Ch 22

Biosynthesis of amino acids, nucleotides and related molecules

Essential amino acids

- A.A. can not be synthesized by human body.
  - ✓ Must be provided in the diet

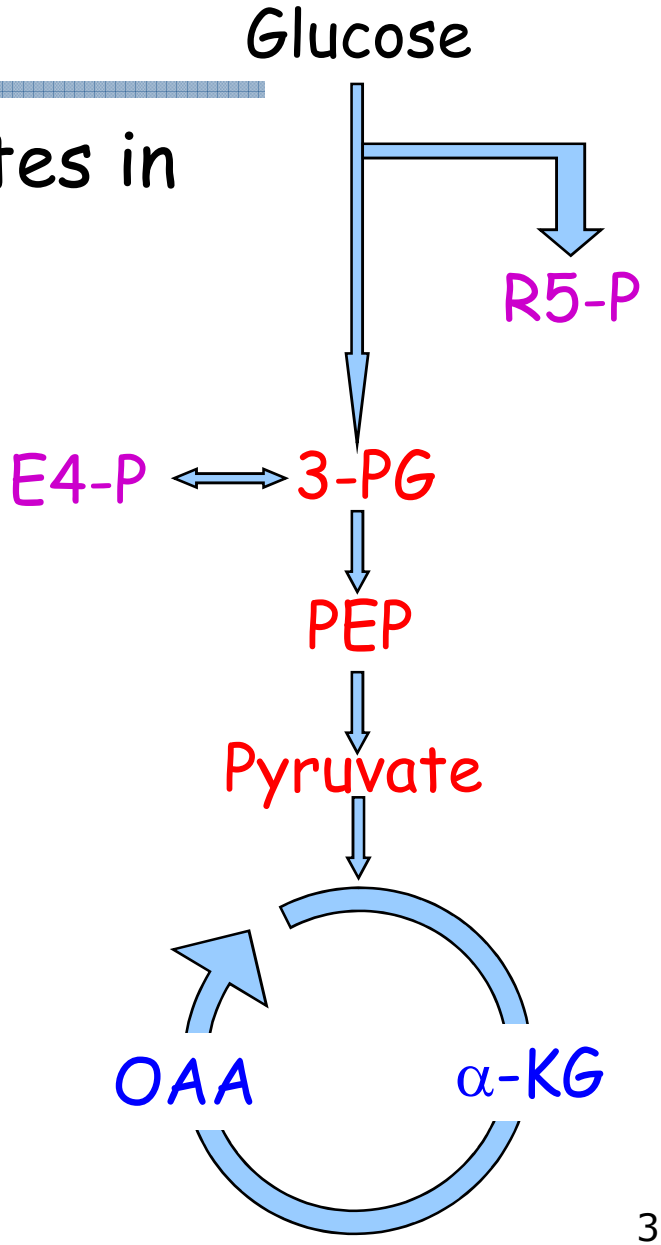
Table 18-1

Nonessential	*Conditionally essential**	*Essential*
Alanine	Arginine	Histidine
Asparagine	Cysteine	Isoleucine
Aspartate	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine

Modified Fig 22-9 p. 861

Biosynthesis of A.A.

- All C derived from intermediates in
  - ✓ Glycolysis
    - 3-Phosphoglycerate (3PG)
    - Phosphoenolpyruvate (PEP)
    - Pyruvate
  - ✓ The citric acid cycle
    - α-Ketoglutarate
    - Oxaloacetate
  - ✓ The pentose phosphate pathway
    - Ribose 5-phosphate
    - Erythrose 4-phosphate
- N enters these pathways as
  - ✓ Glu (aminotransferase)
  - ✓ Gln (amidotransferase, p. 859)



Precursors of amino acids

- Table 22-1

TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate
Glutamate
Glutamine
Proline
Arginine

3-Phosphoglycerate
Serine
Glycine
Cysteine

Oxaloacetate
Aspartate
Asparagine
Methionine*
Threonine*
Lysine*

Pyruvate
Alanine
Valine*
Leucine*
Isoleucine*

Phosphoenolpyruvate and erythrose 4-phosphate
Tryptophan*
Phenylalanine*
Tyrosine†

Ribose 5-phosphate
Histidine*

*Essential amino acids.
†Derived from phenylalanine in mammals.

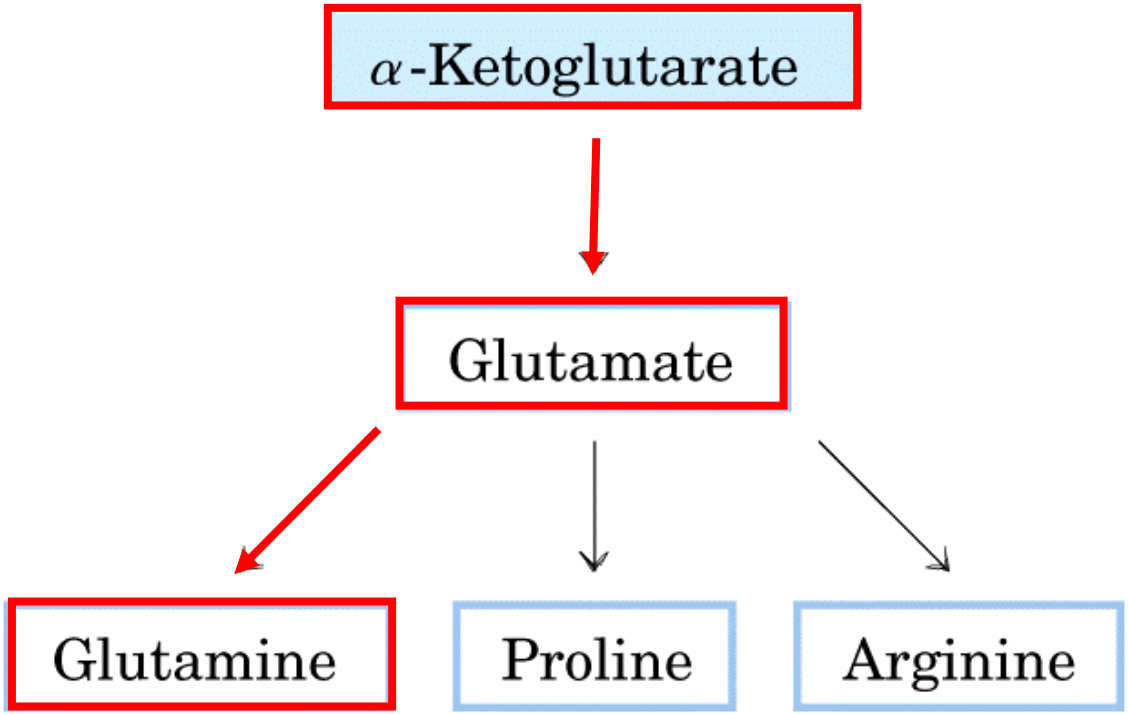
p. 861

α-ketoglutarate

p. 842, left column:

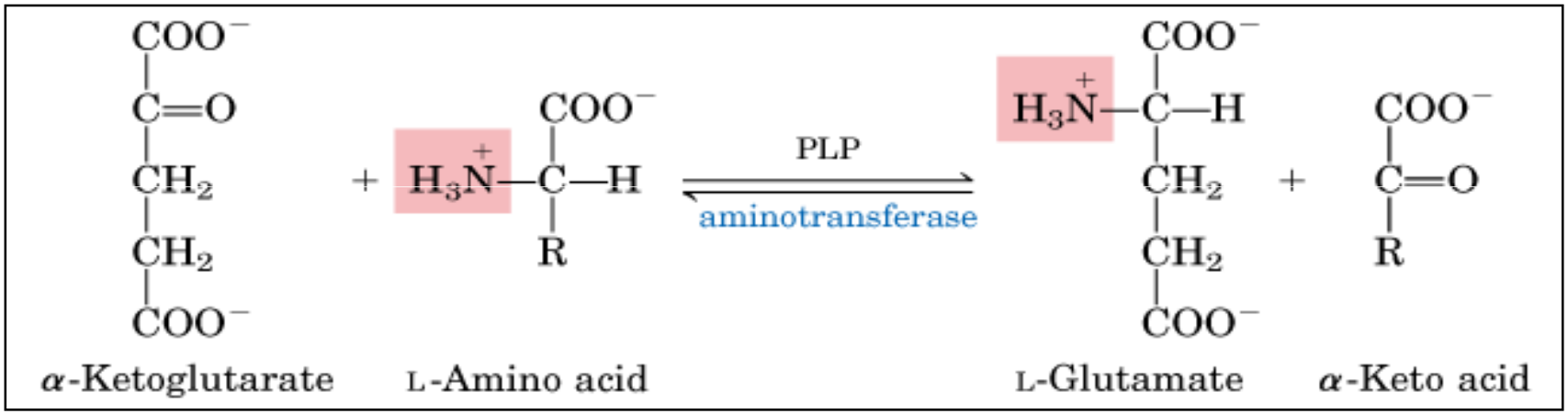
"We have already described the biosynthesis of **Glutamate** and **Glutamine**."

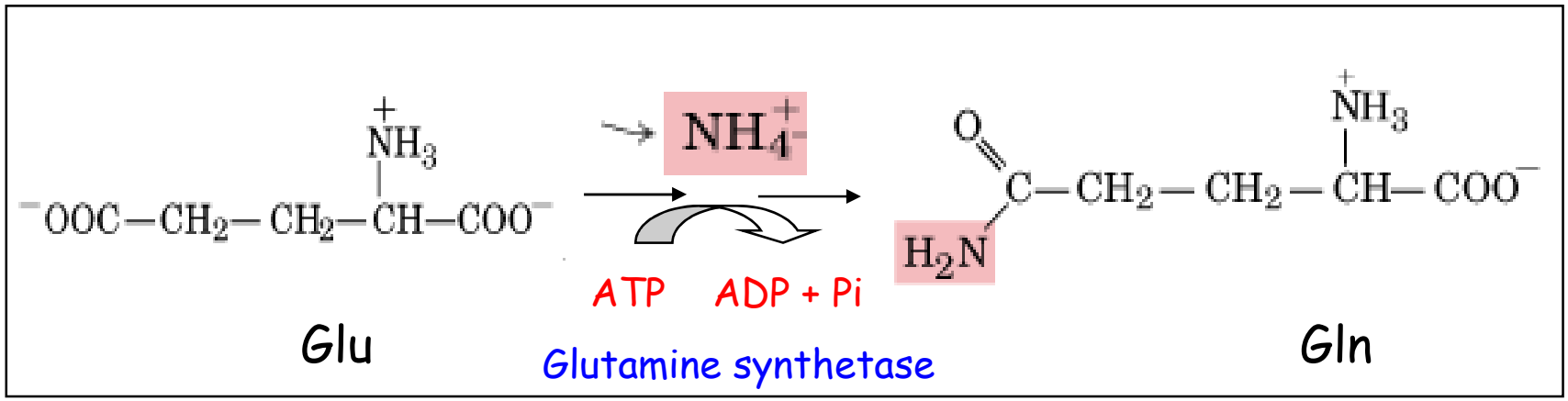
p. 861



α-ketoglutarate → Glu → Gln

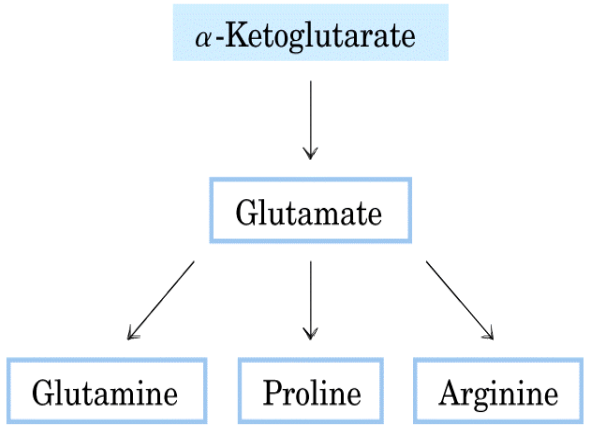
- Glu
  - ✓ By aminotransferase (transamination) (Fig 18-4)
  - ✓ By glutamine synthetase (Fig 18-8)





α-ketoglutarate → Arg

- Arg (in mammals)



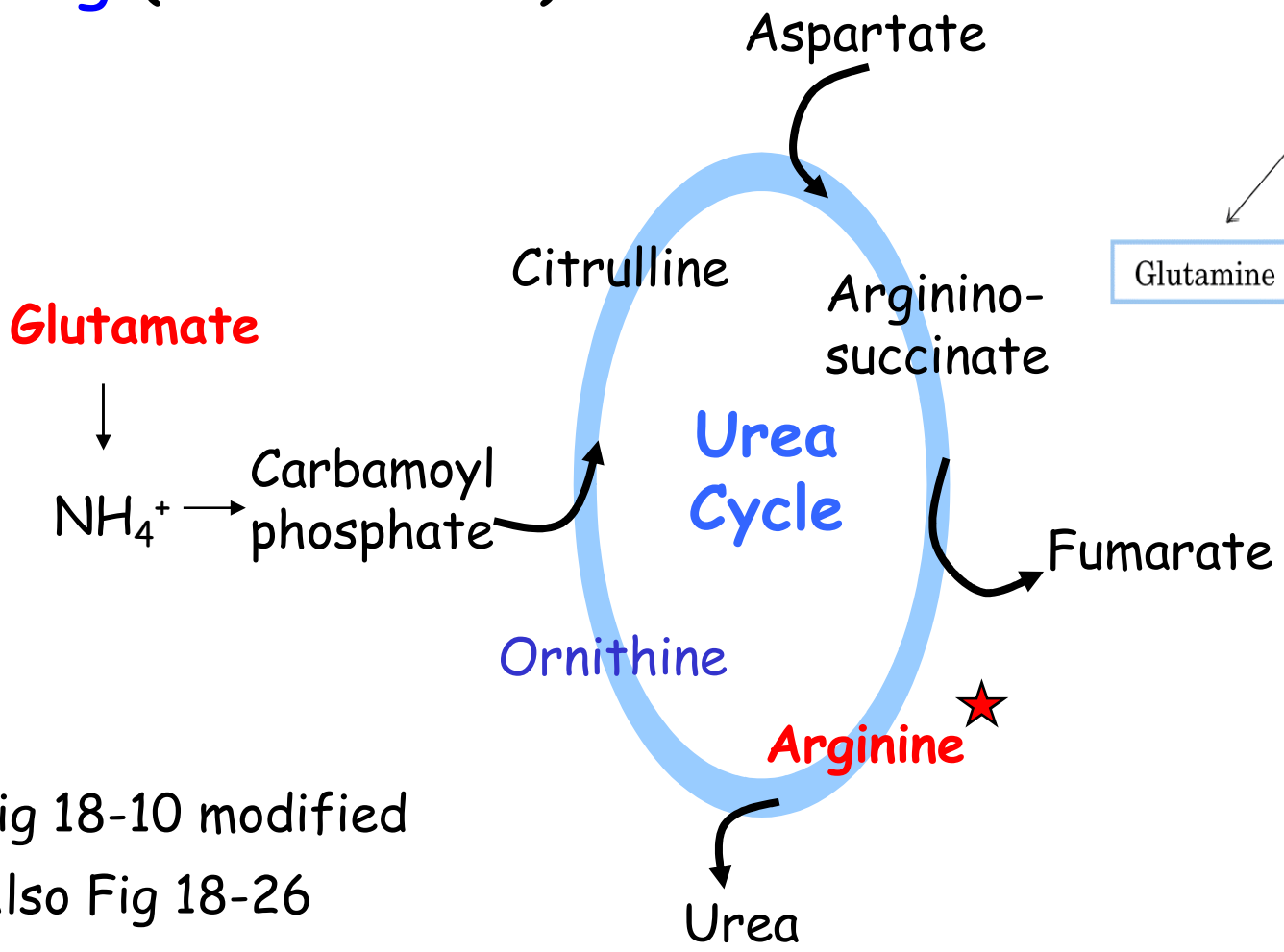


Fig 18-10 modified

Also Fig 18-26

α-ketoglutarate → Pro

- Pro (I) (In mammals)
  - ✓ Fig 22-10 left (as in bacteria)
  - ✓ Spontaneous cyclization (also in Fig 22-11 right)

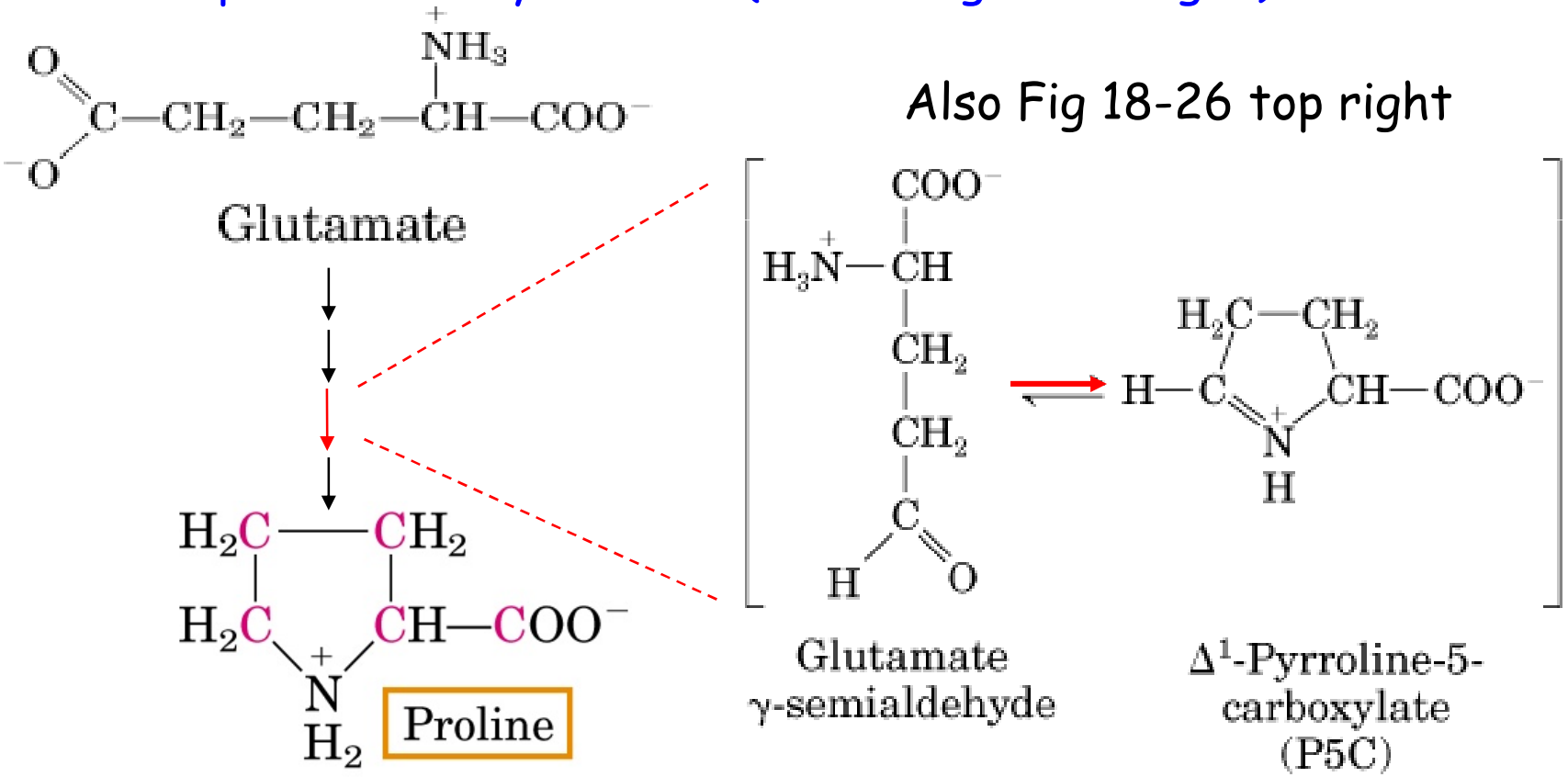
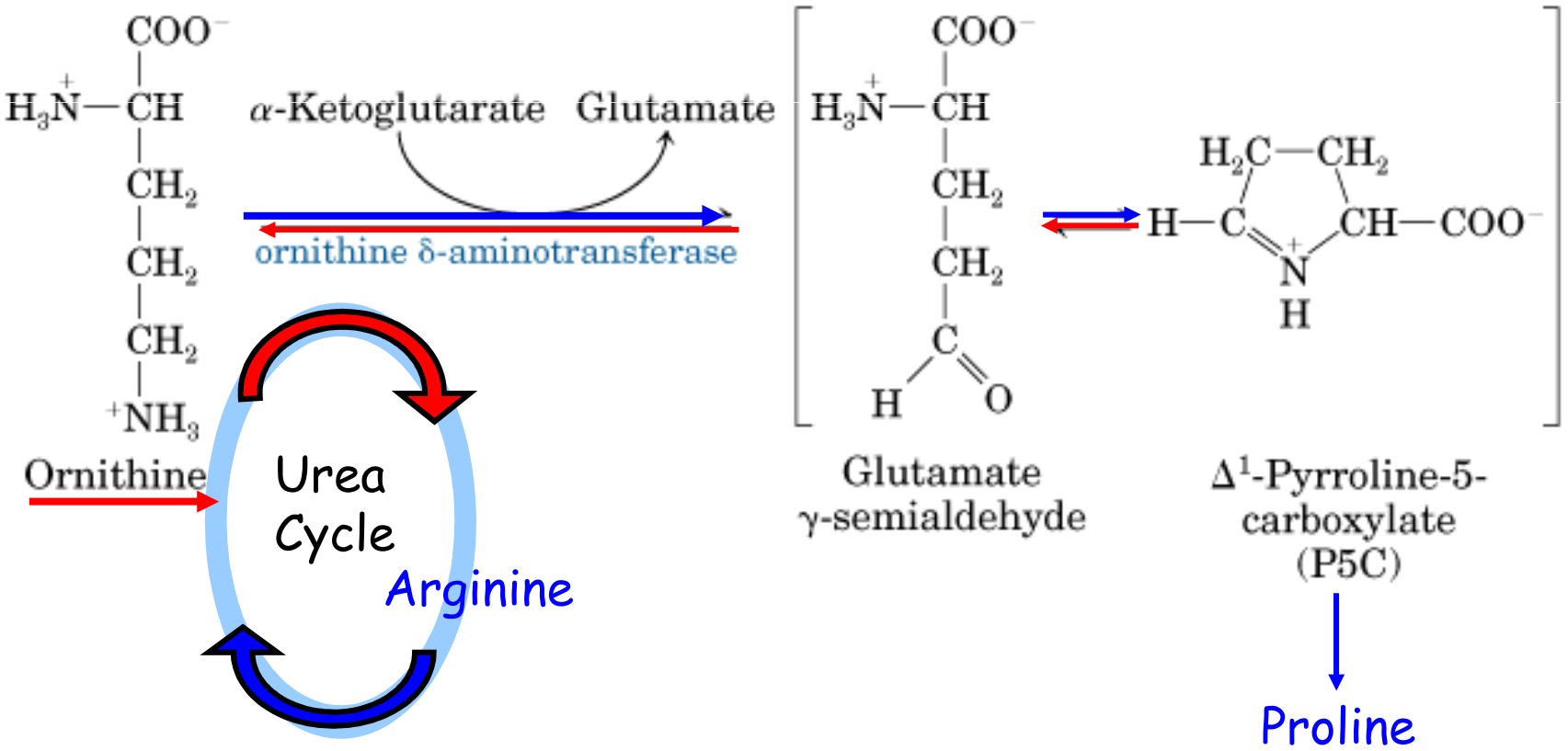


Fig 22-10 left modified

Arg ←→ Pro

- In mammals
  - ✓ Pro – (I) Fig 22-10 left or (II) Fig 22-11 forward
  - ✓ Arg – Fig 22-11 backward

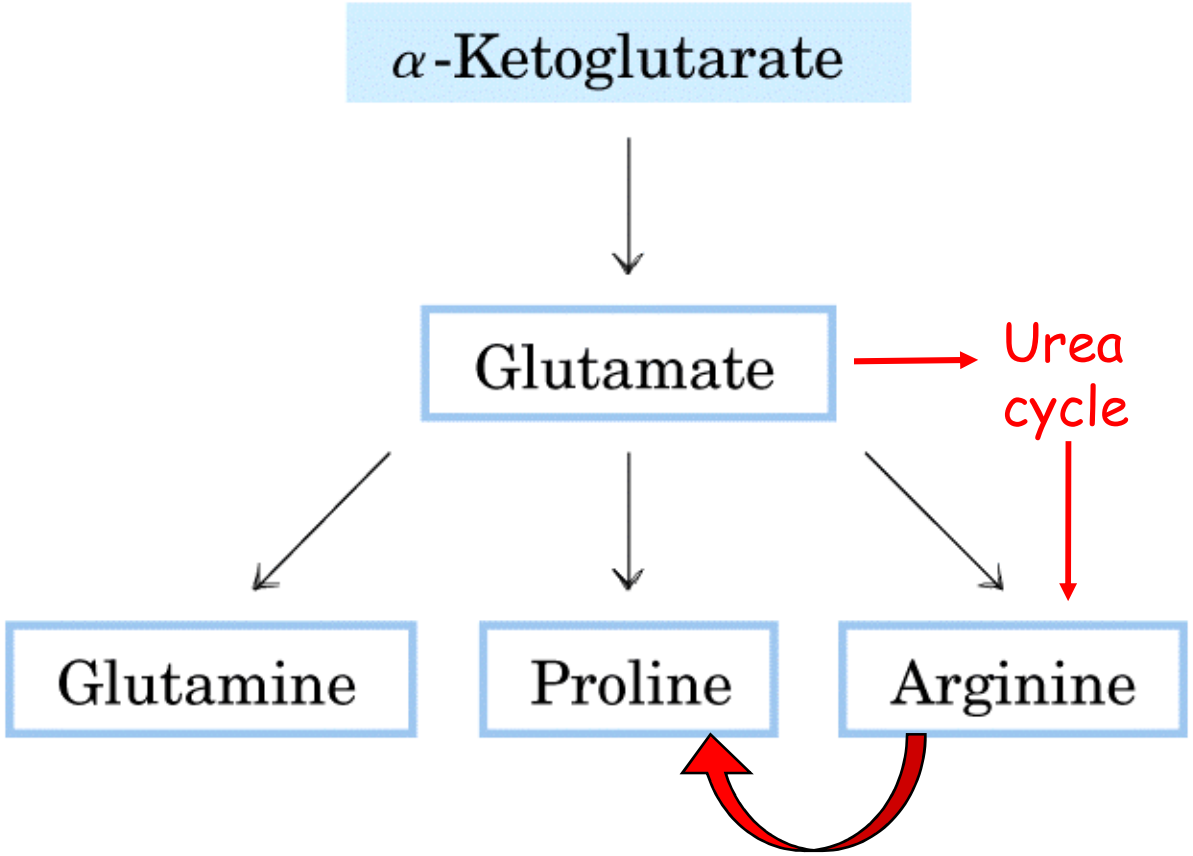
Fig 22-11



α-ketoglutarate

- Bacteria (Fig 22-10)
- In mammals

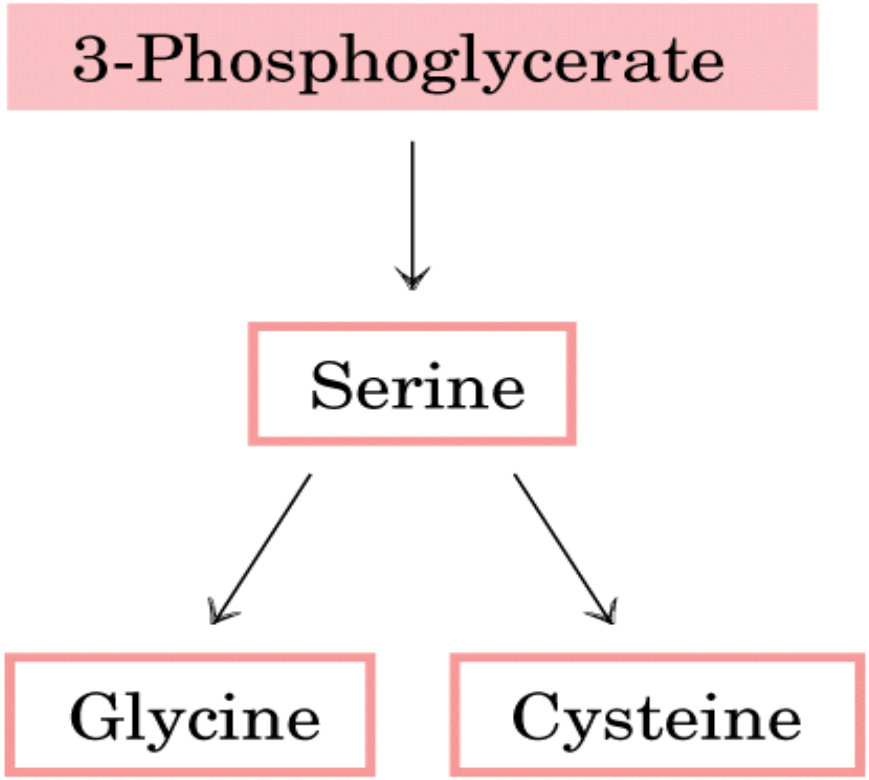
p. 861



3-phosphoglycerate

- In bacterial, plants
- In mammals

p. 863



3-PG → Ser → Gly

- Ser
  - ✓ The synthetic pathway is the same in all organisms (Fig 22-12)
- Gly
  - ✓ Derived from Ser (3C→2C, Fig 22-12)
  - ✓ From CO_2, NH_4^+, and 1 C-transfer (Fig 18-19)

Fig 22-12, p. 863

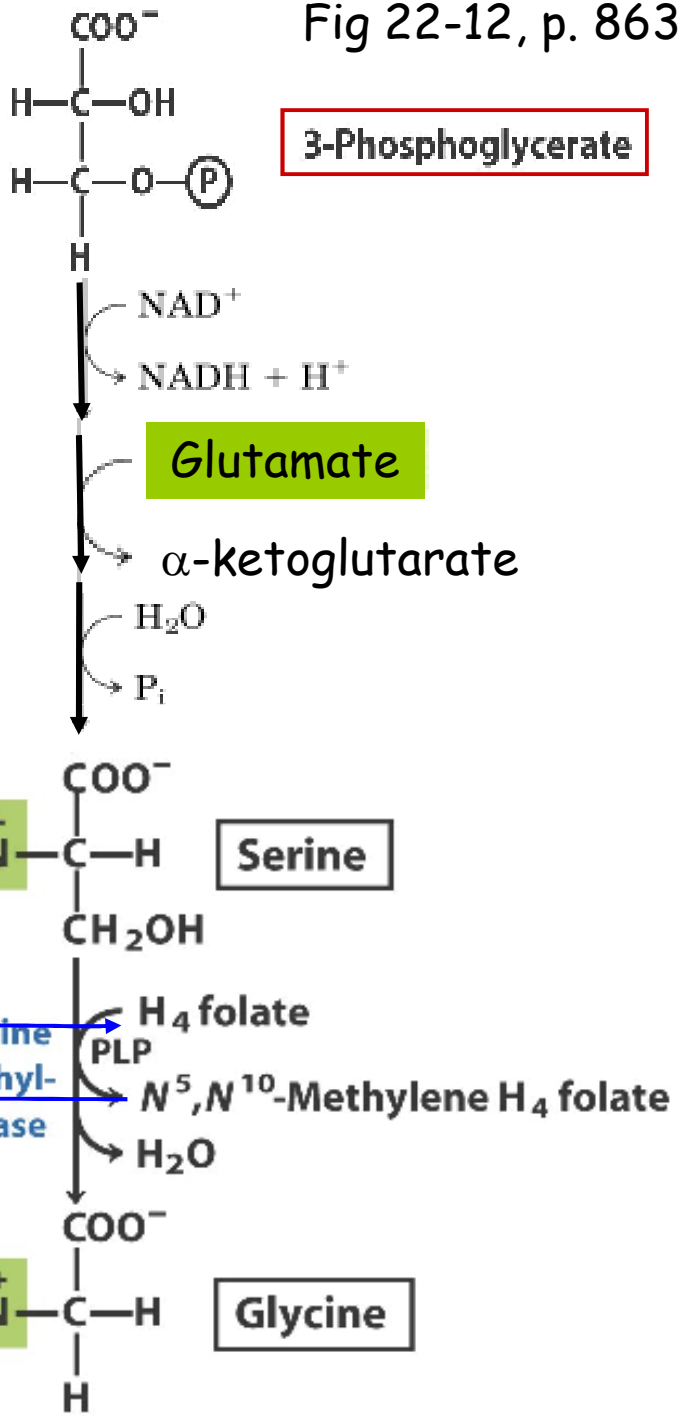
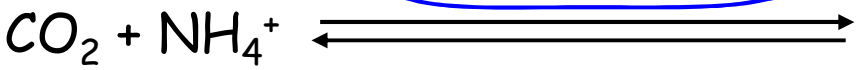
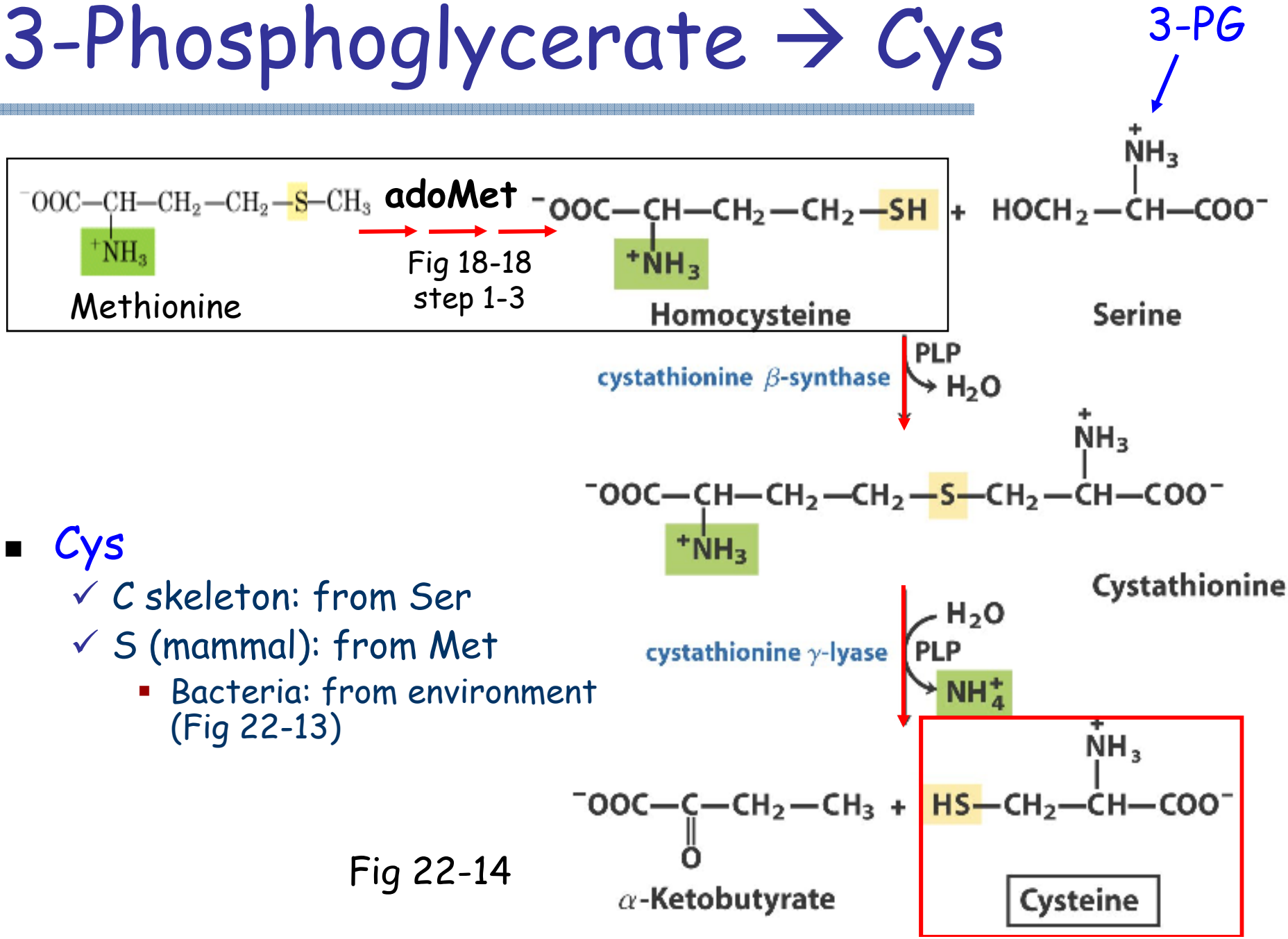


Fig 18-19, p. 692



3-Phosphoglycerate → Cys



- Cys
  - ✓ C skeleton: from Ser
  - ✓ S (mammal): from Met
    - Bacteria: from environment (Fig 22-13)

Fig 22-14

Met(S) + Ser(C) → Cys

- Cys
  - ✓ By-product in C-skeleton degradation of Met
  - ✓ Fig 18-27 (top)
    - Met → Succinyl-CoA

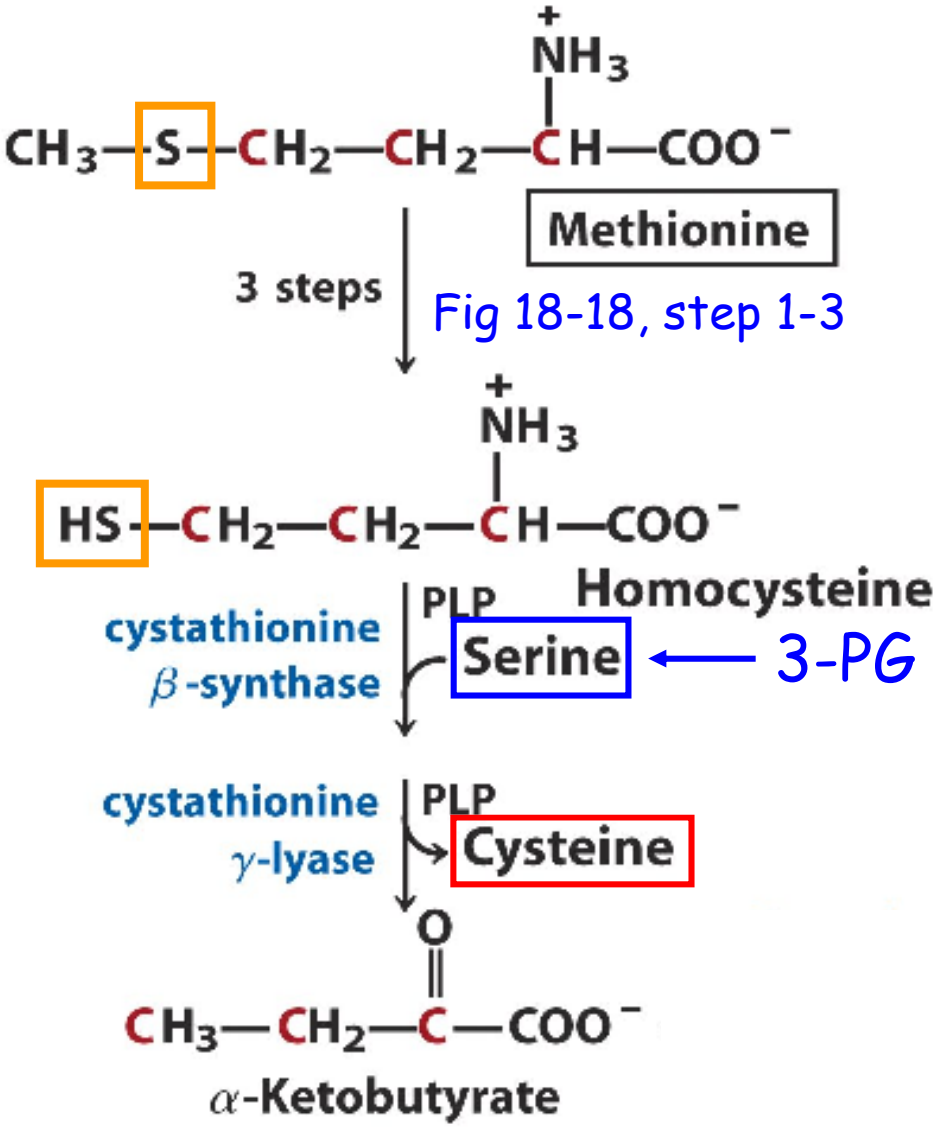
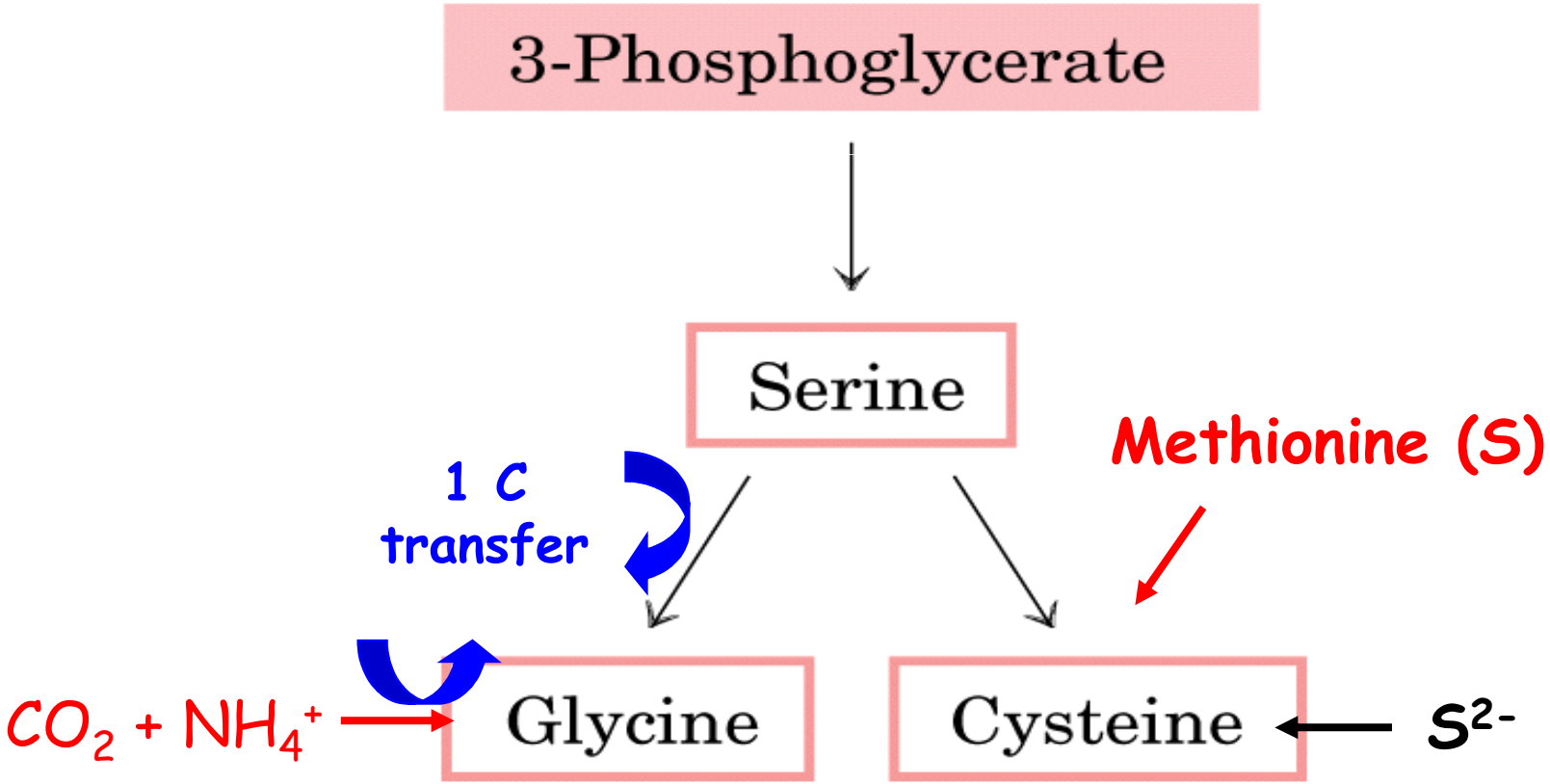


Fig 18-27 top
p. 699

3-phosphoglycerate

- Plants and bacteria
- Mammals

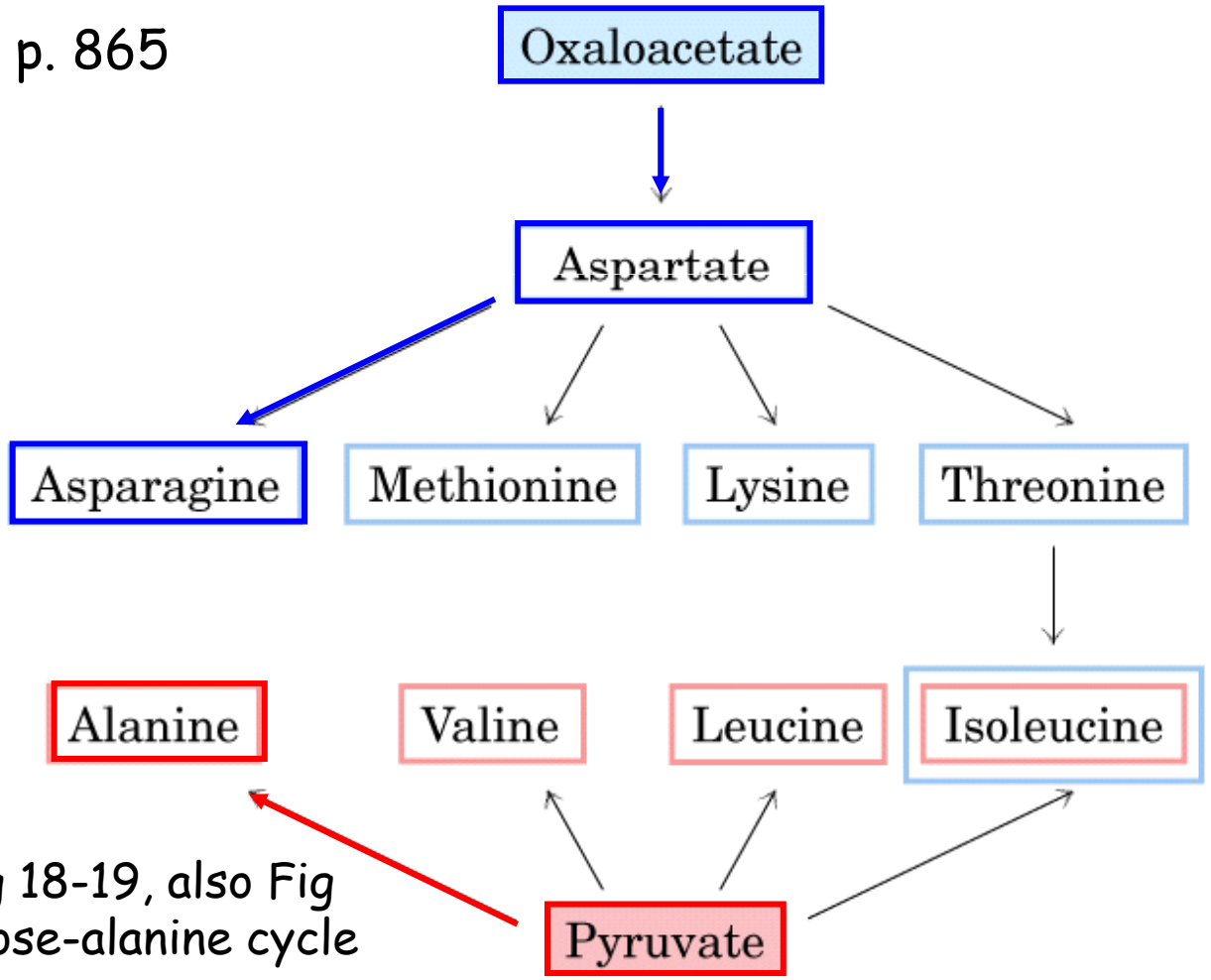
p. 863



Next:

- From oxaloacetate and pyruvate
  - ✓ 3 nonessential a.a. (same pathway in all organisms)

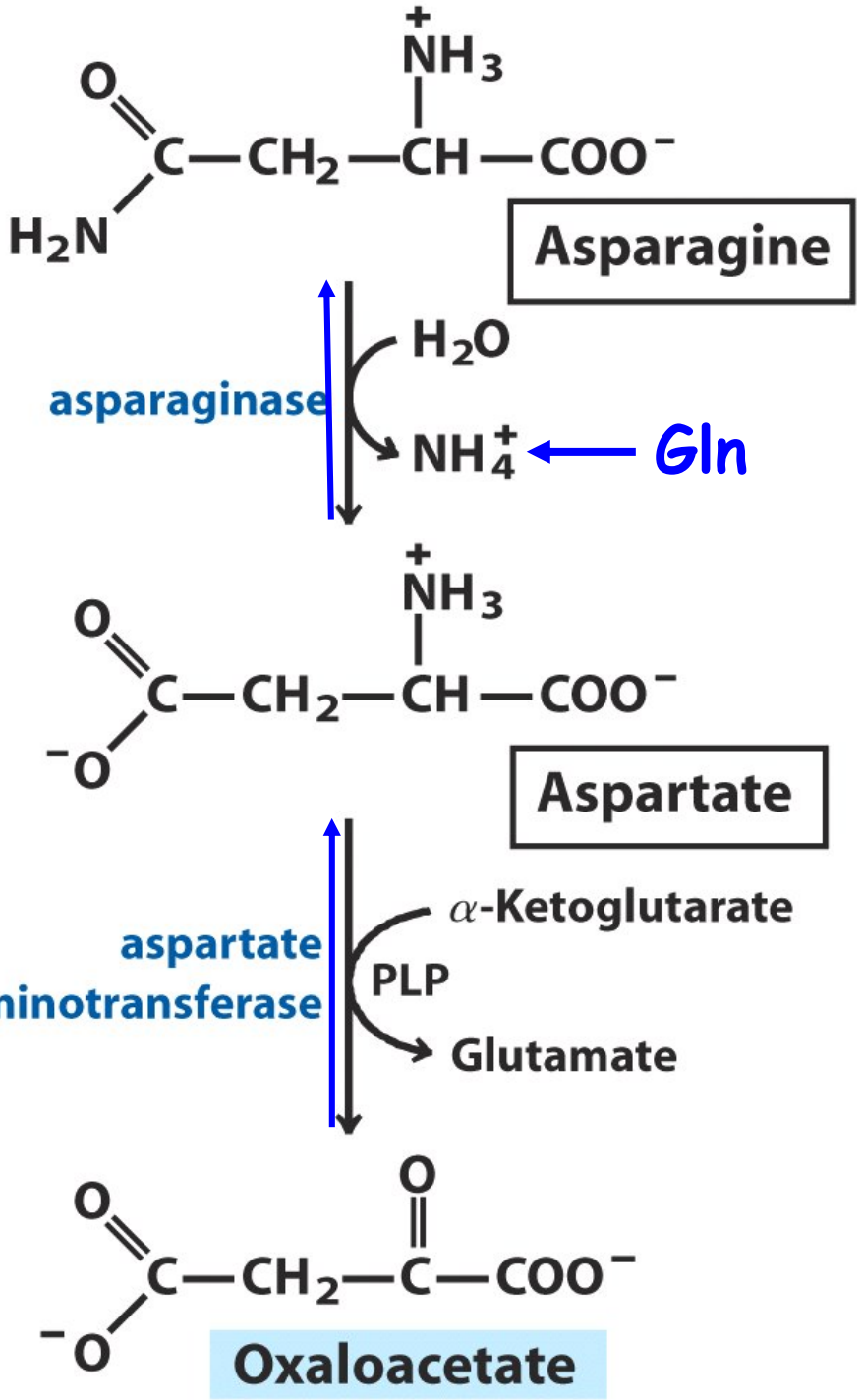
p. 865



Review Fig 18-19, also Fig 18-9, glucose-alanine cycle

Review:

- Oxaloacetate
  - ✓ Asp (transamination)
  - ✓ Asn (transamidation)



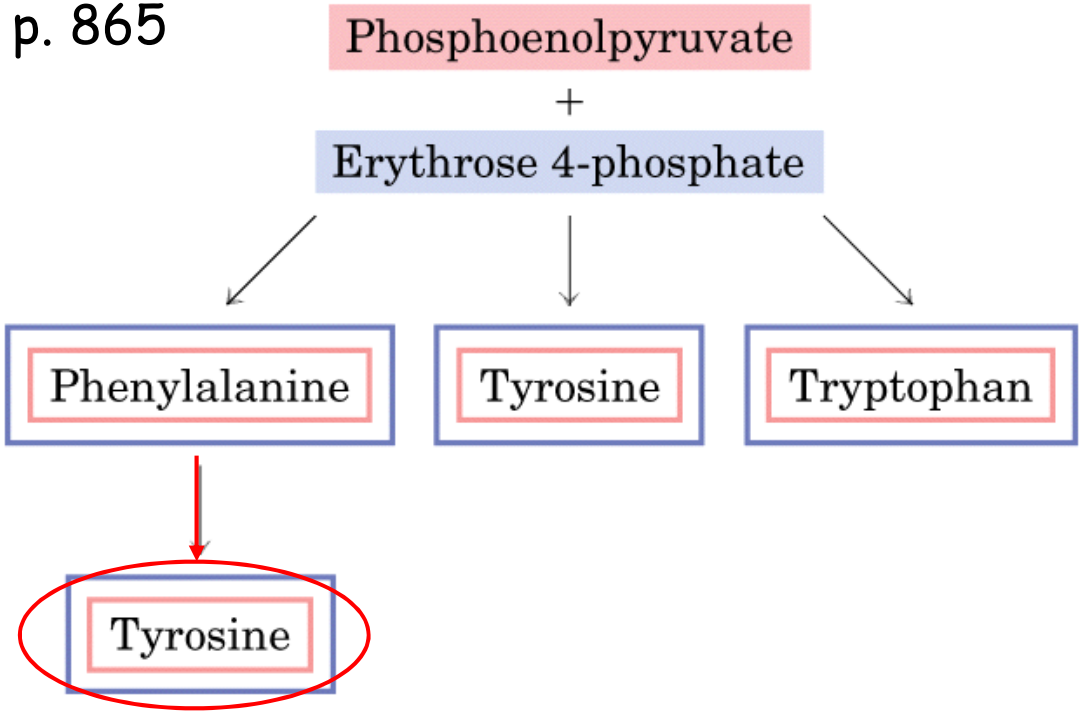
Also Fig 18-10, in mitochondria

Review Fig 18-29

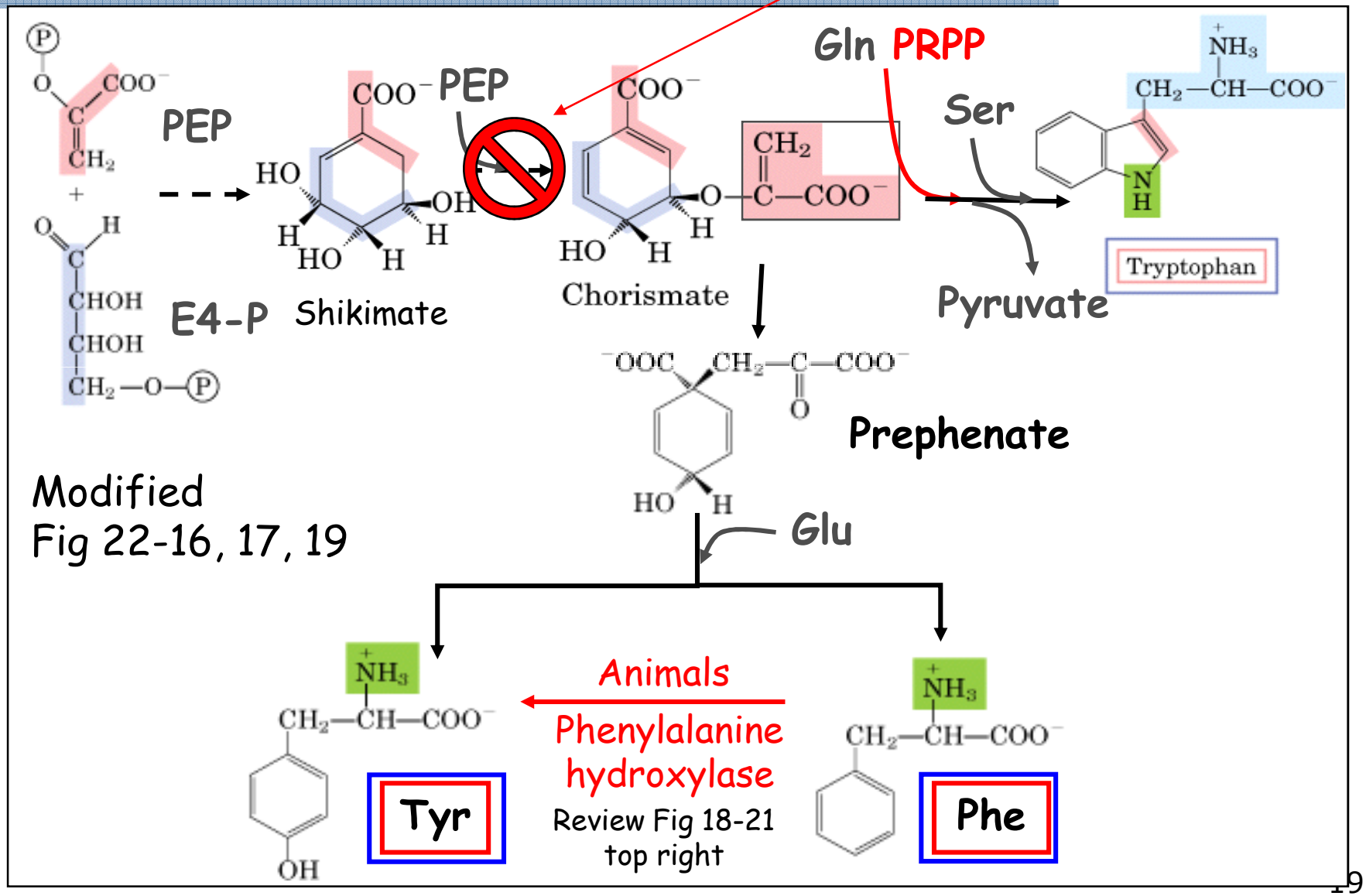
Aromatic a.a.:

- Phe, Tyr, Trp
  - ✓ From PEP and E4-P in bacteria and plants
  - ✓ Key intermediates: shikimate and chorismate:

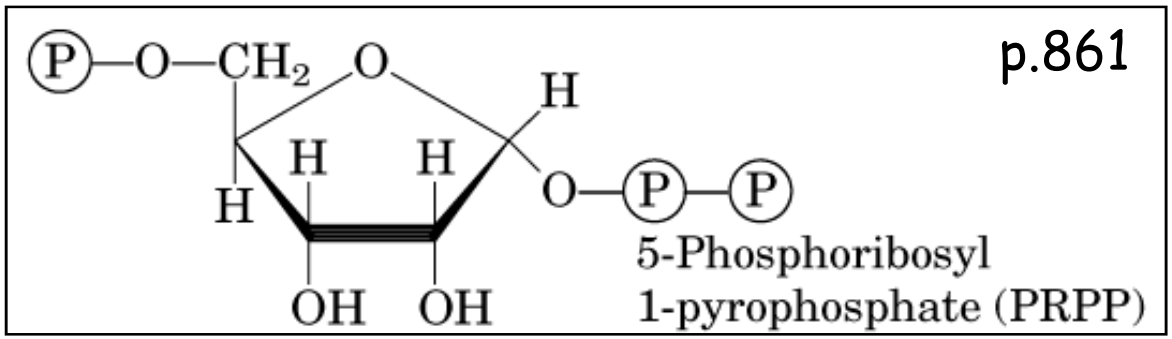
p. 865



Trp, Phe, and Tyr



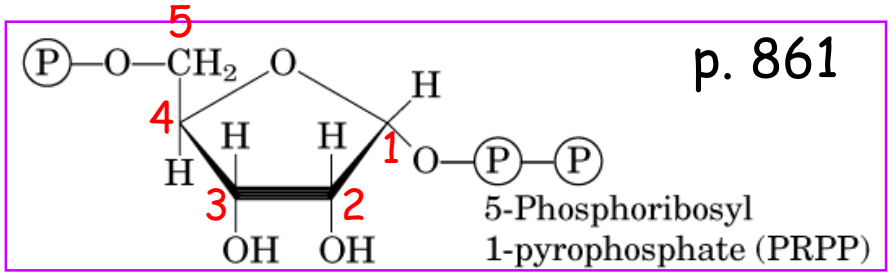
PRPP

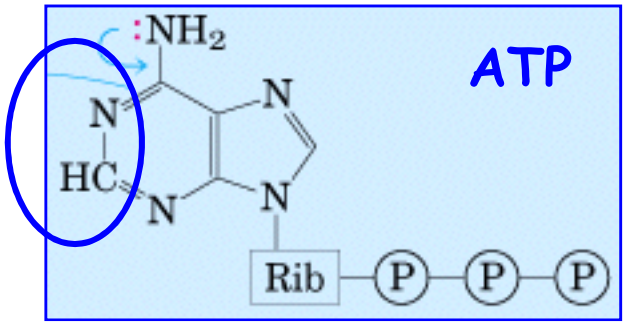


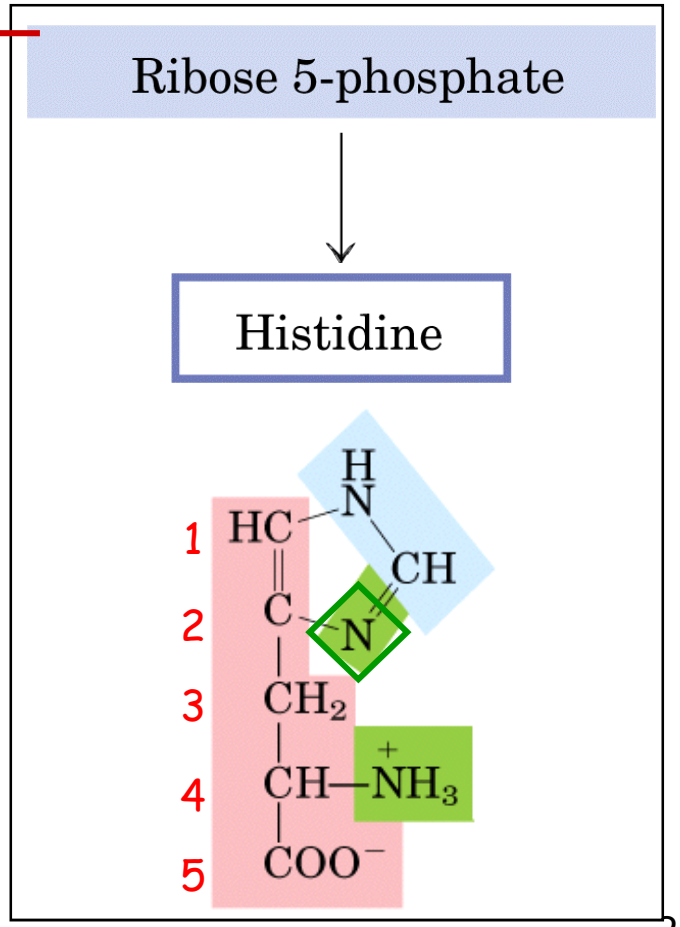
- Ⓟ = PO_4^{3-}
- PRPP = 5-phosphoribosyl-1-pyrophosphate
- Ribose 5-phosphate (from pentose phosphate pathway)
- R5-P + ATP → PRPP + AMP
- An important intermediate in several a.a. (Trp and His) and nucleotide synthesis.

His biosynthesis

- In plants and bacteria (Fig 22-20)
- Derived from 3 precursors:
  - ✓ PRPP (5 C)
  - ✓ Purine ring of ATP (1 N, 1 C)
    - ATP as a metabolite, not as fuel
  - ✓ Gln, Glu (2 N)

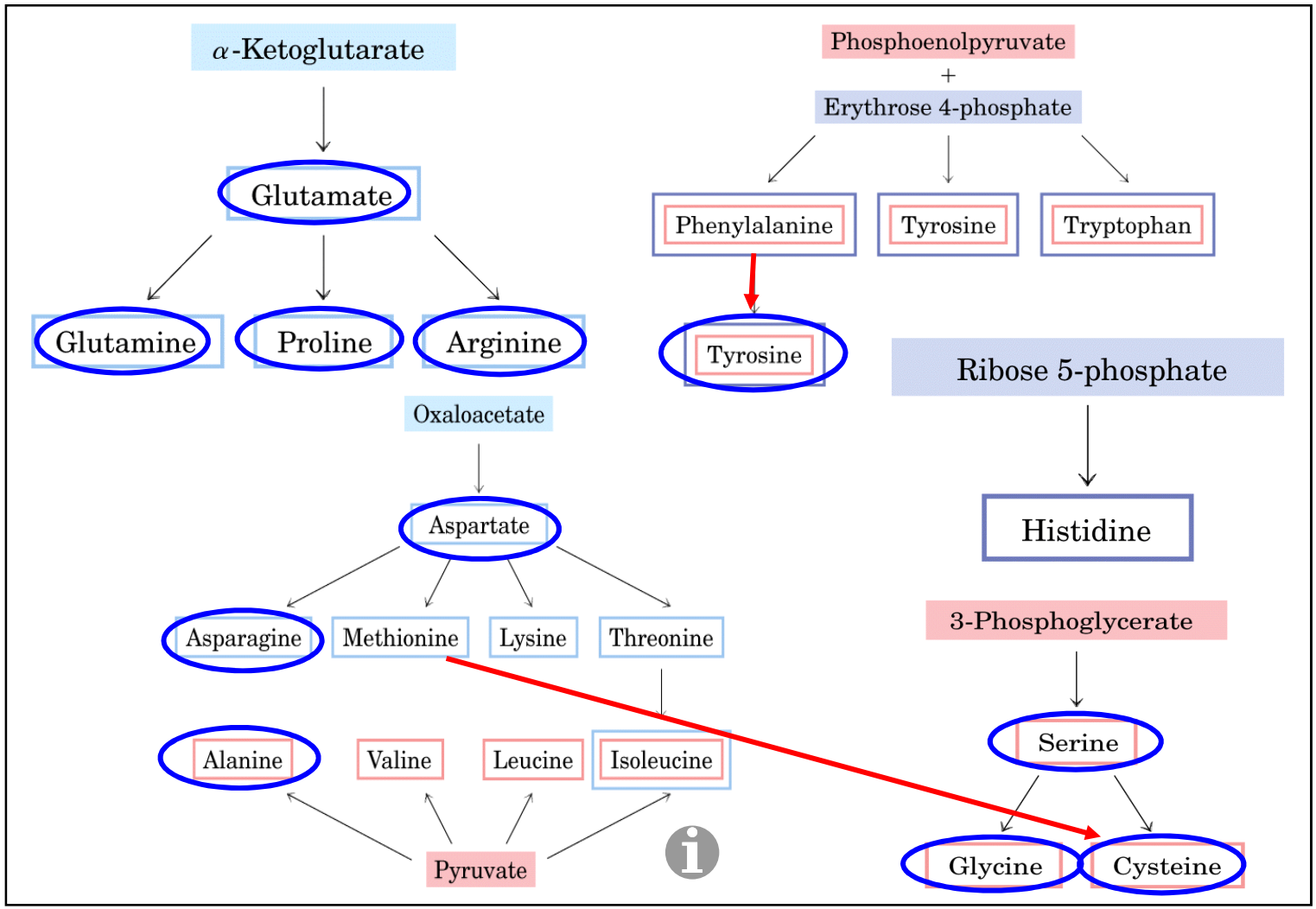






A.A. biosynthesis

- 11 nonessential a.a. in human



Regulation of a.a. synthesis

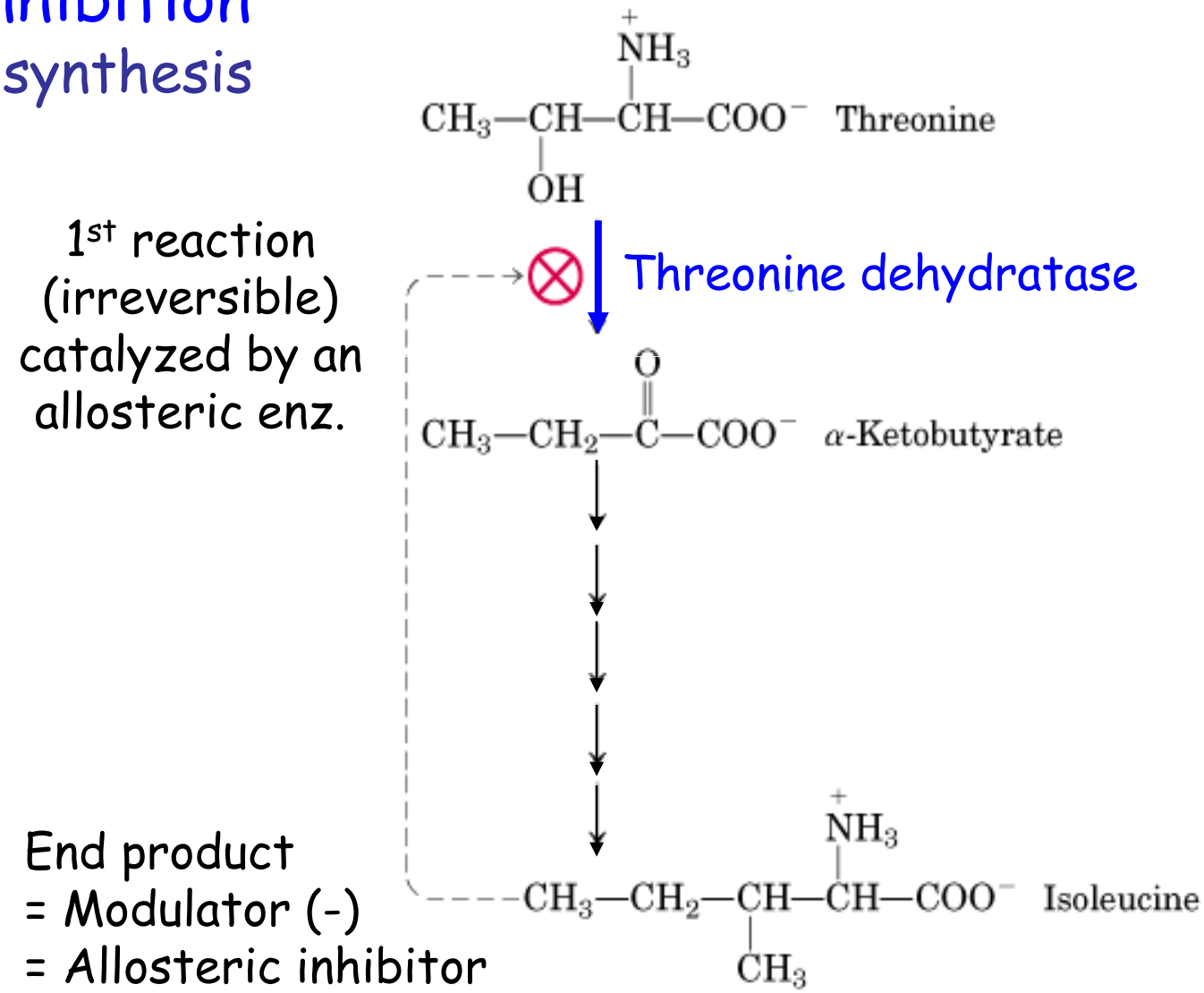
Allosteric regulation
Covalent modification

See also p.220-227
Regulatory Enzymes

Regulation (I)

p. 872,
Fig 22-21
or 6-33

- Feedback inhibition
  - ✓ e.g. Ile biosynthesis



Regulation (II)

- Concerted (cumulative) feedback inhibition
  - ✓e.g. glutamine synthetase
  - ✓Multiple allosteric inhibitors (I)
    - One I → partial inhibition
    - Multiple Is → ***more than additive***
    - All (8) → shut down
  - ✓Continuous adjustment of Gln levels to match immediate metabolic requirements

p. 857-8, and exercise 9 (p.898)

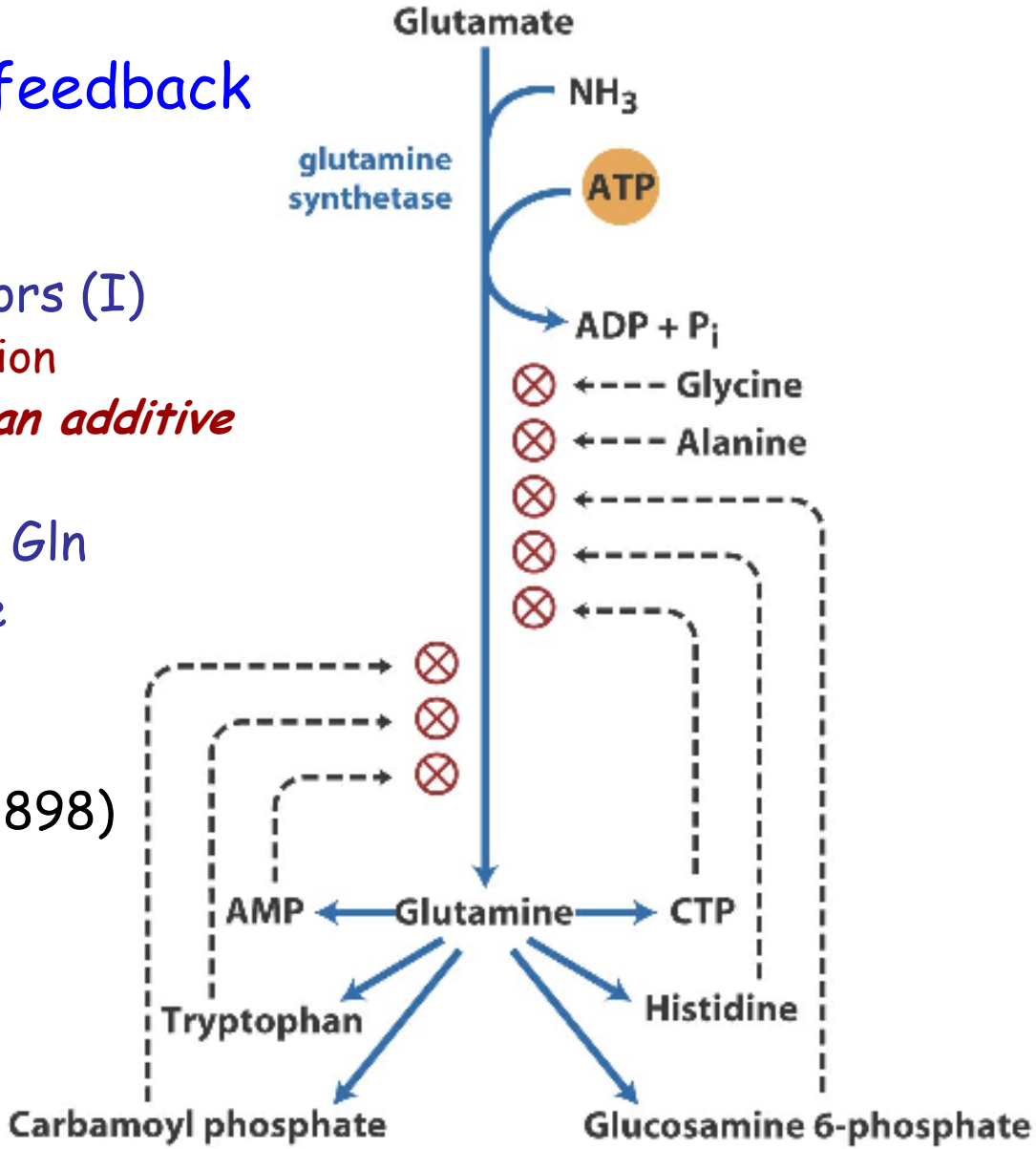


Fig 22-6
p. 858

Regulation (III)

- e.g. Asp derived a.a. in *E. coli*
  - ✓ Enz. multiplicity ($A_{1\text{-}3}$, $B_{1,2}$, $C_{1,2}$)
    - Isozyme (Box 15-2, p.577)
  - ✓ Concerted inhibition (A_1, B_2)
- Sequential feedback inhibition
  - ✓ Multiple + overlapping negative feedback inhibition
    - Rate of each a.a. synthesis
    - Coordinated synthesis

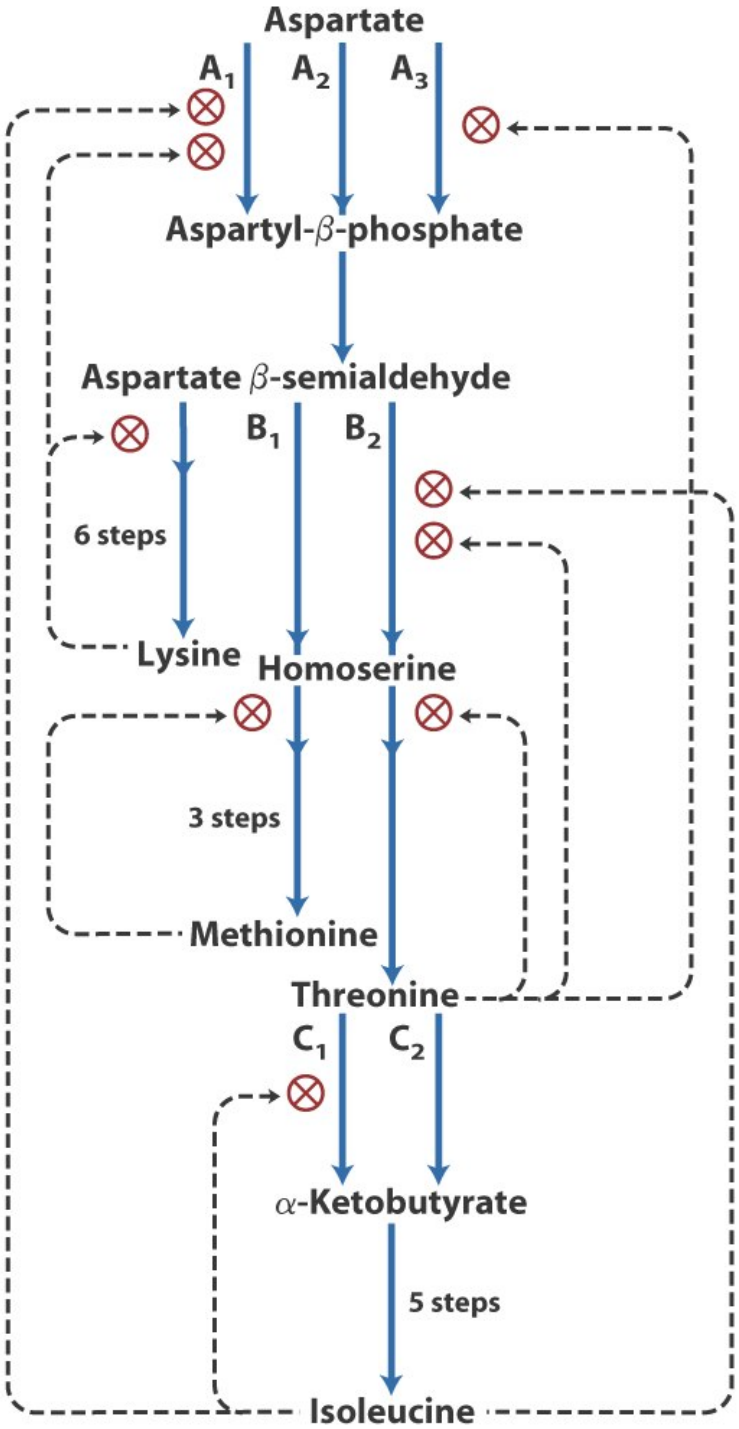
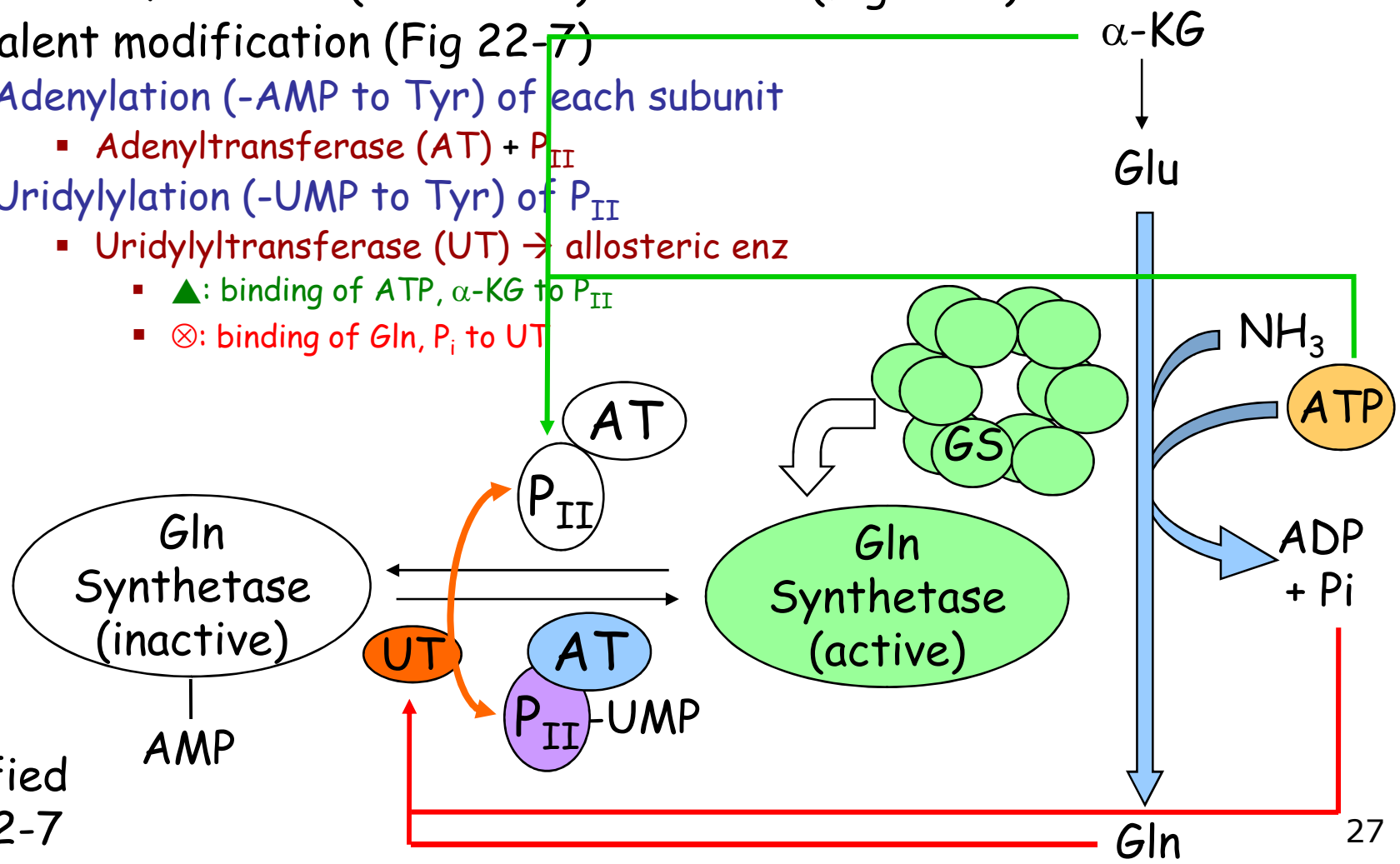


Fig 22-22, p. 872

Gln synthetase (p. 859)

- Consists of 12 identical subunits of 50 kDa
- Allosteric feedback (concerted) inhibition (Fig 22-6)
- Covalent modification (Fig 22-7)
  - ✓Adenylation (-AMP to Tyr) of each subunit
    - Adenyltransferase (AT) + P_{II}
  - ✓Uridylylation (-UMP to Tyr) of P_{II}
    - Uridylyltransferase (UT) → allosteric enz
      - ▲: binding of ATP, α-KG to P_{II}
      - ⊗: binding of Gln, P_i to UT



Modified Fig 22-7

A.A derived molecules

p. 873

Porphyrins

Creatine and Glutathione

D-amino acids

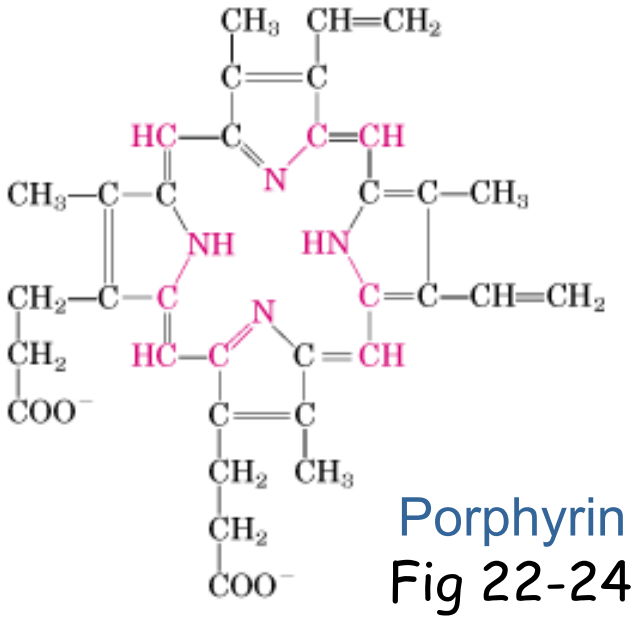
Plant substances

Biological amines

Nitric oxide

Synthesis of heme

- Porphyrin precursors: *glycine* + succinyl-CoA
- Feedback inhibited by heme product
- Congential erythropoietic porphyria (Box 22-2):
  - ✓ Porphyrin precursor accumulation, excreted in urine (red)
  - ✓ Deposited in skin (light sensitive)
  - ✓ Fluorescent teeth under UV
  - ✓ Often anemia (insufficient heme produced)



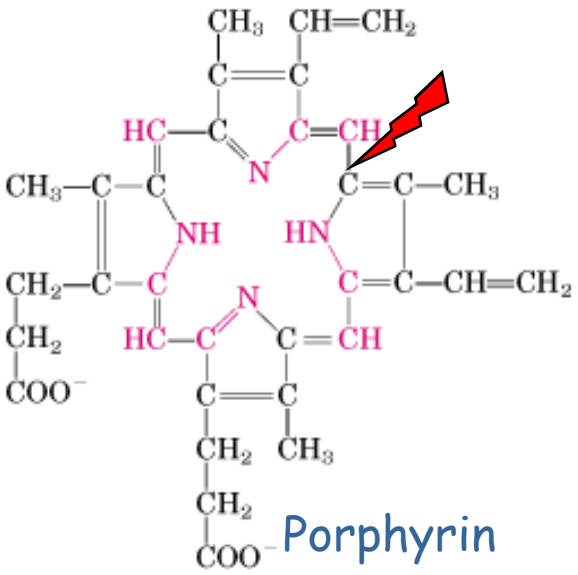
Porphyrin
Fig 22-24

Porphyrin + Fe^{2+} = Heme (Fig 5-1)

Heme breakdown

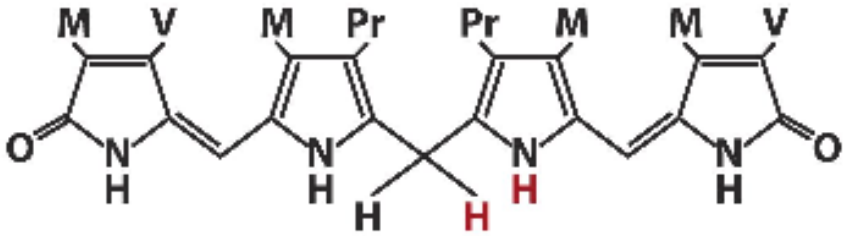
p. 875-6

- Hb = globin (protein) + Fe^{2+} + bilirubin (in spleen)
- *Bilirubin* (reddish-yellow pigment), insoluble
  - ✓ Transported to liver by serum albumin
  - ✓ Transformed to bile pigments (add glucuronide, becomes soluble) in liver
  - ✓ Excreted in the bile
- Impaired liver function or blocked bile secretion:
  - ✓ Bile leak into the blood
  - ✓ Yellowing of the skin and eyeballs
  - ✓ Jaundice



- Bilirubin (insoluble)
- Bilirubin diglucuronide (soluble)

Carbohydrate



Bilirubin (in blood)

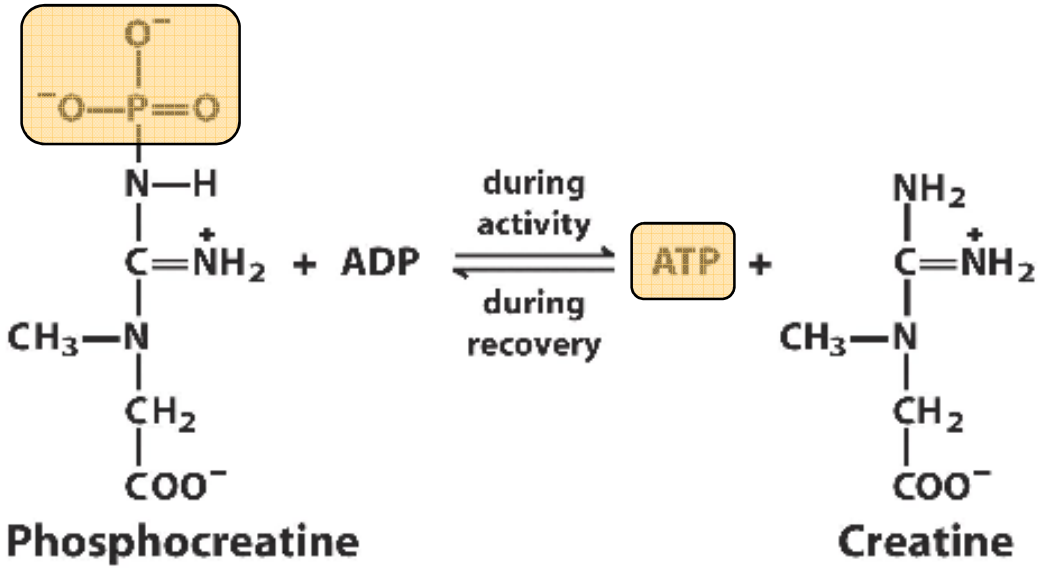
M: methyl;
V: vinyl;
Pr: propionyl

Fig 22-25 modified

Creatine and phosphocreatine

- Creatine (Cr) = Gly + Arg + Met (adoMet) p. 876-7
- Creatine + ATP → Phosphocreatine + ADP
  - ✓ Catalyzed by creatine kinase
- Phosphocreatine (PCr) = Creatine phosphate (CrP)
  - ✓ Very high [PCr] in skeletal muscle (10 x of [ATP])
  - ✓ Source of Ⓟ for ATP synthesis from ADP
  - ✓ PCr as a phosphoryl reservoir (energy buffer)

4th ed.
Fig 23-19
or 22-26



In resting muscle:

[ATP] = 4 mM

[ADP] = 0.013 mM

[PCr] = 25 mM

[Cr] = 13 mM

Energy sources for muscle

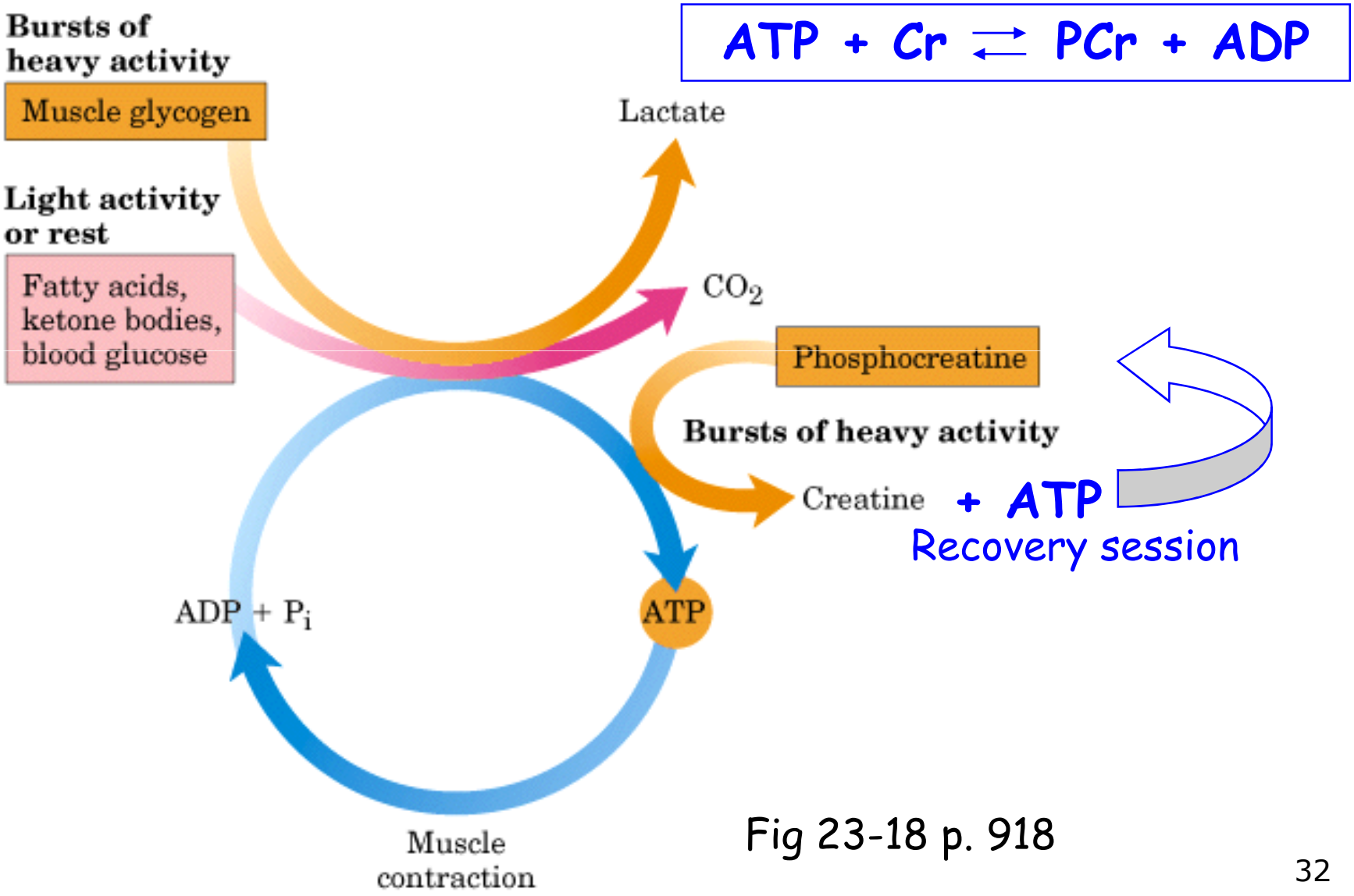
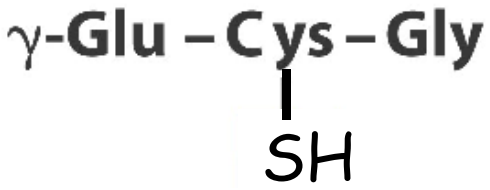


Fig 23-18 p. 918

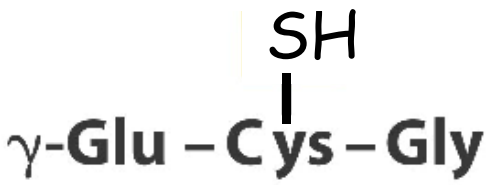
Glutathione (GSH, GSSG)

- GSH (reduced) = Gly + Glu + Cys p. 876-7
- Present at high levels as a redox buffer
  - ✓ Maintain the –SH of protein in reduced state
  - ✓ Maintain iron of heme in Fe^{2+} state
  - ✓ As a reducing agent for glutaredoxin in dNT synthesis (Fig 22-39)
  - ✓ Remove toxic peroxides under aerobic condition
    - 2 GSH + R-O-O-H → GSSG + H_2O + R-OH
    - Catalyzed by glutathione peroxidase (containing selenium, Se, in the form of selenocysteine (Fig 3-8a)



Glutathione (reduced, GSH)

Fig 22-27



Glutathione (reduced, GSH)

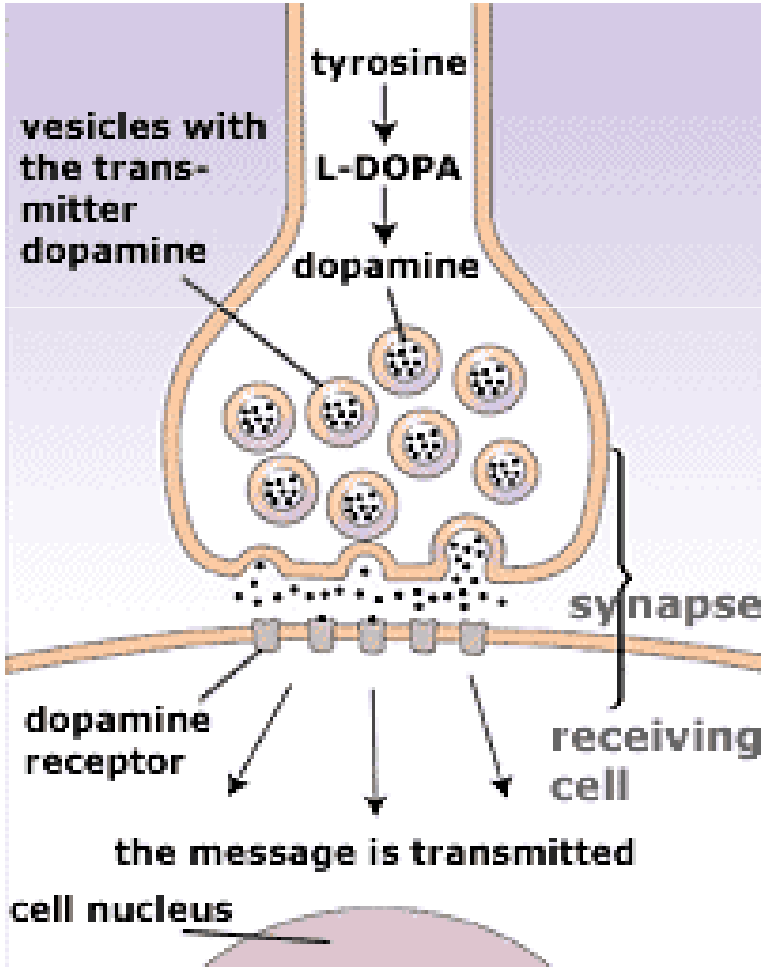
Biological amines

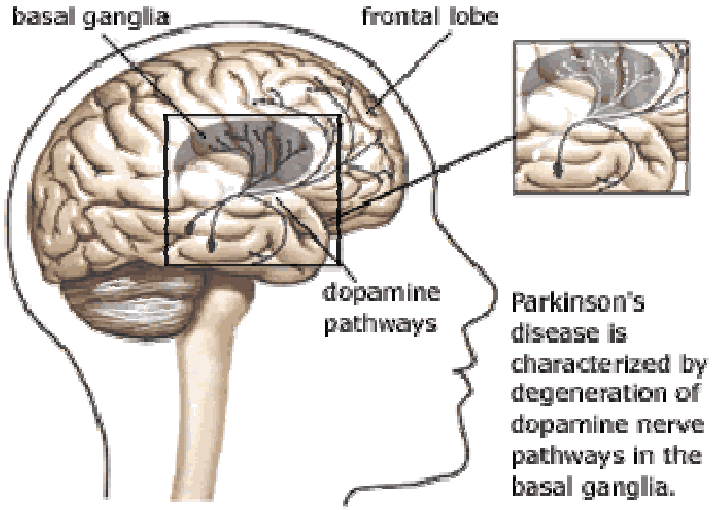
- A.A. are converted to amines by decarboxylation (requiring PLP as a cofactor, Fig 18-6, 22-29)
- Catecholamines (Tyr)
  - ✓ Dopamine, norepinephrine, epinephrine
  - ✓ Affects blood pressure
  - ✓ Parkinson's disease: underproduction of dopamine
  - ✓ Schizophrenia: overproduction of dopamine
- γ-aminobutyric acid (GABA) (Glu)
  - ✓ An inhibitory neurotransmitter (NT)
  - ✓ Epileptic seizures: underproduction of GABA
    - Treatment: increase GABA level
      - GABA analogs
      - Inhibitor of GABA degrading enzyme (GABA aminotransferase)
- Serotonin (Trp)
  - ✓ Neurotransmitter

p. 879

Dopamine and Parkinson Disease

- Tyrosine as precursor





http://www.nobel.se/medicine/educational/poster/2000/index.html

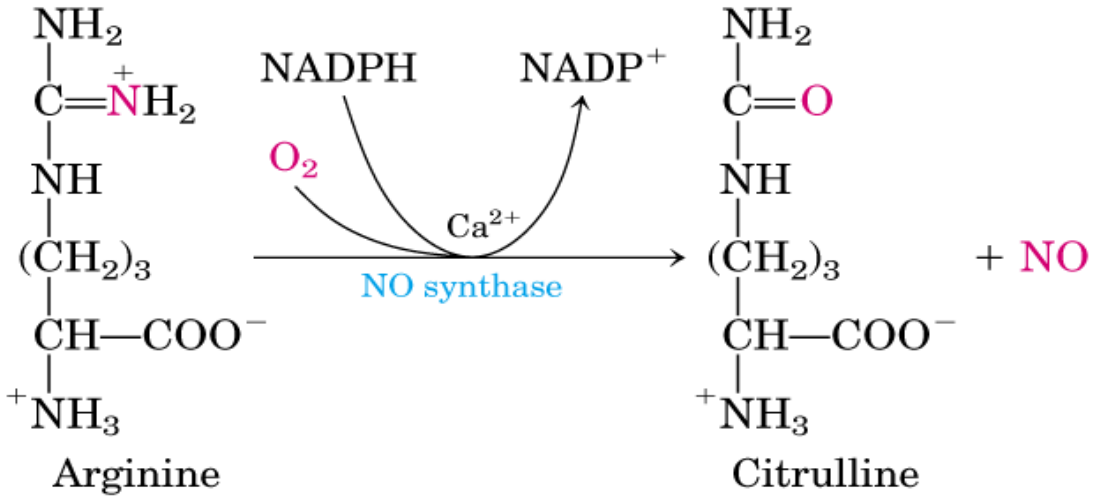
More amines by decarboxylation

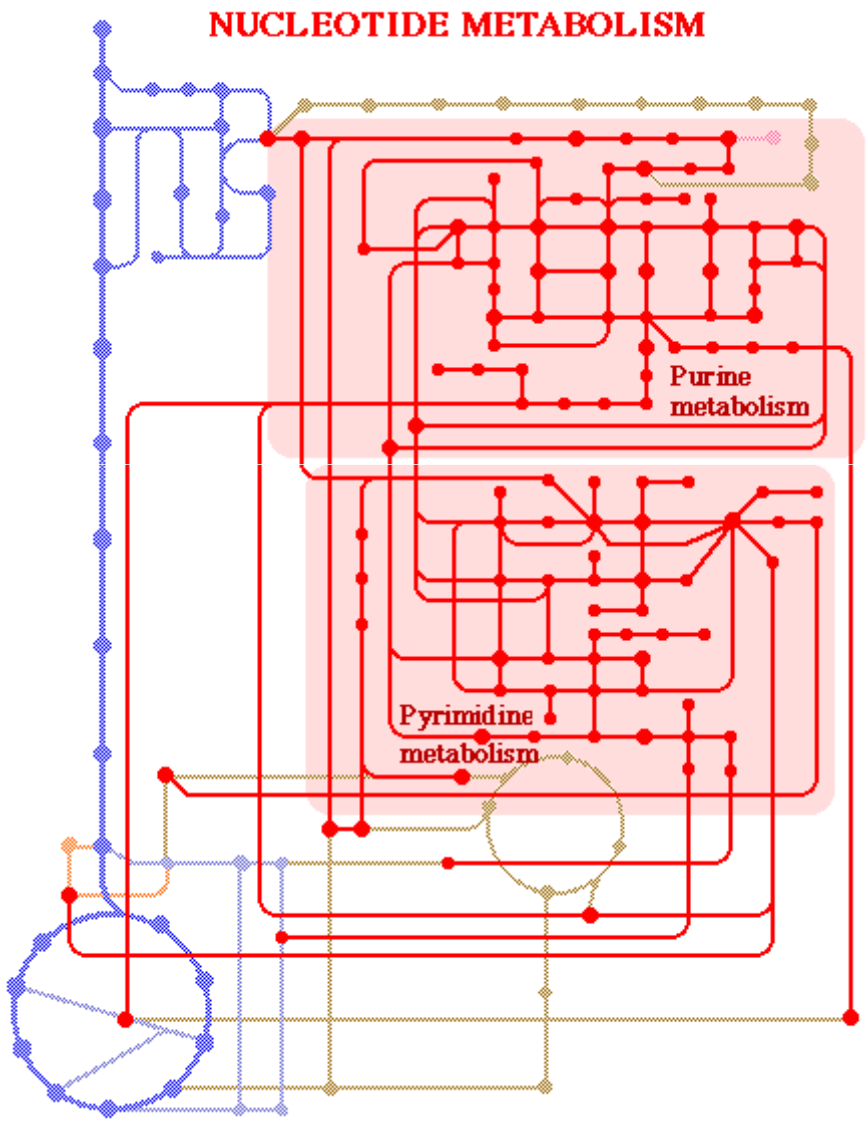
- Histamine (His) p. 879-80
  - ✓ Vasodilator in animal tissue, involved in allergy
  - ✓ Stimulate stomach acid secretion
    - Cimetidine (Tagamet)
      - Structural analog of histamine = histamine receptor antagonist
      - Promoting healing of duodenal ulcers by inhibiting gastric acid secretion
- Polyamine: spermidine and spermine (Met and ornithine)
  - ✓ Used in DNA packaging
  - ✓ Required in large amounts in rapidly dividing cells
    - African sleeping sickness (trypanosome-caused disease, Box 22-3, 錐蟲病):
      - Ornighine decarboxylase has a much slower turnover rate in trypanosome than in human (human, fast turnover, less side-effect of enzyme inhibitor)
      - DMFO (difluoromethylornithine): suicide inhibitor or mechanism-based inhibitor

Nitric oxide (NO)

- Derived from Arg
- Unstable gas, diffuse through membranes
  - ✓ Neurotransmission
  - ✓ Blood clotting
  - ✓ Regulating blood pressure
    - Muscle relaxant (p.446, Ch 12)
      - Cardiac muscle: heart disease and nitroglycerine
      - Smooth muscle: erectile dysfunction and Viagra

Fig 22-31 or p.446





Nucleotides

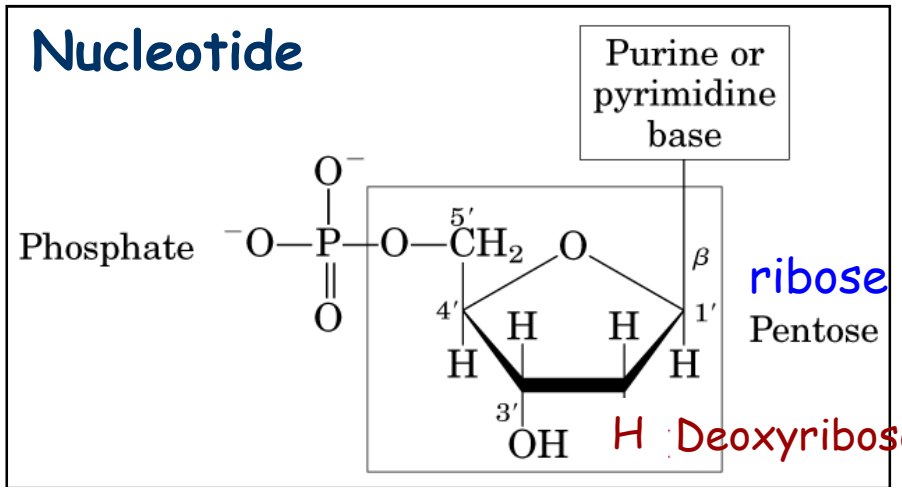
Biosynthesis and Degradation

p.882

Nucleotide (核苷酸)

- Cellular functions
  - ✓ Precursor of nucleic acid (核酸)
    - RNA (ribonucleic acid): A, U, C, G
    - DNA (deoxyribonucleic acid): A, T, C, G ⟹ dA, dT, dC, dG
  - ✓ Carrier of chemical energy
    - ATP and GTP
  - ✓ Act as cofactors and activated intermediates
    - NAD, FAD, S-adoMet, CoA
    - UDP-glucose, CDP-diacyglycerol
  - ✓ Act as cellular second messengers
    - cAMP, cGMP
- Basic structure
  - ✓ Base
  - ✓ Phosphate
  - ✓ Pentose
    - Ribose
    - Deoxyribose

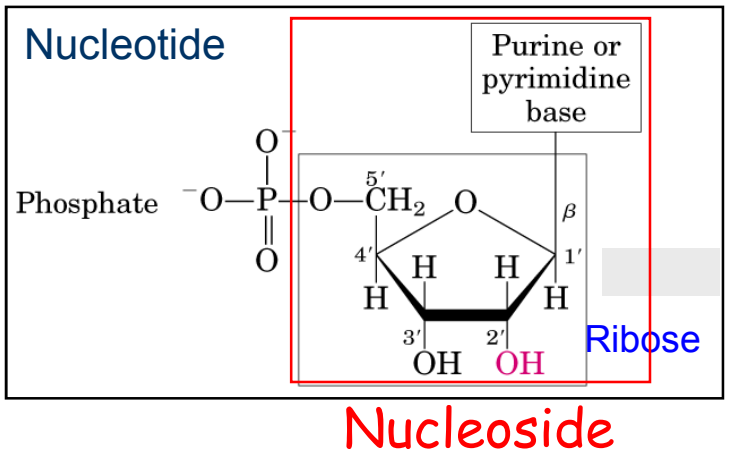
Fig 8-1a on p.271



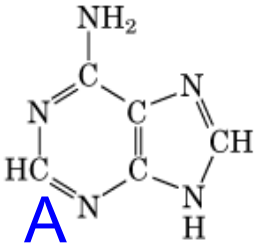
Nucleotide Synthesis

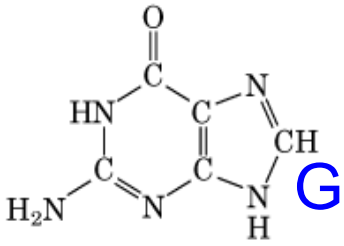
p.882

- de novo pathways
  - ✓ From small molecules readily available in cells
  - ✓ A.A., ribose 5-phosphate, CO_2, and NH_3
  - ✓ The bases are *not* intermediates in this pathway
- Salvage pathways
  - ✓ Recycle the free bases and nucleosides released from nucleic acid breakdown

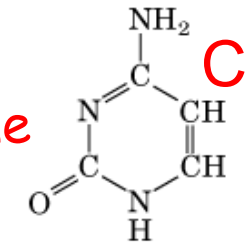


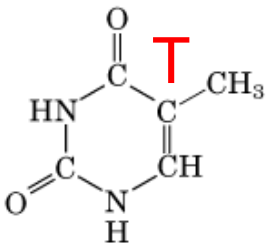
Purine





Pyrimidine





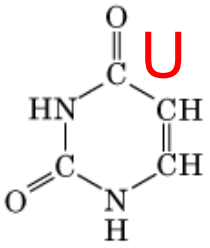
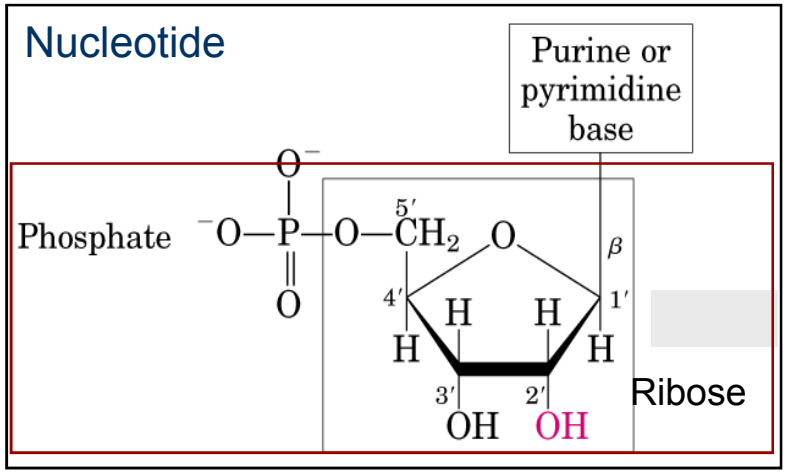


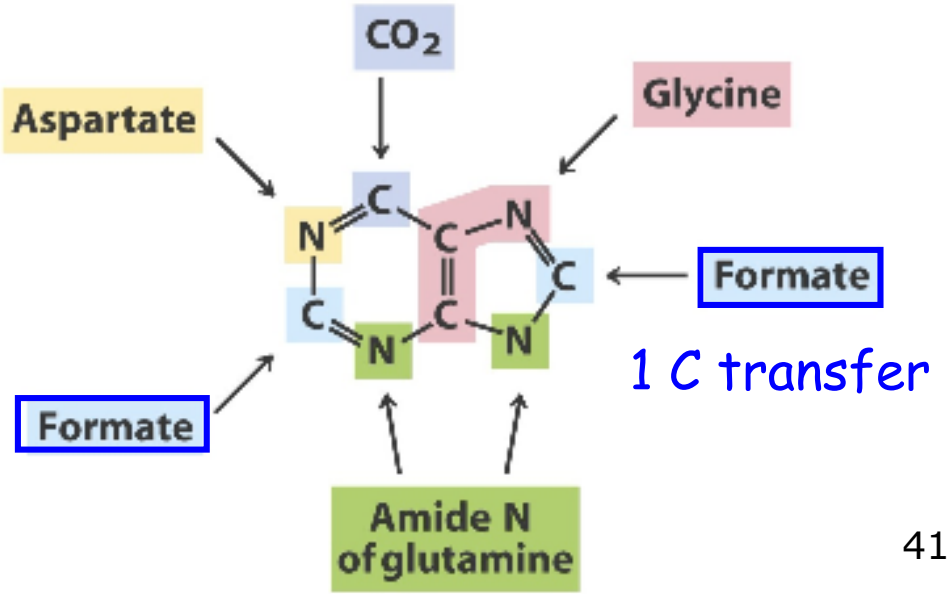
Fig 8-2 on p.272

Purine synthesis (I)

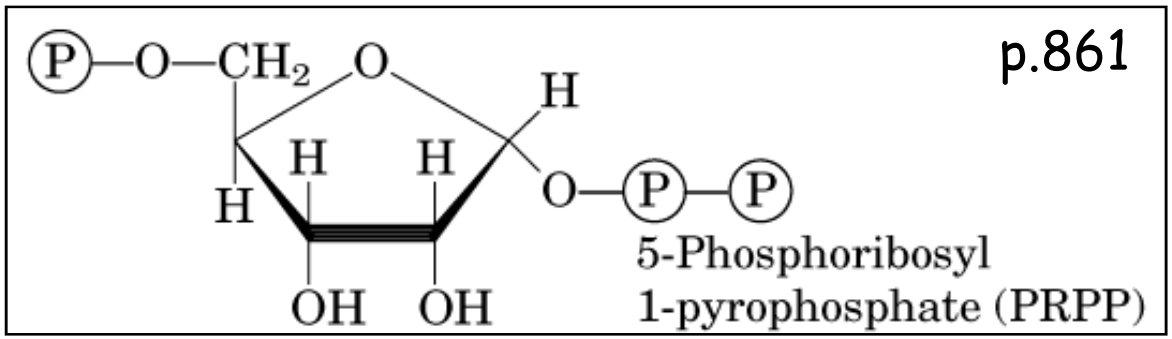
- A (AMP), G (GMP)
- Adding functional groups *one by one* onto a preexisting ribose phosphate → inosinate (IMP)
- Fig 22-33 (p.884):
  - ✓ **PRPP**, Gln, **Gly**, **1-C**, Gln, CO_2, **Asp**, **1-C** → IMP
  - ✓ In steps 8-9, Asp has an analogous role in the urea cycle

Fig 22-32





PRPP

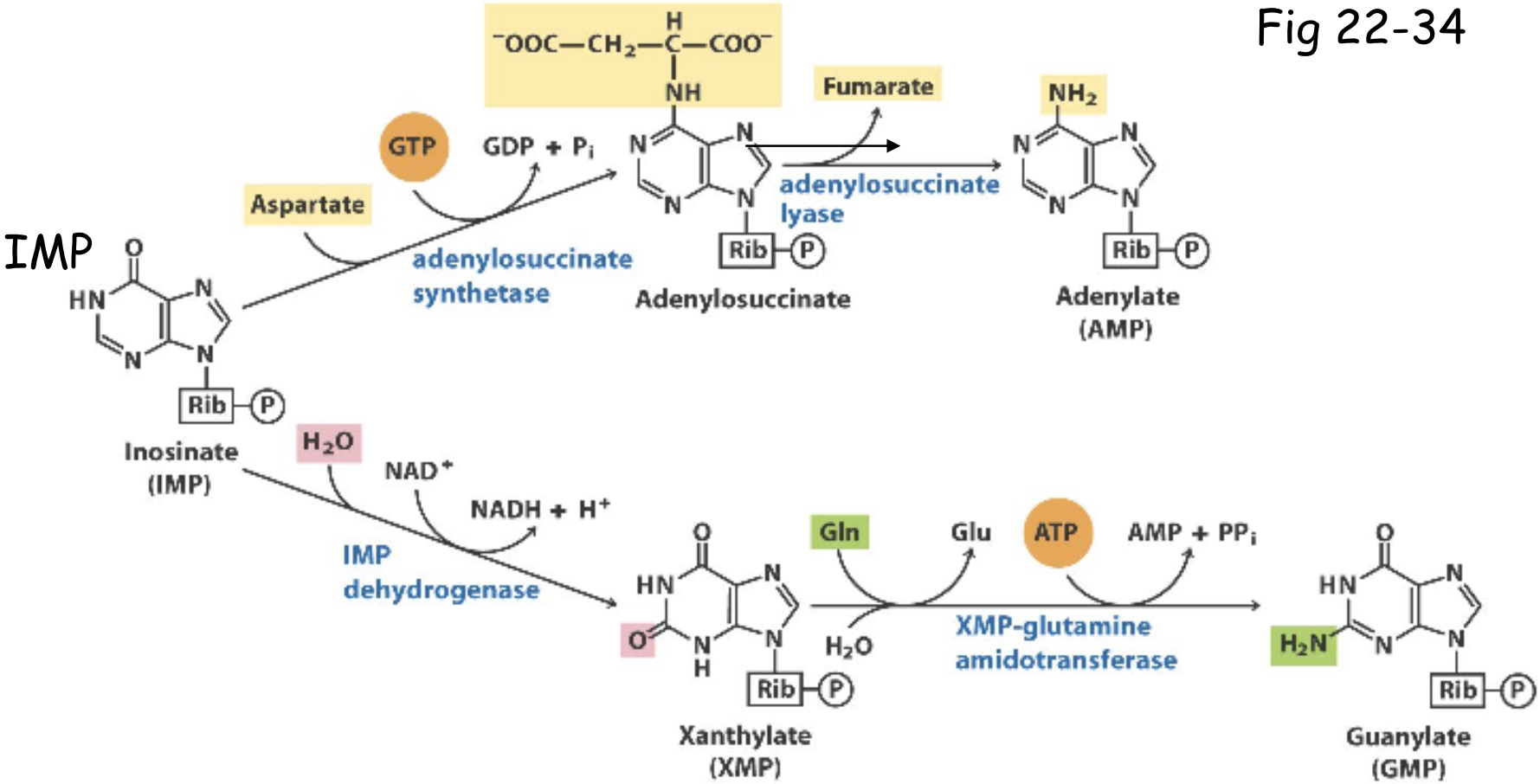


- Ⓟ = PO_4^{3-}
- PRPP = 5-phosphoribosyl-1-pyrophosphate
- Ribose 5-phosphate (from pentose phosphate pathway)
- R5-P + ATP → PRPP + AMP
- An important intermediate in several a.a. (Trp and His) and nucleotide synthesis.

Purine synthesis (II)

- IMP (inosinate, inosine monophosphate)
  - ✓ IMP + Asp → AMP (GTP → GDP + Pi)
  - ✓ IMP → oxidized IMP + Gln → GMP (ATP → AMP + PPi)

Fig 22-34



Regulations

- Rate of synthesis
  - ✓ Overall rate
  - ✓ Relative rate
- 3 major feedback mechanisms
  - ✓ 1st – Concerted
  - ✓ 2nd – A, G inhibition independently
  - ✓ 3rd - a reciprocal energy arrangement (Fig 22-34)

p. 885

Q: Can you identify the "sequential inhibition" in this example ?

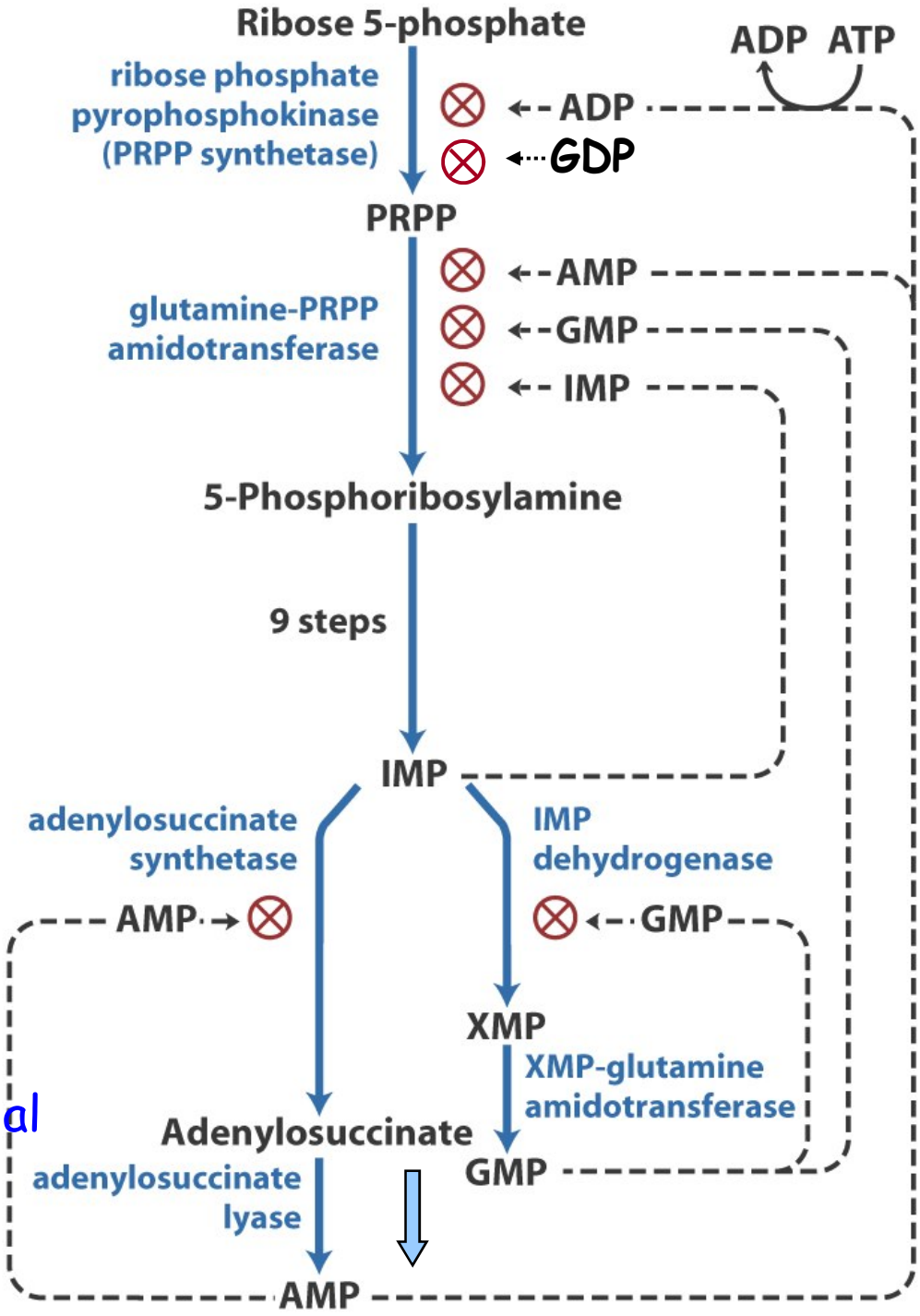
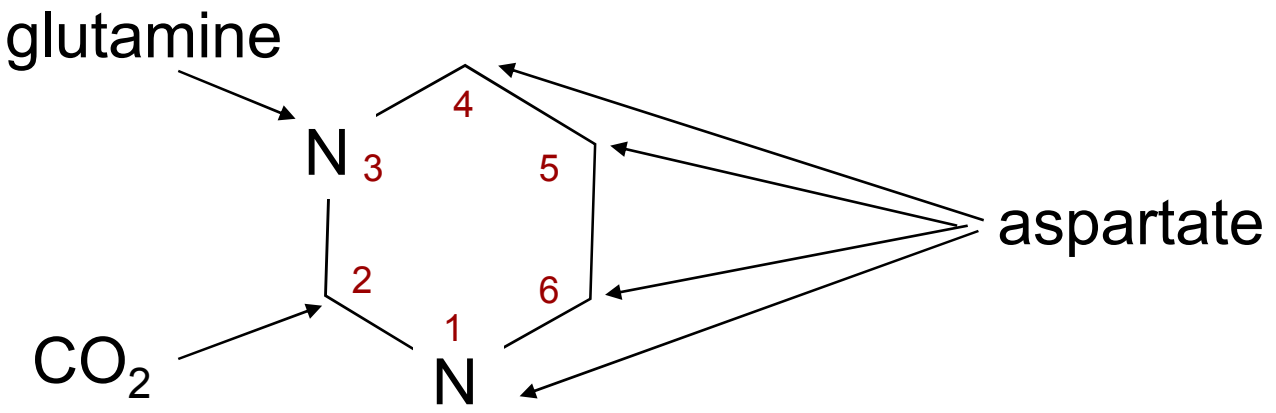


Fig 22-35

Pyrimidine synthesis (I)

p. 886-7

- U (UMP), C (CMP), T (dTMP)
- The ring (orotate) structure is *synthesized first, then attached to PRPP*. (Fig 22-36, center)

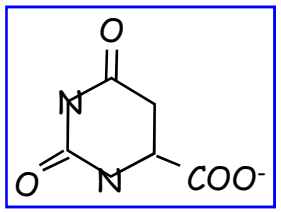


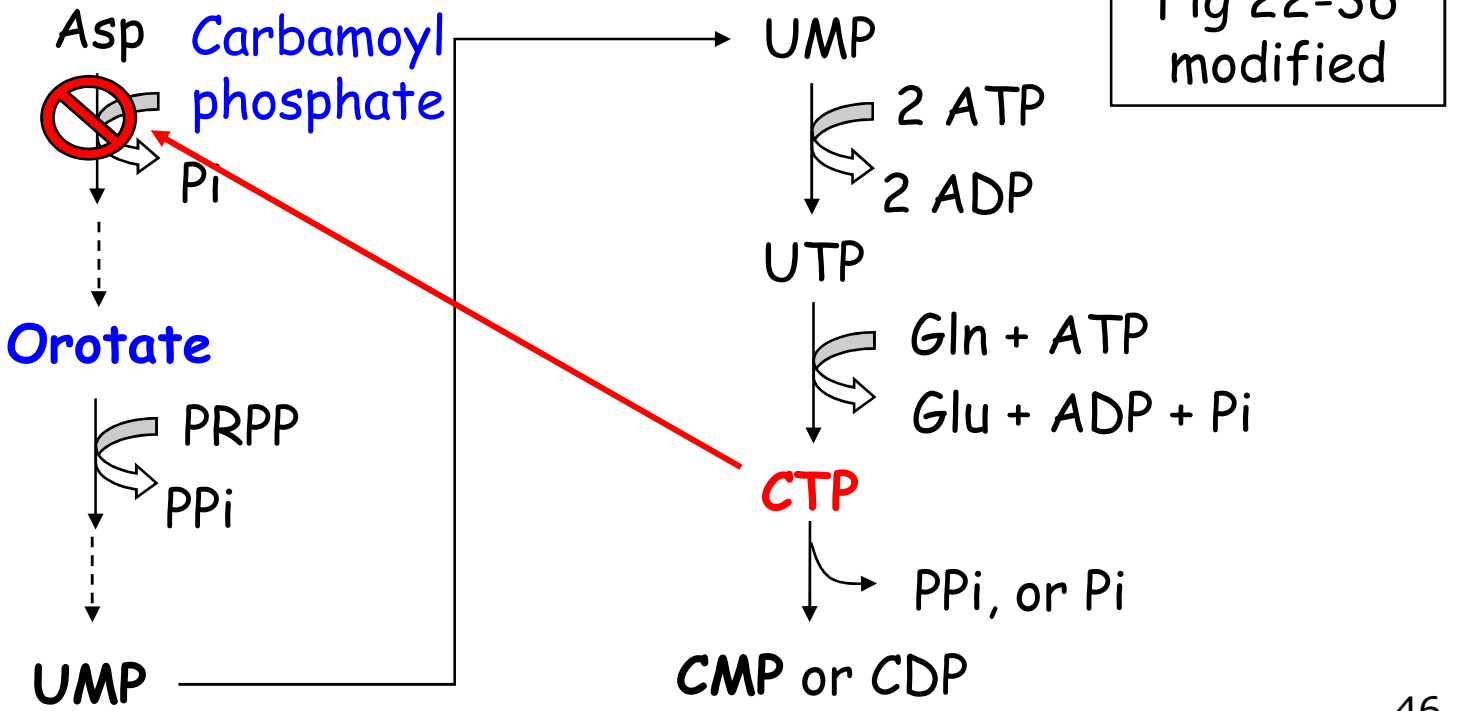
Source of the atoms of the pyrimidine ring.

Pyrimidine synthesis (II)

- Ribonucleotides: U, C
  - ✓ **Carbamoyl phosphate**, aspartate → → → orotate
  - ✓ Orotate + PRPP → → UMP
  - ✓ UMP → UTP + Gln → CTP → CDP, CMP
  - ✓ Regulated by feedback inhibition (end product: CTP)

Fig 22-36 modified

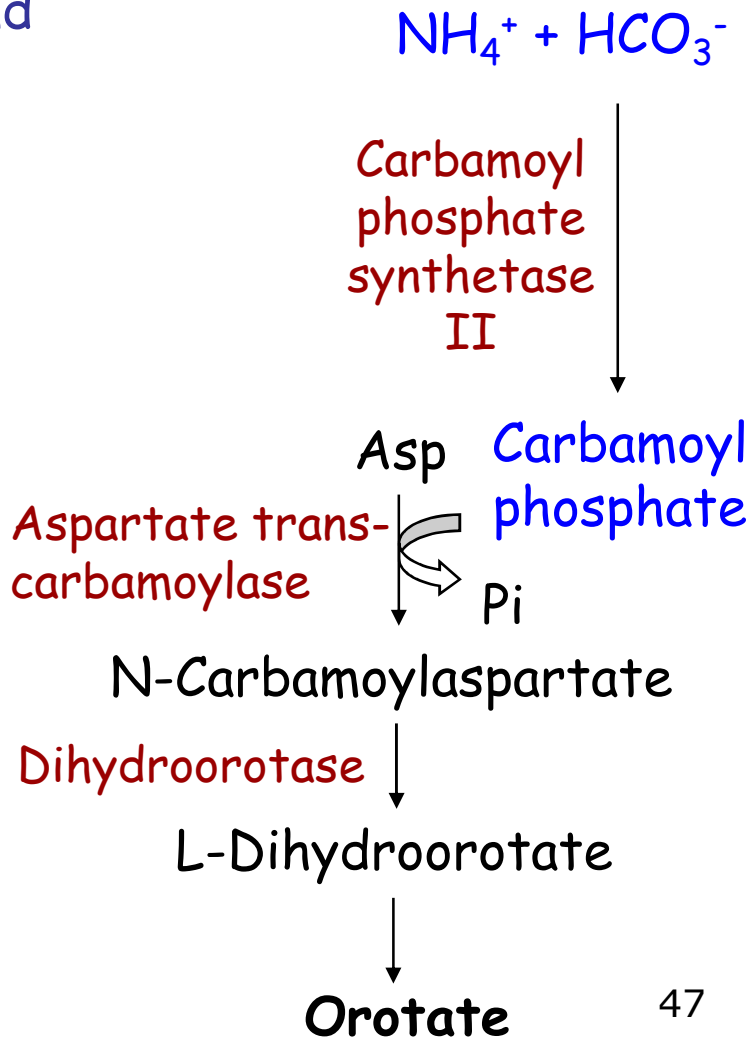




p. 886-7

Carbamoyl phosphate synthetase

- In bacteria:
  - ✓ Carbamoyl phosphate for both Arg and pyrimidines
  - ✓ One carbamoyl phosphate synthetase
- In eukaryotes:
  - ✓ CP synthetase I (mitochondria)
  - ✓ CP synthetase II (cytosol)
    - A single trifunctional protein → CAD
      - Carbamoyl phosphate synthetase II
      - Aspartate transcarbamoylase
      - Dihydroorotase
    - Large, multienzyme complexes



p. 887

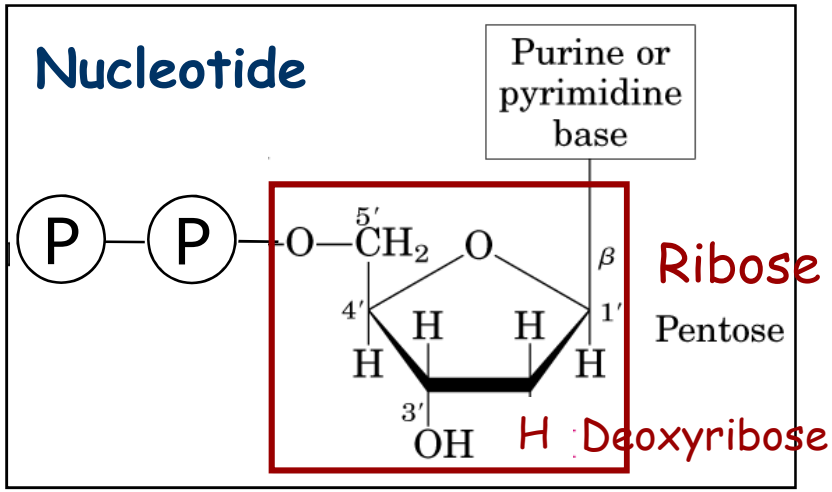
Fig 22-36 Top

CP synthetase I vs. II

- Comparision CP synthetase I and II (from Schumm):
  - ✓ The enzyme catalyzing CP for urea cycle needs a cofactor (N-acetylglutamate), but the enz. for pyrimidine biosynthesis does not.
  - ✓ Mitochondrial [CP synthetase] is 10x greater than cytosolic [CP synthetase]
    - Reflecting a much greater need to synthesize urea than pyrimidines

Deoxyribonucleotide synthesis

- Precursors: ribonucleotides p. 888
  - ✓ Reduction only occur at the level of ribonucleoside diphosphate by ribonucleotide reductase
  - ✓ AMP → ADP → dADP → dAMP
  - ✓ GMP → GDP → dGDP → dGMP
  - ✓ CMP → CDP → dCDP → dCMP
  - ✓ UMP → UDP → dUDP → ? → dTMP



Synthesis of dTMP

- Thymidylate (dTMP) is derived from dUMP

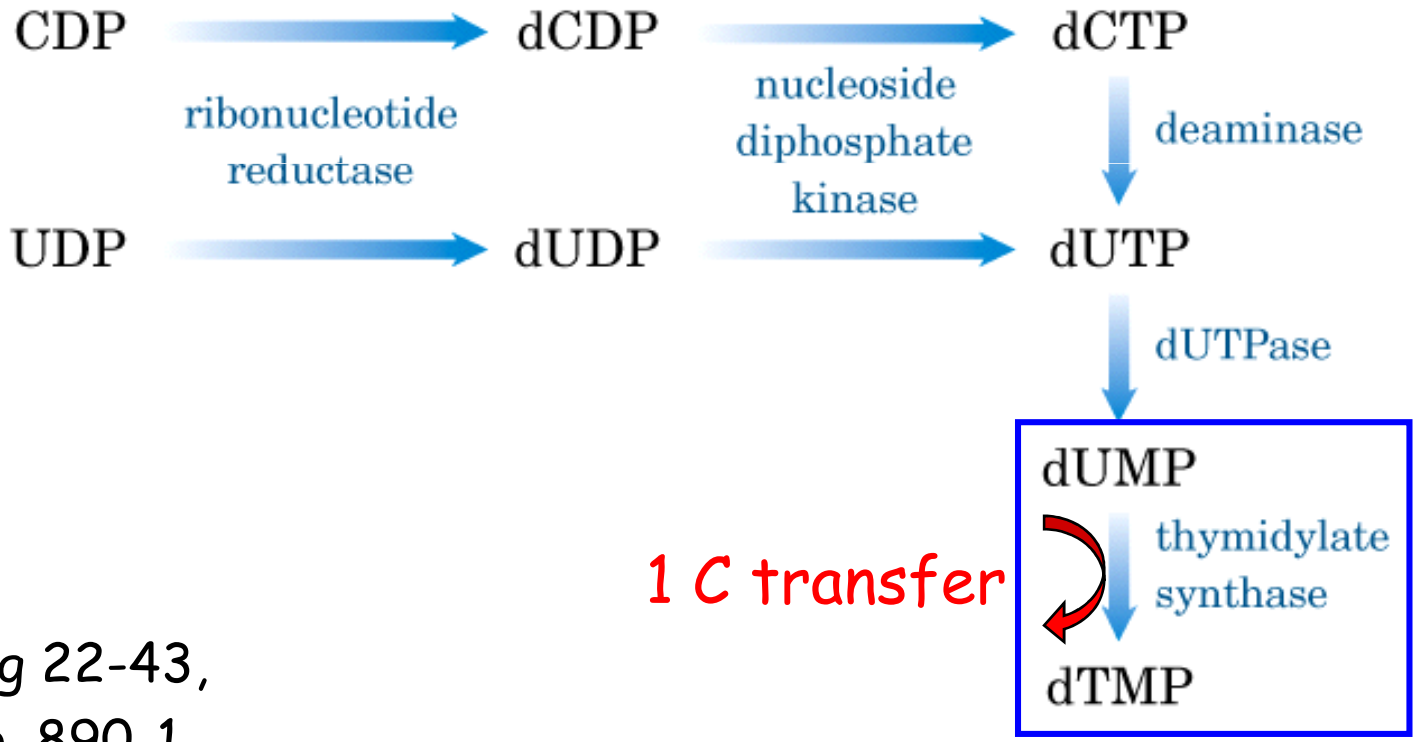


Fig 22-43,
p. 890-1

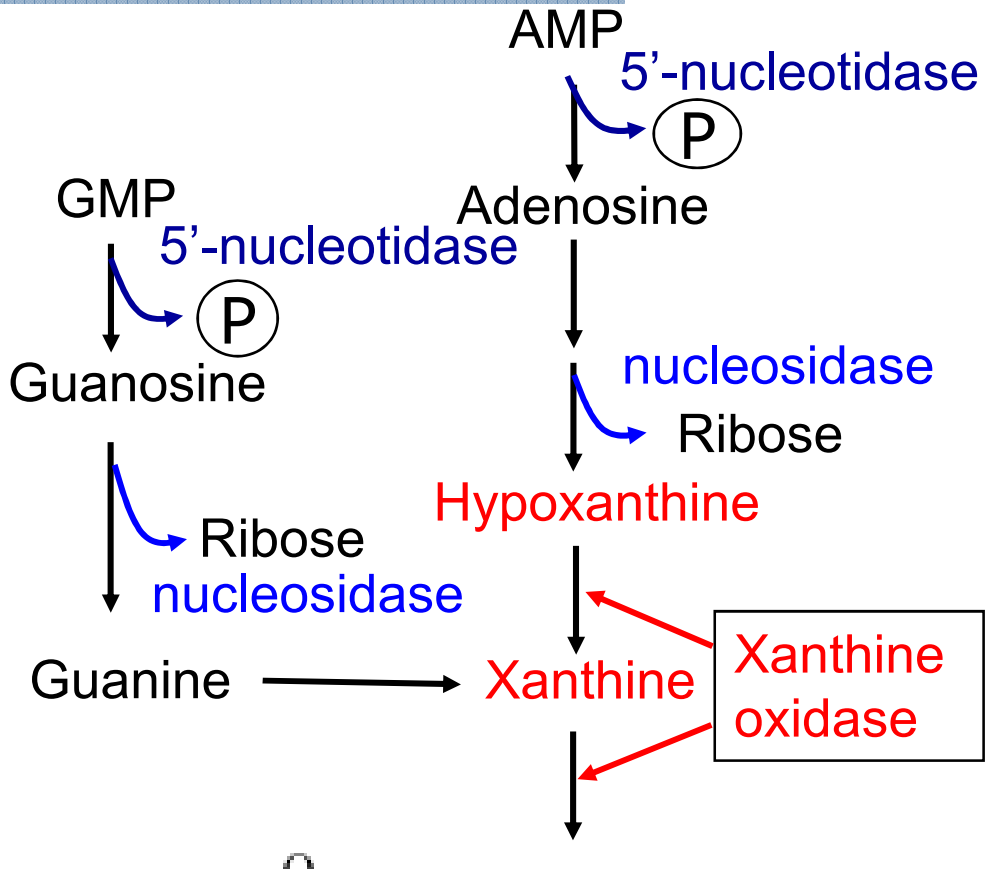
Nucleotide salvage (p. 893)

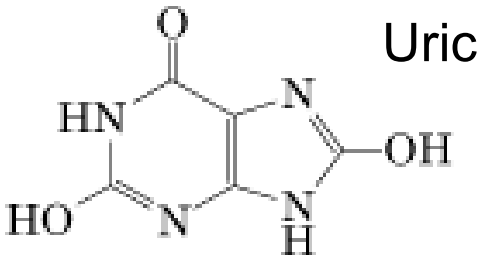
- Purine salvage
  - ✓ One-step reaction
  - ✓ The purine bases (adenine, guanine) + PRPP → AMP, GMP
    - Adenosine phosphoribosyltransferase
    - Hypoxanthine-guanine phosphoribosyltransferase
      - Lesch-Nyhan syndrome
- Pyrimidine salvage
  - ✓ Two-step reaction
  - ✓ The pyrimidine bases (uracil, cytocine) + ribose → nucleosides (uridine, cytidine)
  - ✓ Nucleosides (uridine, cytidine) + Pi → nucleotides (UMP, CMP)

Nucleotide degradation

p. 892-3

- Release bases can be salvaged for reuse
- Pyrimidine degradation
  - ✓ NH_4^+ → urea
  - ✓ Produce all soluble compounds
- Purine degradation
  - ✓ Uric acid
  - ✓ Low solubility





Purine degradation
Fig 22-45 left modified

Uric acid overproduction

p. 893, bottom right

- Gout
  - ✓ Excessive purines → uric acid ↑
  - ✓ Gout – occurs mainly in males
  - ✓ Medication: allopurinol (xanthine oxidase inhibitor)

Fig 22-45

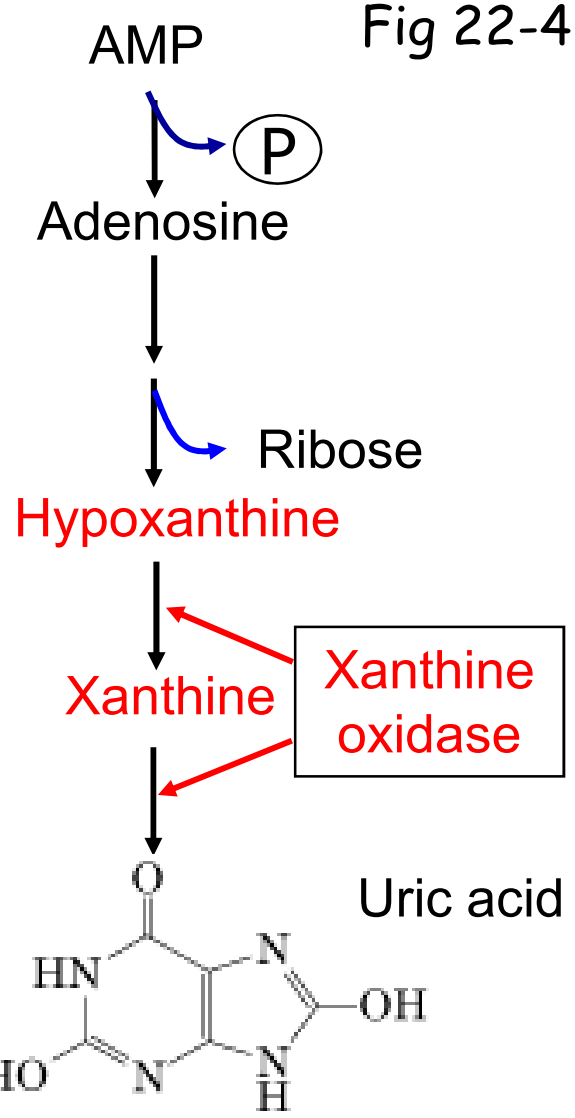
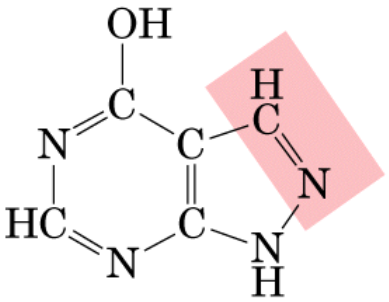
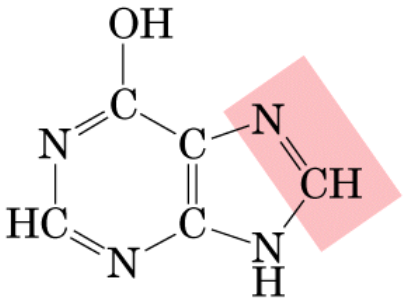


Fig 22-47



Allopurinol



Hypoxanthine (enol form)

Inhibitors and anticancer drugs

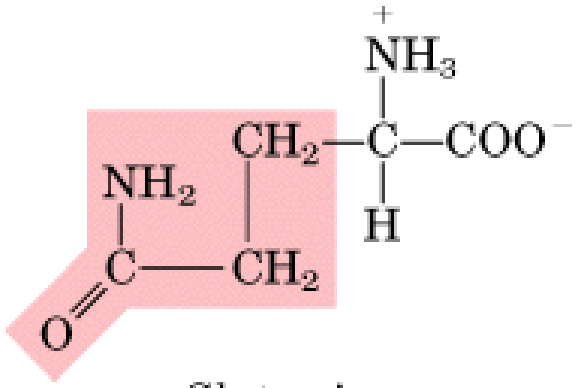
p. 894-6

- Growing cells need to synthesize both DNA and RNA.
  - ✓ Drugs inhibiting nucleotide biosynthesis affect not only tumor cells but normal ones as well.
    - Side effects of cancer chemotherapy
    - Stem cells: require DNA and RNA synthesis
    - Inhibits the formation of erythrocytes, lymphocytes, cells of the intestinal epithelium, and hair-forming cells.
- Most tumor cells possess a more active salvage pathway than do normal cells.
  - ✓ Drugs entering metabolism via the salvage pathways obtain a higher conc. in tumor cells and have a therapeutic advantage.

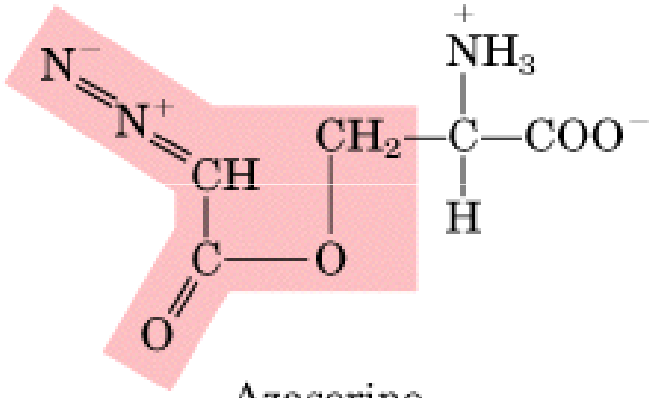
Chemotherapeutic agents

- Azaserine and acivicin
  - ✓Inhibit glutamine amidotransferases
  - ✓Gln analogs

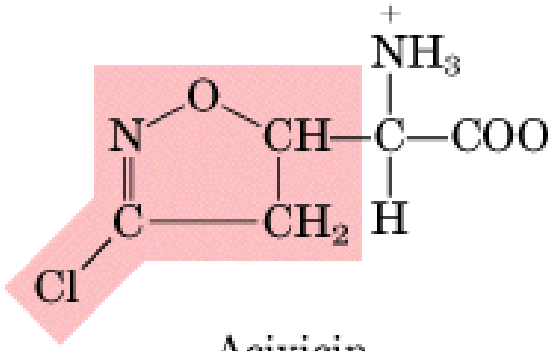
Fig 22-48



Glutamine



Azaserine



Acivicin

Summary

- Non-essential amino acid biosynthesis
- Regulation of a.a. biosynthesis
- A.A. derived biomolecules
- Nucleotide biosynthesis and degradation
  - ✓ De novo synthesis and regulation
  - ✓ Salvage pathway
  - ✓ Inhibitor and chemotherapeutic agent
- Problems
  - ✓ 7, 9, 10, 16