REVIEW

SSB and the RecG DNA helicase: an intimate association to rescue a stalled replication fork

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Abstract: In *E. coli*, the regression of stalled DNA replication forks is catalyzed by the DNA helicase RecG. One means of gaining access to the fork is by binding to the single strand binding protein or SSB. This interaction occurs via the wedge domain of RecG and the intrinsically disordered linker (IDL) of SSB, in a manner similar to that of SH3 domains binding to PXXP motif-containing ligands in eukaryotic cells. During loading, SSB remodels the wedge domain so that the helicase domains bind to the parental, duplex DNA, permitting the helicase to translocate using thermal energy. This translocation may be used to clear the fork of obstacles, prior to the initiation of fork regression.

Keywords: RecG; SSB; stalled replication fork; DNA repair; DNA replication; helicase; atomic force microscopy; OB-fold; SH3 domain; PXXP motif

Introduction

The accurate and faithful duplication of the genome relies on the DNA repair and genetic recombination machinery working closely together.[1–4] This intricate interplay is necessary because the advancing replisomes frequently encounter roadblocks that have the potential to stall or collapse a replication fork.[5,6] The types of impediments to fork progression that exist include proteins bound to the DNA ahead of the replication fork, noncoding lesions in the template DNA, unusual secondary structures that arise in the DNA and either single- or double-strand breaks.[3,7–11] Each of these roadblocks to DNA replication likely utilizes different subsets of repair enzymes and this is highlighted by the varied recombination and repair gene requirements for dealing with exposure to different types of DNA damaging agents.[8,12–16] Whatever its source, the roadblock has to be removed or bypassed, and replication must be restarted.

In bacteria, stalled replication forks can be reversed (regressed) or directly restarted (Fig. 1 and [6,8,12,17–20]). During the process of regression, the fork is moved in a direction opposite to that of replication away from the

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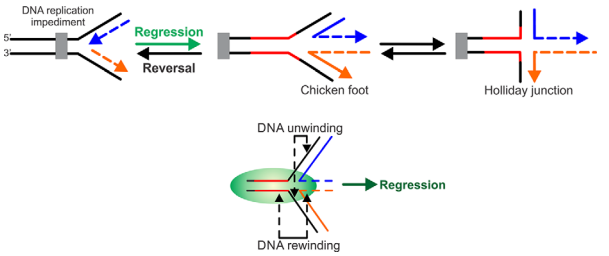

Figure 1. Fork regression. A schematic of the fork regression reaction is presented in the top panel. The fork is shown impeded and the nascent leading (blue) and lagging (orange) strands indicated. Fork regression (green arrow) results in rightward movement of the fork, away from the site of the replication impediment, concomitant with the extrusion of a duplex region resulting from the reannealing of the nascent leading and lagging strands. The resulting DNA structure has been termed the "chicken foot" and is structurally similar to a Holliday junction. Fork reversal (not catalyzed by RecG) would result in leftward movement of the fork following regression and subsequent repair. For regression to occur, a specialized DNA helicase is required as shown in the bottom panel. This enzyme must be able to unwind fork arms while simultaneously rewind duplex DNA regions. RecG efficiently catalyzes both of these reactions simultaneously.[31,109]

site of damage to a region where the nascent, replicated genome is undamaged. This enables the repair machinery to have access to the damage and facilitate repair. This process is analogous to the clearing of train tracks following a derailment/collision. Once repair and/or removal of the roadblock has occurred, the replisome can be reloaded and replication restarted, initiating from within the undamaged region of the genome.

Although replication fork regression can in principle be spontaneous as shown by the Cozzarelli group,[21,22] it was also proposed to be catalyzed by a number of proteins including RecA, RecG, and RuvAB.[23–28] Over the past few years it has become increasingly clear that RecG is the enzyme responsible for regressing stalled DNA replication forks as elaborated.[29–32] Central to the processing of stalled replication forks, is the single strand DNA binding protein (SSB), its association with the RecG DNA helicase and the focus of this review.

SSB: Mediator of Fork Processing

The single stranded DNA binding protein (SSB) is essential to all aspects of DNA metabolism in *Escherichia coli*.[33–37] SSB has two seemingly disparate roles: to bind and stabilize single-stranded DNA (ssDNA) intermediates generated during DNA processing and, to bind to fourteen proteins in temporal and spatial fashion, to both store and target enzymes to the DNA when needed.[38,39] These binding partners are known as the "SSB-interactome" and include Exonuclease I, the X-subunit of DNA polymerase, DnaG, RecO, uracil glycosylase, topoisomerase III, and the PriA, RecG, and RecQ DNA helicases.[38,40,41] Interactome protein binding by SSB is necessary to facilitate replication, recombination or repair.[33,38]

SSB exists as a stable homo-tetramer with a monomer MW of 18, 843 Da.[42] Each 178 amino acid length monomer can be divided into two domains defined by proteolytic cleavage: an N-terminal portion comprising approximately the first 115 residues and a C-terminal domain that includes residues 116–178 [Fig. 2(A) and Ref. 43]. The N-terminal domain which has a pI of 8.01 is responsible for tetramer formation and for binding to ssDNA which occurs via the wrapping of the polynucleotide around the SSB tetramer [Fig. 2(B) and Refs. 44–46]. DNA binding is mediated by the oligonucleotide/oligosaccharide binding-fold (OB-fold). The disordered, C-terminal domain has received considerable attention over the past few years as it is critical for mediating interactions with interactome partners and to the cooperative binding of ssDNA [Fig. 2(C) and Refs. 29,47–55]. It can be further subdivided into two additional regions: a sequence of approximately 50 amino acids that is the least conserved region among prokaryotic SSB proteins, has been called the intrinsically disordered linker (IDL) and has a *pI* of 9.60 [Fig. 2(A) and Refs. [33,37,56,57]]. This is immediately followed by the last 8–10 residues which are very well conserved and

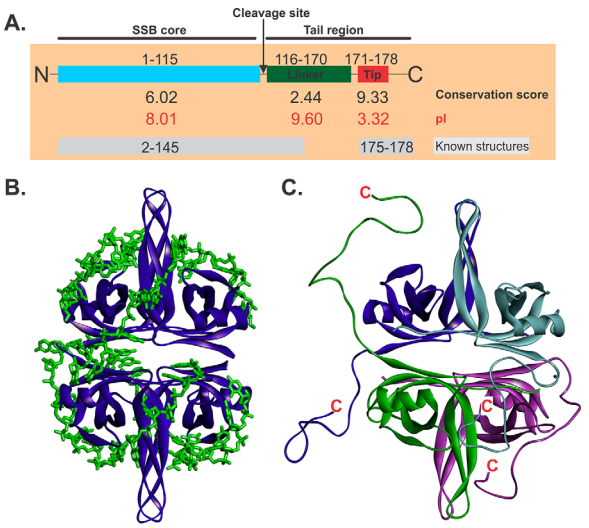

Figure 2. Organization of the SSB protein. (A) Schematic of SSB divided into the core and tail regions by proteolytic cleavage. The conservation scores for each region were calculated from alignments using Praline.[110] The pI of each region is shown in red and was calculated using the ProtParam tool of Expasy.[111] The pI of the intact protein is 5.44. (B) The SSB tetramer is intimately associated with ssDNA. The image was generated using PDB file 1EYG.[45] The DNA is colored green and the tetramer, shown as a ribbon, is colored purple. (C) The linker domain of SSB can adopt different conformations. The image was generated using PDB file 1QVC.[112] Each subunit is presented in a different color with C-termini labelled in red.

is overall, acidic in nature with a *pI* of 3.32 [Fig. 2(A); "tip"; Refs. [33,56]]. This acidic tip is thought to be essential for protein–protein interactions as *in vivo* and *in vitro* studies showed that when it is removed, target protein binding is eliminated, and it can bind to target proteins.[29,39,40,52,58–61] One interpretation of the deletion mutants is that these residues contact the target proteins directly so that once eliminated, binding cannot take place.[40,60,62–64] An alternative proposal suggests that acidic tip plays a regulatory role in linker function and that target protein binding utilizes another region of SSB.[65]

Recently we demonstrated that this other region responsible for mediating protein–protein interactions is in fact the intrinsically disordered linker.[66,67] This makes sense because the IDL is unique to each bacterium, and may explain species specificity in interactome function.[29,56,57] Historically this region of SSB was proposed to be nonessential, and as a result, it was largely ignored until a recent study from the Lohman laboratory demonstrating that the IDL plays a very important role in cooperative ssDNA binding.[43,56] Cooperativity is critical to SSB function enabling rapid single stranded DNA binding resulting in its protection and/or conversion into a more suitable substrate for subsequent processing.[37,68] One component of cooperative binding likely involves SSB–SSB interactions mediated by the intrinsically disordered linker(s) of one tetramer which then make contact with a neighboring tetramer, and possibly even the next tetramer thereby enhancing the cooperative effect.[66] It follows then that this region may also be involved in binding to interaction partners, possibly using a common mechanism and with different regions of the linker possibly mediating binding to different partners.[57]

How might this occur? When we analyzed the sequence of the IDL we found it to be over-represented for Gly 24.6%, Gln 17.4%, and Pro 14.5% (Fig. 3). The presence and spacing of these residues in the N-terminal half of this region, ending at residues F148 and S149 is consistent with the formation of polyproline II helices (PPII) found in proteins such as collagen [Fig. 3(A) the region underlined in black[69]]. However, the spacing of the proline residues in collagen occurs at every fourth position which is not the case for SSB. Instead, glycine, glutamine, and arginine occur in those positions in SSB. A recent study has shown that these amino acids can substitute for proline without perturbing a model PPII helix.[70] As one strand of a collagen fiber is essentially a PPII helix, we wanted to determine whether this region of SSB could be modeled on a collagen-like peptide.[71,72] Indeed, the SSB sequence can adopt a PPII helix that superimposes well with the peptide, with an RMSD = 0.8 Angstroms for the backbone atoms [Fig. 3(B)]. Consequently, we proposed that it plays an important role

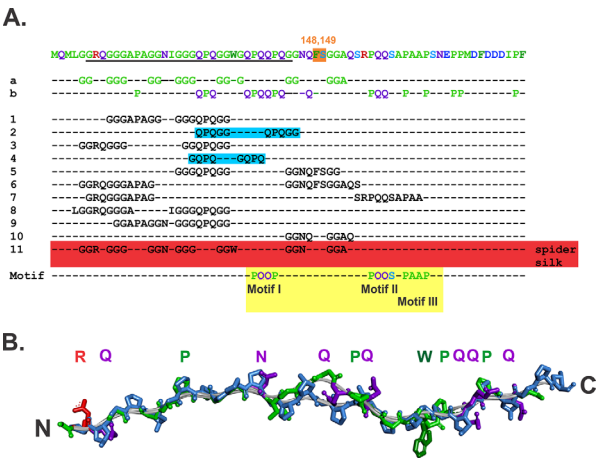

Figure 3. The IDL contains sequence elements critical to its function. (A). The sequence of the C-terminal 69 residues of *E. coli* SSB is presented in the first line, with the most over-represented residues highlighted in lines a and b. The black line under the sequence corresponds to the putative polyproline type II helix. Residues 148 and 149 are highlighted to indicate where the PPII-helix would terminate as these are known to disrupt these helices.[70] Sequence analysis of the protein was done using REPRO at http://www.ibi.vu.nl/programs/ to determine the presence of repetitive elements. Lines 1–11 show a subset of the repeated sequence elements identified with identical repeats colored blue.[73] The spider silk sequence motifs which are repeated seven times, are highlighted in the red box. The analysis of the IDL sequence also revealed the presence of three PXXP motifs indicated in the Motif line. (B). Homology modeling reveals that residues 116–145 can adopt a collagen-like structure. The amino acids underlined in panel were modeled on to collagen as described in the text. The model is shown superimposed onto a collagen fiber. For both peptides, the backbone is colored in grey and collagen side chains are colored blue. The coloring of the SSB sequence matches that in panel A, top line.

in protein function. When its sequence is mutated, or its position relative to the core domain altered, the consequences for both partner and ssDNA binding are disastrous.[56,67]

Second, and as the Gly, Pro, and Gln residues seemed to appear in clusters in the SSB C-terminal domain, we analyzed the entire 69 residue region for repeats using REPRO.[73] This program identifies repeats in the following way. First, it uses an extensive search protocol based on a variation of the Smith–Waterman (SW) local alignment strategy to find a set of best-scoring and nonoverlapping pairwise local alignments. This is followed by an iterative graph-based clustering procedure to define the boundaries of the repeats set(s) using the top alignments identified in step 1. In the third and final step, iterative multiple alignment of the repeats set(s) is carried out along with profile sliding along the input sequence to detect more distant but related sequence fragments missed in the first two steps.

Using REPRO, several repeats were identified and 11 of these are shown in Figure 3(A). Some are these are identical (numbers 2 and 4), while the remaining are quasi-repeats, for example, repeats 1 and 10. Intriguingly there are also seven hydrophilic GGX repeats commonly found in spider silk proteins (line 11[74]). In addition, of the first ten repeats, nine cluster in the N-terminal half of the IDL sequence upstream of F148. Likewise, six of the seven GGX repeats also occur in this region of the protein.

Similar repeats containing glycine, proline and glutamine have been identified in several eukaryotic proteins such as spider silk, the X-type HMW subunit of wheat gluten and ω-protein where they have been shown to confer important structural features and special functions in addition to conferring elastomeric properties to that region of the protein.[75] The over-representation of gly, gln, pro, and ser residues and the presence of the repeats, may impart similar elastomeric properties to the C-terminus of SSB, enabling it to interact with a large range of partners of different sizes, ranging from 16.6 (χ-subunit of DNA polymerase III) to 82 kDa (PriA) as suggested previously.[67] Importantly, we identified a repeat common to spider silk protein that is present seven times [Fig. 3(A), red box[74]]. Consequently, we propose that these impart tensile strength to SSB–SSB complexes which, when assembled on DNA, would be connected by an extensive IDL/OB-fold network of interactions.

Finally, there are also three PXXP motifs in the IDL: PQQP, PAAP and the similar, PQQS [Fig. 3(A), motif]. These motifs are most well known for their ability to bind structurally conserved Src homology 3 (SH3) domains in eukaryotic systems. These domains are ~50 residue modules that often occur in signaling and cytoskeletal proteins.[76–80] Notably, the SH3 domain has a characteristic fold which consists of five or six beta-strands arranged as two tightly packed antiparallel beta sheets and is similar in structure to the OB-fold present in many single-strand DNA binding proteins.[81] SH3-like domains have been identified in several *E. coli* proteins, including Exonuclease I, an SSB–interactome partner.[82–85] Recently, we identified OB-folds in most of the proteins comprising the SSB-interactome (P. Bianco, personal communication). This suggests that interactions between either one, or more, PXXP motifs in the IDL of SSB and the OB-fold (i.e., SH3 domain) in the partner, may play a critical role in protein–protein interactions and in regulation of DNA metabolic processes. Further, these interactions may also play a role in mediating cooperative binding of SSB to ssDNA where critical tetramer-tetramer interactions mediated by the PXXP motifs in one tetramer and the OB-fold of an adjacent protein as alluded to above. This would of course enhance cooperative binding to ssDNA and when coupled to the tensile strength imparted by the spider silk repeats, would provide an incredible combination to ensure rapid protection of ssDNA resulting in an exceptionally stable complex. Consequently, the intrinsically disordered linker would be responsible for linking protein and ssDNA binding by mediating protein–protein interactions as shown recently.[67] When these interactions occur between an SSB tetramer and an interactome partner, loading of that protein onto DNA takes place. When the interactions take place between SSB tetramers, cooperative ssDNA binding occurs.

RecG: A Powerful, Monomeric, Molecular Machine

recG was identified as a mutation that mildly affected recombination and survival following UV-irradiation.[86] Several years later, the purified protein was demonstrated to be a DNA helicase that translocates in a 3′→5′ direction.[87,88] Subsequent studies showed that the enzyme functions as a monomer and binds with high affinity to stalled replication fork substrates with a preference for a fork with a gap in the nascent leading strand.[29,30,89–91]

In addition to being able to process a variety of branched DNA structures *in vitro*, RecG exhibits significant ATPase activity on negatively supercoiled DNA, single stranded DNA (ssDNA), and SSB-coated M13 ssDNA.[29,91] This suggests different ways for RecG to access a stalled replication fork, dictated by the types of DNA that might be available. The strong preference that the enzyme exhibits for negatively supercoiled DNA *in vitro*, suggests that DNA must first be converted from positively to negatively supercoiled for RecG to function.[91] Once the DNA is in this form, RecG catalyzes fork regression efficiently.[92] Activity on SSB-coated M13 ssDNA is intriguing as it involves a species-specific, protein–protein interaction between RecG and SSB.[29,39,67] This interaction is mediated through the C-terminal tail of SSB, similar to that observed for Exonuclease I, PriA, RecQ, and Topoisomerase III.[48,52–55] Further, this interaction is key to RecG function at a stalled fork since the enzyme can be directly loaded onto the DNA in the vicinity of single-stranded regions and is consistent with the role of SSB in targeting repair helicases to active forks *in vivo*.[29,93,94]

The crystal structure of the enzyme bound to a model fork substrate shows how RecG can process a fork [Fig. 4 and Ref. 95]. The structure shown is of the *T. maritima* protein and, with the exception of the N-terminal extension (function unknown) is very similar to that of *E.coli* RecG.[96,97] The enzyme is divided into three domains, highlighted in different colors. Domain I comprises the N-terminal half of the protein and contains both the wedge domain and the so-called N-terminal extension [discussed below; Fig. 4(A), orange and brown, respectively]. The wedge domain is joined to domains II and III, via an

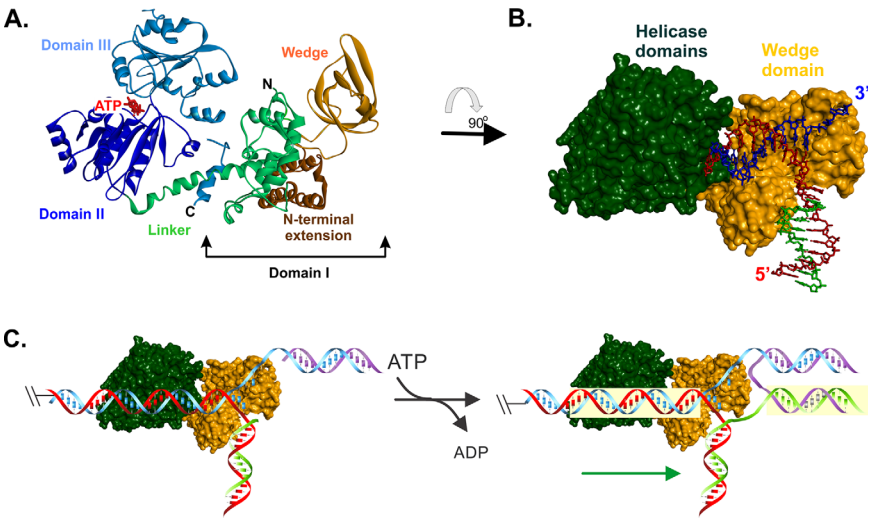

Figure 4. RecG is intimately associated with a fork to drive regression. (A) A ribbon diagram showing the three domains of RecG. The N-terminal extension (brown) which has no known function, is present in *T. maritima* RecG and not in the *E. coli* protein. (B) The protein is shown bound to its preferred fork substrate with parental DNA strands in red and blue and nascent DNA in pale green. The complex is viewed from the top, rotated 90° relative to panel A. The enzyme is shown as a Connolly surface with helicase domains in blue and the wedge domain in orange.[113] (C) RecG is shown in the process of catalyzing fork regression. Here the helicase domains are shown in dark green and the wedge domain in orange. Parental DNA is colored red and blue, nascent leading, and lagging single strands in purple and pale green, respectively. The nascent duplex regions resulting from reannealing are highlighted by the pale yellow boxes. The green arrow indicates the direction of fork regression.

α-helical linker [Fig. 4(A), light green]. These C-terminal domains make up the remainder of the protein, contain the characteristic helicase motifs, and couple the energy released from ATP hydrolysis to drive the enzyme on DNA.[95,98–100] Specific binding to branched DNA structures is clearly mediated by the wedge domain which also facilitates DNA strand separation[101] [Fig. 4(B,C)].

Once loaded at a stalled replication fork, RecG catalyzes an efficient reaction known as fork regression [Figs. 1 and 4(C)]. In the images shown in Figure 4(C), RecG would translocate from left to right and with translocation coupled to ATP hydrolysis occurring in the helicase domains. Intimate interactions between the wedge domain and DNA at the fork would cause strand separation. At the same time, as the helicase moves duplex DNA would be reannealed both in the wake of the advancing enzyme as well as ahead of RecG [regions highlighted in pale yellow; Fig. 4(C)]. While not visible in the original structure, it is likely that nascent duplex binding would occur with the helicase domains.[95,100]

The consequence of this set of interactions is the net backward movement of the fork, away from the site of DNA damage. This is the reaction known as fork regression (Fig. 1). Remarkably, and to do this, RecG couples the unwinding of nascent heteroduplex arms to the rewinding of DNA both ahead of itself as well as in its wake.[31,32] The rate of fork regression is 240 bp/s with the enzyme translocating on average, 500bp before dissociating.[31,91] The product of this reaction is a 4-stranded, Holliday Junction-like structure or "chicken foot".[23,31] The similarity of the resulting regressed DNA structures to Holliday Junctions indicates that following the action of RecG, further processing is carried out by RuvAB.[23,30,89,91,102]

While it is clear that RecG regresses stalled forks, how it gains access to the fork is also an important issue. In the absence of DNA damage, RecG is localized to the inner membrane by SSB.[39] This is proposed to be the storage form of the enzyme. RecG is also known to associate with active replication forks.[103] Once forks are impeded, it is likely to be loaded onto the fork by SSB, with which it is known to interact both physically and functionally.[29,30,91]

A Model for the Interaction of SSB with RecG

To visualize how SSB and RecG might bind to one another via the linker/OB-fold model, we overlaid an SH3 domain bound to its ligand onto the structure of *E. coli* RecG. Here, a model of EcRecG was built using Swiss-Model and the *T.maritima* protein PDB file 1GM5 as a template.[71,72,95,104] Then the SH3 domain bound to its 17 residue peptide ligand (PDB file 2KXC) was superimposed onto the OB-fold in the wedge domain of *E. coli* RecG using TM-align.[105] This structure was selected as the ligand contains PXXP motifs with two in proximity of one another similar to what is observed in SSB.[106] As expected, the SH3 domain overlays on the wedge domain of RecG. Once this process was complete we removed the SH3 domain only, leaving the ligand in place to

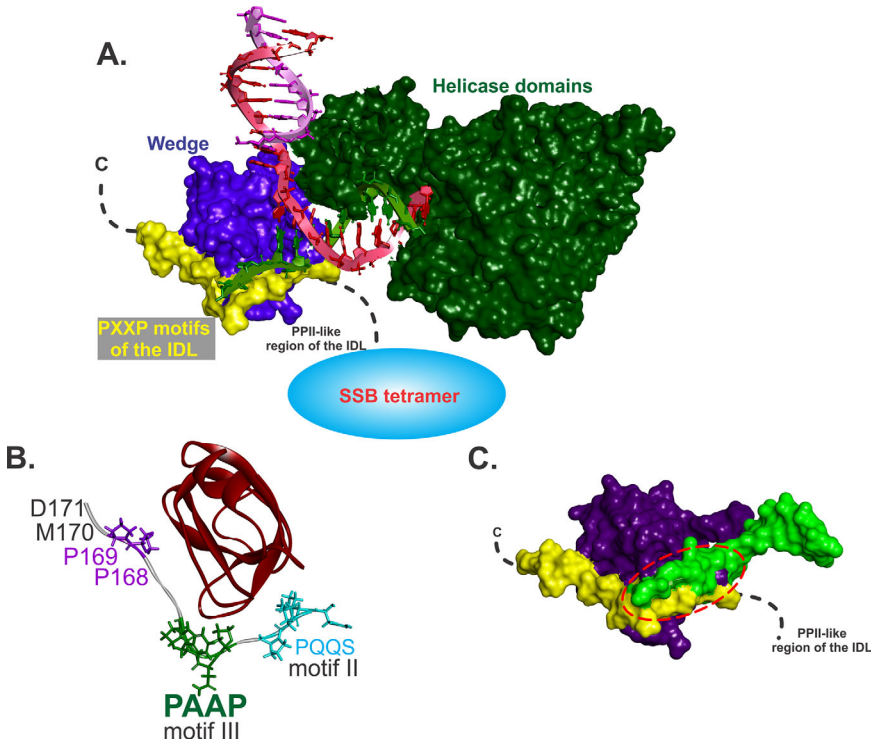

Figure 5. SSB IDL-RecG wedge binding impedes fork binding. (A) RecG is shown as Connolly surfaces with the wedge in purple and helicase domains in green. The complex is viewed from the top. The PXXP ligand from PDB structure 2KXC is shown as a yellow Connolly surface. The fork DNA is represented in cartoon fashion with parental DNA colored green and red and the nascent lagging strand in pink. The proposed positions of the PPII-like helix of SSB, the SSB tetramer core and the C-terminus of the SSB protein are indicated. (B). Modeling predicts that PXXP motifs would bind to the wedge domain (red ribbon) positioning the acidic tip away from RecG. The putative positions of motifs II and III of the IDL are shown in blue and green, with the first residues of the acidic tip highlighted in black. (C), IDL binding to the wedge domain prevents fork binding. The RecG wedge domain (purple) s shown bound to the PXXP ligand (yellow), and the parental leading strand arm of the fork (green). For clarity the remainder of the structure in A is not shown and the complex is viewed from the top. The region in which the DNA and IDL would clash is indicated by the dashed red oval. Black dashed lines, remainder of the IDL of SSB.

illustrate the potential binding location of the SSB IDL on RecG [Fig. 5(A)].

In the model shown, several features can be seen. First, the IDL of SSB is positioned with its N-terminus between the wedge and helicase domains of RecG and the C-terminus on the opposite face of the wedge. Second, when positioned in this way, the proposed polyproline type II region of the IDL would be positioned between the wedge and helicase domains so that the remainder of the SSB tetramer would be in close proximity. The location of the PPII-helix in the model is consistent with recent studies showing changes in the sequence of this region of SSB, as well as alteration of its position relative the core domain of SSB, have disastrous consequences for protein function.[56,67] Due to the close proximity of the remainder of the SSB tetramer to RecG, it is conceivable that additional stabilizing interactions may occur between the helicase domains and the N-terminal OB-fold domain of SSB and/or C-termini of the remaining subunits of the tetramer, but this remains an open question. Second, binding of motif II of the IDL (PQQS) to the wedge domain would likely occupy the DNA binding site of the leading strand arm of the fork [Fig. 5(B,C)]. Third, motif III would be located in the center of this interaction interface and this would place prolines 168 and 169 of the IDL so that the remainder of the acidic tip is pointed away from RecG. Consequently, the acidic tip would likely not be making significant contact with the wedge domain whereas motifs II and III would make contact with the third motif possibly being the more important of the three [Fig. 5(B)]. The model suggests that SSB–RecG binding would be competitive with fork-RecG binding, since the position occupied by the IDL occludes part of the DNA binding site on the wedge domain [Fig. 5(C)]. In the model shown, it is clear that the Connolly surfaces of the ligand and ssDNA arm occupy the same space when bound to wedge domain of RecG. Finally, our model is consistent with previous work showing that the IDL is responsible for mediating SSB protein–protein interactions and with the acidic tip having a regulatory role in SSB function, and not a direct role in target protein binding.[65,67]

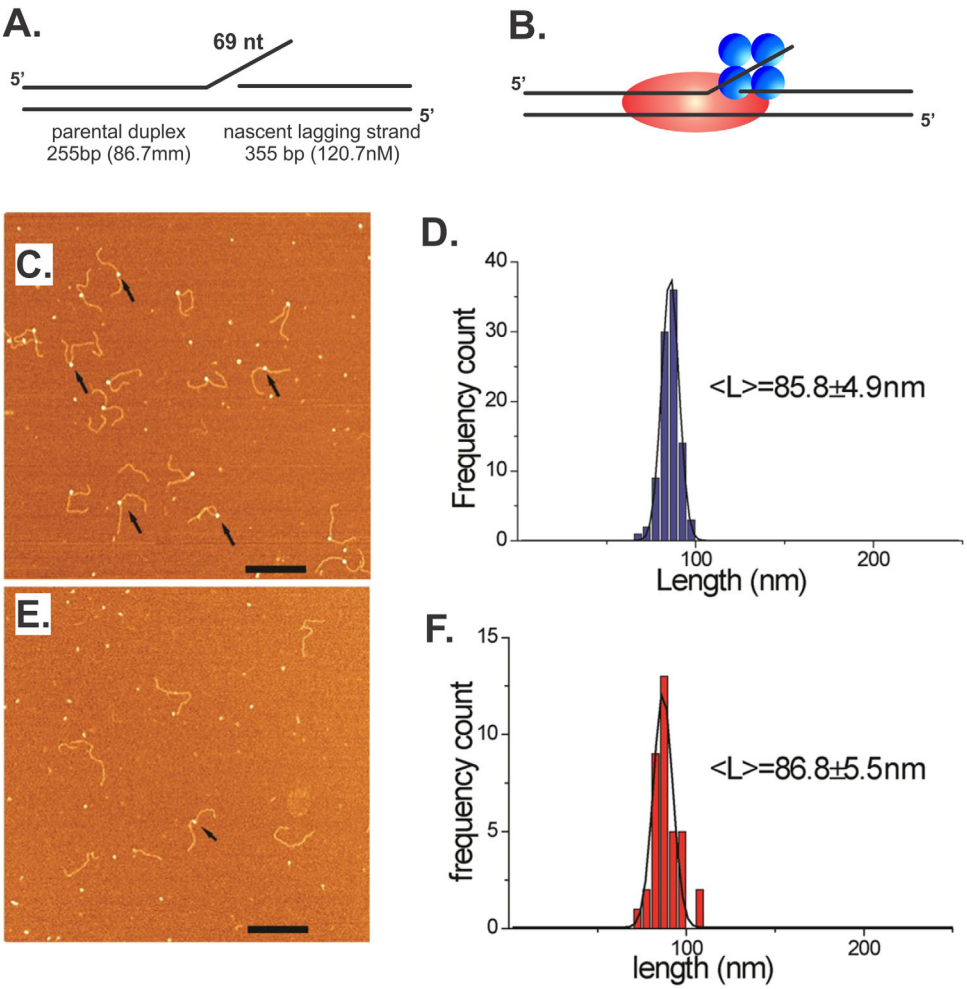

Figure 6. Both SSB and RecG bind specifically to the fork. (A) Schematic of the fork substrate and (B), anticipated binding sites for SSB and RecG. (C) and (E), AFM images of SSB and RecG bound to DNA. Arrows indicate protein–DNA complexes. Bar, 200 nm. (D) and (F), the distribution of SSB and RecG positions relative to the left end of the substrate, respectively. Images are from Ref. [94] and are used by permission.

The intimate association of SSB and RecG in this manner suggests that in the absence of DNA, these two would proteins would from a tight complex. Consistent, the SSB–RecG complex can be purified intact and is stable through several chromatography steps.[39] Finally, the model predicts that during helicase loading by SSB, RecG would be incapable of binding directly at the fork. This is exactly what happens as shown using atomic force microscopy.[94]

SSB Loads RecG onto a Fork

As explained above, the *in vivo* and *in vitro* data show that SSB and RecG bind to one another.[29,39] In addition, there is also a functional interaction with SSB resulting in stabilization of the helicase on ssDNA.[29,91] However, it was not known how RecG could take advantage of its interaction with SSB to enable efficient loading onto a stalled DNA replication fork. This question was answered in a recent atomic force microscopy (AFM) study.[94] In this study, a fork with gap in the leading strand was used to study loading of RecG. This fork was selected as it is the preferred substrate for RecG *in vitro* and its design placed the fork off-center to enable clear visualization in the AFM [Fig. 6(A) and Refs. [29,30,107,108]]. In theory, SSB should bind to the 69nt ssDNA arm (the gapped, nascent leading strand) and RecG should bind at the fork in preparation for translocation from left to right in the figure as shown [Fig. 6(B) and Ref. [95]].

Using this model fork, binding of SSB and separately of RecG was assessed. As expected, each protein binds to the substrate but with different efficiencies [arrows, Fig. 6(C,E)]. For SSB, 90% of the DNA molecules examined were protein–DNA complexes, whereas for RecG, the yield was only 10%.[94] Further analysis of each protein–DNA complex revealed that SSB was bound at 85.8 ± 4.9 nm and RecG was bound at 86.8 ± 5.5 nm from the left end of the substrate [Fig. 6(D,E)]. This distance corresponds exactly to the position of the fork which is 86.7 nm from the left end [Fig. 6(A)]. RecG and

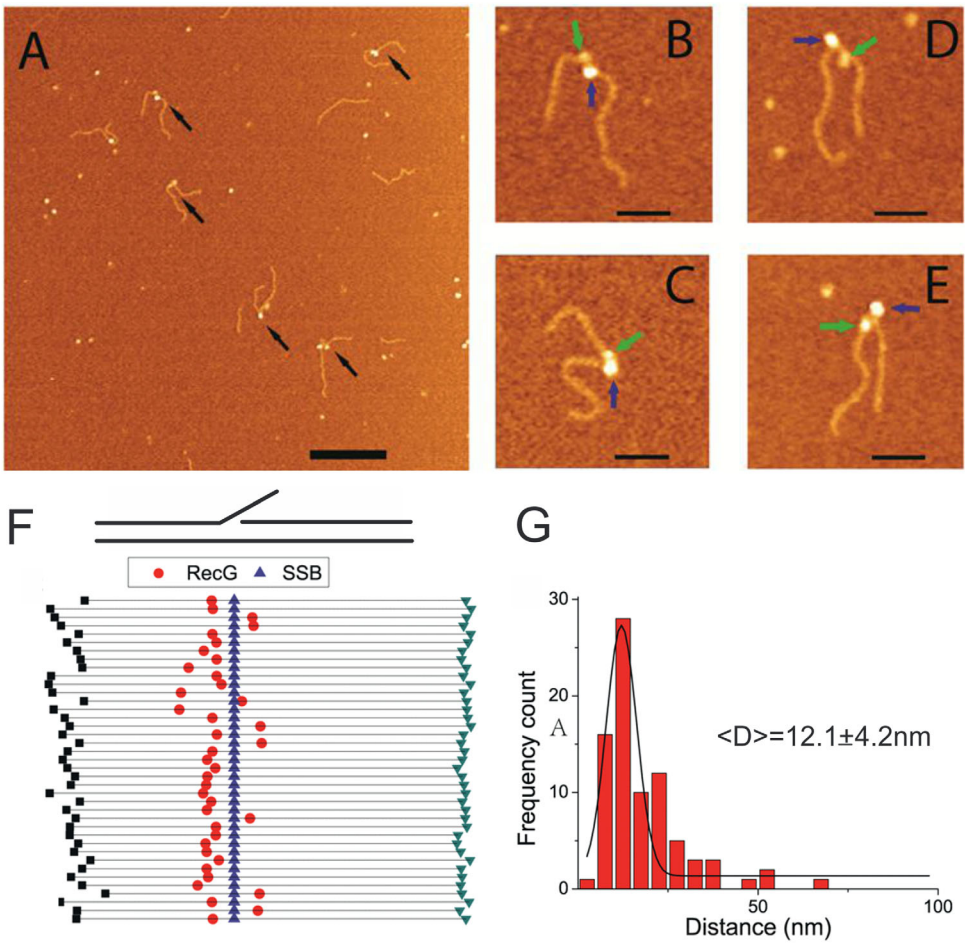

Figure 7. SSB loads RecG and remodels the helicase in the process. (A) Large scale image of SSB–RecG–DNA complexes. Proteins are indicated by arrows. Scale bar is 200 nm. (B–E) Zoomed images of four double-blob complexes. Large (SSB) and small (RecG) blobs are indicated with blue and green arrows (scale bars 50 nm). (F) Maps of RecG position (red circles) relative to the SSB bound to the same DNA. A schematic of the fork substrate is shown at the top to orient the viewer. (G) The histogram of distributions of distances between SSB and RecG. Images are from Ref. [94] and are used by permission.

SSB appear with different contrasts on AFM images, with SSB appearing larger than RecG by a factor of 2.[94] This difference was used to distinguish the proteins from one another in subsequent experiments where both were present.

To determine whether SSB facilitates loading of RecG, the same fork substrate was preincubated with SSB. Then RecG was added and 30 min later, imaging was done. The results show while 90% of the complexes contained SSB, the number containing RecG as well had increased to 30%, up threefold relative to RecG only [Fig. 7(A)]. The analysis of each "blob" on the DNA confirmed that there was one large (SSB; blue arrows) and one small (RecG; green arrows) blob on the DNA. Close inspection of these complexes revealed that SSB was always positioned exactly at the fork [blue arrows; Fig. 7(B–E)]. This was confirmed by measuring the distance SSBs were positioned relative to the left end of the DNA. In contrast, RecG was not bound at the fork but was instead positioned some distance away from SSB.

To understand the positions of RecG and SSB relative to one another, each of the protein–DNA complexes were aligned relative to SSB bound at the fork [Fig. 7(F)]. When viewed in this way, it is clear that RecG is positioned on the parental duplex DNA region of the fork, on average, 36 bp upstream [Fig. 7(F,G)]. As RecG bound specifically to the fork in the absence of SSB, the data suggest that SSB bound to RecG and then loaded it onto the DNA and this is followed by sliding to different positions ahead of the fork. This motion is thermally driven as it was found to be independent of a nucleoside triphosphate.[94] In addition, the authors proposed that the function of this thermal sliding by RecG could facilitate clearing of proteins in the immediate vicinity of the fork.

As RecG was not found bound to the fork, and the wedge domain of the helicase is essential for fork binding,[101] the following model was proposed and is shown schematically in Figure 8. SSB binds to the fork first, onto the ssDNA arm. Then SSB

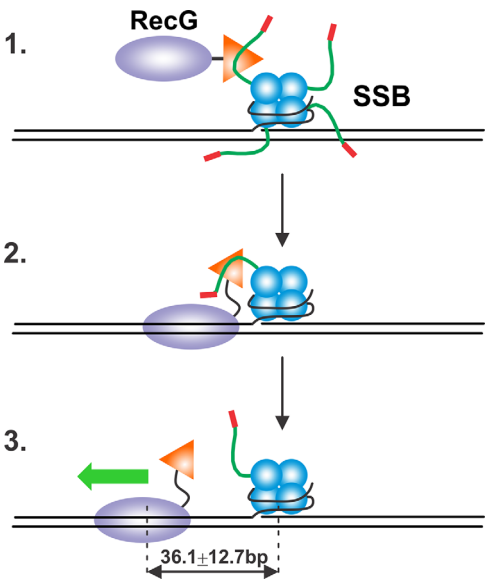

Figure 8. SSB remodels RecG facilitating thermal translocation. 1. SSB binds to the fork and to RecG via the intrinsically disordered linker (green) from one monomer within the tetramer. 2. Loading occurs concomitant with remodeling of the wedge domain (orange) so that only the helicase domains (purple) can bind DNA. 3. Once bound, RecG slides, using thermal energy, on average, 36 bp ahead of the fork. The coloring of SSB matches that of Figure 2 and is core domain in blue, IDL in green and acidic tip in red. Helicase domains of RecG are colored in purple and the wedge domain is in orange.

binds to RecG and loads the helicase from solution onto the DNA. During loading, the single strand binding protein must have remodeled the helicase to enable to bind to the parental duplex DNA. Binding in this case would not be via the wedge domain but instead would involve only the helicase domains.

For this remodeling to occur, intimate interactions between SSB and RecG must be taking place. Consistent, the modeling presented in Figure 5 showing the binding of the PXXP motifs in the SSB IDL to the wedge domain of RecG, provides an explanation of how this might occur.

Summary

Stalled DNA replication fork rescue is an essential process. It requires intimate association between SSB protein and the DNA helicase, RecG. This association is critical to loading of RecG onto a fork, further enabling to out compete other proteins. During the loading process, the helicase is remodeled by SSB so that the wedge domain is inaccessible to the fork. Consequently, the helicase domains of RecG mediate binding to the DNA duplex followed by thermal sliding ahead of the fork, possibly clearing bound proteins in its immediate vicinity. A model for the association of SSB and RecG has been proposed that involves interactions between the PXXP motifs of the intrinsically disordered linker of SSB and the oligosaccharide-oligonucleotide binding fold of RecG present in the wedge domain. This is currently being tested.

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