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DNA requirements for interaction of the C-terminal region of Ku80 with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs)

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ABSTRACT

Non-homologous end joining (NHEJ) is the major pathway for the repair of ionizing radiation induced DNA double strand breaks (DSBs) in human cells. Critical to NHEJ is the DNA-dependent interaction of the Ku70/80 heterodimer with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to form the DNA-PK holoenzyme. However, precisely how Ku recruits DNA-PKcs to DSBs ends to enhance its kinase activity has remained enigmatic, with contradictory findings reported in the literature. Here we address the role of the Ku80 C-terminal region (CTR) in the DNA-dependent interaction of Ku70/80 with DNA-PKcs using purified components and defined DNA structures. Our results show that the Ku80 CTR is required for interaction with DNA-PKcs on short segments of blunt ended 25 bp dsDNA or 25 bp dsDNA with a 15-base poly dA single stranded (ss) DNA extension, but this requirement is less stringent on longer dsDNA molecules (35 bp blunt ended dsDNA) or 25 bp duplex DNA with either a 15-base poly dT or poly dC ssDNA extension. Moreover, the DNA-PKcs-Ku complex preferentially forms on 25 bp DNA with a poly-pyrimidine ssDNA extension.Our work clarifies the role of the Ku80 CTR and dsDNA ends on the interaction of DNA-PKcs with Ku and provides key information to guide assembly and biology of NHEJ complexes.

1. Introduction

DNA double strand breaks (DSBs) are considered the most lethal form of DNA damage. If unrepaired or mis-repaired, they can lead to chromosomal loss or rearrangements, reducing cellular viability and increasing the potential for cellular transformation. In eukaryotes, DSBs can be repaired by either non-homologous end joining (NHEJ), alternative non-homologous end-joining (A-NHEJ) or homologous recombination repair (HRR) [1]. NHEJ is the major pathway for repairing ionizing radiation (IR)-induced DSBs in human cells and mechanistically, can be divided into three steps: (i) DSB detection and synapsis, (ii) DNA end processing, and (iii) DNA end ligation. The Ku70/80 heterodimer plays a critical role in NHEJ, detecting DSB ends and recruiting other NHEJ pathway components to the break including the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), X-ray repair cross-complementing 4 (XRCC4), XRCC4-like factor (XLF), Aprataxin and polynucleotide kinase/phosphatase-like factor (APLF) and Paralog of XRCC4 and XLF (PAXX). Following end processing, the DNA ends are ligated by the XRCC4-DNA ligase IV complex [2,3]. Whereas early models envisioned a pathway in which individual NHEJ components are sequentially recruited to DSBs, recent studies suggest that NHEJ proceeds via the coordinated assembly of NHEJ proteins into a dynamic multi-protein complex rather than in a stepwise fashion [4,5]. Nevertheless, each of these models stresses the importance of the Ku70/80 heterodimer in the initial detection of the DSB as well as for interaction with and recruitment of other NHEJ proteins, including DNA-PKcs.

Human Ku is composed of 69 kDa and 83 kDa subunits, also called Ku70 (*XRCC6*) and Ku80 (*XRCC5*), respectively. The Ku70/80 heterodimer binds with high affinity to ends of double stranded (ds) DNA in a largely DNA sequence independent manner [6]. Eukaryotic Ku70 and Ku80 subunits contain three domains: an N-terminal von Willebrand A (vWA) domain, a central core domain required for DNA binding and dimerization and unique C-terminal domains (Supplementary Fig. 1A). The vWA and core domains of Ku70/Ku80 form an asymmetric ring with an expansive base and a narrow bridge that encircles dsDNA [7]. This preformed ring is capable of accommodating two turns (approximately 14 base pairs) of dsDNA. Ku loads onto the DNA such that Ku70 is proximal to the DNA end and Ku80 is distal, further away from the end [7]. The C-terminal region (CTR) of Ku70 contains a SAP (SAF-A/B, Acinus and PIAS) domain, which is a putative chromatin/DNA binding domain [8], while the Ku80 CTR (residues 545–732) is composed of an α-helical bundle (residues 594–704) flanked by two disordered, flexible linkers (residues 546–593 and 705–732) [9,10] (Supplementary

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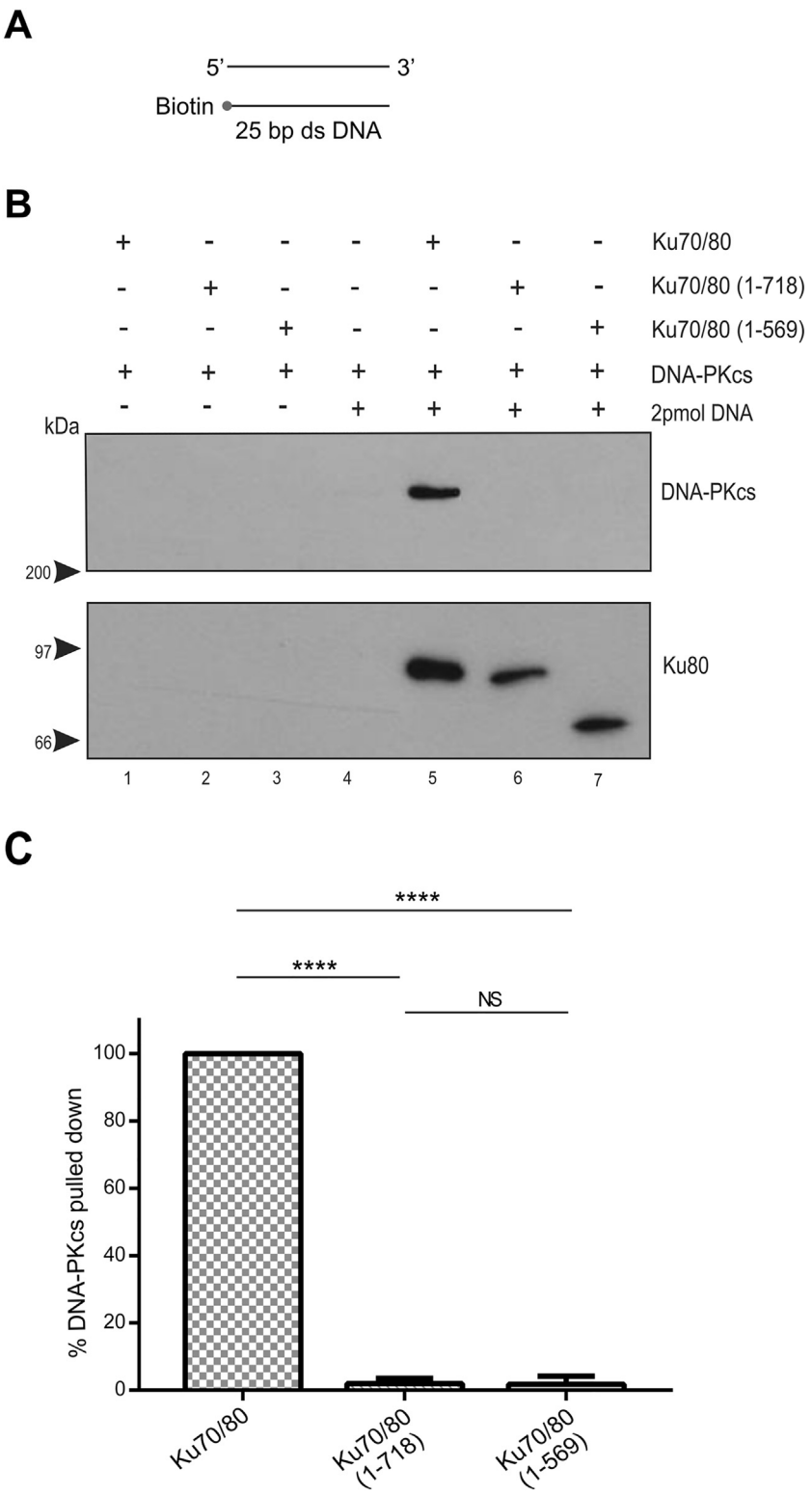

Fig. 1. Ku80 CTR is required for interaction with DNA-PKcs on 25 bp blunt ended double stranded (ds) DNA.
(A) Schematic of the 25 bp blunt ended dsDNA with a 3′-biotin group. Oligonucleotide sequences are shown in the Supplementary Material. (B) Biotin pull-down assays were carried out using 3′ biotin labelled 25 bp blunt ended dsDNA to determine the interaction between DNA-PKcs and Ku wild type and mutant heterodimers. DNA bound-streptavidin coated magnetic beads were incubated with purified Ku70/80 heterodimer (full-length or mutants) as indicated. Beads were pulled down, washed with binding buffer and analyzed by SDS PAGE followed by immunoblot using antibodies to DNA-PKcs or Ku80 as indicated on the right-hand side. Lanes 1 and 5 contained full-length Ku70/80, lanes 2 and 6 contained Ku70/80 (1–718), and lanes 3 and 7 contained Ku70/80 (1–569). DNA was present in lanes 4–7 as indicated. Lanes 1–3 contained DNA-PKcs incubated with Ku plus beads in the absence of DNA. Blots were probed with antibodies to Ku80 and DNA-PKcs as shown.
(C) Quantitation of three independent experiments as described in panel B. In each case, the amount of DNA-PKcs bound was normalized to the amount of Ku in the pull down and expressed as a percentage of the amount of DNA-PKcs bound to full length Ku. Error bars indicate mean ± S.D. **** = $p \leq 0.0001$. NS = non-significant.

Fig. 1B), and forms a highly flexible arm that extends away from the DNA binding core [11]. The extreme C-terminus of Ku80 contains a conserved region (residues 719–732) shown to interact directly with DNA-PKcs in vitro [12,13]. The recent crystal structure of DNA-PKcs in complex with the Ku80 CTR supports the importance of the Ku80 CTR in interaction with DNA-PKcs [14] and our small angle X-ray scattering (SAXS) structures and structure-based models of DNA-PKcs-Ku70/80-dsDNA and NHEJ complexes suggest that the extended conformation of the Ku80 CTR is critical to the flexible tethering and dynamic recruitment of DNA-PKcs to DNA ends [5]. Moreover, Ku80-deficient *xrs*6 cells engineered to re-express a human Ku80 C-terminal truncation mutant composed of residues 1–569 were as radiosensitive as Ku null *xrs*6 cells [15] consistent with the importance of the Ku80 CTR in vivo. Furthermore, work by Turchi and colleagues has revealed the importance of the Ku80 CTR in activation of DNA-PKcs in in vitro kinase assays using purified proteins, synthetic dsDNA oligonucleotides and a short