A time for promiscuity in a eukaryotic recombinase

Maria Spies
From the Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242
Edited by Norma Allewell

Homologous recombination is one of the most important cellular processes because, on one hand, it preserves the integrity of the genome, and, on the other hand, it generates genetic diversity in evolution. The central steps in recombination involve the search for homology between two DNA molecules and the subsequent exchange of the DNA strands, orchestrated by the RecA family of the evolutionary conserved DNA strand exchange proteins (recombinases). The active species in this process is a nucleoprotein filament formed on ssDNA by the ATP-bound recombinase, with each recombinase monomer occluding 3 nucleotides of single-stranded DNA (ssDNA)\(^2\) (see Fig. 1) (1). RecA-like recombinases participate in accurate repair of the most genotoxic DNA lesions, including DNA double-strand breaks, interstrand DNA cross-links and damaged replication forks, and, in organisms that utilize sexual reproduction, also mediate recombination in meiosis. Prokaryotes and archaea typically have a single RecA-like recombinase: RecA in bacteria and RadA in archaea. Eukaryotes are believed to have at least two distinct bona fide recombinases, Rad51, which is involved in DNA repair and protection of damaged replication forks in mitosis, and meiosis-specific Dmc1. Why are there duplicate recombinases? Lee and colleagues provide a new clue in their single-molecule analysis of the three recombinases that demonstrates how different enzymes are specialized in different tasks (2).

During the past few years, groundbreaking single-molecule studies by several groups have tackled the mechanism of the two steps in the DNA strand exchange reaction. First, Ragunathan, Ha, and colleagues utilized single-molecule FRET to demonstrate that the RecA nucleoprotein filament formed on ssDNA undergoes 1D diffusion along the DNA duplex when searching for homologous sequence (3, 4). The limitations of a FRET-based experiment restricted their observations to local, short distance events. Forget and Kowalczykowski used optical tweezers and much longer DNA duplexes to demonstrate that a RecA nucleoprotein filament assembled on a short oligo undergoes a 3D search for homology (5). Thus, it seems that RecA combines a 3D search with local 1D sliding followed by docking and propagation of the joint molecule formation in increments of three base pairs (3).

Lee, Qi, Greene, and colleagues previously explored the mechanism of this process at the level of individual nucleotide triplets with a clever approach involving DNA curtains, a single-molecule high throughput methodology that visualizes numerous DNA molecules aligned on a surface of a total internal reflection fluorescence microscopy flow cell (6, 7). By studying the DNA strand exchange reaction between the recombinase nucleoprotein filaments assembled on long ssDNA molecules and a short dsDNA (70 bp) containing an even shorter region of limited homology, they showed that homology sampling proceeds in 3-nucleotide steps, with robust kinetic selection and that a minimum of eight base pairs were needed to initiate a stable pairing, with the ninth pair contributing additional stabilization to the heteroduplex. The mechanism and its energetic signature seem to be conserved between RecA, Rad51, and Dmc1, but only Dmc1 was shown to stabilize mismatched triplets. However, it was not known how these enzymes deal with the range of imperfections that must be rejected in DNA repair but are tolerated in meiosis.

In their new work, Lee and colleagues (2) extended the DNA curtain assay to probe the impact of imperfections. They assessed the abilities of Escherichia coli RecA and yeast Rad51 and Dmc1 recombinases to tolerate imperfect triplets within a homologous region that is long enough for the recombinase to initiate pairing but short enough to have an experimentally tractable life time (Fig. 1A). Fluorescently labeled DNA duplexes were used as probes for strand invasion events, with probes ranging from a fully complementary sequence to those containing single or multiple imperfections in different positions relative to the ends of the homologous sequence. The stability of the strand invasion was determined from the survival probability of the three-strand structure, which is reflected in the time between the appearance of the fluorescently labeled probe and its dissociation from the curtain. When analyzed for many single molecule events, the average life times of the three-strand structures can be converted into free energies, providing an energetic signature for each probe. Not surprisingly, Dmc1 (a meiotic recombinase) was shown to stabilize and bypass single mismatches, multiple mismatches,
and even abasic sites. None of the three proteins were able to stabilize out-of-register triplets, which would require a significant distortion of the DNA duplex or of the nucleoprotein filament. These observations offer a new perspective on the critical decision point that defines the fidelity of the DNA strand exchange reaction, when a recombinase either engages the incoming duplex when it is deemed homologous or rejects it by reversing the reaction. Because the contribution of individual imperfections to the overall strand exchange energetics is quite small, one can suggest a structural explanation for the stabilization of the mismatches by Dmc1. Mismatches within the Rad51-bound recombination intermediate are readily recognized by the DNA mismatch repair machinery, which controls the fidelity of recombination by initiating heteroduplex rejection (8). In meiosis, a more promiscuous control would allow for differences between the two parental sequences.

Another interesting observation relates to the role of ATP hydrolysis in the recombinase reactions. ATP is an important co-factor for all recombinases, and the active nucleoprotein filament cannot be formed without it. The role of ATP hydrolysis is less clear and is typically thought of as important for bypassing regions of heterology or for recycling the recombinase by promoting its dissociation from the DNA. Lee et al. (2) show that, in the presence of ATP, but not when it is substituted by the slowly hydrolyzable ATP analog ATPγS, RecA can stabilize a homologous triplet that lies beyond the internal triplet that has multiple mismatches, supporting the heterology bypass proposal. These data also suggest a new experimental way to explore ATP utilization by the nucleoprotein filament. The studies of ATP utilization will require observation of the heterology bypass during the DNA strand exchange reaction for longer distances as well new orthogonal strategies that will correlate ATP hydrolysis with the nucleoprotein filament dynamics and activities.

Single-molecule studies of the DNA strand exchange reaction, including this new work by Lee and colleagues (2), have been instrumental in developing a more comprehensive understanding of the mechanics of homologous recombination. It will be exciting to add more complexity by including the many proteins and enzymes that regulate recombination and also to broaden the studies of the strand exchange energetics to other phylogenetic lineages.

References

1. Chen, Z., Yang, H., and Pavletich, N. P. (2008) Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. Nature 453, 489–494
2. Lee, J. Y., Steinfeld, J. B., Qi, Z., Kwon, Y., Sung, P., and Greene, E. C. (2017) Sequence imperfections and base triplet recognition by the Rad51/RecA family of recombinases. J. Biol. Chem. 292, 11125–11135
3. Ragunathan, K., Joo, C., and Ha, T. (2011) Real-time observation of strand exchange reaction with high spatiotemporal resolution. Structure 19, 1064–1073
4. Ragunathan, K., Liu, C., and Ha, T. (2012) RecA filament sliding on DNA facilitates homology search. eLife 1, e00067
5. Forget, A. L., and Kowalczykowski, S. C. (2012) Single-molecule imaging by the RecA family of recombinases. eLife 1, e00067
6. Lee, J. Y., Terakawa, T., Qi, Z., Steinfeld, J. B., Redding, S., Kwon, Y., Gaines, W. A., Zhao, W., Sung, P., and Greene, E. C. (2015) DNA RECOMBINATION. Base triplet stepping by the Rad51/RecA family of recombinases. Science 349, 977–981
7. Qi, Z., Redding, S., Lee, J. Y., Gibb, B., Kwon, Y., Niu, H., Gaines, W. A., Sung, P., and Greene, E. C. (2015) DNA sequence alignment by microhomology matching during homologous recombination. Cell 160, 856–869
8. Honda, M., Okuno, Y., Hengel, S. R., Martín-López, J. V., Cook, C. P., Amunugama, R., Soukup, R. J., Subramanyam, S., Fishel, R., and Spies, M. (2014) Mismatch repair protein hMSH2-hMSH6 recognizes mismatches and forms sliding clamps within a D-loop recombination intermediate. Proc. Natl. Acad. Sci. U.S.A. 111, E316–E325