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# Dynamics of *E. Coli* Single Stranded DNA Binding (SSB) Protein-DNA Complexes

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# Dynamics of E. coli single stranded DNA binding (SSB) protein-DNA complexes

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## Abstract

Single stranded DNA binding proteins (SSB) are essential to the cell as they stabilize transiently open single stranded DNA (ssDNA) intermediates, recruit appropriate DNA metabolism proteins, and coordinate fundamental processes such as replication, repair and recombination. _Escherichia coli_ single stranded DNA binding protein (_Ec_SSB) has long served as the prototype for the study of SSB function. The structure, functions, and DNA binding properties of _Ec_SSB are well established: The protein is a stable homotetramer with each

subunit possessing an N-terminal DNA binding core, a C-terminal protein-protein interaction tail, and an intervening intrinsically disordered linker (IDL). *Ec*SSB wraps ssDNA in multiple DNA binding modes and can diffuse along DNA to remove secondary structures and remodel other protein-DNA complexes. This review provides an update on these features based on recent findings, with special emphasis on the functional and mechanistic relevance of the IDL and DNA binding modes.

## Abbreviations

ssDNA single stranded DNA

*Ec*SSB *Escherichia coli* single stranded DNA binding protein

OB oligonucleotide/oligosaccharide

IDL intrinsically disordered linker

SIPs SSB interacting proteins

RPA replication protein A

## Keywords

SSB; ssDNA; RPA; Intersegmental transfer; Diffusion

## 1. Introduction

The genetic code is encoded and protected within double-stranded DNA (dsDNA). To duplicate DNA, or to repair damage, dsDNA must be unwound by enzymes to expose single-stranded DNA (ssDNA). These transiently exposed ssDNA intermediates are rapidly sequestered and protected by a class of proteins called single-stranded DNA binding (SSB) proteins [[1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], [12]]. SSBs play three essential roles in the cell: (a) they bind to ssDNA with high affinity in a sequence-independent manner to protect the ssDNA from nucleolytic degradation [[2],[10],[13], [14], [15]], (b) through specific protein-protein interactions, they recruit a number of DNA metabolic enzymes to the ssDNA [[16]], and (c) in eukaryotes, they trigger the DNA damage cell cycle checkpoint response [[17],[18]]. The assembly of SSBs demarcate the nucleoprotein substrates upon which factors that coordinate DNA metabolic processes bind and initiate DNA replication, repair and recombination [[19]]. *Escherichia coli* SSB (*Ec*SSB) was one of the early SSB proteins to be functionally and structurally characterized and has long-served as the prototype for mechanistic studies of this class of proteins [[1],[4],[6],[13],[20], [21], [22]]. Comprehensive reviews on the DNA binding properties, structure, and function of *Ec*SSB are available [[11],[16]]. In this review, we provide an update on the mechanism of action of SSB, with an emphasis on recent studies revealing the dynamic properties of these complexes, the potential roles of the different SSB-ssDNA binding modes, and regulation of SSB activities by the intrinsically disordered C-termini.

## 2. Structural organization of EcSSB

SSBs are found in all kingdoms of life and while they serve common functional roles, they are structurally divergent (Fig. 1) [[23], [24], [25], [26], [27], [28]]. All SSBs use oligonucleotide/oligosaccharide-binding (OB) domains to bind ssDNA [29]. *Ec*SSB functions as a homotetramer with each subunit containing a single OB-domain (Fig. 1C) [23]. SSBs in thermophilic bacteria such as *Deinococcus radiodurans* and *Thermus aquaticus* function as homodimers (Fig. 1B), but each subunit contains two OB-domains each [[24],[30],[31]]. Several viral and bacteriophage SSBs are known to function as monomers (GP32, Fig. 1A) or dimers (T7 gene 2.5; see the review on the T7 SSB in this volume by Hernandez and Richardson) [[25],[32]]. The SSB protein from *Sulfolobus solfataricus* (crenarchaea) also functions as a monomer with a single OB-domain [27]. The eukaryotic SSB, Replication Protein A (RPA), appears to be the most complex and is a heterotrimer with RPA70, RPA32 and RPA14 subunits [[33],[34]] (see the review on RPA in this volume by Byrne and Oakley). RPA70 harbors three OB-domains and a fourth resides in RPA32 (Fig. 1D) (there are six total OB-domains in RPA with 4 primarily interacting with DNA) [[33], [34], [35]]. hSSB1, another eukaryotic single stranded DNA binding protein that functions in DNA repair, has one OB-fold and functions as a monomer, and in complex with other DNA repair factors. Under conditions of oxidative stress, hSSB1 forms stable higher order oligomers[[36], [37], [38]] (see the review on hSSB1 in this volume by Croft et al.). The OB-domain interacts with ssDNA through a combination of non-specific base-stacking with aromatic amino acids and electrostatic interactions [[23],[26]]. While the binding of multiple OB-domains provides the high affinity of SSB to ssDNA, remodeling and displacement is achieved through selective displacement of one or more OB-domains [[18],[39], [40], [41], [42], [43], [44], [45], [46], [47]].
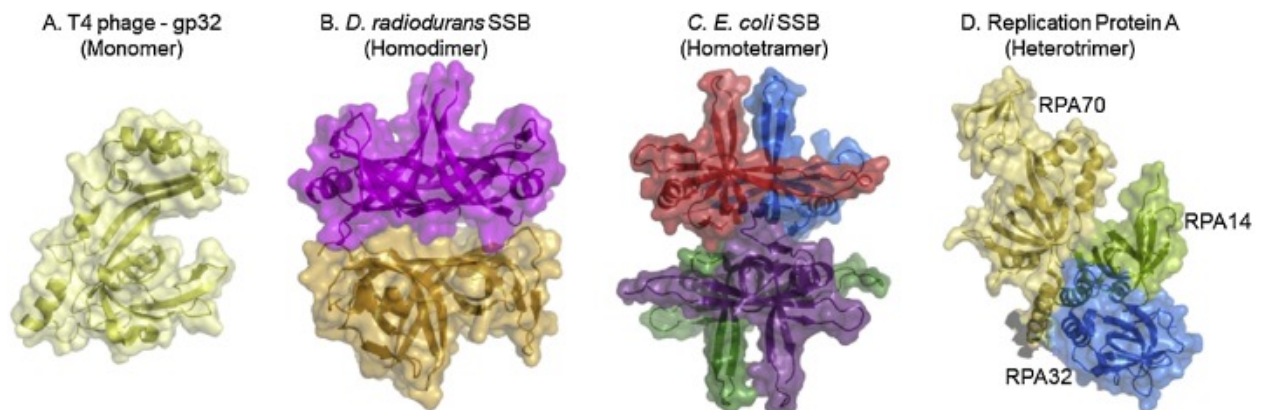


Fig. 1. Subunit composition of single strand DNA binding proteins.

Crystal structures of SSB proteins from various organisms and their respective oligomeric states are depicted. Structures were generated from the following PDB IDs: 1GPC, 3UDG, 1EYG and 4GNX.

*Ec*SSB is structurally organized into an N-terminal DNA binding domain, a C-terminal conserved 9 amino acid tip (TIP) that mediates protein-protein interactions, and an intervening non-conserved intrinsically disordered linker (IDL) (Fig. 2A). The OB-domains interact to form

the tetrameric DNA binding core around which ssDNA wraps (Fig. 2B) [23]. Among the extensive network of protein-DNA contacts, three Trp residues (W40, W54 and W88) mediate base-stacking interactions with ssDNA and are important for the stability of the *Ec*SSB-ssDNA complex [23,48]. The IDL region (residues 113–168) is poorly conserved and is not observed in any crystal structure [23,49,50]. However, the amino acid composition and the length of the IDL influence the binding mode preferences of *Ec*SSB [51,52]. Computational analysis of the IDL region predicts it to exist as an ensemble of globular conformations [52], and an overall compaction of these structures has been observed in solution angle X-ray scattering measurements (SAXS) [53]. Precise functional roles for the IDL region have also been elusive as truncation of the IDL or complete deletion of residues 113–168, leaving behind the C-terminal tip fused to the DNA binding core, appear to be sufficient to complement cell survival *in vivo* [54]. However, the IDL has recently been shown to be crucial for inter-tetramer SSB cooperative binding to ssDNA [51,52]. The final structural feature of *Ec*SSB is its 9-amino acid C-terminal tip (168–177; TIP). SSB interacting proteins (SIPs) bind to the TIP and are recruited to the ssDNA [16,55, 56, 57, 58]. Short peptides corresponding to the TIP have been crystallized in complex with SIPs, and show the last three amino acids (I175, P176 and F177) docked into a hydrophobic binding pocket of the SIPs [55, 56, 57, 58, 59, 60, 61]. The TIP of each SSB subunit represents the dominant site for SSB interaction with other proteins (SIPs). However, it has recently been suggested that the IDL might also mediate protein-protein interactions [62,63], although direct evidence for this is lacking. More than a dozen *S*IPs have been identified thus far and these interactions serve as attractive candidates for the development of small molecule inhibitors to perturb SSB function in the cell [16,59,61,64,65].
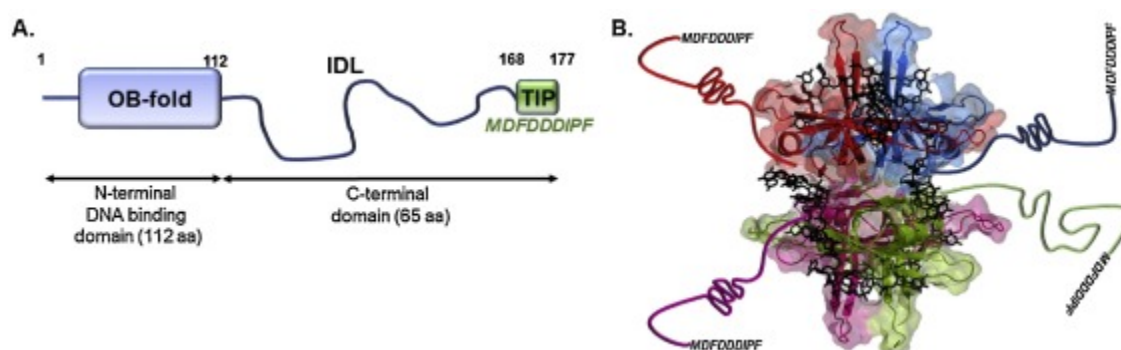


Fig. 2. Architecture of *Ec*SSB.

(A) Schematic of the DNA binding oligonucleotide/oligosaccharide-binding (OB) domain, the C-terminal TIP and the intervening intrinsically disordered loop (IDL) of *Ec*SSB. (B) Crystal structure of *Ec*SSB (cartoon) bound to ssDNA (sticks; 1EYG) is shown with each subunit colored. The IDLs are shown extending away from the DNA binding core and the sequence of the TIP are denoted.

## 3. EcSSB transitions between DNA binding modes

ssDNA can wrap around an *Ec*SSB tetramer with a topology resembling the seams on a tennis ball [23]. Due to the presence of four OB-domains in the tetrameric structure, the number of

SSB subunits interacting with ssDNA can vary and this is influenced by solution conditions [7, 22, [66], [67], [68], [69]]. The variability in the number of ssDNA nucleotides that can interact with an SSB tetramer is exemplified by the observation that SSB can form multiple, distinct binding modes on ssDNA. The population distribution of these binding modes *in vitro* is sensitive to salt concentration and type, pH, temperature and SSB protein to DNA ratio, as well as $Mg^{2+}$, and the polyamines, spermidine, and spermine [7, 22, [66], [67], [68], [69]]. Analysis of *Ec*SSB binding to poly-(dT) revealed the presence of three distinct DNA binding modes: $(SSB)_{35}$, $(SSB)_{56}$, and $(SSB)_{65}$, where the subscript denotes the average number of ssDNA nucleotides occluded by the tetramer [67]. In the $(SSB)_{65}$ mode, all four SSB subunits are bound to ssDNA forming a "fully wrapped" structure. In the $(SSB)_{35}$ mode, the ssDNA interacts with an average of only two SSB subunits, while the SSB remains tetrameric. Less is known about the details of the intermediate $(SSB)_{56}$ structure.

With the exception of the fully wrapped $(SSB)_{65}$ mode, the precise wrapping of ssDNA in these DNA binding modes and the path of the DNA across the OB-domains during transitions between the modes is not fully understood. Single molecule analysis of binding mode transitions show that *Ec*SSB exists in a dynamic equilibrium between multiple, well-defined structural and functional states [44]. Suksombat et al. recently examined the energetics of ssDNA unwrapping from a $(SSB)_{65}$ complex using optical tweezer and fluorescence single molecule approaches [45]. This led to further insights into the topologies of ssDNA wrapping across the four OB-domains. As expected, *Ec*SSB displays the three dominant DNA binding modes $(SSB)_{65}$, $(SSB)_{56}$ and $(SSB)_{35}$. The transitions among the modes occurs without tetramer dissociation, but SSB shows an ability to diffuse along the DNA while releasing segments of ssDNA [43, 44, 70, 71]. Such transitions that free ssDNA from *Ec*SSB-DNA complexes provide opportunities for proteins such as RecA to gain access to the ssDNA and further displace *Ec*SSB. Both the IDL and C-terminal TIP of *Ec*SSB modulate the transitions among the various DNA binding modes [43, 51, 72], and SIPs that interact with the TIP affect the transitions between the DNA binding modes. PriA, PriC, and RecQ, three SIPs, have been shown to interact with SSB in its $(SSB)_{65}$ mode and facilitate partial unwrapping of the ssDNA [56, [73], [74], [75], [76]].

## 4. Conformations of the intrinsically disordered linker (IDL) of EcSSB

While the importance of the DNA binding domain and TIP region for *Ec*SSB function are well established, the role of the IDL is poorly understood. The IDLs are generally conserved in bacteria, but can vary in length (25–135 residues) and composition [16, 52, 63]. Interestingly, the human mitochondrial SSB, which is structurally similar to *E. coli* SSB, is missing an IDL [77]. As we have noted, computational and experimental comparisons of the IDLs from *Ec*SSB and the *Plasmodium falciparum* SSB (*Pf*SSB) have shed light on its functional roles [51, 52, 78]. The *Ec*SSB IDL is 56 amino acid long and glycine-rich with few charged residues, whereas the *Pf*SSB IDL is 80 amino acid long, asparagine-rich and contains significantly more charged residues. Computational studies of the conformational properties of the IDLs suggest that the *Ec*SSB IDL forms heterogeneous conformations that are globular in nature [51, 52]. In contrast, the IDL from *Pf*SSB is predicted to form more extended structures resembling Flory random coil distributions. These predictions agree with hydrodynamic properties measured in solution for these two proteins [51, 52]. Complete deletion of the IDL of *Ec*SSB eliminates highly

[cooperative binding](#) of SSB to ssDNA [51,52]. Interestingly, replacement of the 56 amino acid *Ec*SSB IDL with the 80 amino acid IDL from *Pf*SSB also eliminates cooperative binding, as well as the (SSB)$_{35}$ DNA binding mode [51]. The current model posits that the globular nature of the *Ec*SSB IDLs promote physical interactions among SSB tetramers when bound to ssDNA and facilitates cooperative binding ([Fig. 3](#)) [51]. The IDLs of the majority of the bacterial [SSB proteins](#) are homologous in amino acid compositions to that of *Ec*SSB and are also predicted to adopt globular conformations similar to *Ec*SSB. *E. coli* strains carrying *Ec*SSB variants that lack the IDL region are viable and replicate; however, they show an increased sensitivity to UV irradiation, suggesting that the IDL length and composition is important to recruit [DNA repair proteins](#) [51,52,79]. One explanation could be that removal of the IDL hinders accessibility of the acidic TIP region to interact with some of the SIP proteins. In support of this explanation, strains carrying SSB with only partial deletions of the IDL respond to UV irradiation with sensitivities similar to [wild type](#) [52].

**A. IDL and TIP mediated cooperativity in the (SSB)$_{35}$ mode**
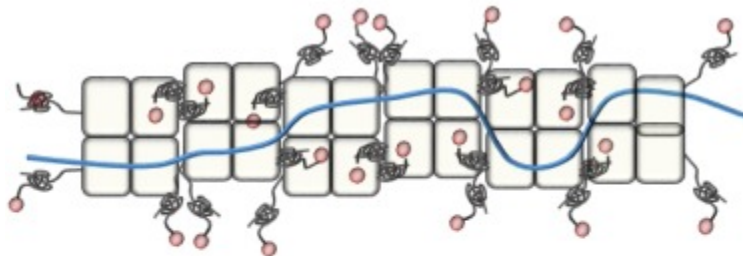
**B. IDL mediated cooperativity in the (SSB)$_{65}$ and (SSB)$_{56}$ modes**
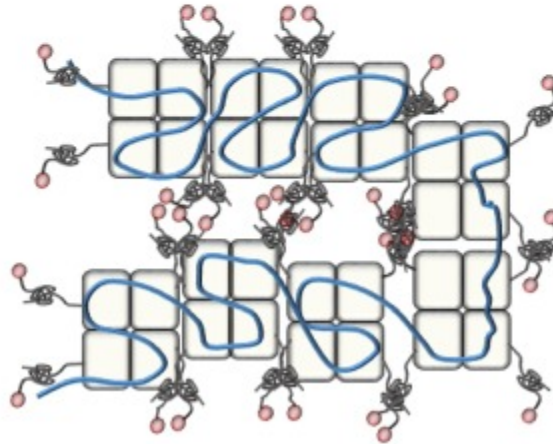


Fig. 3. Models of IDL and TIP mediated cooperativity in *Ec*SSB.

(A) [Cooperative binding](#) of SSB tetramers in the (SSB)$_{35}$ mode is shown. Proposed interactions between the IDLs of neighboring tetramers along with TIP interactions with free ssDNA binding regions in the OB-domains are denoted. (B) Similar cooperative binding to ssDNA in the (SSB)$_{65}$ and (SSB)$_{56}$ modes are proposed to be facilitated through interactions between IDLs of multiple tetramers.

## 5. IDLs mediate cooperativity in SSB-DNA interactions

*Ec*SSB forms cooperative nucleoprotein filaments on long ssDNA substrates that were first visualized by electron microscopy in 1972 [1]. These filaments form under both high and low SSB binding densities, and this cooperative feature was subsequently reproduced in buffers containing low [NaCl] (<10 mM) [66]. Under these conditions, SSB adopts the $(SSB)_{35}$ binding mode, and hence, it was thought until recently that this binding mode was essential for highly cooperative binding behavior. However, recent evidence shows that at physiological salt concentrations containing either acetate or glutamate, which is the dominant monovalent anion in *E. coli,* highly cooperative binding is promoted even when SSB is in a fully wrapped $(SSB)_{65}$ or $(SSB)_{56}$ mode [51]. This was previously obscured because high [NaCl] had typically been used to selectively populate the $(SSB)_{65}$ mode and high [Cl⁻] inhibits cooperativity [7,22,68,80]. The length and composition of the IDL plays a key role in promoting cooperativity. Single molecule studies of SSB-ssDNA interactions in acetate salts show evidence for additional compaction of SSB-DNA complexes beyond that expected from ssDNA wrapping in the $(SSB)_{65}$ mode [81]. This additional compaction likely reflects cooperative binding that is promoted in acetate salts.

Cooperative binding is not observed for the *Pf*SSB protein which shares a high degree of homology with *Ec*SSB in the DNA binding core [50,78]. This appears to be due primarily to the very different IDLs of the two SSB proteins. The *Ec*SSB IDL contains only 3 charged residues (2 R and one E) in addition to the 4 negatively charged residues in the TIP region and is predicted to adopt a compact globular conformation [52]. In contrast, the *Pf*SSB IDL contains 26 charged residues in addition to 3 in its different acidic tip and is predicted to form an ensemble of more expanded Flory random coil configurations [52]. The cooperativity observed in *Ec*SSB is stable even under high concentrations of glutamate (0.5 M) indicating that electrostatic interactions are not a major stabilizing factor for cooperativity [51]. In addition, a chimeric version of *Ec*SSB in which the IDL from *Pf*SSB is substituted for the EcSSB IDL no longer shows cooperative DNA binding [51]. Hence, the more globular, uncharged IDL is needed to promote highly cooperative binding indicating a functional role for the IDL in *Ec*SSB.

A role for the IDL and the acidic TIP region was proposed in facilitating cooperative interactions within the $(SSB)_{35}$ mode [82]. In this model, the TIP from one tetramer interacts with unoccupied DNA binding sites in a neighboring tetramer (Fig. 3A) [83]. However, such a scenario would be prevented in the $(SSB)_{65}$ mode since all subunits are occupied by ssDNA. Since cooperative binding has now been observed in the fully wrapped binding mode, it is possible that high cooperativity is promoted primarily through direct interactions between IDLs of tetramers (Fig. 3B). It is likely that a combination of these features is utilized during DNA binding mode transitions and further modulated by interactions with SIPs.

## 6. SSB interacting proteins (SIPs)

More than one dozen enzymes involved in DNA repair, replication and recombination interact with the TIP of *Ec*SSB. All of the direct SIP:SSB interactions characterized to date are mediated through the TIP, which in *Ec*SSB is – Met-Asp-Phe-Asp-Asp-Asp-Ile-Pro-Phe, with

Phe being at the C-terminus (Fig. 2). A detailed list of SIPs, their functions, and structural features have been reviewed previously by Shereda et al. [16]. Three additional SIPs (RNaseHI, RadD and DinG) have since been identified and an updated list of SIPs is presented (Fig. 4A) [55,84,85]. Briefly, SIPs can be categorized according to their functions in DNA replication, replication restart, recombination and repair. Crystal structures of several SIPs bound to short TIP containing peptides have been solved [55,57,58,60,75,86]. In all of these structures, the last three amino acids of the TIP (Ile-Pro-Phe) are well ordered and bind to a hydrophobic pocket in the SIP. An alignment of all the TIPs in these structures is shown in Fig. 4B. The proline and phenylalanine adopt a rigid conformation akin to a hook binding into the active site of the SIPs. The other residues away from the active site are more mobile as evidenced from the deviations in the B-factors upon alignment (Fig. 4B). Direct binding studies suggest that most of the binding free energy for SSB-SIP interactions is due to interactions with the SSB TIP [87].



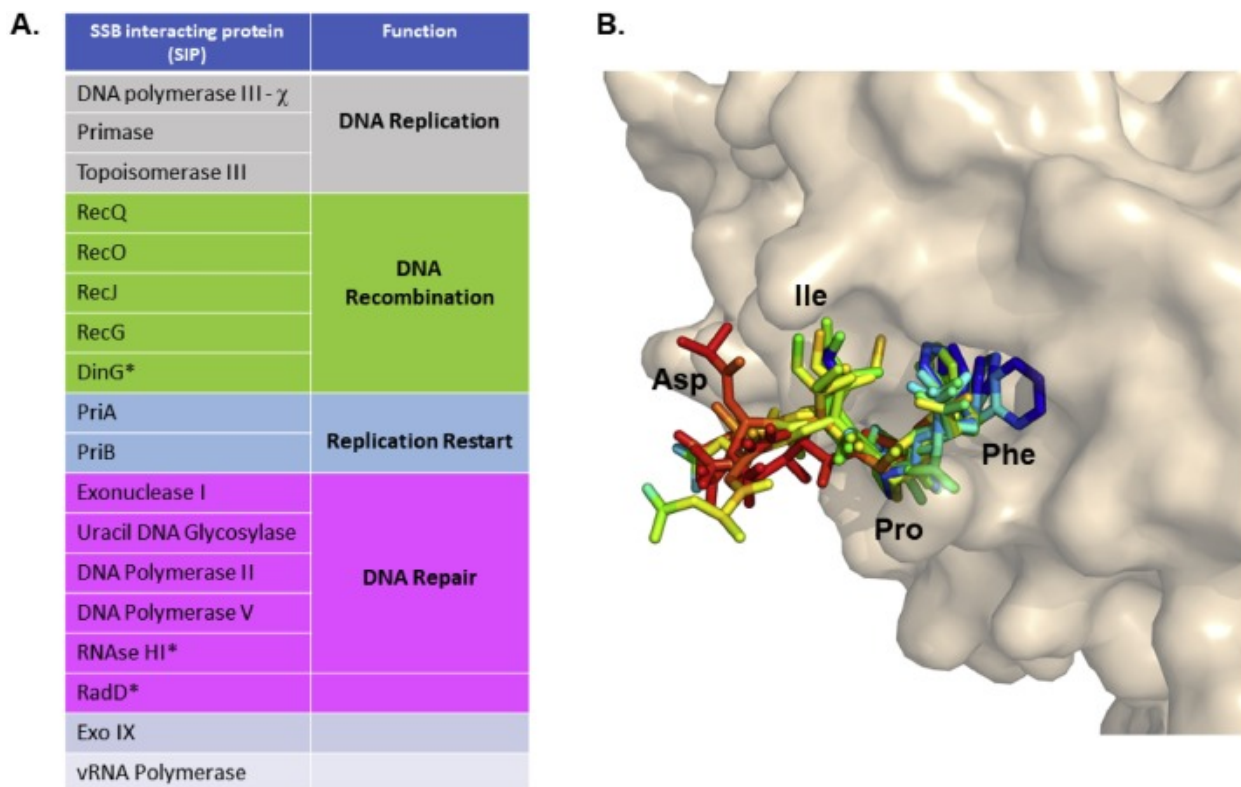| SSB interacting protein (SIP) | Function |
| --- | --- |
| DNA polymerase III - χ | DNA Replication |
| Primase | |
| Topoisomerase III | |
| RecQ | DNA Recombination |
| RecO | |
| RecJ | |
| RecG | |
| DinG* | |
| PriA | Replication Restart |
| PriB | |
| Exonuclease I | DNA Repair |
| Uracil DNA Glycosylase | |
| DNA Polymerase II | |
| DNA Polymerase V | |
| RNAse HI* | |
| RadD* | |
| Exo IX | |
| vRNA Polymerase | |

Fig. 4. SSB interactions with SSB interacting proteins (SIPs).

(A) An updated list of the SIPs identified to date are categorized according to their cellular function. The asterisks denote newly identified SIPs. (B) The crystal structure of exonuclease I in complex with the terminal four residues in the EcSSB TIP (PDB ID: 3C94) is shown. Similar TIP regions from multiple SIP-TIP peptide structures were aligned and shown here colored according to conformational flexibility (B-factors); red and blue denote extremes of high and low flexibility, respectively. The Ile-Pro-Phe residues adopt similar conformations in all these structures, whereas the Asp residue situated away from the active site can adopt multiple conformations.

While almost all SIP interactions to SSB identified to date occur through the TIP region, a recent report suggests that deletions of the IDL regions lead to a loss of SSB interaction with the RecO and RecG proteins in pull down experiments [88]. The authors posit potential roles for three Pro-X-X-Pro motifs in the IDL region being responsible for mediating interactions with RecO and RecG and possibly neighboring SSB tetramers leading to cooperative binding [62,63,88]. However, it is known that the TIP region is essential for SSB interactions with RecO and RecG [[89], [90], [91]]. A variant of SSB, SSBΔC8, lacking the TIP does not support RecG- or RecO-related biochemical activities [92,93]. Further biochemical analysis is required to better understand this potential mode of binding.

## 7. SSB-SIP interactions affect SSB-DNA binding modes

In the $(SSB)_{65}$ mode, the ssDNA is fully wrapped around the tetramer, yet during DNA repair and recombination, numerous SIP proteins need to gain access to the SSB-bound DNA. Recent studies show that upon interaction with SSB, SIPs can facilitate a partial ssDNA unwrapping that promotes a transition from the $(SSB)_{65}$ to the $(SSB)_{35}$ mode (Fig. 5A, B) [43,56,74]. One example is the situation when the DNA replication machinery is prematurely displaced when DNA damage is encountered leading to a stalled replication fork. Replication restart under these conditions is coordinated by the PriA DNA helicase, which is recruited to stalled sites through its interaction with SSB [56]. Using FRET to monitor the $(SSB)_{65}$ versus $(SSB)_{35}$ modes, Bhattacharyya et al., showed that PriA binds to SSB and promotes a transition from the fully wrapped $(SSB)_{65}$ mode to a partially wrapped state, likely similar to the $(SSB)_{35}$ mode [74]. This transition is necessary for PriA to bind ssDNA and initiate replication restart. PriC, another SIP within this replication restart machinery, also binds to $Ec$SSB and can facilitate formation of the $(SSB)_{35}$ binding mode [56]. Another example of SIP-induced reordering of the SSB binding mode has been observed with the RecQ helicase. RecQ is a helicase/translocase that catalyzes dsDNA strand separation to resolve complex DNA structures such as double-Holliday junctions, displacement loops (D-loops) and converging replication forks. RecQ is recruited to these structures through its interaction with SSB, and similar to PriA, needs to displace SSB, at least partially, to gain access to the buried ssDNA [76]. In a recent study, Mills et al. show that RecQ binds to SSB and promotes its transition from the $(SSB)_{65}$ to the $(SSB)_{35}$ mode to gain access to the ssDNA, ultimately displacing SSB in the process [76].
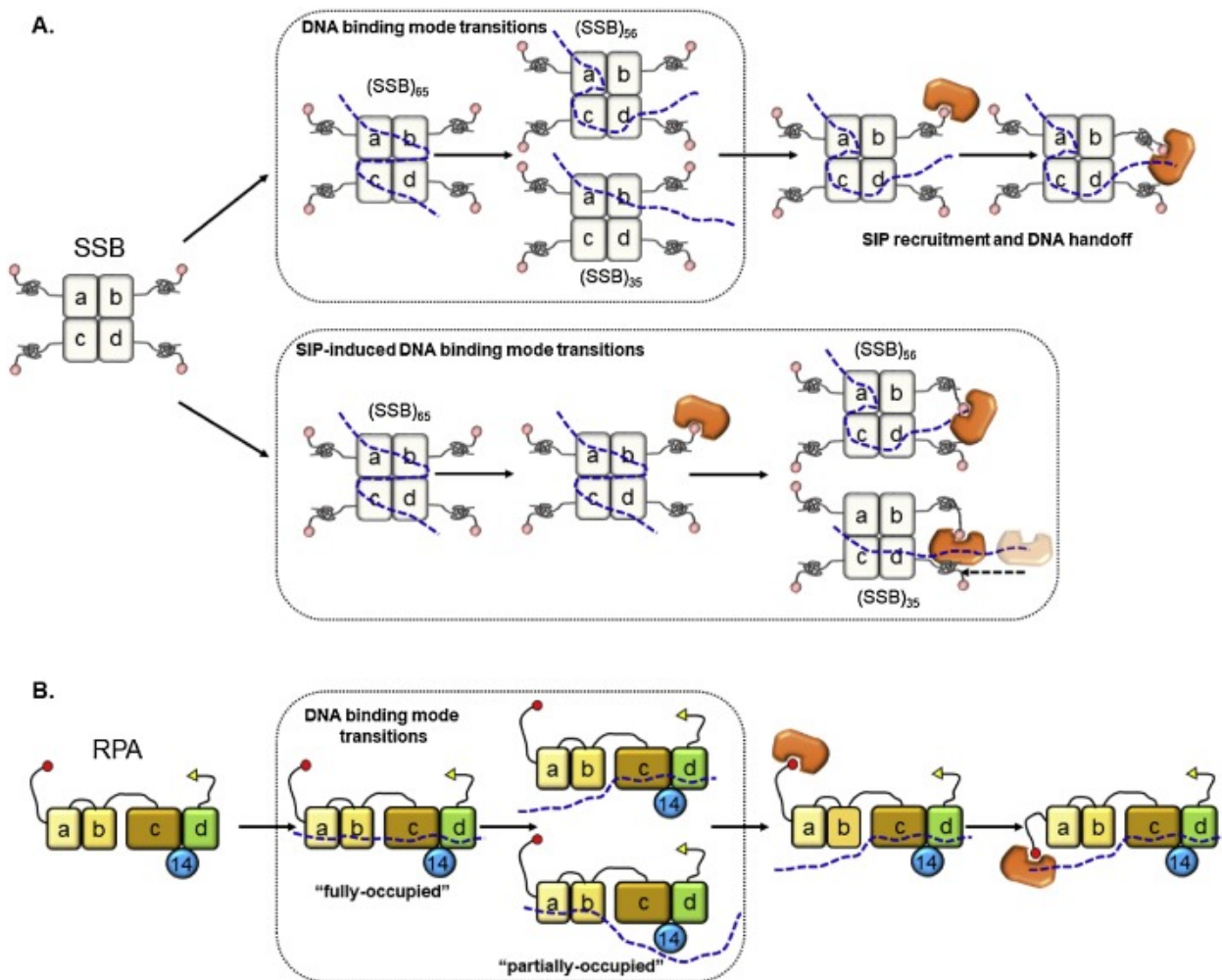
Fig. 5. DNA binding mode transitions in SSB.

(A) SSB can spontaneously transition between the $(SSB)_{35}$, $(SSB)_{56}$, and $(SSB)_{65}$ binding modes, and the transiently open ssDNA allow binding of SIPs. A model where SIPs facilitate transitions between binding modes is also depicted. (B) The four-OB domains in RPA that are primarily responsible for ssDNA binding are shown. DNA binding domains (DBD) a, b and c reside in RPA70 and are connected by flexible linkers. DBD c, d and the RPA14 subunit form the trimerization core. RPA is also proposed to transition between multiple binding modes enabling the binding of RPA interacting proteins (orange) to ssDNA vacated by one or more DBDs.

## 8. Role of SSB-ssDNA dynamics in remodeling of high-affinity SSB-DNA complexes

The rapid and high-affinity binding of EcSSB to ssDNA protects transiently exposed ssDNA during the various DNA metabolic processes. However, the SSB protein must eventually be removed from the ssDNA by other DNA processing enzymes, which have lower affinities for

DNA. How do cells displace the high affinity SSB? Three key features of *Ec*SSB help in this matter: (a) an SSB tetramer is capable of diffusing along ssDNA even when bound tightly in the fully wrapped (SSB)$_{65}$ mode [44,46]. As such it can be moved directionally from a particular site on ssDNA by the action of a nucleic acid motor protein such as an ATP-dependent translocase or a polymerase [94], (b) an SSB tetramer can undergo a direct (inter-segment) transfer between sites on ssDNA without dissociation from the ssDNA [42]. This allows the SSB to readily move between sites on a ssDNA molecule and may be important in recycling of SSB between Okazaki fragments on the lagging strand during DNA replication, (c) the interaction between the TIP of *Ec*SSB and the SIPs can function to load a specific SIP onto the ssDNA substrate (Fig. 5A).

## 9. SSB diffusion along ssDNA

In the (SSB)$_{65}$ binding mode, all four OB-domains interact with ssDNA. However, an *Ec*SSB tetramer is able to diffuse along ssDNA, with an apparent one-dimensional diffusion coefficient of 270 nt$^2$/s at 37 °C [43,46]. Zhou et al. proposed a '*reptation model*', where SSB movement would occur through formation of a transient bulge in the DNA which progressively moves through the four OB-folds (Fig. 6A) [46]. This propensity of *Ec*SSB to diffuse is retained when it is bound to SIPs such as RecO, suggesting that this is a property important for cellular function.

**A. 'Reptation model' for SSB diffusion/sliding**

$\sim 270\,nt^2/s$
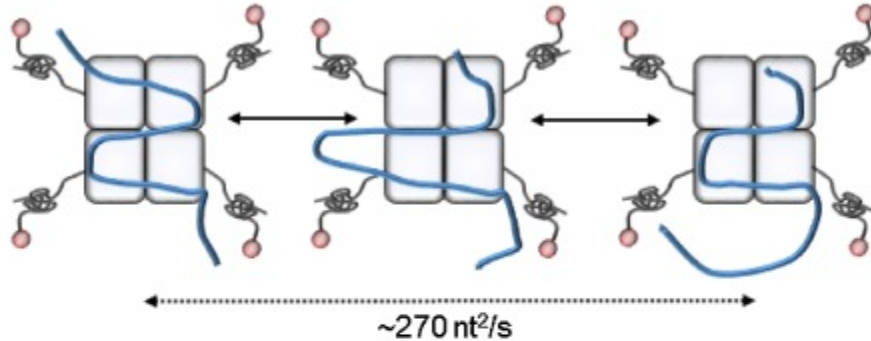
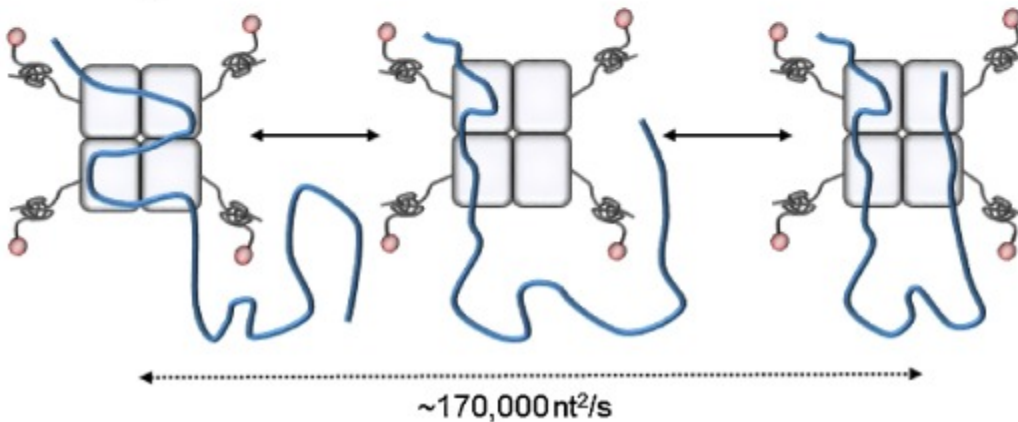**B. 'Intersegmental transfer' of SSB**

$\sim 170,000\,nt^2/s$

Fig. 6. Diffusion of SSB.

(A) The reptation model for *Ec*SSB diffusion/sliding is shown where segment(s) of ssDNA-OB domain interactions are perturbed allowing another OB-domain to bind to the free DNA. This enables the entire tetramer to shift or diffuse along the DNA lattice. (B) On long ssDNA, SSB achieves rapid diffusion using principles of direct transfer where transiently dissociated ssDNA are replaced by DNA from a distant location. The respective rates for the two processes are denoted.

The ability of SSB proteins to diffuse along ssDNA provides the mechanism by which they can transiently invade a DNA duplex to partially melt a duplex DNA hairpin [43,95]. Motor proteins can also rectify the movement of SSB along ssDNA. For example, a 5′ -> 3′ translocase such as Pif1 can push *Ec*SSB in the same direction, whereas a 3′ -> 5′ translocase such as UvrD can push *Ec*SSB in the opposite direction [94]. The diffusional properties of *Ec*SSB can also be utilized by the RecA protein to facilitate formation of a nucleoprotein filament on ssDNA. *Ec*SSB diffusion provides the mechanism by which it can remove secondary structures such as hairpins that hinder RecA filament formation [43].

The ability of an SSB protein to diffuse along ssDNA also is a property of other multi-OB domain proteins such as its eukaryotic homolog replication protein A (RPA; see the review on

RPA by Byrne and Oakley in this issue). RPA also harbors four OB-domains, but are arranged within a heterotrimeric subunit architecture (Fig. 5B) [34]. The RPA-70 subunit has three OB-domains and the fourth OB-domain resides in the RPA-32 subunit [26,28]. RPA also binds to ssDNA in multiple DNA binding modes (occluded site size 18–28 nt/RPA) that are influenced by salt concentration [35]. Similar to EcSSB, RPA also diffuses on ssDNA (diffusional coefficient of ~5000 $nt^2 s^{-1}$ at 37 °C) [95]. The models proposed for EcSSB diffusion and sliding may also be applicable for RPA (Fig. 5B) and its remodeling by RPA-interacting proteins.

## 10. Redistribution of SSB on DNA via direct transfer mechanisms

EcSSB tetramers can also be redistributed along ssDNA via direct transfer or intersegment transfer mechanisms without proceeding through a free (fully dissociated) SSB intermediate (Fig. 6B). Kozlov et al. showed that this direct transfer proceeds through a doubly ligated SSB intermediate where a SSB tetramer is bound to two ssDNA molecules (or different segments of the same DNA) [47]. One key requirement for direct transfer is the need for multiple DNA binding sites on the SSB and the availability, at least transiently, of free ssDNA binding sites on a DNA bound SSB tetramer. With this in mind, direct transfer occurs much more rapidly for SSB in its (SSB)$_{35}$ binding mode, with 2 free ssDNA binding sites, than in either the (SSB)$_{65}$ binding mode [47]. The direct transfer process could be used to recycle SSB during lagging strand DNA synthesis. SSB is thought to bind primarily to the lagging DNA strand during DNA replication, but must then be displaced by DNA PolIII to finish replication [96]. Direct transfer could be used as a mechanism to recycle SSB between Okazaki fragments to promote rapid DNA replication. Consistent with this hypothesis, several SIPs are efficient at shifting SSBs into various DNA binding modes (where one or more OB-domains are free to bind an additional DNA molecule) [56,74].

An "intersegment" transfer of SSB within a single ssDNA molecule has been hypothesized to explain the roughly 600-fold higher apparent diffusion coefficient of an SSB tetramer on long ssDNA (Fig. 6B) [42]. While one dimensional diffusion of SSB on short ssDNA occurs with an apparent one-dimensional diffusion coefficient of ~270 $nt^2$/s at 37 °C (Fig. 6A), a much higher apparent diffusion coefficient of 170,000 $nt^2$/s was measured for much longer ssDNA [42]. This large difference could be explained if SSB can undergo long range intersegmental transfer in addition to its short-range diffusion/sliding. An SSB tetramer bound to one segment of a long ssDNA could transiently unwrap ssDNA from one or more of its OB-domains which could then bind to a more distant segment of the DNA strand (Fig. 6B).

Intersegment transfer would allow SSB to move around and scan a larger span of DNA more efficiently than one-dimensional diffusion [42]. Such long-range movement might be utilized to recruit and position appropriate SIPs at distant locations from where it resides. In addition, when SSB-ssDNA complexes are encountered by a helicase/translocase, the hinderance could be relieved by moving the SSB to a distant spot through intersegment transfer [94]. Such protein relocation mechanisms have been observed for histone repositioning on dsDNA during transcription by RNA polymerase [97]. Local diffusion and intersegment transfer can potentially be coupled as local short-range diffusion/sliding facilitate a transition from a fully wrapped (SSB)$_{65}$ mode to a partially wrapped (SSB)$_{56}$ or (SSB)$_{35}$ mode, making available a ssDNA binding site to participate in intersegment transfer by binding to a distantly located ssDNA site.

## 11. How many SSB tails are needed for function?

While *Ec*SSB is a [homotetramer](#), the homologous [SSB protein](#) in thermophilic organisms function as homodimers ([Fig. 1](#)). However, a comparison of their structures reveals that they are also composed of four OB-domains with two OB-domains residing in each subunit [[24](#),[31](#)]. This architecture results in a key difference in the number of [C-terminal](#) tails and thus two TIP regions per complex. For example, the SSB protein from *Deinococcus radiodurans* (*Dr*SSB) has two C-terminal tails capable of interacting with up to two SIPs, whereas *Ec*SSB has four C-terminal tails capable of interacting with up to four SIPs. Is there a functional advantage to having four versus two tails? What is the minimum number of tails needed per SSB "tetramer"? We addressed these question by generating linked *Ec*SSB proteins where either two or all four OB-domains were linked using short [peptide](#) linkers yielding SSB proteins with four OB-folds, but only either two or one free C-termini [[79](#)]. Most of the DNA binding properties of these linked SSB proteins were not greatly affected, although the binding mode transitions were shifted so that the SSBs with fewer tails showed a progressive promotion of the $(SSB)_{35}$ binding mode. In addition, the one-tailed tetramer showed a reduction in cooperative DNA binding. More significantly, the two-tailed tetramer complemented growth *in vivo*, whereas the one-tailed tetramer was [dominant negative](#) due to decoupling of leading and lagging strand replication [[79](#)]. This suggests that SSB must utilize two of its tails simultaneously during some essential process. Strains carrying the two-tailed tetramer grew faster, accumulated spontaneous mutations at a seven-fold faster rate, and were able to survive high doses of UV-irradiation [[79](#)]. These features are surprisingly similar to that of *Deinococcus radiodurans* suggesting that the interaction with the SIPs are a critical component of [DNA repair](#) and survival.

## 12. Cellular roles of DNA binding modes

[SSB protein](#) binding to ssDNA using multiple DNA binding modes is not a unique feature of *Ec*SSB. Other bacterial SSB proteins and the eukaryotic RPA complex also display multiple DNA binding modes that are sensitive to solution conditions [[35](#),[95](#),[98](#)]. In contrast, *Pf*SSB from the apicoplast of *Plasmodium falciparum*, does not populate an $(SSB)_{35}$ mode, but rather favors the fully wrapped $(SSB)_{56}$ and $(SSB)_{65}$ DNA binding modes [[50](#),[78](#)]. This seems to be a direct consequence of the vastly different and more highly charged *Pf*SSB IDL. The precise cellular functions of these DNA binding modes have been elusive. It is likely that all of the SSB binding modes are populated at least transiently *in vivo.* This seems obvious since the partially wrapped SSB-DNA structures must be intermediates along the path to forming a fully wrapped structure. In fact, the dynamic transitions among the different SSB-DNA binding modes is likely key to some of its functions. The $(SSB)_{65}$ binding mode has been proposed to be associated with events in [DNA recombination](#), as conditions that promote this mode *in vitro* correlate with the propensity of the RecA [recombinase](#) to perform strand exchange [[43](#)]. Similarly, the $(SSB)_{35}$ mode has been proposed to be used during DNA replication, and two recent studies lend support to this model. A single molecule investigation conducted under near-physiological buffer conditions, and where the generation of ssDNA is coupled to DNA replication on a fork substrate, has shown that the human [mitochondrial](#) SSB (*Hm*SSB) binds primarily using a low site size binding mode, likely the $(SSB)_{35}$ mode [[99](#)]. Using linked *Ec*SSB subunits, Waldman et al. investigated the effect of preventing the formation of a fully wrapped ssDNA-SSB tetramer

on cell viability [72]. By covalently linking two SSB subunits, they generated $EcSSB$ "dimers" in which each subunit contains two OB-domains. This allowed them to make SSB variants in which two OB-domains were selectively rendered defective for ssDNA binding through mutagenesis yielding $Ec$SSB variants unable to form the $(SSB)_{65}$ or $(SSB)_{56}$ modes. These variants were able to functionally complement wild type $Ec$SSB, indicating that the SSB protein can carry out its essential functions without the fully wrapped DNA binding mode [72]. These experiments provide the first *in vivo* evidence that the $(SSB)_{35}$ binding mode can function in DNA replication. However, these cells do show a hyper-resistance phenotype to DNA damage inducing agents and an accumulation of mutations as a trade-off for survival. These results suggest that adequate DNA repair mechanisms are poorly coordinated and thus a role for the $(SSB)_{65}$ mode in DNA repair and recombination cannot be ruled out.

## 13. Conclusions

While recent studies have expanded our knowledge of the dynamics of SSB-ssDNA complexes and how these can be remodeled during DNA metabolism, many questions remain to be addressed: (i) what is the molecular basis for how the IDL of $Ec$SSB promotes cooperativity? (ii) What is the role of cooperativity *in vivo?* (iii) How and when does SSB interact with its many binding partners (SIPs) to achieve selectivity during DNA replication, repair, and recombination?

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