BIOPHYSICS

Helicase snaps back

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Helicase enzymes can move along DNA or RNA, unravelling the helices as they go. But simply travelling along a nucleic acid in one direction seems not to be enough for some of these molecular motors.

Proteins of the helicase family are essential for almost all biological processes involving nucleic acids such as DNA, from the replication of the genome to the production of proteins[1,2]. Although these enzymes are mainly known for their ability to unwind DNA or RNA helices, it has become increasingly clear that they can have many other activities. For example, helicases can displace proteins from nucleic acids and remodel nucleosomes — the protein complexes around which DNA coils[3,4]. To account for some of the diverse activities of helicases, it has been proposed, and in a few cases shown, that these enzymes behave more like motor proteins, which move along a strand of DNA or RNA, fuelled by ATP (the cell's energy currency)[5]. On page 1321 of this issue, Myong *et al.*[6] add an unexpected twist to the repertoire of proteins that travel along nucleic acids — the astonishingly complex movement of the bacterial helicase Rep along single-stranded DNA.

Rep from *Escherichia coli* is involved in the replication of the bacterial genome, where it helps to restart stalled replication intermediates[7]. Quite how it does this is not known. However, the DNA helicase activity of Rep is well understood, and it is clear that Rep must double up to form a dimer before it can unzip DNA[1,8]. A single Rep molecule — a Rep monomer — cannot unwind helices, but it can move along single-stranded DNA[8]. Whether Rep exists in the cell as a dimer, a monomer, or both, is not known.

To investigate how Rep travels, Myong *et al.*[6] used a single-molecule fluorescence technique to follow the movement of individual Rep monomers along single-stranded DNA (Fig. 1). In the presence of ATP, Rep monomers slid along DNA in the expected 3′ to 5′ direction. But the authors wondered what would happen if a Rep monomer encountered an obstacle on the DNA. They therefore added a second piece of DNA to form a helix in front of the monomer. A helix creates an insurmountable barrier for the Rep monomer[8], and Rep would be expected either to fall off the DNA or to grind to a halt, remaining in front of the helix. Neither happens. Instead, Rep reaches behind itself, grabs the stretch of DNA it has just traversed, forms a loop with it, and then releases the DNA near the blockade — ending up close to the spot on the DNA where it began its journey (Fig. 1). The authors call this series of coordinated events 'snap-back', and Rep goes through many cycles of sliding forward and snapping back. Such 'shuttling' cycles also occurred when Rep encountered other obstacles on single-stranded DNA, such as bound molecules of the protein streptavidin.

To find out how Rep snaps back, Myong *et al.* monitored changes in the shape of the protein during the shuttling cycle. Throughout the sliding leg of the cycle, Rep seems to change conformation rapidly; but as it runs into the blockade, it favours one particular conformation. The authors hypothesize that this conformation exposes or creates an additional binding site for single-stranded DNA that allows Rep also to bind the DNA over which it has just travelled. As a result, a DNA loop forms, causing Rep to release the DNA at the blockade and to snap back (Fig. 1).

Myong and colleagues also show that Rep does not require a free DNA-end to snap back. The relocation of Rep to near the end of the single-stranded DNA fragments is presumably dictated by the stiffness of the DNA strand. Although further experiments are required to discover where Rep binds to the DNA strand, and how the various DNA-binding events are coordinated, the data clearly indicate that a complex movement of a single enzyme molecule on DNA can occur in a few, relatively simple steps.

Why would it be useful for a motor to shuttle on its track? Myong *et al.* suggest that Rep might repeatedly strip off other proteins from DNA as it restarts stalled DNA replication. The ubiquitous DNA-binding protein RecA, for example, has been proposed to bind to DNA replication intermediates and so interfere with the restart of replication[9]. If true, RecA binding might need to be actively prevented, but it is not known how this is accomplished. The authors show that shuttling Rep can efficiently clear RecA from single-stranded DNA. It remains to be seen whether this process occurs in the cell. However, Rep's ability to continually displace RecA while shuttling on single-stranded DNA provides an attractive hypothesis for a physiological function for Rep.

Because the removal of proteins from nucleic acids is assumed to be a core function of enzymes of the helicase families, it will be illuminating to test whether other family members also shuttle back and forth like Rep. It will also be useful to discover whether the snap-back mechanism allows Rep or other enzymes to switch from one nucleic-acid strand to another, which could simplify the coordination of complex structural changes during DNA or RNA transactions. Therefore, when considering possible roles for a 'helicase' in the future, we should not immediately search for the helix that the enzyme unzips, but instead remember how Rep snaps back. ■

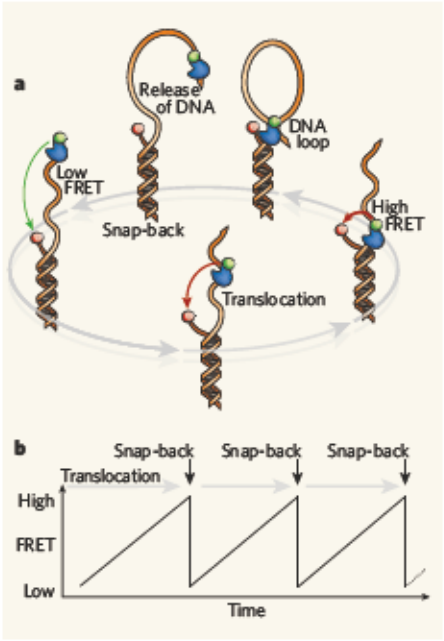

Figure 1 | Watching a helicase shuttle. a, Myong *et al.*[6] followed the movement of single Rep monomers on a DNA strand by monitoring the distance between the Rep helicase and a fixed position on the DNA. They attached one fluorescent label to the DNA (red) and a second label to the helicase (green). If the fluorophores are close enough together, changes are observed in the fluorescence intensity of the two dyes, resulting from fluorescence resonance energy transfer (FRET). The FRET efficiency is highly sensitive to the distance between the two labels; low FRET shows a greater distance, whereas high FRET indicates a lesser one. Rep moves gradually along the DNA until its passage is blocked. At this point a DNA loop forms transiently, then Rep snaps back and begins its journey again. Myong *et al.* directly show the formation of the DNA loop using an alternative scheme of dye-labelling the DNA. **b**, FRET time trajectories for single Rep–DNA complexes show a characteristic sawtooth pattern, with FRET gradually increasing during the translocation phase and then suddenly decreasing during the snap-back.

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