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Name: total document.fi ...
Size: 8.644Mb
Format: PDF

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Permanent Link:	http://hdl.handle.net/1805/2517
Date:	2011-03-16
Committee Chair:	Wek, Ronald C.
Committee Members:	Turchi, John Lee, Suk-Hee Takagi, Yuichiro
Degree:	Ph.D.
Degree Year:	2010
Department:	Department of Biochemistry & Molecular Biology
Grantor:	Indiana University
Keywords:	DNA repair, non-homologous end joining
LC Subjects:	DNA-protein interactions ; DNA repair

Abstract:

DNA double strand breaks (DSB), particularly those induced by ionizing radiation (IR) are complex lesions and if not repaired, these breaks can lead to genomic instability, chromosomal abnormalities and cell death. IR-induced DSB often have DNA termini modifications including thymine glycols, ring fragmentation, 3' phosphoglycolates, 5' hydroxyl groups and abasic sites. Non-homologous end joining (NHEJ) is a major pathway responsible for the repair of these complex breaks. Proteins involved in NHEJ include the Ku 70/80 heterodimer, DNA-PKcs, processing proteins including Artemis and DNA polymerases μ and λ, XRCC4, DNA ligase IV and XLF. The precise molecular mechanism of DNA-PK activation and Artemis processing at the site of a DNA DSB has yet to be elucidated. We have investigated the effect of DNA sequence and structure on DNA-PK activation and results suggest a model where the 3' strand of a DNA terminus is responsible for annealing and the 5' strand is involved in activation of DNA-PK. These results demonstrate the influence of DNA structure and orientation on DNA-PK activation and provide a molecular mechanism of activation resulting from compatible termini,

an essential step in microhomology-mediated NHEJ. Artemis, a nuclease implicated in processing of DNA termini at a DSB during NHEJ, has been demonstrated to have both DNA-PK independent 5'-3' exonuclease activities and DNA-PK dependent endonuclease activity. Evidence suggests that either the enzyme contains two different active sites for each of these distinct processing activities, or the exonuclease activity is not intrinsic to the Artemis polypeptide. To distinguish between these possibilities, we sought to determine if it was possible to biochemically separate Artemis endonuclease activity from exonuclease activity. An exonuclease-free fraction of Artemis was obtained that retained DNA-PK dependent endonuclease activity, was phosphorylated by DNA-PK and reacted with an Artemis specific antibody. These data demonstrate that the exonuclease activity thought to be intrinsic to Artemis can be biochemically separated from the Artemis endonuclease. These results reveal novel mechanisms of two critical NHEJ proteins, and further enhance our understanding of DNA-PK and Artemis activity and their role in NHEJ.

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