Joint CQSE and CASTS Seminar

Weekly Seminar
Oct. 24, 2014 (Friday)

TIME Oct. 24, 14:30 ~ 15:30
TITLE Single Molecule Observation of Direct Transfer of Escherichia coli Single-Strand Binding Protein (SSB) Between Single-Stranded DNA Molecules
SPEAKER Prof. I-Ren Lee*
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PLACE Rm716, CCMS & New Physics Building, NTU

Abstract

Echerichia coli (E. *coli*) Single-Strand Binding (SSB) protein is essential in DNA replication and repair processes owing to its ability to bind the intermediate single stranded DNA (ssDNA), thereby preventing unwanted reannealing or degradation. It was proposed that SSB is recycled by redistributing itself along long ssDNA, for example between two adjacent Okazaki fragments. The mechanism of this process was investigated by the Lohman group using ensemble kinetics methods and a previously proposed a "direct transfer" mechanism - forming a transient intermediate composed of SSB and two ssDNA prior to the transfer was concluded. We developed a single molecule assay to examine this direct transfer in detail through real-time observation of single molecule fluorescence resonance energy transfer (smFRET) signals. The introduction of competitor ssDNA oligonucleotides to an SSB protein transiently bound to a surface immobilized ssDNA ultimately led to the dissociation of SSB from the surface immobilized DNA. The rate of SSB transfer is linearly dependent on the competitor DNA concentration, suggesting that only one ssDNA molecule is involved in the rate-determining step of the transfer process. Prior to the full unwrapping of the original DNA bound to an SSB protein, fast FRET fluctuations with ~100 ms time scale were observed with occasional sudden drops of FRET signal shifted toward the lower value, indicating the structural destabilization of the ssDNA-SSB complex induced by multiple events of competitor ssDNA binding. At the given condition of dT_{50}-SSB complex with dT_{40} competing ssDNA molecules, the final dissociation event measured as a high to low FRET transition occurs rapidly. These findings are consistent with the kinetic model of short-lived and partially-unwrapped intermediate states were induced by the fast binding and unbinding of competing oligonucleotides and only fraction of the intermediate state molecules can achieve complete transfer of SSB.

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