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Abstract:

Homologous recombination is a ubiquitous DNA metabolic process involved in the repair and tolerance of DNA damage, the recovery of stalled or broken replication forks, and the faithful segregation of chromosomes during meiosis. A class of enzymes known as DNA-strand transferases carry out the signature reactions of recombination: homology search, DNA-strand invasion, and DNA-strand exchange. These proteins are found in all domains of life, and their active form is a right-handed protein-DNA filament that utilizes Watson–Crick base-pairing principles to exchange DNA strands.

Keywords: D-loop; DNA-strand exchange; DNA-strand invasion; DNA-strand transferase; Homologous recombination; Homology search; Paranemic joint; Plectonemic joint; Rad51; RadA; RecA; UvsX

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Recombination: DNA-Strand Transferases

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Glossary

DNA joint molecule Two formerly distinct DNA molecules that are joined by base-pairing interactions and/or the DNA-strand transferase filament. Unlike heteroduplex DNA, this term includes synapsed DNA structures whose strands have not yet intertwined (paranemes) or where the heteroduplex DNA has been repaired by mismatch repair.

DNA translocase A motor protein whose ATPase cycle is coupled to movement on DNA.

Heteroduplex DNA The duplex DNA product of DNA-strand exchange, where the two component single strands were originally part of two separate DNA molecules.

Paralog A type of homolog where genes are related by duplication within an organism's genome that led to their specialization of functions.

Homologous recombination is highly reliant on the DNA-strand transferases that catalyze the key reactions of homology search and DNA-strand exchange. The prototype for this class of enzymes is RecA protein from *Escherichia coli*. Other members include UvsX from T4 bacteriophage, RadA from Archaea, Rad51 from eukaryotes, and the meiosis-specific Dmc1 protein, found in most, but not all, eukaryotes. Defects in DNA-strand transferases lead to a severe reduction in homologous recombination associated with DNA damage sensitivity as well as impaired replication and meiotic chromosome metabolism.

Homology Search and DNA-Strand Invasion by DNA-Strand Transferases

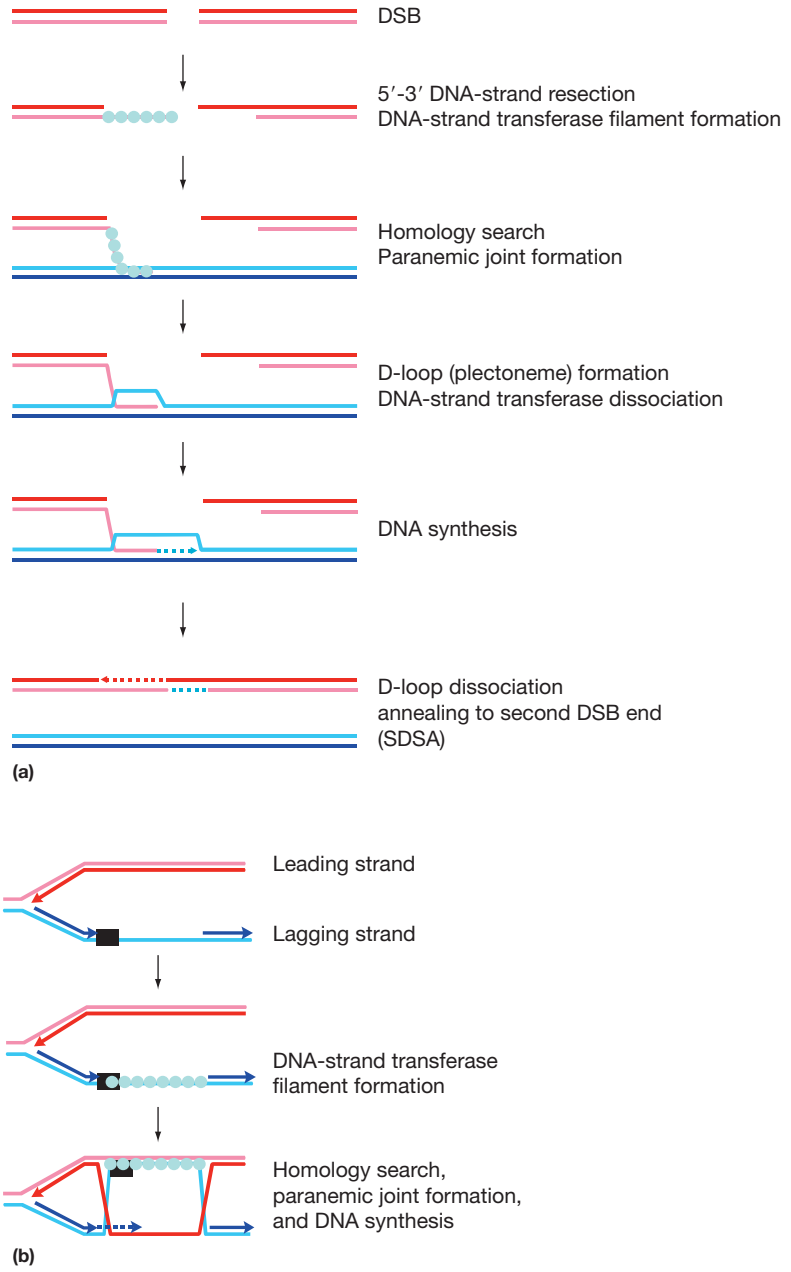
DNA-strand transferases catalyze homologous pairing between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA, or duplex DNA), followed by the exchange of one strand of the dsDNA with the incoming ssDNA. The product of the DNA-strand exchange process is heteroduplex DNA, in which two single strands of DNA that were formerly from distinct DNA molecules form duplex DNA, displacing the original complimentary duplex strand in the region where strand exchange has occurred. This DNA-strand invasion reaction is fundamentally different from DNA-strand annealing, where two ssDNA regions come together to form duplex DNA, because it requires DNA-strand displacement and the original base pairs to be broken. The DNA substrates required for recombination are typically the 3′-overhanging ssDNA from a processed DNA double-strand break (DSB) or ssDNA gaps formed during replication fork stalling (Figure 1) or other processes. The fundamental goal of a recombination reaction is a template switch to provide a 3′-OH primer a suitable template for DNA synthesis. This synthesis allows the cell to reference and recover sequence from an intact copy of a genetic locus when the other has been damaged.

DNA-Strand Transferase Filament Formation

The catalytic form of DNA-strand transferases is a specialized, right-handed helical filament that forms on ssDNA with approximately six monomers per turn. The first challenge of homologous recombination is to assemble these nucleoprotein filaments so the homology search can begin. *In vivo*, the ssDNA substrate for filament formation is coated with single-stranded DNA-binding protein (bacterial SSB, phage T4 Gp32, or eukaryotic RPA) as soon as it is generated. Although the binding of successive DNA-strand transferase monomers to filament ends is cooperative, competition for ssDNA binding with the ssDNA-binding proteins limits the initiation of filament formation, a process termed nucleation. In the cell, nucleation of the first monomers and the replacement of ssDNA-binding protein by growing DNA-strand transferase filaments are assisted by additional proteins, termed recombination mediators (see below). The eukaryotic DNA-strand transferases Rad51 and Dmc1 have added reliance on accessory proteins, as they lack the strong binding preference for ssDNA over dsDNA exhibited by RecA and UvsX. Consequently, *in vivo*, Rad51 or Dmc1 needs to be targeted to ssDNA, and filaments that have formed on dsDNA must be dissociated. The evolutionary constraints that led to these differences between DNA-strand transferases from eubacteria and eukaryotes are not understood yet, but likely are adaptations to the more complex genomes and regulatory requirements of eukaryotic organisms. After generation of ssDNA, nucleation and extension to form an active filament on ssDNA is the primary challenge of the early stage of homologous recombination, generally termed presynapsis. The presynaptic filament is necessary for all subsequent steps of HR.

Homology Search and Paranemic Joint Formation

The presynaptic filament on ssDNA searches for homology in the genome. The homology search process is poorly understood, but a key feature is the stretching of the bound DNA in the confines of the filament by 150% relative to B-form DNA. Presumably, homology search involves many rounds of association/dissociation between the ssDNA filament and duplex DNA to sample homology by Watson–Crick base pairing. Finally, a region of DNA within the strand transferase-ssDNA filament is homologously aligned with duplex DNA, marking the beginning of the synapsis stage of homologous recombination. The initial



f0010 **Figure 1** Involvement of DNA-strand transferases in DSB and gap repair by homologous recombination. (a) Double-strand break repair by synthesis-dependent strand annealing (SDSA). After DSB formation, nucleolytic DNA end-processing creates 3′-ended ssDNA that becomes the substrate for DNA strand transferase filament formation (spheres; not to scale or form). Homology search leads to the formation of a paranemic joint, which transitions to a plectonemic joint (D-loop) after strand intertwining. Upon dissociation of the DNA strand transferase from the heteroduplex product, the invading strand 3′ end is extended by DNA polymerase. SDSA is one pathway of DSB repair, and alternative pathways differ only in the steps after DNA synthesis (see suggested readings). In SDSA, the extended invading ssDNA retracts from the D-loop and anneals to the complementary strand of the other DSB end. Synthesis to fill in the remaining gaps and sealing of the nicks by ligation results in a fully restored chromosome. (b) Gap repair by homologous recombination. Recombination from a gap on the lagging strand can displace the newly synthesized leading strand for use as a template for DNA synthesis past a blocking DNA lesion (black box). Notice that the initial joint molecule that forms is an obligatory paraneme because there is no free DNA end to allow strand intertwining.

synaptic complex is termed a paranemic (from Greek, literally 'beside thread') joint DNA molecule, and it is stable only in the continued presence of the DNA-strand transferase protein. At this stage, there is no net intertwining of the incoming ssDNA with its complement that has been found in the dsDNA.

Plectonemic Joint Formation s0025

After homology search and homologous alignment in the paranemic joint, the invading strand must intertwine with its complement in the duplex DNA, thereby displacing the p0030

original complementary strand in the duplex from base-pairing interactions. The resulting intermediate is known as a displacement loop (D-loop). The 3′-end must intertwine to generate a suitable primer/template junction that can be extended by a DNA polymerase. Once intertwined, the existence of the homologously paired joint DNA molecule is no longer dependent on the continued presence of the DNA-strand transferase filament, as the newly formed heteroduplex DNA stabilizes the joint DNA molecule. Such intermediates where strands from two homologous DNA molecules have intertwined are termed plectonemic (literally, 'knotted threads') joint DNA molecules. They have been experimentally shown to form after paranemic joints, their direct precursors.

p0035 The transition from a paranemic to plectonemic joint requires the presence of a homologous, free DNA end in either the incoming ssDNA or duplex DNA donor to allow the free rotation required for DNA-strand intertwining. Therefore, the initial joint molecule that forms from an ssDNA gap, where there is no free DNA end, is an obligatory paraneme. Conversely, the ssDNA substrate for DSB repair has a free 3′-end, such that paranemes are likely kinetic intermediates on the way to becoming plectonemic joint molecules.

s0030 ## Filament Dissociation

p0040 The continued presence of the DNA-strand transferase on the region of intertwined homology within the plectonemic joint (heteroduplex DNA) can actually inhibit further steps in HR, because it obstructs the 3′-ssDNA′ end from access by a DNA polymerase to initiate DNA repair synthesis. Therefore, the DNA-strand transferase must either self-dissociate or be removed by an external factor. Both types of turnover have been observed. DNA-strand transferases are DNA-dependent ATPases, and the ATPase cycle modulates the affinity of the DNA-strand transferase for DNA. The adenosine triphosphate (ATP)-bound form has the highest affinity for DNA, and for RecA the adenosine diphosphate (ADP)-bound form has the lowest, even lower than the nucleotide-free form. ATP hydrolysis is thought to occur throughout the filament, but because of cooperative interactions between monomers within, dissociation occurs primarily from ADP-bound monomers at the ends. For DNA-strand transferases with higher ATPase activities such as RecA ($\sim$30 min^{-1}) and UvsX ($\sim$200 min^{-1}), this appears to be sufficient for autonomous turnover from heteroduplex DNA. However, eukaryotic Rad51 proteins have a relatively low ATPase activity ($<$1 ATP hydrolyzed per minute), and their ADP-bound form has considerably higher affinity to DNA than RecA leading to very inefficient turnover from dsDNA. These biochemical differences between the RecA and Rad51 proteins explain the necessity for external turnover factors that dissociate Rad51 from dsDNA.

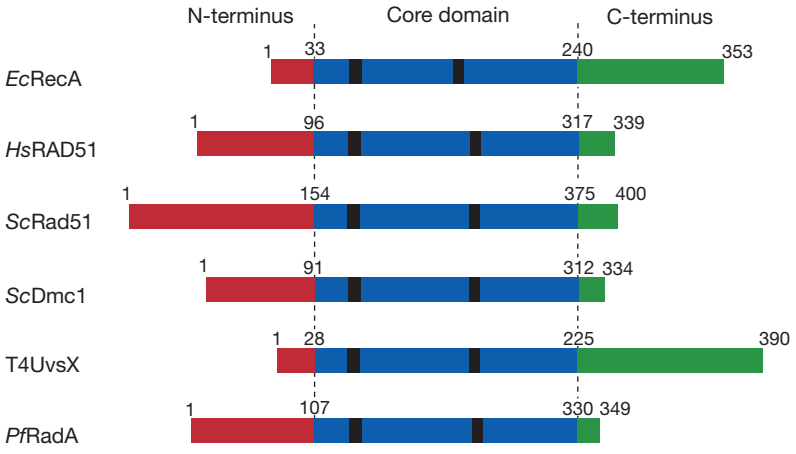
Restoration of Information and Chromosome Resolution s0035

The fundamental purpose of homology search and DNA-strand invasion by the DNA-strand transferase filament is to position the 3′-end of a DSB onto a suitable template for repair DNA synthesis that ultimately leads to the restoration of chromosomal integrity (Figure 1(a)). Similarly in gap repair, DNA-strand exchange leads to a template switch that provides an intact template for a 3′-end that could not otherwise be extended due to a damaged template (Figure 1(b)). p0045

Structure and Properties of DNA-Strand Transferase Filaments s0040

General Properties of DNA-Strand Transferases s0045

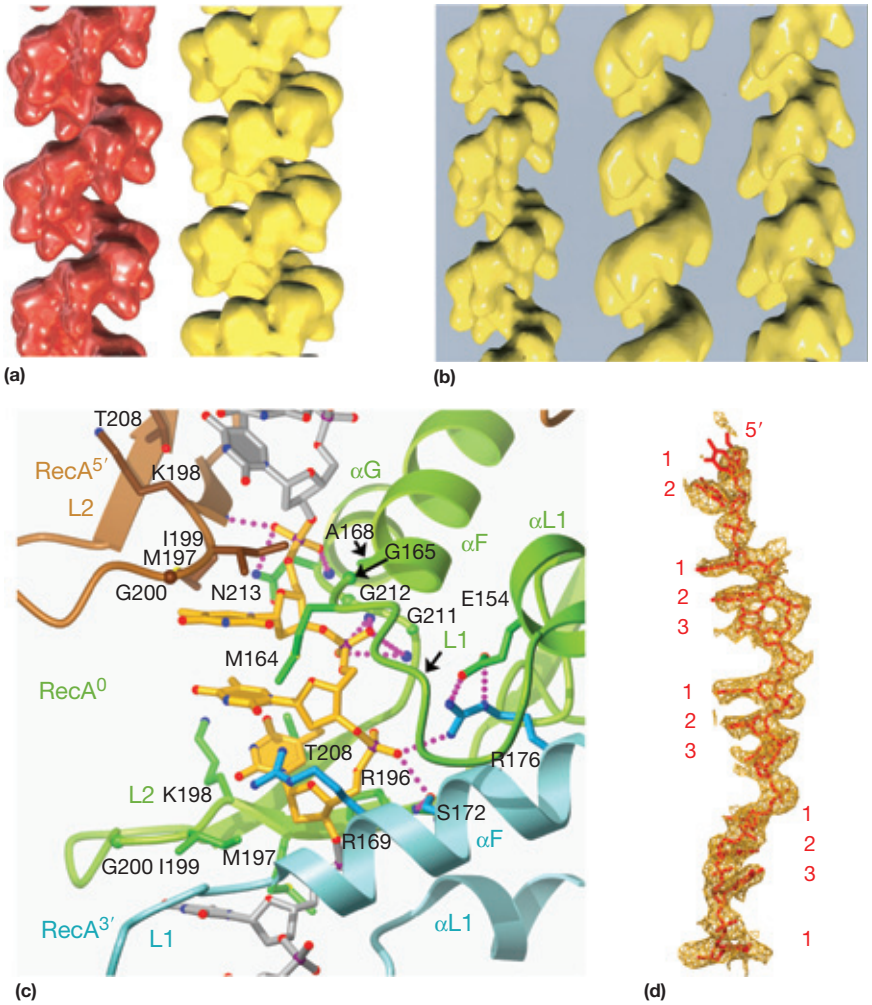
DNA-strand transferases share homology in their central core, called the RecA domain, that shows a considerable degree of sequence conservation between species and contains the ATPase active site as well as residues involved in DNA binding (Figure 2). Amino and carboxy-terminal domains are also present which are more variable between species. RecA has an N-terminal domain (aa 1–33), followed by the core domain (aa 34–240), and a C-terminal domain which contains a DNA-binding site (aa 241–352). In comparison, Rad51 proteins have an extended N-terminal region containing a DNA-binding site and lack the C-terminal domain found in RecA. p0050



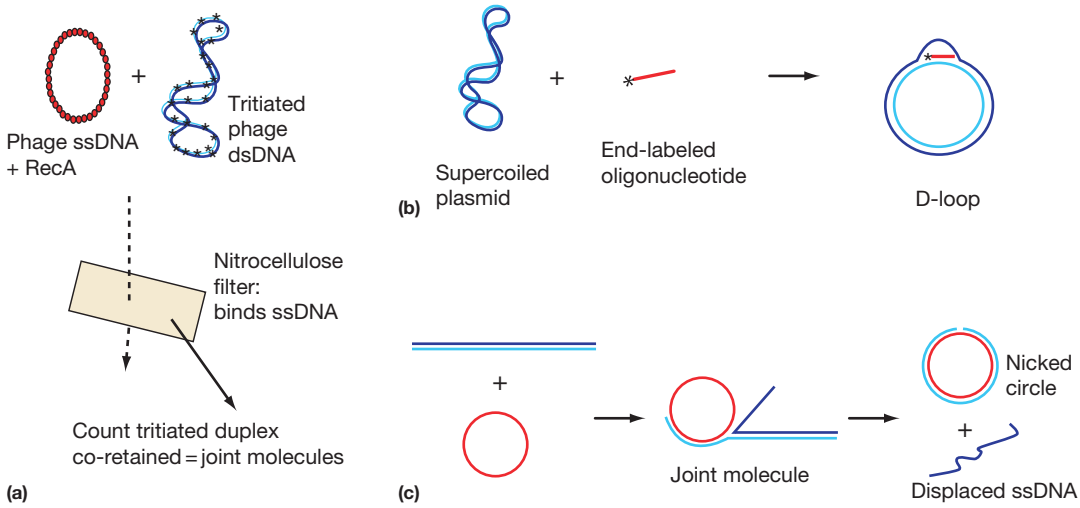
f0015 **Figure 2** Schematic alignment of DNA-strand transferases from Eubacteria, Eukaryotes, Archaea, and T4 virus. Black boxes indicate the location of the conserved Walker A and B ATP-binding motifs within the core domain. Numbers denote amino acid residues. Sequence representations are approximately to scale. *Ec*, *Escherichia coli*; *Hs*, *Homo sapiens*; *Sc*, *Saccharomyces cerevisiae*; T4, T4 bacteriophage; *Pf*, *Pyrococcus furiosus*.

s0050 ## ATP Hydrolysis within the Filament

p0055 ATP hydrolysis is coupled to changes in the conformation of the DNA-strand transferase filament. X-ray crystallography and electron microcopy studies have observed RecA and Rad51 filament structures with distinctly different pitches, correlating to the identity of the bound nucleotide cofactor (Figure 3). An ATP-bound-like state exists in an extended filament conformation with a pitch of about 94 Å/turn, stretching the DNA within to about 150% relative to B-form DNA. The ADP-bound state, however, is in comparison more compressed, with a pitch of 65–85 Å/turn and does not appreciably extend DNA. The extended filament, trapped by the use of slowly hydrolyzable ATP analogs, has been correlated with activity using *in vitro* assays such as the D-loop assay which measures plectonemic joint formation between an ssDNA and a homology-containing supercoiled plasmid (Figure 4(b)). Consequently, and because conditions favoring formation of the compressed filaments do not lead to DNA-strand exchange, the extended filament has been described as the 'active' form of DNA-strand transferase filament, and the compressed form 'inactive'. However, single DNA molecule studies with Rad51 protein have shown that ATP hydrolysis, while leading to partial contraction of the filament, does not necessarily lead to DNA-strand transferase dissociation. The same filament is able to rebind ATP and again adopt a fully extended conformation. Therefore, it appears that ATP hydrolysis, ADP dissociation, and the rebinding of ATP lead to dynamic changes in the pitch of the DNA-strand transferase filament. It is not known if this plays a part in the DNA-strand transferase reaction, but DNA extension appears crucial for the homology search step.



f0020 **Figure 3** Structure of DNA-strand transferase filaments. (a) EM reconstructions of human RAD51 filaments in the extended (left, 99 Å pitch) and compressed (right, 76 Å pitch) conformations. (b) EM reconstructions of extended filament conformations of three DNA-strand transferases, left to right: human RAD51, *S. cerevisiae* Rad51, and RecA. (c) RecA crystal structure snapshot showing a nucleotide triplet bound by the primary monomer ($RecA^0$). The triplet also makes contacts with RecA monomers 5′ and 3′ of the primary monomer. Notice the insertion of loop 2 (L2) residues between triplet base stacks. (d) RecA extended filament ssDNA electron density, with bases in triplets numbered 1–3. Notice the stretched and negatively twisted intertriplet base step between nucleotide triplets. (a, b) Modified from Yu X, Jacobs SA, West SC, et al. (2001) Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. *Proceedings of the National Academy of Sciences USA* 98: 8419–8424. (c, d) Modified from Chen Z, Yang H, and Pavletich NP (2008) Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. *Nature* 453: 489–484.



f0025 **Figure 4** *In vitro* assays for DNA strand transferase activity. (a) Nitrocellulose filter assay for paranemic joints; (b) D-loop assay; and (c) DNA-strand exchange assay. See text for details.

s0055 ## Structure of the RecA-ssDNA Extended Filament

p0060 The X-ray crystal structure of RecA bound to ssDNA in the extended filament conformation (pitch of 94 Å/turn) provides key insights into the cooperative interactions within the filament between ATP, DNA, and adjacent monomers of RecA (Figure 3). Hydrogen bond networks link bound ssDNA to the ATPase active site, such that without DNA binding there would be no ordering of critical residues for ATPase activity. The active site is located at the RecA–RecA interface, with the ADP-AlF_4 (ATP transition state analog) completely buried between monomers, in contrast to the compressed filament structure where the nucleotide is partly solvent exposed. In the extended filament, the core domains undergo a rotation that creates a new interaction surface between monomers. Now, critical lysine residues from the 3′-adjacent RecA monomer, K248 and K250, are positioned to make charge-stabilized H-bonds to the ATP γ-phosphate and E96, another important residue thought to activate a water molecule for nucleophilic attack on the γ-phosphate. ATP hydrolysis destabilizes the inter-monomer interface, because it eliminates these favorable interactions and introduces a net charge in a buried environment.

p0065 Perhaps the most surprising information from the RecA-ssDNA crystal structure is the way in which the DNA is extended, which has profound implications for the homology search process. The DNA inside the filament is stretched to 5.1 Å per base (compared to 3.4 Å per base pair in free dsDNA), and untwisted to 18.5 bases per turn (compared to 10.5 base pairs per turn in free dsDNA). However, this stretching of DNA is not accomplished uniformly along the length of the DNA. Instead, each RecA monomer binds three nucleotides of DNA and holds them with close to B-DNA rise values (3.8–4.2 Å per nucleotide; B-DNA is 3.4 Å). Most of the 1.5-fold extension relative to B-DNA is accomplished by the stretched (7.1–7.8 Å rise) and negatively twisted (−42°) base steps between these nucleotide triplets, from the 3′-most base of one triplet to the first base of the next triplet. This stretching is accomplished by the insertion of hydrophobic residues in the L2 loop region into the base stacks between nucleotide triplets.

Implications of Filament Structure for the Homology Search s0060

p0070 The structure of the RecA-ssDNA filament suggests that for greater than three nucleotides in dsDNA to be consecutively base-paired with nucleotides in the extended filament, the dsDNA too must extend (untwist) in the same pattern of triplet stacked bases intervened by unstacked and stretched intertriplet gaps. To accomplish this, RecA has a secondary DNA-binding site that is thought to interact with one strand of duplex DNA to destabilize the helix and allow bases to be sampled for complementarity. Because net unstacking of base pairs is necessary to pair consecutive triplets, this may favor correct homologous alignment by requiring that the next triplet of dsDNA paired be fully homologous in order to energetically favor base unstacking. Furthermore, triplet isolation limits cooperative base stacking interactions with nonhomologous bases as the heteroduplex region extends, requiring the selectivity be based more on Watson–Crick hydrogen bond interactions.

p0075 Although there are now crystal structures of RecA available bound to both ssDNA and dsDNA, one intermediate remains elusive – the three-stranded, synapsed paranemic joint. It is not known how the DNA strands are arranged in the joint molecule to allow homologous pairing without net intertwining of DNA strands. It is thought that the bases in the target duplex are flipped out for sampling, that is, they temporarily and reversibly break their Watson–Crick hydrogen bonds with the other duplex strand, assume a new orientation, and sample base pairing with nucleotides inside of the DNA-strand transferase filament. The extension of bound DNA by the presynaptic filament reduces stacking interactions to facilitate base flipping, and the structure suggests that the basic sampling unit for base flipping and homology search is the nucleotide triplet.

Recombination Mediators and Other Factors s0065

p0080 Nucleation is the rate-limiting step for Rad51 filament formation and, consistent with other filament-forming proteins such as actin and tubulin, nucleation is a key regulatory target

involving recombination mediators and other protein factors. Some of these proteins may also be involved in maintaining filament stability to increase the steady-state level of active DNA-strand transferase filaments.

s0070 ## Mediators for Presynaptic Filament Formation

p0085 DNA-strand transferases are impeded from nucleating filaments when ssDNA-binding proteins saturate ssDNA. Recombination mediators were defined biochemically as proteins that allow filament formation of DNA-strand transferases on ssDNA coated by the cognate ssDNA-binding protein. The prototypical recombination mediator is UvsY protein from bacteriophage T4, which overcomes the block to ssDNA binding imposed by Gp32 SSB protein to allow UvsX filament formation. RecFOR provides this function in *E. coli* for RecA protein, and in eukaryotes, Rad52 protein mediates filament formation for Rad51 on RPA-coated ssDNA. All three mediators bind their cognate ssDNA-binding protein and DNA-strand transferase.

s0075 ## Rad54, a Specialized DNA Translocase Working with Rad51

p0090 Rad54 is a member of the Snf-2 family, a class of proteins that remodel specific protein-dsDNA complexes, such as nucleosomes or the complex between TATA-binding protein and the TATA box. Rad54 and its paralogs Rdh54 in *Saccharomyces cerevisiae* or RAD54B in human exhibit robust dsDNA-specific ATPase activity, and translocate on DNA (in the case of the yeast enzyme at 300 base pairs per second). Rad54 and its paralogs interact with Rad51 or Dmc1 and stimulate recombination in a species-specific manner. *In vitro*, Rad54 exerts multiple functions, including stabilization of the Rad51 filament on ssDNA (presynapsis), enhancing D-loop formation or DNA-strand exchange (synapsis), as well as branch migration and dissociation of the DNA-strand transferase filament on dsDNA (postsynapsis). Dissociation of Rad51 or Dmc1 is required to allow access by DNA polymerase to the invading 3′-end. In addition, Rad54 and Rdh54 are able to move nucleosomes on dsDNA. While Rad54 is critical for recombination *in vivo*, it is unclear which specific biochemical activity(ies) is most required for its *in vivo* function(s).

s0080 ## Other Eukaryotic DNA-Strand Transferase-Enhancing Proteins

p0095 In eukaryotes, new classes of proteins have been discovered that functionally and structurally interact with Rad51 or Dmc1. These include the Rad51 paralogs, Rad55–Rad57 and Shu1–Psy3, two heterodimers found in *S. cerevisiae*. In humans, RAD51 paralogs include five members – RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 – forming two main complexes (B-C-D-X2 and C-X3). In addition, the human breast and ovarian tumor suppressor BRCA2 bind multiple molecules of RAD51 and other proteins, including PALB2 and DSS1, that are expected to play a role in recombination. While it is known that these factors interact with Rad51, directly or indirectly, their exact function remains to be determined. The multitude of factors that interact with Rad51 and that are genetically implicated in recombination suggests complex regulation of the assembly, stability, and function of the Rad51–ssDNA filament.

s0085 ## Anti-Recombinases Target the Rad51-ssDNA Filaments for Disassembly

p0100 While recombination is an active DNA repair mechanism, inappropriate recombination can interfere with other cellular processes such as DNA replication or lead to toxic intermediates. In eukaryotes, anti-recombinases have been identified genetically that enforce negative control on homologous recombination. The Srs2 DNA helicase was identified genetically in *S. cerevisiae* as an anti-recombinase. Translocation of Srs2 dissociates Rad51 from ssDNA leading to disassembly of presynaptic filaments. Srs2 is the archetype for such an anti-recombinase activity, and other eukaryotic proteins, including FANCJ, FBH1, RECQ5, and BLM, were shown to dissociate Rad51 from ssDNA. In summary, the DNA-strand transferase presynaptic filament appears to be controlled in a meta-stable balance between processes that control its assembly and disassembly.

s0090 ## *In Vitro* Assays for DNA-Strand Transferase Activity

p0105 Functional *in vitro* assays for DNA-strand transferases have generated a detailed mechanistic understanding of their function and have provided test systems for the identification and characterization of recombination mediator and other accessory proteins (Figure 4).

s0095 ## Filter-Binding Assay for Detection of Paranemic Joint Molecules

p0110 The formation of the initial product of synapsis, the paranemic joint, is difficult to study because of its metastable nature, existing only in the presence of the DNA-strand transferase filament. The D-loop and DNA-strand exchange assays (discussed below), while being invaluable for the study of DNA-strand transferase reactions, are limited in that they can only detect stable plectonemic joint molecules where the DNA strands have already intertwined. A filter-binding assay, developed for RecA protein, can detect paranemic joints because it does not require deproteinization of reactions (Figure 4(a)). RecA filaments are first formed on circular ssDNA and then are paired with homologous supercoiled plasmid DNA that has been labeled with tritium. Lack of a free DNA end in the DNA substrates ensures that all joint molecules formed are paranemic, since they cannot intertwine. To detect the presence of joint molecules, the reactions are diluted in a high-salt buffer and passed through nitrocellulose filters. The filter selectively binds ssDNA and any tritiated plasmid that is complexed to it by RecA in paranemic joint DNA molecules. The level of joint molecules is determined by scintillation counting of the washed filters. A major limitation to this assay is that protein binding to dsDNA causes the plasmid to bind the filter regardless of it being complexed in a joint molecule with ssDNA or not. Therefore, the assay is not suitable for DNA-strand transferases that lack an ssDNA-binding preference, such as the Rad51 or Dmc1 proteins.

s0100 ## The D-Loop Assay

p0115 The D-loop assay (Figure 4(b)) is a versatile and sensitive way to detect DNA-strand transferase activity *in vitro*. The ssDNA substrate is commonly a synthetic oligonucleotide that has been 5′-end-labeled with ^{32}P, while the dsDNA homology donor is a small (~3 kb), negatively supercoiled plasmid. DNA-strand transferase filaments are first formed on the oligonucleotide. The supercoiled DNA is then added and D-loops form when the oligo is incorporated into the plasmid as a plectoneme. After deproteinization, the reactions are analyzed by agarose gel electrophoresis, where the fast-migrating free ssDNA is separated from the slow-migrating D-loops, allowing precise quantitation of the proportion of oligonucleotide found in the product D-loop. The deproteinization sidesteps the requirement of the DNA-strand transferase to release its product. Since the plasmid is covalently closed, the amount of DNA-strand exchange that can occur (heteroduplex DNA tract length) is limited by topological constraints. The dsDNA must be supercoiled for productive D-loop formation, as it aids DNA-strand transferase filament-mediated dsDNA unwinding and stabilizes the D-loop during gel electrophoresis.

s0105 ## The DNA-Strand Exchange Assay

p0120 Similar to the D-loop assay, the three-strand DNA exchange assay (Figure 4(c)) detects plectonemic joint molecules formed by DNA-strand transferases. However, the ssDNA substrate is relatively long (5.4 kb for the frequently used phiX174 virion form DNA) and circular, while the dsDNA partner is linear and of the same sequence (phiX174 Replication Form I). Consequently, the region of DNA-strand exchange can now be very long, up to the entire length of the DNAs.

p0125 First, DNA-strand transferase filaments are formed on the ssDNA with the help of the cognate ssDNA-binding protein to remove secondary ssDNA structure. Then, the dsDNA is added and the homology is aligned, likely via the formation of a paranemic joint. Prerequisite to the appearance of products on agarose gels, a plectonemic joint molecule forms, starting with DNA intertwining one strand of a dsDNA end with the incoming ssDNA to begin strand exchange. Lastly, polar branch migration extends the heteroduplex region around the circle, until DNA-strand exchange is complete, with nicked circular dsDNA and displaced linear ssDNA as final products. Upon deproteinization of the reactions followed by agarose gel electrophoresis, two product species can be distinguished – joint molecules and nicked circles. The linear ssDNA product usually cannot be resolved from the circular ssDNA substrate. The joint molecules include all plectonemes that have begun but not completed strand exchange, whereas the nicked circles represent the product with the maximum extent of heteroduplex DNA.

s0110 ## Single DNA Molecule Studies

p0130 The development of techniques to track single DNA molecules and to visualize recombination proteins acting on them has advanced our understanding of DNA-strand transferase filament nucleation, growth, and disassembly. Study at the single molecule level eliminates the averaging and consequent loss of information that results from experiments that employ a large ensemble of molecules. Many different techniques have the sensitivity to gain information on single molecules of DNA. One classical method employed to study DNA-strand transferases involves the use of linear DNA (usually linear 48 kb lambda dsDNA) end-bound to a 1-μm-diameter bead. The bead is held in place inside of a multi-channeled flow cell by an optical trap. By moving the stage, the bead and bound DNA can be transferred into different channels within the flow cell, thereby changing buffer conditions or adding additional proteins. Nucleoprotein filament formation, stability, and disassembly can be monitored by chemically labeling the DNA-strand transferase with a fluorophore. Alternatively, the DNA end opposite the bead is labeled with a fluorophore and DNA-strand transferase binding is monitored by the movement of the fluorophore that results from the extension of the DNA that accompanies DNA-strand transferase binding. Single molecule approaches to study DNA-strand transferases are still in the early phase of their development, but have already provided unprecedented insights into their function.

DNA-Strand Transferases in Signaling and Translesion Synthesis s0115

p0135 Although DNA-strand transferases perform a primary function in the mechanism of homologous recombination, they are also involved in other cellular processes, including DNA damage signaling and translesion DNA synthesis. In *E. coli*, the SOS damage response is dependent on the RecA-ssDNA Au3 filament to act as a coprotease to cleave and deactivate the LexA repressor. This, in turn, leads to the expression of many derepressed genes (SOS operon) required for the damage response. Independent of the SOS signaling function, RecA protein is also required for translesion DNA synthesis by DNA polymerase V. In *S. cerevisiae*, a single unrepairable DSB leads to a checkpoint-mediated G(2)/M arrest, but a phenomenon termed adaptation allows the cell cycle to resume even in the presence of the DSB. Adaptation requires Rad51 protein through an unknown mechanism involving its interaction with Rad52 protein. Thus, it appears that eubacteria and eukaryotes have evolved feedback mechanisms to monitor the status of DNA-strand transferase filaments as a way to assess genotoxic stress conditions.

See also: 00474; 00419; 00496; 00257; 00278; 00248; 00272; 00298; 00242; 00237.

Further Reading

Beernink HT and Morrical SW (1999) RMPs: Recombination/replication mediator proteins. *Trends in Biochemical Sciences* 24: 385–389.

Bianco PR, Tracy RB, and Kowalczykowski SC (1998) DNA strand exchange proteins: A biochemical and physical comparison. *Frontiers in Biosciences* 3: D570–D603.

Chen Z, Yang H, and Pavletich NP (2008) Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. *Nature* 453: 489–484.

Cox MM (2007) Regulation of bacterial RecA protein function. *Critical Reviews Biochemistry and Molecular Biology* 42: 41–63.

Egelman EH (2003) A tale of two polymers: New insights into helical filaments. *Nature Reviews Molecular Cell Biology* 4: 621–630.

Ehmsen KT and Heyer WD (2008) Biochemistry of meiotic recombination: Formation, processing, and resolution of recombination intermediates. *Genome Dynamics and Stability* 3: 91–164.

Forget AL and Kowalczykowski SC (2010) Single-molecule imaging brings Rad51 nucleoprotein filaments into focus. *Trends in Cell Biology* 20: 269–276.

Gupta RC, Folta-Stogniew E, O'Malley S, Takahashi M, and Radding CM (1999) Rapid exchange of A:T base pairs is essential for recognition of DNA homology by human Rad51 recombination protein. *Molecular Cell* 4: 705–714.

Heyer WD, Ehmsen KT, and Liu J (2010) Regulation of homologous recombination in eukaryotes. *Annual Review of Genetics* 44: 113–139.

West SC (2003) Molecular views of recombination proteins and their control. *Nature Reviews Molecular Cell Biology* 4: 1–11.

Yu X, Jacobs SA, West SC, et al. (2001) Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. *Proceedings of the National Academy of Sciences USA* 98: 8419–8424.