Structural and functional study of primosomal proteins PriA and DnaD involved in DNA replication restart

Huai-Yuan Wang[†], Yi-Ching Li[†], Min Guan Lin[†], and Chwan-Deng Hsiao[†*]

[†]Institute of Molecular Biology, Academia Sinica

During the DNA replication elongation, this process is always encountering some problems that might eject the replication complex and form an abandoned replication fork DNA such as DNA damage. In *bacillus subtilis,* they evolve a pathway call "DNA replication restart" to resume DNA replication. DNA replication restart is done with several proteins PriA, DnaD and DnaB. First, PriA can recognize this abandoned replication fork DNA and recruit others primosomal proteins DnaD and DnaB to assemble a primosome. Once primosome is assembled, it loads a replisome onto DNA and restarts DNA replication. However, the molecular machinery of these primosomal proteins in DNA replication restart is still unclear. To understand the biochemical property and primosome assembly activity of these proteins, we expressed *priA* and *dnaD* genes which are isolated from *Geobacillus stearothermophilus* and *Streptococcus pneumoniae* and purified these proteins. We used fluorescence polarization assay to identify the protein-protein and protein-DNA interaction. The results showed that PriA carried a high affinity to DnaD and both proteins have DNA binding ability. To explain a mechanistic model of replication restart and elucidate their regulatory roles, we are trying to study the relationship between structure and function by X-ray crystallography and solved the crystal structure of DnaD at 2.5 Å resolutions by multi-wavelength anomalous diffraction. Unfortunately, we did not observe a continuous density of DnaD C-terminal region because of its flexibility. All in all, these preliminary results of initial protein structure and biochemical property give us a positive feedback for further structure determination and discussion of DNA replication restart.