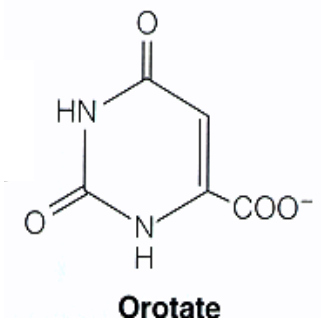
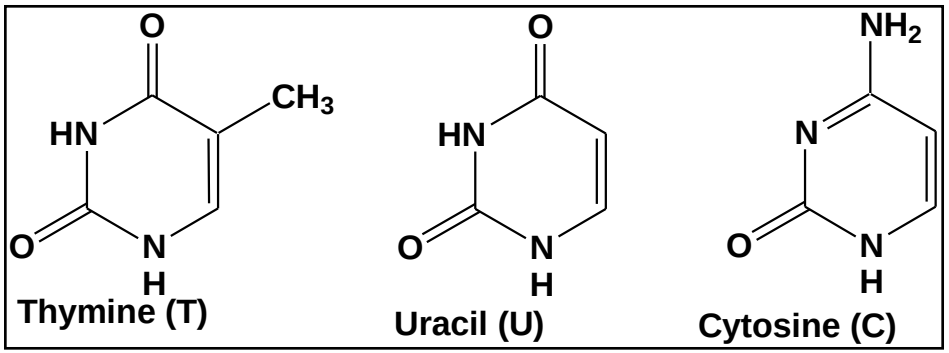
Pyrimidine synthesis

Bread mould



Orotate

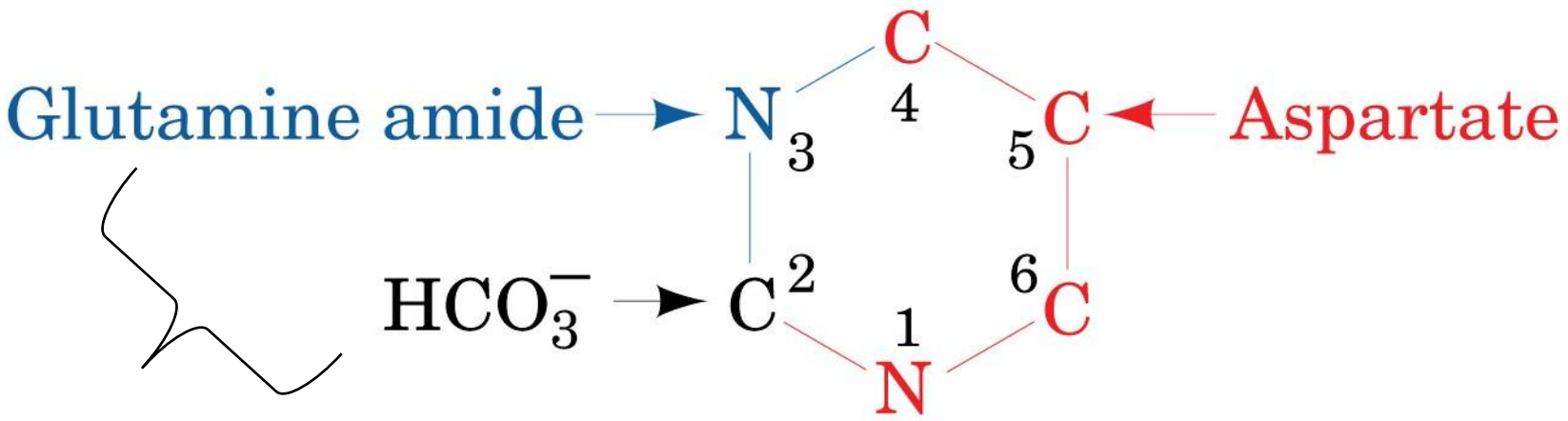


Ring built on ribose-5-phosphate

- Pathway is simpler and involves less steps
- Six steps to UMP
- Ribose-5-phosphate: compare synthesis of purine vs. pyrimidine
- Pathway is identical in many organisms
- Pathway begins with aspartate

Pyrimidine ring made FIRST THEN ribose attached to ring

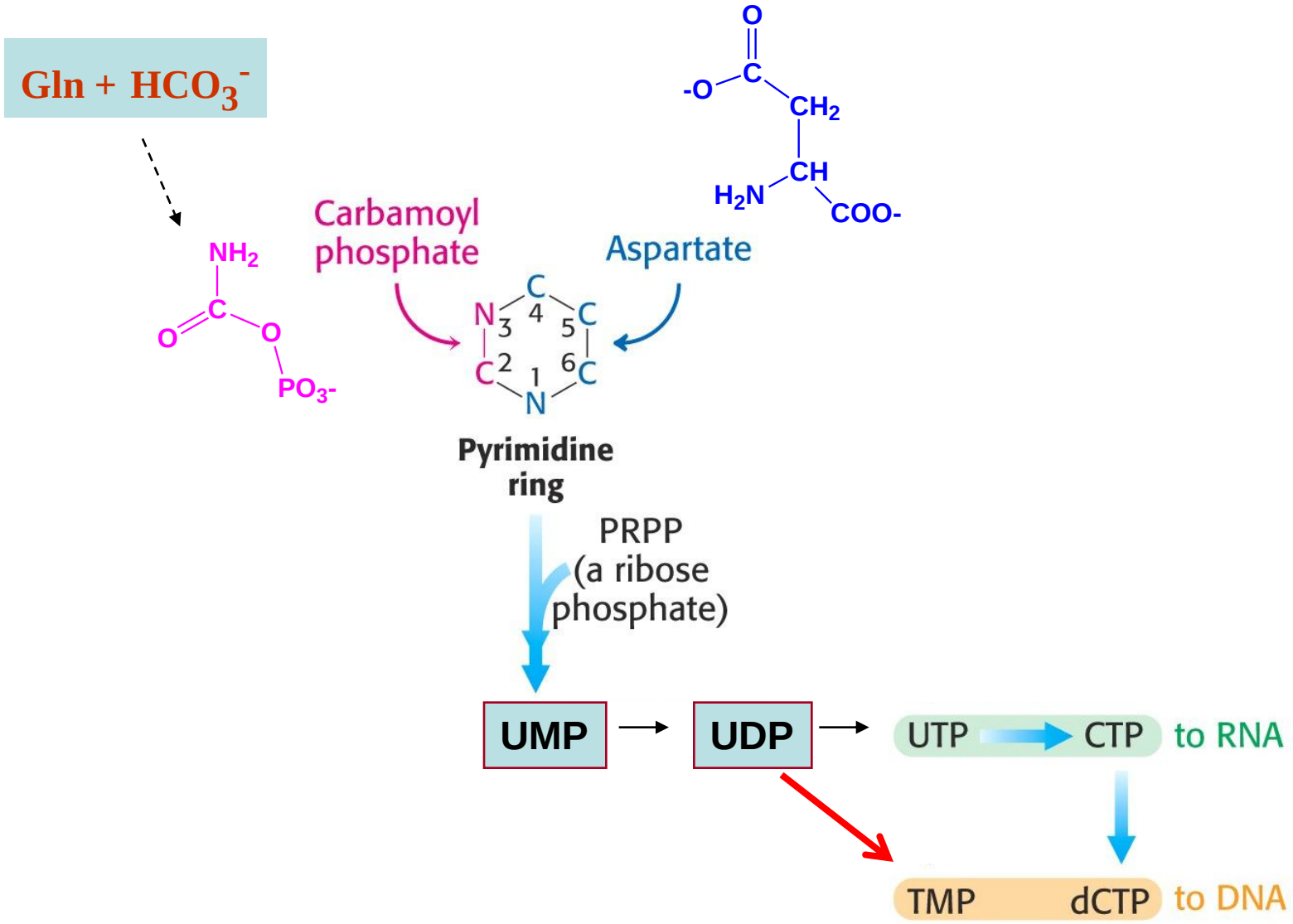
The biosynthetic origins of pyrimidine ring atoms.



Carbamoyl phosphate

Only aspartate and carbamoyl phosphate are involved in the pyrimidine ring formation.

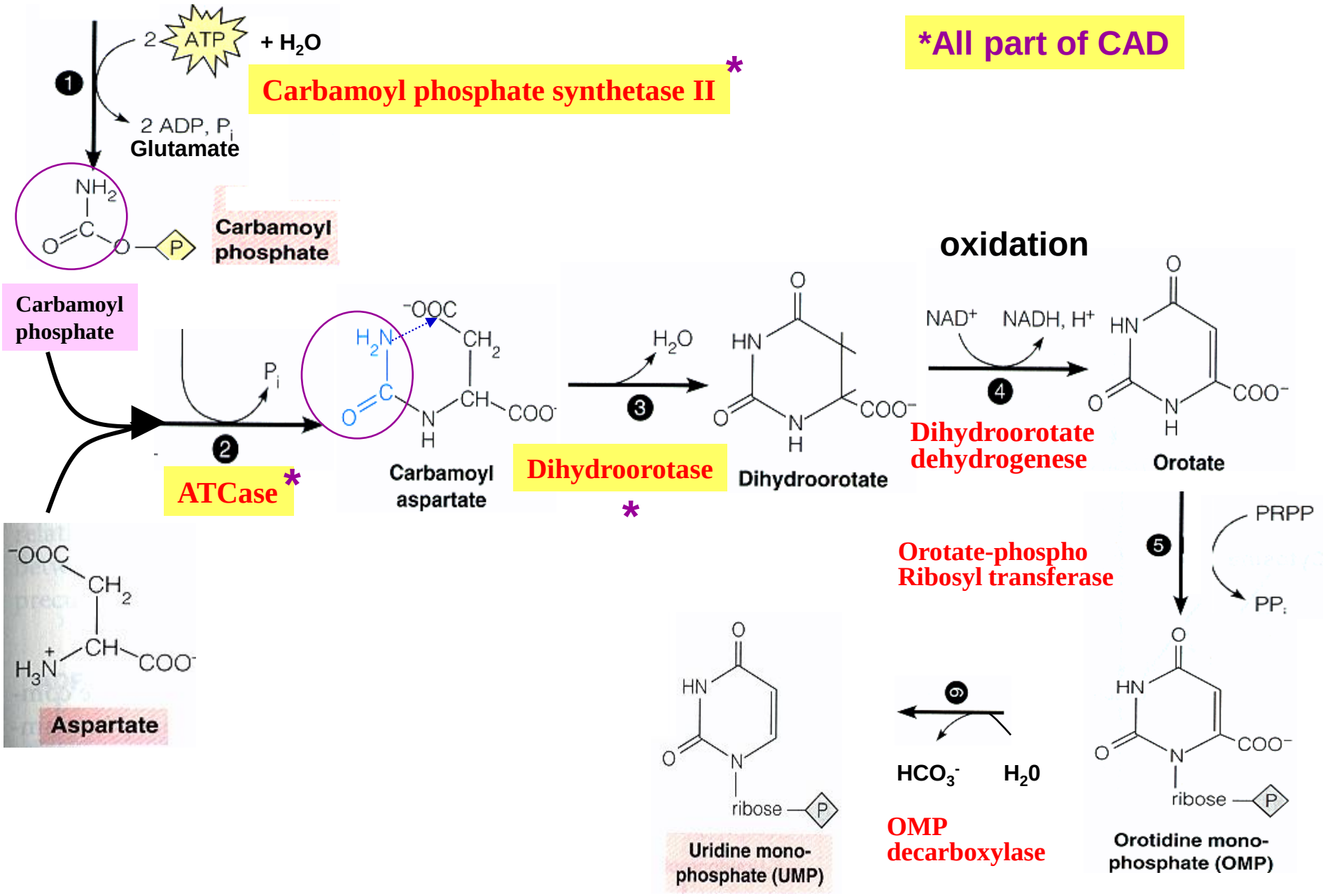
Overall equation: Pyrimidine biosynthesis



Pathway of Pyrimidine Synthesis

$Gln + HCO_3^-$

*All part of CAD



Multi-enzyme complex

- **In bacteria, six different enzymes catalyze six reactions.**
- **In animals, multi-enzyme proteins**

  -Reactions 1-3 are catalyzed by a large multi-enzyme protein. (CAD= Carbamoyl phosphate synthetase II, ATCase, Dihydroorotase: single gene product)

  -Reactions 5 and 6 are catalyzed by a single polypeptide (UMP synthase).

  -NB for channeling of intermediates and joint regulation

CPSase contains synthetase and glutaminase domains - this ensures that hydrolysis of glutamine is synchronised with availability of $HCO3^-$ and ATP

CAD:

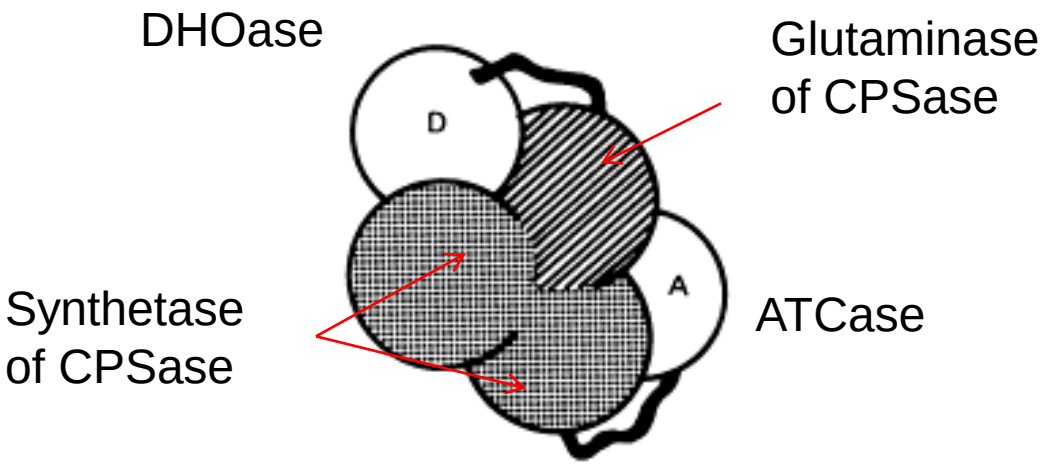


Fig. 6. Model for the conformation of CAD

In this model it is proposed that ATCase (A) and DHOase (D) activities reside in single globular domains at the *C*-terminus and *N*-terminus respectively of the CAD polypeptide, and the central CPSase moiety of 155 kDa comprises three domains of approximately equal size. The flexible linking regions (thick black lines) allow the glutaminase domain (hatched) and the two nucleotide-binding domains (stippled) to contact the A and D domains. Evidence is given in the text that ligand-induced changes in the ATCase or nucleotide-binding domains stabilize the conformation of the glutaminase domain.

Carrey et al, (1986) Biochem J, 236(2): 327–335

How would you begin to prove the existence of a “pyrimidinosome”?

Where (location within the cell) could you expect clustering?

-The enzyme that catalyzes reaction 1 is carbamoyl phosphate synthetase II, which is different from another enzyme (CPSI) in the Urea cycle.
-Two enzymes locate at different cellular compartments.

Carbamoyl Phosphate Synthesis

	Carbamoyl Phosphate Synthetase I	Carbamoyl Phosphate Synthetase II
Tissue Distribution	liver (primarily)	all
Cellular Location	mitochondrion	cytosol
Metabolic Pathway	Arginine synthesis via urea cycle	pyrimidine biosynthesis
Source of Nitrogen	ammonium ion	amide group of glutamine

Evans D. R., Bein K., Guy H. I., Liu X., Molina J. A., Zimmermann B. H. *(1993) Biochem. Soc. Trans. 21, 186–191*

Reminder of Salvage pathway: Phosphoribosyl transferases convert free bases to nucleotides

Uracil + PRPP → UMP + PPi

Orotate phosphoribosyl-transferase

Cytosine + PRPP → CMP + PPi

Orotate phophoribosyl-transferase (reaction 5) involved in de novo and salvage pyrimidine pathway

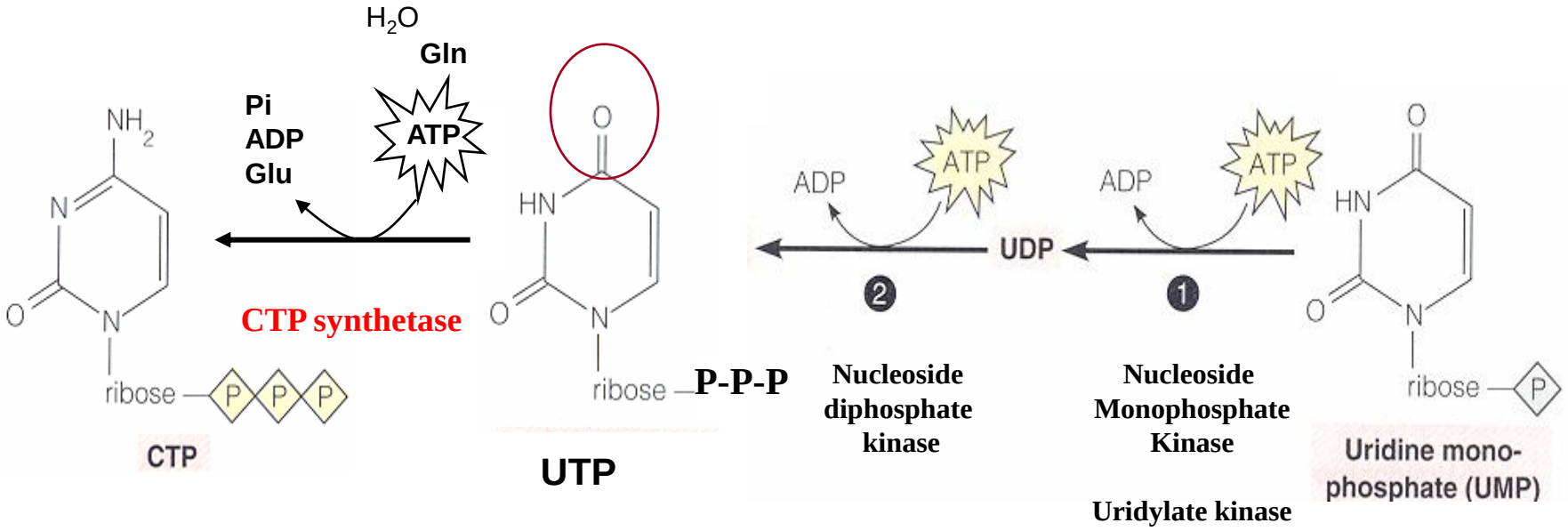
Synthesis of CTP from UMP

Unlike in AMP and GMP synthesis, UMP is not directly converted to CMP.

Rather UMP is first changed to UTP.

UTP is converted to CTP.

Glutamine is the donor for the amino group.



Enzymes and reactions

1. Carbamoyl phosphate synthetase II

formation of carbamoyl phosphate

2. Aspartate transcarbamolase (ATCase)

transfer of carbamoyl to aspartate

3. Dihydroorotase

dehydration reaction - removal of water

closure of the ring

4. Dihydroorotate dehydrogenase

oxidation reaction

5. Orotate phosphoribosyl transferase

transfer of phosphoribosyl

6. Orotidylate decarboxylase

removal of carbon dioxide (decarboxylation)

7. UMP/UDP kinases

addition of phosphate - phosphorylation reaction

8. Cytidylate synthetase

amino group transfer

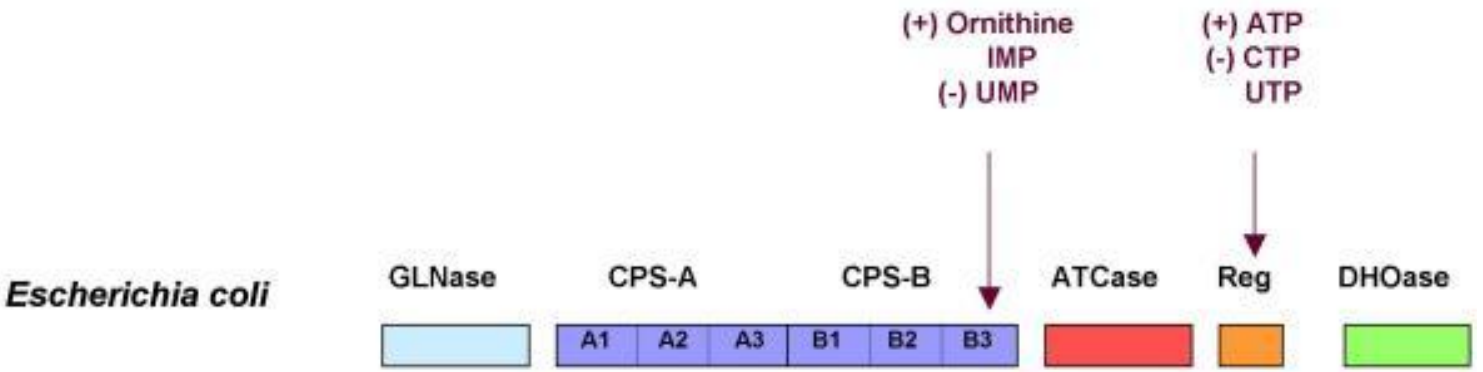
requires energy (ATP)

NOTE:

-N comes from glutamine in animals or $NH4^+$ in some bacteria

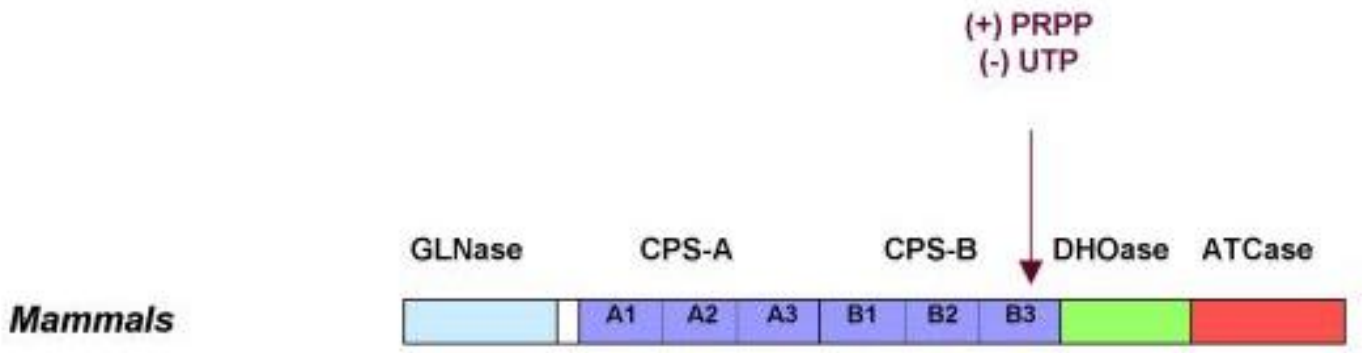
-Note steps involving ATP and steps involving NAD^+, compare the pathways of purine and pyrimidine synthesis.

Prokaryotes: CPSase, ATCase and DHOase are on different proteins



ATCase has reg unit

Eukaryotes: CPSase, ATCase and DHOase are on same protein



...implications for regulation

Serre et al (2004), *BMC Biochemistry* 2004, **5:**

Pyrimidine biosynthesis regulation differs between *E.coli.* and animals

Prokaryotes (*E.coli.*)

- ATCase
  - Inhibited by CTP alone or CTP+UTP
  - Activated by ATP

Eukaryotes

- ATCase
  - No feedback inhibition

Carbamoyl Phosphate Synthetase II and CTP Synthetase are allosterically regulated in both

E.Coli

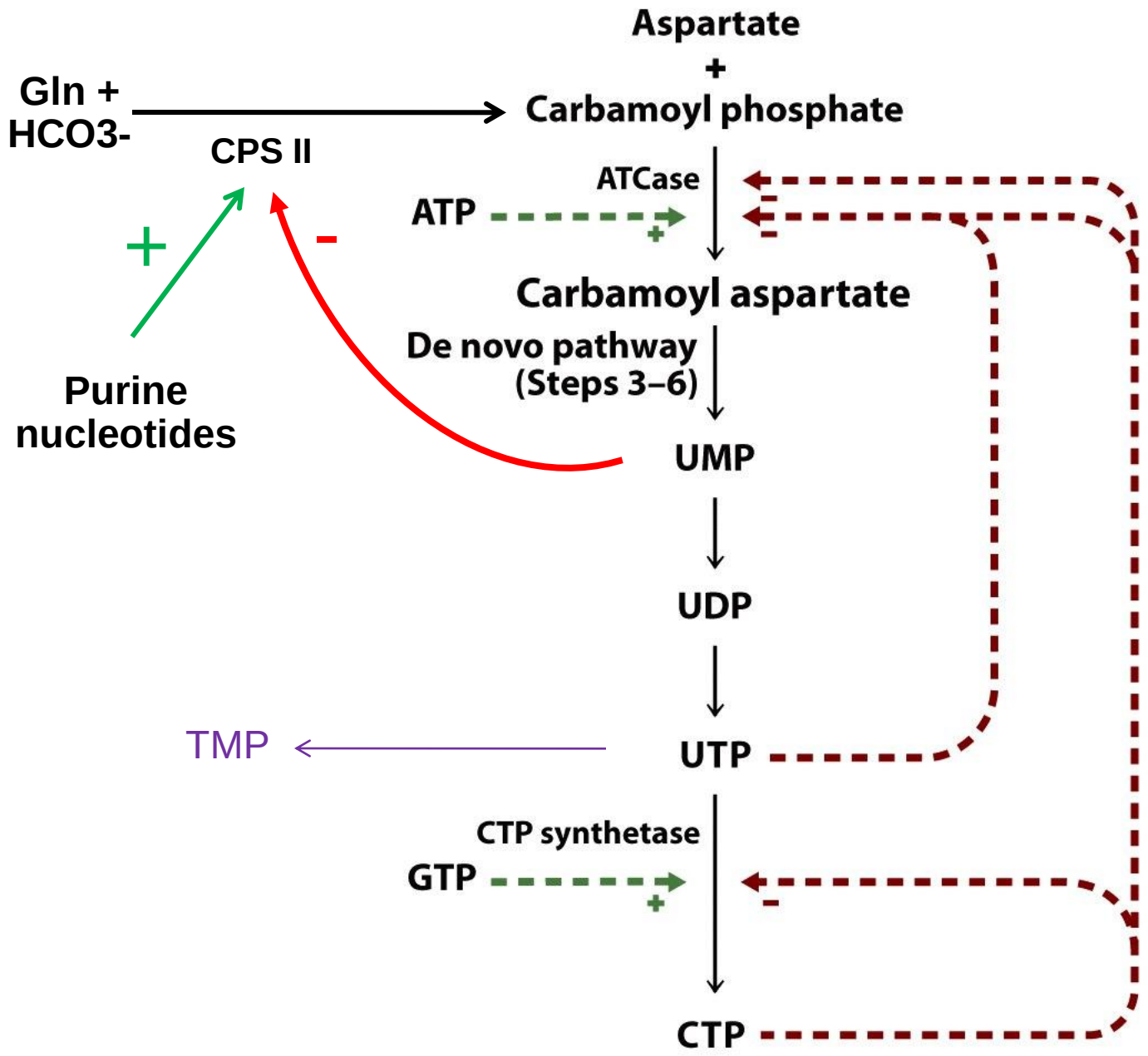
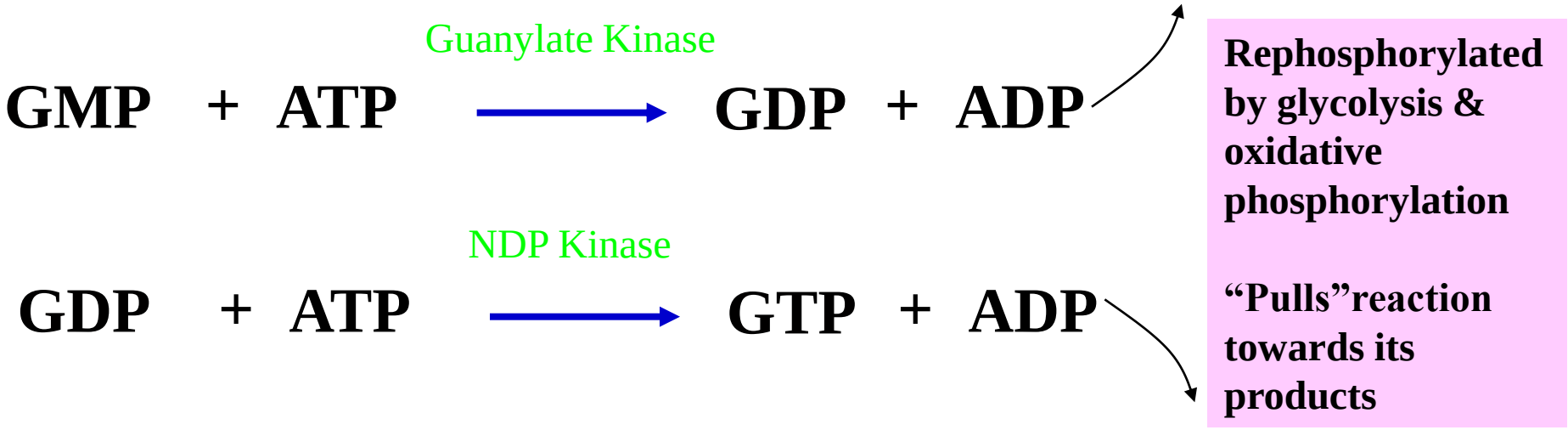


Figure 18-13 Principles of Biochemistry, 4/e

Nucleosides mono, di, & tri-phosphate: Inter-convertible



All nucleoside diphosphates are converted into nucleoside triphosphates by pathways common to ALL cells

Phosphorylation of NMP to NDP is catalyzed by a nucleoside specific kinase

Both reactions: non-specific with respect to ribose/deoxyribose

Main Differences in Purine and Pyrimidine Synthesis

1. Pyrimidine ring: assembled as a free base before attached to the ribose ring.
2. Purine ring: assembled on the ribose.
3. There is a big difference between bacteria and eukaryotics in enzyme regulation

   ATCase + CPSaseII + CTPSase- bacteria

   CPSaseII +CTPSase - animals
4. Pyrimidine synthesis follows an unbranched pathway.
5. Purine synthesis follows a branched pathway.
6. CTP is synthesized from UTP. (unbranched)
7. AMP and GMP are synthesized from IMP. (branched)

Summary.

1 Physical and chemical properties of nucleic acids
2 Nucleotide metabolism
   a) salvage pathways
   b) de novo synthesis
3 Purine synthesis
   a) from PRPP & glutamine to IMP
   b) from IMP to AMP &GMP
   c) control of the pathway
4 Pyrimidine synthesis
   a) from aspartate & carbamoyl phosphate to UMP & CTP
   b) control of the pathway

Question 1

- Azaserine and DON are glutamine analogs. They form covalent bonds to nucleophiles at the active sites of the enzymes that bind glutamine, thereby irreversibly inactivating these enzymes. Identify the nucleotide biosynthesis intermediates that accumulate in the presence of either of these glutamine antagonists.

Question 1- answer

- The reactions in which glutamine participates are
  - Reaction 1 and 4 of IMP synthesis
  - GMP synthetase reaction of GMP synthesis
  - Reaction 1 of pyrimidine synthesis
  - Reaction from UTP to CTP
- Therefore intermediates are
  - PRPP
  - FGAR
  - XMP
  - UTP

If a cell has an adequate supply of adenine nucleotides but requires more guanine nucleotides for protein synthesis:

1. Glutamine-PRPP amidotransferase will not be fully inhibited.
2. AMP will be a feedback inhibitor of the condensation of IMP with aspartate.
3. ATP will stimulate the production of GMP from IMP.
4. ATP will inhibit nucleoside diphosphate reductase.

A. 1, 2 and 3
B. 1 and 3
C. 2 and 4
D. 4 only
E. All four

The correct answer is (A). Why:

1. The synergistic effect of both AMP and GMP is needed for complete inhibition.
2. This assures that the limited amount of IMP formed will be channeled to the production of the guanine nucleotides.
3. ATP provides the energy for this branch.
4. The formation of dATP is not applicable in this situation.

Similarities and differences in the synthesis of purines and pyrimidines

	purine	pyrimidine
• aspartate		
• prpp		
• dehydration		
• carboxylation		
• decarboxylation		
• oxidation/reduction		
• transamination		
• ATP		
• feedback inhibition		
• glutamine		
• formyl group		
• glycine		