ssb gene expression is SOS-dependent in *Escherichia coli*

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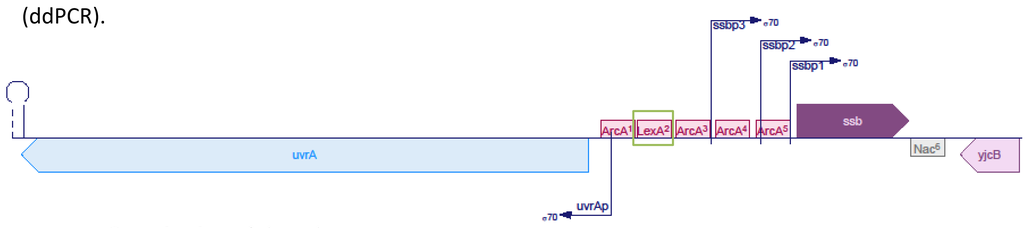
BACKGROUND

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, transcription, and repair. The prototypic *Escherichia coli* SSB protein is encoded by an *ssb* gene. The *ssb* contains three promoters and a single LexA repressor binding sequence (SOS box), which implicates that *ssb* belongs to the SOS regulon. The SOS regulon is a set of about 50 genes in *E. coli* chromosome whose expression is repressed by a LexA repressor, until coordinately activated in stress conditions. Since SSB is such an important governor of DNA metabolism, it should be unsurprising that it is a part of the regulon that regulates DNA replication, repair, mutagenesis, and cell division in bacteria under stress. However, the issue of SOS regulation of *E. coli ssb* expression turns out to be far from settled. Namely, LexA may repress only one *ssb* promoter, whereas the other two enable constitutive, though low, *ssb* expression. The LexA binding box is rather distant (-170 nt) from the *ssb* coding region and is divergent from the consensus SOS box.

Whether the *E. coli ssb* is inducible under SOS response was explored by several laboratories in the 1980s and then again in the 2000s. None of the more than 10 studies showed SOS-dependence of *ssb* expression.

We determined a time-course of *ssb* and *sulA* (as an indication of SOS induction) gene expression by using sensitive and reliable methods, such as quantitative real-time PCR (qPCR) and droplet digital PCR (ddPCR).



- Organization of the *ssb* operon.

RESULTS

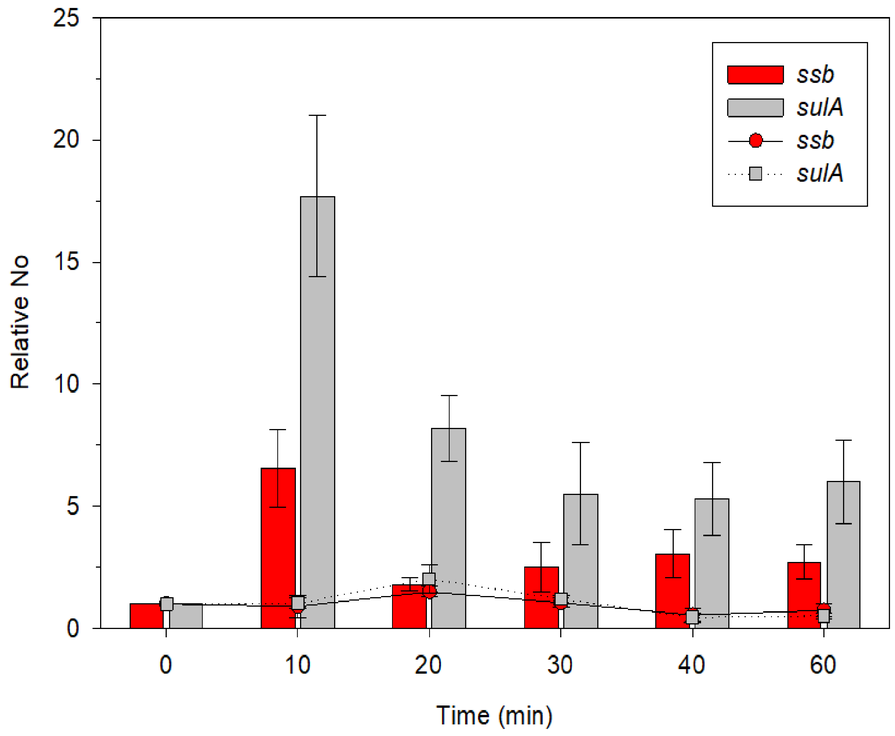


Fig. 1 Increased expression of the ***ssb*** and the ***sulA*** gene in **wild-type** *E. coli* **γ-irradiated** with 400 Gy and incubated at 37 °C. Bars represent expression in irradiated, and dots and lines in unirradiated bacteria. The results show that expression of both *ssb* and *sulA* (SOS system) are induced in stress conditions. No represent normalized average No value for the two genes. Each value is a mean of the three independent qRT-PCR experiments, with error bars representing standard deviation.

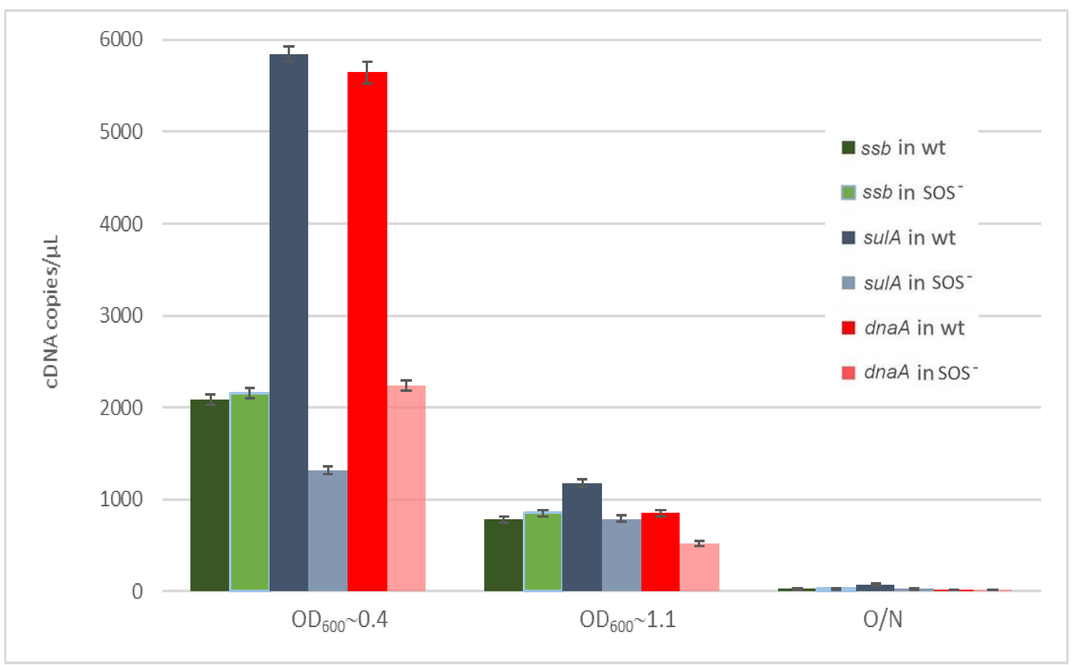


Fig. 3 Absolute quantification of the ***ssb***, ***sulA***, ***dnaA*** genes' transcripts during **undisturbed** growth of **wild-type** (wt) and ***lexA3*** (SOS^-) mutant bacteria in LB medium. Different expression od ***sulA*** in wt and SOS^- mutant indicate induced SOS response, which is not associated with increase in ***ssb*** expression. Measurements were taken at mid-exponential growth phase (OD_{600} ~0.4), early stationary growth phase (OD_{600} ~ 1.1) and overnight (O/N) grown bacterial cultures. Three independent ddPCR experiments at three different concentrations gave the same relative results. Error bars represent Poisson confidence interval at a 95% level.

METHODS

Bacterial growth and irradiation: *E. coli* wild type strain AB1157 and its derivatives were grown in rich, LB medium at 37°C. The logarithmically grown (OD_{600}~0.4) bacteria were irradiated with 400 Gy of gamma radiation at 0 °C, from a ^{60}Co source, or with 40 Jm^{-2} of UV irradiation (254 nm), at room temperature.

RNA isolation and reverse transcription: The irradiated cultures were incubated at 37°C and their mRNA isolated using RNeasy® Plus Mini Kit (Qiagen) and RNAprotect™ Bacteria Reagent (Qiagen) according to the manufacturer's instructions. Approximately 0.5 µg of RNA was reverse-transcribed by the PrimeScript RT reagent kit with genomic DNA Eraser (perfect Real Time, Takara) using random and oligo dT primers mix in 10 µl reaction. Negative controls without reverse transcriptase were used for all samples.

Quantitative real-time PCR (qPCR) analysis: Primers for the ssb and sulA gene expression analysis by qPCR were: fw-GTTGTGCTGTTCGGCAAACT and rev-GCGATCCTGACCGGATTGAT, and fw-GCCGGGCTTATCAGTGAAGT and rev-CCTGAACCCATTCCCGACTC, respectively. Endogenous control genes for normalization were: 16S ribosomal gene and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Both endogenous controls gave the same results. We used the following thermal cycling conditions: 50 °C 2 min, 95 °C 7 min, 95 °C 15 s, 60 °C 1 min for 40 cycles followed by dissociation stage: 95 °C for15 s, 60 °C for 1 min, 95 °C for 15 s and 60 °C for 15 s.
LinRegPCR software v.11.1 was used for post-run data analysis, which enables calculation of the starting concentration of amplicon ("No value"). "No value" is expressed in arbitrary fluorescence units and is calculated by considering PCR efficiency, ranging between 90-100%, and baseline fluorescence. "No value" determined for each technical replicate was averaged and such averaged "No values" were divided by "No values" of endogenous control.

Absolute quantification of RNA levels by ddPCR: Microfluidic droplet digital PCR (ddPCR) procedure was performed using the QIAcuity 2-plex instrument (Qiagen, Hilden, Germany). The ddPCR reaction mixture was assembled using QIAcuity 3X Eva Green PCR Master Mix, 10X primer mix (4µM), RNase-free water and a fixed concentration of cDNA template in a final volume of 15 µL per sample. After accurate vortexing, 12 µL of the above prepared mixture was transferred into the 24-well 8.5 K nanoplate and sealed with the nanoplate seal. The sequence of primers for transcript detection of *ssb* and *sulA* are the same as that used in qPCR. The amplification cycling protocol include 95 °C for 2 min for enzyme activation and the following 40 cycles of 15 s at 95 °C for denaturation, 15 s at 60 °C for annealing and 15 s at 72 °C for extension, and then a final step at 40 °C for 5 min. Fluorescence light is emitted by positive partitions which have a target molecule, as compared to those without target, the negative partitions. The experiments were performed using a negative control without reverse transcription enzyme. Data were analyzed using the QIAcuity Suite Software V1.1.3 (Qiagen) and the results are expressed in copies of cDNA/µL based on Poisson statistics analyses.

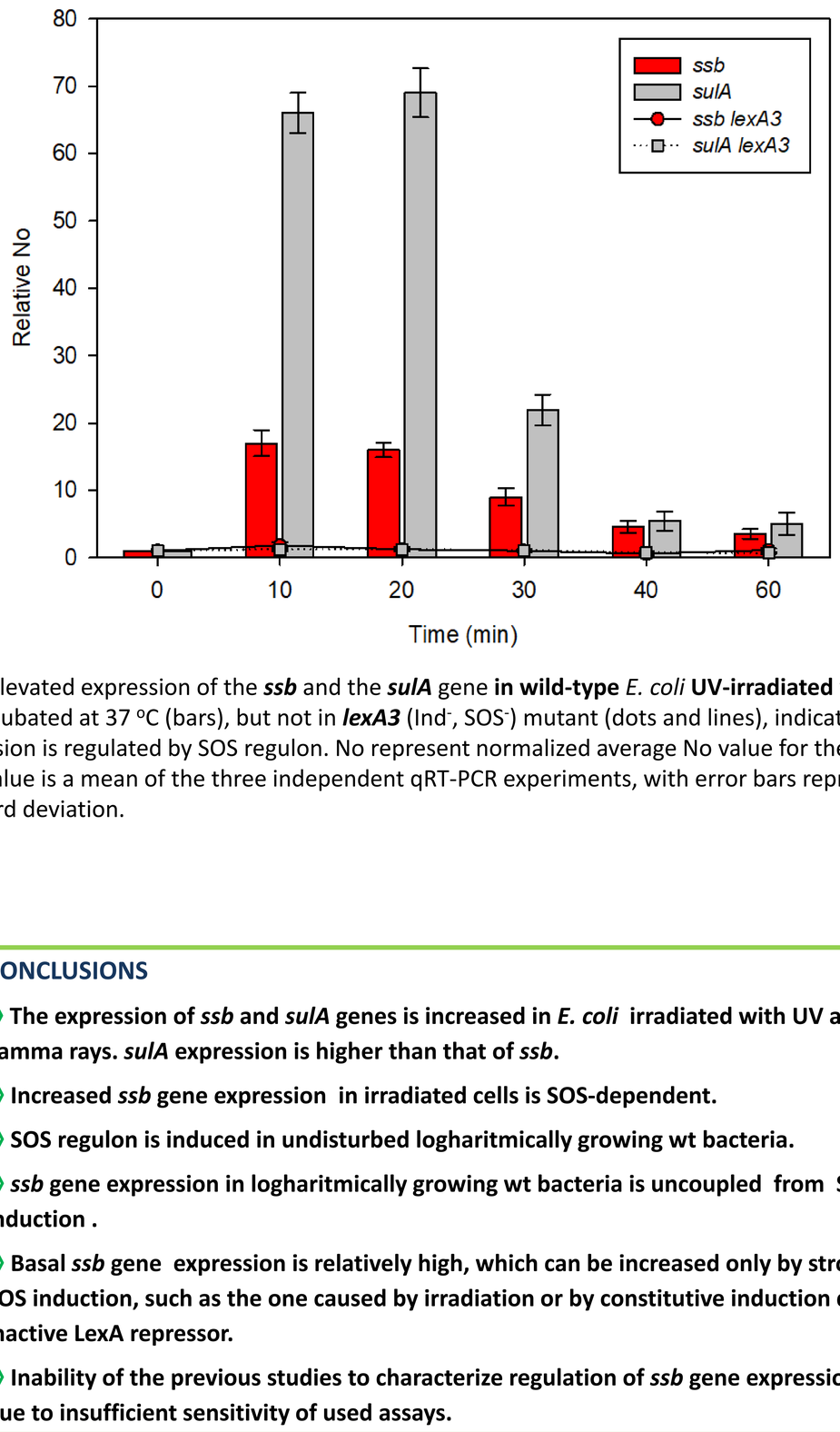


Fig. 2 Elevated expression of the ***ssb*** and the ***sulA*** gene **in wild-type** *E. coli* **UV-irradiated** with 40 Jm^{-2} and incubated at 37 °C (bars), but not in ***lexA3*** (Ind^-, SOS^-) mutant (dots and lines), indicating that *ssb* expression is regulated by SOS regulon. No represent normalized average No value for the two genes. Each value is a mean of the three independent qRT-PCR experiments, with error bars representing standard deviation.

CONCLUSIONS

- **The expression of *ssb* and *sulA* genes is increased in *E. coli* irradiated with UV and gamma rays. *sulA* expression is higher than that of *ssb*.**
- **Increased *ssb* gene expression in irradiated cells is SOS-dependent.**
- **SOS regulon is induced in undisturbed logharitmically growing wt bacteria.**
- ***ssb* gene expression in logharitmically growing wt bacteria is uncoupled from SOS induction .**
- **Basal *ssb* gene expression is relatively high, which can be increased only by stronger SOS induction, such as the one caused by irradiation or by constitutive induction due to inactive LexA repressor.**
- **Inability of the previous studies to characterize regulation of *ssb* gene expression is due to insufficient sensitivity of used assays.**