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## DNA repair depends on specialized DNA-binding by replication Protein A

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Replication protein A (RPA), the major single-stranded DNA-binding protein in eukaryotic cells, is required for processing of single-stranded DNA (ssDNA) intermediates found in replication, repair, and recombination. RPA is a highly flexible complex composed of three subunits, RPA1, RPA2 and RPA3 that contain six DNA binding domains (DBDs). One of the recurring mysteries of RPA is that the affinity of RPA for ssDNA does not directly correlate with cellular function. There are RPA mutations that reduce the affinity of the complex by two orders of magnitude are fully active *in vitro* and in cells, while other mutations that display a higher affinity for ssDNA are partially or completely inactive. In particular, we have characterized a set of separation-of-function mutants in RPA1 that have mutations in pairs of aromatic residues in the DNA binding sites of DBD-A or -B. These mutants support DNA replication but are defective in multiple DNA repair pathways including NER and double-strand break repair. These results suggest that replication and repair require different RPA-DNA interactions. We utilized single molecule total internal fluorescence microscopy (smTIRF) and ensemble assays to determine the molecular basis of this separation of function phenotype. We analyzed the kinetics of binding to different DNA structures including single-stranded intermediates found at sites of damage and replication. Our studies demonstrated that RPA binds to ssDNA in at least two modes characterized by different dissociation kinetics. We also showed that the aromatic residues contribute to the formation of the longer-lived state, are required for stable binding to short ssDNA regions, and are needed for RPA melting of partially duplex DNA structures. We conclude that stable binding and/or the melting of secondary DNA structures by RPA is required for DNA repair but is dispensable for DNA replication. It is likely that the binding modes are in equilibrium and reflect dynamics in the RPA-DNA complex. This suggests that dynamic binding of RPA to DNA is necessary for different cellular functions. Research supported by NIH GM44721 and NCI P30CA086862.

### Biography

Marc Wold laboratory has been studying cellular DNA metabolism for more than 20 years. My lab has experience with a wide range of biochemical, molecular and cellular approaches for analyzing DNA metabolism *in vitro* and *in vivo*. We have extensive experience with purification and biochemical analysis of proteins involved in DNA replication and repair. We have either developed or applied many methods for examining DNA binding and functional analysis of RPA in DNA replication and repair. We also carried out a structural analysis of RPA and in cell methods for analyzing the function of different forms of RPA or other proteins in human cells. These studies have developed a methodology for determining the functions of RPA in cellular DNA replication, DNA repair (including methods for analyzing the efficiency of specific DNA repair pathways) and checkpoint activation. My lab has generated and purified a large number of mutant forms of RPA and analyzed the mechanism of RPA-DNA interactions. We have domain deletion and point mutations that disrupted the function of individual domains of RPA, alter DNA binding properties and knockout or mimic phosphorylated RPA. We have made forms of RPA that can be fluorescently labeled on individual domains without affecting function. We have also recently identified several separation-of-function mutations in RPA that support DNA replication but not DNA repair.

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