

W0422

Structural Analysis of Escherichia Coli ThiF. D.M. Duda, H. Walden, J. Sfondouris and B. A. Schulman, Dept. of Structural Biology and Dept. of Genetics/Tumor Cell Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105 USA

E. coli ThiF is an enzyme in the biosynthetic cascade for generating the essential cofactor, thiamin pyrophosphate. In this cascade, ThiF catalyses adenylation of the Cterminus of ThiS. We report here the crystal structures of ThiF, alone and in complex with ATP. The structures provide insight into a preference for ATP during ThiS adenylation. Additionally, the structures reveal an ordered crossover loop predicted to clamp the flexible tail of ThiS into the ThiF active site during the adenylation reaction. The importance of the crossover loop for ThiF activity is highlighted by mutational analysis. Comparison of ThiF with the structural homologues MoeB, APPBP1-UBA3, and SAE1-SAE2 reveals that the ATP-binding site, including an arginine-finger, is maintained throughout evolution, and shows divergence occurring in protein substrate-binding sites and regions devoted to unique steps in each enzyme's specific function.

We thank APS (SER-CAT), NSLS (X25 and X12C) and ALS (8.3.1) for beamline access. This research is supported by ALSAC, the NIH (P30CA21765, R01GM69530), the Philip and Elizabeth Gross Foundation, the Beckman Foundation, the Pew Scholar's Program, and a postdoctoral fellowship from the American Cancer.