Ku protein binds broken DNA ends, triggering a double-strand DNA break repair pathway. The spatial arrangement of the two Ku subunits in the initial Ku-DNA complex, when the Ku protein first approaches the broken DNA end, is not well defined. We have investigated the geometry of the complex using a novel set of photocross-linking probes that force Ku protein to be constrained in position and orientation, relative to a single free DNA end. Results suggest that this complex is roughly symmetric and that both Ku subunits make contact with an approximately equal area of the DNA. The complex has a strongly preferred orientation, with Ku70-DNA backbone contacts located proximal and Ku80-DNA backbone contacts located distal to the free end. Ku70 also contacts functional groups in the major groove proximal to the free end. Ku80 apparently does not make major groove contacts. Results are consistent with a model where the Ku70 and Ku80 subunits contact the major and minor grooves of DNA, respectively.

Ku protein has a general role in the metabolism of DNA ends in eukaryotic cells, including the repair of double-strand DNA breaks (reviewed in Refs. 1 and 2). Ku binds tightly to broken ends and initiates a repair pathway. Biochemical studies in higher eukaryotes, as well as genetic analysis in yeast, have helped to identify many participants in this pathway. In mammalian cells, these include a protein kinase (DNA-PKcs), a ligase (DNA ligase IV), a nuclease (hMRE11), and various protein cofactors (XRCC4, hRAD50, p95/NBS1) (reviewed in Ref. 1).

One of the functions of Ku protein is to recruit the 470-kDa DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to DNA (3, 4). However, in a number of instances, Ku has been shown to have independent functions in repair that do not require DNA-PKcs. These include the alignment of DNA ends in noncovalent complexes visible in the atomic force microscope and the stimulation of eukaryotic DNA ligases (5, 6). Studies with mutant cell lines further support the idea that there are functions of Ku that are independent of DNA-PKcs. For example, cells lacking Ku are hypersensitive to the action of the double-strand DNA break-inducing topoisomerase II inhibitor, etoposide, whereas cells lacking DNA-PKcs are not (7). Cells lacking Ku are unable to perform either V(D)J coding or signal joint formation (8, 9), whereas most cells lacking DNA-PKcs remain competent to perform signal joint formation (10–12). Finally, Ku homologues have been identified in a variety of fungal and metazoan organisms, including yeast, roundworms, insects, and vertebrates. By contrast, DNA-PK activity has so far been detected only in one branch of the higher eukaryotic evolutionary tree (the Cnidaria), and, among these, the DNA-PKcs gene or polypeptide has been identified unequivocally only in the taxon that includes echinoderms and vertebrates (the Deuterostoma) (reviewed in Ref. 13). It thus appears that DNA-PKcs appeared later in evolution than Ku itself.

Because of the central and evolutionarily ancient role of Ku protein in double-strand DNA break repair, it is important to understand the mechanism by which it recognizes DNA ends. Human Ku is a heterodimer of 70- and 83-kDa subunits, neither of which contains known DNA binding motifs. In vitro binding studies show that the interaction of Ku with DNA is determined primarily by features of the nucleic acid structure (13). Ku binds blunt, overhanging, and hairpin ends (14–16). End-bound Ku can translocate inward, forming a sliding clamp (17). In addition to DNA ends, Ku is capable of binding artificial DNA structures containing single- to double-strand transitions (15, 18). There are also certain sequences that appear to function as entry sites for Ku in double-stranded DNA (19).

Previous studies have shown different requirements for the Ku70 and Ku80 subunits in DNA end binding, depending on the assay used. Ku70 binds DNA in the absence of Ku80 in immunoprecipitation and Southwestern blot analysis (20–22). On the basis of these results, it has been suggested that the major part of the Ku DNA binding domain may reside in the Ku70 subunit. However, DNA binding activity requires sequences in the C-terminal half of both Ku subunits in an electrophoretic mobility shift assay (23). Furthermore, sequence analysis reveals that Ku70 and Ku80 share weak homology, most evident in lower eukaryotes, over a large C-terminal region encompassing the DNA binding domains defined in electrophoretic mobility shift assay (13). This suggests that the subunits derive from duplication of a common ancestral gene and are likely to share a similar tertiary folding pattern. Consistent with this, recent studies have shown that the sequences required for dimerization map to precisely homologous positions in each subunit of Ku (21, 22, 24). The existence of sequence homology in Ku70 and Ku80 suggests, but does not prove, that both subunits may have equivalent roles in contacting DNA.

We have performed site-specific cross-linking experiments to distinguish between the different models for the role of the two

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Ku subunits. These experiments define the spatial arrangement of the Ku subunits in the Ku-DNA repair complexes assembled on defined oligonucleotides. Some cross-linking studies with Ku have been performed before (4, 17, 19, 25, 26). In particular, a report by Zhang and Yaneva (17) indicated the relative positions of Ku70 and Ku80 subunit at the DNA end, based on the pattern of protection of probes that were radiolabeled by different methods. However, this early study relied on nonspecific UV cross-linking chemistry and did not involve the introduction of defined cross-linking groups at specific positions in the DNA. In the present work, we have synthesized a novel set of probes that force Ku to be trapped in a unique position and orientation relative to a single free DNA end. Incorporation of aryl azide groups at a number of different backbone positions allows unambiguous determination of the proximity of Ku70 and Ku80 to different points on the DNA surface. The results of these studies show that Ku protein binds DNA as a roughly symmetric, oriented heterodimer, with each subunit making approximately equal numbers of backbone contacts with DNA. Ku70 makes contacts functional groups in the major groove of DNA, while Ku80 apparently does not.

MATERIALS AND METHODS

Construction of Recombinant Baculovirus—To express non-histidine-tagged Ku70, a DNA fragment encoding histidine-tagged Ku70 was excised from pBC70H1 (27) with BamHI and subcloned into pGEM3zf(+) (Promega, Madison, WI). The open reading frame, without the histidine tag, was amplified by polymerase chain reaction using as primers: 5'-GTAAATACGACTCACTATAGGGC (a T7 primer) and 5'-GCCGCGCCGGATCCTAGCTGGAAGTTGCTGTTG-3'. The amplified fragment was digested with BamHI and subcloned into pVL1393 (PharMingen, San Diego, CA). Baculovirus expressing non-histidine-tagged Ku (VBB2–70Ku) was generated by co-transfection of Sf9 cells with this plasmid and linearized baculovirus DNA (BaculoGold, PharMingen).

Protein Purification—Native Ku was purified from HeLa cell nuclear extracts as described previously, except that the phenyl-Superose and Mono S steps were omitted (28). To produce recombinant Ku protein, Sf9 cells (1 liter, 10^6 cells/ml) were co-infected with VBB2–86Ku (27) and either VBB2–70H1 or VBB2–70Ku with a multiplicity of infection of 5–10. After 72 h, cells were harvested and suspended in 30 ml of CB buffer (50 mM Tris-HCl, pH 7.9, 12.5 mM MgCl2, 1 mM EDTA, 5% glycerol, 0.02% Tween 20, 1 mM dithiothreitol, 0.1 M KCl), and centrifuged at 150,000 g for 30 min. The supernatant was applied to a HR 26/60 Superdex 200 column and eluted in Buffer CB with 0.35 M KCl. Ku-containing fractions were diluted to 0.1 M KCl, applied to a 5-ml heparin-agarose column (Life Technologies, Inc.). Ku was gel-purified and dialyzed overnight in Buffer CB with 0.35 M KCl, and centrifuged for 1 h at 150,000 g.

To purify Ku without a histidine tag, extracts were pooled from three batches (1 liter each) of infected Sf9 cells. After treatment with DNase I and centrifugation, (NH4)2SO4 was added to the supernatant (0.33 g/ml), and the mixture was stirred at 4 °C for 30 min. The pellet was collected by centrifugation at 150,000 g, resuspended in 5 ml of TM buffer (50 mM Tris-HCl, pH 7.9, 12.5 mM MgCl2, 1 mM EDTA, 5% glycerol, 0.02% Tween 20, 1 mM dithiothreitol, 0.1 M KCl), and centrifuged at 150,000 g for 30 min. The supernatant was applied to a HR 26/60 Superdex 200 column and eluted in Buffer CB with 0.35 M KCl. Fractions containing Ku were diluted to 0.2 M KCl and applied to a 5-ml single strand DNA-agarose column (Life Technologies, Inc.). Ku was eluted with a gradient of 0.2 M-1 M KCl in Buffer CB. Ku-containing fractions were diluted to 0.1 M KCl, applied to a 5-ml heparin-agarose column pre-equilibrated in Buffer CB, and eluted with KCl steps of 0.1, 0.2, 0.3, and 1 M in buffer CB. Recombinant Ku prepared by this method is functional in kinase and mobility shift assays (data not shown). Ku with a histidine tag was purified essentially as described previously (29).

Electrophoretic Mobility Shift Assays—To prepare singly biotinylated 14-mer probes without cross-linking groups, a nonbiotinylated top strand and a bottom strand with a 5' biotin group were synthesized with the sequence shown in Fig. 1. The DNAs were annealed, gel-purified, and radiolabeled using [γ-32P]ATP and T4 polynucleotide kinase. To prepare doubly biotinylated 14-mer probe, a bottom strand was synthesized that had a 5' biotin group and lacked the final 3' nucleotide. This was annealed to a 5' biotinylated top strand. The DNA was gel-purified and radiolabeled using [α-32P]dGTP and reverse transcriptase. Binding reactions were initiated by incubating DNA (7–15 nm) and streptavidin (1 μM) (Sigma) at room temperature for 30 min in 25 mM Tris-HCl, pH 7.9, 0.5 mM EDTA, 10% glycerol, and 5 mM MgCl2. Ku (20 nm) was added and allowed to incubate for 30 min. Aliquots were analyzed by 5% nondenaturing polyacrylamide gel electrophoresis in a buffer containing 25 mM Tris-HCl, pH 8.3, 190 mM glycin, and 1 mM EDTA.

Azido-Modified Probes—To prepare top strand DNA probes (Fig. 1A, probes 1–13), oligonucleotides with a single phosphate modification were synthesized and annealed to unmodified, complementary, bottom strand DNA containing a biotin group at the 5' end. Duplex DNAs were gel purified and dissolved in a buffer containing 10 mM Tris-HCl, pH 7.9, 1 mM EDTA, and 100 mM NaCl. Probes were radiolabeled at the 5' end of the top strand using [γ-32P]ATP and T4 polynucleotide kinase. To prepare bottom strand DNA probes (Fig. 1A, probes 1–11), oligonucleotides were synthesized that lacked the final 3' nucleotide, that had a single phosphorothioate modification, and that contained a biotin group at the 5' end. These were annealed to unmodified complementary top strand DNA, gel-purified, and radiolabeled by reaction with [α-32P]dGTP and reverse transcriptase. Radiolabeled DNAs were incubated for 3 h in the dark at room temperature in a reaction containing 150 mM DNA, 40 mM MOPS, pH 7.0, 60% MeOH, and 12 mM 4-azidophenacyl bromide (Sigma). Unreacted reagents were removed using Sephadex G-25 spin columns (5 Prime–o Prim Inc., Boulder, CO).

Aryl Azide Photocross-linking—Protein-DNA complexes were formed in Eppendorf polyethylene microcentrifuge tubes that were placed inside a disposable polystyrene cell culture plate. The plates were then irradiated with 302 nm ultraviolet light (AlphaImager 2000, Alpha Innotech, San Leandro, CA) for 25 s. Reactions were terminated by addition of SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8, 5% glycerol, 2% SDS, 5% 2-mercaptoethanol, 1% bromophenol blue) and heated for 3 min at 100 °C. Products were resolved by 7% SDS-PAGE and detected by PhosphorImager (Molecular Dynamics) analysis.

5-Iodopyrimidine-substituted Probes—Oligonucleotides containing double substitutions of 5-iodo-2'-deoxyuridine or 5-iodo-2'-deoxycytidine (Fig. 1B) were synthesized (Cybersyn, Lenni, PA). The oligonucleotides were annealed to bottom strand DNA containing a 5' biotin group (Fig. 1B). Duplex DNAs were isolated, radiolabeled, and purified as described above. Cross-linking was carried out in Eppendorf microcentrifuge tubes for 30 min using a 325-nm helium-cadmium laser (Omnichrome, Chino, CA).

RESULTS

Oligonucleotide Probes with a Single Free End—When a double-strand DNA break occurs in vivo, each chromosomal fragment has one broken end. These newly created ends provide an entry site for Ku and other repair proteins. The repair complex generated has an inherent orientation, with some components proximal and others distal to the free DNA end.

![Fig. 1. Sequences of DNA probes used in this study.](http://www.jbc.org/doi/fig)
We sought to develop photocross-linkable oligonucleotide probes that would mimic the in vivo situation. Ordinarily, repair complexes can assemble at either of the two ends of a short oligonucleotide, creating ambiguity when mapping proteins (Fig. 2). To this end, a series of oligonucleotides (8–36 nt in length) was tested for formation of a stable ternary complex with streptavidin and Ku. A 14-mer was the shortest oligonucleotide able to form a stable complex, shown in Fig. 2A. This ternary complex was readily distinguishable from streptavidin-DNA and Ku-DNA binary complexes (Fig. 2A, lanes 2–4). To demonstrate the efficacy of streptavidin in blocking the DNA ends, a 14-mer oligonucleotide with biotin at both ends was also tested. In the absence of streptavidin, this DNA formed a complex with Ku, as expected (Fig. 2B, lane 3). In the presence of streptavidin, Ku did not bind to this probe (compare lanes 2 and 4), demonstrating that streptavidin did indeed block both ends. Taken together, these data suggest that the singly biotinylated 14-mer probe can be used to map the spatial arrangement of Ku70 and Ku80 in an oriented complex formed when the Ku protein initially recognizes the broken DNA end.

Identification of Ku70 and Ku80 Adducts—A set of 14-mer probes was synthesized, each with a single aryl azide group at a different position. Our strategy was to derivatize every second backbone position on either strand of the DNA. Since each aryl azide is capable of reacting with proteins within a 9 Å radius (30), this strategy assured saturation coverage of the DNA surface. Probes were radioiodinated, allowed to bind to Ku, and irradiated with ultraviolet light. Cross-linked products were analyzed by SDS-PAGE.

Fig. 3 shows a representative cross-linking experiment using a probe attached near the center of the 14-mer, which allows contact with both Ku subunits. Two well resolved, UV-dependent, radioiodinated bands were seen (Fig. 3A, lanes 2 and 3), corresponding to possible Ku70 and Ku80 adducts. Since Ku70 and Ku80 are fairly similar in size, it was important to determine whether the bands indeed represented cross-linking to the two subunits, rather than cross-linking to the same subunit at different sites to give products of distinct mobility. To distinguish these alternatives, the experiment included one reaction containing a Ku70 subunit with a histidine tag and associated linker sequence. This caused a mobility shift of the lower cross-linked band, relative to nontagged recombinant Ku and native human Ku (Fig. 3A, lanes 1–3). This shift is comparable with that seen with Coomassie-stained preparations of these proteins (Fig. 3B) and identifies the lower band as a Ku70 adduct. The upper adduct band has the same mobility in all reactions (Fig. 3A) and is therefore a Ku80 adduct. For subsequent cross-linking experiments, nontagged recombinant Ku protein was used.

Cross-linking of Ku to Aryl Azide-derivatized 14-Mer Oligonucleotides—We then tested the full set of 14-mer probes. After labeling the probes, we performed a preliminary electrophoretic mobility shift assay, which showed that none of the aryl azide modifications significantly interfered with Ku-DNA binding under the conditions used (data not shown). Cross-linked complexes were then analyzed by SDS-PAGE.

In the absence of streptavidin (Fig. 4A and B, lanes labeled ‘−’), we expected to find a mixed population of complexes assembled in both possible orientations on the DNA. Consistent with this, under these conditions, the majority of the probes cross-linked to Ku70 and Ku80 in approximately equal proportions (probes 3, 7, 9, 11, 13, 3’, 5’). Other probes showed either a modest or significant preference for Ku70 over Ku80 (probes 1, 5, 1’, 7’, 9’, 11’). When streptavidin was present to block one end of the DNA (Fig. 4A and B, lanes labeled ‘+’), there was a marked change in the pattern of Ku-DNA cross-linking. Under these conditions, there were five probes that cross-linked predominantly, and in some cases almost exclusively, to Ku80: 11, 13, 1’, 3’, 5’. The locations of these five probes are marked by squares in the summary diagram in Fig. 4C. They are all within the distal half of the DNA, relative to the free end, and define a region of Ku80 contact. There were two probes, 7 and 9, that did not cross-link to either subunit of Ku in the presence of streptavidin. These define a region of noncontact near the center of the oligonucleotide on the top strand. There were six probes that cross-linked predominantly (but not exclusively) to Ku70 in the presence of streptavidin: 1, 5, 7’, 9’, and 11’. The locations of these probes are marked by circles in Fig. 4C. They lie within the proximal half of the DNA, relative to the free end, and define a region that is contacted primarily by Ku70 and, to a lesser extent, by Ku80. Taken together, these results show that Ku bound in a defined orientation relative to the free DNA end and that strong contacts with Ku70 and Ku80 are roughly symmetric about an axis perpendicular to the DNA helical axis (dashed vertical line, Fig. 4C).
Quantitation of the cross-linking results provides further insight (Fig. 5, A and B). In these graphs, relative cross-linking to different positions on the top strand is indicated by bars extending above the horizontal axis, and cross-linking to equivalent positions on the bottom strand is indicated by bars extending below the axis. A shows cross-linking of Ku70. There is a cluster of cross-linked sites proximal to the free end. Interestingly, the sites of maximum cross-linking are not directly opposite each other on the top and bottom strands, but are rather offset by about three nt in the 5′ direction on each strand (probe 3 on the top versus probes 7′ and 9′ on the bottom). In B-form DNA, the phosphates that most closely approach each other across the minor groove are displaced by 2–3 nt to the 3′ side on each strand. Thus, a 3′-staggered pattern of contacts is strongly indicative of a protein binding site that spans the minor groove (31).

There are two clusters of Ku80 cross-linking apparent in Fig. 5B. One cluster, in the distal half of the fragment relative to the free end, consists of those probes that cross-link only to Ku80 (probes 11, 13, 3′, 5′). Within this distal cluster, the sites of maximum cross-linking on the two strands are offset by about 3 nt in the 3′ direction. Because the phosphates that most closely approach each other across the minor groove of B-form DNA are displaced by 2–3 nt to the 3′ side on each strand, a 3′-staggered pattern of contacts is strongly suggestive of a binding site that spans the minor groove. A second cluster of Ku80 cross-linking is located in the proximal half of the fragment (probes 3, 5, 9′). Cross-linking is about 50% weaker than in the distal cluster, and all of these probes also cross-link to Ku70 more strongly than to Ku80. There are several possible interpretations of the Ku80 cross-linking seen with these probes. One is that the 9 Å cross-linker spacer arms are long enough to permit some reactivity with Ku80 bound in the distal site; another is that Ku80 sequences are partially interdigitated with Ku70 in the folded heterodimer.

Cross-linking of Ku to Iodopyrimidine-substituted 14-Mer Oligonucleotides—Ku-DNA contacts were further investigated using 5-iodopyrimidine-substituted DNA probes. Iodopyrimidines cross-link with amino acid residues that are in close contact with the C5 position of cytidine or thymidine in the major groove of DNA (32). Because Ku is a sequence-specific DNA-binding protein, we expect that adventitious contacts with particular bases will differ depending on the exact sequence that is present in the probe. Our strategy, therefore, was to synthesize probes where several nearby pyrimidines were modified simultaneously. This increases the chances that a structural element of Ku that penetrates the major groove will make at least one contact with a cross-linkable group.
Cross-linking was performed in the presence and absence of streptavidin, and results are shown in Fig. 6A. In the absence of streptavidin (lanes labeled 2), Ku70 cross-linked to probes with substitutions near the ends of the DNA (I-1/5 and I-11/14), but not to a probe with substitutions in the middle (I-7/8). In the presence of streptavidin to orient the complexes (lanes labeled 1), Ku70 cross-linked only to a probe substituted at positions near the free end (probe I-1/5), and not to the other two. These results are consistent with the aryl azide data and confirm that Ku70 makes contacts with the major groove at one or more sites within the proximal half of the fragment, relative to the free end. Ku80 did not cross-link to any of the three iodopyrimidine-substituted probes that were tested. This is consistent with the suggestion that Ku80 binds a site that spans the minor groove, rather than the major groove.

**DISCUSSION**

Although the interaction of Ku protein with nucleic acids has been extensively studied, the essential details of Ku-DNA contacts remain unknown. One of the difficulties is that, because Ku can bind to either end of a conventional DNA probe, there is a potential ambiguity in the orientation of the resulting Ku-DNA complexes. Ku is also known to translocate along the DNA, potentially blurring the pattern of contacts. In the present study, we have adapted a cross-linking method that has been widely used to study sequence-specific DNA binding. This technique is particularly suited to defining the geometry of multisubunit complexes. In this method, aryl azide cross-linking groups are incorporated at specific DNA sites. We designed and synthesized a set of novel aryl azide-containing probes in which one end of the DNA is blocked, forcing the Ku protein to bind to the other end of DNA, in a specific orientation. Moreover, we have used the smallest oligonucleotide that supports formation of a stable Ku-DNA complex, preventing the Ku from sliding along the DNA and also assuring that a high proportion of the contacts are likely to be essential. This approach has allowed us to generate a comprehensive map of the Ku-DNA contacts, showing the proximity of each subunit to different points on the DNA surface.

**Geometry of the Minimal Ku-DNA Complex**—A three-dimensional representation of the DNA probe used in this study, modeled as B-form DNA, is shown in Fig. 7A. The sites of attachment of the aryl azide groups that cross-link predominantly to Ku70 and Ku80 are shown in black and light gray, respectively. The C5 position of iodopyrimidines that cross-link to Ku70 are shown in dark gray.

To help visualize the location of the Ku-DNA contacts, the sites of maximum cross-linking are marked by the heavy ellipses, and the corresponding probe numbers are indicated in boldface. Ku70 makes contacts with the major groove at one or more sites within the proximal half of the fragment, relative to the free end. Ku80 did not cross-link to any of the three iodopyrimidine-substituted probes that were tested. This is consistent with the suggestion that Ku80 binds a site that spans the minor groove, rather than the major groove.

**FIG. 6.** Cross-linking of Ku to iodopyrimidine-substituted 14-mer probe. A, cross-linking reactions were performed using radiolabeled probes with iodopyrimidine substitutions on the top strand at the positions indicated (I-1/5, I-7/8, or I-11/14). Reactions were performed in the absence (−) or in the presence (+) of streptavidin (SA). Cross-linked products were analyzed by SDS-PAGE and visualized by PhosphorImager analysis. Positions of Ku70 and Ku80 adducts are indicated. B, summary of Ku70 (circles) contacts on the iodopyrimidine-substituted 14-mer DNA.

**FIG. 7.** Three-dimensional representation of Ku-DNA contacts. A, the 14-mer probe, modeled as B-form DNA, shaded to indicate Ku70 aryl azide (black), Ku70 iodopyrimidine (dark gray), and Ku80 aryl azide (light gray) cross-links. The free DNA end is at the top and the blocked end at the bottom. Probe numbers are indicated, with stronger cross-links in boldface. Heavy ovals denote regions of maximal cross-linking of Ku70 and Ku80 as indicated. B, same as A, rotated 90° about the helical axis. Regions of noncontact defined by probes 7 and 9 are indicated.
boldface. It appears that the sites of maximum Ku70 contact are in a pocket in the major groove defined by probes 3, 7', and 9. The I-5 probe also lies within this pocket. Since the aryl azide probes have a 9-Å spacer arm, a Ku70 secondary structural element bound within the major groove pocket would still be partially reactive with probes at nearby sites, including 1, 5, and 11'. As noted earlier, both the 5’ stagger of the backbone contacts and the reactivity with iodoaziridines are consistent with a major groove binding model for Ku70.

The sites of maximum Ku80 contact appear to correspond to a region of the minor groove defined by probes 11, 13, 3’, and 5’ (in the view shown in Fig. 7A, this region wraps partly around the DNA molecule to the left). Ku80 bound in this region would be partially reactive with probe 1’, consistent with the observed pattern of cross-linking. Although minor groove-specific cross-linking probes are not available, both the 3’ stager of the backbone contacts and the absence of iodoaziridine reactivity are consistent with minor groove localization for Ku80.

It is interesting that Ku-DNA contacts are located primarily on one face of the helix. This is apparent from inspection of Fig. 7B, where the DNA model has been rotated 90° relative to the view in Fig. 7A. The Ku70 binding pocket now faces to the left, and the Ku80 binding surface faces to the left and behind. In this view, there are few, if any, strong contacts on the right side of the molecule. Probes 7 and 9 show almost no cross-linking above background. Cross-linking to probes 1’, and 11’ is also weak. The arrangement of Ku protein on one face of the helix potentially allows other repair proteins to access the DNA termini, including DNA-PKcs, the MRE11 endonuclease, or the DNA ligase IV-XRCC4 complex.

A Role for Both Ku70 and Ku80 in DNA Binding—The finding that each Ku subunit contacts DNA to a similar extent in the minimal Ku-DNA complex stands in distinction to an earlier proposal that the interaction of Ku with DNA depends primarily on the Ku70 subunit (20–22). That proposal was based on observations that Ku70, but not Ku80, binds DNA independently in immunoprecipitation and Southwestern blot assays. It appears, however, that both subunits contribute to binding in the native Ku heterodimer.

The presence of sequence homology between the two subunits suggests that both may share a common folding pattern. Our results show, however, that at three-dimensional level the details of the DNA contacts are different for the two subunits. Ku70 binds in the major groove, whereas Ku80 apparently binds in the minor groove. The crossover between major and minor grooves occurs at the center of the complex (near probe 7’) and is geometrically necessary in order for Ku to remain primarily on one face of a B-form 14-mer.

A recent study described a low resolution structure of Ku-DNA complexes obtained using a neutron contrast variation technique (33). These authors, who used 24-mer and 30-mer DNAs, suggested that these DNAs bind within a preformed channel that penetrates deep into the Ku protein. This model is somewhat at variance with our 14-mer cross-linking result, which strongly suggests a region of noncontact on one face of the DNA. Although the differences in the experimental methods make direct comparison difficult, one intriguing possibility is that the complex between Ku and the 14-mer represents an initial interaction with the DNA end and that additional regions of Ku come into contact with DNA when a longer sequence is present. If such contacts exist, they do not appear to influence the Ku-DNA binding equilibrium, as measured both in our laboratory and in a previous study (15). It is possible, however, that the assembly of Ku-DNA contacts may play a functional role in the assembly of higher order complexes containing multiple copies of Ku and/or other repair proteins.

Genetic studies have identified a number of additional proteins that participate in a Ku-dependent repair pathway (2), and human homologues of most of these proteins are now known. The system described here, using site-specific DNA probes with a single free end, is potentially applicable to the study of higher order repair complexes. Such studies, using longer oligonucleotides to permit the recruitment of additional repair proteins, will allow the geometry of the fully active double-strand break repair complex to be further elucidated.

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