An Unusual Mechanism for the Antimicrobial Target Flavine-dependant Thymidylate Synthase (FTDS)

Classical thymidylate synthases, encoded by the *thy*A and *TYMS* genes, are present in most eukaryotes, including humans, and are frequently targeted by chemotherapeutic and antibiotic drugs. A recently discovered class of thymidylate synthases, the FDTSs encoded by the *thy*X gene has been found primarily in prokaryotes and viruses including several pathogens and biological warfare agents (see http://www.cdc.gov). Several organisms, including human pathogens, rely solely on *thy*X for thymidylate synthesis. FDTSs share no structure or sequence homology with classical thymidylate synthases (Fig 1), and thus present a promising new frontier for antibacterial/antiviral drug development.

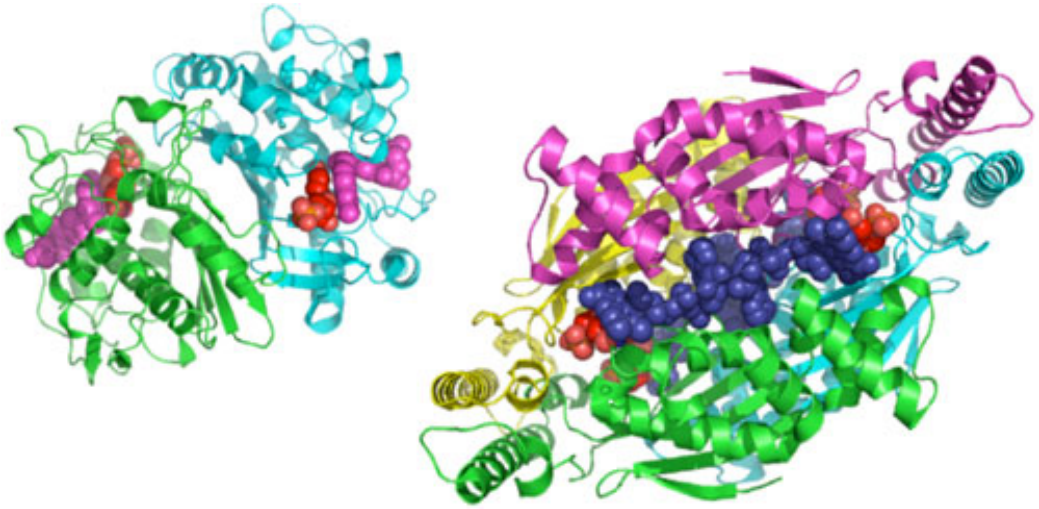
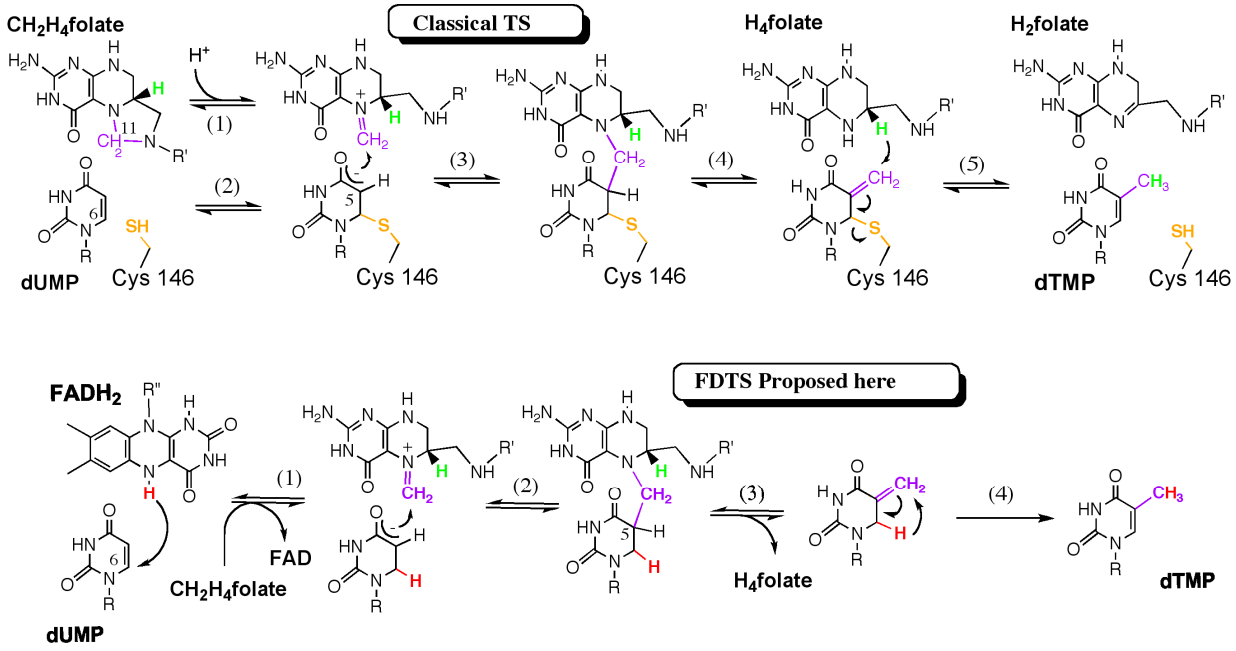


Figure 1. Structural comparison of classical TS and FDTS: Left: Ribbon diagram of an *E. coli* TS (*ec*TS) dimer (PDB entry 2KCE). Both ligands are highlighted as space filling shapes. The substrate dUMP is red and the cofactor analogue (Zd1694, Ralitrexed), is magenta. **Right:** Ribbon diagram of a *tm*FDTS tetramer (PDB entry 1O26). FAD (blue) and dUMP (red) are highlighted as space filling shapes. It is clear that the active site is exposed to solvent and that all four adenine rings interact at the center of the complex.

In an article published in *Nature*, research team lead by Prof. Kohen has unraveled an unusual mechanism for the FTDS catalysis. A significant component of the study involved structural data collected at SSRL Beam Line 9-2 by Dr. Mathews following his successful crystallization of the wild type and two critical mutants of FDTS. Other experimental components include kinetic and isotopic analysis of the enzyme and mutants. The novel mechanism is an example of thymidylate biosynthesis that occurs without an enzymatic nucleophile (Scheme 1). The findings indicate that the putative active site nucleophile is not required for FDTS catalysis, and no alternative nucleophilic residues capable of serving this function can be identified. This study suggests that a hydride is transferred from the reduced flavin cofactor directly to the uracil ring, followed by an isomerization of the intermediate to form the product thymidylate as illustrated in Scheme 1). The observations indicate a very different chemical cascade than that of classical thymidylate synthases or any other known biological methylation. The findings and chemical mechanism proposed here, together with available structural data, suggest that selective inhibition of FDTSs, with little effect on human thymine biosynthesis, should be feasible. Because several human

pathogens depend on FDTS for DNA biosynthesis, its unique mechanism makes it an attractive target for antibiotic drugs.



Scheme 1: Top, the chemical mechanism of classical TS. **Bottom**, the newly proposed mechanism for FDTS.

Primary Citation

Eric M. Koehn, Todd Fleischmann, John A. Conrad, Bruce A. Palfey, Scott A. Lesley, Irimpan I. Mathews, and Amnon Kohen (2009) "A Novel Chemical Mechanism of Thymidylate Biosynthesis in Human Pathogens Containing the *thy*X Gene" Nature 458, 919-923 (2009).

Related Press Release

UI Chemists' DNA Biosynthesis Discovery could Lead to Better Antibiotics, University of Iowa News Release, April 15, 2009
http://news-releases.uiowa.edu/2009/april/041509biosynthesis_discovery.html

Biochemistry: Anchors Away, News and Views, Nature 458, 840-841 (16 April 2009) doi:10.1038/458840a

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SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program, and the National Institute of General Medical Sciences.