

Novel insight from crystallographic studies on an archaeal RadA recombinase

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RecA-like recombinases, which also include a distantly related group of eukaryal Rad51 / DMC1 and archaeal RadA (also called Rad51), form helical nucleoprotein filaments in which a hallmark strand exchange reaction occurs between homologous DNA substrates. A conserved ATPase domain harbors a canonical P-loop and putative DNA-binding loops L1 and L2. Recently determined crystal structures of a RadA recombinase from *Methanococcus voltae* revealed a dynamic nature of the recombinase filaments. The activation of RecA-like recombinases is known to require Mg^{2+} and ATP cofactors. Our functional and structural studies on RadA suggest that the formation of active recombinase filaments requires additional metal ions for proper disposition of the DNA-binding loops and catalytic groups in the ATPase center. Consistent with functional requirement on potassium, two K^+ ions located by anomalous signals make contacts with the terminal phosphate of AMP-PNP, a non-hydrolysable analogue of ATP. One of the K^+ -liganding residue, Asp-302, has a Lys counterpart in bacterial RecA which does not require the presence of any monovalent cation. A RecA-mimicking RadA D302K mutant protein no longer requires K^+ for activation. The recently discovered (by others) functional roles of Ca^{2+} is rationalized by the structural elucidation of one Ca^{2+} ion displacing both K^+ ions while stabilizing an essentially identical conformation of the protein. In comparison, the twin K^+ stabilize an eclipsed conformation of the ATP analogue, while the Ca^{2+} stabilizes a staggered conformation. As such, the calcium ion's inhibitory role on ATP hydrolysis is rationalized. A second Mg^{2+} -binding site unexpectedly revealed in the crystal structure has been characterized by functional studies and site-directed mutagenesis at the Mg^{2+} -liganding residue Asp-246. Our ATPase data indicates that the binding of a second Mg^{2+} is highly cooperative with a Hill coefficient close to 4. Documented studies on human Rad51 homologue have suggested that the lack of cooperativity in binding ATP contrasts the presence of such cooperativity in bacterial RecA. Our study suggest that cooperativity is restored by binding a second Mg^{2+} distant (~ 10 Å) from ATP.

References:

1. Wu, Y., He, Y., Moya, I. A., Qian, X., and Luo, Y. (2004) *Mol Cell* **15**(3), 423-435
2. Wu, Y., Qian, X., He, Y., Moya, I. A., and Luo, Y. (2005) *J Biol Chem* **280**(1), 722-728

3. Qian, X., Wu, Y., He, Y., and Luo, Y. (2005) *Biochemistry* **44**(42), 13753-13761