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# A Ku Bridge over Broken DNA

Minireview

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## Summary

**The Ku heterodimer is essential for the nonhomologous end-joining pathway of DNA double-strand break repair; it both protects the broken ends and recruits some of the many proteins required to complete repair. The recently determined structure of Ku provides insights into how it can both bind to the DNA ends and allow access by the other proteins required to rejoin them.**

## Introduction

Double-stranded breaks (DSBs) in chromosomal DNA occur when cells are exposed to oxidation or ionizing radiation, as well as during normal cellular processes such as meiotic recombination and immunoglobulin gene rearrangement. If not repaired, DSBs can kill cells or lead to potentially oncogenic chromosomal translocations [1, 2]. At least two well-conserved pathways exist in eukaryotes for the repair of DSBs: homologous recombination, in which the intact homologous chromosome or sister chromatid is used as a template for repair, and nonhomologous end joining (NHEJ), in which the broken ends are rejoined to one another (Figure 1). NHEJ is believed to be the predominant form of DSB repair in vertebrates [3], particularly during the G0 and G1 phases of the cell cycle [4]. Genetic evidence indicates that both the 70 and 86 kDa subunits of the Ku heterodimer, as well as the XRCC4/DNA ligase IV protein complex (XRCC4/LigIV), are absolutely vital for efficient and accurate NHEJ (see review [5]). The catalytic subunit of the DNA-dependent protein kinase (DNA-$PK_{cs}$), which together with Ku makes up the functional form of DNA-PK, also plays an important role (see reviews [5, 6]). Because most breaks that result from oxidation or irradiation cannot be immediately rejoined due to chemical damage, additional proteins are required to remove this damage and fill any gaps that are left. The recently determined structure of the Ku heterodimer [7] suggests how it can control access of these many factors to the ends and allow for their limited processing and ligation.

## The Multiple Roles of Ku

The Ku heterodimer promotes efficient and accurate NHEJ, in which the two ends at a DSB are rejoined with minimal loss of genetic information. Ku binds with high affinity to DNA ends regardless of their sequence or structure (i.e., blunt, recessed, or hairpinned), and it can only enter or exit DNA molecules at an end [6]. After binding to an end, Ku can diffuse internally for several kilobases along the DNA molecule, and it can become trapped if the DNA molecule is circularized by ligation [8]. This observation suggested that Ku might form a ring around the DNA. Ku can bridge DNA ends, even if those ends are not competent for joining [9, 10]. This bridging activity could account for the high-fidelity repair pathway seen in cell extracts [11, 12] if Ku restricts the activity of end-processing enzymes such as exonucleases and polymerases to the minimum required for rejoining.

Ku stimulates the end-joining activity of the XRCC4/LigIV complex. XRCC4 can bind to relatively large pieces of DNA [13], and these substrates can be joined by the XRCC4/Lig IV complex in the absence of Ku. Ku is necessary for recruiting XRCC4/LigIV to smaller DNA molecules and stimulating their joining by the ligase complex [10, 14]. The relatively short inter-nucleosomal regions of DNA that would be accessible on either side of a DSB in the cell suggest that Ku's role in recruitment of the ligase complex could be vital. The DNA bridging activity of Ku is also likely to contribute to the stimulation of ligation [14], particularly of blunt ends [10].

## The Ku Structure

Two crystal structures of the human Ku heterodimer, one of Ku alone and one of Ku bound to DNA, have recently been determined by J. Goldberg and his colleagues [7]. They accomplished this by removing the flexible C-terminal domain of the Ku86 subunit and coexpressing it and the full-length Ku70 subunit in insect cells. A DNA molecule with a three-way junction and a single accessible end was used as a binding substrate to select a preferred orientation for Ku relative to the DNA end and prevent Ku from sliding along the DNA. The phase problem was solved by the metabolic incorporation of selenomethionine (SeMet) into the Ku subunits during their expression in insect cell culture. About 70% of the Met residues were replaced by SeMet, which was sufficient to phase the 150 kDa Ku heterodimer by multiwavelength anomalous scattering at 3.5 Å resolution.

The structure of the Ku heterodimer resembles a basket with a large base and a narrow "handle," described by the authors as a bridge (Figure 2a). When Ku is bound to DNA, a 14 base pair segment fits snugly between this base and handle (Figures 2a and 2b). The two subunits of the heterodimer have weak primary sequence homology but are very similar in structure. They each include three domains: an N-terminal $\alpha/\beta$ globular domain with the Rossmann fold, a centrally located DNA binding $\beta$ barrel domain, and the extended $\alpha$-helical arm near the C terminus (Figure 2b). Within each subunit, these three domains are quite separate and barely contact one another (Figure 2b), but inter-subunit interactions integrate them. In the heterodimer, the extended C-terminal $\alpha$-helical arm

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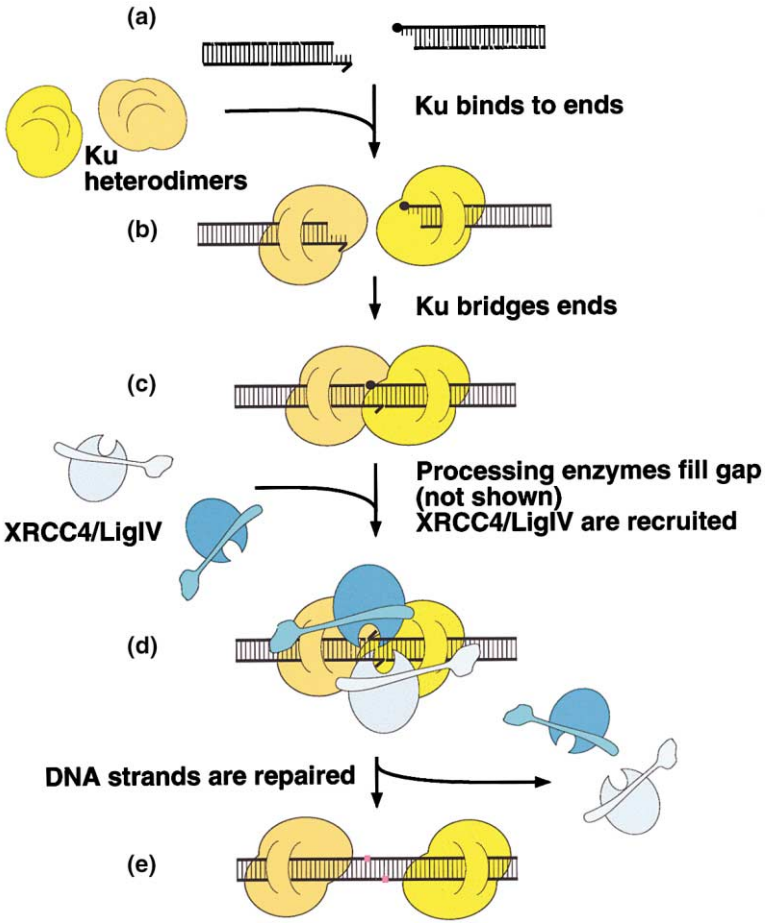


Figure 1. Model for Nonhomologous End Joining

The broken DNA molecule is missing a base and is blocked by a non-ligatable moiety (black circle) on the right-hand side. Ku heterodimers are depicted in orange and yellow. XRCC4/Ligase IV complexes are depicted in blue. The newly repaired DNA backbone is depicted in pink. See text for details.

of Ku86 wraps tightly around the central β barrel domain of Ku70 and vice versa, as if dimerization resulted from swapping of the β barrel domains (Figure 2a).

The β barrel domain is central to interactions with DNA (Figure 3). This domain comprises seven mostly anti-parallel β strands, with the exceptionally long (approximately 70 residue) loop connecting β strands G and N and forming the handle that encircles the DNA duplex (Figure 3c). The two loops from the Ku70 and Ku86 subunits form anti-parallel β strands on the top of the handle (Figure 3a). The Ku DNA binding loop is formed by a continuous polypeptide chain that encircles the DNA and is embedded in the fully folded tertiary structure, which is consistent with the observation that Ku can only bind to DNA via an open end. Even though the crystal structures of Ku alone and Ku bound to DNA are quite similar, the extended conformation of this handle suggests structural flexibility. The handle is characterized by three hairpin-like loops, which restrict the diameter of the DNA binding channel (Figure 3c). In the DNA bound form, this channel is too tight for DNA to slide freely (Figures 3a and 3b), but if these hairpin-like loops were unzipped, the channel could be enlarged. In particular, the β hairpin inserted between the α-helical elements in the handle (Figure 3c, left side of loop) is locked in place by intersubunit interactions with the C-terminal α-helical arm, which in turn is contacted by the N-terminal globular domain (Figure 2a). Thus, although the C-terminal α-helical arm is not directly involved in DNA binding, together with the N-terminal domain it could potentially regulate the conformation of the DNA binding channel.

The β barrels from each subunit in the heterodimer point toward the minor groove of the DNA, with loops connecting the β strands extending into both the major and minor grooves (Figure 3b). Together these domains form a DNA binding platform at the base of the channel, and this platform protects more than 14 bases on one face of the DNA duplex. The opposite face of the DNA molecule is only partially covered by the narrow handle and is relatively accessible to the solvent (Figures 2 and 3). While the overall structure of the heterodimer suggests pseudo 2-fold symmetry, the handle leans toward the Ku70 subunit and is slightly off center relative to the pseudo-dyad axis relating the two β barrels (Figures 2b and 3b). Both the crystal structure and photo crosslinking data [15] suggest that the DNA end enters the binding channel from the Ku86 side and winds up proximal to the Ku70 subunit.

## Biological Implications

The structure of the Ku heterodimer, in which one face of the DNA molecule is protected while the other is left fairly accessible, can be readily integrated into current models for NHEJ. In the cell, DSBs frequently produce partially cohesive overhanging ends that may be missing

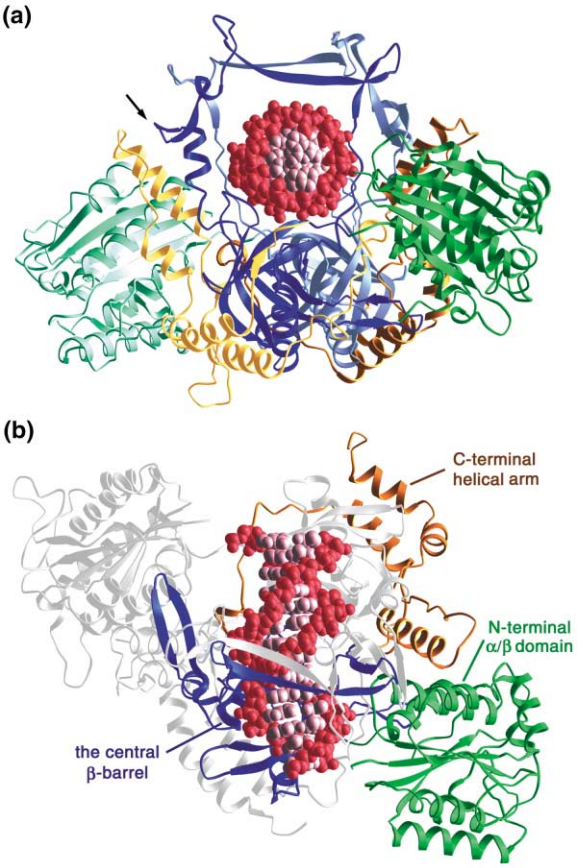


Figure 2. Structure of the Ku-DNA Complex

(a) The Ku heterodimer is represented by the ribbon diagram, and the 14 bp DNA duplex is represented by the space-filling model. The three-way junction portion of the DNA is removed for clarity. Ku70 is colored in deep green (the N-terminal $\alpha/\beta$ domain), blue (the central $\beta$ barrel domain including the handle), and brown (the C-terminal $\alpha$-helical arm); the corresponding structural domains of Ku86 are shown in light blue, light green, and yellow. The backbone of DNA is shown in red, and the bases are shown in pink. The arrowhead marks the lock-and-latch interactions between Ku70 and Ku86. (b) An orthogonal view of panel (a). Ku86 is colored in light gray.

1 or 2 bases and be blocked by nonligatable moieties, as depicted on the right-hand side of Figure 1a. To initiate NHEJ repair, Ku heterodimers would bind to both ends at the break (Figure 1b). Bridging of the ends could be accomplished by interactions between the Ku heterodimers bound at each end (Figure 1c). Although direct evidence for such interaction in cells is lacking at present, the ability of Ku to bridge blunt ends and ends with noncohesive overhangs ([10] and see references therein] suggests that it is likely. Recruitment of DNA-$PK_{cs}$ (not shown) could assist in promoting limited internal translocation of Ku [16] and the assembly of an aligned bridging complex in which the DNA binding platforms of the two heterodimers are coplanar. Walker et al. calculate that in a hypothetical repair complex consisting of a heterodimer bound to each end, the distance between the DNA binding loops of the two Ku heterodimers would be 50-60 Å [7]. This would make approximately 12 base pairs accessible to processing enzymes required to remove damaged nucleotides and fill gaps prior to ligation (Figure 1d; these enzymes are not shown). Once the ends have been made competent for joining, Ku specifically recruits the XRCC4/LigIV complex [14, 17], whose entry into the repair complex could be stabilized by both protein-protein interactions and DNA binding by XRCC4 (Figure 1d [13]). After closure of the nicks on both strands to rejoin the broken ends, XRCC4 and ligase IV may be able to leave the repair complex (Figure 1e), although it is possible that additional disassembly or remodeling would be required to remove them. The Ku heterodimers would probably be trapped on the DNA after repair [7] and would be extremely difficult to remove by any means other than proteolysis.

## Concluding Remarks

The Ku structure will be invaluable in developing targeted mutagenesis strategies to explore the mechanism of DNA end bridging and interaction with other repair factors such as XRCC4 and ligase IV. However, many questions remain. For example, the region of Ku86 that interacts directly with DNA-$PK_{cs}$ [18] is missing from the current structure. In addition, Ku is known to play important roles in cellular metabolism outside of its requirements for NHEJ repair. The Ku heterodimer is required for proper maintenance of the telomeres at the ends of linear chromosomes [5]. Telomeres are not believed to exist as free DNA ends but instead are kept in "T-loops" [19], which may be stabilized by specific proteins and repetitive DNA sequences. It will be interesting to determine how Ku associates with the telomere in the absence of a free DNA end. Nevertheless, the Ku structure provides a milestone to our understanding of the assembly of repair complexes at the ends of broken DNA molecules.

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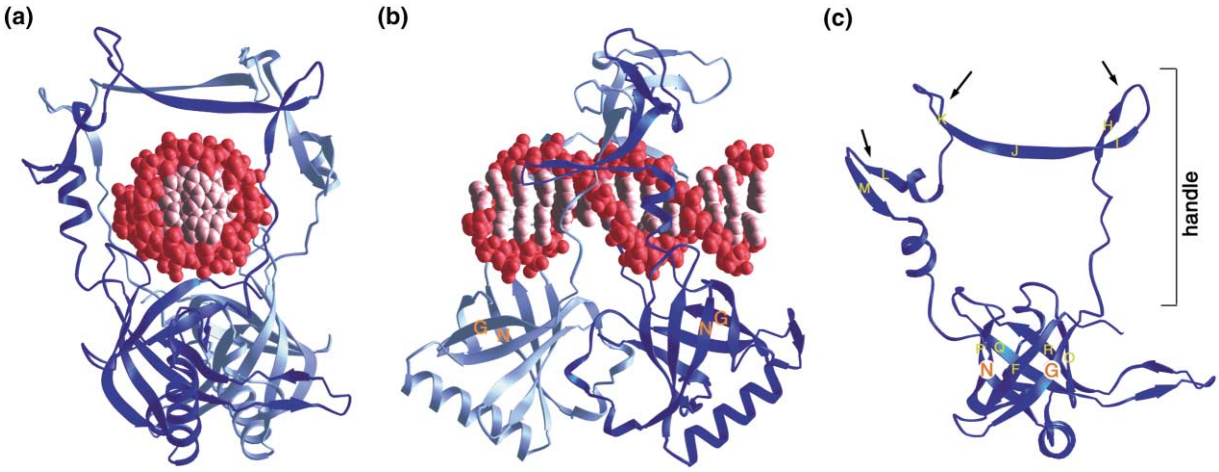


Figure 3. Interactions between Ku and DNA

(a) The β barrel domains, essential for DNA binding, are shown in a ribbon diagram with the 14 bp DNA duplex in the same orientation as in Figure 2a. The β barrels of Ku70 and Ku86 are shown in dark and light blue, respectively.
(b) An orthogonal view of panel (a). (c) A ribbon diagram of the β barrel domain of Ku70. The β strands are labeled according to the nomenclature by Walker et al. (2001). The hairpin-like structural elements in the handle are marked by the arrowheads.

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