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Biophysical and biochemical insights into the mechanisms of action by Redβ during homologous recombination

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Repair of DNA breaks by single-strand annealing (SSA) is a major mechanism for the maintenance of genomic integrity. SSA is promoted by proteins (single-strand-annealing proteins [SSAPs]) such as eukaryotic RAD52 and λ phage Redβ. These proteins use a short single-stranded region to find sequence identity and initiate homologous recombination. Using biophysical single molecule techniques, we have shown that homology is recognized by Redβ monomers that weakly hold single DNA strands together. Upon annealing, homodimerization of Redβ clamps the double-stranded region and nucleates nucleoprotein filament growth. In this manner, DNA clamping ensures and secures a successful detection for DNA sequence homology. Redβ clamp is characterized by a structural change and a remarkable stability against force up to 200 pN. Our findings not only present a detailed explanation for SSAP action but also identify the DNA clamp as a very stable, non-covalent, DNA-protein interaction. Using protein biochemistry and recombination assays, we have shown that C-terminally truncated Red β, whilst still able to promote annealing and nucleoprotein filament formation, is unable to mediate homologous recombination. As evaluated by co-immunoprecipitation experiments, the dsDNA recombination function relates to the Redα-Redβ interaction, which requires not only contacts in the C-terminal domain but also at the N-terminus. Mutations of critical amino acids affected either dsDNA recombination or both ssDNA and dsDNA recombination, indicating two separable functions: one critical for dsDNA recombination and the other for recombination per se. These data further advance Red recombination model and show that Redβ and RAD52 SSAPs share ancestral and mechanistic roots.

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