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McNeil, Sara M.

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Committee Members: Harrington, Maureen A.; Georgiadis, Millie M.
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Abstract:

DNA double strand breaks (DSB) are among the most lethal forms of DNA damage and can occur as a result of ionizing radiation (IR), radiomimetic agents, endogenous DNA-damaging agents, etc. If left unrepaired DSB' s can cause cell death, chromosome translocation and carcinogenesis. In humans, DSB are repaired predominantly by the non-homologous end joining (NHEJ) pathway. Ku, a heterodimer consisting of Ku70 and Ku80, functions in the recognition step of this pathway through binding DNA termini. Ku recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to create the full DNA-PK heterotrimer. Formation of DNA-PK results in autophosphorylation as well as phosphorylation of downstream

proteins of the NHEJ pathway. Previous work shows that the extreme C-terminus of Ku80 stimulates the kinase activity of DNA-PKcs, and Ku DNA binding is regulated as a function of redox via stimulation of a conformational change when oxidized resulting in a decrease in DNA binding activity. To further understand these methods of regulation of Ku and DNA-PK, a pair of mutants has been constructed; one consisting of full length Ku70 and truncated Ku80 (Ku70/80ΔC) lacking 182 C-terminal amino acids. The removal of these amino acids was shown to have little to no effect on the proteins expression, stability or DNA binding, as determined by SDS-PAGE, western blot analysis and electrophoretic mobility shift assay (EMSA). When oxidized Ku70/80ΔC showed a decrease in DNA binding similar to that seen in wild type, however when re-reduced the mutant did not recover to the same extent as wild type. A second mutant was constructed, containing amino acids 590-732 of Ku80 (Ku80CTR), to further understand the mechanism by which Ku80 C-terminus interacts with the rest of the Ku heterodimer. Possible protein-protein interactions were evaluated by Ni-NTA affinity, gel filtration chromatography, fluorescence polarization and two forms of protein-protein cross-linking. Ni-NTA agarose affinity, and gel filtration chromatography failed to reveal an interaction in the presence or absence of DNA. However, photo-induced cross-linking of unmodified proteins (PICUP) as well as EDC cross-linking demonstrated an interaction which was not affected by DNA. The work presented here demonstrates that the interaction between Ku80CTR and Ku is rather weak, but it does exist and plays a relatively large role in the NHEJ pathway.

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