A Genetic Analysis of the Functional Interactions within *Mycobacterium tuberculosis* Single-Stranded DNA Binding Protein

Kervin Rex¹*, Sanjay Kumar Bharti¹*, Shivjee Sah¹, Umesh Varshney¹,²*

1 Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India, 2 Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

**Abstract**

Single-stranded DNA binding proteins (SSBs) are vital in all organisms. SSBs of *Escherichia coli* (*EcoSSB*) and *Mycobacterium tuberculosis* (*MtuSSB*) are homotetrameric. The N-terminal domains (NTD) of these SSBs (responsible for their tetramerization and DNA binding) are structurally well defined. However, their C-terminal domains (CTD) possess undefined structures. *EcoSSB* NTD consists of β₁-β₅-β₂-β₃-x-β₆-β₄-β₅₃-β₅₂-β₅ secondary structure elements. *MtuSSB* NTD includes an additional β-strand (β6) forming a novel hook-like structure. Recently, we observed that *MtuSSB* complemented an *E. coli* Δssb strain. However, a chimeric SSB (mβ₄-β₅), wherein only the terminal part of NTD (β₄-β₅ region possessing L₄₅ loop) of *EcoSSB* was substituted with that from *MtuSSB*, failed to function in *E. coli* in spite of its normal DNA binding and oligomerization properties. Here, we designed new chimeras by transplanting selected regions of *MtuSSB* into *EcoSSB* to understand the functional significance of the various secondary structure elements within SSB. All chimeric SSBs formed homotetramers and showed normal DNA binding. The mβ₄-β₆ construct obtained by substitution of the region downstream of β₅ in mβ₄-β₅ SSB with the corresponding region (β₆) of *MtuSSB* complemented the *E. coli* strain indicating a functional interaction between the L₄₅ loop and the β₆ strand of *MtuSSB*.

**Introduction**

Single-stranded DNA binding protein (SSB) binds single-stranded DNA in a sequence independent manner during major DNA transactions such as DNA replication, repair and recombination [1–5]. Besides their crucial function in DNA transactions, they protect transiently generated single-stranded DNA (ssDNA) from nucleases or chemical attacks [6]. The eubacterial SSBs contain subunits with a similar basic fold, but may exhibit variations in their quaternary association [7]. SSBs possess an oligonucleotide-binding fold (OB-fold) in the N-terminal domain responsible for their oligomerization and DNA binding. The conserved C-terminal acidic tail of SSBs is important in protein-protein interactions [8–11]. One of the features of EcoSSB, important for its in vivo function, is the dynamic transition in its modes of DNA binding [6,12]. SSB binds to ~35 nucleotides by two of its subunits known as SSB₁, and is required for unlimited cooperatively. While all the four subunits bind to ~56 or ~65 nucleotides in a limited cooperative manner known as SSB₅₆ or SSB₅₆, respectively [13–16].

The crystal structures of SSB in free and DNA bound forms have provided valuable information to understand their function [17,18]. *EcoSSB* monomer consists of an N-terminal domain (~115 amino acids) of defined structure, and the C-terminal domain whose three dimension structure is not available. The tertiary structure of the N-terminal domain of *EcoSSB* is defined by the presence of β₁-β₁'-β₂-β₃-β₄-β₄₃-β₄₅₂-β₅ secondary structure elements (Fig. 1). In the X-ray crystal structure, one of the β hairpin loops (L₄₅) with well-defined electron density connects β₄ and β₅. Structural studies of *EcoSSB* suggested that its quaternary association is mediated by the L₄₅ loop as well as by the six-stranded β-sheets formed by the dimers [17]. Furthermore, the L₄₅ loop undergoes a significant change upon binding to DNA [18]. Functional importance of this movement, however, remains unclear.

*MtuSSB* shares ~30% identity and ~39% similarity with *EcoSSB* in its primary sequence. The secondary structure involved in OB-fold is very similar in the two SSBs except for the presence of a novel β₆ strand (numbered according to *EcoSSB*, 17) downstream of the β₅ in *MtuSSB* (Fig. 1). While both the SSBs possess overlapping tertiary structures, there are notable variation in their quaternary associations due to the presence of the β₆ strand in *MtuSSB* [19]. Although a role for β₆ strand in providing stability through the formation of a clamp like structure has been suggested in the mycobacterial SSBs [19–21] its biological importance is unknown.

Recently, using an in vivo assay wherein replication of the resident *sbb* support plasmid in an *E. coli* strain deleted for its chromosomal copy of *sbb* gene could be selectively blocked, we showed that overexpression of *MtuSSB* complemented *E. coli* [22].
However, a chimeric SSB (m₄-b₅), wherein the b₄-b₅ region (which possess the L₄₅ loop) of EcoSSB was replaced with the corresponding secondary structure elements of MtuSSB, did not complement the strain [22]. This suggested that the L₄₅ loop might be involved in specific interactions within MtuSSB. In this study, we have designed additional chimeric constructs to uncover the importance of such interactions between the MtuSSB L₄₅ loop and the novel b₆ strand for its function in E. coli.

Material and Methods

DNA oligomers, bacterial strains and media

DNA oligomers (Table 1) were obtained from Sigma-Aldrich, India. E. coli strains (Table 1) were grown in Luria-Bertani (LB) medium. LB-agar contained 1.6% (w/v) agar (Difco, USA) in LB. Ampicillin (Amp, 100 µg/ml), kanamycin (Kan, 25 µg/ml), tetracycline (Tet, 7.5 µg/ml), or chloramphenicol (Cam, 15 µg/ml) were added to growth media as required.

Cloning, overexpression, purification and gel filtration analysis of SSBs

Standard recombinant DNA methods and site directed mutagenesis [23] were used to generate chimeric SSBs (Table 1, and Methods S1). SSB open reading frames were subcloned into pTrc99C, pBAD/HisB and pET11d vectors, purified and stored in 50 mM Tris-HCl, pH 8.0, 0.1 mM Na₂EDTA, 500 mM NaCl and 10% glycerol [22]. Oligomeric status of SSBs was determined by gel filtration chromatography [22,24].

Electrophoretic mobility shift assays (EMSA)

SSB tetramers (0.2, 2 and 10 pmol) were mixed with 5’-[³²P]-end labeled 79mer DNA oligomer (1 pmol, ~20,000 cpm) in 15 µl reactions containing 20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5% glycerol (v/v) and 50 µg/ml BSA, incubated for 30 min at 4°C and electrophoresed on 8% native-PAGE (30:0.5, acrylamide:bisacrylamide) using 1X TBE (Tris-Borate-Na₂EDTA) for 1–2 h at 15 V cm⁻¹ in cold room, and visualized by BioImage Analyzer (FLA5000, Fuji).

Complementation analysis

The complementation assays were performed using a recently described revised plasmid bumping method [22]. Briefly, the pBAD based expression constructs were introduced into E. coli RDP317-1 harboring pHYD-EcoSSB as support plasmid (ColE1 ori, CamR, whose replication is dependent on the presence of isopropyl-β-D-thiogalactopyranoside, IPTG) and the transformants were selected on LB agar containing Kan, Amp and 0.02% arabinose (or Kan, Amp and 0.5 mM IPTG, as control). The isolated colonies were streaked on LB agar containing Kan and Amp with various concentration of arabinose.
lysine treated multi-well slide, washed with PBS and visualized in

centrifugation, fixed with 4% paraformaldehyde, kept on poly-L
2 ml LB containing arabinose). Bacterial cells were collected by
pBAD based SSB constructs were grown to log phase (7–9 h in

Growth curve analysis

Freshly isolated transformants were inoculated in LB containing
Kan, Amp and 0.02% arabinose to obtain late stationary phase
cultures; and inoculated at 0.1% level in LB containing Kan, Amp
and 0.02% arabinose (as indicated) in the honeycomb plates. The growth
was recorded at 600 nm using Bioscreen C growth reader (OY
growth, Finland) at 37

Microscopic studies

Freshly isolated transformants of E. coli Δssb strain harboring
pBAD based SSB constructs were grown to log phase (7–9 h in
2 ml LB containing arabinose). Bacterial cells were collected by
centrifugation, fixed with 4% paraformaldehyde, kept on poly-L-
lysine treated multi-well slide, washed with PBS and visualized in

fluorescence microscope (ZEISS, Axio Imager) with a 100×
objective lens [22].

Results

Experimental rationale and generation of SSB chimeras

The N-terminal domain of EcoSSB is defined by β1-β1’-β2-β3-
α-β4-β451-β452-β3 as its secondary structure elements (Fig. 1A). The N-terminal domain of MtuSSB, in addition possesses a β6
strand (Fig. 1), which causes a notable variation in its quaternary
structure by the formation of a clamp like structure at the dimeric
interface of the interacting subunits [19]. The C-terminal domains of
both the SSBs possess acidic tails important in protein-protein
interactions during various DNA transactions [8–11].

Recently, we observed that MtuSSB sustained E. coli for its
essential function of SSB [22]. However, the mβ4-β5 SSB,
wherein amino acids 74 to 111 (comprising β4, β451, β452 and β5 strands) were replaced with the corresponding region of *Mtu*SSB, failed to sustain *E. coli* despite its normal oligomerization and DNA binding properties. Another chimera, mβ1-β5 wherein the β1-β5 elements of *Eco*SSB were replaced with the corresponding elements of *Mtu*SSB, conferred filamentation phenotype to *E. coli*. However, the mβ1-β6 SSB with the entire N-terminal domain of *Mtu*SSB (i.e., including the β6 strand) fused to the C-terminal domain of *Eco*SSB, functioned well in *E. coli* [22]. These observations suggested specific interaction of β4-β5 region of *Mtu*SSB with the β6 region of *Mtu*SSB. To study the functional importance of such an interaction and to further our understanding of the structure-function relationship of eubacterial SSBs, we generated additional chimeric SSBs (Fig. 2).

The mβ4-β5 SSB was modified to generate mβ4-β5 (acidic), and mβ4-β6 SSBs. One of the distinctive features of the region between the β4 and the β3 strands of *Mtu*SSB is that, unlike *Eco*SSB, it possesses a number of acidic residues (Fig. 1A). Hence, these residues were changed to *Eco*SSB specific sequences in a chimera designated mβ4-β5 (acidic) by mutating E90, T91, E95, K96, E103, D105, and E106 within *Mtu*SSB region of β4-β5 to T90, D95, Q95, D96, V93, N105 and V106, respectively. To generate mβ4-β6, *Mtu*SSB sequence corresponding to amino acids 74-111 in mβ4-β5 was extended to 131 to include β6 of *Mtu*SSB. Among other constructs, mβ1-α contained the first 73 amino acids (consisting of β1-α structural elements) from *Mtu*SSB and the amino acid 74 to the end from *Eco*SSB. In mβ6 SSB, the β6 strand and the downstream spacer sequences of *Mtu*SSB (amino acid 114 to 133) substituted the corresponding region of *Eco*SSB. The remainder of the sequences (the N-terminal region consisting of the first to 113 amino acids and the C-terminal region (amino acids 134 to the end) were from *Eco*SSB. The mβ6-CTD contains the β6 strand and the C-terminal region (amino acid number 114 to the end) from *Mtu*SSB whereas, the N-terminal region (the first 113 amino acids) from *Eco*SSB. Lastly, the mCTD construct contains only the C-terminal region from *Mtu*SSB (amino acid number 129 to the end) and the N-terminal and the spacer sequences (first 128 amino acids) of *Eco*SSB. More details of generation of these constructs are provided in Methods S1 and Table S1.

**Oligomerization of the chimeric SSBs**

All SSBs were purified and analyzed by gel filtration chromatography to determine their oligomerization status (Fig. 3). Elution profile of the chimeric SSBs was very similar to those of the wild-type *Eco*SSB and *Mtu*SSB suggesting that they folded properly and formed homotetramers.

**DNA binding properties**

To demonstrate the DNA binding abilities of various SSB constructs, we performed electrophoretic mobility shift assays (EMSA) using 32P labeled 79mer DNA. Using this assay (Fig. 4), *Eco*SSB and *Mtu*SSB form a faster migrating complex under limiting SSB concentration (Complex I). As the concentration of SSB increases, a second slower migrating band (Complex II) appears. Based on their mobility, these complexes potentially correspond to the SSB56/65 and SSB35 modes of DNA binding, respectively. More importantly, within the detection limits of this assay, all the chimeric SSBs reveal DNA binding similar to the parent SSBs (compare panels 4A and 4B with 4C to 4H), suggesting that the quaternary structures of the chimeric SSBs are largely unaffected by the mutational manipulations performed to generate them.

**Fig. 2. Schematic representation of various SSB constructs.** Secondary structure elements of N-terminal domain of *Mtu*SSB and *Eco*SSB are represented in red and green, respectively. The beginning and end of structural unit are also numbered in the same color. The C-terminal domains are shown by discontinuous lines. SSB chimeras are shown in respective colors. doi:10.1371/journal.pone.0094669.g002
Functionality of SSB chimeras in E. coli

Recently, we described a sensitive assay to assess the functionality of a test SSB using a modification of the original ‘plasmid bumping method’ [22,25]. In the revised assay, the test ssb construct (on a ColE1 ori plasmid, AmpR) is introduced in a Δssb (ssb::kan) strain of E. coli (RDP317-1, KanR) harboring a plasmid borne support of wild-type ssb on another ColE1 ori plasmid, pHYD EcoSSB (Cam R). The replication of pHYD EcoSSB is dependent on the presence of IPTG. Hence, withdrawal of IPTG from the growth medium results in the loss of the support plasmid (pHYD EcoSSB) and failure of the strain growth unless sustained by the test SSB. Growth of the original transformants of the test ssb plasmid on plate lacking IPTG, together with the loss of CamR phenotype, suggests that the test ssb complemented the Δssb strain of E. coli for its function of SSB. An advantage of this assay is that the in vivo activity of even a weakly functioning SSB can be assessed (fitness disadvantage of the test ssb, if any, is avoided by selectively blocking replication of the original ssb support plasmid).

Using this method, we checked the in vivo activity of various SSB constructs subcloned into a ColE1 ori (AmpR) plasmid wherein their expression was inducible by arabinose (the pBAD series of constructs, Table 1). As shown in Fig. 5A, all constructs showed expression of the corresponding SSBs in E. coli TG1. Subsequently, to check for their in vivo function, the ssb constructs were introduced into RDP317-1 strain (KanR) harboring pHYD EcoSSB (CamR), and the transformants were selected on Kan, Amp and 0.02% arabinose plates either containing or lacking IPTG. An analysis of the plating efficiencies (obtained from the ratios of transformants on the –IPTG to +IPTG plates) is shown in Table 2. The mb4–mb5(acidic) SSB did not complement the Δssb strain of E. coli suggesting that conversion of mb4–mb5 SSB to mb4–mb5(acidic) SSB does not make it functional in E. coli. However, transplantation of the mb6 region of the Mtu SSB into the mb4–mb5 construct in mb4–mb6, resulted in an efficient rescue of the Δssb strain of E. coli suggesting a functional interaction between the mb4–mb5 and the mb6 regions of MtuSSB. Interestingly, substitution of the unstructured region of Eco SSB downstream of its mb5 region with the mb6 region of MtuSSB in mb6 SSB, maintained its activity suggesting that the mb4–mb5 region of EcoSSB is tolerant of its downstream sequences.

In vivo complementation by various SSB constructs was further validated by streaking of the freshly obtained transformants (Fig. 5B) on plates containing either IPTG (as control) or varying concentrations of the inducer (0.002–0.2% arabinose). As expected from the replication of the pHYD EcoSSB support plasmid in the presence of IPTG, all transformants showed growth on the +IPTG plate. Like the vector control (sector 1), neither the mb4–mb5 nor the mb4–mb5(acidic) complemented the Δssb strain at any of the arabinose concentrations (sectors 4 and 5). Further, the results of the growth curve analyses (Fig. 6) of the strains harboring SSBs

![Fig. 3. Oligomerization status of SSB proteins. Standard curve Ve/Vo versus log molecular size markers is shown in the left most panel. Ve represents the peak elution volume of proteins and Vo represents the void volume of the column determined using blue dextran (2,000 kDa). Protein size markers [β-galactosidase (116 kDa), elongation factor-G (77 kDa), bovine albumin (66 kDa), egg albumin (44 kDa) and carbonic anhydrase (29 kDa)] were used to make the plot. The tetramer peak corresponding to EcoSSB is indicated. Panels (A) to (l) show the gel filtration chromatography elution profiles of SSB proteins. Vo and Ve of each SSB is indicated. doi:10.1371/journal.pone.0094669.g003]
that sustained *E. coli* are also consistent with the plating efficiency data. Weakly functioning SSBs, in general, resulted in longer lag phases when expression of SSBs was induced with 0.002% arabinose (panel ii). These differences were, however, lost in cultures induced with 0.02% or 0.2% arabinose (panels iii and iv) which result in higher level of expression of these SSBs (Fig. S1). As a control, when the growth curve analyses were carried out in the absence of inducer, arabinose (Fig. 6, panel i) none of the cultures grew confirming that the phenotypes observed in Table 2, and Figs. 5B and 6 (panels ii and iii) are due to the plasmid borne SSBs. The longer lag phases in Fig. 6 (panel ii) could be a stress related phenomenon. Interestingly, we observed that the weakly functioning SSBs also conferred temperature and cold sensitive phenotypes to *E. coli* for growth at 42°C and 30°C, respectively (Fig. 7). These phenotypes could also be suppressed upon induction of SSB expression with higher concentrations of arabinose. It may also be noted that even under these conditions (temperatures of 42°C or 30°C), the mβ4-β5 and mβ4-β5 (acidic) failed to complement the *E. coli Δssb* strain (Figs. 7A and 7B, sectors 4 and 5, respectively).

**Microscopic analyses**

In our earlier study microscopic analyses of the fixed *E. coli* cells revealed that the mβ1-β5 SSB, a poorly functioning SSB, resulted in a notable filamentation phenotype [22]. On the other hand, SSBs that functioned, but not as well as *EcoSSB*, resulted in a slightly elongated cell phenotype. As before, *MtuSSB* showed a phenotype of slightly elongated cells (Fig. 8, compare panels d and a). However, the mβ4-β6 SSB showed a more pronounced phenotype of the elongated cells (compare panel j with a). The mβ1-α SSB showed a weak phenotype of the elongated cells (compare panels m with a). Interestingly, as in Figs. 6 and 7, overexpression of the SSBs suppressed these phenotypes (compare panels d with e and f; j with k and l; m with n and o).

**Discussion**

Determination of the three-dimensional structure of *MtuSSB* by X-ray crystallography revealed that while its structure at the tertiary level is very similar to that of *EcoSSB*, it shows significant variations at the level of quaternary interactions [19]. A notable difference seen at the level of tetramerization of *MtuSSB* is the presence of a clamp like structure formed by the β6 strand of the mycobacterial SSB [19]. However, it has so far remained unclear as to what the biological significance of this unique structural element of *MtuSSB* is.

The L45 loop in *EcoSSB* has been shown to undergo a conformational change upon DNA binding and suggested to be
important for its cooperative binding [17,18]. In addition, the computational analyses suggested that the movements of L45 loop in *E. coli*, *Mtu*SSB, and *Streptomyces coelicolor* SSB are different [21]. Our observation shows that the mβ4-β5 construct wherein the L45 loop (of *Mtu*SSB origin) is intact does not function in *E. coli* but the mβ4-β6 SSB wherein a small region (β6) downstream of β3 was also included, does. Together with the biophysical and computational analyses [17,18,19,21], these observations highlight the importance of the functional interactions of the L45 loop with its downstream sequence. This is further indicated by the observation (Fig. 6, panel ii) that the construct mCTD (of *Mtu*SSB) functioned nearly as well as the mβ6 (harboring only the β6 of *Mtu*SSB) or the mβ6-CTD (harboring the entire region downstream of L45, from *Mtu*SSB). An availability of the three-dimensional structures of the chimeric SSBs may further our understanding of the interactions L45 establishes within SSB.

**Table 2.** Plating efficiencies of various SSBs.

| Strain      | Plating efficiency (%) |
|-------------|------------------------|
| EcoSSB      | 82±3                   |
| MtuSSB      | 40±4                   |
| mβ4-β5      | 0                      |
| mβ4-β5 (acidic) | 0                 |
| mβ4-β6      | 49±7                   |
| mβ1-α       | 29±4                   |
| mβ6         | 76±3                   |
| mβ6-CTD     | 48±2                   |
| mCTD        | 46±4                   |

*Plating efficiencies were determined by taking ratios of number of transformants obtained with various SSB constructs in *E. coli* RDP317-1/pHYDEcoSSB by plating equal volumes from the same transformation mixes on Kan, Amp and 0.02% arabinose plates vs Kan, Amp and IPTG plates. The values have been tabulated from five independent experiments (with three replicates each). Averages with S.D. values are shown. doi:10.1371/journal.pone.0094669.t002*
Fig. 6. Growth analysis. Growth of *E. coli* RDP317 (Δssb:kan) supported by various SSBs in the absence (panel i) or presence of 0.002, 0.02% or 0.2% arabinose (panels ii, iii and iv, respectively). Averages of the growth of three independent colonies together with SEM are plotted.

doi:10.1371/journal.pone.0094669.g006

Fig. 7. Growth of *E. coli* Δssb:kan supported by various SSB constructs at 30°C (A) and 42°C (B). Transformants of *E. coli* RDP 317-1 harboring chimeric SSBs were obtained in the presence of IPTG and processed as in Fig. 5B. Sectors: 1, pBAD vector; 2, pBAD-EcoSSB; 3, pBAD-MtuSSB; 4, pBADm-b4-b5; 5, pBADm-b4-b5(acidic); 6, pBADm-b6; 7, mβ1-α; 8, mβ6; 9, mβ6-CTD; and 10, mCTD.

doi:10.1371/journal.pone.0094669.g007
Finally, the modification [22] of the ‘plasmid bumping’ assay [25] we recently developed has been useful in determining the efficacy of SSB mutants in sustaining \textit{E. coli} even when they are compromised in their function, and provided with a convenient approach to study the structure-function relationship of the various structural elements of the eubacterial SSBs.

**Supporting Information**

**Figure S1** SSB expression in response to increasing arabinose concentration in the medium.

**Table S1** Nucleotide and amino acid sequences of the EcoSSB, MtuSSB and various chimeric SSBs.

**Methods S1** Details of chimeric SSB constructions.

**Acknowledgments**

We thank our laboratory colleagues for their suggestions on the manuscript. UV is a J. C. Bose fellow of DST. SS is Dr. D. S. Kothari post-doctoral fellow.

**Author Contributions**

Conceived and designed the experiments: UV SB SS KR. Performed the experiments: KR SB SS. Analyzed the data: UV KR SB SS. Contributed reagents/materials/analysis tools: KR SB SS. Wrote the paper: UV SB KR.

---

**Fig. 8. Microscopic observations of \textit{E. coli} \texttt{Dssb::kan} supported by various SSB constructs.** Cultures of \textit{E. coli} RDP317 (\texttt{Dssb::kan}) transformants harboring various SSB constructs were grown in the presence of indicated concentrations of arabinose and analyzed by phase contrast microscopy. Bars at the lower left of each panel indicate a scale of 2 \textmu m.

doi:10.1371/journal.pone.0094669.g008
References

1. Meyer RR, Glassberg J, Kornberg A (1979) An Escherichia coli mutant defective in single-strand binding protein is defective in DNA replication. Proc Natl Acad Sci USA 76: 1702–1705.

2. Lieberman HB, Witkin EM (1981) Variable expression of the ssb–1 allele in different strains of Escherichia coli K12 and B: differential suppression of its effects on DNA replication, DNA repair and ultraviolet mutagenesis. Mol Gen Genet 183: 549–553.

3. Kumar NV, Varshney U (1997) Contrasting effects of single-stranded DNA binding protein on the activity of uracil DNA glycosylase from Escherichia coli towards different DNA substrates. Nucleic Acids Res 25: 2536–2543.

4. Purnapatre K, Hanada P, Venkatesh J, Varshney U (1999) Differential effects of single-stranded DNA binding proteins (SSBs) on uracil DNA glycosylases (UDGs) from Escherichia coli and mycobacteria. Nucleic Acids Res 27: 3487–3492.

5. Lavery PE, Kowalczykowski SC (1992) A postsynaptic role for single-stranded DNA-binding protein in recA protein-promoted DNA strand exchange. J Biol Chem 267: 9315–9320.

6. Lohman TM, Ferrari ME (1994) Single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities. Annu Rev Biochem 63: 527–570.

7. Arif SM, Vijayan M (2012) Structural diversity based on variability in quaternary association. A case study involving eubacterial and related SSBs. Methods Mol Biol 922: 23–35.

8. Hanada P, Acharya N, Varshney U (2001) Chimeras between single-stranded DNA-binding proteins from Escherichia coli and Mycobacterium tuberculosis reveal that their C-terminal domains interact with uracil DNA glycosylases. J Biol Chem 276: 16992–16997.

9. Cadman CJ, McGlynn P (2004) PriA helicase and SSB interact physically and functionally. Nucleic Acids Res 32: 6378–6387.

10. Lohman TM, Overman LB, Datta S (1986) Salt-dependent changes in the DNA binding co-operativity of Escherichia coli single strand binding protein. J Mol Biol 187: 603–615.

11. Bujalowski W, Overman LB, Lohman TM (1988) Binding mode transitions of Escherichia coli single-strand binding protein-single-stranded DNA complexes. Cation, anion, pH, and binding density effects. J Biol Chem 263:4629–4640.

12. Bujalowski W, Hanada P, Venkatesh J, Varshney U (1999) Differential effects of single-stranded DNA binding proteins (SSBs) on uracil DNA glycosylases (UDGs) from Escherichia coli and mycobacteria. Nucleic Acids Res 27: 3487–3492.

13. Lohman TM, Overman LB, Datta S (1986) Salt-dependent changes in the DNA binding co-operativity of Escherichia coli single strand binding protein. J Mol Biol 187: 603–615.

14. Bujalowski W, Overman LB, Lohman TM (1988) Binding mode transitions of Escherichia coli single-strand binding protein-single-stranded DNA complexes. Cation, anion, pH, and binding density effects. J Biol Chem 263:4629–4640.

15. Raghunathan S, Ricard GS, Lohman TM, Waksman G (1997) Crystal structure of the homo-tetrameric DNA binding domain of Escherichia coli single-stranded DNA-binding protein determined by multiwavelength x-ray diffraction on the selenomethionyl protein at 2.9-A resolution. Proc Natl Acad Sci USA 94: 6652–6657.

16. Raghunathan S, Kozlov AG, Lohman TM, Waksman G (2000) Structure of the DNA binding domain of E. coli SSB bound to ssDNA. Nat Struct Biol 7: 648–652.

17. Saikrishnan K, Jeyakanthan J, Venkatesh J, Acharya N, Sekar K, et al. (2003) Structure of Mycobacterium tuberculosis single-stranded DNA-binding protein. Variability in quaternary structure and its implications. J Mol Biol 331: 383–393.

18. Saikrishnan K, Manjunath GP, Singh P, Jeyakanthan J, Jauret Z, et al. (2005) Structure of Mycobacterium smegmatis single-stranded DNA-binding protein and a comparative study involving homologous SSBs: biological implications of structural plasticity and variability in quaternary association. Acta Crystallogr D Biol Crystallogr 61: 1140–1148.

19. Kaushal PS, Singh P, Sharma A, Maniyappa K, Vijayan M (2010) X-ray and molecular-dynamics studies on Mycobacterium leprae single-stranded DNA-binding protein and comparison with other eubacterial SSB structures. Acta Crystallogr D Biol Crystallogr 66: 1048–1058.

20. Bharti SK, Rex K, Sreedhar P, Krishnan N, Varshney U (2011) Chimeras of Escherichia coli and Mycobacterium tuberculosis single-stranded DNA binding proteins: characterization and function in Escherichia coli. PLoS One 6: e27216.

21. Sambrisk JF, Fritzch EF, Maniatis T (1989) Molecular cloning: A Laboratory manual. 2nd Edn edit, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

22. Purnapatre K, Varshney U (1999) Cloning, over-expression and biochemical characterization of the single-stranded DNA binding protein from Mycobacterium tuberculosis. Eur J Biochem 264: 591–598.

23. Carlini LE, Porter RD, Curth U, Urbanke C (1995) Viability and preliminary in vivo characterization of site-directed mutants of Escherichia coli single-stranded DNA-binding protein. Mol Microbiol 10: 1067–1073.

24. Hanada P, Acharya N, Thanedar S, Purnapatre K, Varshney U (2000) Distinct properties of Mycobacterium tuberculosis single-stranded DNA binding protein and its functional characterization in Escherichia coli. Nucleic Acids Res 28: 3823–3829.