

Department of Chemistry and Molecular Biology
Biochemistry Candidate Seminar
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Structure-Function Studies of DNA Polymerases Responsible for Base Excision Repair

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ABSTRACT

Initially, I will focus on the studies of small-angle X-ray scattering (SAXS) and sedimentation velocity (SV) on enzyme·DNA complexes of mammalian DNA polymerase β (Pol β) and African swine fever virus DNA polymerase X (Pol X). Our results indicated formation of a 2:1 Pol β ·DNA complex, whereas only 1:1 Pol X·DNA complex was observed. The structural models for the 2:1 Pol β ·DNA and 1:1 Pol X·DNA complexes were generated from the SAXS data to correlate with the functions of the DNA polymerases. The former shows interactions of the 8 kDa 5'-dRP lyase domain of the second Pol β molecule with the active site of the 1:1 Pol β ·DNA complex, while the latter demonstrates how ASFV Pol X binds DNA in the absence of DNA binding motif(s).

Next, I will cover the studies for the fidelity of DNA polymerases. Understanding how DNA polymerases control fidelity requires elucidation of the mechanisms of matched and mismatched dNTP incorporations. Little is known about the latter because mismatched complexes do not crystallize readily. Here, we employed SAXS, crystallography, structural modeling, NMR, stopped-flow fluorescence, and rapid chemical quenched studies to probe the conformations of different intermediate states of Pol β in its wild-type and an error-prone variant, I260Q. Our structural studies indicate that the mismatched ternary complex lies in-between the open and the closed forms, but more closely resembles the open form for WT Pol β and the closed form for I260Q, and our functional studies show that dNTP-induced conformational change is fast by WT Pol β and I260Q. Together, these results suggest that mismatched dNTP incorporation does not follow a conformational distinct pathway from that of matched dNTP incorporations, and that the dNTP-induced conformational closing is not a major contributor to fidelity.

Reference:

1. **Tang, K.H.**, Niebuhr, M., Tung, C.S., Chen, S.C., Chou, C.C., and Tsai, M.D. Mismatched dNTP incorporation by DNA polymerase β does not proceed via globally different conformational pathways. *Nucleic Acids Res.* in press (doi:10.1093/nar/gkn138).
2. **Tang, K.H.** and Tsai, M. D. Structure and Function of 2:1 DNA polymerase·DNA complexes. *J. Cell Physiol.* in press (doi:10.1002/jcp.21458).
3. **Tang, K.H.**, Niebuhr, M., Aulabaugh, A., and Tsai, M.D. Solution structures of 2:1 and 1:1 DNA polymerase-DNA complexes probed by ultracentrifugation and small-angle x-ray scattering. *Nucleic Acids Res.* **36**, 849-860 (2008).
4. Showalter, A.K., Lamarche, B.J., Bakhtina, M., Su, M.I., **Tang, K.H.**, and Tsai, M.D. Mechanistic comparison of high-fidelity and error-prone DNA polymerases and ligases involved in DNA repair. *Chem. Rev.* **106**, 340-360 (2006).