Nucleotide Biosynthesis: Pyrimidine Synthesis; how does this pathway evolve from prokaryote to eukaryote?

The pathway consists of 6 enzymatic steps to form UMP. Unlike in purine biosynthesis the pyrimidine ring is first formed and the the R5Pis added as PRPP. The N1,C4, 5 & 6 are all derived from aspartate. C3 is from HCO_3^- and N3 from glutamine.

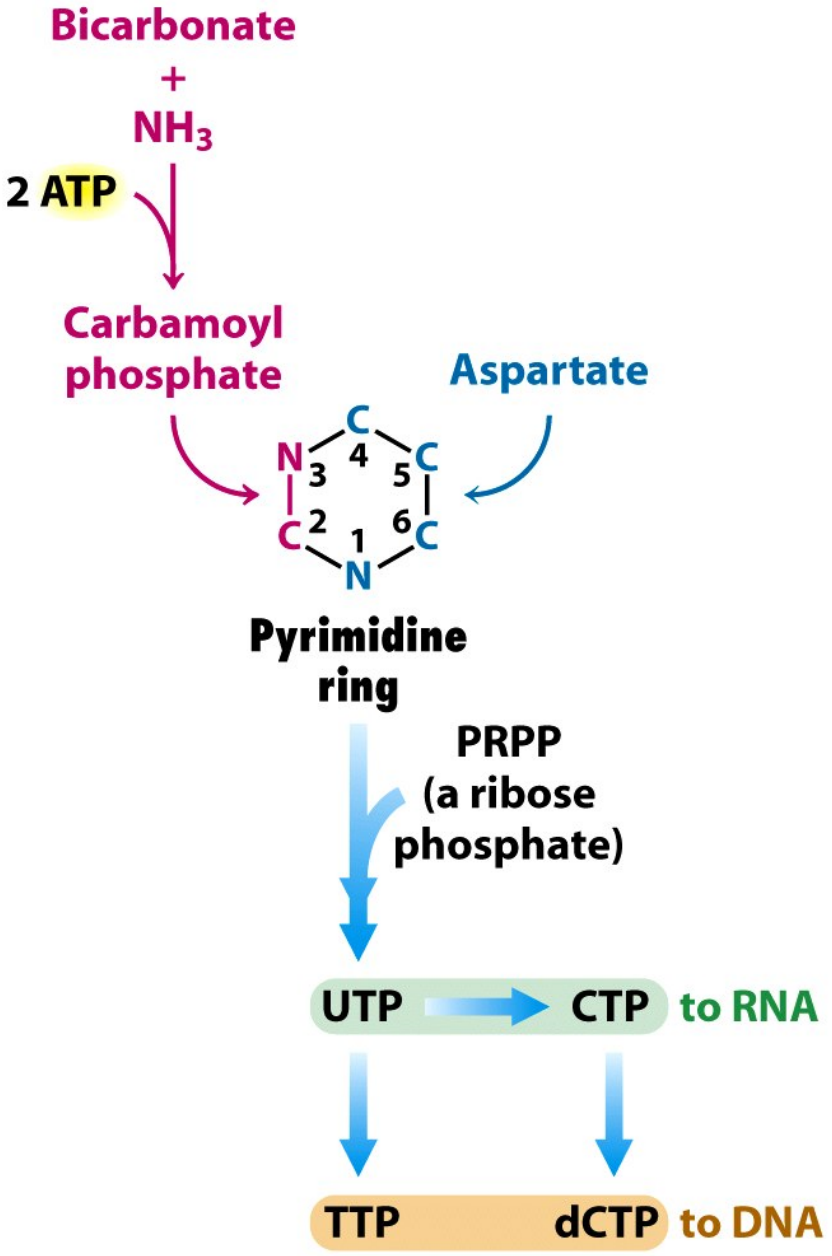
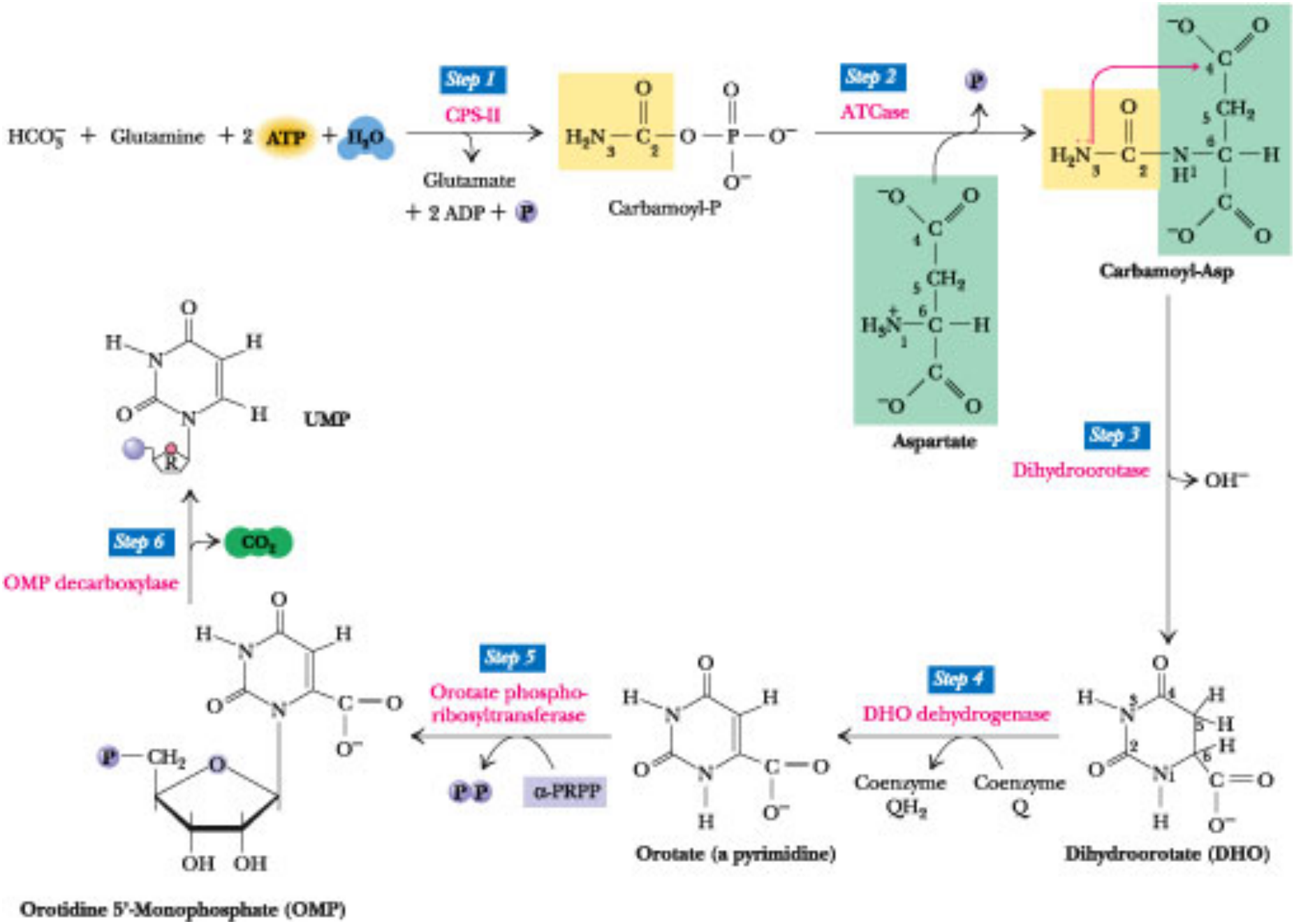
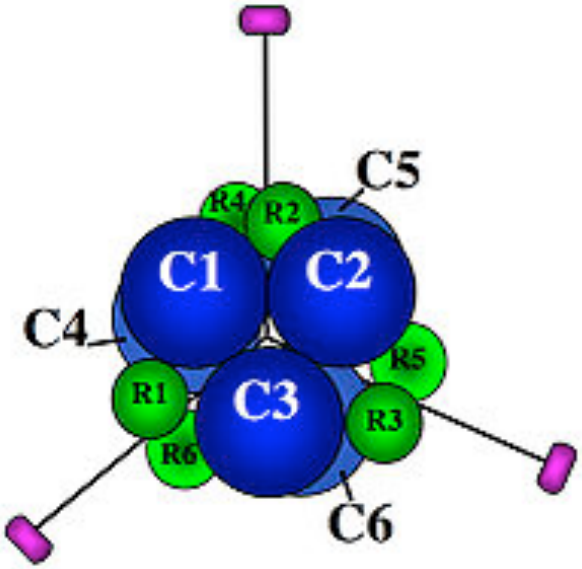
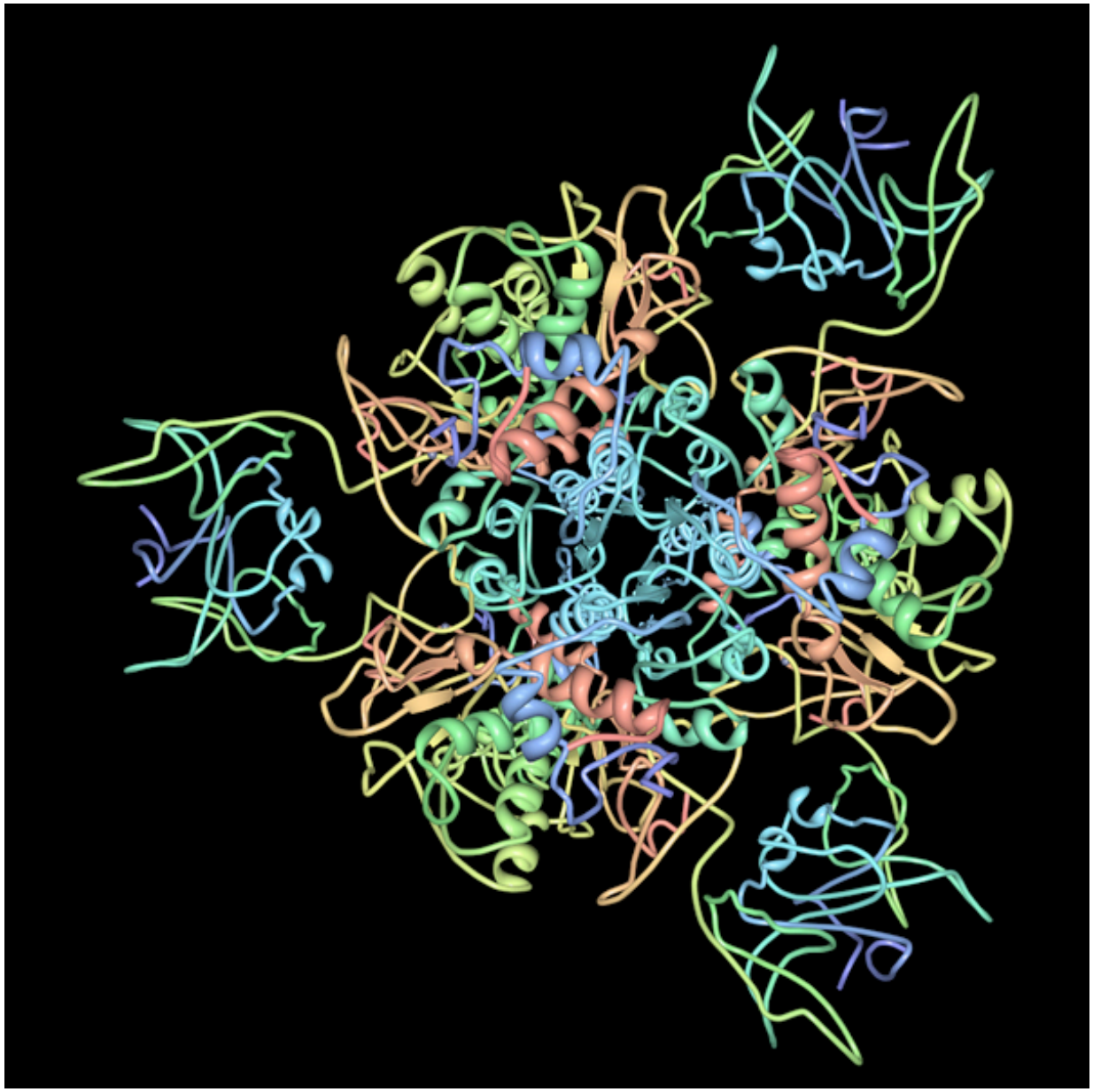


Figure 25-2
Biochemistry, Sixth Edition


HCO3− + Glutamine + 2 ATP + H2O
Step 1
CPS-II
Glutamate
+ 2 ADP + P
Carbamoyl-P
Step 2
ATCase
P
Carbamoyl-Asp
Aspartate
Step 3
Dihydroorotase
OH−
Dihydroorotate (DHO)
Step 4
DHO dehydrogenase
Coenzyme QH2
Coenzyme Q
Orotate (a pyrimidine)
Step 5
Orotate phospho-ribosyltransferase
P P
α-PRPP
Orotidine 5'-Monophosphate (OMP)
Step 6
OMP decarboxylase
CO2
UMP



Schematic diagram of prokaryote ATCase structure, depicting spatial arrangement of green regulatory (R) and blue catalytic (C) subunits.



Aspartate carbamoyltransferase from Escherichia coli.

The allosteric site, found in the allosteric domain of the R chains of the ATCase complex binds to the nucleotides ATP, CTP and/or UTP. There is one site with high affinity for ATP and CTP and one with 10- to 20-fold lower affinity for these nucleotides in each regulatory dimer. ATP binds predominantly to the high-affinity sites and subsequently activates the enzyme, while UTP and CTP binding leads to inhibition of activity. UTP can bind to the allosteric site, but inhibition of ATCase by UTP is possible only in combination with CTP.

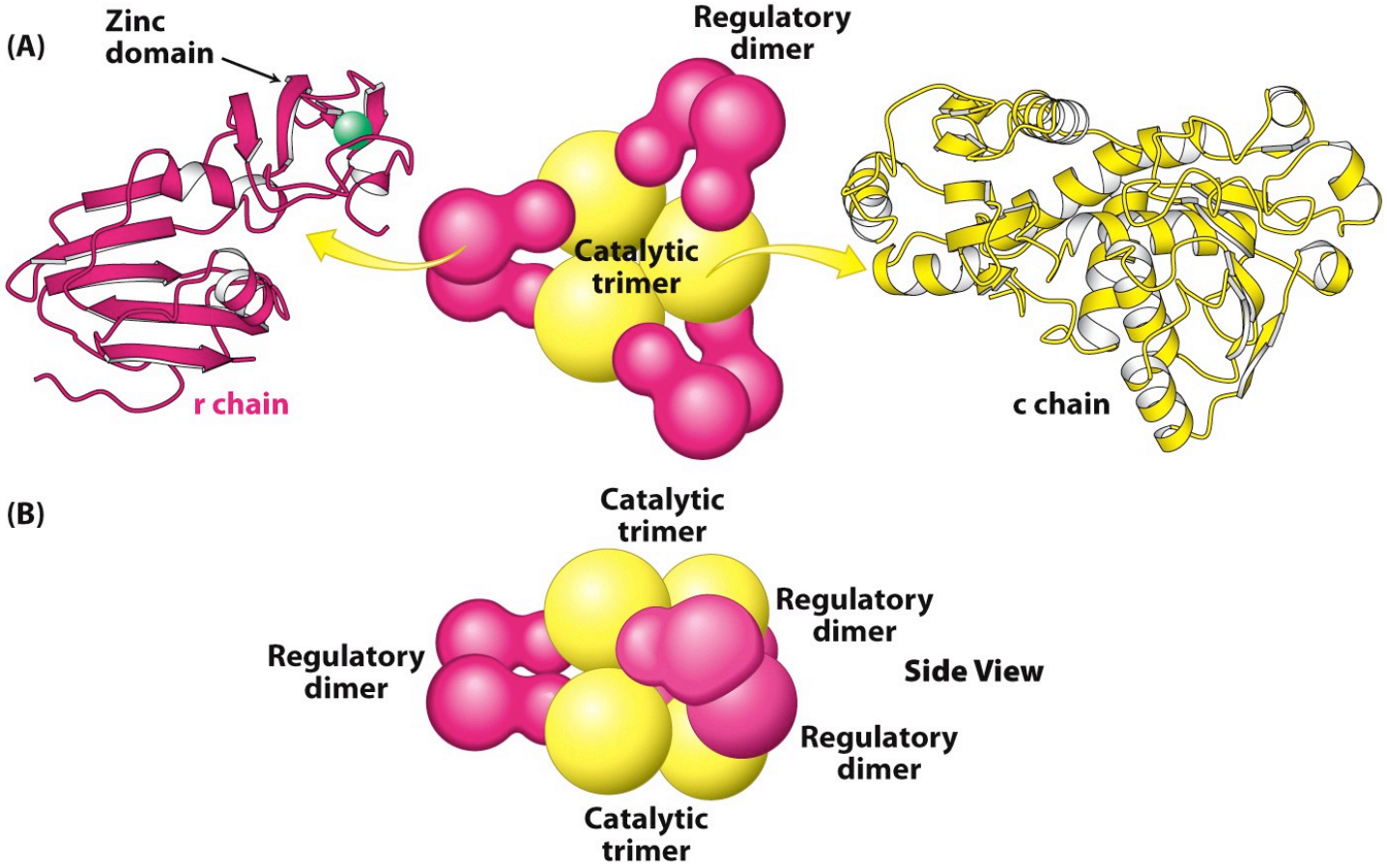
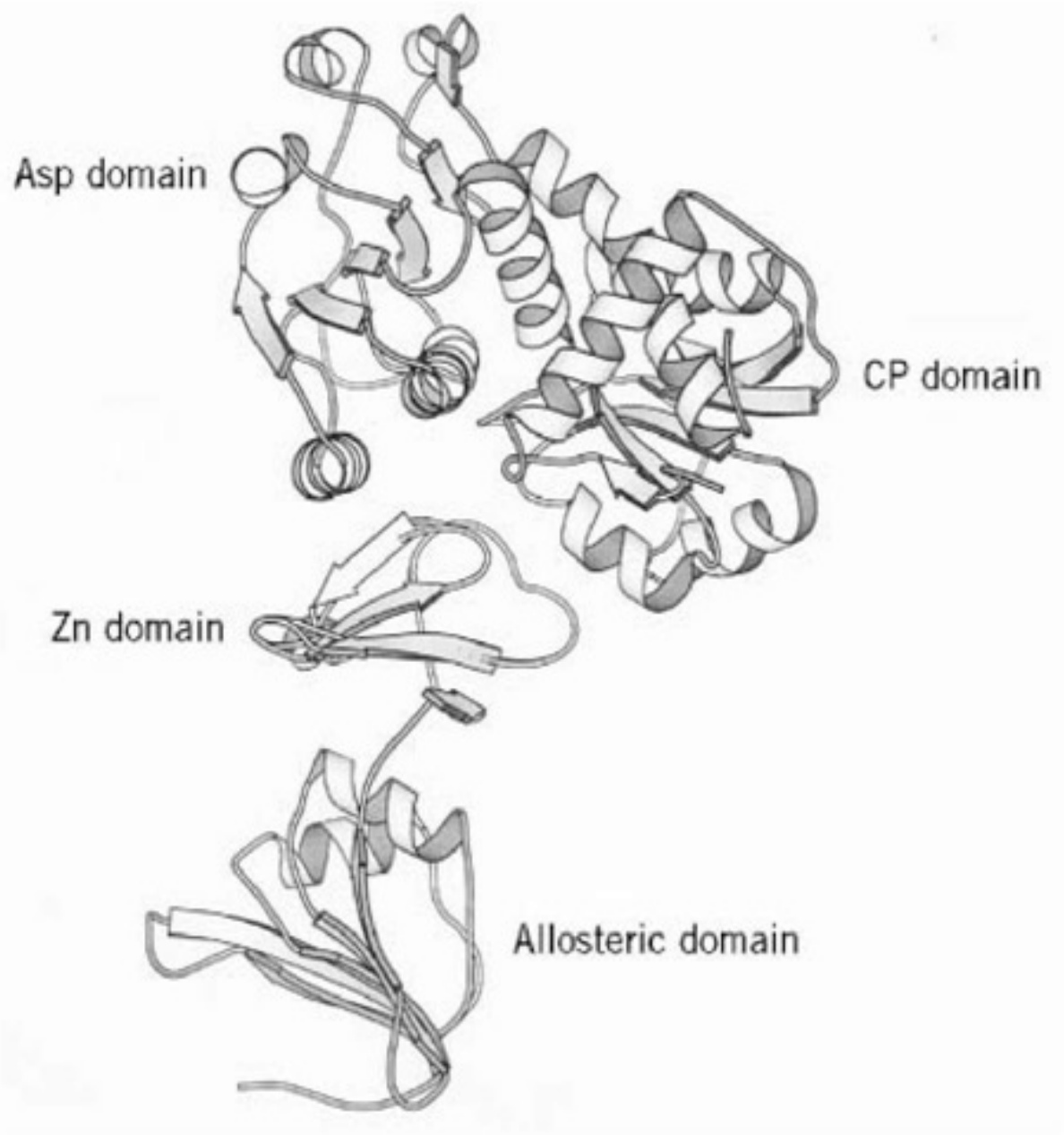
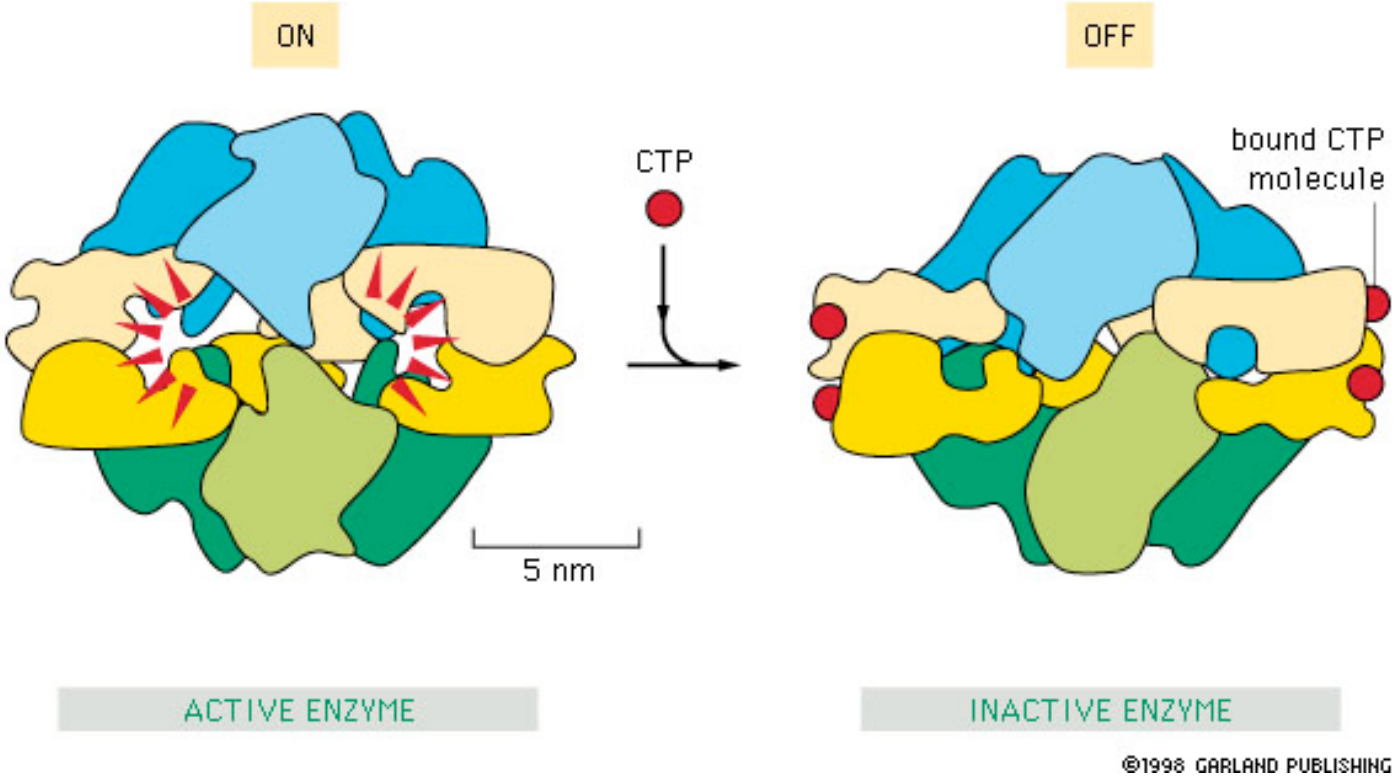


Figure 10.6
***Biochemistry,* Seventh Edition**

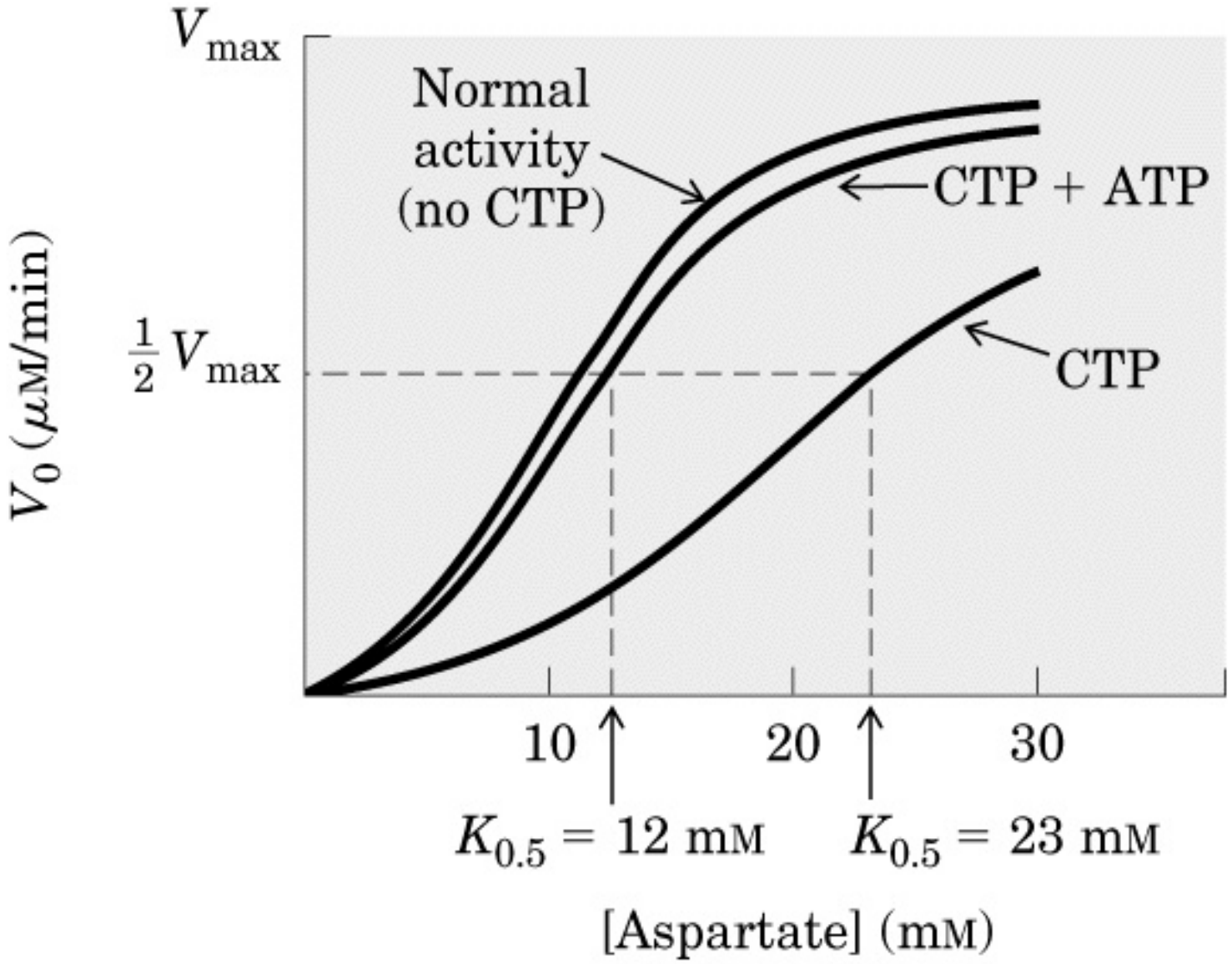


Domain organization of the E. coli ATCase illustrated with one catalytic chain (C1) and its associated regularly is composed of two independently folding domains: the aspartate-binding (Asp) domain and carbamoyl phosphate-binding regulatory chain is composed of the zinc-binding (Zn) domain and nucleotide-binding (allosteric) domain.

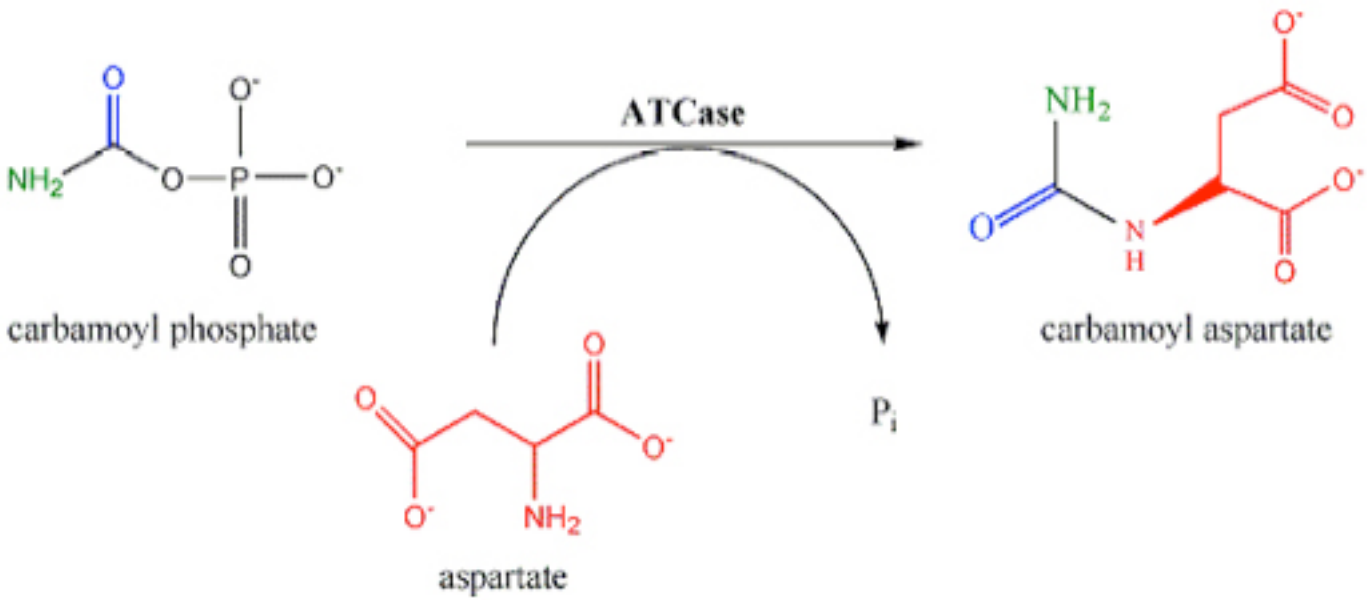
With CTP present, UTP binding is enhanced and preferentially directed to the low-affinity sites. On the converse, UTP binding leads to enhanced affinity for CTP at the high-affinity sites and together they inhibit enzyme activity by up to 95%, while CTP binding alone inhibits activity to 50% to 70%. Comparison of the crystal structures of the T and R forms of ATCase show that it swells in size during the allosteric transition, and that the catalytic subunits condense during this process. The two catalytic trimers move apart along the threefold axis by 12 Å, and they rotate about this axis by 5° each, ultimately leading to a reorientation of the regulatory subunits around their twofold axis by 15°. This quaternary structure change is associated with alterations in inter-subunit and inter-domain interactions.

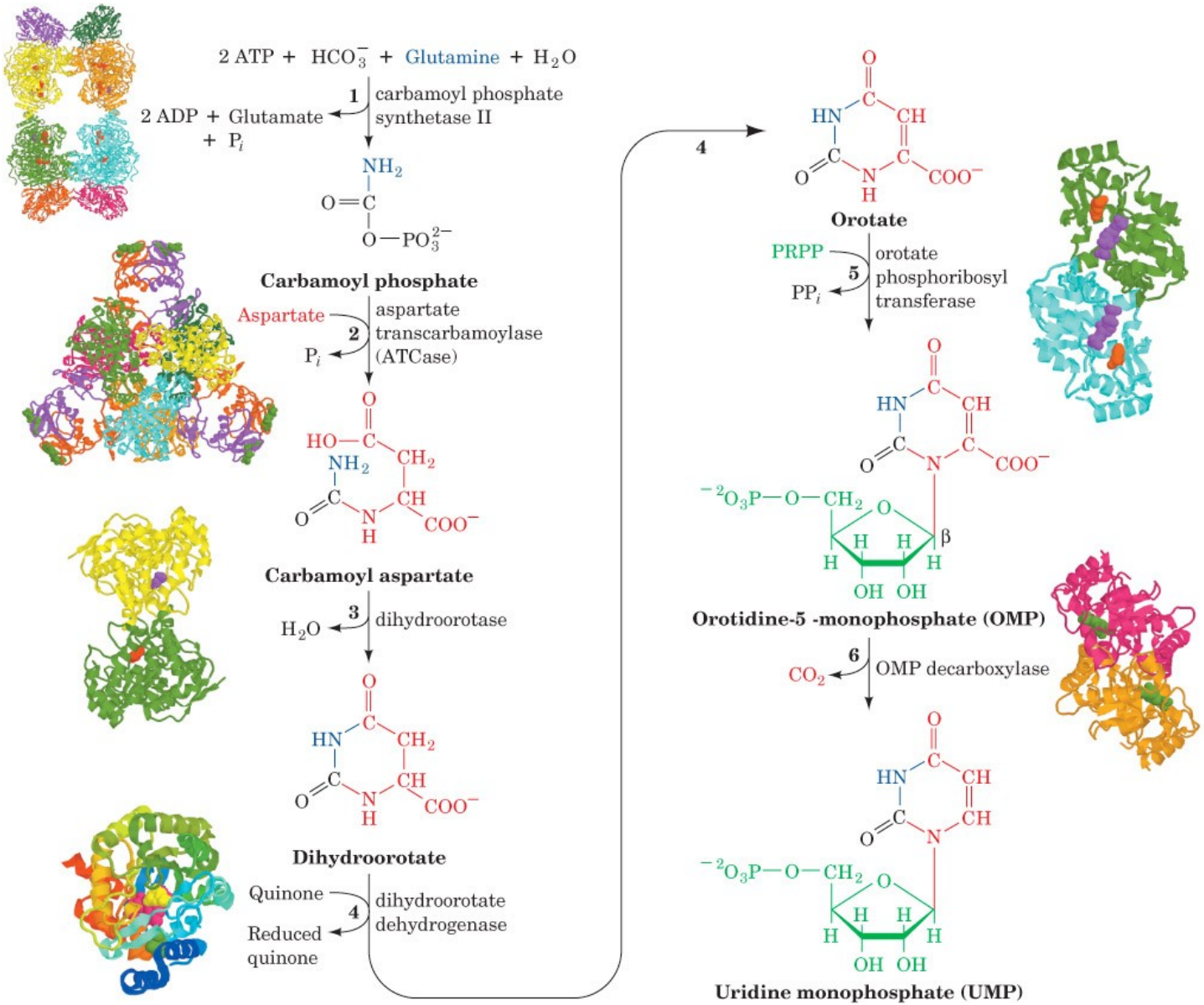


The regulation of pyrimidine synthesis occurs mainly at the first step which is catalyzed by ATCase. Inhibited by CTP and activated by ATP



ATCase is a highly regulated enzyme that catalyses the first committed step in pyrimidine biosynthesis, the condensation of aspartate and carbamyl phosphate to form N-carbamyl-L-aspartate and inorganic phosphate. ATCase controls the rate of pyrimidine biosynthesis by altering its catalytic velocity in response to cellular levels of both pyrimidines and purines. The end-product of the pyrimidine pathway, CTP, induces a decrease in catalytic velocity, whereas ATP, the end-product of the parallel purine pathway, exerts the opposite effect, stimulating the catalytic activity.





Metabolic pathway for the *de novo* synthesis of UMP. Note that in pyrimidine biosynthesis the ring is formed before the PRPP is used as a substrate to attach the R5P. Rxn 1 is a hetero-octomer in *E.coli* of $\alpha_4\beta_4$ and Rxn 2 is a c_6r_6 hetero-dodecamer with the c being the catalytic subunits and the r the regulatory subunits.

This protein is present in bacteria and the mitochondria.

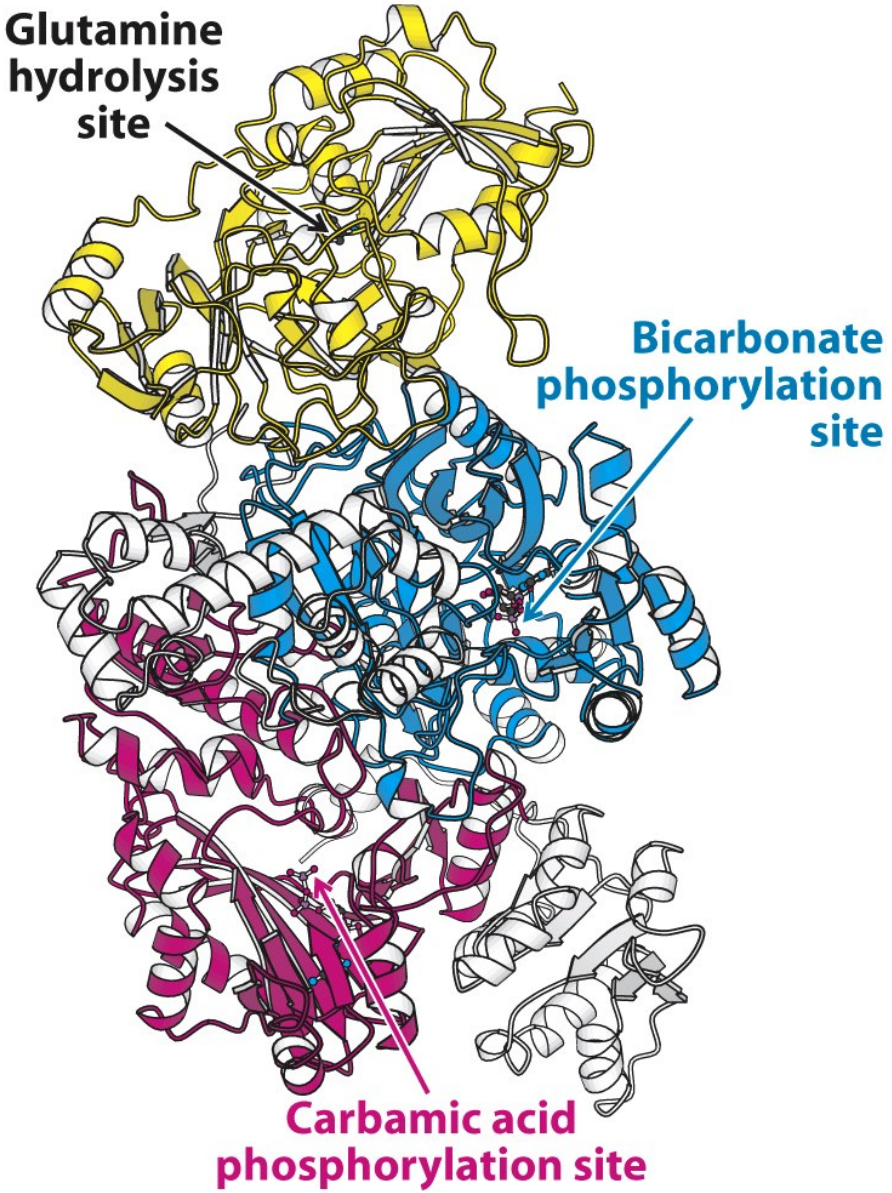


Figure 25-3
Biochemistry, Sixth Edition

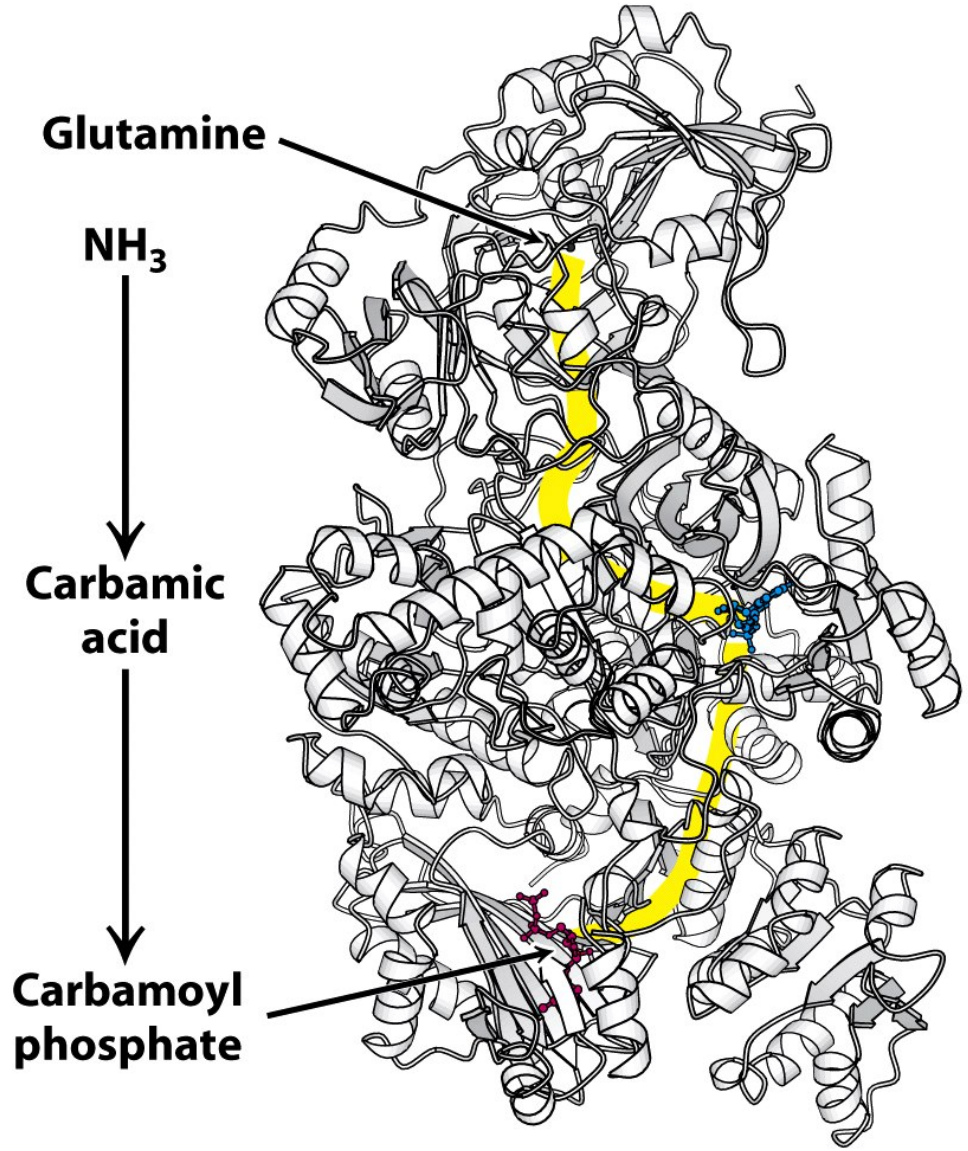
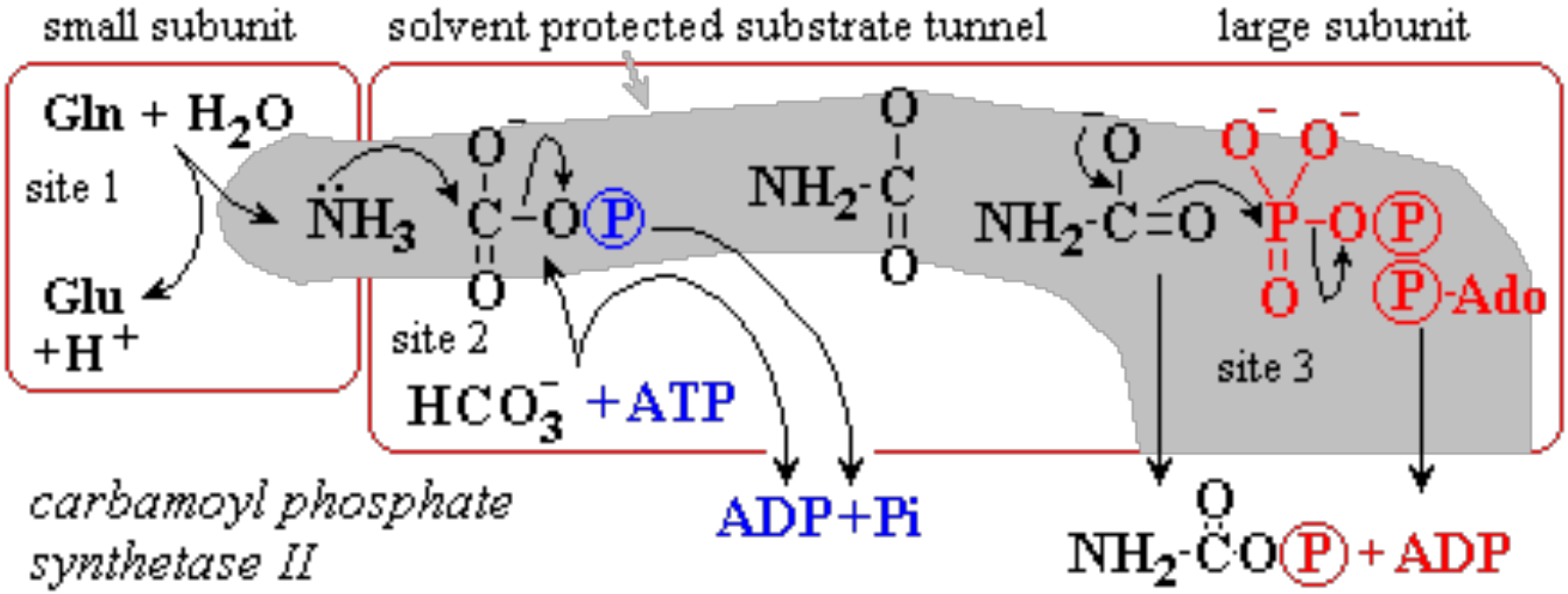


Figure 25-4
Biochemistry, Sixth Edition



Carbamate remains in the protected tunnel, and passes to site 3 for final phosphorylation. This means that carbamate never enters the surrounding medium, and is presented to the ATP at site 3 at a high effective concentration. This is important, since for hydrolysis of carbamoyl phosphate is -51.5 kJ/mol, making it difficult for ATP to phosphorylate in a simple solution phase reaction.

If the small subunit is missing, the carbonyl phosphate is solvent exposed and is simply hydrolyzed back to HCO3- and Pi.

Carbamoyl phosphate synthetase 2 represents one of the common strategies for addition of amino N in synthesis:

X-OH → X-OP → X-NH2

ATP->ADP Gln-> Glu + Pi

In animal cells ATCase is a domain of a multifunctional protein in mammalian cells. It is capable of catalyzing the formation of **carbamoyl phosphate**, **carbamoyl aspartate**, **and dihydroorotate (CAD)**.

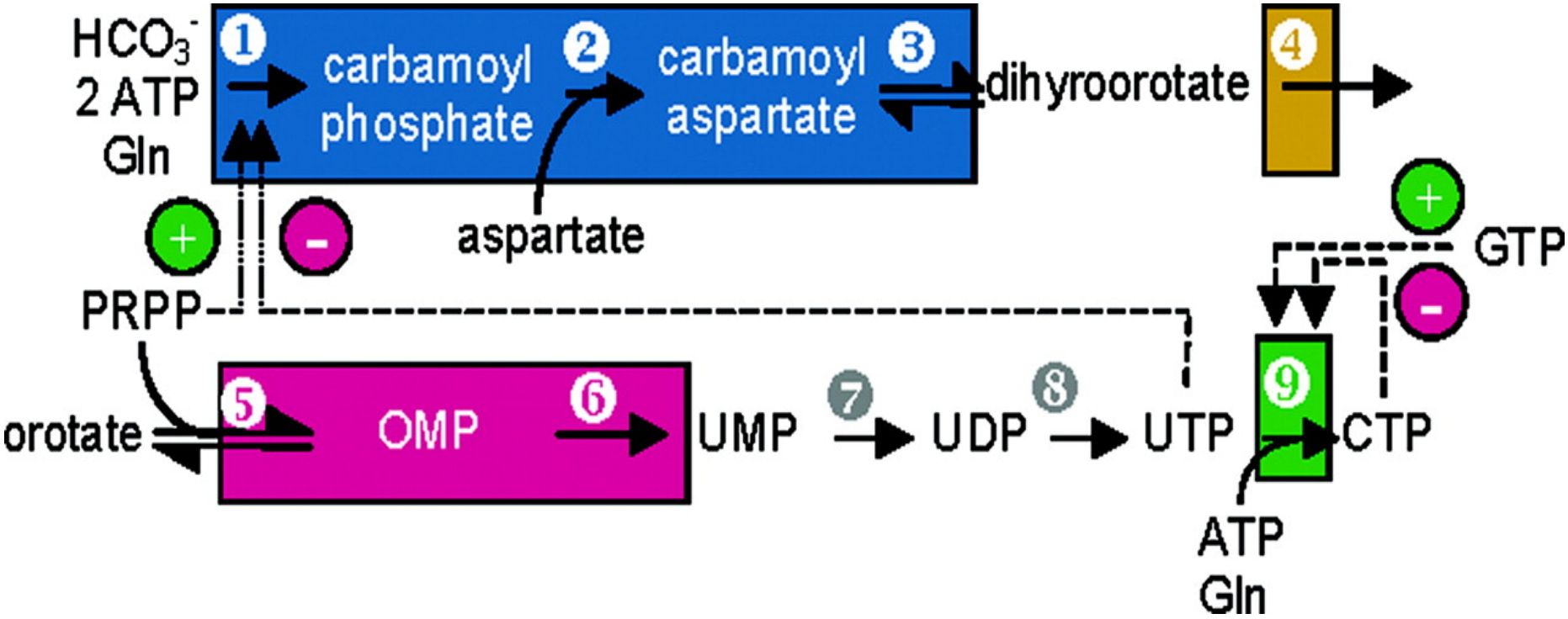
The carbamoyl synthetase activity of this complex is termed *carbamoyl phosphate synthetase II* (CPS-II) as opposed to CPS-I, which is involved in the **Urea Cycle.**

ATCase, and therefore the activity of CPS-II, is localized to the cytoplasm and prefers glutamine as a substrate.
CPS-I of the urea cycle is localized in the mitochondria and utilizes ammonia.

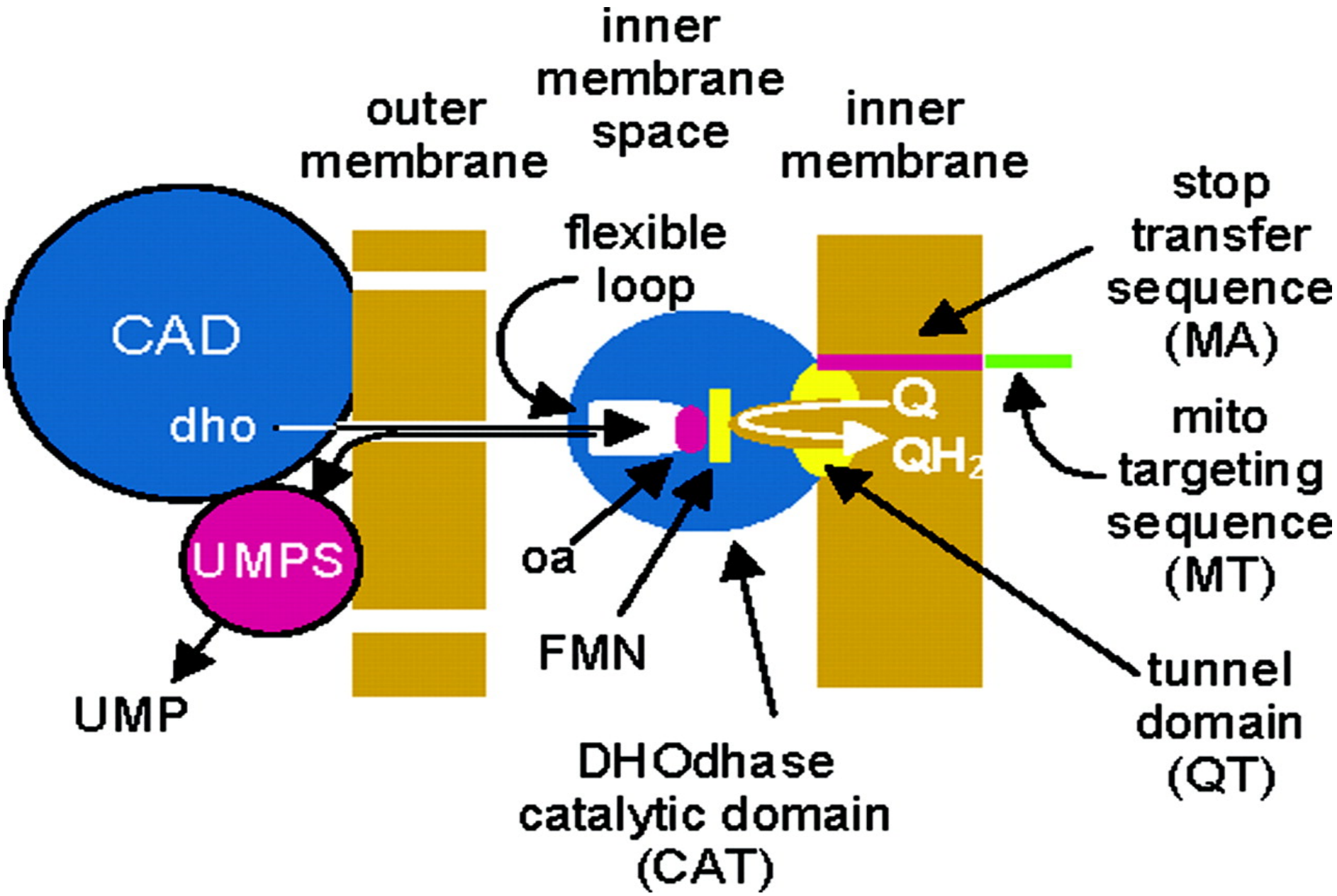
The CPS-II domain is activated by ATP and inhibited by UDP, UTP, dUTP, and CTP. The role of glycine in ATCase regulation is to act as a competitive inhibitor of the glutamine binding site.

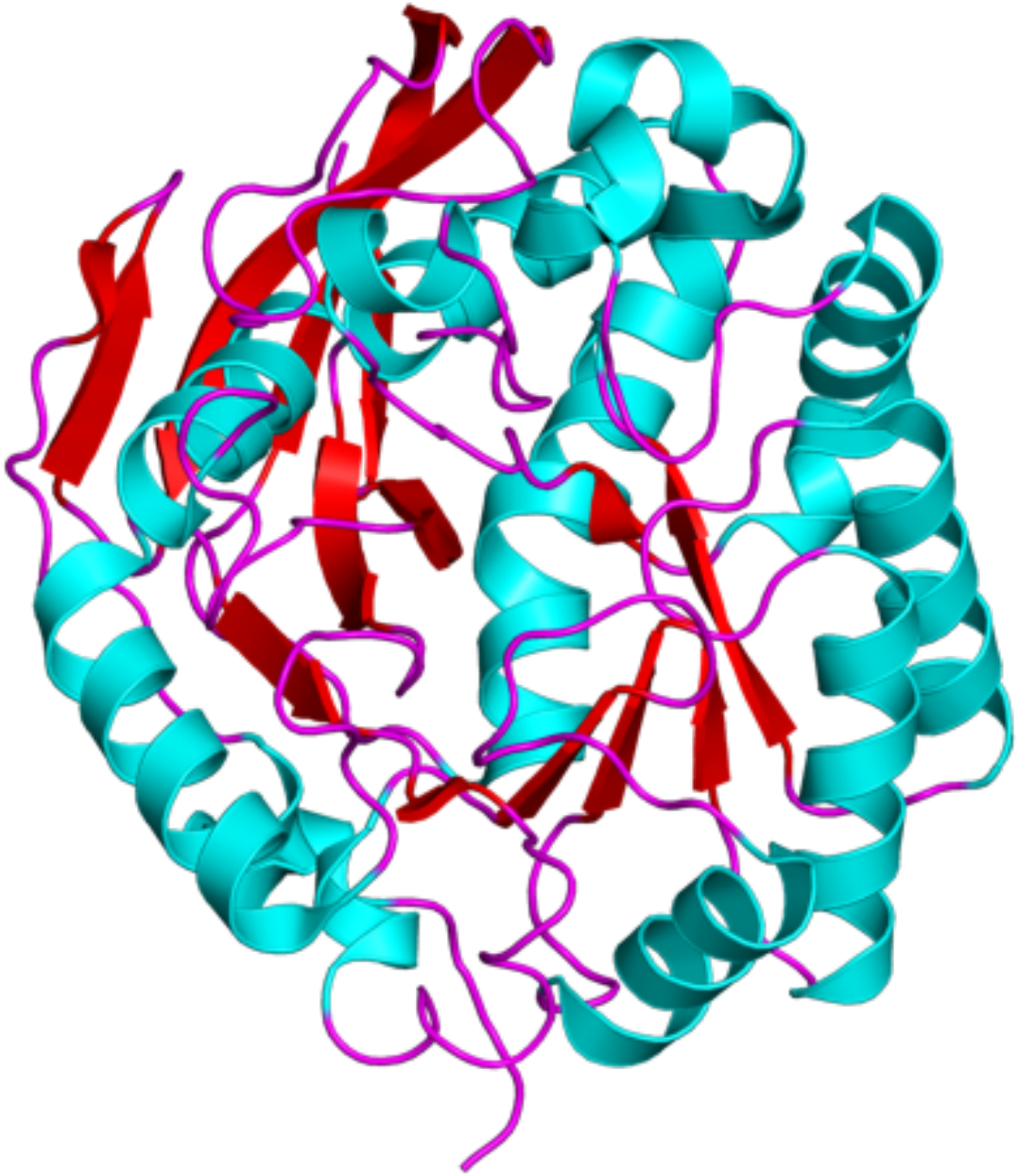
CPS II is unusual in that it does not require biotin to bind the HCO_3^- & requires 2ATP.

The de novo pyrimidine biosynthetic pathway in eukaryotes, steps 1-6, and the subsequent formation of CTP. UTP is formed from UMP by sequential reactions catalyzed by UMP/CMP kinase (circled 7) and nucleoside-diphosphate kinase (circled 8). All 3 activities of CAD single polypeptide, 243kD.

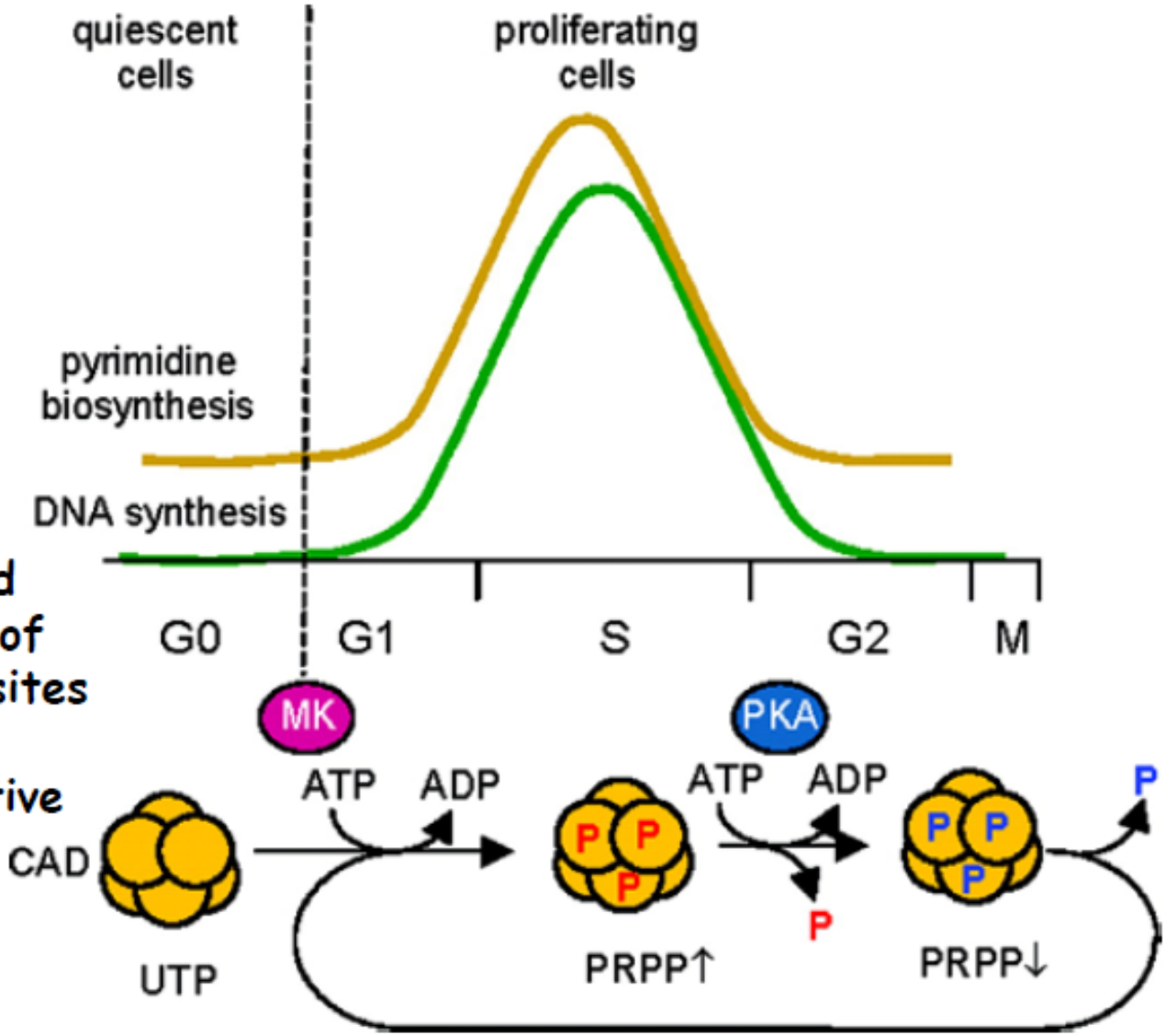


Schematic representation of DHOdhase bound to the inner mitochondrial membrane. oa, orotate; Q, ubiquinone; UMPS, UMP synthase.

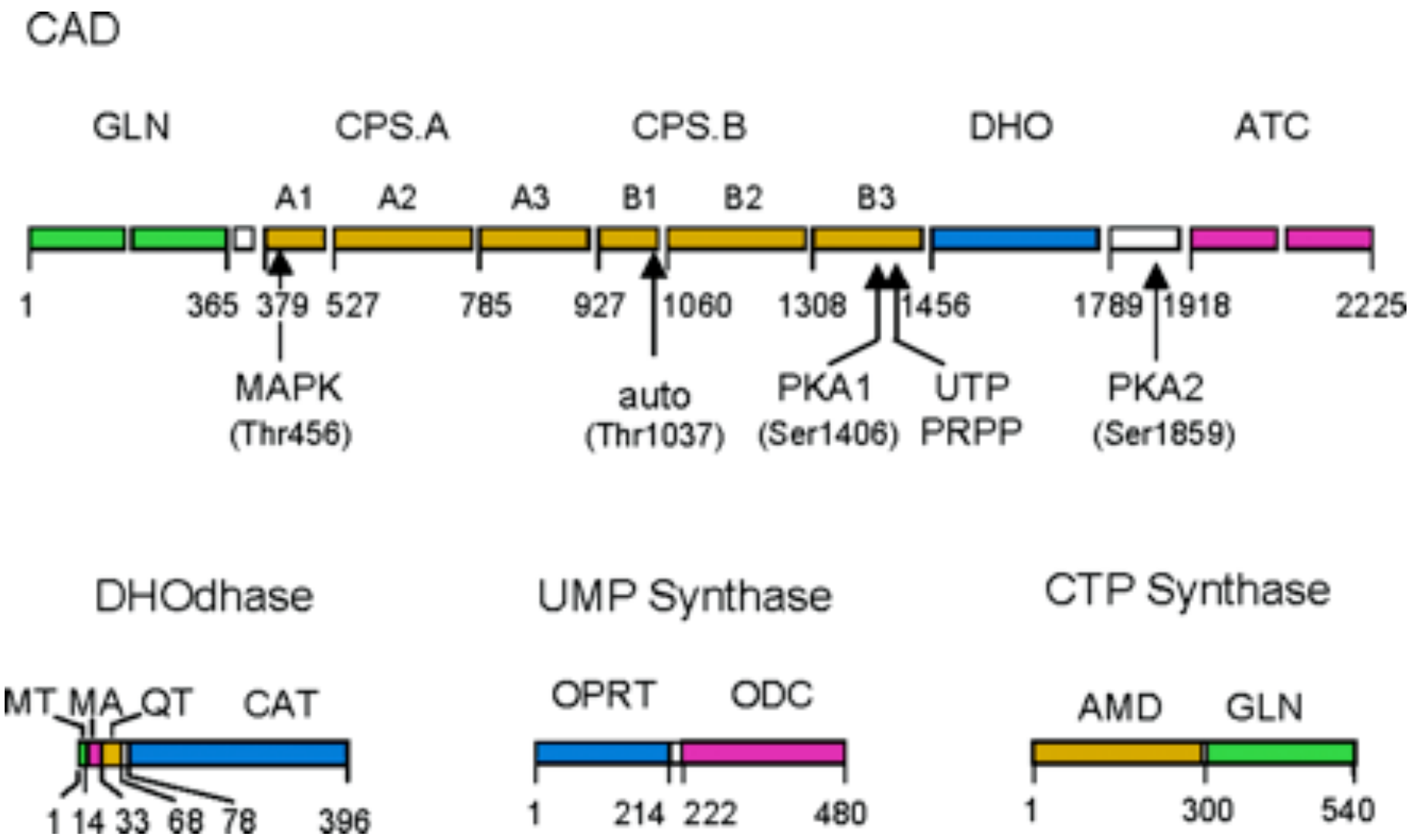




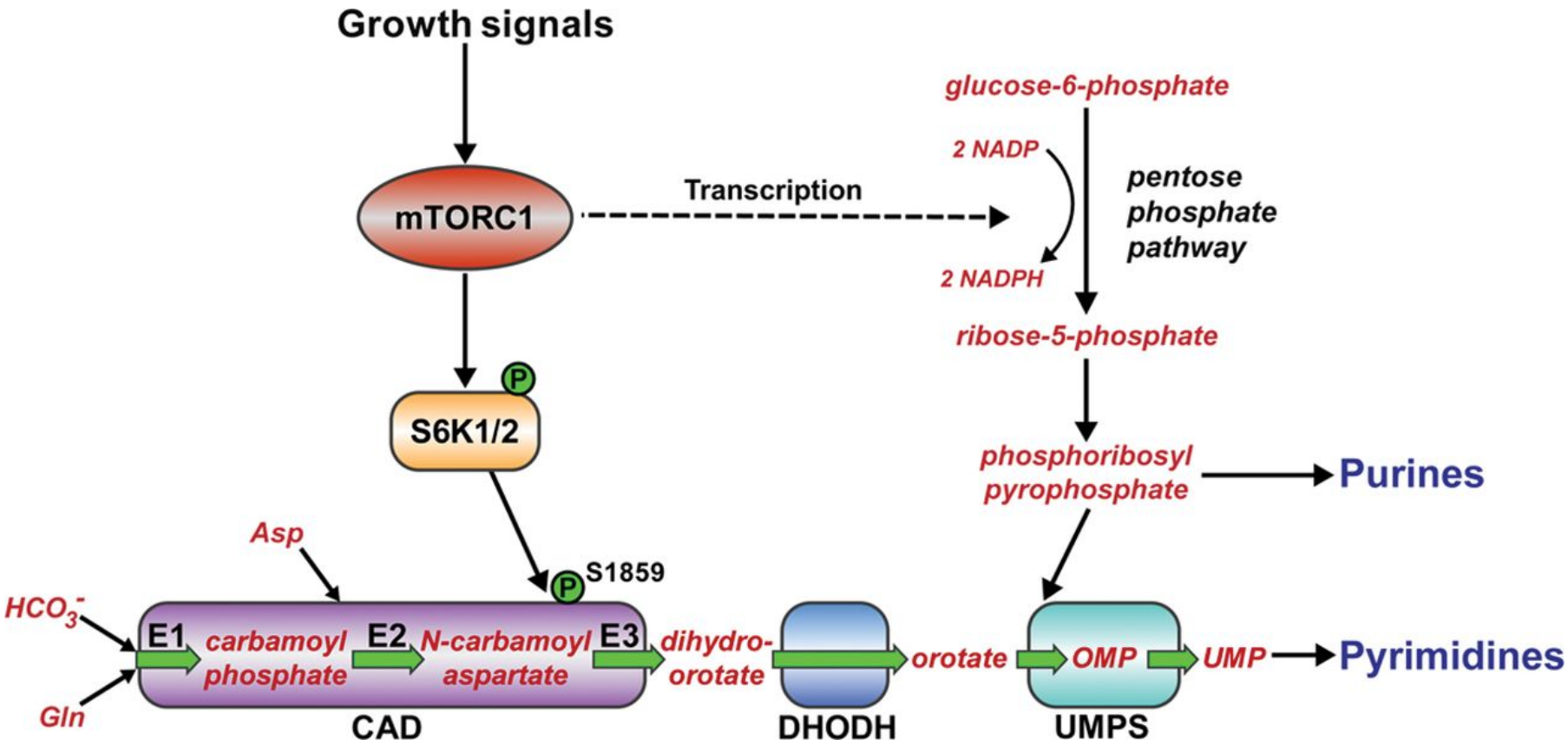
Cell Cycle Regulation of pyrimidine biosynthesis] In eukaryotes



Phosphorylation and Dephosphorylation of CAD at different sites make the enzyme more of less sensitive to activation by PRPP

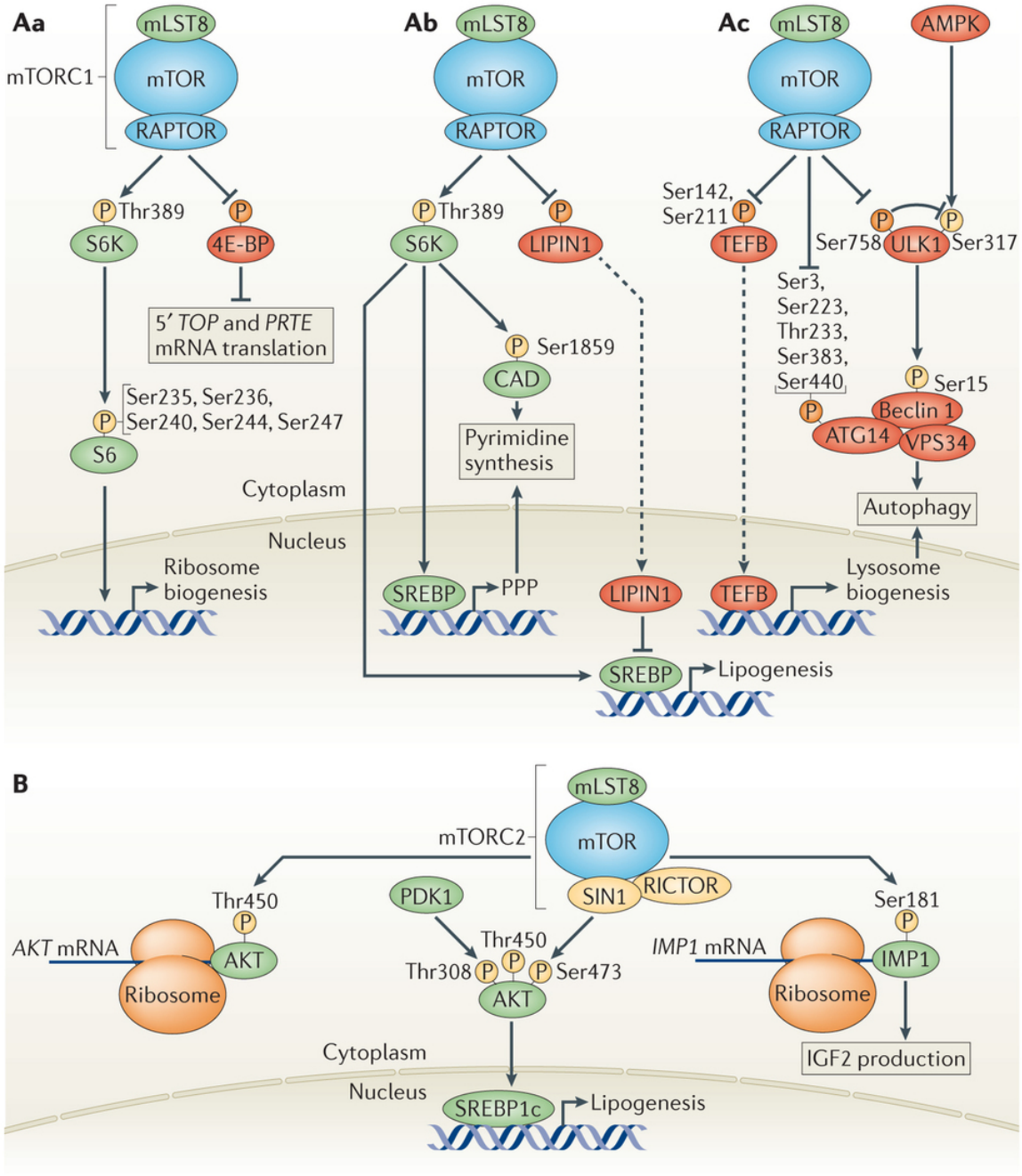


The domain structure of the multifunctional proteins and enzymes that catalyze pyrimidine biosynthesis. The region of CAD that binds UTP and PRPP, the CAD autophosphorylation site (auto), and the CAD sites phosphorylated by MAP kinase (MAPK) and PKA are indicated.



mTORC1 stimulates de novo nucleotide synthesis

Through transcriptional mechanisms, mTORC1 induces the expression of genes encoding enzymes of the PPP, and preferentially stimulates flux through the oxidative branch. This promotes production of NADPH, as well as phosphoribosyl pyrophosphate, required for the synthesis of purine and pyrimidine nucleotides. In parallel, mTORC1 specifically and acutely stimulates flux through the de novo pyrimidine synthesis pathway via S6K1-dependent phosphorylation of Ser1859 on CAD, a multifunctional enzyme that catalyses the first three steps in the pathway. Thus growth signals (e.g. growth factors and nutrients) stimulate an mTORC1-dependent increase in de novo synthesized nucleotides, and their incorporation into RNA and DNA. OMP, orotidine monophosphate; UMPS, UMP synthase.


Aa
mTORC1
mLST8
mTOR
RAPTOR
P Thr389
S6K
P
4E-BP
5′ TOP and PRTE mRNA translation
Ser235, Ser236, Ser240, Ser244, Ser247
S6
Cytoplasm
Nucleus
Ribosome biogenesis
Ab
mLST8
mTOR
RAPTOR
P Thr389
S6K
P
LIPIN1
P Ser1859
CAD
Pyrimidine synthesis
SREBP
PPP
LIPIN1
SREBP
Lipogenesis
Ac
mLST8
mTOR
RAPTOR
AMPK
Ser142, Ser211
P
TEFB
Ser758
P
ULK1
P
Ser317
Ser3, Ser223, Thr233, Ser383, Ser440
P
Ser15
Beclin 1
ATG14
VPS34
Autophagy
TEFB
Lysosome biogenesis
B
mLST8
mTORC2
mTOR
SIN1
RICTOR
Thr450
P
AKT
AKT mRNA
Ribosome
PDK1
Thr450
Thr308 P P Ser473
AKT
Ser181
P
IMP1
IMP1 mRNA
Ribosome
IGF2 production
Cytoplasm
Nucleus
SREBP1c
Lipogenesis

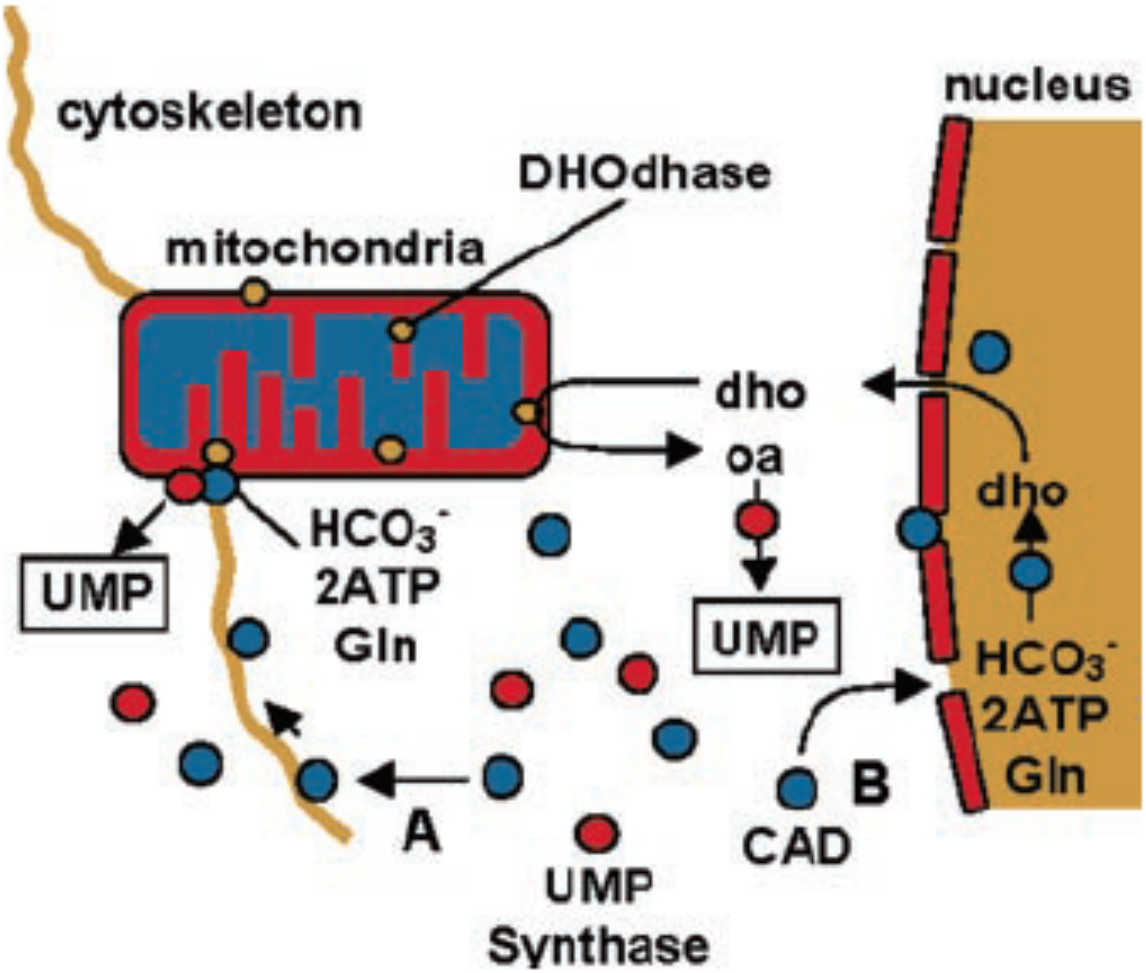
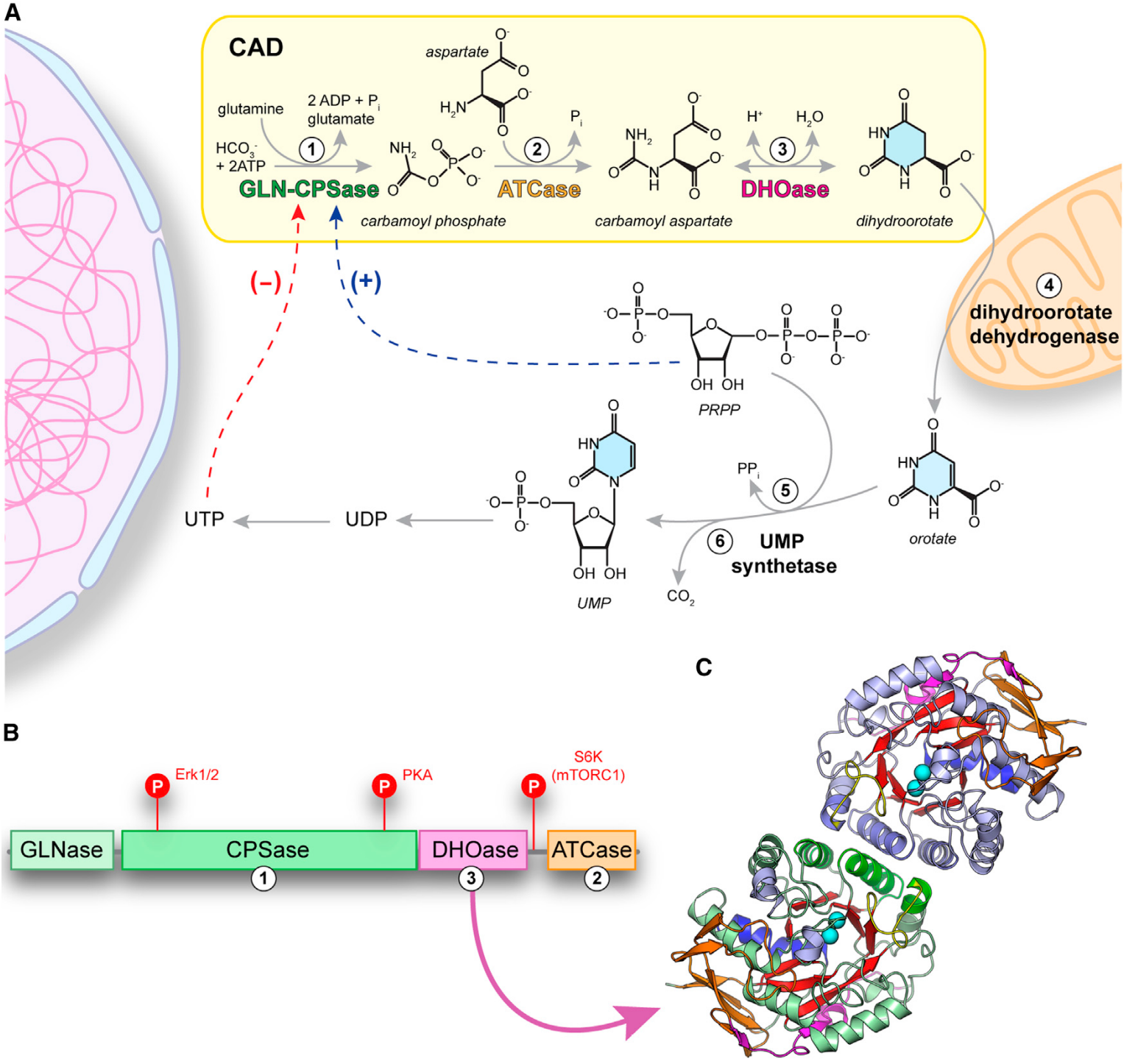


FIG. 4. **Intracellular distribution of CAD (*blue spheres),*DHOdhase (*yellow spheres), and UMP synthase (red spheres)* and putative interactions with the nucleus, mitochondria, and cytoskeleton.**
The paths of CAD, both translocation along the filament (A)*and entry into the nucleus* **(B)** *are shown. oa, orotate.*
Evans D R , and Guy H I J. Biol. Chem. 2004


A
CAD
aspartate
glutamine
2 ADP + Pi
glutamate
HCO3- + 2ATP
1
GLN-CPSase
carbamoyl phosphate
2
Pi
ATCase
carbamoyl aspartate
H+
H2O
3
DHOase
dihydroorotate
4
dihydroorotate dehydrogenase
(–)
(+)
PRPP
orotate
PPi
5
6
UMP synthetase
CO2
UMP
UDP
UTP
B
Erk1/2
PKA
S6K (mTORC1)
P
GLNase
CPSase
DHOase
ATCase
1
3
2
C

From previous slide;

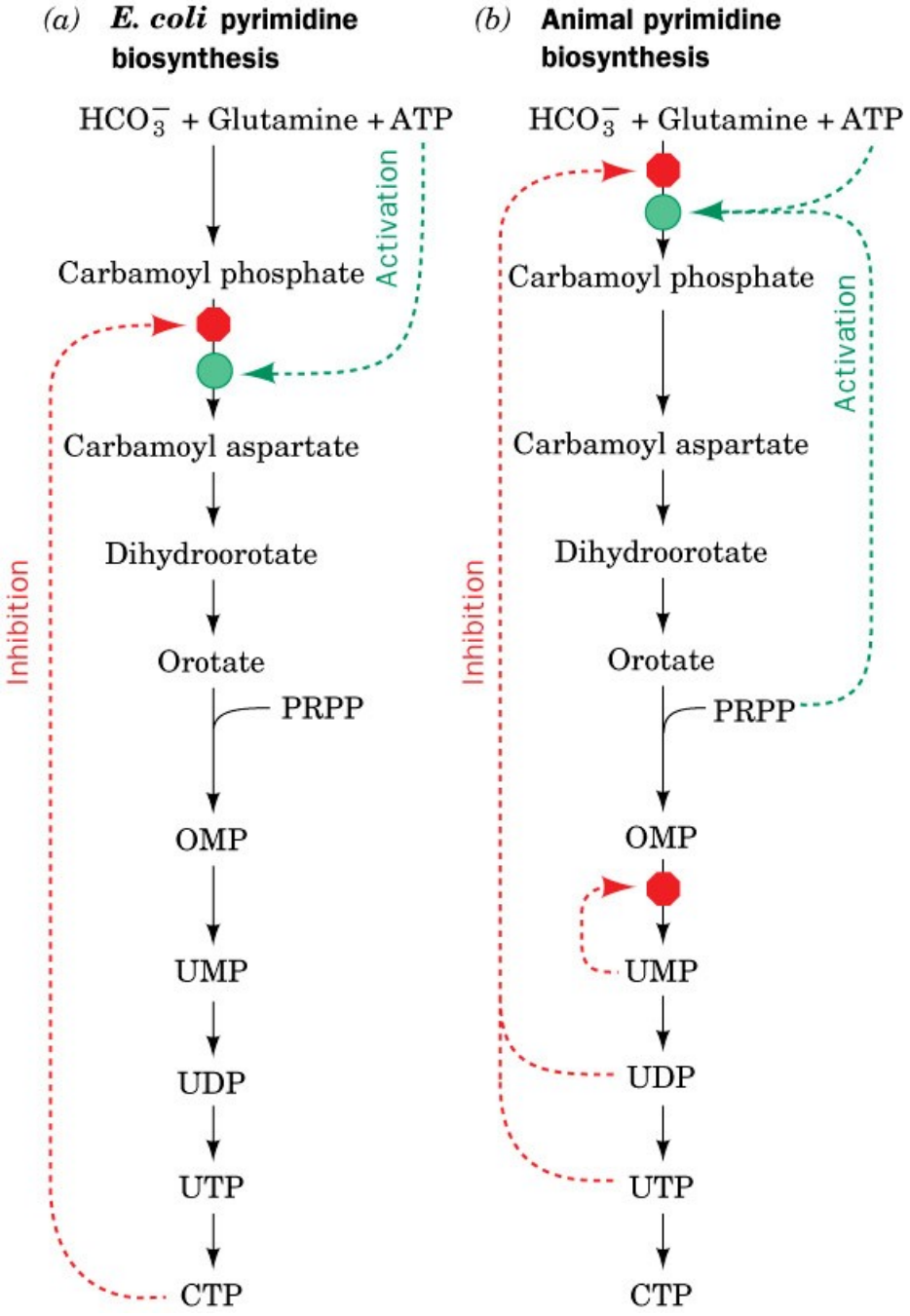
CAD and De Novo Biosynthesis of Pyrimidines; Hermoso *Structure 22, February 4, 2014*

(A) The synthesis of UMP involves six reactions. In animals, the first three steps are catalyzed by CAD (highlighted in a yellow box). The fourth step is catalyzed by a dehydrogenase located in the inner mitochondrial membrane. The transfer of PRPP and the decarboxylation of the pyrimidine ring are catalyzed by a bifunctional enzyme, UMP synthetase. The CPSase activity of CAD is the rate-limiting step of the pathway and is also allosterically inhibited by the final product, UTP, and activated by PRPP (dashed lines).

(B) Arrangement of the functional domains in CAD. The phosphorylation sites for the corresponding kinases are indicated.

(C) Structure of human DHOase dimer.

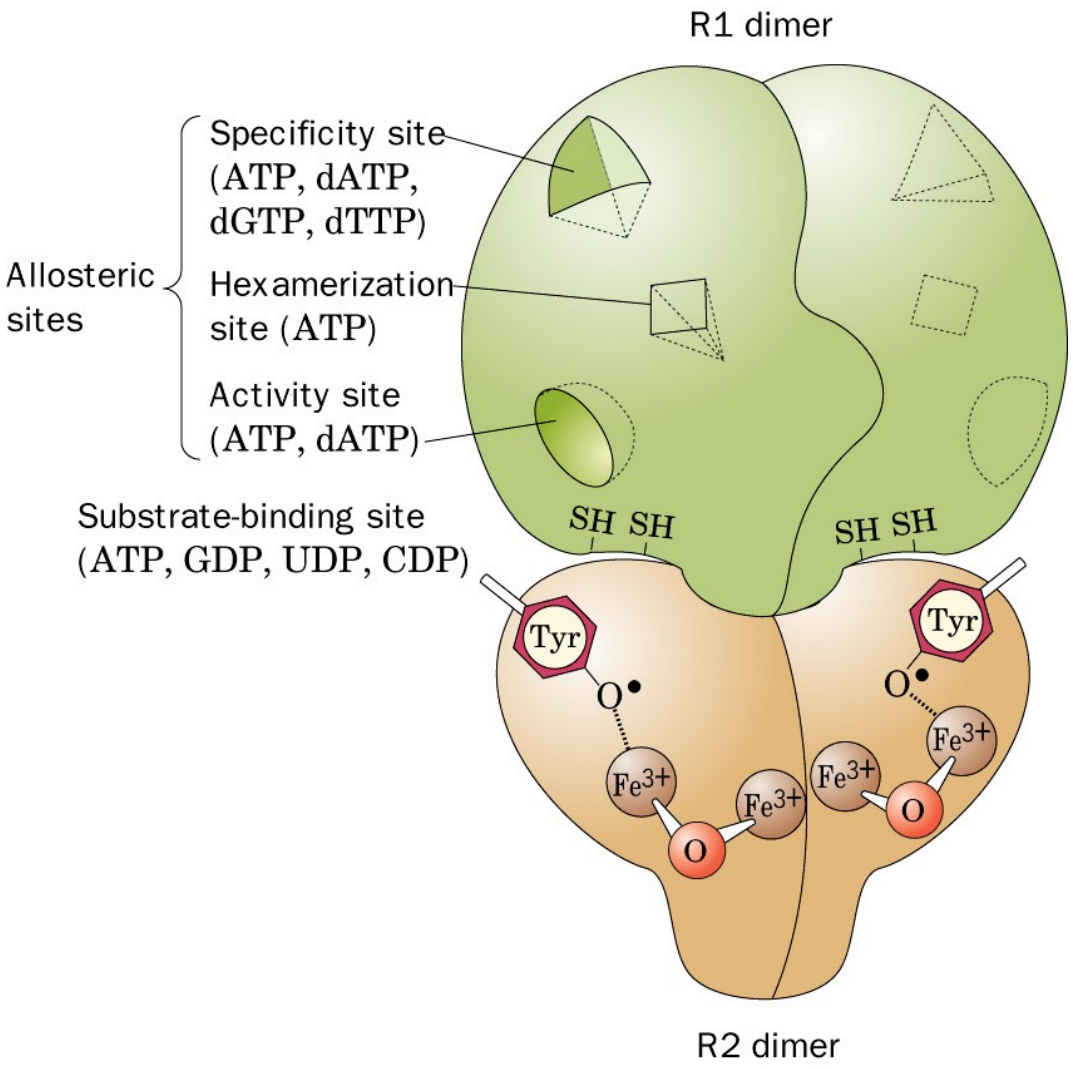
In bacteria pyrimidine biosynthesis is regulated by ATCase Rxn 2, control is exerted by the allosteric stimulation of ATP and it is inhibited by CTP. In many bacteria though UTP is an inhibitor.
In animal cells ATCase is not regulatory, rather pyrimidine biosynthesis is controlled by the activity of CPSII. Another level of control is in the formation of OMP, and due to the availability of PRPP. Pyrophosphate kinase is inhibited by ADP and GDP.



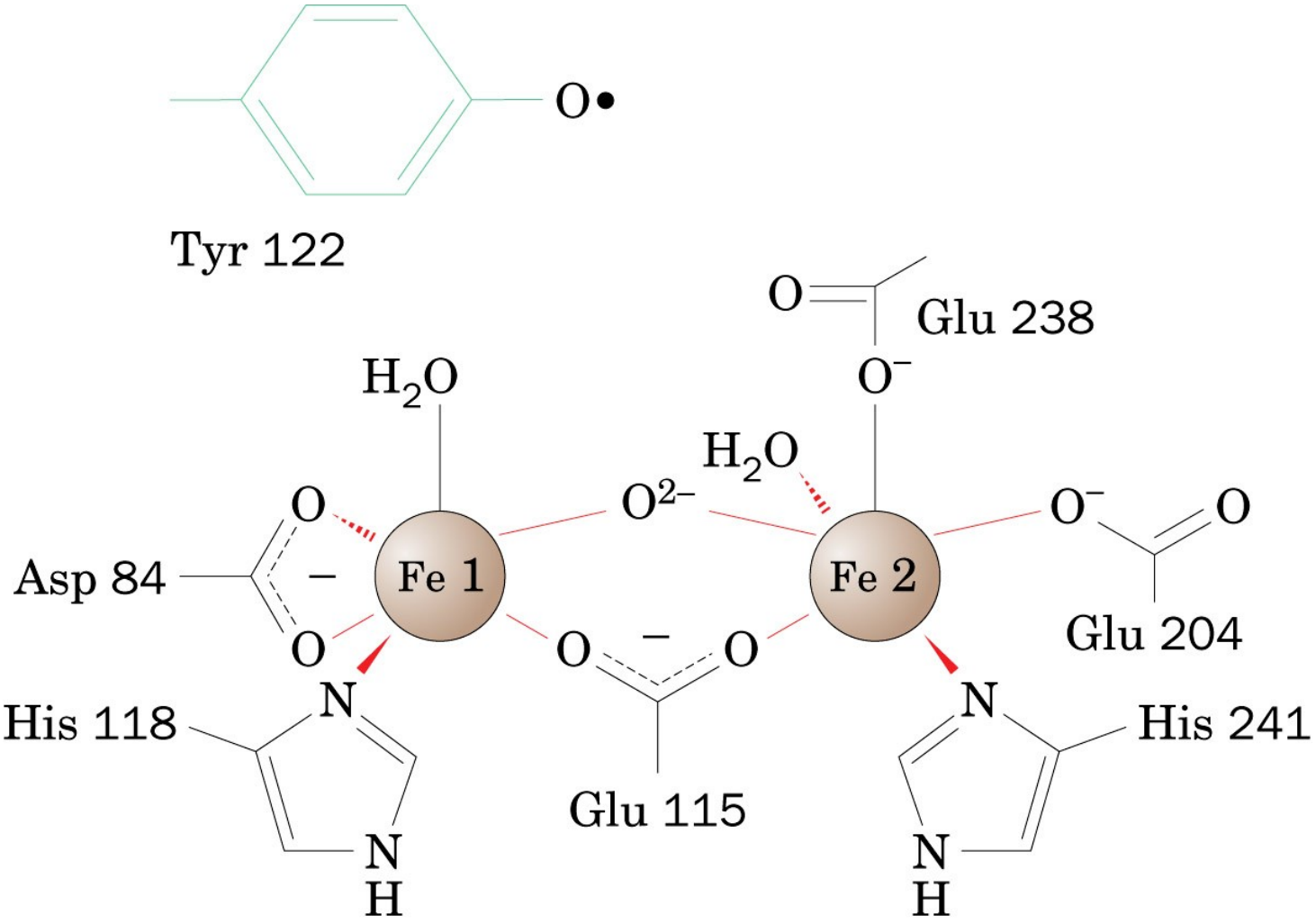
Model of Ribonucleotide Reductase

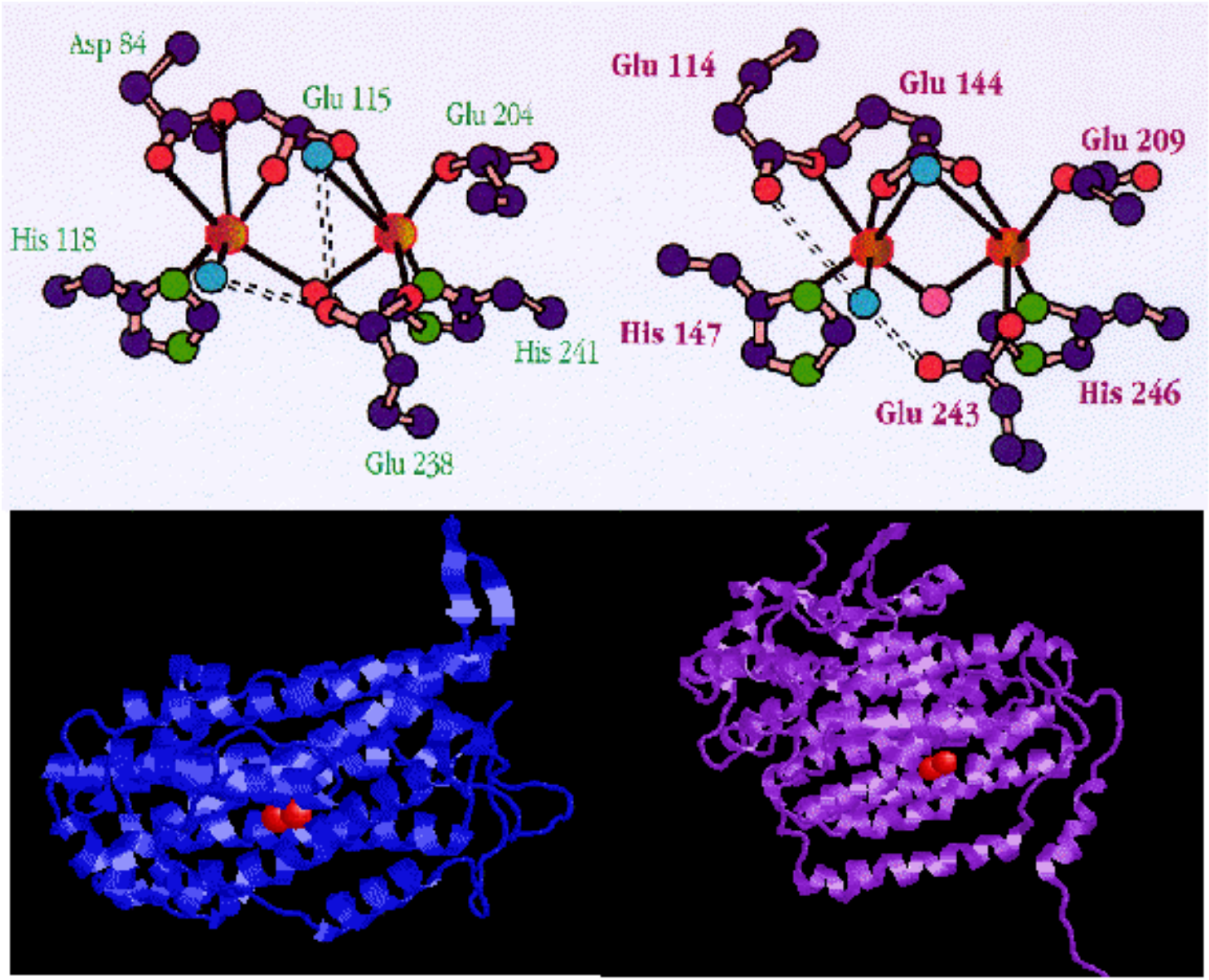
The substrates for RNR are ADP, GDP, CDP and UDP. dTDP (deoxythymidine diphosphate) is synthesized by another enzyme (thymidylate kinase) from dTMP (deoxythymidine monophosphate).

The iron-dependent enzyme, ribonucleotide reductase (RNR), is essential for DNA synthesis. Class I RNR enzymes are constructed from large RNR1 and small RNR2 subunits which associate to form an active heterodimeric tetramer. Since the enzyme catalyses the de novo synthesis of deoxyribonucleotides (dNTPs), precursors to DNA synthesis, it is essential for cell proliferation.



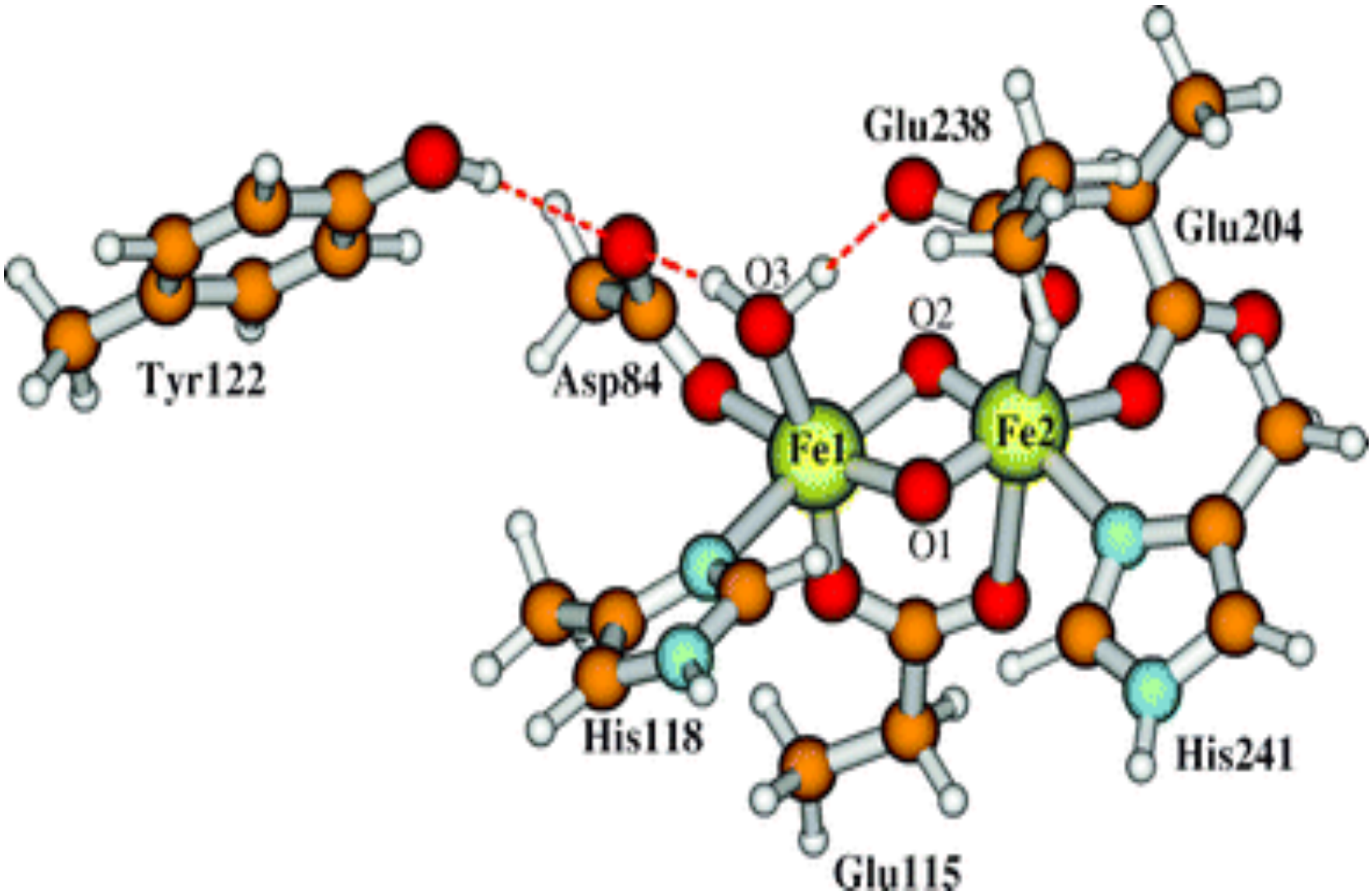
The binuclear Fe(III) complex of R2. Tyr 122 is very similar in it structure to cyt c oxidase.

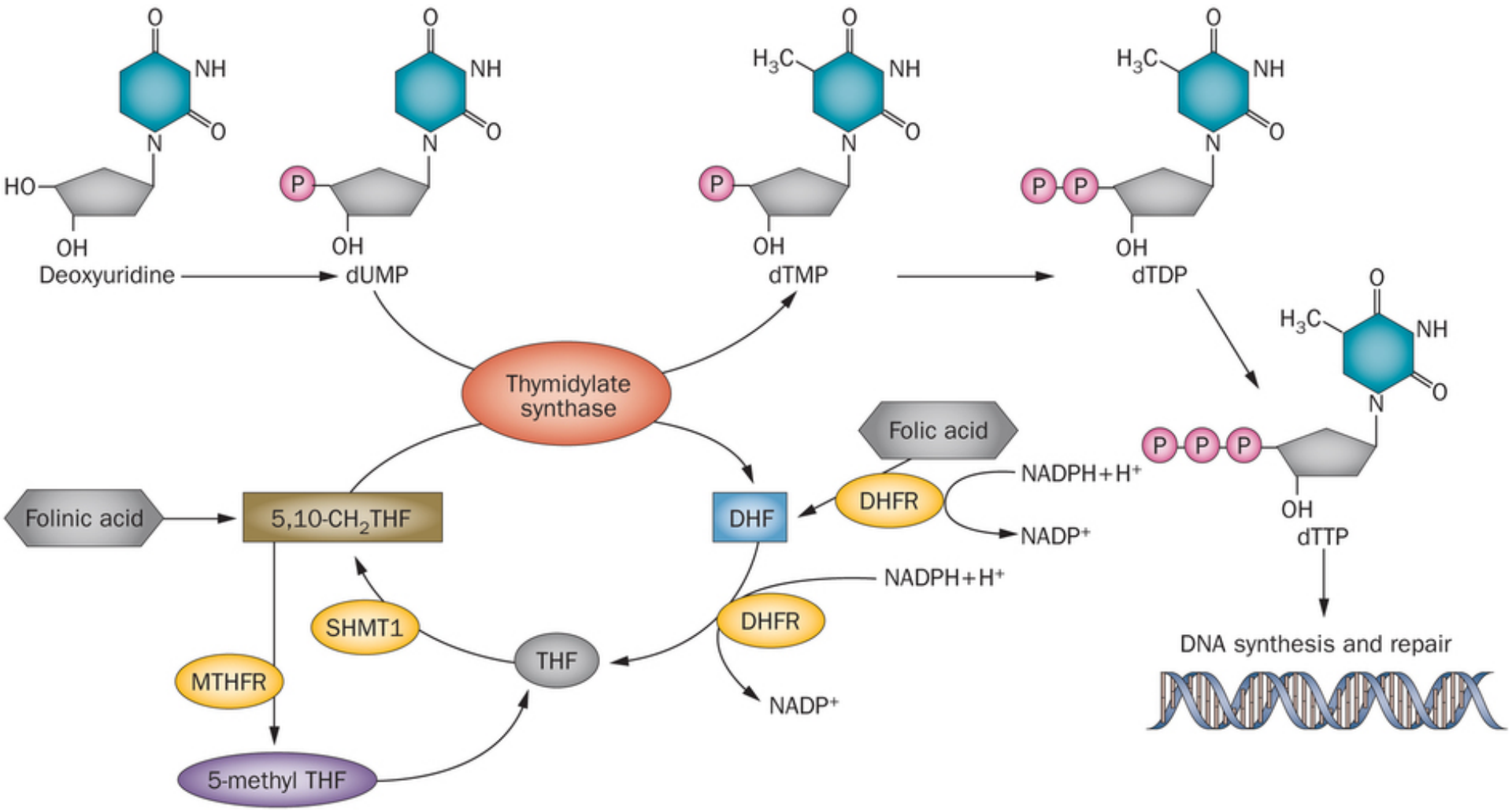


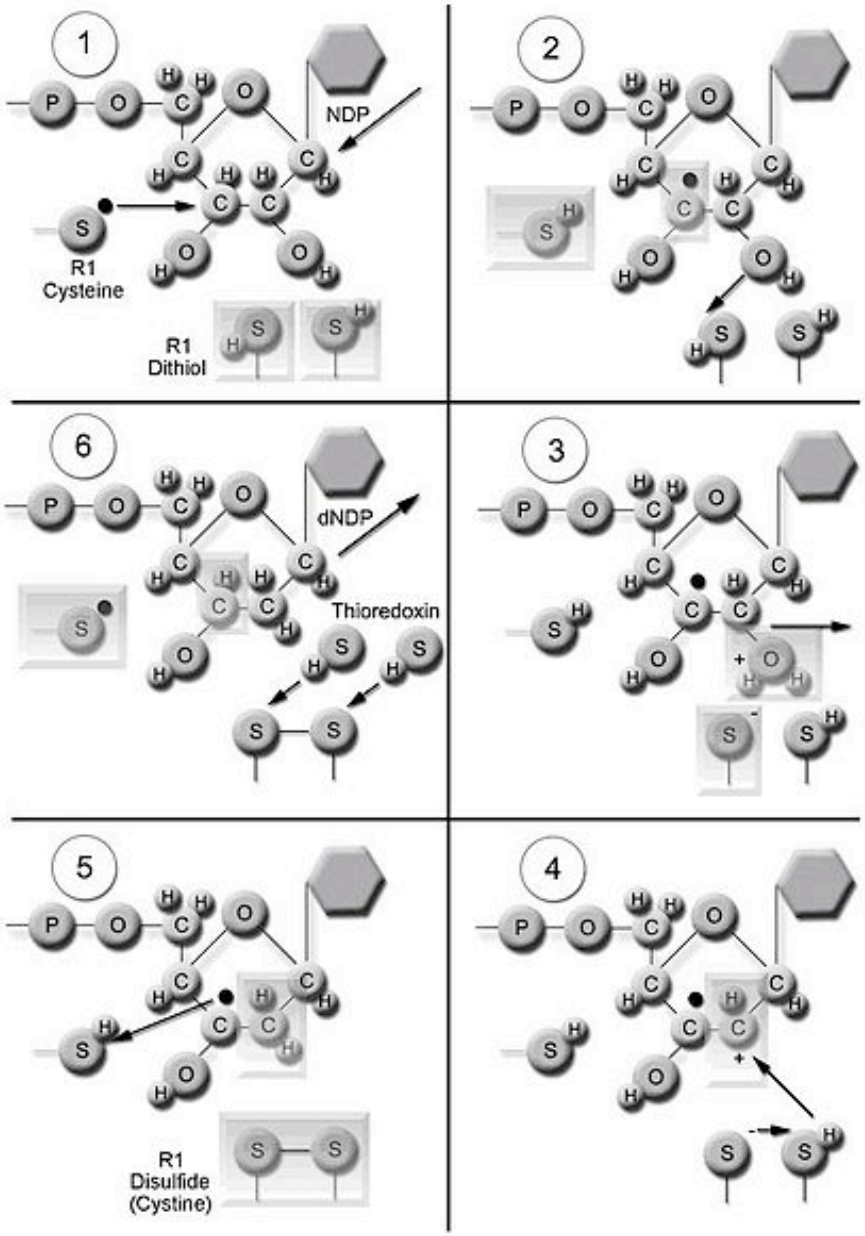


R2 Subunit of
Ribonucleotide Reductase

Hydroxylase Component of
Methane Monooxygenase


Glu238
Glu204
O3
O2
Tyr122
Asp84
Fe1
Fe2
O1
His118
His241
Glu115


Deoxyuridine
dUMP
dTMP
dTDP
dTTP
Thymidylate synthase
Folic acid
DHFR
NADPH+H+
NADP+
DHF
Folinic acid
5,10-CH2THF
SHMT1
THF
MTHFR
5-methyl THF
DNA synthesis and repair



Mechanism to catalyze the conversion of ribonucleotides to deoxyribonucleotides. (1) an electron transfer on the RNR2 subunit activates a RNR1 cysteine residue in the active site with a free radical; (2) the free radical forms a stable radical on C-3, and cysteine radical removes a hydrogen atom; (3) cation is formed on C-2 by transferring a hydrogen from a dithiol group and is stabilized by the radical, resulting in the loss of H2O from C-2; (4) a hydrogen is transferred from the dithiol group to reduce the cation C-2; (5) the C-3 radical is reduced by the hydrogen removed in step 2, and the tyrosyl radical is generated; (6) redoxins transfer two hydrogen to the disulfide group that restores the original configuration.

Electron-transfer pathway for nucleoside diphosphate (NDP) reduction.
Electrons are transmitted (blue arrows) to the enzyme from NADPH via (a) glutaredoxin or (b) thioredoxin. The sulfide groups in glutaredoxin reductase are contributed by two molecules of bound glutathione (GSH; GSSG indicates oxidized glutathione). Note that thioredoxin reductase is a flavoenzyme, with FAD as prosthetic group.
The final step in the RNR catalytic cycle is the reduction of the enzymes disulfide bond to reform the redox active sulfhydryl pair, cys135 & cys138. The terminal reducing agent in this cycle is NADPH.

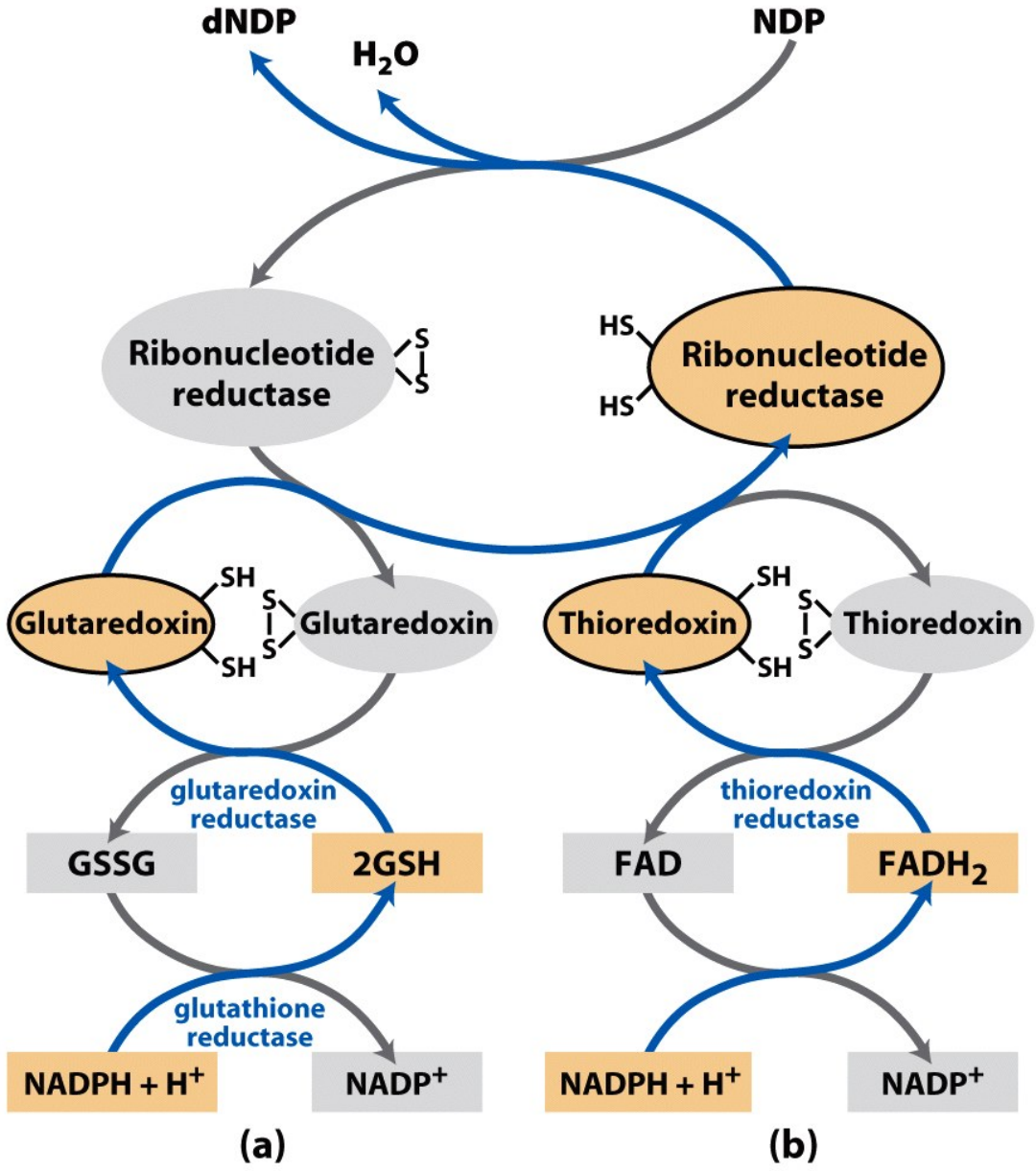


Figure 22-39
Lehninger Principles of Biochemistry, Fifth Edition