

The process of displacing the single stranded DNA binding protein from single stranded DNA by the RecF pathway proteins

Jin Inoue^{1,2}, Masayoshi Honda^{1,2}, Shukuko Ikawa¹, Takehiko Shibata^{1,2,3}, and Tsutomu Mikawa^{1,2,3}

¹RIKEN Discovery Research Institute, ²International Graduate School of Arts and Sciences, Yokohama City University, ³RIKEN Spring-8 Center
e-mail: jinoue@postman.riken.jp

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. However, the function of each of these proteins in this process is unclear. In this study, we examined the mechanism of SSB displacement from ssDNA using purified *Thermus thermophilus* RecF-pathway proteins. To date, RecO and RecR are thought to act as the RecOR complex. However, our results indicate that RecO and RecR have distinct functions. We found that RecR binds both RecF and RecO, and that RecO binds RecR, SSB and ssDNA. The electron microscopic studies and pulldown assays of the protein-ssDNA complex indicated that, although SSB is displaced from ssDNA by RecO, it still remains indirectly attached to ssDNA through its interaction with RecO in the RecO-ssDNA complex. In the presence of both SSB and RecO, the ssDNA-dependent ATPase activity of RecA was inhibited, but was restored by the addition of RecR. Interestingly, the interaction of RecR with RecO affected the ssDNA-binding properties of RecO. These results suggest a model of SSB displacement from the ssDNA by RecF pathway proteins.