BIOMATERIALS

Forces in Biomolecular Systems SFB 863

Single replication machines at work: the coordination of daughter strand synthesis

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Abstract:

To efficiently replicate DNA, all replisomes must solve a directionality problem. Daughter-strand templates generated at the replication fork have opposing polarities, but polymerases can only synthesize in one direction. One solution to this problem, famously known as the textbook 'trombone model' (Alberts et al, 1983), is the formation of replication loops which permit one polymerase to advance in a continuous fashion on the leading strand, while the other polymerase on the lagging strand synthesizes discontinuously with repeated cycles of priming and polymerase loading. How a single protein assembly coordinates the slow enzymatic steps on the lagging strand with continuous leading-strand synthesis is not understood. To study this process, we have developed a novel single-molecule approach to simultaneously monitor the rates of loop growth and leading-strand synthesis by single replisomes. Using this technique, we have unexpectedly discovered that – in contrast to the 'trombone model' – most loop growth events occur only during priming. Fluorescence imaging of single polymerases provides an explanation by showing that most polymerases are not recycled at the replication fork, as previously thought, but instead are released to complete synthesis behind the replisome. Examination of individual replication events revealed numerous reaction cycle types and pausing events, supporting the notion that, while an array of critical interactions and regulatory circuits guide replisome function, a multitude of kinetic pathways are utilized.

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