

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 R5P is added as PRPP. The N1, C4, 5 & 6 are all derived from aspartate. C3 is from HCO_3^- and N3 from glutamine.

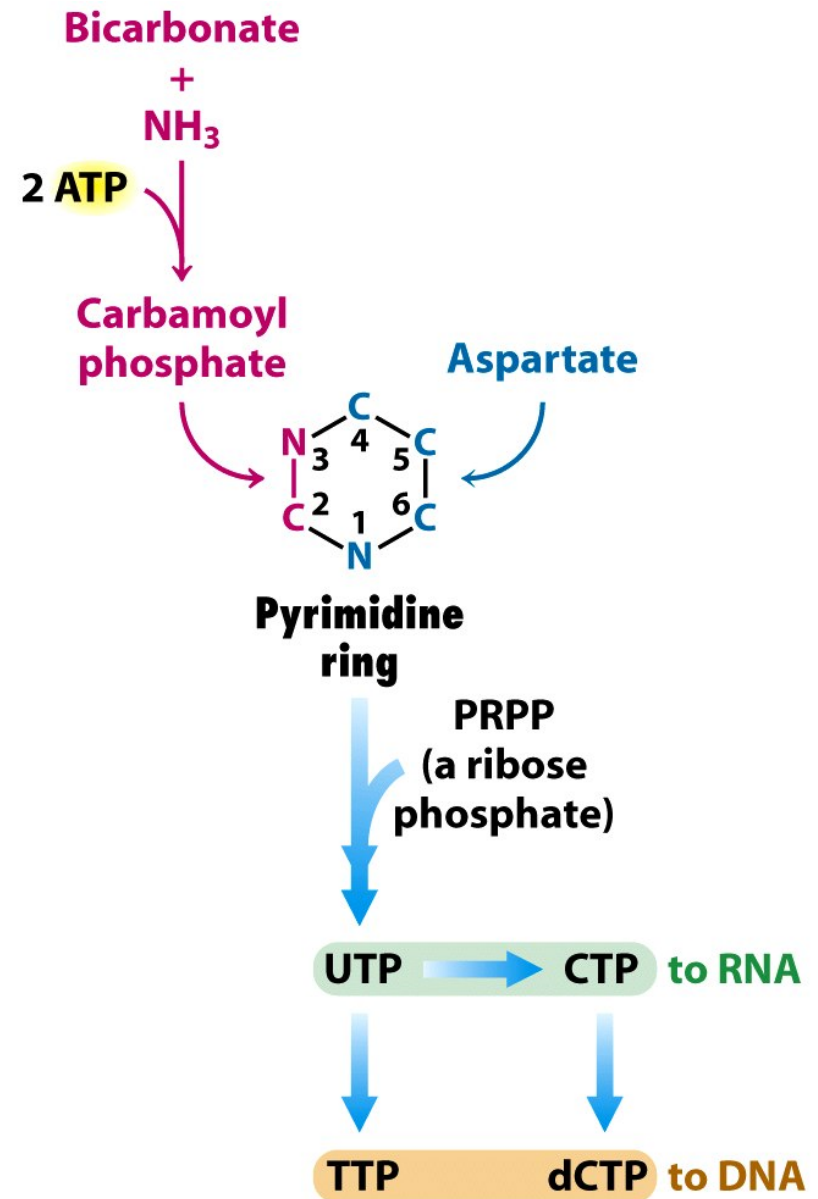
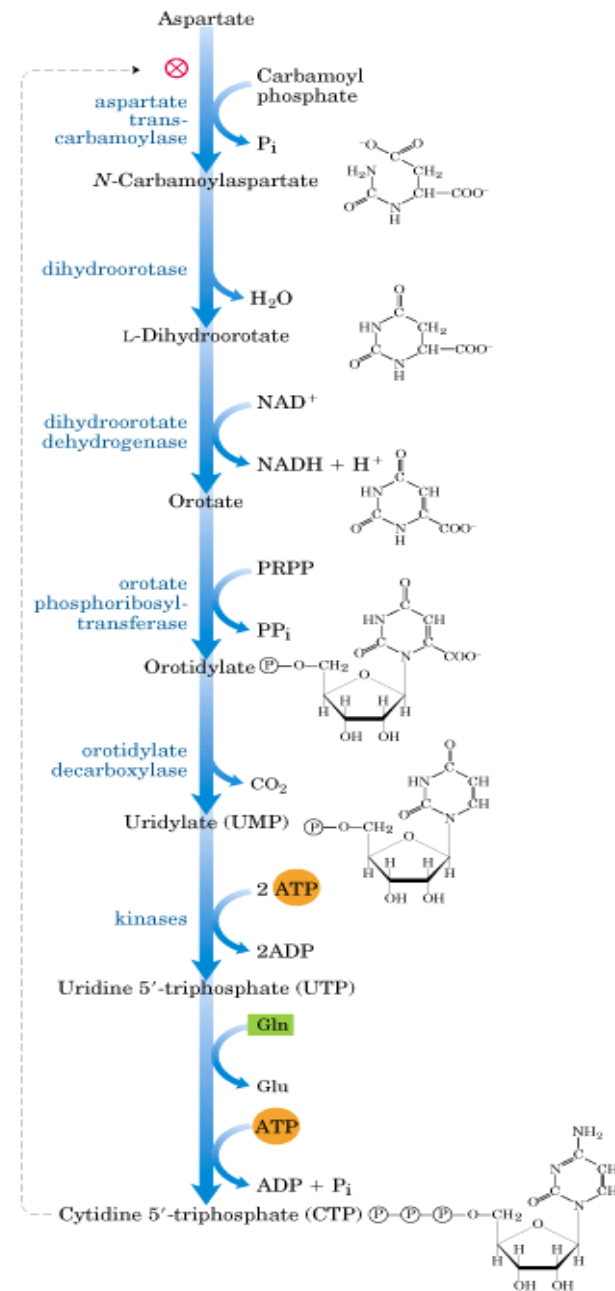
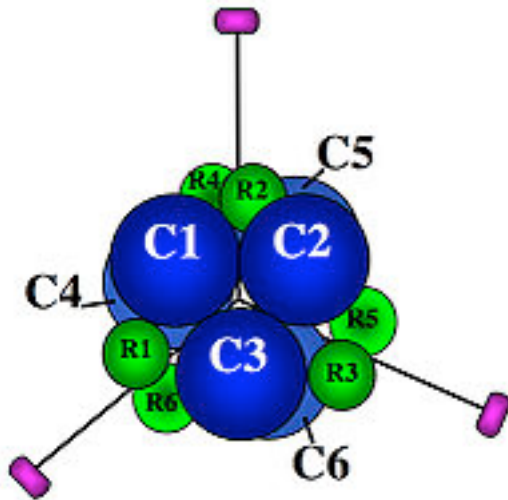


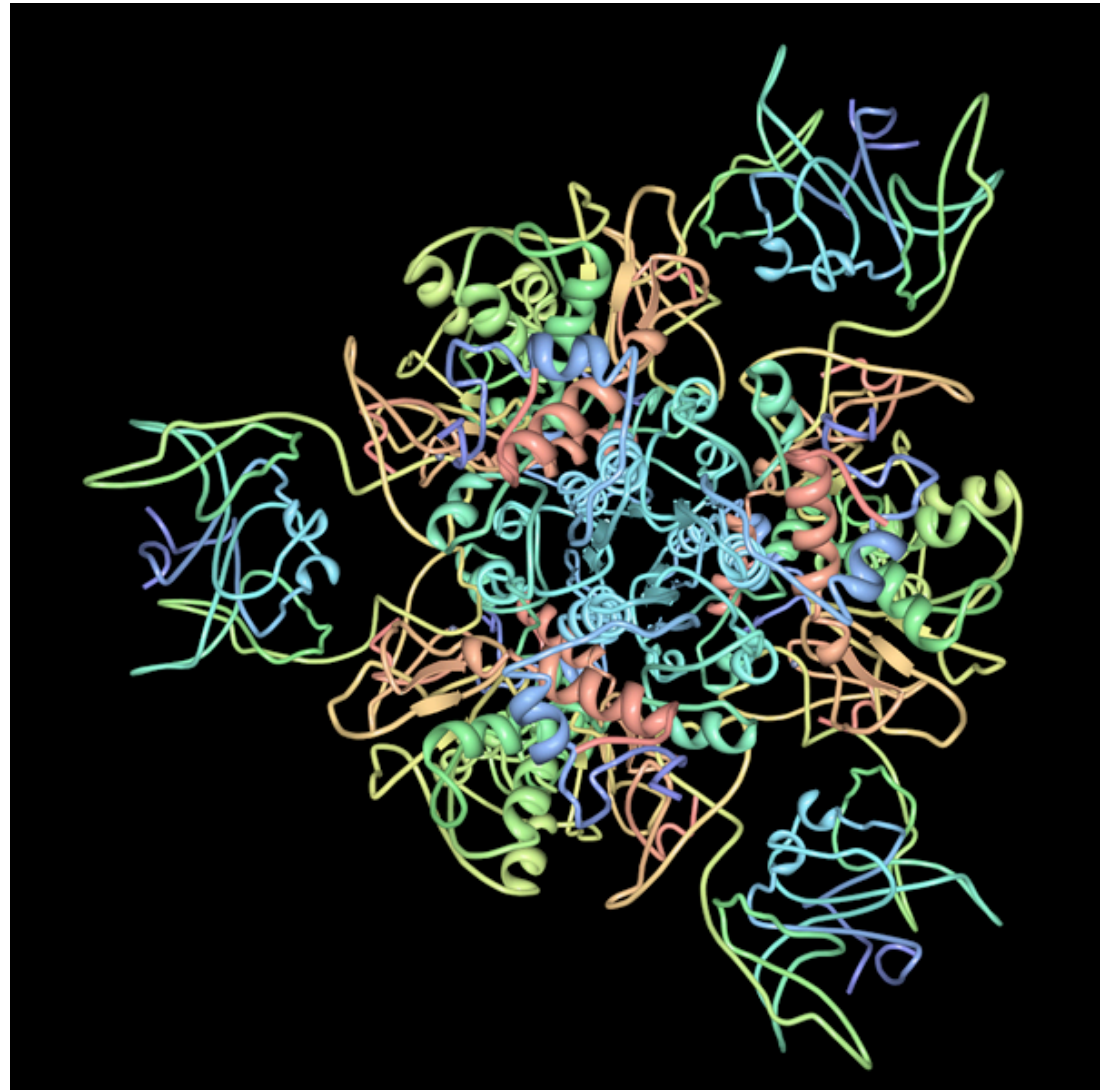
Figure 25-2
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Synthesis of the pyrimidines is less complex than that of the purines, since the base is much simpler. The first completed base is derived from 1 mole of glutamine, one mole of ATP and one mole of CO_2 (which form **carbamoyl phosphate**) and one mole of aspartate. An additional mole of glutamine and ATP are required in the conversion of UTP to CTP. The carbamoyl phosphate used for pyrimidine nucleotide synthesis is derived from glutamine and bicarbonate, within the cytosol, as opposed to the Urea Cycle carbamoyl phosphate derived from ammonia and bicarbonate in the mitochondrion. The Urea Cycle reaction is catalyzed by CPS-I whereas the pyrimidine nucleotide precursor is synthesized by CPS-II. Carbamoyl phosphate is then condensed with aspartate in a reaction catalyzed by the rate limiting enzyme of pyrimidine nucleotide biosynthesis, *aspartate transcarbamoylase* (ATCase). DHODh oxidizes dihydro-orotate and in eukaryotes the enzyme contains FMN, FAD and [2Fe-2S] cluster. The FMNH₂ is oxidized by tunneling that allows it to be past to the mitochondrion ubiquinone. The enzyme associates with the inner mitochondrion membrane by a hydrophobic patch.





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



Aspartate carbamoyltransferase from *Escherichia coli*.

Early studies demonstrated that ATCase consists of two different kinds of polypeptide chains, which have different roles. The catalytic subunits catalyze the carbamylation of the amino group of aspartate but do not have regulatory properties, while the regulatory subunits do not have any catalytic activity but contain the regulatory sites for effector binding. The ATCase holoenzyme is made of two catalytic trimers that are in contact and held together by three regulatory dimers, so the native form of the enzyme contains six chains of each type, with a total molecular weight of 310 kDa.

Each of the catalytic domains is composed of two structural domains, the aspartate domain, which contains most of the residues responsible for binding aspartate, and the carbamoyl phosphate domain, which contains most of the residues that bind to carbamoyl phosphate. Each regulatory domain is also composed of two domains, the allosteric domain, which has the binding site for the nucleotide effectors, and the zinc domain, consisting of four cysteine residues clustered in its C-terminal region. These residues coordinate a zinc atom that is not involved in any catalytic property, but has been shown to be essential for the association of regulatory and catalytic subunits.

The three-dimensional arrangement of the catalytic and regulatory subunits involves several ionic and hydrophobic stabilizing contacts between amino acid residues. Each catalytic chain is in contact with three other catalytic chains and two regulatory chains. Each regulatory monomer is in contact with one other regulatory chain and two catalytic chains. In the unliganded enzyme, the two catalytic trimers are also in contact.

The allosteric site 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.

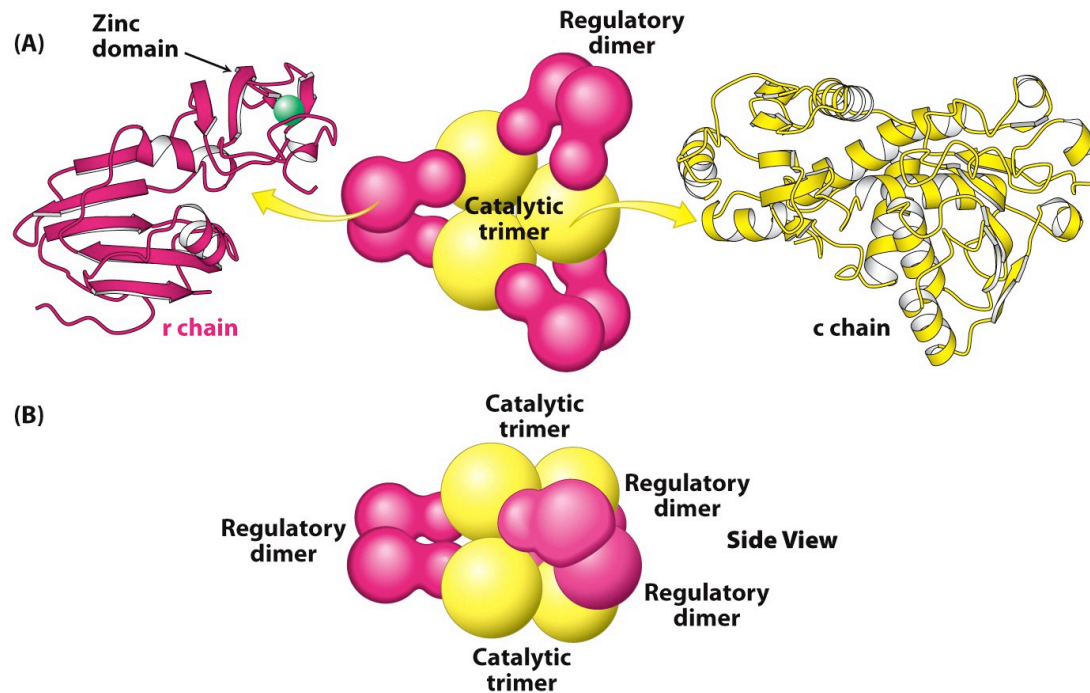
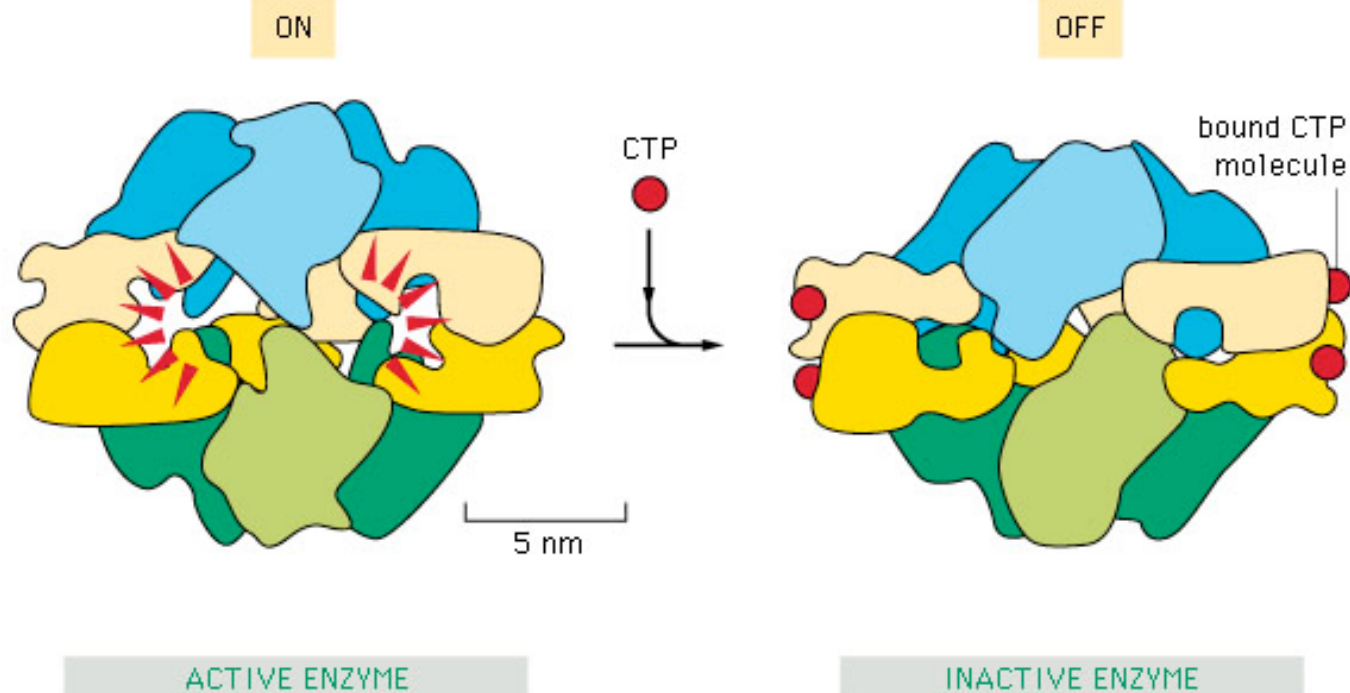
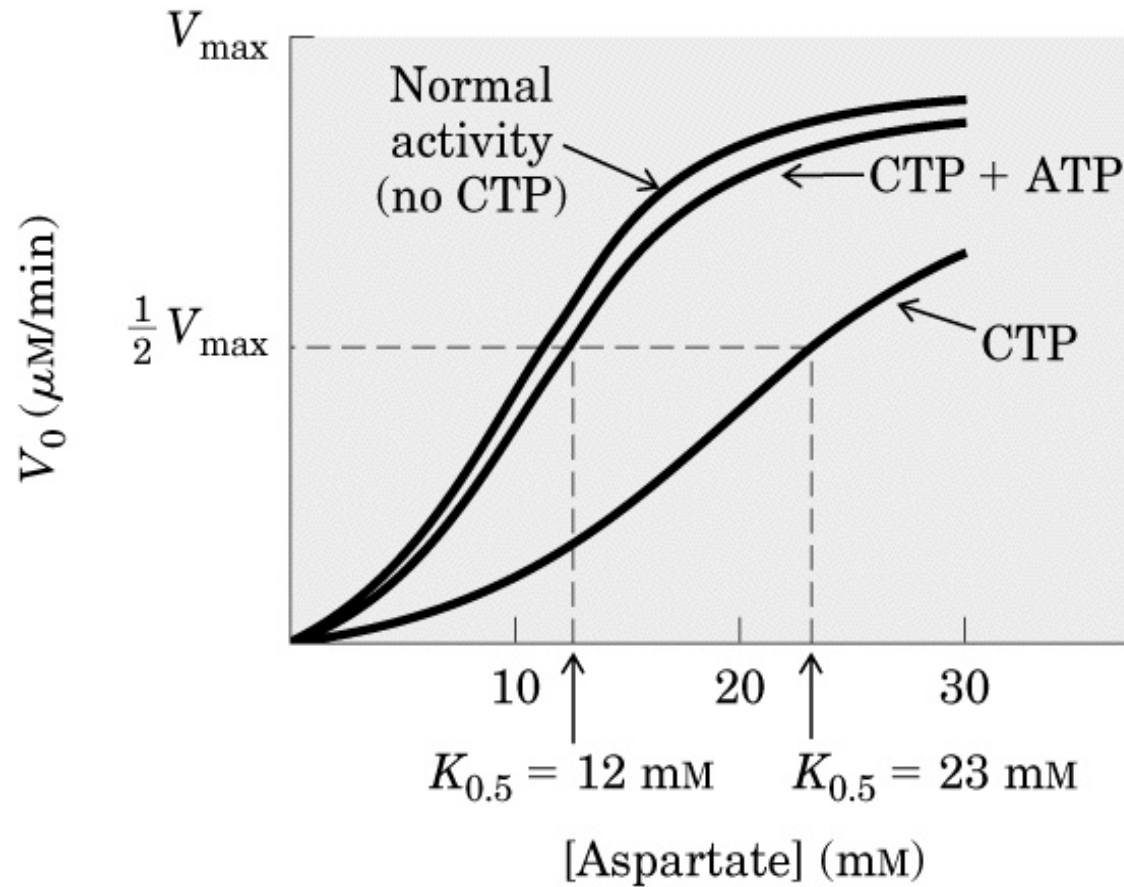


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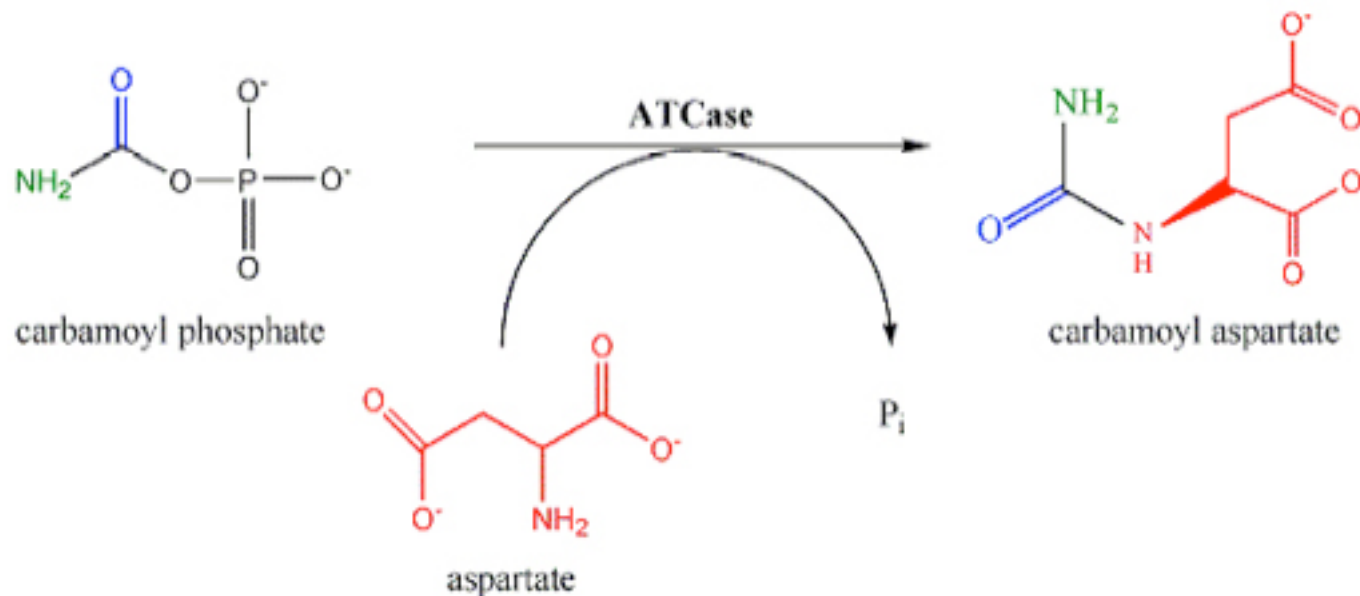
With CTP present, UTP binding is enhanced and preferentially directed to the low-affinity sites. On the converse, UTP binding leads to enhanced affinity for CTP at the high-affinity sites and together they inhibit enzyme activity by up to 95%, while CTP binding alone inhibits activity to 50% to 70%. 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.

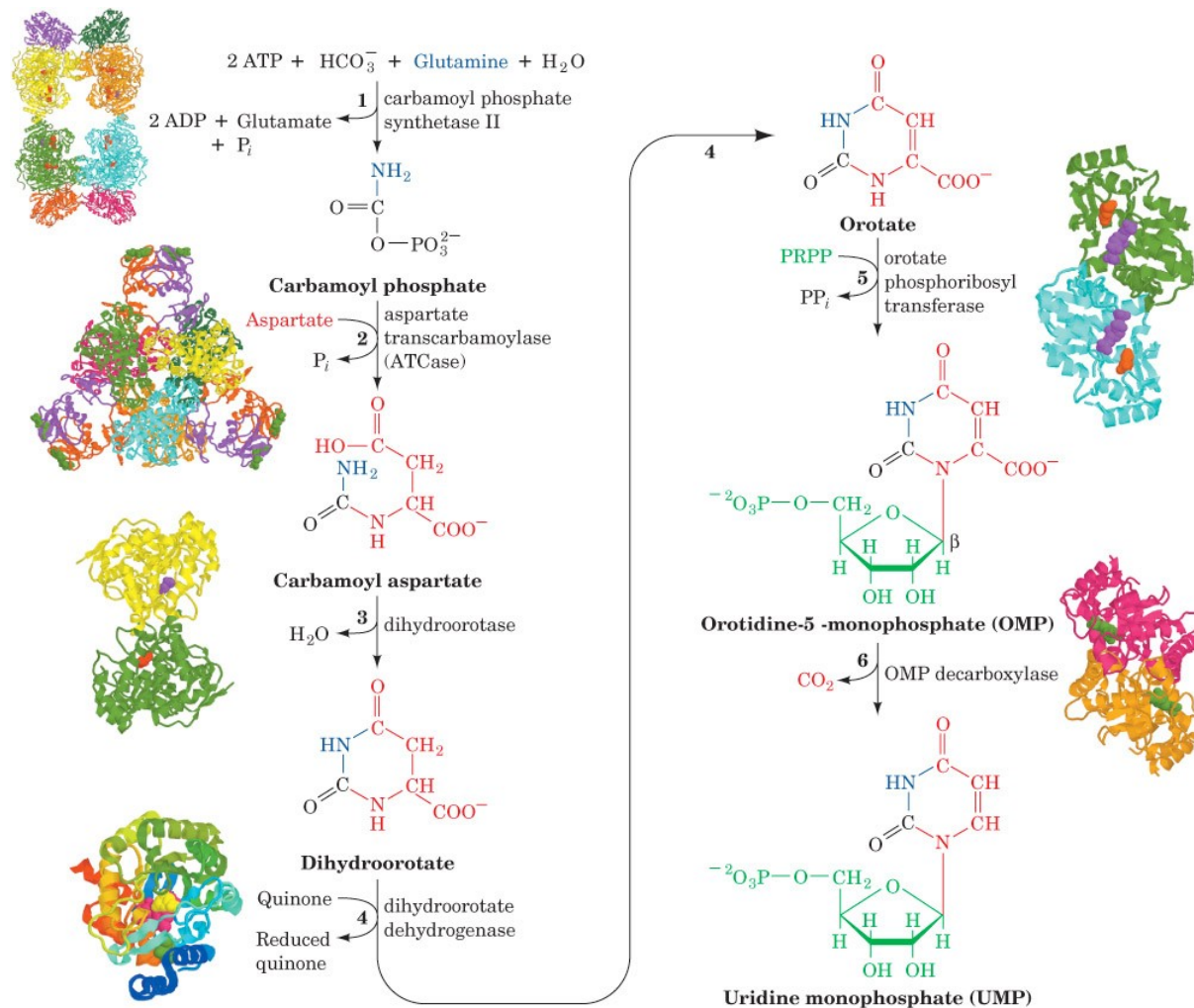


The regulation of pyrimidine synthesis occurs mainly at the first step which is catalyzed by *aspartate transcarbamoylase*, ATCase. Inhibited by CTP and activated by ATP



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.

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.

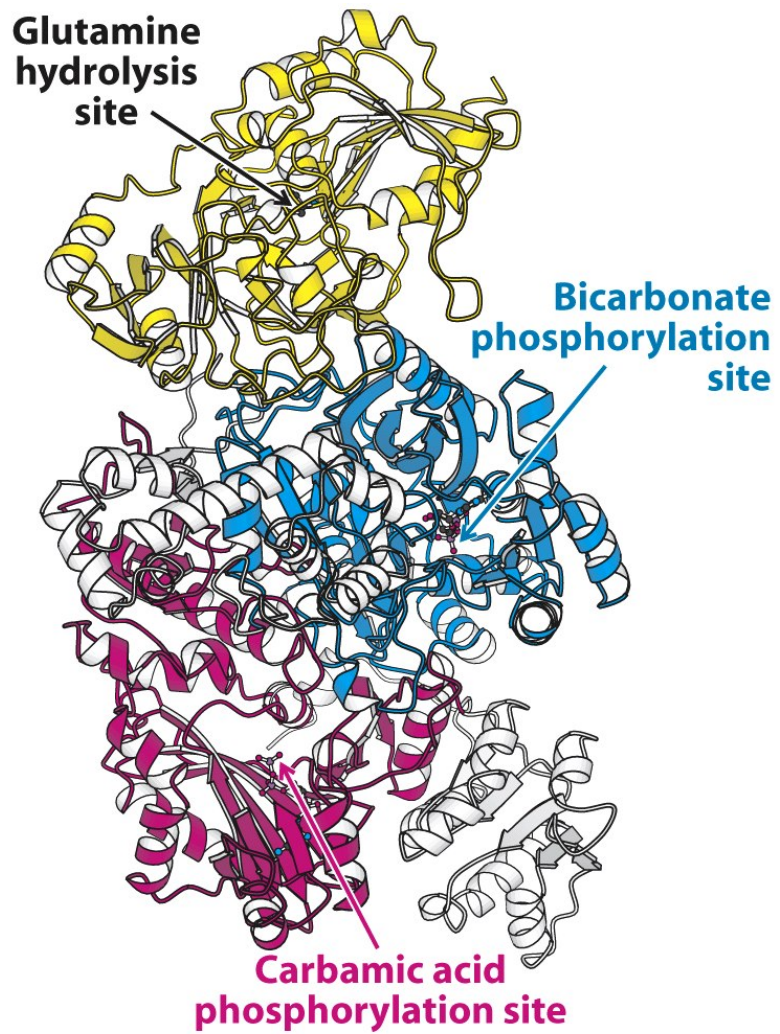


Figure 25-3
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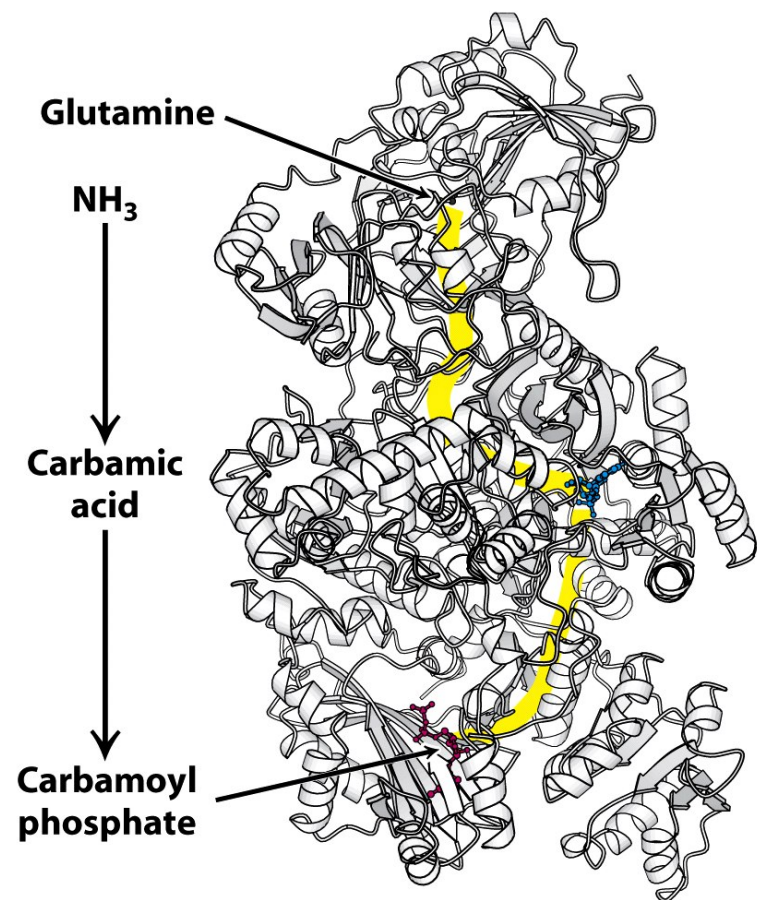


Figure 25-4
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The de novo pyrimidine biosynthetic pathway in eukaryotes, steps 1-6, and the subsequent formation of CTP. UTP is formed from UMP by sequential reactions catalyzed by UMP/CMP kinase (circled 7) and nucleoside-diphosphate kinase (circled 8). All 3 activities of CAD single polypeptide, 243kD.

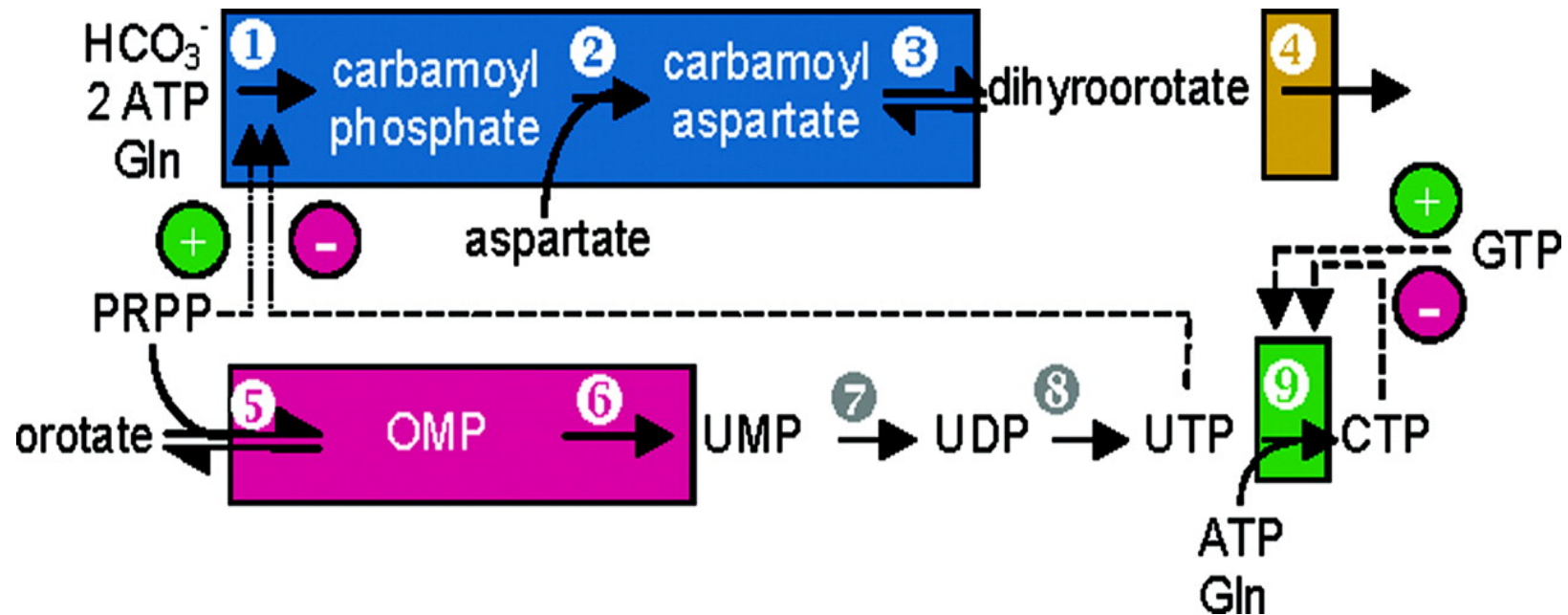


Figure 1

The Multifunctional Protein CAD1 *August 6, 2004 The Journal of Biological Chemistry*, 279, 33035-33038.

Since the pioneering discoveries of Jones, Hoogenraad and others in 1971, the physical association of the first three enzymes of the de novo pyrimidine pathway, carbamoyl-phosphate synthetase (CPSase), aspartate transcarbamylase (ATCase), and dihydroorotase (DHOase) has been documented in many animal cells. Stark and his associates made the remarkable discovery that all three activities are carried on a single polypeptide. The 243-kDa CAD polypeptide associates to form hexamers and higher oligomers so that the mass of the complex exceeds 1.4 MDa or about one-half the size of the ribosome. The domain structure (Fig. 2) has been mapped, and the function of each domain has been assigned . **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.

CAD

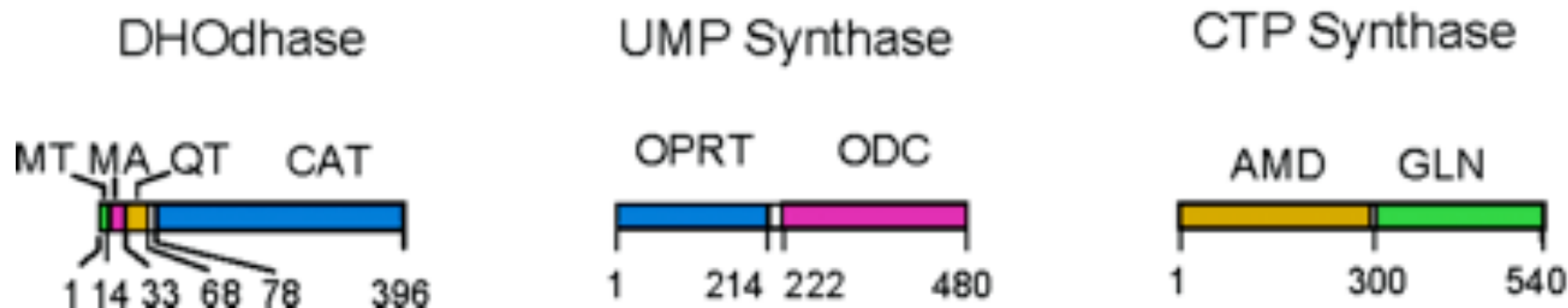
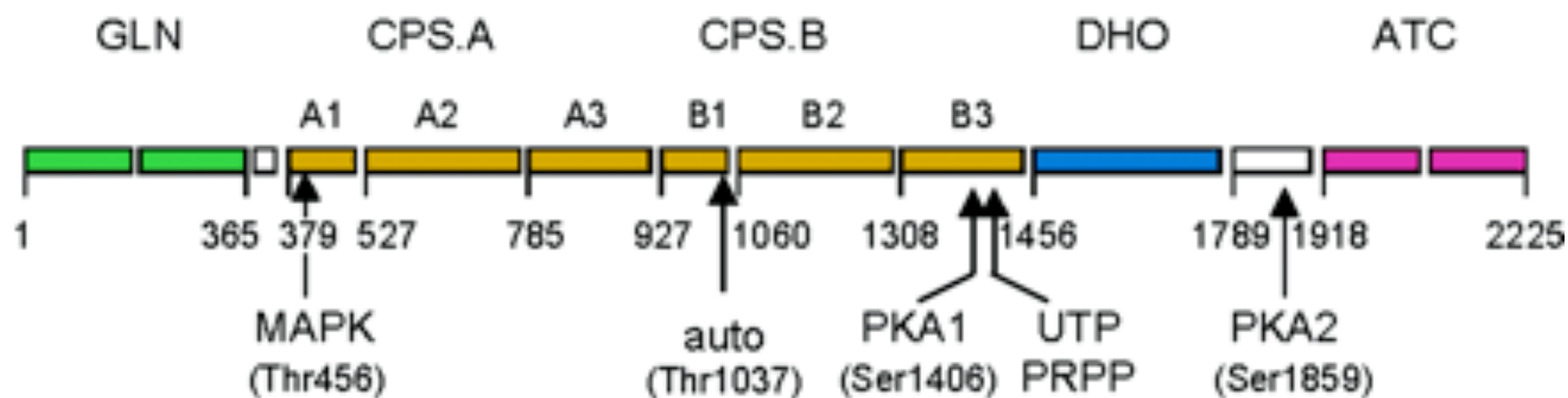
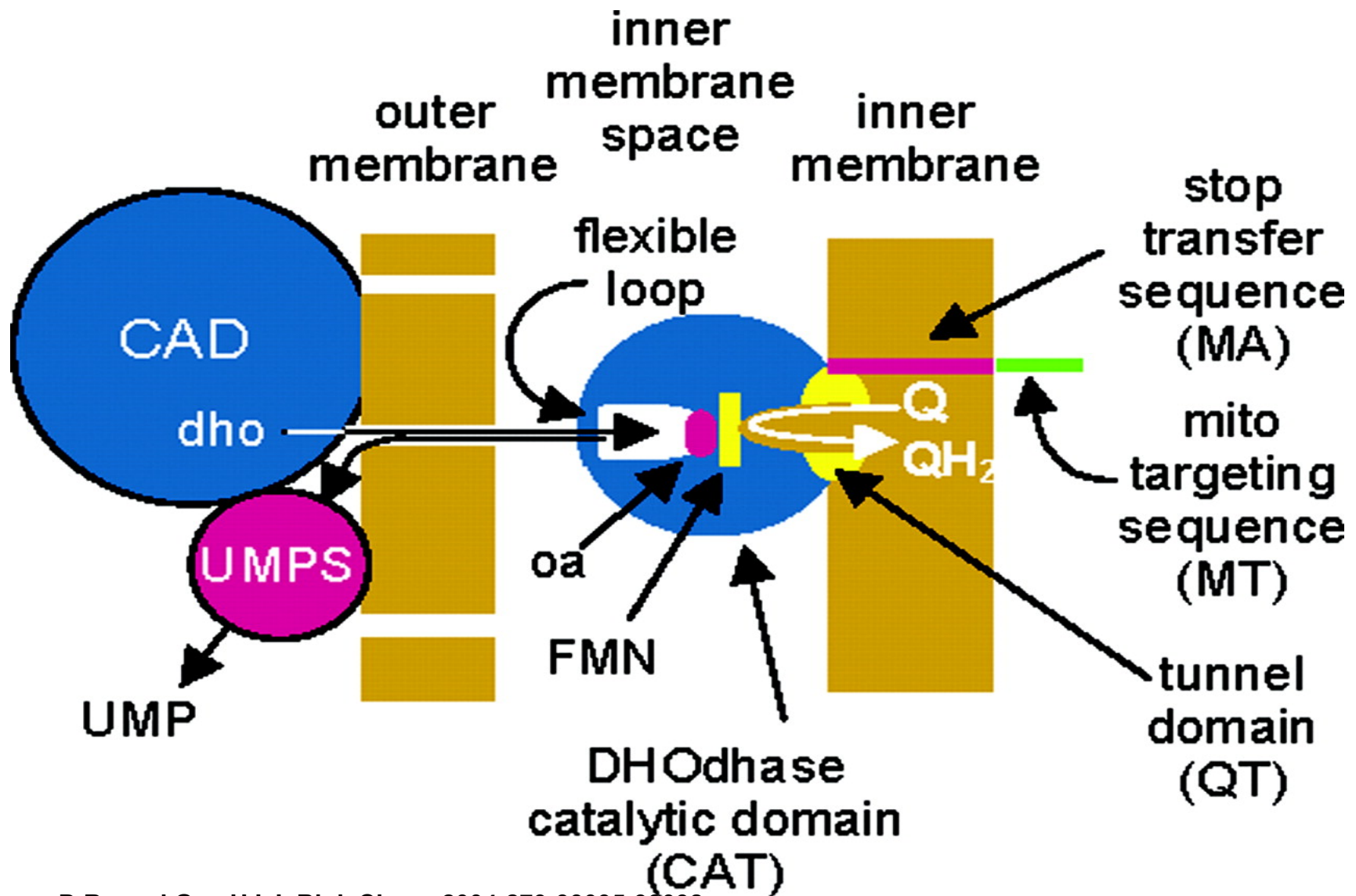


Figure 2

Schematic representation of DHOdase bound to the inner mitochondrial membrane. oa, orotate; Q, ubiquinone; UMPS, UMP synthase. Figure 3



Evans D R , and Guy H I J. Biol. Chem. 2004;279:33035-33038

CAD is primarily cytosolic with a smaller fraction in the nucleus. In the cytosolic compartment, CAD and UMP synthase are localized around and outside the mitochondria, and CAD appears to be associated with the cytoskeleton. Mitochondria are known to be anchored to the cytoskeletal network, so an interesting possibility is that CAD binds to and translocates along the filament to the mitochondria where DHODhase is located (Fig. 4, *path A*). *The physical association of CAD with the mitochondria is an attractive idea because under physiological conditions, the equilibrium strongly favors the formation of carbamoyl aspartate over dihydroorotate . Docking CAD near the mitochondria may allow a more efficient capture of dihydroorotate by DHODhase and prevent the accumulation of carbamoyl aspartate in the cell.*

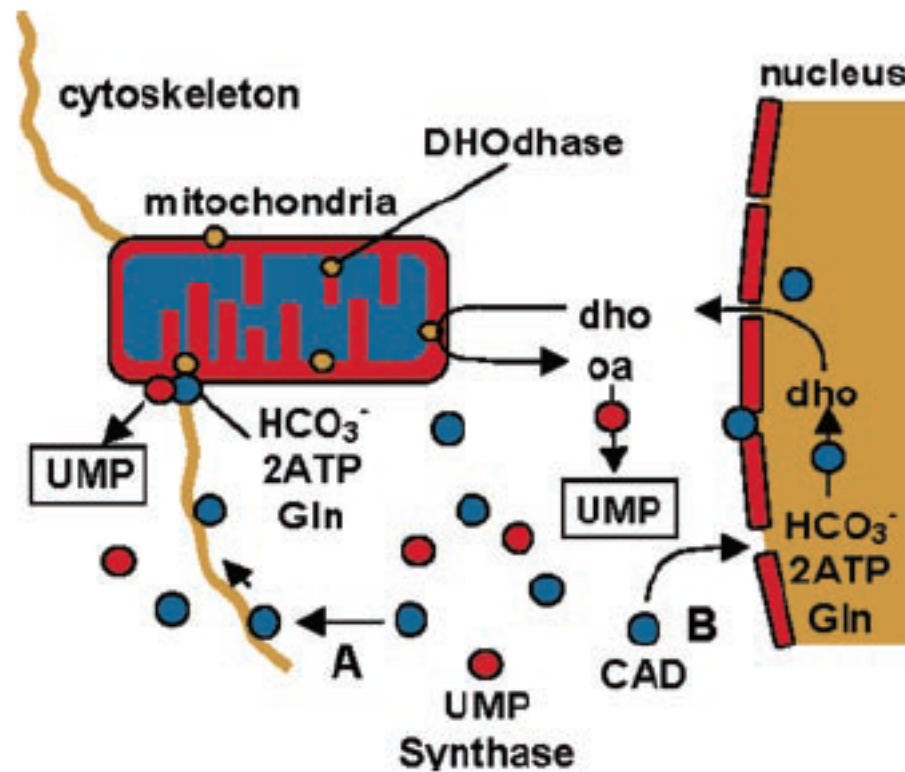
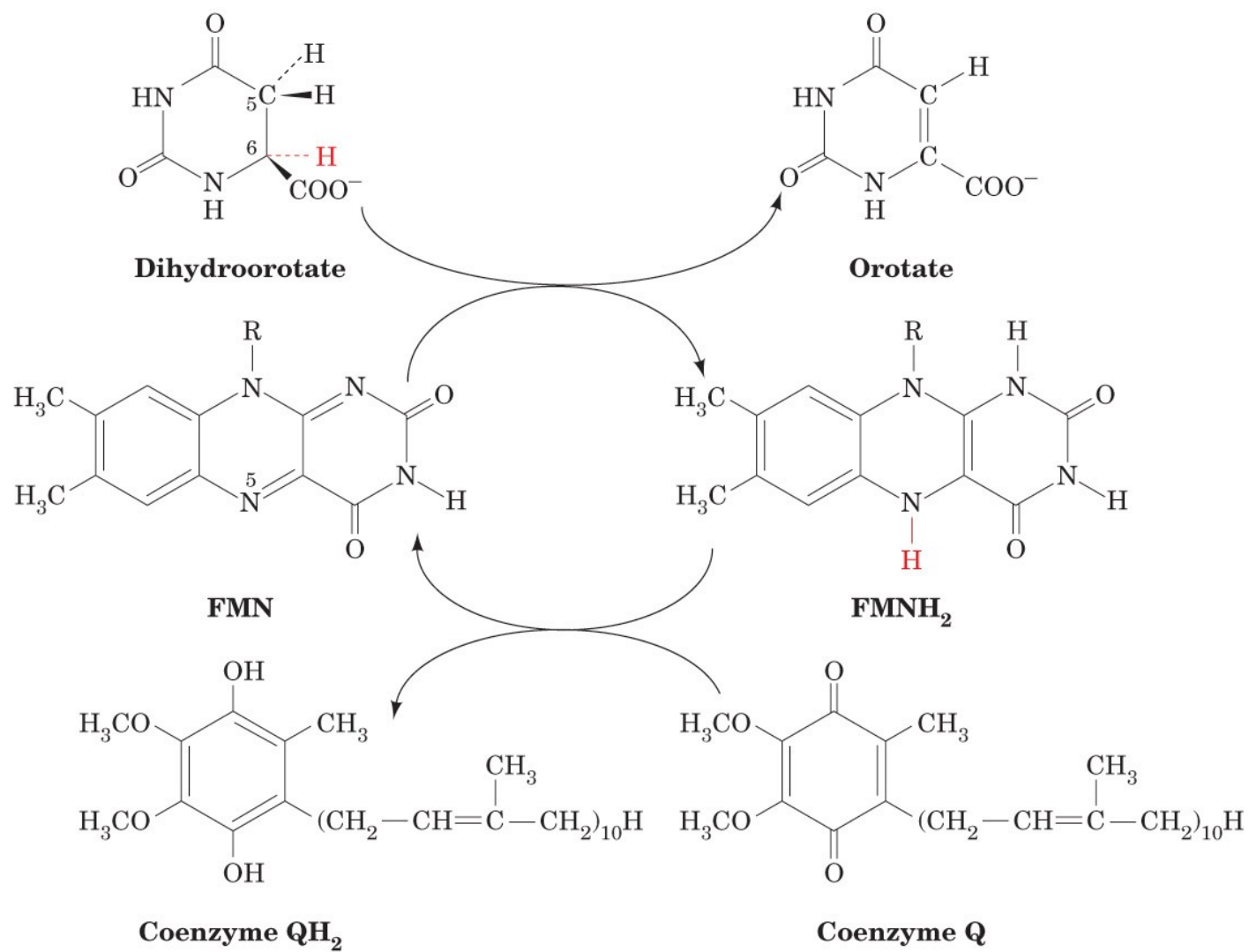
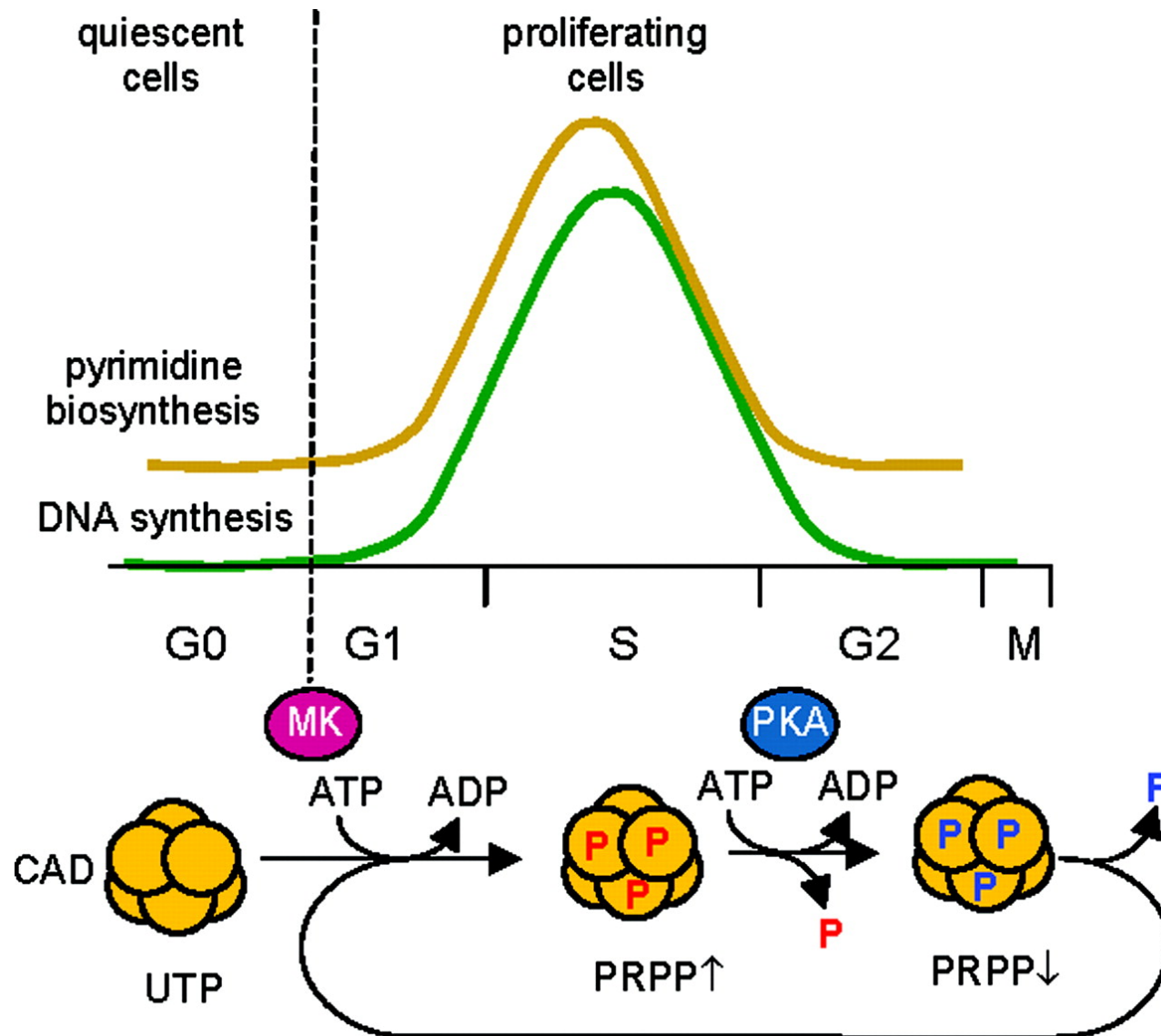


FIG. 4. Intracellular distribution of CAD (blue spheres), DHODhase (yellow spheres), and UMP synthase (red spheres) and putative interactions with the nucleus, mitochondria, and cytoskeleton. The paths of CAD, both translocation along the filament (A) and entry into the nucleus (B) are shown. oa, orotate.
 Evans D R , and Guy H I J. Biol. Chem. 2004;279:33035-33038



The cell cycle-dependent regulation of pyrimidine biosynthesis. In proliferating cells, the sensitivity of CAD to the activator PRPP sequentially increases (\uparrow) and decreases (\downarrow).

MK, MAP kinase **Figure 5**

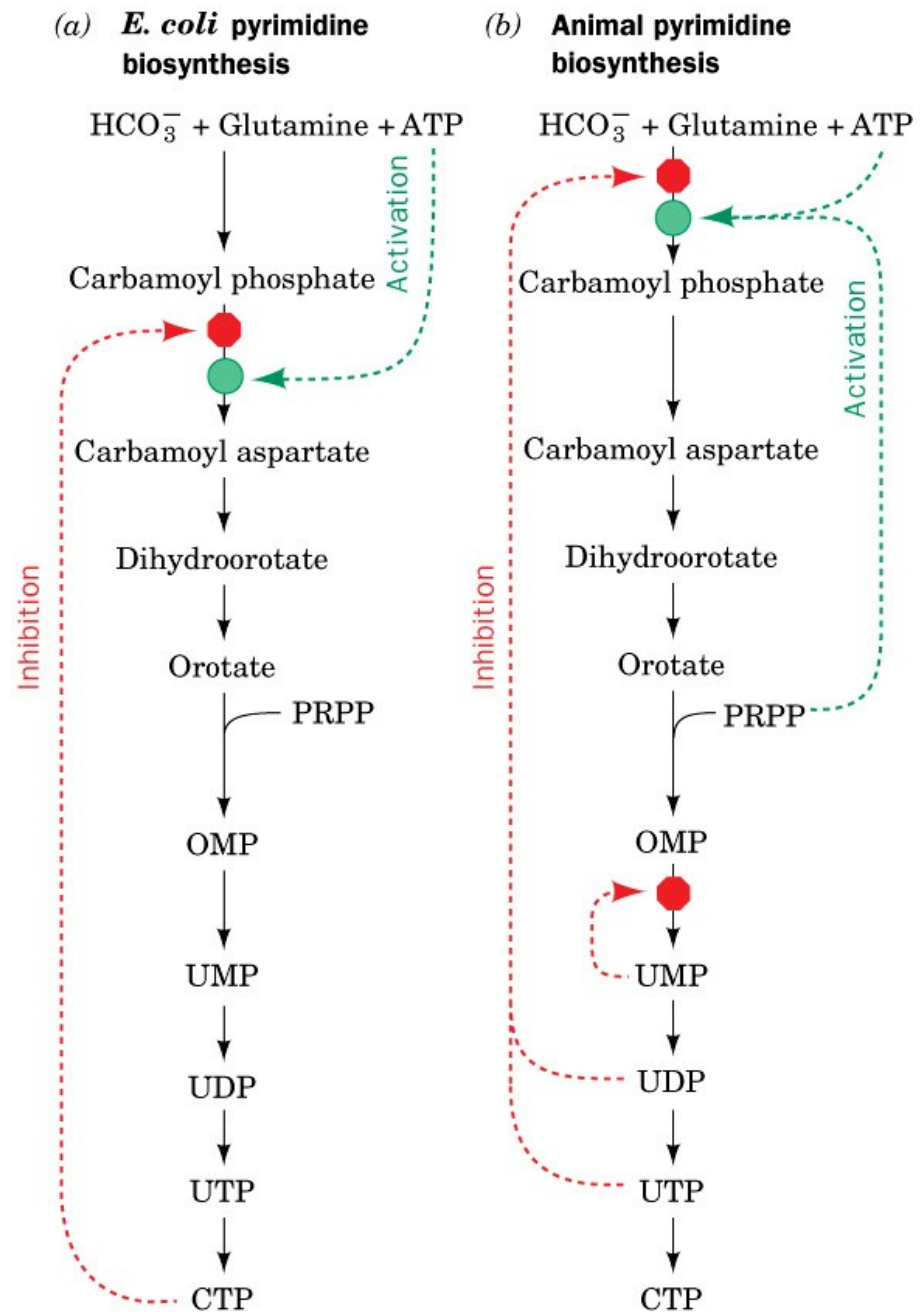


*Gene Expression—CAD gene expression is controlled at both the transcriptional and posttranscriptional level and is upregulated when resting cells enter the proliferative phase . Myc binding to an upstream E box was found to be responsible for the increase in CAD gene transcription that occurs at the G1/S boundary as cells traverse the cycle. Metabolic Control—The CPSase activity of CAD is the major locus of control of de novo pyrimidine biosynthesis . The enzyme is subject to feedback inhibition by the end product UTP and is allosterically activated by PRPP. PRPP, a UMP synthase substrate (Fig. 1), is a feedforward activator and helps coordinate pyrimidine and purine production because it is also a substrate in the first step of purine biosynthesis. The allosteric effectors bind to a regulatory subdomain (Fig. 2, B3) at the carboxyl end of CPS.B in CAD (52) and other CPSases. Several studies suggest that allosteric regulation of CAD governs the rate of pyrimidine biosynthesis *in vivo*.*

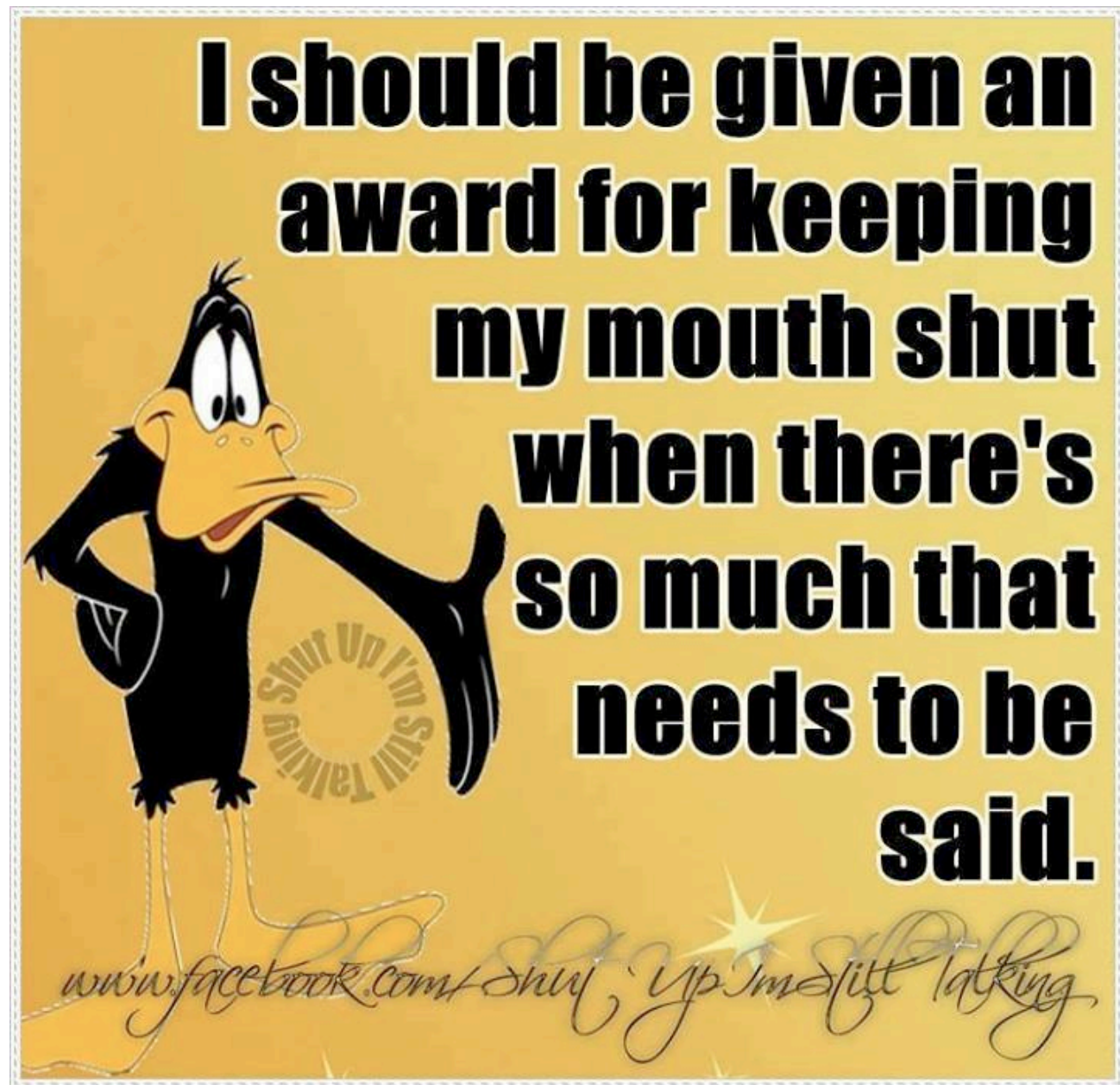
In bacteria pyrimidine biosynthesis is regulated by ATCase Rxn 2, control is exerted by the allosteric stimulation of ATP and it is inhibited by CTP. In many bacteria though UTP is an inhibitor.

In animal cells ATCase is not regulatory, rather pyrimidine biosynthesis is controlled by the activity of CPSII.

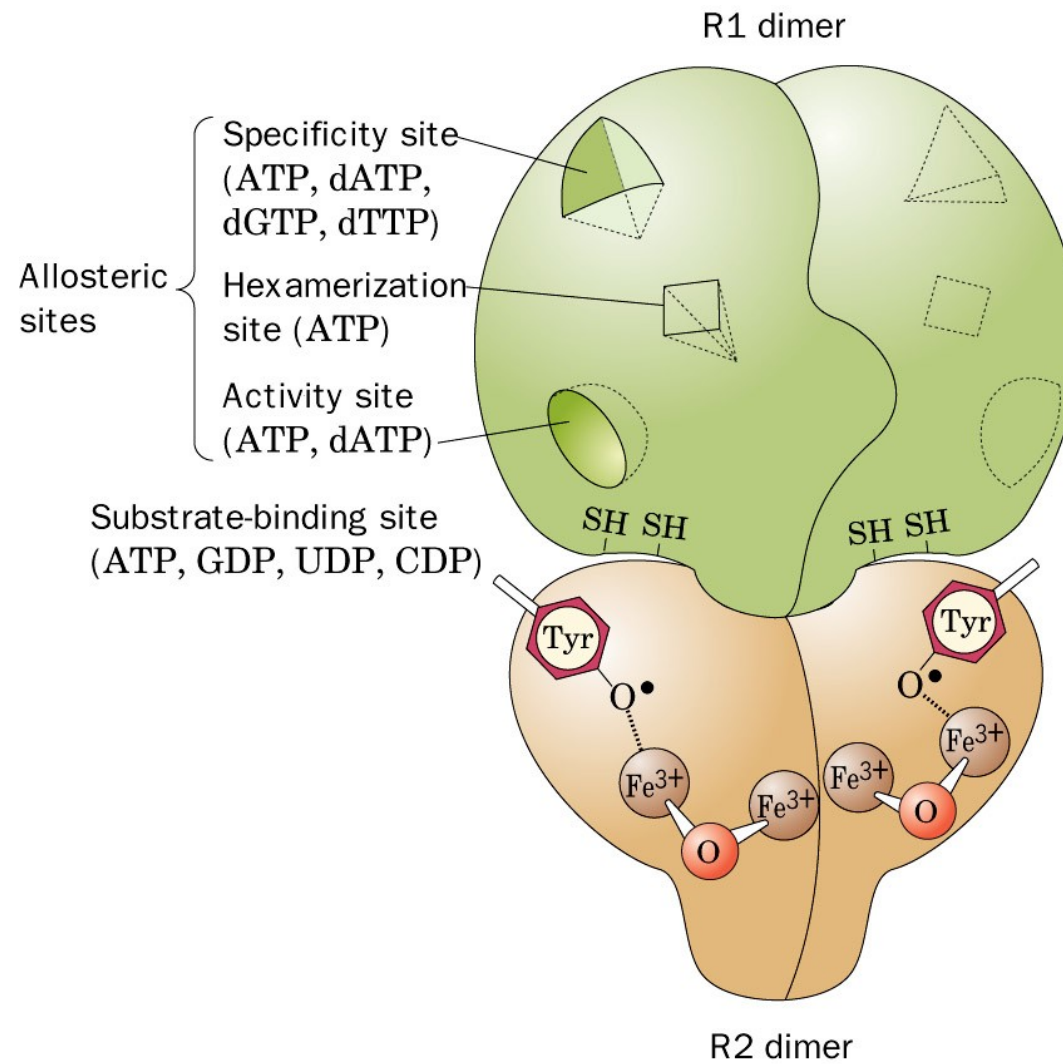
Another level of control is in the formation of OMP, and due to the availability of PRPP. Pyrophosphate kinase is inhibited by ADP and GDP.



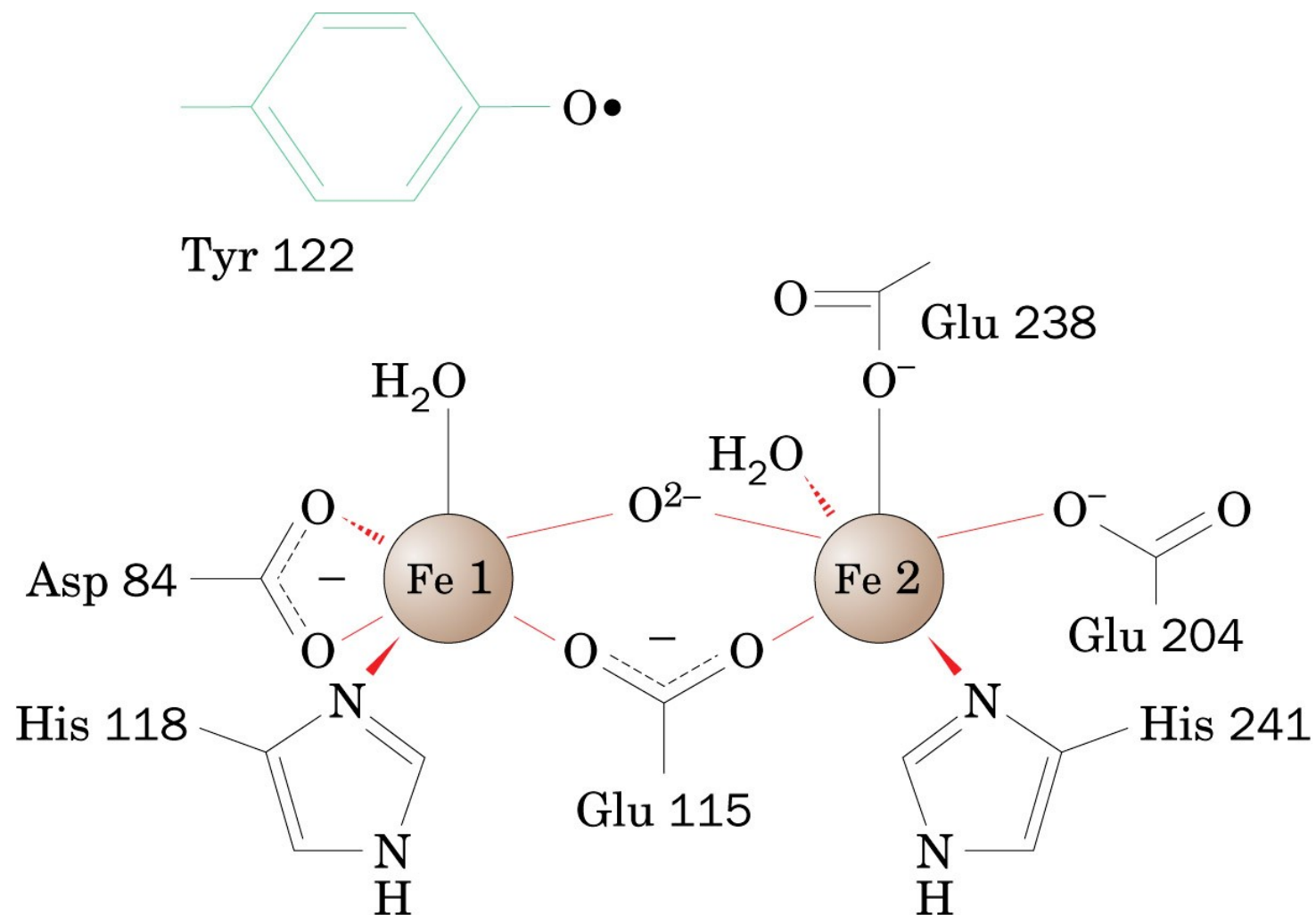
*I only wish
I had
Daffy's
self
control!*

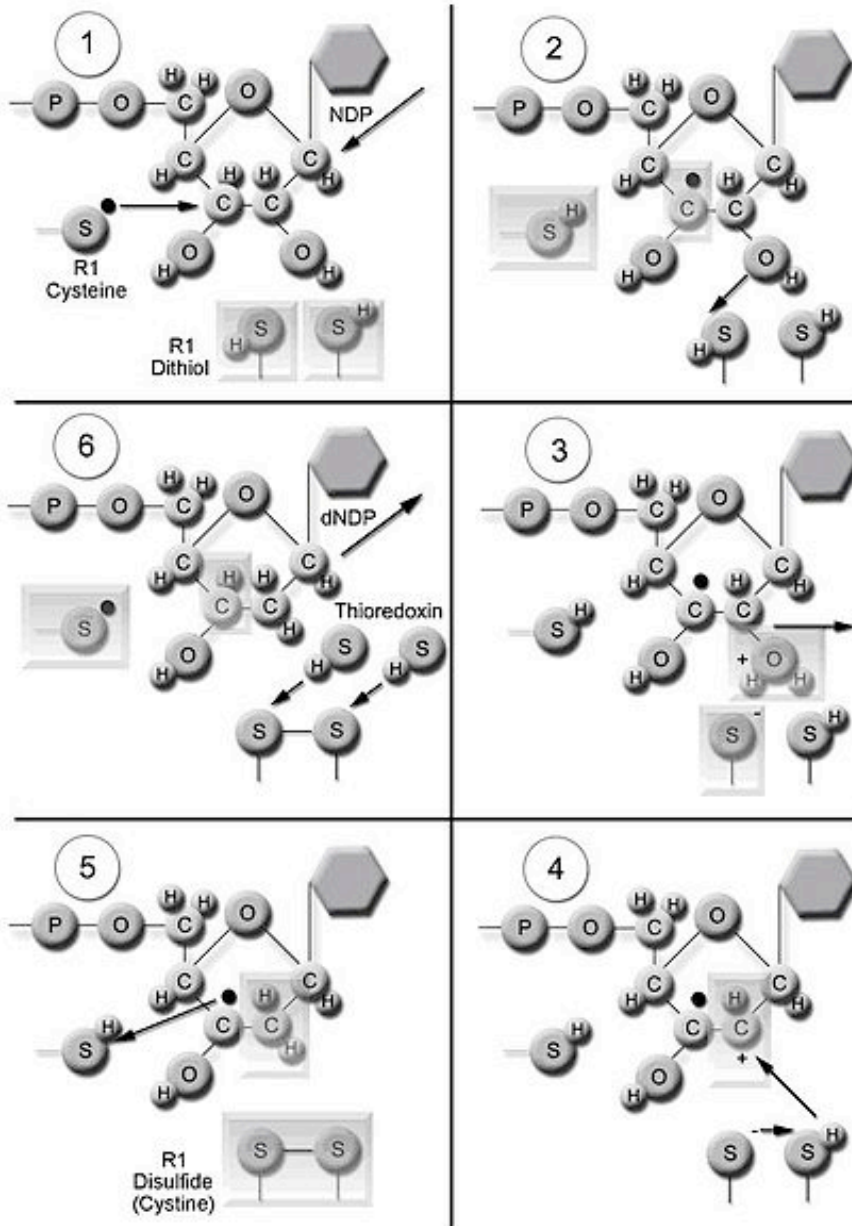


Model of Ribonucleotide Reductase



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





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 hydrogens 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.

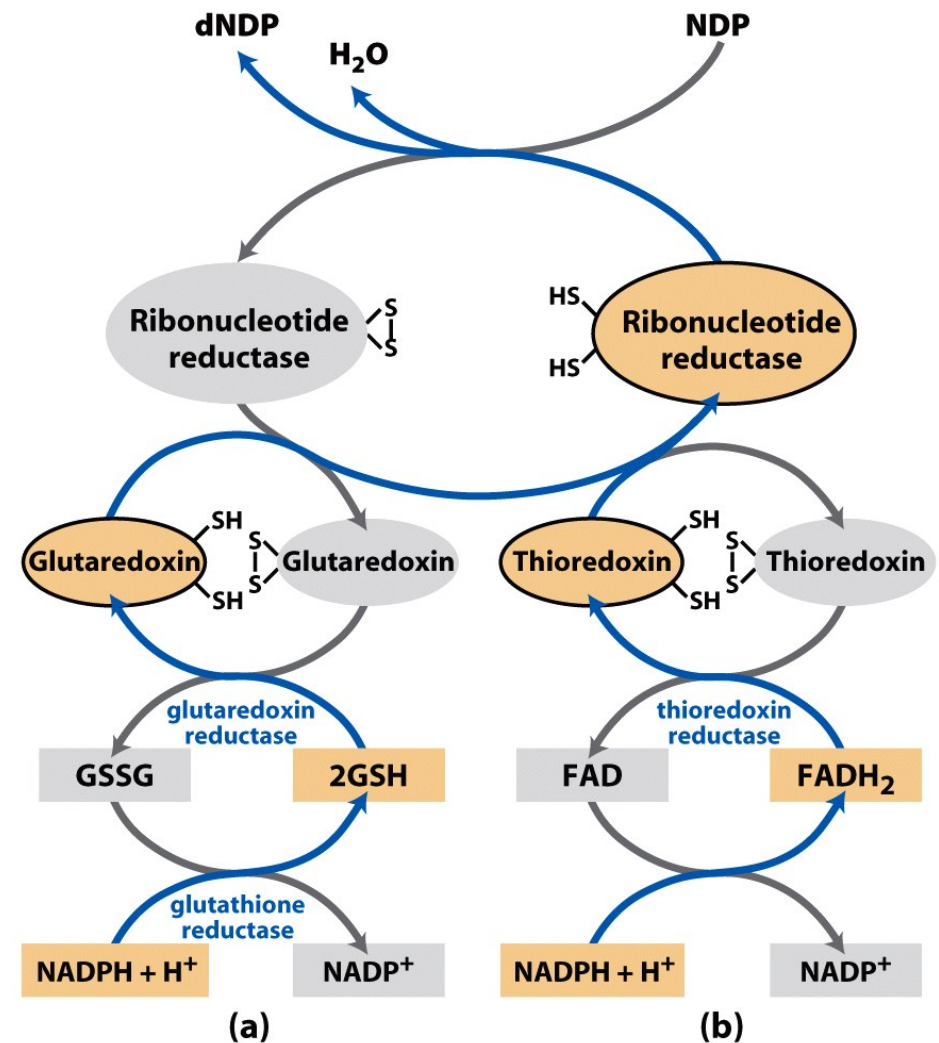


Figure 22-39
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