BIOLOGY AT THE NANOSCALE, ONE MOLECULE AT A TIME
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ABSTRACT
Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual proteins and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these proteins function in concert with other proteins in large complexes, so an important direction is the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies. I will discuss the development of such tools to study DNA replication. In particular, I will focus on the role of micromanipulation techniques, microfluidic approaches, and imaging methods in such single-molecule studies.

KEYWORDS: Single-molecule detection, fluorescence, DNA replication

Complex molecular pathways that consist of a large number of sequential kinetic transitions are extremely challenging to study using ensemble-averaging methods. The dephasing caused by the stochastic nature of molecular transitions will cause kinetic details to be lost and mechanistic information difficult to gain. The ability to observe individual molecules allows us to obtain “molecular movies” and enable us to directly monitor complex sequences of molecular states and transitions [1-3]. We are applying a single-molecule approach to study DNA replication, a process that is supported by a large, multi-protein complex containing a number of different activities. I will present recent results of single-molecule studies of replication in bacterial and eukaryotic systems, both in vitro and in vivo. By combining the mechanical stretching of individual DNA molecules with the fluorescence observation of individual proteins, we visualize the dynamic behavior of phage and bacterial replication complexes during replication in vitro [4]. Further, I will present data from single-molecule replication studies in X. laevis oocyte extracts [5-7]. We have developed a novel imaging scheme that permits single-molecule fluorescence experiments at concentrations of labeled protein that were hitherto inaccessible [8]. Using this method, we visualize, in real time, origin firing and fork movement. Finally, I will present imaging studies that aim to visualize in live bacterial cells the interplay between DNA replication and repair at the single-molecule level.

REFERENCES

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