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Human pyrimidine nucleotide biosynthesis as a target for antiviral chemotherapy

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The development of broad-spectrum, host-acting antiviral therapies remains an important but elusive goal in anti-infective drug discovery. To replicate efficiently, viruses not only depend on their hosts for an adequate supply of pyrimidine nucleotides, but also up-regulate pyrimidine nucleotide biosynthesis in infected cells. In this review, we outline our understanding of mammalian *de novo* and salvage metabolic pathways for pyrimidine nucleotide biosynthesis. The available spectrum of experimental and FDA-approved drugs that modulate individual steps in these metabolic pathways is also summarized. The logic of a host-acting combination antiviral therapy comprised of inhibitors of dihydroorotate dehydrogenase and uridine/cytidine kinase is discussed.

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Introduction

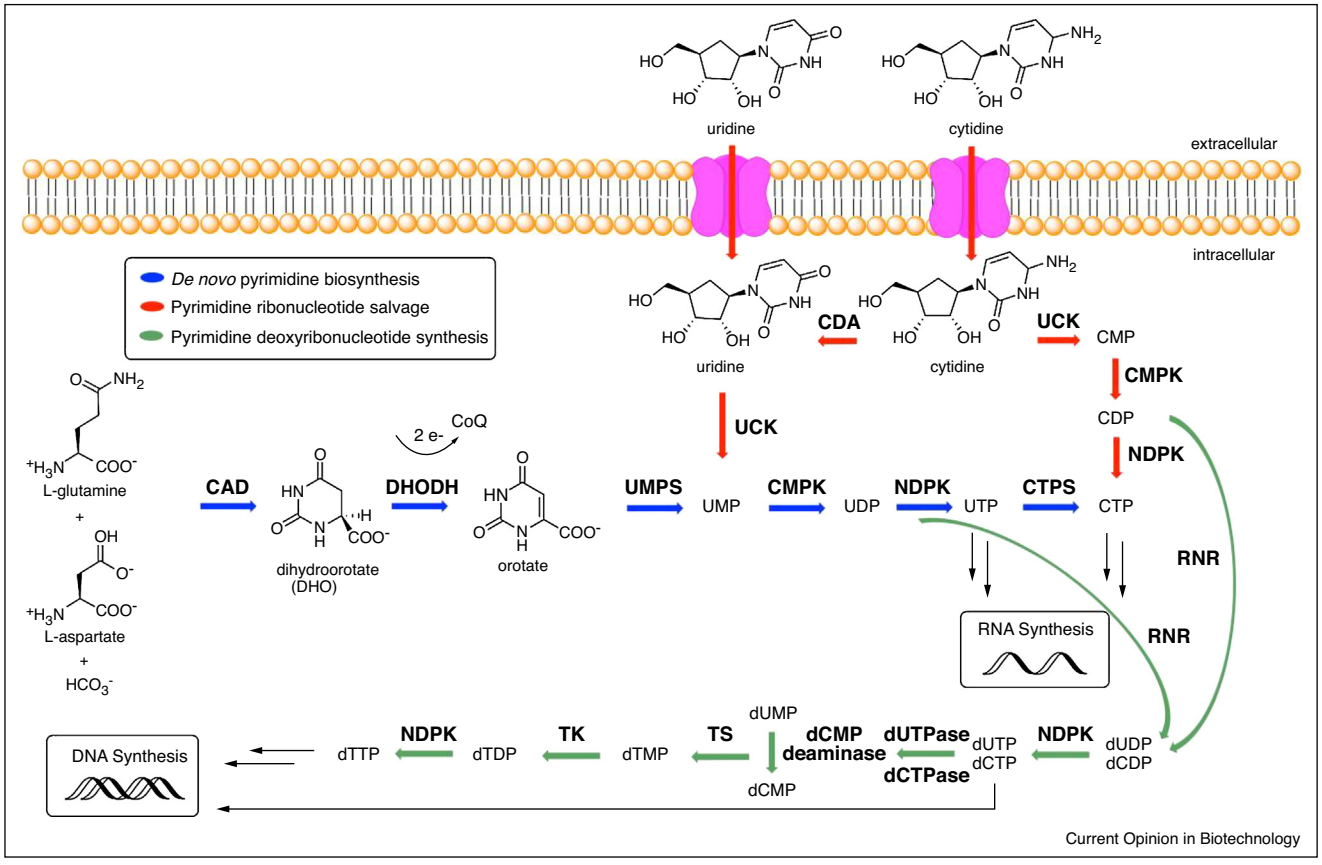
Pyrimidine nucleosides are heterocyclic aromatic metabolites that include uridine, cytidine and thymidine. In addition to their fundamental role in nucleic acid biosynthesis, they are required for carbohydrate and lipid metabolism. For example, a number of glycosyltransferases utilize UDP-sugars, while CDP-diacylglycerol is an intermediate in the biosynthesis of glycerophospholipids. Although pyrimidine analogs such as azidothymidine (AZT), 5-fluorouracil (5-FU), and arabinosylcytosine (ara-C) have been used to target HIV reverse transcriptase or as anti-cancer chemotherapeutic drugs for decades, the potential for rationally targeting human pyrimidine nucleoside metabolism for antiviral chemotherapy has not been generally recognized. Here we review the rationale for such a chemotherapeutic strategy as well as the relevant features of mammalian pyrimidine nucleoside metabolism and its regulation.

Pyrimidine nucleotide biosynthesis through *de novo* and salvage pathways

Mammalian cells derive pyrimidine nucleotides through a combination of *de novo* biosynthesis and salvage [1]. *De novo* biosynthesis is initiated by a multifunctional enzyme (CAD) harboring carbamoyl phosphate synthase, aspartate transcarbamoylase, and dihydroorotase activities [2]. CAD uses an equivalent of L-glutamine, aspartate, and bicarbonate along with two equivalents of ATP to make dihydroorotate (DHO) (Figure 1). A mitochondrial membrane protein, dihydroorotate dehydrogenase (DHODH), then reduces DHO to orotic acid while transferring $2e^-$ to Coenzyme Q (CoQ, ubiquinone) [3•]. Not only does DHODH catalyze the first committed step in *de novo* pyrimidine nucleoside biosynthesis, but it also links this pathway to the electron transport chain of aerobic respiration. Orotic acid is converted into uridine monophosphate (UMP) by a bifunctional protein, uridine monophosphate synthetase (UMPS). The N-terminal domain of UMPS transforms orotic acid into orotidylate (OMP) using phospho-α-D-ribosyl-1-pyrophosphate (PRPP) as a cosubstrate, while its C-terminal OMP decarboxylase converts OMP into UMP [4]. UDP and UTP are synthesized by cytidine monophosphate kinase (CMPK) and nucleoside-diphosphate kinase (NDPK), respectively [5,6]. UTP is converted into CTP by CTP synthetase (CTPS) in an ATP-dependent reaction that uses glutamine as an amine donor [7•]. Alternatively, UDP and CDP are deoxygenated into deoxy-UDP (dUDP) and dCDP, respectively, by ribonucleotide reductase (RNR), and further phosphorylated by NDPK [8]. To avoid misincorporation into DNA, dUTP is rapidly broken down by dUTPase into dUMP. dUMP is a substrate of thymidylate synthase, yielding deoxy-TMP (dTMP) that can be phosphorylated into dTTP [9]. Thus, the *de novo* biosynthetic pathway in mammals is capable of supplying all pyrimidine ribonucleotides (CTP, UTP) and deoxyribonucleotides (dCTP, dTTP) for RNA and DNA biosynthesis, respectively.

In addition to *de novo* biosynthesis, pyrimidine nucleotides can also be salvaged from intracellular nucleic acid degradation or from extracellular nucleosides, which circulate in the bloodstream. The latter pathway depends on several nucleoside transport channels and pumps in mammalian cells. The relative importance of *de novo*

Figure 1



De novo and salvage biosynthesis of pyrimidine nucleotides in humans. For details, see text.

biosynthesis and salvage varies from organ to organ and is also highly dependent on the physiological state of cells. RNA catabolism yields UMP and CMP, which can be converted into the corresponding NTPs via the successive action of CMPK1 and NDPK. With a plasma concentration of ~5 μM, uridine is the dominant circulatory nucleoside in mammals [10]; the plasma concentrations of all other pyrimidine nucleosides are at least an order of magnitude lower [11], and are therefore insufficient to support cellular demands of the corresponding nucleotides via direct salvage. Uridine/cytidine kinase (UCK) converts transported pyrimidine nucleosides into the corresponding NMPs, which can be further phosphorylated and modified as discussed above. Since both *de novo* biosynthesis as well as intracellular and extracellular salvage require CMPK1 activity, this enzyme is essential for pyrimidine utilization in all cells.

As an alternative to salvage, pyrimidine nucleosides can also be irreversibly degraded. Uridine and cytidine catabolism is initiated by the action of uridine phosphorylase (UPase) and cytidine deaminase, respectively, giving rise to uracil, while thymidine phosphorylase releases thymine from thymidine. In principle, these phosphorylases can also catalyze the reverse reactions to convert circulatory bases into nucleosides (as in OMP biosynthesis), although mammals appear to predominantly utilize these enzymes in the catabolic direction [12].

Intracellular regulation of pyrimidine nucleotide biosynthesis

The multifunctional CAD protein is the primary site for regulation of *de novo* pyrimidine biosynthesis. Transcription factors such as Myc are known to induce its gene expression [13]. The enzyme is activated by MAP kinase-catalyzed phosphorylation before the S-phase of the cell cycle, and is inhibited by protein kinase A-catalyzed phosphorylation at a distinct site at the end of S-phase [14,15]. CAD is also activated by phosphorylation at a third site by the mammalian target of rapamycin complex 1 (mTORC1) or the ribosomal protein S6 kinase 1 (p70S6K), thus enabling post-translational control in response to increased anabolic activity in the cell [16,17•].

The importance of coordinately regulating intracellular pyrimidine nucleotide biosynthesis at multiple sites is underscored by our recent observation that genetic knockout of a negative regulator of mTORC1 activity