Structural and Functional Analyses of Five Conserved Positively Charged Residues in the L1 and N-Terminal DNA Binding Motifs of Archaeal RadA Protein

Li-Tzu Chen (陳立慈), Tzu-Ping Ko (柯子平),
Andrew H.-J. Wang (王惠鈞), and Ting-Fang Wang (王廷方)

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

RecA family proteins engage in an ATP-dependent DNA strand exchange reaction that includes a ssDNA nucleoprotein helical filament and a homologous dsDNA sequence. In spite of more than 20 years of efforts, the molecular mechanism of homology pairing and strand exchange is still not fully understood. Here we report a crystal structure of *Sulfolobus solfataricus* RadA overwound right-handed filament with three monomers per helical pitch. This structure reveals conformational details of the first ssDNA binding disordered loop (denoted L1 motif) and the dsDNA binding N-terminal domain (NTD). L1 and NTD together form an outwardly open palm structure on the outer surface of the helical filament. Inside this palm structure, five conserved basic amino acid residues (K27, K60, R217, R223 and R229) surround a 25 A° pocket that is wide enough to accommodate anionic ssDNA, dsDNA or both. Biochemical analyses demonstrate that these five positively charged residues are essential for DNA binding and for RadA-catalyzed D-loop formation. We suggest that the overwound right-handed RadA filament represents a functional conformation in the homology search and pairing reaction. A new structural model is proposed for the homologous interactions between a RadA-ssDNA nucleoprotein filament and its dsDNA target.

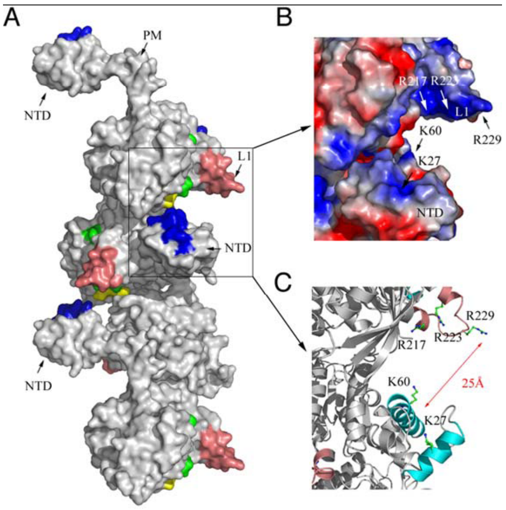

Figure 1. Spatial arrangement of the L1 motif and the NTD along the 31 overwound right-handed SsoRadA filament. (A) Quaternary structure.The putative dsDNA binding regions in the NTD are shown in blue. The L1 and L2 ssDNA binding motifs are shown in pink and green, respectively.ATP binding sites are shown in yellow. The polymerization motif (PM) is indicated by an arrow. (B) A local surface charge potential of the L1 motif and the NTD region. Positive and negative charges are indicated by blue and red, respectively. (C) A ribbon diagram of two neighboring protomers (grey)showing the L1 motif (pink) and the NTD (cyan). The side chains of key basic residues K27, K60, R217, R223, and R229 are depicted in ball-and-stick representations.

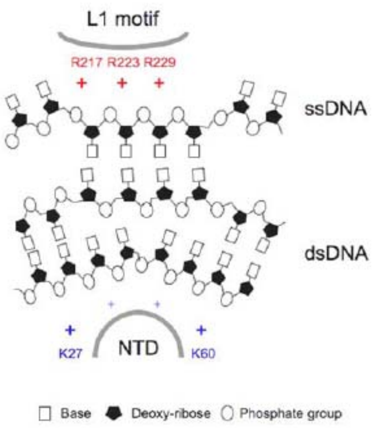

Figure 2. A new hypothesis for homology interactions mediated by RadA protein filaments. Interactions between three arginine residues of the L1 motif and sugar-phosphate backbone of ssDNA result in the nucleotide bases of ssDNA facing the NTD (Figure 1). An anionic dsDNA associates with the NTD along the border of a 92° basic arch via electrostatic interactions or hydrogen bonding. Lys27 and Lys60 are located at each end of this arched basic patch (Figure 3). As a result, NTD-dsDNA association may lead to DNA bending or distortion or flipping of base pairs. L1-ssDNA and NTD-dsDNA interactions function in unison to mediate homologous search and pairing between a 3_1 overwound right-handed RadA-ssDNA nucleoprotein filament and dsDNA.

The original paper was published in: PLoS ONE **2(9),** e858. doi:10.1371/journal.pone.0000858 (2007).