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MOLECULAR BIOLOGY:

Slip sliding on DNA

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Abstract

Dedicated binding proteins stabilize single-stranded DNA, protecting it from breakage and distortion. Once thought to form inert complexes with DNA, such proteins are now shown to be remarkably mobile.

The cell's genetic information is maintained as a double helix of interwoven DNA strands in which the information content (the nucleotide sequence) is tucked away between sheltering sugar-phosphate backbones. Although this stable structure benefits long-term information storage, the two strands of DNA must be unwound to allow DNA replication, genetic recombination and repair. Unfortunately, DNA unwinding comes with inherent risks: single-stranded DNA (ssDNA) is susceptible to environmental insults, and is prone to forming aberrant structures that impede subsequent DNA-processing reactions. To alleviate these problems, cellular ssDNA-binding proteins (SSBs) have evolved that bind, protect and stabilize the DNA^{1,2}, providing a platform that allows enzymes to process the single strand. SSBs have long been thought to form relatively immobile complexes with ssDNA. But Roy and colleagues³ report in this issue (page 1092) that an SSB from the bacterium *Escherichia coli* forms a surprisingly dynamic structure with its DNA strand — one in which SSB slides spontaneously and rapidly along ssDNA tracks.

SSBs bind with high affinity and low sequence specificity to ssDNA^{1,2}. Most bacterial SSBs resemble the prototypical *E. coli* protein, which wraps ssDNA around its tetrameric (four-subunit) protein core (Fig. 1). Bacterial SSBs also bind to other proteins involved in DNA metabolism². Eukaryotic SSBs (those of plants and animals), however, have different DNA-binding-domain arrangements and protein-association mechanisms from their bacterial counterparts⁴.

By virtue of the transience of ssDNA species in cellular DNA-processing reactions, SSB-ssDNA complexes have long been thought to be short-lived but essential structures in normal cellular processes. For example, during DNA replication in *E. coli*, the binding protein not only binds to ssDNA, but is also jettisoned from the DNA strand as the DNA template is copied by the cellular replication machinery. This feat requires that multiple SSBs bind to and dissociate from long stretches of ssDNA (in excess of 1,000 nucleotides) within seconds⁵, implying dramatic and rapid dynamics.

Early microscopic and biochemical studies⁶ showed that *E. coli* SSB forms beaded structures on ssDNA that resemble those of histone proteins (the proteins around which DNA folds to form nucleosomes in the eukaryotic cell nucleus). The movement of histones away from stably

bound DNA sequences often requires energy-consuming cellular machinery⁷, and hence one might infer a model for an SSB–ssDNA complex in which there is limited spontaneous sliding of SSB on its DNA. However, kinetic studies of *E. coli* SSB^{8,9} and of a related SSB from a bacterium-infecting virus, bacteriophage T4 (ref. 10), have supported a more dynamic arrangement in which binding proteins are envisaged to move more freely on ssDNA. Thus, the questions of whether SSB moves along its DNA, and how enzymes that process the DNA in SSB–ssDNA complexes might engage their substrates and dislodge the binding protein, have gone unanswered.

In their study, Roy and colleagues³ show that *E. coli* SSB translocates spontaneously and rapidly along the DNA. The finding comes from a set of single-molecule experiments that followed the mobility of the binding protein on ssDNA over time. The protein was estimated to slide spontaneously at a brisk 60 steps per second at 37 °C, moving at a step size of about 3-nucleotide intervals. The authors show that individual SSB tetramers moved distances in excess of the size of their DNA-binding site (65 nucleotides) in either direction along the ssDNA molecules.

These observations beg the question of how binding-protein dynamics influence enzymes that process the DNA in SSB–ssDNA complexes to maintain the genome. To address this problem, the authors³ examined the relationship between SSB and RecA — an enzyme involved in *E. coli* DNA repair and recombination. RecA forms filaments on ssDNA in a directional manner, and nudges the binding protein along the DNA as the filament elongates. In turn, SSB disrupts hairpin structures in the ssDNA that lie ahead of the growing RecA filament, thereby removing potentially obstructive DNA secondary structures and assisting RecA-filament propagation. Taken together, these studies show how SSB promotes RecA-filament formation through directional diffusion along ssDNA.

The discovery that SSB moves along its DNA partner raises many questions. First, how does SSB diffusion influence the activity of proteins with which it forms complexes? It binds to more than a dozen different proteins and, in many cases, stimulates the activity of its partners². As *E. coli* RecA does not seem to bind to SSB directly, it is unclear whether the current findings apply to other SSB–ssDNA-processing enzymes. For example, if an enzyme interacts with both SSB and ssDNA, does it bind to only one SSB protein and push downstream SSBs like the carriages of a train, or does it traverse from one binding protein to the next?

Second, how is the binding protein removed from DNA? Considering the high affinity of SSB–ssDNA interactions, this is uncertain. If a long train of ssDNA-bound SSBs is pushed by an SSB–ssDNA-processing enzyme, does the binding protein simply fall off the end of the DNA (as seems to be the case for RecA3), or can it be sequentially removed by the enzyme as SSB–ssDNA complexes are processed (Fig. 1)? In the former model, how is the SSB that is bound to gap structures (ssDNA flanked by double-stranded DNA, as would be found in DNA-replication intermediates) removed without a free DNA end? With the clearer understanding of SSB–ssDNA dynamics provided by Roy and colleagues, these fundamental questions can begin to be addressed.

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Figure 1. Binding and removal of SSBs from DNA

Single-stranded DNA-binding proteins (SSBs) bind to single-stranded DNA (ssDNA) by wrapping the single DNA strand around the tetrameric protein core. Roy and colleagues³ report that the binding protein from the bacterium *Escherichia coli* diffuses spontaneously and rapidly along ssDNA. Two possible models for SSB dissociation from DNA are shown. Cellular enzymes that process the DNA in SSB–ssDNA complexes, such as RecA (not shown), might push the binding protein off a free end of ssDNA (top). Alternatively, the protein could be sequentially removed from ssDNA by other enzymes, such as those that bind directly to SSBs, as SSB–ssDNA complexes are processed (bottom). The blue and red arrows indicate the hypothetical direction of movement of the processing enzymes.