Title: Structure and Function of Bacterial RecN Protein

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Summary:
The overall goal of this research is to reveal the molecular function of RecN, a protein critical to the repair of DNA double-stranded breaks (DSBs) in bacteria. RecN is a member of the Structural Maintenance of Chromosomes (SMC) family of proteins. Eukaryotic SMC proteins have essential, albeit poorly understood functions in a variety of house-keeping DNA metabolic processes including chromosomal condensation, sister chromatid cohesion and recombinational DNA repair. Although extensive genetic evidence underscores the importance of RecN to bacterial genome maintenance, there is a lack of functional information regarding its cellular roles. This situation reflects the difficulties working with the *Escherichia coli* (a model bacterial organism) RecN protein stemming from insolubility of the recombinant protein. Importantly, we have identified a RecN homolog from *Deinococcus radiodurans* that is soluble in *E. coli* and are well positioned to conduct studies to formulate testable hypotheses for the biochemical function of RecN in recombinational DNA repair pathways. We will begin the biochemical characterization of RecN using the *D. radiodurans* RecN construct. We will also screen the effect of *recN* mutations highly conserved sequence motifs in both *E. coli* and *D. radiodurans* genetic systems. This work will provide valuable models for understanding the molecular roles of eukaryotic RecN orthologs in genome maintenance.

Define the biochemical properties of the RecN protein: An understanding of RecN function will require a detailed biochemical characterization, describing the binding and kinetic parameters of the substrates of RecN, DNA and ATP. Furthermore, we will test for potential effects RecN may have on the activity of other known DSB repair pathway enzymes, notably the
RecA recombinase. In these regards, we have purified soluble *D. radiodurans* RecN protein. We have confirmed its intrinsic ability to hydrolyze ATP and identified a cohesion-type activity suggesting that RecN can tether DNA double strand ends.

Determine the role of conserved RecN sequence motifs: RecN harbors globular N- and C-terminal domains that contain common ABC-ATPase superfamily motifs involved in ATP binding and hydrolysis and a predicted central coiled-coil domain. Our preliminary results suggest that RecN function requires ATP hydrolysis in vivo. Alignment of RecN orthologs from several hundred bacterial organisms has enabled us to

identify additional highly conserved regions. We will construct and analyze mutations in several key motifs to determine their role in RecN function in vivo and in vitro.

Examine protein complexes involving RecN in vitro and in vivo: We hypothesize that protein-protein interactions target or modulate the function of RecN proteins. We will first define the in vivo complexes containing RecN and determine whether assembly of these complexes is dependent on DNA damage. Next, using purified proteins, we will confirm these interactions and determine whether the associations are direct. Elucidating these interactions will be valuable for understanding the biology of RecN and associated proteins.