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Single Molecule Visualization of Protein-DNA Complexes: Watching Machines at Work

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We can now watch individual proteins acting on single molecules of DNA. Such imaging provides unprecedented interrogation of fundamental biophysical processes. Visualization is achieved through the application of two complementary procedures. In one, single DNA molecules are attached to a polystyrene bead and are then captured by an optical trap. The DNA, a worm-like coil, is extended either by the force of solution flow in a micro-fabricated channel, or by capturing the opposite DNA end in a second optical trap. In the second procedure, DNA is attached by one end to a glass surface. The coiled DNA is elongated either by continuous solution flow or by subsequently tethering the opposite end to the surface. Protein action is visualized by fluorescent reporters: fluorescent dyes that bind double-stranded DNA (dsDNA), fluorescent biosensors for single-stranded DNA (ssDNA), or fluorescently-tagged proteins. Individual molecules are imaged using either epifluorescence microscopy or total internal reflection fluorescence (TIRF) microscopy. Using these approaches, we imaged the search for DNA sequence homology conducted by the RecA-ssDNA filament. The manner by which RecA protein finds a single homologous sequence in the genome had remained undefined for almost 30 years. Single-molecule imaging revealed that the search occurs through a mechanism termed "intersegmental contact sampling," in which the randomly coiled structure of DNA is essential for reiterative sampling of DNA sequence identity: an example of parallel processing. In addition, the assembly of RecA filaments on single molecules of single-stranded DNA was visualized. Filament assembly requires nucleation of a protein dimer on DNA, and subsequent growth occurs via monomer addition. Furthermore, we discovered a class of proteins that catalyzed both nucleation and growth of filaments, revealing how the cell controls assembly of this protein-DNA complex.