeast, >20 in mammals

E3: ubiquitin-protein ligase

- Many species of E3 specific to a set of proteins
- 2 families containing HECT domain or RING finger
- Each E3 is served by one or a few specific E2s
- Really Interesting New Gene (RING)
- Homologous to the E6-AP Carboxyl Terminus (HECT)

Human ubiquitin (76 a.a) (96% sequence identity with yeast protein)
MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQD**K**EGIPPDQQRLIFA
G**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRLRGG

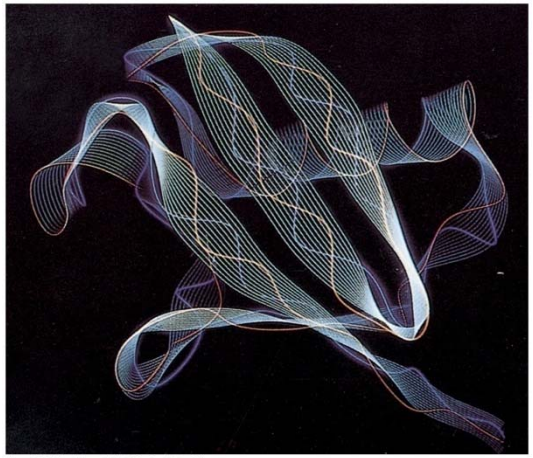


Figure 20-1 Fundamentals of Biochemistry, 2/e

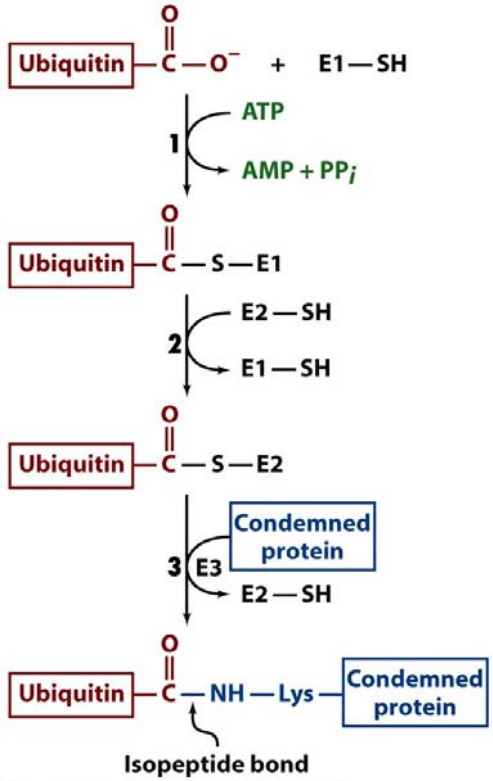
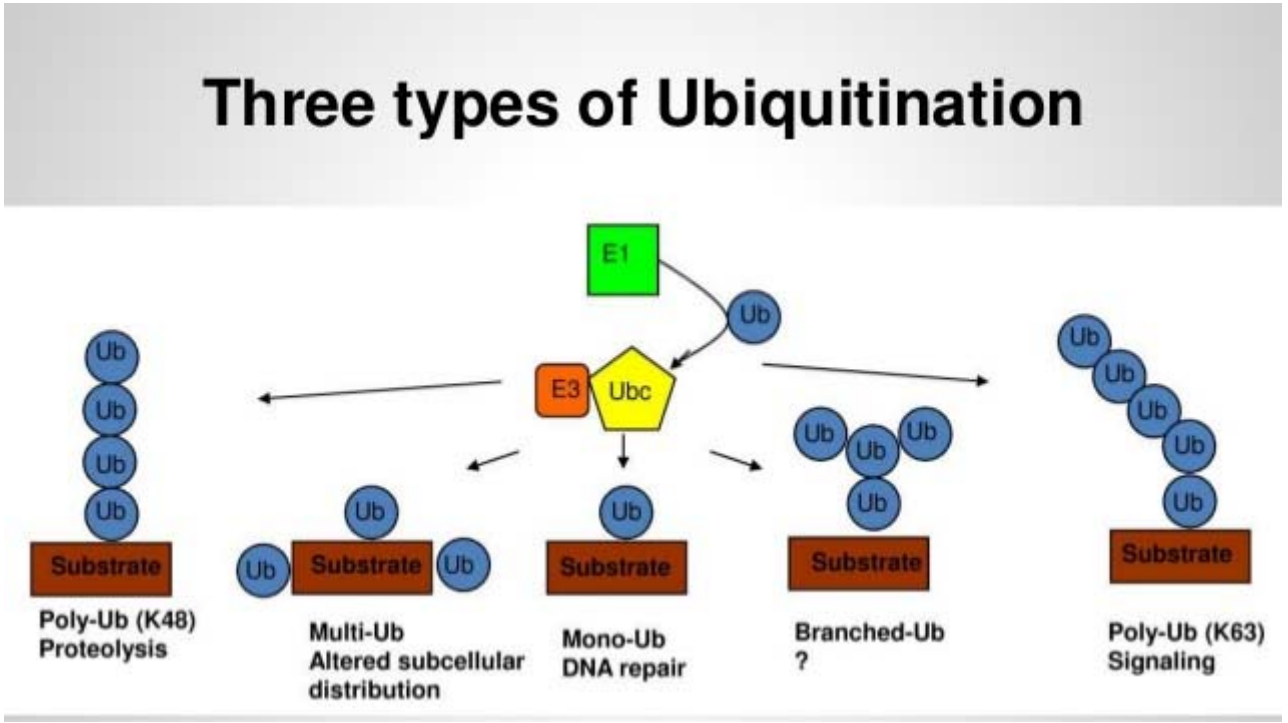


Figure 20-2 Fundamentals of Biochemistry, 2/e

Polyubiquitin

At least 4 (50 or more)

Isopeptide link: Lys 48 with C-terminal carboxyl group



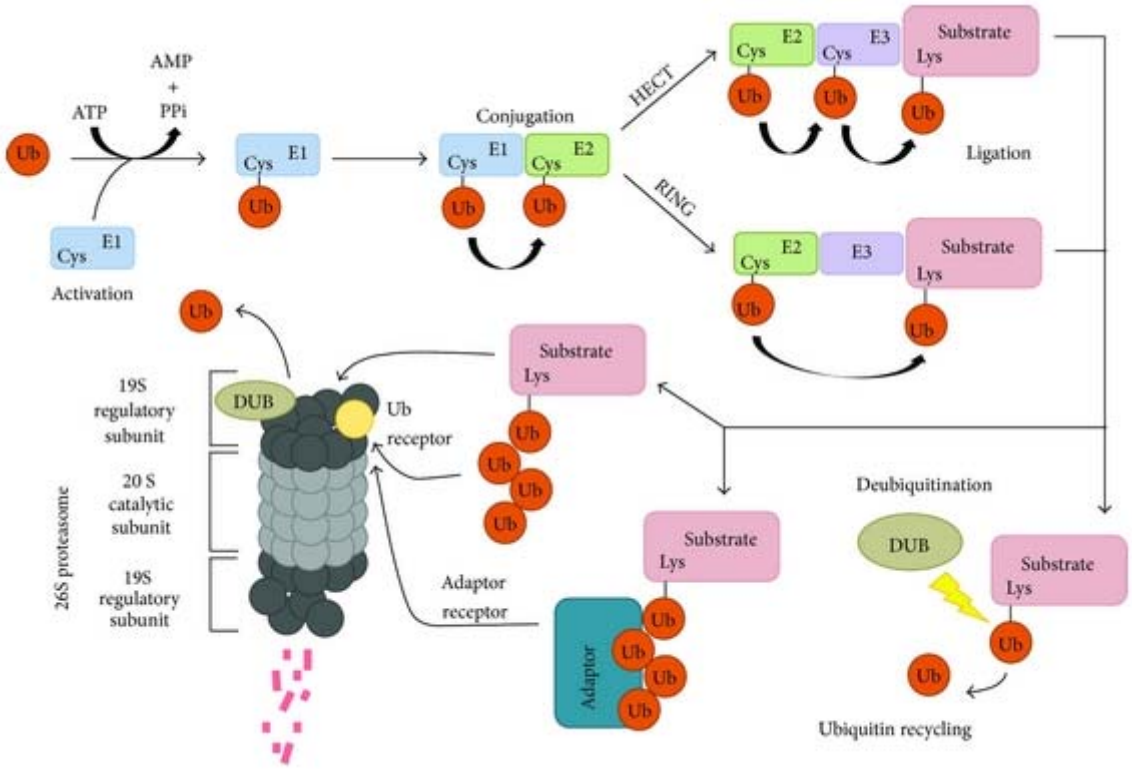


Figure 1: Enzymatic cascade leading to substrate ubiquitination. Three sets of enzymes are required for ubiquitination of a targeted substrate: ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligase (E3) enzymes. There are two main classes of E3 enzymes, the RING and HECT classes, which differ in the manner by which they transfer Ub to a target substrate. Once a Ub molecule is conjugated to its target protein, additional Ub molecules can be attached to form chains (see Figure 2 for a more detailed illustration of Ub binding). However, since ubiquitination is a reversible process, once Ub is attached, deubiquitinating enzymes (DUBs) can then hydrolyze the isopeptide bond between Ub and its target protein (shown by the small lightning bolt) and thus return the protein to its previous state and release Ub. Substrates that contain polyUb chains are often targeted to the proteasome, where they are bound and subsequently degraded. The proteasome is composed of a catalytic 20S core particle structure and two 19S regulatory caps which together are collectively termed the 26S proteasome. While some polyubiquitinated proteins can be bound directly through polyUb binding subunits on the proteasome, others must be shuttled to the proteasome via adaptor proteins (the binding site for Ub and adaptors is represented by a yellow circle). Once the substrate is bound to the proteasome, many ATPase subunits that make up the proteasome utilize ATP to unfold the protein, simultaneously deubiquitinating the protein and releasing Ub while cleaving the protein into small peptide fragments.

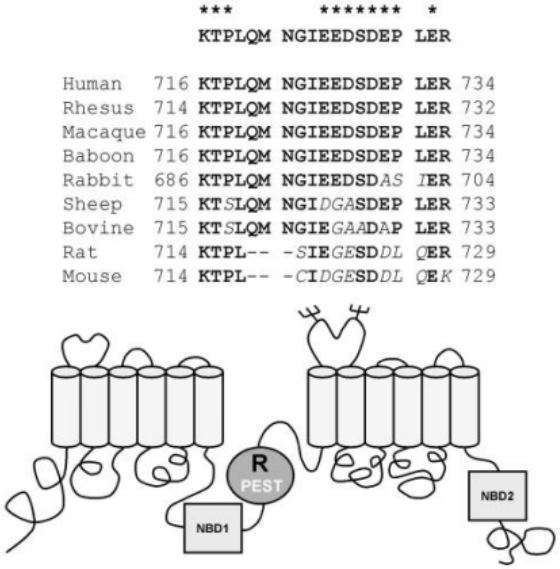
Ubiquitin system has both housekeeping and regulatory functions

The N-end rule

- Half-lives of many proteins depend on their N-terminal residues
- Conserved in both prokaryotes and eukaryotes
- Destabilizing residues: D R L K F, half-lives of 2~3 min
- Stabilizing residues: A G M S T V, half-lives of >10 hrs (in pro) or >20 (in Eu)

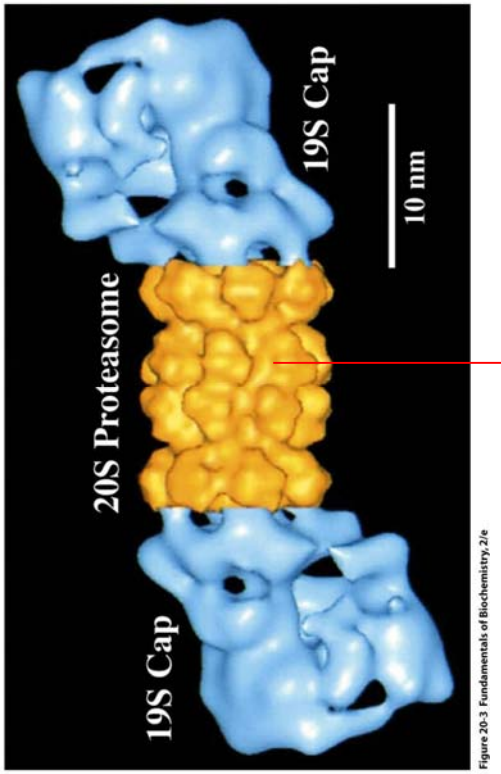
Destabilizing signal in eukaryotes

- Ubiquitination action of E3α (Ring finger E3)
- Variety of ubiquitination signal by more E3s
- PEST proteins are rapidly degraded



The proteasome
Degradation of ubiquinated proteins
Multiprotein complex: ~2100 kD (26S proteasome)
7 different types of α-like and β-like subunits

EM-image of 26S proteasome



X-ray structure of 20S proteasome
C2 & pseudo-sevenfold rotational symmetry

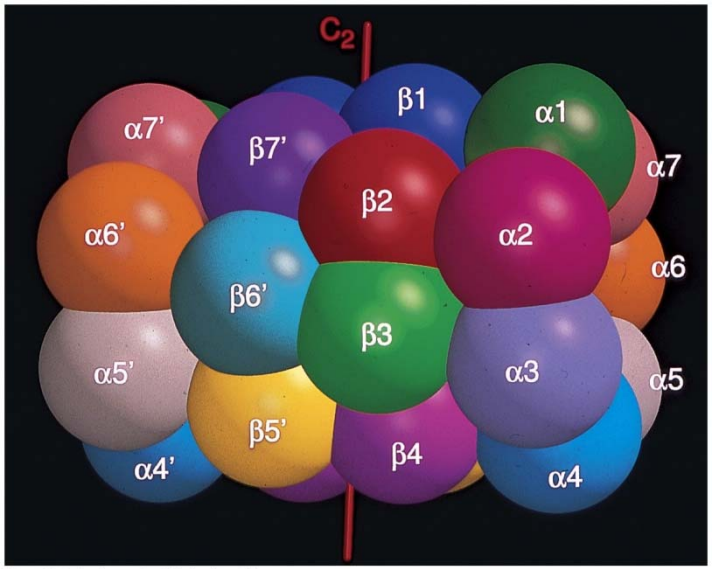


Figure 20-4a Fundamentals of Biochemistry, 2/e

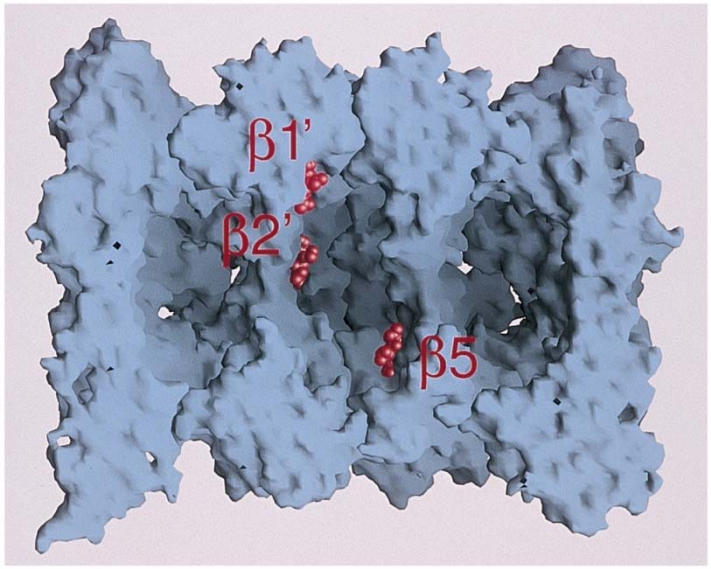


Figure 20-4b Fundamentals of Biochemistry, 2/e

Three proteolytic sites

- β1 subunit: cleaving after acidic residue
- β2 subunit: basic residue
- β5 subunit: hydrophobic
- Yielding fragments of ~8 residues

19S caps

~18 different subunits: Base complex + lid complex

Base complex: 9 subunits, 6 are ATPases

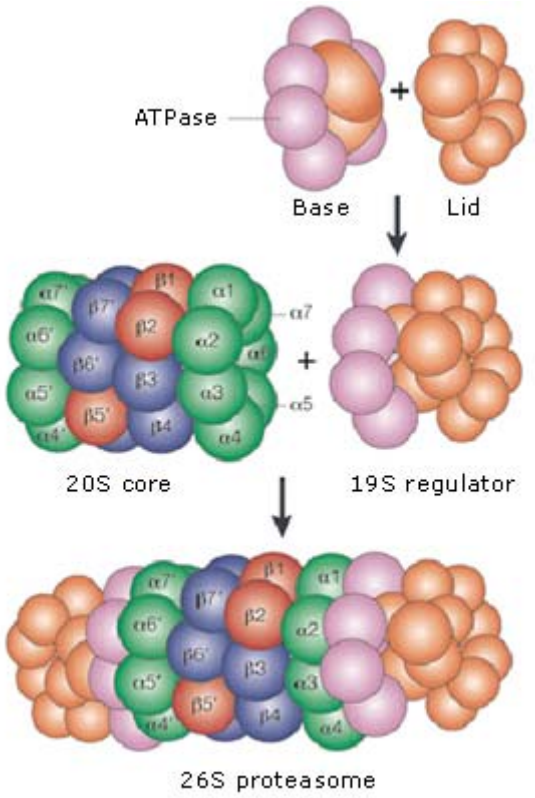
Lid complex: 8 subunits

Control the access of ubiquinated proteins to the 20S proteasome

Recognize ubiquinated proteins

Unfold them

Feed them into 20S in an ATP-dependent manner



Eubacteria

lack 20S proteasome

but also contain *self-compartmentalized* proteases

similar shape and function

meaning early evolutionary history

E. coli Lon and Clp

E. coli CLP

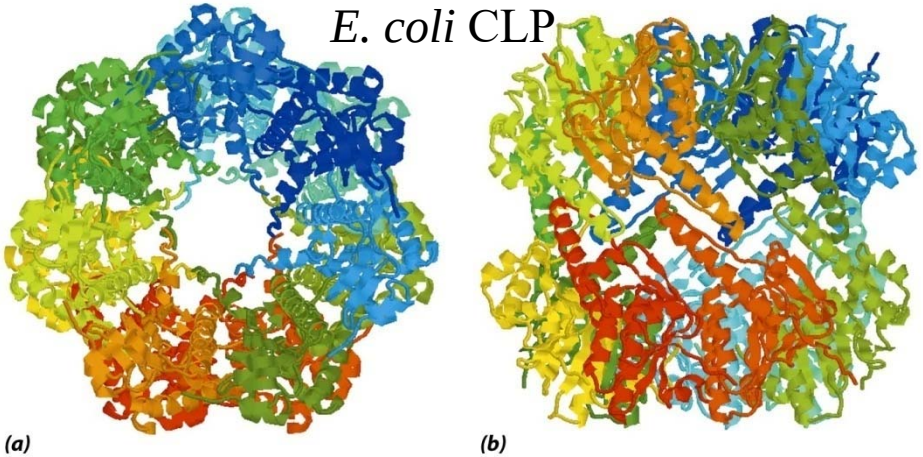


Figure 20-5 Fundamentals of Biochemistry, 2/e

Amino acid deamination

Amino group to ammonia and to urea
Carbon skeleton (α-keto acid)

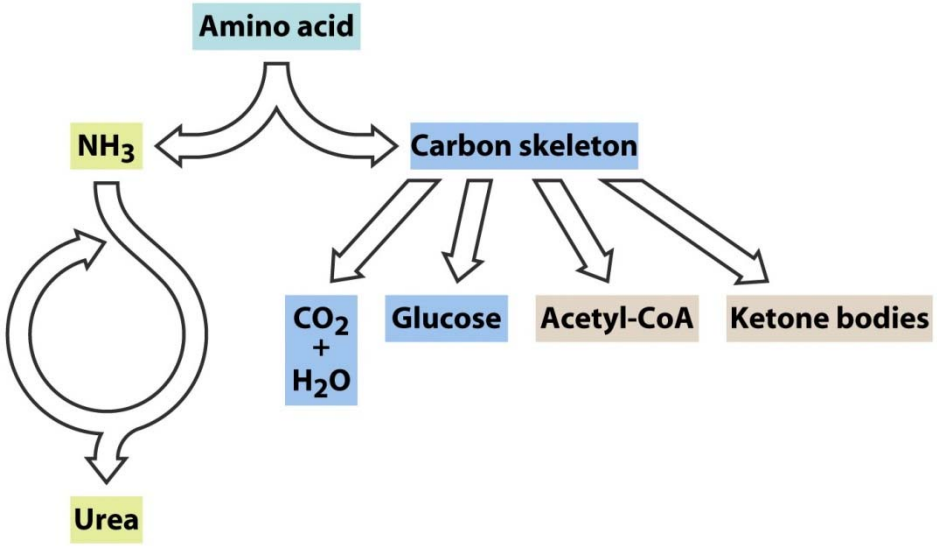
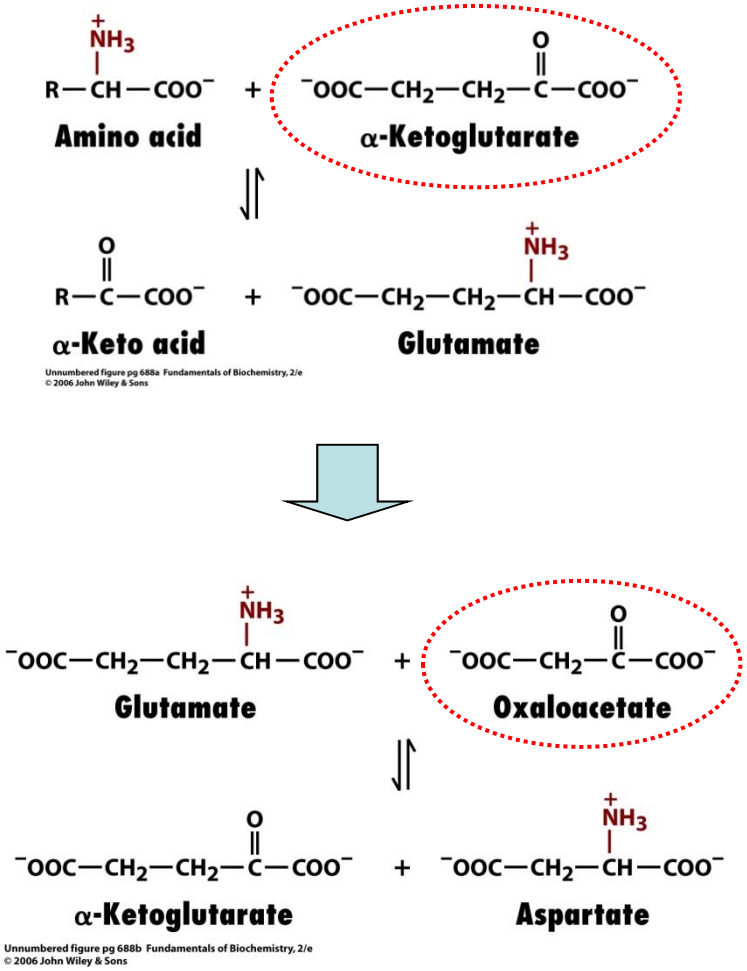


Figure 20-6 Fundamentals of Biochemistry, 2/e

Transamination

The transfer of amino group to an α-keto acid

The predominant amino group acceptor



Unnumbered figure pg 688a Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Unnumbered figure pg 688b Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Aminotransferases
(transaminases)

exist for all amino acid except thr and lys

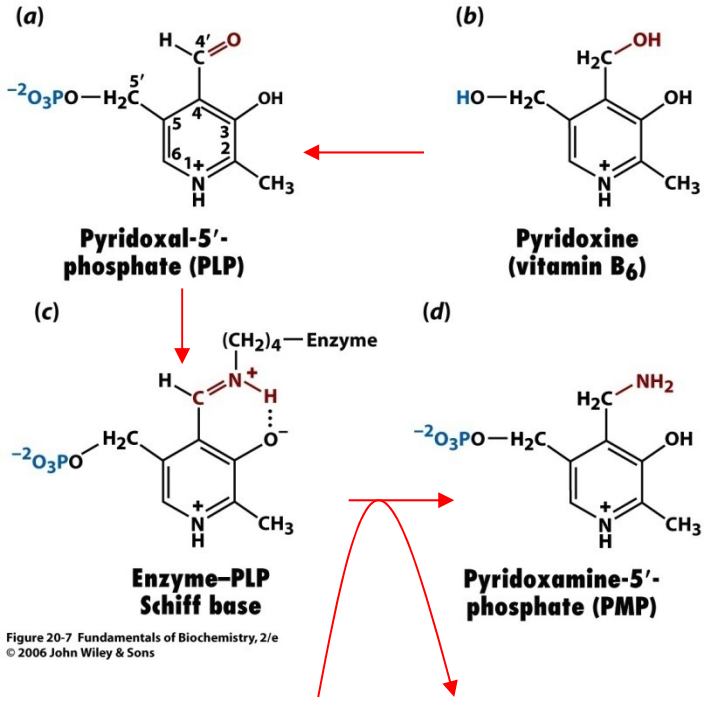


Figure 20-7 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

α-amino acid α-keto acid

The mechanism of
PLP-dependent transamination

1-2-3: Conversion of an amino acid to a keto acid
3'-2'-1': Conversion of an α-keto acid to an amino acid

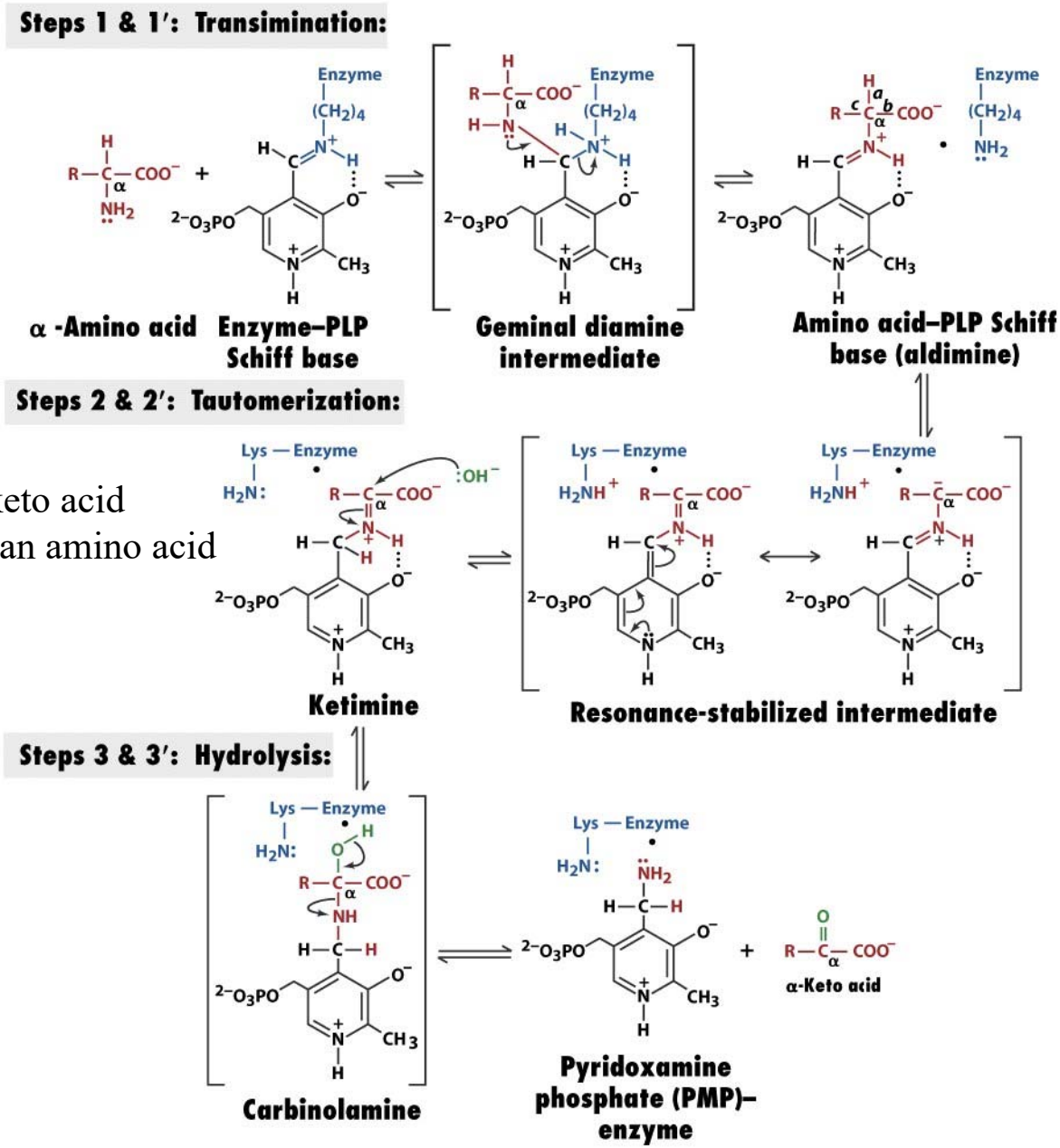


Figure 20-8 Fundamentals of Biochemistry, 2/e

Transaminases are freely reversible in rxn

Participate in both degradation and synthesis

Transaminases as a clinical marker

SGOT (serum glutamate-oxaloacetate transaminase)

= AST (aspartate transaminase)

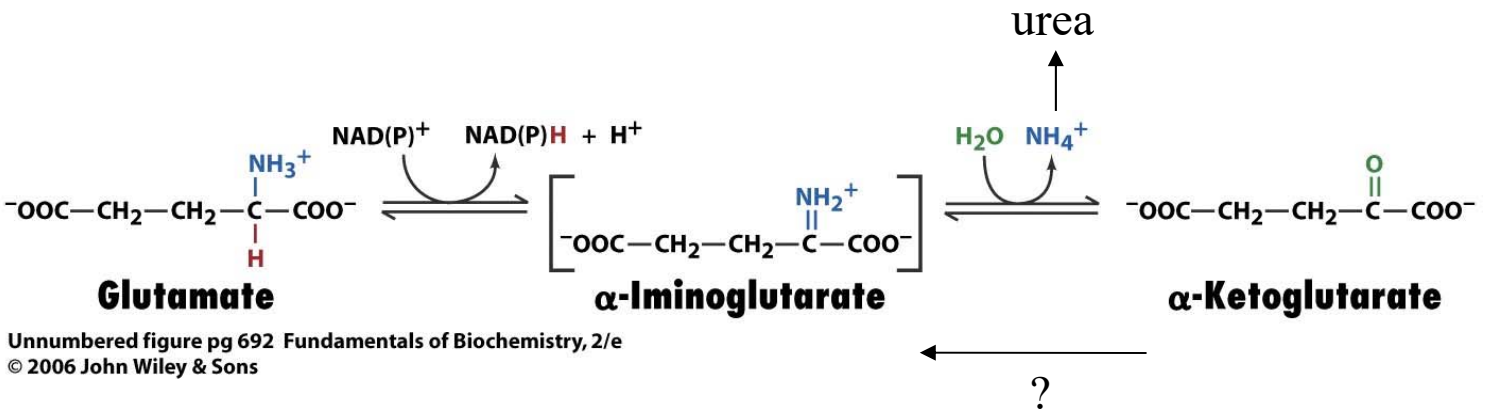
SGPT (serum glutamate-pyruvate transaminase)

= ALT (alanine transaminase)

Heart or liver damage: increase of SGOT and SGPT

GOT (AST, aspartate transaminase): Aspartate + α-ketoglutarate ⇌ oxaloacetate + glutamate
GPT (ALT, alanine transaminase): alanine + α-ketoglutarate ⇌ pyruvate + glutamate

Oxidative deamination by glutamate dehydrogenase (GDH)



Unnumbered figure pg 692 Fundamentals of Biochemistry, 2/e

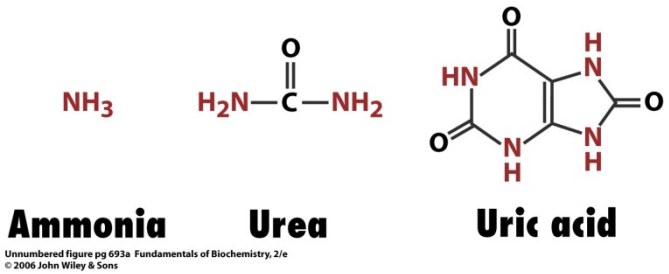
Mitochondrial enzyme
Accept either NAD^+ or $NADP^+$
Near equilibrium reaction under physiological condition (?) ($\Delta G^{o\prime}$=30 kJ/mol)
Allosteric inhibition by GTP and NADH: α-ketoglutarate is an intermediate of CAC

GDH mutation causes hyperinsulinism (hypoglycemia & hyperammonemia)
decreased sensitivity to GTP inhibition & therefore increased GDH activity

Reversible ADP-ribosylation (inactive by ADP-ribosylation)

The urea cycle

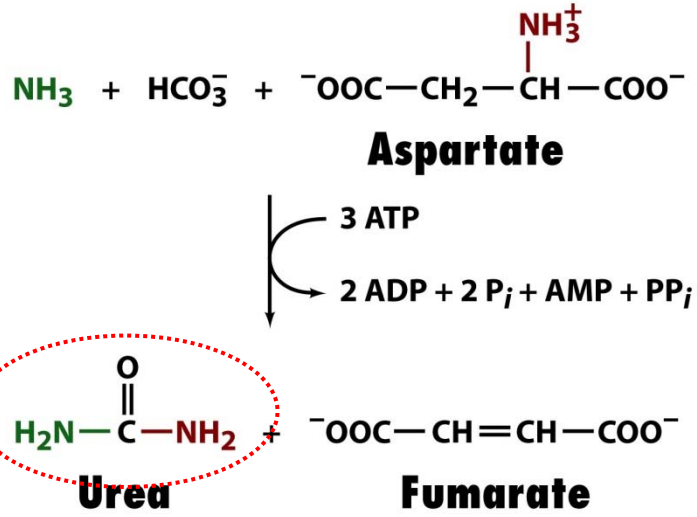
Excess nitrogen to ammonia, urea, uric acid



Unnumbered figure pg 693a Fundamentals of Biochemistry, 2/e

synthesized in liver
secreted into the blood
sequestered by the kidney for excretion in the urine

The overall reaction



Unnumbered figure pg 693b Fundamentals of Biochemistry, 2/e

The urea cycle

Two mitochondrial reactions
Three cytosolic reactions

Carbamoyl phosphate synthetase I
Ornithine transcarbamoylase
Argininosuccinate synthetase
Argininosuccinase
Arginase (I)

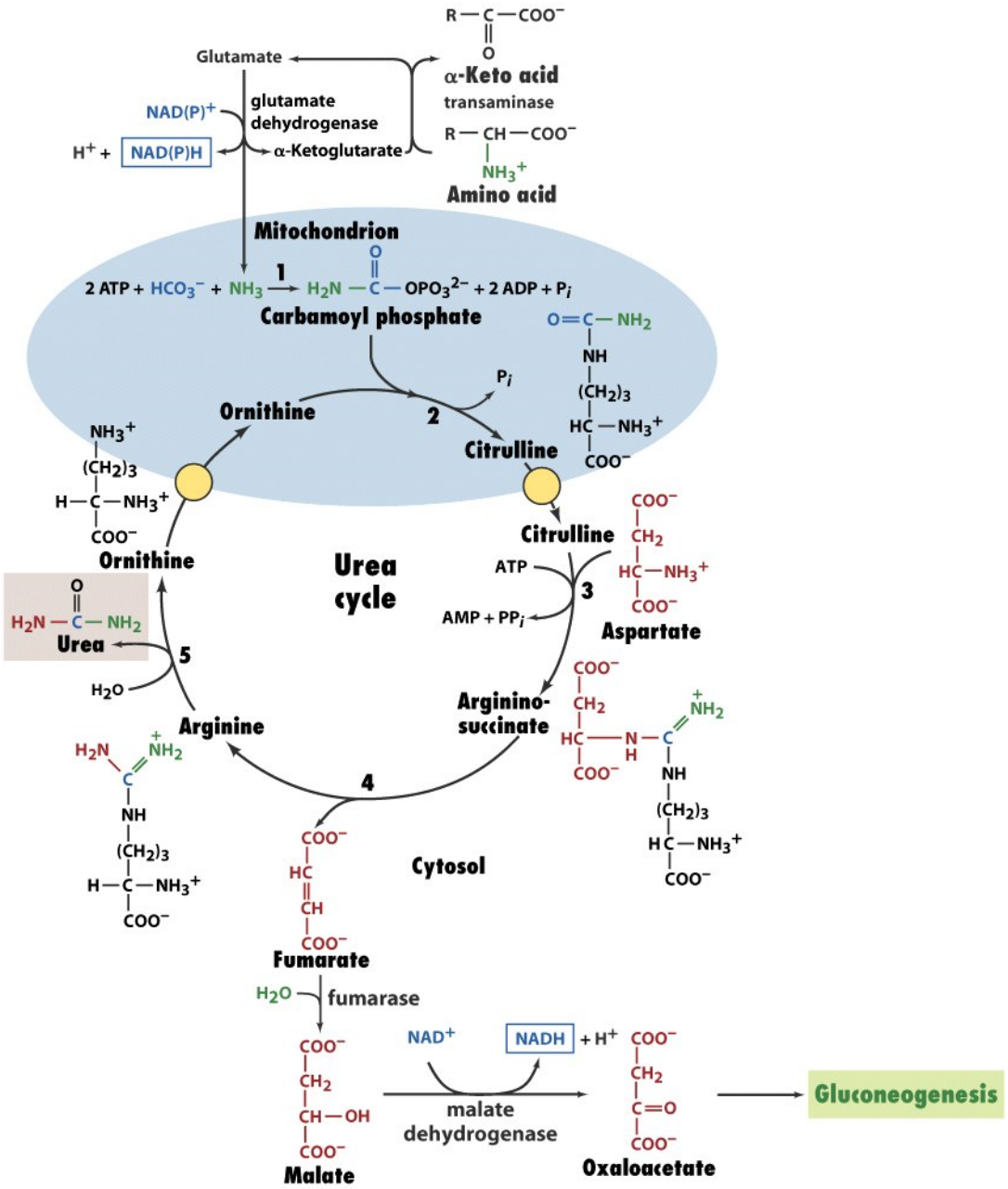


Figure 20-9 Fundamentals of Biochemistry, 2/e

Carbamoyl phosphate synthetase (CPS)

Eukaryotes have two CPS

Mitochondrial: CPS I, uses ammonia as its nitrogen donor and involves in urea synthesis

Cytosolic: CPS II, uses glutamine as its nitrogen donor
and involved in pyrimidine synthesis

The mechanism of action of CPS I: rate-limiting step

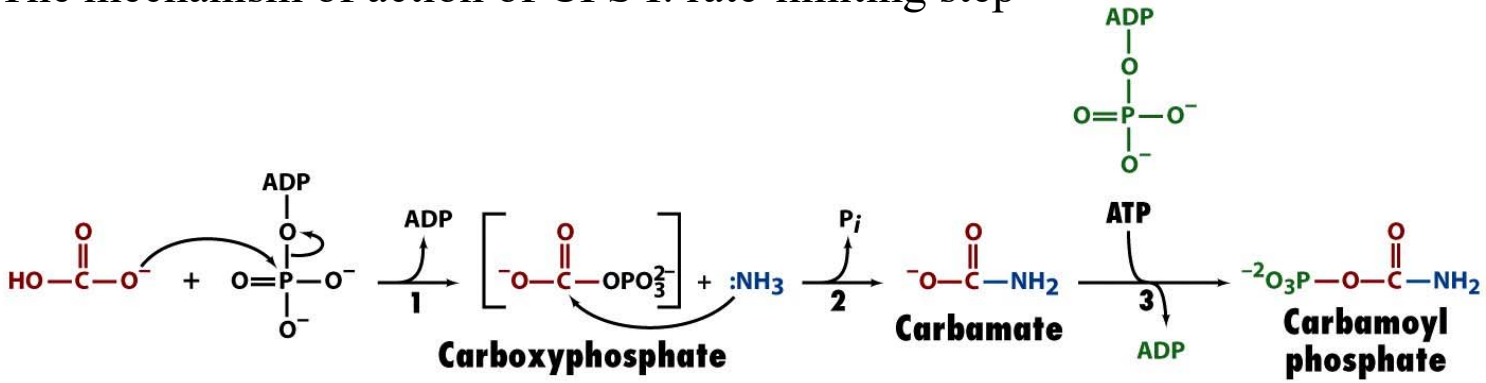
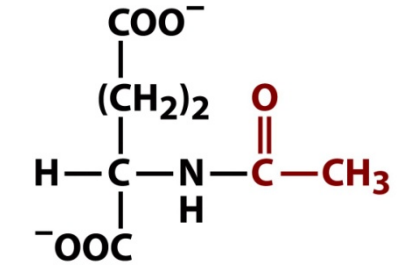


Figure 20-10 Fundamentals of Biochemistry, 2/e

Regulation of the urea cycle

CPS I: allosteric activation by N-acetylglutamate

- Amino acid breakdown
- Increased glutamate
- Increased N-acetylglutamate
- Increased urea synthesis



***N*-Acetylglutamate**

Inherited deficiency in urea cycle enzymes other than arginase

- Substrate buildup (including ammonia)
- Increased rate & normal urea production
- Hyperammonemia
- Brain damage

Breakdown of amino acids

Glucogenic amino acids

Glucose precursor

Ketogenic amino acids

Precursors of fatty acids or ketone bodies

Purely ketogenic: Lys, Leu

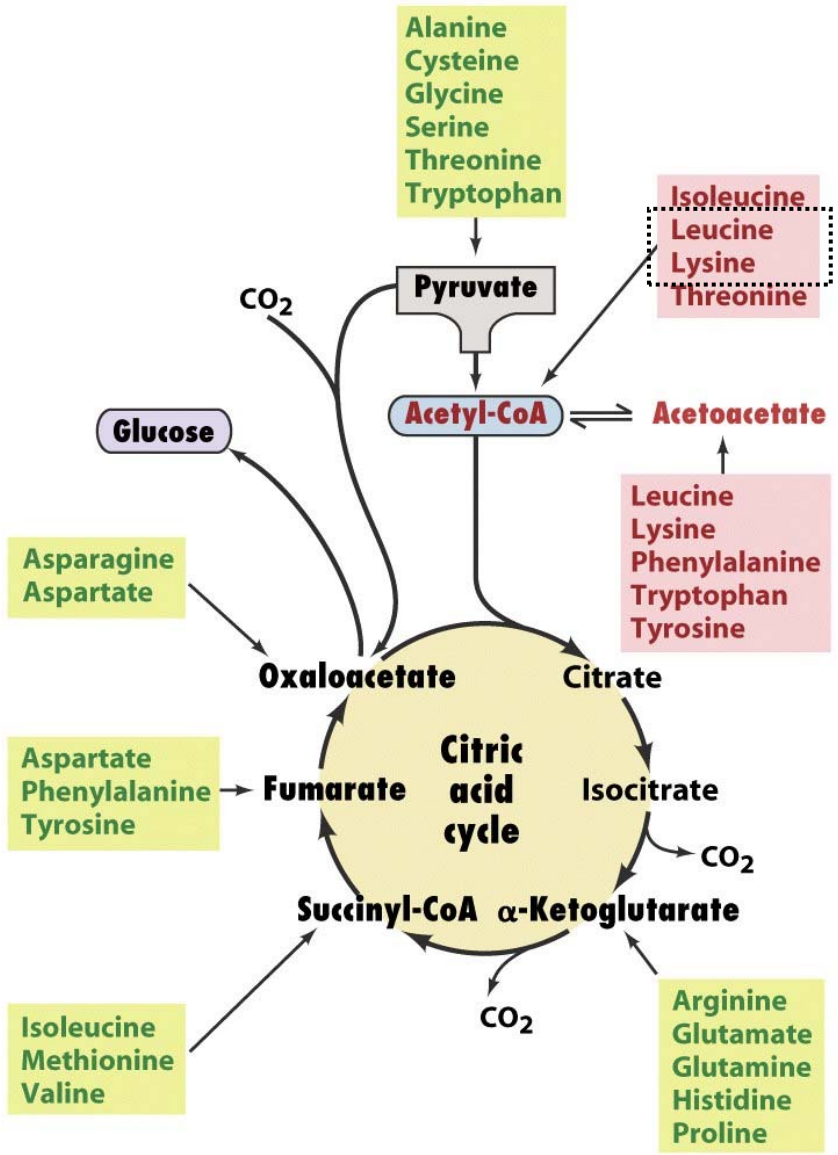


Figure 20-13 Fundamentals of Biochemistry, 2/e

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/aacarbon.htm

Degradation to pyruvate

ACGST

PLP containing enzyme

Serine dehydratase: 2

Serine hydroxymethyltransferase: 4

Glycine cleavage system (rxn 3)

A major route of glycine degradation in mammals

Inherited deficiency: nonketotic hyperglycinemia (glycine encephalopathy)

Elevated glycine levels may be harmless in blood, but lethal in brain (glycine is a neurotransmitter).

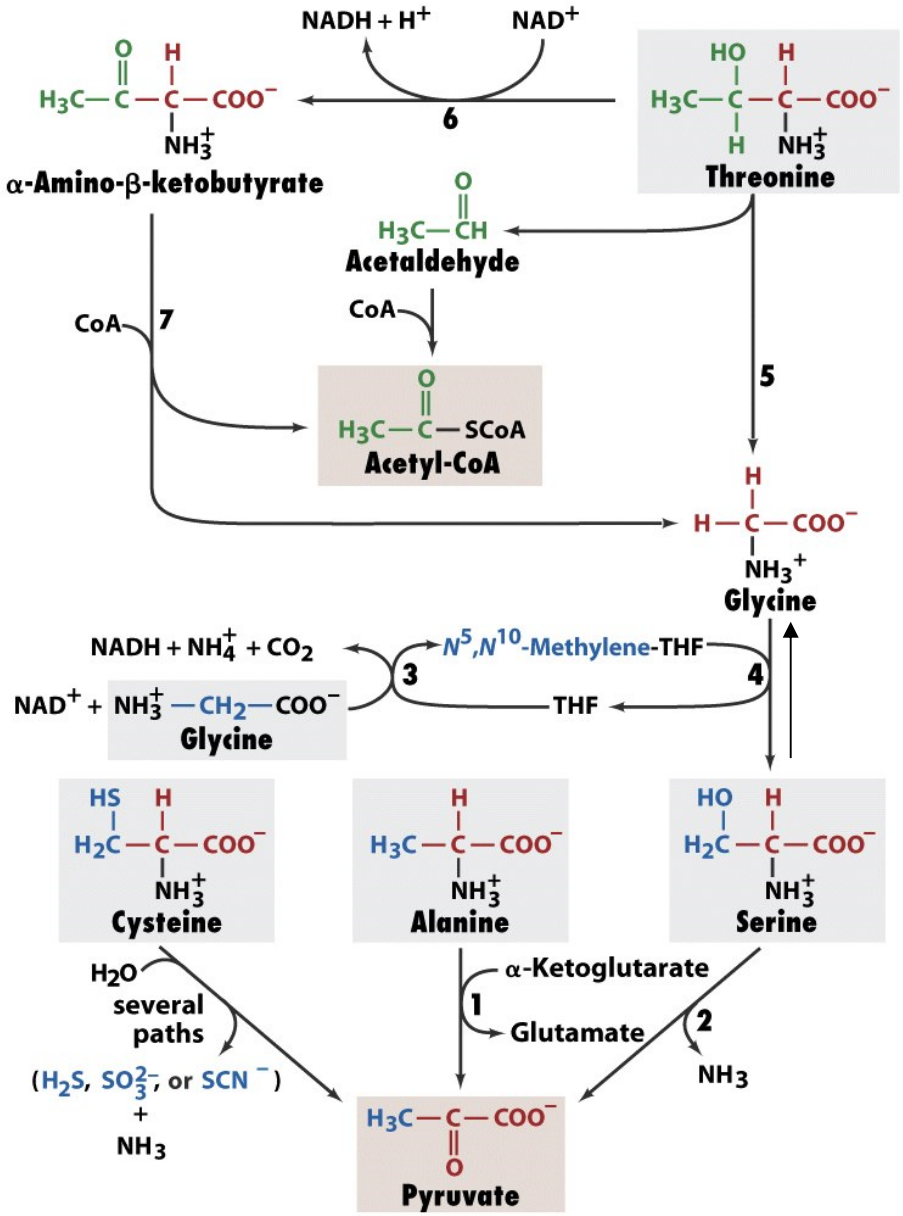


Figure 20-14 Fundamentals of Biochemistry, 2/e

PLP-dependent enzymes

1. The serine dehydratase reaction
   PLP-dependent elimination of water

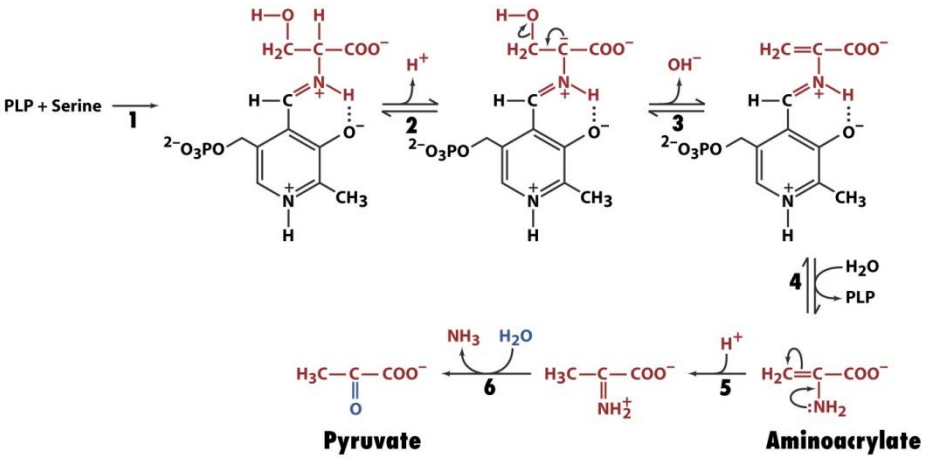
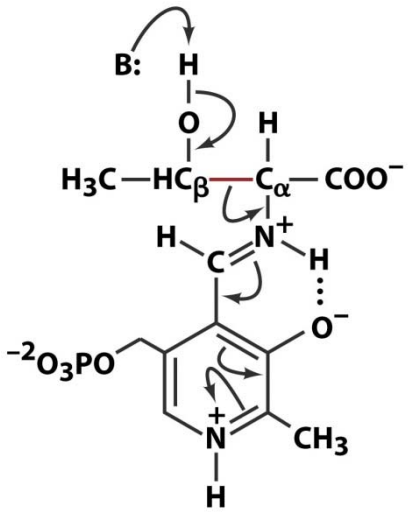


Figure 20-15 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

2. Serine hydroxymethyltransferase
   PLP-dependent C_α-C_β bond formation and cleavage

PLP acts as a coenzyme in all transamination reactions, and in some decarboxylation and deamination reactions of amino acids.



Unnumbered figure pg 700 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

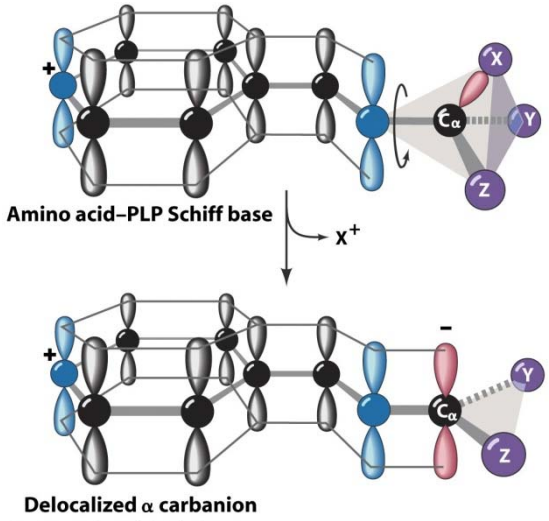
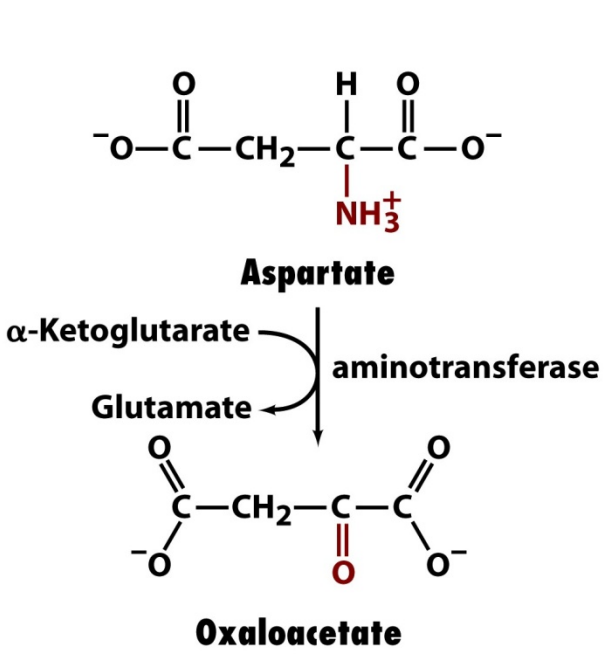
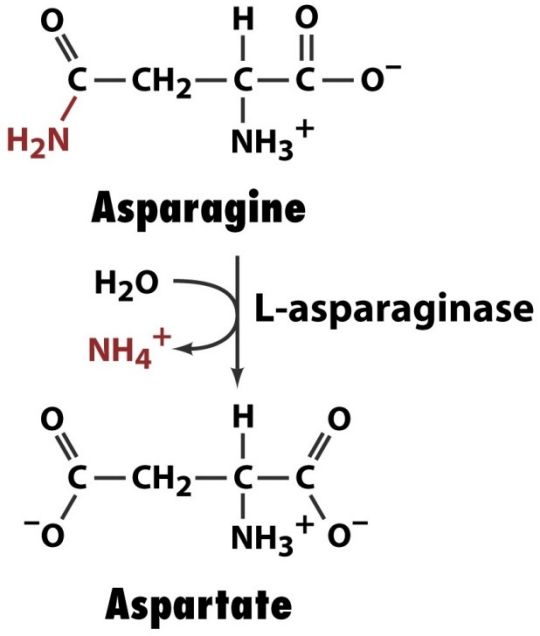


Figure 20-16 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Asn and Asp to oxaloacetate



Unnumbered figure pg 701a Fundamentals of Biochemistry, 2/e



Unnumbered figure pg 701b Fundamentals of Biochemistry, 2/e

Degradation to α-ketoglutarate

REQHP

Gln acts as an ammonia transport system
between
the liver (synthesis) and
the kidney (hydrolyzed by glutaminase)

During metabolic acidosis
Glutaminase eliminate excess acid
By combining ammonia with a proton

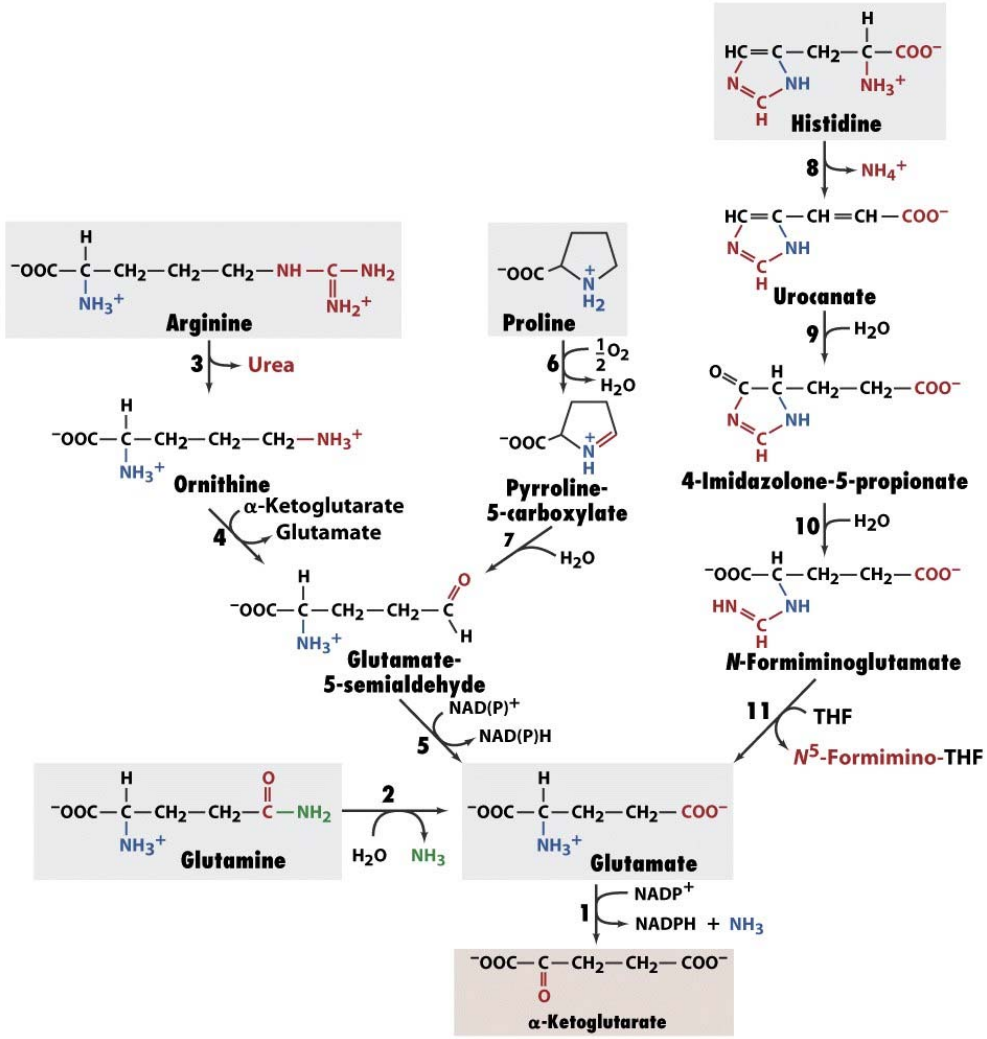
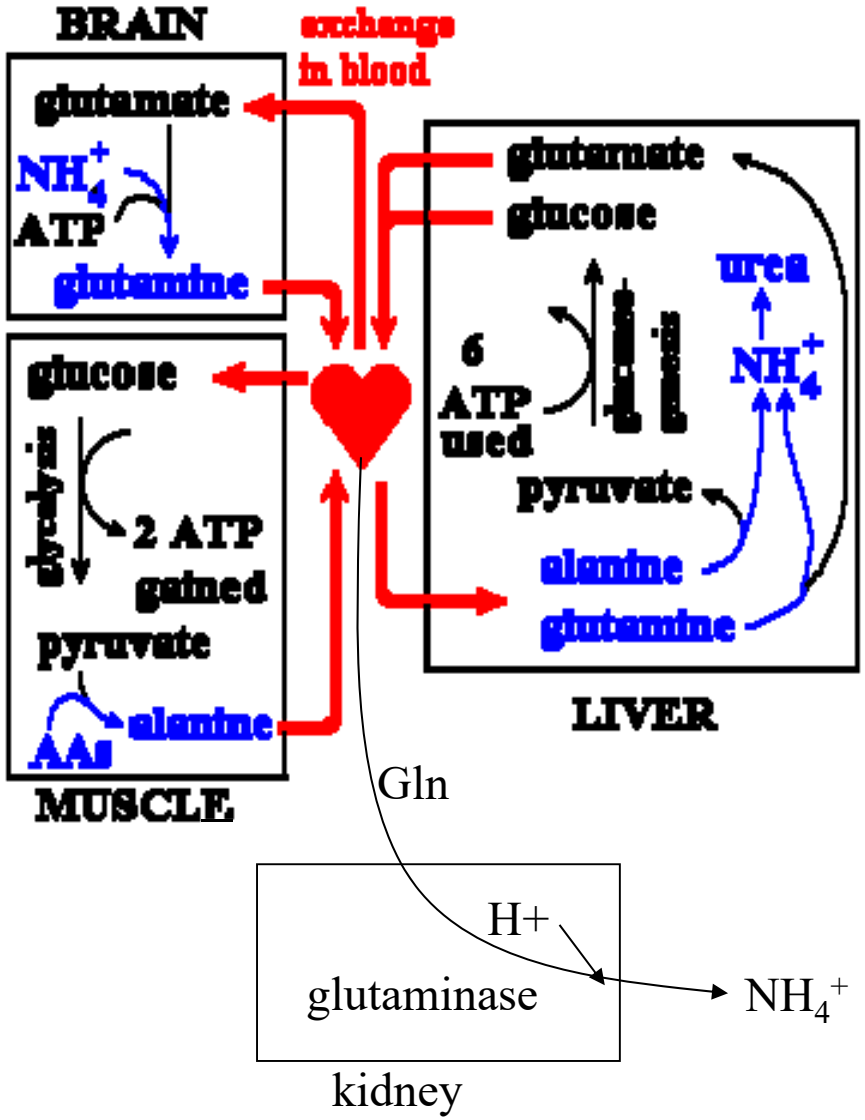


Figure 20-17 Fundamentals of Biochemistry, 2/e



Glutamine + H_2O → Glutamate + NH_3

Degradation to succinyl-CoA

IMV

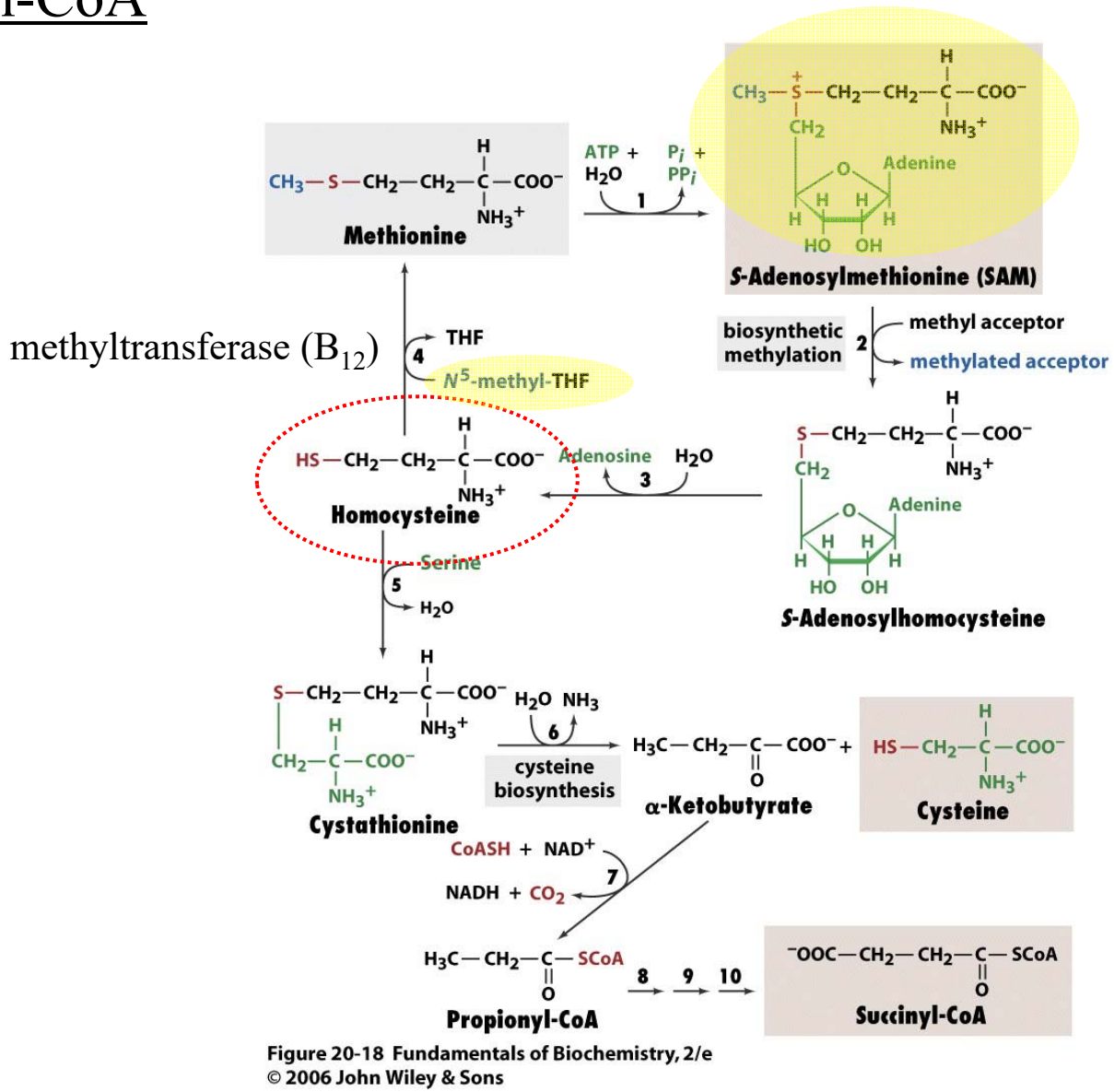


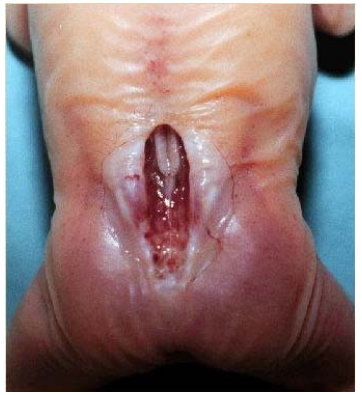
Figure 20-18 Fundamentals of Biochemistry, 2/e

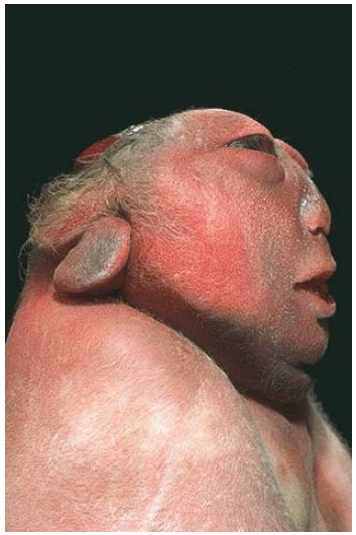
Homocysteine is a marker of atherosclerosis

Homocysteine conc is determined by the rates of
    rxn 2,3,4 and rxn 5
Hyperhomocysteinemia (homocysteinuria)
    associated with cardiovascular disease
    due to oxidative damage to endothelial cells
    (deficiency of folate or vit. B12)

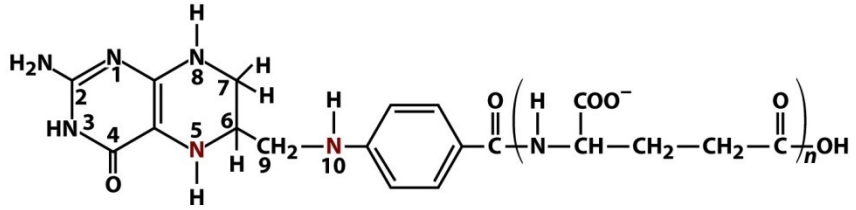
Associated with neural tube defects (NTD)
    Spina bifida
    Anencephaly (http://www.path.sunysb.edu/neuropath/developmental.htm)
    High incidence

MTHFR mutations ($q^2 = 0.01$)
N^5,N^{10}-methylene-THF to N^5-methyl-THF (cofactor for step 4)





Tetrahydrofolates (THFs): one-carbon carriers



Unnumbered figure pg 704 Fundamentals of Biochemistry, 2/e

Dihydrofolate reductase (DHFR)

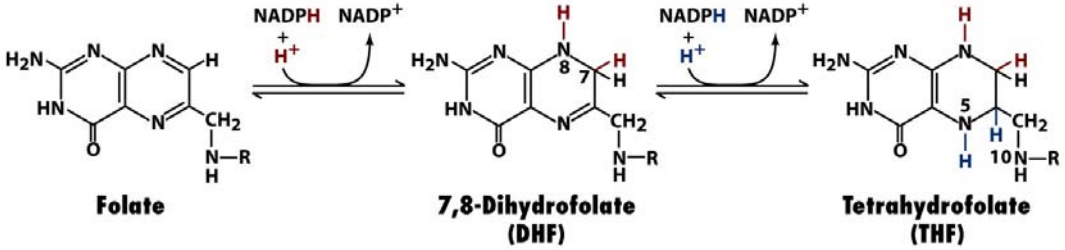


Figure 20-19 Fundamentals of Biochemistry, 2/e

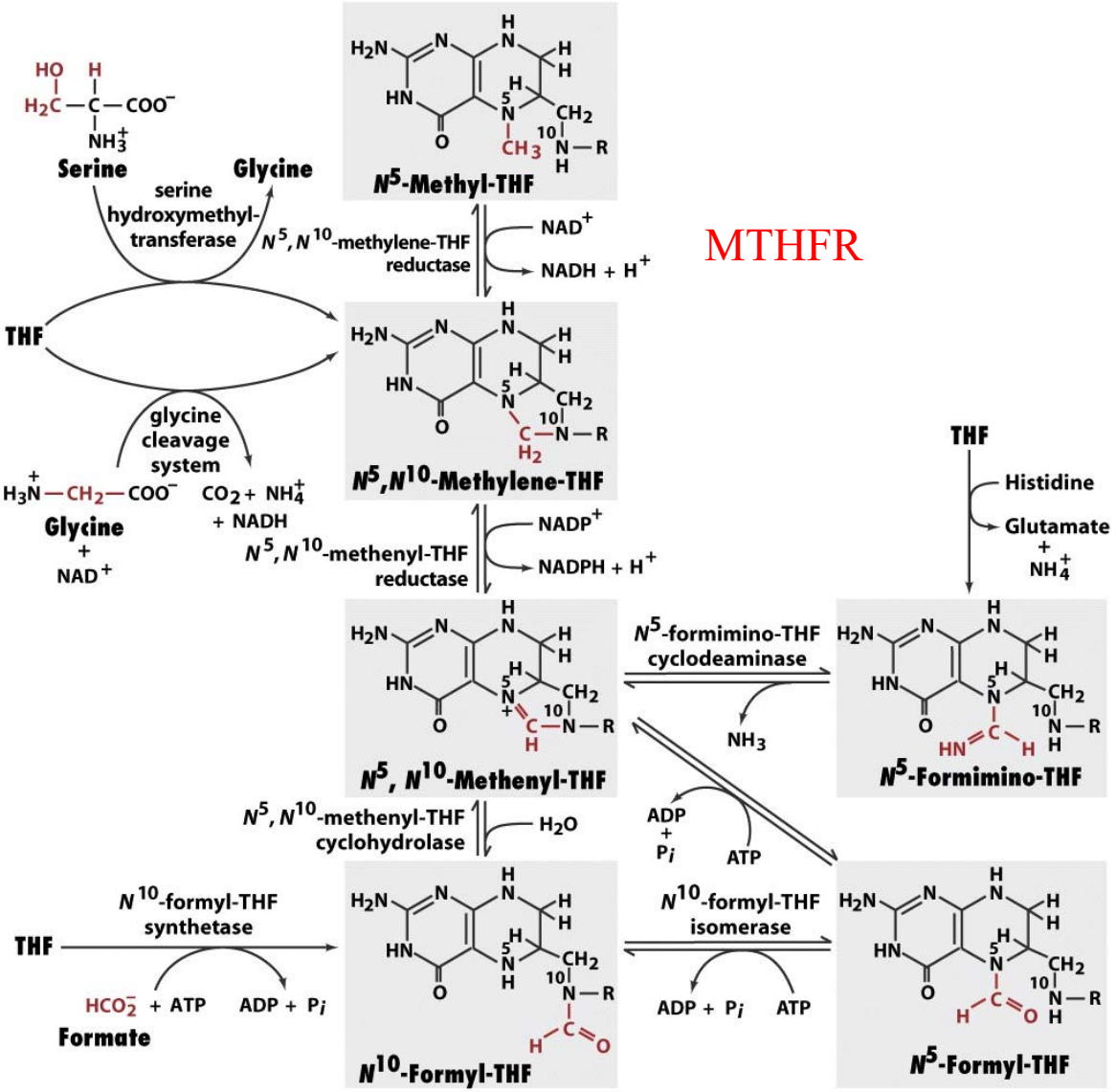
Biotin: CO_2
SAM: CH_3-
THF: various C1 groups

Table 20-2 Oxidation Levels of C_1 Groups Carried by THF

Oxidation Level	Group 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 20-2 Fundamentals of Biochemistry, 2/e

Interconversion of the C1 units carried by THF



MTHFR

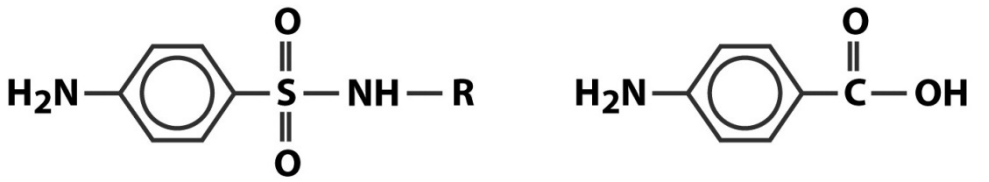
Figure 20-20 Fundamentals of Biochemistry, 2/e

Sulfonamides are antibiotics

Analog of the *p*-aminobenzoic acid of THF

Inhibits folic acid synthesis

Mammals lack folic acid synthesis



Sulfonamides (R = H, sulfanilamide)

***p*-Aminobenzoic acid**

Unnumbered figure pg 706 Fundamentals of Biochemistry, 2/e

Degradation of the branched chain amino acids

Branched-chain α-keto acid dehydrogenase (BCKDH) →

A genetic deficiency: maple syrup urine disease

a fatal disease

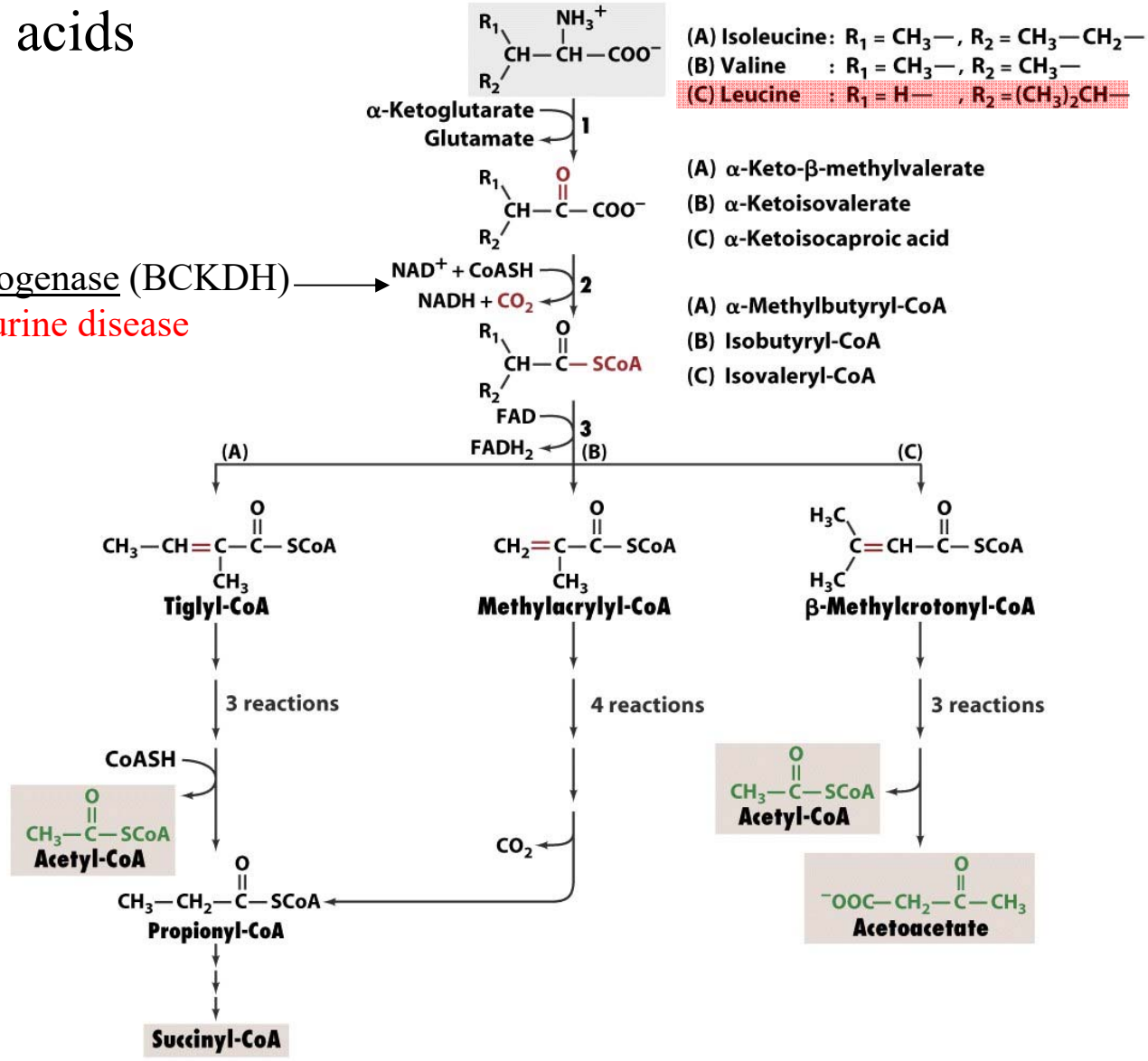


Figure 20-21 Fundamentals of Biochemistry, 2/e

Lysine degradation in mammalian liver

7 reactions were encountered previously (rxn 4,5,6,8-11)

<u>Deficiency in rxn 1</u>

Hyperlysinemia (in blood)

Hyperlysinuria (in urine)

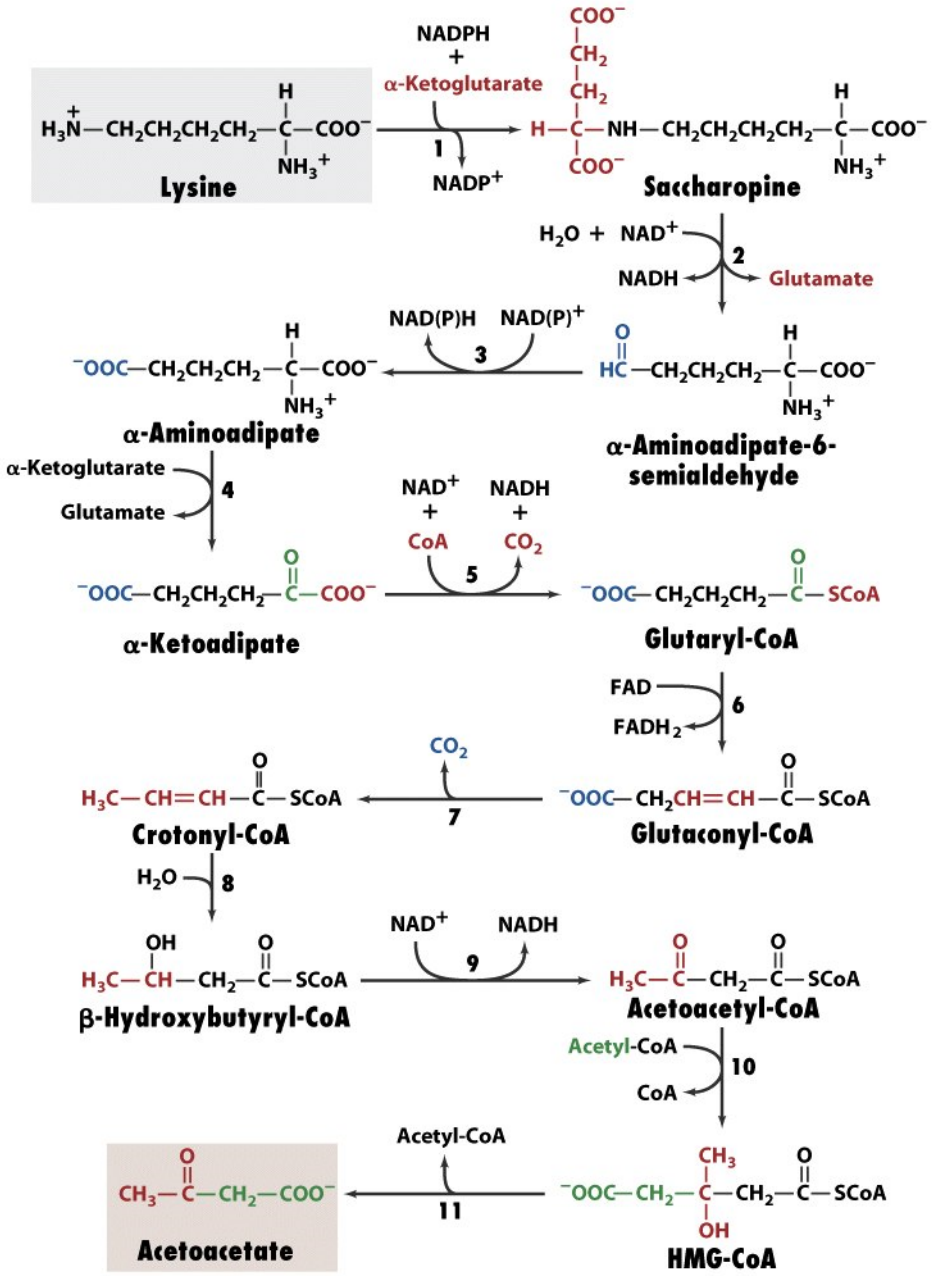


Figure 20-22 Fundamentals of Biochemistry, 2/e

Tryptophan degradation

IDO (indoleamine 2,3-dioxygenase)

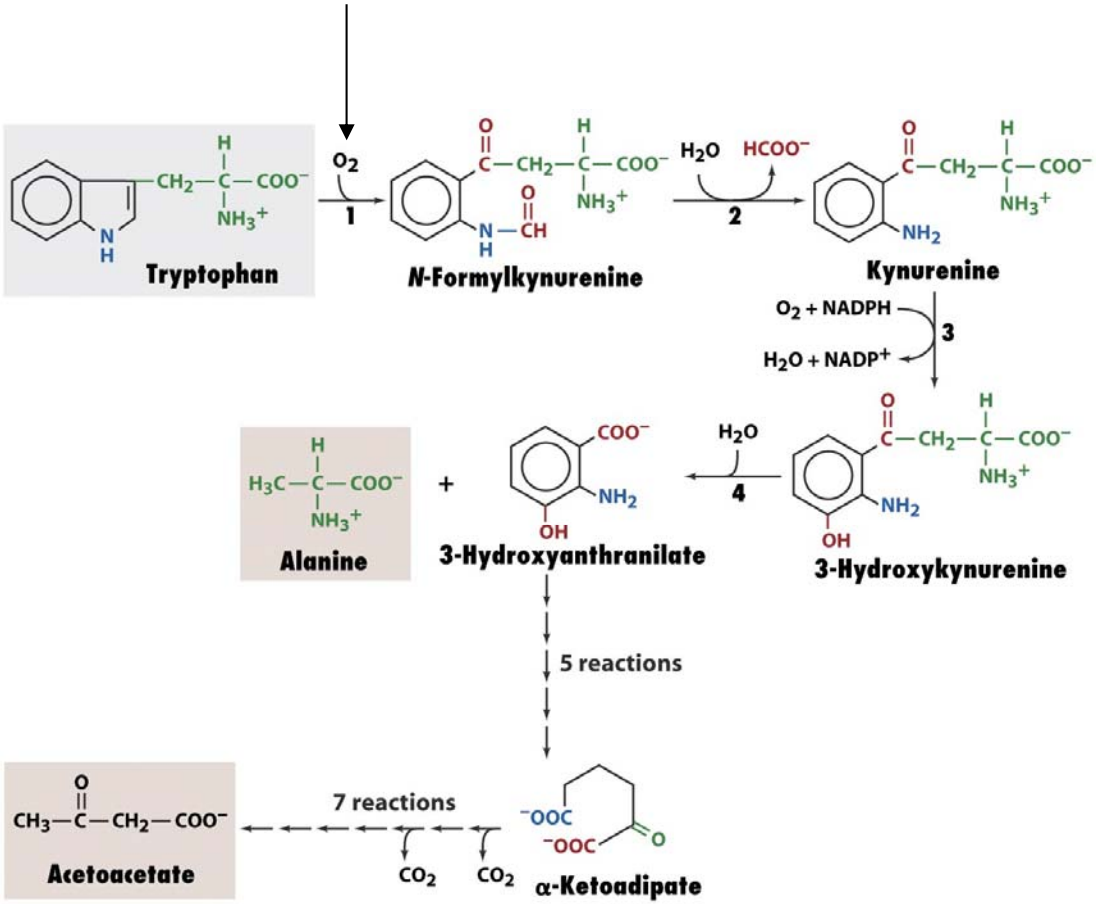
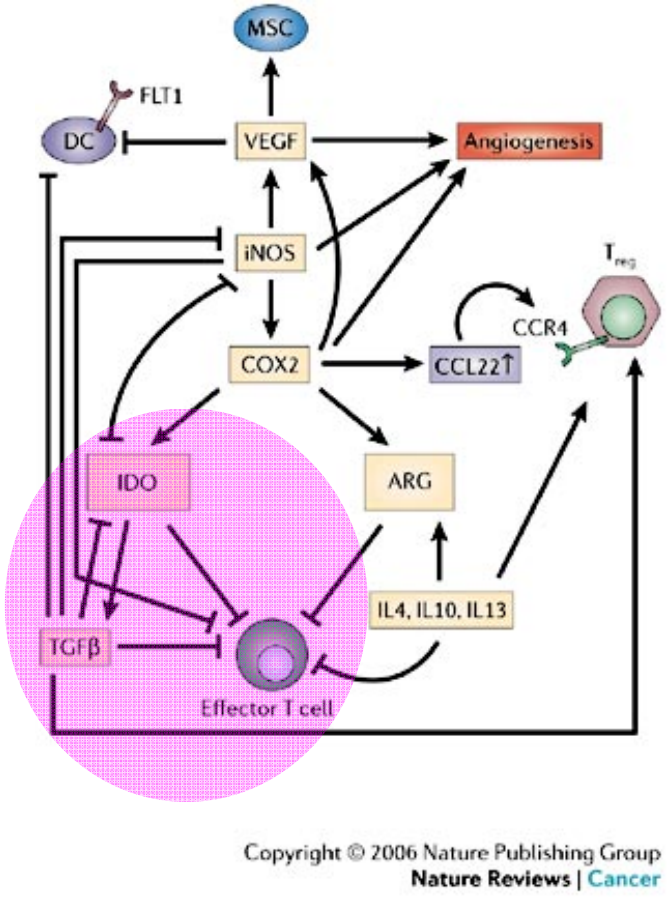


Figure 20-23 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Immunosuppression activity of IDO
tryptophan depletion or its metabolite?



Nature Reviews Cancer **6**, 613–625 (2006)

Phenylalanine degradation

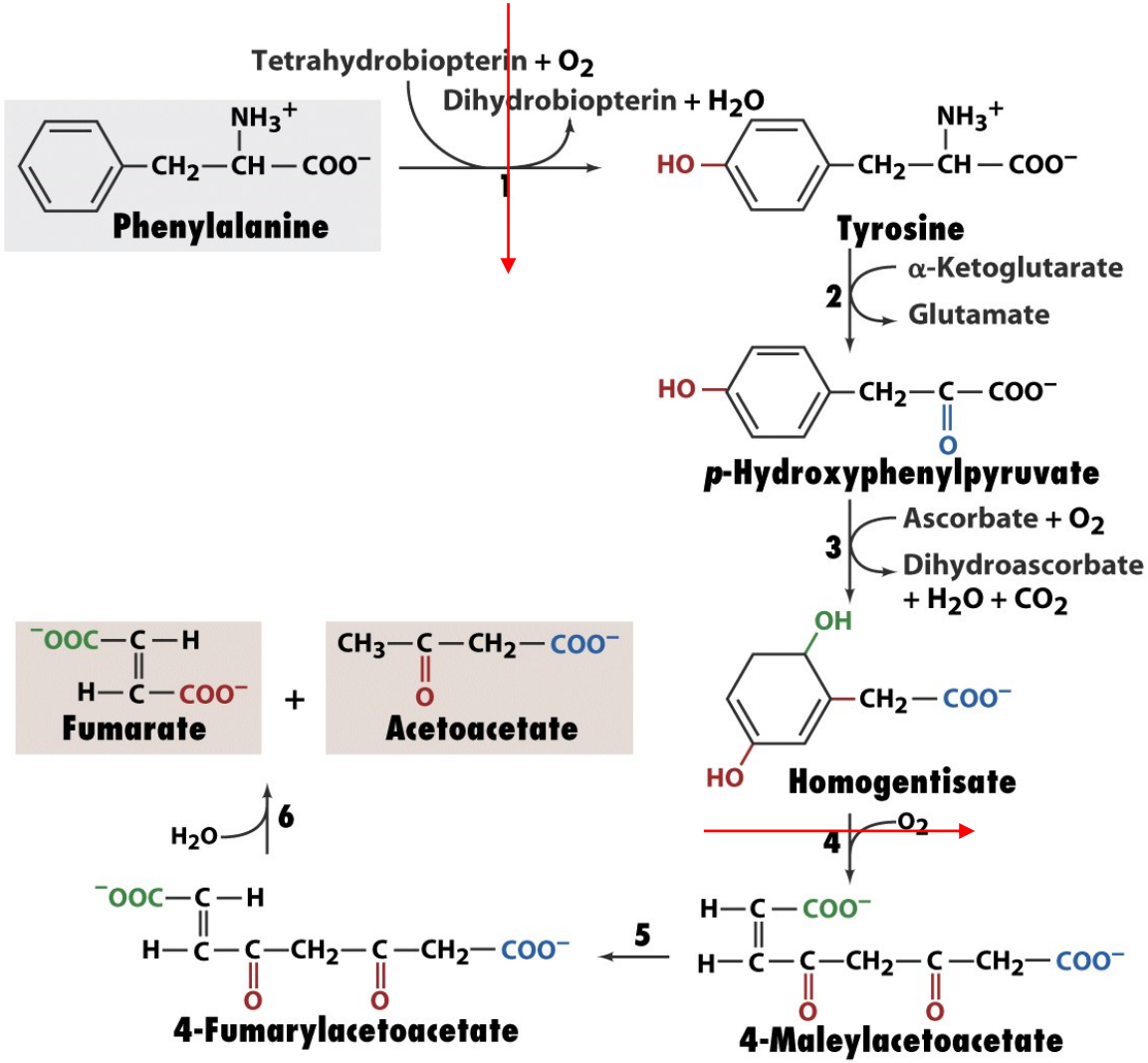


Figure 20-24 Fundamentals of Biochemistry, 2/e

Pteridine ring nucleus of biopterin and folate
Pterins are redox cofactors

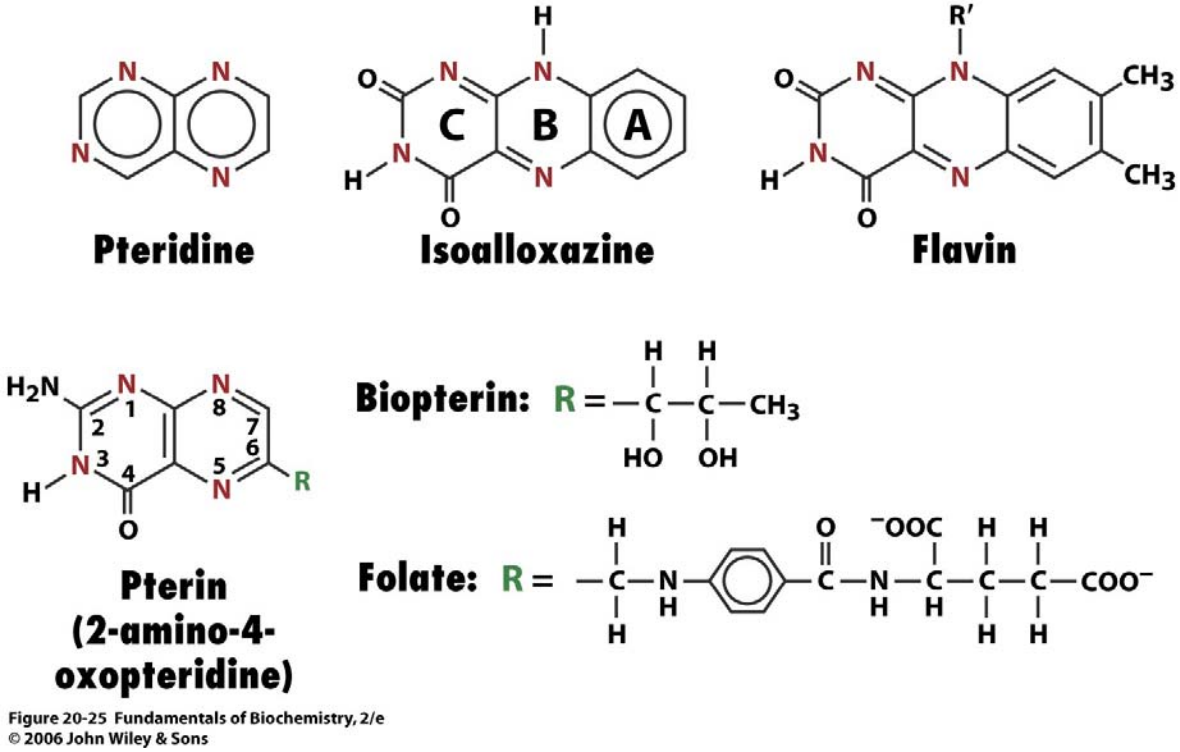


Figure 20-25 Fundamentals of Biochemistry, 2/e

Tetrahydrobiopterin In PAH reaction

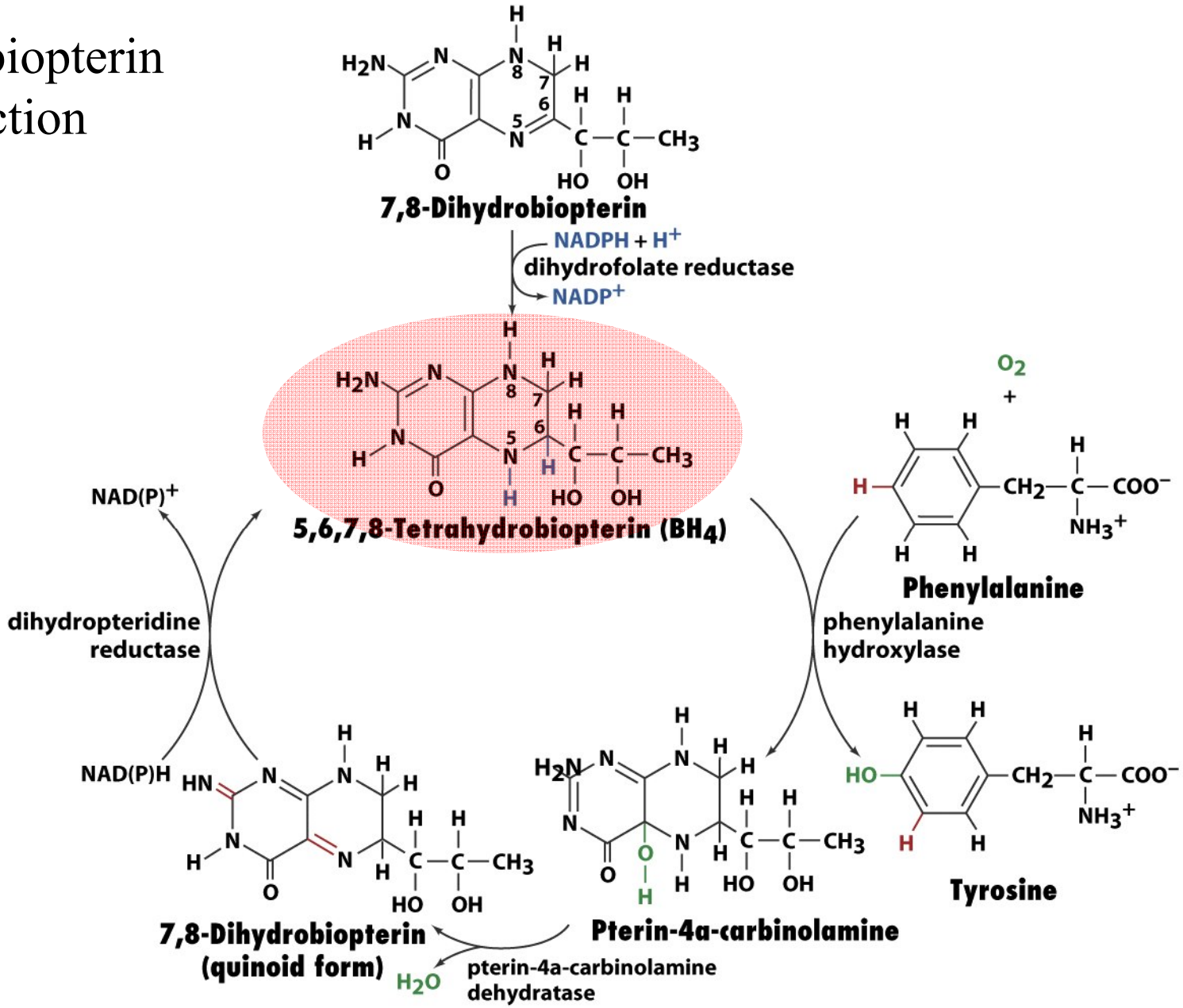


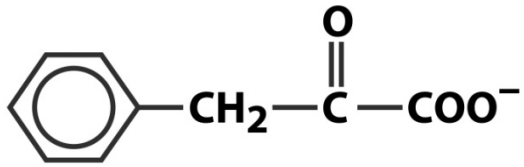
Figure 20-26 Fundamentals of Biochemistry, 2/e

Phenylketonuria and alkaptonuria

Alkaptonuria: deficiency of homogentisate dioxygenase
excretion of homogentisic acid

Phenylketonuria (PKU)
hyperphenylalaninemia: converted to phenylketo compounds
high phe inhibits tyrosine hydroxylation: reduced melanin
high phe saturates LNAAT and blocks transport of LNAA into brain

BH4 synthesis deficiencies



Phenylpyruvate

Box 20-2 Fundamentals of Biochemistry, 2/e