



## **Sak4 of phage HK620 is an SSB-stimulated annealase that is involved in the lytic development of the phage**

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## Sak4 of phage HK620 is an SSB-stimulated annealase that is involved in the lytic development of the phage

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A renewed interest has focused on the study of phages due to their potential use in phage therapy. A possible drawback for their use is the rapid evolution of these entities, related to the presence in their genome of genes coding for particularly diverse and effective recombination protein that, for most of them, remain to be characterized. Their characterization may allow finding ways to control the evolution of phages of interest. Moreover, it may help understanding how similar proteins work in other organisms. For instance, the family of phage Sak4 is composed of proteins similar to Rad51 paralogs acting in homologous recombination but for which the molecular mechanism remains poorly characterized.

Here, we report that Sak4 from phage HK620 that infects *Escherichia coli* TD2158 and encoded by *hkaL* is important for the life cycle of the phage as an *hkaL* mutant is impaired in lytic development. We also show that Sak4 is able to promote recombineering events *in vivo*, albeit at a lower extent compared to the Red $\beta$  protein. To characterize Sak4 at the molecular level, we undertook its biochemical characterization. Sak4 is an ATPase that binds single-strand DNA in an ATP-dependent way, its ATPase activity being stimulated in the presence of ssDNA. Sak4 performs annealing of complementary ssDNA with a low efficiency. The weakness of Sak4 annealase activity can be explained by its dependence on another protein partner. Indeed, we showed that a distant homolog of the single stranded binding protein, SSB, encoded by a gene present almost systematically next to the *sak4* gene, stimulates the recombineering activity of Sak4 *in vivo*. *In vitro*, the binding of Sak4 to ssDNA is stimulated by its cognate SSB, as well as its annealase activity. Interestingly, these positive effects are strictly dependent on the C-terminal 6 amino acids of its cognate SSB, residues that are known to be involved in protein-protein interaction in other bacterial SSBs. Finally, the stimulation of the ATPase activity of Sak4 is decreased in the presence of ssDNA when its cognate SSB is present. We propose that the phage SSB facilitates the recruitment of Sak4 on DNA via a direct interaction. This recruitment probably stabilizes Sak4 on DNA, a prerequisite to an efficient annealase activity of this recombinase. To date, Sak4 of phage HK620 is the first described single-strand annealing protein that uses an SSB to enhance its activity.