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I E C B

Single-molecule study of helicases and polymerases using a hairpin substrate, possible application to single molecule sequencing.

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In the cell many molecular motors collaborate with other enzymes to achieve a specific task. In the T4 replisome the polymerase is coupled with the helicase to drive DNA synthesis. Whenever the replication fork encounters a lesion, a repair mechanism is triggered which might involve UvsW to correct the problem. In E. coli the repair helicase RecQ is known to interact with SSB to unwind the damaged DNA. Observing those multi-enzyme processes at the single molecule level is challenging since the rate of success of this multi enzyme process is usually low. Using magnetic tweezers on typically 50 beads simultaneously, we investigate these mechanisms using a hairpin substrate. We have used this substrate to investigate the coupling between helicase and polymerase in the T4 replisome but also repair mechanism by the helicase UvsW and RecQ with SSB. We shall show that it is possible to follow these collaborative processes in real time. In particular we demonstrate that the replicative polymerase can go backward and is also an active helicase which couples to the replicative helicase to produce an efficient copying machine. This property can be used to sequence a single DNA molecule. With UvsW, we have been able to follow a complete repair mechanism in vitro involving its coupling with a polymerase in a stochastic process.