A Sisyphean helicase

Some helicases continually bang their heads against the wall, if a new report from Sua Myong, Taekjip Ha (University of Illinois, Urbana-Champaign, IL), and colleagues is any indication. The Rep helicase, the group finds, repeatedly motors along a track of DNA, hits an obstacle, and returns to square one. Not all helicases can unwind DNA, but they can all motor along it. While studying how Rep motors in a 5’ direction on single-stranded (ss) DNA, Ha’s group noticed that a duplex DNA obstacle did not knock Rep off its template. Their FRET analysis instead suggested that the helicase returned to its original binding site and tried again.

Unlike the 5’ motoring, the return step was almost instantaneous. “At first it seemed like Rep was doing some sort of quantum tunneling,” says Ha, who is a physicist by training. But more FRET studies cleared up the situation—Rep, it seemed, was transiently bound both to DNA near the obstacle and to its initial 3’ binding site, creating a ssDNA loop.

Closing of a regulatory region of Rep called domain 2B coincided with the sudden 3’ end capture. As Rep approached the obstacle, 2B was increasingly likely to be in a closed conformation. Ha guesses that collisions with the duplex—which would be more frequent as Rep gets closer—might push 2B into its closed position. This closed conformation might then trigger high-affinity binding to 3’ ends of ssDNA—a form of DNA that is, says Ha, “very flexible, like spaghetti.”

In addition to free 3’ ends, Rep also has a high affinity for the three-way junctions at stalled replication forks. The group found that Rep shuttled repeatedly between a fork structure and an Okazaki fragment (the equivalent of the duplex obstacle).

The ssDNA at stalled replication forks is a target for RecA binding, which promotes recombination. But Rep prevented RecA filament formation. The findings might thus explain why Rep mutations lead to increased recombination in bacteria. If so, perhaps the clearing, not the unwinding, of DNA is this helicase’s main duty. JCB

Reference: Myong, S., et al. 2005. Nature. 437:1321–1325.

Actin adhesion controls bacterial movement

Actin comet tails propel bacteria through their host cells. Now, Frederick Soo (University of Washington, Seattle, WA) and Julie Theriot (Stanford University, Stanford, CA) suggest that a bug’s speed is determined by adhesion between actin and bacterium, not rates of actin polymerization.

The twist was revealed when Soo measured the temperature dependency of Listeria movement and thereby measured the apparent activation energy ($E_a$) of the rate-limiting step. He noticed that each bacterium had a different $E_a$. This finding is not predicted by simple polymerization-based models of Listeria movement, which assume that the rate-limiting factor (such as actin concentration) is the same for every bacterium.

Knowing the $E_a$ range for a given population, the authors then predicted the range of speeds for that group at a given temperature. But the actual range of speeds they observed was much smaller than predicted—something was systematically speeding bacteria with high $E_a$, so that they did not move as slowly as expected.

Polymerization-based models cannot explain this compensation simply. But Soo found that it is explained by a model that suggests that bacteria advance via the cooperative breakage of small groups of adhesive bonds. Each bond contributes both entropy and enthalpy components to the energy needed to free a bacterium. With more bonds, more thermal energy is needed to break them. But this increase is compensated by the greater entropy that is released upon their breaking.

The authors suggest that bacteria vary in the number of bonds that must break at once for the bug to move (and thus vary in $E_a$). “Perhaps 10 of those bonds might be stretched,” says Theriot. “If the 10 break simultaneously, the bacterium can move forward 2 or 3 nanometers.” It is then recaptured by the actin comet tail.

Other models also incorporate adhesion, but assume that only one bond must break at a time. “The real insight,” says Theriot, “is thinking of things in a group.” She hopes this thinking will be applied to other force-generating elements that act in parallel, such as spindle microtubules or actin filaments at the leading edge. JCB

Reference: Soo, F.S., and J.A. Theriot. 2005. Proc. Natl. Acad. Sci. USA. 10.1073/pnas.0507022102.