

FA1-MS13-O3**How a Y-family Polymerase can Produce Mutations from a Benzo[a]pyrene DNA Lesion.**

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Erroneous replication of lesions in DNA by DNA polymerases leads to elevated mutagenesis. To understand the molecular basis of DNA damage-induced mutagenesis, we determined X-ray crystal structures of the Y-family polymerase Dpo4 in complex with a DNA substrate containing a bulky DNA lesion and incoming nucleotide triphosphates. Like all Y-family polymerases, Dpo4 possesses the three classic polymerase domains of thumb, finger, and palm, as well as an additional little-finger domain, which is unique to the Y-family. The DNA lesion is derived from an environmentally widespread carcinogenic polycyclic aromatic hydrocarbon, benzo[a]pyrene (B[a]P). In the body, this potent carcinogen is metabolized to diol epoxides which then form covalent adducts with cellular DNA by binding to purine bases. In this study, the major DNA B[a]P diol epoxide adduct in mammalian cells, trans 10S B[a]P-N2-deoxyguanosine (B[a]P-dG), was placed at a template-primer junction opposite dA, the nucleotide most commonly inserted opposite this lesion by Dpo4. Three polymerase-DNA-nucleotide triphosphate ternary complexes reveal replication blockage, extension past a mismatched lesion, and a -1 frameshift mutation. In the productive structures, the bulky adduct is either flipped or looped out of the DNA helix into a structural gap between the little finger and the core polymerase domains. The sequestering of the hydrophobic B[a]P adduct in this new substrate-binding site permits the DNA to exhibit normal geometry for primer extension. Extrusion of the lesion by template misalignment allows the base 5' to the adduct to serve as the template, resulting in a -1 frameshift mutation. Subsequent strand realignment produces a mismatched base opposite the lesion. These structural observations, in combination with replication and mutagenesis data, suggest a model in which the additional substrate-binding site stabilizes the extrahelical nucleotide for lesion bypass and generation of base substitutions and -1 frameshift mutations. This research was previously reported in [1] and was supported by the Terry Fox Foundation and by Intramural Research Program of the NIH. Participation in ECM25 was supported by grant APVV-0024-07 from the Slovak Research and Development agency.

[1] Bauer J., Xing G., Yagi H., Sayer J.M., Jerina D.M., Ling H. *Proc. Natl. Acad. Sci. USA.* **2007**, 104, 14905.

Keywords: translesion synthesis; mutagenesis; DNA polymerases; replication proteins

FA1-MS13-O4**Structural Insights into the Catabolism of Fatty Acids in Plants.** Valerie E. Pye^a, Susan Arent^a, Caspar E. Christiansen^a, Anette Henriksen^a. ^a*Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.*

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Fatty acids are essential biomolecules for all life forms which are degraded, two carbons at a time, by the beta-oxidation pathway. In higher plants beta-oxidation occurs in the peroxisomes and is essential for seed germination and hormone biosynthesis. We are in the process of thoroughly characterizing the enzymes involved in the beta-oxidation pathway of the oil seed plant *Arabidopsis thaliana*, using a variety of biochemical and structural biology approaches, with the aim of understanding the mechanisms by which metabolites are channeled. The crystal structures of 3-ketoacyl-CoA thiolase (KAT2) from *Arabidopsis thaliana* and *Helianthus annuus* have been solved to 1.57Å and 1.8Å respectively. The dimeric structures are very similar and exhibit a typical thiolase fold; dimer formation and active site conformation indicate that these structures are in an open, active, reduced state. These structures confirm the potential of plant KATs to be regulated by redox when compared with the previously solved KAT2 from *Arabidopsis thaliana*, which was in a closed, inactive, oxidised state [1]. The crystal structure of multifunctional enzyme (MFP2) has been solved to 2.4Å revealing the tandem hydratase and dehydrogenase domains. We propose a model for an interaction between MFP2 and KAT2 based on the structure of the bacterial tri-functional multienzyme complex [2].

[1] Sundaramoorthy, R., Micossi, E., Alpey, M. S., Germain, V., Bryce, J. H., Smith, S. M., Leonard, G. A. & Hunter, W. N. **2006.** *J Mol Biol* 359, 347-357. [2] Ishikawa, M., Tsuchiya, D., Oyama, T., Tsunaka, Y. & Morikawa, K. **2004.** *Embo J* 23, 2745-2754.

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FA1-MS13-O5

Structural Insight into the Quinolone-DNA Cleavage Complex of Topoisomerase IV from *Streptococcus Pneumoniae*. Mark R. Sanderson^{a*}, Ivan Laponogov^{a,b}, Maninder K. Sohi^a, Dennis A. Veselkov^a, Xiao-Su Pan^b, Ritica Sawhney^b, Andrew W. Thompson^c, Katherine McAuley^d, L. Mark Fisher^{b*}. ^a*Randall division of Cell and Molecular Biophysics, King's College London, 3rd Floor New Hunt's House, Guy's Campus, University of London.* ^b*Molecular Genetics Group, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St. George's, University of London, UK.* ^c*Synchrotron SOLEIL, L'Orme de Merisiers BP, 48 St. Aubin 91191, Gif sur Yvette, France.* ^d*Diamond Light Source, Didcot, Oxford.* ^{*}*joint corresponding authors.*

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Eighteen percent of all clinical bacterial infections are now treated with quinolone based antibiotics (1), which target the decatenating enzyme topoisomerase IV in gram-positive bacteria such as *S. pneumoniae*. This enzyme is a class II DNA topoisomerase which cuts both DNA strands and changes the linking number in steps of ± 2 . By contrast in gram-negative bacteria such as *E. coli*, the site of action is the topoisomerase Gyrase which controls the overall superhelical density within the bacterial cell.

Topoisomerase IV in its entirety consists of both ParE and ParC domains. It is with a view to addressing the question of how quinolone acts at a molecular level in its interaction with the DNA complex that we have crystallised the complex of ParC55 (55 kDa.), ParE (30kDa.) with a 32 base-pair DNA binding site fragment and quinolone drug. Crystals were grown in space group $P3_2$ with cell dimensions $a=b=118.30$ Å, $c=177.90$ Å $\alpha=90^\circ, \beta=90^\circ, \gamma=120^\circ$ both by conventional hanging drop vapour diffusion in 24-well limbro plates and by sitting drop in 96 well MRC crystallisation plates. The structure has been solved by molecular replacement (CNS) using as search models our ParC55 structure (2) and a ParE domain homology modelled on the basis of the structure of the TOPRIM domain of the yeast type IIA (3). The DNA has been positioned from difference Fourier maps following refinement using CNS. The veracity of the model was confirmed by the ability of this phase set to determine the correct Pt sites for a K_2PtCl_4 heavy atom derivative. The structure gives the stoichiometry and position of quinolone binding in complex and resolves an issue which has been a contested for many years. The structures of two drug-DNA-topoIV complexes with different fluoroquinolones have been solved and fully refined.

[1] Quinolone-mediated bacterial death. Drilca,K., Malik,M., Kerns,R.J. and Zhaol X.L., **2008**, Antimicrobial agents and Chemotherapy 52 385-392. [2] Breakage-Reunion domain of Streptococcus Pneumoniae Topoisomerase IV: Crystal structure of a Gram-Positive quinolone target. Laponogov, I., Veselkov, D., Sohi, M.K., Pan, X.-S., Achari, A., Laponogov, I., Fisher, L.M. and Sanderson, M.R. PLoS ONE, **2007**, e301. [3] Structural basis for gate-DNA recognition and bending by type IIA topoisomerases Dong, K.C. and Berger, J.M., **2008**, Nature 450 1201-1206.