

Nucleotide Metabolism

Nucleotide sources

In humans, dietary nucleotide bases are rarely incorporated into nucleotides. As a result, humans must synthesize their own nucleotide bases. (With the exception of a few parasitic prokaryotes, all organisms can synthesize nucleotides.) Although all nucleated eukaryotic cells can synthesize nucleotides, most human synthesis occurs in the liver.

Nucleotide synthesis is tightly regulated. Nucleotide synthesis is somewhat expensive in that the pathways use several molecules with other uses. In addition, although pyrimidines can be degraded into standard metabolic intermediates, purine catabolism does not alter the basic purine structure, and excessive levels of purines can be toxic.

Purine synthesis

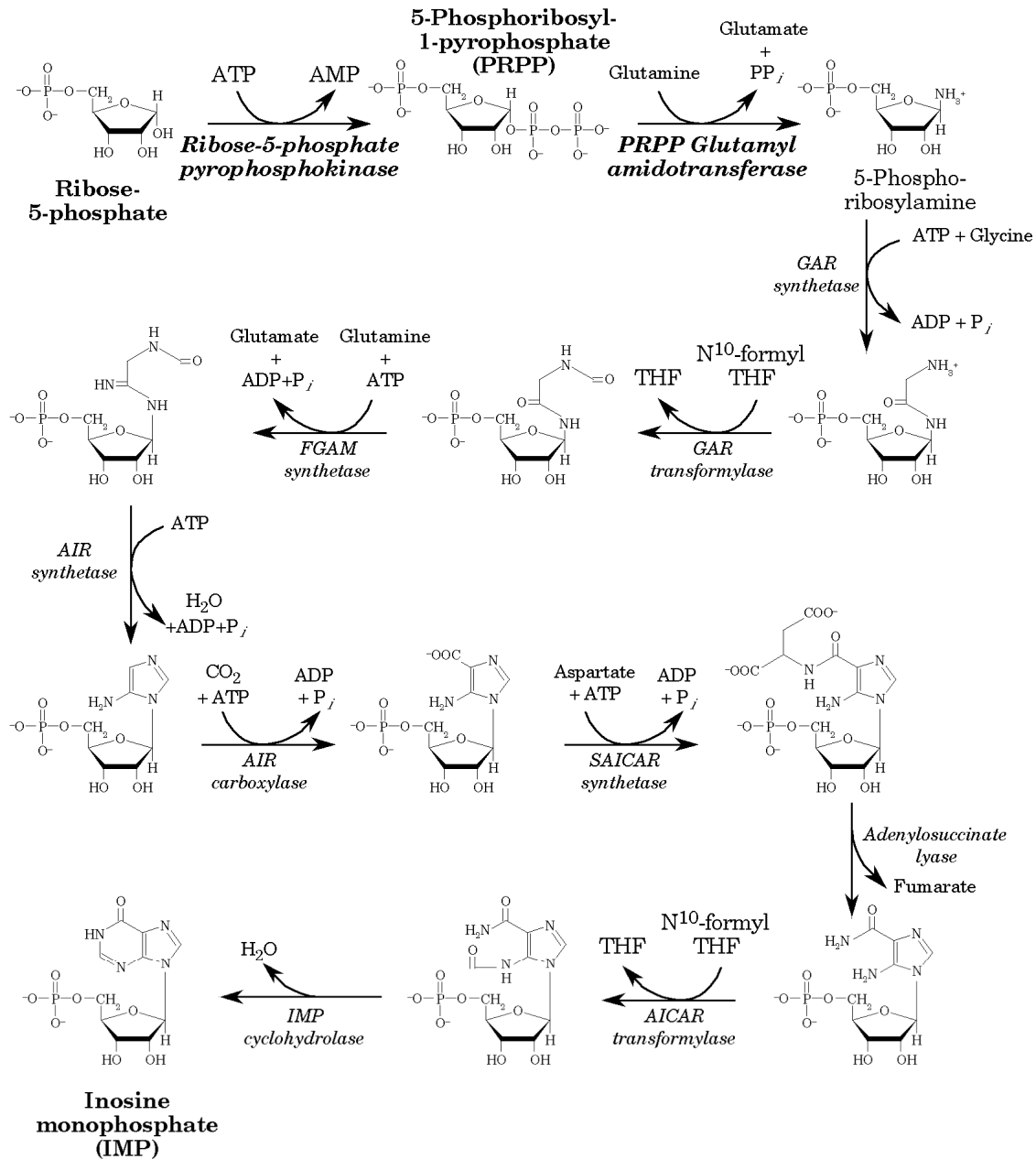
Purine production occurs primarily in the liver, although most tissues produce at least small amounts. Purine biosynthesis begins with ribose-5-phosphate (a product of the hexose monophosphate pathway), and ends with the hypoxanthine-containing inosine monophosphate. Both of the first two reactions in the purine biosynthetic pathway are tightly regulated.

The first reaction of the purine synthesis pathway, catalyzed by **ribose-5-phosphate pyrophosphokinase**, produces phosphoribosylpyrophosphate (PRPP). PRPP is **not** committed to purine biosynthesis; it is also used for other processes (such as pyrimidine biosynthesis, histidine biosynthesis, and nucleotide base salvage). However, availability of PRPP is critical for purine biosynthesis.

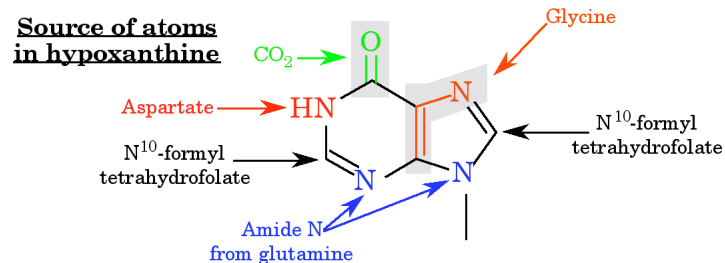
The second step, the loss of the PRPP pyrophosphate, and the addition of an amino group from glutamine, is catalyzed by **PRPP glutamyl amidotransferase**. This step is the committed step for purine biosynthesis, and is also a regulated step.

The pathway includes nine additional enzymatic reactions; it uses the ribose-5-phosphate as a platform for the construction of the purine ring structure. The pathway first builds the 5-membered ring, and then builds the six-membered ring.

As is generally the case in biosynthetic reactions, free ammonium is not used as a nitrogen donor in the pathway; instead the requisite organic nitrogen is obtained from the amino acids glutamine, glycine, and aspartate. One of the reactions in the pathway involves the incorporation of carbon dioxide; the carboxylase responsible for the reaction is somewhat unusual in that it is not a biotin-dependent enzyme.



The product of the purine biosynthesis pathway is inosine monophosphate (IMP), which contains the base hypoxanthine.



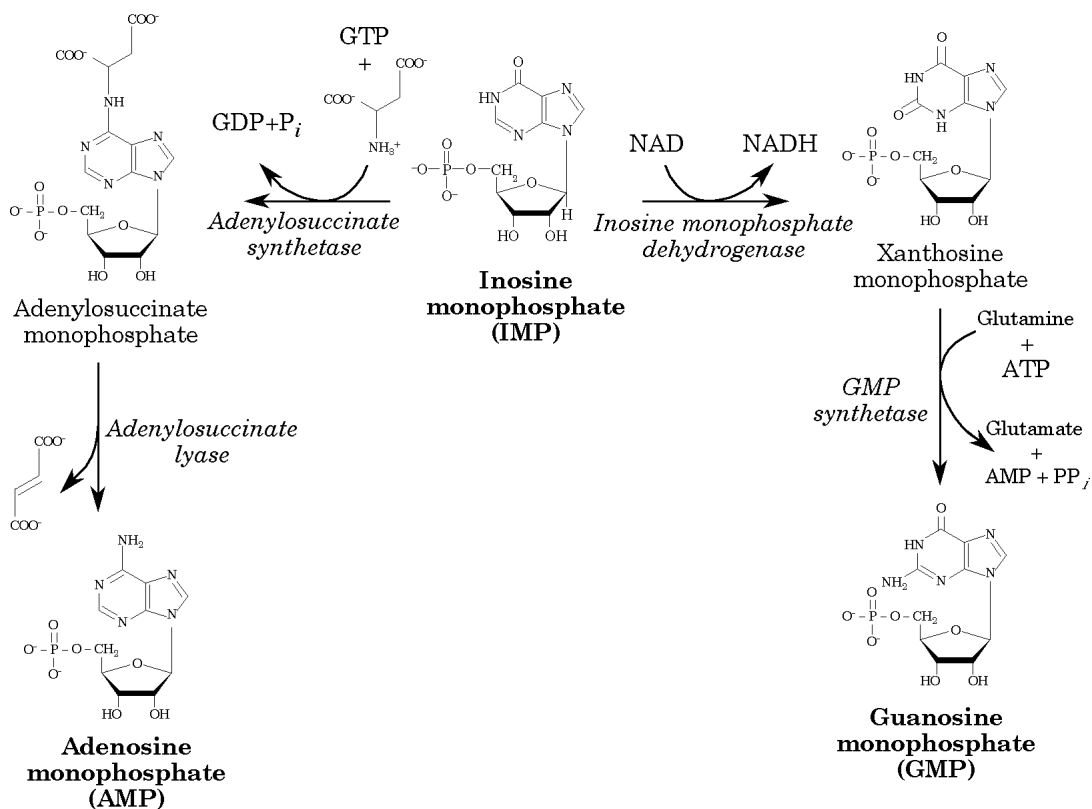
Three of the ring atoms in purines are derived from glycine; the remaining six ring atoms come from six different molecules. The purine structure includes nitrogens derived from glutamine (yielding glutamate) and aspartate (yielding fumarate). One carbon in the purine ring comes from carbon dioxide. Two of the carbons in the purine structure come from N¹⁰-formyl tetrahydrofolate, which is one reason why tetrahydrofolate is so important for nucleotide biosynthesis.

Ribose-5-phosphate pyrophosphokinase is feedback inhibited by nucleoside diphosphates: especially ADP and GDP and dTDP. **PRPP Glutamyl amidotransferase** is **inhibited** by any adenosine or guanosine phosphate. It is **stimulated** by its substrate PRPP, which is an important factor in regulation of purine synthesis. Regulation of purine metabolism will be discussed further below.

AMP and GMP synthesis

Inosine monophosphate does not accumulate; instead it is rapidly converted to adenosine monophosphate (AMP) and guanosine monophosphate (GMP). Each of these processes is comprised of two reactions.

The first step in each pathway is inhibited by the final product (**IMP dehydrogenase** by GMP, and **adenylosuccinate synthetase** by AMP). Note that ATP is required for GMP synthesis and GTP is required for AMP synthesis; in addition, each nucleoside triphosphate acts as a stimulator of the synthesis of the other purine derivative, allowing high levels of one purine nucleotide to favor diversion of IMP toward production of the other.



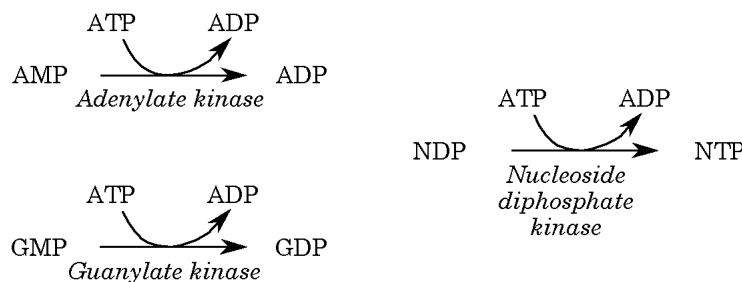
The second enzyme in the AMP pathway, **adenylosuccinate lyase**, catalyzes a similar reaction in the IMP pathway. In both cases, the reaction involves the cleavage of the aspartate added, releasing fumarate while leaving the aspartate nitrogen attached to the base.

The base in xanthosine monophosphate is **xanthine**.

Fumarate is generated from aspartate as part of the IMP synthesis pathway and during conversion of IMP to AMP. This pathway can result in physiologically significant levels of fumarate release (in addition, muscle may cycle AMP back to IMP to enhance fumarate generation). Fumarate generation increases the net amount of carbon in the TCA cycle.

NTP generation

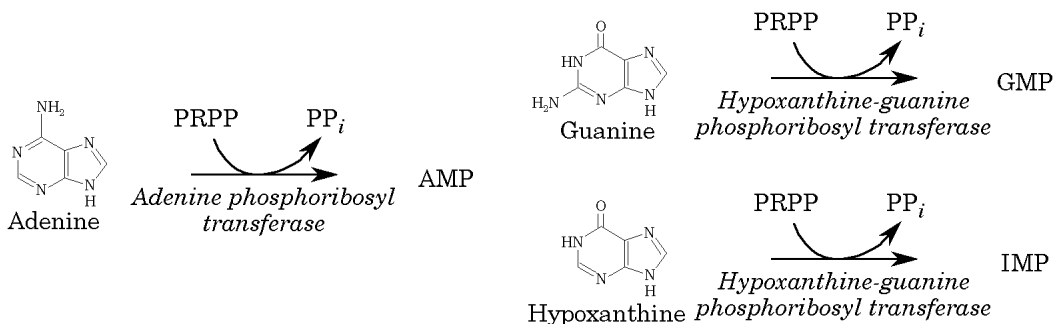
The purine nucleoside monophosphates produced in the biosynthetic pathway need to be converted to nucleoside triphosphates. Enzymes specific for the nucleoside monophosphate catalyze the conversion of the monophosphate to the diphosphate. **Adenylate kinase** is specific for AMP and **guanylate kinase** is specific for GMP.



The enzyme **nucleoside diphosphate kinase** allows donation of terminal phosphate from any NTP to any NDP. Because free ATP is present in higher levels than any other nucleotide, and because ATP can be generated by metabolic processes (by the substrate-level phosphorylation steps of glycolysis and by oxidative phosphorylation), ATP is usually used as the phosphate donor. GTP is occasionally used also, because it can also be generated from GDP by the succinyl-CoA synthetase reaction of the TCA cycle.

Salvage pathways

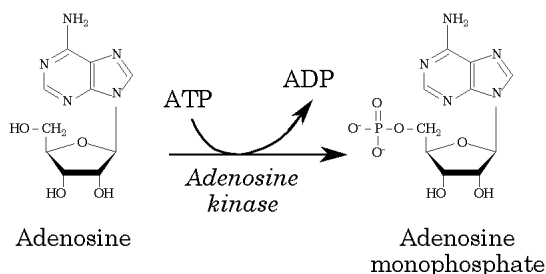
Although humans tend **not** to use dietary nucleotides for nucleic acid synthesis, humans do recycle nucleotides and free nucleotide bases in circulation. This important process is termed the “purine salvage pathway”. It is important because free purines are somewhat toxic, and because the liver, which synthesizes most nucleotides, releases the compounds as either free bases or free nucleosides.



Free purines are attached to the ribose ring (using PRPP as the base acceptor) by two enzymes. Adenine is attached by **adenine phosphoribosyl transferase**. Both hypoxanthine and guanine are attached to the ribose ring by **hypoxanthine-guanine phosphoribosyl transferase** (HGPRT).

The salvage pathway decreases the levels of PRPP, and therefore decreases the rate of purine synthesis. This is an important regulatory mechanism for purine metabolism.

A second pathway allows recycling of nucleosides by initiating the process of adding the 5'-phosphates. **Adenosine kinase** is specific for adenosine; an equivalent reaction occurs for guanosine. (Note that adenosine is a signaling molecule, and tends to be present in significantly larger amounts than guanosine.)



Disorders of purine production

Purine deficiencies are rare in humans (significant deficiencies being rapidly fatal). Most are due to either inhibitors of enzymes in the pathway, or to deficiencies in folic acid (or Vitamin B₁₂ [cobalamin], which is necessary for removing the methyl from N⁵-methyl tetrahydrofolate and thereby regenerating THF).

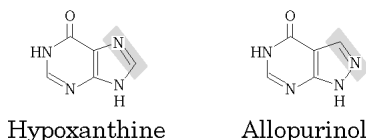
Microorganisms synthesize folate, and convert it to tetrahydrofolate. Animals, however, require folic acid in the diet. The first antibiotics, the sulfonamide drugs, work by inhibiting folate biosynthesis, killing the bacteria without affecting the host. Sulfonamides are analogs of *p*-aminobenzoic acid, a component of folic acid. The inability to produce purines as a result of lack of folate is incompatible with life (note: a few parasites obtain nucleotides from their host organisms, and are therefore exceptions).

Overproduction of purines or lack of salvage of purines leads to significant disorders. **Gout** is a relatively well-known disorder of purine overproduction. Gout

is most commonly due to mutant forms of **ribose-5-phosphate pyrophosphokinase**. These mutations have one of several possible effects, including increasing the V_{max} of the enzyme, decreasing the enzyme K_m , or removing its sensitivity to feedback inhibition. All three mutations result in elevated PRPP production, and therefore in overproduction of purines. Gout results in the painful formation of uric acid crystals in certain joints (especially the large joint at the base of the big toe).

Lesch-Nyhan syndrome results from a complete lack of **hypoxanthine-guanine phosphoribosyl transferase** (HGPRT); the absence of HGPRT reduces recycling of the purines, and results in severe overproduction of purines due to reduced feedback inhibition of PRPP production. The genes for both ribose-5-phosphate pyrophosphokinase and HGPRT are on the X-chromosome, so both of these disorders are essentially exclusively found in males.

Overproduction of purines is less severe in gout than in Lesch-Nyhan syndrome. Gout is treated with inhibitors of purine synthesis (such as allopurinol, a hypoxanthine analog, which inhibits uric acid production by inhibiting xanthine oxidase, and possibly inhibits purine synthesis) and with colchicine, a toxic alkaloid produced by some plants that inhibits microtubule formation.

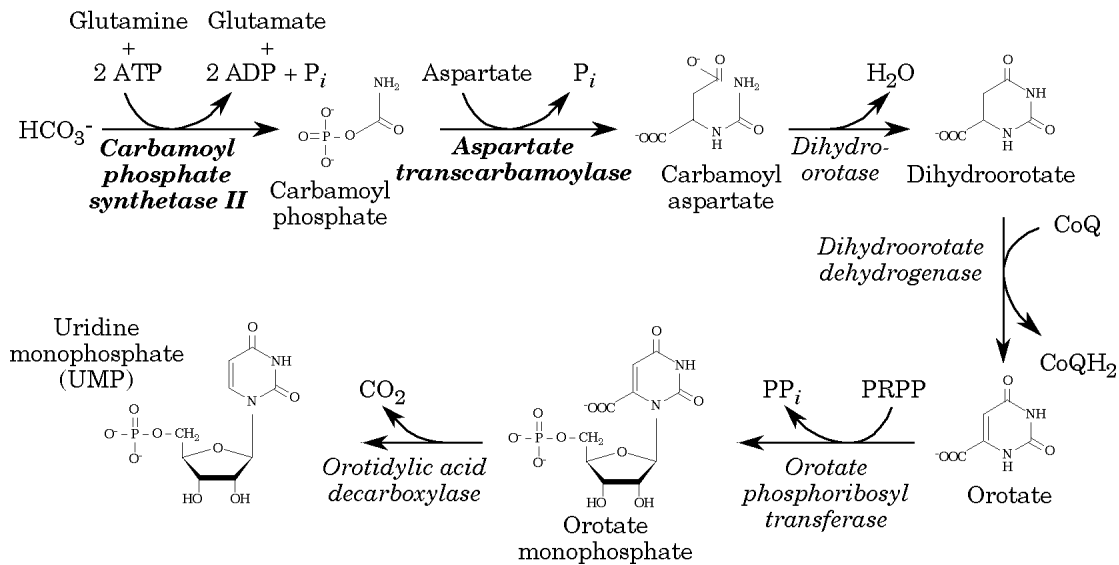


In Lesch-Nyhan syndrome the levels of purines are much higher than in gout; in addition to gout-like symptoms, the high levels of purines in Lesch-Nyhan syndrome result in neurological symptoms (mental retardation and uncontrollable self-mutilation). Allopurinol reverses the gout-like symptoms, but not the neurological effects.

Pyrimidine synthesis

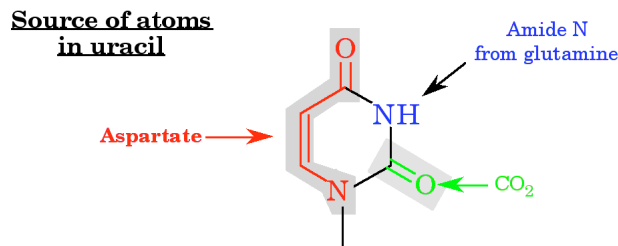
Purines are synthesized by building the ring system on the ribose. In contrast, the pyrimidine ring is constructed first, followed by attachment of the pyrimidine base to ribose using a phosphoribosyltransferase similar to those used for purine salvage reactions. In both purine and pyrimidine synthesis, PRPP is used as the ribose donor, but the stage of the pathway is different.

The first step of the pyrimidine synthesis pathway is the condensation of bicarbonate with a nitrogen derived from glutamine to form carbamoyl phosphate. The enzyme involved, **carbamoyl phosphetase II** is different from the enzyme catalyzing the equivalent step in the urea cycle. Carbamoyl phosphetase II has three **major differences**: 1) it uses nitrogen from glutamine rather than from ammonium, and 2) it is a cytosolic rather than a mitochondrial enzyme, and 3) its regulation is completely different.



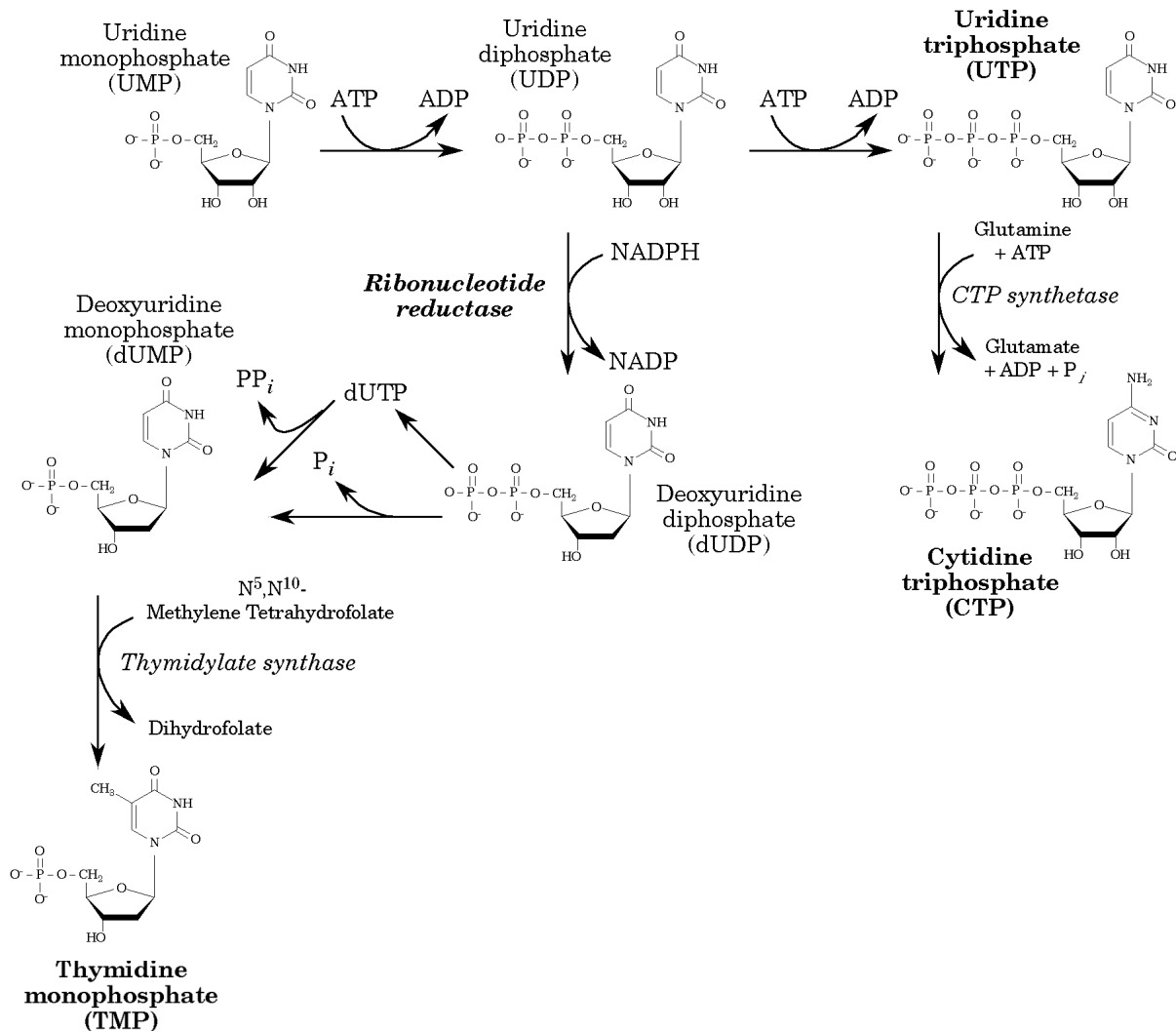
Animals have two separate pools of carbamoyl phosphate: the mitochondrial pool, used for the urea cycle, and the cytosolic pool, used for pyrimidine synthesis. (Bacteria have a single pool used for both purposes, and therefore their pathways are regulated slightly differently.) In bacteria, and to a lesser extent in animals, the second reaction, catalyzed by **aspartate transcarbamoylase** is also regulated.

The **pyrimidine ring skeleton** comes from two molecules, the **carbamoyl phosphate** from the first step, and the **aspartate** added in the second step. (This contrasts with purines, where the nine ring atoms come from seven substrate molecules.)



Dihydroorotate dehydrogenase is a little unusual in that (in animals) it donates electrons directly to coenzyme Q of the electron transport pathway, rather than to NAD (although, in bacteria, NAD is the electron acceptor).

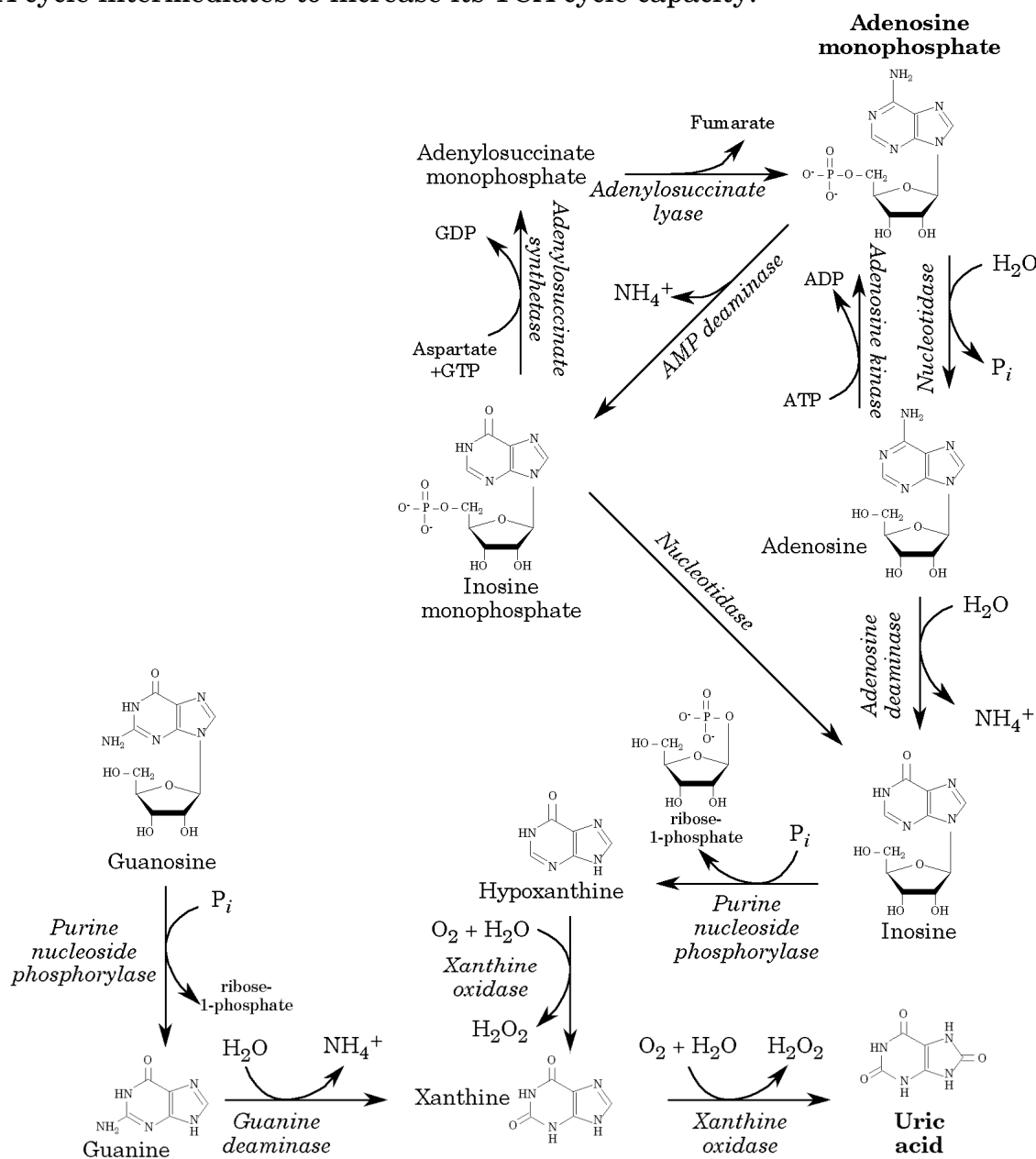
The ribose ring is not added until the synthesis of the pyrimidine orotic acid is complete. This orotic acid is then attached to PRPP with release of pyrophosphate. **UMP** is the first “completed” product. UMP can then be phosphorylated to produce UDP. **UDP acts as a branch point**; it can be converted to UTP and used as a nucleotide, or it can serve as a substrate for the synthesis of the two other major pyrimidine nucleotides.



CTP is produced from UTP by using glutamine as a nitrogen donor. In contrast, **thymidine monophosphate** is produced from UDP. Since thymidine is *only* present in DNA, the first step in the conversion of UDP to TMP is catalyzed by **ribonucleotide reductase**, which removes the 2'-hydroxyl, to create the deoxynucleotide. The dUDP (and any dUTP) formed is rapidly converted to dUMP to prevent the inadvertent incorporation of deoxyuridine into DNA.

Once formed, dUMP is methylated to produce TMP. (Note: strict nomenclature suggests that this is dTMP; however, thymidine ribonucleotides are not produced, so it is not necessary to specify the deoxyribose ring for TMP.) The methylation of dUMP requires N⁵,N¹⁰-methylene tetrahydrofolate (usually generated by conversion of serine to glycine and glycine to CO₂ + ammonium). The reaction releases dihydrofolate, which must be reduced to tetrahydrofolate by **dihydrofolate reductase**. Regeneration of THF is crucial for DNA synthesis; inhibitors of dihydrofolate reductase (such as methotrexate and aminopterin) are potent inhibitors of cell growth.

roles: it allows conversion of adenosine to guanosine if necessary, and allows generation of fumarate. Under low energy conditions, muscle depends on the AMP-IMP cycle as a method for net generation of TCA cycle intermediates. An increase in muscle AMP levels is frequently a signal that energy generation by the TCA cycle is insufficient. The muscle can then use the AMP-IMP cycle to generate additional TCA cycle intermediates to increase its TCA cycle capacity.



The final product of the purine degradation pathway in humans and birds is uric acid. Uric acid is essentially insoluble in water, although it is more soluble at higher pH. Elevated levels of uric acid can result in the uric acid crystal formation in the kidney or in the joints that characterizes gout.

Birds excrete uric acid and free guanine (guano is a mixture of the two) as their major mechanism for nitrogen excretion, because the excretion of these compounds does not require the loss of the significant amounts of water associated with the excretion of urea.

Inhibitors of xanthine oxidase such as allopurinol are drugs used for treatment of gout. These alter the symptoms because hypoxanthine, xanthine, and guanine are more soluble than uric acid.

Other than humans and birds, most animals can breakdown uric acid to other, more soluble, molecules, which are then excreted.