***de novo* synthesis of pyrimidine ribonucleotides**

The common pyrimidine ribonucleotides are cytidine 5'- monophosphate (CMP; cytidylate) and uridine 5'- monophosphate (UMP; uridylate), which contain the pyrimidines cytosine and uracil. *De novo* pyrimidine nucleotide biosynthesis proceeds in a somewhat different manner from purine nucleotide synthesis; the six-membered pyrimidine ring is made first and then attached to ribose 5-phosphate. The pyrimidine ring is assembled from bicarbonate, aspartate and glutamine. Carbamoyl phosphate required in this process is also an intermediate in the urea cycle.

1. In animals, the carbamoyl phosphate required in urea synthesis is made in mitochondria by carbamoyl phosphate synthetase I, whereas the carbamoyl phosphate required in pyrimidine biosynthesis is made in the cytosol by a different form of the enzyme, carbamoyl phosphate synthetase II. In bacteria, a single enzyme supplies carbamoyl phosphate for the synthesis of arginine and pyrimidines. The bacterial enzyme has three separate active sites, spaced along a channel nearly 100 Å long. Bacterial carbamoyl phosphate synthetase provides a vivid illustration of the channeling of unstable reaction intermediates between active sites.
2. Carbamoyl phosphate reacts with aspartate to yield N-carbamoylaspartate in the first committed step of pyrimidine biosynthesis. This reaction is catalyzed by aspartate transcarbamoylase. In bacteria, this step is highly regulated, and bacterial aspartate transcarbamoylase is one of the most thoroughly studied allosteric enzymes.
3. By removal of water from N-carbamoylaspartate, a reaction catalyzed by dihydroorotase, the pyrimidine ring is closed to form L-dihydroorotate.
4. This compound is oxidized to the pyrimidine derivative orotate, a reaction in which NAD^+ is the ultimate electron acceptor. In eukaryotes, the first three enzymes in this pathway—carbamoyl phosphate synthetase II, aspartate transcarbamoylase, and dihydroorotase—are part of a single trifunctional protein. The protein, known by the acronym CAD, contains three identical polypeptide chains (each of M_r 230,000), each with active sites for all three reactions. This suggests that large, multienzyme complexes may be the rule in this pathway.
5. Once orotate is formed, the ribose 5-phosphate side chain, provided once again by PRPP, is attached to yield orotidylate.
6. Orotidylate is then decarboxylated to uridylate, which is phosphorylated to UTP.
7. CTP is formed from UTP by the action of cytidylate synthetase, by way of an acyl phosphate intermediate (consuming one ATP). The nitrogen donor is normally glutamine, although the cytidylate synthetases in many species can use NH^{4+} directly.

Regulation

Pyrimidine Nucleotide Biosynthesis Is Regulated by Feedback Inhibition. Regulation of the rate of pyrimidine nucleotide synthesis in bacteria occurs in large part through aspartate transcarbamoylase (ATCase), which catalyzes the first reaction in the sequence and is inhibited by CTP, the end product of the sequence. The bacterial ATCase molecule consists of six catalytic subunits and six regulatory subunits. The catalytic subunits bind the substrate molecules, and the allosteric subunits bind the allosteric inhibitor, CTP. The entire ATCase molecule, as well as its subunits, exists in two conformations, active and inactive. When CTP is not bound to the regulatory subunits, the enzyme is maximally active. As CTP accumulates and binds to the regulatory subunits, they undergo a change in conformation. This change is transmitted to the catalytic subunits, which then also shift to an inactive conformation. ATP prevents the changes induced by CTP.

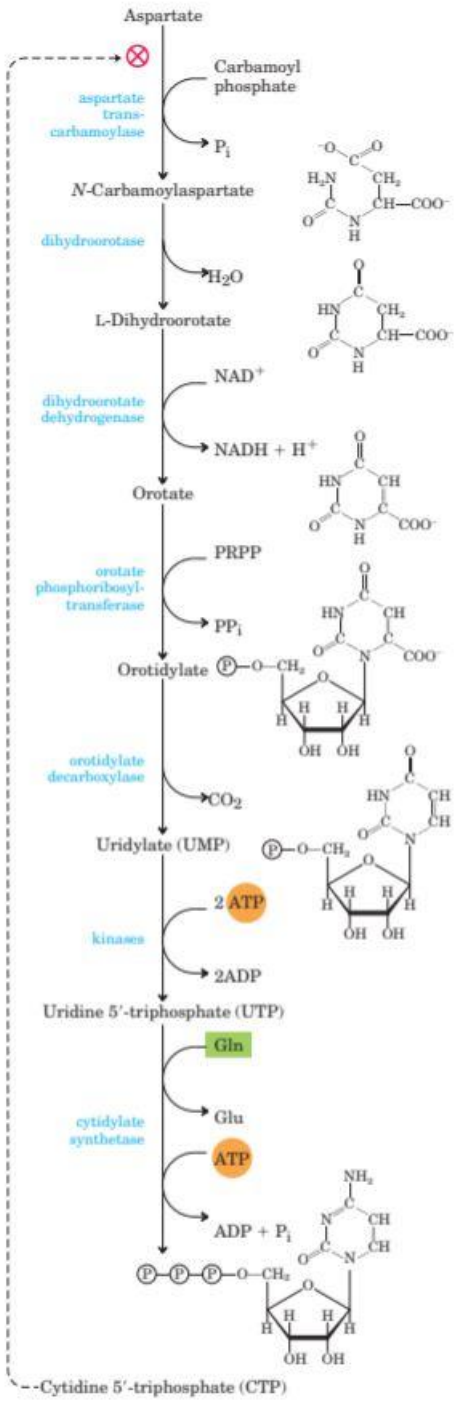

Fig. De novo synthesis of pyrimidine nucleotides: biosynthesis of UTP and CTP via orotidylate. The pyrimidine is constructed from carbamoyl phosphate and aspartate. The ribose 5-phosphate is then added to the completed pyrimidine ring by orotate phosphoribosyltransferase. The first step in this pathway is the synthesis of carbamoyl phosphate from CO2 and NH^{4+}, catalyzed in eukaryotes by carbamoyl phosphate synthetase II

Nucleoside Monophosphates Are Converted to Nucleoside Triphosphates

Nucleotides to be used in biosynthesis are generally converted to nucleoside triphosphates. The conversion pathways are common to all cells. Phosphorylation of AMP to ADP is promoted by adenylate kinase, in the reaction

$$ATP + AMP \rightleftharpoons 2ADP$$

The ADP so formed is phosphorylated to ATP by the glycolytic enzymes or through oxidative phosphorylation. ATP also brings about the formation of other nucleoside diphosphates by the action of a class of enzymes called nucleoside monophosphate kinases. These enzymes, which are generally specific for a particular base but nonspecific for the sugar (ribose or deoxyribose), catalyze the reaction

$$ATP + NMP \rightleftharpoons ADP + NDP$$

The efficient cellular systems for rephosphorylating ADP to ATP tend to pull this reaction in the direction of products. Nucleoside diphosphates are converted to triphosphates by the action of a ubiquitous enzyme, nucleoside diphosphate kinase, which catalyzes the reaction

$$NTP_D + NDP_A \rightleftharpoons NDP_D + NTP_A$$

This enzyme is notable in that it is not specific for the base (purines or pyrimidines) or the sugar (ribose or deoxyribose). This non specificity applies to both phosphate acceptor (A) and donor (D), although the donor (NTP_D) is almost invariably ATP, because it is present in higher concentration than other nucleoside triphosphates under aerobic conditions.

Thymidylate Is Derived from dCDP and dUMP

DNA contains thymine rather than uracil, and the *de novo* pathway to thymine involves only deoxyribonucleotides. The immediate precursor of thymidylate (dTMP) is dUMP. In bacteria, the pathway to dUMP begins with formation of dUTP, either by deamination of dCTP or by phosphorylation of dUDP. The dUTP is converted to dUMP by a dUTPase. The latter reaction must be efficient to keep dUTP pools low and prevent incorporation of uridylate into DNA.

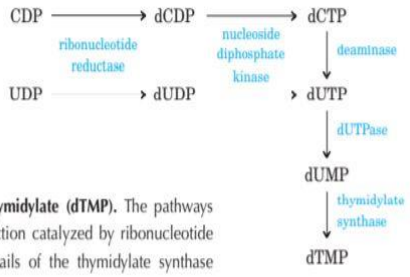

FIGURE 22–43 **Biosynthesis of thymidylate (dTMP).** The pathways are shown beginning with the reaction catalyzed by ribonucleotide reductase. Figure 22–44 gives details of the thymidylate synthase reaction.

Conversion of dUMP to dTMP is catalyzed by thymidylate synthase. A one-carbon unit at the hydroxymethyl ($-CH_2OH$) oxidation level is transferred from N^5, N^{10}-methylenetetrahydrofolate to dUMP, then reduced to a methyl group. The reduction occurs at the expense of oxidation of tetrahydrofolate to dihydrofolate, which is unusual in tetrahydrofolate-requiring reactions. The dihydrofolate is reduced to tetrahydrofolate by

dihydrofolate reductase—a regeneration that is essential for the many processes that require tetrahydrofolate. In plants and at least one protist, thymidylate synthase and dihydrofolate reductase reside on a single bifunctional protein.

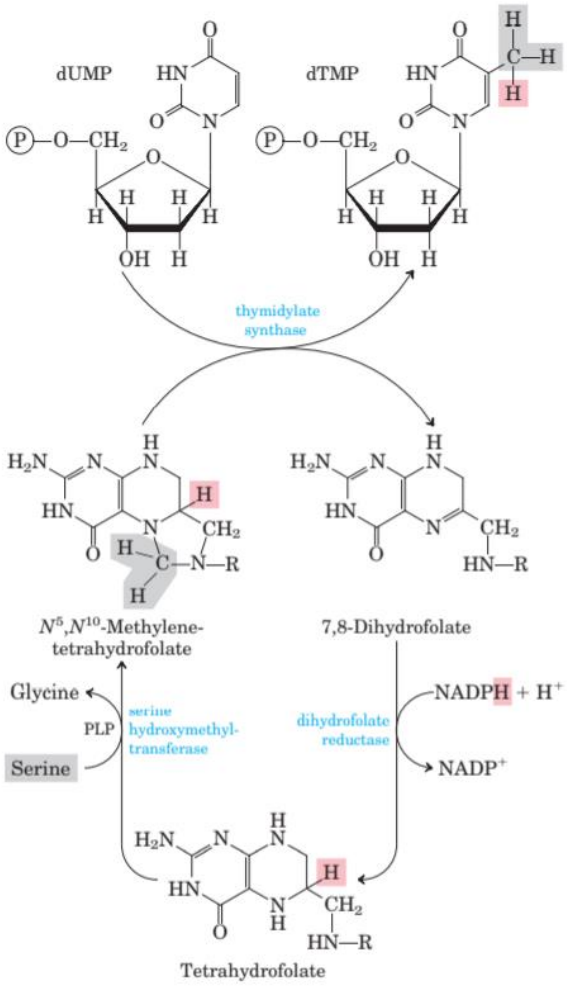

FIGURE 22–44 Conversion of dUMP to dTMP by thymidylate synthase and dihydrofolate reductase. Serine hydroxymethyltransferase is required for regeneration of the N^5,N^{10}-methylene form of tetrahydrofolate. In the synthesis of dTMP, all three hydrogens of the added methyl group are derived from N^5,N^{10}-methylenetetrahydrofolate (pink and gray).