Biochemistry

Metabolism

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Nitrogen Fixation
Urea Cycle

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Nitrogen metabolism

- N_2 assimilation via reduction to NH_3 (nitrogenase complex)
- NH_3 metabolism: *glutamate-dehydrogenase*
  glutamate synthase
  glutamine synthetase
  glutamine amidotransferase
- urea cycle
- C_1 metabolism (PLP, THF, SAM, homocysteine)
- nucleotide metabolism: biosynthesis of purines and pyrimidines
  from RNA to DNA (NDP reductase)
  salvage pathway, HGPRT deficiency
  cytostatic drugs
  catabolism (ADA-deficiency, urate)

Characteristics of Nitrogen Components

Soluble biologically utilisable nitrogen components are generally **scarce** in natural environments.
Thus most organisms maintain **strict economy** in their use of ammonia, amino acids and nucleotides.
These components are often **salvaged and reused.**

Unlike carbohydrates and lipids, amino acids and nucleotides are **not stored** in cells.
Thus the amount of free amino acids and nucleotides is low and their **metabolism** is **accurately controlled and regulated**.
These molecules are **charged** thus affecting the electro-chemical balance in the cell.

The Nitrogen Cycle

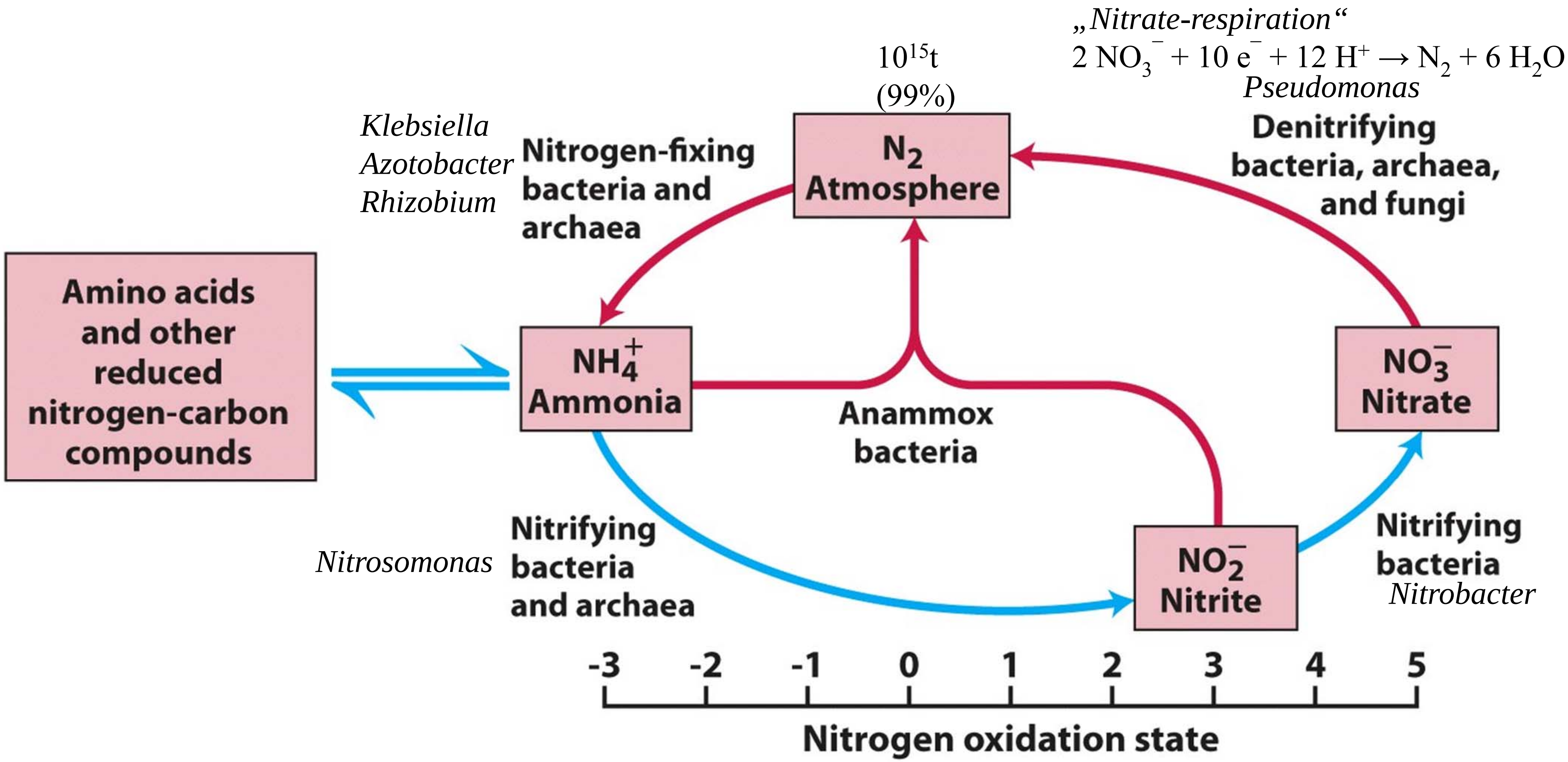
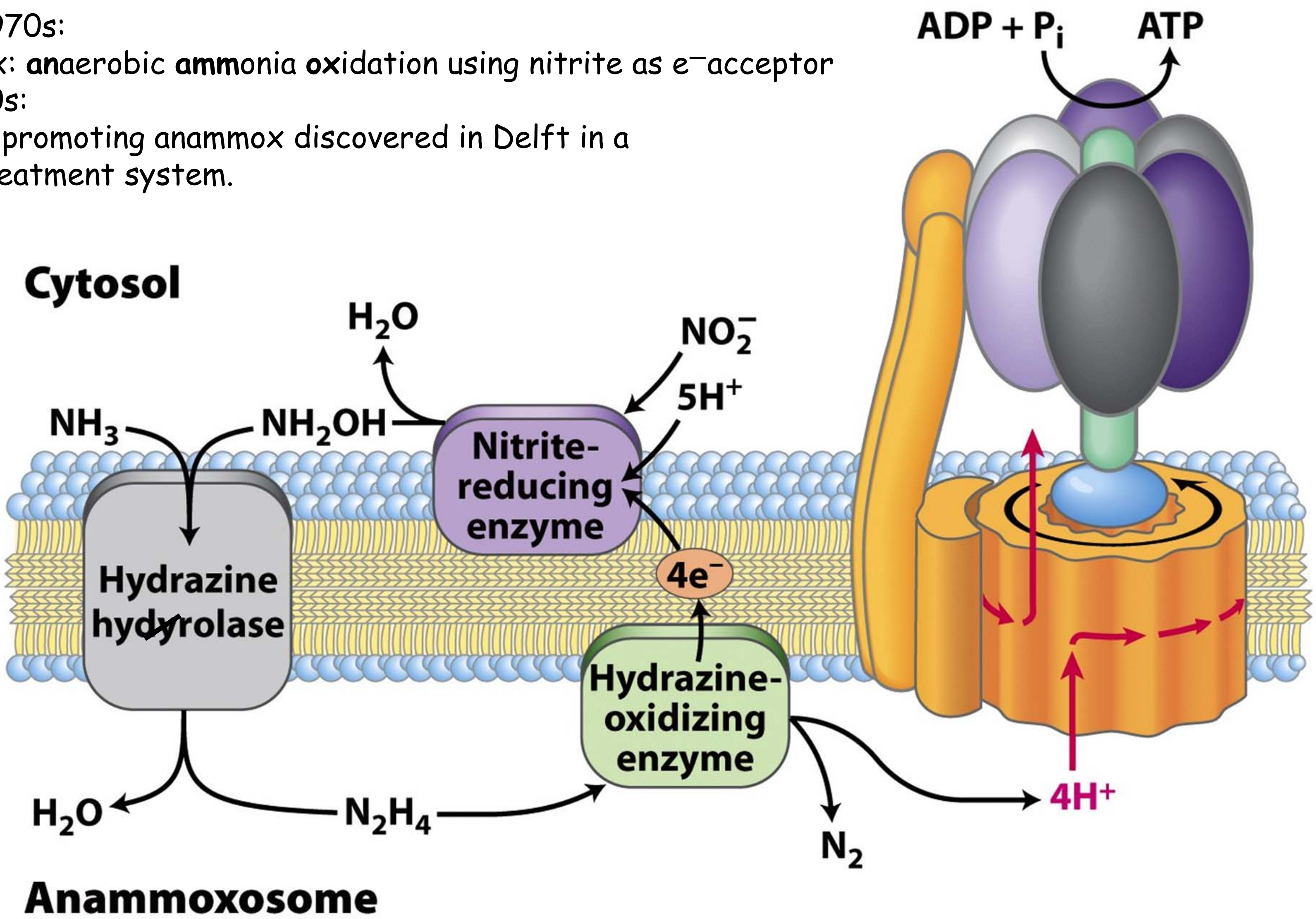


Figure 22-1
Lehninger Principles of Biochemistry, Fifth Edition

The Anammox Reactions

1960s/1970s:
Anammox: **an**aerobic **amm**onia **ox**idation using nitrite as e^-acceptor
Mid 1980s:
Bacteria promoting anammox discovered in Delft in a waste-treatment system.



Box 22-1 figure 1
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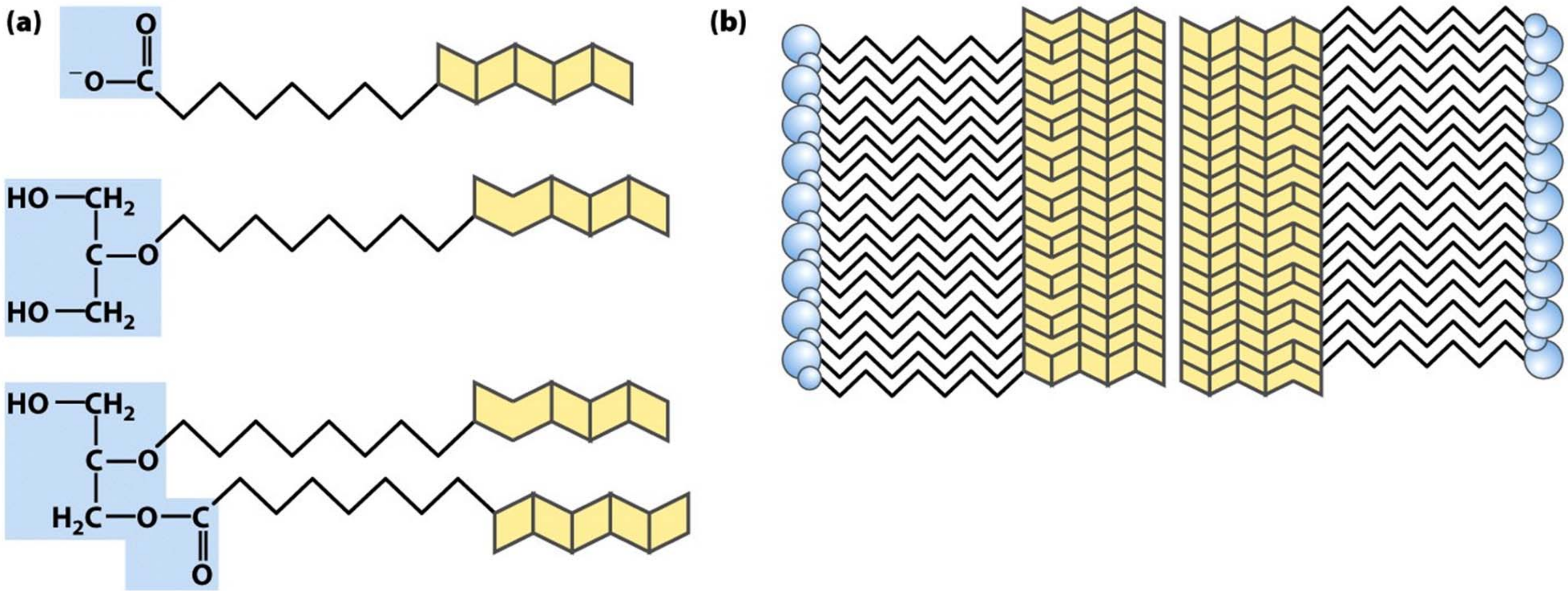
Brocadia anammoxidans
(Planctomycetes)

Ladderane Lipids of the Anamoxosome Membrane



Box 22-1 figure 2
Lehninger Principles of Biochemistry, Fifth Edition

Nitrogen Fixation by the Nitrogenase Complex

Figure 22-4a
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Nitrogen-fixing nodules
Leguminous plant – leghemoglobin
Bacteria – nitrogenase complex
(oxygen lability)

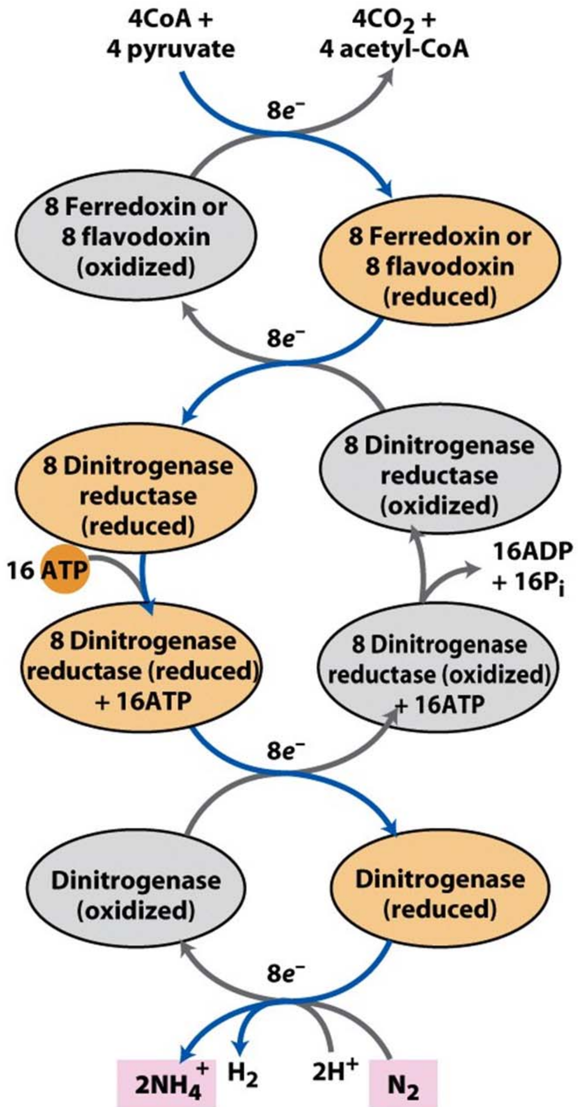

Figure 22-2
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Dimer, Fe-S
30 sec half-life in presence of O_2

Tetramer, Mo-Fe-S
10 min half-life in presence of O_2

Glutamine synthetase:

Glu + ATP $\rightarrow$ γ -glutamyl phosphate + ADP

γ -glutamyl phosphate + NH_4^+ $\rightarrow$ Gln + P_i + H^+

Glu + NH_4^+ + ATP $\rightarrow$ Gln + ADP + P_i + H^+ (a)

Glutamate synthase (not present in animals):

α-KG + Gln + NADPH + H^+ $\rightarrow$ 2 Glu + $NADP^+$ (b)

(a) + (b):

α-KG + NH_4^+ + ATP + NADPH $\rightarrow$ Glu + $NADP^+$ + ADP + P_i

Glutamate dehydrogenase:

α-KG + NH_4^+ + $NADPH^+$ + H^+ $\rightarrow$ Glu + $NADP^+$ + H_2O ($K_{M(NH4^+)}$ = 10mM)

$\leftarrow$

$\longleftarrow$

Glutamine: $-NH_2$ donor

Proposed Mechanism of Glutamine Amidotransferases

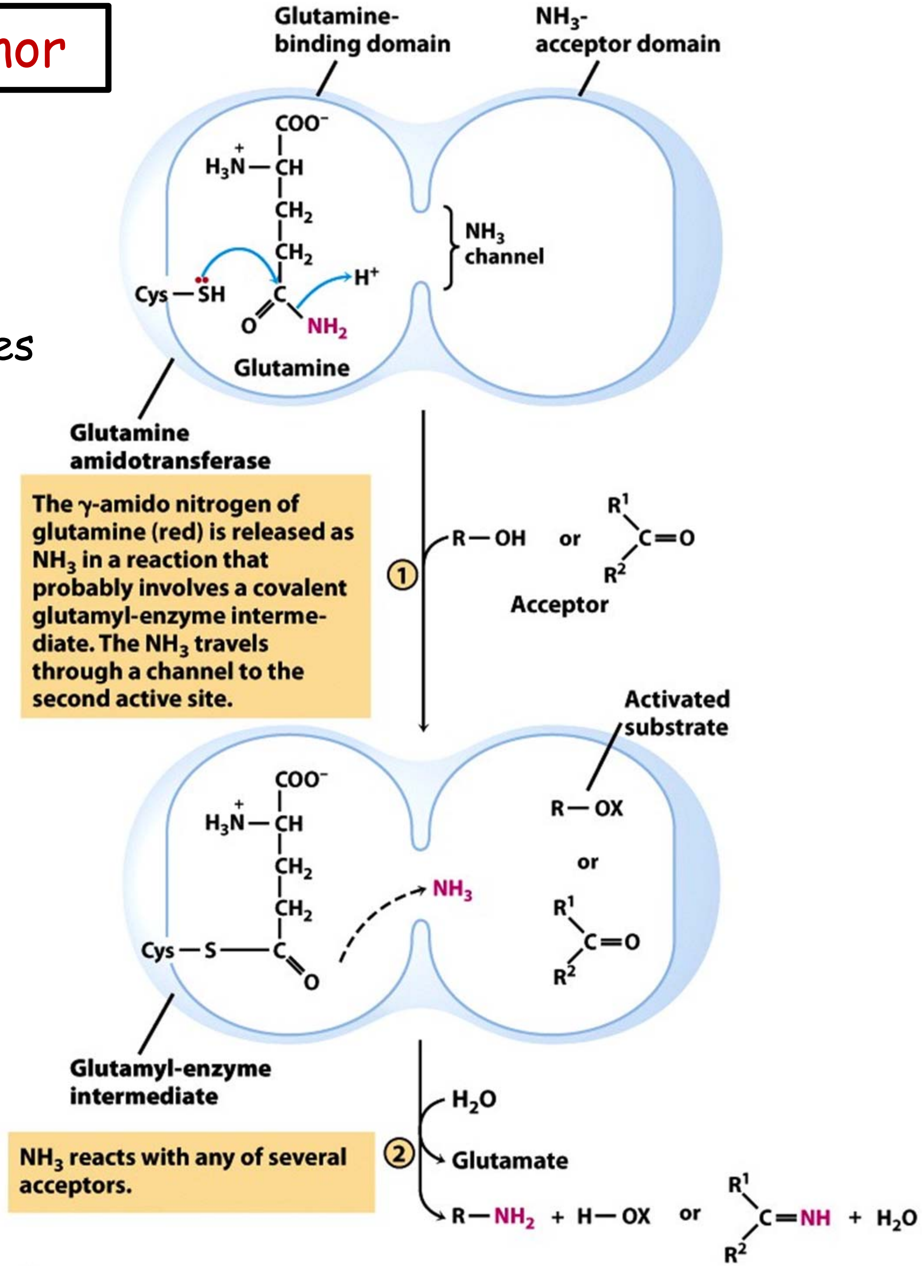

Figure 22-8

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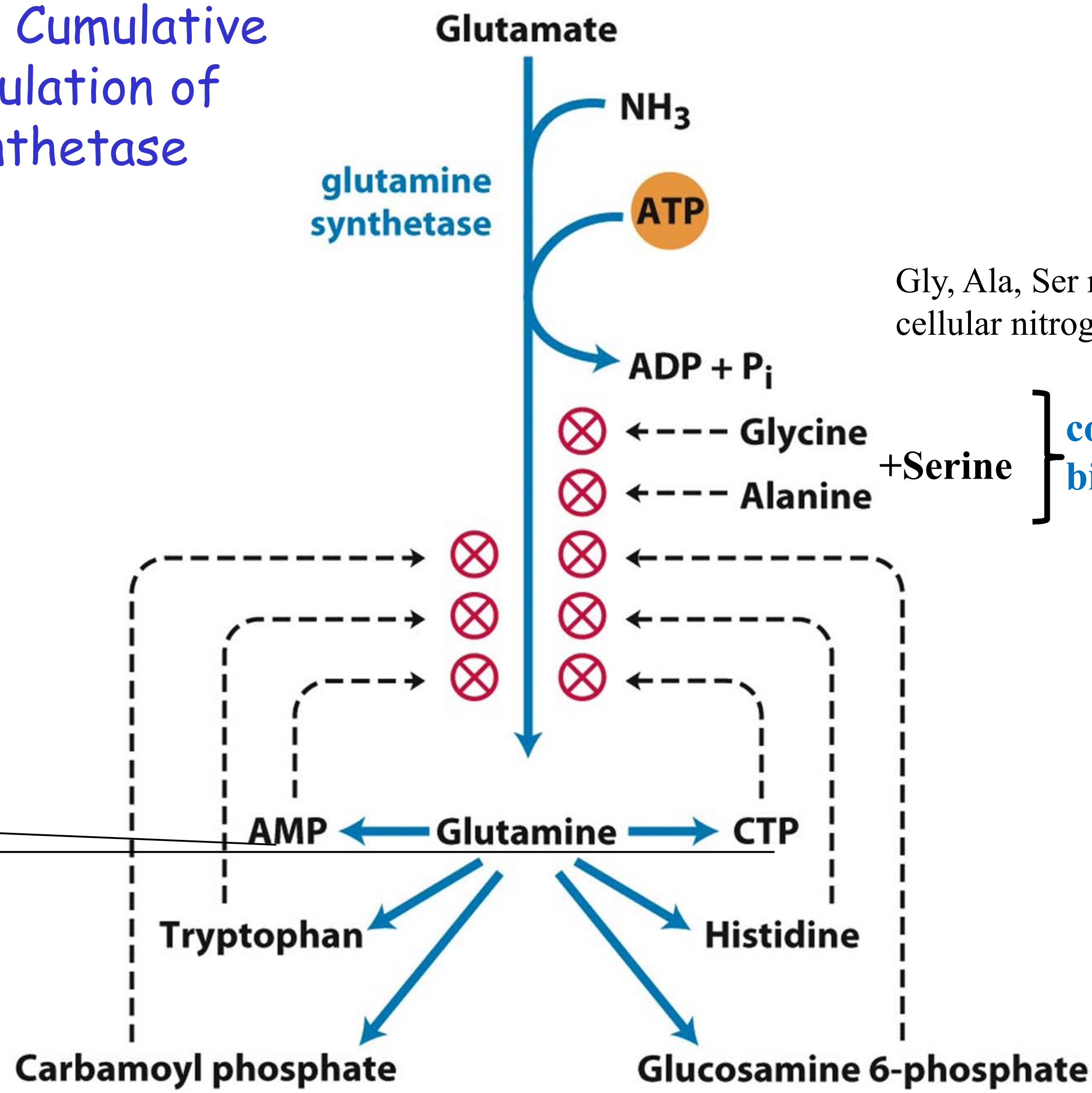
Allosteric and Cumulative Feedback Regulation of Glutamine Synthetase

Gly, Ala, Ser reflect cellular nitrogen level



compete with Glu binding site

compete with ATP binding site

Figure 22-6
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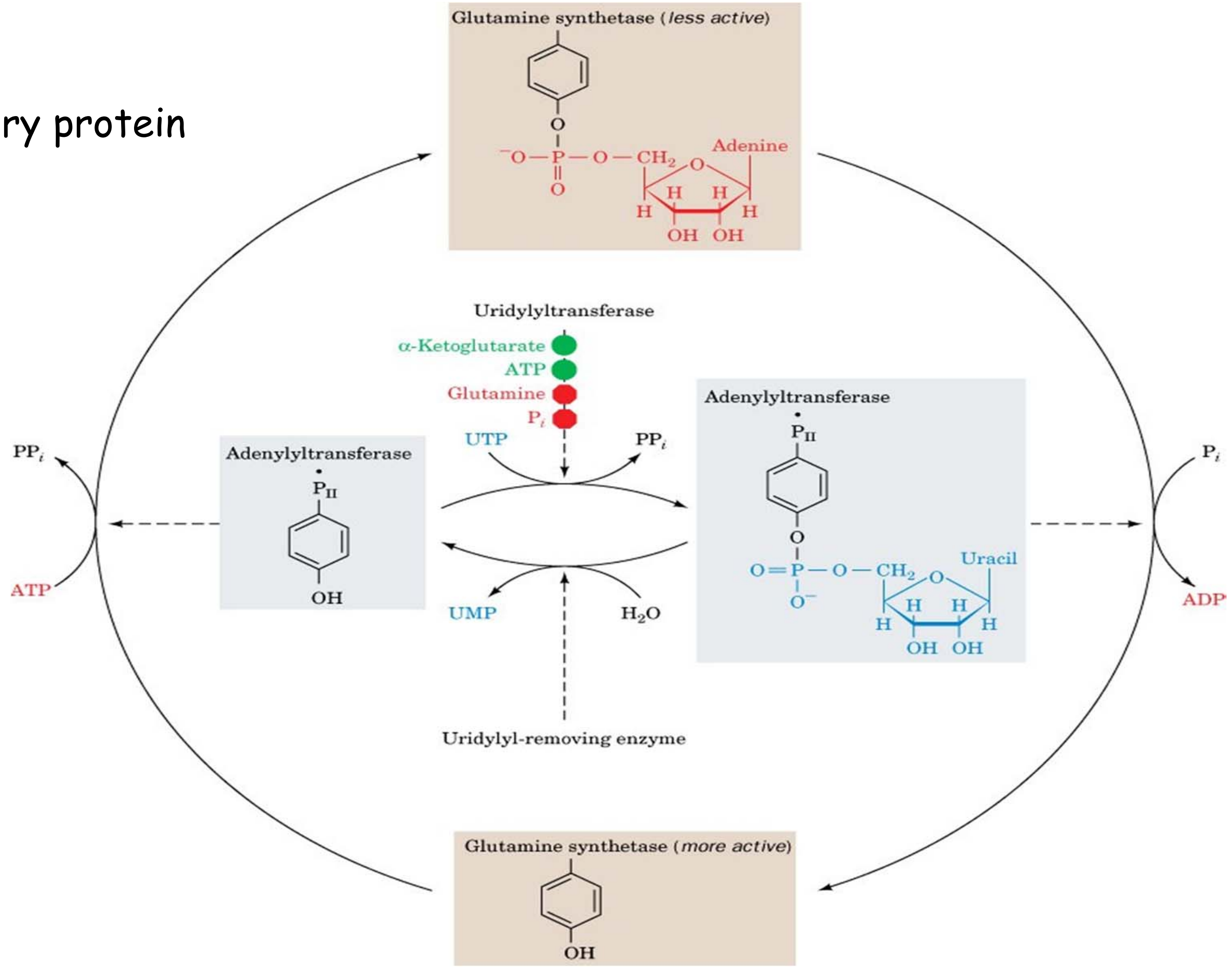
Regulation of bacterial glutamine synthetase by covalent modification

P_{II}: regulatory protein



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Biochemistry, Voet & Voet, 3rd ed.

Regulation of bacterial glutamine synthetase by covalent modification

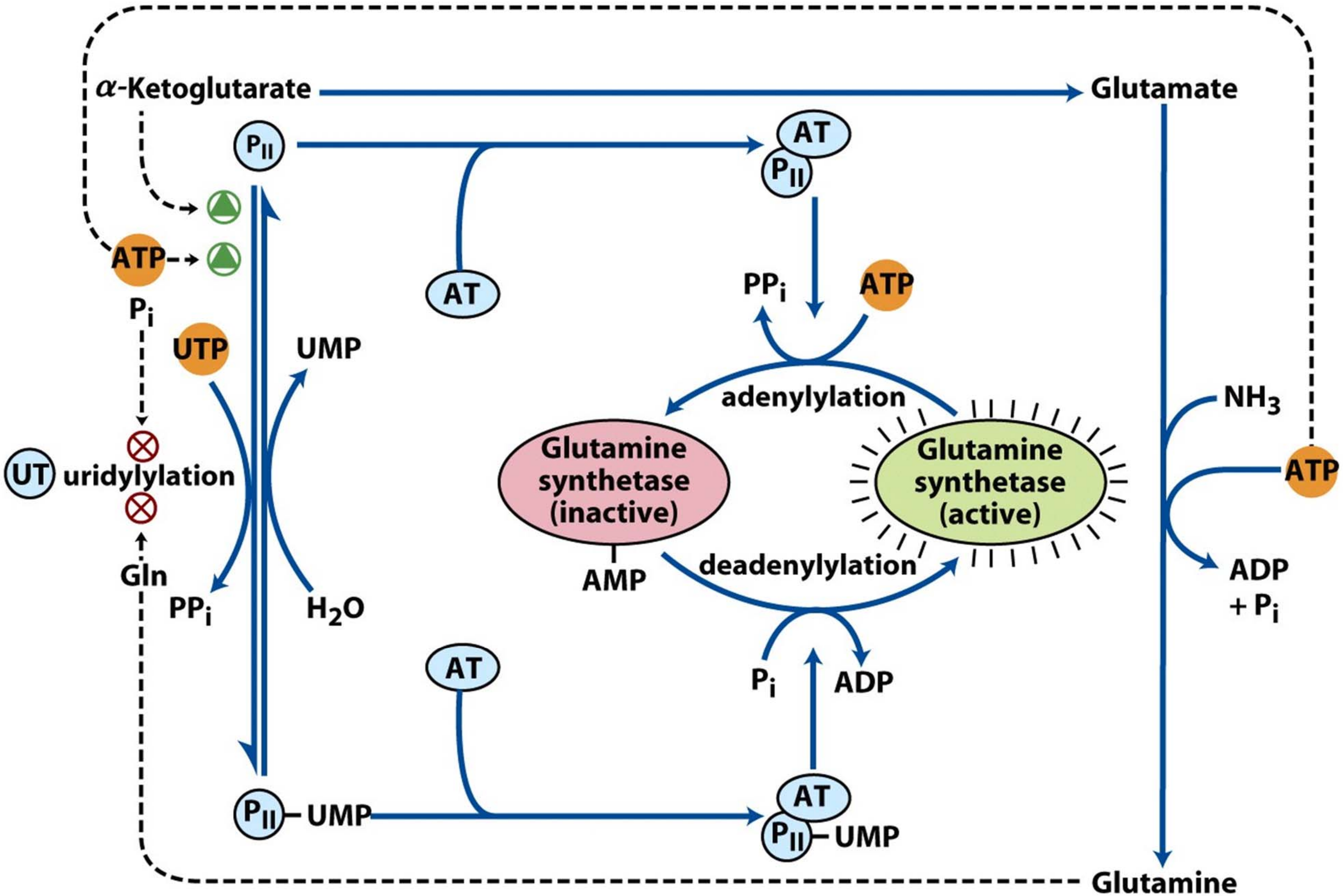

Figure 22-7b
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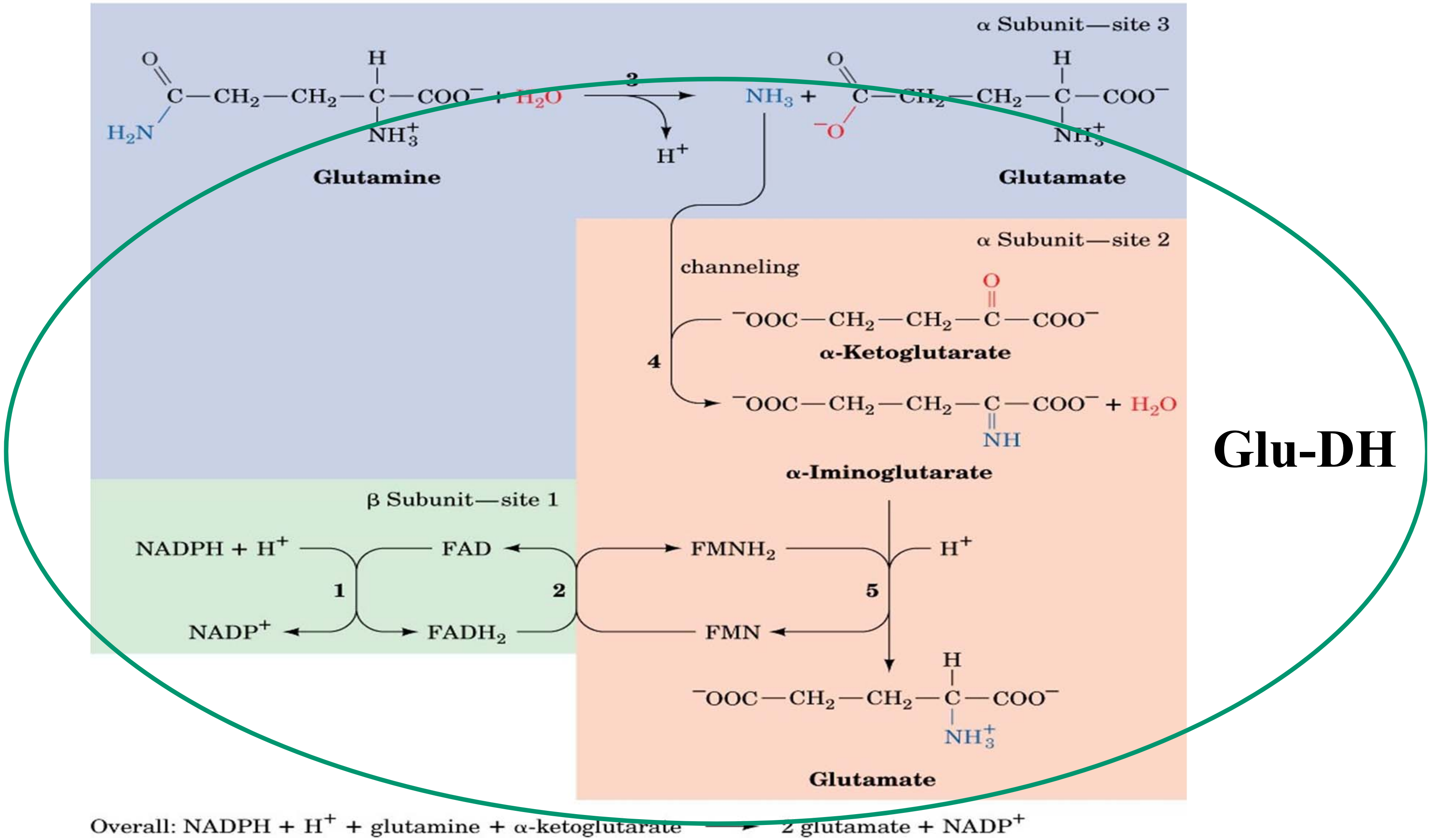
The sequence of reactions catalyzed by glutamate synthase.



Page 1031

Biochemistry, Voet & Voet, 3rd ed.

Overview of the catabolism of amino groups in vertebrate liver

1: Transaminase
2: Transaminase
3: Glutaminase
4: Glu-DH

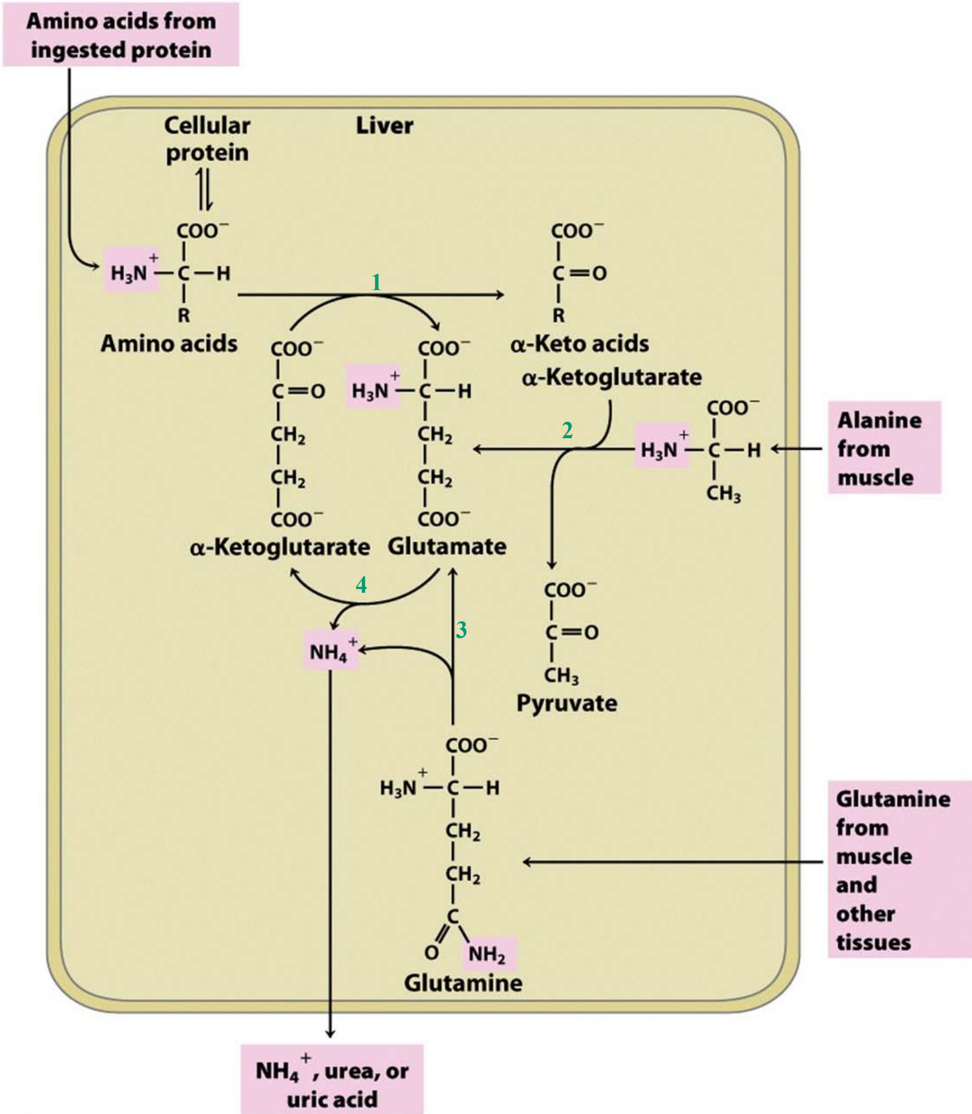


Figure 18-2a
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Urea cycle
&
reactions feeding amino groups into the cycle

Carbamoylphosphate synthetase 1 (CPS)

1. Ornithine transcarbamoylase (OTC)
2. Argininosuccinate synthetase (AS)
3. Argininosuccinate lyase (AL)
4. Arginase (A1)

NH_3 from intestin
via vena porta

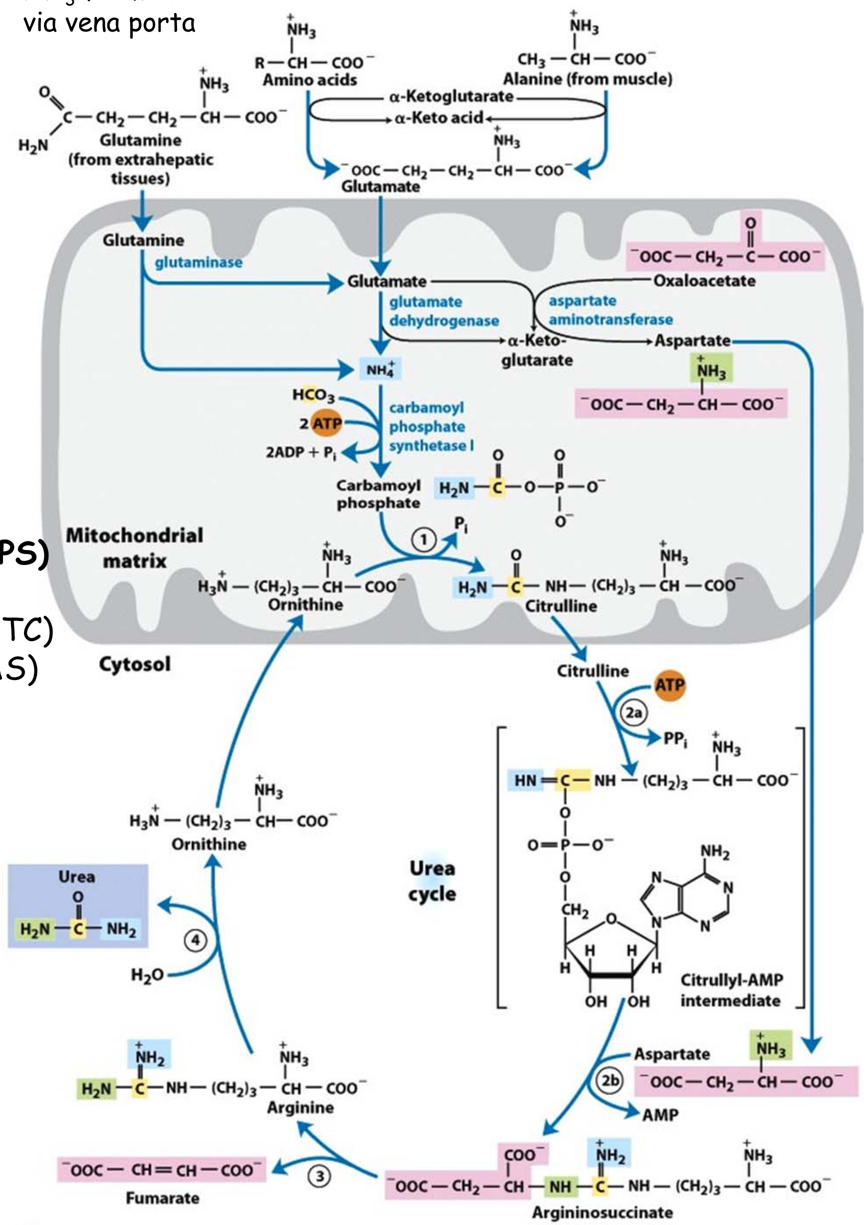

Figure 18-10
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Nitrogen-acquiring reactions in the synthesis of urea

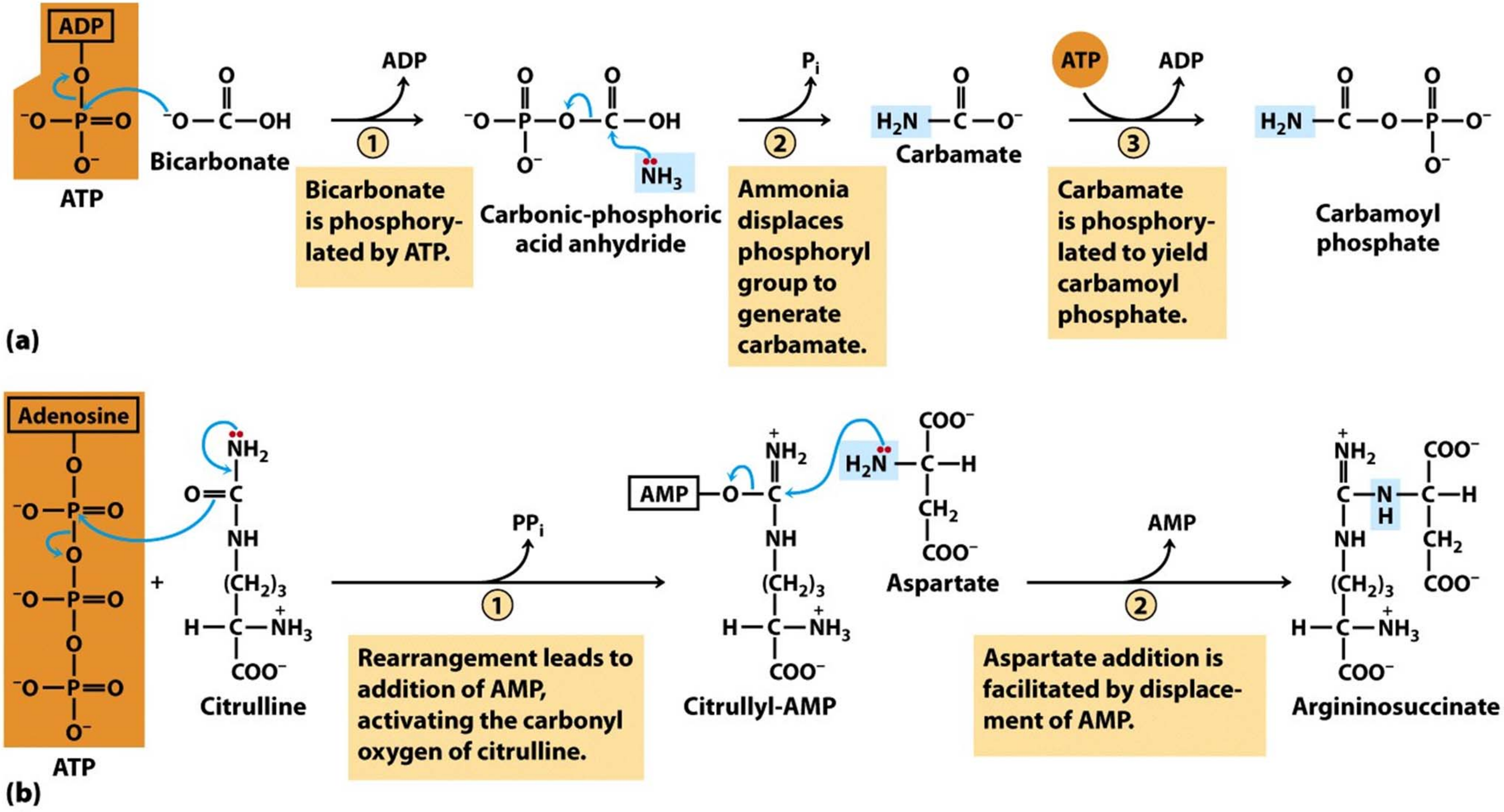

Figure 18-11

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Short-time (allosteric)
regulation of the urea cycle

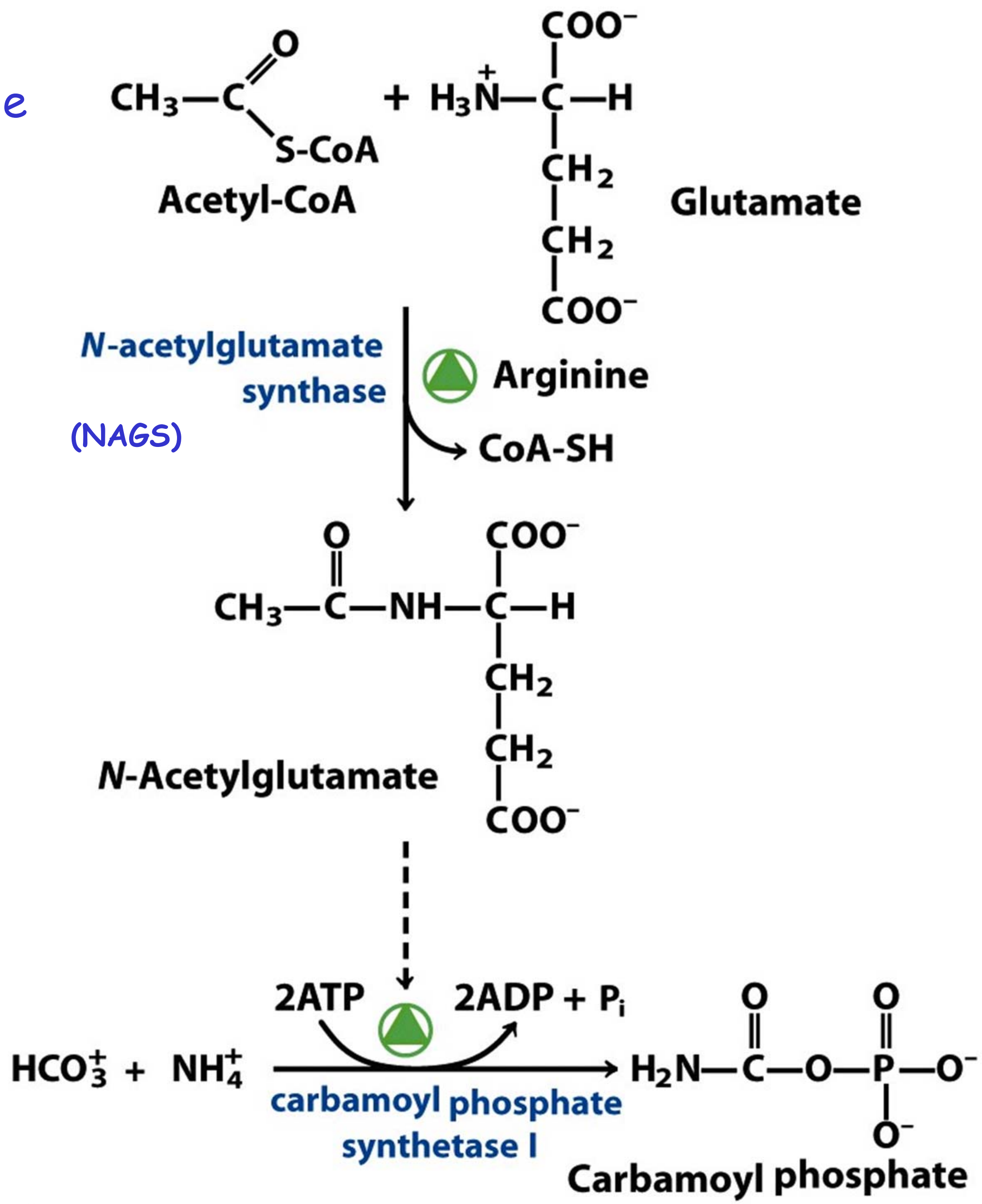

Figure 18-13
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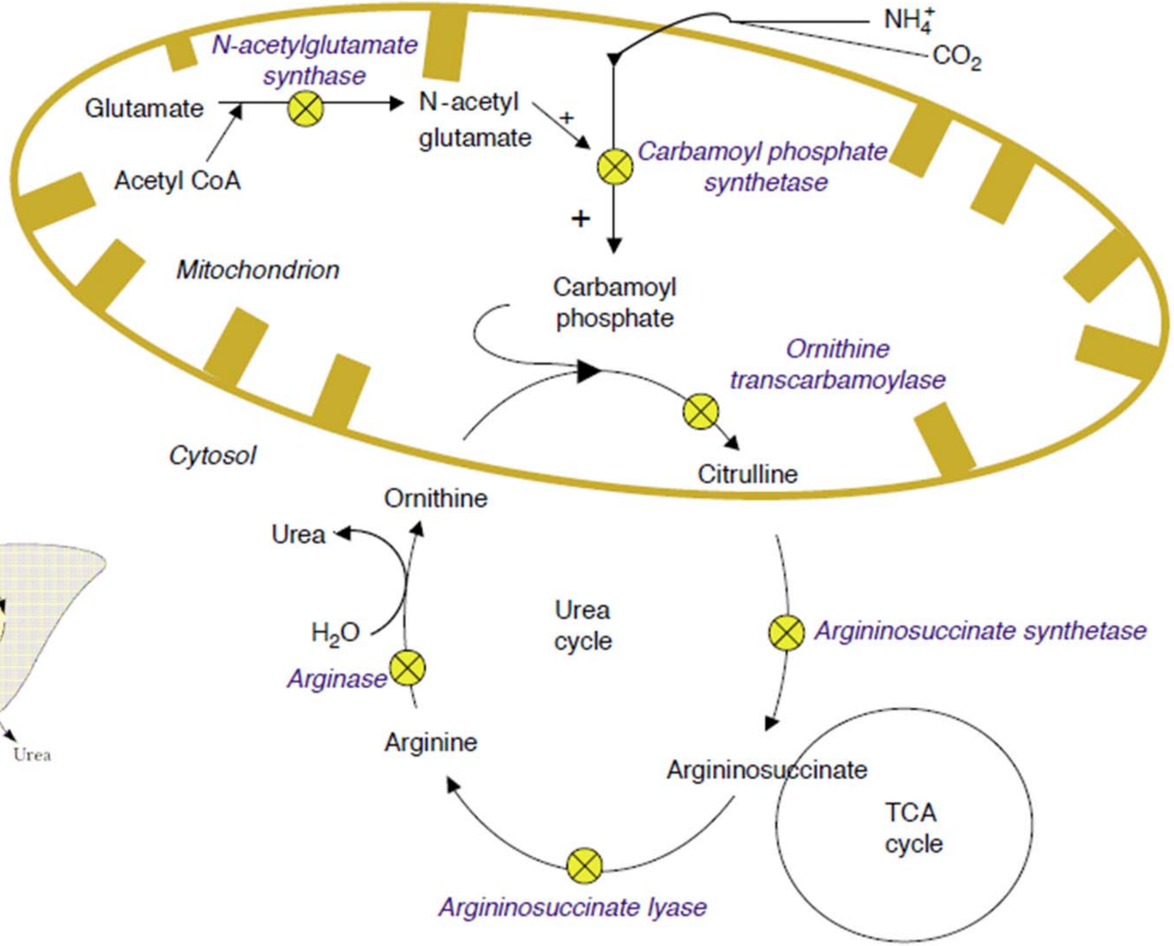
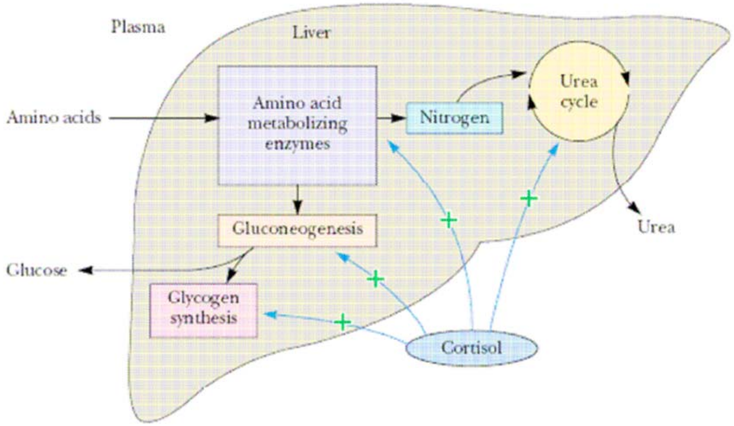
Long-time (rate of expression
of the 5 enzymes of the cycle)
regulation of the urea cycle
is diet-dependent.

Urea cycle disorders





Triple H-Syndrom: Hyperornithinemia, Hyperammonemia, Homocitrullinuria
Defect in Orn mitochondrial transport (no Orn recycling); symptoms: developmental delay, mental retardation, vomiting, ataxia, lethargy, irritability, coma.

Fearing & Shih, Encyclopedia of Biol Chem IV

The six inborn urea cycle defects

Location	Abb.	Enzyme	Disorder	Measurements
Mitochondria	NAGS	N-Acetylglutamate synthase	N-Acetylglutamate synthase deficiency	+**Ammonia**
Mitochondria	CPS1	Carbamoyl phosphate synthetase I	Carbamoyl phosphate synthetase I deficiency	+**Ammonia**
Mitochondria	OTC	Ornithine transcarbamoylase	Ornithine transcarbamoylase deficiency	+**Ornithine**, +**Uracil**, +**Orotic acid**
Cytosol	AS	Argininosuccinic acid synthetase	AS deficiency or citrullinemia	+**Citrulline**
Cytosol	AL	Argininosuccinase acid lyase	AL deficiency or argininosuccinic aciduria (ASA)	+**Citrulline**, +**Argininosuccinic acid**
Cytosol	A1	Arginase	Arginase deficiency or argininemia	+**Arginine**

Inherited autosomal recessive disorders except the X-linked OTC deficiency.
Similar clinical features: Hyperammonemia, respiratory alkalosis, seizures, acute encephalopathy, coma, death.
Unique to AS deficiency: short friable hair, liver fibrosis

Fearing & Shih, Encyclopedia of Biol Chem IV

Therapeutic strategy to overcome deficiencies in urea cycle enzymes

Administration of aromatic acids (benzoat or phenylbutyrate) in the diet lowers blood ammonia level.

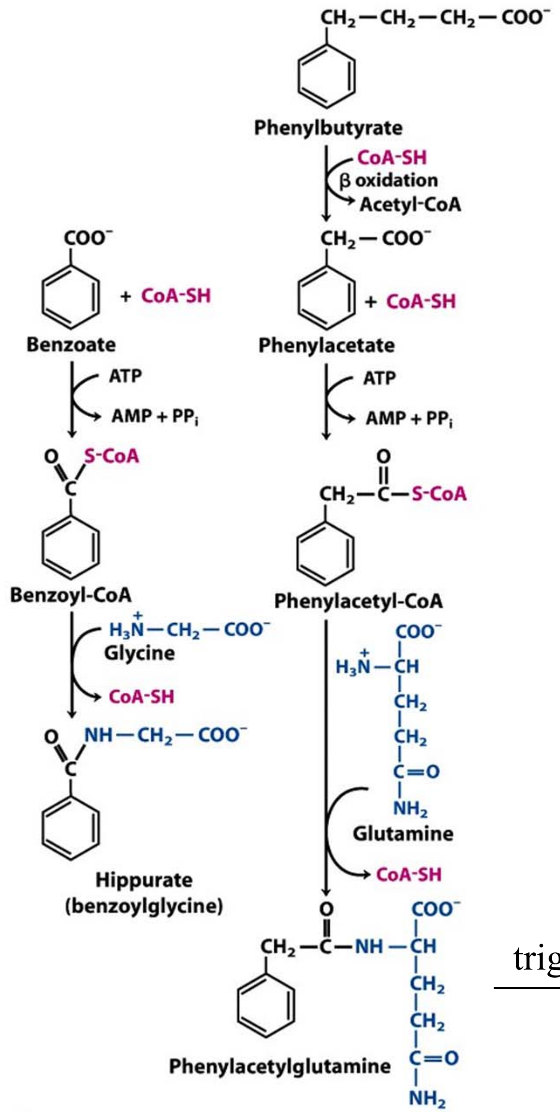


Figure 18-14
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Gly-synthase ← triggers

triggers → Gln-synthetase

Removal of Gly and Gln triggers NH_3-consuming reactions, thus lowering its blood content.

Links between the urea cycle and the citric acid cycle

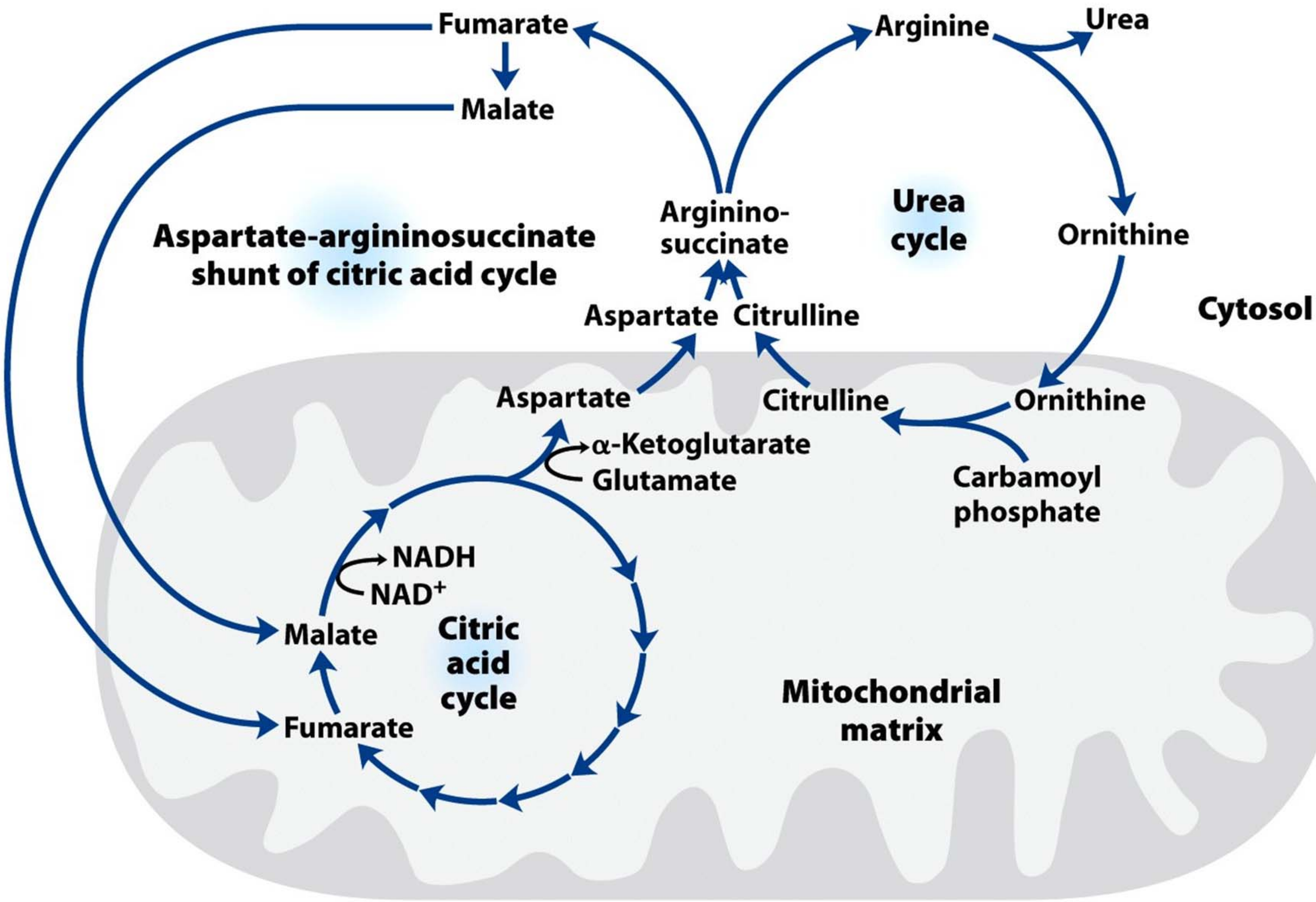

Figure 18-12
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