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Ku Autoantigen: A Multifunctional DNA-Binding Protein

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ABSTRACT: Ku is a heterodimeric protein composed of ~70- and ~80-kDa subunits (Ku70 and Ku80) originally identified as an autoantigen recognized by the sera of patients with autoimmune diseases. Ku has high binding affinity for DNA ends and that is why originally it was known as a DNA end binding protein, but now it is known to also bind the DNA structure at nicks, gaps, hairpins, as well as the ends of telomeres. It has been reported also to bind with sequence specificity to DNA and with weak affinity to RNA. Ku is an abundant nuclear protein and is present in vertebrates, insects, yeast, and worms. Ku contains ssDNA-dependent ATPase and ATP-dependent DNA helicase activities. It is the regulatory subunit of the DNA-dependent protein kinase that phosphorylates many proteins, including SV-40 large T antigen, p53, RNA-polymerase II, RP-A, topoisomerases, hsp90, and many transcription factors such as c-Jun, c-Fos, oct-1, sp-1, c-Myc, TFIID, and many more. It seems to be a multifunctional protein that has been implicated to be involved directly or indirectly in many important cellular metabolic processes such as DNA double-strand break repair, V(D)J recombination of immunoglobulins and T-cell receptor genes, immunoglobulin isotype switching, DNA replication, transcription regulation, regulation of heat shock-induced responses, regulation of the precise structure of telomeric termini, and it also plays a novel role in G2 and M phases of the cell cycle. The mechanism underlying the regulation of all the diverse functions of Ku is still obscure.

KEY WORDS: V(D)J recombination, DNA-protein kinase, telomeres, helicase, ATPase, immunoglobulin isotype switching, double-strand break repair.

I. INTRODUCTION

Ku is a heterodimeric protein originally identified as an autoantigen recognized by sera from patients with autoimmune diseases (Mimori et al., 1981; Mimori et al., 1986; Francouer et al., 1986; Reeves, 1985). Patients with systemic lupus erythematosus (SLE), Sjorgen's syndrome, scleroderma, and polymyositis have been reported to have some levels of anti-Ku antibodies (Reeves, 1992). The name 'Ku' derives from the surname of the first Japanese patient. Ku is present in various mammalian systems and variously termed as nuclear factor IV (NF IV), transferrin receptor promoter element binding factor (TREF), proximal sequence element 1 (PSE 1), end binding protein (EBP), and human DNA helicase II (HDH II) (deVries et al., 1989; Stuiver et al., 1990; Roberts et al., 1989; Knuth et al., 1990; Falzon et al., 1993; Tuteja et al., 1994). Since its discovery in 1981, very little was known about Ku protein. However, in the last few years extensive studies have been undertaken to understand the structural and functional aspects of Ku.

Ku is an abundant protein (estimated 4×10^5 copies per HeLa cell nucleus) found in most human cells studied so far (Mimori et al., 1981; Francouer et al., 1986). Homologues of Ku have also been reported in monkeys (Paillard and Strauss, 1991), *Xenopus laevis* (Higashiura et al., 1993), yeast (Feldmann et al., 1993; Feldmann et al., 1996), *Drosophila melanogaster* (Jacoby and Wensink, 1994), and rodents (Porges et al., 1990; Kim et al., 1995; Errami et al., 1996; He et al., 1996; Lee et al., 1996), although in rodents the concentration of Ku protein is not as high as humans. Ku from plants has not been isolated yet. However, the antibodies against human Ku70 and Ku80 recognize the homologous proteins in pea nuclear extract on Western blotting (Tuteja et al., unpublished observations). These findings show that Ku or Ku-like protein must be existing in plants.

Initial studies into the mechanism of interaction of Ku with DNA showed that Ku binds to the ends of double-stranded DNA fragments without any preference for the nature of ends: Ku does not seem to discriminate between blunt ends and ends with either a 5′ or 3′ overhang (Mimori and Hardin, 1986; deVries et al., 1989; Griffith et al., 1992; Blier at al., 1993; Falzon et al., 1993). It has a much lower affinity for circular DNA, denatured DNA, yeast transfer RNA and poly (rA).poly (dT) (Mimori and Hardin, 1986). The DNA binding characteristics of Ku suggested a role for this protein in V(D)J recombination and DNA repair. The Ku plays an integral role in mammalian DNA double-strand break repair as the DNA binding component (or regulatory subunit) of the DNA-dependent protein kinase (DNA-PK) complex (Jeggo et al., 1995; Anderson and Carter, 1996). The yeast homologues of Ku proteins (yku) were identified and also shown to be critical for joining ends of DNA (nonhomologous end-joining) consistent with the results from mammalian cells (Boulton and Jackson, 1996a; Boulton and Jackson, 1996b; Milne et al., 1996). Ku is also known to be required for immunoglobulin isotype switching (Casellas et al., 1998). Ku acts as a potential transcription factor for RNA polymerase II (Kuhn et al., 1993). It has been shown to play an important role during the G2 and M phases of the cell cycle (Munoz et al., 1998). Two independent groups found that yku70 and yku80 mutants have abnormally short telomeres (Boulton and Jackson, 1996b; Porter et al., 1996), implying that Ku helps to maintain the telomere structure. Several recent reports reinforce the view that Ku is an active player at telomeres in yeast and suggest that the protein participates in telomere replication as well as silencing (Shore, 1998). Recently, it has been shown that the Ku and Sir (silencing information regulators) pro-

teins residing at telomeres relocalize in response to DNA damage, and this process is under the control of the cell's DNA damage check point genes (Martin et al., 1999; Haber, 1999).

Direct and indirect evidence imply that Ku is not only a DNA binding protein, but in fact it is a multifunctional protein and a member of a multigene family. This article attempts to describe the structural and functional aspects of Ku in detail. It also covers all the associated or intrinsic activities of Ku that clearly indicate that Ku is more than a DNA binding protein.

II. STRUCTURE OF THE KU PROTEIN

The Ku protein of almost all the systems studied so far has been reported to consist of two subunits of ~70- and ~80-kDa polypeptides referred to as Ku70 and Ku80, respectively (deVries et al., 1989; Knuth et al., 1990; Falzon et al., 1993). The cDNAs for both the subunits have been cloned and sequenced from a variety of species (Dynan and Yoo, 1998). It has been shown that p70 consists of 609 amino acids with an estimated molecular weight of 69,581, whereas p80 contains 732 amino acids and an estimated molecular weight of 81,914. Both the subunits have periodic repeats of leucines or leucines alternating with serines similar to the leucine zipper structural motif described for a family of DNA binding proteins that are involved in the regulation of transcription (Landschulz et al., 1988; Reeves and Sthoeger, 1989; Yaneva et al., 1989; Wu and Lieber, 1996). The human gene coding for Ku p70 maps to chromosome 22q13 (Cai et al., 1994) and Ku p80 gene maps to 2q33-2q34 (Cai et al., 1994; Blunt et al., 1995b). Based on cross-hybridization and protein sequencing data, it has been suggested that the human Ku70 protein might be part of a gene family (May et al., 1991; Oderwald et al., 1996). Recently it has become apparent that homology exists between Ku70 and Ku80, suggesting that they arose through the duplication and subsequent divergence of a single polypeptide that presumably functions as a homodimer (Dynan and Yoo, 1998). The sequences of the two subunits of Ku protein are more divergent from each other in higher eukaryotes than in yeast and the relationship between the subunits was not apparent until yeast, and other lower eukaryotic sequences became available.

Both the subunits Ku70 and Ku80 contain domains that typically mediate protein/protein and protein/DNA interactions. Weak ATP binding site homologies are also present in the sequences of both Ku70 and Ku80, which may be essential for the proposed ATPase and helicase functions of Ku (Cao et al., 1994; Tuteja et al., 1994). However, the significance of the ATP-binding motif is not clear because mutation of these have not affected Ku function *in vivo* (Jin and Weaver, 1997; Singleton et al., 1997). Studies conducted to map the regions of the Ku subunits that make contact with one another and with DNA have shown that a carboxy-terminal region of ~150 amino acid residues in both Ku70 and Ku80 are essential for dimerization, and larger regions of both proteins are required for effective interactions with DNA ends. The results define a minimum domain of 28 amino acids from the central region of Ku80 (residues 449 to 477) that is required for Ku70-Ku80 interaction (Wu and Lieber, 1996; Jin and Weaver, 1997; Osipowich et al., 1997; Cary et al., 1998; Wang et al., 1998).

III. ACTIVITIES ASSOCIATED WITH KU

The following activities are known to be present in the Ku molecule.

A. DNA-Binding Activity of Ku

The DNA-binding properties of Ku have been characterized extensively (Paillard and Strauss, 1991; Griffith et al., 1992). The early studies on DNA binding by Ku revealed that it takes place with high affinity and that end binding is independent of the structure or sequence of the end (Mimori and Hardin, 1986; Paillard and Strauss, 1991; Falzon et al., 1993). Studies on the mechanism of interaction of the Ku protein with DNA showed that it binds to the termini of double-stranded DNA (dsDNA) and that when additional molecules bind the protein slides along the termini (like beads on a string) with a 25 base pairs periodicity and without an energy requirement (Mimori et al., 1986; Toth et al., 1993; Blier et al., 1993; Woodgett, 1993; Tuteja et al., 1994). Several lines of evidence indicate that Ku binds to the ends of dsDNA without specificity for particular nucleotide sequences or end configurations, that is, binding occurs to blunt ends, to ends with 5′ and 3′ overhangs, and to hairpin ends (Mimori and Hardin, 1986; Griffith et al., 1992); however, it does not bind to closed circular DNA. Ku protein can also bind to the chemically heterogeneous ends produced by ionizing radiations (Pang et al., 1997), or the ends of cisplatin-damaged DNA (Turchi and Henkels, 1996).

Further supporting evidence from our unpublished observations and others (Griffith et al., 1992) show that Ku was highly effective in inhibiting the conversion of linear blunt end DNA with cohesive ends into covalently closed circles. The function of prokaryotic ligase in these reactions is inhibited due to the presence of the Ku protein on the ends of linear DNA. In contrast to the above findings, it has been shown in a recent study that Ku protein stimulates DNA end joining by mammalian DNA ligases (Ramsden and Gellert, 1998). This stimulation of ligation by Ku is probably due to the ability of Ku to bridge two DNA molecules and stabilize an intermolecular association. This stimulation is observed only with eukaryotic ligases, indicating that the ability to interact with the bridged intermediate is specific to eukaryotic ligases. The structure of Ku-bridged DNA ends may be such that eukaryotic ligases can 'fit' in and perform ligation, but prokaryotic ligases cannot (Ramsden and Gellert, 1998).

The affinity of this protein for blunt or staggered ends is approximately the same, although the binding increases proportionately with the increase in the number of dsDNA ends (Griffith et al., 1992). Further studies have shown that Ku protein can interact with DNA by a two-step mechanism, where Ku protein first recognizes the DNA ends, then translocates to internal sites (Paillard and Strauss, 1991; deVries et al., 1989; Zhang and Yaneva, 1992). *In vitro*, many Ku complexes bind to and translocate on a single DNA fragment, thereby covering the fragment with protein. This allows binding of multiple Ku molecules to a single DNA fragment. The resulting complexes can be visualized by electrophoretic mobility shift assay, by protection assays, and by electron microscopy. Each Ku molecule makes contact with 13-21 base pairs of DNA and successive Ku molecules bind at 25-30 base pairs intervals when present in a multimeric array (deVries et al., 1989). In addition, atomic force microscopy studies have revealed the existence of internal as well as DNA end bound DNA-protein kinase complexes and have shown that Ku can juxtapose two DNA ends via a DNA looping mechanism (Cary et al., 1997).

The DNA binding activity of human Ku autoantigen using linear duplexes is shown in Figure 1 S-Z*. Binding to duplex DNA was much higher (60%, Figure 1 S-X and Z) when compared with ssDNA (<5%, Fig-

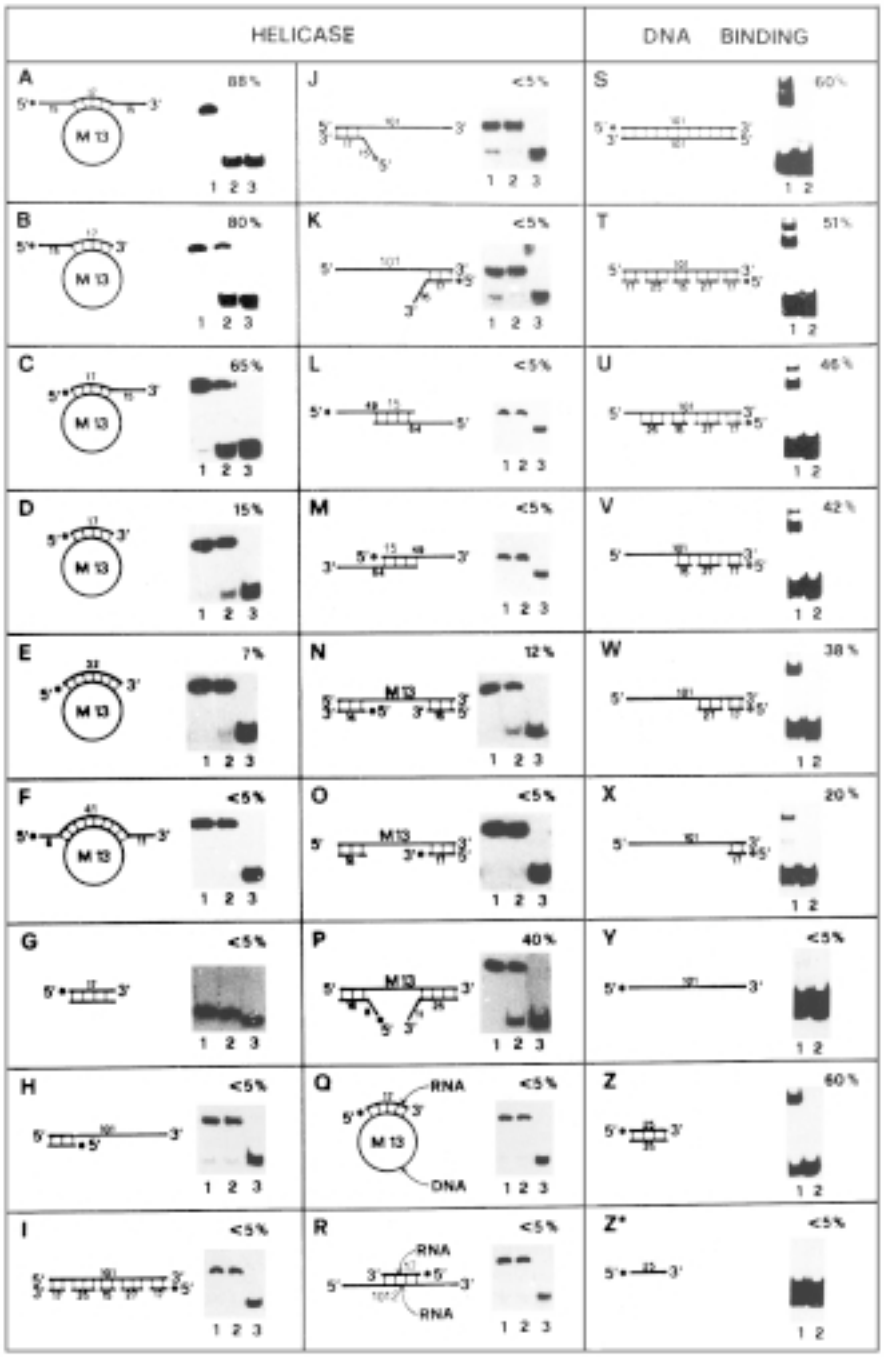

FIGURE 1. DNA helicase and DNA binding activities of Ku/HDH II with different substrates. For helicase, 90 ng of pure Ku and 1 ng of substrate, whereas for DNA binding, 4 ng of pure Ku and 0.2 ng of substrate were used. The asterisks denote the ^{32}P-labeled end. Each panel shows the structure of the substrate used, an autoradiogram of the gel, and the percentage unwinding (A-R) or percentage binding (S-Z*). From (A) to (R), lane 1 is control without enzyme, lane 2 is reaction with enzyme, and lane 3 is heat-denatured substrate. From (S) to (Z*), lane 1 is reaction with enzyme, and lane 2 is control without enzyme. (Reproduced by permission of Oxford University Press and *EMBO* journal [Tuteja et al., 1994].)

ure 1 Y and Z*). The binding was higher and higher after increasing the duplex length. For the 101 basepairs duplex DNA the dissociation constant of Ku was ~1 n*M* and for 25 base pairs duplex it was ~2 n*M*, which were almost similar to the previous observations (Blier et al., 1993). The difference was in accordance with the fact that 25 base pairs contained only one binding site for Ku (Blier et al., 1993), whereas the longer duplex contained more.

Ku has also been reported to recognize single-strand to double-strand transitions in DNA and to bind to a variety of additional structures, including gapped and nicked molecules and closed DNA hairpins (Blier et al., 1993; Falzon et al., 1993; Tuteja et al., 1994). It has also been shown that Ku can actually transit directly from one linear DNA molecule to another if the termini of the two DNAs are capable of base pairing (Bliss and Lane, 1997). There are reports that indicate that the Ku protein binds to single-stranded DNA with very low affinity, whether it is an oligonucleotide or an M13 ssDNA circle (Blier et al., 1993; Tuteja et al., 1994). The dissociation constant for duplex DNA is 1 to 2 n*M*, whereas for single-stranded DNA the affinity of Ku is much lower with a dissociation constant of ~300 n*M* (Tuteja et al., 1994). Direct and indirect evidences indicate that binding to nicks and ends may occur by identical mechanisms (Blier et al., 1993; Tuteja et al., 1994).

DNA binding studies using recombinant protein have shown that full activity, defined as an ability to form stable complexes in an electrophoretic mobility shift assay, requires both subunits of Ku protein (Griffith et al., 1992; Ono et al., 1994; Wu and Lieber, 1996; Ochem et al., 1997). It was shown that the renatured Ku70 subunit, by itself, binds DNA that is attached to the nitrocellulose membranes (Allaway et al., 1990). Both Ku70 and Ku80 make contact with DNA, although it appears that Ku70 makes the more intimate interactions, with a carboxy-terminal stretch of 73 amino acids residues in the Ku70 having been shown to make contact with DNA by Southwestern blot analysis (Chou et al., 1992). These data suggest that amino acids 536 to 609 of Ku70 may comprise a core DNA binding domain in the Ku70 subunit. It has been shown that Ku70 and Ku80 are functionally dependent on each other, neither subunit alone can bind DNA effectively (Wu and Lieber, 1996; Ochem et al., 1997). Studies using *in vitro*-translated Ku protein show that slightly more than half of each subunit is required for DNA binding in the electrophoretic mobility shift essay, that is, the C-terminal 40 kDa of Ku70 and the C-terminal 45 kDa of Ku80 (Wu and Lieber, 1996). A core region within Ku80 (amino acids 210 to 531) has been identified recently that is necessary for binding of Ku to DNA ends (Osipovich et al., 1999). It has been shown that the protooncogene vav associates with Ku70. This interaction requires only the 150-residue carboxy-terminal portion of Ku70, which binds to the 25-carboxy-terminal residues of the carboxy SH3 domain of vav (Romero et al., 1996).

In *Drosophila melanogaster*, the Ku homologue, yolk protein factor 1, binds to a 31 base pairs sequence located a short distance downstream from the transcription initiation site of the yolk protein 1 gene (Mitsis and Wensink, 1989; Jacoby and Wensink, 1994). The heterodimer of alpha and beta yolk protein factor 1 binds to DNA and both subunits make contact with DNA (Jacoby and Wensink, 1994; Jacoby and Wensink, 1996).

The DNA binding properties of a related protein, EPB-80, have been studied in detail, and it was shown that it binds efficiently to linear double-stranded DNA fragments, DNAs with single-stranded tails, DNAs with bubbles and gaps, and to dumb-

bell structures (Falzon et al., 1993). The ability of Ku protein to recognize a diversity of structures *in vitro* leaves open the possibility that it could have some role in recognizing all types of DNA damage. It can be hypothesized that, due to the nonspecific DNA binding activity of Ku, it acts as an 'anchor' to which other subunits of repair machinery bind.

B. Sequence-Specific DNA-Binding Activity of Ku

Ku has been reported on many occasions to bind DNA in a sequence-specific manner. Putative binding sites for Ku protein or Ku protein-containing complexes have been identified in a variety of genes, often in association with transcriptional regulatory elements. Binding of Ku has been reported to the negative regulatory element-1 (NRE-1) in the long terminal repeat of the mouse mammary tumour virus (Giffin et al., 1996) and human T-cell leukemia virus (Okumura et al., 1994; Okumura et al., 1996). Besides these, a number of other genes have been studied for sequence-specific binding. These include a mouse retroviral-like element (Falzon and Kuff, 1989; Falzon et al., 1993), transferrin receptor (Roberts et al., 1989; Roberts et al., 1994), U1Sn RNA (Knuth et al., 1990), T-cell receptor beta chain (Messier et al., 1993), parathyroid hormone (Chung et al., 1996), collagen IV (Genersch et al., 1995), c-myc (Giffin et al., 1997), immunoglobulin octamer motif (May et al., 1991), the AP-1 binding element (Quinn and Farira, 1991), heat shock element (Kim et al., 1995), sequence containing a replication origin (Toth et al., 1993), and a sequence corresponding to a BCL2 major breakpoint (DiCroce et al., 1995). These studies have not resulted in determining a general consensus recognition sequence for Ku binding. It might be possible that some of the earlier studies used preparations of Ku protein purified on DNA affinity columns, and these preparations may have been contaminated with small amounts of other sequence-specific DNA binding proteins. It now appears that in most cases this was artifactual and resulted from the high abundance of Ku and its unique end-binding properties.

At least for the case of Ku binding to the NRE-1 element in the long terminal repeat of the mouse mammary tumour virus, it appears that this example of sequence specific binding by Ku does have some functional basis (Giffin et al., 1996). In a related study, it has been reported recently that the activation of catalytic subunit of DNA-PK (DNA-PKcs) and the repression of mouse mammary tumor virus transcription from NRE-1 are dependent on Ku conformation, the manipulation of DNA structure by Ku and the contact of Ku80 with DNA (Giffin et al., 1999).

C. RNA-Binding Activity of Ku

There are very few studies that show that Ku protein interacts with RNA, although this interaction has been less well characterized than the interaction with DNA. Ku is localized in both the nucleoplasm and nucleolus of mammalian cells as documented by immunostaining (Reeves, 1985). It has been shown that association of Ku protein with the nucleolus is RNA-dependent because it is sensitive to RNAse treatment (Reeves, 1985). Ku protein binds very weakly to total HeLa cell RNA, but it binds selectively to RNA containing the HIV transactivation response element (Kaczmarski and Khan, 1993). It shows no binding to tRNA (Mimori and Hardin, 1986). Recently, RNA binding properties of Ku antigen have been characterized by expo-

nential enrichment technology (Yoo and Dynan, 1998). Many RNAs were identified that bound to Ku protein and competed with DNA for a common binding site in Ku protein. The identification of diverse RNAs that bind to the Ku protein is consistent with the idea that natural RNAs may serve as modulators of DNA-PK activity (Yoo and Dynan, 1998).

D. Ku as ATPase and Helicase

Enzymes catalyzing the hydrolysis of ATP in a DNA-dependent manner has been isolated from both prokaryotes and eukaryotes (Kornberg and Baker, 1991). Many reactions in DNA transactions such as recombination, repair, transcription, and replication are driven by chemical energy derived from the hydrolysis of ATP. A protein with ssDNA-dependent activity has been reported from HeLa cells and was shown to stimulate the DNA polymerase α primase activity (Vishwanatha and Baril, 1990). Later the same protein was identified as Ku autoantigen (Cao et al., 1994).

At the same time it was also reported that Ku is not only the ssDNA-dependent ATPase, but it also contains ATP-dependent DNA unwinding activity that was classified as human DNA helicase II (HDH II) (Tuteja et al., 1994). The DNA helicase activity of Ku/HDH II using a variety of different DNA and RNA substrates is shown in Figure 1 A-R. The Ku prefers to unwind DNA duplexes containing fork-like structure of the substrate (Figure 1 A-C) when compared with the substrate without fork or hanging tail (Figure 1 D). The substrate with a 5′ tailed fork was preferred (Figure 1 B) to the 3′ tailed one (Figure 1 C). If the length of the duplex was increased, the unwinding activity of Ku decreased (Figure 1 D-F). Ku failed to unwind the blunt ended duplex (Figure 1 G) that shows that for helicase activity Ku needs free ssDNA as a loading zone adjacent to the duplex. However, for DNA binding activity, as described before, Ku can use blunt ended duplex DNA as a substrate. Ku was unable to use small linear DNA substrate for its unwinding activity (Figure 1 H-M). Ku cannot unwind DNA from nicks (Figure 1 I). However, it can bind to the nicks (Figure 1 T). The direction of translocation of Ku/HDH II was exclusively 3′ to 5′ (Figure 1 N-P). The efficiency of unwinding by Ku increased if the 3′ to 5′ direction-specific substrate had a 5′ end tail (Figure 1 P). Ku failed to unwind DNA-RNA or RNA-RNA substrates (Figure 1 Q and R) (Tuteja et al., 1994). Both the subunits of Ku bind to ATP as shown by its UV mediated photo-cross-linking with α^{32}P ATP (Vishwanatha and Baril, 1990; Tuteja et al., 1994). The ATPase activity of Ku has been shown to be upregulated by its phosphorylation with DNA-dependent protein kinase (Cao et al., 1994).

Several DNA-interacting anti-cancer drugs have been tested on the DNA-dependent ATPase and ATP-dependent DNA helicase activities of Ku, and it was observed that actinomycin C1, daunorubicin, nogalamycin and ethidium bromide were inhibitory to both the activities of Ku (Tuteja et al., 1997). This inhibition could be due to binding of these drugs to DNA, thereby impeding the movement of Ku helicase for unwinding reaction that could be the drug's most important pharmacological function against cancer cells (Tuteja et al., 1997).

It is important to know whether separate subunits of Ku contain enzymatic activities. It has been shown that the DNA helicase activity resides in the smaller subunit (i.e., Ku70). On the other hand, the ATPase activity was present in both the separate subunits as well as in the heterodimer (Ochem et al., 1997).

E. Ku As a Regulatory Subunit of the DNA-Dependent Protein Kinase

1. Discovery and History

So far, only one modifying enzyme has been found that requires DNA to function, namely, the double-stranded DNA-dependent protein kinase (DNA-PK). The DNA-PK has been purified from HeLa nuclei (Carter et al., 1988; Carter et al., 1990). Initially, a 300- to 350-kDa protein was isolated and shown to phosphorylate several proteins *in vitro* in a DNA-dependent manner, including hsp90, SV40 large T antigen, p53, and human autoantigen Ku (Carter et al., 1990; Lees-Miller et al., 1990). It was noticed that two smaller proteins copurified with the 350-kDa protein and peptide sequencing identified one of these as the Ku autoantigen (Lees-Miller et al., 1990). Later it was shown that Ku is the component of DNA-PK that confers binding to DNA (Dvir et al., 1992; Dvir et al., 1993; Gottlieb and Jackson, 1993; Morozov et al., 1994). Biochemical and genetic data have shown DNA-PK to be composed of a large catalytic subunit, termed DNA-PKcs, and the regulatory factor termed Ku. In recent years, mammalian DNA-PK has been shown to be a crucial component of both the DNA double-strand break repair machinery and the V(D)J recombination apparatus (Smith and Jackson, 1999). In addition, recent work has implicated DNA-PK components in a variety of other processes, including the modulation of chromatin structure and telomere maintenance.

2. The Structure of DNA-PK

Cloning of the DNA-PKcs cDNA revealed that it corresponds to a ~470-kDa polypeptide, and with 4127 amino acids it is among the largest polypeptides in the cell. The amino-terminal ~3500 amino acid residues do not have significant homology to other characterized proteins (Hartley et al., 1995). The human gene maps to chromosome 8q11 (Sipley et al., 1995). DNA-PKcs, however, appears to be restricted to higher eukaryotes, clear homologues have been identified in mouse (Araki et al., 1997), horse (Shin et al., 1997) and *Xenopus laevis* (Labhart, 1997), but it is not present in the genome of *Saccharomyces cerevisiae* and has not been identified in the genomic sequences available for *Caenorhabditis elegans*. The carboxy terminal residues between amino acids 3719 to 4127 of DNA-PKcs comprise a catalytic domain that falls into the phosphatidylinositol-3 kinase family (Hartley et al., 1995; Poltoratsky et al., 1995). The available evidence indicates that DNA-PK has protein but not lipid kinase activity (Hartley et al., 1995; Smith et al., 1999).

Outside the kinase domain, DNA-PKcs has little or no similarity with other proteins and besides the presence of a putative leucine zipper motif, which is required for interactions with the high-affinity DNA binding protein C1D (Yavuzer et al., 1998), it has no clear features that might hint at its molecular functions. Sequences near the kinase homology domain, between amino acids 3002 and 3850, have been reported to interact with the Ku protein (Jin et al., 1997). The structure of DNA-PKcs has been resolved at a resolution of ~20 Å and 22 Å (Chiu et al., 1998; Leuther et al., 1999). Biochemical analysis based on these structures suggests that activation of the kinase requires interactions with both double- and single-stranded DNA (Suwa et al., 1994; Leuther et al., 1999).

3. Ku and Mechanism of Action of DNA-PK

In vitro, DNA-PK has been shown to phosphorylate several nuclear, DNA-bind-

ing, regulatory proteins such as p53, SV40 large T antigen, RNA polymerase II, serum response factor, RP-A, topoisomerases, BPV E2 protein and transcription factors like c-Jun, c-Fos, oct-1, sp-1, c-myc, CTF/NF-1, TFIID etc. (Anderson, 1993). These observations suggest that DNA-PK may have a role in regulating transcription, replication, recombination or DNA repair. DNA-PK also 'autophosphorylates' the 350-kDa catalytic subunit and both Ku70 and Ku80 proteins are phosphorylated in a DNA- and ATP-dependent manner. Autophosphorylation inhibits kinase activity (Lees-Miller et al., 1990; Chan and Lees-Miller, 1996). A few non-DNA-binding proteins can also be phosphorylated by DNA-PK, including heat shock protein 90 (hsp90) and microtubule-associated tau protein (Wu et al., 1993). The sites phosphorylated by DNA-PK have been identified in only a few substrates, including hsp90, the SV40 large T antigen, Jun, and the serum response factor (Anderson and Lees-Miller, 1992; Bannister et al., 1993; Liu et al., 1993). In these substrates, DNA-PK primarily phosphorylates serines or threonines that are followed immediately in the linear peptide sequence by glutamine.

The DNA-PKcs/Ku complex has been shown to phosphorylate proteins most effectively when it is bound to the same DNA molecule as DNA-PK itself, indicating that part of the activation produced by DNA is through the juxtaposition of DNA-PK and its target (Gottlieb and Jackson, 1993). However, DNA also stimulates the ability of DNA-PK to phosphorylate non-DNA-binding peptide substrates, implying that binding to DNA must directly or indirectly induce an activating conformational change in DNA-PKcs.

The impact of Ku deficiency on the sensitivity of anti-cancer drugs has been tested in a recent study (Kim et al., 1999). Ku-null mutant cell lines Ku70-/- and Ku80-/- were highly sensitive to anti-cancer drugs compared with their wild-type cells. Ku-deficient cells were more sensitive to bleomycin-induced DNA fragmentation and exhibited a higher level of c-jun NH_2-kinase/stress-activated PK activity than wild-type cells, whereas R7080-6 cells overexpressing both human Ku70 and Ku80 were resistant to bleomycin-induced apoptosis and exhibited a lower level of c-jun NH_2-kinase/stress-activated PK activity. These data suggest that Ku can affect the susceptibility to anti-cancer drug-induced apoptosis (Kim et al., 1999).

It has been shown recently using ATP as noncompetitive inhibitors of DNA-PK that such a conformational change is unlikely to correspond to an unmasking of the ATP-binding site of DNA-PK (Izzard et al., 1999). DNA-PKcs appears to make direct contact with DNA in the active complex, as evidenced by its UV cross-linking properties (Gottlieb and Jackson, 1993). Consistent with this idea, DNA-PKcs activity can be stimulated to some degree *in vitro* by DNA in the absence of Ku (Yaneva et al., 1997; Hammersten and Chu, 1998; West et al., 1998), but Ku is likely to be required for stabilization of DNA binding by DNA-PKcs *in vivo* (Hammarsten and Chu, 1998). It has been shown recently that the DNA-PK catalytic activity regulates DNA end processing by means of Ku entry into DNA (Calsou et al., 1999). It was also shown that addition of Ku protein stimulated activity 5- to 10-fold over that attained with the addition of DNA alone (Yaneva et al., 1997). According to a recent study the carboxy terminus of Ku80 is responsible for activation of the DNA-dependent protein kinase catalytic subunit (Singleton et al., 1999). Based on these studies, it can be postulated that Ku protein stimulates the DNA-PK activity by two mechanisms. Ku recruits DNA-PKcs to the DNA, when the concentration of DNA ends

is low and the other mechanism by which Ku stimulates DNA-PKcs is through direct protein-protein interactions.

The function of DNA-PKcs can be modulated by other proteins that affect the activity by altering the ability of the Ku protein to interact with DNA-PKcs. High-mobility group (HMG) protein 1 and 2 have been shown to be capable of stimulating DNA-PK activation *in vitro*, suggesting the possibility that DNA-PK activation *in vivo* is influenced by chromatin context (Watanabe et al., 1994; Yumoto et al., 1998). Another protein-protein interaction implicated in regulating DNA-PK function is that between DNA-PKcs and the Lyn tyrosine kinase, which is capable of disrupting the DNA-PKcs/Ku complex *in vitro* (Kumar et al., 1998). The interactions between heat shock transcription factor 1 (HSF1) and Ku and weaker interactions between HSF1 and DNA-PKcs also result in stimulation of DNA-PK activity *in vitro*. These findings suggest that HSF1 could cooperate with Ku and DNA PKcs possibly through stabilizing interactions between the DNA-PK holoenzyme and DNA (Peterson et al., 1995; Huang et al., 1997).

It has been shown recently that poly (ADP-ribose) polymerase and Ku autoantigen bind to matrix attachment sequences with high affinity and specificity in an end-independent manner (Galande and Kohwi-Shigematsu, 1999). It has been demontrated that poly (ADP-ribose) polymerase and Ku autoantigen form a molecular complex *in vivo* and *in vitro* in the absence of DNA and as a functional consequence their affinity to base unpairing regions are synergistically enhanced. These results provide a mechanistic link toward understanding the functional overlaps of poly (ADP-ribose) polymerase, Ku autoantigen, and DNA-PK and suggest a novel role for these proteins in the regulation of chromatin structure and function (Galande and Kohwi-Shigematsu, 1999).

Human Ku80 autoantigen-related protein (KARP-1) is only present in primates, is expressed from the Ku80 locus, and corresponds to a 9-kDa amino-terminally extended derivative of Ku80. It has been proposed that this protein regulated DNA-PK in a manner distinct from ordinary Ku80 (Myung et al., 1997; Myung et al., 1998).

IV. ROLE OF KU AND DNA-PK IN DOUBLE-STRAND BREAK REPAIR

Genetic and biochemical evidence suggest that Ku DNA-end-binding protein complex (which includes DNA-PK also) is involved in double-strand break repair events occurring following ionizing radiation (IR) damage or as a result of B and T cell V(D)J recombination in progenitor stages of immune system development (Jin et al., 1997). The use of the same recombination factors in different processes is clearly illustrated in SCID (severe combined immune-deficiency) mice, which are radiosensitive as well as having immune defects (Fulop and Phillips, 1990). Their immune deficiency relates to an inability of their precursor lymphocytes to perform correct V(D)J DNA recombination (Malynn et al., 1988; Lieber et al., 1988), the accompanying radiosensitivity is caused by inefficient rejoining of X-ray-induced double-strand DNA breaks (Biedermann et al., 1991).

Analysis of a specific series of mutant rodent cell lines showed the involvement of Ku and DNA-PK in double-strand break repair. Early studies into these cells found them to be hypersensitive to ionizing radiation and radiomimetic agents with little or no cross-sensitivity to other types of DNA-damaging agent and showed them to be defective in the repair of chromosomal DNA double-strand breaks (Zdzienicka, 1995).

Subsequent cell fusion studies allowed these cells to be placed into three distinct complementation groups, termed IR4, IR5, and IR7, and the human genes complementing them are called XRCC (X-ray cross-complementing) (Thompson and Jeggo, 1995; Zdzienicka, 1995).

Mammalian cells that are deficient in catalytic subunit of DNA-PK or Ku protein show highly characteristic defects. The most noticeable of these is sensitivity to ionizing radiation that induces double-strand DNA breaks (Taccioli et al., 1994; Rathmell and Chu, 1994a; Rathmell and Chu, 1994b; Smider et al., 1994; Getts and Stamato, 1994; Boubnov et al., 1995; Kirchgessner et al., 1995; Lees-Miller et al., 1995; Blunt et al., 1995a; Peterson et al., 1995b; Li et al., 1995).

Direct evidence linking double-stranded break repair, V(D)J recombination and DNA-PK came from two directions. Members of IR group 5 mutants were shown to lack a DNA end-binding activity that corresponded to Ku (Getts and Stamato, 1994; Rathmell and Chu, 1994b). Parallel studies, aimed at cloning the gene defective in xrs cells, localized a complementing human gene, designated XRCC5 to the region 2q33-2q35, which included the Ku80 gene (Cai et al., 1994; Hafezparast et al., 1993). The ability of Ku80 cDNA to complement the radiosensitivity and V(D)J recombination defects of xrs mutants provided confirmation that Ku80 is the product of XRCC5 (Boubnov et al., 1995; Smider et al., 1994; Taccioli et al., 1994; Blunt et al., 1995b). xrs mutants were also shown to lack DNA-PK activity, which was restored in xrs cells bearing the Ku80 gene, suggesting that Ku represents the predominant mechanism for activation of DNA-PK (Finnie et al., 1995).

Consistent with this, a radiosensitive human cell line (MO59 J) was found to be defective in DNA-PKcs expression and DNA-PK activity (Lees-Miller et al., 1995). Subsequent work showed cells of IR5 and IR7 to harbor inactivating mutations in the genes for Ku80 and DNA-PKcs, respectively. These results revealed that the inactivation of Ku80 leads to a dramatic destabilization of both itself and Ku70 (Errami et al., 1996; Errami et al., 1998a; Errami et al., 1998b; Blunt et al., 1996; Danska et al., 1996; Araki et al., 1997; Singleton et al., 1997; Peterson et al., 1997; Fukumara et al., 1998; Priestley et al., 1998). It was interesting to note that none of the original rodent cell lines was defective in Ku70, but it was assumed that cells lacking it would have a similar phenotype to those in IR4-7. This has been further confirmed by targeted disruption of the gene for Ku70 in mouse cells, allowing such cells to be designated IR6 and the gene for Ku70 to be designated XRCC6 (Gu et al., 1997a; Gu et al., 1997b). According to a recent study, the *Drosophila melanogaster* DmRAD54 gene plays a crucial role in double-strand break repair after P-element excision and acts synergistically with Ku70 in the repair of X-ray damage (Kooistra et al., 1999). The relative contributions of recombinational repair and nonhomologous end joining in double-strand break repair were investigated in a double mutant, DmRad54(-/-)/DmKu70(-/-). Compared with both single mutants, a strong synergistic increase in X-ray sensitivity was observed in the double mutant (Kooistra et al., 1999).

Mammalian Ku may also be involved in the heat shock response, as suggested by its interaction with the chaperone protein hsp70 (Kim et al., 1995) and heat shock transcription factor 1 (HSF1). Yeast cells that are defective in the Ku70 or Ku80 subunits are temperature sensitive (Huang et al., 1997) for growth and sensitive to various DNA-damaging treatments (Boulton and Jackson, 1996a; Boulton and Jackson, 1996b; Milne et al., 1996; Feldmann et al., 1996; Barnes and Rio, 1997; Mages et al., 1996). Because yeast have an efficient recombinational re-

pair pathway, the radiation-sensitive phenotype of Ku mutants is much more evident when this alternative pathway is inactivated (Boulton and Jackson, 1996a; Boulton and Jackson, 1996b; Milne et al., 1996; Mages et al., 1996; Siede et al., 1996).

V. ROLE OF KU AND DNA-PK IN V(D)J RECOMBINATION

As stated previously, DNA-PK clearly functions in the repair of the site-specific breaks introduced during V(D)J recombination. V(D)J recombination consists of a DNA cleavage reaction catalyzed by RAG1 and RAG2, followed by an end-joining reaction that utilizes the cells double-strand break repair machinery.

There is now strong evidence that indicates that the first half of V(D)J recombination produces DNA intermediates with two types of DNA ends. First, blunt DNA ends adjacent to RSSs (recombination signal sequences) were detected in wild-type mouse thymocytes, which actively undergo V(D)J recombination of T-cell receptor loci (Roth et al., 1992; Grawunder et al., 1996). Second, hairpin coding ends were detected in a pre-B cell line induced to undergo very high levels of V(D)J recombination (Ramsden and Gellert, 1995).

The end-joining reaction in V(D)J recombination requires the action of the cells DNA double-strand break repair machinery (Chu, 1996; Rathmell and Chu, 1996). This was first recognized when cells from severe combined immunodeficiency (scid) mice were found to have defects in V(D)J recombination also (Lieber et al., 1988) that were associated with deficient coding joint formation. Subsequent screening of additional IR-hypersensitive hamster cell lines revealed that other cells defective in double-strand break repair were also defective in V(D)J recombination (Pergola et al., 1993; Taccioli et al., 1993). XRCC4 and XRCC5 mutant cells were severely defective in both coding and signal joint formation. XRCC7 mutant cells, which include scid and V3 were severely defective in coding joint formation but only mildly defective in signal joint formation (Lieber et al., 1988). DNA-PK is essential only for coding joint formation in V(D)J recombination (Kulesza and Lieber, 1998). The importance of Ku is further strengthened by the fact that transfection of XRCC5 cells with an expression vector for Ku86 restored the V(D)J recombination activity (Smider et al., 1994; Taccioli et al., 1994). Furthermore, mutations in the Ku80 gene leading to an internally deleted or frame-shifted protein product have been found in XRCC5 cells (Errami et al., 1996). Finally, Ku80 knockout mice were immunodeficient due to an absence of B and T cells (Nussenzweig et al., 1996; Zhu et al., 1996). These data suggest that Ku80 is identical to the XRCC5 gene, which is required for the formation of both coding and signal joints. Analyses of Ku80 knockout mice revealed that coding and signal ends accumulate and fail to be efficiently processed into coding and signal joints, causing a profound failure of lymphocyte development (Nussenzweig et al., 1996; Nussenzweig et al., 1997; Zhu et al., 1996). These mice are also dwarfed and their fibroblasts have prolonged doubling times and abnormal loss of proliferating cells *in vitro* (Nussenzweig et al., 1996), implicating Ku80 in growth control. Ku70 knockout mice are impaired in immunoglobulin rearrangements but not T cell receptor rearrangements (Ouyang et al., 1997).

It has been suggested that Ku can bind to hairpin structures that arise directly at coding ends following endonucleolytic cleavage and Ku binding is an early event, serving to stabilize the ends and prevent nucleolytic degradation (Jeggo et al., 1995).

Consistent with this idea, linear DNA transfected into IR5 cells is more susceptible to end degradation than in control cells (Liang and Jasin, 1996), and the rare products that are generated in mammalian or yeast cells lacking Ku have generally suffered large deletions of terminal sequences before ligation has taken place (Taccioli et al., 1993; Boulton and Jackson, 1996b). However, V(D)J recombination intermediates are relatively stable in the absence of Ku or DNA-PKcs, indicating that in this case at least Ku or DNA-PKcs are not required for DNA end stabilization (Zhu et al., 1996). Another way that Ku/DNA–PKcs could potentiate end ligation is by tethering two DNA ends together. It has been shown that Ku is able to promote interactions between two DNA termini (Cary et al., 1997) and can enhance end ligation by eukaryotic DNA ligases *in vitro* (Ramsden and Gellert, 1998).

Ramsden and Gellert (1998) have suggested that the introduction of a double-stranded break would result in a recruitment of a Ku heterodimer to each end. Subsequent association of the two heterodimers would stabilize the interaction between the two ends. If the broken ends are compatible, ligation would be rapid and efficient. However, if the ends are not a competent substrate for ligation, such as hairpins from V(D)J cleavage, one or both of the Ku heterodimers could translocate internally, continuing to stabilize the intermolecular association of the ends but permitting processing to occur until a substrate that is competent for ligation is produced. According to their model, processing of ends and ligation are competitive; Ku acts to increase the frequency of accurate end joining principally by increasing the rate of ligation, rather than by protecting ends from degradation (Ramsden and Gellert, 1998).

According to the hypothesis put forward by Smider and Chu (1997), Ku helicase activity plays an important role in V(D)J recombination. They suggest that Ku helicase unwinds the hairpin-coding end, making it accessible to a hypothetical hairpin endonuclease. Disruption of the Ku or DNA-PKcs genes would result in loss of this helicase activity, thus explaining the persistance of hairpin ends in thymocytes from scid or Ku80 knockout mice (Ramsden and Gellert, 1995; Zhu et al., 1996). After the hairpin is opened, N-nucleotide addition may occur, either enzymatically by terminal deoxynucleotide transferase or nonenzymatically by nucleotide capture.

Ku helicase activity may unwind the open DNA ends to facilitate a search for regions of microhomology in the two coding ends. The search may be coordinated by a second DNA binding site on Ku. After occupying both the ends, the ends are juxtaposed by two Ku molecules in conjunction with a helicase-dependent search for microhomology. As a result, the end-joining reaction is regulated nicely by the binding of Ku to one DNA end and the activation of helicase activity by the opposing DNA end. The regions of microhomology are annealed, joining the two coding ends by hydrogen bonds (Smider and Chu, 1997; Gottlich et al., 1998). It has been shown that Ku is required for signal joint formation also, because XRCC5 cells have a severe defect in signal joint formation. The process of signal joint formation appears to be slower than coding joint formation, because signal ends are only detectable in wild-type thymocytes (Roth et al., 1992). The rate difference between signal and coding joint formation may arise from the coupling of Ku binding to RAG cleavage. After nicking the DNA, the RAG complex may remain bound to the recombination signal sequences, so that when Ku binds to the nicked DNA, steric interference forces it to translocate to the coding side of the nick. When the cleavage is completed, Ku may already bind the hairpin-coding end, but signal end would be

naked and would need to be bound by Ku before end joining could occur. This additional step results in delaying signal joint formation (Smider and Chu, 1997). The signal joint reaction has now been reported in a cell-free extract (Cortes et al., 1996).

According to a recent study, Ku80 is also required for immunoglobulin isotype switching (Casellas et al., 1998). Isotype switching is the DNA recombination mechanism by which antibody genes diversify immunoglobulin effector functions. During an immune response, B lymphocytes maintain their antigen-binding specificity but can change the antibody constant region subclass they produce by a DNA recombination process known as class switching (Lorenz and Ravruch, 1996; Stavnezer, 1996; Shimizu et al., 1982). Developing B cells in mice that carry mutations in double-strand break repair gene fail to complete V(D)J recombination and lymphocyte development is arrested at early precursor stages (Bosma et al., 1983; Zhu et al., 1996; Nussenzweig et al., 1996; Nussenzweig et al., 1997; Ouyang et al., 1997); therefore, there are no mature B cells.

To reconstitute the B-cell compartment in Ku80 -/- mice, prearranged Vb1-8 DJH_2 (mi) and V3-83 DJK_2 (Ki) genes were introduced into the Ku 80 -/- background (Sonoda et al., 1997; Pelanda et al., 1996). The resultant mice develop mature mIgM+ B cells but are unable to produce immunoglobulins of secondary isotypes and fail to complete switch recombination. These observations suggest that Ku80 is essential for switch recombination *in vivo*, suggesting a significant overlap between the molecular machinery that mediates DNA double-strand break repair, V(D)J recombination, and isotype switching (Casellas et al., 1998).

Ku70 is also required for late B cell development and immunoglobulin heavy chain class switching (Manis et al., 1998). The results from these class-switching studies raise the possibility that the Ku is also involved in other genomic rearrangements and related events in eukaryotes. In line with this, work in *Drosophila* has revealed a function for Ku in P-element transposition (Beall and Rio, 1996; Beall et al., 1994).

VI. ROLE OF KU IN TELOMERE MAINTENANCE

Recently, Ku has been shown to be a likely component of the telomeric complex in yeast, suggesting the possiblity of a similar role for Ku at mammalian telomeres (Boulton and Jackson, 1998; Gravel et al., 1998). Telomeres — the physical ends of eukaryotic chromosomes — are composed of simple tandem repeats, generally bearing short runs of guanines in the strand that runs with 5′ to 3′ polarity toward the tip of the chromosome (e.g., 5′ -TTAGGG-3′ in vertebrates). Telomeres are replicated by a special reverse transcriptase, called telomerase, which can synthesize the tracts of short repeat sequences (TG1-3 in yeast, T2AG3 in most higher eukaryotes) that mark the ends of eukaryotic chromosomes (Shore, 1998). Telomeres carry out at least two primary functions. First, through the action of telomerase, they circumvent the replicative problem of linear DNA ends (Greider, 1996). Second, they protect the ends of the chromosome from being treated as broken ends by the double-strand break repair pathway (Sandell and Zakian, 1993; vanSteensel et al., 1998).

In addition to functioning in the maintenance of chromosome integrity, yeast telomeres exert profound influences on the transcription of adjacent genes. Thus, genes placed within or near to telomeres are subject to transcriptional repression as a result of the phenomenon termed the telomere position effect (TPE) (Gottschling et al., 1990; Aparicio et al., 1991; Shore, 1995).

The yeast Ku homologs (YKU70 or HDF1 and YKU80 or HDF2) play some role in telomere metabolism because it has been discovered that yku70 and yku80 mutants have abnormally short telomeres (Boulton and Jackson, 1996; Porter et al., 1996). Hdf1p/Hdf2p may be an integral component of the yeast telomeric complex. HDF1 and HDF2 affect telomeric position effect, which is dependent on a multiprotein complex that includes Sir2p, Sir3p, and Sir4p (Grunstein, 1997). Loss of HDF1 or HDF2 has a dramatic effect on telomere position effect, equivalent to impairment of SIR2, SIR3, or SIR4 (Nugent et al., 1998; Boulton and Jackson, 1998; Laroche et al., 1998). An attractive explanation for the available data on telomeric silencing is that Ku helps to establish the transcriptionally silent heterochromatin-like state that normally exists in yeast telomeric DNA. However, because of the interaction of Hdf1p with Sir40 in yeast two-hybrid assays (Tsukamoto et al., 1996; Tsukamoto et al, 1997), it is possible that in yeast the interaction of Ku with the telomere is mediated by protein-protein interactions. Sir4p forms a complex with Sir2p and Sir3p, and through associations with Rap1p and the amino termini of histones H3 and H4 can establish a heterochromatin-like structure at the telomere (Grunstein, 1997).

Although the precise function of Ku in telomeric functions is as yet unclear, Ku does appear to be associated physically with telomeric DNA and regulates the precise structures of telomeric termini, possibly via controlling the access of nucleases and recombinases (Gravel et al., 1998; Polotnianka et al., 1998). It has been shown that mutations in either Ku subunit lead to enhanced instability of elongated telomeres by increasing their sensitivity to either degradation or recombination reactions (Polotnianka et al., 1998).

Significant differences exist between yeast and mammalian telomeres. Mammalian telomeres terminate in long (130 to 270 base pair) single-stranded G-rich overhangs (Wright et al., 1997; McElligott and Wellinger, 1997; Makarov et al., 1997), whereas long (720 base pairs) overhangs occur at yeast telomeres only briefly in late S-phase (Dionne and Wellinger, 1996; Wellinger et al., 1996). Thus, differences in the requirements for telomere end binding activities might exist in the two organisms. In particular, the G-rich tails found at the mammalian telomeres might adopt G-G base paired conformations, such as G-quartets, that might prevent Ku from recognizing telomeric ends and thus offer a mechanism to sequester the telomere from the prevalent double-strand break repair pathway in mammals. Recently, it has been shown that Ku binds to mammalian telomeric DNA ends *in vitro,* and G-quartet conformations are unable to prevent Ku from binding with high affinity to the DNA (Bianchi and Lange, 1999).

There are recent studies (Martin et al., 1999; Mills et al., 1999) that suggest that the Ku and Sir proteins residing at telomeres relocalize in response to DNA damage, and this process is under the control of the cells DNA damage checkpoint genes. Immunofluorescent marking of yku80p confirms that it co-localizes with Sir proteins and Rap1p at the several clusters of telomeres at the periphery of the yeast nucleus (Martin et al., 1999). They have shown by quantitative chromatin precipitation analysis that the Ku proteins are found along the silenced adjacent regions, similar to the Sir proteins. The absence of Ku proteins results in the loss of telomere-associated Sir proteins. This gives Ku proteins a more prominent role in heterochromatin assembly at telomeres. There is cytological and molecular evidence to confirm that both Ku and Sir proteins respond to the presence of even a single double-stranded break, by delocalizing from telomeres and binding to the ends

of double-strand breaks. As a result of this delocalization, genes near telomeres that were epigenetically silenced by Sir and Ku proteins are now more strongly transcribed, but genes with adjacent silencer sequences, located more internally on the chromosome, become more repressed (Martin et al., 1999).

VII. ROLE OF KU IN CELL CYCLE REGULATION

As far as localization of Ku during cell cycle is concerned, it has been shown that it is localized in the nucleoplasm and also in the nucleoli of interphase cells (Reeves, 1985; Francoeur et al., 1986). A very little amount of Ku is present in the G1/S boundary, while a moderate amount of Ku is reported to accumulate at late S and G2 phases of cell cycle, which results in the disintegration in prophase and suggested a possible role of Ku in the nucleolar disintegration process (Li and Yea, 1992). In yeast Ku70 (HDF-1)-deficient strain the cell cycle is reported to be blocked at G2 phase, indicating the potential role of Ku in the regulation of cell cycle (Feldman and Winnacker, 1993). Ku is also present in the S-phase of cell cycle in nucleoli of human interphase cells that strengthens the observation that Ku acts as potential RNA polymerase I transcription factor (Higushira et al., 1992; Zhang and Jacob, 1990). Ku is also reported to be localized in the nucleus and nucleolus in quiescent lymphocytes and in early G1, late G1, S and G2 phases the Ku was only present in the nucleoplasm (Yaneva and Jhiang, 1991). The differences in localization of Ku in different studies might be due to the different antibodies and/or different methods used for its detection.

The subcellular localization and nuclear localization signal of Ku70 have been studied (Koike et al., 1999). Ku70 staining was distributed throughout both the nucleus and the cytoplasm in late telophase to early G1 phase cells. The nuclear localization signal of Ku70 was located at the region composed of 18 amino acid residues (position 539 to 556). The Ku 70 nuclear localization signal consisted of two basic subregions and a nonbasic intervening region. The structure of the Ku70 nuclear localization signal resembled the consensus of a bipartite-type nuclear localization signal. The Ku70 nuclear localization signal was mediated to target to the nuclear rim by two components of the nuclear pore-targeting complexes (Koike et al., 1999).

In *Drosophila* the expression of Ku70 (YPF1β) is reported to be developmentally regulated with maximum expression in the ovary (Jacoby and Wensink, 1994). A prolonged cell cycle was reported in fibroblasts from Ku80-deficient mouse embryos (Nussenzweig et al., 1996), and the growth of DNA-PK defects in SCID mice (where Ku was normal) was affected (Nussenzweig et al., 1996). Therefore, it can be said that Ku is involved in cell cycle and growth control.

Recently, Munoz et al. (1998) have demonstrated that topoisomerase II (topo II) mediated functions were inhibited at significantly lower doses of topo II inhibitor (ICRF-193) in Ku86-deficient Chinese hamster cell lines than in wild-type cells. This difference was not observed when DNA-PKcs-deficient cells were analyzed. The ICRF-193 inhibits DNA topo II activity without inducing double-strand breaks (Tanabe et al., 1991). Mutant cells were blocked in G2 at ICRF-193 drug doses, which had no effect on wild-type cells. These data suggest a novel role for Ku antigen in the G2 and M phases of the cell cycle, a role that was not related to its role in DNA-PK-dependent DNA repair (Munoz et al., 1998).

VIII. OTHER POSSIBLE ROLES OF KU

Because Ku is a member of a multigene family and it is abundant in different cell systems, it also belongs to multifunctional protein family. Some of its other functions might be involvement in replication, transcription, and cell signaling.

A. Replication

Because Ku binds to the DNA ends during S-phase, Paillard and Strauss (1991) predicted a possible role of Ku in replication forks movement during DNA replication. Ku is also known to interact with human DNA containing replication origin and an active gene promoter (Toth et al., 1993). It also binds to origin of replication of type 2 adenovirus (deVries et al., 1989). In *Saccharomyces cerevisiae* the origin binding factor 2 (a Ku-like protein) was reported to be required *in vitro* for the assembly of a stable multiprotein complex at a eukaryotic origin of replication (Shakibai et al., 1996). The findings suggested that the Ku protein might have an important role in the initiation of DNA replication at eukaryotic origins of replication. Ku is a subunit of DNA-PK and an additional role of DNA-PK in DNA replication has been suggested by its ability to phosphorylate replication protein A (Pan et al., 1994).

This is the indirect evidence for its role in DNA replication; however, a direct evidence is still necessary.

B. Transcription

The transcriptional regulation of different genes by Ku or Ku-like proteins has been shown in many studies. Some examples are mentioned below:

1. U1 gene transcription was activated by Ku-like protein PSE-1 (Knuth et al., 1990).
2. The TREF may be involved in transcriptional regulation because it is known to bind to transferrin receptor promoter (Roberts et al., 1994).
3. Ku-like protein E1BF binds to the rat rRNA gene promoter, rat rRNA gene non-repeat enhancer, and rat rDNA repeat enhancer and is shown to stimulate RNA polymerase I transcription (Zhang and Jacob, 1990; Hoff and Jacob, 1993). When E1BF was purified from cells grown in serum-free media then it acted as a repressor of rDNA transcription; this effect could be due to posttranslational modification (Niu and Jacob, 1994).
4. The inhibition of RNA polymerase I-directed transcription in mouse by Ku has also been reported (Kuhn et al., 1993). However, DNA-PK (Ku+DNA-PKcs) was shown to be more potent inhibitor of RNA polymerase I-directed transcription than Ku alone (Kuhn et al., 1995).
5. Ku-related CHBF (constitutive HSE-binding factor) binds to the HSE (heat shock element) of heat shock genes and acted as a negative regulator of heat shock genes *in vivo* (Li et al., 1995).
6. A Ku-related protein, CTCBF (CTC-box binding factor), is known to bind to internal CTC-box of human collagen gene and thereby is involved in the control of its transcription (Genersch et al., 1995).
7. The TATA-box binding protein is also known to be associated with Ku in the CTCBF complex and possibly plays a role in combination with Ku and other factors in the transcription complex.

8. Ku is reported as a transcription factor that recruits DNA-PK directly to negative regulatory element 1 specific sequence (-394 to -381 in the long terminal repeat of mouse mammary tumour virus MMTV) and represses glucocorticoid-induced MMTV transcription (Giffin et al., 1996).
9. In *Drosophila melanogaster* the Ku-related yolk protein factor 1(YPF 1) binds to a 31 base pair sequence within the translated region of yolk protein-1 (yp-1) and acts as a positive transactivator in the transcription of YPF 1 (Jacoby and Wensink, 1994).
10. In *Chironomous* polytene chromosome the Ku-related antigens were known to be associated with the transcriptionally active loci (Gorab et al., 1996).
11. Ku is involved in negatively regulating the transcription of the calcium-responsive element-bearing human parathyroid hormone gene (Chung et al., 1996).
12. Ku is reported to a bind to U5-responsive element of the human T-cell leukemia virus type 1 long terminal repeat and thereby represses its basal transcription (Okumura et al., 1994).
13. It has been shown that hypermethylation of metallothionein-I-Promoter and its resultant silencing occur in response to overexpression of the large subunit of Ku autoantigen in a rat fibroblast cell line (Majumdar et al., 1999).
14. Ku associates with Ty1 virus-like particles in *Saccharomyces cerevisiae* and potentiates retrotransposition. Specifically by using a galactose-inducible Ty1 system, it has been found that *in vivo* Ty1 retrotransposition rates were substantially reduced in the absence of Ku. These results suggest that Ku might function in the life cycle of retroelements in other systems (Downs and Jackson, 1999).

C. Cell Signaling

Both the subunits of Ku (Ku70 and Ku80) contain sequences that make them hydrophobic in nature and help to insert into the membrane. The Ku70 was detected on the plasma membrane of human cells (Prabhakar et al., 1990), and Ku80 was present at the surface of human T-cells (Dalziel et al., 1992). It could be predicted that the Ku protein might be involved in cell signaling. In fact, Ku is reported as an important component of signal transduction pathways based on its response to different external stimuli, for example, Viral infection, TGF-β treatment and Ca^{2+} and serum deprivation (Fewell and Kuff, 1996).

Ku regulates the DNA-PK activity and the DNA-PK belongs to the phosphatidylinositol-3 kinase superfamily that is reported to play an important role in signal transduction pathway (Hartley et al., 1995). DNA-PK could also interact with other components of the intracellular signal transduction machinery. DNA-PK interacts physically and functionally with the *c-Ab1* protooncogene product that suggested its role in intracellular signalling (Kharbanda et al., 1997). In addition, Ku protein binds to the *p95vav* oncoprotein, which contains motif characteristic of signal transduction proteins (Romero et al., 1996).

IX. CONCLUSION AND FUTURE PROSPECTS

The involvement of Ku protein in various metabolic reactions in the cell might have many implications for general interest. Many essential structural and functional roles of Ku are mainly due to its DNA-binding property. The involvement of Ku in recombination, repair, and transcription sug-

gest that these events are coordinated to each other. Especially the possible link between the repair and recombination function of Ku is an efficient way for the cell to repair the double-stranded breaks induced by ionizing radiation or by radiomimetic chemicals or oxidative radicals or during meiosis, V(D)J recombination, and mating type switching. Whether specialized domains within each subunit perform different functions of Ku still needs to be confirmed. However, how these multifunctions are regulated appears to be a major question facing Ku research as well as much of biology. A great deal of work still needs to address this question. Ku being a regulatory subunit of DNA-PK is also phosphorylated by DNA-PK. The effect of phosphorylation of Ku on its ATPase and DNA helicase or other activities has not been studied yet. The multifunctionality of Ku may be due to its interactions with several different protein factors in the cell. There is a need to study the expression of the Ku and its modification during cell cycle for a better understanding of its role. Furthermore, cloning of the genomic DNA of Ku and the characterization of its promoter and other cis-acting elements will answer the questions about its regulation. It is also required to identify the various Ku-interacting proteins that may give some clues regarding the mechanism behind its involvement in many diverse cellular functions. Additional studies are also needed to ascertain its role in DNA replication.

Further insights are expected from three-dimensional structural studies of the individual subunits and their complexes with the respective ligands such as DNA or RNA. Electron microscopy and other more sophisticated approaches such as mutational analysis, transient expression studies, and *in vivo* crosslinking are likely to reveal the nature of the assembly of Ku with other cell components. It is important to study the possible link of all the main functions of Ku in different cell systems that will help to answer the intriguing question of generation of autoantibodies against Ku in various autoimmune diseases like systemic lupus erythematosus, scleroderma, polymyositis syndrome, and rheumatoid arthritis. As most of the functions of Ku are not directly related to each other, the evolutionary history of this protein might be challenging. The future also lies in understanding the nature and function of Ku in other systems especially in plants and its role in growth and development.

REFERENCES

Allaway, G. P., Vivino, A. A., Kohn, L. D., Notkins, A. L., and Prabhakar, B. S. (1990). Characterization of the 70-kDa component of the human Ku autoantigen expressed in insect cell nuclei using a recombinant baculovirus vector. *Biochem. Biophys. Res. Commun.,* **168**, 747–755.

Anderson, C. W. (1993). DNA damage and the DNA-activated protein kinase. TIBS, **18,** 433–437.

Anderson, C. W. and Carter, T. H. (1996). The DNA-activated protein kinase, DNA-PK. In Jessberger, R. and Lieber, M. R. (Eds.) *Current Topics in Microbiology and Immunology Lymphocyte Neoplasia and DNA Rearrangements in the Immune System.* Springer-Verlag, Berlin, pp. 91–111.

Anderson, C. W. and Lees-Miller, S. P. (1992). The nuclear serine/threonine protein kinase DNA-PK. *Crit. Rev. Eukaryot. Gene Expr.,* **2,** 283–314.

Aparicio, O. M., Billington, B. L., and Gottschling, D. E. (1991). Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. Cerevisiae. Cell,* **66,** 1279–1287.

Araki, R., Fujimori, A., Hamatani, K., Mita, K., Saito, T., Mori, M., Fukumura, R., Morimyo,

M., Muto, M., Itoh, M., Tatsumi, K., and Abe, M. (1997). Nonsense mutation at Tyr 4046 in the DNA-dependent protein kinase catalytic subunit of severe combined immune deficiency mice. *Proc. Natl. Acad. Sci. U.S.A.*, **94,** 2438–2443.

Bannister, A. J., Gottlieb, T. M., Kouzarides, T., and Jackson, S. P. (1993). c-jun is phosphorylated by the DNA-dependent protein kinase in vitro: definition of the minimal kinase recognition motif. *Nucleic Acids Res.*, **21,** 1289–1295.

Barnes, G. and Rio, D. (1997). DNA double-strand-break sensitivity, DNA replication and cell cycle arrest phenotypes of Ku-deficient *Sacchromyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.*, **94,** 867–872.

Beall, E. L., Admon, A., and Rio, D. C. (1994). A *Drosophila* protein homologous to the human p70 Ku autoimmune antigen interacts with

the P. Transposable element inverted repeats. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 12681–12685.

Beall, E. L. and Rio, D. C. (1996). *Drosophila* IRBP/Ku p70 corresponds to the mutagen sensitive mus 309 gene and is involved in P. Element excision in vivo. *Genes Dev.*, **10,** 921–933.

Bianchi, A. and Lange, T. L. (1999). Ku binds telomeric DNA in vitro. *J. Biol. Chem.*, **274,** 21223–21227.

Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M., and Brown, M. (1991). Scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 1394–1397.

Blier, P. R., Griffith, A. J., and Hardin, J. A. (1993). Binding of Ku protein to DNA. Measurement of affinity for ends and demonstration of binding to nicks. *J. Biol. Chem.*, **268,** 7594–7601.

Bliss, T. M. and Lane, D. P. (1997). Ku selectively transfers between DNA molecules with homologous ends. *J. Biol. Chem.*, **272,** 5765–5773.

Blunt, T., Finnie, N. J., Taccioli, G. E., Smith, G. C. M., Demengeot, J., Gottlieb, T. M., Mizuta, R., Varghese, A. J., Alt, F. W., Jeggo, P. A., and Jackson, S. P. (1995a). Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine SCID mutation. *Cell*, **80,** 813–823.

Blunt, T., Gell, D., Fox, M., Taccioli, G. E., Lehmann, A. R., Jackson, S. P., and Jeggo, P. A. (1996). Identification of a nonsense mutation in the carboxyl terminal region of DNA-dependent protein kinase catalytic subunit in the SCID mouse. *Proc. Natl. Acad. Sci. U.S.A.*, **93,** 10285–10290.

Blunt, T., Taccioli, G. E., Priestley, A., Hafezparast, M., Mc Millan, T., Liu, J., Cole, C. C., White, J., Alt, F. W., Jackson, S. P., Schurr, E., Lehmann, A. R., and Jeggo, P. A. (1995b). A YAC contig encompassing the XRCC5 (Ku 80) DNA repair gene and complementation of defective cells by YAC protoplast fusion. *Genomics*, **30,** 320–328.

Bosma, G. C., Custer, R. P., and Bosma, M. J. (1983). A severe combined immunodeficiency mutation in the mouse. *Nature*, **301,** 527–530.

Boubnov, N. V., Hall, K. T., Wills, Z., Lee, S. E., He, D. M., Benjamin, D. M., Pulaski, C. R., Band, H., Reeves, W., Hendrickson, E. A., and Weaver, D. T. (1995). Complementation of the ionizing radiation sensitivity, DNA end binding and V(D)J recombination defects of double-strand break repair mutants by the p86 Ku autoantigen. *Proc. Natl. Acad. Sci. U.S.A.*, **92,** 890–894.

Boulton, S. J. and Jackson, S. P. (1996a). *Saccharomyces cerevisiae* Ku 70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. *EMBO J.*, **15,** 5093–5103.

Boulton, S. J. and Jackson, S. P. (1996b). Identification of a *Saccharomyces cerevisiae* Ku 80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. *Nucleic Acids Res.,* **24,** 4639–4648.

Boulton, S. J. and Jackson, S. P. (1998). Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.,* **17,** 1819–1828.

Cai, Q. Q., Plet, A., Imbert, J., Lafage-Pochitaloff, M., Cerdan, C., and Blanchard, J. M. (1994). Chromosomal location and expression of the genes coding for Ku p70 and p80 in human cell lines and normal tissues. *Cytogenet. Cell. Genet.* **65,** 221–227.

Calsou, P., Frit, P., Humbert, O., Muller, C., Chen, D. J., and Salles, B. (1999). The DNA -dependent protein kinase catalytic activity regulates DNA end processing by means of Ku entry into DNA. *J. Biol. Chem.,* **274,** 7848–7856.

Cao, Q. P., Pitt, S., Leszyk, J., and Baril, E. F. (1994). DNA-dependent ATPase from HeLa cells is related to human Ku autoantigen. *Biochemistry,* **33,** 8548–8557.

Carter, T.H., Kopman, C. R., and James, C. B. L. (1988). DNA-stimulated protein phosphorylation in HeLa whole cell and nuclear extracts. *Biochem. Biophys. Res. Commun.,* **157,** 535–540.

Carter, T., Vancurova, I., Sun, I., Lou, W., and Deleon, S. (1990). A DNA activated protein kinase from HeLa cell nuclei. *Mol. Cell. Biol.,* **10,** 6460–6471.

Cary, R. B., Chen, F. Q., Shen, Z. Y., and Chen, D. J. (1998). A central region of Ku80 mediates interaction with Ku70 in vivo. *Nucleic Acids. Res.,* **26,** 974–979.

Cary, R. B., Peterson, S. R., Wang, J. T., Bear, D. G., Bradbury, E. M., and Chen D. J. (1997). DNA looping by Ku and the DNA-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 4267–4272.

Casellas, R., Nussenzweig, A., Wuerffel, R., Pelanda, R., Reichlin, A., Suh, H., Qin, X-F., Besmer, E., Kenter, A., Rajewsky, K., and Nussenzweig, M. C. (1998). Ku80 is required for immunoglobulin isotype switching. *EMBO J.,* **17,** 2404–2411.

Chan, D. and Lees-Miller, S. (1996). The DNA-dependent protein kinase is inactivated by autophosphorylation of the catalytic subunit.. *J. Biol. Chem.,* **271,** 8936–8941.

Chiu, C. Y., Cary, R. B., Chen, D. J., Peterson, S. R., and Steward, P. L. (1998). Cryo E M imaging of the catalytic subunit of the DNA-dependent protein kinase. *J. Mol. Biol.,* **284,** 1075–1081.

Chou, C. H., Wang, J., Knuth, M. W., and Reeves, W. H. (1992). Role of a major autoepitope in forming the DNA binding site of the p70 (Ku) antigen. *J. Exp. Med.,* **175,** 1677–1684.

Chu, G. (1996). Role of Ku autoantigen in V(D)J recombination and double-strand break repair. **In:** Jessberer, R. and Lieber, M., Eds., *Molecular Analysis of DNA Rearrangements in the Immune System,* Springer-Verlag, Berlin. pp. 113–132.

Chung, U., Igarishi, T., Nishishita, T., Iwanari, H., Iwamatsu, A., Suwa, A., Mimori, T., Hata, K., Ebisu, S., Ogata, E., Fujita, T., and Okazaki, T. (1996). The interaction between Ku antigen and REF1 protein mediates negative gene regulation by extra cellular calcium. *J. Biol. Chem.,* **271,** 8593–8598.

Cortes, P., Weis-Garcia, F., Misulovin, Z., Nussenzweig, A., Lai, J-S., Li, G., Nussenzweig, M. C., and Baltimore, D. (1996). In vitro V(D)J recombination: signal joint formation. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 14008–14013.

Dalziel, R. G., Mendelson, S. C., and Quinn, J. P. (1992). The nuclear autoimmune antigen Ku is also present on the cell surface. *Autoimmunity,* **13,** 265–267.

Danska, J. S., Holland, D. P., Mariathasan, S., Williams, K. M., and Guidos, C. J. (1996).

Biochemical and genetic defects in the DNA-dependent protein kinase in murine SCID lymphocytes. *Mol. Cell. Biol.,* **16,** 5507–5517.

de Vries, E., Driel, W. V., Bergsma, W. G., Arnberg, A. C., and van der Vliet, P. C. (1989). HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA multimeric protein complex. *J. Mol. Biol.,* **208,** 65–78.

Di Croce, P. A. and Krontiris, T. G. (1995). The BCL2 major breakpoint region is a sequence and cell-cycle-specific binding site of the Ku antigen. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 10137–10141.

Dionne, I. and Wellinger, R. J. (1996). Cell cycle-regulated generation of single-stranded G-rich DNA in the absence of telomerase. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 13902–13907.

Downs, J. A. and Jackson, S. P. (1999). Involvement of DNA end-binding protein Ku in Ty element retrotransposition. *Mol. Cell. Biol.,* **19,** 6260–6268.

Dvir, A., Peterson, S. R., Knuth, M. W., Lu H., and Dynan, W. S. (1992). Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. *Proc. Natl. Acad. Sci. U.S.A.,* **89**, 11920–11924.

Dvir, A., Stein, L. Y., Calore, B. L., and Dynan, W. S. (1993). Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. *J. Biol. Chem.,* **268,** 10440–10447.

Dynan, W. S. and Yoo, S. (1998). Interaction of Ku protein and DNA-dependent protein kinase catalytic subunit with nucleic acids. *Nucleic Acids Res.,* **26,** 1551–5559.

Errami, A., Finnie, N. J., Morolli, B., Jackson, S. P., Lohmann, P. H. M., and Zdzienicka, M. Z. (1998a). Molecular and biochemical characterization of new X-ray-sensitive hamster cell mutants defective in Ku80. *Nucleic Acids Res.,* **26,** 4332–4338.

Errami, A., He, D. M., Friedl, A. A., Okerkamp, W. J. I., Morolli, B., Hendrickson, E. A., EckardtSchupp, F., Oshimura, M., Lohman, P. H. M., Jackson, S. P., and Zdzienicka, M. Z. (1998b). XRC1 a new CHO cell mutant which is defective in DNA PKcs is impaired in both V(D)J coding and signal joint formation. *Nucleic Acids Res.,* **26,** 3146–3153.

Errami, A., Smider, V., Rathmell, W. K., He, D. M., Hendrickson, E. A., Zdzienicka, M. Z., and Chu, G. (1996). Ku86 defines the genetic defect and restores X-ray resistence and V(D)J recombination to complementation group 5 hamster cell mutants. *Mol. Cell. Biol.,* **16,** 1519–1526.

Falzon, M., Fewell, J. W., and Kuff, E. L. (1993). EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single to double-strand transitions in DNA. *J. Biol. Chem.,* **268,** 10546–10552.

Falzon, M. and Kuff, E. L. (1989). Isolation and characterization of a protein fraction that binds to enhancer core sequences in intracisternal A particle long terminal repeats. *J. Biol. Chem.,* **264,** 21915–21922.

Feldmann, H., Driller, L., Meier, B., Mages, G., Kellermann, J., and Winnacker, E. L. (1996). Hdf2, the second subunit of the Ku homolog from *Saccharomyces cerevisiae*. *J. Biol. Chem.,* **271,** 27765–27769.

Feldmann, H. and Winnacker, E. L. (1993). A putative homologue of the human autoantigen Ku from *Saccharomyces cerevisiae*. *J. Biol. Chem.,* **268,** 12895–12900.

Fewell, J. W. and Kuff, E. L. (1996). Intracellular redistribution of Ku immunoreactivity in response to cell-cell contact and growth modulating components in the medium. *J. Cell. Sci.,* **109,** 1937–1946.

Finnie, N. J., Gottlieb, T.M., Blunt, T., Jeggo, P. A., and Jackson, S. P. (1995). DNA-dependent protein-kinase activity is absent in xrs-6 cells

implications for site-specific recombination and DNA double-strand break repair. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 320–324.

Francoeur, A-M., Peebles, C. L., Gompper, P. T., and Tan, E. M. (1986). Identification of Ki (Ku, p70/p80) autoantigens and analysis of anti-Ki autoantibody reactivity. *J. Immunol.,* **136,** 1648–1653.

Fukumara, R., Araki, R., Fujimori, A., Mori, M., Saito, T., Watanabe, F., Sarashi, M., Itsukaichi, H., Eguchikasai, K., Sato, K., Tatsumi, K., and Abe, M. (1998). Murine cell line SX9 bearing a mutation in the DNA PKcs gene exhibits aberrant V(D)J recombination not only in the coding joint but also in the signal joint. *J. Biol. Chem.,* **273,** 13058–13064.

Fulop, G. M. and Phillips, R. A. (1990). The scid mutation in mice causes a general defect in DNA repair. *Nature,* **347,** 479–482.

Galande, S. and Kohwi-Shigematsu, T. (1999). Poly (ADP-ribose) polymerase and Ku autoantigen form a complex and synergistically bind to matrix attachment sequences. *J. Biol. Chem.,* **274,** 20521–20528.

Genersch, E., Eckerskorm, C., Lottspeich, F., Herzog, C., Kuhn, K., and Posehl, E. (1995). Purification of the sequence-specific transcription factor CTCBF involved in the control of human collagen IV genes: subunits with homology to Ku antigen. *EMBO J.,* **14,** 791–800.

Getts, R. C. and Stamato, T. D. (1994). Absence of a Ku like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant. *J. Biol. Chem.,* **269,** 15981–15984.

Giffin, W., Gong, W., Schild-Poulter C., and Hache, R. J. G. (1999). Ku antigen-DNA conformation determines the activation of DNA-dependent protein kinase and DNA sequence-directed repression of mouse mammary tumor virus transcription. *Mol. Cell. Biol.,* **19,** 4065–4078.

Giffin, W., Kwast-Welfeld, J., Rodda, D. J. Prefontaire, G. G., Traykova-Andonova, M., Zhang, Y., Weigel, N. L., Lefebvre, Y. A., and Hache, R. J. (1997). Sequence-specific DNA binding and transcription factor phosphorylation by Ku autoantigen/DNA-dependent protein kinase. Phosphorylation of Ser 527 of the rat glucocorticoid receptor. *J. Biol. Chem.,* **272,** 5647–5658.

Giffin, W., Torrance, H., Rodda, D. J., Prefontaine, G. G., Pope, L., and Hache, R. J. G. (1996). Sequence-specific DNA binding by Ku autoantigen and its effects on transcription. *Nature,* **380,** 265–268.

Gorab, E., Botella, L. M., Quinn, J. P., Amabis, J. M., and Diez, J. L. (1996). Ku-related antigens are associated with transcriptionally active loci in *Chironomus polytene* chromosomes. *Chromosoma,* **105,** 150–157.

Gottlich, B., Reichenberger, S., Feldmann, E., and Pfeiffer, P. (1998). Rejoining of DNA double-strand breaks in vitro by single-strand annealing. *Eur. J. Biochem.,* **258,** 387–395.

Gottlieb, T. M. and Jackson, S. P. (1993). The DNA dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell,* **72,** 131–142.

Gottschling, D. E., Aparicio, O. M., Billington, B. L., and Zakian, V. A. (1990). Position effect at *S. cerevisiae* telomeres: reversible repression of pol II transcription. *Cell,* **63,** 751–762.

Gravel, S., Larrivee, M., Labrecque, P., and Wellinger, R. J. (1998). Yeast Ku as a regulator of chromosomal DNA end structure. *Science,* **280,** 741–744.

Grawunder, U., Finnie, N., Jackson, S. P., Riwar, B., and Jessberger, R. (1996). Expression of DNA-dependent protein kinase holoenzyme upon induction of lymphocyte differentiation and V(D)J recombination. *Eur. J. Biochem.,* **241,** 931–940.

Greider, C. W. (1996). Telomere length regulation. *Ann. Rev. Biochem.,* **65,** 337–365.

Griffith, A. J., Blier, P. R., Mimori, T., and Hardin, J. A. (1992). Ku polypeptides synthesized in vitro assemble into complexes which recognize ends of double-stranded DNA. *J. Biol. Chem.,* **267,** 331–338.

Grunstein, M. (1997). Molecular model for telomeric heterochromatin in yeast. *Curr. Opin. Cell Biol.,* **9,** 383–387.

Gu, Y. S., Jin, S. F., Gao, Y. J., Weaver, D. T., and Alt, F. W. (1997a). Ku 70-deficient embryonic stem cells have increased ionizing radiosensitivity, defective DNA end binding activity and inability to support V(D)J recombination. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 8076–8081.

Gu, Y. S., Seidl, K. J., Rathbun, G. A., Zhu, C. M., Manis, J. P., Vanderstoep, N., Davidson, L., Cheng, H. L., Sekiguchi, J. M., Frank, K., Stanhopebaker, P., Schlissel, M. S., Roth, D. B., and Alt, F. W. (1997b). Growth retardation and leaky SCID phenotype of Ku 70-deficient mice. *Immunity,* **7,** 653–665.

Haber, J. E. (1999). Sir-Ku-itous routes to make ends meet. *Cell,* **97,** 829–832.

Hafezparast, M., Kaur, G. P., Zdzienicka, M., Athwal, R. S., Lemann, A. R., and Jeggo, P. A. (1993). Subchromosomal localization of a gene (XRCC5) involved in double-strand break repair to the region 2q 34-36. *Som. Cell. Mol. Genet.,* **19,** 413–421.

Hammarsten, O. and Chu, G. (1998). DNA dependent protein kinase : DNA binding and activation in the absence of Ku. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 525–530.

Hartley, K. O., Gell, D., Smith, G. C. M., Zhang, H., Divecha, N., Connelly, M. A., Admon, A., Lees-Miller, S. P., Anderson, C. W., and Jackson, S. P. (1995). DNA-dependent protein kinase catalytic subunit a relative of phosphatidyl inositol 3-kinase and the ataxia telangiectasia gene product. *Cell,* **82,** 849–856.

He, D. M., Lee S. E., and Hendrickson, E. A. (1996). Restoration of X-ray and ectoposide resistance, Ku-end binding activity and V(D)J recombination to the Chinese hamster sxi-3 mutant by a hamster Ku86 cDNA. *Mutat. Res.,* **363,** 43–56.

Higashiura, M., Shimizu, Y., Tanimoto, M., Morita, T., and Yagura, T. (1992). Immunolocalization of Ku-proteins (p80/p70): localization of p70 to nucleoli and periphery of both interphase nuclei and metaphase chromosomes. *Exp. Cell. Res.,* **201,** 444–451.

Higashiura, M., Takasuga, Y., Yamashita, J., and Yagura, T. (1993). A protein homologous to human Ku p70-protein is required for reconstitution of *Xenopus* sperm pronuclei. *Chromosome Res.,* **1,** 27–36.

Hoff, C. M. and Jacob, S. T. (1993). Characterization of the factor E1BF from a rat hepatoma that modulates ribosomal RNA gene transcription and its relationship to the human Ku autoantigen. *Biochem. Biophys. Res. Commun.,* **190,** 747–753.

Huang, J. R., Nueda, A., Yoo, S., and Dynan, W. S. (1997). Heat shock transcription factor 1 binds selectively in vitro to Ku protein and the catalytic sub unit of the DNA-dependent protein kinase. *J. Biol. Chem.,* **272,** 26009–26016.

Izzard, R. A., Jackson, S. P., and Smith, G. C. M. (1999). Competitive and non-competitive inhibition of the DNA-dependent protein kinase. *Cancer Res.,* **59,** 2581–2586.

Jacoby, D. B. and Wensink, P. C. (1994). Yolk protein factor 1 is a *Drosophila* homolog of Ku, the DNA-binding subunit of a DNA-dependent protein kinase from humans. *J. Biol. Chem.,* **269,** 11484–11491.

Jacoby, D. B. and Wensink, P. C. (1996). DNA binding specificities of YPFI, a *Drosophila* homolog to the DNA binding subunit of human DNA-dependent protein kinase, Ku. *J. Biol. Chem.,* **271,** 16827–16832.

Jeggo, P. A., Taccioli, G. E., and Jackson, S. P. (1995). Menage a trois: double strand break repair, V(D)J recombination and DNA-PK. *BioEssays,* **17,** 949–957.

Jin, S., Inone, S., and Weaver, D. T. (1997). Functions of the DNA-dependent protein kinase. *Cancer Surveys,* **29,** 221–261.

Jin, S. F., Kharbanda, S., Mayer, B., Kufe, D., and Weaver, D. T. (1997). Binding of Ku and c-Ab1 at the kinase homology region of DNA-dependent protein kinase catalytic sub unit. *J. Biol. Chem.,* **272,** 24763–24766.

Jin, S. F. and Weaver, D. T. (1997). Double-strand break repair by Ku70 requires heterodimerization with Ku80 and DNA binding functions. *EMBO J.,* **16,** 6874–6885.

Kaczmarski, W. and Khan, S. A. (1993). Lupus auto-antigen Ku protein binds HIV-1 TAR RNA in vitro. *Biochem. Biophys. Res. Commun.,* **196,** 935–942.

Kharbanda, S., Pandey, P., Jin, S. F., Inoue, S., Bharti, A., Yuan, Z. M., Weichselbaum, R., Weaver, D., and Kufe, D. (1997). Functional interaction between DNA-PK and cAb1 in response to DNA damage. *Nature,* **386,** 732–735.

Kim, S. H., Kim, D., Han, J. S., Jeong, C. S., Chung, B. S., Kang, C. D., and Li, G. C. Ku autoantigen affects the susceptibility to anticancer drugs. *Cancer Res.,* **59,** 4012–4017.

Kim, D., Ouyang, H., Yang, S. H., Nussenzweig, A., Burgman, P., and Li, G. C. (1995). A constitutive heat shock element-binding factor is immunologically identical to the Ku autoantigen. *J. Biol. Chem.,* **270,** 15277–15284.

Kirchgessner, C. U., Patil, C. K., Evans, J. W., Cuomo, C. A., Fried, L. M., Carter, T., Oettinger, M. A., and Brown, J. M. (1995). DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science,* **267,** 1178–1183.

Knuth, M. W., Gunderson, S. I., Thompson, N. E., Strasheim, L. A., and Burgess, R. R. (1990). Purification and characterization of proximal sequence element-binding protein I, a transcription activating protein related to Ku and TREF that binds the proximal sequence element of the human U1 promoter. *J. Biol. Chem.,* **265,** 17911–17920.

Koike, M., Ikuta, T., Miyasaka, T., and Shiomi, T. (1999). The nuclear localization signal of the human Ku70 is a variant bipartite type recognized by the two components of nuclear pore-targeting complex. *Exp. Cell Res.,* **250,** 401–413.

Kooistra, R., Pastink, A., Zonneveld, J. B., Lohman, P. H., and Eeken, J. C. (1999). The *Drosophila melanogaster* DmRAD54 gene plays a crucial role in double-strand break repair after P-element excision and acts synergistically with Ku70 in the repair of X-ray damage. *Mol. Cell. Biol.,* **19,** 6269–6275.

Kornberg, A. and Baker, T. A. (1991). DNA Replication, 2nd edn, W.H. Freeman and Co., New York.

Kuhn, A., Gottleib, T. M., Jackson, S. P., and Grummt, I. (1995). DNA-dependent protein kinase: a potent inhibitor of transcription by RNA polymerase I. *Genes Dev.,* **9,** 193–203.

Kuhn, A., Stefanovsky, V., and Grummt, I. (1993). The nucleolar transcription activator UBF relieves Ku antigen-mediated repression of mouse ribosomal gene transcription. *Nucleic Acids Res.,* **21,** 2057–2063.

Kulesza, P. and Lieber, M. R. (1998). DNA-PK is essential only for coding joint formation in V(D)J recombination. *Nucleic Acids Res.,* **26,** 3944–3948.

Kumar, S., Pandey, P., Bharti, A., Jin, S. F., Weichselbaum, R., Weavers, D., Kufe, D., and Kharbanda, S. (1998). Regulation of DNA-dependent protein kinase by the Lyn tyrosine kinase. *J. Biol. Chem.,* **273,** 25654–25658.

Labhart, P. (1997). mRNA encoding the catalytic subunit of DNA-dependent protein kinase is widely expressed in *Xenopus* cells. *Gene,* **203,** 235–240.

Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science,* **240,** 1759–1764.

Laroche, T., Martin, S. G., Gotta, M., Gorhan, H. C., Pryde, F. E., Louis, E. J., and Gasser, S. M. (1998). Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. *Curr. Biol.,* **8,** 653–656.

Lee, S. E., He, D. M., and Hendrickson, E. A. (1996). Characterization of Chinese hamster cell lines that are X-ray sensitive, impaired in DNA double-strand break repair and defective for V(D)J recombination. In Jessberger, R. and Lieber, M. R. (Eds.) *Current Topics in Microbiology and Immunology, Lymphocyte Neoplasia and DNA Rearrangements in the Immune System,* Springer-Verlag, Berlin, pp. 133–142.

Lees-Miller, S. P., Chen, Y-R., and Anderson, C. W. (1990). Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53 and the human Ku autoantigen. *Mol Cell. Biol.,* **10,** 6472–6481.

Lees-Miller, S. P., Godbout, R., Chan, D. W., Weinfeld, M., Day, R. S., Barron, G. M., and Allalunisturner, J. (1995). Absence of p350 subunit of DNA-activated protein kinase from a radio-sensitive human cell line. *Science,* **267,** 1183–1185.

Leuther, K. K., Hammarstan, O., Kornberg, R. D., and Chu, G. (1999). Structure of DNA-dependent protein kinase: implications for its regulation by DNA. *EMBO J.,* **18,** 1114–1123.

Li, G. C., Yang, S-H., Kim, D., Nussenzweig, A., Ouyang, H., Wei, J., Burgman, P., and Li, L. (1995). Suppression of heat-induced hsp70 expression by the 70-kDa subunit of the human Ku autoantigen. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 4512–4516.

Li, L. L. and Yea, N. H. (1992). Cell cycle-dependent migration of the DNA-binding protein Ku80 into nucleoli. *Exp. Cell. Res.,* **199,** 262–268.

Li, Z., Otevrel, T., Gao, Y., Cheng, H. L., Seed, B., Stamato, T. D., Taccioli, G. E., and Alt, F. W. (1995). The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. *Cell,* **83,** 1079–1089.

Liang, F. and Jasin, M. (1996). Ku80-deficient cells exhibit excess degradation of extrachromosomal DNA. *J. Biol. Chem.,* **271,** 14405–14411.

Lieber, M. R., Hesse, J. E., Lewis, S., Bosma, G. C., Rosenberg, N., Mizuuchi, K., Bosma, M.J., and Gellert, M. (1988). The defect in murine severe combined immune deficiency: joining of signal sequences but not coding segments in V(D)J recombination. *Cell,* **55,** 7–16.

Liu, S. H., Ma, J. T., Yueh, A. Y., Lees-Miller, S. P., Anderson, C. W., and Ng, S. Y. (1993). The carboxyl-terminal transactivation domain of human serum response factor contains DNA-activated protein kinase phosphorylation site. *J. Biol. Chem.,* **268,** 21147–21154.

Lorenz, M. and Radbruch, A. (1996). Developmental and molecular regulation of immunoglobulin class switch recombination. *Curr. Top. Microbiol. Immunol.,* **217,** 151–169.

Mages, G. J., Feldmann, H. M., and Winnacker, E. L. (1996). Involvement of the *Saccharomyces cerevisiae hdf1* gene in DNA double-strand break repair and recombination. *J. Biol. Chem.,* **271,** 7910–7915.

Majumdar, S., Ghoshal, K., Li, Z., and Jacob, S. T. (1999). Hypermethylation of metallothionein-I promoter and suppression of its induction in cell lines overexpressing the large subunit of Ku protein. *J. Biol. Chem.,* **274,** 28584–28589.

Makarov, V., Hirose, Y., and Langmore, J. P. (1997). Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell,* **88,** 657–666.

Malynn, B. A., Blackwell, T. K., Fulop, G. M., Rathbun, G. A., Furley, A.J.W., Ferrier, P.,

Heinke, L. B., Phillips, R. A., Yancopoulus, G. D., and Alt, F. W. (1988). The scid defect affects the final step of the immunoglobulin V(D)J recombinase mechanism. *Cell,* **54,** 453–460.

Manis, J. P., Gu, Y. S., Lansford, R., Sonoda, E., Ferrini, A. Davidson, L., Rajewsky, K., and Alt, F. W. (1998). Ku 70 is required for late B cell development and immunoglobulin heavy chain class switching. *J. Exp. Med.*, **187,** 2081–2089.

Martin, S. G., Laroche, T., Suka, N., Grunstein, M., and Gasser, S. M. (1999). Relocalization of telomeric Ku and SIR protein in response to DNA strand breaks in yeast. *Cell,* **97,** 621–633.

May, G., Sutton, C., and Gould, H. (1991). Purification and characterization of Ku-2, an octamer-binding protein related to the autoantigen Ku. *J. Biol. Chem.,* **266,** 3052–3059.

McElligott, R. and Wellinger, R. J. (1997). The terminal DNA structure of mammalian chromosomes. *EMBO J.,* **16,** 3705–3714.

Messier, H., Fuller, T., Mangal, S., Brickner, H., Igarashi, S., Gaikwad, J., Fotedar, R., and Fotedar, A. (1993). P70 lupus autoantigen binds the enhancer of the T cell receptor beta-chain gene. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 2685–2689.

Mills, K. D., Sinclair, D., and Guarentc, L. (1999). MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell,* **97,** 609–620.

Milne, G. T., Jin, S., Shannon, K., and Weaver, D. T. (1996). Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. *Mol. Cell . Biol.,* **16,** 4189–4198.

Mimori, T., Akizuki, M., Yamagata, H., Irada, S., Yoshida, S., and Homma, M. (1981). Characterization of a high molecular weight acidic nuclear protein recognised by autoantibodies from patients with polymyositis-schleroderma overlap. *J. Clin. Invest.,* **68,** 611–620.

Mimori, T. and Hardin, J. A. (1986). Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem.,* **261,** 10375–10379.

Mimori, T., Hardin, J. A., and Steitz, J. A. (1986). Characterization of the DNA-binding protein antigen Ku recognised by autoantibodies from patients with rheumatic disorders. *J. Biol. Chem.,* **261,** 2274–2278.

Mitsis, P. G. and Wensink, P. C. (1989). Purification and properties of yolk protein factor I, a sequence-specific DNA-binding protein from *Drosophila melanogaster*. *J. Biol. Chem.,* **264,** 5188–5194.

Morozov, V., Falzon, M., Anderson, C., and Kuff, E. (1994). DNA-dependent protein kinase is activated by nicks and larger single-stranded gaps. *J. Biol. Chem.,* **269,** 16684–16688.

Munoz, P., Zdzienicka, M. Z., Blanchard, J. M., and Piette, J. (1998). Hypersensitivity of Ku-deficient cells toward the DNA topoisomerase II inhibitor ICRF-193 suggests a novel role for Ku antigen during the G2 and M phases of the cell cycle. *Mol. Cell. Biol.,* **18,** 5797–5808.

Myung, K., Braastad, C., He, D. M., and Hendrickson, E. A. (1998). KARP 1 is induced by DNA damage in a p53 and ataxia telangiectasia mutated dependent fashion. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 7664–7669.

Myung, K., He, D. M., Lee, S. E., and Hendrickson, E. A. (1997). KARP 1: a novel leucine zipper protein expressed from the Ku86 autoantigen locus is implicated in the control of DNA-dependent protein kinase activity. *EMBO J.,* **16**, 3172–3184.

Niu, H. F. and Jacob, S. T. (1994). Enhancer 1 binding factor (E1BF), a Ku-related protein is a growth-regulated RNA polymerase I transcription factor: association of a repressor activity with purified E1BF from serum

deprived cells. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 9101–9105.

Nugent, C. I., Bosco, G., Ross, L. O., Evans, S. K., Salinger, A. P., Moore, J. K., Haber, J. E., and Lundblad, V. (1998). Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr. Biol.*, **8,** 657–660.

Nussenzweig, A., Chen, C., daCosta Soares, V., Sanehez, M., Nussenzweig, M., and Li, G. (1996). Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature*, **382,** 551–555.

Nussenzweig, A., Sokol, K., Burgman, P., Li, L., and Li, G. C. (1997). Hypersensitivity of Ku80-deficient cell lines and mice to DNA damage: the effects of ionizing radiation on growth, survival and development. *Proc. Natl. Acad. Sci. U.S.A.*, **94,** 13588–13593.

Ochem, A. E., Skopac, D., Costa, M., Rabillovd, T., Vuillard, L., Simoncsits, A., Giacca, M., and Falaschi, A. (1997). Functional properties of the separate subunits of human DNA helicase II/Ku autoantigen. *J. Biol. Chem.*, **272,** 29919–29926.

Oderwald, H., Hughes, M. J., and Jost, J. P. (1996). Non-histone protein 1 (NHP1) is a member of the Ku protein family which is upregulated in differentiating mouse myoblasts and human promyelocytes. *FEBS Lett.*, **382,** 313–318.

Okumura, K., Sakaguchi, G., Takagi, S., Naito, K., Mimori, T., and Igarashi, H. (1996). Sp1 family proteins recognize the U5 repressive element of the long terminal repeat of human T cell leukemia virus type I through binding to the CACCC core motif. *J. Biol. Chem.*, **271,** 12944–12950.

Okumura, K., Takagi, S., Sakaguchi, G., Naito, K., Minoura-Tada, N., Kobayashi, H., Mimori, T., Hinuma, Y., and Igarashi, H. (1994). Autoantigen Ku protein is involved in DNA binding proteins which recognise the U5 repressive element of human T-cell leukemia virus type 1 long terminal repeat. *FEBS Lett.*, **356,** 94–100.

Ono, M., Tucker, P. W., and Capra, J. D. (1994). Production and characterization of recombinant human Ku antigen. *Nucleic Acids Res.*, **22,** 3918–3924.

Osipovich, O., Duhe, R. J., Hasty, P., Durum, S. K., and Muegge, K. (1999). Defining functional domains of Ku80: DNA end binding and survival after radiation. Biochem. Biophys. Res. Commun., **261,** 802–807.

Osipovich, O., Durum, S. K., and Muegge, K. (1997). Defining the minimal domain of Ku80 for interaction with Ku70. *J. Biol. Chem.*, **272,** 27259–27265.

Ouyang, H., Nussenzweig, A., Kurimasa, A., Soares, V. D., Li, X. L., Cordon-Cardo, C., Li, W. H., Cheorg, N., Nussenzweig, M., Iliakis, G., Chen, D. J., and Li, G. C. (1997). Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination in vivo. *J. Exp. Med.*, **186,** 921–929.

Paillard, S. and Strauss, F. (1991). Analysis of the mechanism of interaction of simian Ku protein with DNA. *Nucleic Acids Res.*, **19,** 5619–5624.

Pan, Z.-Q., Amin, A. A., Gibbs, E., Niu, H., and Hurwitz, J. (1994). Phosphorylation of the p34 subunit of human single-stranded-DNA-binding protein in cyclin A-activated G1 extracts is catalyzed by cdk-cyclin A complex and DNA-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 8343–8347.

Pang, D., Yoo, S., Dynan, W. S., Jung, M., and Dritschilo, A. (1997). Ku proteins join DNA fragments as shown by atomic force microscopy. *Cancer Res.*, **57,** 1412–1415.

Pelenda, R., Schaal, S., Torres, R. M., and Rajewsky, K. (1996). A prematurely expressed Ig (kappa) transgene but not V (kappa) J (kappa) gene segment targeted into the Ig (kappa) locus, can rescue B cell development in lambda5-deficient mice. *Immunity*, **5,** 229–239.

Pergola, F., Zdzienicka, M. Z., and Lieber, M. R. (1993) V(D)J recombination in mammalian

cell mutants defective in DNA double-strand break repair. *Mol. Cell. Biol.,* **13,** 3464–3471.

Peterson, S. R., Jesch, S. A., Chamberlin, T. N., Dvir, A., Rabindran, S. K., Wu, C., and Dynan, W. S. (1995a). Stimulation of the DNA-dependent protein kinase by RNA polymerase II transcriptional activator proteins. *J. Biol. Chem.,* **270,** 1449–1454.

Peterson, S. R., Kurimasa, A., Oshimuta, M., Dynan, W. S., Bradbury, E. M., and Chen, D. J. (1995b). Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand break repair mutant mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 3171–3174.

Peterson, S. R., Stackhouse, M., Waltman, M. J., Chen, F. Q., Sato, K., and Chen, D. J. (1997). Characterization of two DNA double-stranded break repair-deficient cell lines that express inactive DNA-dependent protein kinase catalytic subunits. *J. Biol. Chem.,* **272,** 10227–10231.

Polotnianka, R. M., Li, J., and Lustig, A. J. (1998). The yeast Ku heterodimer is essential for protection of the telomere against nucleolytic and recombinational activities. *Curr. Biol.,* **8,** 831–834.

Poltoratsky, V. P., Shi, X. Y., York, J. D., Lieber, M. R., and Carter, T. H. (1995). Human DNA-activated protein kinase (DNA-PK) is homologous to phosphatidylinositol kinases. *J. Immunol.,* **155,** 4529–4533.

Porges, A. J., Ng, T., and Reeves, W. T. (1990). Antigenic determinants of the Ku (p70/p80) autoantigen are poorly conserved between species. *J. Immunol.,* **145,** 4222–4228.

Porter, S. E., Greenwell, P. W., Ritchie, K. B., and Petes, T. D. (1996). The DNA-binding protein Hdf1p (a putative Ku homolog) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. *Nucleic Acids Res.,* **24,** 582–585.

Prabhakar, B. S., Allaway, G. P., Srinivasappa, J., and Notkins, A. L. (1990). Cell surface expression of the 70-kDa component of Ku, a DNA-binding nuclear autoantigen. *J. Clin. Invest.,* **86,** 1301–1309.

Priestley, A., Beamish, H. J., Gell, D., Amatucci, A. G., Muhlmann Diaz, M. C., Singleton, B. K., Smith, G. C. M., Blunt, T., Schalkwyk, L. C., Bedford, J. S., Jackson, S. P., Jeggo, P. A., and Taccioli, G. E. (1998). Molecular and biochemical characterization of DNA-dependent protein kinase-defective rodent mutant irs 20. *Nucleic Acids Res.,* **26,** 1965–1973.

Quinn, J. B. and Farira, A. R. (1991). Autoimmune antigen Ku is enriched on oligonucleotide columns distinct from those containing the octamer binding protein DNA consensus sequence. *FEBS Lett.,* **286,** 225–228.

Ramsden, D. A. and Gellert, M. (1995). Formation and resolution of double-strand break intermediates in V(D)J rearrangement. *Genes Dev.,* **9,** 2409–2420.

Ramsden, D. A. and Gellert, M. (1998). Ku protein stimulates DNA end joining by mammalian DNA ligases: a direct role for Ku in repair of DNA double-strand breaks. *EMBO J.,* **17,** 609–614.

Rathmell, W. K. and Chu, G. (1994a). A DNA end-binding factor involved in double-strand break repair and V(D)J recombination. *Mol. Cell. Biol.,* **14,** 4741–4748.

Rathmell, W. K. and Chu, G. (1994b). Involvement of Ku autoantigen in the cellular response to DNA double-strand breaks. *Proc. Natl. Acad. Sci. U.S.A.,* **91**, 7623–7627.

Rathmell, W. K. and Chu, G. (1996). Mechanisms for DNA double-strand break repair in eukaryotes. In *DNA Damage and Repair: Biochemistry, Genetics and Cell Biology* (Nickoloff, J. A., Hoekstra Med) Humana Press.

Reeves, W. H. (1985). Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognised by human autoimmune sera. *J. Exp. Med.,* **161**, 18–39.

Reeves, W. H. (1992). Antibodies to the p70/p80 (Ku) antigens in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.,* **18,** 391–414.

Reeves, W. H. and Sthoeger, Z. M. (1989). Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. *J. Biol. Chem.,* **264,** 5047–5052.

Roberts, M. R., Han, Y., Fienberg, A., Hunihan, L., and Ruddle, F. H. (1994). A DNA-binding activity, TRAC, specific for the TRA element of the transferrin receptor copurifies with the Ku autoantigen. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 6354–6358.

Roberts, M. R., Miskimins, W. K., and Ruddle, F. H. (1989). Nuclear proteins TRE1 and TRE2 bind to the transcriptional control element of the transferrin receptor gene and appear to be associated as a heterodimer. *Cell Regul.,* **1,** 151–164.

Romero, R., Dargemont, C., Pozo, F., Reeves, W. H., Camoris, J., Gisselbrecht, S., and Fischer, S. (1996). P95vav associates with the nuclear protein Ku-70. *Mol. Cell. Biol.,* **16**, 37–44.

Roth, D., Menetski, J., Nakajima, P., Bosma, M., and Gellert, M. (1992). V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. Cell, **70**, 983–991.

Sandell, L. and Zakian, V. (1993). Loss of a yeast telomere: arrest, recovery and chromosome loss. *Cell,* **75,** 729–739.

Shakibai, N., Kumar, V., and Eisenberg, S. (1996). The Ku-like protein from *Saccharomyces cerevisiae* is required *in vitro* for the assembly of a stable multiprotein complex at a eukaryotic origin of replication. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 11569–11574.

Shimizu, A., Takahashi, N., Yaoita, Y., and Honjo, T. (1982). Organization of the constant-region gene family of the mouse immunoglobulin heavy chain. *Cell,* **28,** 499–506.

Shin, E. K., Perryman, L. E., and Meek, K. (1997). A kinase negative mutation of DNA-PKcs in equine SCID results in defective coding and signal joint formation. *J. Immunol.,* **158,** 3565–3589.

Shore, D. (1995). *Telomere Position Effects and Transcriptional Silencing in the Yeast Sacchromyces cerevisiae Telomeres.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shore, D. (1998) Telomeres — unsticky ends. *Science,* **281,** 1818–1819.

Siede, W., Friedl, A. A., Dianova, I., Eckardt-Schupp, F., and Friedberg, E. C. (1996). The *Saccharomyces cerevisiae* Ku autoantigen homolog affects radio sensitivity only in the absence of homologous recombination. *Genetics,* **142,** 91–102.

Singleton, B. K., Priestley, A., Steingrimsdottir, H., Gell, D., Blunt, T., Jackson, S. P., Lehmann, A. R., and Jeggo, P. A. (1997). Molecular and biochemical characterization of xrs mutants defective in Ku80. *Mol. Cell. Biol.,* **17,** 1264–1273.

Singleton, B. K., Torres-Arzayus, M. I., Rottinghaus, S. T., Taccioli, G. E., and Jeggo, P. A. (1999). The C-terminus of Ku80 activates the DNA-dependent protein kinase catalytic subunit. *Mol. Cell. Biol.,* **19,** 3267–3277.

Sipley, J. D., Menninger, J. C., Hartley, K. O., Ward, D. C., Jackson, S. P., and Anderson, C. W. (1995). Gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the XRCC7 gene on chromosome 8. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 7515–7519.

Smider, V. and Chu, G. (1997). The end-joining reaction in V(D)J recombination. *Semin. Imm.,* **9,** 189–197.

Smider, V., Rathmell, W. K., Lieber, M. R., and Chu, G. (1994). Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science,* **266,** 288–291.

Smith, G. C. M., Divecha, N., Lakin, N. D., and Jackson, S. P. (1999). DNA-dependent protein kinase and related proteins. *Biochem. Soc. Symp.,* **64,** 91–104.

Smith, G. C. M. and Jackson, S. P. (1999). The DNA-dependent protein kinase. *Genes Dev.,* **13,** 916–934.

Sonoda, E., Pewzner-Jung, Y., Schwers, S., Taki, S., Jung, S., Eilat, D., and Rajewsky, K. (1997). B cell development under the condition of allelic inclusion. *Immunity,* **6,** 225–233.

Stavnezer, J. (1996). Immunoglobulin class switching. *Curr. Opin. Immunol.,* **8,** 199–205.

Stuiver, M. H., Coenjaerts, F. E. J., and van der Vliet, P. C. (1990). The autoantigen Ku is indistinguishable from NF IV, a protein forming multimeric protein-DNA complexes. *J. Exp. Med.*, **172**, 1049–1054.

Suwa, A., Hirakata, M., Takeda, Y., Jesch, S. A., Mimori, T., and Hardin, J. A. (1994). DNA-dependent protein kinase (Ku protein-p350 complex) assembles on double-stranded DNA. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 6904–6908.

Taccioli, G. E., Gottlieb, T. M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P., and Jeggo, P. A. (1994). Ku80 product of the Xrcc5 gene and its role in DNA repair and V(D)J recombination. *Science,* **265,** 1442–1445.

Taccioli, G., Rathbun, G., Oltz, E., Stamato, T., Jeggo, P., and Alt, F. (1993). Impairment of V(D)J recombination in double-strand break repair mutants. *Science,* **260,** 207–210.

Tanabe, K., Ikegami, Y., Ishida, R., and Andoh, T. (1991). Inhibition of topoisomerase II by antitumor agents *bis*(2,6-dioxopiperazine) derivatives. *Cancer Res.*, **51,** 4903–4908.

Thompson, L. H. and Jeggo, P. A. (1995). Nomenclature of human genes involved in ionizing radiation sensitivity. *Mut. Res. DNA Rep.,* **337,** 131–134.

Toth, E. C., Marusic, L., Ochem, A., Patthy, A., Pongor, S., Giacca, M., and Falaschi, A. (1993). Interactions of USF and Ku antigen with a human DNA region containing a replication origin. *Nucleic Acids Res.,* **21,** 3257–3263.

Tsukamoto, Y., Kato, J., and Ikeda, H. (1997). Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae. Nature,* **388,** 900–903.

Tsukamoto, Y., Kato, J. I., and Ikeda, H. (1996). Hdf1, a yeast Ku-protein homologue, is involved in illegitimate recombination, but not in homologous recombination. *Nucleic Acid Res.,* **24,** 2067–2072.

Turchi, J. J. and Henkels, K. (1996). Human Ku auto-antigen binds Cisplatin-damaged DNA but fails to stimulate DNA-activated protein kinase. *J. Biol. Chem.,* **271,** 13861–13867.

Tuteja, N., Phan, T. N., Tuteja, R., Ochem, A., and Falaschi, A. (1997). Inhibition of DNA unwinding and ATPase activities of human DNA helicase II by chemotherapeutic agents. *Biochem. Biophys. Res. Commun.,* **236,** 636–640.

Tuteja, N., Tuteja, R., Ochem, A., Taneja, P., Huang, N. W. Simoncsits, A., Susic, S., Rahman, K., Marusic, L., Chen, J., Zhang, J., Wang, S., Pongor, S., and Falaschi, A. (1994). Human DNA helicase II — a novel DNA unwinding enzyme identified as the Ku autoantigen. *EMBO J.,* **13,** 4991–5001.

van Steensel, B., Smogorzewska, A., and de Lange, T. (1998). TRF2 protects human telomeres from end-to-end fusions. *Cell,* **92,** 401–413.

Vishwanatha, J. K. and Baril, E. F. (1990). Single-stranded DNA-dependent ATPase from HeLa cells that stimulates DNA polymerase alpha-primase activity: purification and characterization of the ATPase. *Biochemistry,* **29,** 8753–8759.

Wang, J. S., Dong, X. W., Myung, K. J., Hendrickson, E. A., and Reeves, W. H.

(1998). Identification of two domains of the p70 Ku protein mediating dimerization with p80 and DNA binding. *J. Biol. Chem.*, **273,** 842–848.

Watanabe, F., Shirakawa, H., Yoshida, M., Tsukada, K., and Teraoka, H. (1994). Stimulation of DNA-dependent protein kinase activity by high mobility group protein 1 and protein 2. *Biochem. Biophys. Res. Commun.*, **202,** 736–742.

Wellinger, R. J., Ethier, K., Larecque, P., and Zakian, V. A. (1996). Evidence for a new step in telomere maintenance. *Cell*, **85,** 423–433.

West, R. B., Yaneva, M., and Lieber, M. R. (1998). Productive and non-productive complexes of Ku and DNA-dependent protein kinase at DNA termini. *Mol. Cell. Biol.*, **18,** 5908–5920.

Woodgett, J. R. (1993). A kinase with Ku-dos. *Curr. Biol.*, **3,** 449–450.

Wright, W. E., Tesmer, V. M., Huffman, K. E., Levene, S. D., and Shay, J. W. (1997). Normal human chromosomes have long G-rich telomeric overhangs at one end. *Genes Dev.*, **11,** 2801–2809.

Wu, J. M., Chen, Y., An, S., Perruccio, L., Abdel-Ghang, M., and Carter, T. H. (1993). Phosphorylation of protein tau by double-stranded DNA-dependent protein kinase. *Biochem. Biophys. Res. Commun.*, **193,** 13–18.

Wu, X. T. and Lieber, M. R. (1996). Protein-protein and protein-DNA interaction regions within the DNA end-binding protein Ku 70/Ku86. *Mol. Cell. Biol.*, **16,** 5186–5193.

Yanewa, M. and Jhiang, S. (1991). Expression of the Ku protein during cell proliferation. *Biochem. Biophys. Acta.*, **1090,** 181–187.

Yaneva, M., Kowalewski, T., and Lieber, M. R. (1997). Interaction of DNA-dependent protein kinase with DNA and with Ku: biochemical and atomic force microscopy studies. *EMBO J.*, **16,** 5098–5112.

Yaneva, M., Wen, J., Ayala, A., and Cook, R. (1989). cDNA derived amino acid sequence of the 86-kDa subunit of the Ku antigen. *J. Biol. Chem.*, **264,** 13407–13411.

Yavuzer, U., Smith, G. C. M., Bliss, T., Werner, D., and Jackson, S. P. (1998). DNA end-independent activation of DNA-PK mediated via association with the DNA-binding protein C1D. *Genes Dev.*, **12,** 2188–2199.

Yoo, S. and Dynan, W. S. (1998). Characterization of the RNA binding properties of Ku protein. *Biochemistry*, **37,** 1336–1343.

Yumoto, Y., Shivakawa H., Yoshida, M., Suwa, A., Watanabe, F., and Teraoka, H. (1998). High mobility group proteins 1 and 2 can function as DNA binding regulatory components for DNA-dependent protein kinase in vitro. *J. Biol. Chem.*, **124,** 519–527.

Zdzienicka, M. Z. (1995). Mammalian mutants defective in the response to ionising radiation-induced DNA damage. *Mut. Res. DNA Rep.*, **336,** 203–213.

Zhang, J. and Jacob, S. T. (1990). Purification and characterization of a novel factor which stimulates rat ribosomal gene transcription *in vitro* by interacting with enhancer and core promoter elements. *Mol. Cell. Biol.*, **10,** 5177–5186.

Zhang, W. W. and Yaneva, M. (1992). On the mechanisms of Ku protein binding to DNA. *Biochem. Biophys Res. Commun.*, **186,** 574–579.

Zhu, C., Bogue, M., Lim, D. S., Hasty, P., and Roth, D. (1996). Ku86 deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. *Cell*, **86,** 379–389.