

MAR11-2010-000846

Abstract for an Invited Paper
for the MAR11 Meeting of
the American Physical Society

Single-molecule measurements of replisome composition and function reveal the mechanism of polymerase exchange

JOSEPH LOPARO, Harvard Medical School

A complete understanding of the molecular mechanisms underlying the functioning of large, multiprotein complexes requires experimental tools capable of simultaneously visualizing molecular architecture and enzymatic activity in real time. I will describe a novel single-molecule assay that combines the flow-stretching of individual DNA molecules to measure the activity of the DNA-replication machinery with the visualization of fluorescently labeled DNA polymerases at the replication fork. By correlating polymerase stoichiometry with DNA synthesis of T7 bacteriophage replisomes, we are able to quantitatively describe the mechanism of polymerase exchange. We find that even at relatively modest polymerase concentration (2 nM), soluble polymerases are recruited to an actively synthesizing replisome, dramatically increasing local polymerase concentration. These excess polymerases remain passively associated with the replisome through electrostatic interactions with the T7 helicase for 50 seconds until a stochastic and transient dissociation of the synthesizing polymerase from the primer-template allows for a polymerase exchange event to occur.