Replication Protein A prevents promiscuous annealing between short sequence homologies: Implications for genome integrity

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Abstract

Replication protein A (RPA) is the main eukaryotic single-stranded DNA (ssDNA) binding protein, having essential roles in all DNA metabolic reactions involving ssDNA. RPA binds ssDNA with high affinity, thereby preventing the formation of secondary structures and protecting ssDNA from the action of nucleases, and directly interacts with other DNA processing proteins. Here we discuss recent results supporting the idea that one function of RPA is to prevent annealing between short repeats that can lead to chromosome rearrangements by microhomology-mediated end joining or the formation of hairpin structures that are substrates for structure-selective nucleases. We suggest that replication fork catastrophe caused by depletion of RPA could result from cleavage of secondary structures by nucleases, and that failure to cleave hairpin structures formed at DNA ends could lead to gene amplification. These studies highlight the important role RPA plays in maintaining genome integrity.

Keywords

chromosome rearrangements; DNA replication; end joining; homologous recombination; Mre11; RPA; Rad51; Sae2

Introduction

RPA is a heterotrimeric complex consisting of 70, 32 and 14 kDa subunits that binds with high affinity to single-stranded DNA (ssDNA) [1, 2]. RPA was originally identified as a factor required for in vitro reconstitution of simian virus 40 (SV40) replication [3]. These studies demonstrated an important role for RPA in stabilizing the unwound ssDNA generated by SV40 large T antigen at the replication origin, and in stimulating the elongation phase of DNA synthesis [2]. RPA is highly conserved among eukaryotes and the genes encoding each subunit, *RFA1, RFA2* and *RFA3* in budding yeast (*RPA1, RPA2* and *RPA3* in vertebrates), are essential for viability [4]. In addition to its essential role during DNA synthesis, RPA functions in most DNA repair reactions that involve ssDNA

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intermediates, including nucleotide excision repair, mismatch repair and homologous recombination (HR), and plays critical roles in DNA damage signaling [2, 5]. The role of RPA in these diverse DNA metabolic processes is to remove secondary structures from ssDNA that would interfere with DNA processing, to shield ssDNA from nucleases, and to interact physically and functionally with other DNA processing proteins. Here, we will discuss how RPA bound to ssDNA stabilizes fragile intermediates during DNA double-strand break (DSB) repair and thereby prevents genome rearrangements.

RPA: A modular DNA binding protein

RPA interacts with ssDNA with a defined 5′-3′ polarity using four OB (oligonucleotide/oligosaccharide binding) folds, also referred to as DNA binding domains DBD-A, -B –C and –D located in the RPA70 and RPA32 subunits (Fig. 1) [1, 6–11]. Two additional OB domains are present in RPA70 and RPA14, but do not contribute to DNA binding [1, 7]. RPA binds to ssDNA in two modes: the low-affinity mode has an occluded binding site of ~8 nucleotides (nt) and involves DBD-A and DBD-B; the high-affinity mode engages all four DBDs and has an occluded binding site of ~30 nt [6]. As an individual domain, DBD-A has the highest affinity for ssDNA and the affinity increases by ~100-fold for the DBD-A-DBD-B peptide [1, 6]. A stepwise transition to the high-affinity mode has been suggested: this involves sequential binding of the DBD-A and DBD-B domain, DBD-C and then DBD-D [1]. The DBD-C and DBD-D domains of RPA70 and RPA32, respectively, are also required for inter-subunit interaction, and the OB fold of RPA14 interacts with DBD-D connecting the three subunits and stabilizing the complex (Fig. 1).

Binding and extension of ssDNA through the removal of secondary structures has been directly visualized by single molecule imaging using a fluorescently tagged variant of yeast RPA (RPA-eGFP) [12]. RPA rapidly extended ssDNA and remained bound to the substrate with little or no dissociation for more than 60 min when free protein was absent from the solution [12, 13]. However, the bound RPA-eGFP could be rapidly replaced with free RPA-mCherry, or with another ssDNA binding protein, when added to the flow cell [13]. The slow dissociation of RPA, but ability to be replaced, suggests RPA undergoes constant local dissociation of the DBDs, and that complete dissociation of the complex only occurs when free protein present in solution competes with the bound form as a result of mass action. This mode of binding is consistent with the modular organization of the DBDs, and ensures that ssDNA remains protected by RPA, but allows RPA to be displaced by other ssDNA binding proteins when necessary.

In addition to DNA binding, RPA interacts directly with a large number of DNA replication and repair proteins via the N-terminal OB fold of RPA70 and the winged helix domain in the C-terminus of RPA32. The N-terminal region of RPA32 undergoes extensive phosphorylation during the cell cycle and in response to DNA damage [14]: indeed, antibodies directed against specific RPA32 phosphorylation sites can be used to monitor activation of the ATR and ATM kinases in human cells by immunoblotting [15]. RPA1 is also SUMOylated after treatment of human cells with agents that generate DSBs, and this modification increases the interaction between RPA and RAD51 to promote homology-directed repair [16].

Many ways to repair a broken chromosome

Chromosomal DSBs are considered one of the most toxic lesions to the cell, and their accurate repair is essential to maintain genome integrity. Failure to repair DSBs or inaccurate repair can result in loss of genetic information, gross chromosome rearrangements (GCRs) or even cell death. DSBs are repaired by one of two main mechanisms: homologous recombination (HR) or non-homologous end joining (NHEJ) (Fig. 2). HR requires a homologous DNA duplex to template repair of both broken strands, and is generally restricted to the S and G2 phases of the cell cycle when a sister a chromatid is available as a donor duplex [17]. NHEJ involves the direct ligation of the DNA ends and is considered to be more error-prone than HR. NHEJ is the primary mechanism to repair DSBs in human cells, whereas HR is the predominant mechanism in bacteria and yeast. The biased use of HR in organisms with compact genomes may be to minimize mutations caused by erroneous NHEJ in coding DNA. On the other hand, for organisms with many repeated sequences HR needs to be tightly regulated because associated crossovers can generate chromosome rearrangements.

An essential first step for HR is the 5′-3′ nucleolytic degradation of the DNA ends to produce 3′ ssDNA tails, a process referred to as end resection [18]. The Mre11-Rad50-Xrs2/NBS1 (MRX/N) complex and Sae2 (CtIP in mammalian cells) initiate resection by endonucleolytic cleavage of the 5′-terminated strand ~50–100 nt from the end, creating a short overhang sufficient for high affinity RPA binding [19]. More extensive resection to generate long 3′ ssDNA tails requires the Exo1 nuclease or Sgs1 helicase in conjunction with Dna2 endonuclease [20]. RPA binds to the ssDNA generated by end resection and is frequently used as a cytological marker for resection of DSBs [21, 22]. Because efficient recombination requires >200 nt homology [23], RPA stabilization of ssDNA intermediates is particularly important for this cellular process. In order for HR to proceed, Rad51 has to displace RPA from the ssDNA, a reaction that requires the Rad52 or BRCA2 mediator proteins [24–28]. Once Rad51 is bound to the 3′ ssDNA tail it catalyzes pairing with a homologous duplex, and exchanges one strand of the duplex with the incoming ssDNA tail to form a displacement loop (D-loop) [18]. The 3′ end of the invading strand is then used to prime DNA synthesis, templated by the donor duplex. In the simplest version of recombination models, the extended invading strand is displaced by the action of a helicase and can then pair with the resected end at the other side of the DSB [29]. Alternatively, the displaced strand of the D-loop pairs with the other DSB end and after gap filling and ligation a double Holliday junction intermediate is formed, which is subsequently processed to form crossover or non-crossover products [30].

NHEJ involves direct joining of the fragmented chromosome through end protection by the Ku complex and ligation via DNA ligase IV [31]. NHEJ can join blunt or complementary cohesive ends (such as those produced by restriction endonucleases), or more complex ends with the assistance of end processing factors. The initiation of end resection is inhibitory to end joining because Ku has reduced affinity for long ssDNA overhangs relative to blunt ends [32]. RPA binding to ssDNA-tailed duplexes is likely to create an additional impediment to Ku binding.

In the absence of Ku and Ligase IV, end joining can still occur at a significant frequency in mammalian cells and at a much lower frequency in budding yeast [33–36]. The more efficient use of MMEJ in mammalian cells is likely due to proteins that can synapse and ligate ends, such as PARP-1 and DNA ligase III, respectively [37–40], that are absent from budding yeast. The alternative end joining mechanism in yeast utilizes microhomologies (MH) in the range of 5–20 nucleotides internal to the ends to align them for repair, and consequently has been named microhomology-mediated end joining (MMEJ) [35, 41]. Alternative end joining in mammalian cells also entails use of MH; however, these tracts tend to be shorter than those observed in yeast cells [34, 42, 43]. Interest in the mechanism of MMEJ has grown with the recognition that many chromosome rearrangements associated with human genetic disease, including translocations, copy number variants and chromothripsis, have MH at the breakpoints [44–46]. End resection is needed to expose MH internal to the ends; thus, MMEJ has some of the same requirements as those for HR (Fig. 2). MMEJ is similar to another DSB repair mechanism called single-strand annealing (SSA) that operates between long direct repeats flanking a DSB [18]. SSA requires sufficient resection to expose complementary strands of each repeat, the homologous sequences are annealed and, if necessary, 3′ heterologous flaps are removed by nucleases to allow DNA synthesis and ligation. In yeast, SSA is highly dependent on *RAD52* and is independent of *RAD51* [18].

RPA plays a critical role in homologous recombination

Much of our understanding of how RPA functions during HR derives from reconstitution of early steps of DSB repair with purified components. In vitro reconstitution of the extensive resection mechanisms revealed an essential role for RPA in Sgs1-Dna2 catalyzed resection, and a stimulatory role with Exo1. RPA stabilizes DNA unwound by the Sgs1 helicase and specifically targets the 5′-terminated strand for degradation by Dna2 [47, 48]. RPA acts indirectly to promote Exo1-mediated resection by preventing non-productive association of Exo1 with ssDNA [49]. Biochemical reconstitution of Rad51-catalyzed strand exchange demonstrated a crucial role for RPA to remove secondary structure from the ssDNA; thus facilitating polymerization of Rad51 to form the Rad51-ssDNA nucleoprotein filament (Fig. 2) [50, 51]. For RPA to promote Rad51 binding to ssDNA, Rad51 must be added to the reaction before RPA. If the proteins are present at the same time, resembling physiological conditions, then Rad52 or BRCA2 is required to facilitate RPA displacement by Rad51 [24–28]. Rad52 binds directly to both Rad51 and RPA, providing a high local concentration of Rad51 to compete with RPA for DNA binding [52–54].

Rad52 also promotes annealing of ssDNA in vitro and this activity is likely important for capture of the second end in HR and for the SSA mechanism of recombination [55–58], RPA is inhibitory to ssDNA annealing in vitro; however, Rad52 is able to overcome this inhibition [59]. Short complementary oligonucleotides (~50 nucleotides in length) undergo efficient spontaneous annealing and the rate is greatly accelerated by Rad52, but for longer ssDNA with the potential to form extensive secondary structure, Rad52-catalyzed annealing requires RPA [55, 56, 59].

Because all of the subunits of RPA are essential for cell viability, studies to elucidate RPA function during HR in vivo have relied on use of conditional or hypomorphic alleles to determine the cellular consequence of RPA loss or malfunction. The budding yeast, *Saccharomyces cerevisiae*, has been particularly informative for the genetic studies. Random mutational analysis of the *RFA1* and *RFA2* genes identified alleles with a temperature sensitive growth defect and/or sensitivity to DNA damaging agents [60–62]. The *rfa1-t11* mutant is extremely defective for both *RAD51* dependent strand invasion and *RAD51*-independent SSA [62, 63]. Biochemical analysis revealed much slower displacement of RPA^{t11} by Rad51, indicative of higher binding to ssDNA [64]. In contrast, the RPA^{t33} and RPA^{t48} mutant complexes show less stable binding to ssDNA [65], but are also defective for DSB-induced HR [62, 66]. Hypomorphic alleles of *RFA1* have also been recovered from genetic screens for altered frequencies of HR. The *rfa1-44* mutant is defective for DSB-induced recombination and exhibits genetic interaction with *RAD52* [67], whereas the *rfa1-D228Y* mutation was recovered as a suppressor of the SSA defect of the *rad52* mutant [68]. *RAD52* is required to promote annealing between the resected repeats during SSA; however, when Rfa1 is impaired by the D228Y substitution, SSA can occur in the absence of *RAD52*. Presumably the RPA^{D228Y} complex is still able to remove most of the secondary structures from ssDNA, but is less efficient in preventing spontaneous annealing [69]. It is interesting to note that the lower limit for Rad52 to promote annealing in vivo is ~14 nt [70], the transition point at which most sequences are unique in yeast. Thus, there is a balance in which RPA prevents strand annealing and Rad52 promotes annealing when the homologous stretches are sufficiently long that the sequences are likely to be unique. This minimizes illegitimate recombination events.

GCRs are elevated when RPA is dysfunctional

The yeast *CAN1* gene is frequently used to measure forward mutation rates because loss of function results in resistance to the drug canavanine (Can^R). RPA was first recognized as a suppressor of GCRs by the unusual spectrum of spontaneous Can^R mutations recovered from *rfa1* mutants [71]. Although the mutation rate in *rfa1* mutants was only moderately elevated compared to wild type, several of the Can^R events analyzed from *rfa1* mutants were generated by large deletions or translocations, in contrast to *CAN1* inactivation by point mutations in wild-type cells. The assay was refined to specifically detect GCRs by inserting a second counter-selectable marker (*URA3*) adjacent to *CAN1* and then measuring simultaneous loss of both markers [72]. Because *CAN1* is within a non-essential region of chromosome V, complete loss of the terminal fragment encompassing the *CAN1* locus is compatible with viability in haploid cells. The frequency of GCRs was extremely low in wild-type cells, and most of the events recovered resulted from loss of the terminal chromosome fragment containing the *URA3* and *CAN1* genes followed by de novo telomere addition. Several *rfa1* and *rfa2* mutants were tested in this assay and showed a 50–5,000-fold elevated rate of GCRs relative to wild type [72]. Furthermore, many of the GCR events recovered from *rfa1* mutants were due to translocations, deletions or inversions that had MH at the junctions. The *rfa1* mutational signature is unique; GCRs also occur at greatly elevated frequencies in the *mre11* mutant, but are not mediated by MH [72].

The role of RPA in suppressing GCRs is not restricted to yeast. The *rfa1-t48* mutation, which confers a 5,000-fold increase in the frequency of GCRs, has been modeled in mouse cells [73]. Although yeast cells harboring the *rfa1-t48* mutation are viable, mice homozygous for the *Rpa1^{t48}* allele display embryonic lethality. The *Rpa1$^{t48/+}$* heterozygous animals were viable, but they had a reduced lifespan and developed lymphomas [73]. Tumors analyzed from the heterozygous animals exhibited multiple genome alterations, including chromosome aneuploidy and segmental gains or losses. The breakpoints of rearrangements were not sequenced, and it would be of interest to determine whether they exhibit increased use of MH.

The increased frequency of chromosome translocations with MH at the junctions in *rfa1* mutants could be due to a defect during DNA synthesis resulting in more DSBs and subsequent channeling of DSBs to a repair mechanism rarely used in wild-type cells. A recent study by Deng et al. supports the notion that RPA suppresses mutagenic repair of DSBs by MMEJ [65]. As noted above, MMEJ requires resection to reveal MH internal to the break ends and is driven by the annealing between short MH (>5 nt) [35]. The length, percent homology and G-C content of the MH are determinants of the frequency of MMEJ in yeast, suggesting that the process is spontaneous and thermodynamically driven [70, 74, 75]. Using a genetic assay to detect MMEJ between 12 bp direct repeats flanking a site-specific DSB, Deng et al. showed that MMEJ occurs at very low frequencies in wild-type cells, whereas several of the *rfa1* hypomorphic mutants tested showed greatly elevated frequencies of MMEJ (Fig. 3) [65]. The *rfa1-t48* mutant displayed the greatest increase in MMEJ (350-fold higher than wild type), consistent with the high frequency of GCRs reported by Chen and Kolodner [72]. The *rfa1-t48* mutation results from substitution of a highly conserved leucine at position 221 of Rfa1 with proline. L221 is within DBD-A and a site of direct contact with ssDNA [6]. The purified yeast RPA^{t48} mutant complex exhibited reduced DNA binding in vitro and was unable to fully extend ssDNA, indicating that it is impaired in removal of secondary structures [65]. The human RPA^{L221P} mutant complex showed a greater defect in DNA binding than observed for the yeast RPA^{t48} complex, and was unable to support SV40 DNA replication in vitro, in agreement with the lethal phenotype conferred by this mutation in mouse cells [73,76]. The *rfa1-D228Y* and *rfa1-t33* alleles, which encode proteins with amino acid substitutions in DBD-A and DBD-B, respectively, that do not make direct contacts with DNA [6], also increased MMEJ, but not to the same extent as *rfa1-t48* [6, 65]. MMEJ was not increased in the *rfa1-t11* mutant, consistent with the RPA^{t11} complex retaining high affinity DNA binding. These studies highlight an important role for RPA in preventing promiscuous annealing between short repeats during DSB repair in yeast.

RPA facilitates extensive resection and shields 3′ ends from degradation

The role of DNA end resection is to create a ssDNA substrate for Rad51 polymerization and a 3′ end that can be extended by DNA synthesis following Rad51-catalyzed strand invasion. Most studies of end resection in budding yeast indicate that the 3′ ends at a DSB remains stable for several hours [77, 78]. To test the hypothesis that RPA protects the ssDNA formed by end resection against degradation, Chen et al. [79] used a heat-inducible *rfa1* degron allele (*td-RFA1*) to rapidly deplete RPA from cells at the same time as the induction of a

site-specific DSB by the HO endonuclease. Resection initiation was unaltered by depletion of RPA, but extensive resection was almost completely abolished. Furthermore, the short 3′ ssDNA tails generated by MRX and Sae2 were unstable, indicating that RPA is indeed directly protecting ssDNA from improper processing.

The block to extensive resection caused by RPA depletion is in part due to failure of Dna2 to localize to DSBs by its direct interaction with RPA [79, 80]. However, the Exo1-dependent extensive resection pathway was also blocked by RPA depletion in vivo, and Exo1 has not been found to physically interact with RPA and can localize to break sites independently of RPA [79]. One possible explanation is that naked ssDNA with secondary structure is a poor substrate for Exo1 nuclease. In support of this view, Cannavo et al. [49] showed that Exo1 acts preferentially on a dsDNA end with a 3′-ssDNA overhang and unprotected ssDNA can sequester free Exo1.

Both Rad52 and Rad51 failed to localize to DSB sites upon loss of RPA [79, 81]; thus, preventing the repair of DSBs by HR. The dependence of Rad52 and Rad51 recruitment on RPA can be partially explained by their direct interactions, but could also be due to the lack of a suitable substrate [53, 82]. Similarly, ATRIP/Ddc2 localization to damage sites is reduced when RPA is depleted from cells [5], likely due to loss of both long tracts of ssDNA and the direct association of the ATRIP/Ddc2 with RPA.

DNA secondary structures are potential targets for nucleases

In addition to 3′ strand loss, RPA depletion also resulted in the formation of fold-back structures by intramolecular pairing between short inverted repeats (5–9 bp) in the 3′ ssDNA overhang formed by end resection, followed by DNA synthesis and ligation to form hairpin-capped ends (Fig. 3) [79]. These DNA secondary structures could be dangerous for cells for several reasons. First, they are likely an unfavorable conformation to be recognized by DNA repair factors that require ends or ssDNA for binding; thus, perturbing end resection and subsequent HR repair.

Second, the hairpin structures at break ends resulting from RPA depletion are potential substrates for structure-selective endonucleases. Eukaryotic cells encode several nucleases that cleave at ssDNA-dsDNA transitions and that could potentially target secondary structures in ssDNA [83]. The Mre11-Rad50 complex is able to cleave hairpins in vitro and genetic studies support a role for MRX in resolving hairpin-capped ends [84, 85]. Sae2 is also required for resolving hairpin structures formed at long inverted repeats in vivo [84], even though MR can cleave model hairpins in vitro in the absence of Sae2 [86]. The hairpin-capped DNA ends formed after RPA depletion were stabilized in *mre11* and *sae2* mutants [79]. Indeed, it is possible that one of the many functions of MRX and Sae2 at DSBs is to remove fold-back structures formed inadvertently at short inverted repeats within the ssDNA tails the result from resection. Although hairpin-capped ends were not detected at the site of a single DSB unless RPA was depleted, fold-back structures have been identified at meiotic DSBs in recombination-defective *rad52* cells [57]. This suggests that hyper-resection at multiple DSBs (in budding yeast, ~150 DSBs are generated during meiosis) due to deficient HR might exhaust the nuclear pool of free RPA.

Pathological RPA depletion can be caused by ATR deficiency in human cells under replication stress, where unscheduled origin firing generates excessive amounts of ssDNA [15]. This global exhaustion of RPA leads to the simultaneous breakage of all active forks, known as replication catastrophe. How depletion of RPA causes DNA breakage at replication forks is not known, but one attractive hypothesis is for MRX/N-Sae2/CtIP to cleave secondary DNA structures formed within unprotected ssDNA. Alternatively, the stalled replication forks resulting from replication stress, or forks remodeled by DNA translocases to form structures resembling Holliday junctions, could be acted on by the Mus81-Mms4/EME1 and/or Slx1-Slx4 endonucleases resulting in DSBs [87–89]. The pool of free RPA also becomes limiting when mouse embryonic fibroblasts deficient for DNA polymerase ζ are treated with UV light due to sequestration of RPA at damaged replication forks, and this interferes with global nucleotide excision repair [90, 91].

Third, it is also possible that certain types of DNA secondary structures formed when RPA is dysfunctional escape cleavage or cannot be processed by nucleases. Replication of a chromosome fragment with a hairpin-capped end would result in the formation of a giant palindromic chromosome (Fig. 3). If the chromosome fragment contained a centromere it would become dicentric and could then be broken at mitosis and undergo further rearrangements. In support of this idea, palindromic gene amplification at telomeres has been reported in telomerase- and recombination-defective yeast as a means to escape senescence [92]. DNA sequence analysis provided evidence for pairing between short (5–9 bp) inverted repeats within the ssDNA formed by resection from the telomeres. We speculate that RPA is depleted as a result of extensive degradation of many chromosome ends, and that the ssDNA forms fold-back structures. Similar types of event have been reported at a site-specific DSB adjacent to a large inverted repeat, but their recovery was only seen in the absence of Sae2 or MRX, presumably because these factors normally process hairpin-capped ends [93, 94].

Conclusions and outlook

Here, we have focused on the important role that RPA plays in preventing inter- and intra-molecular annealing between short repeats during the repair of DSBs. It would be of interest to determine whether this function of RPA is conserved in mammalian cells and acts to prevent MMEJ, as demonstrated in yeast. RPA inhibition of strand annealing likely plays an important role during DNA replication to prevent aberrant processing of secondary structures within ssDNA formed during lagging strand synthesis, or when the replicative helicase and leading strand polymerase become uncoupled due to replication stress. In addition to the threat posed by short inverted repeats, eukaryotic genomes contain many repetitive sequences or motifs that can form stable secondary structures, including trinucleotide repeats, long inverted repeats, AT- and GC-rich mini (10–60 bp repeat) and microsatellites (2–5 bp repeats), and G-rich sequences with the potential to form G-quadruplexes. These sequence motifs are known to stall replication, and induce high levels of chromosome fragility, recombination and chromosome rearrangements [84, 95–99]. Decreased expression of *RFA2*, achieved by using a doxycycline-repressible promoter, resulted in a dramatic increase in GCRs at long $(GAA)_n$ tracts and at palindromic sequences,

suggesting that RPA is indeed a critical safeguard for genomic integrity at trinucleotide and inverted repeats, and possibly at other "at-risk motifs" as well [100, 101].

Acknowledgments

Abbreviations

DSB	double-stranded break
dsDNA	double-stranded DNA
GCR	gross chromosome rearrangement
HR	homologous recombination
MH	microhomology
MMEJ	microhomology-mediated end joining
MRX/N	Mre11-Rad50-Xrs2/NBS1 complex
NHEJ	non-homologous end joining
nt	nucleotide
OB	oligonucleotide/oligosaccharide binding
RPA	replication protein A
ssDNA	single-stranded DNA
SV40	simian virus 40

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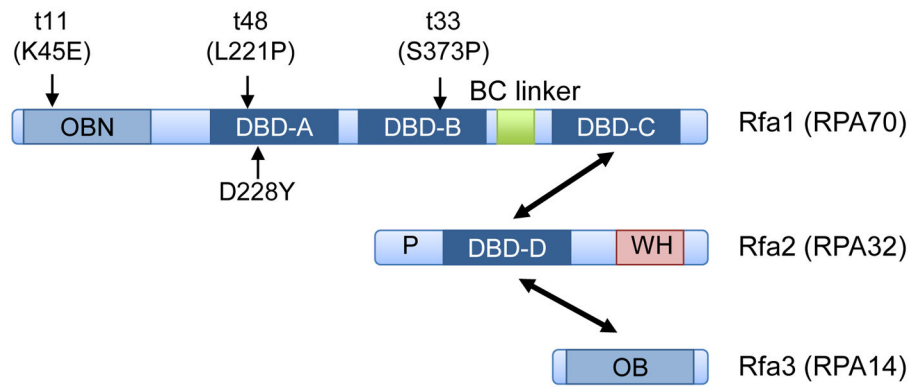
Figure 1.
Schematic showing the domains present in the RPA subunits. The names shown are for the budding yeast proteins with the human protein names in parentheses. The approximate locations of the *rfa1* mutations described in the text are shown. The DNA binding domains (DBD) are indicated by the dark blue boxes and the OB folds that do not interact with DNA are shown in light blue. WH refers to the winged helix domain; double-headed arrows show the domains involved in subunit interactions.
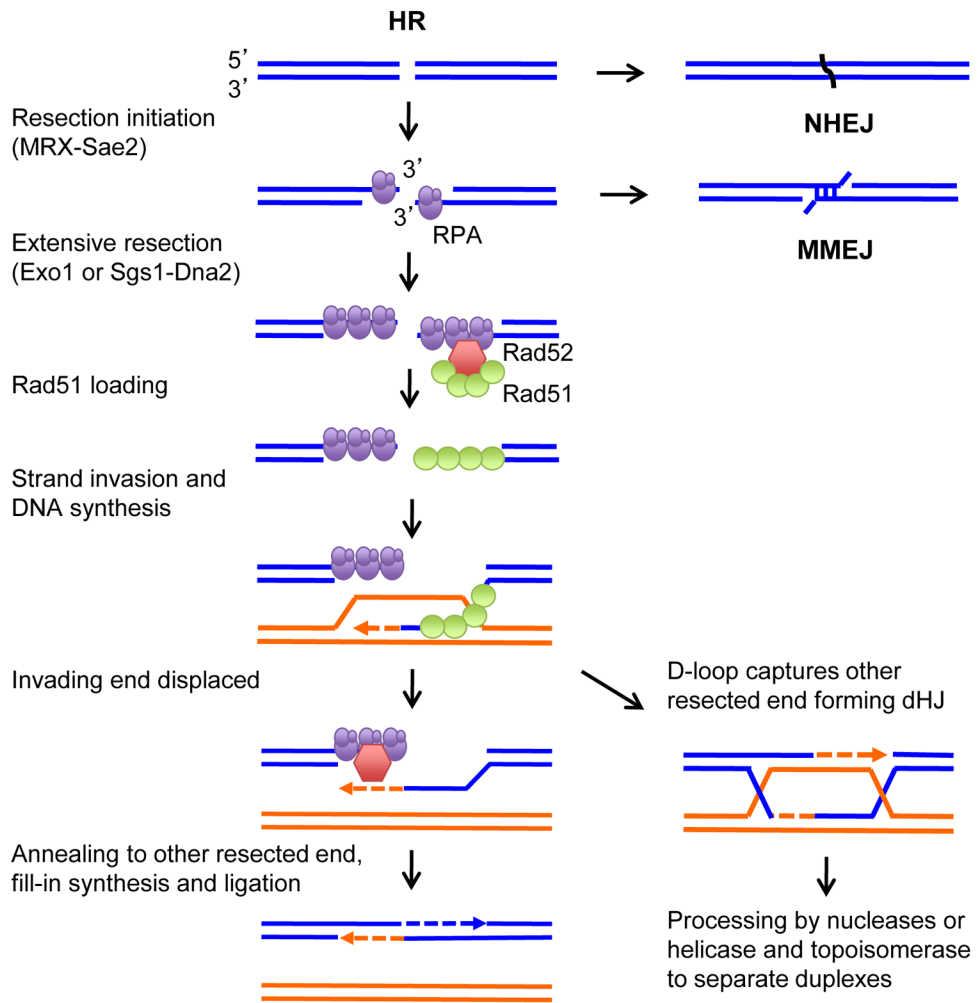
Figure 2.
Models for DSB repair. The DNA ends can be ligated by NHEJ or undergo resection to form 3′ ssDNA tails. The initial processing of the DNA ends has the potential to reveal short homologies internal to the ends that can be used for MMEJ. The long ssDNA tails are initially bound by RPA, which is then replaced by Rad51 with the assistance of Rad52 (or BRCA2). Rad51 promotes invasion of a donor duplex and the 3′ end is extended by DNA synthesis. The extended invading strand can be displaced by a helicase and can pair with the other resected end; alternatively, the displaced strand from the donor duplex pairs with the other break end and after gap filling and ligation a double Holliday junction (dHJ) intermediate is formed. The dHJ is acted on by a helicase and topoisomerase to dissolve the dHJ forming non-crossover products or cleaved by nucleases to generate crossover or non-crossover products.
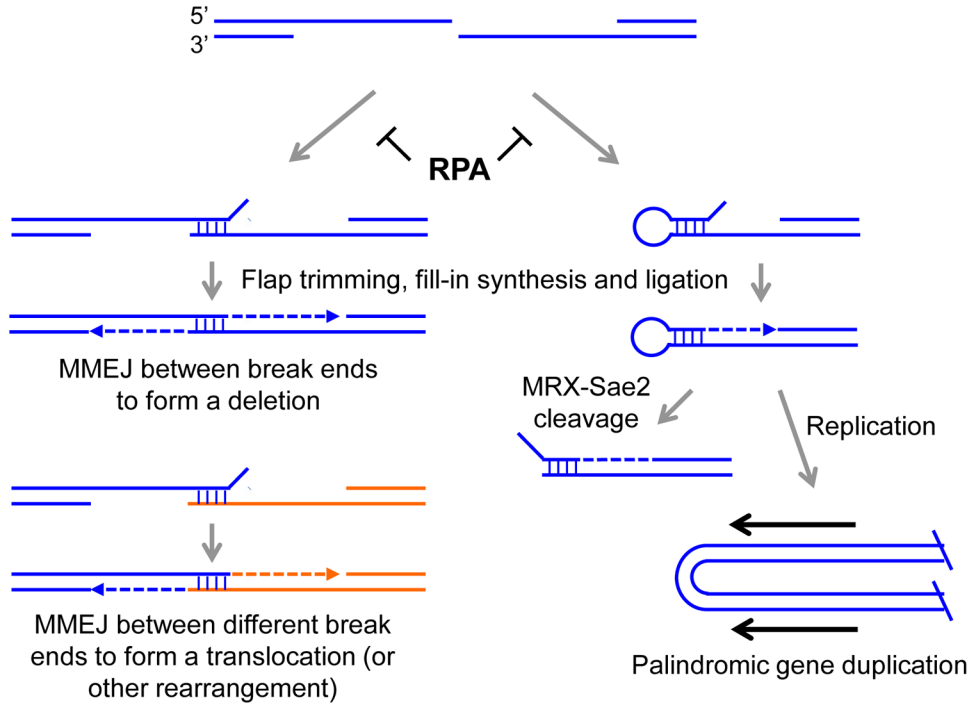
Figure 3.
RPA prevents inter- and intra-molecular annealing between short repeats to prevent MMEJ or formation of hairpin-capped DNA ends. RPA binding to the long ssDNA tracts formed by end resection is required to prevent pairing between short repeats flanking the DSB or potentially with another DSB end elsewhere in the genome. After pairing heterologous flaps are removed, gaps filled by DNA synthesis and the ends are ligated yielding deletions, translocations or inversions. Pairing between short inverted repeats within the ssDNA tail can lead to the formation of hairpin-capped ends. MRX acts with Sae2 to cleave hairpins creating another substrate for end resection and repair. Failure to cleave the hairpin can lead to palindromic gene duplication and possibly more complex rearrangements if the resulting chromosome fragment has two centromeres.