URA7 and URA8 encode CTP synthetase, the enzyme that catalyzes the ATP-dependent conversion of UTP to CTP. This final step in the pathway of CTP biosynthesis, shown here, is important for balancing nucleotide pools and also for synthesizing membrane phospholipids. The two CTP synthetases, Ura7p and Ura8p, share 78% amino acid identity and are functionally overlapping. Null mutations in either gene decrease intracellular levels of CTP, leading to a reduced growth rate, while deletion of both gene products results in lethality. While both URA7 and URA8 are maximally expressed during exponential growth, overall expression of URA7 is two-fold higher than that of URA8. Ura7p is also responsible for the majority of CTP synthesisand the difference in activity between the two isoforms is due to differential regulation in addition to their differential expression.Both CTP synthetases are allosterically regulated by their substrates, ATP and UTP, and their product, CTP. The presence of UTP and ATP cause the inactive homodimer form of the CTP synthetase to oligomerize to an active tetrameric form. The Ura7p CTP synthetase exhibits positive cooperative kinetics with respect to UTP and ATP, but the Ura8p isoform requires the additional presence of CTP for positive substrate cooperativity. CTP is a negative regulator of CTP synthetase, inhibiting enzyme activity by increasing the positive cooperativity of the protein for UTP; Ura8p is more sensitive than Ura7p to CTP inhibition.The URA7-encoded CTP synthetase is also regulated by phosphorylation. Phosphorylation of Ura7p by protein kinases Aand Cincreases CTP synthetase activity by increasing catalytic turnover, facilitating enzyme tetramerization and decreasing sensitivity to CTP inhibition. Although Ura7p activity can be enhanced by phosphorylation by either protein kinase A or C, maximal CTP synthetase activity requires modification by both kinases.Mammalian cell lines mutant in CTP synthetase have abnormally high intracellular levels of CTP and dCTP and an increased rate of spontaneous mutation. Like S. cerevisiae, humans also have two CTP synthetases, CTPS1and CTPS2, and increased enzyme activity has been found in several human tumor types.