

### Identification of the SSB binding site on RecO by NMR analysis

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The regions of single stranded (ss) DNA that result from DNA damage are immediately coated by the ssDNA binding protein (SSB). During the process of DNA recombinational repair, several proteins facilitate the displacement of SSB from ssDNA, allowing the RecA protein to form protein filaments on the ssDNA region, which facilitates the process of recombinational DNA repair. This phenomenon is conserved among all organisms, although the proteins participating in the process are structurally different. In the RecF pathway, RecF, RecO and RecR stimulate RecA filamentation on the SSB-coated ssDNA. Recently, we proposed the model of displacement of SSB from ssDNA by RecO and RecR [ref.]. In this model, the interactions between RecO and SSB/ssDNA appear to be necessary. However, the binding sites of SSB and ssDNA on RecO have yet to be identified.

In this study, we tried to determine the binding site of SSB and ssDNA on RecO by NMR analysis. Result of NMR titration experiment with ssDNA suggested that RecO has two ssDNA binding site on its N-terminal OB-fold and C-terminal domain. Result of titration experiment with SSB suggested that the positively charged region of C-terminal domain of RecO is a binding site for SSB (Fig. 1). We also confirmed that C-terminal acidic region of SSB is involved in interaction with RecO. Based on these results, we constructed a variety of RecO mutants. Result of native PAGE analysis indicated that the R127A mutation decreased the binding affinity for SSB. The mutant RecO could not anneal the SSB-coated ssDNA. In addition, the mutation affected the recovery of the inhibitory effect of SSB on ssDNA-dependent ATPase activity of RecA. These results indicated that the region surrounding Arg-127 is the binding site of SSB.

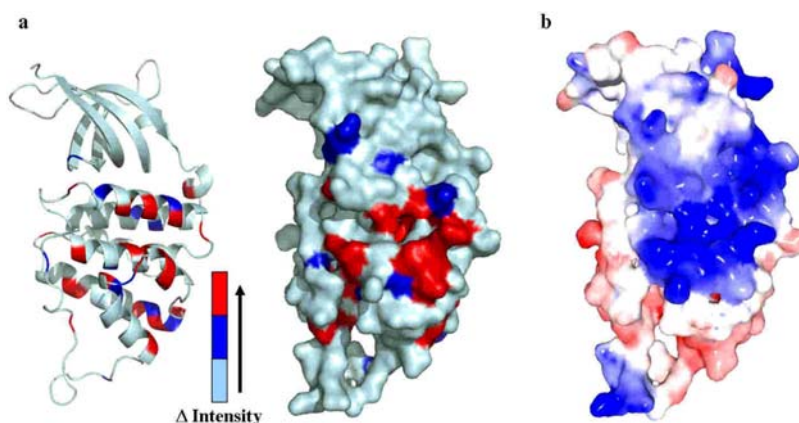


Figure 1. NMR analysis of the SSB binding site on RecO. a. The residues exhibiting significant differences in relative intensity ( $\Delta$  int) by the addition of SSB are color coded and mapped onto the structure of RecO ( $\Delta$  int : red > blue > cyan). b. Electrostatic potential at the molecular surface of RecO.

**Reference** : Inoue J., Honda M., Ikawa S., Shibata T. and Miakwa T. (2008) *Nucleic Acids Res.* **36**:94-109