SSB protein preserves genome stability in *Escherichia coli*

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BACKGROUND

Genome stability is of paramount importance to all living organisms. Genome instability results in severe conditions such as cancer and premature aging in mammals, due to aberrant DNA rearrangements such as deletions, amplifications, translocations etc. Stability of *Escherichia coli* genome is determined by metabolism of 3´-ending tails at DNA double-strand breaks, which are faithfully mended by homologous recombination catalyzed by RecBCD enzyme in wild-type (wt) cells. However, occasionally aberrant DNA transactions occur in *E. coli* genome, which are mediated by microhomologies and result in illegitimate recombination (IR) events. The main genome caretaker in bacteria and eukaryotes is RecQ-class of proteins, which disrupt the aberrant DNA structures. The λ Spi⁻ assay is used to quantify the frequency of IR in *E. coli* genome. It detects aberrantly excised λ prophage that contains part of bacterial genome while lacking part of its own genome (Fig. 1). Such phages can grow on P2 lysogenic bacteria, unlike wt phages. Bacterial SSB proteins, as well as their eukaryotic RPA analogues, are essential and ubiquitous. They avidly bind single-stranded DNA and regulate/coordinate its metabolism, hence enabling essential DNA processes such as replication and repair. Here we assessed the effect of SSB protein on IR occurrence in *E. coli* genome.

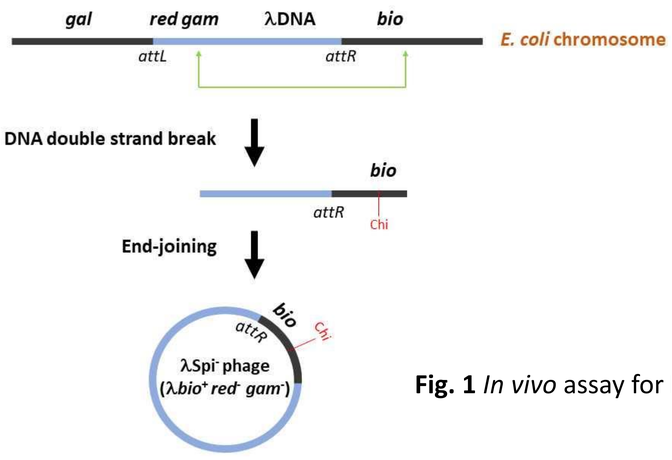

Fig. 1 *In vivo* assay for IR.

RESULTS

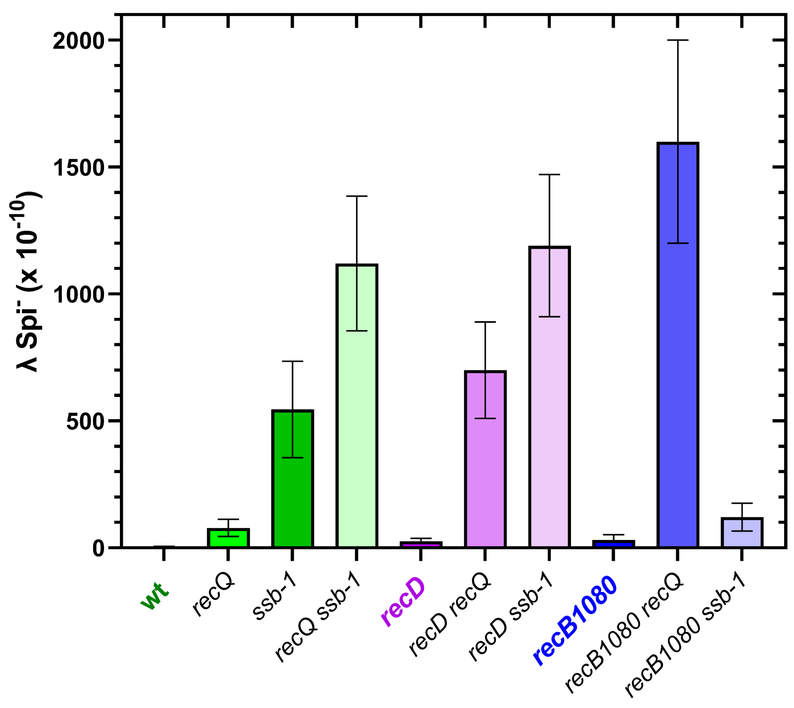

Fig. 2 Inactivation of SSB protein results in increased production of aberrantly excised λ*bio* phages by illegitimate recombination in wt, *recD* and *recB1080* genetic backgrounds of *E. coli.* Each value is a mean of the three independent experiments, with error bars representing standard deviation.

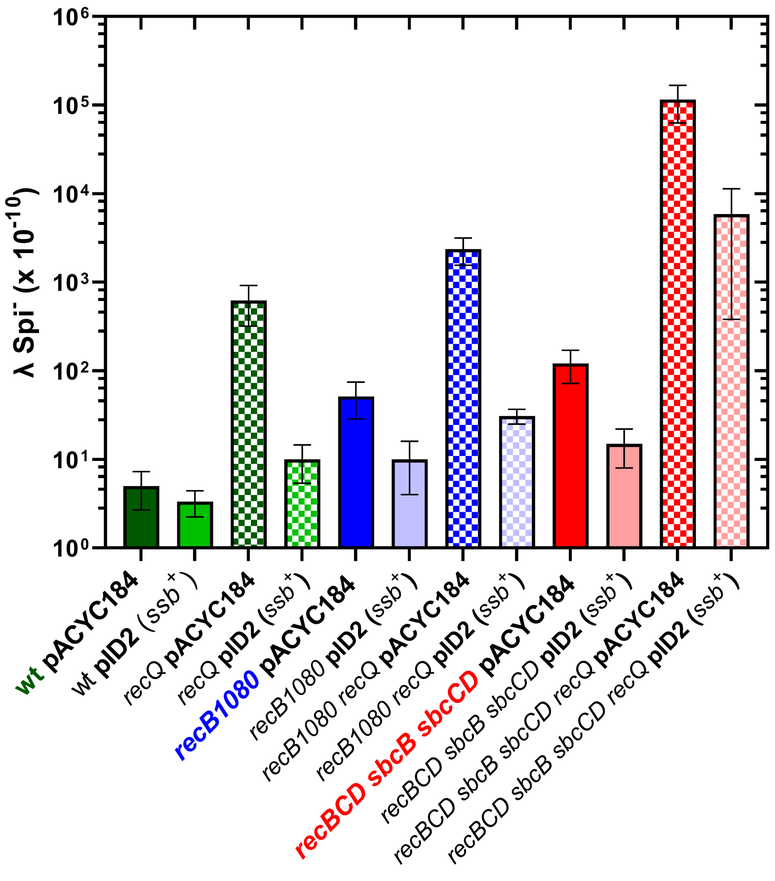

Fig. 5 *ssb* gene overexpression from pID2 plasmid reduces the incidence of illegitimate recombination in various genetic contexts. SSB overproduction largely compensates RecQ deficiency. Each value is a mean of the three independent experiments, with error bars representing standard deviation.

METHODS

Bacterial growth: *E. coli* wild-type strain AB1157 and its derivatives were grown in LB medium (supplemented with 10 mM $MgSO_4$) at 30 °C until reaching mid-logarithmical growth phase (OD_{600}~0.4). All strains were lysogenic with thermo-inducible prophage λ*cI857.*

Preparation of phage lysates: The bacteria (grown to OD_{600}~0.4) were incubated at 42 °C with aeration, for 40 min to induce λ prophage and to inactivate SSB-1 protein. Then, the bacteria were incubated at 37 °C with aeration for 120 min, until the lysis occurred. Chloroform was added to the lysates, which were then centrifuged for 10 min at 10,000 g. The lysates were stored at 4 °C.

Determination of total λ phage titer: The lysates were serially diluted and incubated with AB1157 bacteria for 15 min at 42 °C. The bacteria were then mixed with soft agar, spread on trypticase plates and incubated overnight at 37 °C.

Determination of λ Spi⁻ phage titer: The phages were mixed with P2 lysogenic strain NM767 and incubated for 15 min at 42 °C, after which they were mixed with soft agar, spread on trypticase plates and incubated overnight at 37 °C. The frequency of λ Spi⁻ phage was determined by dividing the titer of λ Spi⁻ phage by the total phage titer.

Plasmid pID2: The chromosomal gene *ssb,* including its natural promoters, was cloned into pACYC184 plasmid. The expression level was determined by RT-qPCR and shown to be about 100-fold above the normal level.

***ssb-1* allele:** Codes for mutant SSB-1 protein (His55 →Tyr), which is temperature-sensitive. SSB-1 gets reversibly inactivated by heating at 42 °C. NaCl content of the rich growth medium affects the balance of SSB-1 monomer-tetramer formation.

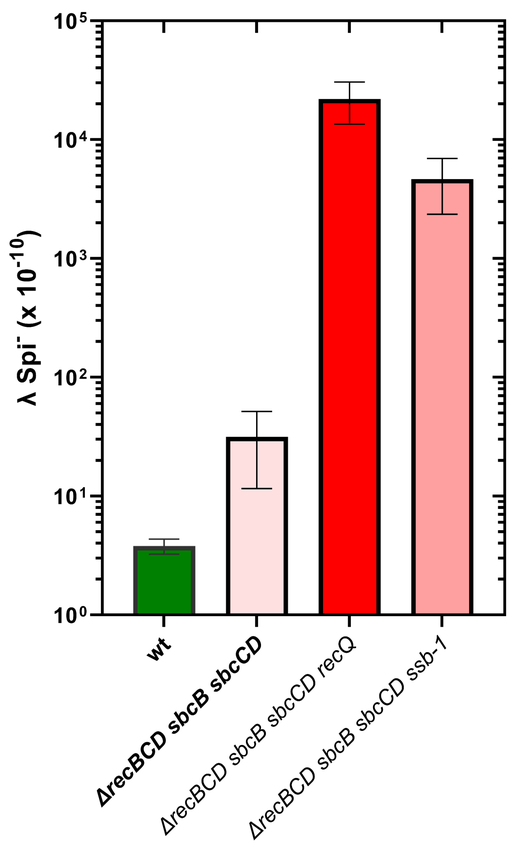

Fig. 3 Highly elevated level of illegitimate recombination in *recBCD sbcB sbcCD* mutant lacking SSB protein function. Each value is a mean of the three independent experiments, with error bars representing standard deviation.

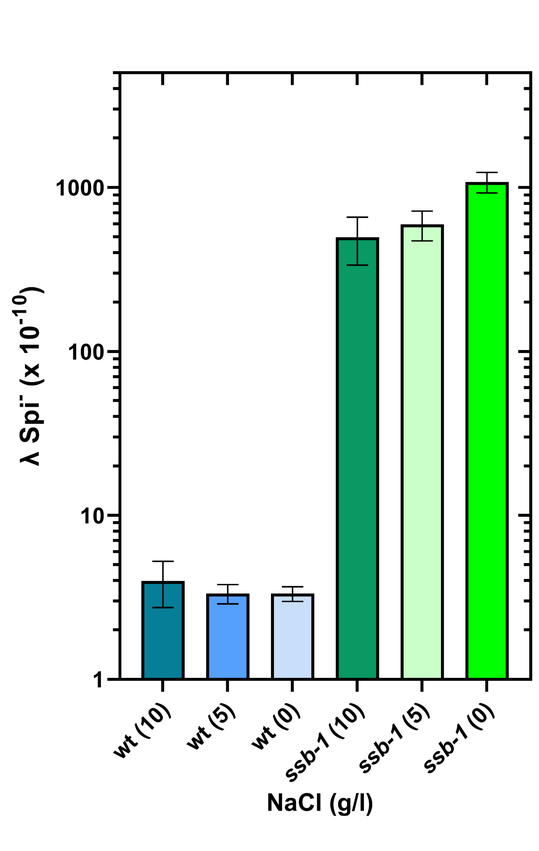

Fig. 4 The effect of *ssb-1* mutation on illegitimate recombination in otherwise wild-type bacteria grown in LB medium with different concentrations of NaCl. Each value is a mean of the three independent experiments, with error bars representing standard deviation.

CONCLUSIONS

- **SSB protein suppresses illegitimate recombination in RecBCD, RecBC, $RecB^{1080}$ and RecFOR pathways of DSB processing, indicating that the effect is of general significance.**
- **The inhibiting effect of SSB on illegitimate recombination is additive to that of RecQ helicase.**
- **SSB protein overproduction lowers the frequency of illegitimate recombination, including in cells lacking RecQ helicase, indicating that the two proteins act in different pathways. Since the RecQ disrupts aberrant DNA structures, SSB role is likely in preventing the appearance of those microhomology-mediated joint exchanges.**
- **NaCl partially suppresses effect of *ssb-1* mutation on illegitimate recombination.**