Identification of Two Domains of the p70 Ku Protein Mediating Dimerization with p80 and DNA Binding

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The Ku autoantigen is a heterodimer of 70 (p70) and ~80 kDa (p80) subunits that is the DNA-binding component of the DNA-dependent protein kinase (DNA-PK) complex involved in DNA repair and V(D)J recombination. Binding to DNA ends is critical to the function of DNA-PK, but how Ku interacts with DNA is not completely understood. To define the role of p70 and p80 and their dimerization in DNA binding, heterodimers were assembled by co-expressing the subunits using recombinant baculoviruses. Two p70 dimerization sites, amino acids 1–115 and 430–482, respectively, were identified. Binding of p70 to linear double-stranded DNA could be demonstrated by an immunoprecipitation assay, and required the C-terminal portion (amino acids 430–609), but not interaction with p80. The p70 mutants 1–600, 1–542, 1–115, and 430–600 did not bind DNA efficiently. However, DNA binding of 1–600, 1–542, and 1–115, but not 430–600, was restored by dimerization with p80, indicating that p70 has two DNA binding sites, each partially overlapping one of the dimerization sites. The C-terminal domain can bind DNA by itself, but the N-terminal domain requires dimerization with p80. These observations could be relevant to the multiple functional activities of Ku and explain controversies regarding the role of dimerization in DNA binding.

EXPERIMENTAL PROCEDURES

Cells and Viruses—K562 (human erythroleukemia, from the American Type Culture Collection, ATCC, Rockville, MD) were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin. The S9 (Spodoptera frugiperda ovary) cell line was obtained from the ATCC and maintained at 27 °C in Grace's insect tissue culture medium supplemented with 3.3 g/liter TC yeastolate, 3.3 g/liter lactalbumin hydrolyzate (University of North Carolina Lineberger Comprehensive Cancer Center Tissue Culture Facility), 10% fetal bovine serum, and penicillin/streptomycin.

Recombinant Baculoviruses—The construction of transfer vectors and recombinant baculoviruses expressing full-length human Ku p70 and p80 has been described (11). Briefly, p70 and p80 Ku cDNAs were subcloned into a modified baculovirus transfer vector pVLB4-mp53 (11) based on pVL1392 (PharMingen, San Diego, CA) and pBlueBacHI (Invitrogen Corp., San Diego, CA). The recombinant vectors express Ku proteins fused to an N-terminal polyhistidine sequence and enterokinase cleavage site. Transfer vectors were co-transfected with linearized wild-type baculovirus (BaculoGold™, PharMingen), into Sf9 cells. Recombinant baculovirus clones were selected by end point dilution assay. Recombinant baculoviruses were identified at m.o.i. of 10. Recombinant baculoviruses expressing mutant p70 proteins were constructed in the same manner. A cDNA sequence encoding p70 amino acids 1–600 (7) was subcloned into the BamHI site of pVL1393 and inserted by homologous recombination into baculovirus. The recombinant baculovirus permits expression of an untagged p70 (amino acids 1–600) protein. The cDNAs for additional p70 mutants were amplified by polymerase chain reaction using primer pairs listed in Table I, and subcloned into pVLB4-mp53 at the BamHI-SmaI or BamHI-EcoRI sites.

Expression of Recombinant Proteins—S9 cells were infected with recombinant baculoviruses at m.o.i. of 10 as described (11). For co-infection, S9 cells were infected with two recombinant baculoviruses each at m.o.i. of 10. 72 h after infection, recombinant p70 or p80 Ku proteins were identified of S9 cell lysates by SDS-PAGE and immunoblotting using human autoimmune serum and specific monoclonal antibodies.

Monoclonal Antibodies—Murine monoclonal antibodies (mAbs) specific for the human Ku antigen were characterized previously (1). Their isotypes and specificities are as follows: 162, IgG2a specific for the p70/p80 dimer (unreactive with free p70 or p80); 111, IgG1 specific for...
human p80 (amino acids 610–705); N3H10, IgG2b specific for p70 (amino acids 506–541). Murine mAbs specific for the polyhistidine tag HIS (lgG2a) and anti-Xpress (lgG1) were obtained from Sigma Chemical Co. (St. Louis, MO) and Invitrogen Corp., respectively.

**Immunoblotting**—Immunoblot analysis of the recombinant p70 and p80 proteins from Sf9 cells or Ku proteins from K562 cells was performed using human autoantibodies or murine mAbs as described (11). Ascitic fluid from mAbs N3H10, S10B1, and 111 or anti-Xpress was formed using human autoantibodies or murine mAbs as described (11). Ascitic fluid from mAbs N3H10, S10B1, and 111 or anti-Xpress was added 1:1000 for probing immunoblots, followed by 1:1500 alkaline phosphatase-conjugated goat anti-mouse IgG antibodies (Tago, Burlingame, CA).

**Immunoprecipitation**—Radioabeled of baculovirus-infected Sf9 cells with [35S]methionine/cysteine and immunoprecipitation was carried out as described (11). Briefly, radiolabeled cells were lysed in NET-Nonidet P-40 buffer (0.15 M NaCl, 50 mM Tris, pH 7.5, 0.3% Nonidet P-40, 2 mM EDTA) with 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5 mM dithiothreitol (DTT). After incubation (10 min at 4 °C), the supernatant was removed, diluted to 0.1 M KCl by adding 50 mM HEPES, pH 7.3, and recovered, diluted to 0.1 M KCl by adding 50 mM HEPES, pH 7.3, and stored at −80 °C.

**Radiolabeled Linear DNA Probe**—Preparation of the DNA probe used for the DNA immunoprecipitation assay and electrophoretic mobility shift assay was described previously (8). Briefly, a 564-base pair linear double-stranded DNA fragment generated by HindIII digestion of bacteriophage A DNA (New England Biolabs, Beverly, MA) was end-labeled with [32P]dATP (3000 Ci/mmol, NEN Life Science Products) using Klenow fragment (Boehringer Mannheim). For gel shift assays, the radiolabeled probe was purified using a Gene-clean III kit from Bio 101 (Vista, CA).

**DNA Immunoprecipitation Assay**—The binding of radiolabeled DNA to affinity purified Ku antigen on protein A-Sepharose beads was measured as described (8) with minor modifications. Cell extract from Sf9 cells infected with recombinant baculoviruses was immunoprecipitated with 5 μl of mAbs 162, N3H10, 111, or anti-Xpress ascites. Approximately equal amounts of different Ku proteins precipitated to the beads, as determined by SDS-PAGE with Coomassie Blue staining in preliminary experiments. Beads were washed with 1.5 M NaCl NET-Nonidet P-40 buffer followed by the same buffer containing 50 mM NaCl instead of 1.5 M NaCl. Radiolabeled DNA probe (25 ng) was added for 1 h, and the beads were washed again with NET-Nonidet P-40 buffer. DNA bound to the purified Ku antigen was recovered by digesting the beads with proteinase K, and an aliquot of the supernatant was used for scintillation counting.

**Gel Mobility Shift Assay**—A gel mobility shift assay for determining Ku DNA end binding activity (13) was used with minor modifications. Radiolabeled DNA was incubated with whole-cell extracts in 20 μl of binding buffer (10 mM Tris, pH 7.5, 1 mM DTT, 5 mM EDTA, 10% glycerol, 150 mM NaCl, 2 μg of closed circular plasmid pX174 RF1 DNA (New England Biolabs as a nonspecific inhibitor) at 22 °C for 30 min. The samples were subjected to electrophoresis in a 4% polyacrylamide gel at 22 °C for 4 h at 100 V. The gel was dried and autoradiographed at −70 °C. For supershift experiments, mAbs (1 μl of ammonium sulfate precipitated ascitic fluid at −1 mg/ml) were added to the binding mixture 5 min after adding probe and incubated for an additional 30 min at 22 °C before electrophoresis.

**RESULTS**

The human p70 and p80 Ku proteins assemble into heterodimers in human cells as well as insect cells infected with recombinant baculoviruses expressing the human Ku subunits (11, 14). The C-terminal portion of p70 protein contains a leucine zipper-like sequence (15), but its role in interactions with p80 is not established. In the present study, dimerization of full-length p80 with p70 deletion mutants in extracts from co-infected S9 cells was investigated using the following criteria: 1) co-immunoprecipitation of p70 by anti-p80 mAbs and vice versa; and 2) immunoprecipitation of both p70 and p80 by mAb 162, which recognizes the p70/p80 heterodimer but not the individual subunits.

**C-terminal Dimerization Domain**—To help define the role of the leucine zipper-like sequence of p70 identified previously (15) in dimerization, deletion mutants were constructed (Fig. 1A) and co-expressed with full-length p80 in Sf9 cells using the baculovirus system. Expression was verified by immunoprecipitation using a polyhistidine-specific mAb (Fig. 1B). To detect heterodimer formation, the p70 mutants were co-immunoprecipitated with anti-p80 MAb 111 (Fig. 1B). In agreement with previous observations (11), full-length p70 was co-immunoprecipitated by mAb 111. Mutant p70 proteins 330–609, 380–609, and 430–609 also were co-immunoprecipitated by mAb 111. In contrast, the mutant proteins 480–609 and 505–609 were not co-immunoprecipitated. These data suggest that a

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**TABLE I**

Primers used to amplify Ku cDNAs for subcloning into baculovirus transfer vectors

| Constructs | 5’ Primer | 3’ Primer |
|------------|-----------|-----------|
| p80.1–728 (full length) | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |
| p70.1–699 (full length) | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |
| p70.2–699 | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |
| p70.3–699 | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |
| p70.4–699 | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |
| p70.6–699 | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' | 5'-GCGGATCCATGTCAGGGTGGGAGTC-3' |

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**Functional Domains of Ku Antigen**

See Table I for primers used to amplify Ku cDNAs for subcloning into baculovirus transfer vectors.
dimerization domain is located on the C-terminal portion of p70 (amino acids 430–609) and that the leucine zipper-like region (amino acids 483–504) is not sufficient for dimerization with p80.

To further localize this domain, a second panel of deletion mutants (Fig. 2A) was co-expressed with p80. As before, protein expression was verified by immunoprecipitating with anti-Xpress (Fig. 2B, Xpress), and dimer formation was demonstrated by co-immunoprecipitating with mAb 111 (Fig. 2B, 111). Although deletion of amino acids 601–609 abrogates the DNA binding activity of p70 in immunoprecipitation and Southern blot assays (7), deleting this sequence had no apparent effect on dimerization (Figs. 3, 4). The calculated sizes of the smallest p70 fragments (amino acids 1–600, 430–600, 1–542, 430–542, 461–542, and 430–482) along with p80 was examined. Positions of full-length recombinant (histidine-tagged) p80 and the p70 deletion mutants are indicated by arrows.

**Antigenic Site Recognized by mAb 162**—The p70/p80 heterodimer is recognized specifically by mAb 162 and protected from dissociation in the presence of 1.5 M NaCl and detergent (16), suggesting that mAb 162 may bind to an antigenic determinant near the p70/p80 dimerization site. The relationship of the epitope recognized by 162 to the dimerization site identified in Figs. 1–2 was investigated by immunoprecipitating p70 deletion mutants co-expressed with full-length p80 (Fig. 3A). The level of recombinant protein expression was verified by anti-Xpress antibody (Fig. 3B). mAb 162 immunoprecipitated full-length p80 when it was co-expressed with p70 mutant 430–609, but not 1–433, localizing the mAb 162 epitope to the C-terminal portion of p70. Further deletions revealed that mAb 162 could immunoprecipitate p80 when it was co-expressed with 430–542 but not with 461–542. mAb 162 did not immunoprecipitate p80 or any of the p70 deletion mutants when expressed alone in SF9 cells (Ref. 16 and data not shown). These data indicate that mAb 162 recognizes an epitope formed by p80 plus amino acids 430–542 of p70. This region overlaps the C-terminal dimerization domain identified in Figs. 1 and 2 as well as a strongly basic sequence adjacent to a leucine zipper-like region.

**Localization of the Dimerization Site by Limited Proteolysis**—To further verify the results obtained using recombinant baculoviruses directing the synthesis of p70 and p80, dimerization was evaluated in human K562 cells. Ku heterodimer was affinity purified onto beads with mAb 162, followed by protease treatment of the beads. mAb 162 recognizes an epitope comprised of p70 plus p80 and stabilizes the dimer under dissociating conditions. Since the binding of antibodies frequently protects proteins from proteolytic cleavage (17, 18), we expected that the dimerization site-associated epitope recognized by 162 might be protected from degradation.

The p70 fragments retained on mAb 162 beads after protease treatment were analyzed by Western blot using mAb N3H10 (Fig. 4). The calculated sizes of the smallest p70 fragments recognized by N3H10 after trypsin or chymotrypsin digestion were 14 and 16 kDa, respectively (Fig. 4, lower arrow in each
Since the N3H10 epitope has been localized to amino acids 529–541 of p70, these studies lend further support to the idea that a dimerization site is located near the C terminus of p70.

Identification of Second Dimerization Domain Near the N Terminus of p70—The identification of a p80 dimerization domain near the C terminus of p70 did not exclude the possibility that additional sequences also could mediate interactions between p70 and p80. To examine that possibility, p70 mutant 1–433 (Fig. 5A) was co-expressed with p80, followed by immunoprecipitation with mAb 111. This fragment dimerized with p80 (Fig. 5B). Mutants 1–214 and 1–115 also were co-immunoprecipitated by mAb 111, but mutants 244–433 and 116–266 were not, suggesting that the second dimerization domain is located on the N-terminal portion of p70 protein, as well as the p70 deletion mutants, or with mAb 162 (specific for the dimer of p70 with p80). Immunoprecipitates were analyzed by SDS-PAGE and autoradiography. Position of full-length p80 is indicated by an arrowhead.

Effects of Dimerization on the Binding of Ku to DNA in Immunoprecipitation Assay—Since it has been reported that p70/p80 dimerization is critical for the DNA binding activity of Ku in gel shift assays (9, 10), but not DNA immunoprecipitation or Southwestern blot assays (5–7), we examined the role of each of the two dimerization sites in the interaction of Ku with DNA. The C-terminal portion of p70 binds readily to linear
dsDNA fragments in a DNA immunoprecipitation assay (7). In agreement with previous observations, recombinant full-length p70 (1–609) bound linear dsDNA comparably with recombinant p70/p80 dimer (Fig. 6B). As shown previously (7), deleting the C-terminal 9 amino acids (mutant 1–600) reduced the DNA binding activity (Fig. 6A). The importance of an intact C terminus was underscored by the fact that p70 mutants 380–609 and 430–609 had DNA binding activity similar to that of wild-type p70/p80 heterodimer (Fig. 6B).

Consistent with this observation, p70 mutants lacking larger portions of the C terminus had low DNA binding activity (Fig. 6B, mutants 1–542, 1–115, and 430–600). Mutants 430–542 and 430–482 also had little binding activity (not shown). Unexpectedly, when p80 was co-expressed with mutant construct 1–542, DNA binding activity was restored to nearly normal, whereas partial restoration was seen with 1–600 and 1–115 (Figs. 6A and B). However, dimerization with p80 had no effect on the DNA end binding activity of p70 mutant 430–600 (Fig. 6B). Consistent with previous observations (5–7), p80 alone did not bind DNA (not shown), indicating that there was little or no dimerization of the recombinant human p80 with endogenous insect p70. Together, these results strongly suggest that, like dimerization, the binding of Ku to DNA in immunoprecipitation assays is mediated by two separate sites. One site, located at the C terminus of p70, does not depend on interactions with p80. The second, N-terminal, site requires dimerization of p80 with the N-terminal dimerization domain of p70.

**Effect of Dimerization on DNA Binding in Gel Mobility Shift Assay**—Different requirements for p80 have been found in gel mobility shift assays (dimerization-dependent) than in immunoprecipitation or Southwestern blot assays (dimerization-independent). To investigate the basis for this discrepancy, binding of recombinant human Ku to DNA was investigated in a gel mobility shift assay (Fig. 7) using the same probe as in the immunoprecipitation assay (Fig. 6).

Consistent with previous findings (9, 10), recombinant Ku heterodimer expressed in Sf9 cells using recombinant baculoviruses bound DNA efficiently, whereas full-length free p70 and p80 both failed to bind DNA in the gel shift assay (Fig. 7A). Interestingly, p70 mutant 1–600 with an intact N-terminal and partially deleted C-terminal DNA binding domain did not bind DNA alone, whereas upon dimerization with p80, DNA binding activity was restored (Fig. 7A). Furthermore, the multi-band pattern visible on the gel (Fig. 7A, 1–600/p80 lane) suggests that dimerization also restores the internal translocation property of Ku (19). A similar, but possibly smaller, effect was seen in the DNA immunoprecipitation assay (Fig. 6A). The p70 mutants 1–542 and 430–609, each carrying one of the two DNA binding domains, did not bind DNA in gel shift assays, even after dimerization with p80 (Fig. 7A). Binding of the recombinant mutant Ku antigens p70/p80 and 1–600/p80 to DNA was verified by supershift assays using anti-Ku mAb 162 and an antibody to the polyhistidine tag of the recombinant
proteins (Fig. 7B). Taken together, the data suggest that Ku DNA binding activity in the gel shift assay requires that both the N- and C-terminal DNA binding domains of p70 be intact. Since p80 is needed for activity of the N-terminal DNA binding site, it is required for binding in the gel shift assay. Finally, the data suggest that dimerization can compensate partially for deletion of a portion (amino acids 601–609) of the C-terminal DNA binding site, even though the intact site binds DNA independently of p80.

**DISCUSSION**

Binding of Ku to DNA is a critical step in the targeting and activation of DNA-dependent protein kinase (1–3). However, the mechanism of DNA binding by Ku has not been defined completely. In this study, two regions of p70 mediating dimerization with p80 were defined, each of which is located in close proximity to one of the two DNA binding sites (Fig. 8). The existence of multiple dimerization and DNA binding sites may explain some of the previous discrepancies regarding the interaction of Ku with DNA. The C-terminal DNA binding domain of p70 corresponds to the minimal domain mapped previously (7), whereas the N-terminal, p80-dependent domain may explain the p80 dependence of DNA binding in gel shift assays (9, 10).

**Mechanism of p70-p80 Dimerization**—One of the main findings of the present study was that p70 has two dimerization sites. One site, localized to amino acids 430–482, is contained within the C-terminal 20-kDa portion of p70, a region implicated previously in subunit interaction using the yeast 2-hybrid system (10). This region does not contain the leucine zipper-like motif near the C-terminal end of p70 (amino acids 483–504), consistent with the previous observation that a leucine zipper-like motif of p80 is not required for interaction with p70 (10). The C-terminal interaction domain of p70 is related to the epitope recognized by mAb 162 (Figs. 3–4), which is specific for the p70/p80 dimer and stabilizes the dimer under dissociating conditions (16). The p70 sequence recognized by mAb 162 (amino acids 430–542) and the dimerization domain mapped using recombinant baculovirus-expressed proteins (amino acids 430–482) correlate well. Limited protease digestion confirmed the importance of this region in dimerization in mammalian cells.

More recently, autoantibodies were identified in autoimmune disease patients’ sera that, like mAb 162, also recognize and stabilize the p70/p80 heterodimer (11). Interestingly, some of these sera appeared to recognize a different epitope than that bound by mAb 162 since they do not compete with mAb 162 for binding to Ku. This raises the possibility that additional subunit interaction domains may be present. The identification of a second dimerization site on amino acids 1–115 of p70 (Fig. 5) is consistent with that possibility. The corresponding dimerization sites of p80 have yet to be defined precisely although the C-terminal 32 kDa of p80 has been reported to exhibit p70 binding activity (10). More precise localization of the p70 interaction sites on p80 is in progress.

The role of the leucine zipper-like motif of p70, if any, remains to be determined. The present studies show conclusively that this region is not required for p70-p80 interactions. Other data suggest that it is not required for interactions of p70 with p96 (20). The catalytic subunit of DNA-dependent protein kinase contains an extensive leucine zipper-like sequence (21) that possibly might interact with the leucine zipper-like motif of p70. Alternatively, this sequence might interact with other proteins or not be of functional significance.

**Mechanism of DNA Binding**—Controversy remains about the role of p80 in DNA binding. Gel shift assays suggest that dimerization of p70 with p80 is required for DNA binding (9, 10). In contrast, DNA immunoprecipitation and Southwestern blot assays suggest that p70 by itself interacts with DNA termini, whereas p80 does not (5–7). The present data may explain this discrepancy. DNA immunoprecipitation assays indicate that two different regions of p70 are involved in DNA binding. One is the previously identified C-terminal DNA binding site (7). This site is carried by p70 mutant 430–609, and its DNA binding activity is unaffected by dimerization with p80 (Fig. 6). However, in agreement with previous gel mobility shift data (9, 10), neither free p70 nor the 430–609 mutant bound DNA in the gel shift assay (Fig. 7). Moreover, dimerization of the 430–609 mutant with p80 did not restore DNA binding activity.

DNA binding activity in the gel shift assay also depended on a second site localized to amino acids 1–542 of p70 (Fig. 6). Unlike the C-terminal DNA binding domain, activity of this N-terminal DNA binding domain required dimerization with p80 via the N-terminal subunit interaction domain (Fig. 6). A shorter fragment (p70 amino acids 1–115) also appeared to have weaker, p80-dependent binding activity. Interestingly, although similar in its requirement for p80 binding, the p70 (1–542)-p80 dimer failed to bind DNA in the gel shift assay (Fig. 7) despite significant binding in the DNA immunoprecipitation assay (Fig. 6). The present studies have not addressed the question of how DNA interacts with this domain. One possibility is that both p70 and p80 participate. Alternatively, the interaction with DNA could be mediated by a conformation of p70 that depends on the binding of p80 or vice versa. Further studies will be needed to address this issue.

We conclude that Ku antigen has at least two domains involved in DNA binding. One of these, the C-terminal portion of p70, does not require interactions with p80. The other forms upon dimerization of p70 (amino acids 1–542) with p80. Neither domain by itself is sufficient for DNA binding activity in gel mobility shift assays, but together they confer activity. Interestingly, although deletion of p70 amino acids 601–609 abrogates p80-independent DNA binding activity of the C-terminal DNA binding site, this activity apparently can be restored by dimerization with full-length p80, since the p70 (1–600)-p80 complex was active in the gel shift assay (Fig. 7).

The discrepancy between the gel mobility shift assay and DNA immunoprecipitation may reflect the ability of the latter
assay to detect lower affinity interactions between Ku and DNA than detected by the gel shift technique. Alternatively, the conditions used for the gel shift assay may induce conformational changes that alter the binding of free p70 to DNA. The DNA binding of both full-length p70 and the C-terminal portion of p70 clearly is conformation-sensitive since a prolonged renaturation step is required after transfer of the denatured proteins to nitrocellulose membranes to demonstrate binding in Southwestern blot assays (6, 7). Finally, it is possible that free p70 is masked by other proteins or nucleic acids in the crude extracts utilized for gel shift assays. In contrast, p70 is affinity purified onto beads in the DNA immunoprecipitation assay, and the high salt pre-washing of the beads prior to DNA binding could release these inhibitory factors.

Functional Significance—The complexity of the interaction between p70 and p80 and between Ku and DNA may be important for the various functions ascribed to Ku antigen. In addition to its role in end binding during DNA double strand break repair and V(D)J recombination (22, 23), Ku antigen has sequence-specific DNA binding activity and may be involved in transcriptional activation (4) or repression (24, 25). The N-terminal acidic domain of p70 identified by sequence analysis (15) has strong transcriptional activation activity (10) although Ku also may repress transcription (24, 25).

It remains unclear whether the portions of Ku mediating end binding activity are the same as those responsible for sequence-specific binding. Previous studies suggest that the C-terminal DNA binding domain of p70 binds preferentially to DNA ends (8). However, we have not yet examined whether the p80-dependent DNA binding site identified here displays a similar preference for ends, nor have we investigated the role of the two DNA binding sites in sequence specific DNA binding to the LTR sequence of mouse mammary tumor virus (4). The answer to these questions may help to further define the mechanisms by which Ku autoantigen functions in DNA repair and transcriptional activation/repression. Ku antigen may be one of a number of multifunctional DNA binding proteins involved in transcription as well as other aspects of nucleic acid metabolism, such as DNA synthesis or repair (26). These distinct functions potentially could be mediated by different DNA binding sites or interaction states of the p70 and p80 subunits.

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REFERENCES
1. Reeves, W. H., Wang, J., Ajmani, A. K., Stojanov, L., and Satoh, M. (1997) in The Antibodies (Zanetti, M., and Capra, J. D., eds.), pp. 33–84, Harwood Academic Publishers, Amsterdam
2. Anderson, C. W. (1993) Trends Biochem. Sci. 18, 433–437
3. Weaver, D. T. (1995) Adv. Immunol. 59, 29–85
4. Griffin, W., Torrance, H., Rodda, D. J., Prefontaine, G. G., Pope, L., and Hache, R. J. G. (1996) Nature 380, 265–268
5. Mimori, T., and Hardin, J. A. (1986) J. Biol. Chem. 261, 10375–10379
6. Allaway, G. P., Vivino, A. A., Kuhn, L. D., Nokine, A. L., and Prabhakar, B. S. (1989) Biochem. Biophys. Res. Commun. 168, 747–755
7. Chou, C. H., Wang, J., Knuth, M. W., and Reeves, W. H. (1992) J. Exp. Med. 175, 1677–1684
8. Wang, J., Satoh, M., Chou, C. H., and Reeves, W. H. (1994) FEBS Lett. 351, 219–224
9. Griffith, A. J., Blier, P. R., Mimori, T., and Hardin, J. A. (1992) J. Biol. Chem. 267, 331–338
10. Wu, X. and Lieber, M. R. (1996) Mol. Cell. Biol. 16, 5186–5193
11. Wang, J., Dong, X., Stojanov, L., Kimpel, D., Satoh, M., and Reeves, W. H. (1997) Arthritis Rheum. 40, 1344–1353
12. Danska, J. S., Holland, D. P., Mairathasan, S., Williams, K. M., and Guidos, C. J. (1996) Mol. Cell. Biol. 16, 5507–5517
13. Han, Z., Johnston, C., Reeves, W. H., Carter, T., Wyche, J. H., and Hendrickson, E. A. (1996) J. Biol. Chem. 271, 14098–14104
14. Ono, M., Tucker, P. W., and Capra, J. D. (1994) Nucleic Acids Res. 22, 3918–3924
15. Reeves, W. H., and Stoegezer, M. Z. (1989) J. Biol. Chem. 264, 5047–5052
16. Wang, J., Satoh, M., Pierani, A., Schmitt, J., Chou, C. H., Stunnenberg, H. G., Roeder, R. G., and Reeves, W. H. (1994) J. Cell Sci. 107, 3223–3233
17. Davidson, H. W. and Watts, C. (1989) J. Cell Biol. 109, 85–92
18. Schweyer, M., Weil, R., Frank, G., and Zuber, H. (1980) J. Biol. Chem. 255, 5627–5634
19. de Vries, E., van Driel, W., Bergsma, W. G., Arnborg, A. C., and van der Vliet, P. C. (1989) J. Mol. Biol. 208, 65–78
20. Romero, F., Dargemont, C., Panz, F., Reeves, W. H., Camonis, J., Gisselbrecht, S., and Fischer, S. (1996) Mol. Cell. Biol. 16, 37–44
21. Hartley, K. O., Gell, D., Smith, G. C. M., Zhang, H., Divecha, N., Connelly, M., Admon, A., Lees-Miller, S. P., Anderson, C. W., and Jackson, S. (1995) Cell 82, 849–856
22. Smider, V., Rathmell, W. K., Lieber, M. R., and Chu, G. (1994) Science 266, 288–291
23. Taccioli, G. E., Gottlieb, T. M., Bhut, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P., and Jeggo, P. A. (1994) Science 265, 1442–1445
24. Kuhn, A., Gottlieb, T. M., Jackson, S. P., and Grummt, I. (1995) Genes Dev. 9, 193–203
25. Kuhn, A., Stefanovsky, V., and Grummt, I. (1993) Nucleic Acids Res. 21, 2057–2063
26. DePamphilis, M. L. (1988) Cell 52, 635–638