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Mini-review

Regulation of DNA strand exchange in homologous recombination

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ABSTRACT

Homologous recombination, the exchange of DNA strands between homologous DNA molecules, is involved in repair of many structural diverse DNA lesions. This versatility stems from multiple ways in which homologous DNA strands can be rearranged. At the core of homologous recombination are recombinase proteins such as RecA and RAD51 that mediate homology recognition and DNA strand exchange through formation of a dynamic nucleoprotein filament. Four stages in the life cycle of nucleoprotein filaments are filament nucleation, filament growth, homologous DNA pairing and strand exchange, and filament dissociation. Progression through this cycle requires a sequence of recombinase–DNA and recombinase protein–protein interactions coupled to ATP binding and hydrolysis. The function of recombinases is controlled by accessory proteins that allow coordination of strand exchange with other steps of homologous recombination and that tailor to the needs of specific aberrant DNA structures undergoing recombination. Accessory proteins are also able to reverse filament formation thereby guarding against inappropriate DNA rearrangements. The dynamic instability of the recombinase–DNA interactions allows both positive and negative action of accessory proteins thereby ensuring that genome maintenance by homologous recombination is not only flexible and versatile, but also accurate.

Contents

1. Introduction

Evolution, the fundamental process that drives biological diversity, demands changes in genomic DNA, the carrier of genetic information. In this light the inherent instability of DNA, a molecule subject to spontaneous hydrolysis reactions and attack from chemicals or radiation, is an advantage. Alterations in the chemical structure of DNA can induce mutations that fuel evolution. However, at the level of the individual, a certain degree of genome stability is required to guard against diseases, including cancer. DNA repair reactions that can restore the structure and functionality of damaged DNA provide a balance between evolution and development of disease [1,2]. We focus on one such repair reaction; homologous recombination, the exchange of DNA strands between homologous DNA molecules.

Due to the chemical complexity of DNA numerous structurally diverse lesions can occur. It is therefore not surprising that multiple DNA repair reactions have evolved [1,2]. Decades of genetic and biochemical experiments resulted in the classification of dis-

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tinct repair pathways and the outline of their mechanisms are emerging. Many DNA repair pathways function by pinpointing the offending covalent chemical adduct modifying DNA through a structure-specific DNA binding protein or protein complex. This recognition event triggers a series of subsequent lesion-processing reactions that eventually restore DNA structure to effect repair. Examples of such pathways include base excision repair, nucleotide excision repair and mismatch repair, which are initiated by recognition of the damaged or incorrect deoxynucleotide, followed by its excision and reinsertion of the correct nucleotide(s) using the complementary DNA strand as a template. The advantage of structure recognition to initiate repair is high specificity, but this also inherently limits the diversity of lesions that can be repaired through each pathway. Homologous recombination does not have this limitation since it does not directly recognize DNA lesions. Instead, the process is initiated on the single-stranded form of DNA, which does not need to contain lesions itself, although it could have arisen as a response to DNA lesions [3–5]. This indirect DNA damage recognition mode and the multiple ways to rearrange homologous DNA strands allows great flexibility in applying homologous recombination to repair many structural diverse DNA lesions or alternative DNA structures, including single-strand gaps, double-strand breaks (DSBs) (Fig. 1), interstrand crosslinks and stalled/collapsed replication forks [71–74].

The multiple applications of homologous recombination in DNA repair imply that this process is subject to multiple control mechanisms [6]. In addition, homologous recombination is only a repair mechanism if the DNA rearrangements catalyzed contribute to DNA genomic stability rather than instability. The exchange of base-paired partners between a DNA segment in need of repair and an undamaged duplex partner of homologous sequence is at the core of DNA repair by homologous recombination [7,25]. Control of homologous recombination repair is focused on controlling this strand exchange step. Notably this reaction is absolutely required for error-free repair of damage involving both strand of duplex DNA. To provide context for this central reaction and

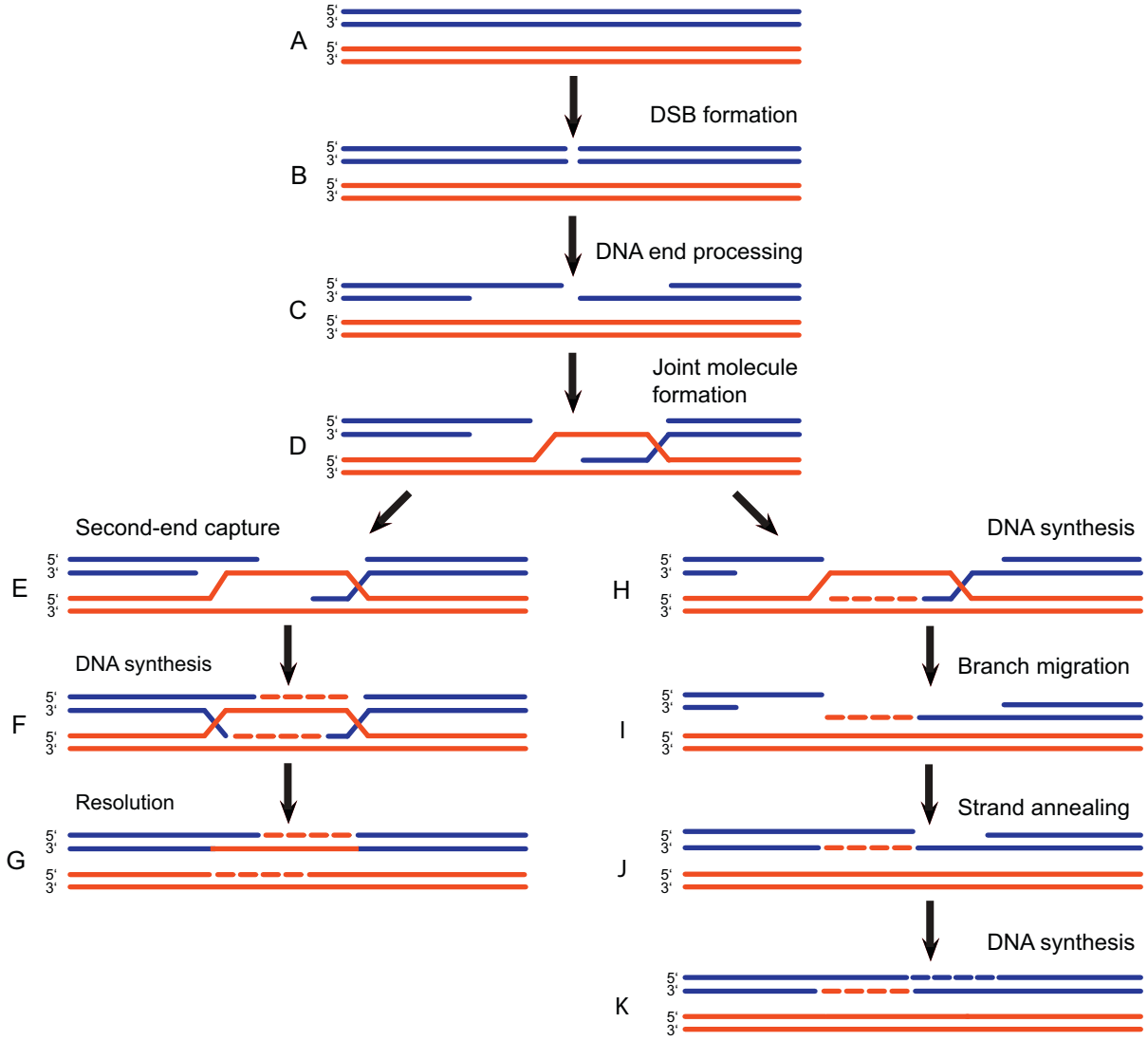

Fig. 1. Schematic representation of a model for DSB-initiated homologous recombination. (A) Two duplex DNAs, representing homologous sister chromatids, are indicated by parallel blue and red lines. This cartoon focuses on the DNA events during recombination and therefore participating proteins are omitted. Upon DSB formation (B), the ends are nucleolytically processed to result in 3′ single-stranded tails (C). (D) Recombinase proteins assembled in a nucleoprotein filament on the single-stranded DNA mediate joint molecule formation between the processed broken DNA and the homologous duplex repair template via homology recognition and DNA strand exchange. Downstream of joint molecule formation, two subpathways of homologous recombination are indicated: DSBR [75] and SDSA [76]. (E) In the DSBR pathway the second DNA end is engaged and upon DNA synthesis, the recombining molecules are joined via Holliday junctions (F). (G) Resolution of the junctions by structure-specific endonucleases releases two repaired duplex DNAs. (H) Upon DNA synthesis in the SDSA pathway the joint molecule is dissolved (I) and the newly synthesized DNA strand anneals with the processed second DNA end (J). Repair DNA synthesis completes repair (K).

its control, we briefly describe seminal events during a complete homologous recombination event leading to the repair of a DSB (Fig. 1).

2. DNA double-strand break repair by homologous recombination

A DSB occurs when covalent bonds in the phosphodiester backbone in both strands of duplex DNA are severed in close proximity. The first order of business is then to keep the break ends, as well as the sister chromatids, nearby so the intact sister chromatid can serve as the repair template for the broken one. In eukaryotes these steps require proteins of the SMC family; the MRN (in vertebrates or MRX in yeast) and cohesin complexes [8,9]. Next, a key intermediate DNA substrate of homologous recombination is generated; single-stranded DNA with a 3′ end (Fig. 1C). This requires the action of nucleases, often aided by DNA helicases. In prokaryotes, such as *Escherichia coli*, the nuclease and helicase functions are provided by the RecBCD complex [10]. More players appear to influence this step in eukaryotes. In yeast, the MRX complex, in cooperation with Sae2, is important for initiating DNA resection [11]. Then more extensive single-stranded DNA is generated through the action of the Sgs1 helicase in cooperation with the Dna2 nuclease, or the Exo1 5′–3′ exonuclease [12]. All these steps are a prelude to the molecular events that define homologous recombination; recognition of homologous DNA sequence to generate a joint molecule between single-stranded DNA and its duplex repair template followed by DNA strand exchange, *i.e.*, switching base-paired partners between these DNAs (Fig. 1D).

The core reactions of homologous recombination, homology recognition and strand exchange, are mediated by recombinase proteins such as RecA in prokaryotes and by Rad51 in eukaryotes, including yeasts and mammals [13]. The DNA rearrangement events that define homologous recombination are catalyzed by RecA and Rad51 assembled into protein filaments on single-stranded DNA. These nucleoprotein filaments are molecular machines promoting specific DNA rearrangement reactions through their sequential assembly, rearrangement and disassembly. The core of the recombination process is controlled by accessory/mediator proteins that modulate the dynamic interaction of recombinase proteins in nucleoprotein filaments [14]. Subsequent steps in homologous recombination can include the engagements of the second DNA end, DNA branch migration, and eventual resolution of repaired DNA strands (Fig. 1E–G). Multiple sub-pathways exist to achieve this, including double-strand break repair (DSBR) with or without associated crossover and synthesis dependent strand annealing (SDSA) [15,75,76].

Upon joint molecule formation, a critical common step in the homologous recombination pathways is the hand off of the 3′ end of the incoming DNA molecule to a DNA polymerase, such that recombination will result in two restored duplex DNAs. At this stage of the reaction the partner DNA molecules are physically joined via branched DNA structures, which upon ligation can be converted into so-called Holliday junctions (Fig. 1F). There appear to be a plethora of structure-specific endonucleases that can resolve these structures resulting in the completion of DNA repair by homologous recombination. Enzymes such as the *E. coli* RuvABC complex and the eukaryotic Gen1 protein incise Holliday junctions producing directly ligatable crossover and non-crossover products [16]. Alternatively, a DNA helicase, BLM, in combination with a type I topoisomerase, can resolve Holliday junctions exclusively in a non-crossover mode [17]. Branched DNA intermediates in homologous recombination can also be acted upon by evolutionary-conserved structure-specific endonucleases, including Mus81/Eme1 and Slx1/Slx4 [16,18]. Since homologous recombination contributes to the repair of many different DNA lesions and obstructed replication forks, each of which might involve subtly different (protein covered) branched DNA intermediates, the variety of structure-specific endonucleases involved is not surprising.

3. The recombinase nucleoprotein filament: structure, variation and dynamics

Homologous recombination is important in many different DNA repair events because joint molecule formation and strand exchange can be used to rescue a wide variety of aberrant DNA structures. These recombinases proteins, RecA and RAD51, accomplish this stunning feat as helical protein filaments bound to the DNAs being acted on. While polymerized around single-stranded DNA in a right-handed filament, they recognize homologous duplex DNA and switch base-paired partners such that the incoming single-strand is now base paired with its complement in the double-stranded DNA partner molecule (Fig. 2) [19]. We view this process as driven by the assembly of recombinase filaments on single-stranded DNA, rearrangement of recombinase filaments and DNA partners in a complex involving both the single-stranded DNA and partner homologous double-stranded DNA and finally disassembly of recombinase filaments from the product double-stranded DNA. Here, we review these events from work describing four stages of recombinase action that can be distinguished during homologous recombination; filament nucleation, filament growth, homologous DNA pairing and strand exchange, and filament dissociation. Strand exchange is catalyzed by this sequence of recombinase–DNA and recombinase protein–protein interactions coupled to ATP binding and hydrolysis. The intervention of different mediator proteins is needed to coordinate strand exchange with the other steps of homologous recombination repair and to tailor the process to the needs of specific aberrant DNA structures.

The recombinase nucleoprotein filament, the catalytic core of homologous recombination, has been extensively studied and its description reflects the tools available for analysis. Initially, electron microscopic (EM) images showed that RecA and Rad51 form extensive right-handed helical filaments around DNA that are highly symmetrical and regular [20]. However, it is difficult to envision how a regular and static nucleoprotein filament can go through the transitions required for recombination, which involve interaction with single-stranded DNA at initiation, three DNA strands during strand exchange and double-stranded DNA near completion of recombination. Not surprisingly, subsequent analyses of three-dimensional reconstructions from EM images and comparison with higher resolution crystal structures revealed structural variation among nucleoprotein filaments [21–23]. Most notably filament pitch varied considerably and could be correlated to status of bound nucleotide cofactor. Scanning force microscopy (SFM) images revealed irregular structure and provided the prelude to more recent insight into the dynamic nature of the nucleoprotein filaments [24]. The ability to quantitatively determine dynamic interactions of recombinase proteins with DNA arrived with the development of tools to manipulate, observe and detect changes in single DNA molecules in real-time [25]. The kinetic details obtained from following recombinase nucleoprotein filament dynamics at different stages reveal steps likely to be influenced by mediator proteins that modify and optimize strand exchange for different circumstances.

4. Recombinase filament nucleation

Nucleation, the initial association of recombinase protomers with DNA, lays the foundation for nucleoprotein filament forma-

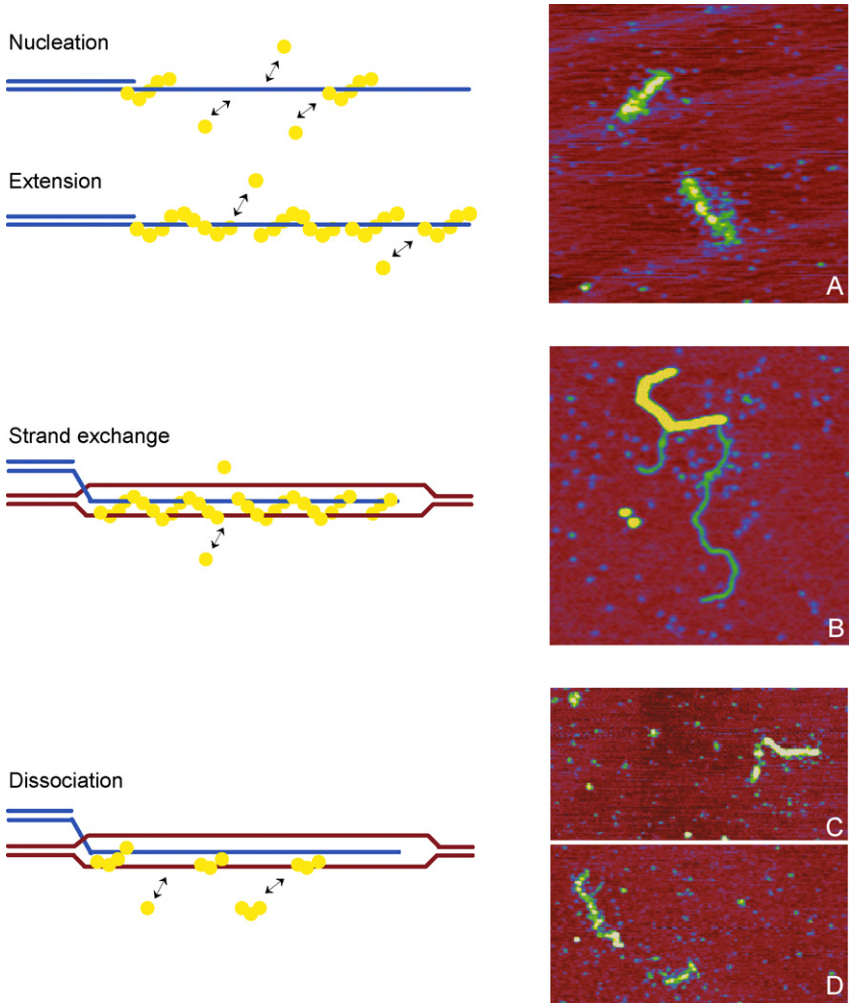

Fig. 2. Recombinase nucleoprotein filament dynamics during homologous recombination. In the panels on the left, the interaction of recombinase protomers with different DNA substrates is indicated during recombinase filament nucleation, extension, strand exchange and dissociation. The reversible interaction (indicated by the double headed arrows) of recombinase protomers (shown in yellow) with DNA (red and blue lines) allows mediator proteins to influence all stages shown as discussed in detail in the text. The events shown are nucleation of clusters of recombinase protomers on single-stranded DNA, recombinase filament growth, joint molecule formation with homologous duplex DNA into a structure in which base pairs are switched between complementary strands, and dissociation of recombinase protomers from double-stranded DNA upon ATP hydrolysis such that downstream steps in homologous recombination can proceed. Panels A–D on the right display SFM images of human RAD51–DNA complexes at the stage indicated by the left-hand panels. (A) Snap shot of a reaction mixture (ATP/Mg^{2+} conditions) containing assembling RAD51 filaments on 810-nucleotide single-stranded DNA molecules. The image is ~1 μm × 1 μm. The color from red to yellow represents height from 0 to 3 nm. (B) Image of a joint molecule with pairing of a 3′ single-strand tailed DNA molecule (240 nucleotides and 500 base pairs) and the homologous region of a 3-kb linear double-stranded DNA molecule formed under conditions of suppressed ATP hydrolysis (ATP/Ca^{2+}). The image is ~1 μm × 1 μm. The color from red to yellow represents height from 0 to 5 nm. (C and D) An early (C) and later (D) snap shot of a reaction mixture (ATP/Mg^{2+} conditions) containing RAD51 filaments disassembling from 1.8-kb double-stranded DNA molecules. The images are ~0.5 μm × 1 μm. The color from red to yellow represents height from 0 to 3 nm.

tion and therefore represents an important control step. Nucleation determines, at least in part, the location on DNA where recombination can occur. New quantitative insight into nucleation came from experiments following single DNA molecules, either measuring the real-time changes in DNA length as recombinase proteins bind using magnetic tweezers [26], by observing binding with fluorescent versions of the recombinase [27,28] or by real-time changes in FRET signal that occur when recombinase binding to DNA spatially separates donor and acceptor fluorophores [29,30].

RecA and RAD51 generally exhibit multiple nucleation events along a DNA molecule in a stochastic manner, although RecA shows a slight bias towards AT-rich regions [27]. However, the proteins differ strongly in nucleation rate. RAD51 nucleates on DNA up to 1000-fold faster than RecA, which implies that RAD51 filament growth is dominated by nucleation at the expense of filament extension. As absolute rates of nucleation depend on reaction conditions this comparisons is for roughly equivalent conditions or those that promote efficient strand exchange activity *in vitro*. Nucleation requires binding of 4–5 recombinase proteins [26]. Although nucleation does not require ATP hydrolysis, it is influenced by ATP binding, which is not surprising since nucleation rather than dissociation requires a stable interaction with DNA. The ATP cofactor binds at the monomer–monomer interface of recombinases in nucleoprotein filaments and stabilizes protein–protein interactions. Therefore, most studies were done in conditions suppressing ATP hydrolysis (Ca^{2+}-ATP) [31] or with the non-hydrolysable ATP analogs, ATP-γ-S and AMP-PNP. This also allows studying filament formation separate from the influence of dissociation. The joint molecule formation and strand exchange steps of homologous recombination require ATP binding but not hydrolysis. In fact suppressing ATP hydrolysis increases the efficiency of these reactions putatively by stabilizing the protein–DNA complexes formed as products.

Since recombinase nucleation is a critical step in homologous recombination this step is likely subject to regulation. It is not surprising that nucleation is sensitive to environmental condi-

tions. Observations of fluorescent protein showed that nucleation rates for RecA as well as RAD51 on double-stranded DNA are dependent on monovalent salt concentration, as expected for protein–DNA interactions largely involving ionic interactions with the DNA backbone. At higher salt concentrations nucleation onto double-stranded DNA is reduced [27,28]. This comprises a biologically relevant putative control step since mediator proteins could locally mimic high salt conditions by inhibiting binding to double-stranded DNA and thereby bias filament formation towards single-stranded DNA. While RecA already preferentially binds single-stranded DNA over double-stranded DNA, RAD51 does not show such a bias. This may indicate that RAD51-mediated recombination utilizes mediators to control loading onto the proper DNA substrate.

Control of recombinase loading must also account for activity needed at specific DNA structures, such as double-strand/single-strand transitions. In the case of human RAD51, the breast cancer-susceptibility gene 2 protein (BRCA2) is expected to direct RAD51 filament nucleation to the single-stranded DNA at the double- to single-stranded junction resulting from resection of a DSB. Structural work on BRCA2 predicts RPA-like OB-folds and a tower-domain imparting single- and double-stranded DNA binding properties, respectively, and potentially junction-binding specificity [32]. Furthermore, BRCA2 contains domains that specifically bind monomeric (BRC domains) or multimeric (TR2, exon 27 C-terminal domain) forms of RAD51 [33–35]. Experiments with peptide fragments of human BRCA2 show inhibition of RAD51 filament formation on double-stranded DNA and enhanced strand exchange activity [36]. The most compelling evidence though is derived from work on *Ustilago maydis* Brh2, which specifically directs *U. maydis* Rad51 filament nucleation to double-stranded/single-stranded DNA junctions promoting filament formation on single-stranded DNA [37]. Several studies have shown that the BRC repeats interact with RAD51 at the same place as the monomer–monomer interface [38], thus preventing filament formation on double-stranded DNA [34,36,39]. However, for single-stranded DNA binding by RAD51 such antagonizing effect by BRC4 was not observed. Hence, it was postulated that the BRC repeats within BRCA2 direct RAD51 filament formation towards single-stranded DNA [36].

For RecA the protein complex that generates the DNA substrate, a single-stranded 3′-overhang, also facilitates recombinase loading. At DNA ends this is accomplished by the RecBCD helicase/nuclease. The current model involves RecBCD unwinding DNA from an end until it encounters a specific sequence, called a χ-site, which modifies the nuclease activity resulting in production of a single-stranded DNA with a 3′ end. Interaction between the RecB subunit and RecA facilitate loading of RecA onto the newly generated single-stranded DNA to from the nucleoprotein filament [40,41].

Depending on the particular substrate onto which a recombinase is to be loaded, different nucleation effectors can be required. While RecBCD requires a DSB, the RecFOR pathway promotes homologous recombination repair of single-strand gaps, present at stalled or collapsed replication forks. RecFOR acts by modulating RecA binding to single-strand binding protein (SSB)-coated DNA. Specifically, RecF is implicated in directing RecA loading to the edges of the gaps [6]. Indeed, loading of recombinases on single-stranded DNA *in vivo* is potentially problematic, since single-stranded DNA is bound by high affinity single-strand DNA binding proteins that can interfere with filament formation. The RecBCD pathway avoids this problem by loading RecA directly onto the single-stranded DNA as it is generated. In the RecFOR pathway RecF facilitates RecA loading but the mechanism is not yet clear. Human RAD51 has to compete with RPA, the human equivalent of *E. coli* SSB, for the single-stranded DNA. As described above displacement of RPA could be mediated by junction specific seeding of a RAD51 filament [37]. For yeast, Rad52 can promote exchange of RPA for Rad51. Substoichiometric amounts of Rad52 suffice to alleviate the inhibitory effect of RPA on homologous pairing and strand exchange reactions. This mediator activity is dependent on interactions between Rad51 and Rad52 [42]. For the human proteins, although not yet demonstrated experimentally, BRCA2 has also been suggested to serve this function.

5. Nucleoprotein filament extension

Formation of a functional nucleoprotein filament requires more extensive coverage of single-stranded DNA than a single nucleation event can achieve. Filament extension formally involves binding of additional recombinase proteins adjacent to nucleated patches on DNA. The biophysical properties observed during filament extension, as additional protomers extensively cover DNA, differ for RecA and RAD51 due to their very different nucleation rates. The absolute rates are influenced by conditions but can be compared between the proteins at roughly equivalent conditions or those that promote efficient strand exchange reactions *in vitro*. While RecA nucleation is relatively infrequent and thus rate limiting, filament growth by extension is a rapid process. This high cooperativity for RecA extension results in long, continuous filaments. For RAD51 nucleation dominates such that extension is limited by the adjacent nucleation. Since nucleation is random along the DNA and every RAD51 monomer covers three nucleotides or base pairs, RAD51 filaments are not continuous, but contain gaps of bare DNA too small for an additional RAD51 protomer to bind [26–28,43,44]. Under conditions of active ATP hydrolysis the average patch size is estimated to be about 30 or 35 monomers for double-stranded and single-stranded DNA, respectively. Thus, due to differences in intrinsic rates of nucleation and extension RAD51 nucleoprotein filaments assembled *in vitro* differ from their RecA equivalents in flexibility since the bare DNA gaps in RAD51 nucleoprotein filaments can act as flexible hinges [24]. Increased flexibility of nucleoprotein filaments might be advantageous during subsequent steps of recombination as we discuss in the section ‘Homologous pairing and strand exchange’.

Currently single molecule experiments are revealing additional details of filament extension. Observations of fluorescent RecA suggest that filaments on double-strand DNA are extended by units of two to seven monomers [27], which encompasses the four to five monomers suggested for RAD51 based on magnetic-tweezers experiments [26]. Another single molecule approach, using RecA, measured differences in FRET intensity, which reports on the distance of DNA-bound acceptor and donor fluorophores that change upon RecA binding. In these experiments RecA filaments extended from double-stranded DNA over a double-stranded/single-stranded DNA junction onto single-stranded DNA. Modeling of the data suggests that RecA filaments extend one monomer at the time [29]. However, following RecA filament behavior in magnetic tweezers indicated that DNA-bound RecA monomers rearrange, a possibility that was not included in the modeling of RecA filament elongation from the FRET data [45]. Elongation of a RecA filament over the DNA junction, however, did provide a mechanism to displace SSB from single-stranded DNA. The FRET studies revealed that SSB can migrate along single-stranded DNA and be displaced by an elongating filament. Diffusing SSB could melt out secondary structures in the single-stranded DNA and transiently facilitate filament extension [29]. It is likely that a RecA filament seeded by RecBCD could displace SSB in the same fashion. In the RecFOR pathway SSB is likely displaced by RecA due to RecO and RecR interactions rather than diffusional migration of SSB.

The RecA FRET studies showed that addition of RecA can occur at both ends of a filament. However, binding of RecA took place with different rates at the respective ends. A 10-fold difference in apparent dissociation constant favored extension at the 3′ end of the RecA filament [30]. In conditions allowing ATP hydrolysis filament growth is affected by concurrent dissociation. The FRET studies revealed that the dissociation rates for both termini were similar. So the net growth of a RecA filament is modulated by a difference in binding affinities. In this way ATP hydrolysis can contribute to rearrangements within the filaments. While previous studies argued that sliding of recombinase proteins does not occur, dissociation of proteins at a 5′ end which may remain bound to single-stranded DNA and re-establishes protein–protein contacts to an adjacent 3′ end provides a plausible mechanism [43,46]. Given that a RecA monomer is reported to bind from three to seven nucleotides of single-stranded DNA depending on bound nucleotide, a change in the number of nucleotides bound in the ATP hydrolysis cycle could result in protein changing position along DNA [47]. This also infers that extension can occur by monomers since that is currently believed to be the unit for dissociation. Such rearrangements will lead to reduction of gaps within a nucleoprotein filament and potentially one continuous filament, *i.e.*, more recombinase on a given DNA molecule. Thus the maximum extension of the DNA length by recombinase would increase in conditions of active ATP hydrolysis. Indeed this was observed in magnetic tweezers experiments after switching assembly conditions from suppressed to active ATP hydrolysis [45]. If the adjacent 3′ filament end was not available, for instance due to capping by the RecA mediator RecX, this would result in a net disassembly as observed in experiments with RecA and substoichiometric concentrations of RecX.

For RAD51 such rearrangements, or rather the extent thereof, are unlikely to happen. The relatively high nucleation rate of RAD51 leads to filaments with more gaps and more ends to disassemble thus less stable filaments. Growth profiles of RAD51 filaments with suppressed ATP hydrolysis are similar on single-stranded DNA and double-stranded DNA. In the presence of ATP hydrolysis there is a difference. RAD51 assembly onto single-stranded DNA is greatly affected by dissociation such that the growth profiles differ [26]. This implies that the RAD51 filament on single-stranded DNA is a very dynamic structure and indeed it can hardly be visualized (without fixation) as a defined structure by SFM or EM [24]. The dynamic nature of recombinase filaments in ATP hydrolysis permitting environments is likely to play an important role in later steps of strand exchange reactions.

6. Homologous pairing and strand exchange

The ability to address mechanistic questions is strongly influenced by technical advances and sensitivity of measurements. As discussed above, single molecule experimental setups allowed new mechanistic questions to be addressed. Magnetic tweezers were also applied to answer questions about homologous pairing and stand exchange. Interestingly, while a signature strand exchange by RecA was observed in real time, no signals were detected of homologous paring, a necessary preceding step. Based on the detection limits of the instrument this suggests that initial homologous paring interactions involve less than 16 base pairs lasting less than one second. Strand exchange was detected as a real-time change in length of a tethered double-stranded target DNA upon interaction of a single-strand RecA nucleoprotein complex. The experiments revealed that, under the employed experimental conditions, the active synaptic region involved in strand exchange was independent of the length of DNA exchanged and remained a constant 80 base pairs [48].

As pointed out above differences in intrinsic nucleation rates between RecA and RAD51 result in nucleoprotein filaments with distinct biophysical characteristics; continuous and relatively stiff for RecA versus interrupted and flexible for RAD51. During homology search flexible filaments can allow multiple sites of interaction between filament segments and the homologous target DNA, while stiffer continuous filaments would be limited in that respect. Additionally, the bare single-stranded DNA in filaments can act as a swivel to relieve potential topological constrains arising during recombination between helical filaments and target DNA. Mediator proteins in the cell might increase the flexibility of RecA filaments *in vivo*. For example, RecX bound in the major groove of a filament as a cap thereby blocking further addition of RecA [6,49,50]. This could introduce gaps in RecA filaments and thereby increase flexibility in the nucleoprotein filament. Mediators such as RecX might not be required for homologous recombination in general but instead could assist RecA-mediated recombination in specific subpathways, such as during recombination-assisted stalled replication fork restart.

In general, differences in DNA/chromatin organization in bacterial and eukaryotic cells may require recombinase nucleoprotein filaments with different biophysical properties. The target DNA substrate in bacteria might retain a certain degree of flexibility itself such that efficient homology search and subsequent strand exchange can occur with stiffer recombinase nucleoprotein filaments. In eukaryotes the situation is different since target DNA is arranged in chromatin. Recent studies suggest that DSBs in heterochromatin can be repaired by homologous recombination [51,52]. In that context the DNA is wrapped around histones in a highly ordered structure. Hence, an increased flexibility of the RAD51 nucleoprotein filament would be required to efficiently probe DNA wrapped around histones.

The size of recombinase filament patches might influence their ability to bypass heterology. The shorter they are the less probable that they can stabilize longer stretches of mispaired bases. In agreement with this hypothesis the longer stiffer RecA filaments are able to bypass larger heterologous stretches [53–55], while Rad51 is very inefficient in bypassing heterologous regions of more than several nucleotides [54]. In diploid eukaryotes it might be important to have a less promiscuous DNA strand exchange machinery to reduced loss of heterozygosity. Crystal structures reveal that RecA–single-stranded DNA complexes have three nucleotides bound per monomer arranged in approximate B-form DNA structure, with DNA stretching occurring between the triplets [56]. Therefore, it is likely that the nucleoprotein filament probes target duplex DNA in units of three nucleotides. RAD51 filaments apparently allow for one such unit to mis-match. Further mis-matches would be expected to destabilize the interactions and stall plectonemic joint formation to favor continued homology search. The size of filament patches may not only be important for the flexibility of the nucleoprotein filament but also for its accuracy in homologous pairing.

During strand exchange and subsequent repair reactions DNA structures arise that can be stabilized by structure-specific binding of proteins, *e.g.*, RAD51AP1 [57,58]. Each of these DNA structure–protein interactions can serve as control points in recombination through stabilization or destabilization of an intermediate. This could promote recombination or limit the extent of recombination as needed. For instance, some proteins specifically favor the formation and stability of Holliday junctions. Additionally, yeast Srs2 helicase promotes SDSA likely by remodeling DNA intermediates of strand exchange in favor of this process. By similar remodeling of strand exchange intermediates, human RAD54 is implicated in both directing repair toward formation of double Holliday junctions or toward SDSA [59].

7. Recombinase dissociation from DNA

Dissociation of recombinase from nucleoprotein filaments is an intrinsic property dependent on ATP hydrolysis [43]. *In vitro* ATP binding, but not hydrolysis, is required for the formation of nucleoprotein filaments, formation of joint molecules, and strand exchange [31,60,61]. Nonetheless dissociation occurs even during filament assembly, especially on single-stranded DNA. This filament formation as a balance between protein association with and disassociation from DNA can result in inefficient formation of filaments on single-stranded DNA *in vitro* and explain why the above mentioned reactions are more efficient in conditions where the ATPase activity is attenuated. One can argue that mediator proteins act *in vivo* to temporarily suppress recombinase ATPase activity at sites of DNA repair or stabilize filaments in a different way. A bacterial candidate for such an activity is DinI, which stabilizes RecA filaments while not directly affecting ATPase activity [62]. This activity of DinI might counteract RecA dissociation from filaments formed on correct DNA substrates in need or recombinational repair, *i.e.*, at a DSB. Recruitment of proteins such as Dinl to sites of DNA damage but not to sites on DNA where homologous recombination would be deleterious would constitute a control point taking advantage of nucleoprotein dynamics, which favor disassembly without the need for other proteins.

Direct observation and quantification of fluorescent variants of RAD51 show intrinsic dissociation behavior that can be interpreted in terms of ATP hydrolysis [43,63]. RAD51 filaments disassemble from an end in a stochastic manner with bursts of varying numbers of monomers separated by pauses of varying time. This is explained by ATP hydrolysis randomly taking place at any position within the nucleoprotein filament but dissociation only occurring when the terminal monomer hydrolyses ATP. Internal RAD51 monomers that have hydrolyzed ATP are kept in place through protein–protein interactions in the filament. Upon hydrolysis of ATP by a terminal RAD51 monomer the interface with the only neighbor is broken. This breaks the structure that stabilized stretched DNA across the monomer interface and the released energy stored in DNA by the filament would drive the dissociation of the RAD51 monomers. Monomers would dissociate from the end of a patch until the point where ATP is still bound [63]. While RAD51 is able to dissociate from double-stranded DNA by itself the dissociation rate is dependent on ATP hydrolysis and slower from double-stranded than from single-stranded DNA. Thus, RAD51 dissociation might be too slow for efficient subsequent reactions *in vivo* or persist at the wrong placed posing a threat for unwanted recombination events. Hence, it has been postulated that other proteins act on recombinase filaments to promote their disruption.

Elevated levels or inappropriate recombination can lead to undesired recombination events resulting in genetic instability. Thus the activity of recombinase filaments to form joint molecules and accomplish strand exchange needs to be carefully controlled and properly targeted. For this purpose there are a variety of remodeling enzymes that antagonize the activities of recombinase nucleoprotein filaments. Globally a variety of helicases can disrupt toxic recombinase filaments. Their redundant activities may reflect the importance of this task but in some cases reveal specificity for certain repair pathways. At persisting stalled replication forks in *E. coli* the RecFOR-loaded RecA can activate the SOS response, which will lead to expression of a set of proteins including UvrD [64]. This helicase is implicated in rescuing replication by fork reversal, a process blocked by the presence of RecA filament or RecA-created DNA structures. UvrD can clearly antagonize homologous recombination *in vivo.* One putative mechanism is the active removal of the RecA filament by ATP-dependent translocation of UvrD along the DNA, thus allowing reversal of replication forks and repair by repair pathways other than recombination [64,65]. *In vitro* many helicases or translocating proteins can disassemble recombinase filaments.

The helicase associated with Rad51 disassembly in yeast is Srs2. It has an important function in controlling inappropriate recombination. Where Rad52 promotes displacement of RPA by Rad51 filaments, Srs2 antagonizes this activity. Thus Rad51 filament formation is controlled by the interplay between Rad52 promoted assembly and Srs2 promoted disassembly [66–68]. Srs2 acts upon Rad51 filaments in a more specific manner than UvrD disruption of RecA filaments. The Srs2 helicase activity can push protein off of DNA but it also specifically enhances Rad51 disassembly. Extensive studies with truncation mutants of Srs2 reveal that specific interactions between Srs2 and Rad51 stimulate ATP hydrolysis by Rad51 promoting its intrinsic dissociation from DNA [66].

While DNA translocating enzymes can have anti-recombination function, such as UvrD and Srs2 discussed above, they can also promote recombination. For example, the Rad54 protein requires double-strand DNA to activate its ATPase activity and thereby trigger its translocation activity. Rad54 can thus remove Rad51 from double-stranded DNA, which is either the product of strand exchange or an inappropriate substrate for filament formation [59]. Rad54-mediated removal of Rad51 from the double-stranded DNA product of recombination actually stimulates recombinational repair because it allows DNA transactions downstream of strand exchange to occur that might otherwise be blocked by bound Rad51. Promoting Rad51 dissociation by Rad54 requires species-specific interactions between the proteins [69]. The detailed studies on intrinsic RAD51 dissociation, described above, suggest a mechanism for RAD54 catalyzed recombinase filament disassembly [28,59]. RAD51 filament stability crucially depends on the nucleotide state of the terminus protein in a filament. When a terminal RAD51 monomer is in the ATP-bound state it functions as a sort of cap that inhibits disassembly. Within a filament, RAD51 monomers appear to be locked on the DNA by their neighbors, independent of nucleotide state [63]. Rad54 can interact at filament ends [70]. The prediction is that protein–protein interactions between RAD54 and the terminal RAD51 would stimulate ATP hydrolysis here and thus RAD51 disassembly from DNA. As a result, RAD54 would accelerate disassembly by substantially decreasing the duration of pauses that occur during unaided RAD51 nucleoprotein filament disassembly.

8. Conclusion

Regulating DNA strand exchange, the salient step of homologous recombination, is obligatory to both actively prevent genome instability and promote genome stability. Modulation at any of the component steps in DNA strand exchange; nucleation of recombinase proteins on DNA, nucleoprotein filament growth, strand exchange, and recombinase dissociation from DNA, provide rich regulatory possibilities and allows controlling recombination to fit different repair requirements. The key to this regulation, essentially providing quality control for homologous recombination, lies in the dynamic instability of the recombinase nucleoprotein filament. The recombinase nucleoprotein filament is not a straightjacket in which homologous pairing and DNA strand exchange take place with machine-like precision. Instead, it is a meta-stable entity undergoing constant association of recombinase molecules with DNA and ATP hydrolysis mediated dissociation from DNA. This dynamic instability of interacting molecular partners allows constructive and destructive processes to occur in competition. The relative success of concurrent structural build-up and tear down tips the balance controlling inappropriate as well as appropriate reaction steps. Quality control or progression down specific homologous recombination subpathways can then be established by factors

that slightly shift the equilibrium to eventually favor appropriate events.

Tight regulation of filament formation itself is an obvious control step early in the reaction. Indeed, the first obstacle to forming a recombinase nucleoprotein filament is the presence of high affinity single-stranded DNA-binding proteins that need to be displaced. Mediator proteins, such as Rad52 in yeast, promote RPA displacement by Rad51, after which single-stranded DNA binding proteins actually promote later steps leading to efficient strand exchange reactions [42]. Thus, a single protein can antagonize filament formation and yet promote strand exchange. This paradox is also evident in studies of BRCA2, where high concentrations of BRC peptide inhibit recombinase filament formation, while lower concentrations can stabilize filaments [34,39]. Even after a nucleoprotein filament has formed on single-stranded DNA, completing homologous recombination is not inevitable because mediators actively dissociated recombinase filaments on single-stranded DNA, such as Srs2 in yeast. Recombination is subject to a balance between positive and negative mediators, in this case Rad52 and Srs2. This theme is present throughout the steps of homologous recombination; intermediate structures arise and can disassemble, either by the effect of mediator proteins or intrinsically. Progression to subsequent steps of recombination requires appropriate stabilization of these intermediates. For instance, the intermediate DNA (branched) structures of strand exchange are also intrinsically metastable and can be stabilized or destabilized by structure-specific DNA proteins, such as RAD51AP1 [57].

The influence of recombination mediators on nucleoprotein filaments assembly, stability, and interactions with partner DNA as well as recombination mediator effects of DNA structures emerging during progression of recombination, ensures that genome maintenance by homologous recombination is not only a flexible process, engaged in repair of multiple structurally diverse lesions, but also an accurate process.

Conflict of interest

The authors confirm there is no conflict of interest, financial or otherwise, in this work.

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