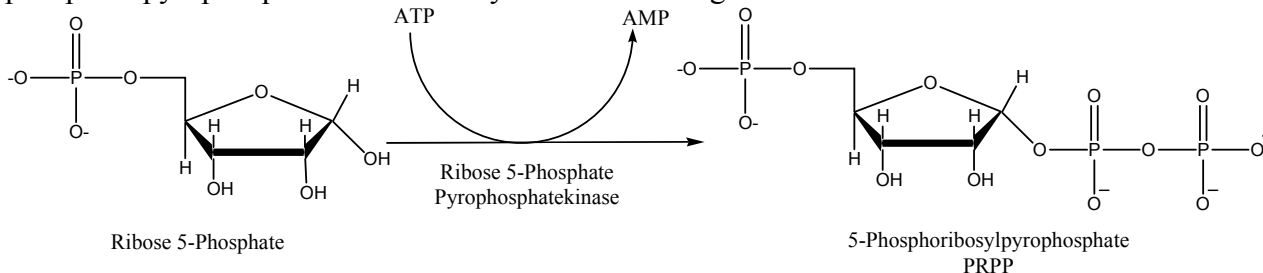


# Ribonucleotide Metabolism

April 25, 2003

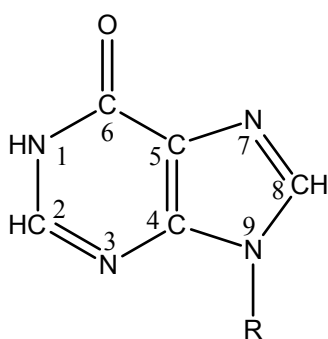
Bryant Miles

PRPP is the center of nucleotide metabolism. PRPP is condensed with orotate to produce oritidylate in the biosynthesis of pyrimidines. The purine ring is built upon PRPP. In addition to the de novo synthesis of nucleotides, there are salvage pathways to recycle the free bases and nucleosides released from ribonucleotide degradation. These salvage pathways also require PRPP. The ribose of PRPP came from ribose 5-phosphate which is produced by the pentose phosphate pathway. The enzyme ribose-5-phosphate pyrophosphate kinase catalyzes the following reaction:

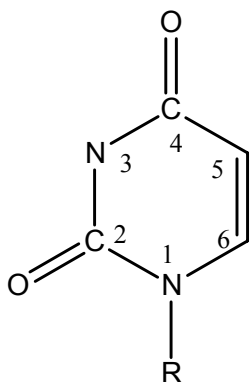


The pyrophosphate group activates the ribose ring for nucleotide biosynthesis.

We just complete the anabolic journey from 5-phosphoribosylpyrophosphate (PRPP) to inosine monophosphate. Starting with ribose 5-phosphate, it takes 12 steps, producing 10 intermediates, many of which are hard to pronounce and spell. Six ATPs were used in the biosynthesis and a pyrophosphate group which is energetically equivalent to an ATP was hydrolyzed by pyrophosphatase. This makes it 7 ATP equivalents consumed in the synthesis of the purine ring. In addition two glutamines and 2 N<sup>10</sup>-formyl-THF molecules were consumed. Shown below is the purine ring. You should be able to tell where every atom of the ring came from.



- N1 was the  $\alpha$ -amino group of aspartate.
- C2 was the formyl carbon of N<sup>10</sup>-formyl-THF
- N3 was the amide of glutamine
- C4 was the carboxylate carbon of glycine
- C5 was the  $\alpha$ -carbon of glycine
- C6 was once bicarbonate
- N7 was the  $\alpha$ -amino group of glycine
- N8 was the formyl carbon of N<sup>10</sup>-formyl-THF
- N9 was the amide of glutamine

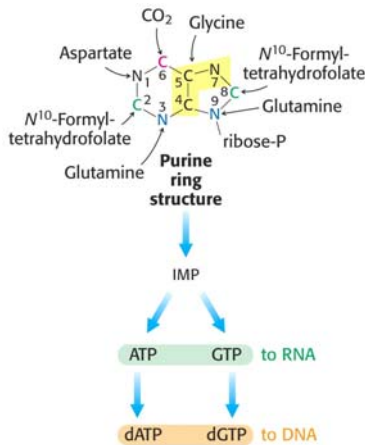


Similarly you should be able to tell me where every atom in the pyrimidine ring came from. In addition the pyrimidine biosynthesis pathway is one I expect you to know for the test.

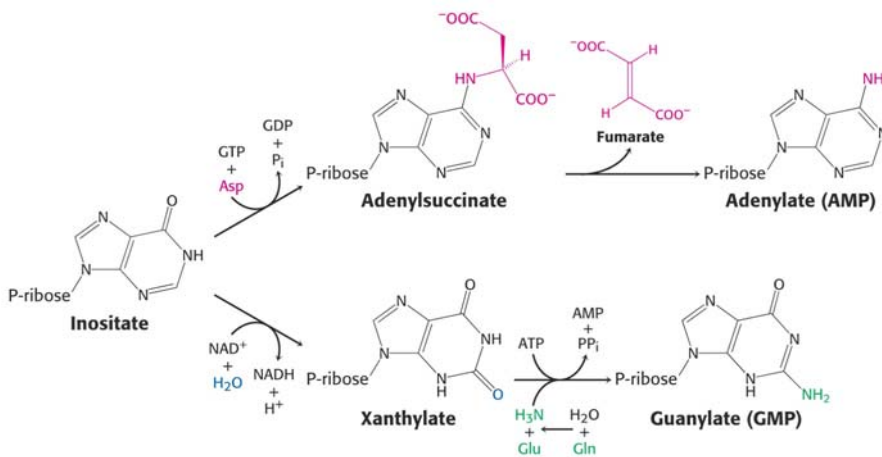
- N1 was the  $\alpha$ -amino group of aspartate.
- C2 was once bicarbonate.
- N3 was the amide of glutamine
- C4 was the carboxylate side chain of aspartate.
- C5 from aspartate
- C6 was the  $\alpha$ -carbon of aspartate.

## I. Biosynthesis of AMP and GMP.

The IMP produced in the purine biosynthetic pathway is a branch point in purine nucleotide synthesis. Inosine monophosphate is converted into AMP or GMP.



**Adenosine monophosphate** is synthesized from inosine monophosphate in 2 steps. The first step is catalyzed by adenylosuccinate synthetase which uses **GTP** to activate the C-6 for nucleophilic attack by the  $\alpha$ -amino group of aspartate to form adenylosuccinate. The enzyme adenylosuccinate lyase then catalyzes the cleavage of fumarate to produce AMP.

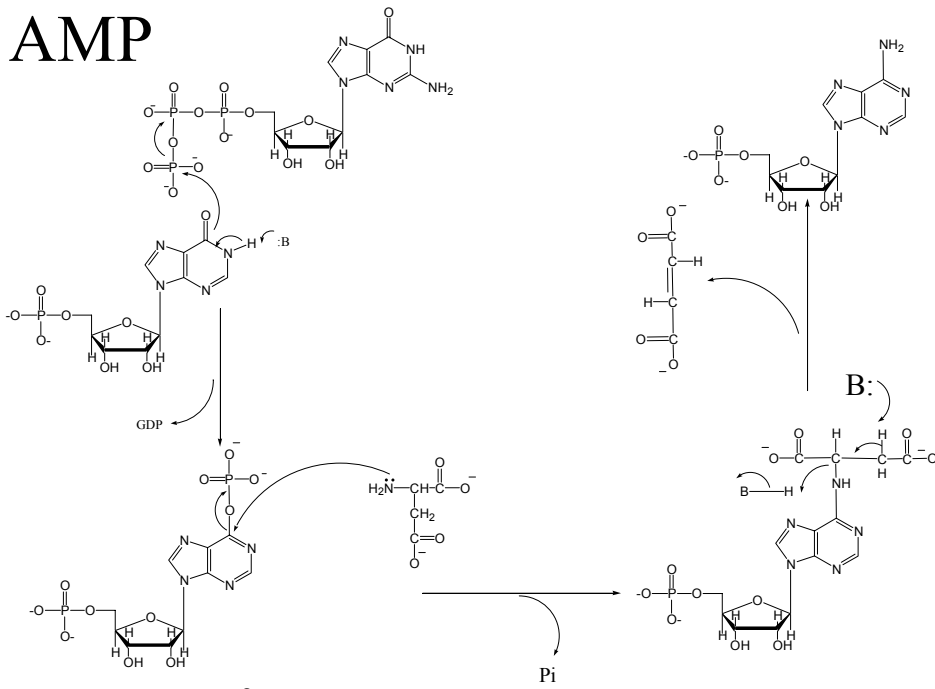


Guanosine monophosphate is also synthesized from IMP in 2 steps. The first step is catalyzed by IMP dehydrogenase which catalyzes the addition of water to the double bond and then the oxidation of the hydroxyl group to form Xanthosine monophosphate. GMP synthetase (another glutamine amido transferase enzyme) transfers the amide nitrogen of glutamine to an activated C2 carbon to produce GMP. This reaction requires **ATP** to activate the C2 carbon for nucleophilic attack by the ammonia.

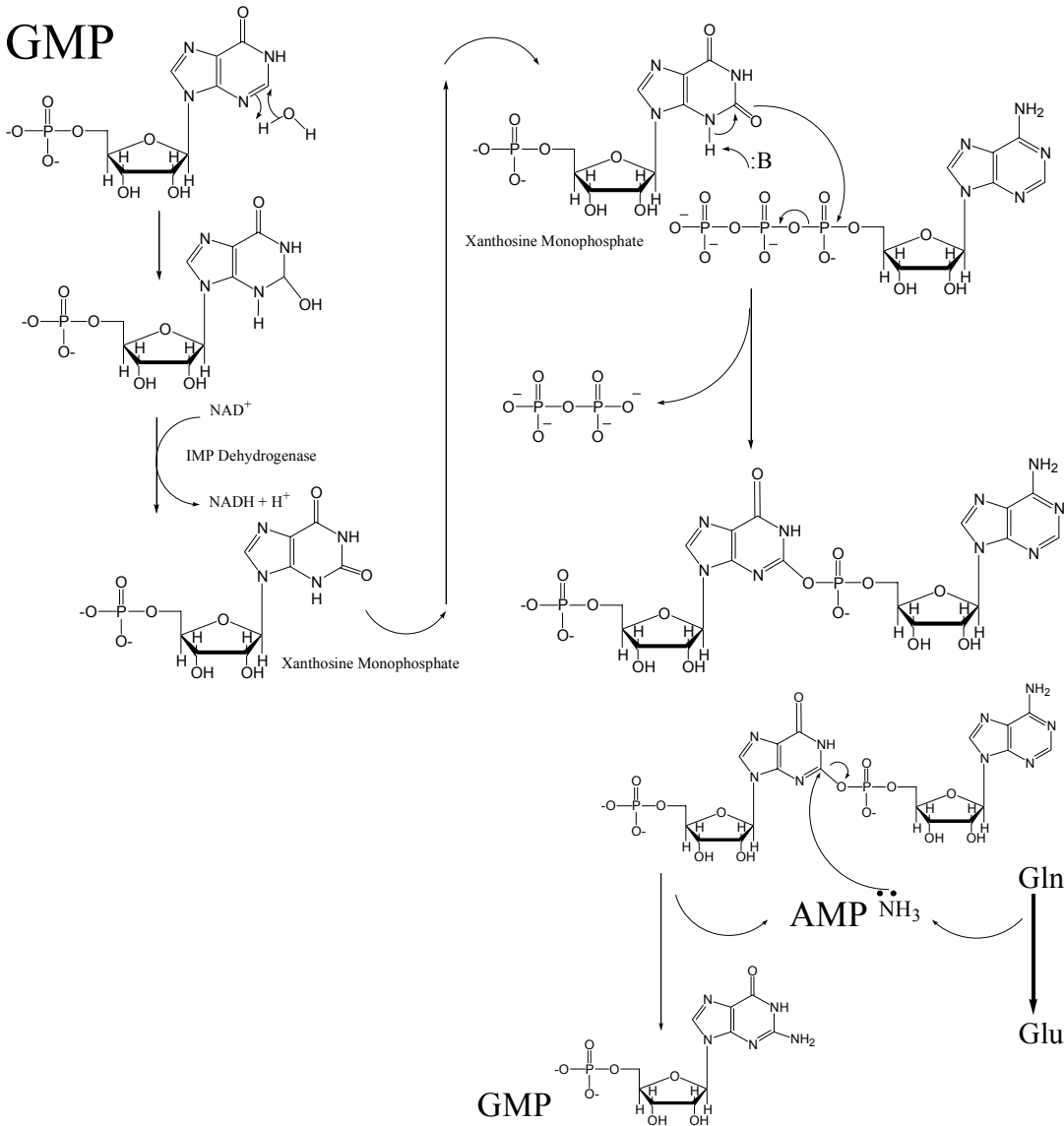
The mechanisms of these enzymes are shown on the following page.

It is important to note that the conversion of XMP to GMP is **ATP dependent** and that the conversion of IMP to adenylosuccinate is **GTP dependent**. This produces a mechanism to control the ratios of ATP to GTP. When the ratio of ATP/GTP is high, IMP is partitioned towards GMP synthesis. If the ratio of ATP/GTP is low, IMP is partitioned towards ATP synthesis.

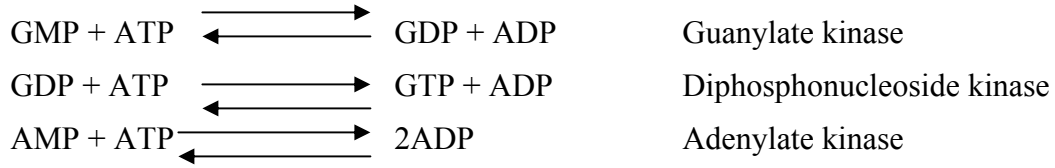
# AMP



# GMP



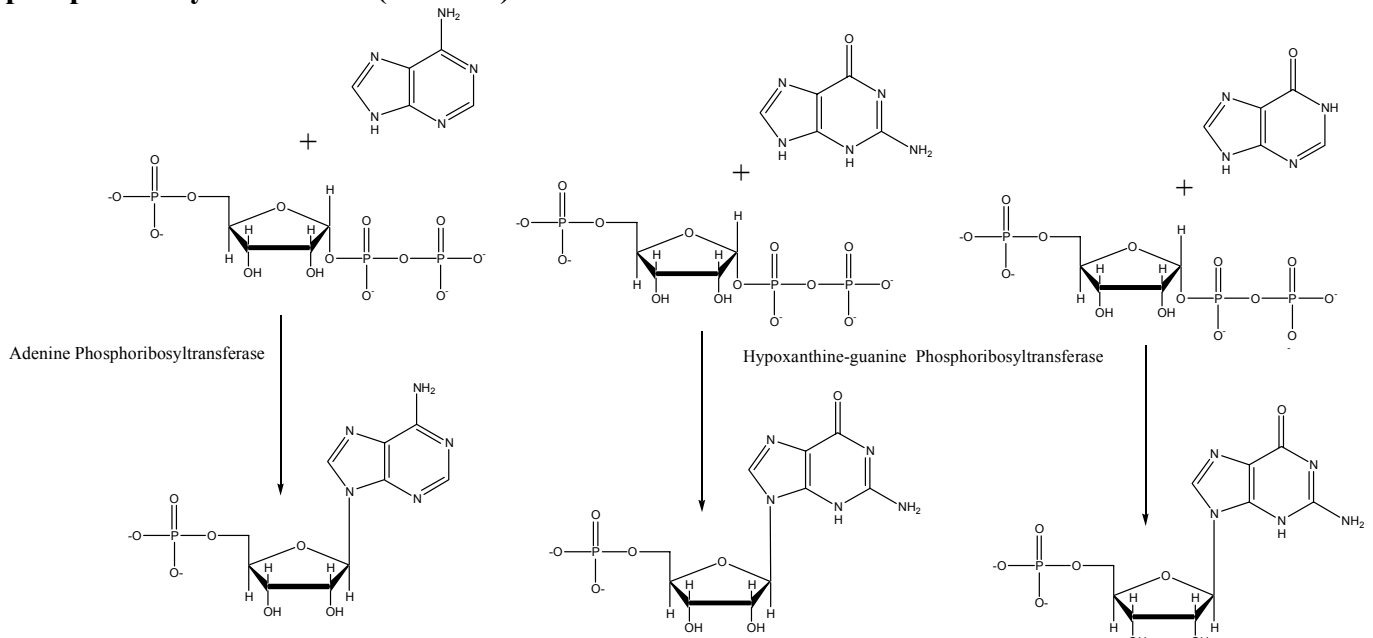
GMP and AMP are converted into the corresponding triphosphates by two sequential phosphorylation reactions.



ATP is produced from ADP by a number of energy producing reactions such as glycolysis and oxidative phosphorylation.

## II. Salvage Pathways

Free purine bases are derived from the turnover nucleotides or from dietary nucleotides. These purine bases can be salvaged. Salvage pathways vary from species to species. In humans there are two enzymes involved in the purine salvage pathway. **Adenine phosphoribosyltransferase** salvages adenine to form AMP. Hypoxanthine and guanine are salvaged by the **enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT)**.



The only free pyrimidine base that can be salvaged is uracil. Cytosine cannot be salvaged. Pyrimidine phosphoribosyltransferase will connect uracil to PRPP to form UMP.

## III. Biosynthesis of the Deoxyribonucleotides.

Your typical cell has 5 to 10 times more RNA than DNA. Ribonucleotides have multiple metabolic roles. Deoxyribonucleotides are used for only one role, the storage of genetic information as DNA. Most of the energy expended on nucleotide biosynthesis goes into the formation of the ribonucleotides, a relatively small fraction goes towards the biosynthesis of deoxyribonucleotides. But this small fraction is of paramount importance because these deoxyribonucleotides are used to replicate our DNA.

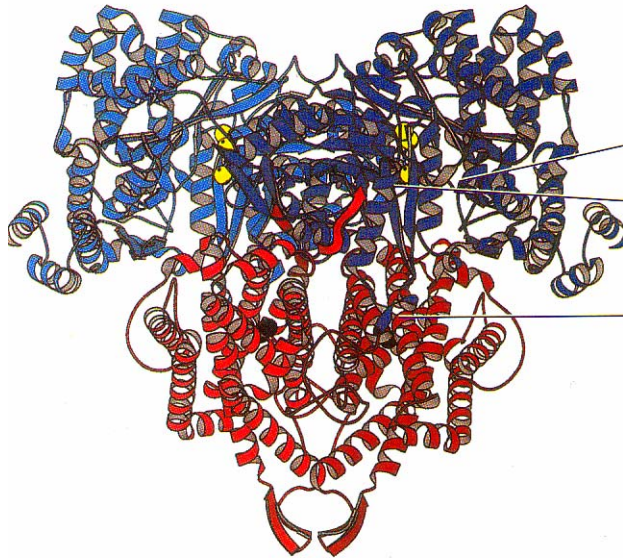
The ribose of ribonucleotides is reduced to the 2'-deoxyribose by the replacement of the 2'-hydroxyl group with a hydride with retention of configuration. The enzyme that catalyzes this reaction is called ribonucleotide reductase. As far as I know, every organism on the planet today uses a single ribonucleotide reductase to reduce all four ribonucleotides into the corresponding 2'-

deoxyribonucleotides. The mechanism involves the generation of a free radical. There are three classes of ribonucleotide reductases.

- Class I ribonucleotide reductase generates an active site free radical on a specific tyrosine residue with the aid of a diferric oxygen bridge. This is the most common class.
- Class II ribonucleotide reductases are found in cyanobacteria and *Euglena*, this enzyme generates a free radical using adenosylcobalamin, Cofactor B12.
- Class III ribonucleotide reductases are found in obligate anaerobes. This ribonucleotide reductase uses S-adenosylmethionine and an iron-sulfur cluster to generate the active site radical.

Animals and plants have a class I ribonucleotide reductase and this is the one we will study.

*E. coli* ribonucleoside reductase is a class I. Happily we have a crystal structure of it. It has a beautiful heart shape.



Specificity site

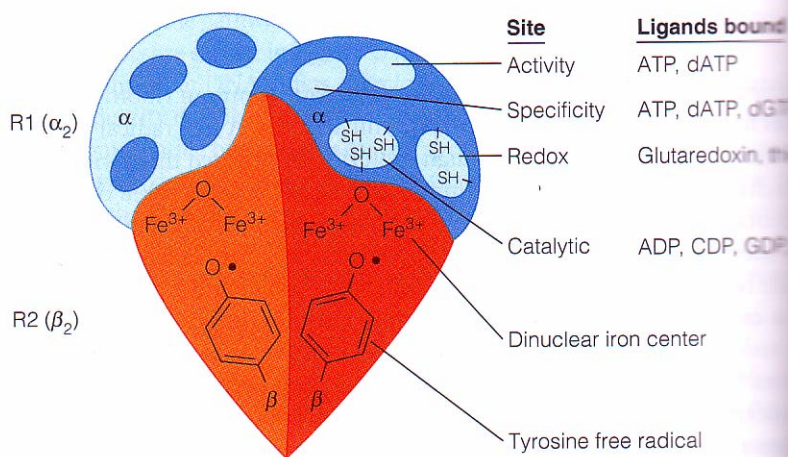
Catalytic site

Dinuclear iron center.

The enzyme is composed of 2  $\alpha$ -subunits and 2  $\beta$ -subunits. The catalytic active sites are located in the  $\alpha$ -subunits. Within the active site are three cysteine residues shown as yellow spheres. 2 of these cysteines function in the redox reaction.

In the  $\beta$ -subunit is the tyrosine free radical that is involved in the reaction. Nearby is the dinuclear iron center that stabilizes the free radical.

(a)



(b)

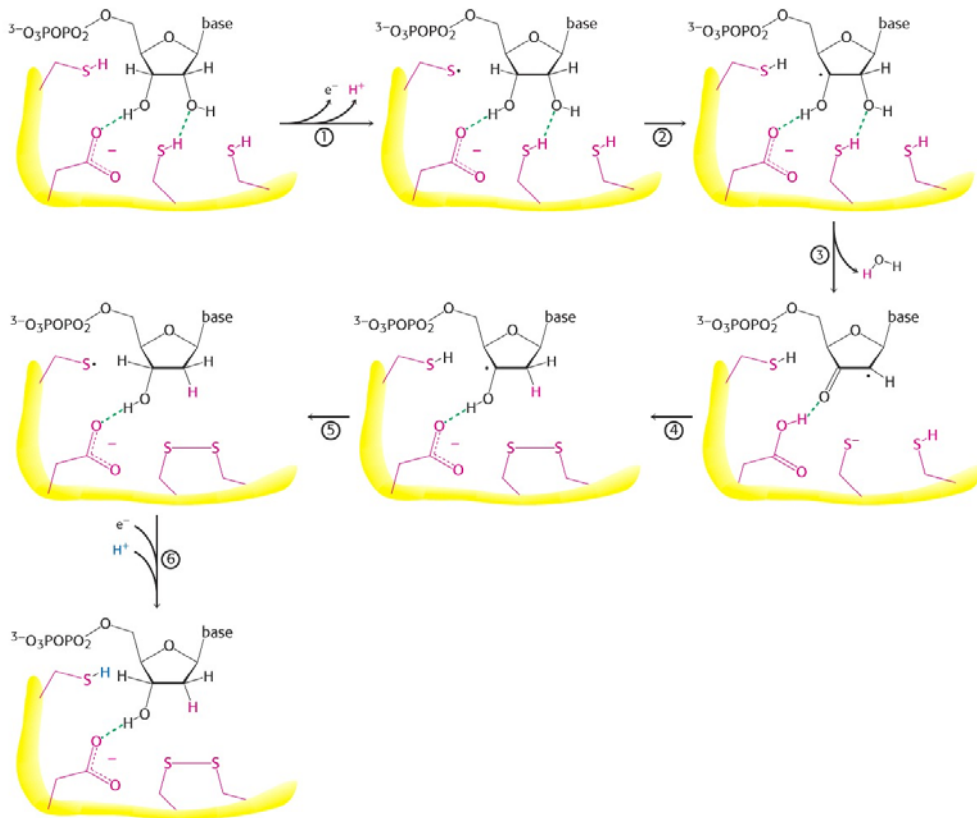
This is a cartoon of the crystal structure. There are three nucleoside binding sites.

1. The activity site which binds ATP or dATP.
2. The specificity site that binds ATP, dATP, dGTP or dTTP.
3. There is a catalytic site which is where the substrate ribonucleotide is bound, ADP, CDP, GDP and UDP.

There is a redox site where thioredoxin or glutaredoxin reduces the disulfide bonds generated during catalysis.

And of course the binuclear metal centers and the tyrosine free radicals.

The mechanism for the reduction is shown on the following page.



First the substrate ribonucleotide binds to the catalytic site. The reaction begins with the tyrosine radical abstracting a hydrogen atom from a cysteine residue to produce a cysteine thiyl radical within the active site.

This thiyl radical abstracts a hydrogen atom from the C-3' of the ribose unit generating a radical on the C-3'.

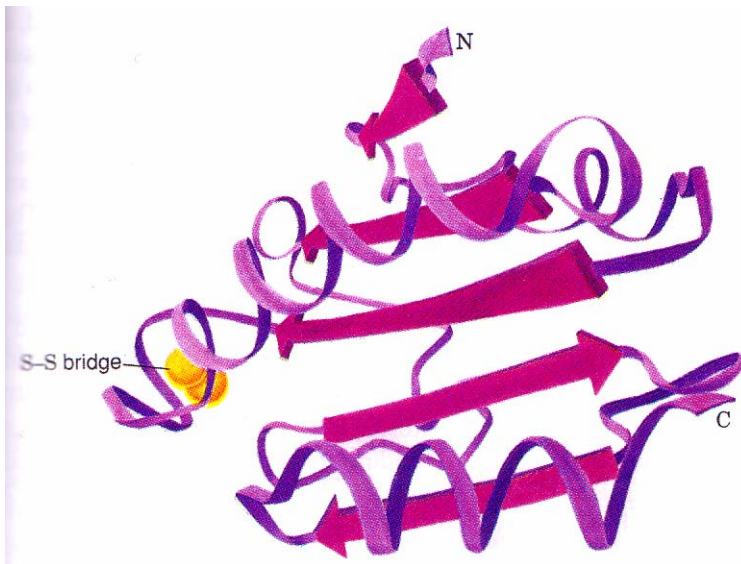
The radical at the C-3' carbon induces release of a hydroxide ion from the C-2' position moving the radical to that position. The hydroxide generated abstracts a proton from a second cysteine residue and leaves as water.

The cysteine anion forms a disulfide bond with the adjacent cysteine residue passing a hydride to the C-2' carbon complete the reduction at this position and shifting the radical back to the C-3' position.

The C-3' radical recaptures the same hydrogen that was originally bonded to it from the first cysteine residue. The deoxyribonucleotide is now free to diffuse from the active site.

The disulfide bond formed during this reaction needs to be reduced before another round of catalysis can occur. There are two disulfide containing proteins that can reduce this disulfide bond. Thioredoxin and glutaredoxin. Remember thioredoxin from photosynthesis?





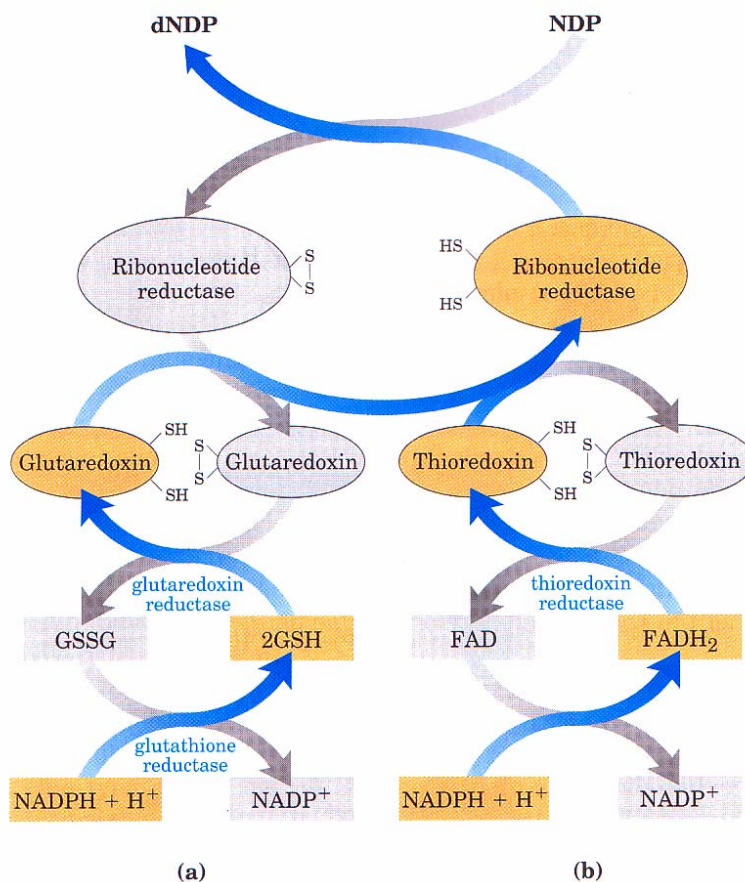
Reduced thioredoxin contains two free sulfhydryls.

These sulfhydryls are located on a protrusion that sticks out from the protein.

This protrusion penetrates into the catalytic active site and reduces the disulfide bond that was formed in the reduction of the ribonucleotide to the deoxyribonucleotide.

The oxidization of thioredoxin results in a disulfide bond. This disulfide bond of thioredoxin is reduced by an enzyme called thioredoxin reductase which has a pair of

free sulfhydryl residues which transfer a pair of electrons to thioredoxin to reduce its disulfide bond back into 2 free sulfhydryls while concomitantly forming a disulfide bond in thioredoxin reductase. The disulfide bond in thioredoxin reductase is subsequently reduced by NADPH. There is a second protein that can reduce the disulfide bond in ribonucleotide reductase. This protein also contains a disulfide and is called glutaredoxin. Oxidized glutaredoxin is reduced by Glutaredoxin reductase which uses glutathione to reduce the disulfide bond generated in the reduction of glutaredoxin. Glutathione reductase uses NADPH as a reductant to generate reduced glutathione.



## Regulation of Ribonucleotide Reductase

Ribonucleotide reductase is regulated in order to maintain the appropriate balance of deoxyribonucleotides. The overall activity must be turned off and on in response to the cell's need for dNTPs.

In order to achieve these functions ribonucleotide reductase has two effector binding sites in addition to the catalytic site. One site is called the **activity site**. This site is an on/off switch. Only ATP and dATP bind to the activity site. ATP bound to this site turns ribonucleotide reductase **on**, dATP bound to this site turns ribonucleotide reductase **off**.

The other effector site is called the substrate specificity site. This site can bind ATP, dTTP, dGTP or dATP. The substrate specificity of the ribonucleotide reductase depends on which of these effectors is bound at this site.

If ATP is bound to the substrate specificity site, then pyrimidine nucleotides are bound to the catalytic active site to produce either dUDP or dCTP.

If dTTP is bound to the substrate specificity site, GDP is bound at the catalytic active site to produce dGDP.

If dGTP is bound to the specificity site, ADP becomes the substrate to produce dADP.

Here is the rationale for this intricate regulation.

Only when the cell's energy state is high does the cell undergo division. Only when the cell is dividing does DNA replication occur. When the cell's energy state is high, the concentration of ATP is high. The concentrations of all the deoxynucleotides are low. ATP binds to the activity site, turning ribonucleoside reductase on. ATP binds to the substrate specificity site and the pyrimidine deoxyribonucleoside diphosphates are produced, dUDP, dCDP. These deoxypyrimidines are precursors for dTTP. The concentrations of dUDP, dCDP go up which causes the concentration of dTTP to go up. High concentrations of dTTP compete with ATP for the substrate specificity site. When dTTP is bound, then GDP becomes the substrate and dGDP is produced. dGDP is rapidly converted into dGTP. The concentration of dGTP increases until it competes with dTTP for the specificity binding site. When dGTP is bound to the specificity binding site, ADP becomes the substrate and dADP is produced. dADP is

phosphorylated to produce dATP. dATP accumulates until it competes with ATP for the activity site. When dATP is bound the ribonucleotide reductase is turned off.

