Research Roundup

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


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

Telomeres avoid checkpoints

The ends of eukaryotic chromosomes look a lot like double-stranded DNA breaks (DSBs). But DSBs induce checkpoints that stall mitosis during DNA repair, whereas chromosomal ends do not. Unnecessary stalls are thwarted, say Rhett Michelson, Saul Rosenstein, and Ted Weinert (University of Arizona, Tucson, AZ), by telomeric sequences that activate “anticheckpoint” measures.

The authors created extra telomeres in yeast by inducing DSBs next to an internally inserted telomeric repeat. Unlike other DSBs, telomere-adjacent breaks induced only short-lived checkpoint delays. This temporary checkpoint delay probably occurs while telomeric proteins assemble at the new DNA ends—a process that only gets started once telomeric sequences are close to a DNA end. In normal cells, the telomeric proteins are already assembled, so checkpoints never get started.

One simple mechanistic explanation calls for a telomere-bound inhibitor that blocks the activity of Mec1, which initiates the checkpoint response from DSBs. Kinase activity of a mammalian Mec1 homologue, in fact, is known to be inhibited by overexpression of the TRF2 telomeric protein.

Anticheckpoints worked on DSBs as far as 600 bp from the telomeric repeat. Thus, says Weinert, “any problems in the DNA near the telomere may not benefit from checkpoint regulation.” This might explain why the DNA internal to telomeres is usually repetitive. “With repeats,” says Weinert, “breaks can be repaired off nearby intact sequences as a way to compensate for having no checkpoint.”


Sigining with tortoise and hare

Findings from Onn Brandman (Stanford University, Stanford, CA) and colleagues suggest that switchlike signal transduction pathways attain speed and reliability by using two positive feedback loops: one fast and one slow.

Just one positive feedback loop is enough to create an all-or-none biological switch. Yet many systems, such as yeast polarization, rely on multiple loops. Removal of the faster loop (GTPase activation) delays polarization, whereas removal of the slower loop (GTPase localization) makes polarization unstable. Using mathematical models, Brandman et al. now show that speed and stability generally require loops of distinct kinetics.

“We can use equations,” says Brandman, “to see what would happen with every combination [of loop kinetics].” One or two fast loops provided a speedy “on” switch, but the system often turned off inappropriately in response to noise. Systems with one or two slow loops, in contrast, remained reliably activated, because short interruptions in a signal were restored before the loop could be shut off. Their slow kinetics, however, created delayed reaction times to the initial incoming signal.

Only the mixture of fast and slow provided both speed and stability. “You can have a switch that is rapidly inducible,” says Brandman. “And if there’s enough stimulus, the system will commit to that state.” Systems with kinetically dis-